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FISHERIES AND AQUACULTURE AN ECOLOGICAL PERSPECTIVE

Editors:

Meera D. Ansal, Asha Dhawan
Vaneet Inder Kaur, Deepjyoti Baruah
Anuj Tyagi, Grishma Tewari
Naveen Kumar B.T. and Prem Kumar



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Collaborating Host Institute

**Guru Angad Dev Veterinary and Animal Sciences University (GADVASU)
Ludhiana, Punjab, India - 141004**

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राष्ट्रीय मात्स्यकी विकास बोर्ड National Fisheries Development Board

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मत्स्यपालन, पशुपालन एवं डेयरी मंत्रालय / Ministry of Fisheries, Animal Husbandry and Dairying
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About NFDB

The National Fisheries Development Board (NFDB) was established in 2006 as an autonomous organization under the administrative control of the Department of Fisheries, Ministry of Fisheries, Animal Husbandry and Dairying, Government of India to enhance fish production and productivity in the country and to coordinate fishery development in an integrated and holistic manner

What We Are

- Providing focused attention to fisheries and aquaculture (Production, Processing, Storage, Transport and Marketing).
- Achieving sustainable management and conservation of natural aquatic resources.
- Applying modern tools of research and development for optimizing production and productivity from fisheries.
- Training and empower women in the fisheries sector and also generate substantial employment.

Mandates

- To bring activities relating to fisheries and aquaculture for focused attention and professional management.
- To coordinate activities pertaining to fisheries undertaken by different Ministries/ Departments in the Central Government and also coordinate with the State/Union Territory Governments.
- To generate substantial employment.
- To train and empower women in the fisheries sector.
- To enhance contribution of fish towards food and nutritional security.

NFDB Mission

- Holistic development of the fisheries sector through enhancement of fish production and productivity; to supplement nutritious protein for the growing population; to accelerate the overall economy of the country, besides improving health, economy, exports, employment and tourism in the country

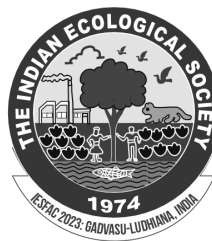
Schemes

- Pradhan Mantri Matsya Sampada Yojana (PMMSY)
- Group Accidental Insurance Scheme (GAIS)
- Fisheries and Aquaculture Infrastructure Development Fund (FIDF)
- Fish Market Price Information System (FMPIS)

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Preface

The Indian Ecological Society, Punjab Agricultural University (PAU) is organizing 3 days conference on “Fisheries and Aquaculture: An Ecological Perspective” (IESFAC-2023) from 22nd to 24th February, 2023 in collaboration with Guru Angad Dev Veterinary and Animal Sciences University (GADVASU) at Ludhiana, Punjab; which is first of its kind in the State to deliberate on strategic innovations and key issues related to eco-sustainability, economic viability and resilience of aqua food production resources. Socio-economic contribution of fisheries sector is evident from its contributions towards food security, malnutrition, livelihood sustenance, employment generation and economic growth at community, regional, national and global levels; in special reference to rural development in developing countries like India. In view of prospective role of the fisheries sector in fulfilling united nations sustainable development goals (SDGs) to attain “Zero Hunger” and “Good Health and Well Being”, it is vital to develop the sector in an eco-responsible manner. Further, conservation and sustainable use of aquatic resources advocated under the SDG- 14 “Life below Water” also relates to economic value of fisheries sector in many ways.

Veteran experts, scientists, young fisheries professionals, farmers and industry representatives will be sharing the conference platform to present latest innovative technologies, requirements, issues and strategies for sustainable development of the sector; under different theme areas covering aquaculture production systems; sustainable aquafeeds and feed technologies; genetics, breeding and biotechnology, aquaculture environment/health management and one health; responsible aquaculture and climate resilience in fisheries; aquatic ecosystem assessment, management and conservation; processing, value addition, quality assurance and marketing; and capacity building and socio-economic empowerment. National and international experts have contributed their research findings and experiences for wider sharing and debate for fruitful linkages and applications. Keynote and lead speakers from esteemed national and international institutes will interact with the participants on important issues related to human resource requirements; sustainable economic utilization of aquatic resources; species and production system diversifications; organic recycling based integrated aquafarming technologies; quality assurance; economic and smart feed technologies; AMR and responsible aquaculture; processing towards circular bioeconomy; marketing and export; and doubling farmers income through food and ornamental fish/shellfish culture in freshwater, brackish water and marine water resources.

An effective forward and backward feedback system is required to address need based regional requirements of the stakeholders to remove weak links within the supply chain and in this context an “Academia-Farmer-Industry” interface during the conference will help in spotlighting required actions to reinforce the sector in interest of stakeholder’s income, national economy, ecosystem services and eco-responsibility. Although, intensive farming systems are inevitable for achieving food production targets to feed the human and livestock population for overall human welfare, but at the same time it is important to monitor environmental wellbeing, public health and sustainability of our food production resources; which have been overexploited and polluted without realizing the future consequences.

The proceedings of this conference consist of two parts, including the keynote and lead lectures covering the conference theme areas in the first part and the contributed original research abstracts received from the participating scientists and fisheries professionals in the second part.

Overall, the conference is expected to put forward climate smart eco-friendly aqua-food production systems to contribute its share towards regional/national/global food and nutritional security, with add on positive impacts on the environment. Optimal economic production from any food system within the realms of environmental wellbeing, climate change, public health and food safety, can only be successful if all that is produced is judiciously utilized as human food, livestock feed, manure or for any other economic purpose through circular bio-economy, with minimal wastage. We hope to achieve the envisioned objectives of sustainability through eco-responsibility and congruent strategic innovations or technological advancements in an equitable manner.

Editors

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Fisheries for Prosperity



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Fisheries Education in India – Human Resource Gap Analysis and Planning for Sustainability

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Introduction

Fisheries and aquaculture remain important sources of food, nutrition, income and livelihoods for hundreds of millions of people around the world. Total fish production of India touched 14.73 million metric tonnes in 2020-21 with a contribution of 11.25 million metric tonnes (76.37%) from inland sector. The sunrise fisheries sector contributes about 1.24 % to National Gross Value Added (GVA) which is Rs 2.11 lakh crores and 7.28 % to agricultural GVA (2021-22) while engaging over 16 million people (National Fisheries Policy 2020) at the primary level and almost double the number along the value chain (Fishermen population India-2,80,63,538- 82% Inland, 18%- Marine, 2020-21). During 2021-22 the export of marine products that includes production from aquaculture reached 13.6 lakh tonnes valued at INR 57,586 crores (US \$ 7.76 billion). The inland sector grew at a compounded annual growth rate (CAGR) of nearly 6.5% between 1979 and 2019, while overall fish production witnessed 4.5% growth, which is double the growth rate of food grains production in India. Post-2014, transformational reforms were brought to the fisheries sector which focused on holistic development of the fisheries sector while also ensuring the socio-economic well-being of fishers, fish farmers and other stakeholders. Recognising the importance of the fisheries sector, the Department of Fisheries was created in February 2019 to provide sustained and focused attention to the development of this sector. This was followed by the creation of an independent Ministry of Fisheries, Animal Husbandry and Dairying in June 2019.

The fisheries potential of Indian marine sector at an estimated 3.72 million metric tons along with the estimated 10.43 million metric tons of inland fisheries potential is combinedly contributing to an overall massive potential of 14.16 million metric tons (in 2019-20) in India. Over the years, the contribution from inland fisheries was almost three-fold higher than the marine sector. However, the utilization of resources from the inland fisheries was much lower (58%) as compared to that of the marine (71%) sector. There is large untapped potential in fisheries and aquaculture, which can immensely contribute towards employment and livelihood security of fishers and fish farmers.

Human Capital in Fisheries Sector

A number of researchers reported manpower requirements in fisheries sector, their estimates widely varying from a few thousand to hundreds of thousands of persons of varying training, education and skill levels (Chidambaram 1985, James 1987, Kohli 1998, Thakur et al 1997, Ayyappan and Biradar 2000). A study by ICAR-Central Institute of Fisheries Education (CIFE) had made a more systematic effort to project the requirement of fisheries graduates for 2020 and estimated need for 10,457 against a supply of 4,570 (Sukham 2010). The enormous potential of fisheries sector can be fully realized only if competent professional human resource is available. This skilled manpower is generated by Fisheries Colleges and Universities across the country which are funded centrally by ICAR and by State Governments. Human capital alters the productive capacity of the economy by changing size or productivity of the workforce, thus structurally impacting the economy. Human resource accounting focuses on the measurement of cost and value of the people in an organization. Universities represent the higher education sector and the primary source of human capital.

Human Resource Gap

Supply of Fisheries Professionals in India

Presently, fisheries education is offered in the country through thirty two professional fisheries colleges in India which constitute State Agricultural Universities and two Central Universities viz. Central Agricultural University (CAU), Imphal, Manipur and Dr. Rajendra Prasad Central Agricultural University (Dr. RPCAU), Samastipur, Bihar and one deemed-to-be university ICAR- Central Institute of Fisheries Education (CIFE), Mumbai in India.

The current intake capacity of all these colleges and institutes is about 1500 for Bachelor of Fisheries Science (B.F.Sc.), about 425 for Master of Fisheries Science (M.F.Sc.) and about 185 for Ph.D. programmes while the outturn is about 80-85% of intake capacity. The B.F.Sc. degree course is offered in 30 out of 32 colleges/institutes. Out





Table 1: Details of Institutions offering degree in fisheries science across the country

No.	College Name	Location	Affiliation	UG Intake	PG Intake	Ph.D Intake	Passed UG's	Passed PG's	Passed Ph.D's	No. of Dep.	Faculty Sanctioned Strength	Faculty Present Strength	Estd
1	College of Fisheries	Mangalore, Karnataka	Karnataka Veterinary Animal and Fisheries Sciences University	42	35	18	1296	642	133	6	81	31	1969
2	Fisheries College and Research Institute	Thoothukudi, Tamil Nadu	Tamil Nadu Fisheries University	40	28	25				10	50	39	1977
3	College of Fisheries	Panangad, Kerala	Kerala University of Fisheries and Ocean Studies	55	45					10	51	24	1979
4	College of Fisheries	Rangailunda, Orissa	Orissa University of Agriculture and Technology	48	10	4	546	103	11		15	13	1981
5	College of Fisheries	Ramagiri, Maharashtra	Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth	40	28	11	960	315	12	3	44	39	1981
6	College of Fishery Science	Pantnagar, Uttarakhand	GB. Pant University of Agriculture & Technology	25	15	10	425			5	17	14	1985
7	College of Fisheries	Dholi, Bihar	Rajendra Agricultural university	25	NA	NA	200	NA	NA	6	18	15	1986
8	College of Fisheries	Raha, Assam	Assam Agricultural University	25	12	3	354	7	NA	6	15	14	1988
9	College of Fisheries	Veraval, Gujarat	Junagadh Agricultural University	79	24	6	300	60	2	5	26	18	1991
10	College of Fishery Science	Muthukur, Andhra Pradesh	Sri Venkateswara Veterinary University	30	10						15	12	1992



11	College of Fishery Sciences	Kolkatta, West Bengal	West Bengal University of Animal and Fishery Sciences	44	34	16	370	233	3	8	32	17	1995
12	College of Fisheries	Lembucherra, Tripura	Central Agricultural University	36	30	3				6	32	27	1998
13	College of Fisheries	Udaipur, Rajasthan	Maharana Pratap University of Agriculture and Technology	30	10	4	54	37	1	5	7	5	2003
14	College of Fisheries	Srinagar, Kashmir	S.K. University of Agricultural Sciences and Technology	40	6		180	12	8	9	49	19	2005
15	College of Fishery Science	Nagpur, Maharashtra	Maharashtra Animal & Fishery Sciences University	32	NA	NA				6	NA	10	2007
16	College of Fisheries	Faizabad, Uttar Pradesh	Narendra Deva University of Agriculture and Technology	40	NA					7	6	5	2007
17	College of Fisheries	Udgir, Maharashtra	Maharashtra Animal & Fishery Sciences University	32	NA	NA				6	NA	8	2007
18	College of Fisheries	Ludhiana, Punjab	Guru Angad Dev Veterinary & Animal Sciences University	40	14	9	145	56	10	5	20	19	2008
19	College of Fisheries	Kawardha, Chhattisgarh	Chhattisgarh Kamdhenu Vishwavidyalaya	45	NA						12	2	2010
20	College of Fishery Science	Jabalpur, Madhya Pradesh	Nanaji Deshmukh Veterinary Science University	30	NA								2012



21	Fisheries College and Research Institute	Ponneri, Tamil Nadu	Tamil Nadu Fisheries University	40					200				10			13	2013
22	College of fisheries Science and Research	Eitawah, Uttar pradesh	Chandra Shekhar Azad University of Agriculture & Technology	35	NA	NA			50	-			-	13		8	2015
23	College of fisheries Engineering	Nagapattinam campus	Tamil Nadu Fisheries University	20					55	-			6			6	2015
24	Institute of Fisheries Post Graduate Studies	Chennai (Vaniyanchavadi), Tamil Nadu	Tamil Nadu Fisheries University	-	4	-										6	2017
25	Institute of Fisheries Biotechnology in Fisheries Biotechnology)	Chennai (Vaniyanchavadi), Tamil Nadu	Tamil Nadu Fisheries University (BTech)	22	-	-										3	2017
26	College of Fisheries	Pebbar, Wanaparthy district, Telangana	P. V. N. R. Telangana State Veterinary University	30	-	-			30	-			7	18		5	2017
27	College of Fisheries, Gumla	Gumla, Jharkhand	Birsa Agricultural University	30	-	-			30	-			7	13		10	2017



Recently established colleges

28	Fisheries College and Research Institute	Thalainayiru, Tamil Nadu	Tamil Nadu Fisheries University	40	-	-	-	-	-	-	-	7	2018
29	College of Fish Nutrition & Food Technology (BTech in Food Technology)	Chennai (Madhavaram), Tamil Nadu	Tamil Nadu Fisheries University (BTech)	40	-	-	-	-	-	-	-	-	2018
30	College of Fisheries, Kishanganj, Bihar	Kishanganj, Bihar	Bihar Animal Science University (BASU)	40	-	-	-	-	12	85	27	2018	
31	Faculty of Marine Sciences	Chidambaram, Tamil Nadu	Annamalai University	40	-	-	-	-	-	36	-	2016	
32	Faculty of Fisheries (Private)	Parlakhemundi, Odisha	Centurion University of Science & Technology	30	-	-	-	-	-	15	-	2017	

(Source: Compiled and updated by Authors, 2022)





of 20 colleges/Institutes that offer PG programmes, only 13 conduct doctoral programmes. Aquaculture, Fisheries Resources Management, Aquatic Animal Health, Aquatic Environment Management and Processing are commonly offered specialisations at PG level. The CIFE offers M.F.Sc. and Ph.D. programmes in 11 specialised disciplines (Table 1)

Estimated Demand for Fisheries Human Capital-2025

There is a lot demand for skilled manpower in fisheries sector. Lakra et al (2014) projected stock requirements of fisheries in various sectors for 2020-30 and it was found that the actual stock from supply was 5,144. Ananthan et al (2017) projected that there is a need for increasing the present annual intake capacity of B.F.Sc. while almost maintaining the intake of M.F.Sc. and Ph.D. programmes till 2025 (Table 2)

Table 2: Demand projections for fisheries human resource by 2025

Sub-sectors	Estimated Demand			
	Diploma	Bachelors	Masters	Doctoral
Fish and shrimp seed hatcheries & disease diagnostic labs	8,670	2525	620	34
Aqua feed industry	2,945	557	180	25
Aquaculture	25,400	5,550	200	
Fish processing	15,800	250	250	
Development and Extension	17,450	4,685	460	15
Research and Academics			670	425
Culture based fisheries	3,540	665		
Mariculture	2,240	597	220	
Coldwater fisheries and aquaculture	1050	250		
Retail fish outlets	950	820		
Financial Institutions		776	32	
Total	78,045	16,675	2,632	499

Demand supply mismatch can be seen. There is no reliable data on the present intake / outturn of Diploma holders, though it is estimated to be <5000. There is a need to strengthen fisheries polytechnics and establish more to increase the intake 10-12 times the present capacity. This has to be done in tandem with vocational fisheries education in schools in light of the New Education Policy 2020.

Planning Sustainability

With half the Indian population comprising of less than 25 years old, demand for more universities and quality higher education becomes obvious. But, public investments in higher education (in real terms as % of GDP spent on education) over the years has neither stagnated nor declined. In this context, it has become imperative to estimate the direct and indirect impact of universities (i.e., their output - human capital) help justify increase/decrease in budgetary allocation to higher education in the India.

The fisheries sector grew at the CAGR of 3.45% and 4.65% during 1992-2016 and 1992-2019 respectively, while the share remained about 1.1% and 1.07%. Translating the percentage to monetary values, it can be indicated that this growth rate translates into Rs. 0.08 lakh crores and 0.55 lakh crores (55,000 crores) as a result of growth of fisheries sector in overall GDP between the two years (2015-16 and 2016-17) and (2020-21 and 2019-20). We have tried to quantify the contribution of CIFE in terms of HRD in fisheries and its contribution to Indian economy using a novel methodology. ICAR-CIFE contributes 8.9% of professional Human Resource Development (HRD) in fisheries that translated to Rs.299-449 crores to India's GDP in 2020-21 in case if it was assumed that only 50-75% of the fisheries sector were actually manned by professional graduates (B.F.Sc, M.F.Sc and PhDs) (Qureshi et al 2021). However, the realistic scenario would be somewhere between 50% and 100%. Also it can be seen that the multiplier effect of investment of CIFE was 4 times during 2008-14 and almost 5.3 times during 2015-2021. A brief representation is given in figure 2. This gives the impact of human capital produced by a University or College. We are trying to assess the same for other fisheries colleges as well so that a macro perspectives can be drawn from the results. These results will help us quantify the input and output of Universities and identify bottlenecks where policy interventions are required; be it intake capacity, seats allocation, expenditure incurred on teaching, research, developmental activities, infrastructure etc or percentage contribution to HRD sector. This will ensure sustainability in producing human capital for fisheries.



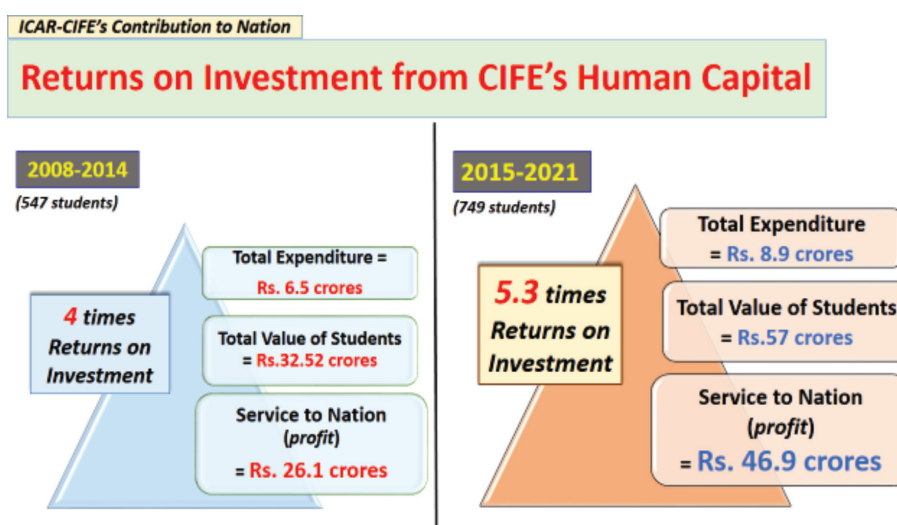


Figure 1: Returns on investment from CIFE's Human Capital (Qureshi, 2021)

Conclusion

Fisheries education in India has rapidly picked up the pace. With the objective of increasing fish production and sustaining the booming seafood exports, the challenge is to increase domestic consumption and cater to the nutritional security of the country and provide cheap fish to all. This requires concerted efforts for providing adequate and professionally skilled human capital. Universities represent the higher education sector which generate skilled/professional human capital and in India there are three fisheries universities and ICAR-CIFE is the first and only National University catering to the higher education sector. There are total 32 fisheries colleges in India. ICAR-CIFE with its world class facilities is envisioned to provide leadership in fisheries education and research. It produces about 8.9% of the professional human capital pool available in the country. In the last 2.5 decades, the country is getting a benefit of 5.3 times the investment made from CIFE's students.

As the next two decades are going to be an era of entrepreneurship, it is recommended that there is a strong scope of developing a niche environment for inculcating the seeds of entrepreneurship among students of all fisheries colleges in India which will ensure job creation for other fisheries professionals as well. Focus has to be given on generating quality students which in turn will add more value to the country. There is a need to address gaps in B.F.Sc. and M.F.Sc. programs and revise course curriculum periodically as demanded by the sector. Emphasis has to be made on enhancing the quality of human capital produced by stressing on upgrading the skills through various capacity building programmes which will further ensure governments' investment into the Fisheries HRD such that it has a substantial multiplier effect on the fishery economy.

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Ecosystem Approaches for Sustainable Economic Utilization of Inland Aquatic Resources

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Introduction

Inland fisheries have a tremendous impact on the livelihoods and food security of people all over the world, and they also offer important economic and recreational benefits. These irreplaceable resources are in jeopardy because there is a lack of research-based understanding of how fisheries impact inland ecosystems and how human activities in inland waterways affect fisheries and aquatic biodiversity throughout inland water resources. The competition for water resources is growing in both rich and developing countries which causes supply shortages and various sorts of contamination. The nutritional potential of aquatic resources is essential in underdeveloped countries. To deliver on this promise, integrated research and management for sustainable water resource use are needed. For this, a solid understanding of the structure and function of aquatic ecosystems is required. A programme emphasizes the interactions between the physical, chemical, and biological components of aquatic systems and their catchments. The following 16 stages—each of which is divided into 5 phases—make up the program: System description, System functioning and modelling, Resource assessment/dynamics, Resource potential and Resource consumption for sustainability. In order to create a better world and better lives, the Sustainable Development Goals (SDGs) of the United Nations serve as a unifying call for action. Therefore, it might be more challenging to realize the “future we seek” if significant natural resources and services, which are not mentioned directly in the SDGs, are not taken into account. If their direct, intrinsic value is not recognised, the sustainability of the services that various natural resources provide and their contributions to the SDGs may be challenged. Here, using inland aquatic ecosystems and the fish and fisheries that live within them as an example, we explore the possibility of acknowledging and including additional natural resources that are missing from the SDGs. Important resources are less likely to be included in global, national, and regional goals, conversations, and policies if the SDGs are not in place. We offer a variety of options on how to better capitalise on the contributions that these underutilised natural resources made while the SDGs and other international instruments were being implemented.

An essential component of the ecosystem management plan is a grasp of the surrounding environment, particularly the cultural variety of “people” (Millennium Ecosystem Assessment 2003). To understand the dynamic of ecosystems, including how they work, the ecosystem approach (EA) requires the adaptive management process. As a result, the management strategy has to be flexible so that it can respond to various uncertainties and incorporate components of “learning by doing” or be based on research input. In order to guarantee that management decisions are made in the framework of a precautionary approach, research was conducted to gain a better understanding of the production function of ecosystems in general, its many components, connectivity, and information requirements. The ecosystem approach is predicated on the “application of relevant scientific approaches focused on layers of biological organisation, which cover the fundamental structure, processes, functions, and interactions among organisms and their environment” (Convention on Biological Diversity 2004). EAs that are important to consider include ecosystem-based management, sustainable forest management, integrated river-basin management, integrated marine and coastal area management, and ethical fishing practises. In what industries and biomes do these techniques help the use of the ecosystem approach? Consider regional initiatives like biosphere reserves, protected areas, and single-species conservation programmes, as well as other national policy initiatives that might be used in the framework of the ecosystem approach to deal with challenging problems. Since the ecosystem approach depends on local, national, regional, and international circumstances, no one method is used in its application (CBD 2004).

The term “ecosystem approach” was initially used in the early 1980s and was officially accepted at the Earth Summit in Rio de Janeiro in 1992, when it was adopted as a concept by the Convention on Biological Diversity (CBD). Later, it was defined as “A strategy for the integrated management of land, water, and living resources that encourages conservation and sustainable use in an equitable manner” (CBD 2004). The CBD is the first international agreement that approaches the preservation of biodiversity and sustainable usage from an ecosystem-based, holistic perspective. The ecosystem approach is described in the Convention on Biological Diversity, 2004, as “a strategy for the integrated management of land, water, and living resources that supports conservation and sustainable use in an equitable fashion. Thus, by utilising the ecosystem approach, it will be possible to balance out the three goals of the Convention—conservation, sustainable use, and fair and equal distribution of the benefits from the use of genetic





resources. Twelve guiding concepts of EA are as follows:

Principle 1: A society's decision will determine the goals for managing land, water, and life resources. Negotiations and trade-offs between stakeholders with varying perspectives, interests, and intentions influence this. It is important to address the interests of all relevant societal groups fairly, which may include delivering various outcomes at various times or in different places.

Principle 2: Decentralize management to the lowest practicable level. Systems that are decentralised might be more effective, efficient, and equitable. Management should consult with all parties and strike a balance between regional and national objectives. Those who represent the relevant communities of interest should make the decisions, and those with the authority to carry them out should manage.

Principle 3: Ecosystem managers should take into account the effects of their actions (actual or projected) on nearby and neighbouring ecosystems. For institutions involved in decision-making, new arrangements or organisational structures are needed to reach, if necessary, the right compromises.

Principle 4: Given the potential benefits of management, it is typically necessary to comprehend and manage the ecosystem from an economic perspective. Any such ecosystem-management programme should (a) lessen market distortions that have a negative impact on biological variety, (b) align incentives to promote biodiversity conservation and sustainable usage, and (c) internalise costs and benefits as much as possible within the given ecosystem.

Principle 5: The ecosystem approach should prioritise maintaining ecosystem structure and function in order to maintain ecosystem services. Dynamic interactions between species, species and their abiotic environment, as well as physical and chemical environmental interactions.

Principle 6: Ecosystems must be regulated within the parameters of how they can operate. The environmental factors that affect ecosystem structure, functioning, and diversity should be taken into consideration.

Principle 7: The ecosystem approach should be used at the proper temporal and spatial scales, according to principle 7. The forces that drive ecosystems, including those brought on by human activity, vary in space and throughout time, necessitating management at multiple scales to achieve management goals.

Principle 8: Long-term goals for ecosystem management should be established in light of the diverse temporal scales and lag effects that define ecosystem processes. The inherent tension with people's propensity to prioritise present advantages over long-term ones.

Principle 9: Management needs to understand that change is inevitable. Ecosystems change naturally and inevitably, so management goals should not be seen as fixed results but rather as preserving natural ecological processes.

Principle 10: The ecosystem approach should aim to integrate the preservation and exploitation of biological variety while also striking the right balance between the two. The commodities and services provided by ecosystems, on which humans ultimately depend, include biological resources.

Principle 11: The ecosystem approach should take into account all pertinent information, including scientific data as well as indigenous and local knowledge, innovations, and practises.

Principle 12: The ecosystem approach should incorporate all pertinent societal spheres and academic fields. Management of biological variety is complicated and requires stakeholders at the local, national, regional, and international levels as necessary. Gomez et al (2016) provided a thorough analysis of the socio-ecological systems that connect ecosystem structure, functions, and benefits to humans and other living things as the ecosystem evolves. According to the authors, social processes such as markets and governing institutions, which are themselves influenced by a number of factors, result in the demand for and use of naturally occurring services (such as population and economic growth, climate change, technological progress, etc.). Ecosystems were put under strain as a result of these needs and services, which led to further modifications in the way that they are structured and operate. These issues can be answered by examining how ecosystems' potential to continue providing ecosystem services—services that are essential to human life, the social system, and the ecological system itself—and how those services impact people's quality of life. This can clarify how ecosystems work as well as how changes brought about by human activity are related to sustainability and human well-being. Consequently, a paradigm change is recognised when management shifts from sectoral to ecosystem-based. Whereas it depicts everything from a single species to an ecosystem, a single spatial scale to multiple scales, a short-term perspective to a long-term perspective, a human-independent ecosystem to a human-integrated ecosystem, management divorced from research to adaptive management, and management commodities to sustained production potential for ecosystem goods and services (Lubchenco 1994, Sherman and Duda 1999). Ecosystem-based management, as defined by Christensen et al (1996), is "management



driven by explicit goals executed by policies, protocols and practises, and made adaptable by monitoring and research based on our best understanding of the ecological interactions and processes necessary to sustain ecosystem structure and function.” When creating the Uganda Water Action Plan, one of the first Integrated Water Resource Management planning programmes launched after the Rio Conference, in 1993, the five-step model was employed. This approach is frequently portrayed as a cycle for Integrated Planning and Management. These are (i) issue assessment and identification, (ii) programme development, (iii) formal adaption and operation, (iii) implementation, and (iv) evaluation (Joint Group of Experts on the Scientific Aspects of Marine Pollution 1996). Following the adoption of these strategies, the Global Water Partnership was developed (Jonch-Clausen 2004).

Inland Fisheries between Ecology and Economy

From less than 0.01% of the total volume of water on earth, inland capture fisheries and aquaculture produce more than 40% of the world’s recorded finfish. Millions of people around the world depend on these fisheries for their livelihood. However, given the challenges of measuring aquatic biodiversity, particularly in developing nations and distant regions, it is possible that inland fish populations are more diversified than previously thought (Cooke et al 2012). Additionally, according to Vörösmarty et al (2010), 65% of inland habitat is moderately or seriously endangered by anthropogenic stressors, meaning populations could vanish even before they are recorded.

According to estimates by Pauly and Christensen (1995), 8% of the world’s primary marine productivity or in other words, the portion of primary productivity that is converted into human food, sustains worldwide fishing. This percentage rises in coastal regions and places with increasing currents (between 24 and 35%) and decreases in the open ocean (around 2%) to confirm the greater productivity of these latter environmental systems in terms of resources used by humans. For maximising the use of resources, inland fisheries still requires more research than that of marine fisheries.

Role of Inland Fisheries towards Sustainable Developmental Goals

The aquatic ecosystems that support inland fish and fisheries, as well as other inland aquatic creatures (Lynch et al 2020, Dickens et al 2020 and Tickner et al 2020), are the frequent underutilised natural resources that provide significant contributions to the SDGs. It is difficult to assess their value and faithfully represent them in policy decisions due to their essential characteristics, such as their great species variety, diverse and divergent fisheries, and definitions of sustainability. However, given the abundance of inland fish and fisheries and their significance in many parts of the world (Cooke et al 2016), it is worthwhile to look for solutions to these problems and chances to raise awareness of their values (Dickens et al 2020). This concept piece has two goals: first, to emphasise that inland fish and fisheries, while widely believed to be covered by the SDGs and other global policies, are actually gravely underrepresented and as a result, continue to be degraded without proper consideration; and second, to note how paying more attention to natural resources, which have been left out of the SDGs, could still help countries implement policies and actions that deliver on the goals. We identify methods through which missing natural resources might be accounted for during the implementation and reporting of the SDGs using inland fish, fisheries, and aquatic ecosystems as examples. We also provide alternative strategies for safeguarding these natural resources by emphasising their value for the SDGs and, more broadly, for achieving global sustainability. These procedures would be very helpful to developing countries because of how important inland fisheries are to them (Funge-Smith and Bennett 2019). The choices considered here might act as a template for other natural resources that are not included by the SDGs.

Inland Fisheries and Food Security

Fish from the inland are especially crucial for resolving “hidden hunger” (micronutrient deficits and the associated health problems) (Kennedy et al 2003). When other food sources are unavailable or too expensive, inland fishes can be a good source of protein, omega-3 fatty acids, calcium, vitamin D, iron, zinc, and lysine (Youn et al 2014). Small fish eaten whole, especially in underdeveloped nations, provides a significant source of minerals (including calcium and vitamin A) that are challenging to obtain from other dietary sources (Roos et al 2007). Inland fish consumption has been demonstrated to lessen the symptoms of various diseases linked to micronutrient deficiencies like rickets in Bangladeshi children (Craviari et al 2008). Small indigenous fish (SIFs) were found to have a significant influence in riparian populations’ nutritional security according to an extensive research by ICAR-Central Inland Fisheries Research Institute (CIFRI) on tribal tribes in the Sundarbans (Mohanty et al 2011). They also offer year-round nutrient sources because these small inland fish may easily be dried or kept, such as dried kapenta (*Limnothrissa miodon*; *Stolothrissa tanganicae*) in Zambia (Musumali et al 2009). In addition to being directly consumed by people, inland fish can also be utilised as feed for animals and aquaculture operations. When dagaa (*Rastrineobola argentea*) is unfit for human food, it is, for instance, sun-dried and used as chicken feed near Lake Victoria.



Inland Fisheries and Economic Security

Fundamental to food sovereignty worldwide are the inland capture fisheries and aquaculture. In many regions of the world, inland capture fisheries are the last alternative when primary revenue sources falter for a variety of reasons, including economic changes, conflict, natural disasters, and water development projects. They act as social safety nets, offering additional or substitute sources of funding, work, and food (Welcomme et al 2010). Along with providing cash and means of subsistence through direct fishing, inland fisheries also create significant amount of money and open up possibilities of employment through ancillary services including the provision and upkeep of fishing equipment as well as jobs in processing and distribution (Welcomme et al 2010). The economic worth of inland fisheries as a whole is increased by secondary operations that raise the market value of the fish products. Recreational fishing and tourism in particular have significant economic multiplier impacts on the experience activities in addition to the market worth of the fish (Southwick Associates 2013). Since fish are frequently traded locally or consumed directly by fishing families, statistics on the economic impact of inland catch fisheries are few (FAO and WorldFish Center 2008). The worth of specific inland fisheries can offer some indication of their economic significance. For instance, a study of six river basins in West and Central Africa discovered local capture fisheries supported 227 000 full-time fishers and had a first-sale value of US\$295 million (Neiland and Bene 2006). With a first sale price of US\$7 billion, the Lower Mekong Basin's entire fish production is estimated to be around 3.9 million tonnes. Perhaps more significantly, the importance of inland fisheries goes beyond economic data since they play a crucial non-financial function in subsistence situations where no money transactions take place.

Ecosystem Function and Biodiversity

Fish live in nearly all significant aquatic ecosystems (Helfman et al 2009). Inland fish can be extremely important to the health of their ecosystems (Dudgeon et al 2006). For instance, the composition of the fish community is significantly impacted by predatory species like northern pike (*Esox lucius*) (He and Kitchell 1990). According to research by Wittmann et al (2014), herbivorous grass carp (*Ctenopharyngodon idella*) can change aquatic flora, and flannelmouth characin (*Prochilodus mariae*) can affect sedimentation rates in Andean streams (Flecker 1997). Migratory fish species including Pacific salmon (*Oncorhynchus* spp.), alewife (*Alosa pseudoharengus*), and *Semaprochilodus* spp. move energy and nutrients to maintain far-off aquatic and terrestrial food webs impacting the habitat on a larger scale (Wipfli and Baxter 2010). When in good condition, inland ecosystems offer people a variety of beneficial services (Pandit et al 2015).

About 40% of all fish species and 20% of all vertebrate species are inland fishes (Helfman et al 2009). At both the species and population levels, inland fish biodiversity offers significant advantages. Natural catastrophes and other disturbances to those ecosystems can be devastating when humans depend on them for their fundamental necessities. Resilient natural ecosystems bounce back swiftly from such disruptions. The relevance of diversified inland fish communities is shown by the ecosystems with higher resilience, having high species richness (Downing and Leibold 2010). The effects of disturbance on fish populations are moderated by a number of other factors in addition to species assemblages. It has also been demonstrated that fish populations of the same species with a diversity of biologically significant traits (such as alternate life histories) are more resilient to disturbances (Schindler et al 2010). For instance, Kovach et al (2015) discovered temporal trends in the timing of the migration of Pacific salmon species in southeast Alaska. When calamities strike, these diversified, resilient inland ecosystems provide dependable sources of food; as a result of climate change, their importance will increase. Aquaculture also benefits from biodiversity. The ability to create transgenic fishes, like the genetically modified Atlantic salmon (*Salmo salar*), which grows faster and consumes less food than non-modified fish, depends on the genetic variety within a species, which serves as the foundation for selective breeding and stock development (Gjedrem 2000). Future inland aquaculture will place significance on biodiversity as a result of technological advancements like transgenic fish that require a variety of genes from the wild. Furthermore, technology is increasingly being employed to protect biodiversity from escaping aquaculture fish.

Inland Fisheries as Aquatic Canaries

Inland fish are excellent indicators of ecological change due to their key role in aquatic environments. Inland fish are utilised as indicators of the present and future effects of environmental change on human well-being, much like the classic "canary in the coal-mine." Beyond overfishing, anthropogenic influences on aquatic ecosystems, both direct and indirect, are present and may have unfavourable effects. 65% of freshwater environments are at danger due to threats such as eutrophication, flow alteration, habitat destruction or degradation, and invasion by exotic species (Dudgeon et al 2006, Vörösmarty et al 2010). Due to the proximity of many anthropogenic activities (such as agriculture, deforestation, and hydropower) to inland aquatic habitats and the fact that aquatic habitats include



environmental influences from all throughout a watershed, these hazards are extremely widespread (Allan 2004).

Some environmental stresses; including chemical and thermal pollution, flow regime change, and temperature change; cause a direct response in fish (Dudgeon et al 2006). Fish also have an indirect reaction to environmental stresses. Large ecological changes taking place in the Laurentian Great Lakes, for instance, were brought to the attention of public and politicians in the 1960s by the catastrophic die-offs of introduced alewives in Lake Michigan. Inland fish populations and species assemblages around the world frequently signal changes in fertiliser inputs to their watersheds (Ludsin et al 2001).

Inland fish are important bioindicators of ecosystem health because they respond to numerous changes in the aquatic and terrestrial environments throughout their watersheds. The Index of Biotic Integrity (IBI) is a frequently used in-situ freshwater evaluation method that takes into account the local fish assemblages, notably the presence or absence of species that are sensitive to habitat perturbations (Karr 1981). Inland fish species are frequently employed as laboratory models to evaluate water quality and environmental toxicology in the chemical and pesticide approval processes because of their representative vulnerability to various toxins and important function in aquatic ecosystems (Barbour et al 1999).

Inland Fisheries as Source of Green Food

Inland fish can be regarded as a component of the “green food” movement for more ecologically friendly food source when they are sustainably caught or raised. When compared to replacement livestock products, the environmentally damaging effects of wild inland fish harvesting can be quite low (Orr et al 2012). In contrast to many other food sources, most inland catch fisheries (both harvest and consumption) show a low dependence on fossil fuels for the production of fishing gear, transportation to and from fishing locations, preservation, and post-harvest fish transportation (Welcomme et al 2010).

The food conversion ratio of sustainable aquaculture is also higher than that of poultry (2:1), pigs (4:1), and cows (7:1) for both herbivorous and omnivorous species (around 2 kg of dry feed every 1 kilogram of growth) (Brown 2002). Inland aquaculture species tend to belong to the lower trophic level to that of the marine aquaculture species, and therefore depend on more sustainably sourced feed, which is another key point to remember (e.g., algae, not wild caught fish). The production of about one million tonnes of fish and nearly 10 million tonnes of rice using more ecologically friendly management techniques is an example of an integrated food system that incorporates sustainable inland aquaculture. This approach is used in China alone (Weimin 2010).

Although not all inland capture fisheries and aquaculture operations have low environmental effect or sustainable management. According to Allan et al (2005), there are several instances of unintended bycatch and overfishing of inland fish populations (Stoot et al 2013). Additionally, some careless inland aquaculture activities, like fertiliser loading, releasing cultivated species, and disease transmission, can have a large negative influence on the environment (Kapuscinski and Brister 2000). Carps, the most widely cultivated species group, for instance, have a long history of having an impact on the environment, including invasions and changes to local aquatic communities that present difficulties for managers of fisheries all over the world (Rasmussen et al 2011). Despite concerns, inland aquaculture and catch fisheries have modest environmental costs when compared to many other food sources that come from animals.

Ecosystem Approach

To assess and manage resources holistically, including their habitat features, taking into account the ecological interactions of targeted creatures and fishes with varying effects on these processes, a thorough ecosystem-based approach is necessary (Zhang et al 2009). For the assessment of fisheries resources in Korean waters, Zhang et al (2009) devised a commendable ecosystem-based approach that focused on three management goals: sustainability, biodiversity, and habitat quality (Fig. 1). Additionally, Alsolami et al (2020) combined three management goals—sustainability, biodiversity, and habitat—with the socioeconomics as extra goals (Fig. 2).

Valuation of Resources

Ecosystem approaches for sustainable management of inland aquatic resources requires proper valuation for rational economic utilization. Economic valuation of inland aquatic resources hints at the interactions between nature and people, the trajectory of resource use patterns in the future to guide relevant policy recommendations locally and globally. Economic valuation for ecosystem-based approach necessitates placing nature first and promotes robust interaction among different stakeholders, knowledge systems and governments. The MEA Approach guides both short term and long-term strategies and interventions for valuing ecosystem services and functions. Four types of

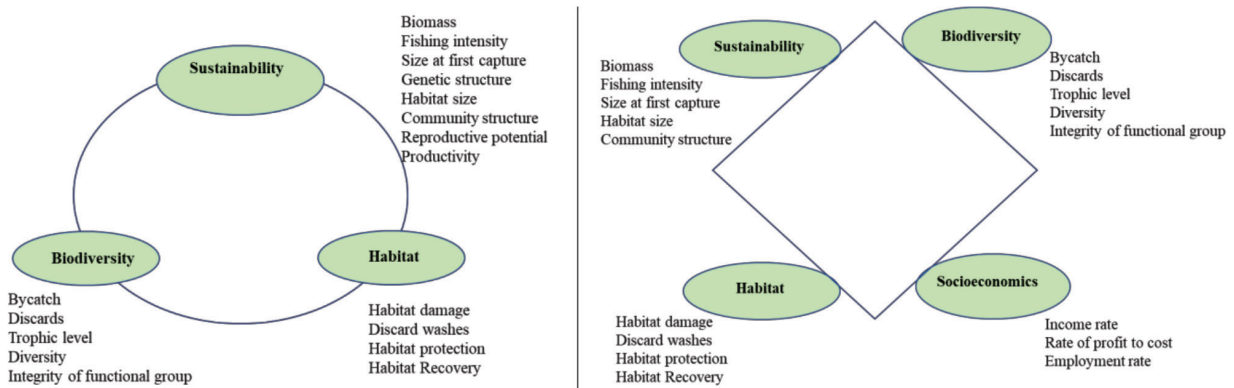


Fig. 1 (left) and Fig. 2 (right) Identification of objectives and attributes for ecosystem-based fisheries management (adopted from Zhang et al 2009, Alsolami et al 2020)

ecosystem services are provided by nature in supporting human well-being such as provisioning (e.g. food, water); regulating (e.g. climate, water, disease regulation); cultural (e.g. spiritual, aesthetic); and supporting (e.g. primary production, soil formation) services. Economic valuation of inland aquatic resources is also based on these principles. The Total Economic Value (TEV, Fig. 3) Framework comprises use and non-use values where TEV refers to the total gain in wellbeing from a policy measured by the net sum of the willingness to pay (WTP) or willingness to accept (WTA). The value that is generally measured for purposes of appraisal by governments is the total value of a marginal change in the underlying ecosystem services.

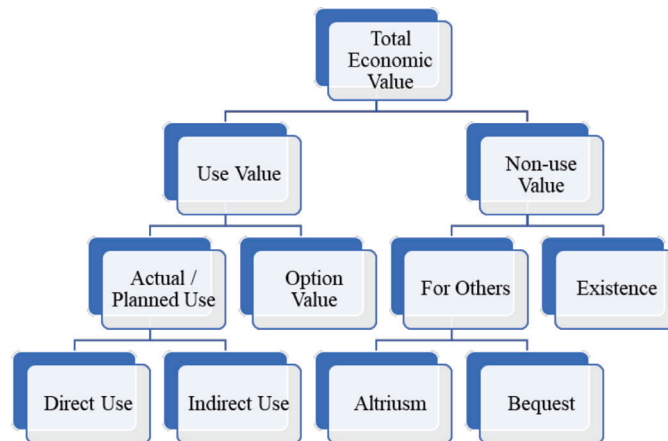


Fig. 3. Total Economic Value Framework

The dollar-based Ecosystem Valuation Method lists eight methods for valuing ecosystem foods and services which are:

1. **Market Price Method:** estimates economic values for ecosystem products or services that are bought and sold in commercial markets
2. **Productivity Method:** estimates economic values for ecosystem products or services that contribute to the production of commercially marketed goods
3. **Hedonic Pricing Method:** estimates economic values for ecosystem or environmental services that directly affect market prices of some other good. Most commonly applied to variations in housing prices that reflect the value of local environmental attributes.
4. **Travel Cost Method:** estimates economic values associated with ecosystems or sites that are used for recreation. Assumes that the value of a site is reflected in how much people are willing to pay for travelling to the site
5. **Damage Cost Avoided, Replacement Cost and Substitute Cost Methods:** estimate economic values based on costs of avoided damages resulting from lost ecosystem services, costs of replacing ecosystem services, or costs of providing substitute services
6. **Contingent Valuation Method:** estimates economic values for virtually any ecosystem or environmental service. The most widely used method for estimating non-use, or passive use values. Asks people to directly state their



willingness to pay for specific environmental services, based on a hypothetical scenario

7. **Contingent Choice Method:** Estimates economic values for virtually any ecosystem or environmental service. It is based on asking people to make trade-offs among sets of ecosystem or environmental services or characteristics. Does not directly ask for willingness to pay, this is inferred from trade-offs that include cost as an attribute.
8. **Benefit Transfer Method:** Estimates economic values by transferring existing benefit estimates from studies already completed for another location or issue.

Valuation of Inland Fisheries Resources by ICAR-CIFRI

ICAR-CIFRI has been working on economic assessments of inland aquatic resources for the past ten years. In terms of livelihood creation for fishermen and through tourism, the Deepor beel in Assam, a Ramsar site, was found to be of great worth in terms of goods and services. The wetland's tourist value, as determined by the travel cost technique, is 16 lakh rupees, although there are other ways to raise the value. The Charan Wetland in Assam's Morigaon district underwent a similar valuation study. The goods and services given by the wetland were assessed at 50.24 lakh, with fish having the biggest part (45%) and natural resource utilisation having the second-highest share (34%). An assessment of 22 km section of the Brahmaputra River in Assam revealed the annual value of products and services to be at 107 crores. According to estimates, Gosaba Island in South 24 Parganas, Sundarbans, West Bengal, generates an annual total economic value of 85.89 crores in goods and services. Ecotourism (39%) was the biggest contributor, followed by dike upkeep (22%), mangrove function (22%) and fisheries (15%). *Avicennia* sp., *Sonneratia* sp., and *Excoecaria* sp. were the three mangrove species whose per-ha carbon storage value was calculated to range from 53,773 to 1,00,824. These three species' combined market prices varied from 564 to 1,058 crores. 167.85 lakh and 125.79 lakh were assessed to be the total value of the products and services provided by Janki Chak Beel in West Bengal for both fish culture and paddy cum fish production systems, with the biggest part going to fish. Paddy, irrigation, and ground water recharge were next in line. The Project Arth Ganga, which aims to improve local economies and create new livelihoods as part of NMCG efforts, is one of the best instances of ecosystem-based sustainable economic usage of inland aquatic resources at the moment. The Arth Ganga Model aims to connect people and the river through economy. In the Ganga basin alone, it aims to contribute at least 3% of the Gross Domestic Product (GDP). The Project Arth Ganga's initiatives are in line with India's pledges to the UN sustainable development goals. The government is focusing on six verticals under Arth Ganga. The first is Zero Budget Natural Farming, which promotes the use of cow dung as fertiliser through the GOBARdhan programme and chemical-free farming for 10 km on either side of the river. The second strategy, called Monetization and Reuse of Sludge and Wastewater, aims to repurpose cleaned water for agriculture, industry, and the generating of income for Urban Local Bodies (ULBs). Arth Ganga would also provide opportunities for livelihood generation by setting up haats where locals may sell goods, medicines, and ayurveda. The fourth goal is to improve public participation by strengthening the relationships between the many river stakeholders. Through boat tourism, adventure sports, and yoga classes, the model also hopes to enhance the cultural history and tourism of Ganga and its surrounds. The concept also aims to support institutional building by strengthening local government for better water governance.

Epilogue

Commercial fishing has already given way to recreational fishing in the industrialised world. This transition happened mostly independent of fisheries and was accompanied by significant societal changes. Participants in the study came to the accurate conclusion that when economic development advances, a stereotyped series of events appears to take place across all aquatic environments. The Ecosystem Approach to Fisheries is a framework for strategy, and its practical implementation must be adjusted to the unique ecological, social, and cultural circumstances in each individual fishery. There is no one way to implement EAF, and it does not require complete knowledge of an ecosystem. Local authorities should decide on the implementation's scope. Although an EAF approach can be maintained straightforward and introduced incrementally from current fisheries management practises, more thorough research on the factors impacting fisheries is required to make it more effective. In areas where they are encouraged and properly managed, inland fisheries may contribute significantly to producing revenue and supporting economic growth. However, the overall idea needs to be explained more, which may be done by discussing implementation experiences as we go. However, other social demands and uses of water resources, such as agriculture, human consumption, power generation, and effluent disposal, frequently have an impact on and compete with inland fish and fisheries. These pressures along with many others, including species invasion, water pollution, habitat degradation, flow modification, and overexploitation threaten the biodiversity of inland fish, which is crucial to ecosystem function and services. As a result, inland fishes are among the most endangered species in the world. These dangers





to biodiversity also pose a threat to the functions that inland fisheries and fish provide. So implementation of effective instrumental governance for sustainable economical utilisation of inland fisheries resources is the need of hour.

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Innovations and Transformations in Brackishwater Aquaculture for Enhancing its Role in the Future Nutritional Security of India

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It has been anticipated that the world's food output will need to double by the year 2050 due to present population growth trends, and scientists think that nutrient-rich foods from aquatic habitats (fisheries and aquaculture) could play a big role. Aquaculture is looked to with a lot of hope and responsibility as the need for seafood increases globally every year, not just for boosting the fish output and feeding the expanding population, but also for achieving a number of other sustainable development goals (SDG). In addition to food production aquaculture can generate employment opportunities, support the food processing industry, offer the chance to reduce carbon emissions, and help conserve biodiversity in diversified fields across the nation.

Aquaculture produced 178 million tonnes of aquatic animals and 36 million tonnes of algae in 2020, making up 57% of the total 214 million tonnes of aquatic food produced worldwide. The aquaculture production statistics of India also stand on par with global standards with production peaking at 9 million tonnes of farmed aquatic animal food for human consumption making it the 2nd largest producer globally. Indian aquaculture production is mainly dominated by freshwater aquaculture comprised of carp and is mainly utilized for domestic consumption. It is inspiring to witness the spectacular growth of this industry in India, spearheaded by shrimp farming which contributed with a seafood export of about INR 38,000 crores in 2021-22, which was only a nascent industry during the early 1990s.

Aquaculture supplies over 60 percent of food fish needs, providing over 20 percent of the total animal protein intake to the Asian population. India is the second largest producer of farmed fish and offers a vast potential for aquaculture development. The aquaculture sector is also confronting severe challenges, such as the impact of climate change and variability, disease outbreaks, environmental degradation, increasing input costs, anthropogenic activities, and related social and economic changes, increasing intra-regional trade and public health concerns over food safety. Therefore, addressing these issues through research and development and policy formulation is of utmost necessity.

Aquaculture in brackishwater

Though aquaculture is possible in all types of water resources, realizing the full potential of the oceans and inland freshwaters requires a paradigm shift to embrace a new, responsible and sustainable approach to present it as more environmentally, socially, and economically effective. Brackishwater, otherwise considered a zero-economic resource (not used in agriculture, drinking or construction), are ideal for aquaculture today and expansion in the future due to its biodiversity richness, high productivity, and negligible footprint on potable water and carbon emission. As an added advantage, the high tolerance of brackishwater flora and fauna for water quality extremes makes them more appropriate for farming under controlled conditions. Therefore, in the future aquaculture development, the scope of expansion of brackishwater aquaculture is significant with the huge potential resources available for horizontal expansion in the country.

Brackishwater resource potential

Out of a total of 3.9 million ha of the estuarine area estimated; 1.2 million hectares of coastal saline waters have been identified as potentially suitable for brackish water farming. Also, about 9 million hectares of salt-affected lands were assessed in the hot semi-arid and arid ecoregion of northern plains and central highlands in the states of Haryana, Rajasthan, Uttar Pradesh, Maharashtra and Gujarat with surface and sub-soil saline water. In India, brackishwater aquaculture is synonymous with shrimp farming, and estimates show that only 11% of the potential area is utilized for farming, which offers tremendous scope for further expansion.

It is inspiring to witness the spectacular growth of this industry in India, spearheaded by the historical highest shrimp production of around 1 million tonnes in 2021-22, which was only a nascent industry during the early 1990s. Besides shrimp, brackishwater has a line-up of seafood choices in its farming basket, ranging from seaweed, clams, mussels, oysters, finfishes of different feeding nature, and gastronomic values, with ready demand in both domestic and export markets. At this juncture, brackishwater aquaculture comes with many scopes and hope due to several positivity's, catering to the country's societal and economic development.





ICAR-CIBA, a specialised institute for the sector

CIBA under the ICAR, has marked over three decades of useful research and development in the brackishwater aquaculture sector focussing on crucial questions to develop strategies and technologies for sustainable aquaculture, we are proud in taking the responsibility of steering the brackishwater aquaculture industry towards sustainability, as well as contributing towards food security and livelihood. Currently, brackishwater sector in this country is centred on the exotic vannamei shrimp, and there is realisation of the risk due to complete on a single species. Therefore, diversification of brackishwater aquaculture with different candidate species of shellfish and finfish to judiciously and responsibly utilise the brackishwater resources sustainably, with broad stakeholder participation is the significant research and development area.

Creditable progress is made, in the multi-disciplinary areas of brackishwater farming covering captive seed production, feed development, farming system development, disease diagnostics and health management, genetics and stock characterisation, climate-smart aquaculture, community engagements using social science tools and policy interventions through government agencies. Cumulatively, these R&D interventions have opened up new diversified farming initiatives and strategies to support the ongoing shrimp farming. Our ultimate goal is achieving sustainable brackishwater aquaculture for food, employment, and prosperity. It is encouraging to see the tremendous support from the farmers, industry people, and government agencies for our efforts. Still, there is a long way to achieve the committed goals and changing needs.

Key Research and Development Areas

Diversification of species for judicious utilisation of resources

Currently, brackishwater farming sector in this country is centred mostly on the shrimp, almost a single species focusses, due to its international market demand, short culture duration, and lucrative market price. When continuing the R&D support for sustainable growth of shrimp farming sector, CIBA continues to stress upon the diversification of brackishwater aquaculture with different candidate species of shellfish and finfish to judiciously and responsibly utilize the brackishwater resources, again with a sustainable mode. Commendable progress in developing technologies for seed, feed, and husbandry of diversified food fishes such as sea bass, hilsa, milkfish, pearlspot, long whiskers catfish, mud crab and five native species of shrimp. CIBA's achievement in breeding the milkfish, mangrove red snapper and grey mullet for the first time in India in captivity has given a considerable hope to the farmers who were looking for a species which they can farm using cost effective feeds, environmentally compatible and profitable. Standardisation of breeding protocols for fishes such as pearlspot and long-whiskered catfish under captive conditions attracted many stakeholders for monoculture as well as in Integrated Multitrophic Aquaculture (IMTA) models. To have a species composition with balanced trophic nature and feeding behaviour, The present focus on the captive breeding of some new species gold lined seabream (*Rhabdosargus sarba*) and rabbit fish (*Siganus javus*) aims for their large-scale production.

As a significant step, pilot scale farming demonstrations of Indian white shrimp, *Penaeus indicus* were carried out all along the Indian coast. Results revealed the potential and economic viability of native Indian white shrimp *P. indicus*, which can be farmed as a candidate complimentary species, along with exotic vannamei. Seeing the encouraging results and call from stakeholders, a genetic improvement program of this potential native species with the financial support of Govt. of India under PMMSY scheme is been put in place.

Efforts in developing seed production of untapped indigenous ornamental species, particularly brackishwater candidates will go a long way for developing a robust ornamental fish industry in India. There is reported successful breeding and larval rearing of important ornamental fishes viz. Spotted Scat (*Scatophagus argus*), Silver Moony fish (*Monodactylus argenteus*), Green Chromide (*Etroplus suratensis*), Orange chromide (*Etroplus maculatus*), and Crescent perch (*Terapon jarbua*).

Research on rearing systems and diversification

Though India has a vast coastal line of 8129 km with estimated potential brackishwater area of 1.2 million ha, it has been assessed that only 14% has been utilized for farming and the development pattern too is not uniform or adequately planned. The real challenge will be to develop plans strategically to utilize the potential resources efficiently. The country has vast areas of open brackishwater resources suitable for cage and pen aqua farming. CIBA in collaboration with coast state fisheries departments and national institutes has widely demonstrated the cage farming of seabass fish (*Lates calcarifer*) in the open backwaters using customized cages. A unique three-tier model comprising nursery, pre-grow out and grow out cages (25m³) got an average production of 460 kg in one cycle at a production cost of Rs.190 per kg of fish. This three-tier cage farming model is a win-win strategy as an





alternative livelihood option for fishers, productive utilisation of water resources and an employment opportunity for the unemployed fisher youths.

Development of indigenous feed technology

Feed is not only just a biological requirement but also an economic factor which determines the success of aquaculture. About 50 to 60% of the operational cost spent on feed. CIBA has involved in development of indigenous cost-effective feeds for the potential candidate species for all their life stages by a strategic approach. Visualizing this as a critical obstacle in realizing the returns by farmers, CIBA has developed a cost-effective grow-out feed branded as Vannami^{Plus} using indigenous ingredients. **Vannami^{Plus}** has been widely tested in farmers' ponds of Andhra Pradesh, Kerala, Haryana and Gujarat. The feed showed impressive performance and the farmers could save Rs. 20-30 rupees in the cost of production. CIBA also involved in the development of cost-effective feeds and feed management for grow-out farming, functional feeds, speciality feeds for maturation & larval rearing and nutrient profiling of fin and shellfishes. In the recent past CIBA commercialized the seral of tis feed technologies such as Seebass^{Plus}, Poly^{Plus}, KOLAR^{Plus} and Larvi^{Plus} for seabss growout farming, polyculture, ornamental fishes and larval rearing.

Health management and disease control

Overall health and disease monitoring is mandatory for successful farming. CIBA as a referral laboratory for screening OIE listed aquatic animal pathogens including emerging pathogens it will continue observing the prevalence of existing and newly emerging aquatic diseases and under the renewed National surveillance programme of aquatic animal diseases (NSPAAD). CIBA has state of the art laboratory facilities for carrying out cutting edge research in frontier areas on aquatic animal health and environment management including diagnostics, prophylactics and health management in brackishwater aquaculture. CIBA has been serving as a National Referral Laboratory to the Department of Animal Husbandry Dairying and Fisheries, Ministry of Agriculture and Farmers' Welfare, Govt. of India. The institute made a pivotal contribution to the import risk analysis (IRA) of Pacific white shrimp into India, which has resulted in quadrupling aquaculture production in the country. CIBA has been carrying out research on various aspects of prophylactics and therapeutics of brackishwater aquatic species. CIBAMOX is a water probiotic containing an innovative combination of autotrophic ammonia oxidizing, nitrite-oxidizing and denitrifying bacterial consortia. LUMI^{Phage} is the phage therapy technology for biocontrol of pathogenic vibrios in shrimp hatcheries. The technology has been developed using bacteriophages (or phages in short, are viruses that selectively infect and kill bacteria) capable of against the luminescent bacteria of genus *Vibrio*. CIBA-Nodavac-R is an injectable recombinant VNN vaccine which can effectively prevent VNN caused by RGNNV in fingerlings and prevent vertical transmission in brooders.

Inland saline shrimp farming: A new avenue

According to Central soil salinity research institute (CSSRI) around 6.7 million hectares of salt-affected soils and 1.9 lakh sq.km of saline groundwater area is present in India. They are present in states like Haryana, Punjab, parts of Rajasthan and Western Uttar Pradesh. Saline aquifers are formed as a result of salt-bearing rocks in the deeper layers or as a result of anthropogenic interventions like excessive irrigation and deforestation. Interestingly, most soils overlying these saline aquifers are also saline. The inland saline areas have long been considered as a hindrance to economic development as the land could not be used for agriculture, animal husbandry and other industrial applications. Saline groundwater, however, may be used to rear brackishwater shrimp and fish after the necessary ionic amendment of the saline water. Shrimp farming in amended inland saline groundwater is already in vogue in the states like Haryana and Punjab. However, the expansion needs to be regulated considering the possibility of salination affecting the freshwater resources.

Road map for development of brackishwater Aquaculture

Sustainable improvements in technological aspects of aquaculture will not be achieved unless they are accompanied by strong R&D, proper planning and appropriate policies that address the social and economic environment within which the aquaculture system is placed. To assist in national planning, management and policy decisions in brackishwater aquaculture, precise data on the potential resource, technological backstopping and societal linkage are crucial. In the near future, the following key elements related to brackishwater sector may be focussed with adequate policy and funding support to achieve the projected blue revolution mission.

- Promote species diversification to have more choices of finfish and shellfish in the Indian farming basket, and its genetic improvement with selected candidates for judicious utilization of resources.
- Promotion and demonstration of diversified farming systems such as cage culture, RAS, pond based farming, biofloc based farming, farming systems for inland saline soils, IMTA, poly farming etc. for better utilization of





available natural water resources.

- Generation of adequate technologies for hatchery production of seeds and indigenous cost-effective feeds for diversified species and demonstrate the technologies with the active participation of the stakeholder.
- Create and strengthen the marketing channels with adequate infrastructure.
- Nationwide disease surveillance, aquatic animal health management, and disease control measures to control the spread of exotic disease and unexpected production losses.
- Promote cooperation of state departments, export promotion agencies, central departments & agencies, R&D institutions for joint action

This all would lead to economically viable, environmentally sustainable, socially acceptable brackishwater farming and ultimately a significant increase in the total farmed seafood production.

Conclusion

At the present context of shrimp aquaculture scenario in India, it is worthwhile to relook into the candidate shrimp species used for aquaculture for a long-term sustainability of brackishwater shrimp farming. Promotion of a native species for aquaculture has always been advantageous as many of the scientific knowledge have already been generated. The present analysis indicates that native Indian white shrimp is similar to the *P. vannamei* in all the aquaculture traits. In the case of *vannamei*, the most advantage, which one can argue, is the availability of genetically selected stock. However, in the case of *P. indicus*, breeding programs have yet to be started. When considering all benefits of the development of domesticated native species, it is an important strategy to achieve the goal of sustainable aquaculture.

As the bulk of brackishwater aquaculture is rural based, it plays a significant role as a provider of direct and indirect employment to the rural poor and, thereby, to poverty alleviation. In this direction, CIBA strengthened its bonds with many stakeholders in the brackishwater sector ranging from farmers to export agencies. This article is expected to give an overall view of the sector, new development brackishwater aquaculture with more focus and new transformations towards sustainable brackishwater farming in this country.



Status, Challenges and Recommendations for Sustainable Shrimp Industry in India

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The shrimp industry in India began with the culture of *P. monodon* and saw the growth during the monodon era which benefited many individuals. However, due to the WSSV (White Spot Syndrome Virus) outbreak, the monodon culture faced severe downfall. Looking into the downfall in the monodon shrimp industry in 2007-2008, *Litopenaeus vannamei* was introduced in the culture ponds of India in 2009 and it brought about tremendous success to the farmers in terms of high production per unit area and high profits. *L. vannamei* culture saw a tremendous growth from 2010 to 2013 and from 2013 to 2017 there was a “Vannamei Tsunami” wave in India where the entire Indian shrimp industry of India flourished and farmers made huge fortunes for themselves. India made its position among the leading shrimp producer and exporter in the world and has contributed more than INR 43,000 crores in foreign exchange value benefiting the nation, farmers and the entire beneficiaries associated with the shrimp value chain.

If one studies the rise of the Vannamei shrimp culture carefully, it can be observed that the success rate at farm level started to decline after the peak growth in 2017 and as of now it can be seen that the success rate at farm level is less than 50% for vannamei culture. Figure (Fig.) 1. shows the production data and Fig. 2. shows the relation between the seed production, shrimp production and farmers success rate.

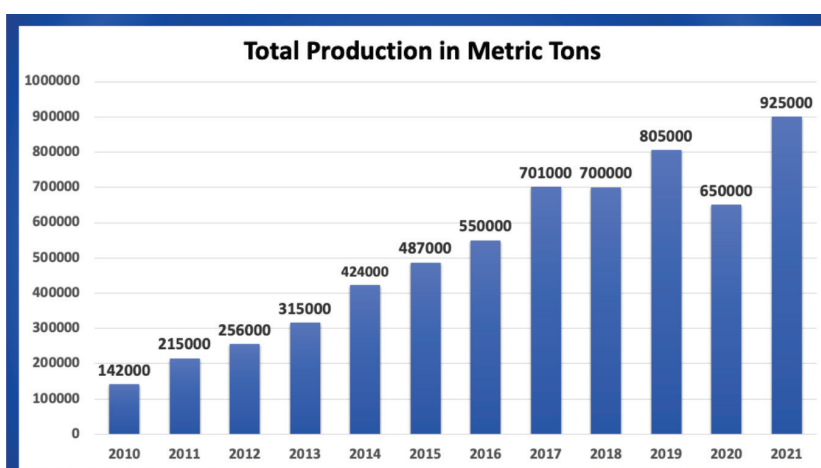


Figure 1 – Shrimp production statistics

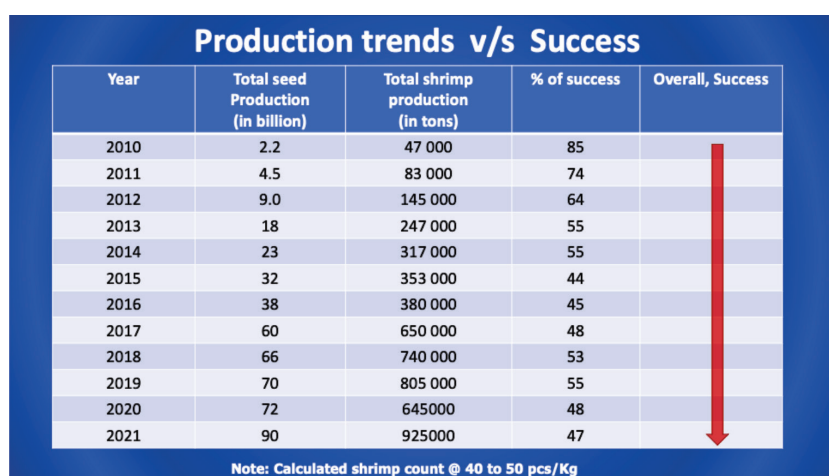


Figure 2 – Shrimp production trend versus its success.

This is primarily because of the outbreak of diseases like *Enterocytozoon hepatopenaei* (EHP), Vibriosis, Acute Hepatopancreatic Necrosis Disease (AHPND), Running Mortality Syndrome (RMS), White Feces Syndrome (WFS) and White Gut Disease (WGD). The mainstay to all these issues was the carrying capacity of the culture ponds as higher the stocking density of shrimps, greater is the feed administration and organic matter accumulation which lead to severe shortcomings in the vannamei shrimp culture in the form of disease, slow growth and mortality. Pond's natural carrying capacity is the vital aspect and should never be neglected as it decides the fate of culture ponds.

There were various external factors such as the COVID-19 pandemic wave, the Russia-Ukraine War which led the world into severe global recession and economic crisis. In addition to that, the increase in raw material price led to increase in production cost at farm level by 27%. Parallely to all these issues, Ecuador saw the rise in vannamei shrimp production and created its place in the world shrimp market giving major competition to India. This created an imbalance in the demand and supply chain across the entire shrimp industry of the world (including India). All these internal and external factors cumulatively had a severe impact on the shrimp industry as farmers could not produce shrimp profitably and processors found difficulties in selling the shrimps due to increase in container cost and competitive pricing offer of Ecuadorian shrimps to the world market. Fig. 3. gives a summarized overview of the major issues that has created trouble for the shrimp industry.

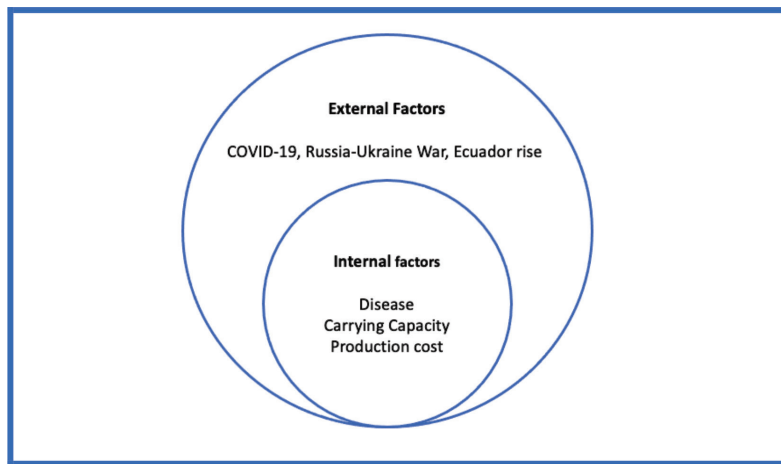


Fig. 3. Factors affecting the shrimp industry and its farmers

Contributing towards the shrimp production for market, farmers are the backbone of the shrimp industry. However, due to the aforementioned difficulties, the shrimp farmers in India are facing severe problems in sustaining themselves as their productivity and profitability has reduced drastically. Apart from having a negligible domestic market, more than 90% of the shrimps are exported from India which makes it very difficult for the entire shrimp industry and its farmers to self-sustain in such difficult times. The reintroduction of Specific Pathogen Free (SPF) *Penaeus monodon* with the benefits of improved growth performance gave slight hope to the farmers who were losing money in shrimp farming. However, the sudden and unorganized increase in the monodon production resulted in a major slump of the farm gate price for monodon. This heavily impacted the shrimp farmers as they merely produced and sold shrimp at break even or incurring losses. Reverting back to monodon in terms of increasing farmers' productivity was a very good idea but did we really "Revert Back to the monodon markets".

The complete dependency on exports and finding market has totally disrupted the entire shrimp industry. Until and unless there is no market and price realization that can sustain the industry, whether one produces vannamei or black tiger, every attempt will be a failure. The entire shrimp industry must come together in this severe crisis or else there will be dire consequences for shrimp farming in India. In countries like China and Brazil, they have a well sustained shrimp industry with a huge domestic market which has helped the entire industry and its farmers during difficult times.

Besides carrying out shrimp production, China even imports shrimp which shows the sizeable domestic market it has created. India's two major markets for shrimp export are the US and China, but Ecuador is now slowly penetrating those marketplaces by exporting their major produce to China and then impressing the US and other buyers. The world shrimp basket is 4.5 million metric tons, but the actual traded shrimps are near about 2.0 to 2.2 million metric tons. India and Ecuador are aggressively catering and competing in this shrimp trade and commerce. Ecuador has marketed themselves as the best shrimp producers in the world and are representing their countries in unity, from



farmers to processors, to boost their exports which has given large benefits to them.

India is technically the most sound nation when it comes to shrimp farming and the shrimp farmers of our country produce one of the best and premium quality shrimps. However, the challenge for our country now is not merely producing shrimps but to produce it in a cost-effective manner and finding better market and price realization for its produce. The shrimp producing states in India have different water source and chemistries, different geographic and climatic conditions, different cost of production and some do not have a processing house in their nearby vicinity. The price disparity among the states is also discouraging many shrimp farmers as the international buyer sees the shrimp, whether be it vannamei or monodon, as the shrimp of India rather than the shrimp from Gujarat, Punjab or Andhra Pradesh.

It is crucial for the shrimp farmers in the respective shrimp farming states to clearly understand their individual strength and weaknesses and plan their shrimp production accordingly rather than focusing on increase in shrimp production per unit hectare as farmers success is not limited to producing only more shrimps, it is also equally important to produce them sustainably by understanding the technical and market acceptance aspects. Thus, it is imperative for the farmers, seed and feed suppliers, industry players and processors to be on the same page and have a transparent mode of communication between them and create a sustainable shrimp industry where every individual in the entire value chain of shrimp industry is prosperous.

There are various ways in which the Indian shrimp and aquaculture industry can move forward to have a better and prosperous future, some of them are:

1. **Domestic market for shrimps** – With the support of government and private players the promotion for domestic market for shrimp consumption should be initiated in the form of celebrity engagement and endorsement, creating marketplace in major cities of India, increasing social media presence and creating awareness about shrimp farming, its impact on rural community, importance of shrimp consumption and illustrating the health benefits of consuming shrimps and arranging shrimp food festival at regional level to make people familiar with eating shrimps. Even the support from government can be very useful for the farmers and private players who want to engage in domestic marketing for shrimps. Though the journey for the domestic market for shrimp consumption is long, but the early steps taken in this area will benefit the future generation of shrimp industry. Benefits of domestic market will be safeguarding the farmers during crash harvest of low counts or where there is no export market for their produce. Farmers will not have a need to plan their produce as per the international market and will have a flexibility to produce smaller size shrimps catering the domestic market. Farmers can be benefited by both domestic and international market for shrimps as they will have two different markets to cater.
2. **Value addition** – The value addition-based exports can also be very beneficial for the shrimp industry as India lacks value addition infrastructure and investment such infrastructure can create a better image for the shrimp processors and bring more market opportunities for the shrimp industry in India.
3. **Creating better brand presence** – Marketing of the shrimps should not be merely done in the form of selling shrimps to the buyers. The Indian shrimp industry and its major players should start campaigns and branding for their shrimps and show how traceable the shrimps offered by India are and how sustainably they are grown. Post harvest infrastructure, collection and handling can give an add on benefit to the shrimp industry. Program marketing will be the future of the shrimp industry where the brand value of Indian shrimps will be increased by showing traceability with all necessary certifications and food safety standards.
4. **Optimizing cost of production** – Farmers must try to purchase feed on cash as the high cash discounts can definitely help farmers in reducing the cost of feed inputs in their culture. Farmers can have a planning for their culture by contacting the processors and growing the shrimps to the size where it is acceptable for the processor as well as the international market. In this way, they can have a prior control to a large extent in their cost of production.
5. **Culturing vannamei and monodon** – The shrimp production in India might see a sharp downfall, but still farmers and industry should not lose hope. The farmers should understand the demand and supply principle of the international market and culture vannamei or monodon as per the demand. Monodon can be 20% of the total shrimp produced for the world shrimp market and the rest can be vannamei. More importantly, the farmers who are doing well in vannamei culture should continue doing vannamei culture and the farmers who are not doing well in vannamei can start practicing monodon culture. Farmers should not get tempted by the high profit margins or short-term success of one crop as overdoing the shrimp production, whether be it monodon or



vannamei, will be of no use. Farmers should produce shrimps by market acceptance as it will be of no use if they cannot sell their produce.

- 6. Species diversification** – The focus should be made on the culture of other shrimp species such as *Macrobrachium rosenbergii*, *Fenneropenaeus indicus* along with brackishwater finfish aquaculture for more diverse the culture species are, more diverse will be the aquaculture product offerings which in turn will create a balance for the farmers and the entire aquaculture industry in the international cycle of demand and supply.

It can be concluded that the Indian shrimp industry has created huge employment opportunities for lakhs of individuals (including women) in the country, especially for the poor rural and the fishermen community. However, as difficulties have arisen due to technical and market issues, the industry players should be on the same page having a common aim of safeguarding the shrimp industry in difficult times and preparing for the future in terms of investing in the domestic market, technical upgradation and species diversification in order to create a better future for the upcoming generation.



Promising Technologies for Sustainable Development of Ornamental Fisheries Sector in Northern India

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Ornamental aquaculture is a significant commercial aspect of aquaculture. It is a promising area of aquaculture that is anticipated to have plenty of growth potential as well as prospects to provide income and employment to the vast number of educated and talented unemployed people in rural areas. In comparison to food fish, most ornamental fish is high value fish that can be raised in smaller spaces. A higher price for ornamental fish can be obtained based on the colour, size and variety of the fish because they are sold individually rather than by weight. A small-scale ornamental fish farm can be started with easy species like live bearers. Different ornamental fishes can be of various colours and forms, such as goldfish, koi, cichlids, angelfish, etc., one can broaden the diversity and improve profitability. It opens up the possibility for creating a small-scale business for unemployed people by rearing, breeding and trading as the export demand of more than 100 varieties of native ornamental fishes in India is increasing. Small-scale farmers now have a market opportunity, thanks to the demand for exotic ornamental fish species including goldfish, cichlids, angelfish, tetras and others in both inside and outside of India.

Major cities like Kolkata, Chennai, Mumbai, Delhi, Hyderabad, Bangalore and Cochin are where the domestic ornamental fish trade is primarily concentrated. With 5.5 lakh individuals employed and generating income, there are already 1.1 million hobbyists raising ornamental fish in their homes nationwide. In the domestic market, there are more than 100 different species traded with 60 of them being freshwater fish. Two biodiversity hotspots, the Western Ghats and North East India, are where most indigenous ornamental fishes are collected and exported. Around 374 freshwater ornamental fishes belonging to 134 genera are mostly found in the North Eastern region and Western Ghats. Also 15 varieties of aquatic plants are traded for planted aquarium as it is gaining a new momentum among hobbyist.

Present Status and Issues for Development of Ornamental Fisheries in Northern India

In northern India, ornamental fish farming is a new endeavor. Most of the ornamental fishes in northern India i.e. Delhi, Punjab, Haryana, Rajasthan, Himachal Pradesh comes from Kolkata, Chennai and Mumbai. The greatest obstacles to the development of ornamental fisheries in Northern India are the extremely difficult environmental conditions, such as hot summers and hard winters. Unlike in West Bengal or Tamil Nadu, the cost of producing ornamental fish in northern India is affected by the availability of live food at higher prices. Although the state fisheries department, fisheries colleges, and ICAR institutes in northern India offer technical assistance and training for ornamental fish farming, it still needs a push to get the stakeholders to step forward for the development of entrepreneurship in this industry.

Contribution of ICAR-CIFA for Development of Ornamental Fisheries in India

In order to ensure that aquarium keeping of ornamental fish as a hobby considerably leads to more popular and enhances the entrepreneurial development, ICAR-Central Institute of Freshwater Aquaculture (ICAR-CIFA) is playing a crucial role in developing captive breeding technology for more than 15 native species, including gourami, barbs, rasbora, danio, and others have had success in breeding and larval rearing. Recent advancements made under the ICAR Network project include broodstock development and captive breeding of native ornamental murrel fish from the North Eastern region, *Channa stewartii*, as well as the Narayan barb (*Pethia narayani*), Tambraparni barb (*Dawkinsia tambraparniei*) and Filament barb (*Dawkinsia filamentosa*). By minimizing the exploitation of natural stock, commercial breeding techniques for many ornamental fish species will significantly aid in the conservation of priceless biodiversity. The technology's commercialization offers enormous potential for the economic growth of those involved in the breeding, selling, and marketing of ornamental fish. A new type of Shining barb (*Pethia conchonius*), whose market worth is higher than the typical rosy barb, has been created through selection programme. CIFACURE is a medication that has been created and made available for sale to control the bacterial and fungal disease. The Institute's ornamental fish culture unit is continuously working on more than 30 commercial species for the domestic trade of the nation. Recently, ICAR-CIFA has established ornamental field schools at Jalpaiguri (North West Bengal), Choudwar, Cuttack (Odisha), and Madurai (Tamilnadu), where nominated and trained progressive farmers of Institute helps other interested farmers by providing them with knowledge. Each year, selected farmers



are trained on new techniques in ornamental aquaculture for upgrading their farming practices. By focusing on breeding and larval rearing of indigenous fish, the institute has currently partnered with seven sister institutes under one platform for Network Project on Ornamental Fish Breeding and Culture. In addition, CIFA will collaborate with the Department of Fisheries, Ministry of Agriculture and Farmers Welfare to implement the Pradhan Mantri Matsya Sampada Yojana Action Plan.

Future Prospectus for Sustainable Development of Ornamental Fisheries in Northern India

In Northern India, the ornamental fisheries business is very diverse. The most effective methods for reducing environmental conditions like scorching summers and cold winters may be modern aquaculture techniques like RAS and Aquaponics. The region may benefit from the selection of hardy species that are crucial for trade, such as goldfish and koi carp, which can withstand the tough environmental conditions. Ornamental Aquaculture sector will grow in northern India if public aquariums, aquarium accessories and ornamental fish feed are produced in Northern India as part of the “Make in India Mission,”. Entrepreneurs can charge premium if they can generate a lot of consumer demand. As a result, it is usually advisable to create a proper, sustainable proposal before starting any ornamental fish breeding units with a solid business plan.

Scope for Women Self-help Group in Ornamental Fisheries Sector

Considering the proven success of involvement of women in development of backyard enterprise in farming of ornamental fish in West Bengal and Kerala, it is necessary that due encouragement is given for creation of women SHGs for such enterprise. It may be interesting to note that Malaysia, with similar climatic condition like that of India, has been able to grow the industry only with small backyard units at individual levels. Thus, development of ornament fish farming in any region, not necessarily requires very large-scale enterprises, but a few successful clusters of back yard units in different regions. In this regard special packages may be provided to these SHGs and unemployed youths or Ex-service men for establishment of such enterprise.

Ornamental Fish Village can be developed in rural areas which are well connected by the cities in northern India by taking the beneficiaries such as women self-help groups, Individual woman (either farmer or housewives) or unemployed youth under PPP (public private partnership) mode. Such cluster-based approach facilitates the marketing of ornamental fishes and buyer gets bulk quantity of fish easily at one spot. ICAR-CIFA has developed three ornamental fish villages in Deogarh district of Odisha. The establishment of ornamental fish village is quite successful in Odisha, West Bengal and other parts of country.

Planning and Implementation for Development of Ornamental Fisheries

After consulting with various stakeholders, ICAR-CIFA and the Department of Fisheries, Government of India established the strategic action plan for the development of ornamental fisheries in India through the PMMSY scheme. Any business person's ability to succeed depends on careful planning, including site selection, efficient organisation, and design of the breeding or rearing unit. Currently, there are several commercial fish farms producing different kinds of ornamental fish.

- The minimum land required is 500-1000 square feet for a small-scale unit whereas, 1 acre and more for large scale farming in which few earthen ponds are required for some species such as Koi carps. Site selection is one of the main criteria where the farmer should select a cool environment for culture and breeding.
- Breeding and rearing unit should be constructed nearby areas having a constant supply of clear water along with the facilities of constant electricity.
- In cold climates, farming is too expensive as the water has to be kept warm to culture tropical fish. The tropical climates favour the production rate because of year-round breeding and probability as successful rearing and better growth. Hence, it is prerequisite for the entrepreneur to select the species accordingly.
- The selection of candidate species depends on the water quality of that area as species prefers either soft or hard water or withstand wide tolerance of water quality for breeding. For example, all the live bearers prefer hard alkaline water. Egg layers such as koi carp, goldfish, gourami, danio, catfish, rosy barb and fighter fish can tolerate wide range of water condition. Species such as angel, discus, tetras, Oscar, loach prefer soft -acidic water. Therefore, water quality needs to be checked.
- In certain areas, some species are difficult to breed where there is a severe problem of water quality like high level of hardness or saline water. Therefore, those sites may be avoided.
- Bio-filtration unit is a necessity for ornamental fish culture and breeding unit. The establishment of either common biofilter unit or individual biofiltration unit, whichever is economical can be done.



- The brood stock selected for breeding should be of superior quality so that good quality fish seed could be produced.
- Brood stocks can be allowed to breed for not more than two years. Fresh stocks from different sources may be added in every two years to the selected parent stocks so as to improve the breeding efficiency and produce healthy offspring.
- The small fish breeder should concentrate preferably on one species so that it helps the breeder to develop expertise on the particular species and good quality fishes can be produced as per the market demand.
- In goldfish and koi carp, if colour reflects in the body within 1 month of rearing (Fry), the stock is considered as optimum.
- The availability of agro-based by-products facilitates the preparation of farm made pelleted diet for the fish. For preparing a pelleted diet, a mini pelletiser may be installed.
- The breeding and rearing unit may be established preferably nearer to an airport/railway station, bus-stand etc. for easy transportation for export and domestic market.
- The breeders should develop market relations with pet/ retail shops, potential farmers, vendors dealing with ornamental fish, marketing network, etc. to facilitate the process of selling/procuring new brood stocks.
- A committed entrepreneur should always ensure regular contact with the recent research developments in the field, attend training programmes and have exposure visits.
- New imported fishes should be quarantined from resident stock. Movement of fishes should be restricted from a suspected / unknown / disease prone areas. Therefore, a few quarantine tanks (away from the unit) are required so that proper observations can be made on health of fishes. If necessary, treatment can be done after due consultation with fish pathologist.
- If any abnormal behaviour is observed in any culture tanks, then the fishes need to be isolated immediately. If mass mortality occurs, expert opinion must be sought for treatment.

Support of PMMSY for Building Ornamental Fisheries

Farmers who have access to water resources and want to expand their farming operations would benefit from ornamental fish rearing. The added financial support will contribute to increasing the sector's trade value, which includes the production of accessories in India. This industry is now in better shape due to the development plans implemented by NFDB and MPEDA during the past ten years. This was mostly concentrated on backyard units with low investment in areas like Kerala, West Bengal, Tamil Nadu etc. Under the Pradhan Mantri Matsya Sampada Yojana, a projected investment of 576 crores is being made to upgrade the ornamental aquaculture sector and create 7 lakh jobs (PMMSY). In the PMMSY plan, the unit cost subsidy for general population is 40%, while it is 60% for SC/ST and women. Additionally, an extra investment of Rs. 500 crore is expected through a World Bank programme to support this sector. This will undoubtedly aid the sector's financial needs to advance farming. Under the PMMSY programme, the following units/projects for the development of ornamental and recreational fisheries are available and interested parties who are eligible can apply through the state government's department of fisheries.

S. No.	Units/Projects	Unit Cost (lakhs)
1	Backyard ornamental fish rearing unit (both marine and freshwater fishes)	3.00
2	Medium scale ornamental fish rearing unit (both marine and freshwater fishes)	8.00
3	Integrated ornamental fish unit (breeding and rearing for freshwater fishes)	25.00
4	Integrated ornamental fish unit (breeding and rearing for marine fishes)	30.00
5	Establishment of freshwater ornamental fish brood bank	100.00
6	Promotion of recreational fisheries	50.00

Economics of Ornamental Fish Breeding and Culture

The economic viability is the foundation of any popular and successful enterprise. On the basis of the business scale, ornamental fish farms can be categorized as backyard/micro scale, small scale, medium scale and large scale. The micro scale and small scale units are low investment backyard enterprises with low intensity of production whereas, the medium scale or large scale commercial enterprises involve higher cost and returns. It is estimated that within a year's time with 2-3 breeding cycle, a beginner may earn upto 10,000 per month from small scale farm. One can earn more with the same infrastructure by developing expertise, improving efficiency and better price. This



income can be considered to be high compared to any other similar enterprises. A summarized economics of different ornamental fish farming model are presented below:

Summary of Economics for Different Models for Ornamental Fish Farming

Particulars	Different Model for Ornamental Fish Farming			
	Micro Scale	Small Scale	Medium Scale	Large Scale
Minimum Land Requirement (sq. ft)	250	700	2000	10,000
Recommended Species	Gourami/Gold Fish/ Cichlid	Gourami/Gold Fish/ Cichlid	Gold Fish/Koi Carp	Gold Fish/Koi Carp
Capital Expenditure (Different type Tanks, bore well, Aeration and filtration system, packaging etc.)	1,30,000	3,00,000	8,00,000	25,00,000
Total Expenditure (Recurring Expenditure for brooders, feed, labour charges including interest and depreciation)	34000	94000	3,65,000	13,00,0000
Gross income/Year (with 2-3 cycle of breeding and culture)	77000	2,30,000	8,00,000	29,00,000
Net Income	43000	1,36,000	4,35,000	16,00,000
Monthly Income	3600	11000	36,000	1,30,0000

Note: The model economic analysis of various types of ornamental fish farms presented here are indicative, and average estimation. The actual cost for farm establishment and operation may vary with price of raw material, labour cost, electricity charges etc. from place to place and type of species selected for farming. The actual profitability varies from place to place depending on the condition and efficiencies in operation and management of the farm.

Conclusion

In ornamental fish farming and trading, several stakeholders’ like fishers, farmers, breeders, hobbyist, traders, vendors, transporters and exporters are involved and the whole business of ornamental fish is based on the supply of fishes from two primary sources i.e. wild collection and captive breeding. There is a wide apprehension on the environmental impact of wild collection which has a damaging effect on the country’s threatened fish biodiversity. Therefore, captive breeding is the foundation of sustainable development of such enterprises. Breeding reduces the pressure from the nature to a considerable degree and provides in-vivo means of germplasm conservation through culture. Therefore, captive breeding technology is identified as a priority area for sustainable development of the sector. There is an ample scope specially in Northern India for developing entrepreneurship through establishment of brood banks of Koi and Goldfish, aquarium manufacturing units including indigenous accessories, feed and medicine manufacturing units.



Unfancy Issues of the Fancy Fishes

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Introduction

The keeping of an aquarium at home or work place is gradually becoming very popular in India like many developed countries. An upsurge was observed in the demand of aquarium and ornamental fishes in the domestic aquarium trade post Covid-19. The number of aquarium shops which was around 5000 in the country during 2015 increased to above 8000 by 2021. The ornamental fisheries have been identified as one of the major focus areas under Pradhan Mantri Matsya Sampda Yojna (PMMSY) of Government of India announced in 2019-20 with proposed investment of Rs. 576 crores and an additional funding support of Rs. 500 crores from the world bank. More than 1800 new ornamental fish production units have been established recently in different states of the country after implementation of PMMSY. The domestic aquarium trade has now shifted from availability based to demand-based trade with increasing demand for high and premium value fishes viz; Discus, Arowana, Flower horns, Japanese Koi carps etc. The aquarium keeping is now not limited to freshwater fishes only but shifted to marine fishes, marine invertebrates as well as aquatic plants. A paradigm change observed in the sector is increasing interest of educated youth of the country to set up ornamental fisheries-based enterprises. Many fisheries professionals, IT graduates, MBAs and other highly educated youth have set up their startups of ornamental fish production, aquarium servicing, aquarium shops, aqua-parks, fish spa, aquarium accessories manufacturing, ornamental fish feed manufacturing etc. The National Skill Development Corporations, Ministry of Skill Development and Entrepreneurship have designed specialised training modules to become a certified “Aquarium technicians” and “Ornamental fish technicians.” However, it is noted that the growth of domestic aquarium trade and ornamental fish keeping hobby in country remains far below of the developed countries. It could be much faster but for certain issues that are retarding the growth of ornamental fisheries development and aquarium keeping hobby.

1. Sale of disease prone fishes

The sale of disease prone fishes to the hobbyists is the most serious problem at present. It is estimated that more than 50% aquarium fishes encounter mortality at different stages of supply chain during their journey from the production centres to the aquarium of the hobbyists. There could be many factors of the fish mortality viz; water quality, improper handling, poor aquarium maintenance, ignorance of biosecurity etc. at any stage of supply chain. The fishes are packed and re-packed again and again in a short period of time, kept in water of different temperature and chemical properties at high stocking densities without acclimatization. The live fish packaging practices in domestic aquarium trade are very inferior to packaging of export-oriented consignments. The transportation stress and poor fish holding facilities at different stages of supply chain make a healthy fish susceptible to diseases mainly white spot and fin rot. Many a times fishes are infected with ectoparasites such as Argulus, Lerneae, Fluke worms etc. but remains unnoticed by hobbyists. The major sufferer of all these poor practices are finally hobbyists as the fish apparently looked healthy when purchased but susceptible to diseases and encounter mortality. It is because of the continuous mortality of the fishes that many hobbyists discontinue to keep the aquarium for ever. A fish dealer in supply chain even if aware of the infection in a fish, try to quickly sale it to next level.

2. Unregulated import

The export of ornamental fishes from the country is regulated by the Marine Products Export Development Authority, Ministry of Commerce and Industry and Animal Quarantine and Certification Service (AQCS), Ministry of Fisheries, Animal husbandry and Dairying (MoFA&D). The designated agencies of the importing countries also check all the live fish consignments as per the rules and regulation of their country. But the import of live fish in India mainly remains unregulated though it is monitored by AQCS at the ports of entry. The monitoring is largely related to fish health and holding of the fishes in quarantine facility of the importers for about 14 days before releasing for sale. However, the import of ornamental fishes in India appears highly unregulated.

The MoFA&D permits import of only 92 varieties of fishes including freshwater and marine but more than 500 varieties of exotic fishes are traded on aquarium shops in the country. The list of exotic ornamental fishes is growing every day as enthusiastic aquarium traders and aquarists always looking for new varieties. The author prepared a list of 218 species of freshwater exotic fishes during January, 2022 which included fishes from the orders *Cichliformes* (65 species), *Cypriniformes* (34 species), *Characiformes* (42 species), *Siluriformes* (23 species), *Anabantiformes* (12





species), *Cyprinidontoformes* (9 species), *Atheriniformes* (13 species), *Gymnotiformes* (2 species), *Osteoglossiformes* (5 species), *Polypteriformes* (6 species), *Perciformes* (3 species), *Synbranchyiformes* (4 species). In addition to this, there are many species of Molluscs, Crustaceans, marine fishes, and marine invertebrates. The adverse impact of few of the exotic edible and ornamental fishes are well documented on aquatic ecosystem, aquatic biodiversity, fish production and introduction of new fish diseases in the country.

Now a days many common and commercially important varieties of Goldfish, Live bearers, Tetras, and Barbs, regularly produced in India are being imported from neighbouring countries at low price and sold in the domestic market. It is adversely affecting the business of local ornamental fish producers.

3. Loss of FOREX

The aquarium and aquarium accessories contribute about 63% to the domestic aquarium trade. Almost 95% of these items are imported from other countries mainly China. A few of these items were being manufactured in the country prior to year 2000 but almost not any presently. The Indian manufacturers could not compete with imported products in terms of cost, quality, and varieties. There is no doubt that the availability of Chinese aquarium products in the domestic market has immensely contributed to popularise the hobby of ornamental fish keeping but it is resulting in loss of FOREX. The country exported ornamental fish worth Rs. 10.84 crores during 2019-20 but our expenses on import of aquarium accessories were much higher during the same period. Consequently, any new addition of an aquarium hobbyist results in loss of few dollars to the country.

4. Threat to aquatic biodiversity

The India is known in global aquarium trade for export of wild caught freshwater ornamental fishes, though the contribution in terms of value was only 0.48% to world ornamental fish export of about US\$ 322 million during 2019-20. The wild caught freshwater ornamental fishes mainly from the rivers of the western ghats and the north-east contributes about 85% to total ornamental fish export from India. The important fishes that were initially exported from India included species of *Channa*, *Botia*, *Puntius*, *Barilius*, *Garra*, *Glyptothorax*, *Horabagrus*, *Schistura* etc. The population of many of these fish species have depleted over a period in nature for many reasons but apparently aquarium trade was blamed by the environment conservationists. As a result, *Sahyadria denisonii* (Miss Kerala) was proposed to be included as Schedule-I species under Wild Life Protection (Amendment) Act 2022 but finally *Channa barca* and *Aenigmachanna gollum* both from north-east were included as Schedule-II species. Both the species remains in high demand in global aquarium trade and captive breeding technology of any of these species is yet not developed.

5. Collection of live fish food

The feeding of aquarium fishes on live food viz; blood worms, tubifex worms, *Moina*, *Daphnia*, *Artemia* etc. is popular among aquarists as they provide a balanced nutrition to the fish for maturation, faster growth, and colour maintenance. These are required regularly not only by ornamental fish producers but many of the hobbyists enjoy feeding their fishes on live food. The sale of live tubifex worms in small plastic packets is very common at aquarium shops in Mumbai, Pune and Kolkata. The blood worms and tubifex worms are mainly collected from sewage channels of the metro cities. As such they carry many types of pathogens with them that are introduced in the aquarium and results in infection to fishes. The other important issue related to live food collection is the health threat to their collectors. The sites of collection are very unhygienic and filthy. The collectors of blood worms and tubifex worms avoid to go to these sites in normal situation but under the influence of alcohol or drugs.

6. Unethical aquarium trading practices

The aquarium traders are first source of knowledge on aquarium keeping to a new hobbyist. The most of the aquarium traders are either themselves not adequately knowledgeable or not willing to share the knowledge and experience with the hobbyists. The aquarium traders treat an aquarium hobbyist as a potential customer and try to sale a product or other every time they are visiting the shop with a problem or for an advice. A few of the very common practices followed by aquarium traders are:

- Sale an aquarium fabricated with glass of less thickness which is not fitted with all essential aquarium accessories so to reduce the cost.
- Sale of stressed and diseased fishes.
- Sale of high value and incompatible fishes to new aquarists.
- Adding of a blue colour liquid to maintain the water quality.



- Frequent cleaning of aquarium for proper maintenance.
- Use of coloured artifacts for aquarium beautification
- Suggesting to keep large sized and a greater number of fishes in a small size aquarium

All these unethical practices followed by aquarium traders greatly discourages new hobbyists to become a matured aquarium lover and resulting as a major drawback in the development of domestic aquarium trade.

7. Unethical aquarium keeping practices

The aquarium keeping is practiced for many reasons viz; hobby, house decoration, ambience enhancement, health benefits, attention diverting objects, belief in Vastu/Fengshui etc. Every one keeping aquarium is not adequately knowledgeable about the basics of aquarium keeping. Generally, an aquarium is known as a glass tank or a glass bowl filled with water, stocked with large size fish in many numbers and fed several times a day. The installation of an air pump is considered the solution to keep the water clean for ever. A few of the unethical practices commonly practiced by new hobbyists include:

- Begin the hobby with a goldfish bowl
- Purchase a large size aquarium fabricated with glass of less thickness
- Do not install essential aquarium accessories viz; heater, air pump, light and filter.
- Stock more numbers of large size fishes in a small aquarium.
- Frequently change the aquarium water for keeping it clean
- Overfeed the fishes and unaware of common fish diseases
- Discarding overgrown or unwanted aquarium fishes in natural water bodies.

It is very a common practice that the hobbyists accuse the aquarium traders for mortality of fish or any other problem of the aquarium. It is very important to popularize ethical practices of aquarium keeping among hobbyists and educate them on the basics of aquarium keeping.

8. Inadequate efforts to promote hobby

The development of ornamental fisheries in country is mainly considered production of ornamental fishes for generating livelihood and employment opportunities in the rural areas of the country and for export promotion. Therefore, the efforts of the all the developmental and funding agencies remains mainly focused on ornamental fish production. The ornamental fisheries do not remain limited to the production of ornamental fish but it also includes manufacturing of aquarium accessories, setting up of aquarium shops, human resource development, skill enhancement and most importantly the promotion of fish keeping hobby. It is the growth of fish keeping hobby that will result in increasing demand of ornamental fish and aquarium products and generate employment opportunities. Hence, there is need of several awareness activities for common man that will promote the fish keeping hobby.

9. Inadequate R & D

The subject of ornamental fish culture and aquarium keeping is taught as 1+1 credit hours course to fisheries undergraduates at all the Colleges of Fisheries in India. It is also taught at several traditional colleges as part of skill enhancement program at undergraduate level. Almost all the fisheries research institute, fisheries colleges and state fisheries departments in the country are presently engaged in some activity on ornamental fisheries but it is mostly limited to skill development trainings only. There is record of producing only one new species *Pethia conchonius* by Dr. Saroj Swain at ICAR-Central Institute of Freshwater Aquaculture, Bhubaneswar, Odisha about a decade back. A few of the institutes have developed breeding technology of several indigenous species which are yet to be commercialised. The major research on ornamental fishes is limited to their use as experimental animal for aquatic pollution related studies throughout the country. The skill enhancement alone will not be helpful to develop the ornamental fisheries, there is an equal need of funding R & D activities on developing commercial technology for breeding of indigenous fishes, development of brood stock of commercially important fishes, developing live food culture technology, development of new products, treatments for ornamental fish diseases etc.

10. Poor presence in global aquarium trade

The Asian countries viz; Japan, Singapore, Indonesia, Malaysia, and Sri Lanka, much smaller than India in terms of geographical area and fisheries resources are the major exporters of ornamental fishes to global aquarium trade contributing from 4-21%. But the contribution of India yet remains less than 1%. It was Rs. 10.84 crores (US\$ 1.55 million) during 2019-20 i.e., 0.48% only of the total global export of US\$ 322 million. The export of ornamental fishes from India began in the year 1969 and the first consignment was valued at Rs. 16.40 lakhs only while other



Asian countries started exporting much later. Secondly, India is mainly exporting wild caught freshwater fishes while other Asian countries are known for captive bred fishes only. The poor quality of captive bred fishes, small size of ornamental fish farms, high air freight and unsupportive government system are the other major reasons of our poor export performance.

Conclusion

The ornamental fisheries developmental programs began in the country with the beginning of 21st century mainly emphasising on export importance of ornamental fishes. It was the Marine Products Export Development Agency (MPEDA), launched the first financial assistance scheme during 2006-07 to increase the production of ornamental fishes mainly from coastal states of India and formulated “Green Certification” program to regulate and monitor collection of wild ornamental fishes. The Kerala state government also launched a public limited company “Kerala Aqua Ventures International Ltd” in December, 2007 to promote export of wild caught ornamental fishes from Kerala. The green certification program was never initiated and the MPEDA closed the funding assistance by 2014-15 as export of ornamental fishes from India could not increase as planned. The KAVIL also could not achieve the desired results. The National Fisheries Development Board (NFDB) launched a pilot project during 2014-15 but it was again restricted to only eight states of India Viz; Kerala, Karnataka, Tamil Nadu, Maharashtra, West Bengal, Gujarat, Bihar, and Assam. It could also not be implemented very successfully. The major reason of the failure of all these schemes were, the thrust on promoting the export of ornamental fish and not the development of domestic aquarium trade. It was for the first time under PMMSY that efforts were initiated for development of domestic aquarium trade which had so far progressed with the individual efforts of ornamental fish producers and aquarium traders only in the country. It was under PMMSY that ornamental fisheries development scheme was launched uniformly in all the states of the country and targeted to grow the domestic trade to Rs. 3000 crores which was about Rs. 500 crores only during 2019-20. The domestic aquarium trade can be increased by encompassing all the components of ornamental fisheries viz; ornamental fish production, manufacturing of aquarium, aquarium accessories and allied product, creating a pool of aquarium service providers, human resource development and most importantly the promotion of ornamental fish keeping hobby. A higher number of aquariums keeping hobbyists will result in increased demand of ornamental fishes and aquarium products. The India should be a dominant player in global ornamental fish export but more attention is required for development of domestic aquarium market which is much bigger with large potential. The estimated potential of domestic aquarium trade of Rs. 3000 crores in country which is as large as total value of global ornamental fish exports, could be achieved subjected to addressing all the unfancy issues of the fancy fishes.

Sustainable Utilization of Coldwater Fisheries Resources for Livelihood and Nutritional Security in Indian Himalayan Region

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Preamble

Indian mountain zone is abounding in numerous rivers, streams and lakes. These high altitudinal aquatic resources in the states of J&K, Himachal Pradesh, Uttarakhand, part of west Bengal, Sikkim, Arunachal Pradesh, Nagaland and Meghalaya in the Himalayan range and Western Ghats like the Munnar sky-scraping ranges in Kerala, Nilgiri hills in Tamil Nadu are various potential areas offering sufficient scope for coldwater fish farming in our nation. Under the control of lower temperature regime, the farming of favored cold water fast growing species i.e. rainbow trout (*Oncorhynchus mykiss*), exotic major carps i.e. grass carp (*Ctenopharyngodon idella*), silver carp (*Hypophthalmichthys molitrix*), common carp (*Cyprinus carpio*) and few minor carps are in practice in Indian Himalayan region.

Nutritive superiority of Rainbow Trout

For humans, fish and fishery products have an important dietary role owing to its high nutritional quality. The content of essential amino acids, long chain polyunsaturated fatty acids (n-3 and n-6 PUFA's) and minerals are high in rainbow trout whereas the cholesterol content of these products is low. Due to the reported health benefits of long-chain omega-3 fatty acids (Eicosapentaenoic acid-EPA, 20:5n-3 and Docosahexaenoic acid-DHA, 22:6n-3) in preventing various chronic diseases like coronary heart disease, rheumatoid arthritis, neurotic disorders in infants, cancer, hyperglycemia, psoriasis, multiple sclerosis and inflammation, people are getting attracted towards a diet rich in fish particularly of trout. It is important to note that long-chain n-3 PUFA's cannot be synthesized readily by human beings and hence, is required through diet, more in case of developing foetus, infants, adolescents, pregnant and lactating women (Fig.1)



Fig. 1. Nutritional benefit of rainbow trout for human health

Flow Through Raceways (FTR) for Rainbow Trout Culture

Rainbow trout is considered as the potential species in hill fish farming. Its mass scale seed production and civilization is being carried out in several fish farms in Kashmir, Himachal Pradesh, Uttarakhand, Arunachal Pradesh and Sikkim. The critical prerequisite for establishing a trout farm is accessibility to a good quality spring water where temperature during the year remains below 20 °C. There are approximately 50 small and big trout hatcheries in India with an estimated eyed ova production capacity of 20 million are there in India, established under various state governments and support of central government plan. J & K and Himachal are the leading state in developing rainbow trout farm in private sector. The maximum trout producer state in India is J & K having production of 900 mt and 15.4 million trout ova. Himachal Pradesh is the second highest producer (850 mt) in the country. Directorate of Coldwater Fisheries Research, Bhimtal is providing scientific support through new technologies, development of best management practices protocol and conducting demonstration programme for expanding trout culture activities

in Ladakh, Sikkim, Arunachal Pradesh, Nagaland and Uttarakhand. Breeding and seed production of trout is mainly happening in the states of J&K and Himachal Pradesh. Nevertheless, due to ICAR-DCFR's continuous efforts, seed production of trout has been comprehensively initiated by Sikkim and Uttarakhand. The state of Arunachal Pradesh is still bringing seed from other states for its culture. At present, the trout production in the country is over 2200 tons (2021-22). Rainbow trout is a costly fish to grow and is considered as a magnificence food beyond the reach of the common man. Being a low volume high value product, the trout has superior potential for local consumptions in Indian market as well as for export. Rainbow trout was introduced in India 120 years back and pristine well oxygenated fluvial water is the major requisite for its farming. Raceways of 30-45 sq m with depth of 1 m are generally constructed for the culture of table sized fish (400-800g). Raceway farming requires continuous flow of water and the farming is based wholly on artificial feed. The stocking density is also dependent on quality and quantity of available water. Quality seed is stocked @ 10-15 fingerlings/sq m (5-10 gm size). The fishes are provided with an artificial feed having more than 40% protein and 10 % lipid. It attains 800-1000 grams in a culture period of 12-14 months during favorable culture conditions. Technology is farm based and eco-friendly where a production of 10-15 kg/sq m. is achieved. There is abundant possibility for improvement of trout production in IHR through PPP mode. The present trout production of the country is around 2200 tons which may be increased up to 10000 tons within a period of 2-3 years with the adoption of recent technology and best management practices. The utilization of abundant raceways and increasing per unit production will be the key strategies for enhancing the production of trout in the country. It provides an excellent opportunity for utilizing the under and unutilized aquatic mountain resources for augmenting the livelihood and nutritional security by fetching an attractive market price in the restaurants (Fig.2)



Fig.2: Flow through raceways

Intensive culture of rainbow trout in RAS

Rainbow trout culture has been a potential livelihood opportunity in IHR due to the copiousness of pristine resources. However, with changes in the demographic outline and struggle for resources, enormous quantity of water required in FTR's, making the action unsustainable in the long term. To retain a kg of trout at a stocking density of 25 kg/m³ in FTR's, flow rate of 600L/minute water is required. Generally 50-200 m³ of water is required to produce 1 kg of rainbow trout in FTR's which is not always possible with reducing water resources. It also restricts rainbow trout farming to the certain pockets of geographical region. Hence, it is necessary to think about climate-resilient intensive farming practices to achieve the targets. The scientific and technological progress in RAS have reduced the water necessity in trout culture by many folds (< 1 m³ of water/ kg fish), having stocking densities up to 100 kg/m³. If the environmental impact of FTR and RAS is compared, it suggests that RAS minimizes water requirement by 93%, eutrophication by 26-38%, and improves FCR by 27%. Also, the bottom surface of high energy requirement in RAS (16 KWH/ kg) can be surmounting by reducing energy usage to a considerable extent by optimizing water re-circulation and oxygenation necessities. In addition, clean energy sources (e.g., solar energy which is adequately available in hilly regions) and biogas produced from fish waste sludge can be utilized to make rainbow trout farming more sustainable and economically viable in RAS. Further, by regulating water temperature in RAS, it is feasible to lessen crop duration up to 5-6 months (in comparison with 12-14 months cycle in FTR). ICAR-DCFR has established a RAS under NICRA project for grow-out production and experimental tanks with rearing capacity of 33 m³. RAS includes radial flow separators and mesh screen drum filter to remove settleable and suspended solids. It also has moving-bed biological filter to remove ammonia and nitrite, and a UV filter for disinfection. With the established

pilot-scale RAS, it is possible to produce 1.2 ton of trout per crop cycle (2.4 ton per year, at the stocking density 40 kg per m³). The production can be increased to ~5.0 ton per year with the help of pure oxygen for oxygenation and addition of CO₂ stripper and protein skimmer to system. RAS system can significantly contribute to increase trout production due to the fact that the RAS farming practices can be done from nearly anywhere in the Indian upland region with limited water availability and lower land footprints which otherwise is impossible with current farming practices in flow through raceways (Fig.3)



Fig.3: RAS of rainbow trout farming

Exotic carp culture in mid-Himalayan region

Chinese carps are generally cultured in the mid-Himalayan region for polyculture. Chinese carp culture were introduced in the polytanks/ irrigation tanks in the mid Himalayan regions as a means of alternate livelihood opportunity. This location specific technology provided opportunities for conservation of water for irrigation and fish culture. The polytanks used for fish culture has demonstrated good growth of fish. Indian major carps do not grow well in the climatic conditions of IHR, due to low thermal regime. Therefore, Chinese carps were taken as the suitable species for polyculture in small land holding farming system. Seed in the form of fry is being used for stocking grow-out ponds, which require 12 months to get the table size fish. The average fish production in the existing system is 2-3 t/ha which can be doubled through technological support, quality seed, feed and improved management practices. Crop duration may be reduced with the exercise of stocking stunted fish of 80-100g. However, provision of seed banks is essential for round the year availability of fish seed to the farmers. There are tremendous opportunities for integration of carp culture with dairy, poultry and horticulture keeping the fish pond as centre in view of enhancing productivity, reducing risk and production cost.

Ornamental fish farming in mid-Himalayan region

Demand of ornamental fishes is growing outstandingly because of their imperative role in the world trade. The export of ornamental fishes from India is only US\$ 0.2 million which is not enough with regard to the increasing requirement of these attractive species in the world aquarium fish market. The potential ornamental fishes of the coldwater resources are *Barilius* species, *Nemacheilus* species, *Botia* species, *Puntius* species, *Garra* species, *Glyptothorax* species etc. Apart from these, few exotic species are unique for aqua gardening in IHR. This diversity is yet to be explored for seed production and propagation having immense coldwater ornamental fish value. Another new idea is to establish culture and breeding of ornamental fishes in the backyard or kitchen garden in upland rural areas which will benefit economic growth. The small landholding in the hills with marginal agricultural and allied activities is directly affecting the economic status of the population through ornamental fish culture. The intervention of ornamental fish culture in the tribal regions will help in upbringing the livelihood opportunities. Ornamental fish keeping in house, hotels, offices had been a general practices due to their fascinating/recreation/aesthetic value which in turn create livelihood (employment) opportunities in the hilly regions.

Paddy cum fish culture in high altitudinal region

Paddy cum fish culture fields are important resources in the state of Arunanchal Pradesh, Nagaland and other states of northeastern region. Paddy cum fish culture is practiced in Apatani plateau, Ziro of lower Subansiri district of Arunanchal Pradesh, in quite a few districts of Nagaland and other northeastern states. The fish production in these fields is comparatively low (200-300 kg/ha) and R & D intervention is required to increase the productivity. Higher

productivity from paddy cum fish culture can be achieved by stocking bigger size (3-4") of quality fingerlings @1-2 fish/m². The redesigning of appropriate sized trenches in the field for providing shelter to the fishes is also needed. Of course, nutrient and natural fish food available have to be taken into account to decide the practices of intensive fish culture. These areas are having great potential to enhance the productivity to many folds. Its worth mentioning that a significant section of hilly population including women are dependent on paddy cum fish culture in NE region and it is providing livelihood and nutritional security to the particular tribal population of IHR.

Sport fishing and eco-tourism as a means of livelihood security

Angling or sport fishing is one of the most fascinating outdoor physical activities which satisfies diverse taste and pursuits. It is a form of eco-tourism promoting sustainable form of resource-use, contributing to environmental conservation while proving accrued socio-economic benefits to the society through non consumptive use and provides high value to natural biological resources. Angling holidays and sport fishing tourism is a booming international business opportunity. It is one of the most sought after adventure tourism activities and there is an ever increasing number of international angling itineraries throughout the world in search of big fish and thrilling sports fishing adventure in an un spool fishing destination. Sport fishing is one of the leading adventure tourism activities in Europe and USA. In NE India, the average fishing trip for golden mahseer in Subansiri river of Arunachal Pradesh cost around Rs. 3000 per kg of which market price is only about Rs. 500/- per kg, calculated on an average of 4 fishing trip from Guwahati (calculated in 2021).

The essential elements of angling tourism (eco-tourism) are natural environment, optimum number of environmental friendly visitors and activities not causing serious environmental and cultural impact on ecosystem with positive involvement of local community. It is essentially a tourism activity which minimizes the conflict between tourism and livelihood of the local inhabitants, their environment and socio-cultural life with major thrust for conservation and preservation of nature and culture. It is for the sport fishing interest itself that the angler likes to conserve nature, comprising of the tree line, the riverbed, the clean and clear water bodies with abundance of fish fauna, their spawning ground, the catchment area of the river as a whole. Although there is tremendous potentiality of angling tourism in many sites, it has not developed to the expected level till date. There is an urgent need to identify the sport fishery areas, policy formulation for sport fisheries including development of platform for promotion. Entrepreneurship development for promotion of fish based eco-tourism with supportive and ancillary service development is a far cry. National and international publicity, awareness and marketing strategies for promoting angling tourism are important factors that need immediate attention for its development which may be important avenues for improving the livelihood security of the common people of IHR living on the bank of the river depending on the aquatic resources (Fig. 4 & 5)



Fig. 4 & 5. Sports fishing

Challenges and opportunities in livelihood and nutritional security

The constraint of coldwater fish farming causing low productivity in IHR is the slow growth rate in almost all fish species except rainbow trout. Fecundity of coldwater fishes is also low. Apart from this, lack of modern infrastructure facilities district wise are also major bottlenecks in the improvement of livelihood and nutritional security in the sector.

Some of the challenges in the fisheries sector of IHR for economic growth

1. Cost intensive activity
2. Slow growth rate leading to less output
3. Lack of infrastructure *e.g.* raceways, hatcheries, and transport *etc.*



4. Costly inputs - less availability of seed, feed for culture
5. Habitat destruction
6. Changing climatic condition
7. Lack of commercial farming.
8. Non adaption of fisheries as main stream profession by local population.
9. Lack of organised market for selling high value fish like trout.

Strategies for improvement of livelihood and nutritional security

1. Promotion of trout farming in areas above 1,500 msl having temperature range of 6-18 °C in a cluster approach with proper marketing channel.
2. Introduction of recirculating aquaculture system for increasing the productivity and as a measure of climate resilient farming practices.
3. Poly culture in hill regions between 700 and 1,500 msl with suitable land and water resources for carp culture with the integration of agriculture, horticulture and animal husbandry.
4. Eco- climatic conditions of the foothills and river valleys located up to 500 msl are suitable for composite culture of carps. Such sites are available in the catchments of the Himalayan and peninsular rivers, streams and their tributaries. Carp farming would be, therefore successful in the valleys and peripheral regions located in the temperature range between 15 to 26°C.
5. Adoption of natural farming with indigenous technical knowledge.
6. Development of eco-tourism platform for attracting tourists to spend time with the pristine eco-system and magnificent fishes.
7. Rearing of ornamental fishes and promoting the concept of eco-gardening in the resort and restaurant.

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Organic Recycling Based Integrated Fish- Livestock Aquafarming Technology for Livelihood Earning of Rural Communities in Gangetic Plains

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In the third world countries where land mass is too much fragmented and livelihood of majority depends on agriculture, multiple use of land is unabated. Integrated aquafarming is a diversified and coordinated way of farming for production of agricultural items in fish farms with fish as main product. The items produced are either used as source of feed and fertilizers, source of additional income or both. The small farmers are normally not financially empowered to get enough nutritious food to lead normal, healthy and active lives. The various types of aquaculture form, a critical component within agricultural and farming systems development that can contribute to the alleviation of food insecurity, malnutrition and poverty through the provision of food of high nutritional value, income and employment generation, decreased risk in production, sustainable resource management and increased farm sustainability. Underlying the importance of fisheries sector, United Nations General Assembly has declared year 2022 as the International Year of Artisanal Fisheries and Aquaculture (IYFA 2022). Livestock production generates by-products that are important inputs for aquaculture. The main linkage between livestock and fish production involves direct use of livestock wastes, as well as the recycling of manure-based nutrients which function as organic fertilizers to stimulate natural food webs.

Integrated fish-livestock farming is a low input, sustainable technology for resource-poor farmers. Pullin (1995) reiterated that mixed enterprise integrated systems involving fish culture are less risky and should produce more fish because they can be more widely adopted. Importance of ducks' excreta as feed and fertilizer in commercial fish farming has been reported by Barash et al (1982), Chauhan et al (1998), Sharma et al (1998) and Latif et al (1993) also advocated the importance of ducks' excreta in fish yield. Little and Muir (1987) found that ducks remained healthier and cleaner in integrated duck-fish farming. Tiwari et al (1999) investigated the economic viability of mixed farming model integrating crop, livestock, poultry, duck and fish farming on 1.5 ha land holding. The model having 2 crossbred cows + 10 goats + 10 poultry birds + 10 ducks on 0.25 ha area including for own living + fish (0.15 ha pond) along with crop cultivation on 1.1 ha was best with cost returns of 1:2 and employment generation of 380 man-days. Fish-livestock integrated system has not received adequate attention. Moreover, scientifically developed package of practices also do not exist. To address these constraints and gaps, an appraisal of integrated fish-livestock and fish-poultry farming has been made. Gebru (2021) dealt with integrated aquaculture with special reference to fish integration with animal husbandry to enhance production and productivity.

The present study was carried out at the Instructional Fish Farm, College of Fisheries, G. B. Pant University of Agriculture & Technology, Pantnagar (Fig. 1). To start with, a survey of fish farmers of the area was conducted to gather information on total water area, fish farming system, extent of adoption of technology, livestock used and degree of integration (Table 1).



Fig.1 Instructional Fish Farm, College of Fisheries, Pantnagar



Table 1 Status of integrated fish farming in plain areas of Uttarakhand

Sl. No.	Details	Small Farmers (< 1 ha)	Large Farmers (> 1 ha)	Total Farmers
1	No. of respondents	20	10	30
2	Trained in fish culture	7	6	13
3	Experience in fish culture			
	a) up to 2 years	9	5	14
	b) 2 – 5 years	6	3	9
	c) above 5 years	5	2	7
4	Total area under fish culture surveyed (ha)	15.25	35.00	50.25
5	Area under integrated fish farming (%)	5.0	0.6	5.6
6	Pond acreage (ha)	0.76	3.50	-
7	Pond size (range in ha)	0.05 – 1.00	0.40 – 12.0	-
8	Average fish production (kg/ha/year)	1926.44	3000.0	-
9	Livestock (no./farmer)			
	a) Cattle	1-3	1-2	
	b) Buffalo	1-2	<1	
	c) Ducks	12	5-6	
	d) Chicken	8-10	4-5	
	e) Pig	<1	<1	

The study was conducted in two phases. In the first phase, two earthen ponds with a water area of 0.4 ha were used. In one pond 'desi' ducks at the stocking density of 200 birds/ha were integrated with fish while the other pond was stocked only with fish to serve as control. The ponds were stocked under composite fish farming with stocking density of fish @ 5000 fingerlings/ha, as detailed in Table 2.

Table 2 Details of stocking, harvesting and production in fish-duck (desi) and fish ponds

Details	Fish-duck pond						
	Duck (Deshi)	Catla	Rohu	Mrigal	Silver carp	Grass carp	Common carp
Species ratio (%)	-	20	30	15	15	5	15
Nos. stocked	80	400	600	300	300	100	300
Nos. harvested	57	322	475	220	234	66	268
Survival (%)	71.3	80.5	79.2	73.3	78.0	66.0	89.3
Initial av. wt.(g)	450	56.0	46.0	32.0	62.0	43.0	98.0
Final av. wt.(g)	1830.0	1265.0	840.0	780.0	1302.0	1225.0	1033.0
Total wt. harvested(kg)	104.31	407.33	399.0	171.60	304.67	80.85	276.04
Gross production/ha/year	4098.72 kg fish; 258.03 kg ducks; 16,625 eggs						
Details	Fish pond (Control)						
		Catla	Rohu	Mrigal	Silver carp	Grass carp	Common carp
Species ratio (%)		20	30	15	15	5	15
Nos. stocked		400	600	300	300	100	300
Nos. harvested		328	503	232	230	61	255
Survival (%)		82.0	83.8	77.3	76.7	61.0	85.0
Initial av. wt.(g)		56.0	46.0	32.0	62.0	43.0	98.0
Final av. wt.(g)		1246.0	803.0	690.0	1044.0	1231.0	950.0
Total wt. harvested(kg)		401.21	381.42	151.80	267.70	81.24	254.60
Gross production/ha/year	3844.62 kg fish						

In the second phase, study on both fish-duck and fish-poultry integrated aquafarming was conducted. Egg laying varieties of duck (Khaki Campbell) and chicken (Bob Cock) were used for integration with fish. Two rectangular earthen ponds of the size of 0.5 ha were used for fish-duck integration and fish culture (control). Six species of carps namely catla (*Catla catla*), rohu (*Labeo rohita*), mrigal (*Cirrhinus mrigala*), silver carp (*Hypophthalmichthys*

molitrix), grass carp (*Ctenopharyngodon idella*) and common carp (*Cyprinus carpio*) were stocked @ 10,000/ha, in both the ponds. One of the pond was completely integrated with rearing of ducks of Khaki Campbell variety @ 600/ha. A pond of 0.0336 ha, with poultry house made over it, was used for chicken-fish integration. The pond was stocked with fish fingerlings @ 8925/ha and 595 nos. of Bob Cock chicken per ha. The ratio of fishes in both the ponds was kept as catla 20%, rohu 25%, mrigal 15%, silver carp 15%, common carp 15% and grass carp 10%. In control pond, the stocked fishes were given supplementary feed @ 2-3% of the body weight daily, while no artificial feeding was done to experimental fishes in fish-duck and fish-poultry integrated ponds. Standard practices of pond fertilization (liming and organic manuring) were resorted to in the control fish pond.

Ducks are aquatic birds, so are very suitable for integration. Fish farmers obtain duck eggs, meat and fish from the same unit area. Various aspects of duck production and its scope to meet egg and meat demand of the country have been discussed by Naik et al. (2022). The ducks were lodged in duck house of the size of 40'X 10' X 10' erected over the pond and made up of bamboo poles and splinters. Space of 1-2 cm was left in between splinters of the floor of the house so that duck-droppings and spilled over feed could get direct entry in to the pond (Figs. 2 & 3). The ratio of male and female ducks (Fig. 4) was kept as 1:5. The ducks were allowed to swim in water after 8 weeks of age. They were allowed to move in run space and dykes during day time. Ducks also help in increasing dissolved oxygen content in pond water by increasing surface area of water (Fig. 5). The floor of duck house was washed every day in the morning.

Ducks feed on snails, fish fry, earthworms, insects, soft vegetation and artificial feed (mash). They get 50-75% of their total food requirement from pond (Table 3). The ducks were given 70-80 g feed (mash)/duck/day in two equal installments, in late morning and late evening. The composition of feed for various stages of duck has been shown in Table 4. Four percent limestone was added in the feed of layers. Giri et al (2015) worked out the effect of dietary protein on reproductive growth of Khaki Campbell ducks. Ducks have difficulty in swallowing dry feed so there



Fig. 2. Duck house erected over fish pond



Fig. 3. Inner view of duck house



Fig. 4. Female and male Khaki Campbell



Fig. 5. Ducks swimming in fish pond

should be enough water in drinkers near feeders in duck house. Average spilled over feed was quantified as 14.37% of the feed given to ducks. The feed conversion ratio (amount of feed consumed in kg to produce one dozen eggs) of Khaki Campbell duck are reported as 1.987-2.038 by Swain et al (2020).

Table 3. Analysis of gut content of Khaki Campbell ducks

Digestive tract	Feed component (g)		
	Pond origin (%)	Duck ration (%)	Stone and others (%)
Crop	8.57 (61.00)	1.24 (9.00)	4.23 (30.00)
Glandular stomach	4.47 (52.00)	2.33 (27.00)	1.80 (21.00)
Gizzard	3.95 (45.30)	3.85 (44.14)	0.92 (10.56)
Total	16.99 (54.18)	7.42 (23.67)	6.95 (22.15)

Table 4. Composition of supplementary feed of Khaki Campbell ducks

Component	Duckling (0-2 weeks)	Growers (3-8 weeks)	Growers (9-20 weeks)	Layers (20+ weeks)
Broken wheat	50	44	36	45
Jowar	15	26	35	25
Rice polish	05	08	14	-
Soybean cake	10	06	05	10
Mustard cake	05	03	03	05
Yeast	05	05	-	03
Fish meal	08	06	05	06
Mineral mix	02	02	02	02
Limestone	-	-	-	04

The profile of physico-chemical parameters has been presented in Table 10 and plankton profile in duck-fish and fish ponds has been presented in Figs. 6 and 7. An adult duck produced 144.875 g excreta/day, thus amounting to total calculated quantity of duck excreta added to pond as 23,051.94 kg/ha/year. Through recycling of duck excreta in fish-duck pond, 314.659 kg nitrogen, 228.372 kg phosphorous, 82.987 kg potassium and 2,881.493 kg organic carbon got entry to duck-fish pond, making it sufficiently productive for growth of the stocked fishes. (Table 5). There was no detrimental effect of duck integration on well being of stocked fishes. The values of condition factor 'K' ranged from 1.15 to 2.43 in duck-fish pond (Table 6).

Table 5. Nutrient content in duck droppings and their recycling in duck-fish pond

Nutrient content	Average quantity in duck dropping (%)	Quantity added to duck-fish pond (kg)	Calculated quantity added to duck-fish pond (kg/ha/yr)
Nitrogen	1.365	68.966	314.659
Phosphorous	1.130	57.093	228.372
Potassium	0.360	18.189	82.987
Ash	3.138	158.547	723.370
Organic carbon	12.500	631.560	2881.493

Table 6. Condition factor 'K' in fishes reared in experimental ponds

Species	Duck-fish pond	Fish pond	Poultry-fish pond
Catla	2.00	1.37	1.36
Rohu	1.39	1.33	1.50
Common carp	1.66	1.40	2.39
Mrigal	1.28	1.32	1.27
Grass carp	2.43	2.80	1.63
Silver carp	1.15	1.15	1.27

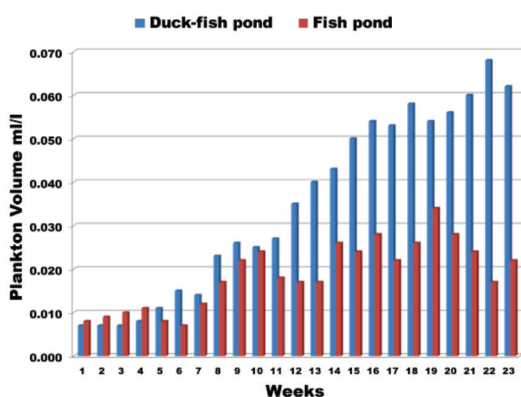


Fig. 6. Comparison of plankton volume in ponds

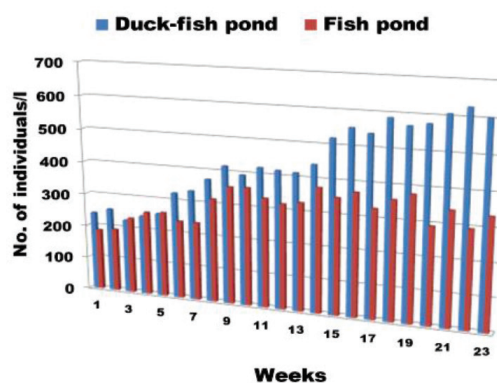


Fig. 7. Status of zooplankton in ponds

The details of growth, harvesting and production in fish-duck and fish pond have been presented in Tables 7 and 8, respectively. The integration of duck with fish resulted in 6.87% enhancement in fish production, in addition to production of 1796.21 kg live weight of ducks per ha/year. As the stocked ducks were not in lay, due to low age, the eggs could not be obtained. However, in another study conducted by the author and co-workers on Khaki Campbell duck-fish integration where ducks were in lay, 30,440 number of eggs per hectare were obtained (Sharma et al 2001).

Table 7. Details of growth, harvesting and production in duck (Khaki Campbell) -fish pond

Details	Duck - Fish pond						
	Khaki Campbell	Catla	Rohu	Mrigal	Grass carp	Silver carp	Common carp
Species stocked							
Species ratio		20	25	15	10	15	15
Nos. stocked	300	1000	1250	750	500	750	750
Nos. harvested	276	907	1004	502	315	461	502
Survival %	92.0	90.7	80.32	66.94	63	61.5	66.94
Initial Avg. wt.(g)	32.5	12.52	44.91	48.25	48.3	33.3	67.1
Final Avg. wt.(g)	1627	380.42	340.6	311.2	375.3	218	394.2
Avg. monthly increment (g)	265.75	61.32	49.34	40.71	54.5	33.3	54.52
Total wt. harvested (kg)	449.052	345.04	341.96	156.23	118.2	100.5	197.89
Gross production in duck-fish pond (0.5 ha)	Fish - 1259.82 kg						
	Duck - 449.052 kg						
Calculated gross production per ha/ year	Fish - 5039.28 kg						
	Duck - 1796.21 kg						

Many authors have emphasized the importance of fish-livestock integration in recycling of waste products, income generation and diversification of products. The integrated farming of chickens or geese with fish is practiced in some countries in Asia, Africa and Latin America. The importance of livestock wastes in enhancement of the fish food organisms has been analyzed by Chauhan et al (1998). Banerjee et al (1979) worked out the manurial potentiality of poultry droppings in aquaculture in aquaculture either alone or in combination with other organic waste. Burns and Stickney (1980) recorded 6.5 fold higher production with 95% survival of tilapia reared in poultry manure ponds of 400 laying hens/ha in comparison to unfertilized water in 150 days of culture period. The details of stocking in fish-poultry pond and physico-chemical parameters in integrated ponds have been included in Tables 9 and 10, respectively. A completely integrated poultry housing system, in which poultry droppings, spilled poultry feed and washings of the housing unit fell directly into the pond, was employed. A poultry house (4x2x2 m) made of split bamboo was erected over the pond. Twenty chicken of 'Bob Cock' variety of the age of about 5 months (ready to lay eggs) were lodged in the poultry house.



Table 8. Details of growth, harvesting and production in fish pond (control)

Details	Fish pond (control)					
	Catla	Rohu	Mrigal	Grass carp	Silver carp	Common carp
Species ratio	20	25	15	10	15	15
Nos. stocked	1000	1250	750	500	750	750
Nos. harvested	833	1065	490	353	474	480
Survival %	83.3	85.2	65.3	70.6	63.2	64
Initial Avg.wt.(g)	12.52	44.91	48.25	48.3	33.3	67.1
Final Avg. wt.(g)	352.4	345.6	265.6	385.3	215.1	310.5
Avg. monthly increment (g)	56.65	50.12	36.23	56.17	30.3	40.57
Total wt. harvested(kg)	293.6	368.06	130.14	136.14	101.95	149.04
Gross production in duck-fish pond (0.5 ha)	Fish - 1178.8 kg					
Calculated Gross Production per ha/ year	Fish - 4715.2 kg					

Table 9. Details of stocking in fish-poultry pond (0.0336 ha)

Species	Number	Av. Length (cm)	Av. Weight (g)
Catla	60	22.09	148.0
Rohu	75	20.73	117.6
Mrigal	45	24.97	178.2
Silver carp	45	22.22	189.4
Grass carp	30	23.40	160.6
Common carp	45	26.50	169.0
Bob Cock chicken	20		1125.0
Stocking rates	Fish - 8925/ha; Chicken - 595/ha		

Table 10. Physico-chemical parameter in duck-fish, poultry-fish and fish ponds

Parameter	Duck-Fish Pond	Poultry-Fish Pond	Fish Pond
Water Temperature (°C)	15.0 – 30.0	12.0-29.1	15.0 – 31.0
pH	7.3 – 8.3	6.7-8.2	7.0 - 8.5
Dissolved oxygen (ppm)	4.5 – 8.0	4.1-8.2	5.1 – 8.0
Free CO ₂ (ppm)	1.5 – 3.5	0.0-9.5	0.0 – 2.0
Total Alkalinity (ppm)	93 - 145	47.0-188.0	90 - 125

The chickens were kept on layer mash (24.6% protein) @ 125g/bird/day. Feed was provided in feed hoppers. Enough amount of water was made available to chicken in drinkers. Three times during the study period, a polythene sheet was spread on the floor of the house for 24 hours to collect chicken droppings and spilled over feed for the assessment of average amount of dropping/bird/day (Table 11) and percentage of spilled over feed (Table 12). The eggs were collected 3-4 times in a day. Ten major genera of plankton were recorded from poultry-fish, out of which 6 belonged to phytoplankton (*Chlorella*, *Euglena*, *Pediastrum*, *Pedorina*, *Phacus* and *Volvox*) and 4 to zooplankton (*Moina*, *Cyclops*, *Diaptomus* and *Brachionus*). The spilled over poultry feed amounted to 19.59% of the feed given to the chicken. Thus, a total amount of 78.53 kg spilled over poultry feed fell directly into the pond during study period which would have been consumed by the fish stocked in the pond. Annual amount of spilled over poultry feed thus could be estimated to the tune of 4674.4 kg/ha. The status of organic carbon in pond bottom soil improved (initial 1.57%; final 3.09%), thereby showing the organic enrichment of pond bottom soil. Based on the findings it can be concluded that addition of poultry droppings resulted in maintaining a healthy population of the fish food



organisms in chicken-fish pond. The spilled over poultry feed also contributed in attaining gross fish production of 5229.76 kg/ha/year in addition to 1363.09 kg chicken and 86,547 nos. of chicken eggs (Table 13). No deterioration in water quality like deficiency of dissolved oxygen, drastic alteration in pH, occurrence of algal blooms, etc. due to integration of chicken with fish was noticed.

Table 11. Production of poultry excreta in fish-poultry pond

No. of Birds	Quantity of excreta/ 24 hrs(g)	Av. Quantity of excreta/ bird/day (g)	Total amount of excreta added to fish-poultry pond (kg)	Total calculated quantity of excreta added to fish-poultry pond (kg/ha/yr)
20	1725.0	86.25	313.918	18685.595
20	2200.0	110.00		
20	1950	97.50		
Av. Quantity of excreta/bird/day : 97.916 g				

Table 12. Nutrient content in poultry dropping and their recycling in fish-poultry pond

Nutrient Content	Quantity in poultry dropping (%)	Quantity added to fish-poultry pond (kg)	Calculated quantity/ha/year (kg)
Nitrogen	1.75	5.493	326.964
Phosphorous	1.20	3.767	223.928
Calcium	2.90	9.103	541.845

Table 13. Growth and production of fish and chicken in fish poultry integration

Species	Survival (%)	Average Monthly Increment (g)	Total Harvested Weight (kg)	Gross Production in Pond (0.0336 ha)	Calculated Gross production/ha/year
Catla	80.00	32.88	16.57	Fish 87.86 kg	Fish 5229.76 kg
Rohu	90.67	27.61	19.26		
Mrigal	55.55	15.19	6.73	Chicken 22.90 kg	Chicken 1363.09 kg
Grass carp	66.67	113.77	16.86		
Silver carp	60.00	52.37	12.08	Eggs 1454 nos.	Eggs 86,547 nos.
Common carp	53.33	72.86	16.36		
Bob Cock chicken	85.00	37.18	22.90		

It is inferred from the results of the studies that integrated duck-fish and poultry-fish aquafarming is technologically sound, economically viable and environmentally congenial farming practices which also helps in the abatement of environmental pollution, employment generation and product diversification.

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Economic Feed Technologies for Sustainable Development of Shrimp Farming in Inland Saline Resources of Northern India

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Introduction

Fisheries and Aquaculture is playing a pivotal role in food security and livelihood generation for hundreds and millions of people around the world, including India. Total fisheries and aquaculture production reached a record of 214 million tonnes in 2020, comprising 178 million tonnes of aquatic animals and 36 million tonnes of algae (FAO 2022). Global aquaculture production continued to grow amid the worldwide spread of the COVID-19 pandemic. Global aquaculture production consisted of 87.5 million tonnes of aquatic animals (predominantly used as food for human consumption) and 35.1 million tonnes of seaweeds and other micro-algae (for both food and non-food uses). The fish production in India stood all-time high of 162.48 lakh tonnes during 2021-22 with an impressive annual growth rate of 10.34% (12.121 MMT from the inland sector and 4.121 MMT from the marine sector). The fisheries sector contributes about 1.24% to the country's gross value added (GVA) and 7.28% to the agricultural GVA. In India, the changing consumer preferences, technological advancements and income growth are the real drivers that augur well for further expansion and demand of food from aquatic origin. To sustain the rapid growth of aqua farming, the feed industry need to support the sector with quality and cost-effective feeds, for feed alone contribute 50-60% of the operative cost in the semi-intensive / improved extensive culture practiced by Indian farmers. Against this backdrop, this sector is poised for unprecedented growth and hence, opening various avenues of entrepreneurship.

Aquaculture

Freshwater carps and brackishwater shrimp are the dominant cultured candidate species in India. Freshwater aquaculture contributes about 90% of the total aquaculture production. The farming of Indian Major Carps (IMC) is the mainstay in fresh water. Though the production is predominantly traditional, the conversion to feed-based aquaculture is gaining momentum. However, feed-based aquaculture is growing rapidly for the species like tilapia, pangasius and pacu. Shrimp farming is the face of brackishwater aquaculture in India and has been carried out as a traditional activity for ages in India. For a developing country like ours, shrimp farming is a high-potential sector with enormous scope for increasing foreign exchange and employment generation.

Further, it is pertinent to note that in India, nearly 8.62 million ha of land area is affected by soil salinity and 2.8 million hectares of salt-affected soils are present within the Indo-Gangetic alluvial plain. Within 8.62 million ha, 40 % of the salt-affected area is concentrated in the north-western, semiarid/arid states of Haryana, Punjab, Uttar Pradesh, and Rajasthan (Allan 2009). There was a considerable momentum among farming communities to utilize the salt-affected lands in these regions, proving to be more profitable than agro farming. To sustain the rapid growth of aqua farming, particularly shrimp farming, the availability of cost effective feed assumes considerable importance. The scientific fish/shrimp farming practiced by the aqua farmers in these hinterlands of North India currently use formulated feed produced by firms located outside the state and are not affordable by the small and marginal farmers of the state. Moreover, substantial quantity of raw materials available for shrimp and fish feed production from this region can be used for the production of cost-effective feed. If cost-effective feeds are available, there is a great scope for improving the productivity of aquaculture enterprise thereby ensuring sustainable aquaculture.

Significance of feed in aquaculture

The significant increase in aquaculture has been possible with the corresponding increase in the availability of formulated feeds. Aqua feeds have been developed for sustainable and commercial culture of fish and shrimps to meet the animal protein needs in developing countries. The principal cost in the manufacture of aqua feeds is that of the raw materials; which could amount to as much as 80 percent or more of the manufacturing costs in commercial feed mills. Aqua feeds in India have evolved commercially in the late 90s for shrimp culture and then for fish culture during the last decade. In the brackishwater aquaculture sector, majority of the feed used is scientifically formulated compounded feed produced by multinational / Indian corporates. Some small players with a capacity to produce 1 tonne per hour are entering the market. In freshwater aquaculture, the traditional farm-made feed consists of rice bran with any of the oil cakes available in their locality. The sinking feed for carps and the availability of floating



feeds for fish seems to make a steady and positive impact in changing the traditional feeding system and increasing productivity. However, farmers are more concerned about the cost of production and the final product quality. Most of the farmers are using formulated feed as supplementary feed but not as complete feed. The price of de-oiled rice bran (DORB) directly affects the use of formulated feed. In freshwater aquaculture, extruder feeds are popular for pangasius, tilapia, and pacu. Though the traditional bag feeding technique is followed for IMCs, about 15% of the production is under sinking and floating extruded feeds, thus opening significant opportunities for the formulated feed market to grow in the freshwater sector.

Most of the corporate and big feed mills are having state of the art facilities for the production of good quality water stable shrimp feed pellets. Most of the shrimp feed produced in India uses a ring die pellet mill to produce a compact sinking pellet. In general, the shrimp feeds for tiger shrimp consist of three grades of crumble (C1, C2 and C3) and three to four grades of pellets in the diameter of 1.8, 2.0 and 2.2 mm. However, there is a gradual shift in the sizes of pellet in the feed meant for pacific white shrimp, *Penaeus vannamei*. The feed pellet sizes of 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 mm are used to feed vannamei (Ambasankar et al 2017). Though the production of feed pellets at sizes less than 1.8mm is a high energy consumption process, feed millers and farmers are of the view that smaller pellets are better and could be able to meet the requirement of the current practice of white shrimp farming at higher densities than *P. monodon*.

A success story on vanami^{Plus}: Doubling farmers' income through inland low saline shrimp farming in Northern India through ICAR- CIBA's initiative under the skill India Programme on fish nutrition

The Pacific white shrimp, *Penaeus vannamei* has become the most sought after shrimp species by the shrimp farming community in India ever since this species was introduced way back in 2009 and has become the main species cultured in brackishwater. The versatility of the species and the amenability to culture in varying geographical conditions resulted in the horizontal expansion of the species beyond the coastal areas and none reached the inland saline areas of North India. The farmers of inland saline regions of Haryana, Punjab and Rajasthan showed an increased interest in the farming of vannamei due to its low productivity and profitability in agricultural farming. Inland shrimp farming in North India is practiced using the amended low saline water as an alternate farming technology option, with crop duration of 90-120 days for doubling farmers' income through the production of high value shrimp along with employment generation and societal development.

Due to escalating prices of farming inputs, production costs of shrimp is also increasing. Formulated pelleted feed is a major recurring cost which often ranges from 50 to 60 % of the total cost of production. At present, the feed cost is about Rs. 80 to 90/kg in intensive shrimp farming regions. The price is further high in newly emerging shrimp farming regions such as Haryana, Rajasthan and Punjab, as the feed is brought from southern states such as Andhra Pradesh, with additional cost on logistics.

In this scenario, ICAR- Central Institute of Brackishwater Aquaculture (CIBA) focused research on the nutrient requirements of shrimp, with expertise in scientific feed formulation and a database on the price and seasonality of locally available ingredients led to the creation of a cost-effective shrimp feed using indigenous feed processing technology. This Desi feed has been branded as vanami^{Plus} and is available to a farmer for Rs. 70-75 when other multinational brands are sold in the range of Rs. 85-95. By using vanami^{Plus} farmers can easily save up to Rs. 15-20/kg in feed cost directly. CIBA's vanami^{Plus} has been widely tested in farmers' ponds under a partnership model in major shrimp farming states of India with better results. Having heard about the success story of the vanami^{Plus}, Mr. Vinod Poonia one of the pioneers in the inland shrimp farming attended a ten days specialised training course on shrimp feed technology. The skilled training has motivated him to establish a small feed mill to produce shrimp feed. He signed an MOU with ICAR-CIBA for the establishment of an indigenous shrimp feed mill using CIBA's technology. In this scenario, a new feed mill, Dr. Attar Aqua Feed adopting vanami^{Plus} feed technology, has been commissioned at Bhiwani, Haryana, as the first of its kind in Northern India. The feed mill was inaugurated on 15th May 2018. The feed produced in this mill is being directly marketed to shrimp farmers in Haryana, Punjab, and Rajasthan states. The cost of CIBA vanami^{Plus} to the farmer is Rs.70 -75 per kg, which is cheaper by Rs. 15-25 in comparison to the commercially available feeds in that region. The farmers who used the feed have realized successful harvests with good growth and an impressive FCR of 1.0 to 1.2. The cost effective white shrimp feed of CIBA vanami^{Plus} resulted in an additional 25 % reduction in the production cost of feed, thereby increasing the profit margin of the farmer. During the current year, more than 50 farmers have used this feed and got benefited to a tune of Rs. 50,000 to Rs.70,000 per acre through the use of CIBA's vanami^{Plus} feed. The joint efforts by the entrepreneur and the ICAR institutions such as CIBA and Government can utilize this model for doubling the farmer's income and also generate employment.



This initiative could lead to the blue revolution drive of the Indian government.

Co-operative feed mills are the need of the hour in this region:

Currently, the country's major feed requirement is produced from the states of Andhra Pradesh and Tamil Nadu. The availability of cost-effective feed in the hinterlands of inland saline regions of North India and central India is a challenge. The farmers are getting the feed at a comparatively higher cost thereby preventing the horizontal expansion of fish and shrimp farming in the newer areas. There were no commercial fish/shrimp feed mills in these regions. CIBA has established a medium-scale shrimp feed mill in the state of Haryana which has paved the way for the expansion of shrimp farming in the inland saline regions of north India and contributed to the sustainability of shrimp aquaculture in this region. These states, which are importing from other states, cause a revenue loss to the state in the form of various tax elements.

A large number of target population is available in the newer and far-off regions where in aquaculture developments are just getting started and beginning to expand. There are no real competitors in the newer locality. Hence, the concept of cooperative feed mill with a mission mode approach will help in the availability of cost effective feed for aquaculture. In this concept, a feed mill to cater to the requirements of 200 hectares of culture area can be planned with the participation of farmers as stakeholders. The required feed can be produced as per demand and can be directly marketed with the concept of "Factory to Farm" to avoid the marketing costs. This will lower the feed costs and directly benefit the farmers. The project may be conceived in the inland saline regions of different states to produce various types of fish and shrimp feeds for the aquaculture sector. This novel proposal envisioned to establish an integrated feed mill with the active support of the state government in collaboration with the CIBA, Chennai. The fish and shrimp feed unit with a capacity to produce one tonnes per hour (TPH) of extruded and pelleted feeds would be ideal for this region. There is plenty of fish feed raw materials available in the newer areas itself, which is the foremost requirement for such an initiative. The unit will be consuming a major portion of locally available raw materials, which otherwise get transferred to the units outside states at lower price for industrial purposes.

Farm made Feed:

Farm-made feeds are made for the exclusive use of a particular farming activity, not for commercial sale or profit. Thus aqua feeds made by the co-operative of farmers and women self help groups would be regarded as farm-made feeds and not commercial feeds even though they are being sold in the nearby area in addition to catering to the needs of their group farming requirement of feed. Thus feeds made by individuals or groups of individuals who are primarily farmers are farm-made. Those manufactured by companies, whose primary business is animal feedstuff production and which have diversified into aqua feeds, or which have set up specific aqua feed mills are commercial. Farm made feed can be prepared for shrimp, fish or crab based on the demand

Selection of site for farm made feed machinery and identification of farmer/Self Help Group – Guidelines for establishment of farm-made feed (FMF)

The location of the farm made feed unit is significant for the success of this technology. While selecting the site two critical things are to be considered

- (i) The unit should be located where aquaculture farming is practiced so that the marketing of feed becomes easy without additional expense on transportation
- (ii) It should be in an area where the ingredients used for FMF should be available at a reasonable price and the majority should be sourced locally.

If it is going to be operated in a cooperative/ self-help group mode then the identification of a suitable location has to be finalized by taking into consideration the following

- a) A common place in the village can be hired or rented
- b) If the place is self-owned by the member of the group agreement ensuring the use of the place by the group for the fixed period should be spelt out in the Memorandum of Agreement
- c) The selected place should have the facility for three phase electricity supply
- d) It should preferably be located away from residential area and it should be easily approachable by road

Feed ingredients used for farm made feed formulations

The central focal element in FMF technology is the type of ingredient used. It is always preferable to use less conventional and more non-conventional feed ingredients in FMF to keep the ingredient costs at minimum possible level. The formulations should be based on the locally available ingredients and cost in addition to their nutritional composition.





We cannot use high cost ingredients like fish meal and fish oil in farm made feed formulations and hence good quality ingredients can be used which are locally available. Plant protein sources like ground nut oil cake, gingelly oil cake, mustard cake, sunflower meal, cotton seed cake, rape seed cake, soybean meal and coconut cake; energy sources like maize, broken rice, broken wheat, tapioca thippi, bajra, jowar and ragi; and apart from these, bengal gram waste, black gram waste, red gram waste, green gram bran, bakery waste, brewers waste, rice bran and wheat bran can also be incorporated in the production of FMF. Additionally, natural binders like gaur gum, tapioca flour, wheat flour and rice flour can be used in FMF.

Conclusion:

Indian aqua feed industry has rapidly evolved over the last five years to cater the needs of the fast-growing carp and shrimp grow-out sector. However, the hinterlands of inland saline regions continue to depend on feeds from far-off places. The availability of locally produced feed using indigenous technology, coupled with the establishment of the co-operative feed mills in strategic locations, will support the growth of this vibrant Indian aquafarming sector and pave the way for the sustainability.

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Smart Feed Management and Use of IoT in Aquaculture

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Shrimp farming is one of the economically important practices in aquaculture and can contribute significantly to the GDP growth of India. High cost of production is the major challenge in traditional shrimp farming. To overcome such challenges, there is a need to adopt new technological advances like 'Internet of Things' (IoT). In recent times, there has been substantial advancement in innovation and use of information technology tools in aquaculture. Sensor-based feeding devices have been developed, automatic water quality assessment has been made possible through pre-installed probes, mobile apps that capture the real-time data and assist to alert the farmers have been brought about. All these tools significantly help the farmers in data driven decision making. Growel has conducted a study on the efficacy of manual feeding vs. timer-based auto-feeding vs. sensor-based (acoustic) auto-feedings. Results have showed that acoustic auto-feeding of extruded feeds has generated higher production efficiency (Biomass-9200 kg), higher harvest weight (31.25g), good FCR (1.13) and high survival (92%) compared to the feeding of extruded or pelleted feeds through the other two methods of feeding systems. The stocking densities, DOC and water quality parameters were kept constant across all treatments while carrying out the above study. Therefore, it is imperative that the use of extruded feeds in combination with sensor-based auto-feeders resulted in higher yields.

Growel Aqua 360° Mobile App is a complete aquaculture management platform for shrimp and fish farmers. It helps farmers manage their farms and ponds effectively by helping them easily logging and keeping track of culture metrics and parameters. The registered farmers can get their farms mapped through a Web Portal by the technical support staff. Farmers can get the nearest Growel seller information across India. The metrics and parameters that can be tracked using the app are tailored by leading aquaculture experts and incorporate the latest advances in aquaculture research and help on board farmers to industrial best practices seamlessly. It includes a chat facility for farmers to connect to technical experts to acquire advice at the right time and maximize their productivity. It keeps farmers to keep update with the latest news and articles from the aquaculture industry around the world. It includes several convenient calculators that are essential for day-to-day proactive farm management. It shows farm gate prices for many species in several important Indian markets that are collected from the field. Standard curve of Daily feed dispenses (kg), ADG (gm), ABW (gm) of each crops helps the farmers to check the optimum growth and feed intake. Facility to switch pro and lite version of Growel Aqua 360° also helps the farmer to keep a track on production value per crop and history of previous crops.

Antimicrobial Resistance in Aquatic Environment: Implications and Mitigation Strategies

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Introduction:

Antimicrobial resistance (AMR) is a global problem and an imminent threat to human and animal health, the global economy, and a burden on public health as well as food and nutritional security. Food demand is increasing in response to the growing population. To achieve a balance between production, consumption, and demand, global food production is primarily dependent on land availability and an adequate supply of water. However, the balance is frequently disturbed by rising populations, climate change, and natural disasters. Aquaculture is a major source of sustenance for the world's population and is essential to achieving the 2030 sustainable development goals, including the goal of ending hunger (Goal 2). It is currently the world's fastest-growing food-producing sector. Aquaculture, which includes the farming of fish, crustaceans, and other aquatic animals and plants, is widely considered to be safe for consumption. It is an important source of dietary protein as well as a key source of income for many artisanal fishers and large-scale farming. With the global increase and reliance on aquaculture, husbandry techniques have undergone rapid change. However, aquaculture produce is associated with food safety issues such as contamination with abiotic and biotic agents such as chemicals, toxins, antibiotic residues and biological agents like bacteria, viruses etc. The most prevalent diseases in warm water aquaculture are associated with *Vibrio*, *Aeromonas*, and *Edwardsiella*. They cause significant morbidity and mortality in fish and shrimp populations and antibiotics are often used as therapeutics or preventatives. Although these treatments are crucial for sustainable aquaculture, using antibiotics indiscriminately increases the risk of drug resistance in pathogens and spread to innocuous microbiota native to the host aquatic environment. With the inexorable rise in the use of antibiotics, all the sectors where they are used irrationally have become reservoirs for the spread of AMR affecting human, animal, fisheries and ecosystem health drastically. The pool of AMR genes in the aquatic environment could be derived from agriculture run-off, the livestock sector, hospital effluents or the human system. The Spread of this resistance in animal and human bacterial pathogens can be through direct selection of resistant variants or transfer of AMR genes within the aquatic environment, from the aquatic to the terrestrial environment, or vice versa. With WHO declaring AMR as one of the top 10 global public health threats facing humanity, there is a dire need for understanding the implications of AMR and developing a comprehensive approach to address AMR in aquaculture and bring out strategies to mitigate it. It is incorrect to perceive aquaculture as a culprit always for AMR. The aquatic environment can also be a victim of the use of antimicrobials in other sectors as all terrestrial effluents reach the aquatic environment.

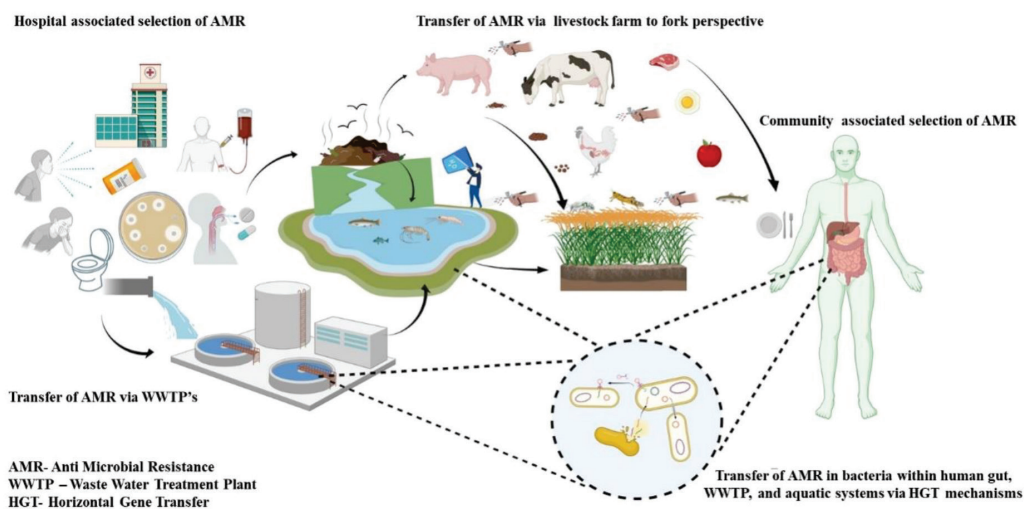
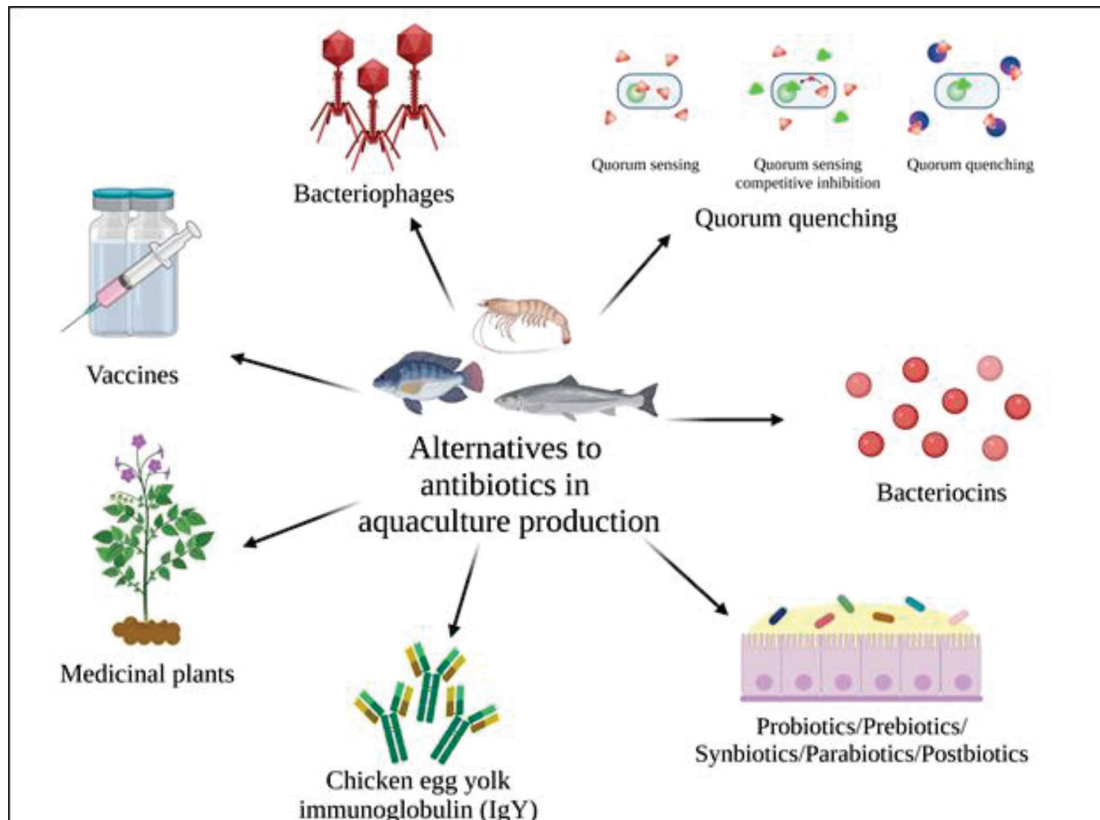


Figure: Aquatic environment as an important source of horizontal transfer of antimicrobial resistant bacterial pathogens

Source : Antimicrobial resistance in fish pathogens and alternative risk mitigation strategies. Reviews in Aquaculture. Deekshit VK, Maiti B, Krishna Kumar B, Kotian A, Pinto G, Bondad-Reantaso MG, Karunasagar I, Karunasagar I. 2023 Jan;15(1):261-73.

Alternative strategies to combat and mitigate AMR in aquaculture

Alternative approaches for combating bacterial infections in aquaculture are required due to the rise in antibiotic-resistant bacteria and antibiotic residues, which have raised concerns on a global scale. Apart from following good aquaculture practices (GAP's) several alternatives to antibiotics have been implemented in aquaculture. Vaccination is an ideal method for preventing infectious diseases. Probiotics and bacteriocins have also been proposed as promising alternative strategies to the use of antibiotics in the aquaculture industry. The use of phytocompounds and essential oils, which are natural components of plants that are generally recognized as safe substances, is another source of alternative treatments. These oils may serve as alternative prophylactic and therapeutic agents in aquaculture due to their antimicrobial properties. Furthermore, phage therapy has received a lot of attention recently because of its benefits in preventing and controlling pathogen infections, and it has been used successfully in aquaculture facilities.

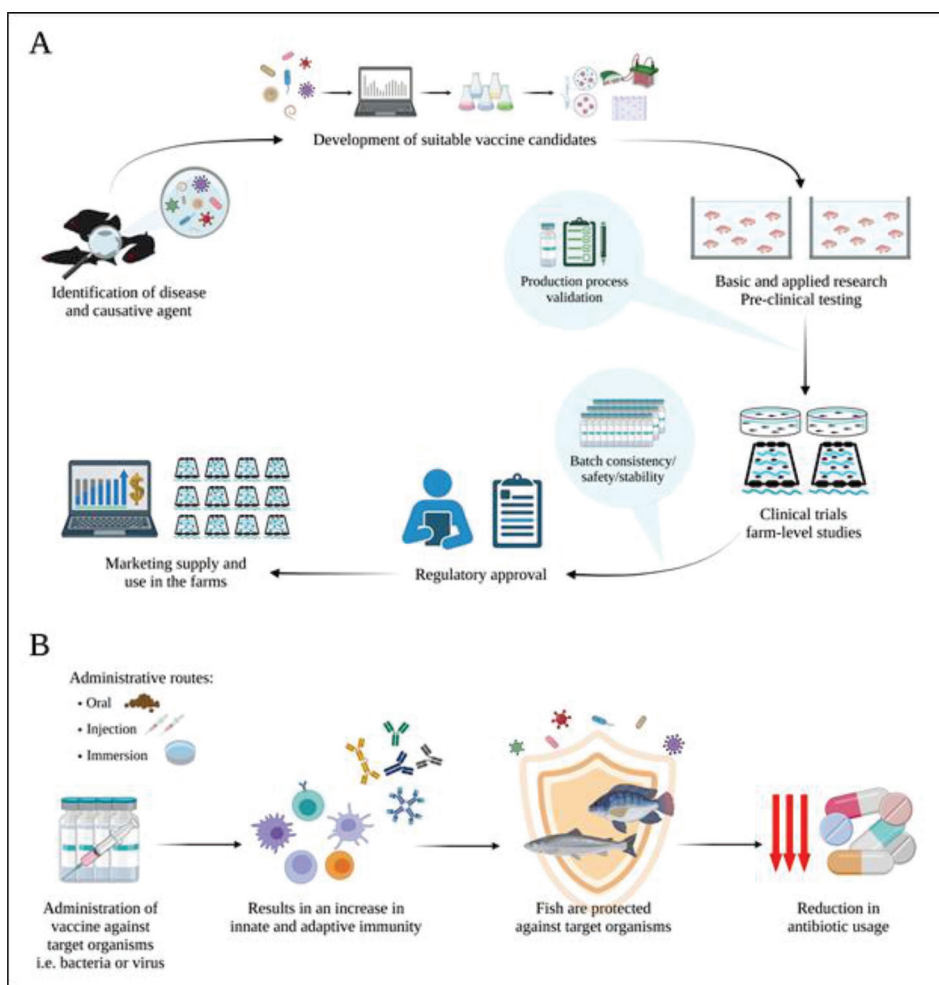


Alternative approaches to reduce the use of antimicrobials in aquaculture, e.g. vaccines, bacteriophages, quorum quenching, bacteriocins, chicken egg yolk immunoglobulin, medicinal plants and microbiomes

Source: Review of alternatives to antibiotic use in aquaculture. *Reviews in Aquaculture*. Melba G. Bondad-Reantaso, Brett MacKinnon, Iddya Karunasagar, SophieFridman, Victoria Alday-Sanz, Edgar Brun, Marc Le Groumellec, Aihua Li, Win Surachetpong, Indrani Karunasagar, Bin Hao, Andrea Dall'Occo, Ruggero Urbani, Andrea Caputo. 2023 Jan; 15(2) (In press)

Vaccines:

Vaccines are recognised as critical tools for the prevention and control of fish diseases and are considered an essential route to the reduction in antibiotic usage within the aquaculture industry. A licensed fish vaccine typically contains attenuated live or killed organisms or their products, antigens of whole-cell proteins or more recent recombinant proteins or subunits such as DNA/RNA, etc. that elicit strong cellular and humoral immunity and provide long-lasting protective immunity. Polyvalent or multivalent injectable vaccines containing adjuvant and multiple antigens are currently used in large-scale commercial aquaculture operations.



Vaccination is a key tool to ensure sustainable aquaculture production. (A) Vaccine developmental stages from identification of disease and causative agent to research, production process validation, clinical trials and farm-level studies, to regulatory approval, marketing and application. (B) Vaccine protects target organisms through increasing innate and adaptive immunity leading to a reduction in antibiotic usage.

Source: Review of alternatives to antibiotic use in aquaculture. *Reviews in Aquaculture*. Melba G. Bondad-Reantaso, Brett MacKinnon, Iddy Karunasagar, Sophie Fridman, Victoria Alday-Sanz, Edgar Brun, Marc Le Groumellec, Aihua Li, Win Surachetpong, Indrani Karunasagar, Bin Hao, Andrea Dall'Occo, Ruggero Urbani, Andrea Caputo. 2023 Jan;15(2) (In press).

List of some of the commercially available vaccines for the prevention of diseases in aquaculture

Sl. No	Developer	Country	Vaccine	Brand Name of the Vaccine	Type of Vaccine	Aquaculture species	Target Diseases
1	Elanco U.S. Inc.	U. S	<i>Aeromonas salmonicida</i> Bacterin	Furogen Dip	Bacterin	Koi carp (<i>Cyprinus carpio</i>) and salmonids	Furunculosis
2	Elanco U.S. Inc.	U. S	Arthrobacter Vaccine	Renogen	Live Culture	Salmonids	Bacterial Kidney Disease
3	Elanco U.S. Inc.	U. S	<i>Aeromonas salmonicida</i> - <i>Vibrio anguillarum</i> - <i>Ordaliisalmonicida</i> Bacterin	Lipogen Forte	Bacterin	Salmonids	Vibriosis



4	Elanco U.S. Inc.	U. S	Infectious Salmon Anemia Virus Vaccine, Killed Virus, <i>Aeromonas Salmonicida-Vibrio anguillarum-Ordaliisalmonicida</i> Bacterin	Forte V1	Inactivated or “killed” vaccine	Salmonids	Infectious Salmon Anemia
5	Elanco U.S. Inc.	U. S	<i>Yersinia ruckeri</i> Bacterin	Ermogen;	Bacterin	Salmonids, trout, eel, minnows, tilapia	Yersiniosis/ Enteric redmouth
	Intervet UK Ltd			AquaVac® ERM; AquaVac® ERM Oral;	Inactivated vaccine	Rainbow trout (Oncorhynchus mykiss)	Yersiniosis/ Enteric redmouth
	Intervet International BV			AquaVac® RELERA™	Inactivated	Rainbow trout (Oncorhynchus mykiss). Salmon and Trout	Yersiniosis/ Enteric redmouth
6	Elanco Canada Limited	Canada	Infectious Hematopoietic Necrosis Virus Vaccine	Apex-IHN (Canada)	DNA	Salmonids	Infectious Haematopoietic Necrosis Virus
7	INTERVET INC	U. S	<i>Edwardsiella ictaluri</i> Vaccine	AquaVac-ESC™	Avirulent Live Culture	Catfish, Channel catfish, freshwater catfish, striped catfish, brown bullhead, Donio spp	Edwardsiellosis/ Enteric septicemia
8	INTERVET INC	U. S	<i>Flavobacterium Columnare</i> Vaccine	Aqua-Vac Col	Avirulent Live Culture	Catfish	Columnaris Disease (Flavobacteriosis/ Columnaris)

Source: Antimicrobial resistance in fish pathogens and alternative risk mitigation strategies. *Reviews in Aquaculture*. Deekshit VK, Maiti B, Krishna Kumar B, Kottian A, Pinto G, Bondad-Reantaso MG, Karunasagar I, Karunasagar I. 2023 Jan;15(1):261-73.

Bacteriophages:

Antibiotic resistance has rekindled interest in bacteriophage therapy. Bacteriophages are a promising alternative to antibiotics, without eradicating other possibly advantageous microorganisms, and offer a possible alternative to antibiotics. Phages can be used as a feed additive or as a water additive. Most of the phages utilized in aquaculture are members of the family *Myoviridae*, *Podoviridae*, and *Siphoviridae*, and belong to the order Caudovirales. However, there are limitations of the phage therapy like the narrow host range and the potential for the emergence of resistant mutants as a result of spontaneous mutations. Phage-derived enzymes or enzybiotics that target bacterial cell wall, bacterial capsules, extracellular biofilm matrix, or lipopolysaccharides are also promising alternatives to antibiotics.

Commercially available phage-based solutions for aquaculture

Sl. No	Developer	Country	Name of the Brand	Aquaculture species	Target Diseases
1	Fixed Phage Ltd,	United Kingdom	aquaPHIX™	Shrimps	Early Mortality Syndrome.
2	Mangalore Biotech Laboratory,	India	LUMI -NIL MBL	shrimp hatcheries	Luminous vibrios
3	Proteon Pharmaceuticals,	Poland	Bafador®	Fin fish aquaculture	<i>Pseudomonas</i> and <i>Aeromonas</i> infections



4	ACD Pharmaceuticals AS,	Norway	CUSTUS®YRS	Yersiniosis, or enteric red mouth disease	Yersiniosis, or enteric red mouth disease
5	Vetophage SAS,	France	VetoPhage	Shrimp	<i>Vibrio parahaemolyticus</i>
6	Intralytix	USA	phage cocktail (under development-2016)	Oyster aquaculture	<i>Vibrio tubiashii</i> and <i>Vibrio coralliiticyis</i> infections
7	Phage Biotech Ltd,	Israel	phage-based treatment	in shrimp hatcheries	<i>Vibrio Harveyi</i>

Source: Antimicrobial resistance in fish pathogens and alternative risk mitigation strategies. *Reviews in Aquaculture*. Deekshit VK, Maiti B, Krishna Kumar B, Kotian A, Pinto G, Bondad-Reantaso MG, Karunasagar I, Karunasagar I. 2023 Jan;15(1):261-73.

Phytocompounds or herbal medicinal plants

The use of natural compounds such as phytocompounds that can outweigh the synthetic drugs commercially available. These phytocompounds are bioactive agents that have antibacterial antiparasitic, antiviral, and antifungal activity and hold much potential. They also stimulate innate or specific immune responses and non-specific immune responses in aquatic animal hosts. Plant extracts like alkaloids, flavonoids, pigments, phenolics, terpenoids, steroids and essential oils are efficacious as well as safe for the host, consumer, environment and handler. However, for on-site farm application, further studies on the practical utility of plant-based products including data on toxicity, and optimal dosage are essential.

Probiotics, Prebiotics and Synbiotics,

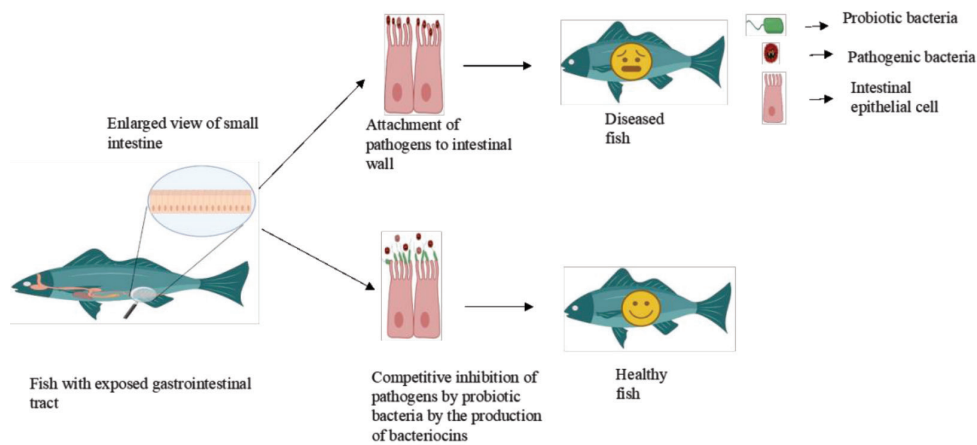
Probiotics are live microbial feed supplements, whereas prebiotics are non-digestible food ingredients that benefit the host by stimulating intestinal bacteria growth and activity. On the other hand, synbiotics is a combination of pre and probiotic. Probiotics boost immunity, growth rates, and disease resistance. On the other hand, prebiotics improve GI microflora thus helping the growth of cultured animals. Synbiotic aids in improved survivability and microbial activity in GI tract. The use of synbiotics can confirm the greater benefits than individual application. Specific strains of yeast, algae and other probiotic microorganisms have been employed in aquaculture. Representatives of *Bacillus sp.*, *Lactococcus sp.*, *Micrococcus sp.*, *Carnobacterium sp.*, *Enterococcus sp.*, and *Lactobacillus sp.*, are commonly used. The surrounding environment greatly influences the health status of aquatic organisms. These microbial feed supplements may include microorganisms that prevent the multiplication of pathogens in the gut, on structural surfaces, and in the growing environment. They also improve the water quality of the culture as well as contribute to food digestion. They have the potential to stimulate host immune responses. One of the major concerns of using probiotics is to make sure that the organism used is free of plasmid-encoded antibiotic resistance genes.

Quorum-quenching:

The formation of biofilms by the bacterial pathogen is one of the major factors for the establishment of disease in aquaculture. A novel strategy used in aquaculture is the disruption of the quorum-sensing (QS) trait in bacterial pathogens is quorum quenching. Bacteria that can degrade QS molecules can be used as biocontrol agents in aquaculture. QS inhibitors from our marine resources like marine microbes are used.

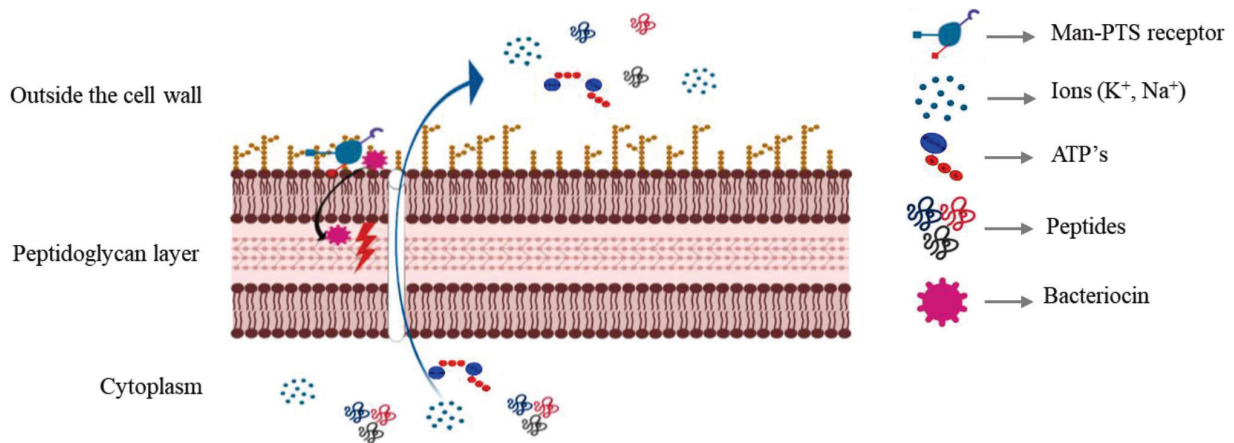
Bacteriocins:

Bacteriocins, bioactive compounds or antimicrobial peptides produced by bacteria possess antibacterial activity against closely related and other bacteria. They are found in all lineages of bacteria and can inhibit or kill closely or distantly related microorganisms. These proteins are potent immunomodulators with broad-spectrum inhibition properties. These peptides are highly specific in action, have a low risk of toxicity and have no adverse impact on the environmental impact. They are encoded either in the chromosome or in extrachromosomal elements.



Common inhibition strategy used by bacteria using various bacteriocins

Source: *Potential application of bacteriocins for sustainable aquaculture. Reviews in Aquaculture. Nayak A, Karunasagar I, Chakraborty A, Maiti B. 2022 Jun;14(3):1234-48.*



Mode of action of class I bacteriocin with pore formation.

ATP= adenosine tri phosphate, K⁺ = potassium, Na⁺ = sodium

Source: *Potential application of bacteriocins for sustainable aquaculture. Reviews in Aquaculture. Nayak A, Karunasagar I, Chakraborty A, Maiti B. 2022 Jun;14(3):1234-48.*

Possible advantages and limitations of bacteriocin applications in the aquaculture system

Advantages	Limitations
<ul style="list-style-type: none"> • Antiviral property by interfering with late stages of viral replication • Antimicrobial activity against <i>Aeromonas</i> toxins (aerolysin and hemolysin) through interaction with binding sites and preventing extracellular cell cleavage • Stability over wide range of temperature and pH • Easy to incorporate along with good binding property with the feed. • Eradicate antibiotic sensitivity problems usually associated with the use of antibiotics 	<ul style="list-style-type: none"> • Usually associated with non-specific inhibition of bacteria • May disrupts the bioremediation process by acting against the microbiota • Cannot be used for diseased and starving fishes • Some bacteriocins known to be cytolytic to higher animals including fishes and some extent to humans • In case lipid II targeting bacteriocins, there is a possibility of resistance among bacteria



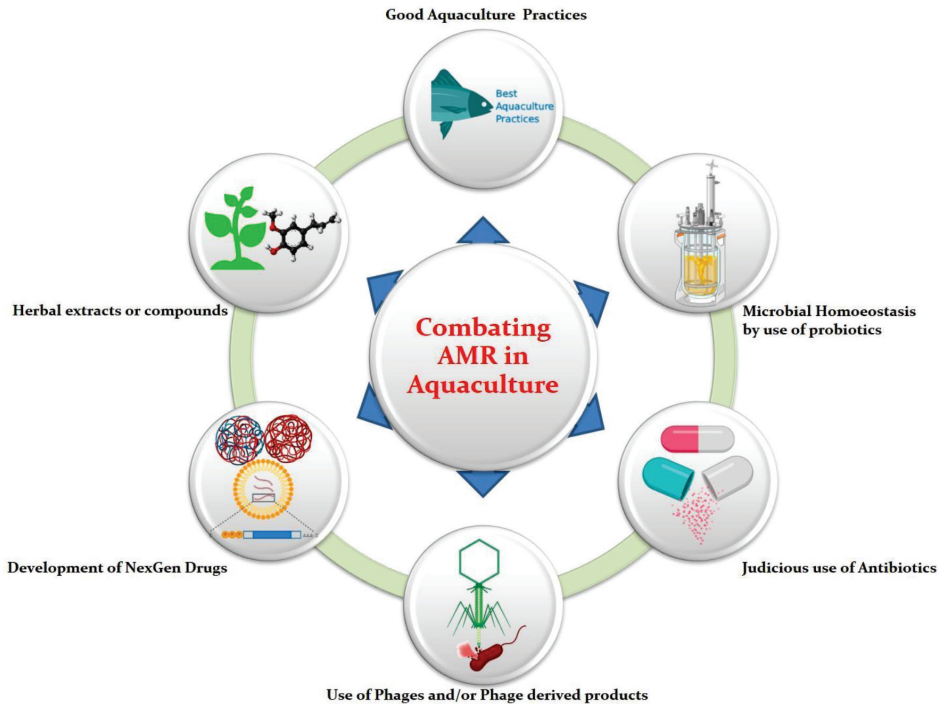
Bacterial strains	Bacteriocins	Class of bacteriocins	Target organisms	Gene	Mode of action	Reference
<i>Lactococcus lactis</i>	Lacticin 481	Class I-lantibiotic	<i>C. tyrobutyricum</i> and wide range of lactic acid bacteria	<i>lctA</i>	Formation of transmembrane pores the depolarising the membranes	Piardet al. 1993
<i>L. lactis</i>	<u>Nisin A</u>	Class I-lantibiotic	<i>Enterococcus</i> , <i>Listeria</i> , <i>Clostridium</i> , <i>Leuconostoc</i>	<i>span</i>	Pore formation in the cytoplasmic membrane	Van et al. 1993
<i>Bacillus subtilis</i>	Sublancin 168	Class I-lantibiotic	<i>B. cereus</i>	<i>sunA</i>	Competitive exclusion	Paik et al. 1998
<i>Lactobacillus plantarum</i>	Plantaricin W β	Class I-lantibiotic	<i>L. lactis</i> , <i>Leuconostoc mesenteroides</i> , <i>S. aureus</i> , <i>Enterococcus faecalis</i> , <i>Listeria monocytogenes</i>	<i>plWbeta</i>	Defence response to Gram-positive organisms	Holoet al. 2001
<i>L. lactis</i>	Nisin Q	Class I-lantibiotic	Lactic acid bacteria, <i>Bacillus</i> , <i>Listeria</i> , <i>Micrococcus</i>	<i>nisQ</i>	Pore formation by depolarising the bacterial cytoplasmic membrane	Zendo et al. 2003
<i>B. licheniformis</i>	Lichenicidin A1	Class I-lantibiotic	<i>L. monocytogenes</i> , methicillin-resistant <i>S. aureus</i> , <i>S. pneumoniae</i>	<i>lanA1</i>	Depolarization of energized bacterial cytoplasmic membranes and formation of pores	Begley et al. 2009
<i>Paenibacillus polymyxa</i>	Paenicidin A	Class I-lantibiotic		<i>paeA</i>	Depolarisation of bacterial cytoplasmic membrane	Lohanset al. 2012
<i>Enterococcus faecium</i>	Enterocin Q	Class IIc bacteriocins	<i>L. sakei</i> , <i>E. faecium</i>	<i>entQ</i>	Not available	Paulsen et al. 2003
<i>E. faecalis</i>	Enterocin Xalpha	Class IIa bacteriocins	<i>L. plantarum</i> , <i>L. lactis</i> , <i>Listeria innocua</i>	<i>enxA</i>	Not available	Hu et al. 2010
<i>L. johnsoni</i>	Lactacin-F	Class IIb	<i>Lactobacilli</i> , <i>E. faecalis</i> , <i>Shigella</i> sp.	<i>lafX</i>	Formation of heat-stable bacteriocin complex (180 kDa) and cytolysis	Sri et al. 2021
<i>E. faecalis</i>	Enterocin Xbeta	Class IIb	<i>Bacillus subtilis</i> , <i>B. circulans</i> , <i>L. plantarum</i> , <i>L. innocua</i>	<i>enxB</i>	Cytolysis	Hu et al. 2010
<i>L. acidophilus</i>	Acidocin A	Class IIb	<i>Lactobacillus</i> sp. <i>L. monocytogenes</i>	<i>acdA</i>	Cell lysis	Kanatani et al. 1995
<i>Lactobacillus plantarum</i>	Plantaricin F	Class IIb	<i>Pediococcus pentosaceus</i> , <i>Lactobacillus</i> , <i>Listeria</i> , <i>Micrococcus</i> , <i>Salmonella</i> , <i>Pseudomonas</i> , and <i>Pediococcus</i>	<i>plnF</i>	Rupture of cell membranes and the loss of cell materials	Fricourt et al. 1994
<i>E. faecalis</i>	Enterolysin A	Class III	<i>L. sakei</i> , <i>L. acidophilus</i> , <i>Clostridium sporogenes</i> , <i>C. butyricum</i> .	<i>enlA</i>	Breaks down the cell wall of target bacteria	Bryan et al. 2021

Source: Potential application of bacteriocins for sustainable aquaculture. *Reviews in Aquaculture*. Nayak A, Karunasagar I, Chakraborty A, Maiti B. 2022 Jun; 14(3):1234-48.

Good Aquaculture Practices (GAQPs):

Good Aquaculture Practices (GAQPs) must be implemented to ensure long-term aquaculture production without an outbreak of infectious diseases. The term “good aquaculture practises” refers to following a set of guidelines, instructions, and protocols. that is designed to foster efficient and responsible aquaculture production. It also involves biosecurity to help ensure product quality, safety, and environmental sustainability. Following the FAO Code of Conduct for Aquaculture by farmers to ensure sustainable, disease free aquaculture production. Apart from this, for the creation of competent antimicrobial stewardship programmes, quantification and tracking of antimicrobial use (AMU) are essential.

Conclusion:



Various preventive strategies to combat the burden of antimicrobial resistance in aquatic environment

Source: Antimicrobial resistance in fish pathogens and alternative risk mitigation strategies. Reviews in Aquaculture. Deekshit VK, Maiti B, Krishna Kumar B, Kotian A, Pinto G, Bondad-Reantaso MG, Karunasagar I, Karunasagar I. 2023 Jan;15(1):261-73.

Innovative strategies to mitigate AMR in aquaculture and decrease diseases are paramount to achieving the global perspectives on food sustainability required by the blue growth philosophy and blue economy goals of new aquaculture 4.0 strategy. The technique chosen should result in the gradual acceptance of policies that support the implementation of good manufacturing and immunization practices, biosecurity and welfare, disease surveillance, and improve the quality of the produce in aquaculture.



Fish Safety and Quality – Global and National Perspectives

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Fish is an important contributor to nutrition and food security at the global level. According to the FAO State of the World Fisheries and Aquaculture 2022, globally, aquatic foods provided about 17% of animal proteins and 7% of all proteins in 2019. In some countries, aquatic foods contribute to half or more of animal protein intake. Fish is also a highly traded commodity and in 2020, the exports of aquatic products was \$151 billion, which is a 7% decline due to Covid-19 from \$165 billion in 2018. Indian seafood exports crossed \$7.0 billion in 2017-18, but covid-19 impacted the exports during subsequent years.

There are very stringent requirements in the international trade of seafood. The international organization dealing with trade issues is World Trade Organisation (WTO). Members of WTO signed the Sanitary and Phytosanitary (SPS) Agreement in 1995, which entitles every country to adopt measures to protect plant, animal and human health according to what they consider an “Appropriate Level of Protection”. But the measures should not be arbitrary and intended only to protect the domestic market, but the measures should be based on a scientific risk assessment carried out according to guidelines from international organisations such as the Codex Alimentarius Commission for issues related to food safety, World Organisation of Animal Health (WOAH, formerly known as OIE) for issues related to animal health and International Plant Protection Convention (IPPC) for issues related to plant health.

Codex Alimentarius Commission is a joint FAO/WHO body responsible for setting international standards for consumer protection and for ensuring fair practices in the trade. Food safety risk assessments are performed by the three main international committees (a) Joint FAO/WHO Expert Meeting on Microbiological Risk Assessment (JEMRA), (b) Joint FAO/WHO Expert Committee on Food Additives (JECFA), (c) Joint FAO/WHO Expert Meeting on Pesticide Residues (JMPR). These expert committees consist of experts drawn from all parts of the globe based on their expertise and they provide independent expert advice to the Codex Alimentarius Commission through risk assessment reports. Codex Alimentarius Commission is the global risk management body and the members of the Commission, who are high-ranking food safety officers of the member countries (eg Chief Executive Officer of Food Safety and Standards Authority of India) will make a risk management decision (Fig 1).

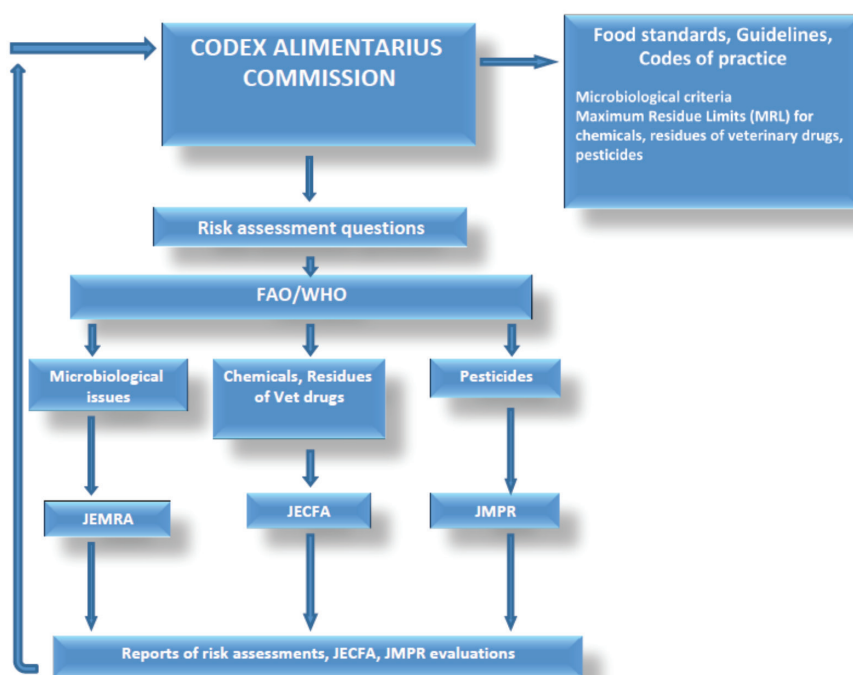


Fig 1. Relation between Codex Alimentarius Commission and Joint FAO/WHO Expert Committees.



Codex Alimentarius Commission may come out with food safety standards eg microbiological limits or maximum residue limits of veterinary drugs or the maximum permitted limits of food additives or contaminants (eg heavy metals, mycotoxins, polychlorinated biphenyls) or pesticide residues. But setting these limits alone will not ensure food safety. It is important to inform or guide the food business operators on how to produce safe food meeting Codex standards. For this purpose, Codex has a series of Guidelines and Codes of Practices. The fundamental requirement is that all food business operators should implement good hygienic practices all along the food chain, from primary production to consumption as outlined in the Codex General Principles of Food Hygiene (CAC 1-1969 last updated 2020). This gives the basic requirement but additionally, there are specific practices required in different sectors. For fish, there is a Code of Practice for Fish and Fishery Products (CXC 52- 2003 last updated 2019). This Code gives guidance on operational aspects of hygiene implementation on board a fishing vessel and in fish farms (primary production), at fish landing centers, fish transportation, and fish processing establishments handling frozen fish, dry fish, smoked fish and other products. In addition to this Code, there are guidelines for specific issues eg: Guidelines on the application of general principles of food hygiene for the control of pathogenic *Vibrio* spp in seafood (CXG 73-2010), Guidelines on the application of general principles of food hygiene for the control of *Listeria monocytogenes* in ready to eat foods (CXG 61-2007 last updated 2009) including smoked fish, Guidelines on the application of general principles of food hygiene for the control of parasites in foods (CXG 88-2016) including fish, Guidelines on the application of general principles of food hygiene for the control of viruses in food (CXG 79-2012) including bivalve molluscs, Guidelines for the sensory evaluation of fish and fishery products in laboratories (CXG 31-1999).

Regarding standards, Codex has a number of them for fish and fishery products eg: Standard for quick frozen finfish (CXS 36-1981), Standard for live and raw bivalve molluscs (CXS 292-2008 updated 2015), Standard for canned prawns (CXS 37-1991), Standard for canned crab meat (CXS 90-1981 updated 2018), Standard for canned fin fish (CXS 119-1981 updated 2018), Standard for smoked fish, smoke-flavoured fish, smoke-dried fish (CXS 311-2013 updated 2018), Standard for fish sauce (CXS 302 – 2011 updated 2018) and many more. The Codex Committee for Fish and Fishery Products is the subject-specific committee that develops Codex documents for fish and fishery products.

Codex has several committees, which deal with different subjects. Some committees like the Codex Committee of Food Hygiene deal with horizontal issues covering all commodities like fruits and vegetables, meat, milk and other foods.

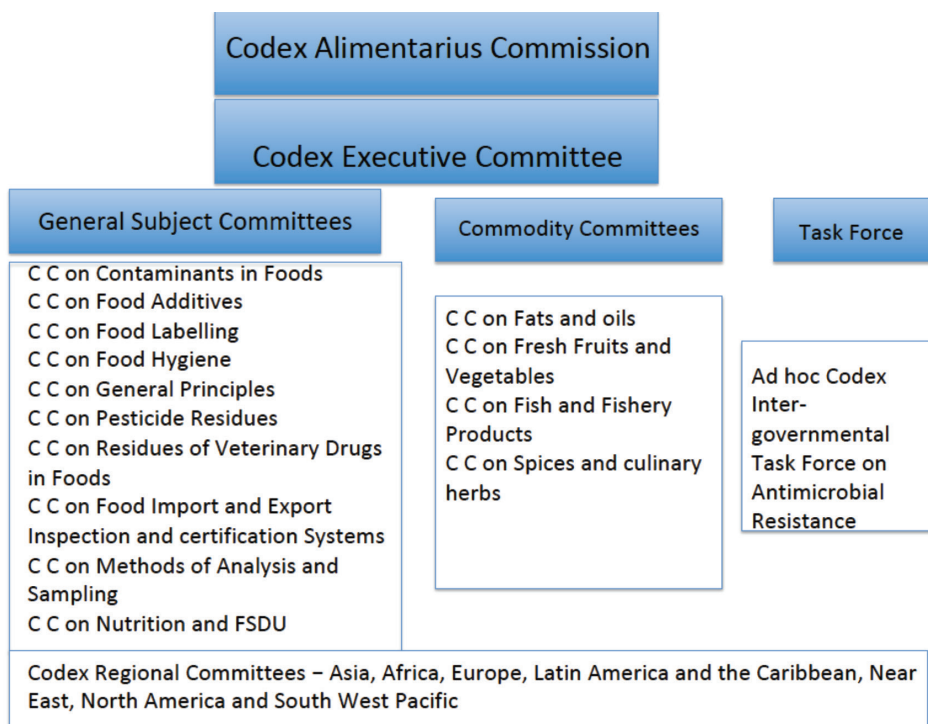


Fig 2. Structure of Codex Alimentarius Commission and the Committees.



Microbiological standards and guidelines related to fish and fishery products are developed by the Codex Committee on Food Hygiene. Risk assessment advice comes from JEMRA. Similarly, the residue limits for veterinary drugs in fish, milk, meat and other foods are developed by the Codex Committee on Residues of Veterinary Drugs in Foods. Scientific advice comes from JECFA. Similarly, residue levels of pesticides in various foods are developed by Codex Committee on Pesticide Residues. Scientific advice comes from JECFA and JMPR. Apart from commodity committees and general subject committees, there can be Ad hoc Task Force dealing with subjects like antimicrobial resistance.

FAO/WHO member countries may directly adopt Codex standards as national standards. In areas, where there are no Codex standards, countries may develop national standards based on national risk assessments. For example, the European Food Safety Authority (EFSA) has scientific panels, which perform risk assessments and provide scientific advice to the European Commission, which makes food safety regulations and standards. In India, the Food Safety and Standards Authority of India (FSSAI) is the national regulatory agency responsible for food safety as per Food Safety Act 2006. The scientific advice for setting standards is provided by scientific panels and scientific committees. There are horizontal scientific panels covering all foods eg Scientific Panel on Biological Hazards, Scientific Panel on Residues of Antimicrobial Residues in Foods. The Scientific Committee consists of Chairs of various scientific panels and additional independent experts. The Scientific Panel recommendations are considered by the Food Authority of India. The Food Authority of India makes risk management decisions.



Role of Genomics in Aquaculture: Status and Prospects in India

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Aquaculture is expected to contribute significantly in future food production to meet the demand coming from increasing population and need for nutritional security. This requires improvement of sustainability of aquaculture operations, which are more productive than before, lower risk of diseases and environment concerns and this cannot be achieved without infusion of modern technologies. Wild relatives of aquaculture species and harvested stocks are still found in nature and still hunted for direct consumption, though use of varieties improved through selection is still limited. Selective breeding programmes have been the foundation for genetic improvements in domesticated species, across the food commodities and contributed to food security. Among aquaculture species, white leg shrimp and GIFT Tilapia are well known globally and in India, Jayanti Rohu, GI Catla, Scampi and Magur are some of the examples. Selective program of Indian white shrimp is also now recently initiated through PMMSY support. In such programs, one of the important lacunae has been the lack of adequate knowledge of genetic diversity below the species level and the means of utilization in selection programs. The molecular genetic resources can be useful tools in such research programmes to document genetic stocks. In India, over 32 species have been characterised using molecular genetic resources such as mitochondrial genomes, microsatellite markers etc.

With the new technological developments leading to assembling whole genomes, transcriptomes and mining for trait specific genes and associate molecular markers are important research areas in agriculture science, including fisheries. The critical considerations for the development of genomic technologies require information of the high-quality whole genome assemblies, which can serve as reference genomes; genome-wide molecular data and its analysis and translate them into meaningful interpretations. Whole genome assemblies would provide insights into structure and organisation of genes as well as discovering the genomic variations, which will in turn facilitates development of genome-wide markers. These markers constitute thousands of markers, dispersed across the most of the regions of the genome. Another important consideration is data analysis; that is, the specific resources and expertise that are available to analyse the huge amount of whole-genome data generated in the process.

The genomic knowledge and their repositories have potential to revolutionise the concept of bio-prospecting of genes and allele mining, which have significant potential for use in improvement of commercially important traits, such as growth, biotic and abiotic stress tolerance/resistance, reproduction in aquaculture species. These may also assist in association mapping, involving a particular trait to a specific chromosomal region or a locus/locus. Thus, it would be useful in marker assisted selection in aquaculture species and would have positive impact on increased productivity from aquaculture. Globally, application of genomic selection in breeding programmes has been initiated in several aquaculture species, major species being Atlantic salmon and rainbow trout (D'Agaro et al., 2021). It is envisaged that genome sequencing in combination with large scale genotyping and phenotyping will elevate its usefulness in many other aquaculture species.

India, with its vast aquatic biodiversity, potentially holds the genomic resources responsible for adaptations accumulated over evolution under diverse climatic conditions. Understanding such adaptations and their underlying mechanisms will be important for future research on bioprospecting of genes and allelic variants. Such advancements need technological capacity and human resource capabilities developed, through actual programs. Realizing this critical gap, during the 12th plan period, in 2015 ICAR launched Consortium Research Platform on Genomics (www.nbfgf.res.in/crpgenomics) and ICAR-NBFGF, Lucknow as the lead centre and eight collaborating ICAR institutes. CRP-Genomics is working across the different commodities, like fish and shellfish, crops, animals, insects, microbes and pathogens. The Platform major objectives are to conduct research into structural and functional genomics in species with commercial potential, to discover variants of genes, from unexplored germplasm, to help cross learning, intensive genomic research related to agriculture by inter-linking all institutes with diverse capabilities and requirements (across commodities) and to develop quality human resources in the field of genomics system biology. The outcome of CRP-Genomics would give framework towards bio-prospecting of useful genes, marker assisted selection and genetic improvement, to enhance productivity.

Globally, whole genomes have been sequenced and assembled for approximately sixteen hundred fish species, as per deposited in NCBI GenBank. In India, some of the important whole fish genomes have been sequenced and



assembled in our country, including magur, rohu, hilsa shad, catla, mrigal, wild variant of zebrafish, pabda, grey mullet and White Shrimp (*Penaeus indicus*), and an oomycete (*Aphanomyces invadens*), a fish pathogen and some important species, like pearl spot, pompano are in the process of sequencing. This has led to discovery of several gene variants and novel genes. Several genes involved in osmoregulation in Hilsa shad (Mohindra et al., 2019) and for adaptations environmental and terrestrial conditions in magur (Kushwaha et al., 2021) were unearthed. Recently, high quality contiguous genome of *P. indicus*, with highest repeat content among the sequenced animal genomes, has been successfully completed (Katneni et al, 2022). Comparison of genomes, known as comparative genomics, have further role in finding evolutionary linkages and phylogenies based on the gene functions. The potential role of the repetitive elements in adaptation to their habitat and environment was reported by comparative genomics analysis of several fish species (Yuan et al., 2018). Similarly, whole mitochondrial genome of more than two thousand fish species, globally has been sequenced and characterized and information is available for 46 Indian fish species. It is widely used for species evolutionary linkages, phylogeny and species identification, especially highly species-specific COI region has been used as a barcode for a species. The recent initiative of deep-sea mission, launched by the Government of India, has documenting marine biological diversity and genomics as an important priority area.

The transcriptome sequencing or RNA-Seq can also be utilised to understand the large-scale expression profiles of the genes, under different environment, life stages or stress conditions, which in turn would explain involvement of the genes in influencing a particular trait. Large scale genomic markers can also be developed from the transcriptome, which may point out to the genetic variability profiles in the genes controlling the particular trait as well as the presence/absence of pressure exerted by the changing environmental conditions, during evolution. There are several studies reporting the candidate genes controlling the important commercial traits in aquaculture species. Several genes related to growth and compensatory growth in Indian major carp, *Catla catla* were identified through transcriptome analysis in different growth stages, which can form potential biomarkers for growth in breeding programmes (Mohindra et al., 2022). In another major carp, *Labeo rohita* (Harshini et al., 2022) and hilsa shad (Mohindra et al., 2023), key genes involved in salinity adaptation and osmo-regulations were reported. Through transcriptome studies in *Clarias magur*, genes interaction in brain and gonads for neuroendocrine inhibition of milt release were discovered (Agarwal et al., 2020). Another study reported the biological processes involved in reproduction through transcriptome analysis and protein interactions of differentially expressed genes (Kushwaha et al., 2023). Jaiswal et al (2021) reported candidate genes for reproductive seasonality as well as its molecular mechanisms in female *Labeo rohita*.

Future prospective and action points

Initiatives on large scale efforts on fish genomics have been undertaken, however targeted efforts are needed to realize its full potential. In this direction, for a particular question to be answered and a target that may have national implications, targeted multi-omics data from multiple projects generated and analysed, in consortium mode research (Murali et al, 2022) with the pooling of expertise from different domains, can provide significant output for public goods. These can also include innovative approaches, to discover new natural products from marine organisms, which are promising for improving the therapeutic strategies. High quality genome information also helps in manipulating genomes of aquaculture importance through various genome editing technologies, by focussing on economic traits, like growth, disease resistance. For example, manipulating the negative regulator gene of muscle growth, myostatin, resulted in increase in muscle mass in Nile tilapia, tiger puffer and red seabream (Hallerman, 2021).

Indian major carps (IMCs) form the major support system of freshwater aquaculture in India and are naturally found in Ganga river system and its tributaries. There is an urgent need to generate comprehensive as well as targeted genomic resources of IMCs for different natural and domesticated populations. This would help in understanding genome variations and diversity evolved under selection pressures during evolutionary processes and identification of selection signatures of adaptation to diverse environments. Identification of link of genomic variations to important production traits and genetic basis for domestication would lead to large-scale markers development, to be utilized for future genomics based selective breeding programs.

Shrimp, an export earning commodity, can be grown in lands affected by high salinity levels and this unproductive land can be converted into economically viable farms through aquaculture. The genomic tools can be applied to develop the strategies for improved performance of growth and minimising the disease outbreaks, through selective breeding programmes.

It is envisaged that with the rapid advancements of genomics research in India, in near future, genomic tools will be utilised for genetic improvement in number of aquaculture species, along with genomic selection and innovations in biotechnology methodologies.

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Aquaculture as a Prospective Diversifying Component for Sustainability and Profitability in Punjab

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Abstract

Aquaculture is considered a sunrise sector in Indian economy and it has witnessed a spectacular growth. Fisheries sector contributed 1.10% to National Gross Value Added (GVA) and 6.72% to the total GVA of agriculture, forestry and fishing. The State of Punjab is emerging as the major carp farming state of India with several farmers diversifying from wheat and paddy cultivation into aquaculture. This article focuses on the economic viability and sustainability of fish farming in Punjab state. Fish farming has an economic potential with annual profitability of Rs. 1.25 to Rs 1.50 lakh/acre/year from carp culture and Rs 2.0 to 2.50 lakh/acre/crop of 6-7 months from pangas culture, respectively. Shrimp culture is also being practiced in medium to high saline areas (10-25 ppt) in south-west parts of the Punjab state with net profit of Rs. 3.5-5.0 lakh/acre/crop of 100-120 days. Moreover, shrimp farming has potential of utilizing lands which are unfit for agricultural production. Diversifying to Aquaculture just by 40% can generate more income i.e. 119% in case of crop-pangas culture followed by 45% increase in net returns in case of crop – carp culture. The Integrated Farming System with Crop- Dairy-Horticulture-Agro-Forestry-Fish has potential to uplift the income of small and marginal farmers with Benefit Cost Ratio of 1.05. Use of wastewater in fish farming is another sustainable alternative.

Key words: Aquaculture, fish farming, economics, sustainability

Introduction:

Fisheries sector secures a pivotal place in socio-economic development of the country. Fisheries sector is the source of generation of livelihood to 28 million fishers and fish farmers and food security to approximately 50% population at national level. Fisheries sector contributed 1.10% to National Gross Value Added (GVA) and 6.72% to the total GVA of agriculture, forestry and fishing during 2020-21. The fish production in India has increased by an annual average growth rate of 12% in last 40 years. After China, India is the second largest producer of aquaculture in the world with total fish production of 16.28 million tonnes (constituting 4.12 MT marine and 12.12 MT inland) and fish seed production of 5,40,690 million fry during 2020-21. Andhra Pradesh (4.8 MT), West Bengal (1.8 MT), Odisha (0.9 MT), U.P (0.81 MT) and Bihar (0.66 MT) are the major states in fish production; and Tripura, Kerala, Manipur, Odisha and Assam are the major states in fish consumption; and the average per capita fish consumption during 2021-22 was 6.31 kg. Freshwater inland aquaculture is growing at preposterous rate in India and Punjab, in particular. Punjab is a predominantly agricultural state having 83% of its area under cultivation with an average cropping intensity of 180%. Green Revolution has changed the overall scenario of agriculture in Punjab. However, stagnation in main grain (wheat and rice) crop yields and drop in agricultural income is major concern along with environmental degradation. Under given conditions, diversification of agriculture is the only way forward in Punjab through various policy measures and incentives.

The Fisheries is considered to be the one of the most remunerative sector for crop diversification. Though Punjab is not among the top fish producers in the nation still it is among the high yielding states of the country. Punjab state is characterized with two distinct topographical and hydro-geological features i.e. fresh ground water regions in northern and central regions while saline ground water regions in south-western region. Due to canal irrigation and inadequate drainage, many salt affected areas in south western region are now affected with the problem of water logging. Thus, making the land unfavorable for agriculture in those regions. Water resources in such areas could be utilized for horizontal expansion of aquaculture in the state for providing dual benefit of food security and economic upliftment. With the rise of blue revolution in landlocked state of Punjab, the area under fish farming has increased from 0.3 thousand ha during 1980-81 to 16.6 thousand ha during 2020-21. Further as shown in figure 1, inland fish production in Punjab has increased by 8.3% and fish seed production have also raised by 1.5% in last 6-7 years respectively.



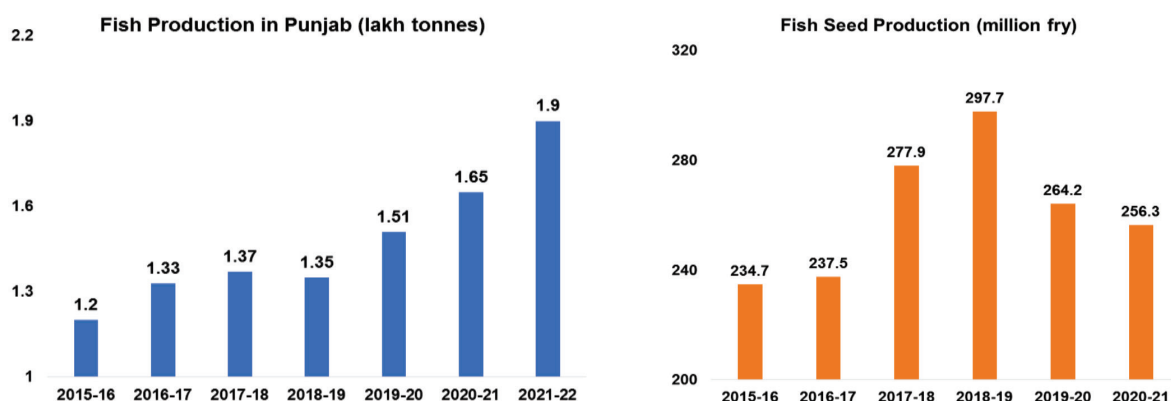


Figure 1: Production of Fish and Fish Seed in Punjab
 (*Source: Handbook on Fisheries Statistics (2022), Ministry of Fisheries, GOI)

Economic Analysis of Aquaculture in Punjab:

Three fish farming production systems are common in the state viz., extensive, semi-intensive and integrated (with agriculture and/or livestock). In last 5 years, about 485 ha of salt affected waterlogged lands in south-western districts have been converted into aquafarms.

Main Aquacultures Models in the State are:

- Carp culture:** It is mainly cultured in fresh water and can also be reared in low saline areas (≤ 5 ppt). The Operational cost for carp culture is 1.20-1.40 lakh/acre/year. It was estimated that net income from carp culture is 1.25-1.50 lakh/acre/year in freshwater and 0.5 to 0.6 lakh/acre/year in low saline water (< 5 ppt).
- Pangas-Catfish:** Due to huge demand for spineless fish in Punjab, Pangas catfish from Andhra Pradesh is sold across the state. Culture technology for rearing pangas catfish under climatic condition of Punjab has been standardized, validated, demonstrated and replicated successfully by GADVASU with an average productivity of 7.0 t/acre (3 times higher than carps) per crop of 6-7 months and corresponding net profit of Rs. 2.0- 2.5 lakh/acre (2.5 times higher than carps).
- Shrimp culture:** Vannamei (*Litopenaeus vannamei*) shrimp culture is practiced in medium to high saline areas (10-25 ppt) in south-west parts of the state. With net profit of Rs. 3.5- 5.0 lakh/acre/ crop of 100-120 days, shrimp farming has potential of utilizing lands which are unfit for agricultural production. This ensures the wise use of natural resources of land and water and stepping towards achieving sustainable goals. Further, on an average 1 ha shrimp pond has been estimated to produce 10 tonnes of shrimp per crop. This ensures support to 3-4 livelihoods/ton at national level including forward and backward linkages. Hence, every 100 ha (1000t) of shrimp farm development is expected to support 3,000 livelihoods. Therefore, utilization of only 1per cent of existing 1.51 lakh ha of salt affected lands in Punjab (1510 ha) will support over 45,000 livelihoods including farmer, farm labor and forward/ backward linkages.

Contribution of Aquaculture in Diversification:

Aquaculture acts as 'synergistic component' when integrated with any agricultural or livestock farming systems. Besides providing high economic returns per unit area as compared to monocultural and conventional agricultural crop rotations, it is an efficient solution to waste management. Aquaculture as source of rain water harvesting can serve the purpose of 'Irrigation tank' for agricultural fields and act as source of groundwater recharging. This would ensure sustainable use of water resources and ensuring food security at the same time.



Table 1: Different Models of Diversification with Crop-Dairy-Aquaculture

(for 5 Acre land holding)

Models	Enterprise	Net Returns/acre (Rs. in Lakhs)	% Increase in net returns
Model 1	Wheat-Paddy-Potato- 5 acre	2.93	-
40% (2 Acre) Diversification with Dairy			
Model 2	Wheat-Paddy-Potato – 3 acre Dairy -2 acre	3.65	25
40% (2 Acre) Diversification with Aquaculture - Carp Culture			
Model 3	Wheat-Paddy-Potato – 3 acre Carp Culture- 2 acre	4.26	45
40% (@ Acre) Diversification to with Aquaculture - Pangas Culture			
Model 4	Wheat-Paddy-Potato – 3 acre Pangas Culture- 2 acre	6.42	119

Table 1 clearly indicated that diversification to allied enterprises significantly increase the net returns of the farmer. Dairy along with agriculture can increase net returns up to 25% and dairy is main allied occupation of the farmer as out of total livestock population of the state, 93% is of bovines. However, the potential of increase in net returns through fisheries is much higher than dairy. Just by diversifying 40% to the fisheries, farmers net returns can be increased by 45 and 119% from carp and pangas culture respectively.

Relative Efficacy of Integrated Farm System Model:

Integrated Model could play a vital role in achieving sustainable development goal also fulfilling the objective of increasing farmers' income. The waste/output of one enterprise could be used as input for the production of the other enterprise and vice-versa. This model shows the case of small farmer who owns less than 2.5 acres of land and by complete integration to different enterprises he can increase his net returns by 81% in per acre.

Table 2: Relative Efficacy of Different Farm Enterprises of Integrated Farming System (IFS) Model

IFS Model Components	Size of the Land (Acre)	Gross Returns (Lakh)	Cost of Production (Lakh)	Net Returns (Lakh)	BC Ratio
Field Crops (Cereals, green fodders etc.)	1.59	1.49	0.56	0.93	1.64
Horticulture	0.25	0.21	0.13	0.08	0.63
Wheat	0.07	0.03	0.01	0.02	
Turmeric		0.07	0.03	0.04	
Total Agro-Forestry	0.07	0.10	0.04	0.06	1.44
Dairy	0.27	2.97	1.66	1.29	0.78
Aquaculture	0.25	0.33	0.09	0.22	2.35
Boundary Plantation (e.g. Galgal, Caneberry)		0.02		0.02	
Kitchen Garden (Cauliflower, Cabbage, Radish, Carrot etc.)	0.05	0.18	0.08	0.10	1.36
IFS Model Total Area	2.48	5.31	2.58	2.73	1.05
Per Acre		2.14	1.04	1.10	

Table 2 clearly depicts that in integrated farming system approach, a farmer can obtain net returns of Rs.1.10 lakh per acre. Moreover, dairy unit provides income on daily basis. Vegetable and fruit component provides balanced nutrition to the family members. Aquaculture has highest BC-ratio which clearly indicates the economic efficiency of this enterprise over the other. Under the gradual shrinking of land holding, it is necessary to integrate land based enterprises like fishery, poultry, field and horticultural crops etc. within the bio-physical and socio-economic environment of the farmers to make farming more profitable for small and marginal farmers. No single enterprise will be able to sustain the growth of small and marginal farmers.



Concluding remarks:

Punjab aquaculture has established itself as a profit making venture. Aquaculture is also playing its role in diversification from agriculture (wheatpaddy rotation). The commercial success of carp culture in some of the other states like Andhra Pradesh has also encouraged some of the farmers to venture in to carp farming in Punjab. This has generated considerable interest in fish farming and persons from all income groups have taken up fish farming either in newly excavated ponds in their own agricultural/non-agricultural land and/or in renovated village ponds through leasing. Government of India is also motivating the enterprise by providing incentives to the fish farmers through 'Pradhan Mantri Matsya Sampada Yojana' for holistic development of fisheries sector as well as welfare of fishers. In view of the positive impact, there is opportunity to expand fish farming in the state and help farmers to derive the benefits from this enterprise.

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Shrimp Farming in Inland Saline Areas of North Western India – Strategic Approaches to Curtail Marketing Snags for Sustainability

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Shrimp farming is a highly lucrative enterprise, if all goes well from production to marketing; where the producer can earn a net profit Rs. 7-10 lakh per hectare (ha) per crop of 100-120 days, depending on the extent to which the best management practices (BMPs) are being followed. The profit margin multiplies further with the number of crops harvested by the farmer in a year, subject to quality shrimp production and stability of demand supply chain at national and international markets. However, the picture is not as rosy as it appears. Until 2021, it went fairly well, but during year 2022 the shrimp farmers suffered many unexpected marketing hiccups and could not even recover the production cost.

Year 2021 - 22	
Major Importers of Indian Shrimp	% Share in Indian Shrimp Export
America	59.05
China	14.59
Europe	8.16
South-East Asia	4.78
Japan	3.61
Middle East	3.17
Other	6.64



The global shrimp market size values at USD 53 .91 billion in 2021, is reported to have reached USD 65.9 billion in 2022 and projected to be 88 billion in 2028. In terms of volume, global shrimp production was 50 lakh tons (t) in 2021, with 65% share from Asian countries and 30% from the U. S., which climbed up to 71 lakh t in 2022. Among the major shrimp producing Asian countries like India, Vietnam, China, Indonesia, Thailand etc., shrimp production increased @ 8-10%, except in China and Thailand; but Ecuador moved ahead very fast with a growth rate of 25-30% for 3 years and produced about 10 lakh t shrimp in 2021. During 2021, with almost 9.30 lakh t of shrimp production, India was Asia's biggest and world's 2nd largest shrimp exporter. Before 2021, India had the honor of being the world's



largest shrimp exporter, while during 2021 Ecuador surpassed India to become world's largest shrimp exporter. Major importers of Indian shrimp include US, China, Europe, South-East Asia and Japan. After introduction of Pacific White Shrimp in 2009, Indian Shrimp production increased from 1.4 lakh t (2010) to 9.3 lakh t (2021) progressively. India exported total 7.28 lakh t of shrimp during 2021-22, in which share of vannamei shrimp was 6.43 lakh t (88.3%).

Shrimp is mainly produced in the southern coastal states/UTs (Gujarat, Maharashtra, Goa, Karnataka, Kerala, Tamil Nadu, Puducherry, Andhra Pradesh, Odisha and West Bengal), where biggest share comes from Andhra Pradesh (~75% during 2020-21). If we talk only about Vannamei shrimp, India produced 8.15 lakh t of Vannamei shrimp during 2020-21, out of which 6.43 lakh t (79%) was exported; It shows that how largely Indian shrimp industry is dependent on the international market.



During the financial year 2021-22, India exported 1.37 million t of Seafood, which is all time high export by value (worth US\$ 7.76 Billion/Rs. 57,586 crores). US and China remained the major importers of Indian seafood and frozen shrimp continued to be the major export item.

During the last 7-8 years, shrimp farming has also developed very fast in salt affected waterlogged waste lands of inland sates like Punjab, Haryana, Rajasthan and Uttar Pradesh (Western UP). Unimaginable income from waste unproductive or underproductive salt affected lands in just 100-120 days lured the farmers towards shrimp farming in these states and it expanded very fast due to complimenting availability of technological and financial support under State and Central government promotional schemes (RKVY, Blue Revolution, PMMSY etc.). If we take an example of Punjab State, area under shrimp farming increased from 1 acre (0.4 ha) in district Fazilka in 2014 (GADVASU's 1st field trial under ICAR-Niche Area Excellence Program) to 1,211 acres (484.4 ha) in 2022, benefiting 366 farmers in 5 southwest districts of the state- Sri Muksar Sahib, Fazika, Mansa, Bathinda and Faridkot.

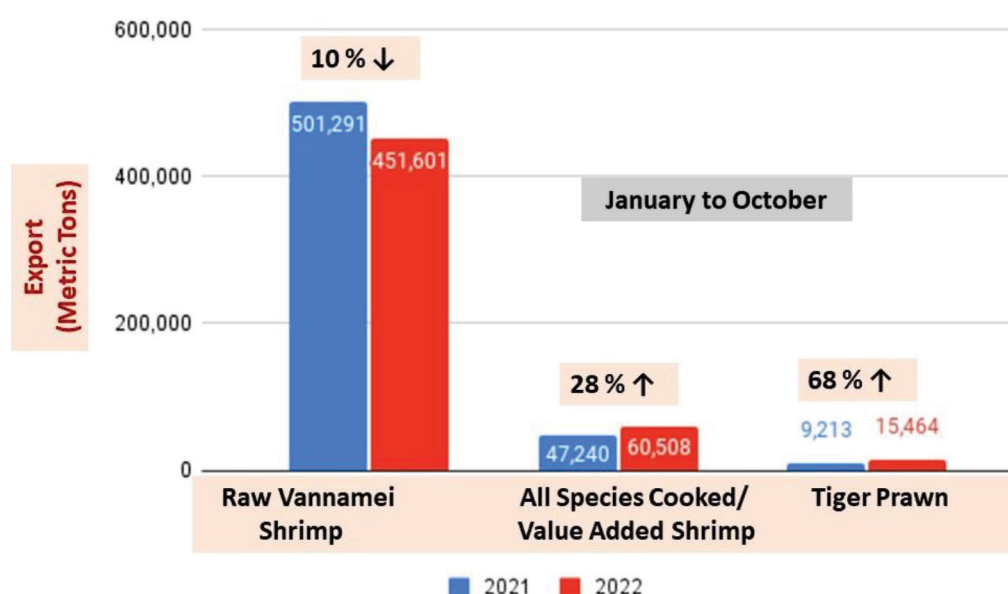
In comparison to the coastal states, farmers of Punjab, Haryana, Rajasthan and U.P. get a smaller window for shrimp farming from April to November/December (~ 8 months), due to climatic limitation (detrimental temperature fall during winter season). Mostly, there is good demand of 20-30 gm Vannamei shrimp (30-50 count) in international market, which can be produced within 100-120 days of culture period. Hence, in Punjab and its neighboring inland states (Haryana, Rajasthan and U.P.) can produce 2 crops during this window and a large underground saline water resource is also available in this region for the said purpose. But the big question is - ***“Do we only want to increase the production levels without thinking of sustainability?”*** It is very important to answer this question as far as India





is concerned, because it is overdependent on international market for shrimp marketing, with negligible domestic market to buffer any unforeseen unexpected breakdown in the production and demand supply chain. Never the less, in view of modern world's cross border socio-economic challenges, stable international trading cannot be expected. COVID- pandemic and Ukraine-Russia War are two big examples, which affected the international cargo services badly in last 3 years. Economic recession and inflation rates also affected import activities in developed countries during 2022.

If we analyze Year-on-Year (Y-O-Y) shrimp export of 2022 (January to October) with 2021, it remained more or less at par with 2021 until July and started declining from August onward; reported to have underperformed by 10% in August, 8% in September, and 27% in October (Source: Ministry of Commerce) resulting in Y-O-Y negative growth of 2022 total shrimp export volume i.e., 2% in August, 3% in September and 5% in October. Fall in shrimp prices in international market further squeezed profit margins of the producers, traders, processors and exporters. Overall, total export of raw (frozen) Vannamei shrimp export (January to October) declined by 10%. Interestingly, demand for cooked and value-added shrimp increased by 28% and Y-O-Y tiger prawn (*Penaeus monodon*) exports also recorded an upsurge by 68% in 2022.



Notwithstanding, U.S. Food and Drug Administration (FDA) refused total 60 entry lines of shrimps in 2022 for presence of banned antibiotics and about half (29) of these antibiotic related entry line refusals belonged to India. Further, the 05 shrimp entry lines refused by FDA recently in November and December, 2022 were for shipments from two Indian exporters. Hence, for sustainable development of shrimp industry in India, we need to focus on the following issues:

- i) Developing a strong domestic market to buffer export related marketing setbacks
- ii) Establishing an International brand name/value by producing quality shrimp to become favorite destination for shrimp importers.
- iii) Maintaining Demand Supply Balance
- iv) Reducing dependence on single species

If we take an overall view of Indian shrimp industry, it is evident that at present India is producing shrimp not for domestic consumption, but for other countries. At world level, 40% of total shrimp production is coming from the two largest exporters - India and Ecuador. In contrast, China consumes its own shrimp production and imports more shrimp from India, Ecuador and other countries to meet its domestic demand. Although, China produced 16 lakh t shrimp in 2021, but its domestic demand was almost 32 lakh t. Obviously another big question is “*Can shrimp industry fail in countries like china, where marketing of domestic produce will not be affected by any cross-border crisis?*”. The answer is “NO”.

Now in view of above, if we talk about India in general and north-western India in particular, shrimp farming can hopefully have a “Golden Future”, if instead of depending on international market alone, a strong domestic market is established within the country. There is a long way to achieve it and till it could be realized, the shrimp farmers and all

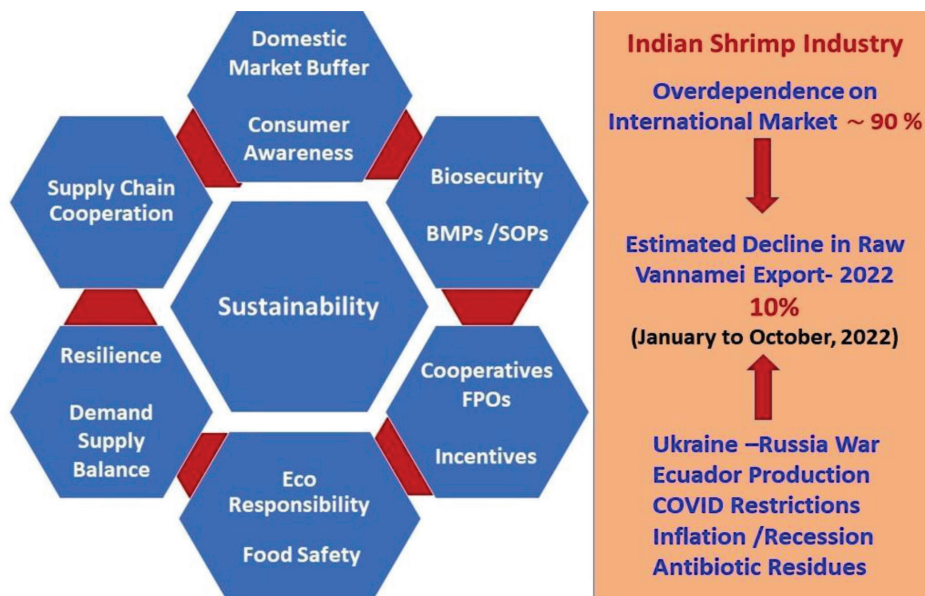


the traders along the supply chain shall come together and work as ‘One Family’ beyond personal interests or greed.

The Inland States have following additional challenges to address:

1. **Harvesting Before Detrimental Fall of Temperature in Winters** – Forced to sell the stock at non-competitive prices due to non-availability of storage facilities within the State/region.
2. **No Processing Industry in Inland States** – Very large distance (1200 to >2000 kms) between the producer and the processor
3. **Meagre Local Consumption** – Fish and shrimp eating population is less as compared to chicken, mutton and pork eaters, especially in Punjab, Haryana and Rajasthan

Owing to above limitations, some traders/middle men try to exploit the shrimp farmers, (having no access to direct marketing) to sell their produce at lesser price without realizing that they can survive only if the farmers get their due stake of profit and maintain the zeal to stay in the sector. If the traders/processors/middlemen exploit the farmers for short term benefits, the economically and emotionally drained farmers will ultimately exit and this will eventually affect the associated industry/stakeholders (hatcheries, feed mills, fishers, pharmaceuticals, processors, traders/exporters etc.), depriving all of sustainable economic growth. On the other hand, if the farmers wish that they may get optimal prices for their shrimp harvest, they also need to follow the recommended BMPs and standard operational procedures (SOPs) honestly to safeguard themselves from any unexpected monetary loss and associated emotional turmoil. They shall understand that **“Optimal Production- Less Stress- More Profit”** is always going to a better concept than **“More Production-More Risk- Less Profit”** ideology in terms of eco-responsibility and sustainability.



Hence, it is very essential to make some personal efforts to solve the existing marketing problems, rather than blaming or depending on the governance, which will certainly help in addressing the issue to some extent. Further, socio-economic growth of the farming community is required to be visualized in a long-term perspective, especially in terms of marketing support and eco- responsibility. In this context, it is proposed that shrimp farmers of inland north western states shall adopt the following recommendations to alleviate stress, save input cost, avert marketing snags and eventually enhance their profit margins:



Recommendation	Benefit
One Pond One Crop	<ul style="list-style-type: none"> • Sustained pond health and productivity • Alleviation of environmental impacts & social conflicts • Reduced risk of monetary losses
Phased Stocking of Ponds (Preferably by a difference of 30-40 days)	<ul style="list-style-type: none"> • Lesser operational cost requirement at a time • Release of bulk marketing pressure • Any untoward disease outbreak incident manageable and with possible minimal losses
No Overstocking (Best 40-50 PL/m ²) Adoption of Less Intensive farming Models (Optimizing Production Cost)	<ul style="list-style-type: none"> • Healthy culture environment within the carrying capacity • Saving on production cost • Saving on disinfectants/sanitizers/other water quality products • Better shrimp growth, quality and production
Priority to Harvest shrimp by 31st August/No Stocking after 15th June	<ul style="list-style-type: none"> • More scope of retaining the stock for longer period, if required • Selling of stock at competitive prices
Phased Harvesting (Higher stocking of 60-80 PL/m ² manageable)	<ul style="list-style-type: none"> • Recovering production cost at the earliest as per market demand of different sized shrimp (15-35g) • Enhanced farmers confidence and mental health, with reduced fear of losing the stock or money.
Capacity Building and Adherence to BMPs/SOPs in Special Reference to Biosecurity Measures	<ul style="list-style-type: none"> • Scientific management of pond and hence, significant saving on input costs • Lesser chances of disease outbreak • Better shrimp quality, growth and production • Reduced risk of refusal of export consignments
Processing and Cold Chain Facility in the Region	<ul style="list-style-type: none"> • Reduced dependence on long distant traders and processors • Long term storage, less wastage and more profit
Cooperatives & Farmer Producer Organizations (FPOs)	<ul style="list-style-type: none"> • Reduced exploitation by middlemen • Direct marketing – More profit • Competitive input (seed/feed/medicines) & marketing deals • More promotional schemes incentives (PMMSY/Blue Revolution/FIDF)
Public Awareness Campaigns to Promote Domestic Consumption of Shrimp*	<ul style="list-style-type: none"> • More domestic demand and consumption • Add on nutritional benefits to consumers • Buffer domestic market to avert unforeseen export crisis • Enhanced market stability and profitability

*Among Public, Hospitals, Armed/Security Forces, Schools, Hotels/Restaurants etc.

It is pertinent to mention that the farmers shall take up shrimp farming in accordance to the climatic/geographical conditions and marketing structure of the State, which can offer optimally satisfying profits and solve the marketing problems to some extent. Greed has no limits and pressurizing yourself as well as the land for more production is no guarantee to more profit. Another question which need to be answered - *Is it really required to undertake intensive shrimp culture involving high investment and high risk?* The answer is again “NO”. The farmer can earn handsome profit even by adopting less intensive shrimp farming models, with lower stocking densities, which will help in conserving the health of shrimp ponds over a longer period of time, besides supporting more numbers of farmer families (for required production targets) and addressing associated environmental issues as well. Hence, it is vital to evaluate that how much shrimp shall be produced in a region/nation, which can support profitability and at the same time safeguard the environment health and quality of produce to stand high in the international market as a sought-after brand.

Shrimp farming is a remunerative enterprise, with recorded 53.18% share by quantity and 74.16% share by value in India's total export earnings from fish and fisheries products (Rs. 57,586 crores) in 2021-22. Further, as per ICAR-Central Institute of Brackish Water Aquaculture (CIBA) study, 1-ha shrimp farming development supports livelihood of 30 people directly or indirectly. In India, if 1/3rd of its population starts consuming only 1kg shrimp per year, 5 lakh t can be consumed within the country and if they consume 2 kg per year, 100% of the present shrimp production will be consumed. The only matter of concern is domestic market shrimp price, which may be expected to be lesser than the international market, but a robust domestic market system needs to support sustained profitability covering unforeseen risks and stock loss in Indian shrimp industry.

Inland states (Punjab, Haryana, Rajasthan and U.P.) have a huge underground saline water resource, which need to be judiciously used for shrimp farming, only in areas totally devastated with waterlogging and not fit for any profitable agricultural activity. Further, regulatory guidelines or authority like Coastal Aquaculture Authority (CAA) also need to be enforced/constituted in the region to monitor shrimp farming activities through an ecosystem approach. Hence, it is our responsibility to see that shrimp industry in north western India is developed in a wholistic manner for economic growth of rural communities and the nation in an equitable manner. For the said purpose, farmers, traders and associated industries need to come together, as it will not only help in responsible utilization of salt affected waterlogged waste lands but also contribute significantly to national shrimp basket, food/nutritional security and economic opulence.



Shrimp Farms in Punjab



Shrimp Harvest



Promotion of Domestic Fish Consumption

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Introduction

Promotion of fish consumption in domestic market is strategically important to sustain the rapidly growing fish production sector in the country and contribute for National Sustainable Development Goals (SDGs) set by the Government. The flagship fisheries development scheme of Government of India – Pradhan Mantri Matsya Sampada Yojana (PMMSY) has targeted in doubling the per capita annual consumption of fish from 5 kg in 2019-20 to 12 kg by 2024-25.

PMMSY envisages to increase the fish production from 14.16 million metric tons (MMT) in 2019-20 to 22 MMT by 2024-25 with a sustained annual average growth rate of 9%. Achieving these targets require paradigm shift in our national strategy and action plan from the usual forward linkage approach of “production to consumption” or “farm to fork” to the backward linkage approach of “consumption to production” or “fork to farm”. This means that, along with the availability (total production), we need to focus on accessibility (distance of fish vending stalls from customer and presenting the fish in different forms and styles according to customer demands) and affordability (different price ranges as per spending power of customers). First step towards this new paradigm is understanding the food habits of consumers.

India is one of the largest fish producing countries in the world and shares 7.58 per cent of the total global production. The importance of fisheries sector can be gauged from the fact that it contributes 1.24 per cent to India's Gross Value Added (GVA) and 7.28 per cent (2018-19) to the agricultural GVA. Known as the country's sunrise sector, fisheries have shown an impressive proliferation with an average annual growth rate of 10.88 per cent during the last few years. Despite high production, fish consumption in the country remains low. Fish and shell-fishes are highly sought after food due to their nutritional components which favour good health of consumer. The synonym of ‘heart food’ and ‘brain food’ for fish is mainly due to the presence of good quantity of essential fatty acids like ‘Eicosapentaenoic acid’ and ‘Docosahexaenoic acid’. Presence of polyunsaturated fatty acids helps in lowering the blood cholesterol level and also helps in increasing the memory. Eating good quality fish at right proportion is also related to reduced risk of cancer and alzheimer's disease. Apart from this, fish is also regarded as one of the best source of protein with good quantity of essential amino acids. Fish is also a good source of vitamins and minerals required for the normal functioning and wellbeing of human beings. Knowing the importance of fish in a healthy diet, many organizations including World Health Organization (WHO) have stipulated the regular consumption of fish. WHO recommends per capita consumption of 11 kg fish per annum for maintaining better health. The American Heart Association has recommended eating fish, particularly fatty fish, atleast two times a week (minimum of two servings or 198 to 226 g). Similarly, Dietary Guidelines for Americans also suggests consuming 226 g of seafood per week (National Institute of Nutrition, 2011).

Fish Production and Per Capita Fish Consumption

India has made a paradigm shift in food availability transforming from a begging bowl to the bread basket during the course of around seven decades. The fisheries sector registered a sustainable growth rate of over 10 per cent and contributed over one per cent of India's annual gross domestic product during the last decade.

Fish production in India has increased by 115% over the last 15 years from 66 Lakh MT in 2005-06 to 142 Lakh MT in 2019-20 (DoF, MoFAHD and GoI, 2020). This means an additional 76 Lakh MT of fish was added per annum to Indian fish basket in 2019-20. During the same period, total quantity of fish consumed in the domestic market has increased by 90% from 58 Lakh MT in 2005-06 to 110 Lakh MT in 2019-20 with additional 52.18 Lakh MT of fish consumption per year in 2019-20 (Handbook on Fisheries Statistics, DoF, MoFAHD and GoI 2020). Rest of the fish have been used for exports and non-food purpose. Population of India has increased from 111 crores in 2005-2006 to 134 crores in 2019-20 (RBI 2022), an addition of 33 Crore people in 2019-20. Therefore, per capita fish consumption per annum has increased from 5.26 Kg in 2005-06 to 8.23 Kg in 2019-20 (an increase of 2.97 Kg over 15 years). This means, the quantity of fish consumed by an individual has increased by 56% over the last 15 years.





On one side, fish can be a poor man's protein (low value fishes) ensuring food security, and on the other hand for the rich, it is a delicacy offered at huge prices incomparable to other protein sources (high value species like shrimps, pomfrets and seer fishes). It has been estimated that around 60 percent of the Indian populace consumes fish and the consumption pattern varies spatio-temporally and across the different social fabric. The annual per capita consumption of fish for the entire population is estimated at 5-6 kg, whereas for the fish eating population it is found to be 8-9 kilogram, which is a mere 50 percent of the global rates.

Intervention required to enhance Domestic Fish Consumption

Globally, fish provides 1.5 billion people with almost 20% of average per capita animal protein intake and 3 billion with at least 15% intake. In the last few years, along with fish production and export, India has also witnessed growth in the domestic fish consumption. However as compared to the total fish production in the country, domestic fish consumption stands low – with an annual fish consumption of 5 Kg per capita. This consumption rate stands considerably low when compared to the global consumption of ~20.5 Kg per capita (2018). Following are some of the initiative that can be undertaken to enhance the domestic fish consumption in India: -

(1) Diversification of Species:

Major thrust is given for the diversification of species in both freshwater and brackishwater culture systems, especially with suitable native fish species having high preference and demand in domestic market by establishing hatcheries, brood stock multiplication centers and nuclear breeding centres. Research and Development programs will focus on developing breeding, hatching, seed rearing and grow-out technology for identified alternative species suitable for aquaculture by bringing in/adoption of advanced technologies and practices to enhance production and productivity. Priority will be for the propagation of area-specific integrated farming systems for adoption after evaluation and field trials.

(2) Product and Market Diversification

Increased attention will be given for product and market diversification in exports with improved infrastructure, trained human resources and support facilities to move up the value chain and meet international standards. Promotion of fish as health food and creation of consumer awareness about increased fish protein consumption would be taken up in a major way for increasing domestic demand-supply for fish.

(3) Encourage the Sales of Preserved and Processed Fish

Steps would be taken to encourage the sales of preserved and processed fish in the national and overseas markets. Development of appropriate packaging material for fish and fishery products will be encouraged. Steps will be taken to facilitate export as well as promotion of domestic consumption of fish, GI (Geographical Indication) in fish like 'Himalayan Trout' and branding of fish like 'Tuna branding, etc.

(4) Organising Fish Festivals/Fish Mela

In India 40% people are vegetarians, whereas in some States/UTs, especially the North-eastern States, fish consumption is quite high. There is a need to promote domestic fish consumption across the country as there are several health benefits. This would create an adequate demand and spur further production and consumption. To popularize fish as food and to increase the per capita consumption of fish, National Fisheries Development Board (NFDB) is extending financial assistance for construction of hygienic wholesale and retail fish markets, fish outlets, mobile fish vending vehicles, processing units, etc. Further, to popularize fish consumption, 'Fish Festivals' are organized in different parts of the country to campaign and create awareness among consumers by exhibiting preparation of different fish menu and showcasing various ready-to-cook and ready-to-eat value-added fish products. NFDB provides 100% financial assistance to the State/UT Fisheries Department to organize Fish Festivals or Melas to create awareness on fish production, adoption of technology and promote fish and fishery products. For organising these events i.e., Fish Festivals and Melas a lumpsum amount of minimum Rs. 3.00 lakh to Rs. 5.00 lakhs per year per State/UT is funded on a case to case basis by NFDB. NFDB has also organized digital campaign, broadcasting the jingles, advertising in print media, preparing posters on nutritional aspects of fish etc.

(5) Online Fish Marketing

Fish vendors doing business online sounds crazy in India where vendors have a virtual monopoly over door sales of both sea and inland fish. Moreover, fish being a highly perishable product, the idea was found to be too difficult to implement unlike other consumables where online marketing rules the roost. However, things changed drastically over the last couple of years particularly in urban areas. E-marketing has gained popularity over the past decade.

In contrast to traditional marketing, E-Marketing takes marketing techniques and concepts, and applies them



through the electronic medium of the internet. Internet marketing offer the possibility to track almost every action a visitor or potential customer takes in response to marketing messages and how they navigate through their buying cycle. Online marketing giants such as Amazon and Flipkart have been showing tremendous growth over the years in Indian e-retail market space. Hence, idea of e-markets is not new for Indian customers and they have become used to it. But fish being a highly perishable commodity, adhering to quality standards makes its sales, marketing and promotion a risky affair.

With an increased knowledge, attitude and better perception about health, quality and safety issues related to fish consumption, customers are rapidly switching to online fish markets. There are various e-commerce sites such as www.freshtohome.com, www.dailyfish.in, www.mathafreshfish.com, www.suvichar.in, www.onedaycart.com, www.freshandhealthy.in, www.wildfish.in, www.biggro.com, www.healthyfishonline.com, www.onedaycart.com, etc. are into business and is expanding their market base day by day. These fish E-commerce sites offer a rich array, mostly the variety available on local coast. Pre-ordered fresh fish reaches consumers' doorstep in curry cut, steak, fully cleaned or even as whole fish at prices affordable to the discerning homemaker. However, they are still far away from disrupting traditional fish vending systems.

(6) Need of Infrastructure Development and Post- Harvest Management

Various schemes and policies of the Government have supported interventions in the best way at each step of the fish value chain for developing fisheries sector across the country through enhanced production and leveraging value addition and export opportunities. It has been observed that, despite having highest production in the country, the per capita consumption of fish in the India is significantly low. Currently, the fish supply chains in India are mostly unorganized and run by multiple private players, entrepreneurs. Moreover, availability of quality aqua products at the right right time and place has been a gap area for the state and is one of the critical factors behind low per capita consumption of aqua products in the India. However, due to change in food consumption habits, nutrition and health consciousness among the people, demand for quality aqua products is increasing at a fast pace in the urban, semi-urban as well as rural area. Hence, there is a need to improve the supply of different aqua species to every corner of the state at a standard price and quality. The emerging markets in nearby cities and rural areas has tremendous potential and capacity to absorb these fish and aqua products. The fish supply chain in India is commonly dependent upon unorganized players, so the fishermen are also dependent upon the local aggregators and traders to sell their produce or catch. This supply chain model lacks standardization in terms of quality of the products and the price realization at primary purchase from the farmers is non-remunerative at times. Hence, there is a need to create a well-structured fish value chain in India where a large number of farmers/fishers can avail a guaranteed market and remunerative price and facilitate supply of standard aqua products at a competitive price to the end consumers.

For example, the State of Andhra Pradesh (AP) has launched a dedicated program for the development of fish and aqua product value chain to enhance the per capita consumption of fish in the State. The primary objective of the program is to enhance the domestic consumption. The model envisages to augment per capita domestic consumption of fish by ensuring easy access to live, fresh, frozen fish and aqua products along with marinated and processed products to the end consumers. With this stated objective, a well-structured Hub and Spoke model has been developed to cater quality aqua products to the households across AP. In this context, establishment of the Aqua Hubs and Spokes or retail units will be the key intervention to meet the State's need under fisheries sector. Aqua Hubs will play the most critical role in the entire value chain and will operate as an aggregation point. It will aggregate different types of products from multiple pre-registered sources and will supply to the registered retail outlets within a defined geography. At the same time these Hubs will conduct multiple activities such as, processing, quality check, etc. These Hubs will be responsible for seamless supply of products to the retail outlets as per the demand. Multiple models of retail outlets have been designed to cater different segment of the consumers and to have a significant outreach across the state.

(7) Promotion of Domestic Fish Consumption and Branding

The Aqua Hubs and the retail outlets (Spokes) will benefit multiple stakeholders of the fish value chain and the end consumer as well. It will provide guaranteed market and remunerative price realization to the fishermen or fish farmers. The consumers are expected to have access of quality and hygienic fish of different variety at a competitive price. To reach out the consumers with proper information and to create a higher degree of awareness among the consumers, there is a need of organized campaigning and awareness drives. To achieve the project's key objective "Improving per capita consumption of aqua products", branding will play a crucial role by communicating the product characteristics, quality parameters, benefits, and unique selling propositions (USP). The brand value will help in easy



identification of products by the customers as this will differentiate the product from others. An Aqua product brand with significant outreach will attract the entrepreneurs, fish farmers and other stakeholders to participate in the value chain and simultaneously the space and opportunity will grow in the fisheries sector.

(8) Standard Fixing Exercises

National Institute of Fisheries Post Harvest Technology (NIFPHATT) is actively participating and contributing to the standard fixing exercises for fish & fishery products at national and international level by serving as an expert member in Bureau of Indian Standards (BIS/ISO), Food Safety and Security Authority of India (FSSAI), Shadow Committee for CODEX and Technical Committee for Risk Assessment for Import of Fish & Fishery Products into India.

(9) Development of Smart Packaging Technology for Indicating Fish Freshness Indicator at Field Level:

Understanding the ever increasing demand for fish in the state, retail marketing and online marketing is flourishing and the price of fish is also increasing. However, fish is being highly perishable in food commodity, freshness decreases continuously from the moment it is taken out of water. Loss of freshness will be more when proper icing or chilling is not followed leading to formation of various chemicals like histamine, lipid oxidized products which pose threat to human health. For fresh and chilled fish, only ice prepared from good quality potable water can be used as preservative. Recently reported frequent incidents regarding the use of adulterants to maintain the freshness of fish poses serious damage to life and health of consumers. This has severely affected the fish business as well as the trust of the consumers. The freshness of fish is either ensured by sensory attributes or by analytical methods. The analytical methods are time consuming, costly and are not real time in nature. This has resulted in relying on sensory quality assessment to judge the freshness and quality of fish being marketed. At many instances consumers are buying inferior quality fish which is found out only while cooking or while consuming. This is true for both whole and cut fish marketed in the fish markets as well as for packed fish marketed in retail outlets.

Availability of simple, low cost and easy to use device will benefit consumers in purchasing quality fish while it is also helpful to the producers or traders to maintain better conditions to retain the freshness. Having known its importance, a simple paper based technology needs to be developed for freshness indicator of fish and packed fish.

At present, ICAR-Central Institute of Fisheries Technology (CIFT) is in the process of developing such technology in which a paper disc is attached inside the pack without coming in contact with the fish. This absorbs the chemical compounds released during the storage period and gives a simple, distinguishing colour change. This colour change can be easily read by anyone to judge the freshness of fish without any elaborate and costly laboratory tests. This freshness indicator can be used for variety of fish and shellfishes, for chilled, refrigerated and iced fish. The use of freshness indicator is beneficial to both producer / manufacturer and consumer. Freshness indicator is helpful to producer / manufacturer in controlling the loss of freshness by proper maintenance by using sufficient quantity of ice or with the desirable temperature and acquires the trust of consumers. It also allows monitoring of supply chain and hence imparts food safety control to the manufacturer and retailers. Consumer will benefit by getting better quality, safe and healthy food products. It is a win-win situation for both without much additional cost.

Conclusion

Fish purchase and consumption is a dynamic process that is constantly evolving and changing. Consumer focus is shifting to quality and convenience rather than price. Online fish marketing is far away from disrupting traditional vending and sustainability of online fish marketing counts mostly on consumer satisfaction and continued patronage. Maintaining the choice of products, better price range, quality and safety guarantees, delivery systems and improved consumer accessibility over mobile and social media platforms are found to drive growth in online fish vending sector. A lot is to be done in the domestic marketing of fish and therefore, it requires to be prioritised to save the industry.



Shaping the Shrimp Value Chain

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Quite contrary to the expectations, shrimp production in India did not go down much in the year 2022. It could be down by 2.5% only as the exports are down by 5% from 7,34,154 tons in year 2021 to 6,97,446 tons in year 2022 and shrimp inventories in the cold stores at the end of 2022 are more than the usual year end inventories. Broodstock imports over the last three months have come down dramatically which in turn could impact the production in the first quarter of this calendar year. Exporters have to cater to the demand in the first quarter essentially from the inventories carried forward from last year.

Shrimp prices have taken a huge beating in year 2022 due to recession like macro economic situation and supply glut. Inflation along with host of diseases in our Indian industry has contributed to the increase in the cost of production considerably. Unless and until the industry comes up with changes at every level of the value chain, it is difficult for the industry to maintain the growth trajectory.

Though hatcheries use SPF broodstock, supply of SPF seed is not ensured if the hatcheries don't practice right husbandry and employ stringent bio security measures. The disinfection and egg washing procedures in the maturation have to be done in the right manner to avoid EHP Spores and bacterial attachments. All the feeds that go in to the maturation tanks to feed broodstock have to be pasteurized or frozen before use. Another alternative would be to use gamma irradiation for frozen feeds. Frequent testing of broodstock would ensure the hatchery operators that their facilities are free of diseases and there by the seed supplied is also free of diseases. We shall make the broodstock suppliers to conduct selection trials in India to identify the better performing families in our conditions and produce broodstock of those families in their breeding facilities. This would help Indian farmers in achieving better efficiencies.

In farming also, there should be lot of changes in the husbandry right from the pond preparation. To disinfect earthen ponds of EHP Spores, apply CaO (quick lime, burnt lime, unstocked lime or hot lime) at 6 tons /ha. Plough the CaO in to the dry pond sediment (10 -12 tons) and then moisten the sediment to activate the lime. Then leave for one week before filling. After application of CaO, the soil PH should rise to 12 or more for couple of days and then fall back to normal range as it absorbs carbon dioxide and becomes CaCO₃. Going for collective break in all the farms around a creek or farms located in a geographical location shall help the farms in combating most of the pathogens. As there is an explosion of shrimp farms on many creeks and emergence of new pathogens, it is prudent to go for lower densities and reduce the stress. Moving forward, the infrastructure of ponds has to be changed, especially the pond sizes have to become small, so that the ponds could be lined effectively and farms could do away with EHP spores. The ponds should be covered on the top with orchid nets to avoid algal crashes and subsequent stress. The existing drains have to be deepened to avoid anaerobic condition below the lining. To enable this change, the farmers may have to go for effluent treatment ponds and from there water could be treated and pumped out in to the outer drains. It would also help in improving the carrying capacity of the creeks. The sludge along with spores could be effectively removed from the pond with regular water exchange. Small farmers could continue with existing infrastructure and go for monodon as this species is showing good tolerance for EHP.

Coming to exports, we need to work on the barriers in several markets. We also need to change our harvesting and purchasing methods to export head on Shell on shrimps to china, as that is the largest segment of the market in china. We need to move up the value chain by going for value addition to enter some of niche markets. As we have large population with good demographic dividend and growing economy, our industry should tap the potential of our domestic market. We also need to come up with a strong narrative in the global market to make our presence felt.

All in All, there should be substantial changes at every level of the value chain and all the stake holders including government shall work in unison to keep the sector sustainable.





Shrimp Exports YOY

Month	2021	2022	%
Jan	38,092	40,402	6.06 ↑
Feb	41,454	39,234	5.35 ↓
March	53,179	51,254	3.61 ↓
April	57,307	59,111	3.14 ↑
May	64,625	64,791	0.25 ↑
June	73,072	76,296	4.41 ↑
July	79,958	77,175	3.48 ↓
Aug	72,685	65,482	9.90 ↓
Sep	62,804	57,100	9.08 ↓
Oct	70,404	52,448	25.50 ↓
Nov	61,872	57,132	7.66 ↓
Dec	58,705	*55,770	4.99 ↓
Total	7,34,157	6,96,195	5.17 ↓

*Estimated

Broodstock imports YOY

Month	2021	2022	%
Jan	26,880	25,303	5.866 ↓
Feb	23,200	23,188	0.051 ↓
March	26,890	17,961	33.20 ↓
April	24,180	16,794	30.54 ↓
May	27,710	23,171	16.38 ↓
June	29,580	18,248	38.30 ↓
July	0	16,657	
Aug	0	13,167	
Sep	23,600	27,044	14.59 ↑
Oct	18,960	23,960	26.37 ↑
Nov	38,950	14,616	62.47 ↓
Dec	40,720	18,876	53.64 ↓
Total	2,81,870	2,38,985	14.85 ↓



Seafood Processing towards Circular Bio-Economy

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The dramatic growth of global population and consumer demands have resulted in the massive production of foods including seafoods and their products to serve for both local and international markets. Simultaneously, the disposal of seafood processing wastes and by-products has also harmed the environment and human health. Therefore, a sustainable food resource production system via effective recycling based on the circular bio-economy for reducing waste has become an important agenda in terms of politics, academia and industries. This can profoundly contribute to environmental and ecological protection. Also, the marine resources have become limited, and the leftover must be converted to the marketable value-added products, especially functional ingredients or nutraceuticals. Those products have gained augmenting interest for the consumers due to their health benefits. A variety of value-added products can be extracted from seafood wastes and the amount of dumped wastes can be reduced. During fish processing, deskinning, evisceration as well as descaling are done. To obtain only meat, filleting process is commonly done, in which the frame or backbone is generated as the leftover. For shellfish such as shrimp, lobster, etc. decapitation or shell peeling are general practices for making ready-to-cook or ready-to-eat products. Fish processing by-products including skins, heads, viscera, bones, scales, and fins, etc. are the potential sources of several valuable molecules, such as proteins and peptides, oils and lipids, vitamins, minerals, pigments, and enzymes. For shellfish processing leftover, cephalothorax and shell are the major byproducts, which can be further used for production of marketable product, e.g. chitin, chitosan, and its derivatives as well as shrimp oil.

Collagen, which is triple helix in structure, can be extracted from defatted skin or demineralized scale or bone with the aid of acids, namely 'acid soluble collagen, ASC'. To increase the yield of collagen, pepsin, especially from fish stomach, has been used to cleave the telopeptide region, thereby favoring the extraction collagen from the collagen fibers. The obtained collagen has been known as pepsin soluble collagen (PSC). To scale up the production, salt removal from precipitated collagen via dialysis is the drawback. Therefore, neutralization in combination with the repeated washing of collagen pellets using cold water has been developed. In general, the yield of collagen varies, depending on the fish species and processes used. However, fish collagen is type I with molecular weight around 300 kDa. Glycine constitutes around 1/3 of total amino acids and imino acids (proline and hydroxyproline) have been known to determine the thermal stability of native collagen. Beta and gamma chains, representing dimer and trimer, respectively can also be present in collagen. Most of collagen is used for tissue engineering or fortified in some acidic drink. To increase the market value, hydrolyzed collagen (HC) can be produced from the aforementioned byproducts. Proteases used and hydrolysis conditions play a major role in characteristics of peptides generated as well as their bioactivities. Chain length and amino acid sequence have the profound impact on the bioactivities of HC. HC exhibits skin nourishment and bone strengthening effects. HC can increase expression of collagen type I and type IV. Furthermore, HC is able to inhibit matrix metalloproteinase. Those mechanisms lead to the skin nourishment and reduced wrinkle formation. HC from Asian seabass has shown to induce the proliferation of fibroblast cell and collagen production, especially when used in combination with vitamin C. HC can accelerate the wound healing by *in vitro* scratch assay. HC also contributes to the induction of preosteoblast cell proliferation as well as cell mineralization. This will be associated with the reduction of osteoporosis.

Bone or scale can be used to produce bio calcium consisting of calcium hydroxyapatite and collagen. Bio calcium is rich in calcium and the calcium/phosphorous ratio is 1.65. When comparing with the calcined bone, bio calcium still has protein, mainly collagen. On the other hand, calcined bone (900°C for 6 h) contained only inorganic matter. In addition, crystallinity of bio calcium was lower (45.19%) than calcined bone (74.69%) when determined using X-Ray diffraction (XRD). Overall, bio calcium shows higher bioavailability and solubility in gastrointestinal tract than commercial calcium products. This would be the advantage of bio calcium over commercial calcium products.

For shrimp shell or squid pen, chitosan and chitooligosaccharide (COS) have been known as the versatile products. Chitosan can act as the fat blocker via formation of thick film surrounding the oil droplets in digestive tract. As a consequence, it can serve as the barrier for lipase or phospholipase to penetrate and further hydrolyze the oil or lipid. Undigested lipids will be secreted without absorption into the body, thus preventing obesity. Since chitosan is an acid soluble substance, it can be limited for the application. The conversion of chitosan to water soluble



low MW derivative, particularly COS, can widen its uses. COS can be produced via the cleavage of chitosan using the specific enzyme namely chitosanase. However, the enzyme is still expensive and non-specific enzymes such as lipase, amylase, protease, etc. have gain more interest. Nevertheless, the chemical reactions via oxidative cleavage of chitosan using oxidizing systems such as hydrogen peroxide or redox pair reaction have been shown to be the potential for production of COS. COS can be produced using 1 M H₂O₂ (COS-HP), or using the redox pair reaction (0.05 M AsA and 0.1 M H₂O₂) (COS-RP). Both reactions were performed at 60 °C for 2 h. However, these chemical processes used have the influence on bioactivities. COS exhibits high antioxidant and antimicrobial activities. Both activities of COS are enhanced when conjugated with plant polyphenols such as epigallocatechingallate (EGCG). COS-EGCG conjugate showed higher DPPH and ABTS radical scavenging activities, ferric reducing antioxidant power (FRAP), oxygen radical absorbance capacity (ORAC) and metal chelating activity than COS counterpart. COS-EGCG could inhibit both *Escherichia coli* and *Listeria monocytogenes*.

Shrimp cephalothorax or hepatopancreas can serve as potential raw materials for production of shrimp oil rich in polyunsaturated fatty acids and astaxanthin. The use of ultrasonication under the nitrogen atmosphere in the presence of tannic acid could lower the negative impact, especially lipid oxidation as induced by the cavitation effect. Also, astaxanthin was more retained. Since shrimp oil is prone to oxidation, encapsulation via several techniques can be implemented to prolong the shelf-life. Microencapsulation using the appropriate wall materials could increase the encapsulation efficiency (EE) and prevent the oxidation of shrimp oil. Sodium caseinate: gelatin: glucose syrup (1: 1: 4, w/w/w) as wall materials could provide EE of 93.32%. Free-flow microcapsules can be fortified in bread or other products. However, the amount added must be optimized to maintain the sensory property or consumer acceptability.

Due to nutraceutical or bioactivities of aforementioned products, they can be fortified in a number of foods. The newly developed products can be launched for the people who have major concern of health. More importantly, the leftover from seafood processing industry can be fully utilized or exploited via the circular bio-economy.

Keywords: Circular bio-economy, Seafood wastes, Bioactivities, Utilization, Value-added products, Nutraceutical, Functional ingredients



Fish Harvest, Handling and Transportation – Advanced Scientific Approaches to Reduce Post Harvest Losses for Increasing Farmers Income

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Introduction

A good and appropriate harvesting technique is one of the keys to obtain good quality live fish. Live fishes are the first choice of fish consumers in almost all inland fish markets in India as it is the best quality that they can receive. Live transportation of fishes acts as a value addition because a live table fish fetches almost double the price to that of its dead counterpart. Most of the fishes usually die if they are kept out of water for few minutes and spoilage begins shortly, provided certain measures of preservation are not taken immediately after harvest. This, unfortunately, adds extra cost to the unit price of fish. Moreover, certain preservation methods cannot retain the quality of fresh live fish. So, fish farmers and vendors strive to transport fish from the production site (pond) to the fish market for fresh consumption in live condition. Fishers often go for crudely made live fish carrier system to transport their produce to the markets which would provide them the best price. The table carps, i.e., catla (*Catla catla*), rohu (*Labeo rohita*), mrigal (*Cirrihinus mrigala*), common carp (*Cyprinus carpio*), bighead carp (*Hypophthalmichthys nobilis*) and silver carp (*Hypophthalmichthys molitrix*) are the species that are cultured in ponds, caught and transported alive and butchered just before primary processing. The life span of such harvested live fishes entirely depends on the method of harvesting, handling, transportation and display methods during retailing.

The techniques of live table carp transportation in India have been reported as Indigenous Technical Knowledge (ITK) by a very few researchers (Kalita et al 2004, Muzaddadi 2011 and Das et al 2013). The studies on oxygen consumption (Arabindakshan et al 2001), application of anaesthesia (Das and Goswami 2003, Pandit and Ghosh 2012), water quality management (Singh et al 2004) during live table fish transportation were conducted individually in different Indian institutes and reported separately. Thus, these findings need to be applied in combination to develop an effective technique for live fish transport.

Harvesting

Harvesting is often a neglected part in the whole process of fish marketing and supply chain (Fig. 1). Fishes are normally harvested from ponds with encircling nets and are sold either in live or dead forms. Net enclosure method is most commonly used for harvesting fish from ponds since ancient times. Such method of harvesting may be very useful for village ponds when fishes are sold either in dead or in iced form. As of late, the entire scenario of fish supply chain has changed and fishes are being marketed in live form, but the method of harvesting remains same.

The way to harvest a pond also greatly depends on the kind of fish to be harvested. For keeping fishes alive, such as juveniles for stocking, brooders for spawning, or table fish to be sold alive, extra care must be taken.

- Stress to the fish should be reduced to a minimum during harvesting, weighing, loading and unloading
- Fish should be transferred to good water as soon as possible, for transport and, if necessary, for storage.
- Best arrangement must be ensured for continuous aeration, water filtration or water exchange during transportation and storage.

Water draining technique is an efficient scientific method of harvesting live fish from ponds. In this method, all the fishes are removed from a specific harvesting area located inside or outside the pond. Unnecessary activities in the pond before harvesting must be avoided and additional clean water from other sources should be used for rinsing the fishes before storage. To increase the survival chances of fishes during transit and storage, one must harvest early in the morning when the temperature is a bit lower. To ensure sound health of fish during transit, fishes should not be fed for 48 to 72 hours prior to harvesting. Bottom slope of the pond should be under good maintenance for efficient draining by its drainage channels. Water should be removed regularly and slowly to concentrate fish at the outlet for collection using scoop nets. Care should be taken in not to injure fish while weighing.

Catch Basin

A small depression built in front of the outlet for concentrating the fish to be harvested is called a catch basin. Fishes can be easily harvested in the deepest part of a completely drainable pond. However, a catch basin needs to





Fig 1. Supply chain of fish from pond to customer

be designed when the outlet pipe across the dike is small (internal diameter under 20 cm) or when the pond is larger than 5000 m² or when the local topography does not easily permit the use of harvesting structures outside the pond. The volume of the catch basin should be enough to contain all the fish to be harvested and its depth should be at least 30 cm. Sizes of catch basin according to the pond size are given in Table 1.

Table 1. Size of catch basin according to the pond size

Pond size (m ²)	Catch basin dimension (m)		
	Length	Width	Depth
100	1.5	1.0	0.3
500	2.5	1.5	0.4
1000	3.0	2.0	0.4
2000	5.0	3.0	0.6
3000	5.0	4.0	0.6
5000*	5.0	4.0	0.6

*Assuming the use of partial harvesting

In larger ponds, it is best to use a totally drainable catch basin. The ponds should be designed such that the level of sloping base is at least 10 cm higher than the bottom of outlet's entrance. Usually, large fishes are harvested from the catch basin, while small fishes are allowed to pass through the pond outlet to be harvested behind it. An ideal catch basin design is given in Fig. 2.

Use of Fish Pump

P/V (pressure/vacuum) pump is used for on-board handling of large quantities of fishes. The principle is that an accumulation tank of size 500-1500 L is alternately put under vacuum and pressure by a water-ring vacuum pump. The fishes, along with some water, are sucked into the tank of the system through a hose and valve. When the tank is full, it is pressurized by changing the vacuum and pressure side connections from the tank to the pump and the fish/

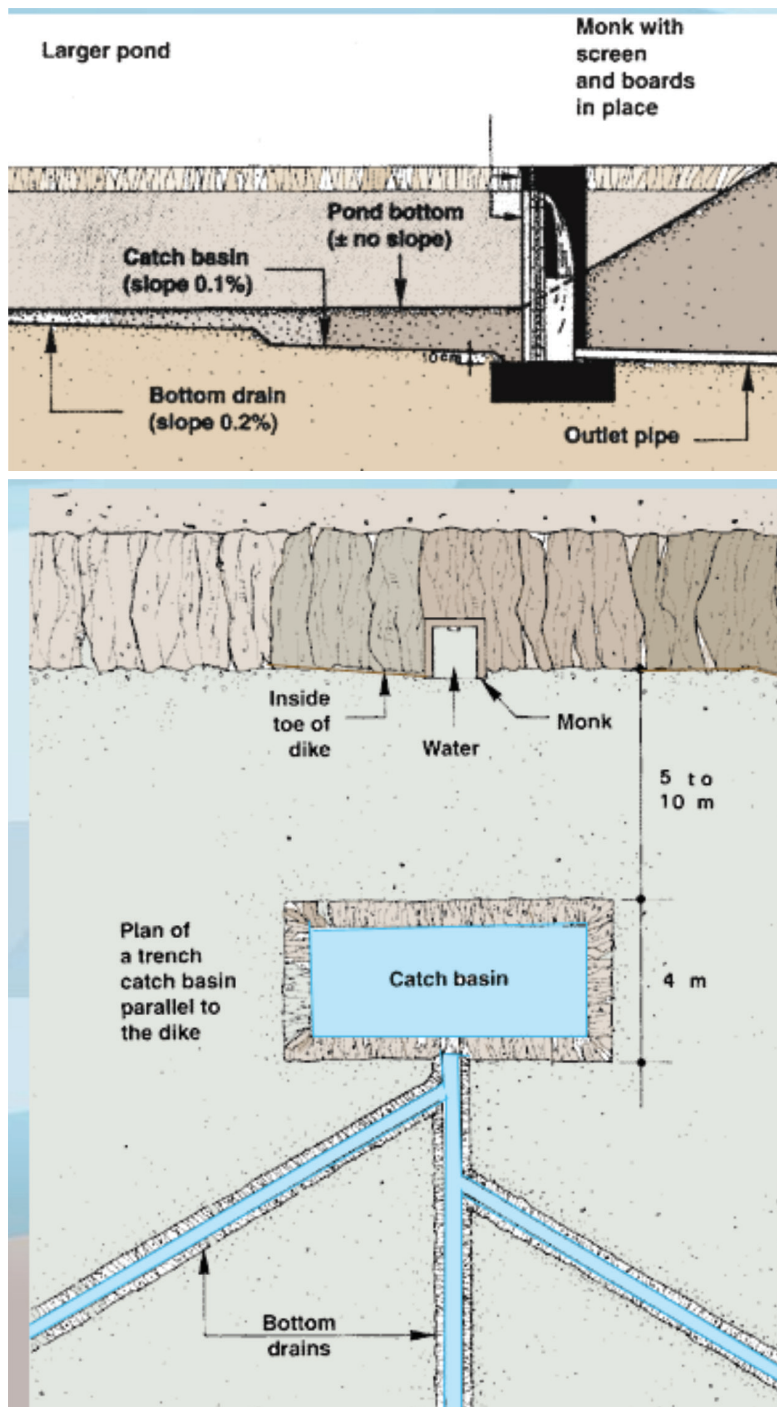


Fig. 2. Design of a catch basin

water mix flows into a strainer through the valve and hose. The P/V-pump is claimed to handle the fish gently (Fig. 3).

It is convenient and necessary to pre-chill the catch during holding in deck-pounds using ice or in tanks using Refrigerated Sea Water (RSW) or a mixture of ice and sea water (Chilled Sea Water, CSW).

Sorting/Grading

The equipment used operates on the basis of thickness of fish using principles such as:

- Vibrating, inclined diverging bars
- Contra-rotating, inclined, diverging rollers
- Diverging conveyors where fishes are being transported along a power driven V-belt.

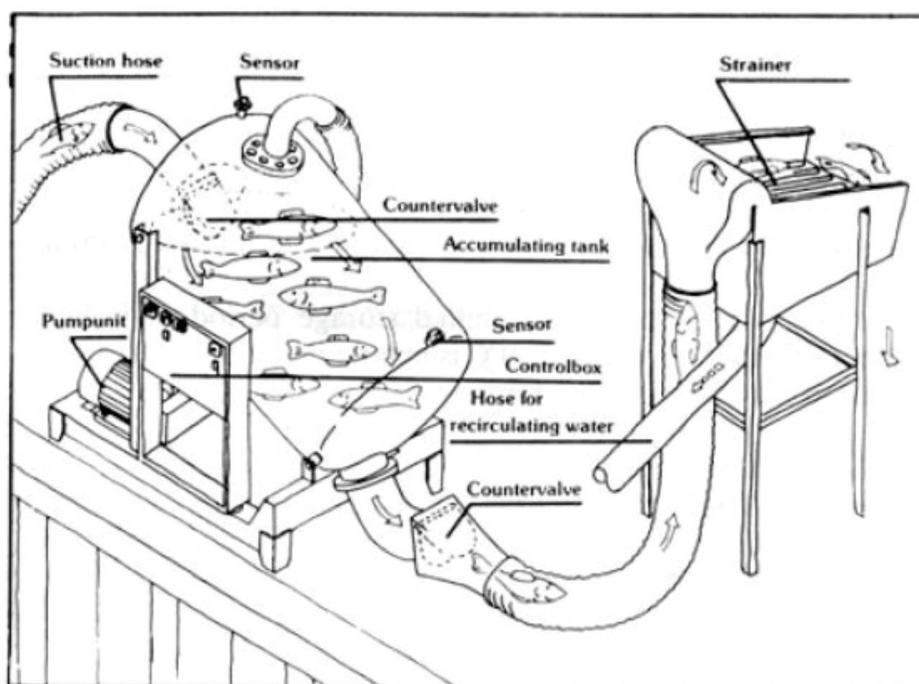


Fig. 3. Modern fish handling through P/V pump

Bleeding/Gutting/Washing

- In order to obtain optimal quality in white fillet, white-fleshed demersal fish (not all) need to be bled and gutted immediately after capture.

Chilling/Chilled Storage

Shelf storage is done by alternating layers of ice and fish (single shelving 25 cm between shelves) up to 100 cm deep.

Bulk stowage: stowage of iced fish in pounds usually formed by fitting portable pound boards into fixed vertical stanchions in the fish room

Boxed stowage: instead of building up pounds in the fishroom the fish is mixed with ice in boxes

Chilled sea water: stowage of fish in sea water chilled by ice and held in insulated fixed tanks

Live Fish Transportation

Problems associated with Live Fish Transportation

1. High fish mortality up to 100%
2. High drudgery, require 4-5 persons per trip
3. Life risks in manual aeration
4. Improper aeration- human error
5. Economic loss due to high mortality
6. Vehicle overturn possibilities

Live Fish Transportation versus Icing

Fishers can earn about 20% more money when they use ice and insulated fish boxes as without ice, fishes will get spoiled and become inedible after 7-8 hours storage at tropical temperatures. In order to maintain optimum fish quality, fish once caught should be chilled at 0 °C as quickly as possible. Ice chills the fish through surface heat transfer either by direct contact between fish and ice or by running cold melt water over the fish surface. Therefore, the cooling rate will be quicker if more ice is in contact with the fish.

Upon landing, the fish should be maintained at or as near as possible to its chilled temperature of 0°C through the market, processing and distribution chain.

Additional ice or re-icing may be required to prevent the fish from spoiling when the fish is laid out on the

market for display prior to sale.

The first obvious way of avoiding spoilage and loss of quality is to keep the caught fishes alive until consumption. Fishes are transported in live condition because

- Fish die out of water and spoil very fast
- General preservation methods such as icing and freezing cannot retain 100% of the freshness of live fish.
- It is a guarantee of freshness
- It acts as a means of value addition to fish as it fetches 30-50% additional value

Fishes are first conditioned in a container with clean water while the damaged, sick and dead fishes are removed. Fishes are made to starve, water temperature is decreased to reduce their metabolic rates and to make them less active. Low metabolic rates decrease the fouling of water by ammonia, nitrite and carbon dioxide that are toxic to fishes and impair their ability to extract oxygen from water. Such toxic substances will tend to increase mortality rates. Less active fishes allow an increase in the packing density of fish in the container.

Live Fish Transportation for Food Purpose

- **Waterless method-** The specimen are held in a cold, moist atmosphere during transportation. Usually molluscan shellfish like clam, mussels, oysters and crustaceans (shrimp, lobster and crab) are used since they can remain alive without water for more than 24 hours if suitable environment is provided. They are packed with pumped oxygen and high relative humidity (approximately 70%) and are kept cool (10-15°C) throughout transportation.
- **Plastic bag method (closed system)-** Fishes are packed in polythene bags pumped with oxygen. This method is usually used for ornamental fish and fry/ larvae of freshwater fish. Fishes are placed in a plastic bag filled partially with clean water. The bag is inflated with oxygen by removing air. Bags are sealed and carried inside a cardboard box insulated with 25-50 thick polystyrene foam. Commonly used for transportation of aquarium fish and fry, fingerlings and post-larvae of cultured fishes and shellfishes. Good water quality, healthy fish, proper loading density, good catching and handling techniques are essential for successful transportation.
- **Tank method-** Fishes are kept in water tanks of various types (open system) and are then transported.
- ICAR-CIPHET Technology of live fish transportation

Live fish carrier system (LFCS) has been developed in ICAR-CIPHET. It is an electric tri-cycle based live fish transportation system for short and medium distance (about 80 km) transportation of fish including the table carps (rohu, catla, mrigal, common carp, grass carp, silver carp and bighead carp) in live condition (less than 1% fish mortality per trip of 40 km with carps of 0.5-2.0 kg individual weight) from one place to another for marketing, rearing, ornamental displaying and breeding purposes. It is devised with water filtration, aeration, drainage, circulation, heating and cooling systems providing good water quality to reduce fish mortality during transportation (Fig. 4 and 5). It is eco-friendly, low-cost, easy to handle and keeps fish alive for more than 4 hours while in transportation.



Fig. 4. Complete Live Fish Carrier System

The carrier is constituted mainly with two components i.e., the Battery Operated Self-contained Aerating Vehicle (BOSCAV) and Non-Stackable Aerating Container (NSAC). The capacity of the present system is 100 kg live fish per trip which can be increased by increasing the size of the container and payload capacity of the vehicle. Moreover, this system provides additional vehicle stability because the water splash breakers protects it from creating imbalance during sudden braking, turning, up-hill climbing or down-hill descending. With low running costs, fish farmers can take benefit of it by carrying their produce to distant markets for fetching better prices.

LFCS has following utilities

1. Live table fish transportation from culture pond to retail market.
2. Live fingerlings transportation for aquaculture.
3. Live brood fish transportation for breeding purposes.
4. Ornamental fish transportation for commercial purposes.
5. Transportation of live fish for research purposes.
6. Transportation of live fish for conservation purposes such as river ranching.
7. Function as mobile fish selling retail.

Features and advantages of LFCS

1. The LFCS has automatic aeration, filtration and evaporative cooling system ensuring continuous availability of good quality water and less fish mortality (<1%).
2. Its water requirement is less than 50% as compared to that of the traditional systems.
3. LFCS requires only 1 worker which is otherwise 4-5 workers in case of traditional systems, thus reducing the cost of labor.
4. It ensures supply of the freshest quality of fish to the consumers.
5. It is a zero polluting system with less running cost for marginal farmers/ entrepreneurs.
6. It is woman-friendly and can be operated by one or two women.

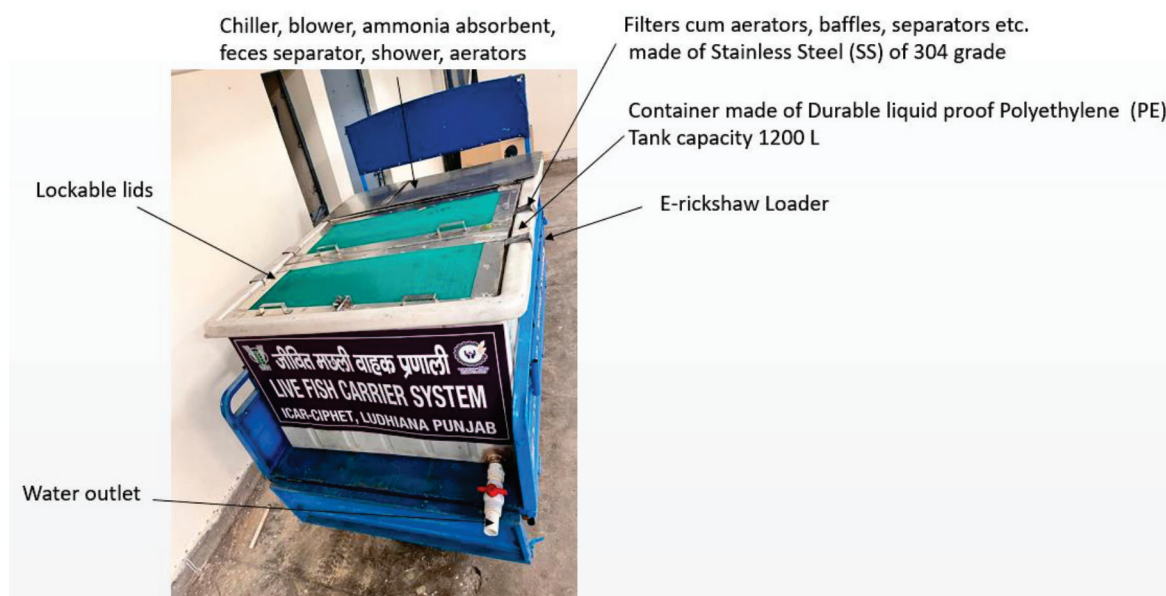


Fig. 5. Complete Live Fish Carrier System

As given in Table 2, it is quite convincing that the live fish marketing provides a lucrative income to the fish farmers, fish traders and retailers. A retailer, using the LFCS can earn about Rs. 50,000/- to Rs.1,50,000/- by selling 100 kg live fish, even if he/she works only for 6 months in year.



Table 2. The economics of entrepreneurship adopting LFCS compared to conventional (existing) method

Net profit per kg of fish sold alive* (₹)	Total profit in existing method of live fish transportation for 100 kg fish/day, (100-20=80) # (₹)	Total profit per 100 kg fish /day in LFCS, (100-2=98) ^ (₹)	Net benefit per 100 kg in LFCS vs existing method /day ** (₹)	Net annual benefit due to the intervention of LFCS ## (₹)	Total profit in LFCS (180 working days, selling 100 kg fish per day) (₹)	Monthly income with LFCS (₹)
A	B=A*80	C=A*98	D=C-B	E=D*180	F=C*180	F/12
35	2800	3430	630	1,13,400	617400	51450
40	3200	3920	720	1,29,600	705600	58800
45	3600	4410	810	1,45,800	793800	66150
50	4000	4900	900	1,62,000	882000	73500
60	4800	5880	1080	1,94,400	1058400	88200
70	5600	6860	1260	2,26,800	1234800	102900
80	6400	7840	1440	2,59,200	1411200	117600
90	7200	8820	1620	2,91,600	1587600	132300
100	8000	9800	1800	3,24,000	1764000	147000

Assumptions:

- *Net profit per kg of fish when sold alive compared to dead fish is calculated by taking the difference i.e. Market Price of live fish- Market Price of iced/dead fish.
- #In existing live fish transportation method, there is 20% Fish Mortality in Transit (FMT), however, normally FMT is 20-100% in existing method.
- ^In LFCS FMT is 2%, normally it is <1% in LFCS.
- **A fish retailer sells 100 kg fish of 1 kg individual weight per day, in a single trip using LFCS.
- ##A fish retailer retails fish using LFCS for 6 months i.e. 180 days in a year.
- Selling of dead fish neither gives profit nor loss, hence nullified, normally it goes in losses.
- The operating costs of selling dead fish and live fish are equal, hence nullified. Normally it will be less in LFCS, because the dead fish requires additional cost of icing and packaging, on the other hand, since live fish needs to be transported with water, less quantity of fish can be transported per vehicle compared to the dead ones.

End Note: The table freshwater fishes especially the carps need special care while transporting them live and these are the group of fishes which are extensively cultured. Fish farmers must be given special attention by the researchers and the policy makers in this particular area. The mechanization of live fish handling, transportation, packing, primary processing (gutting, de-scaling, steaking, filleting) is the key to bring true revolution in fisheries sector which is possible without even increasing the present fish production per hectare. The provision of research facility including an engineering lab, a proper engineering workshop and appropriately educated staff must be made available to all the fisheries colleges and research institutes for mitigating this urgent need.

Acknowledgement: The research funding by National Fisheries Development Board (NFDB) is thankfully acknowledged.

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THEME I

Aquaculture Production Systems and Sustainability





1.1 Survival and Growth Performance of an Indian Major Carp, *Cirrhinus mrigala* (Ham.) Fingerlings Reared in Inland Saline Water

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Keywords: Growth, Inland saline water, Mrigal, Salinity, Survival

Introduction

Carp are the mainstay of freshwater aquaculture activities in India and amongst carps, mrigal (*Cirrhinus mrigala*) is widely cultivated in semi-intensive carp polyculture system. In aquaculture, the productivity is influenced by both abiotic and biotic factors and salinity is considered as a crucial abiotic factor for stenohaline freshwater fish species, influencing their several physiological processes viz. osmoregulation process, digestion and metabolism leading to poor body physiology, growth performance, health and higher mortality beyond the salinity tolerance level (Morgan and Iwama 1991; Enayat et al 2011). Soil salinization is a serious ecological threat at the global level and has major economic, social and environmental consequence making the land unfit for commercial agriculture. However, aquaculture can be used to turn these unproductive, zero-earning salt affected water logged lands into economically viable ones via development of area and species specific viable and sustainable aquaculture technologies. Further, different fish species have different salinity tolerance levels, so specific studies are needed for concerned candidate fish species. Therefore, the present study was carried out to explore the culture possibilities of mrigal in inland saline water.

Materials and Methods

An experimental trial (90 days) was carried out, in triplicate, in glass aquaria to assess the effects of graded salinity levels of 0 (SA0), 2 (SA2), 4 (SA4), 6 (SA6), 8 (SA8), and 10 (SA10) ppt on survival, growth, behavioural responses and morphological changes in mrigal, *C. mrigala* fingerlings during long-term rearing in inland saline water. Healthy fingerlings of mrigal (av. length-11.27 cm, av. weight-11.08 g) were gradually acclimatized to varying salinities by raising the salinity by 1 ppt hourly and then stocked at respective salinity levels @ 10 fingerlings aquarium⁻¹ and were fed with pelleted fish feed (crude protein 26.12%) @ 0.5-1.0% of body weight. Predetermined salinity and water level were maintained in all treatments, and all experimental aquariums were aerated continuously during the trial. Fish growth was studied at monthly intervals and survival (%) and growth performance were calculated at the end of the experiment. Behavioral and morphological changes in fish were examined on daily basis and depicted at fortnightly intervals. The data was analyzed using one way ANOVA and Duncan's multiple range tests using SPSS 20.0 to determine the significant differences ($P \leq 0.05$) between the treatments.

Results and Discussion

In the present study, the temperature, pH, dissolved oxygen, ammoniacal-nitrogen, nitrate-nitrogen and orthophosphate remained well within the recommended range for carps in all the treatments thus depicting that there were no detrimental effect of varying salinity on these water quality parameters. The other parameters like electrical conductivity, total alkalinity, total hardness and ionic composition (Na^+ , K^+ , Ca^{+2} , Mg^{+2} , Cl^- and SO^{-2}) of water differ significantly ($P \leq 0.05$) and increased with increasing salinity levels, being maximum in SA10.

At the termination of the experiment, 100% fish survival was recorded up to 4 ppt indicating that mrigal can survive up to 4 ppt salinity under long term rearing (salinity stress), however, from 6 ppt onwards, mortality was recorded at ≥ 60 days of rearing and at the end of the experiment, fish survival varied significantly ($P \leq 0.05$) and recorded 80.0, 63.33 and 46.67% at 6, 8 and 10 ppt salinity, respectively (Fig 1). Further, growth performance with respect to total body length gain (TBLG), net weight gain (NWG) and specific growth rate (SGR) decreased significantly ($P \leq 0.05$) with the increase in salinity level, being highest in control (3.23 cm, 8.83g and 0.65%, respectively) and lowest at 10 ppt (1.38 cm, 1.05g and 0.10%, respectively). Moreover, feed utilization efficiency with respect to feed conversion ratio (FCR) and protein efficiency ratio (PER) was also observed best in control (Table 1). Fish behaviour with respect to swimming activity and feeding behavior (feed intake) was normal during the entire experimental period up to 4 ppt salinity but afterwards fish showed less active/sluggish swimming activity and reduced/loss of appetite at higher salinity levels at different days during the rearing. Further, morphological appearance of fish was also normal up to 4 ppt, whereas symptoms like darkened fin margins, lose of scales and changes in body colour were observed in fish reared at higher salinities. Hence, it can be concluded that for long term rearing of mrigal, *C. mrigala* in inland saline water, salinity should be maintained at ≤ 4 ppt.

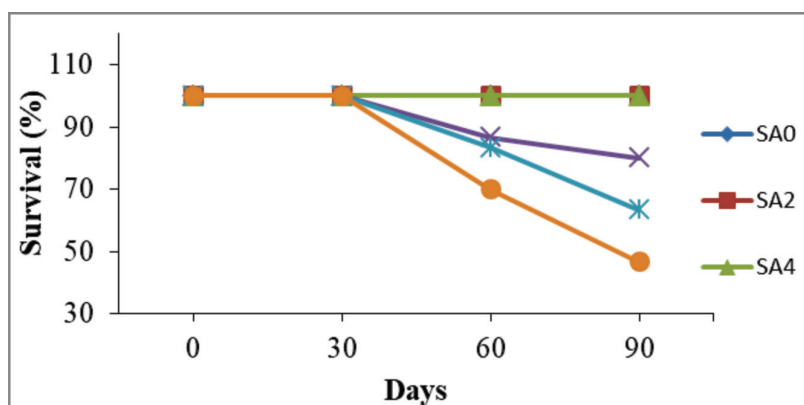


Fig 1: % survival of *C. mrigala* fingerlings at monthly intervals during the experimental period

Table 1: Survival and growth performances of mrigal, *C. mrigala* in different treatments at the experiment termination

Parameters	Treatments					
	SA0	SA2	SA4	SA6	SA8	SA10
Av. TBLG(cm)	3.23 ^a ±0.14	3.01 ^a ±0.05	2.44 ^b ±0.06	1.93 ^c ±0.12	1.79 ^c ±0.08	1.38 ^d ±0.02
Av. NWG (g)	8.83 ^a ±0.58	7.67 ^a ±0.64	5.97 ^b ±0.18	3.81 ^c ±0.16	2.24 ^d ±0.28	1.05 ^e ±0.13
SGR (%)	0.65 ^a ±0.03	0.58 ^b ±0.03	0.48 ^c ±0.01	0.32 ^d ±0.01	0.21 ^e ±0.03	0.10 ^f ±0.01
CF(K- value)	0.67 ^a ±0.03	0.66 ^a ±0.02	0.65 ^a ±0.02	0.62 ^a ±0.01	0.60 ^a ±0.03	0.60 ^a ±0.01
FCR	1.40 ^c ±0.09	1.58 ^c ±0.13	1.89 ^c ±0.05	3.03 ^b ±0.13	3.18 ^b ±0.37	4.29 ^a ±0.40
PER	2.75 ^a ±0.18	2.45 ^a ±0.19	2.02 ^b ±0.05	1.27 ^c ±0.06	1.24 ^c ±0.16	0.91 ^c ±0.09
Survival %	100 ^a ±0.00	100 ^a ±0.00	100 ^a ±0.00	80.00 ^b ±5.77	63.33 ^c ±3.33	46.67 ^c ±3.33

CF= Condition factor

*Values (mean ± S.E.) with same superscripts in a row do not differ significantly ($P \leq 0.05$)

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1.2 Histopathological Alterations in Major Organs of Freshwater Ornamental Goldfish, *Carassius auratus* (L.), Variety Shubunkin Reared in Inland Saline Water

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Keywords: Gold fish, Histopathological alterations, Inland saline water, Salinity Stress

Introduction

Gold fish (*Carassius auratus*) is one of the most demanded ornamental fish species due to its colouration and uniqueness especially finnage, body shape and the hardy nature w. r. to tolerance to drastic changes in physico-chemical parameter of water. Amidst various abiotic factors, salinity is the critical parameters, as it determines the level of osmoregulatory stress to maintain ionic concentration of the body through major organs. Further, adaptability and tolerance of these fish species in water vary greatly due to varied ionic composition of inland saline water (ISW) compared to the sea water (Dhawan *et al.*, 2010). Hitherto, to our knowledge, no reports on the effects of ISW on histopathological alterations of ornamental fish are available. About 1.51 lakh ha area in South-West districts of Punjab is facing dual problem of underground salinity and water logging, in which aquaculture is one of the most viable option and further aquaculture can have wider adaptability among small and medium farmers/entrepreneurs due to operational flexibility. Hence, the present study was designed to assess the histopathological alterations in gills, liver and kidney of *Carassius auratus* (L.) in different salinity levels.

Materials and Methods

Four months experimental period was carried out in glass aquaria (40 L capacity) at Instructional cum Research Farm of College of Fisheries, GADVASU, Ludhiana to assess the histopathological alterations in gills, liver and kidney of *C. auratus* (L.) in different salinity levels prepared from inland saline water (12‰) collected from village Shajrana, district Fazilka (Punjab). The experimental fish were procured from local market, conditioned for 15 days in indoor conditions. After proper conditioning, fish (Avg length, 8.04-8.25 cm) and (Avg weight, 6.30-6.80 g) were acclimatized gradual increase in salinity @ 1‰ (ppt) at 1-hr interval) to five salinity levels (0, 2, 4, 6, 8 and 10‰) and distributed (10 fish/ replicate) randomly in control and experimental salinity treatments in triplicates. All the experimental aquaria were provided with continuous oxygen supply. Histopathological studies were carried out after completion of the experiment. The tissues (gill, liver and kidney) were collected after completion of experiment, stored in 10% neutral buffered formalin and were processed according to the standard protocol.

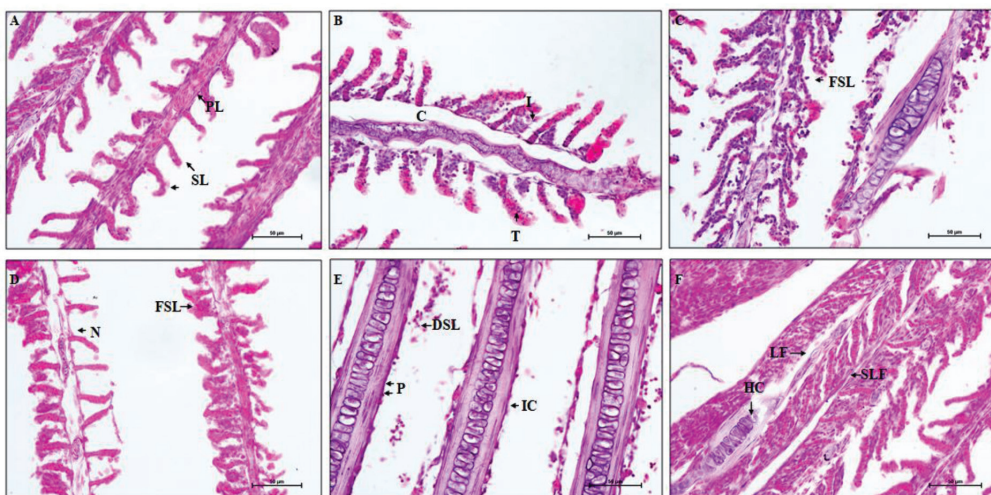


Fig 1. Representative pictures of histomorphology of the gills of gold fish exposed to different salinity conditions for 120 days. A) 0‰ (freshwater) Primary lamellae (PL), Secondary lamellae (SL); B) 2 ‰ Infiltration (I), Telangiectasia (T), Chondrocytes (C); C) 4 ‰ Fused secondary lamellae (FSL); D) 6 ‰ Fused secondary lamellae (FSL), Necrosis (N); E) 8 ‰ Degenerated secondary lamellae (DSL), Increased Chondrocytes (IC), Perichondrium (P) and F) 10 ‰ Lamellar fusion (LF), Hyaline cartilage (HC), Secondary lamellar fusion (SLF). H & E stain. Scale bar= 50 µm.

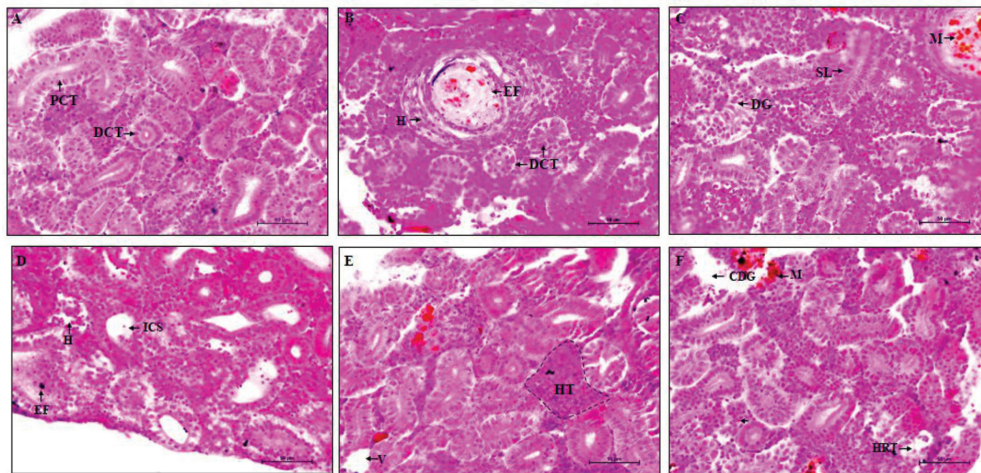


Fig 2. Representative pictures of histomorphology of the kidney of gold fish exposed to different salinity conditions for 120 days. 0‰ (freshwater), Proximal convoluted tubule (PCT), Distal convoluted tubule (DCT); B) 2 ‰ Edmatous fluid (EF), Hyperplasia of tubules (H), Distal convoluted tubule (DCT); C) 4 ‰ Degeneration of glomerulus (DG), Shrunken lumen (SL), Melanomacrophage (M); D) 6 ‰, Edmatous fluid (EF), Haemorrhages (H), Increased capsular space filled with fluid (ICS) E) 8 ‰ Hematopoietic tissue (HT), Vacuolization (V) and F) 10 ‰ Complete degeneration of glomerulus and emptying of Bowman's capsule (CDG), Hypertrophy in renal tubules (HRT), Melanomacrophage (M). H & E stain. Scale bar= 50 μ m.

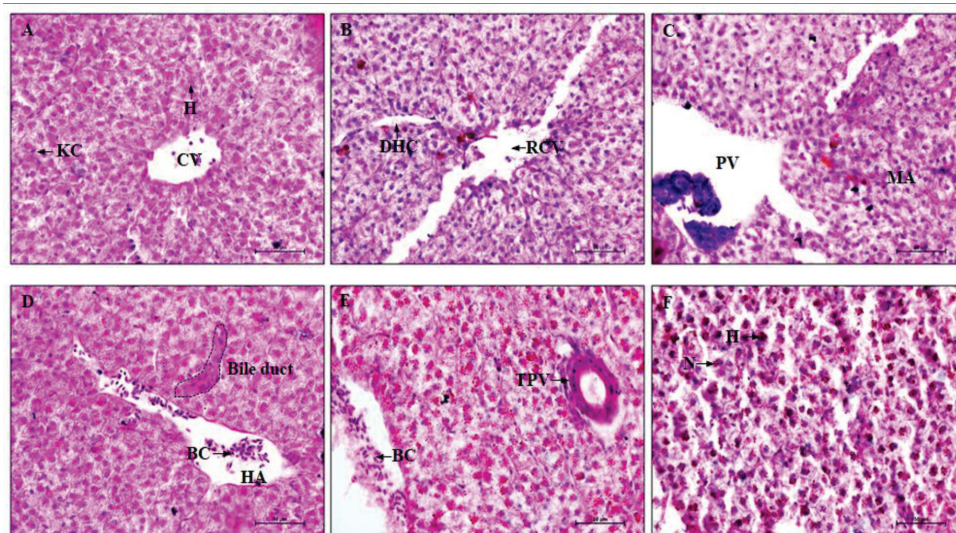


Fig. 3. Representative pictures of histomorphology of the liver in gold fish exposed to different salinity conditions for 120 days. A) 0‰ (freshwater) Hepatocytes (H), Central vein (CV), Kupffer cells (KC); B) 2 ‰ Rupturing of central vein (RCV), Disorganization of hepatic chords (DHC); C) 4 ‰ Melanomacrophage aggregates (MA), portal vein (PV); D) 6 ‰ Blood congestion (BC), Hepatic artery (HA); E) 8 ‰ Thinning of portal vein (TPV), Blood congestion (BC) F) 10 ‰ Haemorrhages (H), Necrosis (N). H & E Stain. Scale bar= 50 μ m

Results and Discussion

Remarkable changes in gills were observed, with lamellar bending and thinning of inter lamellar region (Fig 1B,C,D) to fused secondary lamellae, telangiectasia, hyperplasia, blood congestion and complete degeneration of secondary lamellae in highest salinities (Fig 1E & F). The alterations implicate the defense mechanisms and results in reduced excretory functions of the gills. The major histological alterations in kidney were degeneration of glomerular (Fig 2B,C) and distal convoluted tubules, complete degeneration of glomerulii and vacuolation of Bowman's capsule (Fig 2D, E) along with renal hypertrophy (Fig 2F). In addition, results in terms of shrunken and degenerated glomerulus along with increased exudates were in consistent with what has been found in previous studies (Abdelhamid and El-Ayouty 1991) w.r.to pollutant exposure/water quality alterations. In liver, vacuolization of hepatocytes (Fig 3B), increased melanomacrophage aggregates (Fig 3C, D) along with cellular degeneration, complete blood congestion in portal vein, haemorrhages and necrosis especially at 8‰ and 10‰ (Fig 3E, F), can be described as inhibition of



DNA synthesis required for the growth and maturation of liver under abnormal conditions. It may be concluded that although gold fish can be safely reared for longer duration in ISW by maintaining the salinity $\leq 4\%$, however, as the study was carried out under laboratory conditions, hence the findings need to be validated under field conditions for better understanding of the effect of salinity with special reference to ISW.

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1.3 Comparative Salinity Stress Response of Freshwater Carp Fish Species, *Labeo rohita* (Rohu) and *Cyprinus carpio* (Common carp) Reared in Inland Saline Water

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Keywords: Carps, Cortisol, Histology, Salinity, Stress, Survival

Introduction

In the recent past, shrimp farming gained momentum as an economic aquaculture activity in inland saline areas of north-western India, which is an intensive aquaculture system (Ansal and Singh 2019) concerning high-cost and high-risk. Hence, an alternate low-cost aquaculture option is required for these areas, to safeguard livelihood of small and marginal farmers. In this context fresh water carp culture in low salinity areas have been reported to be an economically viable option (Ansal and Dhawan 2021). Therefore, it is vital to evaluate salinity induced stress responses in stenohaline freshwater carp species, for designing an appropriate economic aquaculture model and species combination to attain optimal production levels. The present study was carried out to compare the stress response of highly economic carp species i.e. *Labeo rohita* (rohu) and *Cyprinus carpio* (common carp), in terms of salinity tolerance, survival and stress induced hormonal and histological changes, when reared in inland saline water.

Material and Methods

Inland saline water, 15 ppt (‰) collected from salt-affected area of village Shajrana (30.3346° N, 74.1196° E), district Fazilka (Punjab) was diluted to prepare different salinity levels (2, 4, 6, 8 and 10 ‰) with underground freshwater (0 ‰). Conditioned fingerlings of *C. carpio* (10 ± 2 cm) and *L. rohita* (11 ± 2 cm) were acclimatised to different salinities, with gradual salinity increase @ 1 ‰ hr⁻¹. Acclimatized fingerlings were transferred to respective salinity levels (triplicate) for 120 days of experimental period and fed with pellet feed @ 1 % of body weight once a day as sustenance ration. Fish survival and salinity induced changes in plasma stress hormone (cortisol) level and histology of tissues (gill, liver and kidney) were recorded at the end of experimental period. The cortisol levels were estimated by using Cayman's Cortisol Kit, while for histological studies, the gill, liver and kidney tissues were collected and preserved in 10% neutral buffered formalin solution (48 hours), followed by preparation of tissue embedded paraffin blocks, tissue sectioning (6-8µm) and staining (haematoxylin and eosin). One-Way Analysis of Variance (ANOVA) was applied (Statistical package SPSS V. 16.0), followed by Duncan's Multiple Range Test for comparison of data (p≤0.05).

Results and Discussion

In case of *C. carpio*, 100 % fish survival was observed up to 6 ‰ salinity, while it was 86.66 and 70.00 % in 8‰ and 10 ‰ salinities, respectively after 120 days of experimental rearing. In *L. rohita*, fish survival was recorded as 100, 94.44, 83.33, 77.77, 61.11 and 50.00 % in 0, 2, 4, 6, 8, 10 ‰ salinity levels, respectively (Table 1), which indicates higher level of salinity tolerance in *C. carpio* than *L. rohita*. Significant increment in cortisol level was recorded at 8-10 ‰ salinity in *L. rohita* and 4-10 salinity in *C. carpio* (Table 1), which indicates better stress regulation in *C. carpio*. Significant structural alterations were also observed in the gills, liver and kidney of both the species at all salinity levels (Fig. 1 & 2). However, clubbing of secondary lamellae and hypertrophy of cellular components of gills were visualised more profoundly in the gills of *L. rohita* than *C. carpio* at salinity levels ≥6 ‰, which could affect respiratory and osmoregulatory efficacy of fish, disturbing its overall metabolism. Degeneration of renal tubules and glomerular degeneration appeared in *L. rohita* at lower salinities (≥6‰) as compared to *C. carpio* (≥8‰). Likewise, vacuolisation of hepatocytes and haemorrhagic damage was less seen in the *C. carpio* than *L. rohita* at salinity ≥ 6 ‰, indicating lesser osmoregulatory stress in *C. carpio*. The results indicate that, *C. carpio* could tolerate higher salinity levels (up to 8 ‰) than *L. rohita* (up to 6 ‰), which need to be considered when both the species are to be cultured together under polyculture system in inland saline water. However, in terms of growth and productivity, it is proposed to keep the salinity levels < 5 ‰, so that the fresh water carps could be reared without any detrimental damage to tissues and fish physiology.

Table 1. Survival and Plasma Cortisol Levels in *Labeo rohita* and *Cyprinus carpio* in different Salinity levels

Parameter	Fish Species	Salinity (‰)					
		0 (Control)	2	4	6	8	10
Survival (%)	<i>L. rohita</i>	100.00 ^a ± 0.00	94.44 ^a ± 5.55	83.33 ^{ab} ± 9.62	77.77 ^b ± 5.55	61.11 ^{bc} ± 5.55	50.00 ^c ± 9.62
	<i>C. carpio</i>	100.00 ^a ± 0.00	100.00 ^a ± 0.00	100.00 ^a ± 0.00	90.00 ^{ab} ± 5.77	86.66 ^b ± 3.33	70.00 ^c ± 5.77
Cortisol (pg ml ⁻¹)	<i>L. rohita</i>	211.30 ^c ± 0.20	213.60 ^c ± 1.00	215.05 ^c ± 1.05	216.70 ^{bc} ± 0.70	222.15 ^b ± 0.05	232.65 ^a ± 4.25
	<i>C. carpio</i>	210.15 ^d ± 2.75	217.05 ^{cd} ± 0.65	223.85 ^{bc} ± 5.65	232.65 ^{ab} ± 2.55	237.60 ^a ± 1.70	244.05 ^a ± 4.25

Values with same superscript in a row do not differ significantly (p<0.05)

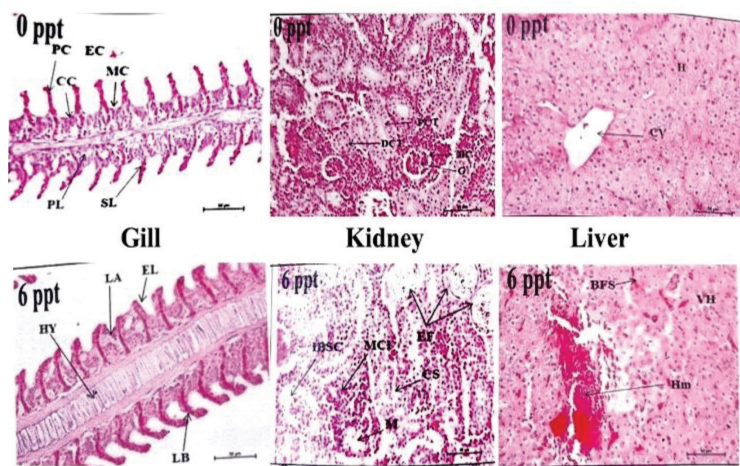


Fig. 1. Photomicrographs of the gill, kidney and liver of *L. rohita* reared in inland saline water. Showing major changes such as Epithelial lifting (EL), Lamellar bending (LB), Hyalinisation (HY) in gills, Melanomacrophage aggregates (M), Edmentous fluid (EF), Cloudy swelling (CS), Mononuclear cellular infiltration (MCI), Increased bowman’s capsule spaces (ICBS) in kidney and Vacuolisation of hepatocytes (VH), Blood filled in sinusides (BFS), Haemorrhages (HM) in liver at ≤6 ‰ respectively. H&E Stained, Magnification 40X.

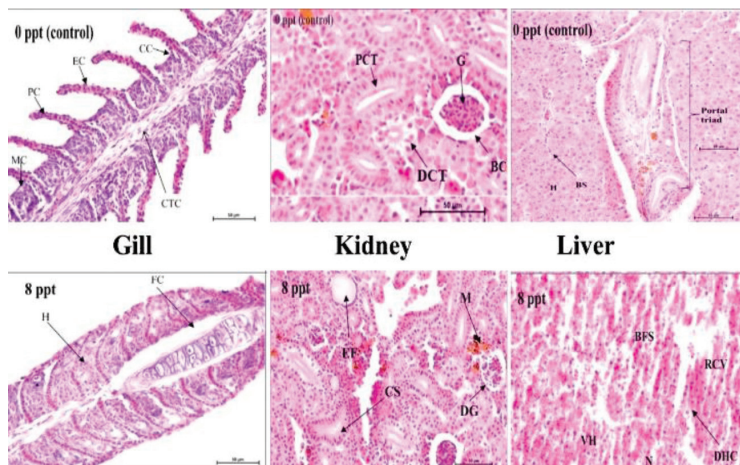


Fig. 2. Photomicrographs of the gill, kidney and liver of *C. carpio* reared in inland saline water. Showing major changes such as Hyperplasia (H), Epithelial lifting (EL), Hyalinisation (HY) in gills, Degeneration of glomerulus (DG), Melanomacrophage aggregates (M), Edmentous fluid (EF), Cloudy swelling (CS) in kidney and Vacuolisation of hepatocytes (VH), Disorganization of hepatic cords (DHC), Blood filled in sinusides (BFS), Ruptured central vein (RCV) in liver at 8 ‰ respectively. H&E Stained, Magnification 40X.

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1.4 Aquaponic Production of Ornamental Koi Carp and Lettuce: Comparison with Traditional Culture System

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Keywords: Aquaponics, Koi carp, Lettuce, Stocking density, Pigment

Introduction

Aquaculture has made tremendous contribution towards livelihood generation and nutritional security, since last two decades across the globe. Department of Fisheries (DOF), MoFAHD, GOI (2020) aims to augment the country's total fisheries production to 22 million metric tonnes by 2024 with a sustainable growth rate of 8-9% and the aquaculture sector is being looked forward to achieve this. However, aquaculture with traditional practices exploits huge quantity of natural resources (water and land), along with surplus discharge of ammonia, nitrate and phosphate in natural resources leading to environmental pollution. To overcome this problem, intensive aquaculture technologies like Recirculatory Aquaculture Systems (RAS), Biofloc and aquaponics are becoming popular. However, among these, aquaponics can easily integrate two systems, i.e. aquaculture and hydroponics (soilless agriculture) with limited use of water and land, as it requires only 10-12% of these resources as compared to traditional aquaculture (Shete et al 2016). With this background, the present study was conducted to optimize the stocking density of ornamental Koi carp, *Cyprinus carpio* in integration with lettuce in an automated aquaponics system, under climatic conditions of Punjab during the winter season.

Materials and Methods

The experimental study (3 months) was conducted in an automated aquaponics unit at Instructional cum Research Farm of College of Fisheries, GADVASU, Ludhiana. Koi carp fingerlings were stocked at three stocking densities i.e. 20 m⁻³ (SD1), 30 m⁻³ (SD2) and 40 m⁻³ (SD3) in circular cemented tanks (10m³) of aquaculture unit and lettuce was planted in hydroponics unit (HM). Water from aquaculture units was circulated into the hydroponic unit at fixed intervals through biological filter, resulting in nutrient uptake by the lettuce plants and optimizing the water quality in the fish tanks. For comparison with traditional system, koi carp fingerlings were stocked in cemented tank of equal size (10m³) at standard stocking rate of 2 m⁻³ and lettuce was planted outdoor in a soil bed system (SM) by following Completely Randomized Design (CRD). Water quality and fish growth was studied at monthly intervals and growth parameters of fish were calculated after the completion of experiment. Lettuce growth was assessed at monthly intervals (total plant height) and after the completion (root length, yield and pigment content - chlorophyll and carotenoids) of the experiment. The data were statistically analyzed by one way analysis of variance (ANOVA) and significant difference between treatment means was determined by CPCS-1.

Results and Discussion

The higher fish growth, in terms of total length gain (TLG), net weight gain (NWG), specific growth rate (SGR) and condition factor (K) was recorded in control tank as compared to aquaponic fish tanks, but the average yield (kg m⁻³) was found significantly higher in all aquaponic tanks, with maximum yield (9.441 times) recorded at stocking density of 40 m⁻³ (SD3) (Table 1).

The lettuce yield (kg m⁻²) increased by 1.58 times in the hydroponic unit, with significant improvement in plant height, root length, plant height gain (PHG) and root length gain (RLG). The chlorophyll a, b and total chlorophyll content were significantly higher (1.15, 1.22 and 1.21 times, respectively) in the lettuce crop harvested from the hydroponic system (HM), as compared to the control lettuce (SM) harvest (Table 2). However, the carotenoid content was found 1.10 times higher in the lettuce crop harvested from the control lettuce (SM), as compared to the hydroponic system (HM). The overall results clearly indicate potential of aquaponic system in enhancing fish productivity substantially (over 9 times) and producing 58% higher lettuce biomass containing higher chlorophyll content. It is attributed to improved water quality maintained in the aquaponic fish tanks through water recirculation and uptake of excessive water nutrients by the lettuce crop in the hydroponic unit. The present study is clear indicative of synergistic integration of koi carp and lettuce with enhanced yield in aquaponics system during winter season in Punjab



Table 1. Comparative growth of koi fish in control and aquaponic tanks

Parameters	Control	SD1	SD2	SD3
Fish Tank ⁻¹	20	200	300	400
Average TLG (cm)	7.99 ^a	6.12 ^c	6.32 ^c	7.11 ^b
Average NWG (g)	17.93 ^a	7.20 ^b	7.47 ^b	7.87 ^b
Yield (Kg m ⁻³)	0.395 ^d	1.727 ^c	2.538 ^b	3.729 ^a
	--	(4.372)	(6.425)	(9.441)
SGR (%)	2.08 ^a	1.28 ^b	1.31 ^b	1.35 ^b
Condition factor (K)	1.46 ^a	1.22 ^b	1.16 ^{bc}	0.98 ^c
FCR	2.85 ^c	4.10 ^a	4.24 ^a	3.87 ^b
Survival (%)	93.33 ^a	82.00 ^b	78.33 ^c	83.25 ^b

* SD = Stocking Density, Control = 2 m³, SD1= 20 m³, SD2 = 30 m³, SD3= 40 m³

Values in parentheses indicate change over control

Values with same superscript (a, b,.....d) in row do not differ significantly (p≤0.05)

Table 2 Comparative growth and pigment content of lettuce in soil based traditional system and hydroponic unit of aquaponic system

Growth Parameters	SM	HM	CD* (=0.05)	CV (%)
IPH (cm)	7.507	7.323	NS**	4.63
PH at 30 DAT (cm)	13.217	19.45	2.6093	4.55
PHG at 30 DAT (%)	76.06	165.60	-	-
PH at 60 DAT (cm)	17.4	24.167	3.118	4.27
PHG at 60 DAT (%)	131.78	230.01	-	-
PH at 90 DAT (cm)	16.8	20.033	1.973	3.05
PHG at 90 DAT (%)	123.79	173.56	-	-
IRL (cm)	3.36	3.457	NS	4.46
FRL (cm)	10.387	32.767	0.328	0.43
RLG (%)	209.14	847.84	-	-
Yield (kg m ⁻²)	0.5728	0.9060	0.1459	5.62
		(1.58)		
Chlorophyll a (mg g ⁻¹)	4.643	5.343	0.388	2.21
Chlorophyll b (mg g ⁻¹)	2.967	3.623	0.30	2.60
Total Chlorophyll (mg g ⁻¹)	2.600	3.140	0.113	1.13
Carotenoids (mg g ⁻¹)	3.886	3.530	0.291	2.23

*CD = Critical Difference, **NS= Non-Significant; Values in parentheses indicate change over control

IPH/FPH – Initial/Final Plant Height, IRL/FRL – Initial/Final Root Length, RLG – Root Length gain,

DAT –Days After Transplanting

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1.5 Quality of Shrimp Reared in Inland Saline Areas of South-West Punjab

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Keywords: Contaminants, Inland, Punjab, Saline, Shrimp, Quality

Introduction

Shrimp aquaculture is a vibrant agri-business sector in India, with record share of 53.18 % in terms of quantity and 74.16% in terms of value in total fisheries export from the country during 2021-22. India produced 9.37 lakh tons of shrimp during 2021 and the shrimp farming which was earlier restricted to southern coastal states, is now spreading very fast in the inland salt affected water logged waste lands of the inland states like Punjab, Haryana, Rajasthan and western Uttar Pradesh (Ansal and Dhawan 2021). A large inland saline water resource is available in these states, which can be potentially utilized for expansion of shrimp farming area with significant contribution to shrimp basket of the country. However, it is pertinent to see that the export quality of shrimp produced is maintained to avert any unforeseen marketing hardships and economical losses thereof. Hence, the present study was conducted to evaluate quality of shrimp reared in inland saline areas of three south-west districts of Punjab.

Materials and Methods

The shrimp samples were collected from 15 farms of three south-west districts (5 per district) viz., Fazilka, Sri Muktsar Sahib and Mansa (Fig. 1), of Punjab in triplicate. The samples were estimated for evaluating quality of shrimp in terms of flesh quality, bacterial load, pesticide residues and heavy metal concentrations. The flesh was analyzed for proximate composition (crude protein, fat, carbohydrate and ash), microbial load (total plate count and presumptive *Vibrio* count), heavy metal (lead, cadmium, chromium, nickel, manganese, iron, copper, zinc and arsenic) concentration and pesticide (organochlorine, organophosphorus and synthetic pyrethroids) residues by standard methods. One-way ANOVA (SPSS 20.0) and DUNCAN's multiple comparison were applied to evaluate significant differences among different shrimp farms in respect to listed parameters ($P \leq 0.05$).

Results and Discussion

The salinity range of selected shrimp farms ranged between 7-16 ppt in district Fazilka, 9-16 ppt in district Sri Muktsar Sahib and 12-15 ppt in district Mansa during the culture period. The shrimp crude protein content (%) ranged between 76.23 -82.58, 60.62 – 80.78 and 65.92 – 78.06 in Fazilka, Sri Muktsar Sahib and Mansa districts, respectively; with corresponding values of 1.42 – 3.85, 0.70 – 2.50 and 1.52 to 2.75 for fat content (%); 9.60- 17.19, 11.37 – 31.25 and 15.65 – 27.37 for carbohydrate content (%); and 4.62 -5.72, 5.02 – 6.37 and 4.47 – 5.67 for ash content (%), respectively. In terms of flesh quality, district Fazilka ranked 1st followed by Sri Muktsar Sahib and Mansa.

In respect to microbial load, including total plate count (TPC) and Presumptive *Vibrio* Count (PVC), shrimp from Fazilka was found the safest (TPC- 5.6×10^5 – 8.7×10^6 and PVC- 3.4×10^3 - 1.3×10^5 cfu g⁻¹), while shrimp from Sri Muktsar Sahib (TPC- 1.4×10^6 - 3.0×10^7 and PVC - 2.5×10^4 – 1.2×10^5 cfu g⁻¹) and Mansa (TPC- 2.3×10^7 - 7.7×10^7 and PVC – 1.0×10^3 – 5.6×10^4 cfu g⁻¹) needed critical monitoring to address food safety concerns. No organophosphorus (Parathion, Dimethoate, Ethion, Chlorpyrifos and Malathion) and synthetic pyrethroid (Permethrin, Cypermethrin and Deltamethrin) pesticide residue was detected in any of the shrimp samples collected from all the districts. However, among the tested organochlorine pesticides (Fipronil, pp-DDE and Beta-BHC), only Beta-BHC was detected in one sample each collected from Sri Muktsar Sahib (60 ng g⁻¹) and Mansa (16 ng g⁻¹) districts, which were though within the permissible limits. The concentrations of all the tested heavy metals (Pb, Cd, Cr, Ni, Mn, Fe, Cu, Zn and As) were found to be within permissible level in the shrimp reared in all the three districts. The overall results revealed that among the three districts, Fazilka ranked first in terms of shrimp quality, while the biosecurity measures, management practices and post-harvest quality measures need to be more stringent in district Mansa and Sri Muktsar Sahib to safeguard the quality of shrimp produce.



GPS - Coordinates		
Fazilka	Sri Muktsar Sahib	Mansa
30.104062°N 74.363053°E	30.073023°N 74.255659°E	29.868807°N 75.27895°E
30.13565°N 74.18587°E	30.264501°N 74.502707°E	29.86627°N 75.27121°E
30.117833°N 74.157999°E	30.287125°N 74.416010°E	29.860255°N 75.242790°E
30.142377°N 74.113811°E	30.1113784°N 74.322471°E	29.871283°N 75.246816°E
30.07557°N 74.05600°E	30.291287°N 74.385320°E	29.867371°N 75.205635°E

Fig. 1 Selected South-West Districts - Fazilka, Sri Muktsar Sahib and Mansa

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1.6 Performance Evaluation of Freshwater Ornamental Black Molly (*Poecilia sphenops*) Reared in Inland Saline Water

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Keywords: Black molly, Inland saline water, Salinity, Ornamental fish, Performance evaluation

Introduction

Inland saline aquaculture has recently opened up a new pathway to turning underused natural resources into productive ones. Punjab has suffered from underground salinity and water logging; aquaculture provides a good opportunity to utilize these degraded lands. Extensive efforts have been made to reclaim and utilize these lands by rearing freshwater (food and ornamental) and brackishwater species. Among ornamental fish species, Koi carp and goldfish have also been successfully cultured in the inland saline waters of Punjab (Sharma et al 2017 and Bhatt et al 2018) with regard to stress responses in terms of physiological changes. It has been observed that black molly ornamental fish can tolerate salinity, when maintained in inland saline waters for a short time period. Based on these considerations, the study explored the feasibility of rearing black molly in inland saline water to assess survival, growth, reproductive performance, and coloration.

Materials and Methods

The study was conducted at the Instructional cum Research Farm of the College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. The inland saline water (16‰) for the study was collected from salt-affected/waterlogged areas in Village Birewala, District Mansa (Punjab). A total of 324 equal-sized fry (3.55 ± 0.05 cm; 3.83 ± 0.12 g) of black molly *Poecilia sphenops*, were randomly stocked @ 12 no./tank in nine treatments in triplicate in the glass aquaria (50L) with continuous aeration (24 x 7 hours a day). Fish were fed *ad libitum* with commercial feed twice (9-10 AM; 3-4PM) a day. The estimation of water quality parameters and ionic composition were carried out by following standard methods. Fish behaviour was observed at monthly intervals throughout the experimental period. Fish survival and growth in terms of total body length and body weight was recorded at monthly intervals. Carotenoid ($\mu\text{g g}^{-1}$) analysis of fish skin and muscle was carried out after completion of experiment. To determine the differences between salinity treatments ($P \leq 0.05$), one-way ANOVA and Duncan's multiple range tests were used to determine the effect of different salinities on growth and breeding performance of experimental fish.

Results and Discussion

The physico-chemical analyses of water during the experiment displayed that electrical conductivity, sodium (cation) and chloride (anion) of water had positive relationship with salinity. The fish were able to adapt up to 14 ppt (>80% survival rate) without adverse effects on growth up to 10 ppt. Fish growth and reproductive performances were significantly affected at the higher salinity treatments (12-16 ppt), while best values were recorded at intermediate salinity treatments (6-10 ppt). Fish behaviour in terms of swimming activity, feeding responses and morphological characters like colouration, body fragility and presence of mucus on skin was recorded at monthly intervals. The results show that fish behaviour is significantly influenced by salinity and after 10 ppt, the fish are slightly stressed. The reproductive performance was studied on the basis of gonado-somatic index (GSI), absolute fecundity, relative fecundity, ovary weight, ovary volume and ovary diameter varied 5.33-11.66%, 10.25-14.68 nos.egg/fish, and 4.67-12.22 nos. of fry/body weight, 0.17-0.34 g, 655.12-876.34 mm³ and 2.21-3.03 mm, respectively. The mean fry production in various treatments ranged between 12.85-34.78, whereas fry survival rate was 85.56-93.61% respectively (Fig. 1). Coloration analysis based on carotenoids and digital imaging showed varying results, with salinity having no direct effect, except at higher salinities. Overall results in terms of survival, growth, reproductive performances, and colouration studies on black molly revealed that, although fish was capable of adapting and growing under salinity conditions up to 12 ppt in inland saline water, but 8 ppt salinity can be considered safe w.r.to overall performance of fish. However, based on the present study, carried out under lab conditions, field trials need to be conducted for better understanding of effect of salinity (inland saline water) coupled with multiple factors under natural conditions.

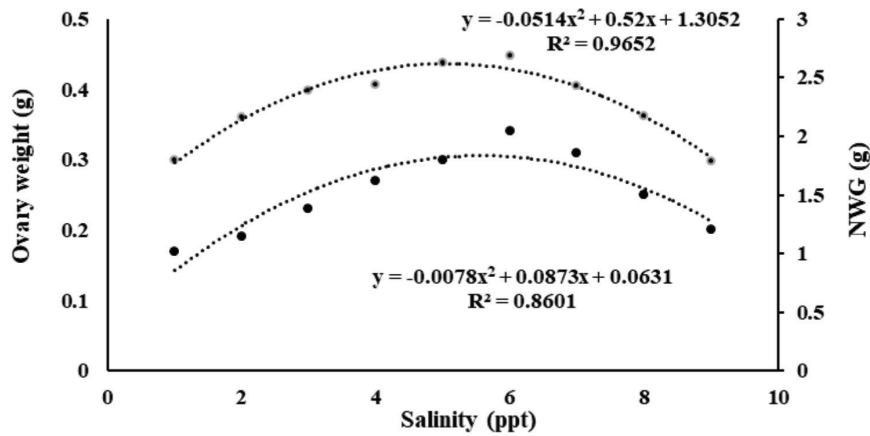


Fig. 1. Polynomial relationship between ovary weight (g) of black molly vs. different salinity levels (ppt) vs. NWG (g) during the experiment

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1.7 A System and Method for Captive Maturation and Multiple Breeding of Endangered Golden Mahseer, *Tor putitora*

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Keywords: Captive maturation, Multiple breeding, Fertilization, Stripping, Golden mahseer.

Introduction

Golden mahseer (*Tor putitora*), a cyprinid fish, is considered a flagship species in upland fisheries of the Himalayan sub-continent by virtue of its economic, ecological, recreational, heritage, cultural and food values. It is distributed in the lacustrine and riverine ecosystems of many Asian countries and manifests higher potential for sports fishery-based eco-tourism and livelihood opportunities. However, due to various anthropogenic and abiotic factors, its wild population declined sharply and was subsequently listed by International Union for Conservation of Nature (IUCN) as an endangered species. Different national and international bodies have advocated its conservation and rehabilitation through stock enhancement/ranching. However, to achieve an effective restoration and conservation of golden mahseer across the country, the availability or the supply of fry should be consistent, sustainable and large-scale in captive conditions. Unfortunately, golden mahseer females fail to complete ovarian development and maturation in captive conditions due to endocrine dysfunctions.

Several efforts have been made during the last four decades to breed golden mahseer in captivity, but they could not succeed or had minimal success, and the issue remained unsolved. Hence, to cater to the high demand for fry, developing captive brooders for mass-scale seed production of golden mahseer was imperative. There are few reports wherein golden mahseer was found maturing in larger ponds with flowing water, but the occurrence of matured females was less than 15 % in a year. Raising brooders in such larger ponds is very labour and cost-intensive, and getting a lesser number of matured female brooders from these ponds further makes it non-sustainable. Therefore, the non-availability of captive matured golden mahseer female brooders in large numbers has been a significant bottleneck for its mass-scale seed production and conservation.

Accordingly, there existed an urgent need for an efficient and cost-effective compact system and method for achieving captive maturation and breeding of golden mahseer to overcome the issue of meagre and inconsistent availability of golden mahseer fry for its rehabilitation and conservation.

Materials and Methods

A series of inter-dependent experiments like the comparative reproductive assessment of the wild and captive golden mahseer, photoperiod and temperature manipulation, substratum manipulation, broodstock diet manipulation, male and female segregation, system design etc., were carried out for several years, and finally developed a technology (system and method) for captive maturation and multiple breeding of golden mahseer.

Results and Discussion

We developed an efficient system and method for multiple breeding of endangered golden mahseer in a compact tank environment, which otherwise failed to complete gonadal development in captivity. The system mimics natural breeding grounds with temperature and light control mechanisms. More precisely, the bottom of the broodstock rearing tank is provided with a breeding bed of specific thickness (a mixture of sand, pebbles and limestone grey grits of a specific size in a particular ratio), and the tank is filled with water to have the desired water column. The broodstock rearing tank is provided with a submersible water heater to maintain specific water temperature and a white or green LED bulb of specific intensity. The tank is also provided with bubbling aeration from a compressed aerator to supply oxygen to the fish, and a specific water flow is also maintained.

Further, the broodstock rearing tank is provided with a network of perforated pipes for turning the tank water through the bed and creating ripples on the top of the water column. The method involves the co-rearing of adult male and female fish (1:2) for 3-4 months in the broodstock rearing tanks on a specific broodstock diet, followed by sex segregation to avoid auto-spawning and egg deterioration. After segregation, the female brooders are monitored regularly based on their behaviour and observation made to their bellies to ascertain the presence of ripened eggs; the females possessing ripened eggs are taken out of the tank and subsequently stripped and mixed with a specific quantity of milt. The fertilized eggs are then transferred into incubation units for further development. This method facilitates the stripping and retrieval of a higher number of eggs. With this technology, breeding is carried out throughout the year.

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1.8 One Paddy-One Fish Culture System for a Green Economy

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Keywords: Anti-Microbial Resistance, Bio-magnification, Bio-accumulation, Geomorphology

Introduction

Aquaculture is one of the fastest growing food sectors across the globe and it has developed in India to such an extent that it is the current top-most producer. All means of food, if cultured in unison will benefit through energy and space conservation. The green concept of the century with acclamation, has taken many forms - Pokkali of Kerala, Bheris of West Bengal, Khazan of Goa and Khar of Karnataka. With science and technology developing and intruding into various fields, more appropriate and more profitable techniques are getting evolved, keeping this tradition as a base. One such fruitful intervention is One Paddy-One Fish culture system, which will form the content of this article whose feasibility and profitability, has been assessed. Shifting to such practices is the need of the hour, as we are struggling to balance the food demand and the environmental palpitations.

Materials and Methods

Being blessed with an indigenous Wetland System, the Alappuzha Fisheries Department of Kerala has created a synergistic rice-fish culture technique, which aims at minimal input and maximum output. The practice is currently popular in Alappuzha, Thrissur and Kottayam districts. As the fish seedlings from hatcheries or fish farms are stocked into the prepared pond (1-3% of the total farmland) with net covering, the field is sown with Uma rice seeds. For good nursery growth, cow dung at 2000 kg/ ha has to be added along with proper liming.

The Indian Major Carps is suitable to be grown in this One Paddy-One Fish Scheme, besides Grass Carp and freshwater prawn. 4-5 cm long carp seeds at 3000/ha is cultivated. In nursery stage, 25-30% protein feed at 5% body weight is provided, which later on should be reduced to 2% body weight.

After paddy harvest and until the paddy field is filled with freshwater, the fish seeds have to be kept in the nursery pond itself, up to 3-4 months, when they attain a length of 8-12 cm. With the rotten paddy, field will be highly enriched with organic and mineral nutrients, thus the pond develops its own planktons for fish to feed. In addition, external feed can also be provided, but is required only in a very lower quantity. Further once the crop gets harvested, the pond embankment broken to let the seeds into the field for further developments.

The practice starts by mid- November with rice harvest within the initial 120 days after. Fish culture lasts for 7-8 months again during which they attain 700-1000 g weight, yielding a total of 1 tonne/ha.

However the only constraint is bund maintenance in heavy rains. Earlier the department used to provide subsidies for bund and nursery pond construction, but now the state department is not lending their hands in the same, but ensuring good seeds for farmers free of cost.

Results and Discussion

The present scenario is such that the farmers are facing repercussions due to insensitive culture practices and hence such organic farming methods hold high relevance. The farmers need not have to prepare the field much for rice and fish cultivation, thereby reducing the total expenses. The production has doubled with 10-15% increase in rice cultivation. No usage of chemical fertilizers and pesticides are an added advantage. The rice stubbles and straw leftover in the fields return the essential nutrients to the soil. Further there is a substantial reduction in the amount of fish feed, reducing the operational cost of fish farming as supplementary feed forms 50% of the total variable cost. Also the nutrient enrichment from the crop residues promotes the growth of beneficial planktons upon which the fish could feed. In addition, the interaction of rice and fish crops has ensured optimized water quality by lowering the amount of unionized ammonia in water during their grow-out in rice field. The system is zero waste generating as well as self-sufficient in terms of nutrition. The farmers are also ensured with year-round production and profit. Since the culture is purely organic, the produce has more market demand and could be sold at a better price. The practice also improves land use efficiency and reduces the chances for land degradation. A study by Nair et al (2014) suggests that a system incorporating rice-fish-prawn rotational culture achieves 20% more net revenue than the conventional techniques of production thus validating its importance to the current ecosystem and economy.



Fig. 1. One Paddy-One Fish Culture Model

Table 1. The Water Quality Requirements of the Pond are

Sl. No.	Water Quality Parameters	Suitable Range
1.	pH	6.5-8.5
2.	Dissolved Oxygen	5 ppm
3.	Temperature	20-32 °C
4.	Water Transparency	25-40 cm

Table 2. The liming requirements of the pond are

Sl. No.	pH	Quantity/Hectare
1.	4-4.5	1000 kg/ha
2.	4.5-5.5	750 kg/ha
3.	5.5-6.5	500 kg/ha
4.	6.5-7.5	250 kg/ha

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1.9 An Attempt to Design Low Cost Filter for Aquariums

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Keywords: RAS, Filter, Media, Water quality, Aquaculture

Introduction

Growth of aquatic organisms are directly related with water quality management. The biota present in water has a great importance for purification of water. Recirculation Aquaculture System (RAS) is a leading culture system nowadays. In RAS, water parameters have to be checked continuously and purification of water should be such that parameters of water should be favorable to the fish culture in the system. This system involves reusing water but before re-circulating water is treated in different filter systems. The biological, chemical and mechanical process of filtering removes solid particles, feces and other impurities from water. Mechanical filter physically traps waste from fish, decaying plant matter and other debris from culture water. With the help of floss, plastic foam and gravel/sand. Biological filter purify the water through N_2 cycle. Through biological filter, the natural population of the good bacteria is enhanced which can control the nitrogen cycle and the system thus get rid of organic waste. Lava rocks, Bio balls, and ceramic shells are a few examples of frequently used filter media. In chemical filter, chemical media also mechanically traps solids. Activated charcoal and foam fractionation are the most frequently used filter media. The overall aim of the study is to design an innovative filtration system which will minimize the effort of performing manual maintenance of freshwater aquarium.

Materials and Methods

The methodology includes designing the filter and testing some water quality parameters. Materials used for present study were air tight container, plastic sheet, media, water pump, and nozzles. In the present study, filter has four compartments which can easily be cleaned and refilled with their respective contents. Plastic sheet were cut and used for compartmentalization of the filter to make a design which was easy to use. The filter container has a lid which can be opened to provide the user with an easy way to clean the filter. Furthermore, the filter was designed in such a way that it was economical and affordable to common people. The first compartment is specifically for the primary filtration of the water and has a foam layer which helps in separating the big physical impurities from it. The second compartment consists of ceramic shells and bio balls which were highly porous media to colonize beneficial bacteria and maintain clean water in the aquarium. Activated charcoal was in the third compartment and the pump was fitted in the fourth compartment.

Results and Discussion

The purpose of the filter in an aquarium is to remove excess food, decaying organic matter, free floating particulate, dangerous chemicals and fish waste product from the water. The present study revealed is the efficiency of the designed filters as compared to normal filter in terms of ammonia and nitrate levels (Table 1) which were found to be very low in DF. This concludes that the designed filter is more efficient than normal filter.

Table 1: Comparative water parameters in aquaria with Normal Filter (NF) and Design filter (DF)

Date of observation	pH (NF)	pH (DF)	Ammonia (NF) in mg L ⁻¹	Ammonia (DF) in mg L ⁻¹	Nitrate (NF) in mg L ⁻¹	Nitrate (DF) in mg L ⁻¹
27/07/2022	7.2	7.2	0	0	0	0
29/07/2022	7.4	7.3	1	0.5	0	0
04/08/2022	7.4	7.2	1	0.5	0	0
06/08/2022	7.4	7.2	1.5	0.5	0	0
08/08/2022	7.4	7.2	1.5	0.5	10	0
10/08/2022	7.4	7.2	1.5	0.5	10	0
12/08/2022	7.4	7.2	1.5	1	10	0
14/08/2022	7.5	7.2	2	0	10	0
16/08/2022	7.5	7.3	2	0	10	0



18/08/2022	7.5	7.3	2	0	10	0
20/08/2022	7.6	7.2	2	0	10	0
22/08/2022	7.6	7.2	2	0	10	0

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1.10 A Comparative Study on the Growth Performance of Tubifex Worms between Normal Water Flow System and Recirculating Water System

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Keywords: Growth performance, Live food, RAS, Tubifex culture

Introduction

One of the most important and high-quality live food used in the ornamental industry is tubifex worms, which are fed to ornamental as well as aquaculture fish larvae in hatcheries. They grow rapidly in mud, which is rich in organic debris and form reddish colonies. They consume sediments, selectively digesting the microorganisms present and absorbing essential nutrients through the body wall for sustenance (Rodriguez et al., 2001). Due to their high food value (energy-5575 calg⁻¹ DW, crude protein-63.32%, crude lipid-28.84%, ash-7.95%), they are regarded as one of the nutrient-dense food for fish (Mollah and Ahamed 1989). In view of importance of tubifex in terms of its nutritive value and utility, the present experiment was conducted to study a comparative evaluation of the growth performance of tubifex worms in a normal running water system and recirculating water system.

Materials and Methods

A 60 days experiment was conducted, for which tubifex worms were collected from Galiff Street, Bagbazar, Kolkata. After conditioning, tubifex worms were stocked in both vertically arranged culture systems (triplicate) at the rate of 30 g per tank (45cm×22 cm) in 9,000 g of media. Each tank was facilitated with an inlet and outlet system. The water flow rate was regulated by adjusting the PVC pipes' stop corks. Continuous water flow was maintained at the rate of 1.20±0.43 L min⁻¹ and dissolved oxygen above 6.10±27 ppm. Media composition for both the system (25% cow dung, 20% fines, 20% mustard oil cake and 35% wheat bran) was as per Mollah and Ahamed (1989). Samples were collected from both systems at 15, 30, 45 and 60 days. From ten randomly chosen locations within each tray, samples were collected and washed with flowing water. With the help of forceps and a dropper, the unwanted particles were separated and cleaned worms were weighed.

Results and Discussion

The standing biomass of tubifex worms at day 15, 30, 45 and 60 in a normal running water system and recirculating system during the experimental period is presented in Table 1. The statistical analysis (ANOVA) of total biomass/standing biomass (g) of tubifex worms in both system showed a non-significant (P>0.05) trend up to 45 days of the culture period. After completing the 60 days of culture, the normal running water system shows significantly (P<0.05) higher biomass when compared with a recirculating system. Throughout the trial, fortnightly water quality measurements (temperature and DO) ranged between 27.37-30.63 °C and 5.9-6.9 ppm, respectively. Tubifex worm is one of the important live food for growing the larvae of hatchery-produced fish, prawn, eel, and ornamental fish due to its high nutritional value. Tubifex collected from sewage fed streams can be contaminated with human pathogens responsible for tetanus, hepatitis, and other bacterial and viral illnesses. In reality, various attempts to produce a pathogen free and sustainable tubifex worm culture technology have shown minimal results with maximum wastage of water. From the current study, it can be concluded that culture of tubifex worms in recirculating water system showed comparable results with normal running water system in terms of growth performance. Hence, culture of tubifex worms can be taken up in the recirculating water system to avoid water wastage as well as maintenance.

Table 1. Comparative tubifex biomass in a recirculating water system and normally flowing water for experimental period 60 days

Days	System-I	System-II	P-Value
15	32.81±0.19	32.88±0.21	0.836
30	36.22±0.15	35.72±0.18	0.109
45	38.88±0.34	37.73±0.25	0.056
60	40.88±0.09	39.83±0.20	<0.001

Data are expressed as mean ± S.E, n=3 (One-way ANOVA) and mean and SEM, n=3

System I -Normal running water

System II-Recirculating water system





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1.11 Complete Utilization of Resources by Integrating Fish and Poultry Farming- A Case Study

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Keywords: Eco-friendly, Fish, Integrated farming, Poultry, Profitability

Introduction

On World Population Day (11th July, 2022), the United Nations has released a report stating that India may surpass China as the world's most populous country by 2023 reaching 1.4 billion people. The enormously increasing population put additional pressure on resources, which are already stressed due to number of activities. Therefore, sustainable integrated farming can be a very good option for achieving optimum productivity with due environmental consideration. In this context, substituting the traditional farming practice with integrated farming can be a profitable approach that involves crop cultivation, dairy, poultry, fishery, vegetable and fruit production etc. together for higher efficiency. Under fish cum poultry integrated farming system, nutrients from the poultry are recycled in the pond and this allows for escalation of production and income while reducing the effluent along with the dumping of the wastes which would have bad impact on the environment (Singh et al 2014). With respect to the above-mentioned aspect of integrated fish farming, the economics of integrated fish cum poultry farming system was worked out to check the feasibility of the practice.

Materials and Methods

It is a case study, which involves comprehensive study of a social unit comprised of a person, a group, a social institution, a district or a community (Young 1996). Sardar Manjeet Singh (age 54) of village Alkara, District Barnala, Punjab has developed a successful integrated farming model at his farm comprising poultry farming and fish together leaving nothing as waste. The integrated model in the present study is of Fish cum poultry farming in 2.5 acres of fish-pond with composite carp culture and rearing 1000 White Leghorn poultry birds in a poultry house constructed over the fish pond. Information was collected from the farmer by personal interview and visiting the farmer's field. Considering the efficacy of this viable production system, the study was purposively carried out at the selected farm during 2021-22 to analyze the farmer's income through adopted system of integrated fish cum poultry farming along with efficacy of available available resources.

Results and Discussion

The data on economic aspect recorded from the integrated fish cum poultry farming unit (2.5 acre) is presented in Table 1. Economic analysis of integrated fish cum poultry farming was made to evaluate the sustainability of the existing integrated farm. It includes year round variable cost and net profit of the integrated model in the study. Totally dependent on poultry droppings, total fish production from 2.5 acres fish pond was 6.3 tonnes (average production of 2.52 tonnes per acre). As a whole, fish and poultry farming have contributed 59.89 % and 40.10 %, respectively of the net farm income. The % return on total variable expense was computed to be 60.87 in the present study. Presently, he gets an average annual net income of Rs. 7.73 lakh with an average of approximately Rs. 64,417.00 per month. For his hard work and success in the field of integrated farming, he was awarded with Best Integrated Farmer by Krishi Vigyan Kendra, Barnala in the year 2022.

Profit

Total Revenue Cost - Variable Expense

= Rs. (20,43,000.00– 12,70,000.00)

= Rs. 7,73,000.00

Percentage return on cost variable expense: 60.87 %

The results proved the efficacy of integrated fish cum poultry farming as a profitable venture for the farmers and can be easily adopted by the young people too for income and self-employment generation to earn more profit in comparison to traditional farming.





Table 1 Economics of Fish cum Poultry Integrated Farming Model (2.5 acre area)

Parameter	Unit amount	Cost (in Rs.)
Variable Input Cost		
Fish Pond		
Fish Fingerling	12000	36,000.00
Supplementary feed	-	-
Medicines, Electricity and Miscellaneous	50,000	50,000.00
Labour	Rs. 12,000 per month (Full time)	1,44,000.00
Input Cost (A)		2,30,000.00
Poultry Unit		
Poultry Birds	1000 (Rs. 300/bird)	3,00,000.00
Feed	1 Quintal per day	7,40,000.00
Input Cost (B)		10,40,000.00
Total Input Cost (A+B)		12,70,000.00
Revenue		
Fish Harvesting	6.3 tonnes (Rs. 110 per Kg)	6,93,000.00
Eggs	Rs. 180 per tray	13,00,000.00
Meat	Rs. 50 per bird	50,000.00
Total Revenue Cost		20,43,000.00
Net Profit		7,73,000.00

Table 2 Income received from different enterprises

Enterprise	Species	Area/ No.	Variable Cost	Gross Income	Net Profit	% contribution in Income
I. Fisheries	Carps	2.5 Acre	2,30,000.00	6,93,000.00	4,63,000.00	59.89
II. Poultry	White leghorn birds	24x36 ft (1000 birds)	10,40,000.00	13,50,000.00	3,10,000.00	40.10

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1.12 Comparative Evaluation of Water Discharge and Growth Performance of Genetically Improved Farmed Tilapia Fry in Biofloc and Control Systems

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Keywords: Biofloc, GIFT tilapia, Carbon sources, Water exchange rate

Introduction

Aquaculture is the leading food-producing sector that supplies nutritious food to the increasing human population around the globe. However, the sustainable expansion of aquaculture is essential by minimizing the effluent discharge from culture practice which will have a minimum impact on the environment. Biofloc technology is a minimal or zero water exchange system in which the water quality is maintained by recycling the waste and nutrients within the system with the production of nutritious feed and some bioactive compounds that will enhance the growth, survival, and defense mechanisms of cultured species.

Materials and Methods

The experiment was carried out in the wet laboratory of the College of Fisheries, Ratnagiri, Maharashtra as per Completely Randomized Design (CRD) with seven treatments and three replicates for 90 days. The fry of Genetically Improved Farmed Tilapia (GIFT) were reared in a biofloc system using carbon sources such as sugar cane molasses (T1), sugar (T2), jaggery (T3), wheat flour (T4), wheat bran (T5) and rice bran (T6) and control (T0). GIFT fry were stocked @ 250 nos. m⁻³ in circular tanks of 110 Litre (L) capacity. In the control tank (T0), daily siphoning was carried out and 10% water was replaced with fresh water. In biofloc treatments (T1-T6), sludge was removed to maintain total suspended solids (TSS) level below 400 mgL⁻¹. At the end of the experiment, the growth of fish was estimated. The quantity of water discharged into the environment and the daily water exchange rate in each treatment were recorded during the experiment.

Water quality parameters such as temperature (°C), pH, dissolved oxygen (mgL⁻¹), total hardness (mgL⁻¹), total alkalinity (mgL⁻¹), free carbon dioxide (mgL⁻¹), total ammonia-N (mgL⁻¹), nitrite-N (mgL⁻¹), nitrate-N (mgL⁻¹) and floc volume (mL⁻¹) were recorded.

Results and Discussion

Growth and water exchange

At the end of the experiment, one-way ANOVA revealed a significant difference ($p < 0.05$) in weight and length gain of fishes reared by using biofloc system (T1, T2, T3, T4, T5, and T6) and control (T0). A higher weight and length gain was recorded in all biofloc treatments as compared to the control, with significantly higher weight gain (3539.66 ± 45.71^a) and length gain (229.33 ± 0.97^a) in T6 treatment. The water exchange rate in biofloc treatments (T1-T6) varied from 0.22-0.45%, whereas in control it was 10% daily. The amount of water discharged into the environment from biofloc treatments ranged between 13.33L to 26.67L, whereas, in T0, 900L of water was released into the environment during the experiment period. All the water quality parameters were within the acceptable range for tilapia fry rearing during the study. The results indicated that the growth performance of GIFT fry was higher in biofloc system with a minimal water discharge into the environment than the control system, thus preventing eutrophication and effluent discharge into the surrounding environment.

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1.13 Economic Analysis of Experimental Cage Culture in Manasbal Lake, Kashmir, India

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Keywords: Benefit cost ratio, Economic viability, Cage aquaculture, Manasbal lake, FCR

Introduction

Cage aquaculture has flourished well across the world. In India, inland open-cage aquaculture is being practiced at an increasing rate. For the last two decades, the country has started to seek potential of cage culture in untapped lakes and reservoirs with the purpose to increase fish production, meet the protein demand and provide alternate livelihood opportunities to local and landless fishers. In this context, the Department of Fisheries, Jammu and Kashmir initiated experimental cage farming practice in Manasbal lake of Kashmir under the Rashtriya Krishi Vikas Yojana (RKVY) sub-scheme; National Mission for Protein Supplements (NMPS) in 2013-14. However, after the completion of project in 2016-17, the cage farming practice in Manasbal lake was stopped. Keeping this in view, an economic analysis of the experimental cage culture in Manasbal lake was performed.

Materials and Methods

The economic indicators like net present value (NPV), benefit-cost ratio (BCR), payback period (PBP) and internal rate of return were used to check cage culture profitability in the region. Feed conversion ratio (FCR) and feed efficiency ratio (FER) were used to analyse the weight gained and feed intake by the fish stock

Results and Discussion

The cost-benefit analysis based on a calculation of net present value (₹ -713844), benefit-cost ratio (-0.97), payback period (-5.94) and internal rate of return (<0%) depicted non-economic viability and poor financial performance of venture in the region. The biomass gained for one crop season of eight months was 3351 kg and the overall feed conversion ratio (FCR) was found to be 2.42. It was found that feed expenses form the highest cost component of ₹ 302170 (86.27%) among variable costs. Gawa et al (2021) and Obiero et al (2022) also found the feed cost as the single most important cost component in inland water cages of Jharkhand, India and Kenya respectively. In case of annual fixed cost, permanent human labour formed the highest cost component of ₹ 2 lakhs (51.53%), followed by depreciation ₹ 112560 (29%) and interest on fixed capital ₹ 75600 (19.48%). Annual fixed cost and variable cost worked out to be ₹ 388160 (52.6%) and ₹ 350253 (47.4%) of the total cost (₹ 738412), respectively. Net income worked out to be negative (₹ -336216), indicating losses from the venture. The cost of production of one-kilogram fish was ₹ 220 which remunerated ₹ 120 as revenue. More research is needed to make cost-effective floating feed, which suits the dietary requirement of exotic carps in the region. Proper utilization of scientific knowledge can help to improve the economic viability of cages in the region. Besides, other livelihood opportunities can also be provided to the local fishers by modifying the cage for recreational purposes.

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1.14 Aquaponics – An Alternative for Food (Fish and Plant) Production

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Keywords: Aquaponics, Water Quality, Rohu, Tilapia, Spinach, Growth

Introduction

Aquaponics is a part of the overall agricultural approach known as integrated agri-aquaculture systems (Gooley and Gavine 2003). Aquaponics is the combination of aquaculture and hydroponic plant cultivation in a re-circulating system, utilizing the nutrients present in the aquaculture effluents to produce plants with commercial value. The aquaponics system was developed to conserve water resources by controlling water quality, production schedule, and fish products. Untreated water containing ammonia discharged into the ecosystem could cause eutrophication and other environmental problems. As a result, aquaponics systems can be used to reduce the effect of inorganic nitrogen accumulation, which can be detrimental to fish growth. Ammonia in the aquaponics system is converted into nitrite and nitrate by nitrification bacteria, and nitrate is absorbed by plants as a nutrient. Furthermore, the plant can be considered as a bio-filter for the fish in a symbiotic relationship with mutual benefit by absorbing nutrients from farming waste and reducing ammonia through the nitrification process.

Materials and Methods

The study was conducted at the Department of Aquatic Biology (VNSGU), Surat, for 60 days in a rectangular aquaponics tank with two different treatments, in which tilapia and rohu were reared in treatment T₁ and T₂ respectively with three replicates. Similarly, for the present study, Spinach (*Spinacia oleracea*) was selected for integration with fish and were transferred to styrofoam sheets on the same day, when fish were stocked in aquaponics tanks. The water quality parameters including water temperature (°C), pH, dissolved oxygen, nitrate, nitrite, phosphate and total hardness were measured at weekly interval following the standard methods. The growth of the fish in terms of total weight gain, survival rate, percent weight gain, SGR, and FCR was recorded/ calculated and data were statistically analyzed by ANOVA and a significant difference between treatment means was determined by SPSS.

Results and Discussion

Growth of the fishes in terms of Total weight gain, Survival rate, Percent weight gain, SGR, and FCR were 9.56±0.01 & 2.96±0.02 gm; 100% & 90%; 267.80±0.73% & 70.46±0.65%; 2.17±0.01 & 0.89±0.01 and 1.26±0.01 & 3.38±0.02 in T₁ and T₂ respectively. The growth of spinach in terms of plant height was (0.650±0.074 cm) in T₁ and (0.463±0.052 cm) in T₂ respectively. Based on the above results it can be preliminarily concluded that the aquaponics system provides a conducive environment for the survival and growth of tilapia and rohu in combination with spinach (which grew better in T₁). This technology has mainly been driven forward by researchers and risk-taking entrepreneurs worldwide, but commercial-oriented production units are emerging with the participation of industry partners from both the aqua and horticultural sectors.

Table 1. Comparative growth of Tilapia and Rohu in different control and aquaponic tanks

Treatment ¹	WG ²	%WG ³	SGR ⁴	FCR ⁵
C ₁	7.24 ^c ±0.03	203.14 ^c ±1.57	1.85 ^c ±0.01	1.66 ^b ±0.01
T ₁	9.56 ^d ±0.01	267.80 ^d ±0.73	2.17 ^d ±0.01	1.26 ^a ±0.01
C ₂	2.83 ^a ±0.03	67.68 ^a ±0.87	0.86 ^a ±0.01	3.54 ^d ±0.03
T ₂	2.96 ^b ±0.02	70.46 ^a ±0.65	0.89 ^b ±0.01	3.38 ^c ±0.02
<i>P Value</i>	< 0.001	< 0.001	< 0.001	< 0.001

Data are expressed as mean ± S.E, n=6 (One-way ANOVA); mean values in the same column with different superscripts differ significantly (p < 0.05)

¹C₁, Control 1 with Tilapia, T₁, Tilapia with Spinach, C₂, Control 2 with Rohu, T₂, Rohu with Spinach;

²WG, weight gain, ³SGR, Specific growth rate, ⁴FCR, Feed conversion ratio

Table 2. Physicochemical parameters of the examined water samples for control and treatments in aquaponics system

Parameters	Observed values									Optimum value (mg/L) except for Temp.
	Control			T ₁			T ₂			
	Min	Max	Mean ± SE	Min	Max	Mean ± SE	Min	Max	Mean ± SE	
Water Temp (°C)	24.00	26.90	25.54 ± 0.42	24.00	27.00	25.64 ± 0.38	23.70	27.00	25.51 ± 0.49	11-42° C (FAO, 2012)
pH	7.20	8.20	7.71 ± 0.13	7.20	8.10	7.76 ± 0.12	7.30	8.20	7.77 ± 0.11	6-9 (Popma and Masser 1999)
Dissolved oxygen (mg/L)	5.00	5.30	5.16 ± 0.05	4.80	5.40	5.10 ± 0.08	4.90	5.30	5.10 ± 0.06	≥5 (Lloyd 1992) 3-5 (Anita and Pooja 2013)
Nitrate NO ₃ ⁻ (mg/L)	0.02	0.08	0.04 ± 0.01	0.02	0.07	0.04 ± 0.01	0.01	0.09	0.05 ± 0.01	≤ 10 (Pillay and Kutty 2005)
Nitrite NO ₂ ⁻ (mg/L)	0.02	0.13	0.05 ± 0.02	0.01	0.08	0.03 ± 0.01	0.01	0.10	0.04 ± 0.02	0.5 (Swann 1997) ≤1 (Pillay and Kutty 2005)
Phosphate (mg/L)	0.05	0.10	0.07 ± 0.01	0.03	0.15	0.07 ± 0.02	0.01	0.11	0.07 ± 0.02	0.03-2 (Anita and Pooja 2013)
Total Hardness (mg/L)	144.00	266.00	196.00 ± 15.44	110.00	266.00	189.14 ± 19.25	142.00	240.00	200.00 ± 12.78	20 –300 (Santhosh and Singh 2007)

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1.15 Supplementing Aquaponics with Black Soldier Fly (*Hermetia illucens*) Larvae Frass Formulated Feed: Effects on the Production and Composition of Stevia and Lavender with Channel Catfish *Ictalurus punctatus*

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Keywords: Aquaponics, Black soldier fly, Channel catfish, Gene expression, Histology

Introduction

There is a rising concern regarding efficiencies of aquaponic systems with respect to availability of enough soluble mineral elements released by fish to assure healthy, consistent plant growth. Adding black soldier fly larvae by-products, which include 'Frass' and chitin (shell of the larvae), in the diets of channel catfish (*Ictalurus punctatus*) may help to supplement plant growth. There is increasing interest in insect farming to improve aquaculture sustainability, and studies have already shown the benefits on the inclusion of larvae as a source of protein and lipids. Present study was conducted with the objectives to supplement aquaponics with black soldier fly (*Hermetia illucens*) larvae (BSFL) frass as fish feed and evaluate its effects on the production and composition of Stevia (*Stevia rebaudiana*) and Lavender (*Lavandula angustifolia*) with Channel Catfish. Comparative growth performance, gene expression and histology of the hepatic tissue of Channel catfish fed with control diet (crude protein of 32%; Rangen, TX, USA) and 10 % BSFL frass feed were also evaluated.

Material and Methods

In this 10-week study, Stevia and Lavender were cultured with or without BSFL frass (10%) supplementations in fish feed in a 2 × 2 factorial design in aquaponics. Each treatment was triplicated with 60/m³ channel catfish (*Ictalurus punctatus*) juveniles and plant growth was periodically monitored and harvested. Fish survival, growth, biomass produced, plant growth, and chlorophyll content of leaves were also estimated. Samples of stevia and lavender leaves, as well as water samples, were measured for iron, calcium, zinc, magnesium, sodium, potassium, phosphorus and manganese using a flame atomic absorption spectrophotometer (AAS) (iCE 3000 series, Thermo Scientific, Santa Clara, CA, USA) with deuterium lamp background correction. Gene expression from the liver samples of channel catfish was performed using total RNA by Trizol (Invitrogen, NY, USA) extraction protocol. For all the isolated RNA samples, the OD260/OD280 absorption ratio was >1.95. Power UP SYBR Green (Applied Biosystems, CA, USA) was used as master mix, and qPCR runs were performed on a QuantStudio 3 qPCR System (Applied Biosystems, CA, USA). Growth hormone receptor (GHR), Insulin-like growth factor I (IGF-I), Insulin like growth factor binding protein 1 (IGFBP1), Glucocorticoid receptor (GR) were studied. EF-1 α was used as housekeeping genes. Histology of the liver was also performed to determine the efficacy of BSFL frass feed as a feed ingredient in diet of Channel catfish

Results and Discussion

BSFL frass feed additions (10 %) had no adverse effect on catfish survival and growth (3.34% higher survival and 26.76 % higher growth), feeding efficiency, SGR and FCR. BSFL frass feed significantly elevated water calcium, potassium and phosphorus levels. Similarly, BSFL frass feed had significant effect on stevia and lavender production. Relative expression of GHR, IGF-I, IGFBP1 and GR genes in the hepatic tissue of Channel catfish fed with 10 % frass (Treatment 2) indicated better performance. The livers of catfish fed with the control diet or diets with 10% BSFL frass showed normal sinusoid organization, clear bile ducts, uncongested central veins, and normal pancreatic islets. The intestine of catfish fed with the control diet showed some inflammatory cells in the villi and lamina propria, while those fed with the 10% BSFL frass diet had longer villi, normal goblet cells and no inflammation. Overall, results show that leafy growth was significantly better in BSFL frass fed fish with enhanced nutritional quality of the aquaponics products without compromising fish production or water quality.

Table 1 Growth parameters (mean ± SE) of Channel catfish reared in aquaponics system.

Growth Parameters	No frass (Treatment 1)	BSFL Frass (10%) (Treatment 2)
Initial weight (g)	5.12±0.01	5.10±0.01
Final weight (g)	42.40±3.02	53.78*±1.05
Weight Gain (%)	726.45±58.80	948.38*±28.02
Survival (%)	87.22±5.88	90.56±5.30
SGR (%)	3.01±0.03	3.35*±0.02
FCR	1.07±0.02	1.04±0.02

Values with asterisk mark among dietary treatments are significantly different (P < 0.05)

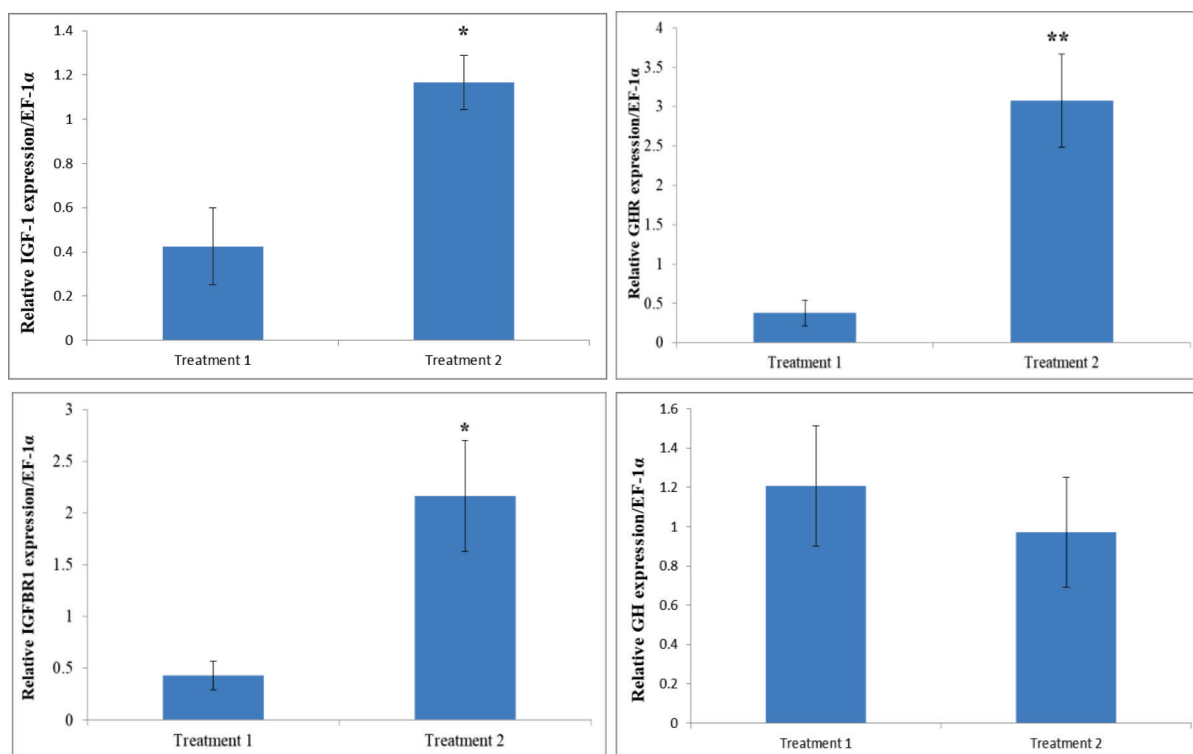


Fig. 1. Relative expression of Growth hormone receptor (GHR), Insulin-like growth factor I (IGF-I), Insulin like growth factor binding protein 1 (IGFBP1) and Glucocorticoid receptor (GR) genes in the hepatic tissue of Channel catfish fed with control diet (Treatment 1) and 10 % frass (Treatment 2) . Values are mean ± SE. Values with asterisk mark among dietary treatments are significantly different (P < 0.05)

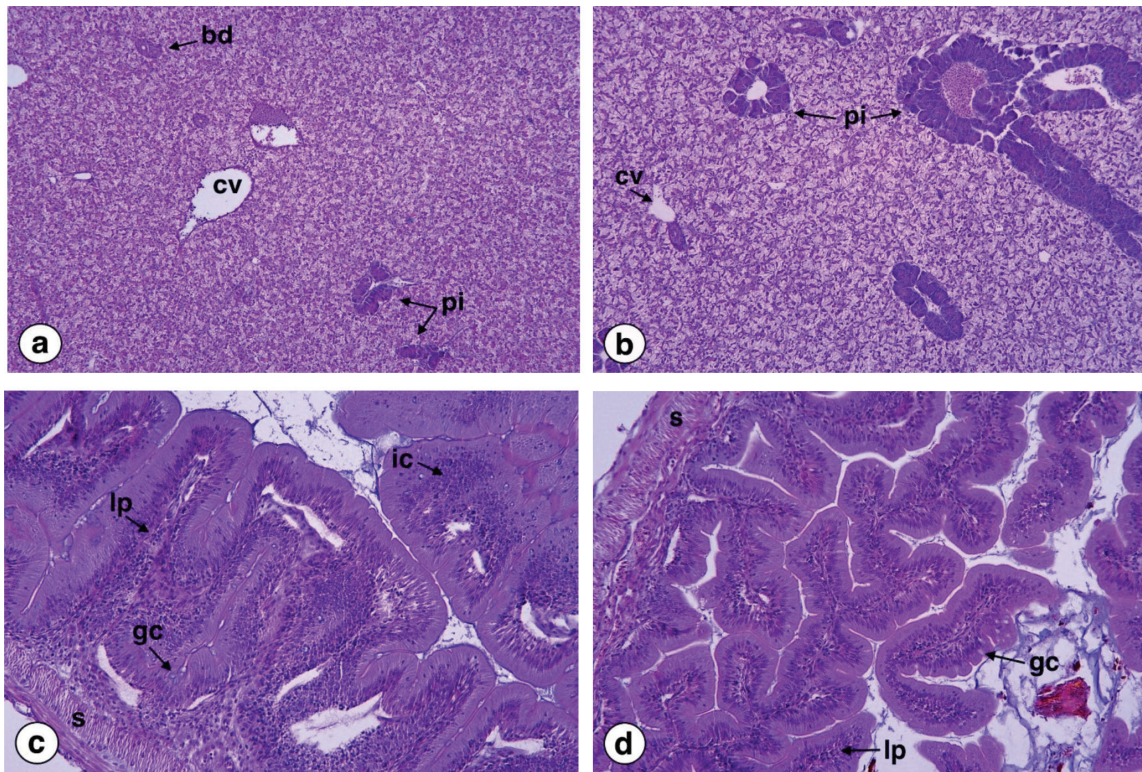


Fig. 2. The livers of catfish fed the control diet (a) or diets with 10% BSFL frass (b) showing normal sinusoid organization, clear bile ducts (bd), uncongested central veins (cv), and normal pancreatic islets (pi). The intestine of catfish fed the control diet (c) showing some inflammatory cells (ic) in the villi and lamina propria (lp) while those fed the 10% BSFL frass diet had longer villi, normal goblet cells (gc) and no inflammation. Magnification x 10 (a & b) and x 20 (c & d); hematoxylin and eosin staining.

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1.16 Assessment of Suitable Carbohydrate Source for Striped Catfish (*Pangasianodon hypophthalmus*) Culture in Biofloc System for Ecological Viability

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Keywords: Biofloc Characteristic, Carbohydrate, Microbes, Striped Catfish, Water Quality

Introduction

The intensive aquaculture system plays a vital role towards mitigating nutritional security across the globe. Among the modern aquaculture systems, biofloc technology (BFT) is considered as the ecofriendly sustainable approach in recent days (Khanzani et al 2022). Though, BFT is getting popularized for wider adaptation in developing countries, poor ecological management in culture system is exhibiting catastrophic changes. The fundamental key in BFT lies on the suitable selection and appropriate application of carbohydrate source in efficient management of total ammonia nitrogen and *in-situ* biofloc development, which further enhance aquaculture production. The high valued striped catfish (*Pangasianodon hypophthalmus*) is considered as a major candidate species in BFT due to omnivorous feeding habit, high growth rate and consumers acceptability. Thus, the present study is designed to access the suitable carbohydrate source for ecological management and production performance of *P. hypophthalmus* in BFT system.

Materials and Methods

The experiment was carried out for 90 days (September-November, 2022) in randomly designed fifteen FRP tanks (1 m³) at the Instructional cum Research Farm, College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India (30°54'21.5" N and 75°48'04.7" E). Four different carbohydrate sources were tested in biofloc system. The experiment comprised of randomized five treatments *viz.* control (no carbohydrate), T1 (tapioca flour), T2 (sugarcane bagasse), T3 (molasses) and T4 (jaggery). Pre-acclimatized 525 fingerlings (7.2±0.03 cm, 7.4±0.003 g) were randomly stocked (35 nos. m⁻³) in the tanks and fed with supplementary diet (30% crude protein) at 2% body weight daily. One liter biofloc media comprised carbohydrate source (200 mg), pond bottom soil (20g), ammonium sulphate (10 mg), yeast powder (25 mg) and curd (500 g). The aerobically fermented inoculum (100 ml l⁻¹) was applied in T1-T4 treatments for *in-situ* biofloc development. Carbon and nitrogen ratio was maintained at 15:1 in T1-T4 through application of the carbohydrate sources. The collection of samples and analysis of water quality and biofloc were carried out at weekly intervals, and microbial parameters and fish performance were carried out fortnight intervals, respectively.

Results and Discussion

The supplementation of carbohydrate sources in T1-T4 significantly ($P \leq 0.05$) changed water quality parameters (Table 1). T4 treatment showed reduced pH (0.58-3.36%), TA (1.09-14.02%) and higher TSS (6.79-38.17%) than T1-T3. pH and dissolved oxygen were >7.5 and >5.0 mg l⁻¹, respectively in all the treatments. All treatments (T1-T4) showed reduced TAN and NO₂-N from day 21 and increased NO₃-N from day 14 onwards during the study period. Jaggery biofloc treatment (T4) showed significantly ($P \leq 0.05$) lower TAN (3.14-18.55%), NO₂-N (2.58-34.48%), and higher NO₃-N (12.82-87.39%), ammonification (NH₃) rate (9.23-158.10%), nitrification (15.71-117.43% NO₂ and 8.33-212.14% NO₃) rate, ammonification (NH₃) potential (9.23-148.67%), nitrification (8.05-74.61% NO₂ and 6.25-156.90% NO₃) potential than other treatments.

Floc characteristics, microbial population and fish performance are presented in Table 2. Jaggery biofloc (T4) showed significantly ($P \leq 0.05$) higher biofloc volume (4.90-30.29%) than T1-T3, lower floc porosity (0.10-0.48%), floc volume index (7.38-23.47%) and floc settling velocity (19.61-40.58%) than T1-T3, whereas, T2 showed 18.72-45.59% higher floc density index than other biofloc treatments. Carbohydrate supplementation significantly enhanced microbial populations in T1-T4. Further, T4 showed higher total aerobic heterotrophic bacteria in water (35.17-9.37 %) and biofloc (10.01-38.73%) as well as higher probiotic bacteria in water (10.01-38.73%) and biofloc (10.83-25.77%) than T1-T3. Also, T4 treatment showed higher higher ammonifying (11.15-52.25%), ammonia oxidizing (6.76-34.61%), nitrifying (7.03-36.59%) and cellulose decomposing (5.81-26.79%) bacteria than other treatments. Fish in T4 treatment showed higher survival rate (10.84-22.67%), body weight gain (32.65-64.07%) and net biomass (51.90-88.30%) than all the treatments. Amongst the carbohydrate sources, jaggery biofloc showed improved water quality, biofloc characteristics and fish performance which may be due to the faster decomposition of jaggery which must have acted as a better substrate for proliferation of aerobic heterotrophic, probiotic and nitrogen mineralizing



bacteria, and active assimilation of nitrogenous compounds. The suitability of jaggery may be recommended for ecological stability for culture of striped catfish culture in biofloc system.

Table 1. Water quality parameters in different treatments.

	Treatments (Mean ± SE)				
	C	T1	T2	T3	T4
pH	7.84±0.05 ^c	7.65±0.04 ^{ab}	7.70±0.04 ^b	7.62±0.04 ^{ab}	7.58±0.02 ^a
TA*	33.38±7.48 ^a	110.74±22.55 ^b	92.65±19.35 ^b	119.86±24.09 ^{bc}	128.01±25.64 ^{bc}
TSS*	182.57±5.88 ^d	160.76±4.62 ^{bc}	164.17±5.24 ^c	158.71±4.81 ^{ab}	156.98±4.46 ^a
DO*	7.15±0.12 ^c	5.94±0.20 ^a	6.65±0.40 ^b	5.90±0.14 ^a	5.89±0.16 ^a
TAN*	0.369±0.04 ^c	0.318±0.04 ^c	0.325±0.04 ^d	0.310±0.04 ^b	0.300±0.03 ^a
NO ₂ -N*	0.173±0.017 ^c	0.128±0.011 ^c	0.134±0.012 ^d	0.116±0.010 ^b	0.113±0.010 ^a
NO ₃ -N*	0.229±0.025 ^a	0.349±0.050 ^c	0.334±0.045 ^b	0.381±0.052 ^d	0.430±0.061 ^c
AR**	0.113±0.009 ^a	0.246±0.024 ^b	0.262±0.025 ^c	0.268±0.025 ^d	0.293±0.027 ^c
NR-NO ₂ **	0.055±0.006 ^a	0.091±0.009 ^c	0.078±0.008 ^b	0.103±0.009 ^d	0.119±0.014 ^c
NR-NO ₃ **	0.198±0.019 ^a	0.540±0.086 ^c	0.498±0.080 ^b	0.571±0.090 ^d	0.619±0.097 ^c
AP**	0.573±0.087 ^a	1.137±0.254 ^c	0.958±0.198 ^b	1.304±0.274 ^d	1.425±0.294 ^c
NP-NO ₂ **	0.078±0.010 ^a	0.119±0.019 ^b	0.115±0.014 ^b	0.126±0.021 ^{bc}	0.136±0.019 ^c
NP-NO ₃ **	0.201±0.027 ^a	0.451±0.083 ^c	0.420±0.080 ^b	0.486±0.089 ^d	0.516±0.095 ^c

*mg l⁻¹; **[mg L⁻¹ (72 hrs.)⁻¹]

pH, potential of hydrogen; TA, total alkalinity; TSS, total suspended solids; DO, dissolved oxygen; TAN, total ammonia nitrogen; NO₂-N, nitrite nitrogen; NO₃-N, nitrate nitrogen; AR, ammonification rate; NR, nitrification rate; AP, ammonification potential; NP, nitrification potential
Mean values superscripted with different letters in each row are significantly different ($P \leq 0.05$).

Table 2. Biofloc characteristics, microbial population and fish performance in different treatments.

	Treatments (Mean ± SE)				
		T1	T2	T3	T4
Biofloc characteristics					
BV (ml l ⁻¹)		17.35±3.14 ^b	15.68±3.04 ^a	19.47±3.55 ^c	20.43±3.65 ^c
BP (%)		98.27±0.31 ^b	98.43±0.30 ^c	98.05±0.36 ^a	97.96±0.36 ^a
FVI (ml g ⁻¹)		52.48±7.93 ^d	43.96±8.17 ^c	43.36±6.00 ^b	40.16±5.36 ^a
FDI (g 100 ml ⁻¹)		3.07±0.73 ^a	4.47±1.14 ^b	3.47±0.73 ^c	3.76±0.81 ^c
FSV (mm sec. ⁻¹)		3.81±0.69 ^d	3.50±0.64 ^c	2.90±0.49 ^b	2.43±0.41 ^a
Microbial population					
*TAHB (water)	0.33±0.07 ^a	0.55±0.08 ^c	0.51±0.05 ^b	0.68±0.05 ^d	0.72±0.08 ^c
*Probiotic (water)	---	0.28±0.04 ^{bc}	0.24±0.04 ^b	0.30±0.07 ^a	0.39±0.06 ^c
*TAHB (biofloc)	---	0.49±0.09 ^b	0.43±0.08 ^a	0.54±0.11 ^c	0.60±0.12 ^d
*Probiotic (biofloc)	---	0.45±0.04 ^b	0.41±0.03 ^b	0.47±0.05 ^a	0.52±0.05 ^c
AB (water)	0.44±0.08 ^a	0.52±0.09 ^{ab}	0.52±0.08 ^{ab}	0.61±0.10 ^{ab}	0.67±0.11 ^{ab}
AOB (water)	0.58±0.05 ^a	0.60±0.05 ^a	0.68±0.06 ^{ab}	0.73±0.07 ^{ab}	0.78±0.07 ^{ab}
NFB (water)	0.56±0.03 ^a	0.62±0.04 ^a	0.66±0.06 ^a	0.72±0.06 ^{ab}	0.77±0.07 ^{ab}
CDB (water)	0.60±0.04 ^a	0.65±0.05 ^a	0.68±0.05 ^a	0.72±0.06 ^{ab}	0.77±0.06 ^b
Fish performance					
SR (%)	71.43±1.65 ^a	84.76±2.52 ^{bc}	79.05±2.52 ^b	82.86±1.65 ^{bc}	87.62±0.95 ^c
BWG (g)	13.97±0.03 ^a	18.73±0.02 ^c	17.28±0.01 ^b	20.93±0.58 ^d	22.92±0.02 ^c
NB (kg)	0.27±0.01 ^a	0.43±0.02 ^c	0.34±0.02 ^b	0.42±0.01 ^c	0.52±0.01 ^d

BV, biofloc volume; BP, biofloc porosity; FVI, floc volume index; FDI, floc development index; FSV, floc settling velocity; TAHB, total aerobic heterotrophic bacteria; AB, ammonifying bacteria; AOB, ammonia oxidizing bacteria; NFB, nitrifying bacteria; CDB, cellulose decomposing bacteria; SR, survival rate; BWG, body weight gain; NB, net biomass

Mean values superscripted with different letters in each row are significantly different ($P \leq 0.05$).





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1.17 Effect of Covid-19 in Inland Salt-affected Areas: Evidence from Jhajjar (Haryana) and Mathura (U.P.)

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Keywords: Covid-19, Salinization, Salt-affected Soils, Haryana, Uttar Pradesh

The outbreak of coronavirus disease in Dec 2019 was declared a Public Health Emergency of International Concern, as the virus has spread across the globe. India imposed a countrywide lockdown in different phases without proper preparedness. It adversely affected all the food system value chain operations including aquaculture sector of the country. The impact was also severe in salt-affected areas as the growing salinity has already posed a serious threat to the farming community. The increasing issue of salinity synergized with the emergence of COVID-19 has seriously impacted the farming sector of these areas. During the Covid-19 lockdown, the farming sector was highly affected both from the forward and backward linkage sides. Keeping this in view, the amount of the loss and problems faced by the farmers during the lockdown were assessed. A household survey was carried out among the farmers of salt affected areas of Mathura and Jhajjar districts of Uttar Pradesh and Haryana and a total of 180 farmers were selected with 90 farmers from each district. For constraint analysis, Garret ranking and Rank Based Quotient analyses were used and the major constraint faced by the farmers was in terms of access to feed and seed from input linkage side and access to transportation from the output linkage side. The lockdown imparted serious impact in terms of increase in cost of seed and feed (81.41%) followed by the decline in the price of the harvested crop (75.12%), reduced production (66.85%), and a decline in the farming area (17.27%) and employment reduction (10.84%) due to a lack of basic input facilities. Comparatively the extent of lockdown was more sever in Haryana than Uttar Pradesh, because of more strict restriction and lack of basic facilities in terms of the nearby areas. Most of the farmers have not received any assistance from government or any other organization and believe that authorities were not prepared and appropriately instructed to handle the lockdown situation. Short and medium-term technical and policy measures are suggested to tide over the impact and loss of COVID-19 related lockdown and related restrictions.

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1.18 Assessment of Growth Parameters of *Labeo rohita* Grown in Aquaponics System with Selected Medicinal Plants

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Keywords: Aquaponics, Rohu, Tulsi, *Aloe vera*, Mint

The study was done to assess the growth parameters of Rohu (*Labeo rohita*) in an aquaponic system with selected medicinal plants namely, Tulsi (*Ocimum tenuiflorum*), Aloe (*Aloe vera*) and Mint (*Mentha spicata*). The experiment was conducted for 6 months in a polyhouse at instructional Fish Farm of the College of Fisheries, GB PUA & T, Pantnagar, Uttarakhand. Tulsi, aloe and mint plants were grown in three FRP (Fibreglass Reinforced Plastics) troughs which were respectively placed on cemented tanks T1, T2 and T3. The troughs were filled with stone gravels of size 8 – 20 mm. Fingerlings of rohu (5.6±0.2 cm, 16±2 g) were collected from the hatchery of the university and stocked in rectangular cemented tank filled with 8000 L of water. Each cemented tank was stocked with 400 numbers of rohu fingerlings. A control with 400 rohu fingerlings, in absence of plants, was also run simultaneously for comparison. 200 L of water from fish tank was used to water the plants of the aquaponic unit daily. Tulsi-extract feed (TEF), aloe-extract feed (AEF) and mint-extract feed (MEF) comprising 1% of the respective plant extract along with a combination of oil cake, rice bran, maize, fish meal (amounting to 28% protein) were prepared. The TEF, AEF and MEF were administered respectively to the fingerlings of tanks T1, T2 and T3. Length and weight of the fishes were recorded every month. Initial and final biomass of plants was weighed at initiation and after completion of the experiment. Aquaponics with aloe showed better growth of rohu (9.4 cm, 143.4 g) as compared to other treatment tanks, tulsi (8.5 cm, 139 g) and mint (7.4 cm, 98.5 g). Through this study, it can be concluded that an aquaponic is a bio integrated, eco-friendly and sustainable system. In locations with scarcity of land and water, such systems can prove to be highly favorable. Additionally, the incorporation of plants having medicinal properties will be beneficial as they are readily available and cost-effective than marketed medicines.

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1.19 Suitability Assessment of Inland Saline Groundwater Resources from North-Western India for Shrimp Farming based on the Production Performance and Water Quality Variables

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Keywords: Hardness, Inland saline groundwater, *Penaeus vannamei*, Mg²⁺/Ca²⁺ ratio, Productivity

Inland shrimp farming in Haryana, Punjab and Rajasthan is growing rapidly and about 3500 acres of land have already been brought under scientific shrimp farming. Site selection for inland shrimp farming based on water quality variables of saline groundwater is imperative for the overall success of the farming operation. Saline groundwater samples from operational farms growing whiteleg shrimp, *Penaeus vannamei* in major farming clusters in the states of Haryana (n=52), Rajasthan (n=50) and Punjab (n=28) were collected and water quality variables viz., salinity, pH, total alkalinity, total hardness, relative hardness (RH=observed hardness/hardness of seawater diluted to the same salinity), magnesium to calcium ratio (Mg²⁺/Ca²⁺), sodium to potassium ratio (Na⁺/K⁺) and potassium equivalence [K⁺(%)=observed aqueous K⁺ levels/aqueous potassium levels in seawater of similar salinity] were estimated. The collected saline groundwater samples were further grouped based on the farm productivity data viz., high (H: yield > 3000 Kg/acre; n=79), medium (M: yield 1500-3000 kg/acre; n=37) and low productivity (L: yield < 1500 kg/acre; n=8). Overall, 63.7% of the surveyed source waters indicated high productivity, followed by farms with medium (29.8%) and low productivity (6.5%). Relative hardness varied significantly (p<0.05) between the sample groups, wherein highly productive farms demonstrated a mean RH of 1.34±0.03, and the medium and low productive groups indicated mean values of 1.79±0.07 and 0.93±0.07 respectively. Mg²⁺/Ca²⁺ ratio was significantly higher (p<0.05) for the highly productive group (1.22±0.04) as compared to samples with medium productivity (1.20±0.06). Though low productive farms indicated significantly higher Mg²⁺/Ca²⁺ ratio (1.94±0.05) in the source waters, did not transform into higher yield. Na⁺/K⁺ ratio were very high compared to typical seawater and not significant in these areas as potassium is supplemented to the raw groundwater. Total alkalinity did not vary significantly (p>0.05) between the groups. K⁺(%) was higher for samples from Punjab and Haryana (30.05-34.14%), whereas those from Rajasthan possessed significantly lower aqueous potassium levels (6.30%). Saline groundwater of inland origin generally possessed higher total hardness than seawater diluted to the same salinity due to disproportionately higher aqueous calcium and magnesium levels. Inland saline groundwater with total hardness exceeding seawater diluted to the same salinity up to RH level of approximately 1.5 has been found to improve the growth and production of *P. vannamei*. Source waters with RH exceeding 1.8 have been observed to reduce productivity and sites with RH beyond 2.5 can be grossly avoided. On the contrary, samples with total hardness less than that of seawater of the same salinity i.e., RH<1.0 is not suitable for growing *P. vannamei* and resulted in total mortality to low productivity. The study also concludes that the relative hardness of the source water is a principal criterion for the selection of a site and a minimum Mg²⁺/Ca²⁺ ratio of approximately 1.5:1 to be suitable for rearing *P. vannamei* in inland saline groundwaters. Overall, the study lays down new horizons for further development of site selection criteria for inland saline groundwaters for the benefit of the shrimp farming community.

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1.20 Evaluation of Water Quality and Fish Growth in Aquamimicry Fish Culture System

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Keywords: Aquamimicry, Plankton, Feed Conversion, Protein Efficiency, Benefit-Cost

The present study was conducted from December 2021 to May 2022 (165 days) to evaluate the effect of aquamimicry culture technology on water quality, plankton production and fish performance. The experiment was carried out in 12 rectangular FRP tanks kept indoor in poly carbonate house (size 4 m³ each) stocked with 40 fingerlings/ m³ in three groups of fishes viz. Catla (T₁), Rohu:Mrigal @ 1:1 (T₂) and Rohu:Mrigal:Silver Carp:Grass Carp:Amur Common Carp @ 30:10:15:15:30 (T₃) in triplicate and one set of control for each group. Water and rice bran in the ratio of 7.5:1 were mixed with probiotics @0.43 mg probiotic/g rice bran, to prepare the inoculum and applied in treatment tanks after 24 hours. In addition, the experimental fishes in aquamimicry units were fed with (24% protein floating feed) half of required feed (2% body weight), while 100% required feed (4% body weight) was given in control units. During the investigation period, the data with respect to water quality, plankton population and fish growth was analyzed and compared. The physical parameters viz. temperature, electrical conductivity and total dissolved solids varied from 16.2-32.3°C, 150-500 µS cm⁻¹ and 220.5-357.8 mgL⁻¹, respectively while chemical parameters viz. pH, dissolved oxygen, total alkalinity, hardness, salinity, ammonia, phosphate and biochemical oxygen demand varied from 7.5- 8.7, 3.22-7.15 mg L⁻¹, 160-220 mg L⁻¹, 170-220 mg L⁻¹, 0.17-0.26 psu, 0.01-0.24 mg L⁻¹, 0.002-0.74 mg L⁻¹ and 1.15-4 mg L⁻¹, respectively. There was no negative impact of aquamimicry fish culture system on water quality parameters. Treatment tanks showed better average phytoplankton: zooplankton ratio with best in T₂ as compared to control tanks, as well as higher plankton diversity *i.e.* 13 zooplankton species in treatment tanks while 9 species in control tanks. The result of fish growth reveals that aquamimicry fish culture shows higher specific growth rate in Catla (1.80 % day⁻¹) as compared to Control (1.60 % day⁻¹). Also, Feed Conversion Ratio improved from 1.443-2.3 (in control) to 0.728-1.4 (in aquamimicry). Protein Efficiency Ratio values ranged from 2.96 to 5.7 in aquamimicry system as compared to 1.7-2.8 in control. Best survival rate among various fish species has also been recorded in T₂. There was 33.40 to 37.87% decrease in fish production cost and benefit -cost ratio reached 3.05 in T₁, 2.29 in treatment T₂ and 2.32 in T₃. This indicates that even without intensification, the production can be increased with increased economic output in the aquamimicry culture system.

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1.21 Integrated Farming System for Income Enhancement and Livelihood Security of Small and Marginal Farmers

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Key words : Employment, Income enhancement, Climate smart agriculture, Integrated farming system

In India, about 85 percent farmer community comes under the marginal and small farmers category. Farmers under these categories are economically poor and work in diverse, risk prone environments. The income from seasonal field crops on small and marginal farms is hardly sufficient to sustain their family. No single farm enterprise is likely to support the small and marginal farmers for generation of adequate income and gainful employment year round. Therefore, integrated farming system (IFS) is a multifaceted whole farm approach and very helpful in solving the problems of marginal and small farmers. The approach aims at increasing employment opportunities and income from small-holding by integrating various farm enterprises, recycling crop residues and by products within the farm itself. This system helps in eco-friendly utilization of waste of one component as input of another, leaving behind nutrient and organic matter rich soil for higher end products for sustaining the soil productivity. An IFS model was developed at the research farm of School of Organic Farming, Punjab Agricultural University, Ludhiana under “All India Coordinated Research Project on Integrated Farming Systems (ICAR)”. The Integrated Farming System model experiment was initiated during Kharif 2010. An area of 1.0 ha (10000 sq m area) comprises of crops, horticulture, aquaculture, dairy, agroforestry, goatry, bio-gas and vermicompost components. The kharif crops viz. paddy, maize and turmeric were grown on 6400 sq m area and in the following rabi and summer season potato, berseem, wheat, gobhi sarson, onion, pearl millet and spring maize were grown. Around 1600 sq m area was utilized for horticulture component comprising guava and citrus plantation and the inter row spacing of 1500 sq m area was utilized for raising vegetable crops while 200, 1000 and 300 sq m was used for dairy (cattle+goats), aquaculture and agro-forestry components, respectively. In addition to this, boundary plantations with karonda and galgal were also done. The data from the study (average of 5 years) indicated that adoption of IFS by inclusion of crop based enterprises, horticulture, dairy and aquaculture have recorded overall average net returns of Rs 4,95,810/ha with the highest been contributed by dairy (Rs 2,86,201) followed by crop (Rs 1,25,070), horticulture (Rs 30,156), aquaculture (Rs 24,891), boundary plantation (Rs 19,341), kitchen gardening (Rs 8,048), and agro-forestry (Rs 2,104). Hence, the 1.0 ha model developed for marginal and small farmers resulted in gross returns of Rs 8,40,114/ha while the costs incurred was Rs 3,44,304/ha and net returns by deducting all variable costs were Rs 4,95,810/ha which were far higher than the prevailing rice-wheat cropping system. The total amount of carbon source and sink were 5938.9 CO₂-e (kg) and 11319.7 CO₂-e (kg), thereby making IFS model a climate smart agriculture system with negative greenhouse gas emission measured at -5380.7 CO₂-e (kg). Moreover, IFS model effectuates 30-60% saving with recycled farm products. It also saves water to the tune of 5.9 kg/m³ versus 0.6 kg/m³ in rice-wheat cropping system. It also provides an employment of 280 man days, which generated a value of Rs 63,100/annum thereby, ensuring 16.9% saving of farm labour engaged. Hence, the adoption of multiple farm enterprises in an integrated manner can ensure a substantial income generation to sustain the livelihood of farmers over the meagre income from self- standing enterprises as revealed from this study. Therefore, IFS proves it's tremendous potential for developing farms to their optimum levels by integrating different enterprises in a farming system model to make agriculture a profitable venture for farmers under different agro-climatic and ecological situations.

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1.22 Biofloc Technology: An Emerging Avenue for IMC Culture in India

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Keywords: Biofloc, IMC, Microbial protein, Fish feed

The global population is expected to reach 9.6 billion by 2050 and as the demand for animal protein is increasing year by year it is a challenge to provide quality protein by safeguarding its natural resources for future generations. In this context, aquaculture plays a key role in promoting health by providing animal protein as well as generating employment and economic growth. As the aquaculture industry intensively develops, its environmental impact increases and there grows a strong dependency on fishmeal in the diet, hence, the use of biofloc technology (BFT) can reduce these problems. The basic principle of the BFT is the retention of waste and its conversion to biofloc as a natural food within the culture system. Nowadays BFT has become very popular in aquaculture sector. The concept of floc formation in activated sludge systems can be used for application in BFT. Number of factors influence the formation of biofloc. Microbes are manipulated in BFT, in order to control and reduce toxic inorganic nitrogen concentrations. Extensive development of microbial biomass is an integral part of this process. Microbial proteins in BFT systems should be considered only as a supplement of feed proteins. Many researchers revealed that net protein uptake by the shrimp from biofloc were equivalent to 25 to 50% of conventional protein feeding. Biofloc contains about 30% crude protein along essential fatty acids and other essential minerals. Therefore, it appears that biofloc can be successfully used as fish feed particularly for herbivorous and omnivorous species. Microorganisms in biofloc might partially replace protein content in diets or decrease its dependence of fishmeal. It is thought that the use of BFT in aquaculture applications will play an active role in minimizing some of the problems in growth and water quality for sustainable aquaculture development.

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1.23 Effect of Variable Stocking Densities of Striped Catfish (*Pangasianodon hypophthalmus*) on Water Quality and Growth Performance under Biofloc-based Aquaculture System

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Keywords: Biofloc, Jaggery, Striped catfish, Water Quality, Growth Performance

Optimum stocking density of fish species in biofloc aquaculture system must be determined to maintain long term sustainability of production system. A 120-day indoor experiment was conducted to evaluate the effect of different of stocking densities of striped catfish (*Pangasianodon hypophthalmus*) on water quality, survival and growth performance in randomly designed fifteen fiberglass reinforced plastic tanks (1 m³ capacity) with minimal water exchange. Seven hundred twenty pre-acclimatized healthy fingerlings of striped catfish (initial weight 2.62 ±0.01g and length 7.74±0.02 cm) were stocked at five stocking densities (SD30, SD40, SD50, SD60, and SD70 fish m⁻³) and three replications under similar feeding regime (commercial feed with 32% CP fed @ 2% body weight daily). Amongst the treatments, the low stocking density (30 fish m⁻³) served as control. Jaggery was applied in the five treatments as the main carbohydrate source at the C:N ratio of 15:1. The increase in stocking density significantly ($P \leq 0.05$) reduced pH and dissolved oxygen and enhanced biological oxygen demand. Amongst the nitrogenous metabolites, maximum concentration of total ammonia nitrogen (0.78 mg l⁻¹) and nitrite (0.41 mg l⁻¹) were recorded at higher stocking density (70 fish m⁻³). Fish showed highest survival rate (>96.89%), net body weight gain (14.98-58.28%↑), specific growth rate (4.20-14.40%↑), protein efficiency ratio (0.97-14.89%↑), apparent net protein utilization (5.16-21.19%↑), net biomass (16.45-61.79%↑) and improved feed conversion ratio (1.64-10.38%↓) at the stocking density of 50 fish m⁻³ compared to rest of the treatments. Moreover, SD50 and SD60 treatment displayed overall better fish performance and net biomass production as compared to other treatments. The above findings of the present experiment suggested that use of the intermediate stocking density i.e. 50 to 60 fish m⁻³ of striped catfish in biofloc aquaculture system could be advisable for better management of water quality for enhanced fish production and sustainable culture system.

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THEME II

Sustainable Aquafeeds and Feed Technologies







2.1 Dietary Protein Replacement of Fish Meal with Black Soldier Fly Larvae Meal: Effects on Growth and Haemato-Biochemical Responses of Juvenile Goldfish, *Carassius auratus*

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Keywords: Black soldier fly, Fish meal, Goldfish, Haemoglobin, Larvae meal, Total protein

Introduction

Aquaculture, a pivotal component of aquaculture, renders aesthetic value and is a reliable source of foreign exchange. Feeding is a significant cost factor in livestock production, in which feed costs contribute 60-80 % of overall production expenses. At present, insect meal draws a flourishing interest as a vital replacement for fish meal in the aqua diets. The black soldier fly (*Hermetia illucens*) larvae had tremendous development due to its preference for organic waste as a growth substrate. The black soldier fly larvae have a protein content of about 35-46 % and a lipid content of about 19- 37% on a dry weight basis. The amino-acid profile and fatty acid profile of black soldier fly larvae further make them suitable for inclusion in animal feeds.

Thus, the present study was focused on estimating the effects of a dietary swap of fish meal with black soldier fly larvae meal (BSFLM) on growth performance and haemato-biochemical responses in juvenile goldfish (*Carassius auratus*).

Materials and Methods

Black soldier fly larvae meal is a propitious animal feed ingredient due to its good protein content and its sustainability in the aquaculture realm. For the potential application of this ingredient in goldfish culture (*C. auratus*), six isonitrogenous and isoenergetic diets were formulated by replacing fish meal with BSFLM at 0 (T0), 20 (T20), 40 (T40), 60 (T60), 80 (T80) and 100 (T) and prepared. Triplicate groups of 20 goldfish (Mean weight: 0.31±0.04 g) per aquaria were stocked and provided ad-libitum feeding of the diet twice a day. The experimental data were examined for normality (Shapiro–Wilk test) and homogeneity (Levene’s test). The data were transformed when they did not show normal distribution. The one-way analysis of variance (ANOVA) was applied to verify the differences between six dietary groups in 95 % confidence value (P < 0.05) using the SPSS software (v.22.0). Tukey’s range test was used to compare means and identified significance letters. All the calculated data were shown as the mean values ± standard deviation (SD) of three replications.

Results and Discussion

Goldfish juveniles fed with the T60 diet exhibited maximum growth and feed utilization. However, escalating the percentage of fishmeal substitution with Black Soldier Fly Larvae Meal above 60 percent led to a significant reduction in growth and feed utilization (Table 1). There was improvement in haematological and biochemical parameters with special reference to haemoglobin and total protein in T60 (Table 2).

Table 1: Effect of replacing FM with BSFLM on growth performance and feed utilization of goldfish juveniles

	T0	T20	T40	T60	T80	T100	p value
Initial body weight (g)	2.92±0.09	2.66±0.18	2.87±0.06	2.68±0.14	2.82±0.22	2.85±0.07	<0.22
Final body weight (g)	4.20±0.07 ^c	4.21±0.18 ^c	4.64±0.06 ^b	5.09±0.16 ^a	4.12±0.20 ^c	4.11±0.08 ^c	<0.001
Weight gain (g)	1.28 ±0.15 ^c	1.55±0.21 ^{bc}	1.77±0.12 ^b	2.40±0.20 ^a	1.30±0.10 ^c	1.25±0.14 ^c	<0.001
SGR (% per day)	0.60±0.07 ^b	0.76±0.11 ^b	0.79±0.05 ^b	1.06±0.09 ^a	0.63±0.07 ^b	0.60±0.07 ^b	<0.001
Survival (%)	95.57±3.54	96.66±2.88	96.66±2.88	96.66±2.88	96.66±5.77	96.66±2.88	0.125
Feed intake (% BW/ day)	1.51±0.005 ^b	1.37±0.005 ^d	1.16±0.005 ^f	1.24±0.005 ^e	1.61±0.005 ^a	1.47±0.007 ^c	<0.000
ADG (g/fish)	0.021±0.002 ^c	0.025±0.003 ^{bc}	0.029±0.002 ^b	0.040±0.003 ^a	0.021±0.001 ^c	0.020±0.002 ^c	<0.001
Protein retention efficiency (%)	2.72±0.010	1.45±0.005	3.2±0.005	3.93±0.005	3.61±0.005	3.31±0.007	<0.000
FCR	2.59±0.14 ^{ab}	2.52±0.16 ^{ab}	2.28±0.10 ^b	1.88±0.14 ^c	2.56±0.17 ^{ab}	2.79±0.18 ^a	<0.001



PER	0.37±0.06 ^c	0.45±0.05 ^{ab}	0.52±0.03 ^b	0.78±0.07 ^a	0.38±0.01 ^{ab}	0.36±0.02 ^c	<0.001
Thermal Growth Coefficient	0.009±0.001 ^b	0.012±0.001 ^b	0.013±0.00 ^b	0.017±0.001 ^a	0.010±0.001 ^b	0.009±0.001 ^b	<0.001

SGR- Specific growth rate; ADG- Average daily gain; FCR- Feed conversion ratio; PER- Protein efficiency ratio

Table 2: Haematological and biochemical parameters of goldfish juveniles fed with BSFLM-based experimental diets

	T0	T20	T40	T60	T80	T100	p value
HAEMATOLOGICAL PARAMETERS							
Hb (g/dl)	4.30±0.36 ^b	4.33±0.05 ^b	4.33±0.05 ^b	4.93±0.05 ^a	4.06±0.05 ^b	4.23±0.05 ^b	<0.001
RBC(million/cu.mm)	0.53±0.00 ^b	0.48±0.01 ^c	0.54±0.00 ^b	0.57±0.01 ^a	0.48±0.01 ^c	0.43±0.0 ^d	<0.001
PCV (%)	7.5±0.20 ^c	7.23±0.05 ^d	7.9±0.10 ^b	8.3±0.10 ^a	6.43±0.05 ^c	6.16±0.05 ^e	<0.001
MCV (µm ³)	125.3±1.65 ^f	145.5±0.15 ^c	153.8±0.52 ^a	151.6±0.10 ^b	133.5±0.10 ^d	129.5±0.05 ^e	<0.001
MCHC (g %)	59.6±0.05 ^d	60.1±0.05 ^c	59.5±0.10 ^d	62.8±0.10 ^a	61.5±0.43 ^b	55.6±0.05 ^e	<0.001
MCH (g %)	84.7±0.10 ^c	87.6±0.10 ^b	91.53±0.15 ^a	91.66±0.05 ^a	82.5±0.10 ^d	80.46±0.05 ^e	<0.001
BIOCHEMICAL PARAMETERS							
TP (gdl ⁻¹)	3.28±0.01 ^e	4.54±0.05 ^b	3.71±0.00 ^c	5.29±0.01 ^a	3.47±0.01 ^d	3.68±0.07 ^e	<0.001
Albumin(gdl ⁻¹)	0.84±0.15 ^c	1.05±0.01 ^{bc}	1.02±0.15 ^{bc}	2.36±0.010 ^a	1.36±0.015 ^b	1.37±0.005 ^b	<0.001
Globulin(gdl ⁻¹)	2.41±0.005 ^d	2.57±0.01 ^c	2.66±0.01 ^b	2.92±0.005 ^a	2.12±0.01 ^c	2.53±0.02 ^c	<0.001
TCHO	111.0±1 ^b	119.0±1 ^a	116.0±1 ^a	75±1 ^d	115.6±0.57 ^a	96.6±1.15 ^c	<0.001
Triglycerides (mgdl ⁻¹)	101.6±1.5 ^d	169±1 ^a	132±0 ^b	99±1 ^d	110.6±0.57 ^c	84.6±1.52 ^c	<0.001

Values were expressed as means ± SD of three replicate aquaria per treatment (n=3), and values with different superscripts indicate significant differences as determined by Tukey's test ($p < 0.05$).

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2.2 Effects of Dietary Partial Replacement of Fish Meal by De-oiled Castor Cake on Growth Performance, Immune-haematological Status, Digestive Enzymes Activity, and Flesh Quality of GIFT Tilapia (*Oreochromis niloticus*)

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Keywords: Aquafeed, Castor cake, Fish meal, Growth, GIFT Tilapia

Introduction

Nowadays, the adoption of intensive feeding practices leads to exponential growth rate in aquaculture. High cost and fluctuating quality of fish meal have aggravated the need to search alternatively available protein sources. Among the various plant derived ingredients, soybean meal is used as an alternative to fish meal in aquafeed. However, high market price, demand in animal husbandry sector and human consumption of soybean, encourages to choose its alternate source in aquaculture. De-oiled castor cake (DCC) can be a good alternative to soybean meal due to its high protein (68%), fiber (18%), ash (7.5%) and lipid (40%) content (Jayant et al., 2021). Genetically improved farmed tilapia (GIFT) has greater potential in low-input aquaculture systems for its higher growth performance. Considering the above facts and figures, the present study was designed to evaluate the efficacy of dietary replacement of fish meal by de-oiled castor cake on growth performance, health status and flesh quality of GIFT tilapia.

Materials and Methods

The experiment was carried out for 90 days in fifteen FRP tanks (1 m³) at the Instructional cum Research Farm, College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India (30°54'21.5" N and 75°48'04.7" E). Castor oil cake was detoxified by addition of Ca(OH)₂ (40 g kg⁻¹). The pre-acclimatized advanced fingerlings of Genetically Improved Farmed Tilapia were stocked at 10 nos. m⁻³ and fed ad libitum upto satiation level with formulated five isoprotenious (350 g. kg⁻¹) diets at different substitution levels of fish meal by DCC as follows: 0 % (C: control), 5% (T1), 10% (T2), 15% (T3) and 20% (T4). Fish body length and weight were checked after fifteen days intervals, whereas, survival rate, net body weight gain, specific growth rate, daily feed intake, protein efficiency ratio, apparent net protein utilization, viscera somatic index and hepatosomatic index were calculated according to the standard formula and immuno-haematological responses and flesh quality were evaluated according to the standard methods after termination of the experiment. Blood samples were collected via the caudal vein puncture from three fish of each tank and stored in EDTA coated vials. Statistical analyses of the data were computed by SPSS 20.0 (IBM Inc.), subjected to one-way ANOVA followed by Duncan's multiple range test (DMRT) post-hoc analysis to get the significant differences among the treatment mean values ($P < 0.05$).

Results and Discussion

The dietary inclusion of DCC significantly ($P \leq 0.05$) enhanced survival rate, fish performance and flesh quality after termination of the experiment (Table 1). Amongst all the treatments, GIFT tilapia showed higher survival rate (>80%), net body weight gain (24.63-66.24%↑), specific growth rate (15.50-37.59%↑) in 2% DCC fed dietary treatment (T4). With respect to feeding efficiency, GIFT tilapia reared in T4 treatment showed improved average daily feed intake (15.51-29.77% ↓), feed conversion ratio (11.03-22.59% ↓) and also enhanced protein efficiency ratio (21.25-42.41%↑), apparent net protein utilization (20.34-52.60%↑) compared to other treatments (Table 1). Moreover, GIFT tilapia in T4 showed significantly ($P \leq 0.05$) improved haematological parameters such as 4.43-19.38%↑ total erythrocyte count, 7.86-26.26%↑ total leukocyte count, 4.02-23.52%↑ haemoglobin, 4.21-49.85%↑haematocrit than other treatments and 0.24-4.11%↓ mean corpuscular volume, 0.38-3.45%↓ mean corpuscular haemoglobin than T1-T3. Similarly, fish reared in T4 treatment showed significantly ($p \leq 0.05$) improved immunological parameters such as 5.89-39.79%↑ total serum protein, 7.69-22.22%↑ albumin, 3.45-51.11%↑ globulin, 3.87-18.81%↑ lysozyme and 0.56-3.34%↓ glucose) and antioxidant responses (8.59-25.82%↑ super oxide dismutase and 15.40-34.55%↑catalase). However, the dietary inclusion of DCC did not show significant effect on both viscera and hepatosomatic indices; fish showed enhanced digestive enzymes activity (10.84-30.60% ↑amylase, 12.22-37.35%↑ protease and 6.12-40.54%↑ lipase) in T4 than others treatments. The flesh quality was also significantly improved in terms of higher crude protein (0.24-5.65%↑), lipid (0.60-4.01%↑) and ash (0.47-3.30%↑) in T4 than other treatments (Table 2). The findings of the present study are similar to the previous reported findings in African catfish fingerlings (Agboola et al., 2019). Overall, the findings of the study revealed that dietary replacement of fish meal by DCC upto 20% level may be



advised to the farmers for feed formulation to enhance GIFT tilapia production performance without deleterious effect on health status and flesh quality.

Table 1 Growth performance, health status and digestive enzymes activity of GIFT tilapia in different treatments.

Parameters	Treatment (Mean±SE)					P Value
	Control	T1	T2	T3	T4	
Survival Rate (%)	80.46±0.02	80.44±0.01	80.51±0.05	80.48±0.06	80.47±0.05	P=0.055
IBW (g)	30.45±0.00	30.43±0.01	30.43±0.01	30.44±0.01	30.45±0.01	P=0.015
FBW (g)	82.75±0.21 ^c	87.33±0.18 ^d	92.26±0.08 ^c	100.21±0.02 ^b	117.39±0.89 ^a	P≤0.001
NBWG (g)	52.30±0.21 ^c	56.90±0.18 ^d	61.83±0.08 ^c	69.77±0.01 ^b	86.95±0.89 ^a	P≤0.001
SGR (%/day)	1.01±0.00 ^d	1.07±0.01 ^c	1.08±0.00 ^c	1.20±0.01 ^b	1.39±0.03 ^a	P≤0.001
ADFI (% body weight day ⁻¹)	1.44±0.01 ^c	1.38±0.00 ^d	1.32±0.00 ^c	1.22±0.00 ^b	1.01±0.01 ^a	P≤0.001
FCR	2.45±0.01 ^e	2.33±0.01 ^d	2.25±0.00 ^c	2.13±0.01 ^b	1.90±0.01 ^a	P≤0.001
PER	1.66±0.01 ^e	1.72±0.00 ^d	1.81±0.00 ^c	1.95±0.00 ^b	2.36±0.02 ^a	P≤0.001
ANPU (%)	25.99±0.16 ^c	29.36±0.09 ^d	30.72±0.09 ^c	32.96±0.00 ^b	39.66±0.33 ^a	P≤0.001
TEC (10 ⁶ cell / mm ⁻³)	1.51±0.03 ^d	1.63±0.02 ^c	1.69±0.03 ^{bc}	1.73±0.03 ^{ab}	1.81±0.04 ^a	P≤0.001
TLC (10 ³ cell / mm ⁻³)	4.08±0.04 ^e	4.34±0.04 ^d	4.57±0.03 ^c	4.77±0.04 ^b	5.15±0.02 ^a	P≤0.001
Hb (g %)	5.87±0.10 ^c	6.76±0.05 ^b	6.80±0.10 ^b	6.97±0.07 ^b	7.25±0.06 ^a	P≤0.001
Ht (%)	18.08±0.04	25.45±0.28	25.99±0.47	26.00±0.26	27.09±0.46	P≤0.001
MCV (μ m ³)	119.56±2.47 ^c	156.49±2.76 ^b	153.95±4.99 ^{ab}	150.41±3.61 ^{ab}	150.05±3.41 ^a	P≤0.001
MCH (g %)	38.77±0.43 ^b	41.56±0.36 ^a	40.29±1.13 ^{ab}	40.29±0.52 ^{ab}	40.13±0.58 ^{ab}	P=0.137
MCHC (%)	32.45±0.64 ^a	26.57±0.24 ^b	26.18±0.22 ^b	26.81±0.53 ^b	26.77±0.61 ^b	P≤0.001
TSP (g dl ⁻¹)	3.77±0.01 ^e	4.51±0.02 ^c	4.22±0.03 ^d	4.98±0.02 ^b	5.27±0.01 ^a	P≤0.001
Albumin (g dl ⁻¹)	1.26±0.01 ^d	1.35±0.01 ^c	1.32±0.01 ^c	1.43±0.01 ^b	1.54±0.01 ^a	P≤0.001
Globulin (g dl ⁻¹)	1.65±0.01 ^d	2.22±0.02 ^c	2.32±0.04 ^b	2.41±0.02 ^a	2.49±0.03 ^a	P≤0.001
A/G	0.76±0.01 ^a	0.61±0.01 ^b	0.57±0.01 ^c	0.59±0.01 ^b	0.62±0.00 ^b	P≤0.001
Lysozyme (mg dl ⁻¹)	6.03±0.03 ^d	6.36±0.06 ^c	6.16±0.06 ^d	6.89±0.09 ^b	7.16±0.06 ^a	P≤0.001
Glucose (mg dl ⁻¹)	65.32±0.48 ^b	65.58±0.02 ^b	66.34±0.05 ^a	64.48±0.14 ^c	64.12±0.02 ^c	P≤0.001
Amylase*	15.52±0.02 ^c	16.98±0.02 ^c	16.47±0.02 ^d	18.29±0.03 ^b	20.27±0.02 ^a	P≤0.001
Protease**	12.88±0.03 ^c	15.15±0.02 ^c	14.86±0.01 ^d	15.76±0.01 ^b	17.69±0.03 ^a	P≤0.001
Lipase [#]	0.12±0.01 ^b	0.15±0.01 ^{ab}	0.16±0.01 ^a	0.16±0.01 ^a	0.17±0.01 ^a	P=0.038
VSI	8.53±0.02 ^a	8.50±0.05 ^a	8.48±0.03 ^a	8.49±0.02 ^a	8.11±0.07 ^b	P≤0.001
HSI	0.91±0.01	0.91±0.01	0.92±0.02	0.91±0.02	0.90±0.03	P=0.948

IBW, Initial body weight; FBW, Final body weight; NBWG, Net body weight gain; SGR, Specific growth rate; ADFI, Average daily feed intake; FCR, Feed conversion ratio; PER, Protein efficiency ratio; ANPU, Apparent net protein utilization; TEC, Total erythrocyte counts; TLC, Total leukocyte counts; Hb, Haemoglobin; Ht, Haematocrit; MCV, Mean corpuscular volume; MCH, Mean corpuscular haemoglobin; MCHC, Mean corpuscular haemoglobin concentration; TSP, Total serum protein; A/G, Albumin and globulin ratio; VSI, Viscerosomatic index; HSI, Hepatosomatic index

*Unit: moles of glucose liberated per hour per mg of tissue protein at 25 °C

**Unit: μ moles of tyrosine liberated per hour per mg of tissue protein at 25 °C

[#]Unit: Unit mg protein⁻¹ hr⁻¹

Note: Mean values superscripted with different letters in each row are significantly different ($P \leq 0.05$).



Table 2 Flesh quality (%wet weight basis) of GIFT tilapia in different treatments.

Parameters		Treatment (Mean±SE)					P Value
		Control	T1	T2	T3	T4	
Moisture (%)	Initial	75.48±0.07 ^c	75.62±0.18 ^c	75.45±0.10 ^b	75.40±0.02 ^a	75.39±0.02 ^a	P≤0.001
	Final	72.18±0.03	72.19±0.01	72.14±0.02	72.16±0.01	72.15±0.01	P=0.287
Crude Protein [CP (%)]	Initial	12.70±0.01	12.73±0.02	12.76±0.01	12.64±0.11	12.72±0.01	P=0.028
	Final	14.51±0.03 ^d	14.79±0.02 ^c	15.08±0.05 ^b	15.29±0.02 ^a	15.33±0.02 ^a	P≤0.001
Crude lipid [CL (%)]	Initial	5.13±0.01	5.13±0.01	5.15±0.01	5.14±0.01	5.17±0.01	P=0.035
	Final	6.49±0.03 ^c	6.55±0.05 ^{bc}	6.64±0.02 ^{ab}	6.71±0.02 ^a	6.75±0.05 ^a	P=0.002
Carbohydrate (%)	Initial	2.67±0.08	2.47±0.18	2.59±0.08	2.77±0.12	2.69±0.03	P=0.034
	Final	2.68±0.08 ^d	2.26±0.07 ^d	1.91±0.05 ^c	1.58±0.03 ^b	1.50±0.06 ^a	P≤0.001
Total ash [TA (%)]	Initial	4.02±0.00	4.05±0.01	4.05±0.02	4.05±0.01	4.03±0.01	P=0.016
	Final	4.14±0.01 ^b	4.21±0.01 ^a	4.23±0.04 ^a	4.26±0.01 ^a	4.28±0.01 ^a	P=0.013

Note: Mean values superscripted with different letters in each row are significantly different ($P \leq 0.05$).

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2.3 Remedial Efficacy of Quercetin Supplemented Feed on Growth, Physiology and Histopathology of *Labeo rohita* Challenged with AFB₁

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Keywords: Aflatoxin B₁, Physiological responses, Oxidative stress, Quercetin, Rohu

Introduction

One of the most serious issues confronting aquaculture industry today is elimination of foodborne aflatoxin B₁ (AFB₁) and there is a great demand for an effective decontamination approach. AFB₁ is the most toxic and harmful produced by *Aspergillus flavus* which affect aquaculture and livestock sectors resulting economic losses. For this purpose, one of the practical bio-control approaches is to use natural bioactive compounds or flavonoids. Flavonoids are reported to inhibit fungal growth through various mechanisms. However, their modes of action underlying the inhibition of mycotoxin biosynthesis remain unclear. So, the current study suggests the ability of flavonoid to alleviate the toxic AFB₁ effects in *L. rohita*. Until now, no validation study at least to our knowledge was conducted to determine the long-term effect of feeding quercetin in combination with aflatoxin toxicity in the diet of *L. rohita*.

Material and Methods

Three months experimental period was carried out at Instructional cum Research Farm of College of Fisheries, GADVASU, Ludhiana. *L. rohita* fingerlings were randomly distributed into five experimental groups with three replicates per group. Each group was maintained under an outdoor condition in a fibre-reinforced plastic (1.5x1.0x0.75 m with water holding capacity of 1.0 m³) at a density of 15 fish per pool. Fishes were divided into five groups (G₁-G₅): G₁ (control), G₂ (100ppb AFB₁), G₃ (25ppb AFB₁+200mg QC), G₄ (50ppb AFB₁+200mg QC) G₅ (100ppb AFB₁+200 mg QC) /kg diet. Fish growth, haematological and stress indices parameters was studied at monthly intervals and calculated after completion of experiment. Haematological and stress indices parameters was assessed and calculated as per standard protocols (Mukherjee 1988 and Nishikimi et al 1972). The histopathology of liver was also performed by standard protocol. The data were statistically analyzed using one-way analysis of variance at a 5% significance level.

Results and Discussion

Lowest fish growth was observed in positive control group (G₂). While among co-administration of AFB₁+QC group highest growth was found in G₃ group which is comparatively less but at par towards G₁ (Table 1). In G₄ diet, fish suffered a significant decrease in growth indices relative to G₅. Overall results suggest that negative effect of AFB₁ seems to be correlated to the amount of toxin and duration of exposure. However, haematological profiles were significantly decreased (p≤0.05) in the treatments which received high dose of AFB₁ in *L. rohita* fingerlings. The highest haematological parameters were observed in G₁ and at par in G₃ (Table 1). The decreased haematology observed indicates anaemia, which is possibly due to hemosynthesis and osmoregulatory dysfunction that occur owing to inhibiting the activities of several enzymes involved in heme biosynthesis. Moreover, the activity of SOD and catalase were found to be increased at higher inclusive levels of aflatoxin while it was in reduced form upon quercetin administration (Fig 1A). That coincide with fact that quercetin prevents against oxidative stress or delay the lipid oxidation that may result in changing activities of endogenous antioxidants to suppress apoptosis. Further, no histomorphological abnormalities were observed in control. But hepatic cell damages were observed specifically in treatment received high concentration of AFB₁ that can disturb homeostasis and physiological balance of fish (Fig 2B). Change in liver histomorphology with dilated sinusoids may be due to loss of structural proteins. In contrast, co-administration of quercetin and AFB₁ showed an improvement of liver histomorphology, aside from mild inflammation by enhancing antioxidant enzyme activity and decreasing pro-oxidant effect.

Table 1 Growth performance and haematological parameters of the *L. rohita* after 90 days of feeding in different treatments

Parameters	G ₁	G ₂	G ₃	G ₄	G ₅
Initial weight (g)	17.36±0.00	17.31±0.00	17.34±0.00	17.31±0.00	17.32±0.00
Final weight (g)	30.62 ^a ±0.00	26.25 ^c ±0.00	30.31 ^b ±0.00	29.15 ^c ±0.44	28.38 ^d ±0.00
Weight gain (g)	13.26 ^a ±0.73	8.94 ^c ±0.12	12.97 ^{ab} ±0.12	11.84 ^b ±0.05	11.06 ^{bc} ±0.04
SGR	0.89 ^a ±0.02	0.59 ^d ±0.01	0.81 ^b ±0.04	0.69 ^{cd} ±0.03	0.64 ^d ±0.07
K	1.23 ^a ±0.01	0.64 ^e ±0.01	1.22 ^b ±0.01	1.04 ^c ±0.01	0.92 ^d ±0.01
FCR	1.19 ^c ±0.01	1.26 ^a ±0.01	1.20 ^d ±0.01	1.21 ^c ±0.01	1.25 ^b ±0.01
PER	0.31 ^a ±0.01	0.21 ^c ±0.02	0.30 ^{ab} ±0.01	0.28 ^{bc} ±0.03	0.27 ^d ±0.04
Survival (%)	100.00 ^a ±0.00	79.76 ^c ±2.77	98.21 ^b ±2.77	94.59 ^c ±0.45	91.54 ^d ±2.77
Haematological parameters					
Hb (g %)	5.37 ^a ±0.01	5.14 ^c ±0.01	5.34 ^b ±0.02	5.32 ^c ±0.01	5.25 ^d ±0.01
PCV/ Hct (%)	21.75 ^a ±0.03	18.51 ^c ±0.01	21.14 ^b ±0.04	20.54 ^c ±0.03	19.91 ^d ±0.02
TEC (x10 ⁶ mm ³ -1)	1.96 ^a ±0.03	1.72 ^c ±0.03	1.91 ^b ±0.03	1.87 ^c ±0.04	1.82 ^d ±0.04
TLC (x10 ³ mm ³ -1)	2.55 ^a ±0.04	1.82 ^c ±0.03	2.27 ^b ±0.04	1.93 ^c ±0.06	1.87 ^d ±0.02
MCV (µm ³)	110.97 ^a ±0.18	107.62 ^c ±0.23	110.68 ^b ±0.21	109.84 ^c ±0.17	109.39 ^d ±0.24
MCH (g %)	27.40 ^c ±0.08	29.88 ^a ±0.06	27.95 ^d ±0.04	28.44 ^c ±0.06	28.84 ^{ab} ±0.07
MCHC (g %)	24.69 ^c ±0.06	27.80 ^a ±0.03	25.29 ^d ±0.01	25.89 ^{cd} ±0.04	26.40 ^b ±0.04

Values are Mean ± S.E. (p≤0.05)

Values with same superscript (a, b, ...d) in a row does not differ significantly (p≤0.05), SGR= Specific growth rate; PER= Protein efficiency ratio; FCR= Feed conversion ratio; K= Condition Factor, TEC= Total erythrocyte count; TLC= Total leucocyte count; Hb= Haemoglobin; PCV= Packed cell volume; MCV= Mean corpuscular volume; MCH= Mean corpuscular haemoglobin; MCHC= Mean corpuscular haemoglobin content

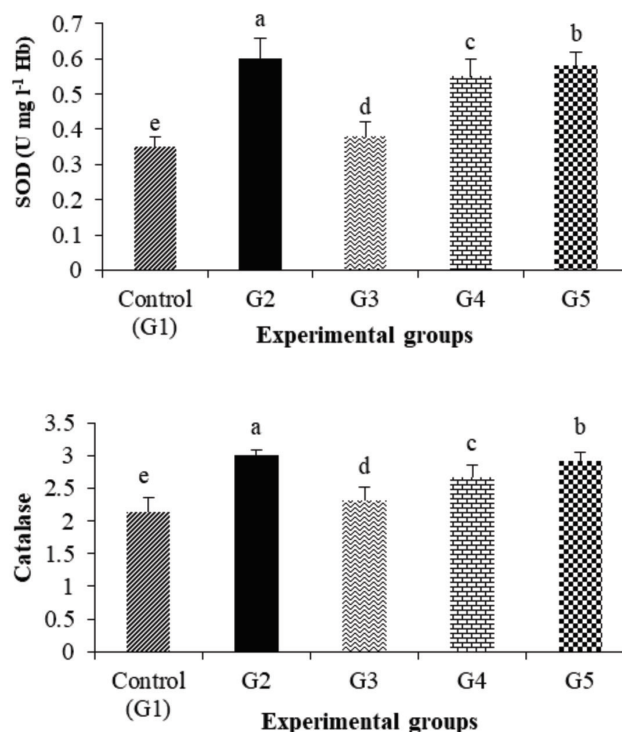


Fig. 1A Effects of feeding various experimental diets for 90 days on the (A) SOD (U·mg·L⁻¹·Hb) enzyme activity and (B) CAT level in the serum of *L. rohita*. Bars with different letters indicate significant differences (p < 0.05). Data are presented as mean ± standard error of three replicates.

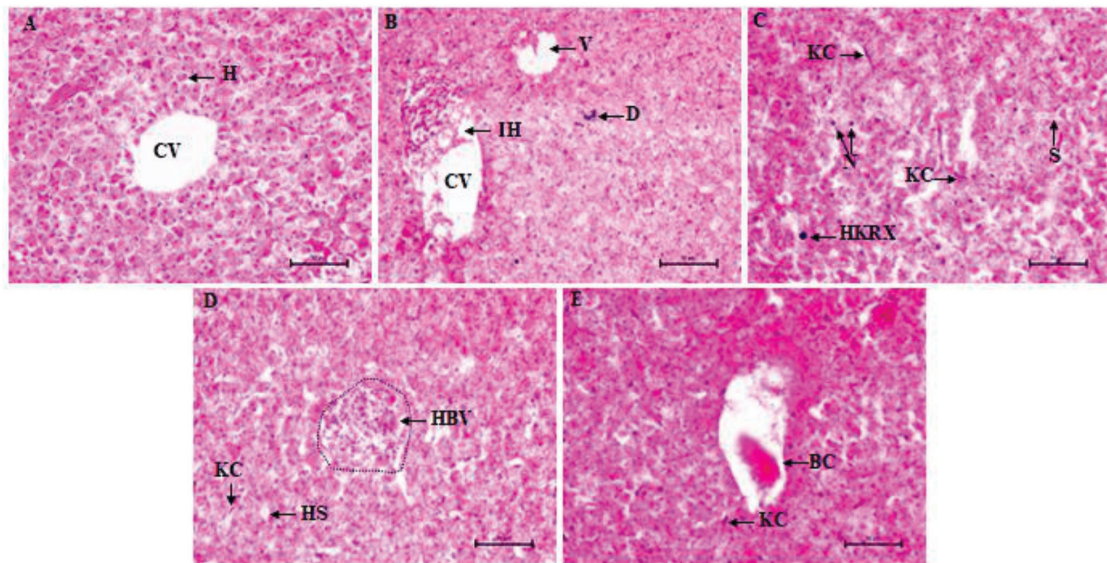


Figure 2B Representative pictures of the liver showing the effect of aflatoxin and quercetin induced alterations in the histopathology of the liver of *L. rohita*. Scale bar= 100µm. H & E staining.

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2.4 Quality Parameters and Fermentation Characteristics of Ensiled Water Hyacinth (*Eichhornia crassipes*)

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Keywords: Fermentation, Moringa, Silage, Unconventional feed, Water Hyacinth

Introduction

Water hyacinth (*Eichhornia crassipes*) is an invasive floating plant found in bodies of water all over the world. The water hyacinth (WH) is well-known for its ability to purify sewage, but known for most noxious water weed due to its rapid growth, interfere fish farming and power generation. Water hyacinths can be fed to fish and pigs; ruminants were fed deferent form of fresh, ensiled or hay water hyacinth. Ensiling can be enhance water hyacinth use in animals and palatability. Therefore, present study has been conducted to preserve water hyacinth with different ingredients and evaluate quality parameters of silage prepared.

Material and Methods

Water hyacinth was ensiled in seven treatment combinations namely; T1 (WH 75 + rice straw 16+ molasses 4%) T2 (WH 75 + rice straw 15.5+ molasses 4%+ Urea 0.5); T3 (WH 75 + rice straw 10+ molasses 4%+ wheat bran 6%); T4 (WH 75 + rice straw 10+ molasses 4%+ moringa 6%); T5 (WH 65 + rice straw 25+ molasses 4%); T6 (WH 65 + rice straw 25+ molasses 4% and Urea 1%) and T7 (WH 95%). Samples were analysed for chemical composition and fibre analysis as per standard protocol. Water extract was prepared as per procedure for pH and ammonia nitrogen analysis (Cai et al 2004).

Results and Discussion

DM, OM, CP, EE and ADF contents of silage prepared with different treatments were statistically at par with each other. DM content was found to be higher in T5 and T6 while lowest in T7 (Table 1). The addition of 10% rice straw + 6 % WB or 6% *Moringa olifera* (T3 and T4) increased ($p < 0.001$) the CP, DM intake (%BW), digestible DM (%), TDN (%), RFV and RFQ, whoever ($p < 0.001$) reduced pH value, NDF, ADF concentration of silage as compare to WH silage added 16 % RS (T1 and T2) and 25% RS (T5 and T6). However, NGP (ml/g), NDFD (%) and TOMD (%) were found to be higher ($p < 0.001$) in T1 and T2 as compared to others.

Successful ensiling of water hyacinth is difficult due to higher moisture and lower water soluble carbohydrate. Therefore, in the present study rice straw was added to complement dry matter and provide absorbent function, whereas wheat bran was added to increase WSC of water hyacinth (Liu et al., 2011). Benefits of adding DM through wheat bran or moringa instead of rice straw that the CP and WSC content with lower NDF and ADF content of wilted WH silage. Possibly *M. olifera* could be a source of soluble protein. Similarly, previous workers has reported addition molasses alongwith wheat bran improved CP, WSC content and silage fermentation characteristics of wilted water hyacinth silage. The present *in-vitro* study revealed that water hyacinth ensiled with rice straw (10%) and wheat bran (6%) or *M. olifera* (6%) and molasses (4%) can be used as an unconventional feed resource for animal.

Table 1 Nutrient composition and feed values of silage prepared from wilted *Eichhornia*

Parameter	Silage-1	Silage-2	Silage-3	Silage-4	Silage-5	Silage-6	Silage-7	SEM	P value
Nutrient composition, % DM									
Dry matter	21.2 ^c	17.2 ^b	21.4 ^b	21.4 ^b	25.0 ^c	25.5 ^c	7.25 ^a	0.638	<0.001
Crude protein	7.92 ^c	8.20 ^c	13.0 ^d	14.9 ^c	7.29 ^b	6.34 ^a	16.3 ^f	0.119	<0.001
Ether extract	2.00 ^b	1.90 ^b	1.90 ^b	2.37 ^c	1.35 ^a	1.35 ^a	1.60 ^b	0.113	0.001
Neutral detergent fiber	66.3 ^d	61.7 ^c	58.1 ^b	53.9 ^a	69.6 ^c	67.9 ^{de}	60.4 ^{bc}	0.770	<0.001
Acid detergent fiber	52.1 ^{cd}	48.8 ^c	37.8 ^b	37.2 ^b	49.7 ^c	55.5 ^d	30.7 ^a	1.307	<0.001
Cellulose	45.4 ^d	41.6 ^c	32.6 ^b	31.2 ^b	44.4 ^{cd}	48.5 ^e	26.6 ^a	0.998	<0.001
Ash	18.0 ^b	20.1 ^d	16.2 ^a	15.6 ^a	18.8 ^c	19.0 ^c	16.0 ^a	0.170	<0.001



Feed values									
Dry matter intake, % BW	1.80 ^{ab}	1.94 ^c	2.06 ^d	2.23 ^c	1.72 ^a	1.77 ^{ab}	2.01 ^d	0.023	<0.001
Digestible dry matter, %	48.3 ^{ab}	50.7 ^b	59.4 ^c	59.9 ^c	50.1 ^b	45.4 ^a	65.0 ^d	1.018	<0.001
Total digestible nutrients, %	50.4 ^{ab}	53.5 ^b	61.4 ^c	61.8 ^c	53.0 ^b	48.7 ^a	66.3 ^d	0.915	<0.001
Relative feed value	67.9 ^a	76.5 ^b	95.1 ^c	103 ^d	67.0 ^a	62.4 ^a	100.0 ^{cd}	2.167	<0.001
Relative feed quality	1.67 ^a	1.86 ^b	2.35 ^{cd}	2.46 ^d	1.63 ^a	1.54 ^a	2.35 ^d	0.048	<0.001
NE _L , M cal/kg	1.14 ^{ab}	1.19 ^b	1.38 ^c	1.39 ^c	1.18 ^b	1.07 ^a	1.50 ^d	0.022	<0.001

DM: dry matter; BW: body weight; NE_L: Net energy lactation; SEM: standard error mean.
 Figures with different superscripts in a row differ significantly, p<0.05.

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2.5 Effect of Partial Substitution of Conventional Protein Source with Duckweed on Digestibility and Nitrogen Retention in Beetal Goats

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Keywords: Duckweed, Goat, Rumen fermentation, Retention

Introduction

One of the most expensive costs to any livestock operation is feed. The protein component is a critical nutrient in ruminant rations. In all diet formulations, soybean meal (SBM) is the most widely used protein supplement. The amino acids present in SBM and the abundance of the feedstuff make it attractive as a universal protein source; however, soybean meal does raise the cost of the diet. The aquatic plant duckweed can have similar crude protein levels and contains the essential amino acids needed in ruminant rations. The aim of the present study is to compare the nutritional quality of TMR containing degraded levels of duckweed replacing the total crude protein of soybean as the protein source in ruminants.

Materials and Methods

The duckweed sample used in the study was obtained from the College of Fisheries, GADVASU, Ludhiana and was sun/ air-dried and then ground in a Wiley mill through a 2 mm screen. The samples were analysed for proximate (AOAC 2000) and cell wall components (Robertson and Vansoest 1981). The total mixed rations (TMR) were prepared by using various duckweed levels i.e. control, 1/3 duckweed 2/3 duckweed and 100% duckweed replacing the total CP of soybean in 50:50 ratio (R: C) as shown in Table 1. All the TMR prepared were iso-nitrogenous having approximately 15% CP.

Table 1 Ingredient and Chemical composition of TMR fed to male goats, % DM basis (R:C 50:50)

Ingredient	Control (TMR1)	1/3duckweed (TMR2)	2/3 duckweed (TMR3)	100% duckweed (TMR4)
Chemical Composition				
DM	92	92	91	91
Ash	10.20	11.80	13.95	14.37
OM	89.8	88.20	86.05	85.62
CP	14.87	15.07	15.27	15.27
NDF	49.70	50.50	51.20	50.50
ADF	26.50	28.35	31.10	34.0
HC	23.20	22.15	20.10	16.50
EE	2.55	2.45	2.55	2.40
Cellulose	17.0	17.80	17.30	17.50
TCHO	72.91	71.11	68.22	67.95
NFC	23.21	20.61	17.02	17.45

Results and Discussion

The CP content of all TMRs varied from 14.87% to 15.27% with different levels of duckweed supplementation. All the rations prepared were iso-nitrogenous in nature (Table 1). The NDF content varied from 49.70% to 51.50%, while ADF values varied from 26.50% to 34.0%. The fat content of ration was between 2.01% to 2.55%. The ash content in all TMRs were between 10.20% to 14.37% and OM content varied from 85.62% to 89.80%. The total carbohydrates (TCHO) in the rations were in between 67.95% to 72.91% with different levels of duckweed supplementation.

The DM intake (g/d) was statistically similar in control and duckweed supplemented groups. Results revealed no significant ($P < 0.005$) difference in digestibility of acid detergent fibre (ADF), crude protein (CP) and cellulose



digestibility, dry matter (DM), neutral detergent fibre (NDF), cellulose, hemicellulose and organic matter (OM). There was significant difference ($p < 0.005$) in the digestibility dry matter (DMD), neutral detergent fibre (NDF), ether extract, organic matter (OM), total carbohydrate and non-fiber carbohydrate in control and duckweed supplemented groups. All these digestibilities were higher in group (control) followed by group II (1/3 duckweed), group III (2/3 duckweed) and lowest in group IV (100% duckweed).

The intake of nitrogen (g/d) was statistically comparable between all the groups. The faecal, urinary and total nitrogen excreted (g/d) were statistically similar among all the four groups as no significant effect was seen in duckweed supplemented groups. The daily total excretion of nitrogen was highest in duckweed supplemented groups as compared to control group. The urinary nitrogen excretion was highest in animals fed with duckweed supplement and lowest UN excretion was observed in control group. Animals in all the four groups were in positive N balances. The nitrogen retention was highest in control group than duckweed supplemented group though the results were non-significant.

Table 2. Digestibility and Nitrogen Retention Parameters, %DM basis

Parameters	Group 1	Group2	Group 3	Group 4	SEM
DM intake (g /d)	458.79	478.54	427.63	458.34	9.48
DMD	65.55 ^c	56.92 ^b	52.26 ^a	52.36 ^a	1.71
NDFD	50.06 ^c	42.84 ^b	40.20 ^{ab}	37.76 ^a	1.43
ADFD	39.32	35.37	38.83	39.20	0.74
EED	78.23 ^b	74.22 ^{ab}	75.41 ^{ab}	72.25 ^a	0.87
Cellulose digestibility	46.51	42.82	42.90	43.86	0.75
HCD	64.52 ^d	52.32 ^b	58.45 ^c	36.83 ^a	3.07
OMD	67.36 ^c	60.12 ^b	54.90 ^a	54.54 ^a	1.60
CPD	80.34	78.12	76.87	77.39	0.59
TCHOD	61.15 ^c	51.43 ^b	44.67 ^a	45.05 ^a	2.08
NFCD	80.90 ^c	78.24 ^{bc}	74.19 ^b	68.37 ^a	1.54
Nutritive Value					
Total TDN digested	274.73 ^c	244.61 ^b	200.90 ^a	214.16 ^a	9.36
TDN%	59.96 ^c	51.10 ^b	46.98 ^a	46.72 ^a	1.66
DCP%	80.34	78.12	76.87	77.39	0.59
DCP g	62.47	63.11	58.07	60.49	1.07
Actual CP intake	77.79	80.76	75.54	78.14	1.19
Nitrogen Retention					
Total nitrogen intake (g/day)	12.44	12.92	12.08	12.50	0.19
Urinary N (g/day)	2.62	2.91	2.87	3.27	0.19
Fecal N output (g/day)	2.45	2.82	2.79	2.82	0.082
Total N outgo (g/day)	5.07	5.73	5.66	6.09	0.22
N retention (g/day)	7.37	7.18	6.42	6.40	0.24
% N retention	59.47	55.63	52.91	51.19	1.73

Means bearing different superscripts in a row differ significantly ($p < 0.05$)

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2.6 Effect of Duckweed as Alternate Protein Source on Rumen Fermentation and Health Status of Beetal Goats

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Keywords: Duckweed, Goat, Rumen fermentation, Retention, Blood parameters

Introduction

One of the most expensive costs to any livestock operation is feed. Protein is an expensive macro-nutrient. In all diet formulations, soybean meal (SBM) which contains 44% to 48% crude protein is the most widely used protein supplement. One such feedstuff is duckweed; the tiny, free-floating, vascular aquatic plant. The duckweed can have similar crude protein levels and contains the essential amino acids required by the ruminant rations. Number of studies have reported crude protein in duckweed as high as 45%. Duckweed has high quality protein with a superior amino acid profile as most plant proteins and is similar to animal protein. The goal of this research is to explore the nutritional quality of duckweed particular as the protein source on rumen fermentation and blood profile in ruminants.

Materials and Methods

The duckweed sample used in the study was obtained from the College of Fisheries, GADVASU, Ludhiana was air-dried and then ground in a Wiley mill through a 2mm screen. The samples were analysed for proximate (AOAC 2000) and cell wall components (Robertson and Vansoest 1981). The total mixed rations (TMR) were prepared by using various duckweed levels sources i.e. control, 1/3 duckweed, 2/3 duckweed and 100% duckweed replacing the total CP of soybean in 50:50 ratio (R:C) as shown in Table 1. Rumen liquor samples were taken by stomach tube and blood samples were drawn from all male goats at 0 day and at the end of experimental feeding.

Results and Discussion

Rumen fermentation parameters

The mean pH values after feeding (4 hrs) were statistically similar as no significant effect of duckweed supplementation on rumen pH was observed. On supplementation of duckweed there was increase in the concentration of TCA-ppt N (mg/100 ml) in group II (1/3 duckweed) than control group although the results were statistically nonsignificant. Non protein nitrogen concentration (mg/100ml SRL) was also lower in group IV (89.81), followed by group III (2/3 duckweed) and group II (104.02) as compared to control group I (128.73) but the results were statistically non-significant. The total nitrogen (mg/dl) was significantly Results showed a significant (P < 0.05) higher in control group I and lower in group III (2/3 duckweed). There was non statistically increase in TVFA concentration in group II (1/3 duckweed) as compared to other groups. The mean percentage of acetate, propionate, butyrate, isobutyrate, isovalerate and valerate and A:P ratio were statistically comparable in control and duckweed supplemented groups.

Blood biochemical aspects

There was no significant effect of duckweed supplementation on triglycerides, GGT, Glucose, BUN, Total protein and ALT and AST in all groups. The results revealed that there was significantly (P < 0.05) increase in serum cholesterol and creatinine concentration in duckweed supplemented groups, but the results were within the physiological range. So it can be concluded that duckweed has no deleterious effect on animal health.

Table 1: Ingredient composition of different concentrate mixtures containing duckweed

Ingredient	Control	1/3 duckweed	2/3 duckweed	100% duckweed
Maize	35	35	35	35
Soybean	28	18.6	9.3	0
Duckweed	0	15.5	31	46
Wheat bran	17	13	8	4
Rice bran	14.75	12.6	11.5	9.5
Mineral Mixture	2	2	2	2



Salt	1	1	1	1
Urea	0	0.3	0.7	1
Bypass fat	2.25	2	1.5	1.5
NFC	26.63	21.43	14.25	15.10

Table 2: *In vivo* Rumen fermentation parameters and blood constituents in male beetal goats

Parameters	Group 1	Group2	Group 3	Group 4	SEM
Total nitrogen mg	250.07 ^c	239.49 ^{bc}	176.57 ^a	189.70 ^{ab}	11.01
NPN mg	128.73	104.02	96.95	89.81	6.97
TCA-N mg	121.34	135.38	79.62	99.89	10.79
NH ₃ mg	49.52	59.67	51.80	50.40	3.64
pH	5.82	5.76	6.14	6.11	0.059
Volatile fatty acids production (mM/dl)					
Acetic acid	5.62	6.64	5.91	5.84	0.17
Propionic acid	1.60	1.85	1.46	1.49	0.060
Iso butyric acid	0.037	0.048	0.036	0.051	0.003
Butyric acid	0.97	1.01	0.87	1.05	0.038
Iso valeric acid	0.055	0.068	0.051	0.061	0.005
Valeric acid	0.063 ^b	0.067 ^b	0.051 ^a	0.064 ^b	0.002
TVFA	8.35	9.68	8.38	8.57	0.24
Relative proportion, %					
Acetate	67.24	68.60	70.62	68.02	0.54
Propionate	19.22	19.08	17.37	17.56	0.38
Iso butyrate	0.45	0.50	0.43	0.59	0.027
Butyrate	11.67	10.42	10.34	12.34	0.35
Isovalerate	0.66	0.69	0.61	0.71	0.041
Valerate	0.75 ^b	0.69 ^{ab}	0.61 ^a	0.76 ^b	0.021
A:P ratio ¹	3.50	3.59	4.11	3.91	0.11
Triglycerides (mg/dl)	15.68	15.27	16.28	15.86	2.03
GGT (U/L)	42.61 ^{ab}	43.26 ^{ab}	38.44 ^a	57.65 ^b	2.86
Glucose (mg/dl)	39.39	34.13	36.37	37.07	2.40
BUN (mg/dl)	24.30	24.65	27.07	26.53	1.04
Creatinine (mg/dl)	0.77 ^a	0.85 ^{ab}	0.93 ^b	0.84 ^{ab}	0.018
Cholesterol (mg/dl)	40.50 ^a	58.16 ^{ab}	63.42 ^b	49.54 ^{ab}	3.69
ALT (U/L)	20.22	19.60	20.37	20.39	0.57
AST (U/L)	101.24	90.72	101.42	101.57	4.38
Total protein (g/dl)	6.29	6.37	6.73	6.74	0.13

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2.7 Change in Fatty Acid Profile of Live Food Rotifer *Brachionus Calyciflorus* through Enrichment with HUFA and Vitamin C

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Keywords: Rotifer, HUFA, Live food, Vitamin-C

Introduction

Live foods are considered as 'living capsules of nutrition' from which fish larvae get all the nutrients. Live foods are easily detected due to their swimming movements in the water column, and they are highly digestible, given their high water content (>80%) and endogenous enzymes. Rotifers are generally used as starter feed for early-stage fish larvae due to their relatively small size, and nutritional value. They contain some amount of highly unsaturated fatty acids (HUFA) such as Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) (Lubzens et al 1989), but not in sufficient quantity for larval growth and survival of all fish species. However, rotifers have nutritional deficiencies for better growth and survival of predator fish larvae, particularly in essential n-3 highly unsaturated fatty acids (HUFA). Several studies have demonstrated the positive effect of enriched live food on the growth performance of various aquaculture species. Keeping the above findings in mind, the present study was conducted to assess the effect of enrichment on the freshwater live food organism, rotifer (*Brachionus calyciflorus*) with Highly Unsaturated Fatty Acid and Vitamin C.

Materials and Methods

The experiment was conducted at ICAR-Central Institute of Fisheries Education (ICAR-CIFE), Kolkata Centre. Enrichment of zooplankton Rotifer (*B. calyciflorus*) was done using prepared emulsions in accordance to Singh et al (2019). Required quantity of zooplankton was collected, thoroughly washed and kept at a concentration of 0.1 million numbers per litre of water in a 6-litre capacity glass container containing 4 litres of water. The emulsion was added @ 2ml/litre of water containing HUFA (T2) or Vitamin C (T3) or HUFA + Vitamin C (T4) and kept undisturbed for 18 hours with mild aeration. One glass container was also maintained containing the same density of zooplanktons without adding any prepared emulsion (T1). Total lipids were extracted followed by esterification of extract from the prey Rotifer (*B. calyciflorus*) following the standard method. Gas Chromatography-Mass Spectrometry measurements were performed following the mass spectrometer. The fatty acids were identified referring to the retention time and GC-MS library.

Results and Discussion

The highest levels of linoleic acid and linolenic acid were found in the rotifer enriched with Vitamin C emulsion (11.94% and 7.03% respectively). The EPA and DHA content of rotifer showed similar trends with the EPA and DHA content of emulsions when enriched with HUFA and HUFA + vitamin C emulsion. The uptake of EPA by rotifer from HUFA and HUFA+vitamin C emulsion was also very high; it was calculated to a level of 61.6% and 65.6% of emulsion respectively. The uptake of DHA by rotifer was not highly efficient as compared to EPA by rotifer. So, HUFA+ Vitamin C enriched rotifer resulted in highest level of both EPA (15.04%) and DHA (9.46%) content (n-3 fatty acids) followed by HUFA enriched rotifer (EPA 13.65% and DHA 8.26%) than other experimental groups. The total n-3 fatty acid content of HUFA (37.52%) and HUFA + Vitamin C (39.69%) emulsion was almost equal, but no n-3 fatty acid was found in the vitamin C emulsion. The enriched rotifer showed the highest level of total n-3 fatty acid content when enriched with HUFA + vitamin C emulsion followed by HUFA emulsion. The total n-6 fatty acid content of HUFA + Vitamin C emulsion was found higher followed by HUFA and vitamin C emulsion. In case of vitamin C enriched rotifer, the n-6 content showed comparatively higher than others. The Vitamin C enrichment increases the total n-6 fatty acid content of the rotifer (13.28%) which was much higher than other emulsions. This indicates that Vitamin C may play an important role in the protection of n-6 fatty acids from oxidation. (Table 1 and 2). From the present study, it can be concluded that the enrichment of rotifer with HUFA and HUFA + Vitamin C could enhance its fatty acid profile significantly.



Table 1. Fatty acid profile (% of total lipid) of the lipid source of emulsions

Fatty acids	HUFA Emulsion	Vitamin C Emulsion	HUFA + Vit. C Emulsion
C 14:0	11.89	-	13.38
C 15:0	0.91	-	1.03
C 16:0	0.17	68.06	3.17
C 16:1 n-7	28.88	6.68	17.98
C 16:2 n-6	1.19	-	1.53
C 17:0	0.34	-	0.55
C 18:0	0.43	-	0.70
C 18:1 n-9	11.20	19.74	13.27
C 18:1 n-7	-	-	-
C 18:2 n-6	2.72	5.51	3.55
C 19:0	0.05	-	0.41
C 18:3 n-3	2.93	-	5.22
C 20:0	0.07	-	0.11
C 20:1 n-9	0.32	-	0.13
C 20:1 n-7	0.39	-	1.44
C 20:4 n-6	1.78	-	1.88
C 20:5 n-3	18.87	-	19.85
C 22:5 n-3	1.05	-	1.22
C 22:6 n-3	14.67	-	13.40
C 24:0	1.69	-	0.72
Total SAFA	15.98	68.06	20.52
Total MUFA	40.79	26.42	32.82
Total PUFA	43.21	5.51	46.65
n-3	37.52	-	39.69
n-6	5.69	5.51	6.96
n-3/n-6	6.59	-	5.70
DHA/EPA	0.77	-	0.67

Table 2 Fatty acid profile of rotifer enriched with different experimental emulsions

Fatty acids	Control (un-enriched)	HUFA enriched	Vitamin C enriched	HUFA + Vit. C enriched
C 14:0	6.46	14.58	5.41	14.60
C 15:0	9.86	1.28	3.72	1.19
C 16:0	39.55	12.29	40.56	10.81
C 16:1 n-7	10.02	22.92	7.50	22.45
C 16:2 n-6	-	-	-	-
C 17:0	5.71	2.50	3.83	0.23
C 18:0	4.76	1.19	4.41	0.94
C 18:1 n-9	4.36	10.96	3.45	15.15
C 18:1 n-7	-	-	-	-
C 18:2 n-6	0.91	2.98	11.94	1.48
C 19:0	-	-	-	-
C 18:3 n-3	0.52	1.89	7.03	2.91
C 20:0	9.11	1.86	3.34	0.58
C 20:1 n-9	0.54	1.55	1.66	1.58



C 20:1 n-7	-	-	-	-
C 20:4 n-6	2.42	1.68	1.34	1.56
C 20:5 n-3	2.02	13.65	2.38	15.04
C 22:5 n-3	2.24	2.13	1.81	1.62
C 22:6 n-3	1.12	8.26	1.39	9.46
C 24:0	0.40	0.28	0.24	0.41
Total SAFA	75.85	33.98	61.51	28.76
Total MUFA	14.92	35.43	12.61	39.18
Total PUFA	9.23	30.59	25.89	32.07
n-3	5.9	25.93	12.61	29.03
n-6	3.33	4.66	13.28	3.04
n-3/n-6	1.77	5.56	0.94	9.54
DHA/EPA	0.55	0.60	0.58	0.62

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2.8 Effect of Selected Vegetable Oils on Growth, Feed Utilization and Digestive Enzyme Activities of *Labeo rohita*

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Keywords: Sunflower, Significantly, Efficiency, Lipase, Protease, Control

Introduction

As feed accounts for 60-70 percent of the entire production cost in aquaculture, hence, cost-effective feed management is the key to success of farming. For growth, reproduction and other body functions, a balanced amount of protein, fat, vitamins and minerals in fish feed is a must. Oils added to the fish diet act as antimicrobial, anti-oxidative and growth promoter agent. They also enhance the palatability, digestibility and binding capacity of feed (Yadav et al 2019). Fish performance such as feed efficiency, growth, feed intake, etc. are not significant in the partial replacement (by 60–75 per cent) of fish oil with substitute lipid source in approximately all finfish species studied, provided that the essential fatty acid requirements are met. This present study was aimed to evaluate the efficacy of selected vegetable oils concerning fish growth, and other feed utilization parameters.

Materials and Methods

Experimental setup: The experiment was conducted in 200-liter rectangular plastic tanks. Each tank was washed before the introduction of fish. All the experimental tanks were cleaned and filled with filtered tube well water. Each tank was stocked with 5 fingerlings of almost in equal size and the experiment was conducted in triplicate following Completely Randomized Design (CRD) for 60 days. The experimental fish were fed @ 3 percent of body weight twice a day (10 AM and 5 PM) in split doses for 60 days.

Growth parameters: At the end of the experiment, all the fishes from experimental tanks were harvested with a hand net. The weight of individual fish was measured on an electronic balance. The growth parameters such as weight gain, percent weight gain, SGR and GCE were calculated as per standard formulae.

Digestive enzyme activities: The enzyme activities viz. lipase, protease and amylase were recorded.

Results and Discussion

The impact of different vegetable oil supplementation in carp (*L. rohita*) diet had a significant impact on fish growth performance as given in Table 2. The net weight gain data obtained for experimental fish are presented in Table 3. From Table 3, it is evident that the net weight was significantly ($p < 0.05$) higher in treatments than that in control. The weight gain was significantly ($p < 0.05$) higher in T_4 (60.076 ± 1.557 %) followed by T_2 (54.67 ± 0.86 %), T_3 (47.711 ± 1.60 %), T_1 (41.02 ± 0.81 %), and lowest control (32.711 ± 0.422 %). Specific growth rate (SGR) was significantly ($p < 0.05$) higher in T_4 (0.784 ± 0.016) and lowest in control (0.472 ± 0.005). The digestive enzyme analysis amylase, protease and lipase are summarized in Table 3. The supplementation of vegetable oil @ 10 percent in fish diet enhanced the activity of protease. Thus, the dose of soybean oil @ 10 percent level is suggested for supplementation in the diet of *Labeo rohita* fingerlings.

Table 1. Feed composition (ingredients g/kg)

Particular	Experimental Diet				
	T ₁	T ₂ (Sunflower oil)	T ₃ (Mustard oil)	T ₄ (Sesame oil)	T ₅ (Soybean oil)
Diet Ingredients (g/100g)					
Basal Diet*	100	90	90	90	90
Vegetable oils**	00	10	10	10	10
Total	100	100	100	100	100
Proximate composition (%)					
Moisture	10.40	10.28	10.23	10.18	9.87
Crude protein	24.56	23.52	23.34	23.29	23.48
Fat	7.58	17.38	17.30	17.25	17.42
Ash	6.40	6.34	6.45	6.24	6.20



Carbohydrates	61.46	52.76	52.76	53.22	52.60
Energy (kcal/100g)	428.37	481.99	480.42	481.55	481.55

*Basal diet constitutes groundnut oil cake, rice bran, wheat flour which was taken in the ratio of 40: 40: 20, respectively.

Table 2 Summary data on growth performance of *L. rohita* fed with different vegetable oils supplementation diet.

Treatments	Parameters							PER
	Initial weight	Final weight	Net weight gain (g)	Per cent weight gain	SGR	FC R	GCE	
T ₁ (Control)	12.27	16.29	4.017 ^a ±.043	32.711 ^a ±0.422	0.472 ^a ±.005	6.22 ^c ±0.05	0.161 ^a ±.001	0.163 ^a ±.002
T ₂	12.29	17.33	5.040 ^b ±.064	41.020 ^b ±0.806	0.573 ^b ±.010	5.10 ^d ±0.07	0.196 ^b ±.003	0.235 ^b ±.020
T ₃	12.32	19.06	6.737 ^d ±.098	54.666 ^d ±0.856	0.727 ^d ±.009	4.06 ^b ±0.04	0.246 ^d ±.003	0.276 ^b ±.009
T ₄	12.29	18.07	5.787 ^c ±.151	47.7110 ^c ±1.601	0.643 ^c ±.018	4.56 ^c ±0.11	0.220 ^c ±.005	0.264 ^b ±.021
T ₅	12.28	19.69	7.417 ^e ±.136	60.076 ^e ±1.557	0.784 ^e ±.016	3.76 ^a ±0.07	0.266 ^e ±.005	0.322 ^c ±.003

Data expressed as mean ± SE (n=3); Mean value in the same column sharing different superscripts are significantly different (P<0.05)

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2.9 Designer Duckweed (*Spirodela polyrhiza*) Production: Biomass Yield and Protein Content Enhancement with Manuring

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Keywords: Biomass, Duckweed, Manures, Protein, Yield

Introduction

Duckweeds (DWs) are surface floating aquatic plants; holding immense potential for utilization as fodder or feed ingredient for livestock, poultry and fish due to fast growth rate, high nutritive value and better digestibility as compared to terrestrial plants. Depending on the nutrient availability, the DW protein content may vary from 15-45% on dry matter (DM) basis (Leng et al 1995), containing all the essential amino acids. Furthermore, DW protein closely resembles animal protein; having lysine and methionine content much higher than many plant feed stuffs, except soybean. However, biomass yield and protein production per unit area from DWs is much higher than soybean and can be potentially utilised in fish/shellfish feeds as prospective replacement for costly animal and plant feed ingredients (Ansal et al 2010). In view of above, growth, biomass production and protein content of DWs can be manipulated through nutrient management. Hence, culture trials were conducted to evaluate efficacy of locally available manures in respect to productivity and protein content of Greater DW, *Spirodela polyrhiza* (L.) Schleid, which is abundantly found in tropical and subtropical regions.

Materials and Methods

S. polyrhiza was cultured in FRP pools (1m²); by using different types of locally available organic manures viz., cow dung (CD), Poultry droppings (PD), CD +PD combination (1:1), vermicompost (VC) and *Azolla* Compost (AC) in a randomly designed experiment (in triplicates). Each pool was manured with 2 kg manure, left for 1 week and then stocked with 1 kg of *S. polyrhiza* inoculum procured from animal shed waste water bioremediation unit.

The DW biomass was harvested once it completely covered the water surface in each FRP pool. After recording total DW biomass, half was released back into the FRP pool and rest was sundried for proximate composition (crude protein, fat and ash content) as per standard methods. Likewise, second DW harvest was also made from each manure treatment.

Time taken by the DW to cover the complete water surface was recorded for each manure treatment during the two harvests. Atmospheric temperature, rainfall and water quality parameters were also recorded during the experimental period. The said experiment was conducted during the month of July for two years. Statistical analysis of data was carried out with the help of One-Way ANOVA (Statistical package SPSS 16.0), followed by Duncan's Multiple Comparison ($p \leq 0.05$).

Results and Discussion

In terms of DW productivity, maximum biomass (1st +2nd harvest) was recorded in case of treatment CD+PD (4.29-4.32 kg/m²) followed by PD (4.03-4.13 kg/m²), CD (3.49-3.73 kg/m²) and VC (2.84-3.04 kg/m²)/AC (2.84-3.01 kg/m²) treatments (Fig. 1). First harvest biomass was significantly higher than the second harvest in each treatment; owing to decline in nutrient concentration with progress of experiment. For 1st harvest, fastest DW growth was recorded in PD treatment, which covered the water surface in 2-3 days, followed by CD+PD (3-4 days), CD (4-5 days) and AC (5-6 days)/VC (5-6 days) treatment. For 2nd harvest, the DW covered the entire water surface in 3-4, 3-4 and 5-6 days in PD, CD+PD and CD treatments, respectively; while it recorded as 6-7 days in case of AC and VC treatments.

Highest protein content (%) was found in DW harvest from PD treatment (26.46-26.72) followed by CD+PD (25.65-25.87), VC (20.79-21.03), AC (18.63-19.88) and CD (18.18-18.93) treatments (Fig. 2). Relative ash content in DW harvest from different treatments were found in the order PD > CD+PD > VC = AC ≥ CD, while the DW fat content was found in the order CD+PD > PD ≥ VC = AC = CD. Significant differences among treatments in reference to availability of ammoniacal nitrogen, nitrates nitrogen and soluble phosphates effected growth and protein content of DW with a positive correlation. The DW biomass and protein content of DW *S. polyrhiza* was enhanced up to 48% and 43% by using CD+PD manure combination and PD manure, respectively.



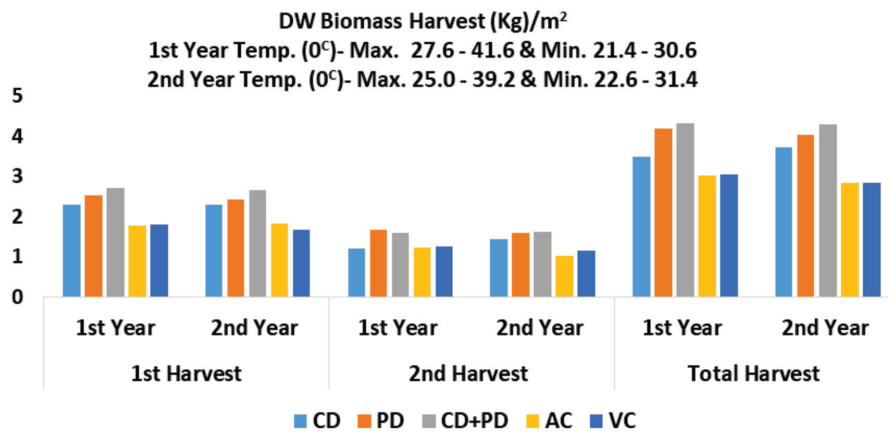


Fig. 1 Duckweed Biomass Harvested from Different Manure Treatments

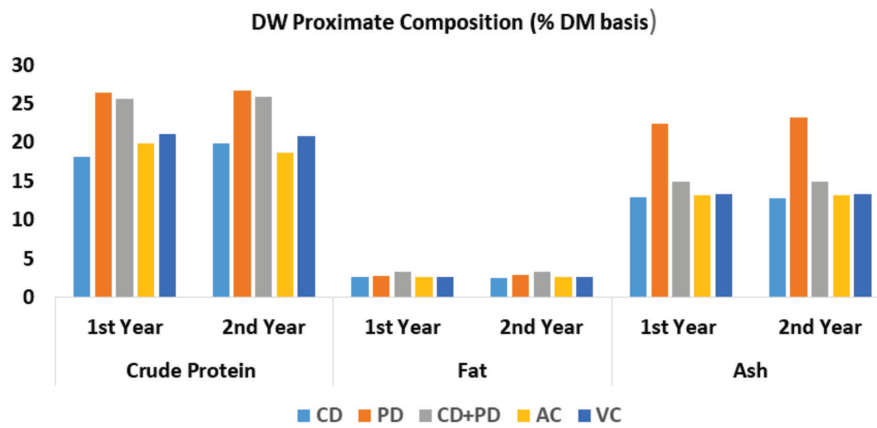


Fig. 2 Proximate Composition of DW Harvested from Different Manure Treatments

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2.10 Synergistic Effect of Prebiotic with Gut Isolated Probiotic Bacteria on Growth and Health of Rohu, *Labeo rohita*

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Keywords: Prebiotics, β -glucan (BG), Mannan oligosaccharide (MOS), Synergistic effect

Introduction

In India, Inland fish production has crossed 11 million tons during the year 2021 contributing over 76% of the total fish production. However, outbreaks of diseases are major hurdle in intensification and harnessing full potential of carp farming. Overuse of antibiotics and therapeutics is causing negative impacts like development of antimicrobial and drug resistance, accumulation of chemical residues in tissue and reduced consumer preference (Anderson 1992). The oligosaccharides prebiotics are reported to reduce β -glucuronidase and nitroreductase activities resulting in the enhancement of immunity and modulation of mucin production and the expression of immune regulatory genes. Moreover, prebiotics can have the additive effects to establish and stimulate probiotic bacterial growth rapidly inside the gut. This study was carried out to investigate the effect of prebiotics compounds on rohu fish in different doses when it is used in combination with probiotics bacteria isolated from fish gut.

Materials and Methods

The study was conducted in outdoor cemented tanks (20 m²). Fingerlings of rohu, *L. rohita* (720 nos.) were distributed randomly in experimental tanks (40/tank) in triplicate. Prebiotics β -glucan (BG) and Mannan oligosaccharide (MOS) was added in basal diet (T1) at 2 different levels (T2 @ 0.2% BG + 10⁸ *Lactobacillus plantarum*, T3 @ 0.5% BG + 10⁸ *L. plantarum*, T4 @ 0.2% MOS+10⁸ *L. plantarum*, T5 @ 0.5% MOS + 10⁸ *L. plantarum*, and T6 @ 10⁸ *L. plantarum*) and fed to the fish for a period of 120 days @ 5% fish body weight daily in two split doses. Hematological parameters were observed at 30, 60 and 120 days intervals while growth parameters were observed at 30, 60, 90 and 120 days intervals. The blood samples were analyzed for hemoglobin (Hb), total erythrocyte count (TEC), total leukocyte count (TLC), Hematocrit (Ht) and Erythrocyte sedimentation rate (ESR). MCV, MCH and MCHC were calculated by the method of Mukherjee (1988). Total length and body weight was assessed at monthly intervals whereas carcass composition was assessed at the completion of the experiment.

Results and Discussion

Fish survival was 100% in control and all the treatments. Among different treatments, total body length (cm) increased from 8.70 to 16.13, 9.00 to 17.25, 8.78 to 18.73, 8.80 to 17.04, 8.67 to 18.21 and 8.79 to 17.01 in T1, T2, T3, T4, T5 and T6 respectively. Total body length (cm) was significantly higher ($p \leq 0.05$) in T3. Among different treatments, body weight (g) increased from 10.50 to 43.84, 10.47 to 56.60, 10.27 to 70.00, 10.38 to 52.16, 10.47 to 63.05, and 10.33 to 50.51 in T1, T2, T3, T4, T5 and T6 respectively. Average final total body weight (g) was maximum in T3 (70.00) and minimum in T1 (43.84). Difference were significant ($p \leq 0.05$) for hematological parameters. Elevated hemoglobin content was observed after 60 days which again reduced after 120 days but remained higher in comparison to control. Other hematological parameters were also observed improved in T3. Among biochemical parameters (carcass composition) flesh protein were significantly higher in T3 followed by T5, T2, T6 and T4. Among all the diets, combination of probiotic *L. plantarum* @ 10⁸ CFU/g and prebiotic β -glucan 0.5% BG was observed best and recommended for field trials at farmers' field.



Table 1. Comparative hematological parameters of rohu, *L. rohita* (Ham.) at different time intervals in different treatments

Parameters	Days	Treatments*					
		T1	T2	T3	T4	T5	T6
Hb (g %)	30	4.23±0.00 ^{e,1}	5.06±0.04 ^{c,3}	5.41±0.0 ^{a,3}	4.91±0.02 ^{d,1}	5.20±0.00 ^{b,2}	4.94±0.03 ^{d,2}
	60	4.33± 0.03 ^{f,1}	5.53± 0.03 ^{c,1}	6.44±0.07 ^{a,1}	5.36± 0.03 ^{d,2}	6.21±0.06 ^{b,1}	5.20± 0.05 ^{e,1}
	120	4.32± 0.04 ^{d,1}	5.22±0.05 ^{b,2}	5.73±0.09 ^{a,2}	5.01± 0.04 ^{c,2}	5.25±0.05 ^{b,2}	4.96±0.03 ^{c,2}
RBC (x10 ⁶ mm ⁻³)	30	1.71±0.02 ^{c,1}	1.75±0.01 ^{bc,2}	1.75±0.03 ^{bc,2}	1.82±0.01 ^{ab,3}	1.86±0.02 ^{a,3}	1.87±0.03 ^{a,2}
	60	1.82±0.01 ^{d,1}	2.06±0.01 ^{bc,1}	2.17±0.02 ^{a,1}	2.07±0.02 ^{bc,1}	2.11±0.02 ^{ab,1}	2.02±0.01 ^{c,1}
	120	1.79±0.04 ^{c,1}	1.96±0.05 ^{ab,1}	2.08±0.04 ^{a,1}	1.91± 0.03 ^{bc,2}	2.04±0.00 ^{a,2}	1.90±0.03 ^{bc,2}
WBC (x10 ³ mm ⁻³)	30	3.87±0.02 ^{c,1}	4.84±0.02 ^{c,1}	6.01±0.08 ^{a,1}	4.55±0.05 ^{d,1}	5.32±0.07 ^{b,1}	4.48±0.05 ^{d,1}
	60	3.95±0.08 ^{e,1}	4.65±0.04 ^{c,2}	5.22±0.06 ^{a,2}	4.35±0.05 ^{d,2}	5.01±0.08 ^{b,2}	4.29±0.02 ^{d,2}
	120	3.83±0.04 ^{c,1}	4.35±0.06 ^{c,3}	4.96±0.12 ^{a,2}	4.16±0.05 ^{cd,2}	4.67±0.02 ^{b,3}	4.07±0.02 ^{d,3}
PCV (%)	30	21.93±0.08 ^{d,1}	23.63±0.31 ^{bc,3}	24.56±0.06 ^{a,3}	22.96±0.08 ^{d,3}	23.83±0.03 ^{b,3}	23.33±0.12 ^{cd,2}
	60	23.50±0.20 ^{d,1}	26.43± 0.14 ^{bc,1}	27.76±0.14 ^{a,1}	26.46± 0.23 ^{bc,1}	26.76±0.38 ^{b,1}	25.93±0.03 ^{c,1}
	120	23.47±0.12 ^{c,1}	25.10±0.15 ^{b,2}	26.45±0.25 ^{a,2}	25.05±0.28 ^{b,2}	25.43±0.46 ^{b,2}	24.84±0.05 ^{b,2}
MCV (µm ³)	30	126.84±1.62 ^{b,1}	134.55±2.16 ^{a,1}	139.95±2.69 ^{a,1}	126.21±1.50 ^{b,1}	127.95±1.69 ^{b,1}	124.42±2.43 ^{b,1}
	60	128.90±1.67 ^{a,1}	127.93±1.77 ^{a,1}	127.79±1.37 ^{a,2}	127.71±2.57 ^{a,1}	126.70±2.54 ^{a,1}	128.39±0.59 ^{a,1}
	120	130.79±2.48 ^{a,1}	128.03±3.32 ^{a,1}	126.85±1.52 ^{a,2}	131.23±1.92 ^{a,1}	124.69±2.62 ^{a,1}	130.36±2.35 ^{a,1}
MCH (g %)	30	24.46±0.39 ^{e,1}	28.81±0.44 ^{b,1}	30.84±0.65 ^{a,1}	26.98±0.09 ^{cd,1}	27.91±0.33 ^{bc,2}	26.35±0.35 ^{d,1}
	60	23.77±0.27 ^{c,1}	26.77±0.30 ^{b,2}	29.66±0.64 ^{a,12}	25.88±0.15 ^{b,12}	29.42±0.51 ^{a,1}	25.74±0.43 ^{b,1}
	120	24.08±0.83 ^{b,1}	26.66±0.82 ^{a,2}	27.51±0.96 ^{a,2}	26.28±0.45 ^{ab,2}	25.73±0.18 ^{ab,3}	26.06±0.48 ^{ab,1}
MCHC (g %)	30	19.28±0.07 ^{c,1}	21.42±0.41 ^{ab,1}	22.03±0.04 ^{a,12}	21.38±0.20 ^{ab,1}	21.82±0.03 ^{ab,2}	21.19±0.24 ^{b,1}
	60	18.44±0.30 ^{d,1}	20.93±0.30 ^{b,1}	23.21±0.38 ^{a,1}	20.28±0.29 ^{bc,2}	23.22±0.12 ^{a,1}	20.05±0.24 ^{c,2}
	120	18.40±0.28 ^{c,1}	20.82±0.12 ^{ab,1}	21.67±0.49 ^{a,2}	20.03±0.35 ^{b,2}	20.66±0.52 ^{ab,3}	19.99±0.16 ^{b,2}
ESR	30	2.36±0.005 ^{a,1}	2.31±0.003 ^{bc,1}	2.27±0.005 ^{c,1}	2.32±0.003 ^{b,1}	2.30±0.008 ^{cd,1}	2.29±0.003 ^{d,1}
	60	2.35±0.00 ^{a,1}	2.18±0.01 ^{b,2}	2.06±0.03 ^{c,2}	2.20± 0.00 ^{b,3}	2.07± 0.01 ^{c,2}	2.21± 0.01 ^{b,2}
	120	2.82± 0.01 ^{a,2}	2.27± 0.01 ^{a,1}	2.21±0.01 ^{b,1}	2.28±0.01 ^{a,2}	2.28±0.01 ^{a,1}	2.30±0.00 ^{a,1}

Values are Mean ± S.E. (p≤0.05)

Values with same superscription in a row do not differ significantly (p ≤ 0.05)

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2.11 Comparative Efficacy of Duckweed, Cane Molasses and Corn Starch as Fish Feed Binder: Proximate Composition, Water Stability, Sinking Time and Storage Quality

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Keywords: Binder, Duckweed, Feed, Fish, Stability, Storage, Quality

Introduction

In pellet fish feed manufacturing, traditionally used binders (molasses and corn starch) are carbohydrate rich ingredients with good binding properties to enhance water stability of the pellets. Some potential protein alternatives need to be explored, which can serve as an equally good binder and also add some extra protein to the feed. Duckweeds (DW) holds immense potential in this context, due to high nutritive value, cuticular waxy components and antifungal properties (Leng et al 1995 and Effiong et al 2009). The protein content of DWs varies from 19 to 48 % on dry matter (DM) basis, with species, nutrient availability and season. The DWs are expected to add value to pellet feeds not just as a cost-effective potential binder due to its waxy properties, but also enhance its nutritive value, physical properties (water retention time/stability and sinking time) and storage life. Hence, efficacy of DW paste (*Spirodela polyrhiza*) as binder was evaluated in comparison to traditional binders, molasses and corn starch.

Materials and Methods

Six (6) experimental compressed sinking pellet feeds i.e. without binder control feed (CF), 5% molasses binder feed (MF5), 5% corn starch binder feed (CSF5), 5% duckweed (DW) binder feed (DWF5), 7.5% DW binder feed (DWF7.5) and 10% DW binder feed (DWF10), were made with the help of a mechanical pelletizer and stored in airtight polybags.

The experimental feeds were tested for proximate composition, water stability and sinking time at monthly intervals for 8 months, while storage spoilage (bacterial load and aflatoxin) was evaluated at monthly intervals for 6 months. The proximate analysis of feeds (crude protein, fat, fibre, ash and nitrogen free extract) was carried out following standard methods. Sinking time of pellets was recorded in terms of time taken by the pellets to travel down a water filled beaker with the help of stop watch. Thereafter, water stability of pellets was evaluated in terms of time in which it totally disintegrated into an undefined mass. The bacterial load in terms of total plate count (TPC) and aflatoxin contamination in the experimental feeds was estimated as per standard methods. Statistical analysis of data was conducted with the help of One-Way ANOVA (Statistical package SPSS 20.0), followed by Duncan's Multiple Comparisons ($p \leq 0.05$).

Results and Discussion

The DW paste appeared as a more effective binder in comparison to molasses and corn starch. DW incorporation did not alter the protein content of the feed (Table 1) and protein content of DW binder feeds was comparable with control feed (CF). However, the 8-month storage average protein content was lowered by 5.35 and 11.77% in case of molasses and corn starch binder feeds. Further, DW paste also improved the water stability of feed pellets significantly. The maximum 8-month average water stability recorded in DWF7.5 binder feed was 42.99, 43.66 and 28.57% higher as than CF, MF5 and CSF5 feeds, respectively (Table 1). The DW binder feeds exhibited higher average sinking time (enhanced column availability). The 8-month average sinking time was recorded as 4.51, 5.17 and 6.29 seconds^{-cm} sink in DWF5, DWF7.5 and DWF10 feeds, respectively as compared to 4.02, 3.90 and 2.68 seconds^{-cm} sink recorded in CF, MF5 and CSF5 feeds, respectively (Table 1). Microbial contamination (TPC) and aflatoxin level in all the DW binder feeds reduced significantly during the storage period of 6 months, indicating antimicrobial and antifungal properties of duckweed (Fig. 1 & 2).

The results indicate that duckweed paste can be used as an effective protein rich binder to develop fish pellet (sinking) feed, without altering its nutritive value; and with add-on benefits of extended feed availability/stability in water and enhanced storage quality of feed.

Table 1. Protein content, Water Stability and Sinking Time of Experimental Feeds

Experimental Feed	Average Protein Content (% DM basis)		Water Stability (Hrs.)		Sinking Time (Seconds ^{-cm sink})	
	Day-1	Day-240	Day-1	Day-240	Day-1	Day-240
CF	26.69 ^a ± 0.21	26.72 ^a ± 0.10	2.12 ^e ± 0.01	2.15 ^e ± 0.00	4.01 ^b ± 0.17	4.03 ^d ± 0.10
MF5	25.66 ^b ± 0.04	25.53 ^b ± 0.02	2.09 ^f ± 0.00	2.18 ^d ± 0.01	3.94 ^b ± 0.10	3.89 ^d ± 0.06
CSF5	24.91 ^c ± 0.19	24.24 ^c ± 0.12	2.31 ^d ± 0.00	2.38 ^c ± 0.01	2.42 ^c ± 0.40	2.62 ^e ± 0.12
DWF5	26.84 ^a ± 0.34	26.91 ^a ± 0.19	2.46 ^c ± 0.00	2.39 ^c ± 0.00	4.18 ^b ± 0.57	4.56 ^c ± 0.28
DWF7.5	26.63 ^a ± 0.01	26.87 ^a ± 0.02	3.07 ^a ± 0.01	3.07 ^a ± 0.00	5.41 ^a ± 0.35	5.14 ^b ± 0.17
DWF10	26.45 ^a ± 0.21	26.77 ^a ± 0.09	2.49 ^b ± 0.00	2.51 ^b ± 0.01	6.49 ^a ± 0.36	6.27 ^a ± 0.10

Values with different superscripts in a row differ significantly (P<0.05)

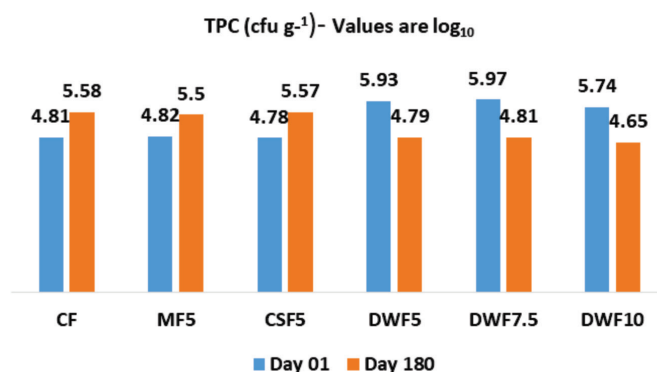


Fig. 1 Microbial Load (TPC) in experimental feeds during the Storage Period

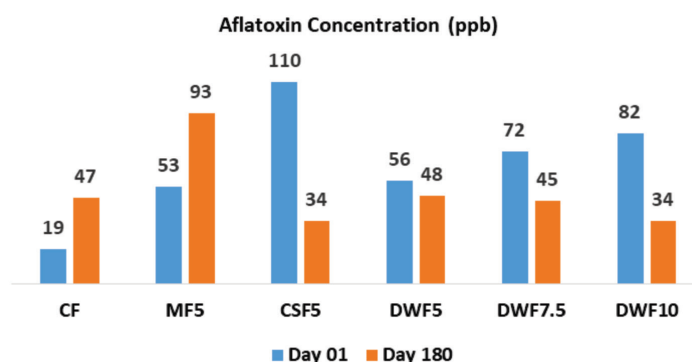


Fig.2 Aflatoxin Concentration in experimental feeds during the Storage Period

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2.12 Effects of Dietary Supplementation with Amla (*Phyllanthus emblica*) Fruit Powder on Survival and Growth of Genetically Improved Farmed Tilapia (GIFT, *Oreochromis niloticus*)

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Keywords: Amla, Herbal, Growth, GIFT,

Introduction

Aquaculture production can be increased through the efficient and effective use of nutrients in feed to meet market demand. It is estimated that 60-70% of the cost of aquaculture is associated with feed, a major factor determining the economic success of the industry. However, herbal supplements can be used to reduce feed costs by enhancing feed efficiency and effectiveness. Few studies have demonstrated that herbs such as garlic, aloe vera, ashwagandha, amla, and ginger have a positive effect on fish growth and also help to improve the quality of fish meat. Thus, this study investigated the effects of amla fruit powder on the growth, feed efficiency, and meat quality of genetically improved farmed tilapia (GIFT, *Oreochromis niloticus*).

Material and Methods

The experiment was carried out in outdoor fiberglass-reinforced plastic (FRP) tanks (water holding capacity of approximately 1.0 m³) at the Instructional cum Research Farm of College of Fisheries, GADVASU, Ludhiana for 90 days. Each tank was stocked with pre-acclimatized healthy, active and equal size of fingerlings of GIFT strain of Nile tilapia (10.13±0.01 g @ 20 numbers) procured from GKVK, Bengaluru, Karnataka. For amla fruit powder (AFP) preparation, fresh good quality amla (*P. emblica*) pieces were dried with freeze drying method using lyophilizer and stored in air tight container. A total of six diets were formulated with various inclusion levels of dietary AFP, including 0, 1, 2, 3, 4, and 5% in T0 (control), T1, T2, T3, T4, and T5, respectively. Fish were fed up to satiation level twice a day with each diet in triplicate. Fish growth was assessed at monthly intervals, whereas mortality was recorded on daily basis. The data were statistically analyzed by one-way analysis of variance and significant difference between treatments means was determined by Duncan's multiple range tests.

Results and Discussions

Fish growth is affected by various factors like fish species, nutrients in feed, feed additives, and the rearing environment. The present study tested five levels of AFP dietary incorporation in fish diets against the control. The incorporation of AFP in the fish diet showed a significant improvement in growth performance and survival of GIFT, *O. niloticus* during 90 days experiment (Table 1). No significant differences were observed in the initial total body length (cm) and initial body weight (g) of fish in the different treatments. The dietary inclusion of AFP in fish diets significantly affected final body length ($P<0.05$). Further, the fish fed with 3% AFP diet had the highest final body weight compared with other AFP diets and control diets. Net weight gain (NWG) and total length gain (TLG) were significantly higher ($P<0.05$) in all AFP-fed treatments than in the control at the end of the experiment. Based on the results of the second-order polynomial regression analysis ($y = -0.0164x^2 + 1.036x + 20.77$; $R^2 = 0.8275$), dietary supplementation of AFP up to 30 g kg⁻¹ appear to be the most effective in enhancing fish growth with respect to net weight gain (Fig. 1). Compared to all other AFP treatments and control, FCR was significantly ($P<0.05$) lower in T3 (1.45), while PER showed a reverse trend and was significantly ($P<0.05$) higher in T3 (2.61) (Table 1). The fish survival was recorded as 90.00, 93.33, 96.67, 100.00, 98.33, and 96.67 % in T0 (control), T1, T2, T3, T4, and T5, diets, respectively. The inclusion of AFP in fish diets showed no significant difference in fish survival ($P>0.05$). However, T3 (3% AFP inclusion level) treatment recorded the highest survival (96.88%) than control and other AFP-fed diets. The results of the proximate composition of GIFT fingerlings in terms of protein, fat, carbohydrates, ash, and moisture contents revealed that the addition of AFP to fish feed significantly improved meat quality ($P\leq 0.05$). The growth-promoting properties of amla have the positive effect of improving digestion, absorption, liver function, and assimilation of foods, as well as improving protein synthesis and flesh quality. The present findings depict that incorporation of 30 g kg⁻¹ AFP in the fish diet might help to enhance the survival and growth performance of GIFT (*O. niloticus*).



Table 1 Growth performance of GIFT, *Oreochromis niloticus* after 90 days of feeding trial

Parameters	C	T1	T2	T3	T4	T5
Initial weight (g)	29.77	29.61	30.34	29.47	30.12	29.87
Final weight (g)	52.82	55.79	63.07	70.14	67.21	59.78
NWG (g)	23.05	26.18	32.73	40.67	37.09	29.91
SGR	0.64	0.70	0.81	0.96	0.89	0.77
FCR	2.58	2.26	1.85	1.45	1.62	2.01
PER	1.45	1.66	2.03	2.61	2.34	1.9
Survival (%)	90.00	93.33	96.67	100.00	98.33	96.67

SGR= NWG= Net weight gain; Specific growth rate; PER= Protein efficiency ratio; FCR= Feed conversion ratio

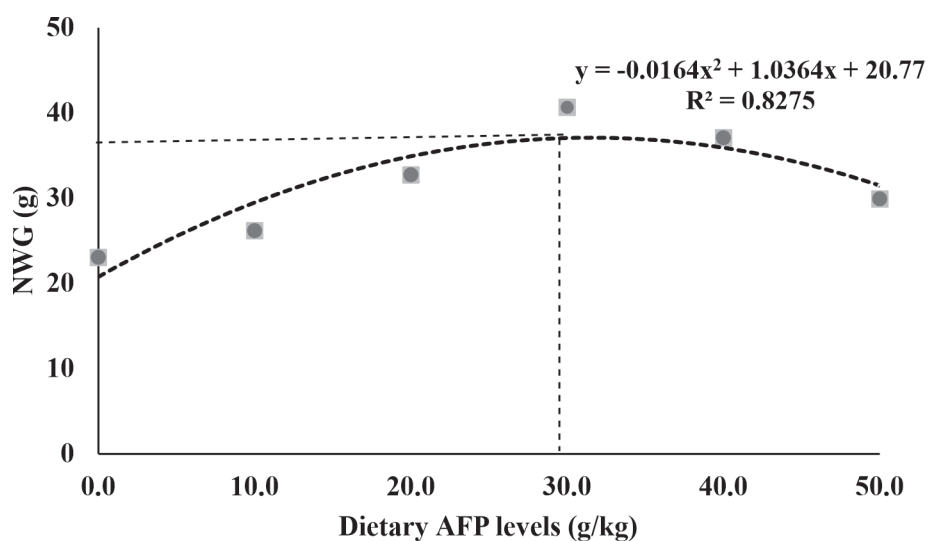


Fig. 1. Second-order polynomial regression analysis of NWG against graded dietary AFP levels

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2.13 Effect of Immunostimulants on Biochemical Parameters of Striped Catfish, *Pangasianodon hypophthalmus* (SAUVAGE, 1878)

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Keywords: Immunostimulants, Striped catfish, Challenge test

Introduction

Freshwater aquaculture sector of India is mainly dominated by Indian major carps. However, with the initiative from the Government of India, pond and cage culture practices of striped catfish are being spread rapidly in the recent past. The species is also well known for its faster growth, high disease resistance and tolerance of a wide range of environmental parameters. Conventionally, antibiotics, vaccines and chemicals are used to prevent or treat fish diseases. Furthermore, the antibiotic residues can be transferred to the aquaculture environment, to fish pathogens and could be accumulated in the fish bodies posing danger to human health. In the present study, some of the common immunostimulants such as brewer's yeast, L-ascorbic acid, α -tocopherol and ginger were used to evaluate their effect on the biochemical parameters of striped catfish, *Pangasianodon hypophthalmus* in an attempt to find out environment-friendly disease prevention strategy.

Materials and Methods

Experimental diets containing brewer's yeast @ 10 g (F1), L-ascorbic acid @ 1000 mg (F2), α -tocopherol @ 80 mg (F3) and ginger @ 10 g (F4) were added, mixed and blended per kilogram of basal feed according to Parmar et al (2012), Ispir et al (2011), Sobhana et al (2002) and Sahan et al (2016), respectively. The control group was fed without any immunostimulants in the diet. The experiment was subjected to five treatments and four replicates using completely randomized design (CRD). The fishes were fed @ 5% of their body weight twice a day. The feeding experiment was conducted for a period of 90 days. After 90 days of feeding experiment, experimental fishes were challenged intra-peritoneally with pathogenic bacteria, *Aeromonas hydrophila*. Analysis of biochemical parameters such as albumin, globulin, total serum protein, glycogen and glucose was carried out during pre- and post-challenge study.

Results and Discussion

The fishes groups fed ginger incorporated diet revealed significantly ($P < 0.05$) higher content of albumin, globulin and the serum protein than that of the other treatments. The maximum serum glucose was found in the vitamin C. With regard to glycogen, no significant difference ($P > 0.05$) among the treatments in pre-challenge group. A similar trend was observed in post-challenge tests. There was a significant difference ($P < 0.05$) between pre- and post-challenged groups for all the treatments. The relative percentage of survival was also found to be the highest in the fishes treated with ginger diet. The findings of the study are expected to help the aquaculturists and the feed manufacturers to make the use of ginger as a natural immunostimulant in feed to enhance the immunity of striped catfish.

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2.14 Liver and Intestinal Histoarchitecture of *Labeo rohita* Fingerlings Fed with Different Inclusion Level of De-oiled Rice Bran

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Keywords: Rohu, Rice bran, Feed, Cotton seed oil cake

Introduction

Aquaculture production depend on many factors but the feed is always at the top and accounts for more than 50% of the total input cost. The formulation and preparation of balanced and cost-effective fish feed, have always been a challenging task for both aquaculturists and nutritionists. Species-specific feed formulations cannot only support maximum fish production but also can reduce the cost of production. Major feed ingredients used by farmers for preparation of feed of Indian major carps are de-oiled rice bran (DORB), groundnut oil cake (GNOC), cotton seed oil cake (CSOC), raw rice bran or rice polish, sorghum, wheat bran, soybean cake or meal (SBC or SBM) etc. DORB is the major and most commonly used feed ingredient for carp feeds and is used by carp farmers in the country due to their easy availability and low cost either singly or in combination with other ingredients.

To our knowledge, only limited studies have been conducted on the histoarchitecture of liver and intestine fed with different level of DORB to *L. rohita*. The aim of the present study is to examine the histological alteration of liver and intestine of *L. rohita* fingerlings fed with different inclusion levels of DORB.

Materials and Methods

A 60 day feeding trial was conducted to study the liver and intestinal histoarchitecture of *L. rohita* fingerlings fed with different inclusion level of de-oiled rice. Six iso-nitrogenous (26%), iso-lipidic (7%) and iso-energetic (374 Kcal/100g) diets were prepared with different inclusion level of DORB viz., T33 (33% DORB), T38 (38% DORB), T43(43 % DORB), T48 (48% DORB), T53 (53% DORB) & T58 (58% DORB). One hundred and eighty fingerlings (Average Weight 9.8 ± 0.5 g) were randomly distributed in seven experimental groups in triplicates following a completely randomized design. The Histology of intestine and liver was done as per the method of Ranjan et al (2021).

Results and Discussion

Histological examination of liver and intestine showed no difference among different treatment groups, and in these organs, only normal cells were observed. The histological examination of the liver revealed round hepatic nuclei with dispersed chromatin and prominent nucleoli and granular cytoplasm. The liver histological section of the fish from all the groups revealed no pathological alteration and showed normal histo-architecture with typical organization of this organ. The histological examination of the liver revealed round hepatic nuclei with dispersed chromatin and prominent nucleoli and granular cytoplasm. The histological section of the intestine showed (Fig 1) normal histo-architecture, well-defined intestinal villi and distinct goblet cells and lymphocyte in the fish fed with different experimental diets. Our earlier study revealed that the inclusion level of de-oiled rice bran above 43% leads to the irrational increase in metabolic activity and compromised immune responses (Kumar et al 2020 and Ranjan et al 2021) however there were no histological changes were observed in the liver (Fig 2) and intestine (Fig 1) of the tissue. This confirm that even a higher dietary inclusion of de-oiled rice bran is not having a tissue specific changes in the *L. rohita*, however, due to metabolic disturbances are inevitable, which is due to the poor absorption of nutrient at high inclusion level of DORB in the diet of *L. rohita*. Similarly, Ranjan et al (2021) also reported a normal histoarchitecture of liver and intestine of *Labeo rohita* fed enzyme supplemented DORB based diet at 90% inclusion level. The present study revealed unaltered histoarchitecture of liver and intestine even at 58 % inclusion of DORB in the diet of *L. rohita*.

Table 1 Ingredient composition of the different experimental diets

Ingredients (%)	T33	T38	T43	T48	T53	T58
Casein	18.70	18.00	17.30	16.40	16.00	15.60
Gelatin	4.50	4.40	4.30	4.40	4.00	3.50
Dextrin	5.00	5.00	5.00	5.00	5.00	5.00
Starch	23.50	20.20	17.00	13.90	9.70	6.60
DORB	33.00	38.00	43.00	48.00	53.00	58.00
Sunflower oil	3.50	3.50	3.50	3.50	3.50	3.50
Cod liver oil	3.50	3.50	3.50	3.50	3.50	3.50
Cellulose	5.28	4.38	3.38	2.28	2.28	1.28
CMC ^a	1.00	1.00	1.00	1.00	1.00	1.00
Vit. min. mix	1.90	1.90	1.90	1.90	1.90	1.90
Vitamin C	0.10	0.10	0.10	0.10	0.10	0.10
BHT	0.02	0.02	0.02	0.02	0.02	0.02

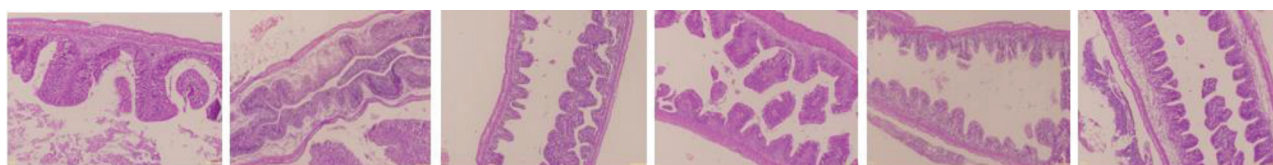


Fig 1 Intestine of *L. rohita* fingerlings fed with different level of de-oiled rice bran

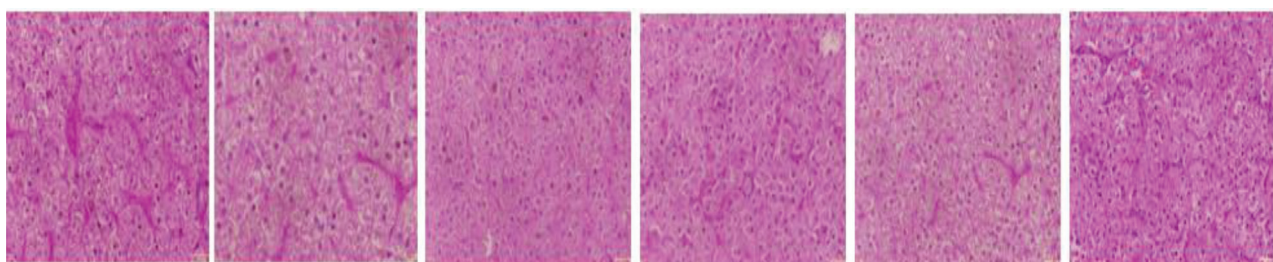


Fig 2 Liver of *L. rohita* fingerlings fed with different level of de-oiled rice bran

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2.15 Harnessing Nutritional Potential of Duckweed as Protein Source in Ruminant Diet

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Keywords: Degradability, Duckweed, Gas production, *In-vitro* studies

Introduction

Feed shortage is the major constraint for optimum production performance of ruminants and these animals are generally fed on high roughage diet to meet out their requirement particularly in tropical countries. Therefore, addition of concentrate mixture is a crucial component to meet the nutrients requirement particularly protein requirement. Global demand for alternative feed ingredients has risen sharply due to steep hike in the prices of traditional feed ingredient in past few years. In this context, duckweed can be considered as an alternative protein source having about 15-35 % crude protein (CP) with a superior amino acid profile than most plant proteins (Demann et al 2023) along with about 10-30 % total ash. Further, duckweed is the fastest-growing aquatic flowering plant with almost exponential growth as it can produce about 10-30 tons of DM/ha/year. Therefore, the aim of this research is to explore the nutritional potential of duckweed as protein source in the diet of ruminants.

Materials and Methods

The duckweed sample used in the *in-vitro* study was obtained from the College of Fisheries, GADVASU, Ludhiana. The samples were air-dried and then ground in a Wiley mill through a 1 mm screen and were analysed for dry matter (DM), crude protein (CP), ether extract (EE) and ash content. In the present study, duckweed was used as protein source replacing concentrate in total mixed ration (TMR) having concentrate mixture and roughage in 70:30 ratio (Table 1). Six different experimental substrates of TMR were prepared with graded duckweed levels i.e., Control (0 duckweed), TMR-5 (5% duckweed), TMR-10 (10% duckweed), TMR-15 (15% duckweed), TMR-20 (20% duckweed) and TMR-30 (30% duckweed).

For study of *in-vitro* gas production (Menke et al., 1979), rumen liquor was collected from fistulated buffalo bulls fed on standard diet (roughage: concentrate in 70: 30 proportion) in the morning (6 AM) before feeding and watering into a pre-warmed thermo-flask and brought to the laboratory. Each substrate was incubated (triplicate) for 24 h in Menke glass syringe and after 24 h of incubation total gas production was recorded in each syringe. For determining the true dry matter (IVDMD) and organic matter (IVOMD) digestibility, the contents of syringes were transferred to spout-less beaker, boiled with neutral detergent. The partitioning factor (PF) and microbial biomass production (MBP) were calculated based on IVTDOM (Blümmel et al 1999). Data from *in-vitro* study was analyzed 1x3 factorial design (Snedecor and Cochran 1994), by using SPSS Version 19. The differences in means were tested by Tukey B.

Results and Discussion

Duckweed used in the present study contained about 7.55% DM, 24.5% CP, 3.5% EE and 20.07% ash. The results of *in-vitro* study showed that total gas production (TGP) was affected linearly ($P < 0.001$) and quadratically ($P < 0.001$) with inclusion of duckweed. It was observed that TGP of TMR-5 and TMR-10 was remained statistically similar to the control diet and decreased significantly thereafter with lowest value at TMR-30. IVDMD and IVOMD were statistically higher in TMR-10 (75.0 and 75.8% respectively) as compared to TMR-30 and remained statistically comparable to all other groups. In contrast, PF value was observed to be statistically higher in TMR-30 (4.82mg/dl) as compared to all other treatments except TMR-20, whereas MBP production remained statistically similar among all the treatment group. PF is a measure of efficiency of MBP *in-vitro* and theoretical range of PF in ruminant diets should be 2.74-4.41 (Blümmel et al 1997). The mean values of PF were ranged within the theoretical range upto 15 % replacement of concentrate mixture. The ME value of various TMR substrates was found to be decreased significantly both linearly ($P < 0.001$) and quadratically ($P < 0.001$) after 10 % replacement of concentrate mixture with duckweed with lowest value at TMR-30 (5.15 MJ/kg). Therefore, it can be concluded that upto 10 % replacement of concentrate mixture can be done by duckweed in a TMR having 70:30 roughage to concentrate mixture ratio without any adverse effect.



Table 1: Ingredient composition of total mixed ration (roughage: concentrate ratio, 70:30) having graded level of duckweed

Attributes	Duckweed	Concentrate mixture	Maize silage	Wheat straw
Control	0	30	50	20
TMR-5	5	25	51	19
TMR-10	10	20	53	17
TMR-15	15	15	54	16
TMR-20	20	10	55	15
TMR-30	30	0	57	13

Table 2: *In vitro* evaluation of different level of duckweed as protein source in total mixed ration (70:30 roughage to concentrate ratio)

Parameters	Control	TMR-5	TMR-10	TMR-15	TMR-20	TMR-30	SEM	P-Value	
								Linear	Quadratic
Total gas (ml)	161.5 ^a	158.2 ^a	156.5 ^{ab}	151.5 ^b	143.2 ^c	128.2 ^d	2.20	<0.001	<0.001
IVDMD%	74.2 ^{ab}	73.3 ^{ab}	75.0 ^a	73.3 ^{ab}	72.5 ^{ab}	70.5 ^b	1.13	0.028	0.052
IVOMD%	74.8 ^a	73.8 ^{ab}	75.8 ^a	74.1 ^{ab}	72.9 ^{ab}	70.9 ^b	1.07	0.003	0.028
PF, mg/dl	4.25 ^b	4.25 ^b	4.37 ^b	4.37 ^b	4.51 ^{ab}	4.82 ^a	0.11	<0.001	0.048
MBP, mg/dl	64.6	63.2	66.3	64.3	64.8	65.7	2.50	0.570	0.939
ME (MJ)	6.12 ^a	6.02 ^a	5.98 ^{ab}	5.83 ^b	5.59 ^c	5.15 ^d	0.063	<0.001	<0.001

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2.16 Dietary Supplementation of *Lactobacillus plantarum* Enhances Survival, Growth and Health Status of *Cirrhinus mrigala* Fingerlings

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Keywords: *Cirrhinus mrigala*, Diet, Growth, Health status, *Lactobacillus plantarum*

Introduction

Since adding probiotics to feed improves animal performance, feed manufacturers have paid close attention to this practise. The maintenance and enhancement of the animal's performance (production and growth), as well as the prevention and management of enteric infections, are the main goals of employing probiotics in animal feed (FAO 2020). The use of probiotics in aquaculture is now widely acknowledged as there is a growing desire for environmentally friendly aquaculture. However, we still need to learn more about gut microbiology, how to prepare it effectively, and how safe probiotics are. Research and development are desperately needed in this area. As a result, the current study's objective is to determine the impact of nutritional supplementation with *Lactobacillus plantarum* on the survival, growth, and general health of mrigal, *Cirrhinus mrigala* fingerlings.

Materials and Methods

The experiment was carried out in outdoor FRP ponds at the Instructional Cum Research Farm of the College of Fisheries, GADVASU, Ludhiana. The basal diet consisted of rice bran, mustard meal, vitamin and mineral mixture, and salt (49, 49, 1.5 and 0.5 % respectively). *L. plantarum* FLB1, a probiotic bacterial strain (already identified, isolated and appropriately stored at COF, GADVASU), was sprayed to the base diet at varying concentrations (0, 10⁶, 10⁷, 10⁸, 10⁹ CFU/g, in T0, T1, T2, T3, and T4, respectively). FRP pools were used for experiment and the fish was stocked @ 15 fingerlings each, and experimental feeds were provided for 120 days (May to August 2022). Haematological and immunological parameters were assessed using established techniques, whilst water quality (APHA 2012) and growth parameters were examined. To determine differences between the treatments, the data were statistically evaluated using one-way analysis of variance (ANOVA) and Duncan's multiple comparisons.

Results and Discussion

All the water quality parameters were well within the acceptable limit for carp culture. At the conclusion of the experiment, probiotic supplementation significantly improved blood Hb, TEC, TLC, PCV/Ht, MCV, MCH, and MCHC levels (P≤0.05), in addition to improving growth performance and feed utilisation. Similar improvements in total serum protein, albumin, and globulin were seen, all of which were significantly better (P≤0.05). The levels of TG, HDL, total cholesterol, and blood sugar were all significantly (P≤0.05) lower. All probiotic-fed groups showed improvements in non-specific immune parameters, including respiratory burst activity and serum lysozyme activity. In all of the probiotic-fed groups, a significant increase (P≤0.05) in the meat protein content was observed. Fish in probiotic-fed groups had a much higher count of lactic acid bacteria in their digestive tracts, which further demonstrated the survival and viability of *L. plantarum* in fish guts. The findings were supported by histomorphological alterations in the intestine, which resulted in increased villi height for better feed absorption, which in turn improved growth and health. Although all of the treatments showed higher relative percent survival and improved biochemical parameters when compared to control in the challenge study against the fish pathogen (*Aeromonas veronii*), the highest value was found in T4 (@10⁹ CFU/g feed), indicating maximum protection against the fish pathogen. *L. plantarum* FLB1 probiotic strain can therefore be supplemented (@ 10⁹ CFU/g diet) for improved growth and health status of *C. mrigala*.



Table 1 Growth and survival of *Cirrhinus mrigala* in different treatments during the experimental period

Parameters	Treatment*				
	T0	T1	T2	T3	T4
TLG	2.49 ^c ±0.03	2.80 ^c ±0.16	3.21 ^b ±0.20	3.52 ^{ab} ±0.09	3.77 ^a ±0.03
TLG%	21.13 ^d ±0.27	23.83 ^{cd} ±1.70	27.36 ^{bc} ±2.14	30.20 ^{ab} ±0.87	31.86 ^a ±0.17
NWG (g)	17.50 ^e ±0.51	19.61 ^d ±0.12	23.75 ^c ±0.14	26.26 ^b ±0.12	29.00 ^a ±0.19
NWG (%)	126.94 ^d ±4.18	141.62 ^c ±2.66	183.34 ^b ±3.66	205.77 ^a ±0.59	214.37 ^a ±1.87
SGR	0.68 ^d ±0.02	0.73 ^c ±0.01	0.87 ^b ±0.01	0.93 ^a ±0.00	0.95 ^a ±0.01
Condition Factor (K)	1.07 ^b ±0.01	1.08 ^b ±0.00	1.10 ^{ab} ±0.01	1.11 ^{ab} ±0.02	1.12 ^a ±0.01
Survival rate (%)	82.22 ^b ±2.22	91.11 ^a ±2.22	93.33 ^a ±0.00	95.56 ^a ±2.22	97.78 ^a ±2.22

*Values (Mean±S.E.) with different alphabetical superscripts in a row differ significantly (P<0.05)

TLG= Total length gain, NWG = Net weight gain, SGR = Specific growth rate

Table 2 Blood metabolic profile of *Cirrhinus mrigala* in different treatments at the end of experiment

Biochemical Parameters	Treatment*				
	T0	T1	T2	T3	T4
Total serum protein (gdl ⁻¹)	3.43 ^c ±0.05	3.85 ^d ±0.07	4.25 ^c ±0.09	4.55 ^b ±0.03	4.87 ^a ±0.06
Albumin (gdl ⁻¹)	1.15 ^c ±0.01	1.32 ^d ±0.01	1.44 ^c ±0.02	1.58 ^b ±0.02	1.70 ^a ±0.03
Globulin (gdl ⁻¹)	2.28 ^d ±0.04	2.53 ^c ±0.08	2.81 ^b ±0.08	2.97 ^{ab} ±0.04	3.17 ^a ±0.08
Alb/Glb ratio (gdl ⁻¹)	0.50 ^a ±0.00	0.52 ^a ±0.02	0.52 ^a ±0.02	0.53 ^a ±0.01	0.54 ^a ±0.02
Glucose (mgdl ⁻¹)	81.22 ^a ±0.34	78.08 ^b ±0.79	74.72 ^c ±0.95	68.62 ^d ±0.87	61.51 ^e ±0.34
Lipid profile					
Cholesterol (mgdl ⁻¹)	129.47 ^a ±0.26	122.76 ^b ±0.18	118.96 ^c ±0.29	113.94 ^d ±0.44	107.79 ^e ±0.45
Triglycerides (mgdl ⁻¹)	138.95 ^a ±0.85	132.31 ^b ±1.23	129.66 ^b ±0.53	122.64 ^c ±1.46	105.14 ^d ±0.89
High- Density Lipoprotein (mgdl ⁻¹)	62.90 ^a ±0.18	52.01 ^b ±0.31	48.09 ^c ±0.24	39.45 ^d ±0.28	28.28 ^e ±0.39

Values are Mean ± S.E., (P≤0.05), n=3

Values with same superscript (a, b,....e) in a row do not differ significantly (P≤0.05)

Alb/Glb = Albumin/ Globulin ratio

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2.17 Digestive Enzyme Profiling in Different Life Cycle Stages of the Rainbow Trout (*Oncorhynchus mykiss*, Walbaum 1792) from Kashmir Valley

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Keywords: Digestion, Enzymes, Fry, Metamorphosis, Trout

Diet, biochemical characteristics, and nutrient utilization during larval-juvenile ‘metamorphosis’ of fish should be considered with respect to enzyme-dependent changes in digestive tract anatomy and physiology. The larval intestine’s capacity and rates to hydrolyze and transport specific nutrients are genetically programmed to correspond to the diet ingested in nature. In this light, we should be able to produce artificial feed that will satisfy the needs of all larval fish. For most fish species, the digestive tract contains enzymes related to metabolism, digestion, absorption and assimilation of molecules including proteins, lipids and glycogen. In comparison to adult fish, small fish exhibit relatively low activity of the enzymes. The study includes the collection of about 200 samples of *O. mykiss* from the national trout fish farm Kokernag to estimate the enzyme profile of fish from fry to brood size stages. The trypsin activity among different life cycle stages of *O. mykiss*, the highest value was achieved in fry stage with a value of 95 U/mg followed by yearling stage (80 U/mg), lowest value was observed in table size stage (0.0098 U/mg). The total ALP activity in all life cycle stages of *O. mykiss* was found highest value in table size stage (85.16 U/mg) followed by yearling stage (39.72 U/mg). The lowest was achieved in fingerling stage (3.44 U/mg). The total lipase activity in all life stages was seen maximum in table size (273.16 IU/L) and minimum was recorded in yearling stage (25.38 IU/L). The total amylase activity in all life cycle stages of *O. mykiss* was highest recorded in yearling stage (208.24 IU/L) followed by the fry (151.57 IU/L) and lowest was seen in brood stage (1.73 IU/L). Various manipulation to trout feeds is now the need of the hour as feeding of trouts is highly cost intensive. So by feed manipulation according to digestive ability of various life cycle stages of *O. mykiss* keeping in view the enzyme activity of that stage, will lead to better growth by proper utilization of nutrients which in turn lead to improved productions of trout fish ultimately contributing not only to the economy but also to per capita consumption adding to health perspective. We now have an understanding that developing artificial diets for fish larvae is more complicated than just finding the right combination of nutrients. In the formulation of microdiets for fish larvae, a ‘holistic’ approach is required. Future research should focus on the chain-of-events of ingestion, digestion, assimilation and absorption, and the application of this knowledge to diet formulation.

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2.18 Effect of Linseed Oil Cake as a Dietary Protein Source on Digestibility, Survival and Growth Performance of the *Cyprinus carpio* (Linnaeus, 1758) Yearlings

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Keywords: Common carp, Dietary protein, Growth, Linseed oil cake

This study was conducted to evaluate the effect of Linseed Oil Cake as a Dietary Protein Source on digestibility, survival and growth performance of the *Cyprinus carpio* (Linnaeus, 1758) yearlings. For this purpose a 60 days of experiment was conducted during October to December. Ten fishes with an average body weight of 11.03 ± 0.14 gm which were distributed randomly in 20 tanks with four replications and fed with four inclusion levels of linseed oil cake which were 10% in T1, 20% in T2, 30% in T3 and 40% in T4. The fishes were fed @ 3% body weight per day in the morning and evening. Range of water quality parameters were found as; temperature $16.3-26.5^{\circ}\text{C}$; pH 7.4 to 8.7; EC 149-210 mS/cm; DO 5.2 to 7.8 mg/l; hardness 603 to 640 mg/l; alkalinity 116.3 to 122.5 mg/L.

The result showed significant ($p < 0.05$) response in growth performance of fish. The growth performance results were in following order:

Weight gain: 62.39 ± 0.59 g (T3) > 53.46 ± 0.59 g (T2) > 47.69 ± 0.59 g (T4) > 47.68 ± 0.59 g (T1) > 42.73 ± 0.59 g (T0).
Percent weight gain: 56.42 ± 0.54 (T3) > 48.44 ± 0.54 (T2) > 43.14 ± 0.54 (T1) > 42 ± 0.54 (T4) > 38.62 ± 0.54 (control).
SGR: 0.799 ± 0.007 (T3) > 0.705 ± 0.007 (T2) > 0.640 ± 0.007 (T1) > 0.638 ± 0.007 (T4) > 0.583 ± 0.007 (T0).
FCR: 5.44 ± 0.095 (T0) > 5.042 ± 0.095 (T1) > 4.928 ± 0.095 (T2) > 4.475 ± 0.095 (T3) > 4.126 ± 0.095 (T4).
The GCE 0.266 ± 0.002 (T3) > 0.235 ± 0.002 (T2) > 0.213 ± 0.002 (T1) = 0.213 ± 0.002 (T4) > 0.194 ± 0.002 (T0)

The average value of apparent protein digestibility was highest (74.415 ± 0.014) in 30% LOC(T3) inclusion level feed and lowest in 70.206 ± 0.014 (T2). Protein content in fish carcass was found in the range of 16.97% to 16.50%. Fat 5.58 to 4.66%, Ash 4.55 to 3.74%, Moisture 70.44 to 69.38%, Carbohydrate 4.27 to 3.21%. Survival rate was observed 100% of test fishes, during the experimental period. The survival rate of each treatment at different inclusion levels of linseed oil cake was unaffected. In conclusion, the present study suggested that Linseed oil cake at level of 30% inclusion level in diet can help to promote in fish growth and aquaculture production.

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2.19 The Synergic Role of Holy Basil and Autochthonous Probiotic Bacterium in Feeds of *Cirrhinus mrigala* for Growth promotion, Immunostimulation and Histoprotection

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Keywords: Autochthonous, Growth, Histoprotection, Holy Basil, Immunostimulation

The purpose of present investigation is to assess the synergic role of holy basil (*Ocimum sanctum*) along with autochthonous probiotic *Bacillus cereus* on growth performance, hematological parameters and non-specific immune parameters in *Cirrhinus mrigala* fingerlings.

It was also attempted to evaluate their effects on histopathology and antioxidant status of fingerlings. Six dietary treatments were designed in triplicate in which fingerlings were fed on diets containing various incorporation level of dried holy basil leaves and probiotic Bacterium diets (C1; Control diet, T1; *Bacillus cereus* @ 2000 CFU g⁻¹, T2; holy basil @ 35 g Kg⁻¹, T3; holy basil @ 35 g Kg⁻¹ + *Bacillus cereus* @ 2000 CFU g⁻¹, T4; holy basil @ 17.5 g Kg⁻¹ + *Bacillus cereus* @ 2000 CFU g⁻¹, T5; holy basil @ 35 g Kg⁻¹ + *Bacillus cereus* @ 1000 CFU g⁻¹) for a period of 90 days. Results clearly showed significantly ($P < 0.05$) high values of growth parameters such as live weight gain, growth % gain in BW, growth per day in % BW, Specific Growth Rate, Gross Conversion Efficiency, Protein Efficiency Ratio and Intestinal enzyme activity in dietary treatment group T3 (Holy basil @ 35 g Kg⁻¹ + *Bacillus cereus* @ 2000 CFU g⁻¹) in comparison to other dietary treatment groups. Similarly, collation of data of hematological and immunological parameter represent significantly ($P < 0.05$) high values of Total Erythrocyte Count, Total Leukocyte Count, Serum Protein, Respiratory Burst Activity, Phagocytic Activity, Lysozyme Activity in dietary treatment group T3 (Holy basil @ 35 g Kg⁻¹ + *Bacillus cereus* @ 2000 CFU g⁻¹) as compared to other treatment groups. Furthermore, an antioxidant enzymatic analysis and histopathological investigation of tissues demonstrate the positive and harmonious effect of holy basil along with probiotic *Bacillus cereus* supplemented diet in *Cirrhinus mrigala* fingerlings. In comparison to control, dietary supplementation of holy basil or probiotic enhanced the growth and immunity; however, probiotics along with holy basil supplemented diets (T3; holy basil @ 35 g Kg⁻¹ + *Bacillus cereus* @ 2000 CFU g⁻¹) showed better results revealing their synergic role in *C. mrigala* growth promotion, immunostimulation and histoprotection.

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2.20 The Effect of Turmeric Supplemented Feed on the Hematological and Biochemical Parameters of *Pangasius hypophthalmus*.

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Keywords: Supplementation, Isoenergetic, Curcumin, Hematological

The disease has become a primary constraint to sustainable aquaculture causing devastating loss to fish industry and enhanced expenditure on use of chemicals as preventive and control measures, which can cause immune suppression in fishes and may also leave residual effects. To tackle the problem of diseases, the use of medicinal and herbal plants for the management practices of aquaculture ponds is gaining momentum in India. The main aim of present study was to evaluate the hematological and biochemical changes in *Pangasius hypophthalmus* treated with turmeric supplemented feed. The experiment was conducted for 90 days, using glass aquaria of 48×15×18” and 240 fingerlings were randomly divided into 3 treatment groups in triplicate along with a control group. Three isoenergetic diets were prepared by supplementing turmeric @3gm/kg (T1), 6 gm/kg (T2) and 9 gm/kg (T3) with basal diet (mustard oil cake and rice bran) and control diet without turmeric. The supplementation of fish feed with 6gm/kg turmeric (T2) showed best hematological results from initial to the final day and non-significant increase in the values of RBC count, haemoglobin (Hb), PCV, MCH and MCV was observed except in WBC count, where significant increase was recorded. Various enzymatic activity including aspartate amino-transferase (AST), alanine amino-transferase (AL/SGPT) and alkaline phosphatase (AL/SGPT) and decreased significantly ($p < 0.05$) in comparison to control. The lowest concentration of SGPT and SGOT recorded in treatment with highest concentration of Turmeric. Low levels of these enzymes indicates that bioactive compounds such as curcumin and others trigger the immune system of fishes, prevents infection and thus improves the overall health and of fishes.

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2.21 Economic Evaluation of Mineral Fortified Diet for Shrimp Farming in Inland Saline Waters

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Keywords: *Litopenaeus vannamei*, Potassium, Magnesium, Ground saline water

The inland saline aquaculture is a fast-growing sector of Indian aquaculture owing to the salinization of land which made it unsuitable for agriculture activities. As inland ground saline water (IGSW) is deficient in potassium with high levels of calcium and variable concentrations of magnesium, it became mandatory to balance these important ions using commercial fertilizers (magnesium chloride, muriate of potash) for growth and survival of *Litopenaeus vannamei*. Since this method needs large quantities of fertilizers and involves high cost, the present study was made to explore the use of desired mineral supplements through feed rather than in water. The farming of white leg shrimp, *L. vannamei* is the most popular farming activity in the inland saline waters. However, the shrimp farming is sustained by the mineral fortification, mainly potassium and magnesium in the water. A 60 days trial was conducted to investigate the effect of different dietary levels of K^+ - Mg^{2+} on *L. vannamei* juveniles in two types of water namely raw IGSW and 100% K^+ - Mg^{2+} fortified IGSW as sea water at constant salinity 10 ppt. Three gelatin coated diet were formulated with varied K^+ and Mg^{2+} levels (K^+ = 5 g/kg and Mg^{2+} = 150 mg/kg, K^+ = 10 g/kg and Mg^{2+} = 300 mg/kg, K^+ = 15g/kg and Mg^{2+} = 450 mg/kg) and commercial shrimp feed serves as basal diet. The present study evaluated the effect of simultaneous supplementation of the minerals in the water as well as in the feed. Further, the authors also attempted to assess the economic viability of optimum mineral fortification (10 g KCl and 300 mg $MgCl_2 \cdot kg^{-1}$ of feed) in feed to achieve 13% more survival, which leads to a corresponding increase in the total production. Further, the economic analysis revealed that 13% more survival led to an additional profit of 9.5%.

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2.22 Comparative Evaluation of Silkworm pupae, Housefly Maggot and Earthworm Meals in the Diet of *Labeo rohita*

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Keywords: Earthworm, Housefly maggot, Insect meal, Rohu, Silkworm pupae

A 60-day feeding trial was attempted to determine the potential of silkworm pupae, housefly maggot and earthworm meals as an alternative of soybean meal in the diet of *Labeo rohita*. Four isonitrogenous (~35% crude protein) and isolipidic (~6% crude lipid) experimental diets were prepared viz. Control (with 30% soybean meal), T1 (with 30% silkworm pupae meal), T2 (with 30% housefly maggot meal) and T3 (with 30% earthworm meal). One hundred ninety-two *L. rohita* fingerlings (initial average body weight: 5.07 ± 0.01 g) were randomly distributed in 12 experimental tanks (16 fish/tank) following a completely randomized experimental design. The fish were fed twice daily (07:00 h and 16:00 h) with the respective experimental diets to the satiation level. About 30% of water from each tank was replaced at every three days intervals to remove the faecal matter and maintain the physico-chemical parameters of the water. At the end of the feeding trial, final body weight (FBW), specific growth rate (SGR), percent weight gain (WG%), feed conversion ratio (FCR) and protein efficiency ratio (PER) were affected significantly ($P < 0.05$) among the experimental groups. The values of FBW, SGR, WG% and PER were significantly higher ($P < 0.05$) in T1 and T2 groups compared to Control and T3 groups. However, there was no significant ($P > 0.05$) differences observed between Control & T3 groups, and T1 & T2 groups. A significant ($P < 0.05$) opposite trend was observed for FCR values. Hence, it can be concluded that the use of silkworm pupae and housefly maggot meals in the diet of *L. rohita* provides better growth performances and feed utilization efficiency than soybean and earthworm meal based diets.

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2.23 Production of Biomass of *Spirulina (Arthrospira) platensis* in Aquaculture Wastewater for Value-added Pigments

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Keywords: Aquaculture, Chlorophyll-a, Carotenoid, *Spirulina*, Wastewater

The purpose of this study was to evaluate the feasibility of aquaculture wastewater cultivation and biomass production of a commercially significant species *Spirulina (Arthrospira) platensis*. Aquaculture wastewater obtained from ornamental fish culture tanks of ICAR-CIFE was used for the experiments after certain amendments. The relative performance of the amended aquaculture wastewaters supplemented with NaHCO_3 (8 g l^{-1}) and NaNO_3 (2.5 g l^{-1}) was determined through the biomass production, pigment quantity (carotenoid and chlorophyll-a) and compared with the prescribed medium (CIFE medium). The dry weight was almost comparable with the control showing a negligible decrease of 0.89 %. In case of carotenoids, cells grown in amended aquaculture wastewater expressed only 22.40 % reduction from that of control. The carotenoid content in aquaculture wastewater supplemented with NaHCO_3 8 g l^{-1} and NaNO_3 2.5 g l^{-1} was $45.42 \pm 0.62 \text{ mg g}^{-1}$ dry weight however, control exhibited a comparatively higher carotenoid content ($58.53 \pm 0.21 \text{ mg g}^{-1}$ dry weight). In the case of chlorophyll, cells grown in amended aquaculture wastewater expressed negligible reduction from that of control. The chlorophyll-a content in aquaculture wastewater supplemented with NaHCO_3 8 g l^{-1} and NaNO_3 2.5 g l^{-1} was $49.7 \pm 1.56 \text{ mg g}^{-1}$ dry weight and in control the value recorded was $117.12 \pm 5.98 \text{ mg g}^{-1}$ dry weight. The highest chlorophyll content was found in control followed by amended aquaculture wastewaters. The present study shows the potential of *S. platensis* cultivation using aquaculture wastewater to make it cost-effective by reducing the cost of biomass production through the reuse of the aquaculture wastewater. The reduction in the cost of the medium through the utilization of aquaculture wastewaters after certain amendments is a novel approach to the cost-effective production of *S. platensis*. The quality of biomass and pigment properties in amended aquaculture wastewater grown cultures were not altered to the extent which affects the quality and purity of the value-added pigments such as chlorophyll-a and carotenoid. The overall findings and results provide evidence for the suitability of aquaculture wastewater for the biomass produced showed an appreciable yield of value-added pigments.

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2.24 Effect of Supplementation of White Button Mushroom, *Agaricus bisporus* (Imbach, 1946) on Disease Resistance in White Leg Shrimp, *Litopenaeus vannamei* (BOONE, 1931)

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Keywords: White button mushroom, *Litopenaeus vannamei*, Growth, Immunity

The present study was formulated to find out the effect of supplementation of *Agaricus bisporus* on disease resistance in *Litopenaeus vannamei* against *Vibrio parahaemolyticus*. This experiment was conducted during the study period from March to May 2022. Six experimental diets were prepared with 35% protein level. The control diet was prepared without supplementation of white button mushroom (WBM) powder, while the treatment diets were prepared with supplementation of WBM powder at the rate of 1%, 1.5%, 2%, 2.5% and 3%. *L. vannamei* post larvae (average weight 0.012g) were stocked at a density of 20 nos. in each 24 aquarium tanks having capacity of 35 litres and fed with treatment diet for 60 days. Challenge test was carried out after the end of 60 days experiment for 4 days. A random sample of shrimps (10 nos.) was taken from each treatment and challenged with 0.1×10^4 cfu/ml of *V. parahaemolyticus* via gentle intramuscular injection with minimal stress to them. The data pertaining to growth and disease resistance against *V. parahaemolyticus* in *L. vannamei* were recorded and evaluated. The result of this study shows that supplementation of 3% WBM powder in diet significantly improved mean weight gain. In the challenge test against *V. parahaemolyticus* lowest mortality (10%) was recorded in the *L. vannamei* fed with the treatment diet having 3% supplementation of WBM. The result of present study recommends a dietary supplement of *A. bisporus* at 3% level that significantly affected the mean weight gain and showed improved survival during challenge test against *V. parahaemolyticus* in *L. vannamei*. This indicates that white button mushroom might have impact on enhancing immune system that prevented mortalities due to *V. parahaemolyticus* infection.

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THEME III

Fish Genetics, Breeding and Biotechnology







3.1 Molecular Characterization and Expression Analysis of GH-IGF System in the Snow Trout

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Keywords: Aquaculture, Coldwater, Snow Trout, GH-IGF gene

Introduction

Snow trout (*Schizothorax richardsonii*) has been identified as a potential candidate species for coldwater aquaculture. To ensure its sustainable availability, the species must be brought into aquaculture. However, due to its slow growth rate and early maturity, the species is not yet domesticated and very little is known about the physiology of the species so as to devise any strategy for its growth enhancement. Hence, this study was undertaken to characterize the growth axis regulatory components, and their expression was evaluated in response to nutritional status (fed, starved and re-fed) and diet composition (different protein and lipid levels).

Materials and Methods

For the cloning and characterization of targeted genes, gene-specific primers were designed based on the conserved regions from closely related cyprinid fishes and were amplified using the cDNA template, gene-specific primers and Phusion polymerase enzyme (Thermo Fisher Scientific, MA, USA), as per manufacturer's instructions. Amplified products were gel eluted, purified, ligated into a pCR-Blunt vector and then transformed into TOPO 10 cells. Positive colonies were screened and sequenced after plasmid extraction. The sequences were assembled using BioEdit. A 42-day starvation and re-feeding experiment was conducted to elucidate the effect of nutritional status on the expression of growth axis regulatory components. Two other feeding trials were also conducted to study the effect of different protein (25, 35 and 45%) and lipid (3, 7 and 13%) levels on the growth. After each feeding trial, fish were killed, liver and muscle tissues from different treatment groups were collected, and total RNA was isolated using TRIzol (Invitrogen, Carlsbad, CA). The RNA was then reverse-transcribed into cDNA using the PrimeScript 1st strand cDNA synthesis kit (Takara, USA). The expression of target genes was carried out in ABI Step One plus real-time PCR detection system (Applied Biosystem, USA) using SYBR Premix Ex Taq II (Tli RNase H Plus) (Takara, USA) and specific primers. Relative quantification of target gene expression was performed using the mathematical model described by Pfaffl (2001).

Results and Discussion

In the present study, we sequenced and characterised the major growth axis regulatory components in the slow-growing Himalayan cyprinid *S. richardsonii*. In addition, we have studied the transcriptional regulation of these regulatory genes in response to nutritional conditions (feed restriction-availability, dietary protein and lipid levels). Sequence analysis of GH revealed a partial ORF of 593 bp nucleotides. Hepatic growth hormone receptor (*ghr*) was expressed in two isoforms where one of the isoforms consisted of a 5'UTR of 280 bp and partial ORF of 981 bp encoding 327 amino acids. Similarly, we have partially characterized hepatic insulin-like growth factors (IGFs). The nucleotide sequence of snow trout GH-IGF axis shows similarities with *Labeo rohita*, *Cyprinus carpio*, *Carassius auratus* and *Danio rerio*. Nutritional status had no significant influence on the expression of *igf2* and *igfbp1*. However, a significant downregulation in the transcript abundance of *igf1* and *igfbp3* was noticed in starved fish, and their expression was highest during short-term re-feeding after the starvation phase. Their expression levels in the long-term re-fed group were similar to that of the fed group. The hepatic mRNA expression of *igf1* and *igf2* did not vary, even though we observed a significant increase in the growth of snow trout with increasing dietary protein levels from 25 to 45%. On the other hand, we found no changes in the hepatic mRNA expression of *igf1* and *igf2* in snow trout fed 3, 7, or 13% lipid diet, which correlated to the similar growth recorded in the four dietary lipid groups. However, growth hormone receptor (*ghr*) expression was significantly higher in fishes fed the highest protein, (45%) and lipid (13%) diet compared to low protein and low lipid fed groups. In the white muscle, different dietary protein levels did not alter the expression of *igf2*, *igfbp1* and *igfbp3*, while the highest transcript level of *igf1* was noticed in the 35% protein fed group. These findings suggest the possible nutritional regulation of the GH-IGF axis in snow trout, which would be helpful in formulating cost-effective grow-out diets for this slow-growing indigenous Himalayan fish.

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3.2 Labelled SSR Libraries: An Effective Method of Finding Polymorphic SSR Marker for Giant-river Catfish *Sperata seenghala* (Sykes, 139) Stocks

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Keywords: *Sperata seenghala*, Polymorphic SSR, Probe Enrichment, Molecular Marker

Introduction

The giant river-catfish *Sperata seenghala* is one of the commercially important freshwater catfishes of India with wide distribution in all major rivers and reservoirs. Conversely, the culture practices for this fish have not yet been standardized and capture fisheries is the only source to meet the demand. Thus, this may lead to over exploitation of resources and subsequent stock depletion. Knowledge on genetic stock structure is prerequisite to formulate sustainable management and conservation measures. Microsatellites are the most suitable markers for characterizing the genetic stocks. Though several methods smeared for microsatellite development but probe enrichment method is very popular for vertebrates and specifically for fishes. The present study was carried out to develop microsatellite markers through probe enrichment method from *S. seenghala* and to characterize the population genetic structure of *S. seenghala* collected from river Brahmaputra, Ganga, Godavari, Mahanadi and Narmada.

Materials and Methods

A total of 30 individuals (including all sampling locations) of *S. seenghala* were collected from each river and dorsal fins were collected aseptically and preserved in absolute alcohol. Total genomic DNA was isolated using phenol-chloroform extraction method as per standard protocol. Agarose gel electrophoresis was performed to check the integrity of the isolated DNA on 1% agarose gel. Although the individuals of *S. seenghala* were identified using morphological characters at field level, DNA barcoding approach was also used to authenticate the fish species. A total of five genomic DNA libraries of *S. seenghala* representing five populations, i.e., Brahmaputra, Ganga, Godavari, Mahanadi and Narmada were prepared prior to SSR identification. Genomic DNA from ten individuals of each population was pooled separately to prepare a population specific genomic library. Each library was constructed with 1 µg of genomic DNA using the Ion Xpress™ Plus Fragment Library Kit (ThermoFisher Scientific, USA) following manufacturer's guidelines. The Ion Torrent Personal Genome Machine® (PGM™) (ThermoFisher Scientific, USA) was used to sequence the SSR enriched DNA libraries of *S. seenghala* following manufacturers protocol. An *in-silico* approach was implemented to screen putatively polymorphic SSRs from contigs.

Results and Discussion

A total of 2184, 9226, 5116, 6541 and 1438 counting's observed from Ganga, Godavari, Mahanadi, Narmada and Brahmaputra River, respectively. All the contigs merged together to make one master contig of all the stocks. Each individual contigs were mapped against the master contig. Each population contig were identical as each population's fragments were ligated with adapter sequences (IonXpress™ P1 Adapter) and subsequently labelled with oligonucleotides (IonXpress™ barcodes) specific to each population. The repeat number of SSRs varies in each stock, so when all the contigs align together, the repeat region overlapped with each other but each repeat number is varied from other. Hence, the polymorphic SSR markers were sorted by following this method easily and effectively. Through this approach, a total of 28 polymorphic loci with an appropriate flanking sequence were chosen for validation. Fifteen microsatellite loci were characterized using 30 samples of *S. seenghala*. Out of the 15 loci, the number of di, tri, tetra and penta nucleotide repeats were 2(13.3%), 7(46.6%), 4(26.6%) and 2(13.3%), respectively. All loci showed polymorphic amplicons with a PIC value of more than 0.37.

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3.3 Effect of Kiss-10 Nanoformulation for Induced Maturation in *Catla catla* (HAMILTON, 1822)

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Keywords: Kisspeptin, HPG axis, Induced maturation, Nanoformulation

Introduction

Kisspeptin has been identified as potent regulator of reproduction, particularly for the onset of puberty. The synthesis and secretion of FSH and LH in the pituitary is stimulated by kisspeptin through hypothalamus and thus regulates gonadal development and reproduction. However, the half-life of the kisspeptin is very short and unstable. Hence, nanoparticle based delivery of the peptides will increase the stability and efficacy. In the present study, the kisspeptin from *Catla catla* that was characterised and sequenced in FGB lab was synthesised commercially and tested for its role in maturation in *C. catla*. The exogenous kisspeptin viz. kiss1 and kiss2 were then conjugated with chitosan nanoparticle. The size of the chitosan and chitosan conjugated kisspeptin particles were 138 nm and 178 nm respectively. The zeta potential of the particles was 40mV. The loading efficiency of the kisspeptin was found to be in the range from 70% to 84%. The nano-conjugated peptin found to be stable while checking through FTIR Spectroscopy. The effect of the nano-formulation was checked in fish by injecting @ 0.2mL/kg b.w. The histological examination of the gonads in immature fish showed advancement in gonadal development compared to control. The gene expression of GnRH was also elevated at different sampling points. The hormonal titre in the treated immature fish showed elevation in LH and FSH level compared to control. The fish injected with the nanoformulation showed increased hormonal surge and gonadal development compared to control. These observations clearly state that the exogenous kisspeptin enhances the gonadal development and thereby helps in decreasing the age of maturity in *C. catla*.

Material and Methods

For better understanding of role of kiss-10 in reproduction and maturation status of *Catla catla*, the experiment was conducted in ICAR- Central Institute of Freshwater Aquaculture; CIFA. Two experiments were set up for carrying out the research. The first experiment was conducted in immature fishes while the second experiment was conducted on mature fish. The sampling design was Completely Randomised Design and the sampling time points were weekly sampling (2nd, 4th and 6th week). In the first experiment, immature fishes (average body weight 350-400g) were procured. The experiment included nine treatments with three replicates. The first group was maintained as control in fishes was administered chitosan nanoparticle. In the second group, intra-muscular injection of naked kiss-1 (100µg) was given @ 0.2 mL / kg body weight of fish. In the third group, intra-muscular injection of naked kiss1 (50µg) was given at the same volume of injection. In the fourth group, intra-muscular injection of CK1-100µg was administered followed by administration of CK1-50µg dose in the fifth group of fishes. In the same way, kiss-2 was injected at two doses 100 and 50 µg, naked, as well as conjugated in the simultaneous sixth, seventh, eighth and ninth group of fishes. The injection was given using 1 mL syringe fitted with 22 gauge needle. Blood was collected after 2, 4 and 6 weeks sampling times in order to see the half-life of pituitary level hormones, namely FSH and LH at such time intervals, from the serum of fish. In the second experiment 1 year old fishes were used. Fishes was divided into three treatment groups, with three replicates. The sampling design consisted of one control group of fishes, other one was chitosan conjugated kiss-100µg and the last group had chitosan conjugated kiss-50 µg treated animals. The sampling regime was 0, 6, 12, 24 and hours in which blood serum was analysed for hormones namely FSH and LH.

Results and Discussion

Two amidated kisspeptin core conspecific *Catla catla* decapeptide sequences were synthesized namely kiss1 and kiss2. In order to develop kiss-10 nanoformulation as well as to see the effect of both the peptides, two doses based on the studies in different fish species, were prepared, viz., 100µg and 50µg per kg body weight. Chitosan nanoparticles of size 139 nm and zeta potential of 40mV was prepared using Calvo et al., 1997. Nanoformulation was developed by encapsulating kiss1 and kiss2 peptides with chitosan nanoparticle followed by confirmation of the prepared conjugate by means of FTIR-Spectroscopy. Bound drug concentration was measured using Lowry method and at particular absorbance, the protein concentration was measured, that in turn formed the basis for calculating drug encapsulation efficiency. After that the nanoformulation was ready to be administered in the fish through intramuscular injection mode of delivery. Hormonal assay for reproductive hormones was also done using ELISA FSH and LH kits. LH as



well as FSH surge was observed at 12 hrs sampling period and it was lowered after 48 hours. For confirmation of any maturation signs, histology analysis was also conducted in fish ovary which depicted the initiation of development of late maturing oocytes after keeping the injected fishes for nearly 20 days. Hence, preparation of this nanoformulation can serve two important purposes. Firstly the development of stable chitosan-kiss10 nanoformulation provides greater insights in development of other peptide hormones for specific and targeted delivery as well as increment in the half-life of short lived peptides. Secondly, the problem associated with late maturation of *Catla catla* under captive conditions can also be ascertained with strong evidence.

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3.4 Population Genetic Structure of Indian Scad, *Decapterus russelli* along Indian Coast Inferred from Nuclear *aldolase b* Gene Sequences

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Keywords: Population genetics, Indian ocean, Pelagic fish, Carangidae, Nuclear DNA

Introduction

The Indian Scad, *Decapterus russelli* is a benthopelagic marine shoaling fish found at depths ranging from 40 to 300 metres. The species' distribution was traced to depths of 200 m along India's west coast and 300 m along east coast. It contributes a significant portion of India's pelagic resources, ranking next to Indian oil sardine. Previous research on the geographical structure of this species using truss morphometric analysis along the Indian coast (Sen et al 2011) demonstrated that the species is composed of spatially structured populations that should be managed independently. However, no attempts have been made to investigate the genetic basis of this structuring. Thus, studying the geographical structure of its population at the genetic level is critical for making effective management recommendations.

Materials and Methods

A total of 125 specimens of *D. russelli* were collected from 5 localities along the India coast. The total DNA was extracted from the muscle tissue using the standard phenol chloroform method (Sambrook 2001). PCR amplifications were carried out in a final volume of 20 μ l containing 50ng of genomic DNA, 10mM of each dNTP, 10 μ M of each primer and the buffer recommended by the polymerase suppliers. PCR cycling consisted 4 min at 94°C, 32 cycles of 30 s at 94°C, 12 s at 52 °C, 20 s at 72 °C followed by 3 min at 72°C. The products were then sequenced completely in both directions by performing Sanger sequencing. 310 bp sequence of *aldolase b* obtained were aligned using clustalW in MEGA X. The genetic diversity indexes including number of alleles (h), haplotype diversity (Hd) and nucleotide diversity (π) were calculated using DnaSPv5.0. Genetic distance was calculated with MEGA X. Pairwise F_{ST} values were estimated also with DnaSPv5.0. In order to test for molecular signatures of demographic expansion pairwise mismatch distribution analyses as well as Tajima's D and Fu's FS were performed in DnaSPv5.0.

Results and Discussion

A 210 bp sequence of intron one of *Aldolase b* was successfully sequenced from 125 individuals (250 alleles). There were 54 unique alleles identified. The overall haplotype diversity was high (0.9075) whereas the global nucleotide diversity value was low (0.02303). We identified 131 loci with only one allele. As observed no obvious population structure was detected either related to the coast or related to the geographical distance. The minimum spanning network showed a star shaped pattern. One haplotype was common for all the geographical location. The common haplotypes within the network are inferred to be ancestral, whereas the tip haplotypes are derived or descended from ancestral (internal) haplotypes. Thus, the occurrence of star like pattern radiating from these major haplotypes suggest that *D. russelli* population have undergone recent demographic expansion.

Genetic differentiation among the population from different location was tested using F_{ST} pairwise comparison and most of them were significant. Genetic variation within population was high at 94.71% revealing most of the variation was present within populations. A non-hierarchical AMOVA analysis showed significant global F_{ST} ($F_{ST} = 0.04655$, $P < 0.05$). The estimated F_{SC} and F_{CT} value in the hierarchical AMOVA based on the two groups failed to reveal statistically significant values ($p > 0.05$). The Mantel test revealed lack of significant correlation between genetic differentiation ($F_{ST} / (1 - F_{ST})$) and geographical distance among tested population, hence no evidence of isolation by distance. The Tajimas D and Fu's Fs values observed for all populations were negative except puri which showed a positive Fu's Fs. It indicates these populations experienced a selective sweep or recent population expansion. In addition, the Harpending's raggedness index, $H_{ri} = 0.01854$, $SSD = 0.0118$ ($p = 0.2$) supported the recent population growth hypothesis. Mismatch distribution of combined population showed unimodal pattern indicating a sudden expansion in population.



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3.5 Transcriptome-Wide Identification and Expression Profile of the *Dmrt* (Doublesex and Mab-3 Related Transcription Factor) Genes in Golden Mahseer (*Tor putitora*)

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Keywords: Golden mahseer, DMRT genes, Sex biased expression, Sex differentiation.

Introduction

The doublesex and mab-3-related transcription factor (*dmrt*) gene family is a class of crucial transcription factors involved in sex determination and neural development across various biological groups. In fish, seven *dmrt* genes are reported, including *dmrt1-6* and *dmrt2b*. Among these, *dmrt1* functions in male sexual fate in zebrafish and reported as essential for the normal development of testis tubules and spermatogenesis. *dmrt2a* and *dmrt2b* are involved in the branchial arch and slow muscle development. *Dmrt3* is speculated to play a role in the developmental processes of the nerves and germ cells. Systematic studies on the expression of these genes in various fish species have revealed a sexually dimorphic expression pattern regardless of their sex-determination mechanism. In bisexual fish species, the increased expression of *dmrt* genes is closely related to male gonadal development and further gender dimorphism. Mutations disrupting these genes and genome editing were reported to lead to sex reversal in some fish species. Hence, knowledge of these genes benefits understanding and manipulating sex in fish species.

The golden mahseer, *Tor putitora* is an important food and sports fish in the family Cyprinidae. The species has great demand in the Himalayan region due to its culinary quality, nutritional value, and fighting traits. However, a skewed sex ratio is a major problem in many teleosts, including the golden mahseer, affecting the population structure and viability of sensitive stocks and negatively impacting reproductive success. Understanding sex-related genes and their expression pattern will provide insight into the molecular mechanisms that establish and maintain the male and female phenotypes in golden mahseer. Further, no information is available on the critical genes involved in the sex determination and differentiation mechanisms of the species. Therefore, in the present study, we identified and analysed the genomic organization of four *dmrt* genes and profiled their sexually dimorphic expression patterns in golden mahseer.

Materials and Methods

The nucleotide sequences of *dmrt1*, *dmrt 2a*, *dmrt 2b* and *dmrt 3* were identified from the de-novo transcriptome assembly of the golden mahseer by blasting the nucleotide sequences of the common carp with CLC Genomics Workbench 7.5.2. BLASTN searches confirmed the coding sequences against the NCBI non-redundant protein sequence database (nr). Then, the conserved domains were searched against the local database by the BLASTp program.

Gonad and brain tissues were sampled randomly from the healthy male and female golden mahseers. Total RNA was extracted from these samples and reverse transcribed into complementary DNA (cDNA). Specific primers for the retrieved genes were designed using the Primer-BLAST suite. The specificity of amplification was confirmed by the melting curve and agarose gel electrophoresis. PCR efficiency was calculated based on the slope of a standard curve generated using two-fold serial dilutions of pooled cDNA. The qRT-PCR was performed to profile the sexually dimorphic expression patterns of these genes in golden mahseer. Statistical analysis for the differences in the expression level of studied genes was performed with GraphPad Prism 5.0.

Results and Discussion

In the present study, four *dmrt* genes were identified namely *dmrt1*, *dmrt2a*, *dmrt2b*, and *dmrt3*, and submitted the nucleotide sequences into the NCBI database (Table 1). Analysis of the NCBI Conserved Domain Search results found that the ORF of these *dmrt* genes had a highly conserved DM domain similar to those of the *Dmrt* superfamily members. Tissue distribution analysis showed that all these genes exhibited sexually dimorphic expression patterns. Amongst these, *Dmrt1* had high expression levels in the testes and male brain, while *Dmrt2b* had more significant expression levels in the ovaries and female brain than in other tissues. The expression pattern observed here is in accordance with the expression pattern of other teleost fish, such as common carp and zebrafish. Overall, the present study provides a comprehensive insight into the *Dmrt* gene family of golden mahseer. The results also suggest that *Dmrt1* may play an important role in testis differentiation/ development, while *Dmrt2a* and *Dmrt2b* are critical in ovary development and maintenance in this species.



Table 1 Summary of all dmrt genes in Golden Mahseer

Gene	Transcript ID	Accession Number	CDS length
dmrt1	TRINITY_DN3507_c0_g1_i4	OP776400	798 bp
dmrt2a	TRINITY_DN48789_c0_g1_i1	OP776401	1320 bp
dmrt2b	TRINITY_DN77483_c0_g1_i4	OP776402	1002 bp
dmrt 3	TRINITY_DN61706_c0_g1_i4	OP776403	1356 bp

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3.6 Determination of the Influence of Dilution Ratio and Freezing Rates on Cryopreserved *Cyprinus carpio* (Linnaeus, 1758) Spermatozoa Quality

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Keywords: *Cyprinus carpio*, Dilution ratio, Freezing rates, Cryopreservation

Introduction

Due to emergence of intensive aquaculture and development of commercial aquaculture, high quality seed is required for grow-out of marketable size under certain period of time which pave way for indiscriminate hormonal manipulations by hatchery operators. Thus, leading to questionable quality and genetic purity of seeds, due to inbreeding and unintentional intraspecific hybridization practices. Therefore, it is a necessity to conserve the gene pool of wild stocks for better quality brood production. In the above light, cryopreservation can be used as a tool for conserving the wild gene pool. The conventional freezing methods causes cryoinjuries on the spermatozoa cryopreserved, thus the choice and concentration of cryoprotectants, rate of freezing, cooling must be optimized as the basis for successful cryopreservation. Hence, the best dilution ratio along with freezing rate was studied to minimise cryo injuries during the cryopreservation of *Cyprinus carpio* milt.

Materials and Methods

Adult male common carp (*C. carpio* L.) brooders with an average body weight of 468±0.5 g were maintained in cemented tanks, fed with supplementary feed @ 2-3% BW. Milt was obtained from donor by gentle stripping. The collected milt was diluted with Freshwater Fish Saline (FWFS) as extender and DMSO at 10% (v/v) as cryoprotectant at different dilution ratios (1:10, 1:20 and 1:40). Further, based on spermatozoa motility, best dilution ratio was obtained and milt again diluted with best dilution ratio and transferred to controlled rate freezer (PLANER, Kryo 560-16) programmable by Planer's MRV controller system fixed at three different freezing rates. Once the curve programming was completed and the temperature reached -80°C, the cryovials were immediately transferred to a BA11 Cryocan (IBP, India) for storage at -196° C in canister containing goblet. The quality of cryopreserved spermatozoa was analysed fortnightly for 30 days.

Results and Discussion

When compared with three different dilution ratios (1:10, 1:20, 1:40), the highest motility duration of 74.3±2.16 s was obtained at 1:10 dilution ratio (Table.1). The milt obtained from *C. carpio* brooder was diluted at 1:10 dilution ratio and was further subjected to freezing trial (3 different freezing protocol with two step cooling profiles). Three sets of three step freezing protocol (FP) were used, FP I: 5°C to -4°C (at the rate of 4°C/min) (Ramp 1 min) and from -4°C to -80°C (at the rate of 10°C/min) and held for 10 min and direct transfer to liquid nitrogen (LN₂); FP II: 5°C to -4°C (at the rate of 5°C/min) (Ramp 1 min) and from -4°C to -80°C (at the rate of 10°C/min) and held for 10 min and direct transfer to liquid nitrogen LN₂ and FP III: 5°C to -4°C (at the rate of 10°C/min) (Ramp 1 min) and from -4°C to -80°C (at the rate of 10°C/min) and held for 10 min and direct transfer to liquid nitrogen LN₂.

The highest initial mean post-thaw motility duration of spermatozoa was observed when subjected to FP-I (of 77.14± 4.38 s) which decreased to 62.28± 2.12 s on 30th day (Table. 2). The lowest post-thaw motility duration was obtained when FP-III was used with initial value of 68.33 ± 1.63 s that declined drastically to 44.9±0.87 s (Table. 2). The study concluded successful cryopreservation of *C. carpio* milt at 1:10 dilution ratio following slow freezing rate. However, to develop a standard freezing protocol the level of cryo-injuries occurred to spermatozoa subjected under various freezing rates is to be studied.



Table 1 Observation of mean motility duration of spermatozoa after equilibration

Dilution ratio	Motility duration (s)	
	Initial	Final
1:10	79.6±1.63 ^a	74.3±2.16 ^b
1:20	77.3±3.74 ^c	70.0±1.63 ^d
1:40	75.6±2.16 ^e	67.3±2.82 ^f

abcdef $p < 0.001$

Table 2 Observations of mean motility duration (s) of cryopreserved milt with three different freezing protocols

Freezing Protocol	Days of cryopreservation			
	Initial	10	20	30
I	77.14± 4.38 ^a	70±4.63 ^b	67.8±1.39 ^c	62.28± 2.12 ^d
II	70.66 ±1.69 ^a	66.5±1.5 ^b	64.8± 0.97 ^c	59.33±1.28 ^d
III	68.33 ± 1.63 ^a	63 ±2.47 ^b	58±2.90 ^c	44.9±0.87 ^d

abcd $p < 0.001$

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3.7 Reproductive Performance and Salinity Tolerance of Ornamental Swordtail (*Xiphophorus helleri*) Reared in Inland Saline Water

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Keywords: Inland Saline Water, Salinity tolerance, Swordtail, Physiology, Colouration

Introduction

The ornamental fish business in Punjab is now in its early stages; the trade is primarily dependent on freshwater fish species obtained from coastal regions. i.e. West Bengal, Kerala, Maharashtra and Tamil Nadu. The technologies for culture, breeding and seed production of some commercially important freshwater ornamental fish species viz., koi carp, goldfish, molly, guppy, platy and swordtail are also available for commercial adoption in the State. The southwest districts of Punjab are severely affected by water logging and salinization. There is a severe problem with agriculture in this region and to boost the socio-economic growth of this area ornamental fish farming is the best possible viable option for marginal/small farmers for economic utilization of salt-affected and water-logged areas of the State. Considering the above facts, the present study was designed to check the reproductive performance and salinity tolerance of swordtails in inland saline water.

Materials and Methods

The present investigation was carried out at the Instructional cum Research Farm of the College of Fisheries (CoF), Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana. The inland saline water (stock) for the study was uplifted from salt-affected/waterlogged regions of Village Birewala, District Mansa (Punjab). The experiment was carried out within glass aquaria (50-litre capacity) with varying salinity (0, 2, 4, 6, 8, 10 and 12 ppt) in triplicate and provided continuous aeration. Swordtail (*Xiphophorus helleri*) fry's were obtained from the backyard ornamental fish seed production unit, CoF, GADVASU, Ludhiana and stocked @ 12 fish/aquaria. Fish were fed *ad libitum* with commercial feed twice (9-10 AM; 3-4PM) a day. The estimation water quality parameters and ionic composition were carried out by following standard methods. Fish behaviour was observed throughout the experimental period. Fish survival and growth in terms of total body length and body weight was recorded at monthly intervals. The reproductive parameters were estimated according to Khairnar and Kaur (2021). To determine differences between salinity treatments ($P \leq 0.05$), one-way ANOVA and Duncan's multiple range tests were used to determine the effect of different salinities on growth and breeding performance of experimental fish.

Results and Discussion

The growth in fish was assessed in terms of total body length (TBL) and body weight (BW) at monthly intervals during 90-day experimental period. At the conclusion of the experiment, the growth parameters, such as TBLG (%), NWG (%) and SGR (%) in fish were significantly higher ($p \leq 0.05$) in all the salinity groups (S2-12) as compared to control (S0). There is an indicative negative impact of salinity on fish growth. Further, there was remarkable decline in growth at higher salinities of 10-12 ppt. The fish survival was 94.44% in S0-S8 (0-8ppt), whereas, at higher salinities, gradual mortality was observed from the 30th day onwards and at the conclusion of the experiment it was recorded as 88.89% in S10, and 80.55% in S12. Fish behaviour in terms of swimming activity, feeding responses and morphological characters like colouration, body fragility and presence was significantly influenced by salinity and after 10ppt, the fish were slightly stressed. The reproductive performance was studied on the basis of GSI, absolute fecundity, relative fecundity, ovary weight, ovary volume and ovary diameter varied 4.76-9.10%, 7.45-10.83nos.egg/fish, and 3.65-7.64 nos. of fry/body weight, 0.135-0.213 g, 545.94-708.94 mm³ and 1.87-2.21 mm respectively (Table 1). The results of the fry performance study revealed that fry production and fry survival rate were significantly affected by salinity levels and ranged between 13.19-18.65, whereas fry survival rate was 83.66-93.33% respectively. Based on the results of the present study, 8 ppt salinity is considered safe in terms of fish performance, even though fish can adapt and grow to salinity levels up to 12 ppt in inland saline water. Hence, it can be concluded that freshwater ornamental Swordtail *X. helleri* can be reared in inland saline water for more extended periods by maintaining the salinity ≤ 8 ppt.



Table 1 Reproductive efficiency and fry production of Swordtail reared at various salinities during the experiment

Reproductive Parameters	Treatments*						
	S0	S2	S4	S6	S8	S10	S12
GSI (%)	4.763±0.063 ^a	5.883±0.119 ^b	7.213±0.058 ^c	8.843±0.159 ^d	9.487±0.246 ^d	9.210±0.318 ^d	9.10±0.329 ^d
Absolute fecundity	7.453±0.250 ^a	8.37±0.607 ^{ab}	8.823±0.292 ^c	10.497±0.464 ^d	12.023±0.537 ^d	11.746±0.115 ^d	10.833±0.136 ^{cd}
Relative fecundity	3.65±0.513 ^a	4.713±0.292 ^a	6.813±0.235 ^b	8.167±0.393 ^c	9.51±0.494 ^d	8.576±0.629 ^{cd}	7.64±0.191 ^{bc}
Oocyte diameter (mm)	1.873±0.258	2.023±0.047	2.116±0.033	2.21±0.128	2.32±0.243	2.293±0.165	2.216±0.121
Ovarian volume (mm ³)	545.943 ±25.230 ^a	618.057 ±16.091 ^b	697.393 ±21.820 ^c	728.49 ±20.380 ^c	745.636 ±14.108 ^c	717.963 ±18.836 ^c	708.943 ±18.399 ^c
Ovarian weight (g)	0.135 ±0.041 ^a	0.147 ±0.017 ^{ab}	0.177 ±0.013 ^{abc}	0.233 ±0.014 ^{cd}	0.263 ±0.014 ^d	0.237 ±0.023 ^{cd}	0.213 ±0.018 ^{bcd}
Fry production per female (nos.)	13.916 ±1.419 ^a	16.083 ±0.885 ^a	20.416 ±1.166 ^b	22.74 ±1.637 ^b	27.64 ±1.537 ^c	22.307 ±1.444 ^b	20.413 ±1.036 ^b
Fry survival number	13.19±0.45 ^a	15.047±0.83 ^{ab}	17.77±0.43 ^{bc}	19.91±0.63 ^{cd}	22.39±2.42 ^d	19.19±0.74 ^{cd}	18.65±0.57 ^c
Fry survival rate (%)	85±2.64	87±2.63	89.33±2.33	93.33±4.09	93.33±4.91	91.66±4.90	83.66±2.90

Values are mean ± S.E., (P≤0.05), n = 3; Values with same superscript (a, b,....d in a row and 1, 2,.....3 in a column) do not differ significantly (P≤0.05)

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3.8 Population Structure and Molecular Diversity of Randall's Threadfin Breems, *Nemipterus randalli* along Indian Coast

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Keywords: *Nemipterus randalli*, Cytochrome b, Population structure, Conservation

Introduction

Understanding stock structure is necessary for the effective management of commercially important species. Limited stock structure data add to the complexity of managing these marine species. In order to conserve genetic diversity, the first step must be to comprehend the structure of fish stocks. Randall's threadfin bream, *Nemipterus randalli* forms the dominant species (54%) in demersal finfish landings contributing significantly to the trawl fisheries of India. Since *N. randalli* is a commercially important demersal species, there are concerns about its stock structure. The current study investigated the population structure and genetic diversity of *N. randalli* along the Indian coast using the mitochondrial Cytochrome b (*Cyt b*) gene sequences, which is critical for the proper management and sustainable fisheries.

Materials and Methods

A total of 125 specimens were collected from five different locations along the Indian coast and were preserved in 95% ethanol. Genomic DNA was extracted using phenol-chloroform method. Approximately 550 bp of *Cytb* gene was amplified using universal primer. PCR reactions were carried out in 20 μ l reaction mixture. The PCR products were visualized on 1.2% agarose gel stained with ethidium bromide and were sequenced. Sequences obtained were aligned and trimmed using MEGA 7 (Kumar et al 2016). Genetic diversity indices including haplotype diversity, nucleotide diversity were estimated in DnaSP 6.0 and Arlequin v3.5. Historical demographic changes were investigated based on neutrality tests (Tajima's D and Fu's Fs statistics), Harpending raggedness index and sum of squared deviation using DnaSP 6.0 and Arlequin v3.5 (Excoffier et al 2005). Mismatch distribution was carried out using DnaSP 6.0 and Arlequin v3.5. While, PopART was used to construct network diagram to visualize geographical distribution pattern of the haplotypes. In order to examine population structure, a hierarchical AMOVA were performed in Arlequin v3.5. Pairwise F_{ST} values between all populations were also calculated using Arlequin v3.5. Mantel test was used to find out the relationship between the level of genetic differentiation and geographic distance.

Results and Discussion

Among 125 individuals obtained from 5 different locations along Indian coast, 58 haplotypes were identified with haplotype and nucleotide diversity of 0.8932 and 0.01205 respectively which indicated considerable genetic diversity. The majority of haplotypes were private haplotype (51/58, 87.93%) i.e. confined to a specific location. Among these private haplotypes, two were found in more than one individual whereas remaining private haplotypes was found in only one individual. Of the 58 haplotypes, seven haplotypes were shared among different locations (H1, H4, H13, H15, H20, H36, H37). Chennai population had the highest haplotypes (16) followed by Veraval (10) and Cochin (10), Puri (9) and Mangalore (8). The haplotype diversity (H_d) ranged from 0.69667 (Mangalore) to 0.92667 (Chennai) and the nucleotide diversity (π) ranged from 0.00318 (Veraval) to 0.00439 (Cochin). AMOVA results indicated presence of genetic structure in *N. randalli* ($\Phi_{ST} = 0.73953$, $P < 0.05$). The pairwise F_{ST} statistics indicated significant genetic differentiation and genetic structure among populations. Neutrality test recorded significant negative value for Tajima's D and Fu's Fs, suggesting population expansion. The haplotype network diagram formed two distinct haplogroups, indicating presence of population structuring in *N. randalli*. A significant pattern of isolation by distance was observed based on Mantel test ($r=0.511$).

Our results provide insights into the genetic structure and diversity of *N. randalli*. This genetic assessment forms a foundation for formulating conservation and management strategies. In our study, we showed evidence of significant population structuring in *N. randalli* populations using mitochondrial *Cyt b* gene sequences. The mitochondrial DNA analysis of *N. randalli* showed genetic differentiation between west and east coast of India. As a consequence, we propose to manage *N. randalli* on the west and east coast as separate units. Data deduced from this work can be utilized in the conservation and management of *N. randalli*.



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3.9 Molecular Characterization of Sperm Bindin Gene Containing Fucose-Binding Lectin Repeats from Indian Oyster, *Magallana bilineata*

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Keywords: Oyster, Bindin, Fucose lectin, Open reading frame

Introduction

Bivalves are ideal organisms for fertilization studies because many of them release sperm and eggs freely into the water column. Because these animals are free spawning, proteins involved in reproduction and, more specifically, in gamete recognition, which control the binding and fusion of gametes may play an essential role in reproductive isolation and speciation. Oyster sperm have ring shaped acrosome vesicles that exocytose to expose an insoluble protein that bonds sperm to microvilli in the egg vitelline envelope. The insoluble acrosomal rings contain oyster bindin, a protein that agglutinates unfertilized eggs. Oyster bindin is a single copy gene with fucose-binding lectin (F-lectin) domains. These F-lectin repeat number and sequence are variable within and between species. The rapid divergence of gamete recognition proteins within species ultimately leads to incompatibility of sperm and egg between species (Springer et al 2008). In this study, we aim to identify and characterise male gonad specific bindin gene from *Magallana bilineata* (Mb-bind).

Materials and Methods

The male gonad tissue from adult *Magallana bilineata* was collected and subjected to sequencing on the Illumina HiSeq X Platform. A partial cDNA encoding bindin was screened from the obtained transcriptome data. Open reading frame (ORF) of the gene was obtained by ORF finder which was reconfirmed by blast search against NCBI database. ExPASy translate tool (<http://web.expasy.org/translate/>) was used to obtain the encoding protein sequence for the gene. ExPASyMW/pI tool (http://web.expasy.org/compute_pi/) was used for determining the theoretical molecular mass and pI. The sequence similarities with other available bivalve's bindin sequences were analyzed by blastP search at NCBI databases (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Domain architecture and conserved domains were identified using Scan-Prosit (<http://prosite.expasy.org/scanprosite/>), SMART (<http://smart.embl-heidelberg.de/>) and InterProScan (<http://www.ebi.ac.uk/Tools/pfa/iprscan/>) online tools. Presence of signal peptide was predicted by using SignalP 5.0 Server (<http://www.cbs.dtu.dk/services/SignalP/>). F-lectin 3D model was constructed by the SWISS-MODEL Protein Modelling Server (<http://swissmodel.expasy.org/>). For phylogenetic analysis, sequences were aligned using ClustalW, aligned fragments were extracted and phylogenetic tree was generated by a neighbor joining method using MEGA11. The phylogenetic trees of the selected F-lectin repeats were also produced with Neighbor-Joining (NJ) method.

Results and Discussion

In this study, based on the results of transcriptome sequencing of the Indian backwater oyster *M. bilineata*'s gonads, we obtained a cDNA encoding a male gonad specific bindin gene with an ORF of 1710 bp. The deduced protein is composed of 570 amino acids, with an estimated molecular mass of 62 kDa and isoelectric point of 10.45. SignalP program analysis revealed that the cDNA contained a putative signal sequence of 24 amino acids. Obtained signal sequences shared great similarity with that of *Crassostrea sikamea* and *C. gigas*. Alignment of amino acid sequences showed that its deduced amino acid sequence shared 100 and 91% identity with that of *C. gigas* and *C. sikamea*, respectively. Domain architecture analysis by SMART and Scan-Prosit server confidentially predicted the presence of three FTP (Fucolectin Tachylectin-4 Pentaxrin-1) Domains. F-lectin is an ancient protein domain which binds specifically to fucose and found in both prokaryotes and eukaryotes. Three sequences of F-lectin repeat have been got from Mb-bind. Phylogenetic tree based on the nucleotide sequences of five F-lectin repeats from *C. gigas*, 11 haplotypes from *C. sikamea*, three haplotypes from *C. angulata*, one F-lectin repeat from *C. nippona*, and three haplotypes from *M. bilineata* were also constructed. The NJ tree suggests that *M. bilineata* is forming a separate clade other than that of *C. gigas* clade and *C. sikamea* clade. It is interesting that three haplotypes of *C. angulata* nest in two different clades with one in *C. gigas* clade and two others closely associated with *C. sikamea* clade. Diversity of bindin may increase the chance of recognition of other species' egg receptors and this could be an evolutionary response by sperm to match egg receptors that have diversified to avoid being fertilized by multiple sperm (Wu et al., 2011). Hence, the study on diversity among the F lectin domain in Mb-bind could enable understanding the complex speciation and adaptation.



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3.10 Strong Genetic Differentiation in *Uroteuthis duvaucelii* along Indian Peninsular Waters: Revealed by Nuclear Marker

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Keywords: Loliginidae, Population genetics, Indian squid, Nuclear marker, Arabian Sea

Introduction

The Indian squid *Uroteuthis duvaucelii* is an Indo-Pacific species, constituting an important component of the inshore cephalopod fisheries in the Indian coast. It has been undergoing irremediable depletion in its population owing to its high demand in global export trade. Therefore, knowledge on their population dynamics is important to substantiate its sustainability. Stock assessment of Indian squid has been conducted from Arabian Sea and Bay of Bengal based on demographic parameters such as Total mortality rate (Z), Natural mortality rate, Longevity (t_{max}), Growth parameters (L_{∞} and K) and others. However, no records are available on the genetic population structure of this species from the Indian Ocean. Besides, paucity of genetic information on population parameters and stock status of the species may cripple any management intervention towards the sustainable exploitation of their resources in India. Therefore, the present study aimed to characterize population status of *U. duvaucelii* from the Indian Ocean using nuclear marker.

Materials and Methods

The samples of *U. duvaucelii* were collected from selected major collection sites across east and west coast of India. The collected samples were identified to the species level based on characters described by Krishnan et al (2022). Genomic DNA was extracted from the fin base tissue using a modified phenol chloroform method and stored at -20 °C. Quantity and quality of DNA were checked by spectrophotometry (NanoDrop) and Agarose gel electrophoresis (1% agarose gel) respectively. Partial regions of the nuclear gene, rhodopsin, were PCR amplified in Veriti Thermal cycler (Applied Biosystems, USA) using Gene Specific Primers designed using Primer3. Sequence assembly and analysis were carried out using the multiple alignment tool ClustalW in MEGA X. Bayesian phylogenetic analysis was carried out using the above data with Bayesian method in BEAST. The Haplotype network diagram was plotted using PopART. Population genetic statistics like haplotype diversity, nucleotide diversity, Tajimas D, Fst etc. were calculated using DnaSP, MEGA and AMOVA were carried out using Arlequin software.

Results and Discussion

Generally, squids demonstrate complex population structure. In spite of complexities reported in various studies regarding to size, growth rate and age of maturity in their populations, no records are available on the genetic population structure of this species from Indian Ocean. In the present study phylogenetic analysis of *U. duvaucelii* from the Indian Ocean (n=60), using rhodopsin gene revealed two genetically distinct lineages corresponding to the eastern and western Indian Ocean population (BPP=1). A median-joining networking analysis of the rhodopsin gene revealed there is no sharing of alleles between these two populations. From the biogeographic perspective, western Indian population shows little genetic exchange with eastern Indian Oceanic region. In addition, very high genetic differentiation was observed in AMOVA ($F_{ST} = 0.97, p = <0.001$) and pairwise genetic distance estimation ($F_{ST} = 0.98, p = <0.001$) between these two populations. Nucleotide diversity (π) and haplotype diversity (Hd) was low in both populations showing a very low level of variation in rhodopsin gene (Anderson 2000). However, two non-synonymous mutations were observed between two clades. Low genetic diversity, positive and significant value of Tajimas D and Fu's F_s (Neutrality test) and unimodal plot from Mismatch analysis indicated signals of declining populations in both Oceanic regions. Geographical structuring in *U. duvaucelii* population along Indian peninsular waters might be an indication of local adaptation and ecological speciation in the Eastern and Western Indian Ocean as these basins exhibit large contrast in their hydrographic characteristics and biological productivity. Further studies with wide geographical coverage and advanced molecular markers are necessary to further characterize these populations, which need specific conservation measures in the warming Indian Ocean.



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3.11 Molecular Characterization of Calmodulin Gene from Indian Oyster, *Magallana bilineata*

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Keywords: Oyster, Calmodulin, Open reading frame

Introduction

Molluscan shells consist primarily of crystalline and amorphous calcium carbonate polymorphs, calcite and aragonite, which are stabilized by proteins in the shell matrix. The organic macromolecules in mollusc shells are thought to play critical roles in nucleation, orientation, morphology, polymorphism and organization of calcium carbonate crystallites of the shell (Belcher et al 1996). Calmodulin (CaM) is a multifunctional calcium metabolism regulator crucial to bivalve shell formation. They are important calcium sensors and buffers and can respond to ocean acidification in marine calcifiers (Wang et al 2022). Reports on accumulation suggest that calmodulin is of vital importance in the regulation of bivalve calcium homeostasis. However, the precise regulatory mechanisms still need to be understood. Hence to understand the molecular mechanism, we focused on the sequence and phylogenetic analysis of the calmodulin (MbCaM) gene from the Indian oyster *Magallana bilineata* for its potential role in oyster calcium metabolism.

Materials and Methods

The mantle tissue from adult *M. bilineata* from control (pH 7.6) and CO₂ (pH 6.9) stressed groups were collected and used for RNA preparation. Sequencing libraries were prepared and sequenced on IlluminaHiSeq X Platform. A partial cDNA encoding calmodulin was screened from the obtained transcriptome data. The sequence data was then compared using the nucleotide BLAST tool- in National Centre for Biotechnology Information (NCBI) with default parameters. Further, Open Reading Frame (ORF) region was predicted using ORF Finder and corresponding protein sequence were predicted and analysed using DNASTAR software (Lasergene, USA). Domain signatures within the protein sequence were identified using Simple Modular Architecture Research Tool (SMART)). Signal peptide sequences were predicted using the software SignalP 6.1 (www.cbs.dtu.dk/services/SignalP/) and potential phosphorylation sites were determined by NetPhos 3.1 Server (www.services.healthtech.dtu.dk/service.php). The three-dimensional structures of Indian oyster calmodulin were obtained by homology modeling (<http://swissmodel.expasy.org/workspace/index.php>). A multiple sequence alignment was generated using homologous calmodulin gene sequences from other species obtained from NCBI blast query, using Bio-Edit multiple alignment tool and clustalW. Finally, a phylogenetic tree was constructed using MEGA software version X with the Neighbor -Joining method.

Results and Discussion

Based on the transcriptome sequence information, the cDNA of *M. bilineata* CaM contains 402 bp long ORF encoding 133 amino acids with a calculated molecular mass of 15.26 KDa. The deduced amino acid sequence showed 92% identity with CaM from *Crassostrea gigas*. CaM is an important calcium-binding protein with typical EF-hand conformation. Like other homologs, Mb CaM also possess four continuous typical EF-hand domains located from 1 to 22 amino acids, 30 to 58 amino acids, 68 to 96 amino acids and 104 to 132 amino acids. EF-hand is one of the most common calcium-binding domains in both vertebrates and invertebrates, and they bind calcium with high affinity causes a conformational change that alters the activity of the protein. MbCaM lacks signal peptides at the N terminus of the deduced amino acids and 27 predicted phosphorylation sites were found. The primary sequence around the phosphorylation site is important for substrate recognition. From an evolutionary perspective, CaM is remarkably conserved. Multiple sequence alignment of the deduced amino acid sequences of MbCaM with CaM from other invertebrates and vertebrates have been depicted. The CgCaM was firstly clustered with calmodulin protein of *C. gigas* and next with *C. virginica* and then joined into the invertebrate group. The results are in accordance with biological classification. The tertiary structure of MbCaM predicted using multiple templates which have high sequence coverage. Calmodulin are multifunctional proteins involved in a variety of functions such as biomineralisation, stress response and have different functions in different tissues. Our study examines the sequence and phylogenetic analysis of calmodulin gene in commercially important oyster species, *Magallana bilineata*. The findings provide a foundation for understanding the mechanisms involved in calcium regulation in Indian oysters.



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3.12 *De Novo* Transcriptome Assembly and Annotation of the Indian Mackerel *Rastrelliger kanagurta* (Cuvier, 1816)

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Keywords: Transcriptome, *Rastrelliger kanagurta*, De novo assembly, Annotation

Introduction

The Indian mackerel *Rastrelliger kanagurta*, is one of the abundant pelagic shoaling fishes commonly found in the Indo-west Pacific Ocean and its surrounding seas. They are distributed all along India's east and west coasts in the inshore waters up to a depth of 25 m. Scombrid fishes are commonly found in tropical and temperate waters. The increase in next-generation sequencing (NGS) techniques has helped in the generation of the genome and transcriptome sequence information for species without prior genetic information, thereby facilitating the study of gene interaction with environmental changes. This study aims to generate a reference transcriptome of *R. kanagurta* using NGS technology.

Materials and Methods

R. kanagurta was captured off the coast of Kochi; four different tissues were collected immediately upon capture, preserved in RNA later, and kept at -20 °C until RNA extraction. RNA extraction was done for each sample according to the manufacturer's instructions. After determining the purity and integrity, RNA was pooled to prepare a single cDNA library and was paired-end sequenced via Illumina HiSeqTM2500. The reads after quality screening were assembled with the de novo assembler Trinity v2.9.1. The final transcriptome assembly was queried against the non-redundant (NR) database of NCBI applying an E-value cut-off of 1 e-5. Blast2GO software was used to assign GO terms to the assembled transcripts.

Results and Discussion

In the present study, *de novo* transcriptome assembly of the Indian mackerel *R. kanagurta* was performed using Trinity. Trans Decoder was used to identify coding regions, and CD-HIT-EST was then used to remove redundant sequences (Gnocchi et al 2020). All metrics have shown that the overall quality of the transcriptome produced in this study is relatively high. The total number of transcripts and N50 is similar to that of *Scomber colias* liver transcriptome (Machado et al 2018). The transcripts have shown more similarity towards *Oreochromis niloticus*, *Larimichthys crocea*, and *Fundulus heteroclitus*. The GO terms were categorised into three main categories: Biological process (25,436), molecular function (36,878) and cellular component (20,959). The results provide an insight into specific processes, functions and pathways that are involved in the Indian mackerel and pave the way for further analysis of this species under various conditions.

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3.13 Chromosome Set Manipulation for the Induction of Triploidy in Rainbow Trout (*Oncorhynchus mykiss*) from Kashmir Himalaya: A Sterile Trout for Sustainable Aquaculture Enhancement

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Keywords: Rainbow Trout, Heat shock, Fertilization, Hatching, Yolk sac absorption, Triploidy

Rainbow trout, *Oncorhynchus mykiss* is one of the promising cultivable fish species and has wide potential for its expansion. Being a low volume, high value commodity the trout has strong potential for both domestic consumption and international export. The current study to produce sterile rainbow trout (triploids) was carried out at the Govt. trout farm at Laribal, Srinagar. Heat shock at 26°C and 28°C for 10 minute duration were applied after 15 and 20 minutes of fertilization using a water bath. No treatment (heat shock) was given to control. The highest fertilization rate (90.393±0.37%) was observed in group T1, heat shocked at 26°C, after 15 minutes of fertilization (TAF) while as the highest hatching rate (81.843±0.88%) among the treatment groups was observed in group T2, heat shocked at 26°C applied after 20 minutes of fertilization. The highest triploidy rate of 76.66±3.33% was estimated in group T4 heat shocked at 28°C, after 20 minutes of fertilization. However, none of the fish from control group were found to be triploid. Temperature intensity showed significant negative correlation with fertilization and hatching rates respectively. However, hatching rates showed significant positive correlation with heat shock time after fertilization. Similarly, triploidy rates showed significant positive correlation with heat shock time after fertilization and heat shock temperature intensity. Control group resulted in higher survival rates at fertilization and hatching compared to treatment lots.

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3.14 Effect of Extenders, Cryoprotectants and Dilution Ratios on the Spermatological Properties of Cryopreserved Milt of Cobia, *Rachycentron canadum*

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Keywords: *Rachycentron canadum*, Extenders, Cryoprotectants, Dilution ratios, Cryopreservation, Spermatological properties

Introduction

Cobia (*Rachycentron canadum*) belonging to the family Rachycentridae is one of the important marine finfish species having very high potential for aquaculture. Cobia has fast growth rate, adaptability for captive breeding, low production cost, good meat quality and high market demand especially for *sashimi* industry. However, the major problem faced in cobia culture is i) availability of seeds due to asynchronous spawning behaviour of this species, ii) maintaining male and female brooders in the farm require long time and high input cost iii) maintaining only female brooders in the farm can reduce the maintenance cost of male brooders which can be achieved by cryopreservation of spermatozoa. The success of cryopreservation depends not only on preserving the motility of the spermatozoa but on maintaining its metabolic function. The most critical factors that need to be optimised for the successful preservation of fish gametes are the composition of the extenders, cryoprotectants, equilibration time, rate of freezing and thawing temperature. Hence the present study analysed the effect of cryopreservation on the spermatological properties of *R. canadum* spermatozoa and also envisaged to standardize the cryopreservation protocol for *R. canadum* spermatozoa for long term storage.

Materials and Methods

R. canadum male brooders (5 nos.) having approximately 115 cm length and 17.5 kg weight were used to collect milt by cannulation. Two extenders such as Marine Ringers Solution (MFRS) and 0.85% Physiological Saline (PS) solution were used for cryopreservation of spermatozoa. Dimethyl Sulphoxide (DMSO) was used as cryoprotectant at 10% (v/v) concentration. The milt was diluted with these cryodilutents at 1:10 ratio and equilibrated for 10 min at 4 °C in the cold handling unit. The cryopreserved spermatozoa was analysed once in 10 days for a period of 30 days after thawing of the straws at 30 °C for 30s. The milt was diluted with DMSO as cryoprotectant at 5%, 10% and 15% (v/v) concentrations at dilution ratio of 1:10 and the milt was cryopreserved. Based on the previous trials, the best performing extender and cryoprotectant concentration was selected and freshly collected milt was diluted at three dilution ratios *viz.*, 1:5, 1:10 and 1:15 and the milt was cryopreserved. Spermatological parameters such as sperm motility, motility pattern, motility score and percentage of live cells were analysed in raw and cryopreserved milt. The collected data was analyzed by one-way ANOVA and statistical significance for all data were compared at 5% probability level.

Results and Discussion

The motility duration of fresh spermatozoa of *R. canadum* was 58s. When PS was used as extender for cryopreserving milt, the highest mean post thaw motility duration on 30th day was 39.19±0.13 s (Table 1). When milt was diluted with MFRS, the highest mean post thaw motility duration obtained was 37.23±0.06 s on 30th day (Table 2). The values were statistically significant between the two extenders when analysed using one-way ANOVA ($p < 0.05$).

Cryopreservation of *R. canadum* spermatozoa with PS gave the highest percentage of live cells. At the start of the experiment, 89% (highest) of the spermatozoa were found alive, and reduced to 61% on 30th day of cryopreservation. The highest percentage of live cells observed in milt diluted with MFRS was 79% which decreased to 49% at the last day of cryopreservation. Since PS had only 145 mM NaCl and no KCl, it would have better retained the spermatozoa and gave higher number of live spermatozoa when compared with MFRS.

The highest mean post thaw motility duration of 55.27±0.09 s was recorded when the milt was cryopreserved with 10% DMSO. The values between different DMSO concentrations were statistically significant ($p < 0.05$). When different dilution ratios were used, the highest mean post thaw motility duration observed on the first day of experiment was 41.37±0.09 s, 39.34±0.11 s and 35.11±0.06 s when milt was diluted at 1:5, 1:10 and 1:15 ratio, respectively



(Table 2). These values decreased to 31.04 ± 0.04 s, 25.23 ± 0.06 s and 24.26 ± 0.21 s at the end of the experiment at 1:5, 1:10 and 1:15 dilution ratio, respectively and the values were statistically significant between the three dilution ratios ($p < 0.05$) when analysed using one-way ANOVA.

From the present study it can be concluded that milt of *R. canadum* when cryopreserved with 0.85% PS solution and 10% DMSO diluted at 1:5 dilution ratio gave better results in terms of spermatological properties such as higher motility duration, motility score, motility pattern and percentage of live cells. Therefore with the present result, further investigation can be made in validating the effect of cryopreserved milt on the fertilization success of cobia.

Table 1 Mean motility duration (s) of *R. canadum* spermatozoa cryopreserved with different extenders

Extender	Days of cryopreservation			
	Initial	10	20	30
FWFS	52.31 ± 0.01^b	44.97 ± 0.03^b	40.03 ± 0.03^b	37.23 ± 0.06^b
PS	53.31 ± 0.22^a	48.03 ± 0.03^a	42.37 ± 0.03^a	39.19 ± 0.13^a

Table 2 Mean motility duration (s) of *R. canadum* spermatozoa cryopreserved with DMSO at 3 different concentrations

DMSO concentrations (%)	Days of cryopreservation			
	Initial	10	20	30
5	50.34 ± 0.01^c	42.35 ± 0.11^c	40.61 ± 0.03^c	36.04 ± 0.04^c
10	55.27 ± 0.09^a	49.19 ± 0.11^a	43.66 ± 0.01^a	39.19 ± 0.13^a
15	53.53 ± 0.11^b	46.12 ± 0.66^b	42.37 ± 0.03^b	37.23 ± 0.06^b
Mean motility duration (s) of <i>R. canadum</i> spermatozoa cryopreserved at 3 dilution ratios				
Dilution ratios	Days of cryopreservation			
	Initial	10	20	30
1:5	41.37 ± 0.09^a	38.34 ± 0.06^a	35.27 ± 0.06^a	31.04 ± 0.04^a
1:10	39.34 ± 0.11^b	35.16 ± 0.03^b	31.48 ± 0.09^b	25.23 ± 0.06^b
1:15	35.11 ± 0.06^c	32.35 ± 0.11^c	28.48 ± 0.09^c	24.26 ± 0.21^c

In all the tables, Data expressed as Mean \pm SE (n=10, r=3); Mean values in the same column with different superscript differ significantly ($p < 0.05$).

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3.15 Algal Meal's Impact on the Fertilisation Abilities of Common Carp (*Cyprinus carpio* var. *communis*)

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Keywords: Algae, Fecundity, Feed, GSI, *Spirulina*

Spirulina is blue - green algae that contain upto 70% dry weight protein and is a good source of vitamins and PUFA. This study's goal was to evaluate the reproductive efficiency of Common carp fed with *Spirulina platensis* supplemented diets at varied levels and to achieve the most effective supplementation of *S. platensis* in feed. The experiment was set up in which 150 *C. carpio* fish were assigned randomly to following treatments in triplicates : T₁ =Spirulina 25g/kg of feed (2.5%), T₂=Spirulina 50g/kg of feed (5.0%), T₃=Spirulina 7.5g/kg (7.5%) of feed, T₄=Spirulina 100g/kg (10%) of feed and T₀ = feed without Spirulina supplementation as control. The results showed that supplementation of *S. platensis* affected reproductive parameters in common carp and better results were obtained at a dosage of 7.5% i.e. T₃.

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3.16 Ovatide and Wova-FH Effect on the Gonadotropin and Steroidal Levels of *Cyprinus carpio* var. *communis* in Kashmir Waters

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Keywords: Wova-FH, Ovatide, Gonadotropin, Estradoil, Progesterone

A global call for foods of aquatic origin has heightened due to the zooming population growth. As the global aquaculture expands, increasing efforts are focusing on induced breeding techniques. Induced breeding is entailed to meet the rising demand of fish. *Cyprinus carpio* is considered the most desirable species to meet the challenge through induced breeding techniques. *Cyprinus carpio* var. *communis* brooders were treated with two synthetic hormones; Wova-FH and Ovatide and the resulting changes in Gonadotropins (GtH-I and GtH-II) and steroid hormones (estradoil and progesterone) were studied. The changes in the breeding performance due to hormone injections were studied. Efficacy of Wova-FH and Ovatide at different graded dosages (Control, T1, T2 and T3) were tested to see the breeding performance in Common carp, *Cyprinus carpio* var. *communis*. The ability of these treatments to elicit the response was further examined by stimulation of GtH-I and GtH-II and steroids (estradiol and progesterone). GtH-I, GtH-II, Estradoil and Progesterone were significantly impacted by hormone type and hormonal treatments. The level of these hormones (GtH-I, GtH II, estradoil and progesterone) also varied significantly with post injection durations (6 hr, 24hr, 36 hr and 48 hr). Efficacy of Ovatide was found higher than Wova-FH in terms of breeding success and eliciting the gonadotropin and steroidal release.

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3.17 Use of Nanotechnology in Fisheries Management

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Keywords: Aquaculture, Nanocapsules, Nanotechnology, Nano-materials

Nanotechnology is the manipulation of matter on a near-atomic scale to produce new structures, materials and devices. Nanotechnology refers to engineered structures, devices, and systems. Nanomaterials have a length scale between 1 and 100 nanometers. At this size, materials begin to exhibit unique properties that affect physical, chemical, and biological behaviour. Nanotechnology can be used in the fisheries and aquaculture industry for rapid disease detection, enhancing the ability of fish to absorb drugs, vaccines and nutrients. Disease outbreak is the major problematic cause in the development and sustainability of aquaculture. Various approaches have been made to resolve disease problems in aquaculture, one among these is vaccination. Some fishes and shellfishes show impermissible levels of side effects with the use of oil emulsion as an adjuvant. Nanoparticle carriers, like chitosan and poly-lactide-co-glycolide acid (PLGA), of vaccine antigens may provide a high level of protection to fishes against bacterial diseases. They also offer advantages in suppressing certain viral diseases with vaccine-induced side effects. Nano-capsules contain short-strand DNA and are resistant to digestion and degradation. These nano-capsules can be used for mass vaccination which when applied to the water gets absorbed into fish cells. The capsules are broken by the ultrasound mechanism, which releases the DNA and causes an immune reaction against fish as a result of the vaccination. Similar to how oral administration of these vaccines and site-specific release of the active agent for vaccination will lower the expense and effort of managing diseases, application of treatment and vaccine delivery, etc., at the same cost of feeding, resulting in sustainable aquaculture. In the expanding field of aquaculture research, the use of nutraceuticals for stress reduction, value addition, and health management in fish and shellfish is highly beneficial. Nutraceuticals are more expensive to incorporate despite having a minimal requirement. Therefore, it must be used in a way that reduces wastage for optimal use and to make the finished product commercially viable. The development of a nano-delivery method for these kinds of compounds may be able to solve the issues with their commercial use in aquaculture activities. The potential for using nanoparticles to deliver nutraceuticals in fish feed and neutrogenomics research is enormous. Additionally, a variety of feed nano-formulations contribute to the uniformity and flavour of the feed being maintained. Nano-materials like carbon and alumina with added compounds like zeolite are used in aquaculture applications for holding aerobic and anaerobic biofilm for the removal of ammonia, nitrites and nitrate contaminants. Similarly, contaminants like trichloroethane, carbon tetrachloride, dioxins, and polychlorinated biphenyls can be cleaned from ultra-fine nanoscale powder which is iron-based to produce simpler carbon compounds that are less harmful, opening the door for nano-aquaculture. Fishing lures are designed to reflect light to capture fish by drawing the fish's attention. These common lures, however, only reflect light in one direction. To solve this issue, the surface of the lure is painted, and then a nano-coated polyamide film is applied, increasing the likelihood of catching fish by two to three times when compared to the use of a lure without a polyamide coating.

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3.18 Cryopreservation and Its Use in Fisheries

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Keywords: Cryobiology, Cryoprotectants, History, Aquatic animals

Cryopreservation is a process that preserves the integrity of living cells, tissues and organisms by cooling these biological constructs to extremely low temperatures. The science of cryopreservation is known as cryobiology. The responses of living cells to ice formation are of theoretical interest, but there are many practical applications of cryopreservation. Stem cells and other viable tissues, which have great potential for use in research and have many medical applications, cannot be stored with simple cooling or freezing for a long time because ice crystal formation and membrane damage during freezing/thawing will cause cell death. Cryoprotective agents help preserve the cell membranes during cryopreservation procedures by protecting them from breaking down at temperatures below -196°C (less than -320°F). They give protection against cell damage, freeze-thaw cycles, and thawing-induced injury and improve the viability of the frozen tissue. The most common cryoprotective agents are Dimethyl sulfoxide (DMSO) and glycerol. DMSO (ATCC 4-X, 5×5 mL) is most often used at a concentration of 5 to 10% (v/v) in freezing media; the optimum concentration varies with the cell line. Glycerol is added to freezing media as a minor component to increase water solubility. The main aim of cryopreservation is to achieve low temperatures without incurring further harm due to ice crystal formation which can be done over the following temperature: Solid CO_2 (at -79°C); deep freezer (at -80°C); vapor nitrogen (at -150°C) and liquid nitrogen (at -196°C). Cryopreservation was initiated in 1948 after accidental discovery of fowl spermatozoa which successfully survive after freezing to -70°C using glycerol. Scientists worldwide have long sought to preserve fish, frogs, and other aquatic species for future use. Some fish sperms have been successfully frozen for years before thawing and resuscitation however, many others do not survive even transient storage of minutes to hours at -101°F (-75°C). Extensive effort has been directed toward the development of methods to improve survival rates, extending storage times, reducing chilling injury and improving quality control procedures. It has enabled researchers to resurrect the fauna, which were extirpated from their original habitats by human activities, or lost as a result of overfishing, pollution and climate change. Though the protocol for cryopreservation for producing high quality sperm has been developed for more than 200 species of fishes, this technique is rarely adopted at commercial level. The cryopreservation of aquatic species is an actively studied area of research. Hence, the application of cryopreservation is the possible hope in overcoming malnutrition, genetic issues affecting quality seed production, and the conservation of aquatic biodiversity.

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3.19 Use of Cryopreservation for Sustainable Aquaculture

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Keywords: Cryopreservation, Sustainability, Aquaculture, Seed production

Besides human and technological resources, land, water, seed, and feed are the four most important resources to aquaculture. Efficient use of these resources is necessary to guarantee the sustainability of aquaculture. Most aquaculture species can be grown year-round and demand for cultured fish is also largely year-round. However, for the majority of species, spawning and thus seed supply is seasonal. The potential negative impacts of genetics-related broodstock management issues such as inbreeding, genetic drift, introgressive hybridization and unconscious selection have been influencing the production of quality seeds negatively and are a major threat to aquaculture. It is well established that many, if not the majority, of aquaculture stocks, have been negatively impacted by poor genetic management. Cryopreservation serves as a way out of these problems and a possible answer to producing quality seeds and genetically improved varieties. It has been considered a major strategy for the conservation of fish genetic resources. Cryopreservation of fish gametes has been vague since 1953 and the technology is well-studied and validated for many species. Successful cryopreservation is of importance to concerns around aquatic sustainability, biodiversity, and environmental conservation. Sperm can be stored (almost indefinitely) and used whenever eggs are available. This is important for the culture of species where males and females tend to mature at different times. Off-season spawning can be induced in females, and frozen sperm can then be used for fertilization. Sperm of high-quality males can be kept for the fertilization of equally high-quality eggs to improve the performance of fish grown in commercial aquaculture. This can drastically reduce the time for the breeding cycle to achieve genetic improvements, such as faster growth rate, disease resistance, adaptability to extreme conditions, and better feed conversion efficiency. However, it is further mentioned that cryopreservation of fish eggs/embryos could not be made successful because of high chilling sensitivity and low membrane permeability.

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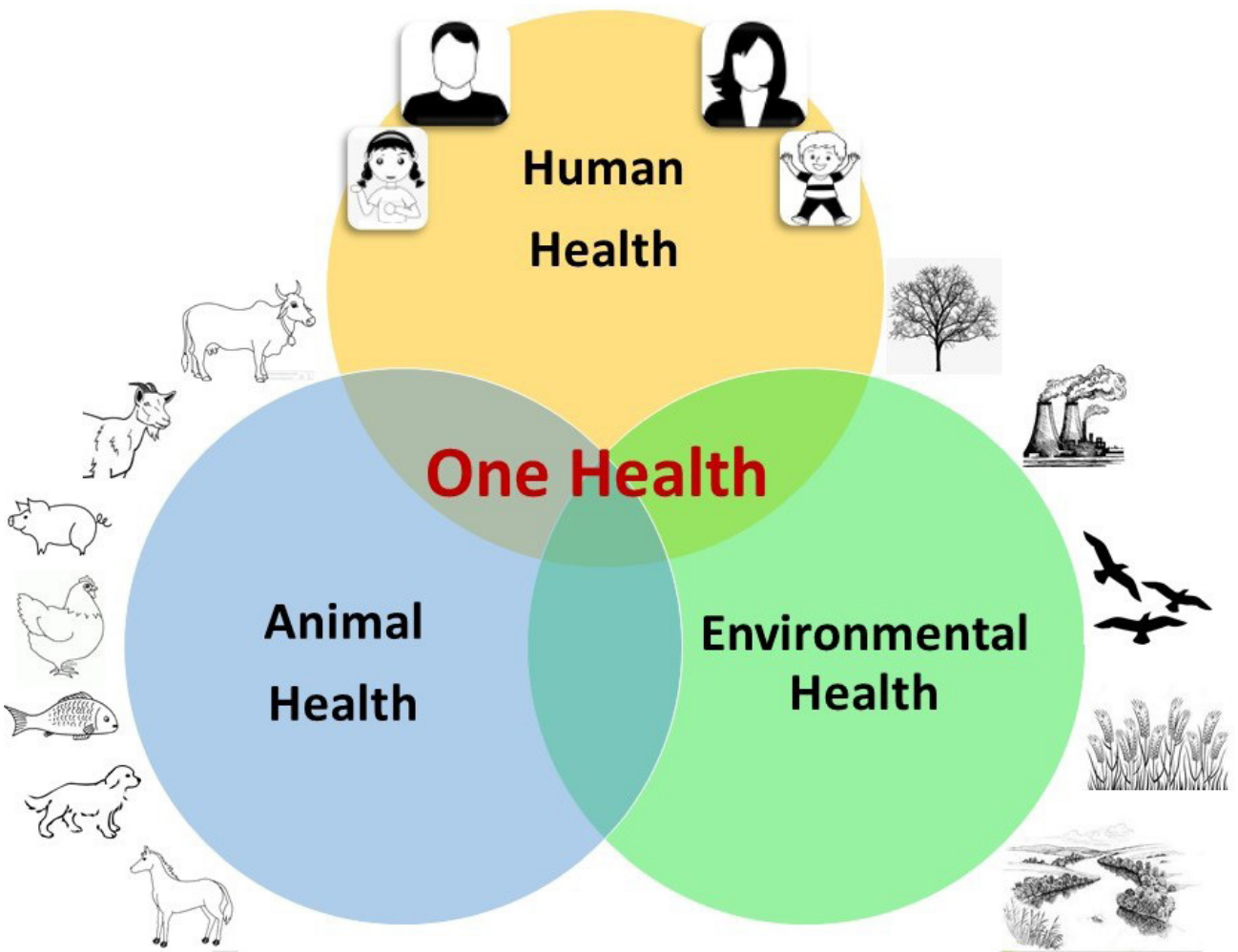




THEME IV

Aquaculture Environment/Health Management and One Health in Aquaculture







4.1 Isolation, Characterization, Genome Sequencing and Stability Study of a Jumbo *Aeromonas veronii* Phage

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Keywords: Aquaculture, Disease outbreaks, Antibiotics, Phage, Host range, Genome sequencing

Introduction

Aeromonas veronii, a Gram-negative, rod-shaped, facultative anaerobic member of the family *Aeromonadaceae*, can cause motile haemorrhagic septicaemia, and tail and fin rot in freshwater fish. Besides, *A. veronii* also has zoonotic potential with the capability to cause human gastroenteritis, soft tissue infections bacteraemia and septicaemia. Antibiotics have often been used as prophylactic and therapeutic agents in human, veterinary and aquaculture medicine. Indiscriminate antibiotic use has led to the global emergence of antibiotic-resistant bacterial pathogens. The rapid emergence of resistance has discouraged pharmaceutical firms to invest in the discovery of novel antibiotics due to “return on investment” concerns. In this context, control of pathogenic bacteria with bacteriophages (phage therapy) has re-emerged as an attractive alternative. Phage characterization in terms of morphology, host range, biochemical properties and genomic characteristics is an important pre-requisite of successful phage therapy. The present study describes the host range, physicochemical and genomic properties of an *A. veronii* phage.

Materials and Methods

For phage enrichment and initial lytic activity detection from dairy farm sewage samples, a previously characterized *A. veronii* A8-AHP isolate was used as the host. Phage host range was determined against a total of 61 field/standard isolates of *Aeromonas*, *Pseudomonas*, *Vibrio*, *Salmonella*, *Escherichia*, *Listeria* and *Staphylococcus*. One-step growth curve was constructed to determine phage adsorption rate, latent period and burst size. The phage genome was subjected to paired-end (2x150 bp) sequencing on Illumina HiSeq 2500 platform. Sequencing reads were quality filtered with Trimmomatic v0.36 and *de novo* assembled with SPAdes v3.7.1. Sequence similarity and phylogenetic relationship of phage genome with other jumbo *Aeromonas* phages (genome size >200 bp) were investigated with BLASTn, VIRIDIC and VICTOR tools. GeneMark Suite, NCBI conserved domain search, BLASTp tools were used for open reading frame (ORF) prediction and functional annotation. The stability of phage preparations was determined at the pH values of 2, 4, 6, 8, 10 and 12. The long-term storage stability of phage AVP1 was determined at temperatures of -80 °C, -20 °C, 4 °C and 25 °C. *In vitro* lytic activity of phage against *A. veronii* host was determined at the multiplicity of infection (MOI) values of 0.001, 0.01, 0.10, 1, 10 and 100. Detailed protocols for above-mentioned analyses have been described elsewhere (Rai et al., 2023).

Results and Discussion

Phage presence was confirmed in 1 out of 10 sewage water samples. This particular phage, named AVP1, could lyse 5 out of 17 *A. veronii* isolates and 1 out of 2 *A. caviae* isolates during host range testing. During one-step growth curve, 95% of phage particles could adhere to host bacterial cells. Phage AVP1 had a latent period of ~20 min within the host cell and a burst size of 86.4±4.1. As *de novo* assembled circularly permuted AVP1 genome consisted of 234,448 bp (NCBI Genbank accession no. – OP889247), it was classified as jumbo phage. BLASTn and VIRIDIC analysis revealed high (>78%) sequence similarity of AVP1 with *Aeromonas* phage pAEv1810 from China and *Aeromonas* phages PS1 and PS2 from India. During VICTOR phylogenetic analysis too, phage AVP1, pAEv1810, PS1 and PS2 clustered together in the same genus. A total of 244 ORFs were predicted in the phage genome. Protein products of 31 ORFs were functionally annotated, whereas rests were classified as hypotheticals. Several proteins essential for phage life cycle were encoded by AVP1 genome (Fig. 1). Besides, 3 tRNA encoding genes were also present in the AVP1 genome. Phage AVP1 showed good stability in pH range of 6 – 12 with a reduction of phage counts by ≤1 log₁₀ unit over a period of 2 h. However, AVP1 could not survive even for 10 min at pH 2 and 4. During storage over a period of 60 days, phage counts remained almost stable at 4 °C and declined by <2 log₁₀ units at 25 °C and -80 °C. Relatively poor stability with phage count reduction of ~ 4 log₁₀ units was observed at -20 °C. Phage AVP1 significantly inhibited *A. hydrophila* growth for up to 10 h at all tested MOIs (*one-way ANOVA*, *p*<0.05). The genomic, host range, biochemical and stability data from the present study indicate that Phage AVP1, either alone or as a part of a phage cocktail, could be used for therapeutic applications.



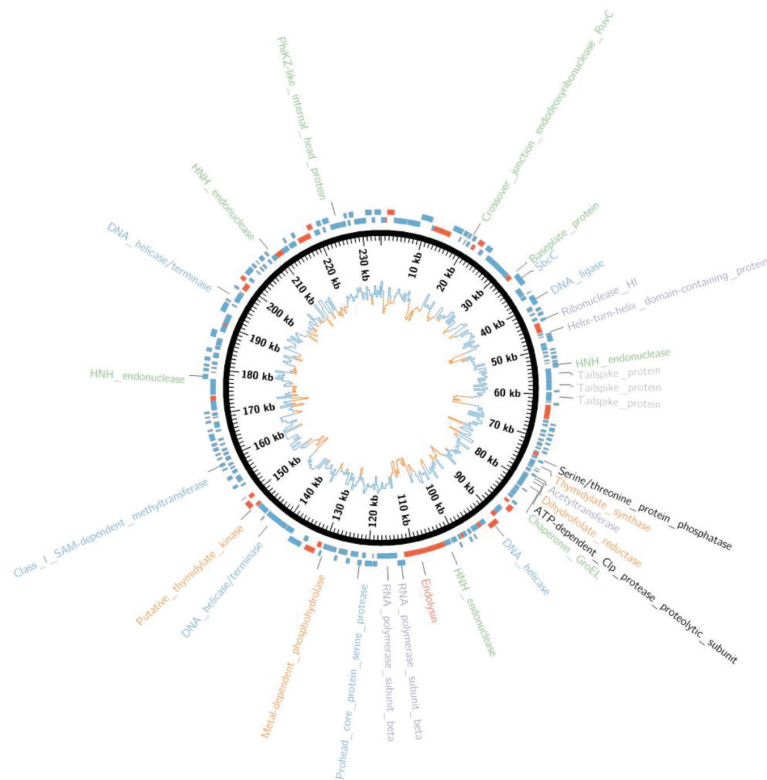


Fig. 1 AVP1 genome representation. The innermost circle depicts positive and negative GC skew in blue and orange colours, respectively. The black circle represents the phage genome in kilobase pair (kb) units. ORFs on positive and negative strands have been shown in blue and red colour, respectively. Label colours of annotated ORFs have been assigned as per functional categories: Light grey – phage adsorption and nucleic acid insertion; Blue – phage DNA replication and repair; Purple – phage DNA transcription; Green – phage structural and packaging; Red – host lysis; Black – unknown.

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4.2 Impact of Dietary Ashwagandha on Various Growth Indices and Gut Histomorphology of *Catla catla*

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Keywords: Ashwagandha, Common carp, Growth performance, Gut histomorphology

Introduction

The aquaculture industry has been growing at an enormous pace on account of the high demand for fish which is an excellent source of protein and so many other nutrients. Of late, numerous chemicals, drugs, antibiotics and other synthetic chemicals are commonly being used as feed additives in fish diets for promoting fish growth (Rico et al., 2013). Recent studies have found that the use of herbal/plant extracts in feed improves fish growth because of the presence of many active components like polysaccharides, alkaloids and flavonoids. Because of the enormous therapeutic relevance of traditional medicinal plants or herbal extracts in stimulating growth, these are being used increasingly in fish feed to supplement growth. The present study aimed to determine the impact of the ayurvedic medicinal herb, *Withania somnifera* (Ashwagandha) as a dietary supplement on the growth, gut histomorphology and postprandial excretory pattern of ammonia and orthophosphate in fingerlings of *Catla catla*.

Materials and Methods

A 90-day feeding trial was conducted on catla fingerlings which were fed with experimental diets in varying concentrations of Ashwagandha powder i.e. 0% (T₀, Control), 2% (T₁), 4% (T₂), 6% (T₃) and 8% (T₄). Fish with an average weight of (5.15±0.034 gms) were divided into five treatment groups (10 fish/tank) in triplicates. Fish were measured for weight before and after the feeding trial to compute various growth performance and feed utilization indices. Fish were fasted for 24 hours and 6 fish i.e. 2 fish per replicate were collected and dissected aseptically at 4°C for histo-morphological studies of intestine. The intestinal tissues were fixed in neutral buffered formalin (10%). An ethanolic dehydration was performed followed by cleaning in xylene. Sections were embedded in paraffin wax, cut at 5 µm and examined under a light microscope. The image measurements were done using ImageJ software. At the end of the 90-day trial, the fish were not fed for 24 hours. Following that, the next day feed was given and after 2 hours, water samples from each tank were collected at 2hr intervals to estimate the excretory levels of total ammonia (N-NH₄) and reactive orthophosphate (o-PO₄) (Raparia and Bhatnagar 2016). All the data were statistically analysed.

Results and Discussion

The results showed a significant improvement (p<0.05) in growth performance indices such as weight gain, specific growth rate, weight gain and daily weight gain and feed utilization indices like feed conversion ratio, feed efficiency rate and protein efficiency ratio in catla fed with Ashwagandha supplemented diets compared to the control diet. Among all treatment groups, the diet containing Ashwagandha @ 6% (T₃) was found to significantly increase growth as compared to other groups. All the treatment groups showed similar basic intestinal histomorphology without any pathological lesions. However, significantly improved villi parameter in terms of height and width of the villi was observed in the T₃ group (6%) compared to the control. The results indicated that the inclusion of ashwagandha in catla fingerlings diets enhances growth performance and improve gut histomorphology. Orthophosphate (O-PO₄) excretion followed a similar pattern across all treatment groups but excretion was minimum in the T₃ (6%) group. Ammonia (N-NH₄) excretion was minimum in T₃ (6%) group whereas the maximum excretion for both ammonia and orthophosphate was in T₀ (control) post-feeding than in groups fed with ashwagandha-supplemented diets. Improved growth performance and nutrient uptake necessarily require a reduction in discharge (N-NH₄ and o-PO₄) in holding water, thus improving the water quality. The use of a suitable plant protein source like ashwagandha has a significant role in the management of excretory levels of ammonia and phosphorous besides improving the growth performance of fish in aquaculture systems.



Table 1: Growth performance indices of *Catla catla* fingerlings fed with different inclusion levels of Ashwagandha

Growth Parameters	T ₀ (0%)	T ₁ (2%)	T ₂ (4%)	T ₃ (6%)	T ₄ (8%)
Initial weight	5.151±0.034	5.097±0.010	5.108±0.0173	5.095±0.032	5.089±0.020
Final weight(g)	7.608±0.217 ^a	7.904±0.029 ^a	8.110±0.315 ^{ab}	8.874±0.102 ^b	8.208±0.435 ^{ab}
Survival rate %	98%	100%	100%	100%	98%
LWG	2.457±0.1845 ^a	2.807±0.0291 ^a	3.001±0.332 ^{ab}	3.779±0.099 ^b	3.118±0.454 ^{ab}
SGR* (% d ⁻¹)	0.432±0.025 ^a	0.487±0.0042 ^a	0.511±0.0476 ^{ab}	0.616±0.0129 ^b	0.527±0.0618 ^{ab}
FCR*	75.644±6.122 ^b	65.414±0.679 ^a	62.870±7.707 ^{ab}	48.642±1.275 ^a	61.151±7.798 ^{ab}
FCE*	1.338±0.1004 ^a	1.529±0.015 ^a	1.634±0.181 ^{ab}	2.058±0.054 ^b	1.698±0.247 ^{ab}
PER*	0.068±0.0051 ^a	0.077±0.0008 ^a	0.083±0.0092 ^{ab}	0.104±0.0027 ^b	0.086±0.0126 ^{ab}
Growth% gain in BW	47.657±3.298 ^a	55.078±.5987 ^a	58.799±6.684 ^{ab}	74.191±2.029 ^b	61.339±9.206 ^{ab}

All the values in the table are mean ± SE of the mean. Data with different superscripts are significantly (p<0.05) different as analysed by Duncans post hoc test. *PER = Protein Efficiency Ratio, BW = Body Weight *SGR = Specific Growth Rate, *FCR = Feed Conversion Ratio, *FCE = Feed Conversion Efficiency

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4.3 Identification of Iron Overload Stress Responsive Genes by Transcriptome Profiling in Aquaculture Species *Labeo rohita*

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Keywords: Transcriptomics, Iron overload, Rohu, RNA-Seq, Stress

Introduction

By 2030, global aquaculture production needs to be increased by 350% to meet the demand of fish. Unremitting fish supplies for an increasing population will depend on maximizing the efficiency and sustainability of the fish production system. But, continuously changing climate conditions have caused severe damage to the environment and aquatic ecosystems are worst affected by these changes. These changes include extreme temperature, high acidity, suboptimal nutrition, infection and high iron content in water bodies. Due to adverse effects of these stressors, aquaculture industry faced significant losses worldwide. A study published by Central Water Commission, India (2014) report that out of 1918 water samples analyzed from different water bodies during the study period, iron concentration was above the acceptable limit in 492 samples. Although, iron is an essential element for many physiological functions of most organisms including fishes but its presence in excess causes toxicity. Fishes tightly regulate the uptake and secretion of iron because they lack regulatory pathway for iron elimination. To keep the balance between its benefits and toxic effects, fishes employ several proteins for handling of absorbed iron which are currently unknown and causing major hindrance in developing mitigation strategy against iron overload stress. Therefore, understanding of expression profile of genes involved in iron overload stress can be vital information in genetic improvement with superior inherited traits like tolerance to iron overload. In view of above, present study was conducted to identify the iron overload stress responsive genes in aquaculture species *Labeo rohita* by whole transcriptome profiling.

Materials and Methods

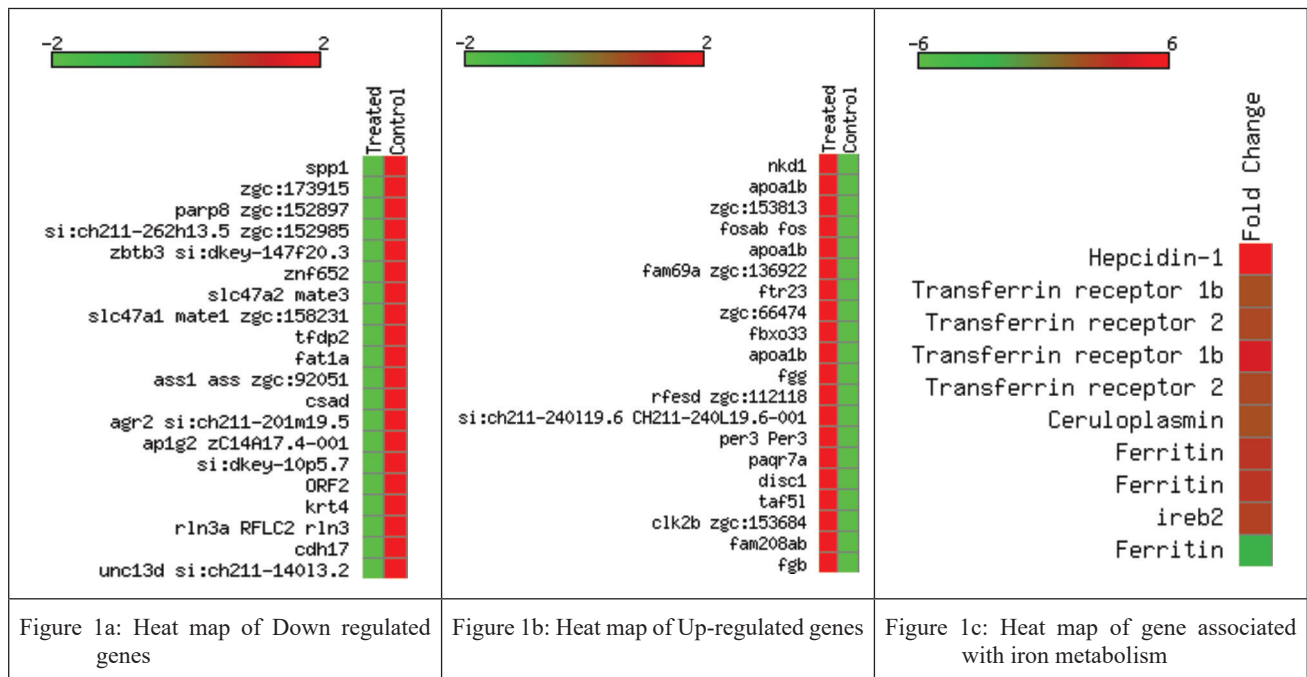
Live specimens of *L. rohita* were procured from local market and were acclimatized in 40 liters of glass aquarium for 2 weeks prior to the experiment. Feeding was stopped before 24 h of *in vivo* exposure experiment. Chemically pure sulphate compound of iron (FeSO₄) was used for *in vivo* exposure of iron. The 96 h LC₅₀ of FeSO₄, i.e. 132.04 mg L⁻¹ for *L. rohita* was determined, using probit analysis. Based on this value, the sub-lethal test concentration of FeSO₄ i.e. 33.01 (SL=1/4th of LC₅₀) mg L⁻¹ and control (0.00 mg L⁻¹) were used for *in vivo* exposure. The both set of experiments i.e. treatment vs. control was performed in duplicate where 40 fishes (20 controls and 20 treatments i.e. 10 in each replication) were exposed. After 96 hrs of exposure, liver tissue from control and treated fishes were aseptically removed and total RNA was extracted. Paired-end cDNA library was prepared from the pooled total RNA (4µg) of liver tissue of control replication 1 & 2 (10 individual per pool) and treatment replication 1 & 2 (10 individual per pool) in equal quantity using mRNA-Seq assay for transcriptome sequencing on Illumina Next Seq 500 Platform. Sequencing was done in one lane to generate 2x150 bp PE reads. The raw sequences generated by Illumina Genome Analyzer were processed with SeqQC-V2.1 for various quality controls. *De novo* assembly of generated transcripts from control and reference guided assembly of control and treatment was done, using CLC Genomics Workbench 9.0. Gene ontology annotation analysis was performed in Blast2GO version 2.5.0 and pathway analyses of unique sequences were carried out based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Differential expression analyses between control and treatment were also performed.

Results and Discussion

A total of 144,393,784 sequences were generated from control and treatment. Differential expression analyses showed up regulation of 5723 genes with highest fold change of +103.43 and down regulation of 5629 genes with highest fold change of -28 in treatment as compared to control. Heat map for top 20 down regulated (Fig. 1a) and up regulated genes (Fig. 1b) were generated and genes involved in iron metabolism with significant fold change were also identified (Fig. 1c). Genes showed the significant (p value <0.01) fold changes in response to the excess iron treatment are mainly ferritin (2.36 fold down regulation), transferrin receptor1b (4.02 fold up regulation), hepcidin (5.34 fold up regulation), and ceruloplasmin (2.01 fold up-regulation). Ferritin is a 450-kDa main iron storage protein whereas transferring receptor helps in transportation of iron-transferrin complex by endocytic pathway. Down-regulation of ferritin coupled with up regulation of transferrin receptor in treatment suggest that during iron overload stress condition liver cells tend to decrease the storage of iron and transport the excess iron out from the liver. Over



expression of ceruloplasmin in treatment may be due to activation of host response against iron overload stress as ceruloplasmin is an acute phase protein found to be induced by the host immune system during stress conditions. Hepcidin is a 25-amino-acid peptide hormone act as the principal regulator of iron absorption and its distribution to tissues. Up regulation of hepcidin in liver tissue of treatment indicates that hepatocytes produce more hepcidin hormone for maintaining iron homeostasis under iron overload stress condition in fishes. Although, present results must be interpreted cautiously as these expression profiles are not validated by quantitative real time pcr. Our results provide the comprehensive insight on differential expression of genes in liver tissue under iron overload in *L. rohita*. Iron overload stress responsive genes identified in present study will also in developing mitigation strategy against iron overload stress in *L. rohita*.



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4.4 Variation in Arsenic Accumulation among the IMC Species: Evidenced through Wetlab Experiment

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Keywords: Arsenic, Bioaccumulation, Carps, Fish, Heavy metal

Introduction

Arsenic (AS), a metalloid having widespread occurrence, toxicity and considered as one of the most dangerous substances to human health. Its odorless and colorless characteristics during the dissolved condition in water and various modes of entry points into the ecosystem through natural, anthropogenic or biogenic sources tagged it as a “Silent Killer” (Sun et al 2022). Moreover, in a trophic food chain, each level can act as an entry point e.g in an aquatic food chain ranging from the primary producers (the phytoplankton) to the tertiary consumers (the fishes) can accumulate arsenic from the surrounding environment (Sarkar et al 2022). Although organisms live in the same environment, the accumulation potency varies with different species. Similarly, in case of IMCs, many authors reported that the Catla fish is more Arsenic accumulating species than the other two species. Therefore, a wet lab experiment was carried out to understand whether Catla is the most AS accumulating species among the IMCs or not.

Materials and Methods

A steady-state wet lab experiment of 60 days duration was designed. Arsenic at two concentrations viz. higher (2 ppm) and lower (0.2 ppm) was used for exposure along with untreated control to the IMC species *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala*. Fishes of 10 – 20 gm were kept in glass aquaria of 100-liter capacity and the experiment was carried out in duplicate having 30 numbers (10 nos @ species) in each aquarium. Prior treatment, the fish were acclimatized in the glass tanks. The fishes were fed once daily. The experiment was continued for 60 days and on each alternate day, 2/3 of the water was exchanged with a proportionate amount of the test material. The first sampling was done on the 30th day and the second on the 60th day. The collected fish were dissected and the organs including skin, gill, liver and muscle were collected for further analysis. The collected samples were digested in tri acid mixture followed by total AS determination in ICP-MS.

Result and Discussion

The inter-organ, as well as inter-species variations in total AS concentration was observed. Among the organs, gill was found as the most AS accumulating organ and the muscles were the least accumulating organ across the species. Particularly, in the lower concentration, the pattern of the deposition of AS was found as gill > skin > liver > muscle; whereas in the higher concentration the deposition pattern was gill > liver > skin > muscle. In fishes treated

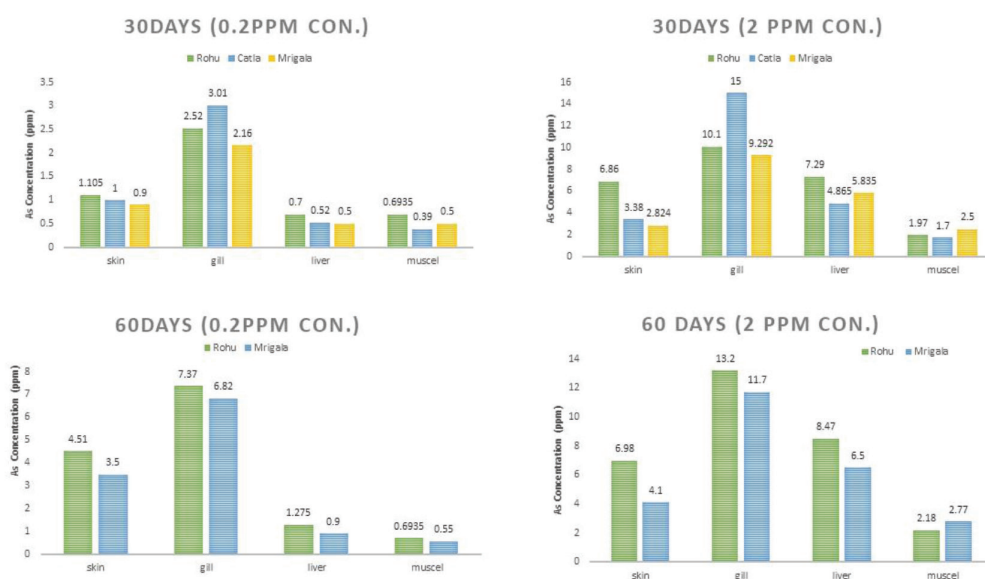


Fig 1.: Bioaccumulation of AS concentration in IMCs



with lower concentration, AS accumulation was 0.39 to 3.01 ppm at 30 days and 0.55 to 7.37 ppm at 60 days. In fishes exposed to higher dose, the concentration range was 1.7 to 15 ppm after 30 days and 1.55 to 10.9 ppm after 60 days. *Catla catla* was found as the most accumulating species with 15 ppm of AS in gill tissue across all the studied concentrations. However, before the 60th-day sampling, all the *C. Catla* fishes had died and from the 60th-day result, it was observed that *Labeo rohita* is having more bioaccumulation potency than *C. mrigala*. In conclusion, the study revealed that gills of Catla accumulated most AS followed by gills of other species. However, for other organs, no specific pattern of AS accumulation was observed among the IMC species. As the muscles were the least accumulating, it is quite encouraging from human food safety point of view.

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4.5 Isolation and Probiotic Characterization of *Lactobacillus plantarum* and *Lactococcus lactis* from Gut Microbiome of Rohu (*Labeo rohita*)

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Keywords: Aquaculture, *Lactobacillus plantarum*, *Lactococcus lactis*, Probiotics

Introduction

Industrialization of the aquaculture sector has led to intensification resulting in stress on aquatic organisms and frequent disease outbreaks leading to huge economic losses. Indiscriminate use of antibiotics as growth promoter and prophylactic agent in aquaculture has resulted in rapid emergence and spread of antibiotic resistance in bacterial pathogens. Over the past few years, use of probiotics (as alternative of antibiotics) in aquaculture has gained attention due to their immuno-stimulant and growth promoting properties. After administration, a probiotic bacterium has to compete and establish itself against native microbiota to show its eventual beneficial properties. Due to their non-fish origin, commercial probiotics sometimes may display poor probiotic functionalities and antagonistic effects. Thus, isolation and characterization of probiotic bacteria from same fish host is very much necessary. In this study, attempts were made to isolate potent probiotic lactic acid bacteria (LAB) from intestinal micro flora of rohu fish.

Materials and Methods

Twenty-five experimental rohu fishes (mean weight 400±20gm, mean standard length 20±3cm) were used to collect fish gut in a sterile condition. The digestive tracts of fishes (1.0 gm) were homogenized in 9 ml of sterile saline solution followed by 10 fold serial dilution and spreading on de Man, Rogosa and Sharpe and *Tryptone soya agar* (HiMedia) plates in triplicates. After incubation at 37° C for 24-48 h, 150 tentative LAB colonies of different morphologies were selected and subsequently sub-cultured from each batch. Gram +ve, non-endospore forming, catalase negative isolates were further shortlisted as tentative LAB. These isolates were subjected to biochemical and molecular testing for genus level identification. Antimicrobial activity spectrums of cells free supernatants of LAB isolates against two fish pathogenic strains *Aeromonas hydrophilla* and *Micrococcus leuteus* was assessed using agar well diffusion method (Ridwan et al. 2008). Species level confirmatory molecular identification of isolates was performed by DNA isolation followed by PCR amplification and sequencing of 16S rRNA gene fragment. Two isolates from each species (FLB1, FLB2 from *L. plantarum*; and FLC1, FLC2 from *L. lactis*) were compared *in vitro* for their hemolytic activity, acid and bile tolerance, auto-aggregation, cell-surface hydrophobicity, tolerance to phenol, cell adhesion, and safety parameters.

Results and Discussion

A total of 17 isolates, identified as *Lactobacillus plantarum* and *Lactococcus lactis*, identified by biochemical tests and PCR amplification and sequencing of 16S rRNA gene fragment, displayed promising antimicrobial activity against both the pathogens. None of the tested isolates, showed any hemolytic activity indicating their potential safety. Antibiotic sensitivity test revealed that all the tested LAB isolates were resistant to vancomycin, gentamicin, streptomycin, and erythromycin and sensitive to erythromycin, chloramphenicol, ampicillin, trimethoprim, and nitrofurantoin. Tetracycline resistance was found in *L. plantarum* (FLB1 and FLB2 isolates), whereas *L. lactis* were susceptible to it. Moreover, these isolates were tolerant to 0.3% bile (75-82% survival), phenol stress (96-99% survival) with 100% viability at pH 3 over a period of 3 h which show their survival capability to stay in gut environment. Intramuscular and intra-peritoneal challenges to fingerlings of rohu fish (5±1gm weight) with FLB1 showed no pathogenicity and occurrence of disease symptoms in fishes over an observation period of 7 days. The study revealed heterogeneity in probiotic properties among the different isolates and facilitate the possibility of using single isolate as well as mixing multiple cultures for the extensive range of useful traits. More specifically, results showed that owing to its good probiotic properties and safety characteristics, *L. plantarum* FLB1 strain would be the best candidate species for inclusion as probiotic in functional food and health products for fishes in aquaculture.

Table 1 Antibiotic susceptibility of selected FLB and FLC isolates

Antibiotics	Concentration		Selected Isolates									
	(µg/disc)		FLB1	FLB2	FLC1	FLC2						
Tetracycline (TE) (only reacted differently in all antibiotics)	30		R	R	S	S						
<i>S – Sensitive; R – Resistant</i>												
Viable cell count of <i>Lactobacillus</i> and <i>Lactococcus</i> strains at different pH (log cfu/ml)												
Isolates	pH 1.0			pH 2.0			pH 3.0			Control		
	Time			Time			Time			Time		
	0 h	1h	2h	0 h	1h	2h	0 h	1h	2h	0 h	1h	2h
FLB1	7.46±0.17	0	0	8.40±0.06	0	0	9.17±0.20	9.22±0.18	9.31±0.12	9.27±0.10	9.34±0.02	9.60±0.05
FLB2	7.12±0.21	0	0	8.38±0.10	0	0	9.13±0.04	9.18±0.01	9.26±0.03	9.26±0.03	9.32±0.05	9.54±0.05
FLC1	6.80±0.23	0	0	8.07±0.16	0	0	9.05±0.05	9.10±0.05	9.20±0.01	9.23±0.02	9.28±0.02	9.44±0.02
FLC2	6.82±0.21	0	0	8.14±0.09	0	0	9.00±0.07	9.07±0.06	9.19±0.02	9.20±0.01	9.25±0.02	9.43±0.03
<i>Values are mean ± SD of three independent determinations (n = 3) of each sample</i>												
Survival of isolates after 3 h at 0.3% bile concentration (log cfu/ml)												
Isolates	0.3% bile				Control without bile							
	Time				Time							
	0 h	1 h	2hr	3hr	0 h	1 h	2hr					
FLB1	8.31±0.02	7.83±0.04	6.93±0.02	6.86±0.02	8.36±0.02	8.45±0.06	8.71±0.08					
FLB2	8.26±0.04	7.77±0.12	6.75±0.15	6.63±0.15	8.34±0.01	8.39±0.03	8.64±0.05					
FLC1	8.19±0.02	7.62±0.07	6.62±0.15	6.40±0.08	8.25±0.02	8.29±0.01	8.50±0.04					
FLC2	8.25±0.03	7.68±0.22	6.27±0.11	6.21±0.14	8.30±0.04	8.34±0.03	8.56±0.05					
<i>The mean of three values of each sample are presented along with ±SD</i>												
Survival of isolates after 24 h at 0.4% phenol							Cell Auto aggregation properties of selected isolates					
Isolates	Viable counts (log cfu/ml)						% Aggregation					
	0 hr			24 hr								
FLB1	9.36±0.01			9.33 ^a ±0.03			83.275 ^a ± 2.199					
FLB2	9.39±0.02			9.21 ^b ±0.11			80.4578 ^b ± 0.15					
FLC1	9.43±0.02			9.19 ^b ±0.01			67.530 ^c ± 0.749					
FLC2	9.45±0.01			9.14 ^b ±0.04			74.426 ^d ± 1.956					

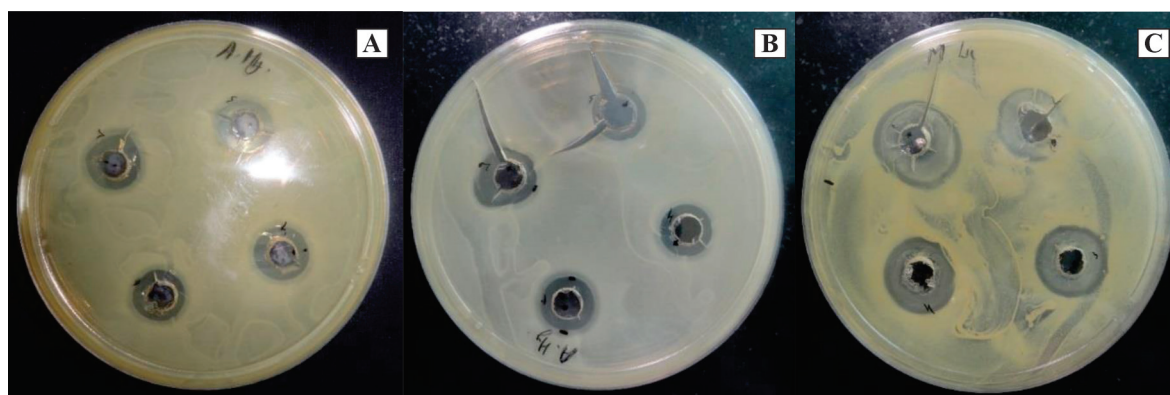


Figure 1 Antibacterial activity of selected FLB and FLC isolates against *A. hydrophila* (Fig. A & B) and *M. luteus* (Fig. C)



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4.6 Comparison of *Oreochromis niloticus* Erythromorphology following Dietary Emamectin Benzoate Administration at the Recommended and Extended Doses

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Keywords: Nile tilapia, Antiparasitic veterinary drug, Emamectin benzoate, Haematology, Erythrocyte morphology, Haematotoxic

Introduction

The aquaculture sector has a substantial impact on the economy, food and livelihood security by its contribution for wealth through the supply of nutritious food. Globally, tilapia has now become the second most cultured species after carps. Emamectin benzoate (EB) from the avermectin family is used to treat marine and freshwater fish lice at the recommended dose of 50 µg/kg biomass/day for 7 consecutive days (MSD Animal Health, 2012). Often, the farmers disregard the recommended dose and dosage of medications to see results quickly, which may affect the health of fish. The haematological responses of freshwater fish under the stress of toxicants may be used as an indicator in biomonitoring programmes to assess fish health (Das *et al.*, 2022). This study compared erythromorphologic alterations of Nile tilapia *Oreochromis niloticus* fed the recommended and extended dose of EB.

Materials and Methods

The experiment was carried out in sequentially labelled fibreglass reinforced plastic tanks with 25 farm-grown *O. niloticus* juveniles having size 13.56 ± 0.46 g. The top-coated EB diets were offered to tilapias at 2% body weight thrice daily for 7 (7D group) and 14 (14D group) consecutive days separately (Das *et al.*, 2022). For a stress-free collection of blood, the clove oil anaesthetized fish were sampled and blood was collected without anticoagulant in 2-mL sterile syringes. A drop of blood was immediately placed on the chemically cleaned slide before it gets coagulated to make thin smears, which were further stained with undiluted Giemsa stain. After drying, the stained smears were observed under an advanced trinocular research microscope using a digital camera attached to the microscope and computer. Descriptive data were obtained by observing the shape alterations of the cells as well as their nuclei with 10× (ocular) 100 (objective) magnifications. On each sampling day, i.e., pre-dosing (Day 0); EB-dosing (Day 7 and/or day 14) and post-EB-dosing (Day 28 post-dosing for each experiment), nearly 100 erythrocytes were observed and the frequency of aberrations in 7D and 14D groups were documented and compared.

Results and Discussion

There were similarities and dissimilarities in the erythrocyte morphological changes of both groups fed EB at 50 µg/kg biomass/day. The 7D group documented 14 and 17% of cellular and nuclear abnormalities, respectively. In the 14D group, both abnormalities hiked proportionately. It signified that when fish were administered the EB diet for an extended time, the EB exerts a severe haematotoxic effect on the blood cells, especially the erythrocyte nucleus. Cellular changes like crenation on the cell wall were markedly higher in the 14D group, which indicated the peroxidation of unsaturated fatty acid in biological membranes. Cytoplasmic extrusions were first noted for the 7D group, which led to the formation of teardrop-shaped erythrocytes (Figs. 1b,d). Notched nuclei indicated aneuploidy from tubular failure, whereas binucleated cells indicated cytokinesis blocking during cell division. Vacuolation was absent in the 7D group, while its presence in the 14D group indicated the internal damage of the erythrocytes. Peripheral or eccentric nuclei indicated the movement of the nucleus inside the erythrocytes, which was noted in about 2% of the cells of the 14D group. The observations on the marked nuclear changes particularly increased micronuclei formation in the 14D group signified the genotoxic effect of extended EB-dosing. With the cessation of dosing, erythrocytes started to recover. However, the D14 group exhibited a higher frequency of cellular and nuclear anomalies (>9%) even after 4 weeks of suspension of dosing (Figs. 1c,e). The observations of the present study indicated that EB-dosing for an extended period can cause more detrimental effects on *O. niloticus* at the cellular, nuclear and even genetic levels than the recommended dosed group. The judicious use of medications at the recommended dose and dosage is, therefore, suggested to maintain the health of fish and to prevent production loss.

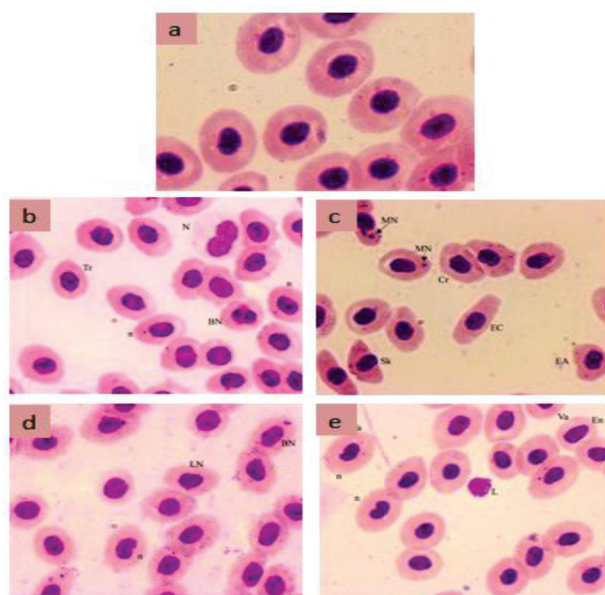


Fig. 1. Photomicrography of blood smears of the Nile tilapia *Oreochromis niloticus* [a] control group, [b] EB-dosed group on day 7 of dosing, [c] on day 14 of dosing, [d] EB-dosed group on day 28 post-dosing for 7D group, [e] on day 28 of post-dosing for 14D group, $\times 1000$ Giemsa staining. Tr: teardrop-shaped cell, N: neutrophil, BN: Binucleated cell, n: notched nucleus; MN: micronucleus; EC: Elongated cell; EA: abnormal-shaped cell; Sk: Sickle cell; LN: lobbed nucleus; Va: vacuolated cell, En: elongate nucleus, Cr: crenated cell.

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4.7 Toxicological Effects of Organophosphate Insecticides on Haemato-Biochemical Indices of *Cyprinus carpio* var. *communis*

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Keywords: *Cyprinus carpio* var. *communis*, Organophosphate, Toxicity, Hematology, Biochemical indices

Introduction

Insecticides are used to kill, mitigate unwanted insect pests. However they reach natural waterbodies through leaching, percolation or drift and pose threat to vital aquatic organisms such as fishes that are used as food organisms for human consumption. Among various classes of insecticides organophosphates are widely used around the world. They have majorly replaced persistent chlorinated compounds on the account of their low persistence and cumulative ability in the environment (Svoboda, *et al.*, 2001). Besides inducing delayed neurotoxicity, organophosphate insecticides alter various haematological and biochemical indices (Qayoom *et al.*, 2017 & 2018). This forms the basis of evaluating hemato-biochemical indices in fishes as a basic tool for assessment of organophosphate toxicity. In this context, present study was carried out to study the effects of two organophosphate compounds, chlorpyrifos and dimethoate on haemato-biochemical indices of juvenile *Cyprinus carpio* var. *communis*.

Materials and Methods

Toxicity tests and test organism

All the toxicity tests were carried out as per Reish and Odisha, (1987). Common carp, *Cyprinus carpio* var. *communis* was taken as test organism in the present study brought from National Fish Seed Farm Manasba

Bioassay

Short term acute bioassays were carried out for both insecticides on *C. carpio* var. *communis*. Fishes were observed for the period of 96 hours and mortality was recorded after 6, 12, 24, 48, 72 and 96 hours. The three same concentrations, each for dimethoate and cpf were selected (C1=1.0ppm, C2=4.0ppm and C3=6.0ppm) based on the earlier literature. *Haemato-biochemical studies*

For estimation of various haemato-biochemical indices, insecticides exposed fishes were kept in 200 ppm benzoquinone solution till they became unconscious for collection of blood. The blood was collected from live fishes from their heart (Lucky, 1977). For biochemical analysis, fish blood was centrifuged @ 4000 rpm and decant the supernatant for analysis of various biochemical indices.

Results and Discussion

Toxicities of dimethoate and chlorpyrifos

In dimethoate exposed fishes, C3 witnessed death of maximum number of fishes (66.67%) followed by C2 in which (16.66). No mortality was recorded in C1. In chlorpyrifos experiments, all fishes died in C2 and C3 while as in C1 (66.67%) were found dead at the termination of experiment. The LC₅₀ values for dimethoate and chlorpyrifos was estimated 5.38 and 0.695 ppm, respectively.

Table 1: Hematological indices of *Cyprinus Carpio* exposed to dimethoate and chlorpyrifos

Hematological Parameters	Dimethoate		P-value	Chlorpyrifos		P- value
	Control	Treated		Control	Treated	
Hb (g/L)	2.54±0.29	1.6±0.4	0.011	2.18±0.105	0.98±0.21	0.010
TEC (×106/mm ³)	2.75±0.12	1.496±0.318	0.010	2.64±0.22	1.34±0.13	0.010
TLC (× 106/mm ³)	2.79±0.395	3.7±0.303	0.011	2.46±0.188	3.74±0.422	0.010
MCV (%)	0.61± 0.045	2.2±0.799	0.576	0.46±0.146	1.85± 1.149	0.034
MCH (%)	0.46± 0.15	1.85±1.25	0.576	7.67±0.815	7.47±0.38	0.833
MCHC (%)	14.18±1.59	1.99±0.64	0.010	14.58±0.42	2.35±1.22	0.010
PCV (%)	46.48±23.87	6.86 ±2.28	0.0120	46.48±2.87	6.64 ±1.07	0.0111



Table 2: Blood biochemical parameters in *Cyprinus Carpio* exposed to dimethoate and chlorpyrifos

S. No	Biochemical indices	Dimethoate			Chlorpyrifos		
		Control	Treated	P-value	Control	Treated	P-value
1	TP (g/l)	3.82±0.13	1.66±0.034	0.0119	5.14±0.40	1.42± 0.73	0.0119
2	SA (g/l)	2.44±0.02	1.58±0.55	0.010	1.66±0.43	0.60± 0.840	0.014
3	SGLO (g/l)	1.3±0.267	1.26±0.278	0.0119	1.66±0.43	0.60± 0.645	0.0119
4	SGLU (mg/dl)	24.4±6.53	29.94±9.73	0.501	11.36±1.86	62.36± 14.36	0.0119
5	SB (mg/dl)	0.1±0.03	1.28±0.196	0.576	0.18±0.063	1.34± 0.706	0.830
6	SC (mg/dl)	16.32±1.44	6.94±1.61	0.010	19.94±1.08	2.62± 0.94	0.010
7	AST (IU/L)	975.4 ±53.95	1155.6±61.6	0.0119	864.4±10.5	1072.0±91.56	0.0119
8	LDH (IU/L)	1119.6±34.68	1830.8±51.04	0.0119	1183.0±34.87	1817.0±53.52	0.0119
9	ALT (IU/L)	619.8 ±10.06	812.4±21.8	0.0119	582.0±34.06	886.0±22.86	0.0119

During present study, the acute toxicity tests were carried out on *C. carpio* var. *communis* exposed to dimethoate and chlorpyrifos commercial. The median lethal concentration (LC₅₀ value) for dimethoate and cpf was 4.34mg/l and 0.6195mg/l respectively.

Reduction in total erythrocyte count were recorded in both the pesticide treated fishes when compared with their respective controls. The significant reduction in TEC and Hb values could be attributed to the destruction of RBC's could be due to the pesticide exposure leading to the haematotoxicity in fishes. The total leukocyte count in the present study was found significantly increased due to the pesticide exposure as compared to the control. During present study the values of MCV level of dimethoate treated fishes was reduced. While in chlorpyrifos treated fishes it increased. In both the experimental specimens, normochromic normocytic anaemia was observed with unusual occurrence of hypochromia in the RBCs of chlorpyrifos treated fishes. The values of MCH level of dimethoate treated fishes was reduced. While as in chlorpyrifos treated fishes the Mean Corpuscle Haemoglobin increased. Thus, the occurrence of normochromic normocytic anaemia is confirmed in relation with the values of MCV obtained in the present study.

The total protein contents were found decreased indicating a severe hypoproteinaemia, in *C. carpio* var. *communis* treated with both the pesticides in comparison with the control. Decrease in total protein content is attributed to the stress conditions that arise due to acute pesticide exposure. The albumin levels were found decreased (hypalbuminaemia) when exposed to dimethoate and chlorpyrifos. However, in chlorpyrifos treated fishes the decrease was more indicative of potential toxicity of the compound as compared to dimethoate. The globulin levels were found decreased in controls (hyperglobulinaemia) of both experiments. The decrease in the globulin levels is probably due to the potential toxicity of organophosphates responsible for the destruction of cells, muscle constituents and protein reserves in the body. The increase in the serum glucose levels was observed in both, dimethoate and chlorpyrifos treated fish groups. Blood glucose is one of the important biochemical parameters determining the pathological or stress mediated alterations in the body. Increase in the bilirubin concentrations depicted liver damage in fishes as observed in the present study. The increase in chlorpyrifos treated fishes was observed more indicating damage of hepatocytes in the fishes. The cholesterol levels showed a significant decrease in both the pesticide treated fishes. It is possible that the organophosphates cause hepatic injury and blockage of enzyme system for steroidogenesis also reported by Ganeshwade (2012 b) in *Puntius ticto* exposed to dimethoate. The increased levels of LDH were found in fishes exposed to dimethoate and chlorpyrifos as compared to the control specimens. The increase in LDH levels is due to the hepatocellular damage caused by organophosphorus pesticide toxicity in fishes leading to the release of the enzyme in the blood stream. High levels of ALAT in the serum of pesticide treated fishes as compared to their respective controls. The increase in the ALAT levels can be attributed to the hepatic injury caused by the acute exposure of pesticides to fishes.

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4.8 Comparative Evaluation of *In Vitro* Proliferation of *Lemna minor* on Different Nutrient Media and Acute Toxicity Testing of Oxybenzone

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Keywords: *Lemna minor*, Nutrient media, Growth, OECD, Oxybenzone Toxicity

Introduction

Lemna minor is a ubiquitous floating plant, inhabiting stagnant to gently-flowing waters and exhibiting rapid vegetative growth. It causes pollutants' degradation and removal from wastewater aided by its unique morphology and physiology. Hence, it has been commonly used as a phytoremediator. Oxybenzone is an emerging pollutant with the potential of endocrine disruption and neurotoxicity and can be phytoremediated using *Lemna minor*. Toxicity assessment using standard OECD testing guidelines is a pre-requisite for phytoremediation studies. However, for *Lemna*, for the test to be valid, the doubling time (DT) of the frond number in control must be less than 2.5 days (OECD, 2006). This study aimed to investigate the proliferation ability of *L. minor* under axenic conditions, in response to two OECD-recommended nutritive solutions for *L. minor* toxicity testing- Steinberg and Swedish Standard Institute (SIS) media. This data would enable trials for toxicity research against oxybenzone, to suffice the said validity criteria.

Materials and Methods

The pure culture of the aquatic plant was sub-cultured in 500ml Erlenmeyer flasks with Steinberg media (pH 5.5 ± 0.2) and SIS media (pH 6.5 ± 0.2) under laboratory conditions (temperature 24 ± 1°C with 30 watts light intensity provided by cool white fluorescent lamps and continuous illumination). The plant growth was assessed manually over 21 days period. The frond number was counted periodically and a standard curve was made establishing the correlation of frond number with days of culture. The plant's average specific growth rate (ASGR) (μ) and DT (t_d) (days⁻¹) were calculated using the OECD's formula (2006).

Growth inhibition test:

The growth inhibition test was conducted for 7 days to determine the IC₅₀ (median percentile inhibitory concentration) of oxybenzone on *L. minor*, following OECD Guideline 221 (OECD, 2006), with modifications, with an exposure to six different concentrations of oxybenzone, i.e., 1, 2.5, 5, 7.5, 10 and 12.5 mg L⁻¹; simultaneously, control cultures were kept without the addition of oxybenzone. The percent inhibition of growth compared to the control culture was calculated as per OECD guidelines, 2006. IC₅₀ values (95% confidence interval) were determined on 7th day using GraphPad prism version software 8 (GraphPad Software, Inc., San Diego, USA).

Results and Discussion

Preparation of growth curve in Steinberg and SIS media

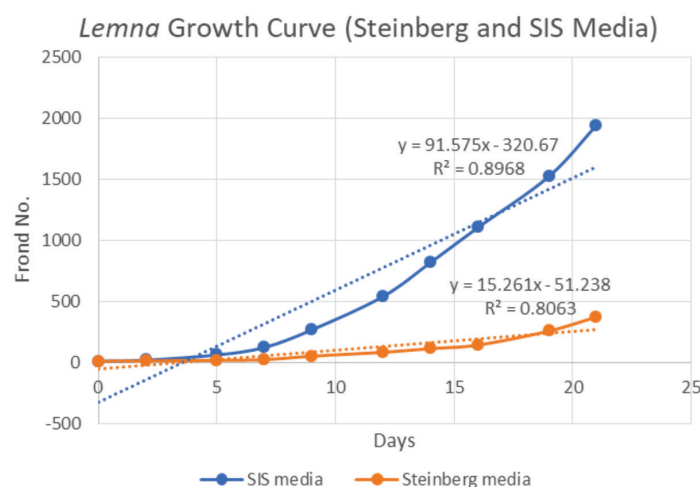


Fig: *Lemna minor* growth curve in Steinberg and SIS Media



The ASGR of *Lemna minor* in Steinberg media was 0.17 day⁻¹ while that in SIS media was 0.25 day⁻¹ and respective DT was 4.02 days and 2.76 days. Also, the growth in SIS media (R²=0.8968) followed a good linear relationship compared to the Steinberg media (R²=0.8063). Hence, SIS media was selected for culture of *L. minor*.

Growth Inhibition Test (As per OECD Guidelines 221, 2006)

Table 1 Effect of varying OBZ concentrations on the growth of *L. minor*

Concentration (mg L ⁻¹)	FronD No.	Avg. Specific Growth Rate (μ) (per day)	Doubling Time (days)	Growth Inhibition (%)
Control	450	0.2889 ± 0.0011	2.405 ± 0.005	-
1	493	0.3004 ± 0.0004	2.3 ± 0.000	-4.5247 ± 0.0046
2.5	417	0.2770 ± 0.0000	2.5 ± 0.000	3.785 ± 0.0051
5	224	0.1891 ± 0.0009	3.685 ± 0.005	34.626 ± 0.0039
7.5	169	0.1490 ± 0.0010	4.675 ± 0.005	48.6126 ± 0.0074
10	140	0.1205 ± 0.0005	5.725 ± 0.005	57.9442 ± 0.0042
12.5	125	0.1024 ± 0.0024	6.605 ± 0.005	63.5765 ± 0.0035

Values are presented as the mean ± SE of three independent replications (n=3)

The DT in control was found to be 2.405 ± 0.005 days. Further, the growth study demonstrated a significant increase at 1 mg L⁻¹ followed by decrease in the frond no. and corresponding ASGR of the plant in a concentration-dependent manner compared to the control. The DT of the plant was the least at 1 mg L⁻¹ and further increased along with increasing concentration. Compared to the control, the highest percent inhibition of growth rate (μ) was observed for 12.5 mg L⁻¹, and the lowest percent inhibition of growth rate was observed at 2.5 mg L⁻¹; with 1 mg L⁻¹ showing a beneficial effect, known as hormesis effect, decreasing subsequently with increasing concentrations. The results revealed that the 7-days IC₅₀ value for oxybenzone based on inhibition of frond number was 8.531 mg L⁻¹ (7.414 to 9.648 mg L⁻¹) for *L. minor*.

Thus, SIS media was found to be more suitable for the growth of *L. minor* with a significant difference in its growth parameters when compared to Steinberg media, while also meeting the validity criteria of the toxicity testing guidelines by OECD against oxybenzone. In the growth inhibition test, hormesis effect was observed at 1 mg L⁻¹ as an adaptation response to the toxic effect of oxybenzone, while a further reduction in the growth in a dose-dependent manner. From the IC₅₀ value, oxybenzone can be categorised as ‘toxic to aquatic organisms’ as per the European directive EC 93/67/EEC.

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4.9 Determination of LC₅₀ Value of Arsenic Trioxide for Fish, *Cyprinus carpio*

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Keywords: Arsenic trioxide, *Cyprinus carpio*, LC₅₀, Toxicity

Introduction

Search for water molecules on other planets, clearly state that water is the most precious natural resource without which we cannot imagine any life on our planet. But natural as well as anthropogenic activities are deteriorating the quality of available water resources. One such agent is heavy metal which accumulate in living organisms and storage is faster than their breakdown or excretion. Fishes make a major contribution to aquatic ecosystem by providing essential nutrients as well as generate employment for millions of people. Fishes are important source of Omega 3 fatty acid which keep our heart and brain healthy. Arsenic, a heavy metal is known to cause detrimental effects on aquatic organisms. Its exposure to human results in skin manifestations, vascular diseases, renal disorders, neurological alterations, teratogenic effects and is carcinogenic. *Cyprinus carpio* is a commercial edible fish in India. It is necessary to evaluate the edible organisms like them from toxicity point of view as human health is directly associated with it. In the present investigation, *Cyprinus carpio* is used to determine the 96-Hr LC₅₀ concentration of Arsenic trioxide (As₂O₃).

Materials and Methods

The experimental fish, *Cyprinus carpio* with an average weight 100±120g and length 20-25cm were collected from Deoli fish farm at District Bilaspur, Himachal Pradesh. The fishes were acclimatized to laboratory conditions in dechlorinated tap water for 15 days in glass aquarium of 150 liters capacity equipped with aeration. The water was dechlorinated by dissolving anti chlorine solution and then exposing it to air for 24 hours. Fishes were given bath for 3-4 minutes in 0.1% KMnO₄ solution to prevent parasitic infestation. Only half of the volume of the water was changed after every 24 hours to maintain osmolarity. Fishes were fed with commercial feed equal to 1/10th of their body weight. Unconsumed feed and excreta were siphoned daily. Feed was not given to the experimental fishes 24 hours before the commencement of experiment. Eight individuals of experimental fish were tested against different concentrations of As₂O₃ for the determination of its lethal concentration. On the basis of mortality in each group, 96-Hr LC₅₀ of As₂O₃ was calculated by using Probit Static Bioassay test system. The data obtained from present investigation were statistically analysed by using IBM SPSS 21 computer program.

Results and Discussion

Eight individuals of *C. carpio* were tested against As₂O₃ concentration for the determination of 96-hr LC₅₀ at constant temperature (22-23°C) and pH (7.00) of water. During the present investigation, the 96-hr LC₅₀ of As₂O₃ for the fish *C. carpio* was found to be 13.74 mg/g at 95% confidence limit, and its lower and upper limits are 9.41 mg/g and 18.47 mg/g, respectively. The probit analysis of the mortality of *C. carpio* at various concentrations of As₂O₃ is shown in Table 1. It is evident from the result that the observed and expected responses in probit analysis do not deviate much. The SPSS software generated regression line between the probit mortality of *Cyprinus carpio* and the log values of the concentrations of As₂O₃ is shown in figure I.

Table 1 Probit analysis of mortality of *C. carpio* at various concentrations of As₂O₃

	Number	Concentration	Number of Subjects	Observed Responses	Expected Responses	Residual	Probability
PROBIT	Probit 1	.602	8	1	.550	.450	.069
	2	.903	8	2	2.059	-.059	.257
	3	1.079	8	3	3.480	-.480	.435
	4	1.204	8	4	4.580	-.580	.572
	5	1.301	8	5	5.392	-.392	.674
	6	1.380	8	6	5.990	.010	.749
	7	1.447	8	6	6.432	.432	.804
	8	1.505	8	8	6.763	1.237	.845

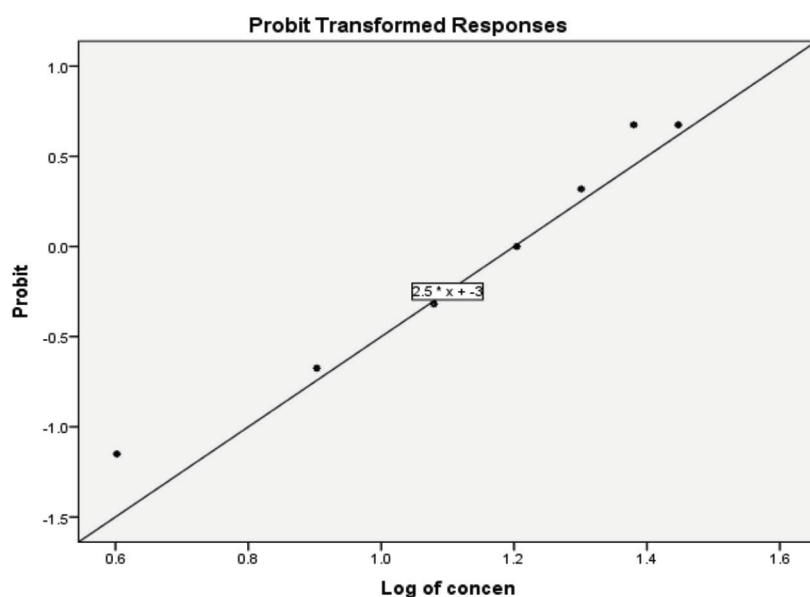


Figure 1 Regression line between the probit mortality of *Cyprinus carpio* and the log concentrations of As_2O_3

These observations are in conformation with various workers who performed bioassays of arsenic compounds in other fish species, like the work of Nassr-Allah H et al (2009) in Nile Catfish, *Clarias gariepinus*. There are differences in the value of LC_{50} found in the same fish species for same heavy metal. Different studies reveal that some fishes are very sensitive towards the toxicity caused by one heavy metal and shows less sensitivity towards another equally toxic heavy metal at the same concentration. Similarly, toxicants which can cause detrimental effects to some organisms even at lower concentrations may be less or more toxic to some other organisms at higher or same concentration. This is due to the fact that several factors including differences in the test species, age, feeding habit, sex, composition of toxicant and also the experimental conditions under which the tests are performed (Latif et al 2013).

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4.10 Utilization of Orange Peel (Fruit waste) as an Anti-Microbial Agent against Selected Fish Pathogens

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Keywords: Stress, Orange peel extract, Antimicrobial, *Aeromonas veronii*, *Edwardsiella tarda*

Introduction

Fishes in a day are exposed to different types of stress like physiological stress, Biological stress, and Environmental stress which leads to susceptibility to the disease under the attack of multiple fish pathogens. The infectious disease caused by *Aeromonas* most frequently occurs in freshwater fishes. Generally, species like tilapia have been encountered with the aeromonad *Aeromonas hydrophilla* but along with this different aeromonads have been found and *Aeromonas veronii* is one among them. *Aeromonas veronii* is a gram-negative rod-shaped bacteria found in water or associated with animals, this bacteria is even harmful to humans. *Edwardsiella tarda* is one of the most serious fish pathogens in aquaculture and also in wild. It has been reported that *Edwardsiella tarda* has a broad host range and geographic distribution, and contains important virulence factors that enhance bacterial survival and pathogenesis in hosts. The aid of this issue earlier time variety of antibiotics and drugs were used, these drugs up to a certain extent ameliorate the infection but also produce residues in water that lead to developing resistance to that antibiotic. Hence antibiotics are completely restricted in aquaculture. So now the issue of infection has to resolve using natural sources which act potentially on microbial communities rather than a specific microbe. With this background in our study, we are using orange peel, which is considered a waste in day-to-day life in fruit juice shops and pulp industries. The orange peel in its different extract forms (Aqueous extract, Methanolic extract, Crude hesperidin extract, and Hesperidin) and its flavanone compound have been tested against *Aeromonas veronii* and *Edwardsiella tarda* (in-vitro).

Materials and Methods

The Mandarin / Tangerine fruits were procured from a Local Fruit market of Seven Bangalow Mumbai Market and processed in FNBP Laboratory CIFE New Campus Mumbai. The methanolic extract and aqueous extract of orange peel were prepared. The Crude hesperidin extract was prepared according to Sharma et al (2013) with some modifications. Antimicrobial assay performed by agar well diffusion method. The Mueller Hinton agar was inoculated with 100 µl of the inoculum and poured into the Petri plate (Parekh et al 2006). A well was prepared in the plates with the help of a cork borer. 100 µl of the test compound was introduced into the well. The plates were incubated overnight at 37 °C and microbial growth was determined by measuring the diameter of the zone of inhibition.

Results and Discussion

The experiment resulted in an extract of orange peel showing better antimicrobial activity than the hesperidin (Flavanone) compound against selected fish pathogens because of the presence of various phytochemicals in the extract rather than single flavanone extract and its pure chemical grade form.

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4.11 Impact of Stinging Nettle Extract Supplemented Diet on Digestive Enzyme Activity in Fingerlings of Common Carp (*Cyprinus carpio*)

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Keywords: Nutrition, Common carp, Stinging nettle, Digestive enzymes, Fish growth

Introduction

The high nutritional quality of aquaculture products has made the aquaculture industry the fastest growing industry with a growth rate of 8% each year. A variety of antibiotics and chemicals are being used in aquaculture to increase the growth and production rate of fish. These chemicals have adverse impacts on human health and cause environmental pollution. Herbal extracts are a promising alternative to these chemicals as they have the potential to stimulate total body metabolism and feed utilisation. Stinging nettle (*Urtica ardens*) considered as a weed which has a long history of traditional medicinal uses in many countries in the world, and the present study has been proposed to evaluate the effects of dietary inclusion of *U. ardens* on digestive enzyme activity of the common carp (*Cyprinus carpio*). Analysis of digestive enzyme content can provide information on the potential effects of nutrients on digestive function and nutritional absorption.

Materials and Methods

Feed was formulated using ethanolic plant extract of *Urtica ardens* and the supplemented extract was added in 0.3, 0.6, 0.9 and 1.2% of the formulated feed. The feeding trial was conducted for 90 days on the fingerlings of common carp and thereafter the fingerlings were deprived of feed for 24 hours. After starvation period, three fishes were randomly selected from each treatment group, dissected and the whole intestine was homogenized in 5 volumes (v/w) of ice-cold physiological saline or ice-cold distilled water using a tissue homogenizer and centrifuged at 10000 rpm for 10 minutes at 4°C. The supernatant was collected and used to determine the activity of digestive enzymes like protease, amylase and lipase. Concentration of protein was determined using standard Lowry assay; protease activity by casein-hydrolysis method (Furne *et al.*, 2005); amylase activity by method of Bernfeld (1955) and lipase activity by spectrophotometric method using para-nitrophenyl palmitate as a substrate.

Results and Discussion

After the feeding trial of 90 days, the protein concentration increased with increase in dietary supplementation of nettle extract in group T₁ group (399.79±12.12 µg/ml), T₂ group (415.20±9.27 µg/ml), T₃ group (477.70±31.42 µg/ml) and T₄ group (573.54±35.86 µg/ml) as compared to the control T₀ group (267.70 ±11.28 µg/ml). Protease activity was significantly higher in T₂ group (247±18.41 µmol/mgmin) than T₀ (159.70±6.47 µmol/mgmin) group and thereafter, the protease activity decreased with further increase in supplementation of nettle extract with T₄ group with significantly lower protease activity (105.28±7.00 µmol/mgmin). Amylase activity showed no significant difference between T₀ group (222.01±9.52 µmol/mgmin), T₁ group (213.95±11.44 µmol/mgmin), T₂ group (256.68±4.71 µmol/mgmin) and T₄ group (214.75±14.77 µmol/mgmin). There was a significant increase in amylase activity only in T₃ group (621.68±41.94 µmol/mgmin). Lipase activity increased in a dose dependent manner as it increased from T₀ group (5.39±.23 µmol/mgmin) to T₄ group (11.13±.63 µmol/mgmin).

The current findings indicate that stinging nettle extract enhances the digestion process till a specific extract concentration between 0.6 to 0.9%. By modulating digestive enzymes, it might help in improving gut microbiota raising the energy required for nutrient digestion and enhance growth performance and overall health of fish.

Table 1 Comparative digestive enzyme activity in common carp (*Cyprinus carpio*) on dietary inclusion of Stinging nettle (*Urtica ardens*)

Groups	T ₀ (0%)	T ₁ (0.3%)	T ₂ (0.6%)	T ₃ (0.9%)	T ₄ (1.2%)
Protein (µg/ml)	267.708±11.280 ^a	399.791±12.126 ^b	415.208±9.279 ^{bc}	477.708±31.427 ^c	573.541±35.86 ^d
Protease (µmol/mgmin)	159.700±6.47 ^b	145.141±4.183 ^b	247.830±18.415 ^c	155.685±11.262 ^b	105.281±7.002 ^a
Amylase (µmol/mgmin)	222.011±9.528 ^a	213.988±6.605 ^a	256.682±4.715 ^a	621.682±41.947 ^b	214.750±43.128 ^a
Lipase (µmol/mgmin)	5.395±0.231 ^a	6.0335±0.184 ^a	10.356±0.235 ^b	10.029±0.624 ^b	11.139±0.660 ^b

All the values in the table are mean ± SE of the mean. Data with different subscript are significantly (P<0.05) different as analysed by Duncan post Hoc test.



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4.12 Isolation and Characterization of Probiotic Properties of Autochthonous Quorum-Quenching Probiotic Bacteria *Enterobacter cloacae* from Fish Gut

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Keywords: *Labeo rohita*, *Aeromonas hydrophila*, Quorum-sensing, Virulence, Quorum-quenching, Probiotic

Introduction

Frequent disease outbreaks result in economic losses for the farmers and threaten the sustainability of the aquaculture industry. Among various aquatic bacterial pathogens, *Aeromonas hydrophila* is of specific concern accounting for 5-10% of cases of disease outbreaks in freshwater finfish culture. Antibiotics are often used for disease control in aquaculture, and their indiscriminate use has resulted in the global emergence of antibiotic-resistant bacterial pathogens. Thus, novel eco-friendly disease prevention and treatment strategies are needed. As cell-to-cell communication through autoinducer signal molecules (quorum-sensing) plays important role in bacterial pathogenicity, inactivation of these signal molecules (quorum-quenching) has emerged as an innovative disease control strategy. Quorum-quenching mechanisms have also been reported in probiotic bacteria (Chu et al 2014). Thus, the application of these autochthonous (host-associated) probiotic bacteria could control disease outbreaks and have beneficial effects on the host. The present study reports the isolation and characterization of quorum-quenching probiotic bacteria *Enterobacter cloacae* from the gut of *Labeo rohita*.

Materials and Methods

Samples of *Labeo rohita* were collected from the Instruction cum Research Farm, College of Fisheries, GADVASU, Ludhiana. For enrichment of potential quorum-quenching probiotics (QQPs), the fish gut homogenate was inoculated into KG media containing 500 µg/ml of C6-HSL (N-Hexanoyl-L-homoserine lactone) followed by incubation at 30 °C for 24 h. A total of three rounds of enrichment in KG media were carried out. The enriched KG media was streaked on Luria Bertani (LB) agar plates and single colonies were randomly picked based on the varying colony morphologies. These isolates were screened for potential quorum-quenching (QQ) properties by polymerase chain reaction (PCR) and quorum-sensing inhibition (QSI) assay against *Chromobacterium violaceum* MTCC 2656. The isolates showing QQ properties were subjected to genus/species-level identification with biochemical tests, 16S rRNA gene fragment sequencing, and MALDI-TOF MS. *In vitro* safety of selected QQ isolate was assessed in terms of antibiotic susceptibility testing (AST) and hemolytic activity testing. For determining the probiotic properties of QQ isolate, antagonistic activity, acid tolerance, bile salt tolerance, and phenol tolerance assays were carried out. The activity of QQ isolate against the planktonic cells and biofilm of fish pathogenic *A. hydrophila* was also investigated with co-culture and biofilm inhibition assays, respectively.

Results and Discussion

Out of 27 bacterial isolates obtained from the fish gut, none was found positive for the autoinducer inactivation homolog (*aiiA*) gene by polymerase chain reaction (PCR). However, a total of 18 isolates among these showed significant QQ properties by inhibiting the purple-coloured violacein pigment in *C. violaceum* during the QSI assay (Fig. 1). After confirmatory identification of all 18 QQ bacterial isolates, *Enterobacter cloacae* RK9 was selected for further downstream characterization. During AST by disc diffusion assay against 14 antibiotics, *E. cloacae* RK9 was susceptible to chloramphenicol and tetracycline; intermediate resistant to Cefepime, Gentamycin and Meropenem; and resistant to ampicillin, amikacin, aztreonam, ceftazidime, ciprofloxacin, imipenem, and kanamycin. Antibiotic resistance in naturally occurring probiotic bacteria is not an issue unless it is present on mobile genetic elements such as transposons and plasmids. *E. cloacae* RK9 did not show any hemolytic activity on blood agar, making it potentially safe as a probiotic. During the antagonistic activity assay, this isolate did not show any antimicrobial activity against *A. hydrophila*. Previous studies have also reported that not all potential probiotic bacteria have antimicrobial properties. During the acid tolerance test, *E. cloacae* RK9 exhibited good viability at pH 3.0 and 4.5 for 18 h. Besides, the isolate could also survive and grow at 0.3% and 0.6% bile salt concentrations. Though *E. cloacae* RK9 was viable at the 0.4% phenol concentration, no growth was observed over a period of 24 h. During co-culture assay, *E. cloacae* RK9 significantly inhibited the growth of pathogenic *A. hydrophila* (one-way ANOVA, $p < 0.05$). Besides, the cell-free filtrate of *E. cloacae* RK9 also significantly inhibited the biofilm formation in pathogenic *A. hydrophila* (one-way ANOVA, $p < 0.05$). Results from the present study suggest that *E. cloacae* RK9 is a good probiotic candidate for field-level studies for the control of *A. hydrophila* infection in fish.

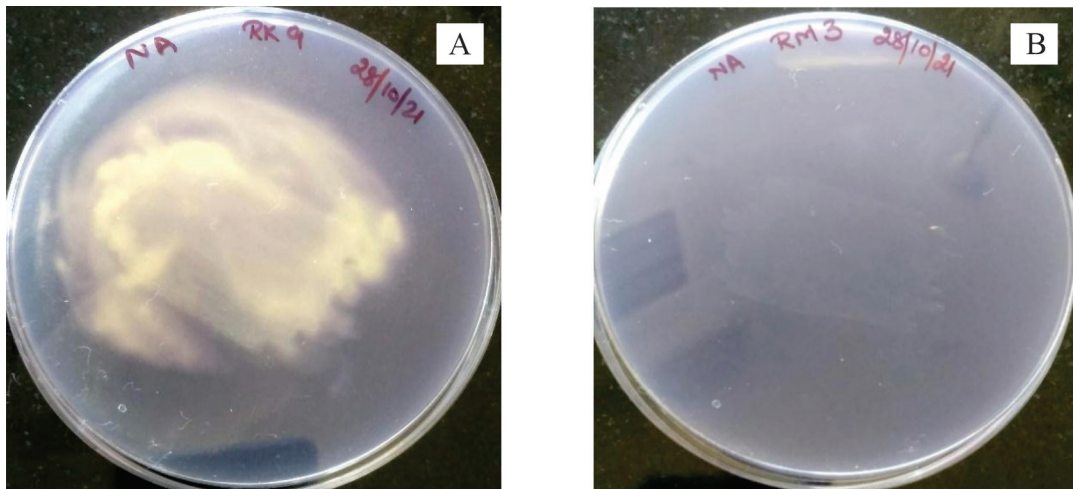


Fig. 1 Quorum-quenching (QQ) activity of *E. cloacae* RK9 during quorum-sensing inhibition assay (QSI) against *C. violaceum* (A). Loss of purple pigmentation in *C. violaceum* around the central lawn of *E. cloacae* RK9 indicates the inhibition of quorum-sensing in *C. violaceum* by *E. cloacae* RK9. Isolate having no QQ activity has also been shown for comparison (B).

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4.13 Isolation and Identification of *Aeromonas hydrophila* from Suspected Carp Fish during Disease Outbreak

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Keywords: Punjab, Fish farm, Carp, Disease outbreak, Bacteria, *Aeromonas hydrophila*

The aquaculture sector has seen vertical and horizontal expansion, and there is a growing need for fish as a source of animal protein. The increased susceptibility of culturable species to infectious diseases as a result of the sector's intensification and unscientific management practices are the significant obstacle to the growth and sustainability of the aquaculture business. Frequent disease outbreaks can generate large financial losses for fish farmers. Therefore, diagnosing the disease and timely treatment followed by best management helps to overcome from disease outbreak.

Through passive surveillance programme, fish samples (20 nos.) were received from Ranjitpura/Awankot in Rupnagar district of Punjab (30°03'03.1" N 76°33'14.7" E). Farmer observed the running mortality (2-2.5 quintals per day) in the farm and clinical signs such as fish moving at the surface and sides, red spot, hemorrhages etc. The submitted samples were diagnosed in the lab using the standard protocol. In the level 1 diagnosis, clinical examination revealed haemorrhages on the body, discoloration, fin rot, red inflammation areas on the flanks, etc.

Further, broth was used to enrich kidney samples taken from suspected samples before they were streaked on the selective media. In the selective media, yellow-colored colonies were observed on the Rimler-Shotts agar plate (Fig. 1A). Then, molecular diagnosis was used to confirm the causative agent. Using pathogen-specific primers, a polymerase chain reaction (PCR) assay was carried out under the level 3 diagnosis. It amplified the PCR product of size about 264 bp using *Aeromonas hydrophila* primers (Fig. 1B). Additionally, it was further verified using matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) method.

The results of the current study showed that the disease epidemic in the fish pond of the Rupnagar district of Punjab is being caused by *Aeromonas hydrophila*. Therefore, advised the farmer to use the appropriate aqua-medicine as well as good management techniques.

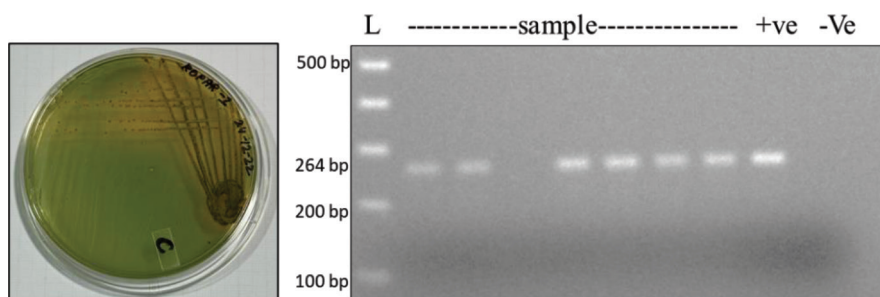


Figure 1 A. Yellow-colored colonies on Rimler-Shotts agar plates showing *Aeromonas* sp. infection in fish. B. Agarose gel electrophoresis of the PCR product amplified from collected fish samples; L: 100 bp DNA ladder, lane 1-7: fish samples, lane 8: positive control, lane 9: negative control.

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4.14 Triclosan and Triclocarban – Antimicrobial Substances Used in Personal Care Products as Emerging Contaminants in Inland Open Waters

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Keywords: Triclosan, Triclocarban, Antimicrobial, Contaminant

Introduction

Triclosan [TCS, 5-chloro-2-(2, 4-dichlorophenoxy)-phenol] and Triclocarban [TCC, 3-(4-chlorophenyl)-1-(3, 4-dichloro phenyl) urea] are two antimicrobial chemical compounds used in many personal care, health & hygiene, and consumer products. Both TCS and TCC are highly toxic to aquatic biota specifically algae, crustaceans and fish (Brausch and Rand 2011). Residues of these compounds in varying concentrations have been reported in surface waters from different countries including India. As a part of our ongoing activities on monitoring aquatic environment for pollution, we studied the occurrence and distribution of TCS and TCC in few inland open water systems.

Materials and Methods

Seasonal sampling of water and fish was carried out in Torsa river and East Kolkata wetlands. The same method of extraction, clean up, and analysis as employed in Nag et al (2018) was followed for detection and estimation of TCS, its methyl derivative (Me-TCS), and TCC.

Results and Discussion

TCS and TCC were detected in water at concentrations 0.055–0.79 µg/l and 0.041–0.84 µg/l, respectively which exceeded the Predicted No Effect Concentration (PNEC) of TCS (0.05 µg/l) and TCC (0.025 µg/l) for algae. In fish, TCS (91.1 - 972 µg/kg) was much higher than that of TCC (29.1 – 440.4 µg/kg). Me-TCS, a more persistent metabolite of TCS was also detected in fish (0.11-596 µg/kg). TCS level in water above the PNEC indicate possible impact to the sensitive biota. But estimated daily intake of TCS and TCC by human through fish was much below the acceptable daily intake.

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4.15 Protective Efficacy of an Orally Administered Phage Cocktail Against *Aeromonas hydrophila* Infection in *Labeo rohita*

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Keywords: Aquaculture, Disease outbreaks, *A. hydrophila*, Phage cocktail, Oral administration

Aeromonas hydrophila, a Gram-negative, facultative anaerobic, motile rod-shaped bacterium, is an important finfish pathogen with the capability to cause haemorrhagic septicaemia, tail and fin rot in both feral and cultured fish. The present study investigates the protective efficacy of an orally administered 2 phage (D6 and CF7) cocktail against *A. hydrophila* infection in *Labeo rohita*. During *in vitro* pH and simulated gastric fluid (SGF) stability studies of the phage cocktail, both D6 and CF7 phages were able to survive and form plaques even after 2 h incubation at pH 4 – 10. However, no phage viability was detected in the SGF of pH 2 and 2.4. During studies to evaluate the suitability of phage cocktail for oral feed-based delivery, it showed good survival in feed for almost 2 months with a decline of phages counts by $\leq 1.66 \log_{10}$ and $\leq 0.34 \log_{10}$ plaque forming unit (pfu) per gram of feed at room temperature and 4 °C storage, respectively. After oral feed-based administration of the phage cocktail, both D6 and CF7 phages were able to survive in the fish gut and also cross the intestinal barrier to distribute into the fish kidney. At the estimated phage cocktail concentrations (D6 and CF7 each) of 1×10^6 , 1×10^7 and 1×10^8 pfu per gram of feed, relative percentage survivals (RPS) in *A. hydrophila* LD50 challenged fish groups were 8.3, 30.6 and 66.7, respectively. At LD₉₀ *A. hydrophila* challenge and phage cocktail concentration (D6 and CF7 each) of 1×10^8 pfu per gram of feed, the RPS was 30.2. From the RPS data, it was evident the protection against *A. hydrophila* infection was directly proportional to the administered phage dose. The findings of the present study are significant as these lead to a practical approach to applying phage therapy for disease prevention in large-scale aquaculture farms.

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4.16 A Case Study Survey on Fish Seed Mortality in the Early Stage of Fish Life Cycle

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Keywords: Survey, Fish seed, Mortality, Hatcheries

The availability of healthy, high-quality seed is crucial for the growth of the aquaculture sector. Fish mortality in the early phases of growth is costing seed producers/suppliers significantly in terms of revenue. Grow out farmers also struggle with seed mortality during the initial stages of culture. In this regard, there are fewer records of fish seed mortality in the early life phases, such as the egg, spawn, fry, and fingerling stages. Therefore, survey was conducted in the present study to record the mortality data.

For the study, a questionnaire was developed that took into consideration all the variables in both the native language and English. The prepared survey Google Form was sent via WhatsApp or email to the fish hatcheries (fish seed producer cum suppliers) and fish seed suppliers. The study was conducted in four different states: Punjab, Gujarat, Chhattisgarh, and Karnataka (Fig. 1A). Based on data acquired from fish hatcheries and fish seed suppliers in each state, specifics have been provided in the Fig. 1B. From the state of Punjab and Karnataka, fish seed mortality data was obtained from fish hatcheries and fish seed suppliers. But for the state of Gujarat and Chhattisgarh, data was obtained only from fish seed suppliers. The data used in this study were given by private hatcheries and seed suppliers, not from the government hatcheries.

High mortality rates of up to 10–30% and up to 10–40% were reported by hatcheries and fish seed suppliers, respectively (Fig. 1C). Additionally, the mortality rate from spawn to fingerling stage was over 40%. In contrast to fish seed suppliers, hatcheries experience lower post-spawn mortality rates. Overall, more fish hatcheries reported mortality of 10–20%, and fish seed suppliers reported mortality of 20–40%. Finally, it may be concluded that the early phases of fish seed rearing reported a 10–40% mortality rate. Therefore, it is necessary to develop the solutions for resolving the aforementioned issue. This will aid in finding a fix for the seed availability problem and further aids in providing the quality and healthy seed to the farmers.

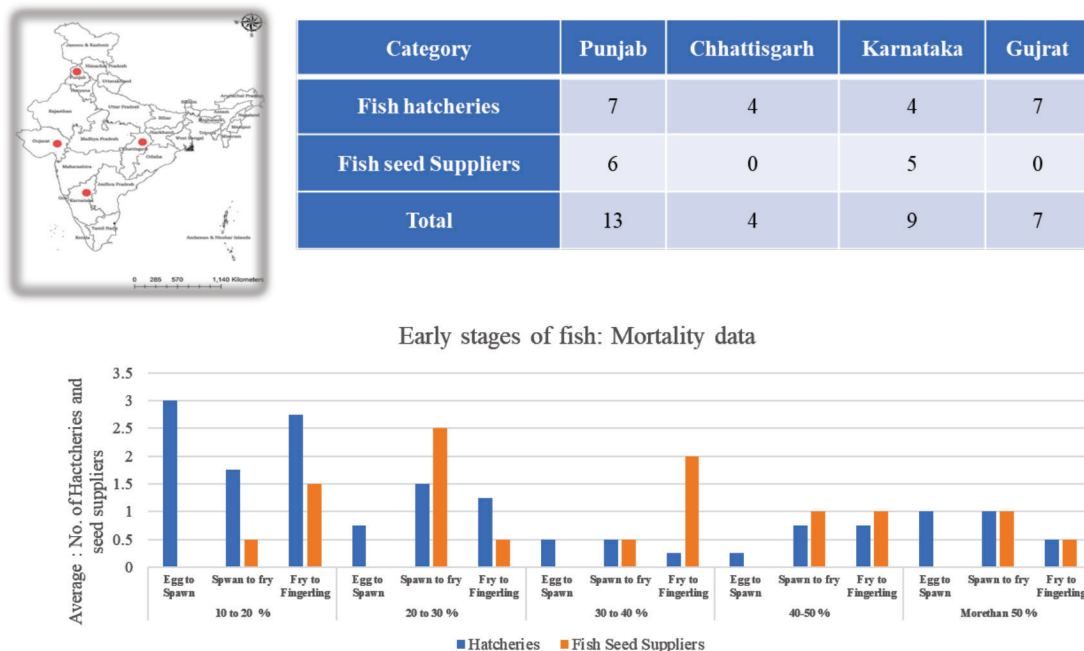


Fig 1 A. Red dots showing the survey areas; B. list of the fish hatcheries and fish seed suppliers employed in this investigation.; C. Data on fish mortality in the early stages from hatcheries and fish seed providers in a few Indian states.

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4.17 Transcriptome Sequencing and Analysis of Common carp (*Cyprinus carpio*) Egg

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Keywords: *Cyprinus carpio*, Egg, Next-generation sequencing, Transcriptome, Pathway analysis

Introduction

Globally as well as in India, aquaculture has been playing an increasingly important role to meet the food and nutritional security demands of rapidly growing populations. Productivity enhancement in a sustainable manner is the key to successful and economically viable aquaculture. Besides best management practices (BMPs), seed quality plays an important role in aquaculture production. As “genotype regulates phenotype”, a deeper understanding of the genetic potential and gene expression patterns in early seed development stages is necessary to develop a good quality seed capable of high growth rate and disease resistance. Besides, these investigations also help to know more about the genetic components transferred from broodfish to the offspring. Globally as well as in India, common carp (*Cyprinus carpio*) is considered an important aquaculture species due to its hardy nature, high growth rate and less demanding culture requirements. The present study reports the transcriptome sequencing and assembly of common carp eggs.

Materials and Methods

Total RNA from three fish egg samples was isolated with Trizol method. After qualitative and quantitative quality checks, total RNA was subjected to mRNA enrichment and sequencing library preparation using NEBNext® Ultra™ RNA Library Prep Kit as per standard protocols. The final quality-checked sequencing libraries were subjected to paired-end (PE) 2x150 bp sequencing on Illumina Novaseq 6000 platform. After quality filtering to remove adapters and poor-quality reads, the PE reads from each sample were mapped against *C. carpio* reference genome (assembly accession no. GCA_018340385.1) using the STAR (v2.7.10a) tool. Sequencing reads mapped to the genome were assembled into transcripts with StringTie (v2.2.1) tool. BLASTx tool was used to annotate assembled transcripts from egg samples against the reference *C. carpio* protein file. Gffcompare (v0.12.6) utility was used to compare assembled transcripts against reference *C. carpio* transcripts to discover novel transcripts in egg transcriptome. Online KEGG automatic annotation server (KAAS) was used for pathway mapping of assembled transcripts against KEGG database using BLASTx with a default bit-score value of 60. Assembled transcripts were also subjected to gene ontology (GO) term assignments.

Results and Discussion

From each fish egg samples, 10.12 – 10.89 µg of total RNA was isolated with Trizol method. The purified total RNA passed all quality checks on 1% agarose gel and Agilent Tape Station 4150. During next-generation sequencing, 28.29 – 30.47 million PE reads, corresponding to 8.54 – 9.20 GB data, were generated for each sample. During alignment, ~94% of sequencing reads were mapped to reference *C. carpio* genome with a unique mapping rate of ~89%. With StringTie, ~74,000 transcripts with mean and median lengths of ~2,900 bp and 96,185 bp, respectively were assembled from reference-mapped reads of each sample (Table.1). A comparison of assembled transcripts with reference *C. carpio* transcripts revealed the presence of 6,834 novel isoforms in fish egg transcriptome. During pathway analysis, mapped transcripts represented metabolic pathways of major biomolecules such as carbohydrates, lipids, nucleotides, amino acids, glycans, etc. The mapped transcripts also represented the genes involved in metabolism, genetic information processing, environmental information processing, cellular processes, and organismal and systems. During GO analysis, 2,585 – 2,638 transcripts from each sample were assigned GO terms. The present study provides important insights into *C. carpio* egg transcriptome. To the best of our knowledge, this is the first study investigating the transcriptome of *C. carpio* from India.

Table 1 Sample-wise assembly statistics of *C. carpio* egg transcriptome

Sample Name	# Assembled transcripts	Total assembled (bp)	Mean transcript size (bp)	Max transcript size (bp)
T1	74,565	219,466,237	2,943	96,185
T2	74,766	220,066,856	2,943	96,185
T3	74,086	218,506,828	2,949	96,185

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4.18 Ameliorative Immunity Benefits of Ashwagandha (*Withania somnifera*) Root Powder in an Indian Major Carp Species *Labeo rohita* (Ham.)

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Keywords: Ashwagandha, Carp, Fish, Health, Herb, Rohu

Introduction

Aquaculture is one of the world's fastest growing food production sector with significant role in food security, livelihood generation and economic growth. Several substances, including antibiotics and disinfectants, are used for health management in intensive aquaculture systems; which may lead to serious environment and food safety issues. Hence, eco-friendly alternatives are essentially required for prevention and management of aquaculture diseases. Several herbal supplements (garlic, amla, *Aloe vera*, shatavari, ashwagandha, ginger, neem, moringa etc.) are reported to boost immunity and overall health in human beings, livestock and fish. Ashwagandha (*Withania somnifera*), is known to possess immuno-modulatory, anti-inflammatory, anti-stress, anti-oxidant, antibiotic, and aphrodisiac properties owing to the presence of several biologically active compounds like alkaloids (isopellertierine, anferine) and steroidal lactones (withanolides, withaferins), flavonoids, tannins etc. and its application in aquaculture is under researched. Therefore, efficacy of ashwagandha root powder (ARP) as an immunity booster in fish was evaluated in respect to an important Indian major carp fish species, *Labeo rohita*.

Materials and Methods

A feeding trial was conducted in cemented tanks (80 m²) to evaluate the ameliorative effect of dietary incorporation of ARP on immunity and overall health of rohu, *L. rohita* (Ham.). Healthy fingerlings of *L. rohita* (average length 9.65±0.10 cm and weight 9.93±0.27 g) were stocked in 4 sets (duplicate) of experimental tanks @ 80 fingerlings tank⁻¹ (10,000 fingerlings ha⁻¹). Four iso-proteinous (24.83-25.37% crude protein) feeds, including control feed without ARP (ARP0) and three experimental feeds containing ARP @ 1% (ARP1), 2% (ARP2) and 3% (ARP3). The fish was fed with different feeds daily @ 2% body weight for 180 days. Water temperature, pH, dissolved oxygen, total alkalinity, total hardness, ammonical-nitrogen, nitrate nitrogen and orthophosphate were monitored at monthly intervals following standard methods. After 180 days of feeding trial, haematological parameters like total erythrocyte count (TEC) and total leucocyte count (TLC) were estimated by using a Neubauer haemocytometer, while haemoglobin (Hb) and haematocrit (Hct) values were assessed by acid hematin method using Sahli's haemoglobinometer and microhematocrit capillary tube, respectively. Mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH), and mean cell volume (MCV) were calculated using the standard formulae. Serum immunological (total protein, albumin, globulin, A:G ratio, total immunoglobulin) and biochemical parameters viz. glucose, cholesterol, triglycerides, alanine aminotransferase (ALT), Aspartate aminotransferase (AST), lipid peroxidation (LPO) and superoxide dismutase (SOD) were analysed using standard diagnostic kits (Erba)/protocols/formulae. At the termination of trial, fish from each treatment were challenged with bacteria *Aeromonas veronii* to assess protection levels in terms of relative percentage survival (RPS). One-way ANOVA and Duncan's multiple range test were performed using SPSS 16 software to analyze significant differences (P≤0.05) among different treatments.

Results and Discussion

The physico-chemical parameters of water remained well within the recommended range for carps culture throughout during the experiment period in all the treatments. Dietary supplementation of ARP improved the haematological profile, serum biochemistry and immunity of fish significantly (P≤0.05) at all the inclusion levels, but the best results were recorded with 2% ARP inclusion level (ARP2). The TEC, TLC, Hb and Hct values increased by 39.47, 11.17, 67.24 and 34.57%, respectively as compared to control (Table 1). Further, serum immunological parameters viz. serum total protein, albumin, globulin and total immunoglobulin recorded maximum increased from 2.86, 1.50, 1.36 and 0.84 g dl⁻¹ in control to 4.11, 1.87, 2.24 and 1.22 g dl⁻¹ in ARP2 treatment, respectively (Fig 1). Furthermore, the serum biochemical parameters like glucose, cholesterol, triglycerides, ALT, AST and LPO decreased significantly from 70.55 mg dl⁻¹, 156.75 mg dl⁻¹, 140.30 mg dl⁻¹, 29.53 U l⁻¹, 132.83 U l⁻¹ and 1.89 nmol MDA gHb⁻¹ in control to 65.50 mg dl⁻¹, 131.20 mg dl⁻¹, 108.10 mg dl⁻¹, 24.83 U l⁻¹, 117.20 U l⁻¹ and 1.17 nmol MDA

gHb⁻¹, respectively at 2% ARP inclusion level (ARP2), whereas the SOD value increased significantly from 0.36 in control to 0.52 U mgHb⁻¹ in the same treatment (Table 1).

Growth of ARP fed fish also increased significantly and maximum increase in net weight gain (74.53%) was recorded in ARP2 treatment fish. After 180 days of feeding trial, mean fish survival (%) in a 10 days of *A. veronii* challenge test was recorded as 50.0, 60.0, 70.0 and 65.0% in ARP0, ARP1, ARP2 and ARP3 treatments, respectively and the RPS with reference to control was found to be 20.0, 40.0 and 30.0% in ARP1, ARP2 and ARP3, respectively. The overall results revealed that ARP can be potentially utilized as an organic growth promoting immuno-modulatory feed additive in carp feed for prospective health benefits and productivity enhancement.

Table 1: Haematological and serum biochemical parameters of *L. rohita* in different treatments

Parameters	Treatments			
	ARP0	ARP1	ARP2	ARP3
Haematological Parameters				
TEC (no.x10 ⁶ mm ³ ⁻¹)	1.52 ^d ±0.01	1.73 ^c ±0.02	2.12 ^a ±0.02	1.92 ^b ±0.05
TLC (no.x10 ³ mm ³ ⁻¹)	2.06 ^c ±0.02	2.18 ^b ±0.02	2.29 ^a ±0.03	2.33 ^a ±0.01
Haemoglobin (g%)	3.48 ^d ±0.07	4.45 ^c ±0.06	5.82 ^a ±0.06	5.03 ^b ±0.05
Haematocrit/PCV (%)	17.50 ^d ±0.29	20.03 ^c ±0.34	23.55 ^a ±0.28	21.53 ^b ±0.27
MCH	22.94 ^b ±0.62	25.78 ^a ±0.34	27.52 ^a ±0.50	26.31 ^a ±0.89
MCV	115.24 ^a ±2.73	116.02 ^a ±1.37	111.45 ^a ±2.33	112.69 ^a ±4.54
MCHC	19.90 ^d ±0.12	22.22 ^c ±0.16	24.70 ^a ±0.15	23.38 ^b ±0.25
Serum Biochemical Parameters				
Glucose (mg dl ⁻¹)	70.55 ^a ±1.88	63.25 ^{bc} ±2.56	65.50 ^{ab} ±1.32	58.84 ^c ±2.19
Cholesterol (mg dl ⁻¹)	156.75 ^a ±3.58	147.96 ^a ±2.87	131.20 ^b ±3.33	124.80 ^b ±3.16
Triglycerides (mg dl ⁻¹)	140.30 ^a ±3.91	129.21 ^b ±2.04	108.10 ^d ±1.44	117.57 ^c ±1.37
ALT (U l ⁻¹)	29.53 ^a ±0.76	28.42 ^a ±0.45	24.83 ^b ±0.75	27.81 ^a ±0.51
AST (U l ⁻¹)	132.83 ^a ±2.19	124.37 ^{bc} ±3.63	117.20 ^c ±3.14	127.42 ^{ab} ±1.40
LPO (nmol MDA gHb ⁻¹)	1.89 ^a ±0.01	1.69 ^b ±0.01	1.17 ^d ±0.02	1.43 ^c ±0.02
SOD (U mgHb ⁻¹)	0.36 ^c ±0.01	0.44 ^b ±0.01	0.52 ^a ±0.01	0.52 ^a ±0.01

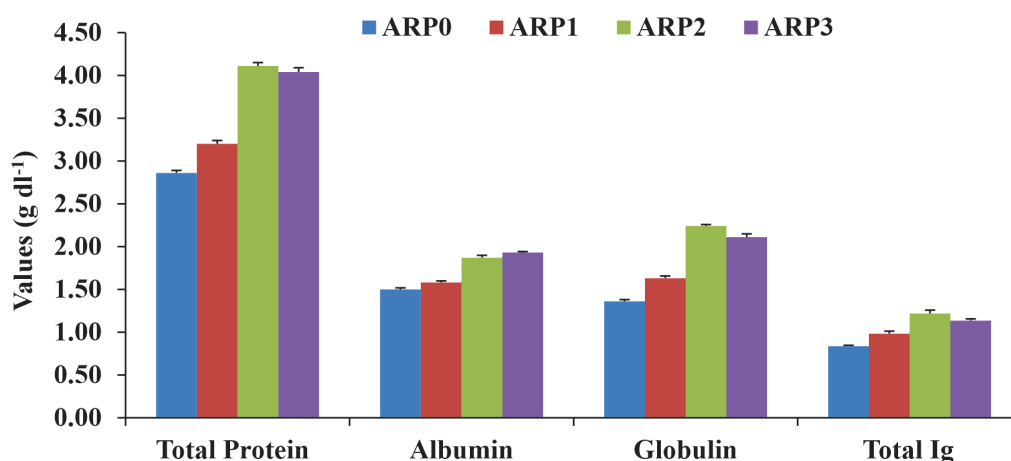


Fig. 1 Immunological parameters of *L. rohita* in different treatments

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4.19 Detection of Tilapia Lake Virus (TiLV) in Maharashtra Fish Farm: A Potential Threat to Indian Aquaculture

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Keywords: Tilapia Lake Virus, Emerging pathogen, Histopathology, *Oreochromis niloticus*

The third-heaviest farmed fish in the world, the Nile River Tilapia (*Oreochromis niloticus*), contributes to the production of 4407.2 thousand tonnes of aquaculture. Tilapia fish can be successfully cultivated even in backyards and provide an affordable source of animal protein. One of the issues endangering the sustainability of tilapia production is infectious diseases. A novel viral infection known as Tilapia Lake virus (TiLV) recently emerged in the tilapia farm. Recently, anorexia and other local haemorrhages were observed in tilapia from the local fish farm in Maharashtra, India. The samples were suspected and screened for the presence of TiLV by Reverse Transcriptase-PCR using segment 1 specific PCR primers. Further confirmation of amplified product was done by Sanger sequencing. The positive amplification of semi-nested RT-PCR of segment 1 of TiLV was observed from the samples showing sudden onset of mortality (clinical) and the subclinical samples were without outward clinical signs. The histological alterations were observed in the liver, spleen, excretory organ, gill, and brain. Alterations such as congested capillary in brain; loss of cellular cytoplasmic integrity and prominent sinusoids in the liver; and increased melanomacrophage centre (MMC) in the spleen were observed. The lumen was constricted and tubular membrane was lost in the kidney. Reduced number of developing eggs in gonad and hyperemia was seen in the gill tissue. The positive correlation of RT-PCR result and histopathological observations confirms the tropism of virus in these vital tissues.

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4.20 Prevailing Fish Diseases in the Aquaculture Systems of Eastern Assam: A Status Review

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Keywords: Assam, Aquaculture, Pathogens, Management

Aquatic animal diseases pose a major threat to aquaculture industry in the Northeastern part of the country. Mortality in fish is often noticed due to the degraded water quality of aquaculture systems and poor management of fish farms and hatcheries. The present study was carried out in two districts of the northern bank of Brahmaputra river namely the Lakhimpur and Dhemaji districts of Assam to identify and document the most commonly occurring fish diseases. Altogether, 24 fish farms and 6 fish seed production units were visited during 2022 and information with samples were collected. Based on the study it has been found that the commonly occurring fish diseases include abdominal dropsy (20%), ulcer or aeromoniasis (15%), columnaris disease (10%), fin and tail rots in bottom-dwelling fish species (10%), white spot disease or ichthyophthiriasis (5%), argulosis or fish lice infection in carps (10%), cotton wool disease (10%), and pin head disease (20%). Most of the diseases were found during the winter season of the year when the immunity of fish was compromised due to the low temperature. It is interesting to find that the pathogens like fungi and pathogens were reported from these aquatic animals. Therefore, an attempt has been made to initiate and to further carry out a systematic study on the aquatic animal diseases of both districts as early detection of the diseases and rapid response to the identified diseases as these indicators will be critical for the effective management of aquatic animal disease emergencies in the area.

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4.21 Gut Histopathological Alterations upon Prolonged Dietary Florfenicol Administration in *Oreochromis niloticus* Juveniles

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Keywords: Approved antibiotic, Intestine histology, *Lamina propria*, Aquaculture

Antibiotics such as florfenicol (FFC) is widely used around the world and is often associated with serious biological responses of *Oreochromis niloticus*. Although being approved for aquacultural use, FFC at higher doses is known to contribute to anorexia and reduced fold change in biomass. However, its effect on teleost gut histology is still unclear although FFC-induced microbial dysbiosis has been reported. The current study was hence taken up to evaluate the behaviour, feed consumption, survival, biomass, and intestinal histological aberrations in *O. niloticus* juveniles upon dietary administration of florfenicol (FFC) at 0-10 times the minimum therapeutic dose of 10 mg/kg biomass/day for 30 consecutive days. The 30 days feeding intended to mimic the long term use of antibiotics and to document the probable changes alongwith. A dose-dependent reduction in survival and feed intake was documented. A decline in fold change in biomass was seen in all the groups in a dose dependent fashion. Mortality was documented in all the dosing groups although the therapeutic dose observed mortalities only on day 30 FFC-dosing. Distinct histopathological changes such as loss of absorptive regions, epithelial degeneration, necrotized areas and swollen lamina propria were observed during the dosing period in a dose-dependent fashion. Qualitative scores implied mild to moderate changes in histopathological alterations. Another striking observation in intestinal histology was the detachment of lamina propria from the epithelium after 30 day dosing implying at the imminent irritability. Furthermore, the goblet cell numbers reduced drastically at the end of the 30 day feeding tenure concomitant with an exponential increase in intraepithelial lymphocytes. However, 13 days of cessation of FFC-feeding saw a substantial increase in goblet cell numbers and absorptive regions, indicating recovery. The histological analyses indicated damage to intestinal villi after FFC treatment that underwent considerable repair after lifting antibiotic pressure proclaiming the tolerability of FFC dietary supplementation in *O. niloticus* juveniles.

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4.22 Impact of Water Quality Parameters on Distributional Pattern of Zooplankton in Dal Lake, Kashmir

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Keywords: Dal lake, Water quality parameters, Zooplankton distribution

The present study was carried to assess the impact of water quality parameters on distributional pattern of zooplankton in Dal Lake, Kashmir. The study was carried on seven sampling sites from the three basins of Dal Lake, namely Hazratbal basin, Nishat basin and Nehrupark basin. Two sites will be selected from Hazratbal basin, namely Dhobi ghat and central site (near island); two sites from Nishat basin, namely Camel bridge and littoral site; and three sites from Nehrupark basin, namely Dalgate exit, Central site and Littoral site. Water samples were collected from march 2021- february 2022. While comparing the past limnological data (1997) with the present study (2022), a progressive increase in various chemical properties viz., Specific conductivity, Calcium hardness, Magnesium hardness and Total hardness was recorded while considerable increase was noticed in ortho phosphate, nitrate nitrogen, total phosphorous, ammonia nitrogen, nitrite nitrogen and total nitrogen. The lake condition continues to deteriorate, thereby, threatening the very existence of the lake besides posing serious health hazard to the people living within and around the lake. A total of 82 zooplankton species were recorded from Dal lake, with 38 species belong to Rotifera, 24 species belong to Cladocera and 20 species belong to Copepoda. A significant relationship between zooplankton abundance and water quality parameters were recorded and indicated that the water quality conditions results in suppression of some zooplankton species while favoured few.

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4.23 Histological and Biochemical Evidence of Zinc Toxicity in White Leg Shrimp, *Litopenaeus vannamei* (Boone)

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Keywords: Biochemical, Haemocytic infiltration, Histological changes, *Litopenaeus vannamei*, Necrosis

In an *in-vitro* study conducted from June to October 2021, the effect of zinc sulphate on the biochemical and histological parameters of *Litopenaeus vannamei* was evaluated at six doses: 0.5, 1, 2, 4, 6, and 8 ppm. The results showed that higher doses of zinc sulphate were toxic to *Litopenaeus vannamei* and it was found that there were significant changes in the biochemical and histological parameters of vannamei exposed to increasing concentration of zinc sulphate. At higher concentration (8 ppm), a decrease in R-cells, F-cells, B-cells, and E-cells, low haemocyte count, high haemocytic infiltration, abnormal lumen, necrosis, and melanization were recorded in hepatopancreas. In gills, lamella was found to be fused, pillar cells became interspersed, and showed large deformities in comparison to control. In the intestine, there was a maximum height of the epithelial cell in control which continuously got decrease with increasing concentration of zinc sulphate. The increasing level of zinc sulphate showed significant differences in ($p < 0.05$) total haemocyte count (THC) of shrimp. The total haemocyte count (THC), total granulocyte counts (TGC), and total agranulocyte counts (TAC) of shrimp were significantly lower in ZnSO₄ (14.45±0.60, 10.51±0.39, and 3.94±0.22) than control (25.18±0.5, 17.50±0.43 and 7.83±0.12). Total haemocyte counts were used as a health marker for shrimp which gradually decrease at high concentrations. Thus, high concentrations of zinc were more potent toxic to shrimp.

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4.24 Role of Epidermal Mucus of Carps as a Stress Indicator

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Keywords: Indian major carp, Biochemical components, Mucosal immunity, Stress

Fish epidermal mucus is regarded as a barrier between fish and infectious microorganisms in their surroundings protecting it against pathogens. Fish mucosal surfaces, and particularly the skin, are one of the most important to protect the integrity and homeostasis of the body and to prevent skin infections by pathogens. This study deals with evaluation of biochemical components, mucosal immunity of epidermal mucus of *Catla catla*, *Labeo rohita*, and *Cirrhinus mrigala* during stress conditions like starvation (14 days) and fish pathogen *Aeromonas hydrophilla* (20 days). Sampling points of starvation were: day 0 (as a control), day 4, day 7, day 10 and day 14 of starving, and then 7 days after food restoration (as a “recovery” measurement). Starvation affect all the biochemical parameters in which level of protein, lipid and carbohydrate were decreased. Mucosal immunity indicating parameters viz., lysozyme, alkaline phosphatase, myeloperoxidase and protease vary from control level after starvation period of two weeks. All selected fish species were also challenged with *A. hydrophila* through water to check, if there is any change in mucus secretion change or its parameters change. Studies have shown that after challenge with *A. hydrophila*, an increase in mucus secretion was observed in all the three species of carps. All the mucosal parameters alter after the inoculation of pathogenic strain and show an enhancement in biochemical, as well as immune parameters also increased. Mucus metabolites composition responded to the different challenges. Thus the study provides an insight for the non invasive method to detect the health status of fishes just by measuring mucosal immunity parameters.

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4.25 Evaluation of Lethal Concentration (LC_{50}) Value of Heavy Metal (Zinc) Exposure in Juveniles of *Cyprinus carpio* var. *communis*

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Keywords: Toxicity, Heavy metal, Common carp, LC_{50}

Zinc is the second most abundant trace element after Fe and is an essential macronutrient in living organisms. Zn is in certain concentrations desirable for plants and animals but its over accumulation is hazardous. Zn reaches the aquatic ecosystems through various sources and thus induces toxicity to the non-target organisms such as fishes. Presence of Zn in the water bodies of Kashmir valley has been reported by several authors but its toxic effect on aquatic animals particularly fishes has not been studied yet. Therefore, the present study was undertaken to investigate the acute toxicity of Zn in juveniles of Common carp. The median lethal concentration (LC_{50}) value of Zn to Common carp was found to be 17.55 ppm. The upper and lower fiducial limits calculated by probit were 12.49 ppm and 20.13 ppm respectively. The results suggest that zinc as common constituent in wastes discharged into water bodies possess possible ecological risk to aquatic lives especially at high concentrations, therefore, it is important that the usage of this heavy metals is controlled and monitored.

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4.26 Genotoxicity of Sewage Water for *Catla catla* and its Bio-Amelioration using Autochthonous Sewage Water Bacteria

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Keywords: Autochthonous, *Catla catla*, Genotoxicity, LC_{50} , Sewage water

A significant hazard to aquatic ecosystem is the uncontrolled flow of untreated municipal sewage water into natural water bodies. The genotoxic effects of these sewage water contaminants, agricultural wastes, pesticides, heavy metals, etc. on aquatic species, particularly fish, are most likely to occur. Thus, the present study was conducted to evaluate the genotoxic effect of sewage water on the fingerlings of *Catla catla* in terms of micronuclei (MN) assay and comet assay. Attempts were also made to study the ameliorative effect of indigenous biodegrading bacteria isolated from sewage water. Lethal Concentration (LC_{50}) of sewage water for *C. catla* was calculated to be 33.9% (Probit analysis). Later the fishes were kept in sub lethal concentrations of sewage water such as 3.39%, 4.23%, 5.65%, 8.47%, 16.95% (v/v) (SL1-SL5 Concentrations) i.e., 1/2, 1/4, 1/6, 1/8, 1/10 of LC_{50} respectively. The results revealed that there was increased in frequency of MN and DNA damage (in terms of tail length and head diameter) with increase in concentration of sewage water and the genotoxicity of the treatments followed the order SL5> SL4> SL3> SL2> SL1>Control. Autochthonous bacteria from sewage water were isolated and then screened for protease production ability and antipathogenic activity against *E. coli*. These bacteria were characterized as *Bacillus cereus*, *Exiguobacterium indicum* and *Bacillus altitudini* via 16S rDNA technology. These three biodegrading bacteria were used to treat the sewage water in the form of pure culture (T1-T3) and in the form of consortium (T4). It was found that there was a reduction in values of BOD, COD and other pollution indicating parameters in treated sewage water and highest reduction was observed in consortium treated sewage water. Further to evaluate the change in extent of DNA damage, fingerlings were kept in these bacterial treated sewage water and the results obtained that in comparison to the fish kept in untreated sewage water, the degree of nuclear abnormalities and DNA damage was significantly reduced in the fish kept in consortium treated sewage water. These bacteria can be further used at commercial level to decontaminate the sewage water before being discharged or used for agricultural purposes.

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4.27 Aquatic Macrophytes Composition in Relation to Water Quality Parameters of Shallow and Deep Lakes of Kashmir

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Keywords: Dal lake, Manasbal lake, CCA, Macrophytes, Water quality parameters

The main goal of this study was to determine the macrophyte composition and their relation with water quality parameters such as dissolved oxygen, pH, nitrate and total phosphorus concentrations in Dal and Manasbal lakes of Kashmir. A total of 19 species of aquatic macrophytes were recorded in Dal lake and 18 species from Manasbal lake. Canonical correlation analysis (CCA) analysis was used to derive the relationship between water quality parameters and macrophytic species and it explained a total variance of 73.69%. The Axis1 was positively contributed by *Phragmites communis*, *Myriophyllum aquaticum* and *Sparganium ramosum* which were positively correlated with Nitrate-nitrogen and Total phosphorous in Dal lake indicating that these species grow in nutrient-rich environments. The Axis 2 was characterized by positive loadings on *P. natans*, *P. lucens*, *Azolla*, *Nymphaea peltata* and *P. australis* which were positively correlated with depth and transparency, indicating that water depth is the most critical element in determining the seedling establishment and growth of these species.

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4.28 Effect on Hemato-Biochemical Indices of *Pangasianodon hypophthalmus* Reared in Different Culture Systems

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Keywords: Aquaculture systems, *Pangasianodon hypophthalmus*, Haematology, Stress, Extensive, Semi-intensive, Intensive

Pangasianodon hypophthalmus farming is considered one of the fastest-growing aquacultures worldwide. Over the years it has contributed to the fishery economy and livelihood of the communities that depend on it. The overall homeostasis and health of fish are highly influenced by their surroundings and culture environments. Knowing how the culture conditions and environmental parameters are affecting the health of stock is very important for growth and sustainability in production. Standard physiological indices are very useful for the proper monitoring of cultured fish. The health status of fish stock can be assessed by observing the hematological and stress parameters. Reference intervals of *P. hypophthalmus* from three different culture systems (extensive, semi-intensive, and intensive) were established to study these parameters following standard methodologies and non-parametric statistics. The result indicated that hemoglobin (7.42 ± 0.03 mg/dl), TEC ($2.45 \pm 0.56 \times 10^6$ /mm³), and PCV (24.24 ± 1.64 %) were found to be higher in the semi-intensive culture system; while TLC ($5.20 \pm 3.01 \times 10^3$ /mm³) and MCH (30.86 ± 0.22 pg) were higher in the intensive culture system, MCV and MCHC did not vary significantly in all culture systems. Glucose and cortisol were found to be higher in intensive and semi-intensive culture systems while ALT was higher in the extensive culture system. SOD and catalase values did not vary significantly in all three culture systems. The study suggested that hemato-biochemical parameters can be good indicators of the health status of fish and can be assessed in a minimally invasive way and this normometric database can be used to observe and study the stress conditions and well-being of cultured pangasius.

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4.29 Study on Emerging Impaired Fertility in Trout Brooders and Post -Hatching Mortality in Selected Central Himalayan Farms: A Case Study

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Keywords: Rainbow trout, Nutritional Disorders, Impaired Fertility, Liver enlargement, Uneven egg

The Himalaya, source of numerous revitalizing rivers, rivulets, and streams, is an abode for biotic diversity. In recent years, owing to growing awareness and high demand for fish-based protein diets, as well as government incentives under the PMMSY, rainbow trout farming has expanded and intensified many folds in the hill states of the country. The current investigation was carried out to find out the probable primary reason for the emerging patterns of rainbow trout (*Oncorhynchus mykiss*) reproductive failure in some Central Himalayan farms and hatcheries, resulting in lower seed production. Baseline data from the seventeen farms and hatcheries located in Himachal Pradesh and Uttarakhand was collected, and laboratory investigations were carried out. Emaciation in brooders with uneven egg size and poor egg fertility haemorrhages in the lower alimentary canal and liver enlargement were clinical features exhibited by affected trout brooders. Poor-quality, moist feed (above 12%) probably induced stress, which was aggravated by opportunistic bacterial pathogens, i.e., *Aeromonas hydrophila* and *Lactococcus garvieae*. The presence of undigested feed in the alimentary canal even after 5–6 hours of feeding was also an interesting symptom observed. Stripped eggs confirmed the poor maturity and fertilization. On some farms, frequent changes in water temperature above and below normal levels might also have an adverse effect on normal ovulation, including egg maturation, and overall reproductive performance. About 35–40% of the prospective female brooders from the studied farms were also revealed to be older and in poorer health. In the post-hatching stages, it was found that hydroelectric and mining activities in the source water are seriously affecting the water quality, with high turbidity and fine silt entering the trout farms. The silt deposition over the incubating eggs was found hampering the normal development of the eggs, as they require an optimum level of oxygen. Based on the above observations made at diverse locations, the lower brooder performance seems to be a cumulative outcome primarily caused by nutritional disorders, followed by infections and environmental stress. However, a more detailed investigation, taking into account all the limiting factor, is desirable for determining the primary causative factor responsible for inducing emerging non-fertility in rainbow trout brooders and affecting the trout seed producer.

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4.30 Virulence and Molecular Characteristics of *Vibrio parahaemolyticus* Isolates from Asian Green Mussel and their Inhibition by Chitooligosaccharide-Tea Polyphenol Conjugates

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Keywords: Antibacterial, COS polyphenol conjugate, *Perna viridis*, *Vibrio parahaemolyticus*, Virulence

Fifty different isolates of the bacteria *Vibrio parahaemolyticus* were studied for their pathogenicity, biofilm formation, motility, and antibiotic resistance. Chitooligosaccharide (COS)-tea polyphenol conjugates were also tested for against all isolates. Forty-three isolates were randomly selected from 520 isolates from Asian green mussel (*Perna viridis*) grown on CHROMagar™ *Vibrio* agar plate. Six isolates were obtained from stool specimens of diarrhea patients. One laboratory strain was *V. parahaemolyticus* PSU.SCB.16S.14. Among all isolates tested, 12% *V. parahaemolyticus* carried the *tdh*⁺*trh*⁻ gene and were positive toward Kanagawa phenomenon test. All of *V. parahaemolyticus* isolates were able to generate biofilm and showed relatively strong motile ability. When COS-catechin conjugate (COS-CAT) and COS-epigallocatechin-3-gallate conjugate (COS-EGCG) were compared against *V. parahaemolyticus*, the former showed the greater bactericidal activity with the MBC value of 1.024 mg/mL toward both pathogenic and non-pathogenic strains. The majority of the representative *V. parahaemolyticus* isolates from the Asian green mussel revealed high sensitivity to all antibiotics, while one of the isolates showed intermediate resistance to cefuroxime. Besides this, the representative clinical isolates were highly resistant to 9 types of antibiotics and had multiple antibiotic resistance (MAR) index of 0.64. To control the disease caused by *V. parahaemolyticus* in Asian green mussel, COS-CAT could be applied as potential antibacterial agent.

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4.31 Oxidative Stress Response in Brain of *Cyprinus carpio* (Linnaeus, 1758) Exposed to Azole Fungicide Tebuconazole

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Keywords: Antioxidant, Azoles, Pesticide, Tebuconazole,

Pesticides are frequently used in agriculture to effectively manage crop diseases, increase quality and quantity hence ensuring food security for the entire world's population. Pesticides are introduced into aquatic ecosystems through evaporation, aerial spraying, drift, and discharge of waste. One of the best fungicides for treating fungal illnesses in humans, animals, and plants is azoles. Concerns regarding azole compounds' impact on aquatic life are raised by their existence in nature and their concentration in fish. Pesticides gradually build up in aquatic organisms' tissue. One of the most typical ways that people are exposed to environmental toxins may be through their consumption of fish. Tebuconazole, a triazole fungicide frequently found in surface and ground waters, was tested for its effects on common carp (*Cyprinus carpio*) antioxidant state and oxidative stress. Fish were administered sublethal doses of tebuconazole (7 and 8 l/L) for 10, 20, and 30 days. Superoxide dismutase (SOD), catalase (CAT), protein and thiobarbituric acid reactive substance (TBARS) levels were assessed in the brain. Results revealed that in fish exposed to tebuconazole, there is an increase in the level of lipid peroxidation, and superoxide dismutase, and a decrease in the level of protein and catalase. A buildup of pesticides reduces fish quality in hatcheries and endangers their survival after release, which costs aquaculture money. The study demonstrated changes in antioxidant indicators and the significance of determining the potential long-term risk that triazole tebuconazole poses to fish and ultimately to people.

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4.32 A Comparative Study on Microbiome of Brackishwater Fish Larval Development in Clear and Green Water Rearing Systems

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Keywords: Larval microbiome, Brackish water, Next generation sequencing

The expansion and intensification of fish production, where healthy and disease-free seeds are given top priority by aquaculture operations, is hampered by a number of factors. Fish larval production is an integral part of aquaculture, and it is vital to comprehend the early microbial interactions during larval development in different rearing systems. Marine larval production faces several constraints from developmental and physiological impairments, including diseases due to microbial agents. In order to understand the fish larval microbiome of brackishwater rearing systems, Pearlscale (*Etroplus suratensis*) larvae reared in clear and green water systems at different time intervals during the development was analysed using 16S rRNA-based next-generation sequencing in the Illumina MiSeq Platform and compared with larval microbiome of viz., milkfish (*Chanos chanos*) and Asian seabass, (*Lates calcarifer*) available from our previous study using Mothur version 1.42 and MicrobiomeAnalyst. The taxonomic analysis revealed the relative abundance of *F. Pseudomonadaceae* and *F. Vibrionaceae*, in early larvae of pearlscale, milkfish, and Asian seabass. The late larvae (18 dph) of *Etroplus* and Milkfish developed a more diverse bacterial population, whereas Asian seabass developed a microbial population dominated by *F. Pseudoalteromonadaceae* (16-42%), and *F. Vibrionaceae* (18-32%) in the late phase of larval development (12 and 18 dph). In the present study, the analysis of three major brackishwater fish larval development revealed significant differences in the larval microbiomes, indicating the feeding and host-species mediated microbial population. The current research on larval microbiome will be helpful in understanding bacterial flora in brackishwater larval development and aids in formulating synergetic bacterial formulation for healthy larval production systems.

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4.33 Comparative Analysis of the Biochemical Parameters of the Seminal Plasma in the Two Species of Trout viz, *Oncorhynchus mykiss* and *Salmo trutta fario* in Cultured Conditions in Kashmir

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Keywords: *Oncorhynchus mykiss*, *Salmo trutta fario*, Seminal plasma, milt quality, Biochemical parameters

The present study was conducted to compare the biochemical parameters of seminal plasma of the rainbow trout and brown trout cultured in Laribal trout fish farm. A total of 45 fish samples of each species were taken for the present study. During the study period the overall seminal plasma of Rainbow trout was found to contain 75.24 ± 10.75 mg/dl glucose, 2.72 ± 1.15 g/dl total protein, 14.99 ± 5.6 mg/dl triglyceride, 2.55 ± 2.47 mg/dl cholesterol and 31.65 ± 4.78 mg/dl urea, whereas Brown trout seminal plasma had 84.00 ± 2.09 mg/dl glucose, 1.32 ± 0.62 g/dl total protein, 11.3 ± 3.92 mg/dl triglyceride, 1.53 ± 1.031 mg/dl cholesterol and 33.45 ± 4.78 mg/dl urea. The results revealed that total protein, triglyceride and cholesterol were found higher in rainbow trout than the farmed brown trout. On the contrary, concentration of glucose and urea were found higher in farmed brown trout. The present study gives us an idea of the effect of environment on milt quality of the two species of trout. The higher concentration of urea and glucose in the milt of brown trout is an implication of its lower productivity under farmed conditions in Kashmir as compared to rainbow trout.

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THEME V

Responsible Aquaculture, Climate Resilience and ICT/AI in Fisheries





Fisheries for Prosperity



5.1 Effect of High Temperature and Social Stress in All Male GIFT Tilapia Reared in a Confined Space

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Keywords: Social stress, GIFT, Temperature

Introduction

Fishes adapt to changes in their environment via an adequate stress response through the activation of physiological processes, behavioral changes, energy homeostasis to cope up with the environmental threat. Fish being poikilothermic has its body temperature equivalent to the water temperature. Temperature, salinity, fish's nutritional state and presence of disease determine the magnitude of the stress response in fishes. Exposure to warm temperatures for shorter or longer duration alters function of the stress axes and response to other stressors, thus compromising the adaptability of the animal. World economic forum, 2020 has predicted that if the average global temperature rises by 5°C, almost 2/3rd of global fish species could be eradicated by 2100. Thus, a better understanding of the effects of elevated temperature on the stress physiology of fish is crucial for predicting the consequences of global warming and climate change on fisheries. Fish are generally reared in groups of similar-sized individuals in farms. However, social animals of same size tend to have similar fighting ability, which enhances the aggressiveness for social ranking and social stress. Thus, studying the interactive effect of social stress and temperature can be beneficial in understanding the fish physiology for sustainable aquaculture practices.

Material and Methods

A study was conducted to evaluate the effect of high temperature and social stress in 4 different treatments designated as T1, Ambient temperature + No social stress; T2, High temperature + No social stress; T3, Ambient temperature + social stress; T4, High temperature + social stress in all male GIFT Tilapia reared in a confined space for 10 days. The temperatures ambient ($25 \pm 1^\circ\text{C}$) and High temperature ($34 \pm 1^\circ\text{C}$) in the tanks was maintained using a thermostat. The social stress was maintained by keeping 1 bigger sized fish (30 percent bigger) per replicate before hand in treatment T3 and T4. One hundred twenty fingerlings with an initial weight of $7.0 \pm 0.5\text{g}$ were randomly distributed in 4 different treatments with three replicates for each treatment and fed with floating feed (1mm) containing $30.02 \pm 0.13\%$ crude protein and $7.92 \pm 0.15\%$ crude lipid. The effect of high temperature and social stress in different treatment was evaluated by focusing on the behavioral changes, physiological stress-induced changes and oxidative damage. The collected data was statistically analysed by statistical package SPSS version 22.0.

Results and Discussion

Both SOD and catalase are the first-line defense against antioxidants in the biological system. Catalase disintegrates the H_2O_2 produced by the action of SOD on the superoxide anion. The present study indicated higher increase in the SOD activity in liver of the T2 treatment group compared to other treatment groups. The liver catalase activity was highest for the T2 treatment followed by T4. However, treatment T1 and T3 had significantly similar catalase activity. The serum glucose was lowest for the T1 treatment. The serum glucose in the treatments T2, T3, T4 did not differ significantly amongst each other but were significantly higher than the T1 group. Higher temperature ($>32^\circ\text{C}$) reduces the performance of all male GIFT-Tilapia. Social animals like Tilapia of same size have similar fighting ability to establish territory and hence higher stress levels and rising water temperature further aggravates this. There certainly exists a social hierarchy in tilapia, thus rearing tilapia in heterogenous size (One big, rest of same size) can help in reducing stress (less fights) upto some extend during its culture in rising temperature due to climate change.



Table 1: Superoxide Dismutase and Catalase activity in the liver of GIFT Tilapia fingerlings reared in different treatments

Treatments	SOD ($\mu\text{mol/mg protein/min}$)	CAT (mmoles of H_2O_2 decomposed/min/mg protein)
	Liver	Liver
T1	9.89 ^b ±1.68	51.73 ^a ±2.16
T2	26.11 ^d ±1.47	201.05 ^c ±2.54
T3	4.6 ^a ±0.13	37.92 ^a ±0.34
T4	20.26 ^c ±0.24	110.71 ^b ±19.42
p-value	<0.001	<0.001

Data are presented as mean \pm SE (n = 6) and overall SEM; Mean values with different superscripts in the same column differ significantly ($p < 0.05$); T1, Ambient temperature+ No social stress; T2, High temperature + No social stress; T3, Ambient temperature+ social stress; T4, High temperature + Social stress

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5.2 Commensurable Analysis of Scientific Communications Published in *Reviews in Aquaculture* Applying Scientometric Analysis

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Keywords - Scientometrics, Aquaculture, Bibliometrics, Collaboration, Visualization

Introduction

Identification of the prevalent scenario of scientific developments in aquaculture is very important to understand and strengthen this industry. *Reviews in Aquaculture* is a pioneer journal enlisted amongst the high-impact journals, publishing reviews on developments in aquaculture techniques, policies, and planning. Scientometric analysis of *Reviews in Aquaculture* can help the academicians, researchers, and scholars to understand the strengths and gaps in aquaculture for determining their future course of action for boosting up the profession by paying attention to the research hotspots and for taking measures to fill the gaps, if any. A few attempts have been made globally to study trends in fisheries and aquaculture applying scientometric analysis (Tao et al 2016 and Du et al 2020). However, none of the studies applied network visualization for better revelations of hidden intricacies amongst the nodes (keywords, institutions, and countries) based on their co-occurrences. The exploration of authorship, institutional and geographical collaborations, identification of sub-domains of aquaculture dealt by *Reviews in Aquaculture* journal based on co-occurrence of keywords, collaboration trends amongst authors, institutions, and countries using network visualization makes this study a first of its kind in the subject of aquaculture.

Materials and Methods

Datasets: The bibliographical data about the articles published in *Reviews in Aquaculture* journal were accessed from *Scopus* for the period 2011-2020. The bibliographical parameters were included while downloading data. A total of 412 records were downloaded in tab-delimited text (CSV) format and were used for scientometric analysis.

Subject analysis: The subject terms representing theme of records enlisted under the heading 'author keywords' field in the *Scopus* data were used for subject analysis (n=1490 terms) having one or more occurrences in records retrieved). Further, the singular and plural terms were standardized to avoid duplicity of appearance of terms.

Institutional productivity-cum-collaboration analysis: Institutional names were examined individually and were standardized manually to bring uniformity for analyzing these through visualization software. The institution(s) with multiple campuses within a country were treated as a single institution(s) for analyzing institutional contributions and collaborations.

Data visualization: The scientometric aspects of records under study viz. prolific authors, institutions and countries, and collaborations amongst them vis-à-vis keywords based subject inferences were mapped using network visualization software 'VOSviewer' (<https://www.vosviewer.com/>) and 'Gephi' (<https://gephi.org/>). Based on the co-occurrence of authors, institutions and countries within same records, respective clusters were developed using cluster schema of *VOSviewer*, each cluster representing inter-related nodes and each node in a network falling under one cluster only. The network visualizations developed have nodes (representing the variable) and edges (representing the links between nodes establishing their inter-connectivity). The thickness of edges indicates the strength of collaboration and size of the nodes represents the number of records to which an author, institution and country has contributed and the frequency of occurrence of keywords in articles also representing theme.

Results and Discussion

The study depicted trends and hotspots in aquaculture based on scientometric analysis and network visualization of metadata of 412 records published in *Reviews in Aquaculture* retrieved from *Scopus* database in terms of chronological trends of publications, most productive authors, major contributing countries, institutional productivity and subject analysis based on keywords.

The results indicated that the distribution of number of records published in *Reviews in Aquaculture* has been found uneven. From the year 2018 onwards, the publication trends of the journal witnessed exponential growth (Table. 1). This could be attributed to the recognition of growing significance of aquaculture globally as correspondingly the scientific outcome on the subject is also growing. *Reviews in Aquaculture* has witnessed contribution of articles from around the globe. A strong international collaboration trend (Fig. 1) was observed amongst nations as except Poland



and Nigeria, all other 60 countries have international collaborations for one or more articles. European nations have contributed maximum number of records and consequently, institutions representing Europe emerged as predominant contributors of articles to *Reviews in Aquaculture*. Moreover, the top five countries having contributed highest number of articles also falls under the European Union with the focus on aquaculture practices with management strategies *vis a vis* environmental impacts. The study revealed that diverse facets of the aquaculture subject are being explored globally for strengthening of the sector in view of its growing and widening significance towards attainment of sustainable food security.

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Table 1: Number of records and their citation pattern

Year	No. of records	Total citations	Average citations
2011	11	511	46.45
2012	17	609	35.82
2013	22	1451	65.95
2014	18	609	33.83
2015	17	491	28.88
2016	19	615	32.37
2017	23	544	23.65
2018	56	1520	27.14
2019	75	960	12.80
2020	154	718	4.66

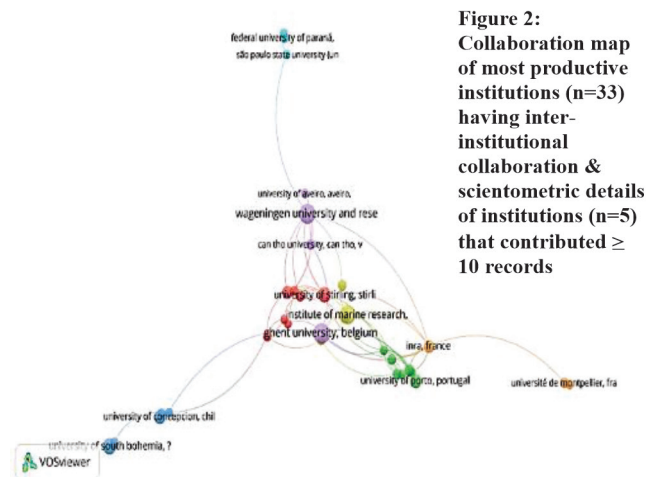


Fig. 1 Seven Clusters Identified Representing Institutional Collaborations

Cluster 1- CSIRO, Australia; Deakin University, Australia; Shanghai Ocean University, Shanghai, China; Universiti Putra Malaysia, Malaysia; University College Cork, Cork, Ireland; University of Stirling, Stirling, United Kingdom and Worldfish, Penang, Malaysia
Cluster 2 - Instituto de Ciencias Marinas de Andalucía, Cadiz, Spain; IRTA, Sant Carles de la Ràpita, Spain; University of Algarve, Faro, Portugal; University of Bergen, Bergen, Norway; University of Crete, Greece; University of Porto, Portugal and University of the Algarve, Portugal
Cluster 3- Interdisciplinary Center for Aquaculture Research, Chile; University of Chile, Chile; University of Concepcion, Chile; University of La Frontera, Temuco, Chile and University of South Bohemia, Czech Republic
Cluster 4- Institute of Marine Research, Norway; Norwegian Institute of Food, Fisheries and Aquaculture Research, Norway; University of Melbourne, Australia and University of Tasmania, Australia
Cluster 5 - Can Tho University, Can Tho, Viet Nam; Ghent University, Belgium; University of Aveiro, Aveiro, Portugal and Wageningen University and Research, Wageningen, Netherlands
Cluster 6 - Federal University of Parana, Brazil; Shantou University, Shantou, China and São Paulo State University (UNESP), Jaboticabal, Brazil
Cluster 7- INRA, France; Université De Montpellier, France and Université Laval, Québec, Canada

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5.3 Revolutionizing Aqua Sciences with Artificial Intelligence

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Keywords: Artificial intelligence, Computer vision, Machine learning, Robotics

Artificial Intelligence (AI) is becoming increasingly important in the field of aqua science, as it offers new ways to study and manage aquatic ecosystems. From identifying and tracking individual fish to monitoring and protecting endangered species, AI has the potential to revolutionize the way we understand and manage our oceans and freshwater systems. AI can be used to automatically identify fish species from images, and can also be used to track fish movements and predict where they are likely to go. AI is also being used to develop better methods for aquaculture, such as automated feeding and water quality monitoring. AI helps fish farmers to increase production and improve the quality of their fish. AI can be used to monitor and optimize the water quality in fish ponds, as well as to identify and track individual fish. AI can also be used to automatically feed the fish and monitor their health. AI-enabled robots are frequently used in different organizations to identify and remove dead fish from aquaculture farms. The robot uses a combination of computer vision and Machine Learning (ML) to identify and remove dead fish from aquaculture farms. Scientists are also working on a robot that can identify and remove algae from aquaculture farms. Fish behaviour and production optimization can be done more accurately with the help of AI technologies. For example, AI-based sensors can be used to monitor water quality and temperature in real time, allowing farmers to make immediate changes to improve conditions for their fish. Additionally, AI can be used to automatically identify and track individual fish, which can help farmers to more effectively manage their stock. AI can assist in forecasting pest attacks through prediction analysis by considering factors like humidity and temperature. Every day millions of data points related to aqua science are generated on each factor influencing their fish cultivation decisions. Using AI and ML models, with real-time data, useful insights can be generated to allow farmers to take preventive measures. AI can effectively be used in aqua sciences for the timely detection of pest attacks at the initial stage and forecast the yield estimation for their proper marketing. However, it is important to note that the use of AI in fishery science is also associated with many challenges. One of the main challenge is ensuring that the data used to train AI systems is accurate and representative, which can be difficult to obtain in the case of fish populations. Additionally, it is also important to ensure that AI-based systems are used in an ethical and transparent manner, to avoid unintended consequences such as overfishing or mismanagement of fish populations.

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5.4 Methane Emissions in Aquaculture Component of Integrated Farming System

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Key Words – Methane, Green house gases, Integrated farming system, Aquaculture

Human activities including fossil fuel combustion, biomass burning, wide spread deforestation, industrial emissions and improper land and water management practices have adversely affected the global atmospheric composition. The key sources of greenhouse gas (GHG) emissions from the agricultural sector are enteric fermentation (63.4 %), rice cultivation (20.9 %), cultivable soils (13.0 %), manure management (2.4 %), and burning of crop residues (2.0%) and there is a need for viable strategy for the mitigation of the problem. Integrated farming system (IFS) have gained a lot of popularity as it aims to maximise productivity, while minimising the environmental damage. Aquaculture is one of the important components of integrated farming system increasing the system productivity and net returns of the farm but adding wastes (cow dung), presence of vegetation, and other dead organisms in the aquaculture pond mainly contribute to organic matter and are merely available to the bacteria for degradation, resulting in a significant amount of greenhouse gases from the water-air interface. Organic matter decomposes and results in reduced oxygen content in water, creating an anaerobic condition in the bottom of a pond. Under an anaerobic environment, methanogenic bacteria consume the OM and lead to CH₄ production through the methanogenesis process. Therefore, to quantify the methane emissions in aquaculture pond, an experiment was conducted at School of Organic Farming, PAU, Ludhiana to quantify the methane emissions in aquaculture component of integrated farming system. Air samples were collected at 10 days interval from the pond and subjected to gas chromatography. The results revealed that methane emissions from aquaculture pond vary between 18.6 g/ha/day to 38.2 g/ha/day during the period of experimentation.

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5.5 Artificial Intelligence in Fisheries Management

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Keywords: Artificial Intelligence (AI), Machine learning (ML), Umitron cell, Aquapods

Fish demand is expected to increase by 30-35% in the range of 112.1- 114.1 million tons in 2030, requiring an additional 30- 32 million tons of fish. To meet this high level of demand, transformation with a fast-track approach using disruptive innovations and technologies for revolutionizing the aquaculture industry is needed. Artificial Intelligence (AI) is the simulation of human ability in computers. In computer science, artificial intelligence (AI) also known as machine intelligence, is machine-confirmed intelligence, as opposed to human-demonstrated natural intelligence. The use of AI in fisheries will enable the sector to expand rapidly, and make aquaculture a less labor-intensive sector. With greater crop productivity, less reliance on manual labor, and improved environmental sustainability, AI can transform the sector and make it globally competitive. AI and ML (machine learning) are two of the most talked about and exciting technologies of this time. AI is generated through Artificial Intelligence and ML is a subset of AI that involves programming computers to learn from data and make decisions based on what they learn. With the use of it, the fisheries farms integrate a high degree of automation, self-regulated control cycles and artificial intelligence within a Farm Management Information System to achieve a productive and environmentally friendly operation. Cloud-based IoT open operating system is available which connects products, plants, systems, and machines, enabling to harness the wealth of data with advanced analytics. Sensors ensure water quality and oxygen level creating a stress-free environment for optimized fish growth. Machine Learning algorithm may calculates biomass and hunger level through videos analysis to optimize feeding using artificial Intelligence. The AI is used to reduce waste feed by controlling release the right amount of food. Umitron cell is a smart automated feeder for aquaculture and the world's first real time ocean-based fish appetite detection system. Camera vision can detect skin anomaly and unusual swimming behavior to prevent outbreak of diseases. There are many uses of AI in the fishing industry, such as biomass estimation. Biomass estimation can help us estimate how much fish have been caught in a certain area, as well as what species are being caught. Using AI, we can model the environmental impact of fishing. We can regulate the fishing zones and ensure that there is a balance between overfishing, underfishing and maintaining healthy marine ecosystems. Offshore fish farms can be monitored from a distance using drones due to their ability to process real-time images. Aquapod (robotic cages) is a free-floating fish farm that can accommodate several hundred or several thousand fish using minimal space and very low impact on the environment. The demands on modern machines and plants are steadily growing in all industries due to maximum efficiency, flexibility, and cost effectiveness.

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THEME VI

Aquatic Ecosystem Assessment, Management and Conservation







6.1 2,4-Dichlorophenoxyacetic acid Induced Modulations in Immune System and Histopathological Alterations in Common carp *Cyprinus carpio*

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Keywords: 2,4- D, Fish, Herbicide, Histomorphology, Immune system, Pesticide

Introduction

Herbicides play a crucial role in agriculture production, and constitute a largest group and receive special attention. Amongst herbicides, 2,4-dichlorophenoxyacetic acid (2,4-D) is the most widely applied for its affordability, wide spectrum and efficiency. The toxic effects of herbicides to non-target organisms (fish) revealed that they disrupt the metabolic activities. In the aquatic environment, fish are highly sensitive to the contaminants, and hence considered as suitable bio-indicators. Amongst the fish responses, the evaluation of biochemical and immunological characteristics in fish blood has become an important means of understanding possible mechanisms of toxicological impacts. In addition, tissue biomarkers share a close link with other stress biomarkers since metabolic activation is a pre-requisite for many pollutants in order to provoke changes in tissues in the exposed organism. Choosing histopathology as a biomarker for pollution impact assessment is advantageous because of its intermediate position between the molecular and individual levels. However, studies on 2,4-D at immunological and histomorphological levels in fishes is scanty, and the present study investigated its impacts on ubiquitously available common carp, *Cyprinus carpio* at lethal and sub-lethal concentrations.

Materials and Methods

2,4-D (EC97%) of commercial grade was used for both lethal and sub-lethal exposures. Fish were exposed to 2.9, 29 and 58 mg/L for 28 days. Following which, immunological and histopathological responses were analysed. Nominal concentrations were used as 2,4-D is relatively stable. However, to overcome any degradation, a 100% renewal was performed every after 48 h. The water quality parameters such as temperature, pH, DO and ammonia were regularly monitored. After termination of the exposure experiment, fish were utilized for immunological and tissue architecture studies. The experiments were conducted as per the national and international guidelines for care and use of animals.

Results and Discussion

The study showed significantly increased SOD and CAT activity with increase in 2,4-D concentrations (Figure 1). Whereas, reactive oxygen species (ROS) in 2,4-D exposure groups declined significantly compared to the control, but no significant differences observed among the 2,4-D groups. Similarly, the lysozyme activity decreased with increase in pesticide concentration and its activity was significantly less compared to positive and negative controls. At the histological observations, study observed a disparaging alterations in the tissues at sub-lethal levels and the responses were dose dependent. Briefly, in tegmentum of the brain of unexposed fish showed a well organized nerve fibres and uniformly scattered nerve granules and clearly visible erythrocytes. While, fish at 2.9 mg/L 2,4-D revealed increased intensity of dark granules, and nerve fibres formed bundles. However, at 29 mg/L 2,4-D, the quantity of nerve fibres decreased and the nerve granules were prominent with pigmentations (Figure 2). The study presented a well organised primary and secondary lamellae along with uniformly distributed chondrocytes in control group. Whereas, fish exposed to 2.9 and 29 mg/L 2,4-D exhibited a minute telangiectasia, increased erythrocytes, necrosis, leukocyte infiltration, fused secondary lamellae, vasodilatations and decreased chondrocyte size. Concurrently, study observed a uniformly distributed proximal convoluted tubules (PCT) and distal convoluted tubules (DCT) in the kidney of the unexposed fish. Whereas with 2,4-D exposures, increased lumen space in PCT was noticeable, and hematopoietic tissue damage was evident. With increased 2,4-D, Bowman's space increased, minute tumour nodules were prominent. While, uniformly distributed hepatocytes and Kupffer cells were observed in the liver of unexposed fish, with exposures, yellowish to black MMCs were noticed. The study showed 2,4-D is immunotoxic, and perturbed multiple tissues. Future studies focussed on molecular insights of either 2,4-D or any other herbicides that could impact either fish or any other aquatic animals are advocated.

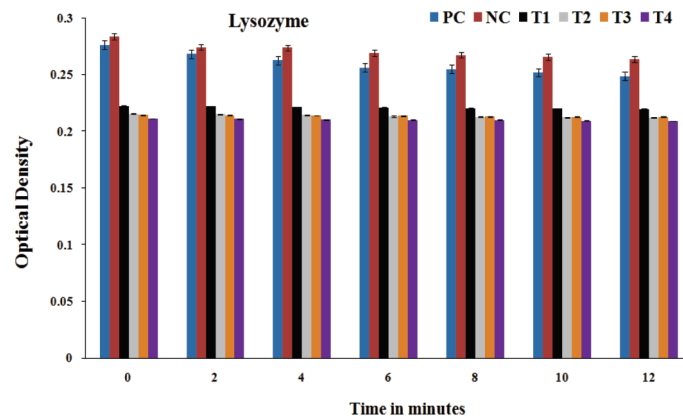


Figure 1. Lysozyme activity in the serum of *C. carpio* exposed to herbicide 2,4-D. PC: Positive Control, NC: Negative Control. T1: Control, T2: 2.9 mg/L 2,4-D, T3: 29 mg/L 2,4-D and T4: 58 mg/L 2,4-D.

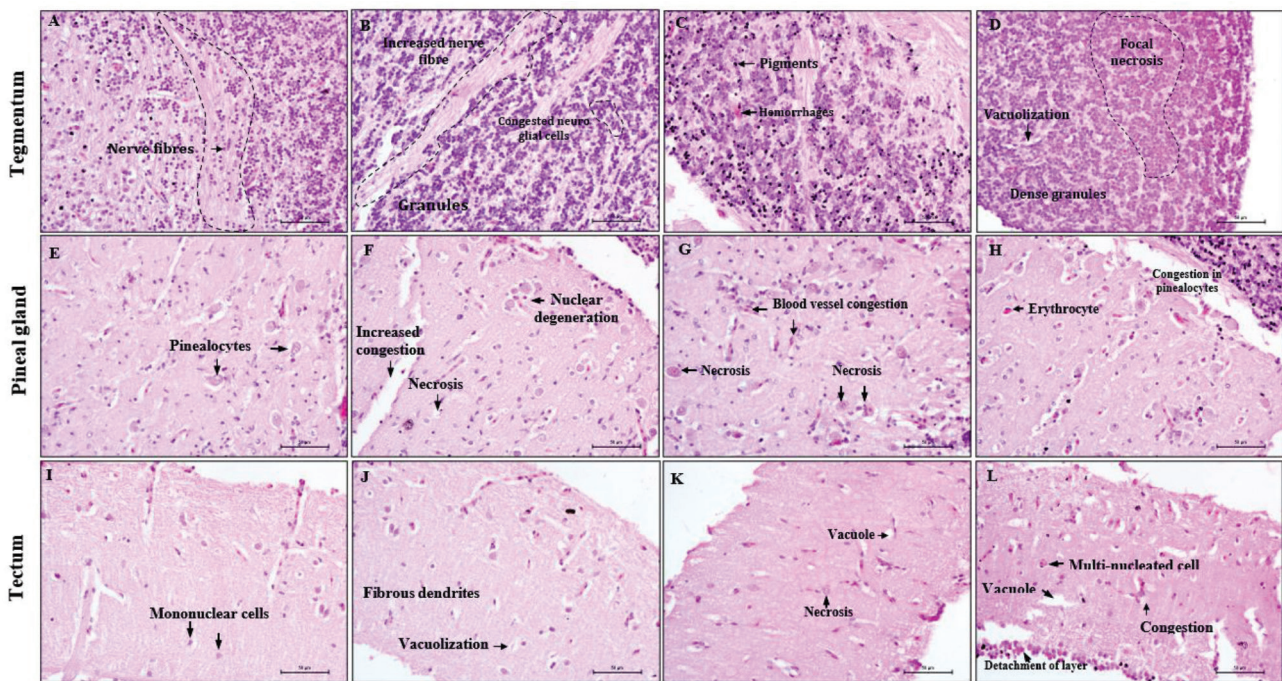


Figure 2 Microphotographs for detection of changes in the tegmentum (A-D), pineal gland (E-H) and tectum (I-L) of the brain of *C. carpio*. Control (A, E, I), Fish exposed to 2.9 mg/L 2,4-D (B, F, J), 29 mg/L 2,4-D (C, G, K), and 58 mg/L 2,4-D (D, H, L). Scale bar=50µm. H & E staining.

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6.2 Diversity of Molluscs and Herpetofauna along River Beas in Punjab, India

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Keywords: River Beas, Molluscs, Herpetofaunal diversity, Bioindicators

Introduction

The River Beas rises in the Himalayas in Himachal Pradesh and flows for 185 km in Punjab State and merge in Sutlej River at Harike (Punjab). During May, 2018 accidental release of Molasses in River Beas from a sugar mill near Gurdaspur District severely impacted aquatic life and ecological environment of river. The diversity of herpetofauna and molluscs along the river bank can act as an index of bio-indicator of the ecological environment. They also form an important link in the food chain. Regular monitoring of faunal diversity is essential especially when anthropogenic activities like industrialization and urbanization are increasing to ensure a healthy ecological regimen. Keeping this in view, surveys were conducted from 2018-19 to 2020-21 to study herpetofaunal and molluscan diversity along River Beas under a project supported by Punjab Pollution Control Board.

Materials and Methods

This study was conducted from 2018-19 to 2020-21 during autumn and spring seasons. Faunal diversity was studied at different sites along River Beas bank at village Dhilwan, district Amritsar, villages Chambha and Harike, district Tarantaran and villages Alampur and Bhait, district Gurdaspur. Both live and dead mollusc species were collected by hand pick method along the bank of river Beas, brought to laboratory, washed and identified (Patil *et al* 2011). Amphibian and reptiles were photographed and identified as per procedure by Ali *et al* (2022). A Cannon Powershot camera was used for the photography of animals along the river bank.

Results and Discussion

The faunal diversity was studied from 2018-19 to 2020-21 along the bank of River Beas during autumn and spring seasons. During 2018-19, four molluscan i.e. *Indoplanorbis exustus*, *Gabbia orcula*, *Lymnaea*, Freshwater clam, one amphibian *Hoplobatrachus tigerinus* and six reptilian species *Varanus varius*, *Craspedocephalus gramineus*, *Eutropis macularia*, *Gerarda prevostiana*, *Fowlea piscator* and rat snake were recorded along river bank. However in 2019-20, Seven molluscan species i.e. *Indoplanorbis exustus*, *Gabbia orcula*, *Bellamya bengalensis*, *Lymnaea*, *Cryptozonia semirogata*, *Cochlicopa lubrica* and Asian calm and two amphibians, *Hoplobatrachus tigerinus* and *Rana limnocharis* were recorded. While in 2020-21, Molluscs of eight different species *Indoplanorbis exustus*, *Gabbia orcula*, *Bellamya bengalensis*, *Lymnaea*, *Cryptozonia*, *Gyraulus*, *Corbicula* and *Bithynia* were recorded along river Beas. Indian bull frog and fresh water turtles were also recorded near river bank during this year. Earlier studies reported the presence of five species of snails, *Indoplanorbis exustus*, *Radix luteola*, *Melanoides tuberculata*, *Bithynia tentaculata*. *Kashmiriensis* and *Cryptozonia bistrialis* in Punjab State. Freshwater molluscs (mussels and snails), like other macro-invertebrates, are vital components of any aquatic ecosystems. Their sensitivity to the habitat's conditions allows them to act as biological indicators of the health of their habitats. Out of the various molluscan species recorded during the present study period, five species were resistant. In 2018-19, 75% molluscan species were resistant. Percentage of resistant species reduced to 52.67% by 2020-21. Reduction in the percentage of resistant species and increase in the number of molluscan species along river Beas from 2018-19 to 2020-21 indicated improvement in the quality of water

Table 1 Diversity of molluscs recorded from 2018-19 to 2020-21

Molluscan species	2018-19	2019-20	2020-21
<i>Indoplanorbis exustus</i>	✓	✓	✓
<i>Gabbia orcula</i>	✓	✓	✓
<i>Bellamya bengalensis</i>	✓	✓	✓
<i>Lymnaea</i>	✓	✓	✓
Freshwater clam	✓	✓	
<i>Cryptozonasemirogata</i>		✓	✓
<i>Cochlico palubrica</i>		✓	
<i>Corbicula</i>			✓
<i>Gyraulus</i>			✓
<i>Bithynia tentaculata</i>			✓

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6.3 Preliminary Studies on Seasonal Variations in Plankton Communities in Relation to Physico-Chemical Parameters in a Central Himalayan stream, Shipra, India

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Keywords: Plankton communities, Diversity index, River, Phytoplankton

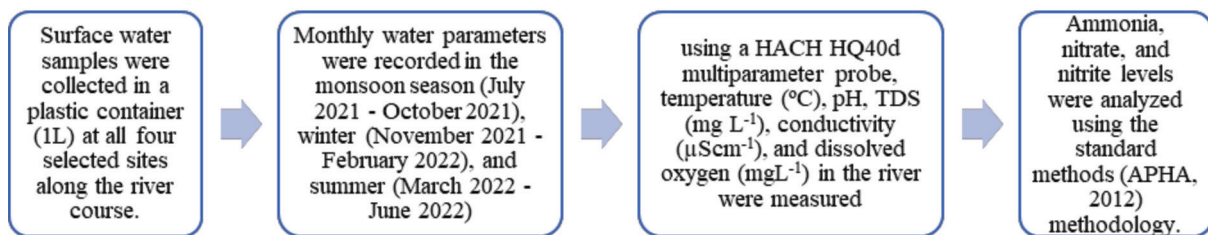
Introduction

The primary components of water bodies for maintaining the aquatic ecosystem or its health are physico-chemical parameters (abiotic factors) and plankton diversity (biotic factors). In the aquatic ecosystem, phytoplankton and zooplankton serve as the primary and secondary producers; hence, these biotic factors help maintain the river's water quality parameters. Plankton is an essential biomarker for water quality assessment and responds quickly when a disturbance occurs in an aquatic ecosystem. Therefore, the plankton distribution study is an essential step among various measures for conserving the aquatic ecosystem. With this background, a preliminary study was conducted on seasonal changes in the plankton communities concerning the river Shipra's physico-chemical parameters.

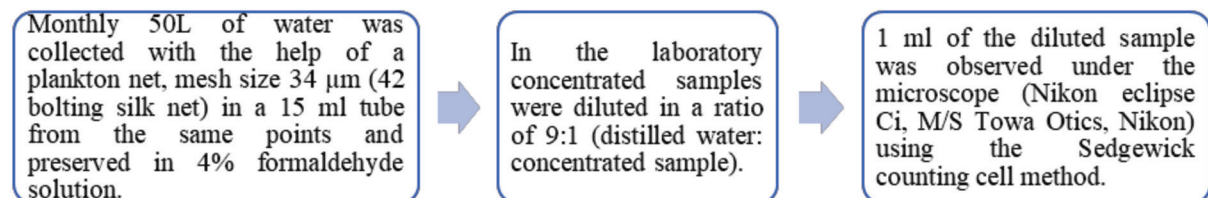
Materials and Methods

Monthly sampling was carried out for one year (July 2021 - June 2022) in river Shipra, Uttarakhand, to measure the value of temperature, pH, TDS, electrical conductivity, dissolved oxygen, ammonia, nitrate, nitrite, and plankton diversity. In the present study, four sampling stations were marked for taking samples from a 10 km stretch, i.e., near Kainchi Dham Mandir to Paadli.

Physico-chemical analysis (abiotic factors)



Plankton analysis (biotic factors)



Plankton identification was made up to the genera level. The total number of plankton in 1L of the water sample was calculated using the formula.

$$\text{Total no. of phytoplankton/zooplankton units/L of water filtered (N)} = \frac{n \times v \times 1000}{V}$$

Where, **n** = average no. of plankton sample (units/L); **v** = plankton concentration volume (1 ml); **V** = total water filtered volume (50 L)

The Shannon-Wiener diversity index method to examine species diversity and richness:

$$\text{Shannon - Wiener Index (H')} = - \sum \frac{ni}{N} \ln \frac{ni}{N}$$

Where N = total number of individuals of all species; ni = total numbers of individuals of species; H = Shannon -Wiener diversity index. The species evenness (J) and Simpson diversity index (D) were also calculated using the following formula (Pielou, 1966):

$$\text{Species evenness (J)} = \frac{H_i}{\ln(s)}$$

Where s = total no. of species; H' = Shannon-wiener diversity index.

$$\text{Simpson diversity index (D)} = \frac{ni (ni-1)}{N(N-1)}$$

Where N = total no. of individuals of all species; ni = no. of individuals of particular species

Results and Discussion

In hilly rivers, variations are reported among physico-chemical parameters in monsoon (July 2021 - October 2021), winter (November 2021 - February 2022), and summer (March 2022 - June 2022) seasons (Figure 1). The water temperature, dissolved oxygen, pH, TDS, conductivity, ammonia, nitrate, and nitrite shows variation in a range of 10 – 21°C, 7.6 -10.3 mgL⁻¹, 7 - 8.1, 55 - 117.8 mgL⁻¹, 70.5 - 175.3 μScm⁻¹, 0 - 0.04 mgL⁻¹, 0 – 1.5 mgL⁻¹ and 0 – 0.02 mgL⁻¹ respectively in different seasons in the selected river stretch. Forty-three genera represent the phytoplankton group under the classes- Bacillariophyceae, Chlorophyceae, Zygnematophyceae, Cyanophyceae, Euglenopyceae, and 4 genera of zooplankton group, i.e., Protozoan, Rotifers, Copepoda and Insecta. During winter, the phytoplankton group was highly diversified, followed by the summer and monsoon months, whereas the richness of zooplankton was observed in summer, followed by winter and monsoon seasons (Figure 2). The correlation among plankton communities and physico-chemical parameters showed that temperature is negatively correlated with DO, pH, and phytoplankton and positively correlated with conductivity and ammonia; dissolved oxygen is positively correlated with plankton communities. During the study, various diversity indices were studied, i.e., Shannon diversity index (H') having a range of 2.9 – 5, Species evenness (J) in the range of 1.4 – 2.8, and Simpson diversity index (D) in the range of 0.35 - 0.9. These seasonal variations showed significant levels (P ≤ 0.05) among plankton diversity and physico-chemical parameters.

Current baseline information of the river Shipra demonstrated that plankton communities are closely interrelated with environmental parameters. The changes in plankton communities may subsequently affect the feeding habits of the inhibiting fish species and other environmental factors, which may result in variation in fish species.

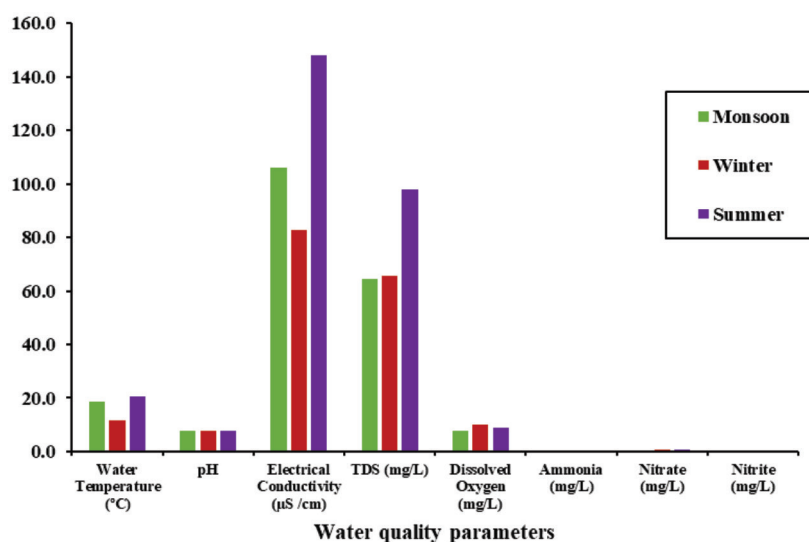


Figure 1 Seasonal Variations in Physico-chemical Parameters of River Shipra for a year (July 2021 – June 2022).

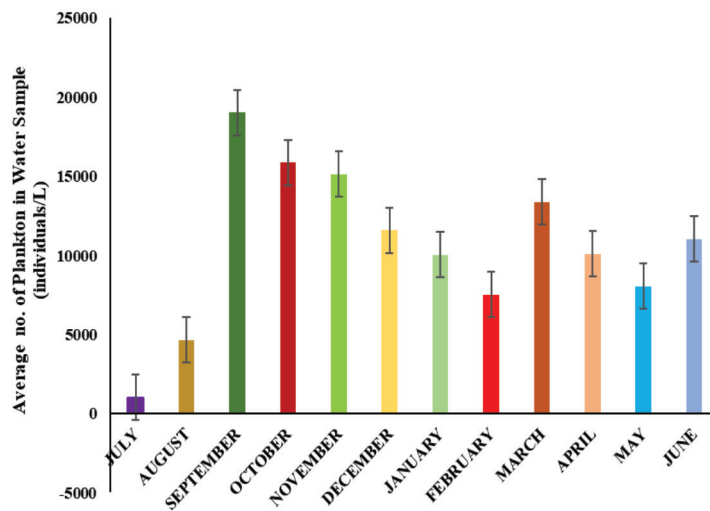


Figure 2 Plankton Diversity at all Selected Sampling Site in River Shipra (Kainchi Dham), Uttarakhand.

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6.4 Geo-informatics Approach for Sustainable Aquaculture Expansion: A Case Study on Water Spread area Mapping and Fish Production Potential in Manar Reservoir, Maharashtra

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Keywords: Geo-informatics, Productivity, Manar reservoir, Water spread area, Bathymetry

Introduction

Fisheries and aquaculture is a key global food production system and sustainable utilization of the same is a major concern. Reservoirs are playing significant role in the inland fish production though their full potential hasn't yet realized. Looking at the production data of the reservoir it has been noticed that the reservoir is not utilized fully and there is a yield gap. Study has been conducted to assess the seasonal water dynamics and depth of the Manar reservoir so that to suggest the measures for increasing fish production. With the help of RS and GIS the effective water spread area for stock enhancement and potential site for enclosure culture has been identified using Normalized Difference Index (NDWI) and Water Presence Frequency (WPF) so that, effective measures for the improved stocking density and appropriate site for the enclosure culture can be suggested.

Materials and Methods

Manar is medium reservoir of Maharashtra, located in the Kandhar taluk of Nanded district. The data used to map the Water Dynamics were extracted from Sentinel 2 Multispectral Instrument images with a 10 m × 10m resolution, which were obtained from the Copernicus Open Access Hub. Images during the period of February and May of the years 2019 to 2022 were selected. Selection of study season was done based on the dam water used for agriculture cropping pattern of the nearby villages. Water extent available in February and May was considered as water available for at least 8 months and 11 months respectively. NDWI was calculated using the digital number value of green and near infra-red (NIR) bands where the positive value and negative of NDWI indicated water and landmass respectively. Based on the number and size of the pixel of the sentinel image area of the water has been calculated from the attribute table. Calculation of the Fingerlings required for the stocking was done as suggested by Anand *et al.*, (2022) For calculation WPF total 62 images for the years 2019 to 2022 were used. Depth of the reservoir was calculated using Digital Elevation Model.

Results and Discussion

Results indicate that the reservoir's maximum water spread area was 2490.86 ha, and that from 2019 to 2022, it kept an average of 2250.41 ha and 1740.19 ha of water in February and May, respectively. Based on the National Fisheries Development Board's recommendation of a minimum stocking density of 500 no./ha, it is estimated that 10 lakh fish should be stocked, yielding an estimated 200 mt. The government-recommended minimum stocking density is currently being used to fill the Manar reservoir, and successful results have been shown in the years 2019 and 2020 where the actual output was 190.9 and 195.7mt, which is pretty close to our predicted production, Production dropped to 150 mt in 2021. The average fish production of the Manar reservoir is 89.3 kg/ha which is lower than the average productivity of medium reservoirs in India (94 kg/ha/year) which can be increased up to 200 kg/ha/year. Analysis of this study shows that the potential of Manar reservoir is underutilized, based on the productivity and availability of the seeds, stocking can be increased up to 1000kg/ha/year to utilize the full reservoir potential. Manar reservoir is not having enough depth to fulfill the minimum depth criteria given by government for cage culture. So, it has been suggested that for effective utilization of waterbody pen culture can be practiced in the suitable site erected in the reservoir and the fingerlings reared in the pens can be stocked in the reservoir for the further grow out. It will increase the survival rate of the fishes and hence the overall fish production.

References



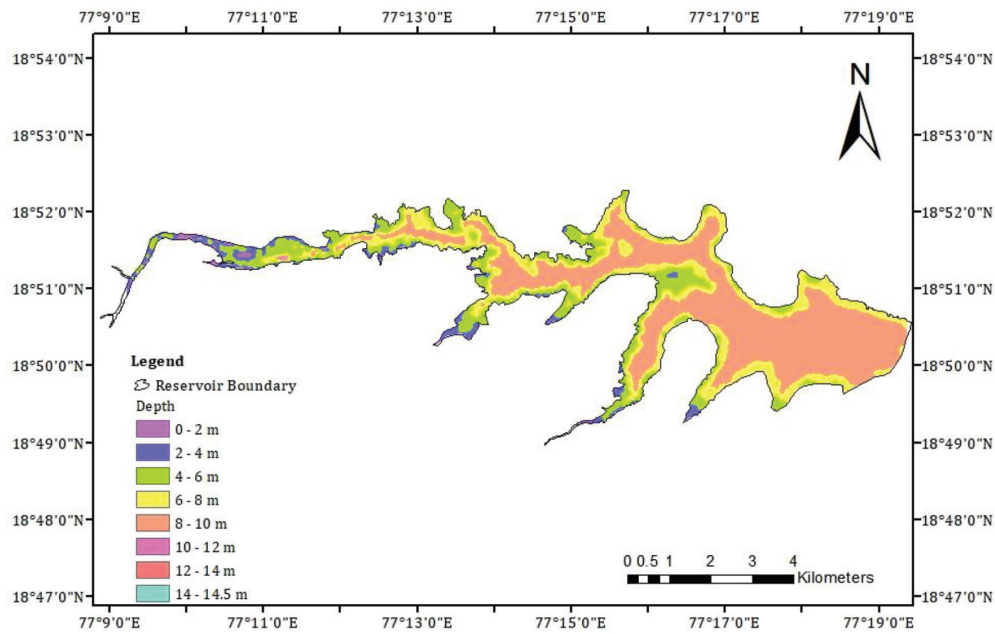


Figure 1. Bathymetry of Manar reservoir

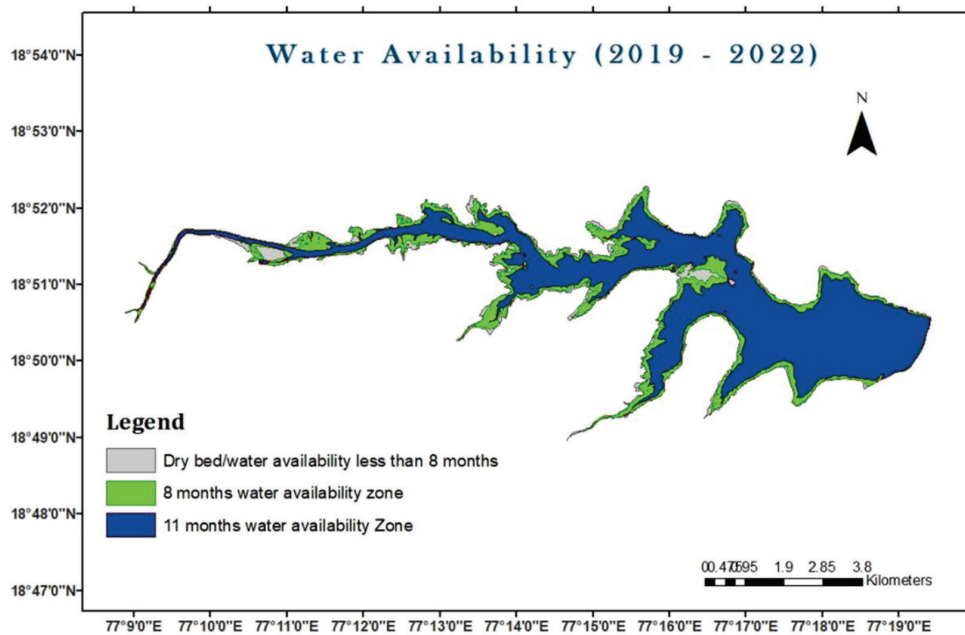


Figure 2. Water dynamics of Manar reservoir

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6.5 Endemic Ichthyofauna of North-East India

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Keywords: Checklist, Conservation, Diversity, Hotspot

Introduction

Endemism is the ecological state of a species being unique to a defined geographic location, such as an island, nation, country or other defined zone, or habitat type. The fishes inhabiting such a particular geographical location or in a defined place are referred as “Endemic fishes”. The North-eastern region of India, comprising the eight landlocked states of Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura, is known for its rich biodiversity and is the convergence of two important fish biodiversity hotspots in the world, the Eastern Himalayas and the Indo-Burma region. Ichthyofaunal diversity reports in the Northeastern region of India can be reviewed from the pioneer works of Hora, 1921-1953; Ghosh and Lipton 1982 (172 species); Sinha 1994 (230 species); Sen 1982-2000 (267 species); Goswami et al 2012 (422 species); Sarma et al 2010 (97 species); Nath and Dey 1997, 2000 (131 species); Bagra et al 2009 (213 species); Barman 1988-2004 (129 species), (48 species), (19 threatened species of India), (258 coldwater fish species of India), (ichthyofauna of Manipur) (fish diversity of Mizoram, Tripura and Barak drainage in Assam). In contrast to the high species diversity of the Indian ichthyofauna, no detailed systematic fish inventory has been available on the endemic ichthyofauna, particularly to the North-eastern region of India. As such, the present communication happens to be one of the maiden detailed systematic checklists of the endemic fishes thrived in the major aquatic bodies of North-eastern India.

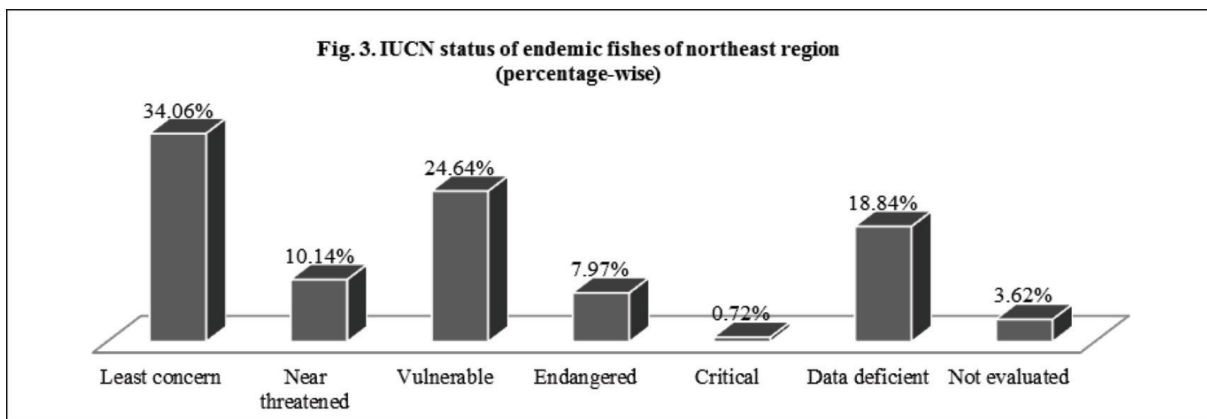
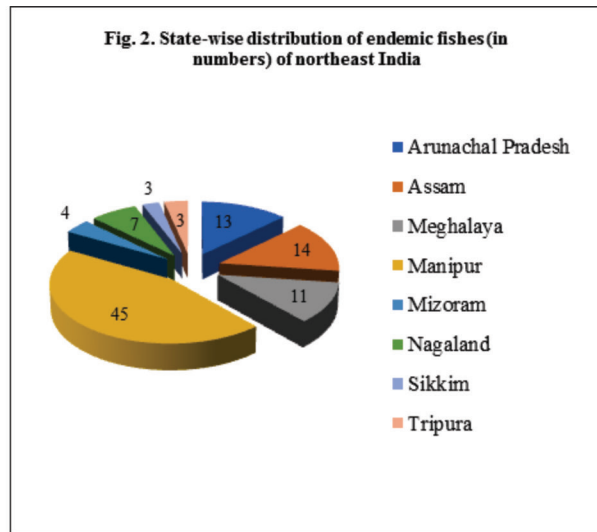
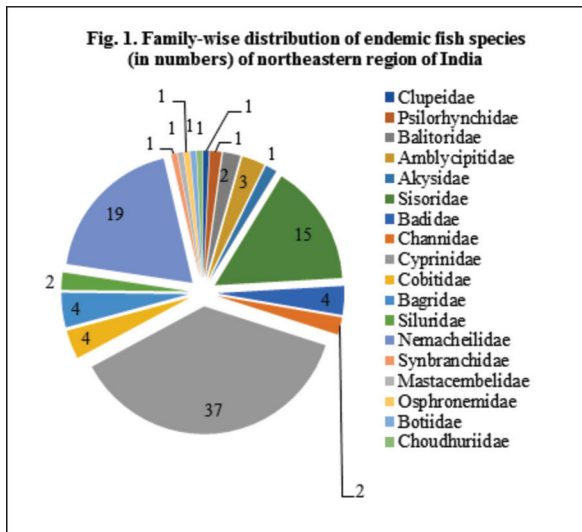
Materials and Methods

Extensive survey was conducted for last two decades in all the eight North-eastern states of India at various aquatic resources, fish landing centres, fishing villages, fishing zones, fish markets etc accessible by road and foot track. Primary information was collected by personal interaction with the fishermen, *mohaldars*, lessess, wholesalers, retailers and anglers at each randomly selected sampling station along the length of the rivers and associated wetlands. The endemism of the species recorded in this present communication is restricted within the Indian political boundary although a few species are also reported from the neighbouring countries. Fish specimens were preserved in 10% formaldehyde for laboratory identification on the basis of morphometric and meristic characters and for voucher specimens. Fish identification, taxonomic review of the identification and preparation of systematic checklist on endemism was prepared based on published literatures and Fish Base. The identified endemic fish in this checklist has been classified as food, sport and ornamental value based on their usage by the local consumers as reported in each of the sampling sites and secondary information collected. Conservation status of the fish species was primarily ascertained from the IUCN Red List of Threatened Species (<http://www.iucnredlist.org>).

Results and Discussion

Altogether, 6 orders, 18 families and 54 genera were recorded, enlisting 138 endemic species which represents about 32.7% of total confirmed fishes from Northeast India (422 species) and about 53.5% of total upland species (258 species) recognized from India. The greatest endemism of the fish species is represented in Fig. 1. In terms of economic importance, 58 species were found to have only food value, 43 species of ornamental value, 27 species with both ornamental and food value and 10 species have both food and sports value. State-wise distribution of endemic fish species is represented in Fig. 2. However, some of the fish species have a wide area of endemism and therefore have a relatively large distribution range, crossing the political boundaries of a state of the North-eastern region.

It is noteworthy to mention that majority of the endemic species (34.06%) are under the Least Concern category as per *The IUCN Red List of Threatened Species. Version 2015-4*. (<http://www.iucnredlist.org>), which indicates their abundance in drainages and stability of the aquatic environment (Fig. 3). The high degree of endemism in the region is thought to have resulted from its long period of isolation and complex evolutionary history, which promoted *in situ* diversification. The presence of different types of habitat and climatic conditions make the North-eastern region an ideal place for diverse ichthyofauna. Freshwater fishes thriving in topographical variations (foothills to high-altitude) exhibit high morphological divergence, suggesting that they evolved to adapt to specific habitats that differ in environmental stressors. Considering the anthropogenic interference and climate vagaries, there is growing concern over the resource assessment, breeding, production and management of the endemic species of the North-



east Himalayan region. However, aquaculture is since one of the fastest growing sectors in the region, many of the hill stream species can be considered as candidate species for diversification in freshwater aquaculture in the hilly region. Farming of these fishes would not only help in generating livelihood for the people but also to conserve germplasm from extinction.

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6.6 Indigenous Snow Trout (*Schizothorax* spp.) Fisheries in Coldwater Habitats of Arunachal Pradesh

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Keywords: Aquatic, Fishing, Habitat, Himalayas, Trout, Upland

Introduction

The natural aquatic resources of the state Arunachal Pradesh lying in the eastern part of Indian Himalayan Region (IHR) is comprised of five major river drainages and numerous upland lakes, supporting a rich diversity of valuable indigenous cold water fishes. The indigenous snow trout are known for their economic importance and are recognized as potential species for food and recreation for the people in Arunachal Pradesh and is mostly confined to the capture fisheries from three major drainages viz., Kameng, Subansiri and Siang, (tributaries of the river Brahmaputra). This group of snow trout belongs to the subfamily Schizothoracinae and family Cyprinidae and are classified under vulnerable (VU) in India by the IUCN (2012 along with scanty information regarding its taxonomy, distribution, biology, and food value. The demand for this group of fish has increased drastically with increasing fishing pressure on natural aquatic resources due to lack of much sustained aquaculture avenues in this hill locked part of India (Baruah et al 2018). Henceforth, an attempt has been made to highlight the status of snow trout fisheries thriving in the upland aquatic habitats in eastern Himalayan State of Arunachal Pradesh.

Materials and Methods

An investigation was carried out in seven snow-fed tributaries of Arunachal Pradesh viz, river Dirang *chu* (92°16'23"E, 27°22'30"N), river Sangti (92°37'22.4"E, 27°18'29.1"N), river Tenga (92°45'58"E, 27°18'15.7"N), river Choskorong Kho (92°27'32.8"E, 27°26'51.5"N) , river Kiile (93°49'53"E, 27°33'18.2"N), river Shei (94°71'90"E, 27°99'08"N) and river Yargyap *chu* (94°09'49.3"E, 28°34'32.4"N) of Arunachal Pradesh to study the distribution and catch composition of snow trout, their gonadal maturity, gut content, abiotic and biotic environmental conditions and the methods of fishing. Sampling was carried out on seasonal basis (winter, pre-monsoon, monsoon and post-monsoon) for two years 2020-2022. Fish identification was done by conventional and molecular methods of taxonomy. Water quality analysis was done by standard methods of APHA. Identification of phytoplankton, periphyton and zooplankton was done by standard identification keys. Detailed information on the fishing gear was recorded at the fishing sites in prescribed Performa and classified based on design, construction and mode of operation.

Results and Discussion

The dominating species of snow trout in the Kameng drainage were comprised of *Schizothorax plagiostomus* followed by *S. richardsonii* whereas *S. richardsonii* dominated in most rivers. In case of river Shei, *S. richardsonii* and *S. progastus* were recorded proportionately. The average length of *S. plagiostomus* was 17.32±4.89 cm with an average weight of 54.36±33.92 g in these snow-fed tributaries of Arunachal Pradesh. *S. richardsonii* recorded an average length of 14.64±2.36 cm with an average weight of 49.33±21.52 g. *S. progastus* recorded an average length of 16.44±1.02 with an average weight of 45.63±14.34 g. The other lesser found species of snow trout identified were *S. molesworthi* and *S. esocinus*. The length at first maturity was 11-12 cm and 16-17 cm for male and female snow trout, respectively, recorded during May to August. The GSI showed 1.3-13.7 in males and 0.8-14.4 in females; relative gut length of 1.8-2.8; Index of preponderance has established periphyton as the most preferred food with the dominance of *Pinnularia* followed with *Navicula*, *Fragillaria*, *Nitzschia*, *Achnanthes*, *Chlorella* and *Spirogyra*. The rivers were perennial in nature and flows through the hilly terrains at an altitude from 1411-1934m msl. The temperature ranged from 12.70±0.20 to 21.31±0.19 (°C), dissolved oxygen: 6.78±0.69 to 8.97±0.32 (mg/l), pH: 5.75±0.16 to 8.07±0.42, alkalinity: 34.00±2.10 to 110.00±2.55 (mg/l), hardness: 12.00±7.31 to 75.00 ± 3.10 (mg/l) and other essential nutrient variables within the optimum level for good fish health. Altogether, 52 phytoplankton species, 19 periphyton genera, 8 zooplankton genera were identified with the density of 68-1370 cells/litre. The fishing gears operated to harvest snow trout are indigenous and specific to a particular area based on the nature of the river and skills of the tribesmen. The major gears were noose and line, cast nets, *kholeya*, *neuta*, *hoap* and *lipums* with a CPUE of 1.8-2.2 kg/hour, 0.5-5.2 kg/hour, 2.0-15.0 kg/day, 0.5-4.2 kg/day and 2-4 kg/day respectively. Snow trout, henceforth is highly valued as a sport and food fish in Eastern Himalayas where the natural aquatic bodies provide conducive environment for self propagation and survival. The local people residing along these drainages could benefit from the fish by adopting to fish based ecotourism avenues. It also fetches high price compared to carps and catfishes in the region. But to raise



the fish in captivity needs proper feeding strategies and seed production protocols and this holds the major challenge for research. Therefore, overcoming the difficulties of ready feed and seed availability in the region, the young ones can be stocked for aquaculture as well as can be released into the natural systems for their self-propagation.

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6.7 Stock Delineation of Spinycheek Grouper Along West Coast of India by Otolith Shape Analysis in Central Eastern Arabian Sea

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Keywords: Arabian sea, Otolith analysis, Spinycheek grouper, Stock

Introduction

Stock differentiation in India is a vital subject in organization of fisheries resource. It is an interdisciplinary area that comprises the documentation of self-sustaining constituents among the natural populations. The stock documentation is a main tool for the execution of stock assessment and population dynamics. Otoliths, made of calcium carbonate are formed arrangements situated in the inner ear of fish, are a secondary way for observing fish populations and assessing the association between the environment and the organisms (Zengin et al., 2015). In various stocks or populations, otolith forms are changing. Sagittal otoliths are typically used in revisions of otolith morphology, so otolith shape analysis can be used as a tool for delineating stocks.

Material and Methods

The fishes are collected from three landing centres of Maharashtra, Kerala and Gujarat. The fishes are cleaned with water to remove mucus and dirt. Afterward reaching the laboratory left sagittal otoliths are collected from the fresh individuals with the aid of a scalpel. Next that otolith was washed in water to eliminate the flesh and kept for drying. Then dried otoliths are stowed in vials and they are named with unique code. Otolith was positioned ventrally and captured a photo by means of stereo zoom microscope Olympus SZX16 which are connected to a DP72 digital camera (Olympus Inc., Tokyo). The microscope magnification was attuned with the dimension of the otolith to safeguard the uppermost resolution changing between 1× and 2×. The digitized copy was then evaluated using Digimizer 5.0.0 image examination software to quantify its area (A), perimeter (Pr), maximum length (ML), and maximum width (MW). Otolith shape indices encompass of circularity, ellipticity, rectangularity, and form factor were then projected using the way of Tuset et al (2003).

Results and Discussion

PCA	Eigenvalues	Total Variance (%)	Cumulative (%)
1	3.129338	3.129338	62.58676
2	1.094291	21.88582	84.47257

The PCA have got a cumulative eigen of around 84.47 %. The otolith shape was differed between the stocks. The reason might be due to changes in environmental condition differences in sampling site.

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6.8 Occurrence of Amphibians and Reptiles at Village Ponds in Ludhiana, Punjab: Diversity, Threats and Conservation Prospects

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Keywords: Amphibians, Ecological indicators, Reptiles, Village ponds

Introduction

Amphibians and reptiles are cold blooded, habitat specific and sensitive to environmental changes; they are considered as indicators of healthy ecosystems. They are important components as both prey and predators in intricate food webs existing particularly in terrestrial and specifically in pond ecosystems. They also have significant role as predators of insects and rodents respectively, thereby help in controlling agricultural pests. In India, a total of 43 species of amphibians (15 genera and 06 families) and 64 species of reptiles (39 genera and 10 families) have been represented in agro-ecosystem. The present investigation on faunal diversity was carried out on ponds from March 2019 to February 2020 in two villages namely Jhammat, Malakpur and sewage treated water pond situated in Punjab Agricultural University (PAU) campus Ludhiana mentioned as pond A, B and C respectively. Overall, amphibian and reptilian diversity accounted for 2 and 4 species respectively at selected ponds.

Materials and Methods

Three selected ponds in village Jhamat, village Malakpur and Punjab Agricultural University (PAU) campus named as pond A, B and C respectively at location I (district Ludhiana). Ponds A (area 1.01 ha) is located in the village Jhamat surrounded by residential houses. Pond B (area 1.21 ha) was located near outskirts of the village having residential village houses on one side and agricultural fields on the other sides. Pond C (area 1.61 ha) is a man-made pond having treated water of sewage plant located near agricultural fields. Selected habitats of ponds were surveyed on fortnightly basis following Line/Point count transect methods.

Survey and identification of amphibians

Frogs were recorded through visual encounter survey. Dead animals were found and preserved in 8% formaldehyde solution for fixation and preservation. The field characteristics were noted down according to the guidelines given by Daniel (2002).

Survey and identification of reptiles

Reptile's survey was taken through visual encounter survey. The snakes were identified with the help of the standard reference book (Whitaker and Captain 2004).

Results and Discussion

Out of the class Amphibia, two species were recorded i.e *Euphlyctis cyanophlyctis* and *Duttaphrynus stomaticus* during the study period. *Euphlyctis cyanophlyctis* was often found along the edges of pond A and B. *Duttaphrynus stomaticus* was noted in shallow water, burrows or under leaves or undergrowth at the pond B.

Four species of class Reptilia namely i.e. *Lygosoma punctata*, *Ptyas mucosa*, *Bungarus caeruleus* and *Varanus bengalensis* were recorded at studied ponds. *Lygosoma punctata* was observed concealed beneath logs or among piles of leaves and twigs at the ponds A and B. *Ptyas mucosa* (Indian rat snake) was recorded along the dung piles or wild vegetation; it was recorded to be solitary during the sightings. *Bungarus caeruleus* (Indian krait) was noted at the pond C. *Varanus bengalensis* (Bengal monitor) was recorded along the diffused walled boundary of pond A.

Class Reptilia was found to be showing positive correlation with Class Amphibia. Both Amphibia and Reptilia had shown positive relation with water temperature, ambient temperature, pond area and fallow land area; whereas values of pH, BOD, DO and vegetation area were found to be negatively related. According to literature, amphibians and reptiles being diverse and rich components of terrestrial and freshwater ecosystems contribute to a varied array of ecological functions.

The major threats to the amphibians and reptiles inhabiting the village ponds of Punjab State include habitat degradation, loss of vegetation, encroachment of banks for agricultural purposes, used as sink for domestic/ sewerage waste and chemical run off from adjoining crop fields. Overall present field work has real time application both for the sustenance of village ponds as water resources and also as biodiversity sites in intensive agricultural scenario of Punjab State.



Table 1 Different species of Class Amphibia and Reptilia observed in different seasons at selected ponds

Name of species	Summer			Monsoon			Winter		
	Pond A	Pond B	Pond C	Pond A	Pond B	Pond C	Pond A	Pond B	Pond C
Frog <i>Euphlyctis cyanophlyctis</i>	+	+	-	+	+	-	-	-	-
Toad <i>Duttaphrynus stomaticus</i>	-	+	-	-	+	-	-	-	-
Skink <i>Lygosoma punctata</i>	+	+	-	+	+	-	-	-	-
Rat snake <i>Ptyas mucosa</i>	-	+	-	-	+	-	-	-	-
Krait <i>Bungarus caeruleus</i>	-	-	-	-	-	+	-	-	-
Monitor Lizard <i>Varanus bengalensis</i>	+	-	-	-	-	-	-	-	-

(+) Observed, (-) not observed

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6.9 Assessment of Drinking Water Quality and Efficiency of Rangil Water Treatment Plant

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Keywords: Rangil water, Water quality parameters, Treatment plant

Introduction

Water, a universal solvent is one of the major resources on this planet without which sustenance of life would be impossible. Availability of drinking water is an important topic that has received great attention since early times due to the high demand. The individual daily demand of drinking water is 2 Litre considering an average weight of 60 kg in accordance to human activity (Frewtrell and Bratram 2001). The drinking water should be pure, sterilized, suitable for human consumption, and free from chemical pollutants such as lead, arsenic and benzene. However, like any other natural resource, the drinking water sources are also facing contamination due to anthropogenic actions. In order to overcome such problems and to avoid health hazards, water treatment plants have been set up almost all over the country to provide potable water to the masses. An important water treatment plant in Ganderbal district, which supplies drinking water to majority parts of district is the Rangil water treatment plant. Rangil water treatment plant is situated at Rangil area of Ganderbal district. The water in the plant comes from Sind River and gets treated before being supplied to the city. There are different sections for treatment of raw water viz: Flash mixer cum stilling chamber, various water filtration units including Alum tanks, chlorination units, 20 MGD (million gallons per day) and 10 MGD water reservoirs besides water testing laboratories. This water treatment plant at Rangil supplies water to more than seven lakh population of Srinagar city through its 51.63 km long pipeline.

Materials and Methods

The present study was carried out on Rangil water treatment plant situated in Rangil, Ganderbal district of Kashmir valley. Water samples were collected from three sites namely inlet (untreated raw water), central (tap water) and outlet (treated water). The water samples were collected from selected sites in the month of May 2022 in plastic bottles for a period of 3 weeks. Separate glass stoppered bottles of 250 ml capacity were used for collection of dissolved oxygen samples. For collection of water sample from household, the tap was turned on at maximum flow rate, and the water was allowed to flow for 2 minutes. The tap was disinfected for a minute using a 70% alcohol and allowed to flow at a medium rate for 2 minutes. Previously sterilized glass and clean bottles were used for collecting water samples by holding the bottle steady under the water jet. Initial fixation was done in the field and chemical analysis of water samples were carried in laboratory within 24hrs. A total of 11 parameters were evaluated, of which Water Temperature was measured on spot while other parameters were measured in AEM laboratory of Faculty of Fisheries using standard methodologies of APHA (2015).

Results and Discussion

The mean limnological parameters of Rangil water treatment plant during the study period is presented in Table 1.

Table 1 Limnological parameters of rangil water treatment plant

Sr no.	Parameters	SITE 1 (Raw water)	SITE 2 (Treated water)	SITE 3 (Tap water)	MEAN±SE
1.	Water temperature °C	11.4	12.3	21.3	15±3.1
2.	pH	7.2	7.3	7.3	7.2±0.03
3.	Conductivity(µS/cm)	221.5	221.5	174	205.66±15.83
4.	Dissolved Oxygen (mg/L)	8.5	12.8	6.8	9.36±1.78
5.	Free carbon dioxide (mg/L)	11.7	18.7	5.2	11.86± 3.89
6.	Total Alkalinity (mg/L)	90.6	77.3	88.6	85.5±4.14
7.	Ca Hardness (mg/L)	33.6	31.0	25.4	30.0±2.41
8.	Mg Hardness (mg/L)	114.3	203.5	148.5	155.43±25.98
9.	Total Hardness (mg/L)	148.0	234.6	174	185.53±25.65
10.	Chloride (mg/L)	15.9	8.5	5.9	10.1±2.99
11.	Orthophosphate (µg/L)	12.3	14.94	11.9	13.04±0.95



Table 2 Comparison between observed mean values and permissible values

S. no.	Water Quality parameters	Min-max values recorded	Permissible range	Standards approved by
1.	pH	7.2 – 7.3	6.5 – 8.5	WHO
2.	Conductivity(μ S/cm)	174- 221.5	1000	WHO
3.	Dissolved oxygen (mg/L)	6.8 – 12.8	6.0	EQS
4.	Total alkalinity(mg/L)	77.3 – 90.6	200-600	BIS
5.	Ca hardness(mg/L)	25.4 – 33.6	75 - 200	BIS
6.	Mg hardness(mg/L)	114.3- 203.5	30 - 100	BIS
7.	Total hardness(mg/L)	148 – 234.6	200-600	BIS
8.	Chloride(mg/L)	5.9 – 15.9	0.2 – 0.5	WHO
9.	Orthophosphate(μ g/L)	11.9 – 14.94	6.0	EQS

Based on the results, it was concluded that almost all physico-chemical parameters except chlorine were within the permissible limits of WHO, BIS and other drinking water quality standards. The efficiency of the treatment plant was in a satisfactory level reflecting good water quality. However, for further improvement in the efficiency of the plant, strategies should be developed from source to household taps to deliver safe water to reducing human health risks.

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Geospatial Analysis for Sustainable Aquaculture Expansion: A Case Study on Water spread area Mapping and Fish Production Potential in Dimbhe Reservoir, Maharashtra

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Keywords: Normalized difference water index, Water spread dynamics, Water presence frequency, Bathymetry, Fish culture

Introduction

Indian reservoirs with an area of 3.91 million ha possess a lot of potential for fisheries that offer economic and social security, especially in rural areas. Poor stocking compliance & lack of information about seasonal water availability for aquaculture activities are mainly causing low productivity in Indian reservoirs. By FY 2024–25, the Government of India aims to increase the current fish production in reservoirs through cage culture from 2.44 lakh metric tonnes to 6.29 lakh metric tonnes. But planning aquaculture and fisheries activities in a reservoir require knowledge of water spread dynamics and potential fish culture areas. Geospatial technologies in inland water resource management and decision-making for fish culture have been widely used around the world. The current study used Sentinel II Multispectral Instrument (MSI) images (2019 to 22) to examine the water spread dynamics and define potential locations for intensive fish culture in Dimbhe reservoir, Maharashtra, India.

Materials and Methods

Mapping of the spatial extent of the water spread area (WSA) for identifying the potential sites, area for stocking, & fish culture in the reservoir has been done using Normalized Difference Water Index (NDWI) and Water Presence Frequency (WPF) of Sentinel II MSI (2019-22) images for the month of Feb (Rabi season) which is considered as water available for at least 8 months, and May (Zaid / summer season) which shows the minimum water extent which is available in a waterbody for at least 11 months, and total average fingerlings requirement, fish culture yield potential was calculated. The WPF was generated and mapped using seasonal and perennial water spread area in the 2019-22 monsoon season. Bathymetry study was also performed using the Digital Elevation Model and kriging interpolation to determine suitable locations for fish culture in the reservoir, which is critical.

Results and Discussion

The results show that the WSA declined to 30% between the two time periods, with 70.44% (1445.39 ha) of the area covered with water for 8 months and 39.60% (812.45 ha) retaining water for 11 months, respectively. Estimated average number of fingerlings required to utilize the available water in Dimbhe reservoir is 0.56 million with a production potential of 112.88 metric tonnes. It was found that the perennial water spread area available in the Dimbhe reservoir is 551.22 ha, comprising 26.9% of the total reservoir which is most suitable for permanent cage culture practices. More than 66% water availability is shown in the perennial area, with maximum depth areas (20-33 meters) suited for cage culture and lower depth regions also suitable for pen culture due to water availability and shallow depth throughout the year. Based on the water dynamics and depth of Dimbhe reservoir, a culture-species matrix has been developed to provide an overall concept of the feasible culture methods and candidate species. In

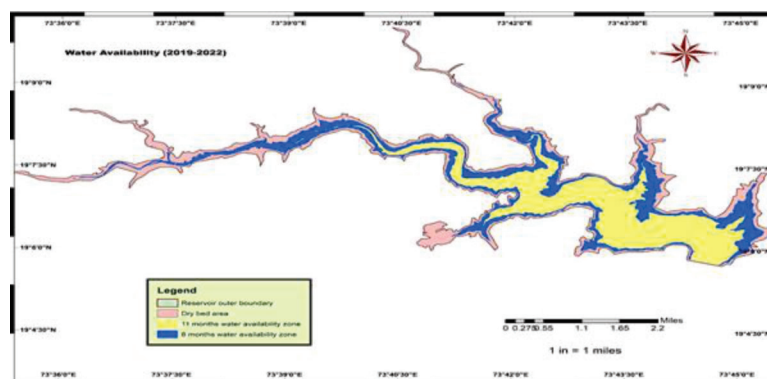


Figure 1 Dynamics of the watershed in Dimbhe reservoir

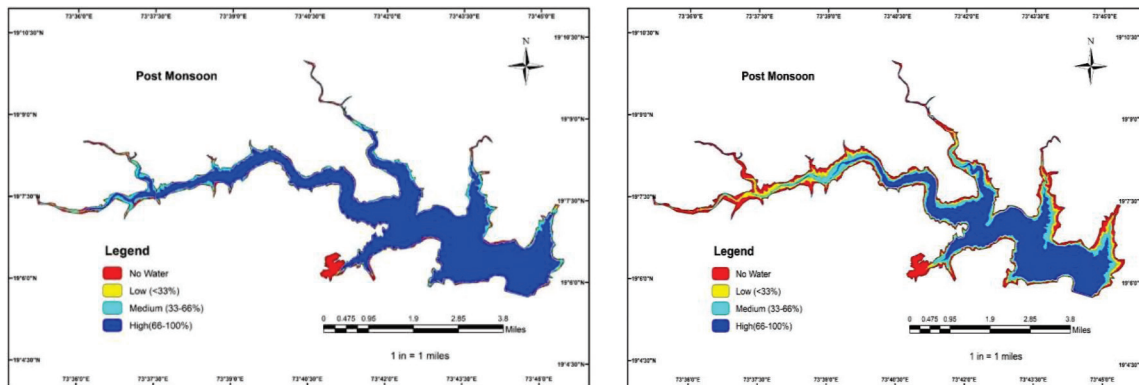


Figure 2 Comparison between post monsoon and pre monsoon water dynamics

2022, the stocking density is 9 lakh, which is significantly lower than the predicted stocking density (0.56 million) & proposed to be increased in future in Dimbhe reservoir. The selection of ideal culture methods and prospective species based on location would aid in the optimal usage of the previously underutilized reservoir. This research can be useful in planning scientific ranching and the advancement of cage culture in areas, which can be replicated in other reservoirs for tapping the fish culture potential and to plan appropriate interventions for reducing their yield gap.

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6.10 Assessment of Ecological Health of Selected Stretches of River Beas in Punjab, India

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Keywords: Beas, Contaminants, Metals, Microbes, Pesticides

Introduction

River Beas is one of the important tributaries of the Indus river system, with total length of 470 km from its source Rohtang Pass, covering 256 km in Himachal Pradesh and about 214 km in Punjab; is reported to be hosting about 31 fish species (total 54) of economic importance (Moza and Mishra 2007). In May 2018, accidental spillage of sugar mill molasses into river Beas at Village Kiri Afgana in district Gurdaspur killed several hundred fish, including catfish and carps, owing to consequential oxygen depletion and rise in BOD following the crisis. Under the State action plan of Punjab Pollution Control Board (PPCB) for conservation and restoration of ecology of River Beas, ecological health of stretch of river Beas passing through Punjab was regularly monitored for a period of 3 years, in terms of physico-chemical parameters, plankton profile (qualitative and quantitative) and presence of key contaminants like microbes, heavy metal and pesticide residues, at multiple sites from its entry point in the State at Talwara in District Hoshiarpur to the point of its confluence with river Satluj at Harike Pattan in district Tarn Taran.

Materials and Methods

Total nine sites (Talwara, Dasuya Kiri Afgana, Tanda, Beas Bridge, Gagrewal, Goindwal, Karmuwala, and Harike wetland) covering complete stretch of river Beas in Punjab was selected for this study. At monthly intervals, water samples (total 3816) and plankton samples (total 954 each for phytoplankton and zooplankton) were collected and preserved/processed in triplicate to analyse physico-chemical parameters, nutrient status, microbial load, heavy metal concentrations, pesticide residues and plankton profile (phytoplankton and zooplankton) from December, 2018 – December, 2021, following standard methods. The physico-chemical parameters, plankton and microbial load were analysed as per standard methods of APHA (2017), while the pesticide residues were analysed following the procedure of Hernandez *et al* (1993) and heavy metals were analysed in Inductively Coupled Plasma Spectrophotometer (Agilent, Germany) following standard protocol. Time series data generated were analysed in ArcGIS and 'R' software packages.

Results and Discussion

Year wise data in respect to critical water quality parameters revealed optimum dissolved oxygen (DO) level throughout the study period ranging 5.75 - 10.18 mg/l. Although, higher BOD levels in river during 2019 (0.57-4.01 mg/l), but subsequently it reduced to 0.71-1.05 mg/l in 2020 and 0.89-1.35 mg/l in 2021. Similarly, higher COD levels in water recorded during 2019 (0.33-15.5 mg/l) subsequently declined to 0.2-8.04 mg/l in 2020 and 3.3-7.63 mg/l in 2021. Overall, NH₃ level was recorded well below the permissible limit of 1.0 mg/l in the River (0-0.42 mg/l in 2019, 0.2-0.29 mg/l in 2020 and 0.17-0.25 mg/l in 2021, respectively). As per average relative abundance (%) of phytoplankton, Chlorophyceae was found to be the dominant phytoplankton group followed by Cyanophyceae, Bacillariophyceae, Dinophyceae and Euglenophyceae. Rotifera appeared as the dominant group among zooplankton followed by Cladocera, Ostracoda, Copepods, Oligocheates and Diptera. Total coliform count was found higher (210 to >1100 MPN/100 ml) than the CPCB desirable level of 500 MPN/100 ml (for B-Category river systems) at 6 sites (Tanda, Beas, Gagrewal, Goindwal, Karmuwala and Harike Pattan) during the study period from January, 2019 to December, 2021. Whereas, the fecal coliform number was also found above desirable limit of 100 MPN/100 ml during pre-monsoon/monsoon period in 2019 at Dasuya site (4 to 232 MPN/100ml); during monsoon period in 2019 and winter period in 2020 /2021 at Tanda site (3 to 270 MPN/100ml); and throughout the study period at Beas (4 to 590 MPN/100 ml) and Karmuwala sites (4 to 464 MPN/100ml). The concentration of metals in water was within the permissible limit throughout the study period and were found in the order Na>Mg>Fe>Zn>Mn>Cu>Ni>As>Cd>Cr >Pb. Among estimated pesticides, although α -HCH, β -HCH, permethrin and malathion were detected from the river water in 2019 at Dasuya, Kiri Afgana, Beas, Karmuwala and Harike Pattan sites during the different periods, but no pesticide residues were recorded in the following years at any of the 09 sites. As per Rapid Fisheries Assessment by Market Survey (RFAMS) methodology, the fish catch from the River was well represented by 29 commercially important fish species.

Overall, the ecological status of river Beas represents a healthy ecosystem to support aquatic biodiversity and if compared with the timeline database, the overall health of river Beas has improved, in special reference to oxygen and BOD levels, since the spillage incident in 2018. However, higher fecal contamination levels at sites downstream to Beas city, including the ‘Dolphin Hotspot’ (Gagrewal, Goindwal, Karmuwala and Harike Pattan sites) is a matter of concern and hence, need to be monitored to safeguard public health and conserve biodiversity richness of the river.



Fig. 1 Nine Sites selected on River Beas Stretch in Punjab during the Present Study

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6.11 Temporal Variations in Microbiological Quality of Water Samples from River Beas: A Case Study 2019-2021

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Keywords : River Beas, MPN-index, Emerging pathogens, Coliform, Heterotrophic plate count

Introduction

Pollution of riverine water is a major risk not only to the aquatic fauna and flora but also to the human health (Leong et al 2018). The present work was funded by Punjab Pollution Control Board (PPCB) to determine the influence of molasses spillage in river Beas by analyzing the bacterial communities at seven sites of riverine flow and observe the change in microbial dynamics during the conservation and restoration process. There is substantial data base available on microbial water quality of underground water being used for potable purposes but scanty information is available on the microbial community structure of irrigation waters. Surface water used for irrigation is monitored much less intensively. A limited monitoring is available on occurrence of indicator organisms rather than on occurrence of actual pathogens. In the present study both indicator organism and pathogenic bacteria were monitored by regular sampling at all selected sites during winter, pre-monsoon and monsoon season.

Materials and Methods

Present investigation was carried out at DST funded FIST Laboratory in Department of Microbiology, College of Basic Sciences and Humanities, P.A.U., Ludhiana. A total of 60 water samples of River Beas were collected from different locations (Harike, Goindwal Sahib, Mukerian, Tanda, Dhilwan, Talwara and Bhet) of Punjab during different seasons. All the samples were collected in sterile glass bottles and were processed on the day of collection. Microbiological attributes of collected water samples like Most Probable Number (MPN) index, heterotrophic plate count, faecal coliform count, indicator and emerging pathogens were analyzed as per standard microbiological methods (APHA 2005). The bacterial isolates were also screened for their heavy metal and antibiotic resistance for their potential use in bioremediation.

Results and Discussion

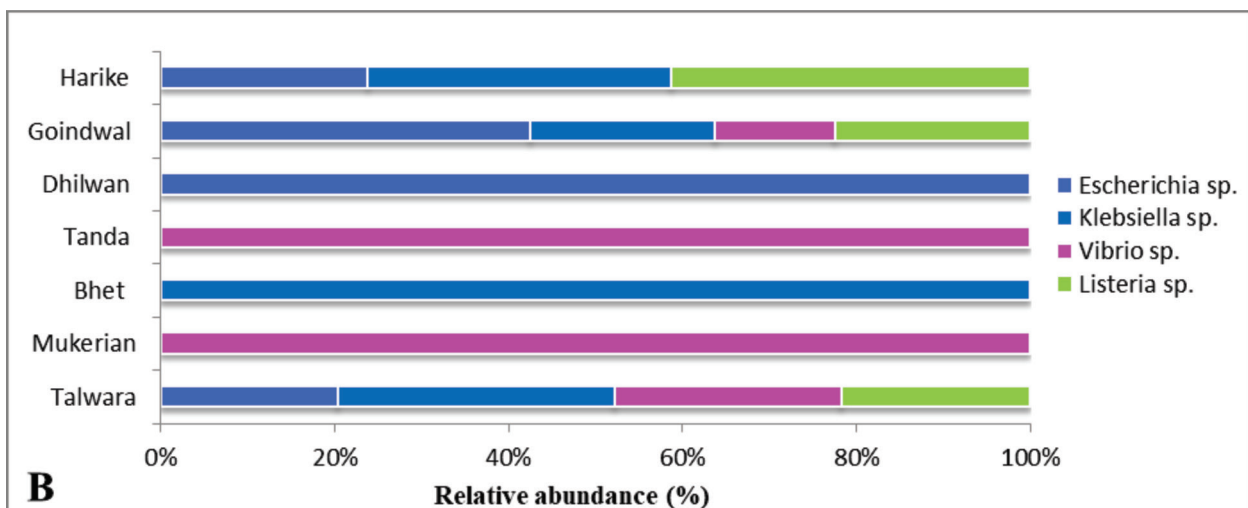
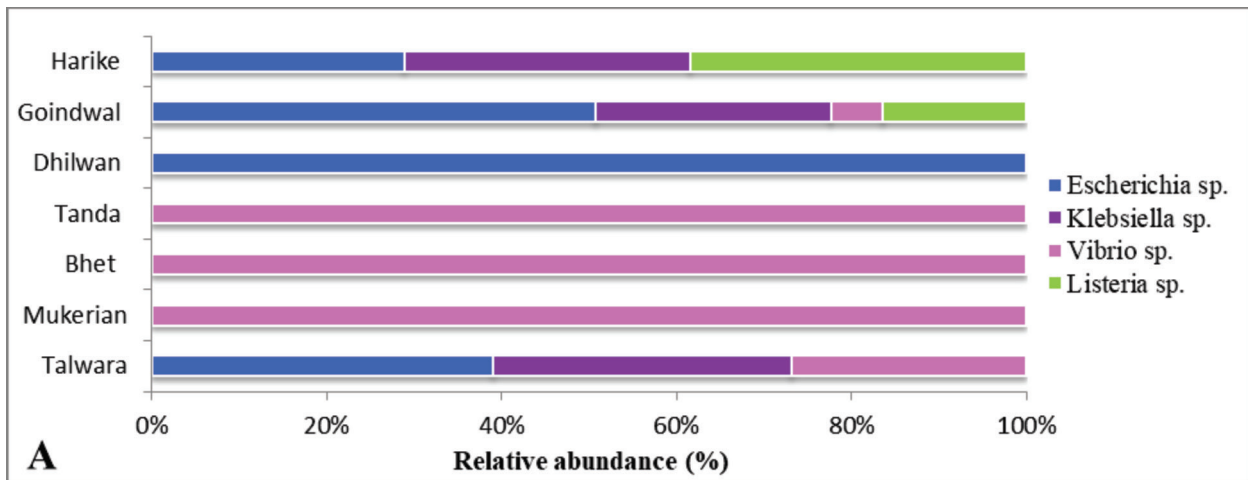
It has been analyzed that there was significant reduction in MPN index and heterotrophic plate count from 2019-20 to 2020-21 at Talwara and Tanda (Table 1). The maximum microbial load was found in Tanda (6.36 log₁₀ cfu/ml) in 2019-20 and at Talwara (4.88 log₁₀ cfu/ml) in 2020-21. The maximum microbial count in River Beas water samples were observed during monsoon as compared to winter and pre-monsoon season. The seasonal variation in heterotrophic plate count has been attributed to the environment factors like temperature and availability of nutrients. The maximum coliforms and fecal coliforms count for majority of water samples were found in monsoon as compared to pre-monsoon and winter season. The maximum log value of total coliform and fecal coliform were ranged between 1.71 (Talwara) and 1.34 (Mukerian), respectively during monsoon. In winter maximum log value of total coliforms and fecal coliforms was recorded as 1.44 (Dhilwan) and 1.04 (Mukerian), respectively. In pre-monsoon season, maximum log value of total coliforms and fecal coliforms was recorded as 1.53 (Dhilwan) and 1.17 (Goindwal) respectively. Due to presence of coliforms and fecal coliforms in water it is unsafe for drinking but can be used for irrigation purposes. Emerging pathogens like urinary infection bacteria (UTI), *Vibrio cholera*, *V. parahaemolyticus*, *Aeromonas hydrophilia*, *Salmonella typhi*, *Shigella sp.*, *Listeria monocytogenes*, *Campylobacter jejuni* and *Staphylococcus aureus* have arisen as a major public health concern. The relative abundance (Figure 1) of these emerging pathogens during different seasons revealed the highest abundance of *Vibrio sp.*

Table 1 Seasonal variation in MPN index and Heterotrophic plate count of River Beas (Dec 2019 to Aug 2020)

Sampling sites	MPN index /100ml			Heterotrophic plate (log ₁₀ CFU/ml)		
	Winter	Pre-monsoon	Monsoon	Winter	Pre-monsoon	Monsoon
Talwara	≥1600	220	31	5.98	3.50	4.68
Mukerian	140	1100	140	6.25	5.61	4.49
Bhet	910	380	22	6.34	3.93	2.30
Tanda	≥1600	1100	110	6.36	5.57	4.95
Dhilwan	240	460	75	5.91	4.61	3.04



Goindwal	220	110	240	5.56	3.80	4.62
Harike	540	140	110	5.62	3.23	2.60
Seasonal variation in MPN index and Heterotrophic plate count of River Beas (Dec 2020 to Aug 2021)						
Sampling sites	MPN index /100ml			Heterotrophic plate (log ₁₀ CFU/ml)		
	Winter	Pre-monsoon	Monsoon	Winter	Pre-monsoon	Monsoon
Talwara	1100	1100	1100	3.62	3.85	4.88
Mukerian	1100	460	1100	3.72	3.73	4.72
Bhet	1100	1100	1100	3.45	3.66	4.40
Tanda	36	36	210	3.64	3.79	4.60
Dhilwan	150	93	1100	3.41	3.69	4.42
Goindwal	>1100	1100	1100	3.51	3.64	4.36
Harike	210	93	1100	3.40	3.68	4.68



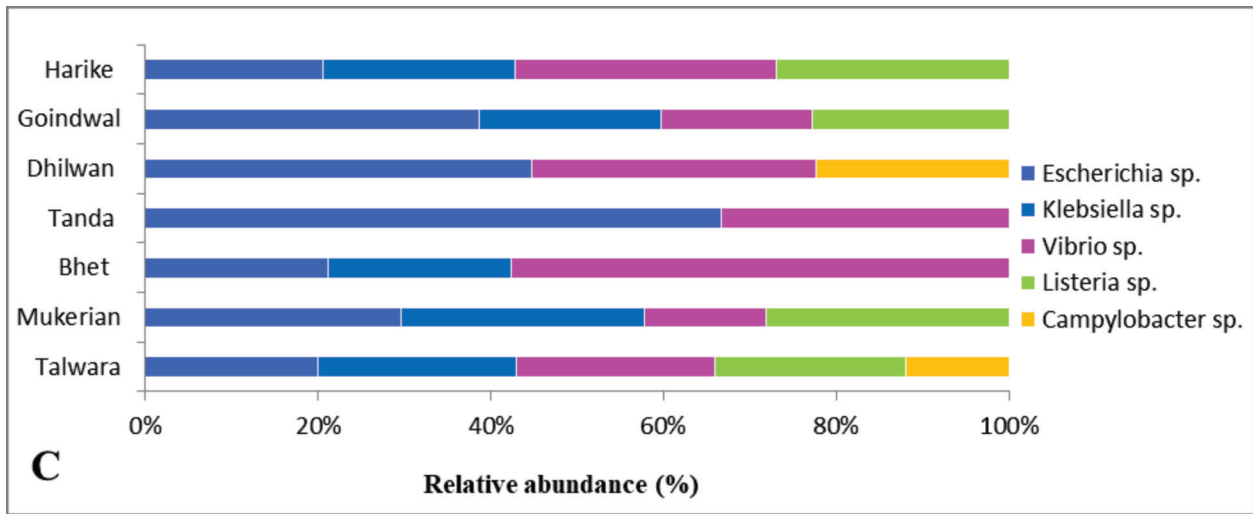


Figure 1 Relative Abundance of indicator and emerging pathogens in River Beas sites during A) winter, B) pre-monsoon and C) monsoon season

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6.12 Sustainability of Fisheries in a Small Reservoir in India: Interactions in a Food-web Context

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Keywords: Food web model, Electivity index, Trophic level

Introduction

The trophic interactions of an ecological community is represented by the food web, which shows the flow of biomass and energy among different organisms in the ecosystem. Food web models take into account population dynamics, organism-to-organism interactions, natural/anthropogenic stressor impacts, direct/indirect influence, energy and biomass flows, biodiversity analysis trends and changes in ecosystem functions across food web attributes and the impacts on ecosystems. This is emphasized in multi-species/ecosystem models that incorporate species interactions. The management of fisheries for sustainable development needs to consider the prey-predator interactions in the ecosystem. This is done through an ecosystem-based approach by constructing food web models. The models thus developed act as a tool to quantify the trophic interactions in the ecosystem and the extent of impact by various processes or stressors.

Materials and Methods

A foodweb model of Manchanabele reservoir in Karnataka was constructed using 15 functional groups to describe the trophic flows. The reservoir with a water spread area of 335 ha at FRL (12°53'55"N 77°19'37"E) in Ramanagara District is built across the river Arkavathi. The Ecopath with Ecosim software was used to construct the trophic model of the reservoir and the 15 ecological groups which include a non- living group (detritus) were used for parametrization. The biomass of fish groups was calculated based on the experimental fishing conducted at the reservoir and also from the commercial fish catch. The biomass for unexploited groups were obtained from our field studies and also from similar ecosystems (Moreau et al 2001 and Panikkar and Khan 2008). The criterion used for balancing the model was ecotrophic efficiency (EE). Flow indices were computed for analysing maturity of the ecosystem.

Results and Discussion

The diet matrix developed showed that the herons and cormorants classified as aquatic birds feed on murrels, gobids, major carps, Nile tilapia, cichlids and to a lesser extent on the catfishes. The groups in the next trophic level were the catfishes which feed mainly on barbs (mainly, *Puntius ticto*, *P. sophore*), aquatic insects, other cichlids and Indian major carps. Murrels (*Channa punctatus* and *C. striatus*) found to feed mainly on fishes and to a very lesser extent on the dipterans. Gobids consumed aquatic insects in larger proportion. The major carps were consumers of groups in lower trophic level groups. Major component in the gut of Nile tilapia and other cichlids consisted of detritus. The exotic carps in the reservoir fed mainly on the molluscan forms and periphyton. The Omnivory index was highest for barbs (0.391) indicating the least diet specialization.

Table 1 Key indices calculated for Manchanabele reservoir ecosystem

Sl. No.	Ecological group	Flow to Detritus (t/km ² /yr)	Net efficiency	Omnivory index
1	Aquatic birds	0.291	0.434	0.291
2	Catfishes	0.922	0.366	0.024
3	Murrels	0.236	0.370	0.018
4	Major carps	4.311	0.361	0.312
5	Nile Tilapia	6.668	0.357	0.338
6	Other Cichlids	2.448	0.432	0.345
7	Barbs	48.790	0.331	0.391
8	Aquatic insects	104.00	0.417	0.287
9	Benthic fauna	126.500	0.446	0.177
10	Zooplankton	740.500	0.490	0.322



11	Macrophytes	734.100		
12	Phytoplankton	668.000		
13	Detritus			

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6.13 Spatio-Temporal Analysis of Commercial Multi-Day Shrimp Trawl Bycatch along the Coast of West Bengal, India

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Key words: Bycatch, GIS mapping, Shrimp trawl, West Bengal

Introduction

Trawling accounts for a higher rate of bycatch among the different fishing gears due to its comparatively low selectivity. Bycatch and discards have become severe threats for sustainable fisheries at all levels, until and unless they are proficiently managed. Studies on bycatch composition, quantification, losses and means of its reduction have been carried in different coastal states of India and very few works have done on the geo-spatial mapping of bycatch. However, no such attempts have been made along the coast of West Bengal. Precise and timely assessment of bycatch and discards would provide necessary data for sustainable fisheries management of the State. The present study is therefore carried out to envisage comparative analysis of faunal composition, bycatch quantification and seasonal variation from a commercial multi-day shrimp trawl along the coast of West Bengal, India.

Materials and Methods

The study was conducted from July 2019 to March 2020 covering three seasons namely post monsoon (July 2019 to September 2019), winter (October 2019 to December 2019) and pre-monsoon (January 2020 to March 2020). A total of 62 hauls at a depth ranging between 9.5 m and 54.5 m were observed. In this study, the following terms and definitions are used for sorting the catch. “Target Catch” refers to the catch of a species or species assemblage primarily targeted by the particular trawl. “Retained bycatch” is that portion which is retained catch of a non-target species. So, “Bycatch” is the retained bycatch (non-target catch) plus discarded catch. Group-wise catch data during different months selected for cluster analysis. Catch data were normalized using the square root transformation function, converted into a lower triangular matrix using the Bray–Curtis Similarity Coefficient and dendrogram plots were constructed using the group average function Plymouth Routines in Multivariate Ecological Research (PRIMER) v.6 computer program. Mapping was done with Arc GIS 10.1 software.

Results and Discussion

A total of 148 species comprising finfishes and shellfishes was recorded during the study period with the maximum in the winter followed by pre-monsoon and post-monsoon periods. Maximum catch in shrimp trawl was contributed by sciaenids followed by prawns, clupeids, Bombay duck and ribbonfish. The percentage bycatch was maximum in November. The overall target catch, retained bycatch and discards formed were 13.1 %, 67.1 % and 19.8, to the total, respectively. There existed a positive correlation between target catch and bycatch during the study period. The GIS mapping showed that maximum fishing operations were carried out in the south-east direction. Maximum and minimum bycatch per hour were found in July and January, respectively, Predictive total catch per haul of shrimp trawl along the West Bengal coast showed maximum catch in the south-east direction at a depth range of 25-30 m. Both overall discards per haul and discards per hour were maximum within 20 m depth. Spatio-temporal catch and discard data with species distribution maps would help in understanding the area and seasonal abundance of catch and bycatch. The geo-database generated by the present study would help the stakeholders to gather exact geospatial information on fishing activities of trawlers along the West Bengal coast.

Table 1: Season wise abundance of target catch, retained bycatch and discards in shrimp trawl

Season	Target catch (%)	Bycatch		
		Retained bycatch (%)	Discards (%)	Total (%)
Post-monsoon	18.2	64.5	17.3	81.8
Winter	7.8	71.3	20.9	92.2
Pre-monsoon	11.3	66.1	22.6	88.7

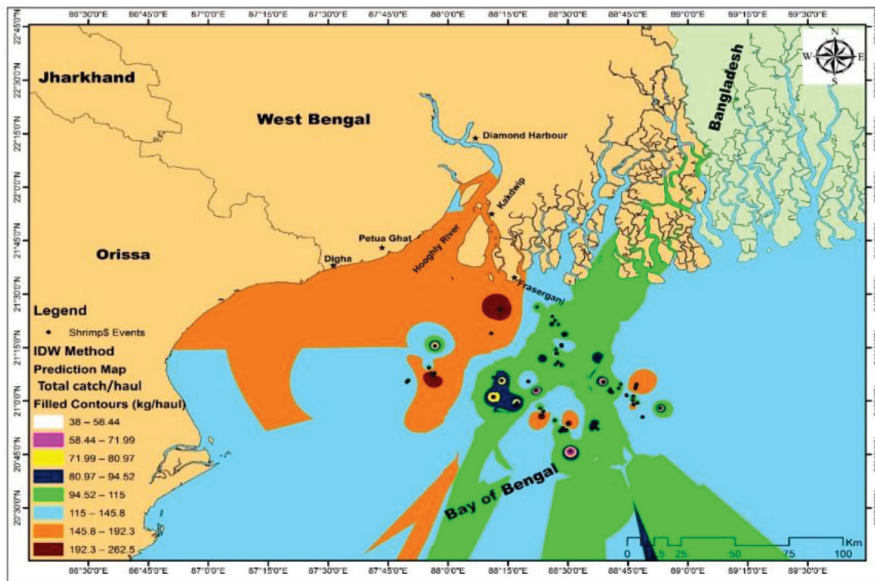


Figure 1: Predictive map of total catch/haul from the shrimp trawl

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6.14 Length-Weight Relationship and Condition Factor of *Mastacembelus armatus* (Lacepède, 1800) from Burhi Gandak River, North Bihar, India

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Keywords: Burhi Gandak, Bihar, Length-weight, *Mastacembelus armatus*

Introduction

The representative fishes of family Mastacembelidae is named spiny eels due to the presence of spines in dorsal fin, *Mastacembelus armatus* are distributed widely across Asia (Talwar and Jhingran 1991) and these are one of the potential candidate species in ornamental fisheries. The northern part of Bihar possesses network of Himalayan originated left bank tributaries of Ganga river like Burhi Gandak and has vast underutilized fisheries resources that produce an enormous diversity of species. The ichthyofaunal diversity of the river Burhi Gandak is dominated by the order Cypriniformes (Sahil 2020). *M. armatus* fetches higher market price (Rs. 300/-per Kg) as food fishes in particularly local markets of North Bihar as well especially when sold alive. However, no work has been reported so far from this region, henceforth, the present investigation has taken up to provide baseline data for further studies, management and conservation of *M. armatus*.

Materials and Methods

The present investigation was conducted from July 2019 to June 2020 in a Burhi Gandak river to study the biology of *M. armatus* from river Burhi Gandak, Bihar. Fish samples were randomly collected representing various classes of lengths on monthly intervals from Pilkhi Ghat, Dholi and Pusa sampling points in the Muzaffarpur and Samastipur using traditional gears like bamboo made traps, Ghanas and nets with floats and sinkers attached for the gear stability. The collected fishes were cleaned with water and morphometric and meristic characters were observed for the identification by using the standard taxonomical keys. Length and weight measurements were taken to the nearest 1.0 mm and 0.01g using vernier calipers and weighing balance, respectively for analysis. The taxonomic identification and biological analysis of collected specimens were done in fish biology laboratory of College of Fisheries, Dholi. The length weight relationship and condition factor was calculated by using the cube formula.

Results and Discussion

About 300 individual samples of *M. armatus* were collected during the study period and subjected to analysis. The length of *M. armatus* was ranged from 6 cm to maximum being 58.4 cm whereas weight ranged from 3g to 654g. In the present study the estimated b value for the *M. armatus* was 2.52. The length weight relationship as represented in figure 1 indicates negative allometric growth pattern for this species, lower value (<3) signifies as the length of the fish grows the body becomes slimmer. The mean value of Fulton's condition factor (Kn) comes out to be 0.29 ± 0.15 while the mean modified condition factor (K) was 1.38 ± 0.55 which may be attributed to different environmental conditions of river.

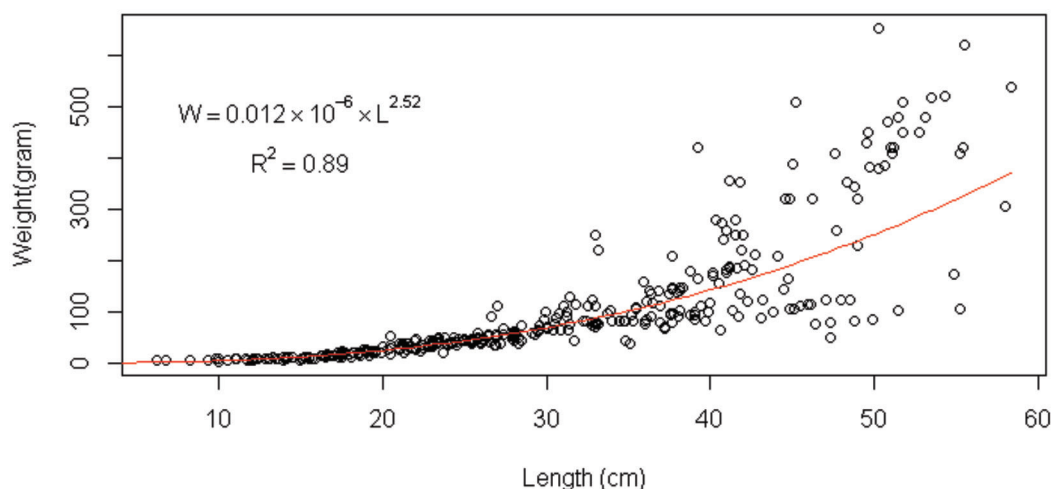


Figure 1 Length weight relationship of *M. armatus*



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6.15 Physico-Chemical Profile of Indus River System Tributary - Sutlej Along its Course of Flow in Punjab, India

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Keywords: Pollution, Sutlej, Water quality, Buddha Nallah, Punjab

Introduction

Growing industrialization/urbanization has increased water pollution alarmingly, with 70% of Indian rivers presumed to be polluted. The majority of the riverine resources of the country are heavily polluted and have turned into open sewers (Anand et al 2007). The largest tributary of the Indus river system in Punjab i.e. Sutlej is among the severely polluted rivers, particularly its stretches passing through Ludhiana town due to direct discharge of partially treated/untreated domestic sewage, agricultural run-off and industrial effluents into it. The river Sutlej sustains diverse fish species, besides supporting the livelihood of fishermen/riparian populations. Moreover, fish harvested from Sutlej is being directly used for human consumption. Due to the immense importance of Sutlej in terms of its rich floral-faunal biodiversity, the major source of capture fisheries of Northern India and livelihood generation for fishermen, it becomes imperative to assess the ecological health of Sutlej to form suitable strategies to control and prevent the menace of pollution, biodiversity conservation and restoring its pristine purity.

Materials and Methods

Water samples (N=approx. 100) were collected (in triplicate) at two-monthly intervals during the study period from February 2018–June 2019 from four designated sites spots i.e. S-1 (River Sutlej at Ropar Headwork), S-2 (River Sutlej before the confluence of Buddha Nallah at Phillaur), S-3 (River Sutlej after the confluence of Buddha Nallah near village Wallipur), and S-4 (Harike-Pattan where river Sutlej meets with Beas). The samples were collected in clean and dry plastic bottles in the early morning hours of the day and brought to the laboratory under iced conditions in insulated boxes for further analysis on the same day. The physio-chemical parameters of water viz., total alkalinity (TA), total hardness (TH), chloride, dissolved oxygen (DO), biological oxygen demand (BOD), and chemical oxygen demand (COD) were analyzed according to the standards methods of APHA (2005). In contrast, ammonia, nitrate, and phosphate were estimated using the DR-900 colorimeter (Hach make). Water temperature and pH were estimated on the spot using a mercury thermometer and digital pH meter, respectively. One-way ANOVA and Duncan Multiple Range Test (DMRT) were applied to find the significant differences in water quality parameters at the different sampling sites of the river. Bivariate correlation analysis was performed to find out the associations among the different water quality parameters.

Results and Discussion

The impairment of water quality due to the introduction of pollutants is a problem faced by most industrial cities worldwide. During the present study, comparatively, elevated levels of temperature (27.77°C), TA (170.51 mg CaCO₃l⁻¹), TH (253.40 mg CaCO₃l⁻¹), chloride (167.82 mg l⁻¹), ammonia (1.85 mg l⁻¹), phosphate (1.81 mg l⁻¹), BOD (48.44 mg l⁻¹) and COD (143.18 mg l⁻¹) were recorded at S-3 indicating higher degree of pollution at this site which is mainly due to direct discharge of industrial effluents, run-off from the catchment area and domestic sewage waste. The pH (8.33, 7.75 and 8.15) and DO (9.09, 7.93 and 7.34 mg l⁻¹) values were recorded within desirable limits at S-1, S-2 and S-4 indicating good ecological health of the river. In contrast, nitrate values were higher at S-2 (5.66 mg l⁻¹) due to the direct discharge of Phillaur sewage into river Sutlej. An inverse relationship was found between the values of DO and BOD ($r = -0.972$), confirming heavy load of pollutants being received by river Sutlej along its course of flow in Punjab.

The results of the present study revealed that river Sutlej is under pronounced pressure of pollution after the influx of Buddha Nallah, which is greatly threatening the aquatic biodiversity and livelihood of hundreds of dependent fishermen population. Additionally, higher levels of pollutants present in water might be interfering with fish physiology coupled with the endocrine disruption and the accumulating level of pollutants above maximum permissible limits is also life-threatening to the fish-consuming human population. Therefore, our findings warrant continuous monitoring, devising of suitable pollution control strategies and their strict implementation to restore, conserve, and sustain the biodiversity of river Sutlej before these changes become irreversible.





Table 1 Comparative variation in water quality parameters at selected sites during the study period

Parameter	Site – 1	Site – 2	Site – 3	Site – 4
Temperature (°C)	21.08 ^d ±0.03	24.45 ^b ±0.05	27.77 ^a ±0.02	23.03 ^c ±0.03
Ph	8.33 ^a ±0.01	7.75 ^c ±0.04	7.05 ^d ±0.51	8.15 ^b ±0.02
Total alkalinity (mg CaCO ₃ l ⁻¹)	109.29 ^c ±0.55	132.89 ^b ±0.56	170.51 ^a ±0.64	108.74 ^c ±0.57
Total hardness (mg CaCO ₃ l ⁻¹)	134.66 ^c ±1.25	156.00 ^b ±1.36	253.40 ^a ±1.75	117.63 ^d ±0.68
Chloride (mg l ⁻¹)	31.58 ^d ±0.35	52.76 ^b ±0.34	167.82 ^a ±0.67	46.40 ^c ±0.33
Ammonia (mg l ⁻¹)	0.02 ^c ±0.006	0.74 ^b ±0.04	1.85 ^a ±0.31	0.05 ^c ±0.001
Nitrate (mg l ⁻¹)	1.91 ^b ±0.27	5.66 ^a ±0.43	1.63 ^c ±0.24	1.78 ^c ±0.25
Phosphate (mg l ⁻¹)	0.51 ^c ±0.07	1.32 ^b ±0.09	1.81 ^a ±0.08	0.53 ^c ±0.07
Dissolved oxygen (mg l ⁻¹)	9.09 ^a ±0.69	7.93 ^b ±0.63	2.43 ^c ±0.49	7.34 ^b ±0.55
Biological Oxygen Demand (mg l ⁻¹)	2.29 ^d ±0.02	4.21 ^c ±0.11	48.44 ^a ±1.14	11.82 ^b ±1.03
Chemical Oxygen Demand (mg l ⁻¹)	14.24 ^d ±1.24	45.78 ^b ±1.41	143.18 ^a ±1.39	23.47 ^c ±1.25

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6.16 Relation of Diatoms with Few Water Quality Parameters of Rangil Stream

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Keywords: Water quality parameters, Diatoms, Correlation, Stream

Introduction

Water is one of the world's most important natural resources, without which life would be impossible. In fact, all living things depend on water for much of their life. It is one of the most important human exploits of all resources. The Kashmir Valley is well-known for its water resources. Planktons, particularly phytoplankton have been used as indicators of water quality. Living in the flowing water can be beneficial for plants and algae, as the stream is usually well ventilated for a long period of time. Some species grow in highly eutrophic waters while others are more sensitive to organic and / or chemical waste. Some of the species are responsible for the noxious blooms, which may in turn create offensive taste and odours. Some of the species may create anoxic or toxic conditions resulting in animal deaths and human illness.

Materials and Methods

The study was carried on Rangil stream situated in Rangil, Ganderbal district of Kashmir valley. It originates from Sindh River and is stretches through Harwan area. For the study, three sites were selected for the assessment of water quality and the diatoms population. The water and plankton samples were collected from selected sites in the month of May 2022. Three samples were collected. The surface water samples were collected from each designated sites in one litre sample bottle. For Dissolved oxygen samples were collected in separate D.O. bottles and fixed on the spot as per Winkler's modified method and further analysis of water samples were carried in AEM laboratory, Faculty of Fisheries. Remaining water quality parameters were analysed following standard methods of APHA (2012) during the study period. For the quantitative and qualitative enumeration of phytoplankton a known volume of stream water (1-2L) was passed through plankton. The filtered plankton samples were collected in the plastic tube and were transferred to separate containers and preserved by few drops of Lugol's iodine. The preserved samples were brought to AEM laboratory at Faculty of Fisheries. The detailed qualitative investigation was done under compound microscope up to genus/species level using standard keys given by Adoni *et al.* (1985). For quantitative enumeration the results are expressed in cells/L. The results were analysed using relevant Statistical methods, including MS excel and SPSS (Ver. 16) software.

Results and Discussion

The total of eleven diatom species were recorded from all the three sites of Rangil stream. 8 diatom species belonging to *Synedra*, *Navicula*, *Cymbella*, *Nitzschia*, *Cymbopleura* genera were recorded from all sites. With an increase in water temperature the diatom cells also recorded an increase in number. Therefore it can be concluded that with an optimum temperature range diatoms also increase and reach their peak. Dissolved oxygen showed a significant positive correlation with total diatoms ($r=0.72$), which is a clear indication that with an increase in the photosynthetic activity of diatoms the concentration of dissolved oxygen increases and subsequently the free CO_2 decreases. A significant positive correlation ($r=0.78$) between orthophosphate and total diatoms indicate that with an increase in the nutrient concentration in the water, the diatom population also increases. The significant negative correlation ($r=-0.52$) between total diatoms and silicate indicates that diatoms take silicate from water for the development of their outer silicified cell wall (frustules) thereby decreasing silicate concentration in water.



Table 1: Summary statics of physico-chemicals parameters of water of Rangil stream

S. no.	Parameters	Units	Sites	Week 1	Week 2	Week 3	Mean
1	Air temperature	°C	1	28.5	22.5	20.6	23.8
			2	27.9	23.2	21.5	24.2
			3	27.8	23.5	20.3	23.8
2	Water temperature	°C	1	11.4	11.9	10.7	11.3
			2	11.6	11.8	11.1	11.5
			3	12.2	12.5	11.6	12.1
3	Depth	M	1	0.8	0.8	0.8	0.8
			2	1	1	1	1
			3	0.2	0.2	0.2	0.2
4	Transparency	M	1	0.4	0.22	0.3	0.30
			2	0.42	0.2	0.3	0.30
			3	0.2	0.2	0.2	0.2
5	pH	-	1	7.4	7.55	7.56	7.5
			2	7.5	7.4	7.5	7.4
			3	7.5	7.3	7.5	7.4
6	Conductivity	µScm ⁻¹	1	300	172	192	221.3
			2	254	167	187	202.6
			3	238	165	191	198
7	DO	mgL ⁻¹	1	9	6	7	7.3
			2	4	9	8	7
			3	10	7	5	7.3
8	Free CO ₂	mgL ⁻¹	1	11	7	12	10
			2	16	14	19	16.3
			3	21	12	12	15
9	Chloride	mgL ⁻¹	1	9	2	3	7.6
			2	7	9	6	10
			3	5	4	4	10.3
10	TA	mgL ⁻¹	1	80	88	72	80
			2	74	94	72	80
			3	66	92	94	84
11	PO ₄	µgL ⁻¹	1	28	32	34	31.3
			2	36	22	18	25.3
			3	15	18	12	15
12	SiO ₂	MgL ⁻¹	1	12.02	11.85	15.28	13.05
			2	13.20	13.56	15.54	14.10
			3	11.26	15.92	16.24	14.47

*temp: temperature; DO: dissolved oxygen; TA: total alkalinity, CO₂: carbon dioxide, Cl: chloride, PO₄: orthophosphate, SiO₂: silicate



Table 2: Diatom density at three sites/L

S.no	Genera/species	Abbreviations	Week 1			Week 2			Week 3		
			S1	S2	S3	S1	S2	S3	S1	S2	S3
1	<i>Synedra tabulate</i>	SYTA	15	25	30	20	15	40	20	30	30
2	<i>Synedra ulna</i>	SYUL	25	75	40	30	60	30	25	75	35
3	<i>Amphora ovalis</i>	AMOV	0	10	25	0	15	25	0	20	30
4	<i>Navicula radiosa</i>	NARA	35	15	60	30	10	50	25	20	65
5	<i>Navicula cuspidate</i>	NACU	0	0	130	0	0	110	0	0	140
6	<i>Navicula sp.</i>	NASP	10	25	15	15	40	20	10	30	35
7	<i>Nitzschia amphibian</i>	NIAM	50	40	90	45	45	80	40	35	75
8	<i>Cymbella sp.</i>	CYM1	25	15	100	20	25	90	15	40	125
9	<i>Cymbella sp.</i>	CYM2	20	25	35	30	15	30	20	10	40
10	<i>Cymbopleura sp.</i>	CYMB	20	30	200	25	35	180	30	40	220
11	<i>Gomphonema sp.</i>	GOMP	10	0	90	15	0	100	25	0	75
			210	260	815	230	260	755	210	300	870

Table 3: Pearson's Correlation coefficient (r) between physico-chemical variables of three sites of Rangil stream and the diatom species present in them

	Air temp	Water temp	Depth	Trans	pH	Cond	DO	Free CO ₂	Chloride	TA	PO ₄	SiO ₂	Total Diatoms
Air temp	1												
Water temp	-0.27	1											
Depth	0.69	-0.88	1										
Trans	0.5	-0.97	0.97	1									
pH	-0.5	-0.69	0.27	0.5	1								
Cond	-0.32	-0.81	0.45	0.65	0.98	1							
DO	-1	0.27	-0.69	-0.5	0.5	0.32	1						
Free CO ₂	0.65	0.53	-0.08	-0.32	-0.98	-0.92	-0.65	1					
Chloride	0.4	0.76	-0.37	-0.58	-0.99	-0.99	-0.4	0.95	1				
TA	-0.5	0.97	-0.97	-1	-0.5	-0.65	0.5	0.32	0.58	1			
PO ₄	0.15	-0.99	0.81	0.93	0.78	0.88	-0.15	-0.64	-0.84	-0.93	1		
SiO ₂	0.19	0.88	-0.56	-0.74	-0.94	-0.99	-0.19	0.86	0.97	0.74	-0.93	1	
Total Diatoms	-0.72	0.86	-0.99	-0.95	-0.22	-0.4	0.72	0.03	0.32	0.95	0.78	-0.52	1

*Cond: conductivity, temp: temperature, DO: dissolved oxygen, TA: total alkalinity, CO₂: carbon dioxide, Cl: chloride, PO₄: orthophosphate, SiO₂: silicate

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6.17 Comparative Biometrics of Commercially Important Fish Species under Channidae Family from River Sutlej in Punjab

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Keywords: Sutlej River, Channidae, Biometrics, Length-weight relationships, Condition factor

Introduction

River Sutlej is one of most important tributaries of Indus river system. In the state of Punjab the total length of river Sutlej is approximately 440 km. The fish species under family Channidae mainly dominated by *Channa striata* (Shoal), *C. marulius* (Sawal) and *C. punctata* (Daula) in India, generally called as murrels/ snake head. *Channa* species have unique flavour, postoperative medicinal application to enhance wound healing and reduce pain and discomfort. Fish eating population also prefers this fish because of negligible intramuscular spines content in flesh. Biometric traits is one of the most frequently employed and cost-effective methods for stock identification. Relationship between the length-weight plays vital roles to estimate population fitness of any fish species. This is the first study to look into biometrics of commercially important fish species under family Channidae from River Sutlej in Punjab.

Materials and Methods

Length-weight data of fish species under Channidae family from the three different sampling sites of Sutlej River i.e. Rupnagar, Rail/road Bridge at Phillaur, Ludhiana and Harike has been evaluated from November, 2020-October, 2021. Multistage stratified random sampling method described by Srinath et al (2005) of CMFRI, Cochin was followed during sampling. A total of 120 specimens out of which *Channa striata* (n=40), *C. marulius* (n=40) and *C. punctata* (n=40) of different age groups were collected from selected sites. A total of 15 morphometric characters including weight of the fish (Wt) and 5 meristic counts were considered in present study. Le Cren (1951) formula $W = aL^b$ was used to represent the relationship between length and weight. The condition factor (K) was used to explain changes in weight as a function of length, assuming that the length-weight relationship follows the cube law.

Results and Discussion

During the present study, total three species under family Channidae were recorded i.e. *C. striata*, *C. marulius* and *C. punctata* from the selected sites of River Sutlej in Punjab. Out of these species, highest average weight was recorded in *C. marulius* (1482.39±290.02 g) followed by *C. striata* (570.58±53.72 g) and *C. punctata* (37.43±5.81 g). Average total length (TL), standard length (SL) and head length (HL) also followed similar trend as *C. marulius* recorded highest average TL, SL, HL (57.70±3.74 cm, 49.94±3.27 cm 11.37±0.82 cm, respectively) followed by *C. striata* (41.00±1.64 cm, 34.97±1.48cm, 10.79±0.51 cm, respectively) and *C. punctata* (16.88±1.46 cm, 14.10±1.32 cm, 3.84±0.46 cm, respectively). The value of exponent “b” for *C. striata* (2.15), *C. marulius* (2.85) and *C. punctata* (2.28) indicated a negative allometric pattern, thus species got slimmer as they grew longer. Relative condition factor (K_n) revealed good health of all three species. *C. striata* had the highest average K_n value (1.17), followed by *C. punctata* (1.15), and *C. marulius* had the lowest (1.01). The study concludes that the fish species under family Channidae maintaining a healthy condition in River Sutlej stretch in Punjab and sufficient number of matured individuals are there to sustain the breeding and recruitment.

Table 1 Average morphometric characteristics of family Channidae during the study period

	<i>C. striata</i>	<i>C. marulius</i>	<i>C. punctata</i>
Morphometric Characters			
WT	570.58±53.72	1482.39±290.02	37.43±5.81
TL	41.00±1.64	57.70±3.74	16.88±1.46
SL	34.97±1.48	49.94±3.27	14.10±1.32
PDL	11.75±0.47	14.22±0.99	5.58±0.55
PPL	10.85±0.46	13.03±0.84	4.61±0.46
PPeL	11.72±0.50	14.56±0.84	5.14±0.47
PAL	19.55±0.70	25.37±1.68	7.81±0.71
DFL	21.40±1.04	32.91±2.76	7.80±0.74



	<i>C. striata</i>	<i>C. marulius</i>	<i>C. punctata</i>
PFL	5.40±0.26	6.97±0.55	2.41±0.30
PeFL	3.61±0.26	4.08±0.31	1.51±0.21
AFL	13.95±0.85	21.73±1.75	5.56±0.49
CFL	5.89±0.32	7.86±0.56	2.96±0.27
HL	10.79±0.51	11.37±0.82	3.84±0.46
HD	4.73±0.36	4.91±0.54	2.56±0.37
BD	5.32±0.29	6.71±0.53	2.85±0.37
ED	0.97±0.03	1.06±0.04	0.45±0.04
Meristic Characters			
DFR	44.52±0.53	52.25±0.81	29.29±0.29
PFR	16.01±0.23	16.79±0.39	15.13±0.32
PeFR	6.00±0.00	5.41±0.17	5.84±0.08
AFR	25.42±0.71	32.75±0.43	20.56±0.23
CFR	14.60±0.65	14.18±0.19	12.85±0.18

*(Weight of fish (WT), Total length (TL), Standard length (SL), Pre-dorsal length (PDL), Pre-pectoral length (PPL), Pre-pelvic length (PPeL), Pre-anal length (PAL), Dorsal fin length (DL), Pectoral fin length (PL), Pelvic fin length (PeL), Anal fin length (AL), Caudal fin length, Head length (HL), Head depth (HD), Body depth (BD), Caudal length (CL) and Eye diameter (ED))

** Dorsal fin rays (DFR), Pectoral fin rays (PFR), pelvic fin rays (PeFR), Anal fin rays (AFR), Caudal fin rays (CFR).

Table 2. Logarithmic Length- weight relationship (LWRs) equation of selected species of Channidae family from Sutlej River

Species	Logarithmic equation (Log W = Log a + b Log L)
Channa striata	Log W= -1.12+2.15 log L
Channa marulius	Log W= -1.89+2.85log L
Channa punctata	Log W= -0.94+2.28 log L

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6.18 Population Dynamics and Stock Assessment of Great Snakehead *Channa marulius* from River Sutlej Stretch in Punjab

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Keywords: Sutlej River, *Channa marulius*, Growth, Exploitation ratio, Overexploitation

Introduction

Fish species under Channidae family are always considered as preferred choice for consumers for their taste, flavour, absence of intra-muscular spine and medicinal values. Great snakehead *Channa marulius* is one which is always being targeted by fishermen from natural water bodies for its demand and values in markets. In natural water bodies, siltation, encroachment, habitat destruction, scarcity in food items of juvenile fishes and extreme fishing pressure are considered as major factors contributing towards declining its number (Kaur et al 2017). This is the first study to look into population dynamics and stock assessment of *C. marulius* from Sutlej River, India. The latest modelling approach is employed in this study to compute growth, recruitment, mortality, yield/ recruit and virtual population analysis (VPA) to understand the current status and formulate future management and conservation plans.

Materials and Methods

Length-frequency data of a *C. marulius* from the three different sampling sites of Sutlej River i.e. Rupnagar, Rail/road Bridge at Phillaur, Ludhiana and Harike has been evaluated from November, 2020-October, 2021 using FISAT II (FAO-ICLARM Stock Assessment Tools) computer software package. Multistage stratified random sampling method described by Srinath *et al* (2005) of CMFRI, Cochin was followed during sampling. A total of 500 numbers of *C. marulius* was considered for population dynamics study. Growth parameters including Von Bertalanffy growth equation, Growth Performance Index (ϕ), Age at Zero Length (t_0), Longevity (t_{max}) were evaluated. Pauly's empirical formula was used to determine natural mortality (M). Relative biomass per recruit (B'/R) was estimated from the relationship $B'/R = (Y'/R)/F$, while E_{max} , $E_{0.1}$ and $E_{0.5}$ were assessed by using the first derivative of this function. The iso-values of Y'/R were plotted to generate the yield isopleth diagram.

Results and Discussion

Using the ELEFAN I algorithm, the asymptotic length (L_∞) and growth coefficient (K) were calculated as 99.75 cm and 0.880 year⁻¹ in *C. marulius*, respectively. The t_0 was estimated 0.013 year in *C. marulius*. The growth equation of von Bertalanffy was estimated as $L_t = 99.75 (1 - e^{-0.88(t - 0.013)})$. The growth performance indices (ϕ) value was calculated to be 3.941 whereas; longevity (t_{max}) was calculated to be 3.42 years. Higher recruitment peaks were detected during June (23.50 %) and July (16.34 %). The length at recruitment (L_r) was found to be 30 cm. Natural mortality (M), fishing mortality (F) and total mortality (Z) for *C. marulius* were estimated to be 1.19 year⁻¹, 1.27 year⁻¹ and 2.46 year⁻¹, respectively. According to virtual population analysis (VPA) the predominant loss in the stock due to natural causes were recorded up to 30 cm. After this size, fish become more sensitive to fishing gear, resulting in an increasing fishing related mortality. *C. marulius* in the size range of 40-50 cm was vulnerable to fishing

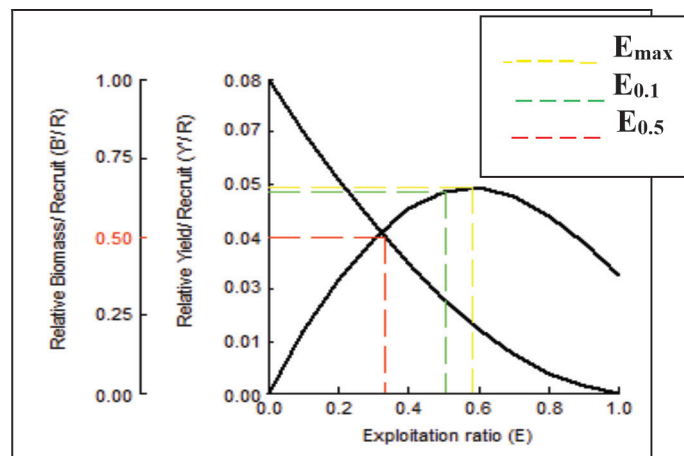


Figure 1 *C. marulius* stock structure using Beverton and Holt's Relative yield per recruit and biomass per model from River Sutlej



mortality. Maximum Y/R could be achieved at an exploitation ratio (E_{max}) of 0.545. In case of *C. marulius*, E_{cur} value (0.52) is greater than $E_{0.1}$ (0.463), thus no further increase in fishing efforts is suggested as the stock is at the verge of overexploitation. The levels of exploitation which will result in the decrease in B/R value of 50% compared to its virgin biomass ($E_{0.5}$) were calculated 0.332 in *C. marulius*. The yield isopleth diagram revealed that an L_c/L_∞ of 0.55 and an E of 0.5 provided the best relative yield per recruit. Finally it is suggested that existing fishing pressure should be reduced to restrict the growth overfishing and recruitment overfishing in *Channa* species in River Sutlej.

Table 1. Population dynamics parameters of *C. marulius* from River Sutlej

S.No.	Parameters	<i>C. marulius</i>
1.	Von Bertalanffy growth equation	$L_t=99.75$ $[1-e^{-0.88(t-0.02)}]$
2.	Asymptotic length (L_∞) (cm)	99.75
3.	Growth coefficient (K) ($year^{-1}$)	0.880
4.	t_0 (year)	0.02
5.	\emptyset	3.941
6.	t_{max} (year)	3.42
7.	Total mortality (Z) ($year^{-1}$)	2.46
8.	Natural mortality (M) ($year^{-1}$)	1.19
9.	Fishing mortality (F) ($year^{-1}$)	1.27
10.	Exploitation ratio (E)	0.52
11.	Exploitation rate (U)	0.38
12.	L_{25} (cm)	26.46
13.	L_{50} (cm)	33.96
14.	L_{75} (cm)	41.63
15.	E_{max}	0.545
16.	$E_{0.1}$	0.463
17.	$E_{0.5}$	0.332
18.	M/K	1.35

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6.19 Population Dynamics of Giant River Catfish, *Sperata seenghala* from River Sutlej in Punjab, India

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Keywords: Population Dynamics, Catfish, *Sperata seenghala*, River Sutlej

Introduction

The population dynamics of Giant river catfish, *Sperata seenghala* from river Sutlej in Punjab (India) was investigated at first time using length frequency data to assess the population parameters as well fishery status of the said species. *S. seenghala* is one of the major commercial fishes captured from river Sutlej with high consumer demand. Furthermore, its aquaculture technology has not yet been commercially developed and the demand for this catfish is entirely reliant on natural resources. In view of that, assessment of present stock status and level of exploitation of the said species is of utmost importance for its sustainable production. Population dynamics studies include the determination of growth and mortality parameters along with the exploitation rate of fishes (Sparre and Venema 1998). Estimation of these parameters leads to the prediction of fish stock assessment, which provides overview of the current state of a given fishery for management purposes to achieve the target of maximum sustainable yield. The present study was conducted to assess the fishery status of the *S. seenghala* from river Sutlej in Punjab in order to ensure the sustainable utilization of resources.

Materials and Methods

A total of about 500 fish specimens of *S. seenghala* were collected from landing centres / fish markets representing catch from selected stretches of river Sutlej in Punjab, covering upstream (near Ropar), midstream (Ludhiana) and downstream (Hariké pattan) courses. The length and weight data were collected on monthly basis from November 2020 to October 2021 and further subjected to analysis using ELEFAN-I method employing FiSAT (FAO-ICLARM) program. Population parameters *viz.* growth parameters (von Bertalanffy growth equation), asymptotic length (L_{∞}), growth co-efficient (K), Fishing mortality (F), Natural mortality (M), Total mortality (Z), Exploitation rate (Beverton and Holt 1957), Exploitation ratio, Probability of capture (L_c), Length-structured Virtual Population Analysis (VPA), Yield per Recruit and biomass per recruit, Yield isopleths diagram (Relative yield/Recruit Analysis knife-edge) were estimated with FiSAT software (Sparre and Venema 1998).

Results and Discussion

The growth parameters were estimated as asymptotic length (L_{∞} = 110.25 cm), growth coefficient / curvature parameter (K = 0.520 /year) and theoretical value of age at birth (t_{0} = -0.21) indicated optimum growth of fish. The value of growth performance index (ϕ' = 3) indicated suitable condition of stock in Sutlej ecosystem. The mortality coefficients for total mortality (Z), natural mortality (M) and fishing mortality (F) were estimated as 1.48 yr^{-1} , 0.81 yr^{-1} and 0.67 yr^{-1} , respectively. The virtual population analysis (VPA) based on pooled length-frequency data represented the highest fishing mortality (F) in the class range of 90-100 and 100-110 cm while least in the length class of 30-40 cm. The length at first capture (L_c) was recorded as 30.6 cm, while length at recruitment (L_r) as 35 cm.

The current exploitation ratio of *S. seenghala* from Sutlej ($E_{current}$ = 0.45) indicating optimum exploitation of stock when compared with standard value of exploitation rate (E = 0.5). The current exploitation ratio ($E_{current}$ = 0.45) is lower than E_{max} (0.501) while slightly higher than $E_{0.1}$ (0.421). The higher $E_{current}$ value (0.45) than more conservative yield concept ($E_{0.1}$ = 0.421) indicated that there is further no scope to increase fishing efforts for *S. seenghala* as it is probably approaching to full exploitation. Combination of technical measures including reduced fishing efforts, mesh size regulation, closed season along with awareness program for fishermen is needed for sustainable production of *S. seenghala* stock from Sutlej.



Table 1 Population parameters of *S. seenghala* from river Sutlej

Sr. No.	Population Parameters	Value
Growth Parameters		
1.	Asymptotic length (L_{∞}) in cm	110.25
2.	Growth coefficient (K), Gulland and Holt's Plot	0.52 year ⁻¹
3.	Theoretical value of age at birth (t_0)	-0.21
4.	Longevity of fish (t_{max})	5.55 years
5.	Growth performance index $\phi = \text{Log } K + 2 \text{ Log } L_{\infty}$	3.79
Mortality Parameters		
6.	Total mortality (Z) by Length converted catch curve method	1.48 year ⁻¹
7.	Natural mortality (M) by Pauly's empirical formula	0.81 year ⁻¹
8.	Fishing mortality (F=Z-M)	0.67 year ⁻¹
9.	$F_{opt} = 0.4 * M$	0.32 year ⁻¹
10.	$F_{limit} = 2/3 * M$	0.54 year ⁻¹
Exploitation Parameters		
11.	Length at first capture (L_c)	30.6 cm
12.	L_c/L_{∞}	0.27 cm
13.	M/K	1.55 year ⁻¹
14.	Exploitation ratio (E) = F/Z	0.45
15.	Exploitation rate (U)	0.34

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6.20 Mammalian Diversity Recorded along River Beas in Punjab, India

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Keywords: River Beas, Mammalian Diversity, Punjab

Introduction

The Beas River rises in the Himalayas in central Himachal Pradesh, India, and flows for some 470 kilometres before merging with the Sutlej River at Harike Wildlife Sanctuary, Amritsar in Punjab. The river flow through extensively cultivated landscape; vegetation composition along the river banks comprises of grasses, herbs with occasional presence of trees like *Azadirachta indica*, Eucalyptus etc. The habitat heterogeneity and a complex land-water association make a floodplain able to maintain disproportionately high biodiversity. In spite of its vital importance, the information on the mammalian biodiversity along river bank is incomplete. Keeping this in view, surveys were conducted from 2018-19 to 2020-21 to study mammalian diversity along River Beas under a project supported by Punjab Pollution Control Board.

Materials and Methods

This study was conducted from 2018-19 to 2020-21 during autumn and spring seasons. Mammalian diversity was studied at different sites along River Beas bank at Dhilwan of district Amritsar, Goindwal Sahib, Chamba kalan and Harike of district Tarntaran, Talwara and Tanda of district Hoshiarpur, and Alampur and Bhet of district Gurdaspur. Mammalian species seen were photographed and identified (Atwal et al 1984). A Cannon Powershot camera was used for the photography of animals along the river bank. Rodent species were identified on the basis of characteristic burrow entrances (Singla et al 2022)

Results and Discussion

The mammalian diversity was studied from 2018-19 to 2020-21 along River Beas. In 2018-19, different species of rodents, Wild Boar, blue bull, Cows and Dogs were spotted at Talwara, Dhilwan, Goindwal Sahib and Harike. Survey of wheat crop after sowing during this period on the bank of River Beas in villages of district Tarntaran revealed the presence of three rodent species i.e. *Bandicota bengalensis*, *Mus booduga* and *Millardia meltada*. Number of burrows ranged from 47.33 to 71.00/acre indicating high rodent population density. At pre-harvest stage, survey of wheat crop fields in districts Gurdaspur, Hoshiarpur, Amritsar and Tarntaran near river bank revealed the presence of four rodent species i.e. *B. bengalensis*, *T.indica*, *M. booduga* and *M. meltada*. However at this stage, rodent population density was medium with number of rodent burrows ranging from 5.67 ± 0.27 to 22.00 ± 4.32 /acre (Plates 1-2). Survey in paddy crop fields revealed very low rodent infestation. Number of burrows were only 4.67 ± 1.89 . Foot prints and damage of Blue bulls, wild boar and rabbits were also seen in villages of district Gurdaspur, Amritsar and Hoshiarpur near river Beas. In 2019-20, four rodent species (*B. bengalensis*, *M. booduga*, *M. meltada* and *T. indica*) and wildboar were recorded near river bank at different locations surveyed. In addition rhesus macaque, common Indian mongoose, Indian hare, palm squirrel were also recorded. In 2020-21, Survey of rodent diversity and damage in paddy fields near river bank and along river bank indicated presence of three rodent pest species. Estimation of their population density indicated low to high rodent infestation in these fields. Number of burrows were less in monsoon season. Wild boar was also recorded in villages near river bank of district Gurdaspur, Tarntaran and Amritsar. However blue bull was seen only near river bank of district Tarntaran. In addition Indian Jackal & Feral horses were also recorded (Table 1). These animals depend upon this habitat for feeding and breeding. Species diversity recorded along river Beas indicated good water quality to support biodiversity.



Table 1 List of Smaller Mammals recorded along River Beas

S. No.	Species	No. of individuals per sighting	Locations	IUCN Status	IWPA Status Schedule
1.	Palm Squirrel <i>Funambulus pennanti</i> Wroughton, 1905	2-5	Goindwal Sahib, Chamba kalan, Harike Bhet, Talwara, Tanda	Least Concern	IV
2.	Soft Furred field Rat <i>Rattus meltada</i> (Gray 1837)	2-14 burrows/acre	Dhilwan, Talwara, Tanda	Least Concern	IV
3	Indian Mole Rat <i>Bandicota bebgalensis</i> (Gray 1835)	7-29 burrows/acre	Dhilwan, Goindwal Sahib, Chamba kalan, Harike	Least Concern	IV
4	Indian Gerbil <i>Tatera indica</i> (Hardwicke 1807)	7-17 burrows/acre	Dhilwan, Bhet, Talwara, Tanda	Least Concern	V
5	Common Indian Field Mouse <i>Mus booduga</i> (Gray 1837)	4-11 burrows/acre	Goindwal Sahib, Dhilwan, Bhet	Least Concern	IV
6	Golden Jackal <i>Canis aureus</i> (Linnaeus, 1758)	1-2	Chamba kalan, Harike	Least Concern	II
7	Indian Grey Mongoose <i>Herpestes edwardsii</i> (E. Geoffroy Saint-Hilaire, 1818)	1-2	Goindwal Sahib, Chamba kalan, Harike Bhet, Talwara	Least Concern	II
8	Black Naped Hare <i>Lepus nigricollis</i> (F. Cuvier, 1823)	3-5	Goindwal Sahib, Dhilwan, Tanda	Least Concern	IV
9	Wild boar <i>Sus scrofa</i> (Linnaeus, 1758)	2	Dhilwan, Bhet, Tanda	Least Concern	III
10	Rhesus Macaque <i>Macaca radiata</i> (E. Geoffroy, 1812)	2-5	Talwara	Vulnerable	II
11	Nilgai <i>Boselaphus tragocamelus</i> (Pallas 1766)	4-10	Dhilwan,	Least Concern	III
12	Feral Horse <i>Equus</i> sp. Linnaeus 1758	4-6	Talwara, Harike	Near threatened	I

IUCN International Union for Conservation of Nature
IWPA Indian Wildlife Protection ACT 1972

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6.21 Length Weight Relationship (LWR) and Condition Factor (K) of Indian Major Carps Collected from Riverine and Domesticated Habitats

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Keywords: Indian Major carps, Allometric growth, Domesticated habitats, Condition factor

Introduction

The study of length-weight relationship (LWR) has an immense implication in fish biology studies as it helps to comprehend growth form, maturity, reproductive status and overall well-being of the fish. LWR can concurrently be used to assess intra species variation on the basis of body form of fish specimen collected from different habitats or varied geographical locations (Moutopoulos and Stergiou 2002). The growth rate of fish species vary as per their genetic makeup, availability of food and the existing ecological conditions. Under such conditions, the cube law is used as one of the important measure to determine the growth of fish. Present study examined the length-weight relationship and condition factor of Indian Major Carps collected from domesticated (private fish farms) and natural (River Sutlej) habitats of Punjab with an aim to see the effects of habitats, prevailing environment condition and availability of food on the well-being of the fishes.

Materials and Methods

The samples of fish were collected from four different sites i.e S-1: River Sutlej at Ropar Headworks, S-2: Harike Pattan at the meeting point of Sutlej and Beas, S-3: Fish ponds at College of Fisheries, GADVASU, Ludhiana and S-4: Fish ponds from nearby farms of district Ludhiana from November 2020 - September 2021. The fish samples (for biological studies) were collected in triplicate while as for morphometric and meristic studies, the sample size for *Labeo rohita* was 134, for *Catla catla* was 103 while as for *Cirrhinus mrigala* it was 119.

Length-weight relationship was calculated using linear regression analysis function of MS-excel.

Results and Discussion

The mean average length ranged from 32.9–51.54 cm, 35.69 to 63.40 cm and 35.86-59.90 cm with corresponding mean weight of 431.91-1926.74 g, 619.47-6569.30 g and 447.37-2679.70 g for *L. rohita*, *C. catla*, *C. mrigala*, respectively at the selected sites. During the present study, 'b' values ranged from 2.70-2.93, 3.02 -3.29, 2.52-2.88 with corresponding 'r' values as 0.90-0.94, 0.89-0.94 and 0.89-0.93 for *L.rohita*, *C.catla* and *C. mrigala*, respectively at the selected sites. *L. rohita* and *C. mrigala* individuals showed negative allometric growth at all the selected sites during the study as evident from values of $b < 3$ clearly indicating that fishes are becoming slimmer with increase in length i.e. the weight of fish is lower than cube of its length while as *C. catla* showed positive allometric growth indicating fish grow lighter or heavier, for a specific length as it grows in size. Similar findings have been reported by several others researchers like Ujjania et al (2012) who stated that negative allometric growth patterns are characterised by fish becoming slimmer with increase in length. The values of correlation coefficient depicted a strong positive correlation between length and weight indicating an increase in length with corresponding increase in weight.

The values of condition factor (K) ranged from 1.11 to 1.54 for *L. rohita*, 1.02-2.57 for *C. catla* and 0.97-1.31 for *C. mrigala*. K values greater than 1 reveal that the environment of the selected habitats were conducive for the growth and survival of the candidate fish species, however the domesticated fish species were getting more conducive environment as revealed by the condition factor value equalling near to 1.

Table 1 Length-weight relationship parameters and Condition factor (K) of Indian major Carps from two different habitats

Length-weight relationship parameters and Condition factor (K) of <i>L. rohita</i> from two different habitats				
Parameters	Natural population		Domesticated population	
	Site- 1	Site- 2	Site-3	Site-4
Mean length (cm)	44.5	51.54	32.9	33.8
Mean weight (g)	1361.75	1926.74	394.7	431.91
Growth coefficient 'b'	2.70	2.81	2.93	2.75
Correlation coefficient 'r'	0.91	0.94	0.92	0.90
Condition factor 'K'	1.54	1.40	1.11	1.11



Growth type	Negative allometric growth	Negative allometric growth	Negative allometric growth	Negative allometric growth
Length-weight relationship parameters and Condition factor (K) of <i>C. catla</i> from two different habitats				
Mean length (cm)	60.94	63.40	40.79	35.69
Mean weight (g)	5265.6	6569.30	692.70	619.47
Growth coefficient 'b'	3.14	3.02	3.12	3.29
Correlation coefficient 'r'	0.94	0.93	0.92	0.89
Condition factor 'K'	2.32	2.57	1.02	1.36
Growth type	Positive allometric growth	Positive allometric growth	Positive allometric growth	Positive allometric growth
Length-weight relationship parameters and Condition factor (K) of <i>C. mrigala</i> from two different habitats				
Mean length (cm)	57.95	59.90	37.95	35.86
Mean weight (g)	2552.08	2679.70	580.85	447.37
Growth coefficient 'b'	2.88	2.79	2.64	2.52
Correlation coefficient 'r'	0.93	0.89	0.92	0.91
Condition factor 'K'	1.31	1.24	1.06	0.97
Growth type	Negative allometric growth	Negative allometric growth	Negative allometric growth	Negative allometric growth

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6.22 Reproductive Traits of a Threatened Loach, *Triplophysa kashmirensis* (Hora, 1922) in Kashmir Himalaya

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Keywords: *Triplophysa kashmirensis*, GSI, Fecundity, Sex ratio

In this study, a total of 180 specimens of *Triplophysa kashmirensis* were studied. The total length of specimens varied from 54.85 mm to 130.17 mm for the weights 2.21 g and 21.03 g respectively. Maximum GSI (gonadosomatic index) value was demonstrated in the month of November with the peak value of 1.23 in males and 20.67 in females. The absolute fecundity varied from 561 to 11,386 eggs and relative fecundity from 914 to 1441 eggs per gram body weight. The average absolute fecundity recorded was $3,851.61 \pm 266.61$ whereas average relative fecundity was 418.45 ± 19.25 per gram body weight. The resultant average sex ratio, male: female was 1:0.8, indicating a significant dominance of males. The length at first maturity (L_{50}) for females of *Triplophysa kashmirensis* was estimated at 86 mm.

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6.23 A Study on Morphological and Anatomical Structure of Gills in *Schizothorax esocinus* (Heckel 1838) from Dal Lake, Kashmir

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Keywords: *Schizothorax esocinus*, Gill arch, Gill filament, Gill raker, Histology

The present work was conducted to study the anatomical structure and variation in gill components with respect to total body weight of the fish. A total of 60 samples of *Schizothorax esocinus* were collected from landing centres around the Dal Lake ranging in body weight from 76.50 g to 427.00 g with a mean weight of 198.66 g. The gill arch length ranged from 70.11 mm to 143.50 mm (mean = 99.10 mm). The total gill filament length ranged from 5651.62 mm to 13284.94 mm (mean = 9238.66 mm) while as the total gill filament number ranged from 1346 to 1928 (mean = 1643.59). The total gill raker length in the fish ranged from 30.22 mm to 70.38 mm (mean = 50.09 mm) while as the total gill raker number ranged from 102 to 183 (mean = 128.17). A significant positive relationship was reported between the total body weight of the fish and gill arch length, total gill raker length, total gill filament length and number ($p < 0.01$). However, a non significant weak relationship was observed between total gill raker number and total weight of the fish ($p > 0.05$). Histologically, gill arch constituted the cartilaginous core structure, supporting two rows of gill filaments. These filaments were lined by epithelial layer which extended to cover the secondary lamellae forming the main sites for gaseous exchange. The gill rakers were observed to be short, rounded and widely spaced covered with epithelial layer interspersed with numerous mucus cells and taste buds.

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6.24 Geospatial Mapping of High-altitudinal Lakes of Arunachal Pradesh for Coldwater Fisheries Development

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Keywords: Geospatial technology, Coldwater, High-altitudinal lakes, Analytical Hierarchy Process

The incorporation of geospatial technology with multi criteria decision-making method using Analytical Hierarchy Process (AHP) in this study has made it possible for the identification of the potential sites in the selected lakes (Sela, Nagula, and Panga Teng Tso) for the development of coldwater fisheries in the district of Tawang, Arunachal Pradesh (India). The highly suitable areas of 6.24 ha (33.50 %), 1.14 ha (33.26 %), and 2.19 ha (26.89 %); moderately suitable areas of 1.45 ha (7.76 %), 0.63 ha (18.53 %), and 5.61 ha (68.95 %) were recorded for Sela, Nagula, and Panga Teng Tso, respectively. The physico-chemical parameters, i.e., temperature ($5.3\pm 0.1^{\circ}\text{C}$ to $8.2\pm 0.05^{\circ}\text{C}$), electrical conductivity ($3.1\pm 0.00\ \mu\text{S}/\text{cm}$ to $52.35\pm 2.35\ \mu\text{S}/\text{cm}$), total dissolved solids ($2.0\pm 0.0\ \text{mg}/\text{L}$ to $33.5\pm 1.5\ \text{mg}/\text{L}$), dissolved oxygen ($6.77\pm 0.01\ \text{mg}/\text{L}$ to $8.05\pm 0.15\ \text{mg}/\text{L}$), carbon dioxide ($2.09\pm 0.01\ \text{mg}/\text{L}$ to $2.50\pm 0.09\ \text{mg}/\text{L}$), pH (6.6 ± 0.10 to 7.3 ± 0.10), phosphate ($0.010\pm 0.000\ \text{mg}/\text{L}$ to $0.270\pm 0.010\ \text{mg}/\text{L}$), nitrite ($0.0025\pm 0.0005\ \text{mg}/\text{L}$ to $0.0085\pm 0.0005\ \text{mg}/\text{L}$), iron ($0.07\pm 0.020\ \text{mg}/\text{L}$ to $0.16\pm 0.005\ \text{mg}/\text{L}$) and ammonium ($0.011\pm 0.001\ \text{mg}/\text{L}$ to $0.020\pm 0.001\ \text{mg}/\text{L}$) were found to be quite conducive for coldwater fisheries development in the selected lake resources. The biological parameters, i.e., phytoplankton and zooplankton numbers were comparatively greater in the highly and moderately suitable areas than that in the unsuitable areas of the lakes. Overall, it was found that coldwater and sports fisheries development can be taken up in the selected lake resources of the district. Therefore, the assessment of such remote lake resources and the application of scientific management methods for exploitation will not only increase the prospects of establishing nutritional security for the local inhabitants of the district but will also increase the potential for sports fishery development. It shall, in turn, promote eco-tourism in the state and will also pave the way towards employment generation.

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6.25 Morphometric and Meristic Variation in the Churru Snow Trout *Schizothorax esocinus* from River Jhelum (Kashmir, India) using Truss Network System

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Keywords: Churru Snow Trout, Meristics, Morphometrics, Truss, Jhelum

Landmark-based morphometrics were examined to evaluate the population status of Churru snow trout, collected from three locations of the river Jhelum in Kashmir Valley, India. Morphometric characters along with truss network measurements and meristic counts were applied. Thirteen conventional morphometric and six meristic traits were analysed along with thirty truss measurements. Significant variability was observed in four out of six meristic characters examined. These were lateral line scales, pectoral fin rays, pelvic fin rays and anal fin rays. Out of thirteen characters, twelve morphometric traits significantly differed to varying degrees. These were total length, standard length, pre-dorsal length, pre-pelvic length, pre-pectoral length, snout length, head length, caudal fin length, eye diameter, pre-anal length, pre-orbital length, post-orbital length. Principal component analysis of 30 truss measurements extracted from three factors with Eigen values > 1 , explained 88.83% of the total variance. The cross validation of this analysis revealed that the percentage of correctly classified fishes was 70.2% from all three sites. It was concluded that all the landmark of truss measurements significantly contributed to the body shape of the fish.

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6.26 Water Quality of Dachigam Stream : A Major Inflow of Dal Lake, Kashmir, J&K, India

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Keywords: Water quality, Stream, Nitrates, Phosphates, Anthropogenic activities

Stream water is one of the most important freshwater resources available for human consumption among various water sources. The present study was carried to assess the water chemistry of Dachigam stream situated in the district Srinagar of Kashmir valley. The Dachigam stream and a host of other streams draining the bordering mountain slopes constitute the drainage network of Dachigam nallah. The stream originates from the lake Marsar (5849 m) and the surrounding snow – bound mountain a feeding it during summer. All along its course the stream is joined by a number of tributaries of a perennial – non perennial origin. The water samples from four different locations covering whole stream were collected for a period of six months covering two seasons viz., winter and spring. The season wise mean values recorded for various physico-chemical parameters were recorded. The maximum to minimum concentration range of water quality parameters such as water temperature: 4.5-16.9°C, Depth: 0.17-1.07m, pH: 6.9-8.1, EC: 120-366 µS/cm, D.O: 5.0-11.0 mg/L, free CO₂: 4.8-21.5 mg/L, chloride: 8-43 mg/L, Ammonical- nitrogen: 13-281.6 µg/L, nitrate: 89.6-477 µg/L, total phosphorus: 113 - 930.7 µg/L was recorded during the investigation. It was concluded that the overall water quality of Dachigam stream falls under good category, however the quality of its water showed gradual decline moving downstream. This gradual deterioration of water quality and an increase in the nutrient concentration was attributed to the increased human inhabitation and interference down the stream especially at the site 3 where locals use the stream water for washing clothes and dispose off domestic wastes which resulted in the high organic matter and high nutrient concentration.

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6.27 Studies on Morphology and Food and Feeding Habits of *Triplophysa kashmiriensis* Hora (1922) in Kashmir Himalaya

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Keywords: *Triplophysa kashmiriensis*, Morphometry, Meristic, Length-Weight relationship, Gut content analysis

Study on the morphometry, meristic characters, length-weight relationship and food & feeding habits of *Triplophysa kashmiriensis* (Ara gurun) Hora (1922) was carried out from September 2018 to February 2019. A total of 180 samples were collected from Dagwan and Lidder streams for this study. Various morphometric characters showed high degree of correlation (R^2) between them and the values of correlation ranged from 0.6998 to 0.9934. Coefficient of variation for various morphometric characteristics was found to be 20.95 % between snout length and total length, 17.65 % between eye diameter length and total length. The fin formula of the fish was found as D(I)8; A(I)5; P1(10-11); P2(7-8); C(17-18). The length-weight relationship was recorded separately for both males and females. The equations found were $\text{Log } W = -5.485 + 3.167 \text{ Log } L$ for males and $\text{Log } W = -5.440 + 3.153 \text{ Log } L$ for females and the combined equation was obtained as $W = -5.491 + 3.174 \log L$. The value of 'b' obtained for the pooled data was found to be 3.174 which is significantly greater than 3 indicating a positive allometric growth in the fish.

Gut contents of *T. kashmiriensis* were analyzed both by occurrence method and volumetric method. On an average, the dominant food items were found to be insects and their larvae followed by their appendages. Analysis of gut contents following the Index of Preponderance method revealed the presence of high percentage of insect and their larvae (24.23%), appendages (20.76%), crustaceans (13.27%) and Diatoms (11.99%) in the diet of fish confirming its carnivorous mode of feeding. Monthly average GaSI was recorded highest in the month of February and lowest in the month of November and the values were 4.28% and 1.37% respectively. Average RLG value was recorded at 0.85 indicating carnivore feeding behaviour of the fish. Feeding intensity indicated that the length-wise feeding was highest in the fishes of length group of 110-130mm; Month-wise feeding intensity of *T. kashmiriensis* was highest in the month of February and lowest in the month of November.

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6.28 Length-Weight Relationship and Morphometrics of an Endemic Snow Trout *Schizopyge niger* (Heckel, 1838) from Dal Lake, Kashmir

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Keywords: *Schizopyge niger*, Length-weight relationship, Morphometry, Dal lake, Kashmir

The study was aimed to investigate the relationship between various morphometric measurements and to establish length-weight relationship of *Schizopyge niger* collected from Dal lake, Kashmir. *S. niger*, locally known as Ael gad, forms an important coldwater fishery resource of the valley. Length-weight relationship (LWR) is one of the standard methods that yield authentic biological information and is of great importance in fishery assessments. A total of 80 fish samples of *S. niger* ranging from 188-388 mm in total length and 61-305 g in total weight were collected for this study. The length-weight relationship was established logarithmically as $\text{Log } W = -3.2131 + 2.2618 \text{Log } L$ ($R^2 = 0.8117$). The b value was estimated at 2.2618 indicating negative allometric growth pattern for *S. niger*. Furthermore, seventeen morphometric characters, analysed in the present study, showed significant correlations across the variables with R^2 values ranging from 0.3466 to 0.925 indicating that the characters were highly correlated to each other. The highest correlation was observed between SL and TL ($R^2=0.925$) followed by PAL and PPvL with TL ($R^2= 0.8833$, 0.8781, respectively) and lowest correlation value was found between ED and HL ($R^2 = 0.3466$).

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6.29 Meristic Variation in Two Congeneric Snow Trout Fishes (*Schizothorax niger* and *S. curvifrons*) from Dal Lake, Kashmir, India

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Keywords: *Schizothorax*, Meristics, PCA, Kashmir, Dal lake

A simple yet useful criterion based on meristics is currently used to differentiate two congeneric snow trout fish species *Schizothorax niger* and *Schizothorax curvifrons* from Dal lake, Kashmir, India. Principal Component Analysis and test of significance were used to investigate meristic differences between these two species. The purpose of this study was to appropriately identify the two species correctly and assign them to distinct genetic groups. Eleven meristic traits (dorsal fin spine, dorsal fin rays, pectoral fin spine, pectoral fin ray, ventral fin spine, ventral fin ray, anal fin spine, anal fin ray, caudal fin rays, lateral line scales, number of gill rakers externally on the first gill arch) were studied in 180 fish specimens. The test of significance of meristic traits among *S. niger* and *S. curvifrons* revealed that meristic character namely dorsal fin rays, ventral fin rays, lateral line scales, number of gill rakers externally on the first gill arch were significantly different ($p < 0.05$) between *S. niger* and *S. curvifrons*. PCA revealed that the first three components together explained 60.51% of the total variance. The meristic characters were also counted to draw the fin formula of these fish species. This study provides useful information to support taxonomic identification based on meristic counts.

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6.30 Studies on Macrophytic Diversity and Water Quality Parameters of Waskura Lake, Kashmir

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Keywords: Waskura lake, Macrophytic diversity, Water quality, Density, Fresh water.

The present study was carried out on Waskura lake, a fresh water rural lake in the state of Kashmir. The study was carried for a period of one year (2017-2018) in order to ascertain vegetation composition of Waskura lake, Kashmir. Monthly surveys were conducted to carry eight different quantitative characteristic viz., number of individuals of macrophytes, frequency, density, abundance, relative frequency, relative density, relative abundance and importance value Index (IVI). Besides this, water quality assessment of few parameters (Water temperature, depth, transparency, dissolved oxygen, pH, free carbon di-oxide and total alkalinity) was also carried on seasonal basis. During the present study total of 13 aquatic macrophytes belonging to 12 different families were found distributed in Waskura Lake. Out of 13 species recorded, *Potamogetonaceae* family has shown the presence of maximum number of species (2), while all other families recorded the presence of only single species. The aquatic macrophytes found in the lake were categorized into three sub-categories viz., (a) Submerged (*Chara* spp., *Ceratophyllum demersum*, *Hydrilla verticillata*, *Utricularia flexouosa*, *Potamogeton lucens* and *Myriophyllum spicatum*) (b) Rooted with floating leaves (*Nymphaea alba*, *Nymphoides peltatum*, *Nelumbo nucifera*, *Trapa natans* and *Potamogeton natans*) and (c) Emergent (*Phragmites communis* and *Typha angustata*). No macrophytic species were found at site 6 (central site) of the lake throughout the year. ANOVA test showed that there was a significant variation ($p < 0.01$) among the different sites of the lake. Correlation coefficient was carried out between lake parameters and macrophytes. A significant positive correlation (+0.468) was recorded between water depth and rooted floating macrophytes. However, significant negative correlations (-0.712) was recorded between water depth and emergent macrophytes. The present study revealed that the diversity of aquatic macrophytes distribution, depend upon the physico-chemical parameters of the water and environment factors. The results clearly depicted that the study area was mesotrophic and moving towards eutrophication, therefore, the present work was aimed to evaluate the study area on the basis of various community features of macrophytes.

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6.31 Evaluation of the Stress of Environmentally Relevant Concentration of Low Density Polyethylene Microplastic in Brain of *Cirrhinus mrigala*

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Keywords: Microplastic, Brain, *Cirrhinus mrigala*, Comet assay, Nucleo-cellular abnormality assay

Omnipresence of microplastics (size<5mm) in the aquatic ecosystems and their accumulation in diverse organisms has caused serious vandalization of its flora and fauna. Low-density polyethylene is one of the prominent contenders of all other types of microplastics found in the water bodies. Present study deals with the determination of behavioural alterations and genotoxic effect (comet assay and nucleo-cellular abnormality assay) in the brain of a food fish, *Cirrhinus mrigala* (70-80g,15-17cm) after 30 days of exposure to the environmentally relevant concentration of low density polyethylene microplastic {50 µg/L, size : 150-250µm [MP₁] and <150µm [MP₂]}. During the course of exposure, MP₂ fishes showed highest pigmentation, scale-less patches, gulping, banging in the aquarium walls, hyperactivity, restless jerky swimming and very frequent movement of operculum compared to MP₁ and control fish. Comet assay showed significant ($p \leq 0.01$) increase over control in tail length, tail DNA, tail moment, olive tail moment of exposed fish but it was higher in MP₂ group. The order of increase in parameters was, tail length > tail DNA > tail moment > olive tail moment. Nucleo-cellular abnormalities were significantly ($p \leq 0.01$) more in the exposed groups than control, however, these abnormalities were more in the MP₁ group. In exposed groups, nucleoids were maximum (more in MP2 group than MP1 group). The results reflect that the environmentally relevant concentration of low density polyethylene microplastic causes degenerative changes in the brain of *C. mrigala* that may become the underlying cause of morbidity and mortality of fish in natural water bodies. The study holds importance as the pollution due to microplastics is extensive but there is hardly any report regarding genotoxicity of polyethylene in freshwater food fishes.

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6.32 Aquatic Ecosystems: Conservation, Restoration and Management

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Keywords: Ecosystem, Conservation, Water resources, Management

Aquatic ecosystems are live communities of organisms that are interdependent and dependent on their environment. The two main types of aquatic ecosystems are marine ecosystems and freshwater ecosystems. Aquatic ecosystems are extremely diverse. This includes lakes, ponds, rivers and streams with varying depths, flow rates and water chemistries. Aquatic ecosystems include wetlands where water is just below or just above the soil surface. The depth and distribution of this water can change with the seasons. Aquatic ecosystems perform many valuable environmental functions. They recycle nutrients, purify water, regenerate groundwater, and provide habitat for a variety of flora and fauna and recreation for people. Rapid population growth, accompanied by unplanned developmental work, has resulted in pollution of surface waters by urban, agricultural, commercial and industrial runoff and a reduction in the number of water bodies. The increasing demand for drainage from wetlands is being met by canalisation, leading to further loss of river habitat, increasing numbers of extinct or endangered aquatic life, and the loss of much of the water for drinking, swimming, fishing, etc. Various human activities have altered the physical, chemical, and biological processes of aquatic ecosystems. Halting further degradation of these fragile ecosystems requires an integrated and accelerated effort to restore and protect the environment. Failure to restore these ecosystems will later result in significantly increased environmental costs, extinction of species and ecosystem types, and permanent damage to ecosystems. The concept of ecosystem services connects people and nature and can provide a valuable approach for advocating for the conservation and restoration of natural ecosystems. Despite the growing interest in this topic, the application of these concepts to water resource management has been hampered by the lack of practical definitions and methods.

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6.33 Geo-Spatial Tools in Fisheries Resource Management

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Keywords: GIS, Remote sensing, Water resources, Reservoirs

In the age of information and technological advancements, location awareness is popping into a key feature in management of natural resources. Geospatial mapping is a location-based study that's anticipated to be an excellent tool for fisheries scientists, aquatic resource managers and policy planners in developing and coming up with ways for management of fisheries resources. Remote sensing and geographic information systems (GIS) play a key role altogether in geospatial aspects of the assessment and management of water resources for inland fisheries and cultivation. Lake, wetland, and reservoirs are variety of the inland water resources for potential fish production which will facilitate in improving the agricultural economy on large scale. Water resource assessment like unfold area, water quality, nutrient analysis, pigment content, and different chemical properties can be done through the mapping. Remote sensing and GIS code and footage like Landsat-ETM+, Landsat-8 and totally different multi spectral footage are utilized at short timeframe for potential fishing zone identification, land cowl, erosion and accretion in reservoirs thus it facilitate in deciding and blueprint preparation for management at an inexpensive rate. Further, the applications of geospatial tools are well acknowledged in data-scarce regions and inside the sector of natural resources management. The remote sensing (RS) and Geographical system (GIS) can be used for characterization of inland water bodies, viz., inventory, observation of water unfolds and quality, surroundings risk, mapping, prediction. Incorporation of field exploration information with landscape information derived from geospatial tools enhance our understanding on aquatic habitats. The demonstration of RS and GIS applications at an even bigger landform scale in inland open water bodies is very important due to its potential for science-based management of inland aquatic habitats for the conservation of fish genetic resources. The current review focuses on the prospects and usage of geospatial tools to analyze and study all the parameters that may be used in fisheries resource management.

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6.34 Assessment of the Water Quality of Selected Religious Ponds in Kurukshetra District with Reference to Suitability for Fish Culture and Conservation

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Keywords: Fish production, Physico-chemical parameters, Pond management, Water quality

The optimum fish production and conservation is totally dependent on the physical, chemical and biological qualities of water to most of the extent. Adverse changes in water quality of aquatic ecosystems are reflected in the biotic things mostly in fish species. Hence, successful pond management requires an understanding of water quality. The present study was conducted to assess the suitability of certain water bodies of Kurukshetra, Haryana for fish culture/ stocking (for conservation). The selected sites include the ponds in Kurukshetra district with religious activity viz Baan Ganga, Braham Sarovar, Sannhit Sarovar, Jyoti Sarovar, Sunhedi Pond. Although fish culture practises are not common in these ponds yet these waters can be used for conserving/stocking specific fish species. The obtained values of physical and chemical parameters of water samples were compared to the desirable, permissible and acceptable ranges for pisciculture recommended standards. The results of the comparative analysis of water quality showed that most of the water quality parameters (temperature, pH, DO, BOD, COD, alkalinity, chloride, hardness, calcium, magnesium, ammonia, orthophosphate) were suitable for fish culture however some parameters such as pH, hardness, TDS, TSS, COD were found inappropriate in a sampling site (Sunhedi Pond) for fish culture. This shows these ponds with religious activity have suitable water quality can be used for conservation the specific fish species as netting is not allowed in these religious ponds for fish culture.

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6.35 Evaluation and Quantification of Phytoplankton Diversity of Ottu Reservoir in Haryana

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Keywords: Ottu reservoir, Phytoplankton, Shannon – Weaver diversity index

The study was carried out in Ottu reservoir situated in Sirsa city of Haryana, India. Evaluation and quantification of phytoplankton of Ottu reservoir were monitored from March to June, 2021. Total 19 genera of phytoplankton belongs to class Bacillariophyceae (3 genera), Cyanophyceae (3 genera), Chlorophyceae (11 genera), Euglenophyceae (2 genera) were observed. In various months -highest 123 species of phytoplankton were observed in site 2 and site 4 and Chlorophyceae found as dominant group. The highest quantity of phytoplankton was observed in site2 while the minimum quantity was observed in site 3. Shannon and Weaver's diversity index for phytoplankton found maximum in site 1 (2.78) and minimum in site 4 (2.05). In site 1 and site 2 significant difference ($P < 0.05$) was observed for the phytoplanktons groups present every month from March to June. However, a non-significant difference was observed in site 3 and site 4.

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6.36 Comparative Growth Evaluation of Different Catfish and Carps under Treated Sewage Water

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Keywords: Growth, Catfishes, Carps, Treated sewage water, Water quality

Environmentalists and aquaculturists have concerns about the disturbance in ecosystem and biodiversity. In inland water resources, pond ecosystem and biodiversity changes as per hydrochemical properties of natural resources. India will likely face a severe shortage in freshwater resources in the foreseeable future. Based on growth parameters, the suitability of treated sewage water to grow different catfish and carps was determined under this experiment. Four different types of fish species; advance fingerlings of *Pangasianodon hypophthalmus*, *Catla catla*, fry of *Cyprinus carpio haematopterus* and fingerlings of *Clarias batrachus* were cultured under sewage treated water and freshwater. The results of the study revealed that the weight of catfishes (*P. hypophthalmus* and *C. batrachus*) reared in sewage treated water showed significant increment from 3.64 and 1.86 g to 9.71 and 6.21 g within 30 days of experimental period. Similarly, length of fish increased to 11.85 and 8.71 cm from 8.17 and 6.46 cm, respectively. Because sewage treated water provides essential nutrients, organic matter, microorganisms that assure the growth of living and non-living food organisms for the better growth and survival of aquatic animals under aquatic environment, even in the absence of supplementary feeding. However, carps (*C. carpio haematopterus* and *C. catla*) were not able to grow well in the sewage treated water under *in vitro* conditions. The weight and length of both the fishes declined within 30 days. Additionally, the fry of *C. carpio haematopterus* released in sewage treated water was not able to survive in spite of feeding as the mortality (50%) recorded at 15th day increased to 100 percent at 30th day. Moreover, the growth (weight, length) of catfishes showed significant positive correlation with various water quality parameters as compared to carps.

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6.37 Agrichemical Induced Oxidative Damage in Kidney of Freshwater Fish *Cyprinus carpio* (Linnaeus, 1758)

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Keywords: Pesticide, Fish, Tebuconazole, Oxidative stress, Antioxidant system

The pollution leads to a steady decline in the aquatic flora and fauna, particularly fishes. Pesticides usage in the world has significantly increased over the past two decades. Pesticides disrupt the ecological balance by becoming the most common contaminant of agricultural lands, underground waters, and surface waters, and exert adverse effects on natural ecosystems. Pesticides generally exert a toxic effect on nontarget organisms, especially fish. Pesticides can have an effects on the biochemical functions of living organisms. Tebuconazole is a broad-spectrum conazole fungicide that has been used in agriculture in the control of foliar and soil-borne diseases of many crops. The present study has investigated the adverse effects of sub-chronic exposure to fungicide tebuconazole on the kidney of common carp *Cyprinus carpio*. After acclimatization for 15 days, the fish were divided into three groups. Group I were designated as control group II and group III were exposed to increasing concentration of azole fungicide tebuconazole (6.47 and 8.29 μ l/l) for 10, 20 and 30 days. A commercial formulation of azole fungicide tebuconazole 25.9% ec used. Oxidative stress induced due to exposure to tebuconazole were evaluated by activity of superoxide dismutase (SOD), catalase (CAT), and protein content, as well as levels of thiobarbituric acid reactive substances (TBARS) in kidney of common carp. Changes were observed in SOD and CAT activity in kidney with increasing exposure time and increasing concentration. Also rise in TBARS level observed. While the decrease in protein content were analyzed. Maximum rise in TBARS level were noticed in the group exposed to higher concentration of tebuconazole. The observed effects on common carp antioxidant systems may be a defense against oxidative damage. In conclusion, the study indicates that azole fungicide tebuconazole exposure adversely affected the health of the fish, attributable to oxidative stress.

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6.38 First Record of Life History Traits of *Puntius terio* (Hamilton 1822) and *Amblypharyngodon mola* (Hamilton 1822) from Indian Waters: The Tributary of Ganga River-Burhi Gandak

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Keywords: *Amblypharyngodon mola*, Burhi Gandak river, Growth, Mortality, *Puntius terio*

In the present study life history traits of *Puntius terio* (Hamilton 1822) and *Amblypharyngodon mola* (Hamilton 1822) was estimated for the first time from the Indian waters. Monthly fish sampling was performed in selected stretches of Burhi Gandak river from June 2019-March 2020 to collect the length frequency and length weight data. A total of 794 samples of *P. terio* with length ranging from 1.2 to 9.8 cm and 1015 specimens of *A. mola* with length ranging from 2.3 to 8.1 cm were observed for the further analysis. The calculated von Bertalanffy growth parameters, asymptotic length (L_{∞}) and growth coefficient (K) values for *P. terio* were 9.77 cm and 0.87 and for *A. mola* the values were 8.72 cm and 1.4 respectively. The total instantaneous mortality co-efficient (Z) of *P. terio* and *A. mola* was 2.62 and 4.12 respectively. Fishing mortality was relatively lower than the natural mortality and it was estimated as 0.37 for *P. terio* and 0.95 for *A. mola*. The higher value of exploitation rate was estimated for *P. terio* (0.47) followed by *A. mola* (0.23). The estimated value of exploitation indicated that these species were under exploited in Burhi Gandak River. Hence worth, the exploitation rate should be increased to reach MSY.

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6.39 Assessing Polychaetes Diversity and its Ecological Importance in Patalganga Estuary, Maharashtra, India

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Keywords: Patalganga Estuary, Maharashtra, Polychaetes, Biodiversity, Ecology

Polychaetes being the most dominant groups in benthic infaunal communities plays significant roles in the trophic system as they exploit all forms of food available in the sediment and form an important link in the energy transfer. Estuaries are considered the nutrient trap among aquatic ecosystems. Patalganga estuary is considered one of the most productive ecosystems in Maharashtra. Polychaete diversity in the Patalganga estuary was studied and 22 species of polychaetes belonging to 19 genera, and 13 families were recorded. Dominant families in the ecosystem were Spionidae, Magelonidae, and Nephtyidae with 5 and 3 species, respectively. It was observed that post-monsoon and winter seasons were suitable for most of the species in the ecosystem. During the pre-monsoon and monsoon periods, the population density of polychaetes decreased. The estuarine mouth was richest with polychaetes (11 ± 10 no./m²), the sediment in the estuarine mouth might be suitable for the polychaetes to settle and grow. Polychaetes showed a strong positive correlation to salinity ($R = 0.502$) in the area. The riverine side of the estuary had the lowest density (8 ± 6 no./m²) where the pollution load and the organic content in the bottom were less compared to the estuarine mouth. Polychaetes are used for biomonitoring programs in order to check the health of the water body. Here in the estuarine ecosystem, *Prionospio spp.* observed indicated the ecosystem is frequently encountered with organic enrichment and the ecosystem is considered healthy. Biodiversity indices like Simpson's index/ Dominance (D), Simpson's diversity index (1-D), Shannon (H') index, and Evenness (J') index were observed at 0.072, 0.928, 2.636, and 0.997 respectively indicated that the ecosystem is healthy and productive. The main aim of the work is to develop a database about the diversity of the region and this will help the policymakers to do necessary actions for the management of the ecosystem.

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6.40 Remote Sensing and GIS in Fisheries Management and Conservation

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Keywords: Aquaculture, Fisheries, GIS, Remote sensing

Geographic Information Systems “GIS” and Remote Sensing (RS) technologies are examples of existing technologies that can be used to improve planning and management of fisheries and aquaculture. By applying these technologies and appropriate approaches; strategies and management plans can be developed for sustainable development of fisheries resources and environmental facilities to achieve short and long term goals. Aquaculture is a highly diverse activity in terms of the species grown, the habitats in which they are reared, the geographical distribution of species, the techniques used and the socio-economic environment in which they are reared. GIS is a tool for creating and analyzing spatial data, primarily concerned with the location of features and their properties and attributes. It helps to collect, analyze and visualize geographic data for a variety of uses. GIS quantifies the location of features by taking coordinates which describe the locations on the earth. Users primarily leverage sensor capabilities developed for a wide range of applications such as land mapping, weather forecasting, and oceanography to support fisheries and aquaculture management. Geographic Information Systems (GIS) is an information technology that provides the ability to collect, analyze and visualize data from various sources. GIS enables users to create spatial databases using features such as roads or boundaries to identify valuable industrial and natural resources that are located in specific areas. The technique helps to identify locations for aquaculture projects and resource management areas for fisheries. The most common applications of remote sensing in fisheries and aquaculture are to support aquaculture development, practices and management, including impact assessment, and to monitor and manage various aspects of marine fisheries.

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6.41 Socio-Economic and Marketing Constraints Faced by Traditional Fishers of River Jhelum, India

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Keywords: Constraints, Traditional fishers, Decline, Pollution, River Jhelum

Since time immemorial, rivers have been a vital source of fish food to inland traditional fishers. These fishers have been performing subsistence fishing activities for their survival. However, industrialization, urbanization, uncontrolled use of agricultural pesticides and fertilizers and other developmental activities like sand extraction from the river has caused a drastic decline in the Jhelum river fish fauna. The ultimate effect of which can be observed in the traditional river Jhelum fishers. There has been a continuous decline in fish catch and an increase in the fishing effort over the years. The marginalization of these traditional river Jhelum fishers has pushed them to a corner, where they feel nearly impossible to go head-on-head with the other agricultural enterprises. Although the traditional and small-scale fisheries contribute more than 90% of the fish production and value chain-related activities, their role in the fisheries sector is being ignored or neglected. There have been very few studies on the problems faced by riverine traditional fishers across the globe. Keeping this in view, the present investigation was carried out to identify and analyze the constraints faced by traditional fishers of river Jhelum, Kashmir. The river flows through six districts of the Kashmir valley *viz*: Anantnag, Pulwama, Srinagar, Ganderbal, Bandipora and Baramulla covering a total length of 165 kilometres in the region. A total of more than 6000 fishers are directly dependent on capture fisheries activity. The proportional multi-stage stratified sampling procedure was followed to collect data from 200 fishers across all the districts through which the river Jhelum flows. For constraint analysis, Garrett ranking method was used. Fishers were asked to rank the constraints from 1 to 15, in which rank 1 indicates the most important constraint and rank 15 implies the least felt constraint. The most important constraint that the fishers faced is a drastic decline in fish catch from the last two decades (83%), followed by pollution in the river (73%). The pollution has occurred due to many developmental activities in the region. High cost of raw materials and equal disbursement of schemes occupy the 3rd to 6th rank. For that, the institution can play a key role to relieve the fishers from it. The seventh highest constraint faced by traditional fishers is the lack of marketing facilities (53%). There is a need to provide a proper marketing place in each district where the fishers would sell their catch with proper hygiene and recognition. The department of fisheries can provide a pivotal role in augmenting their monthly income by providing them with proper training on post-harvest and related activities. Besides, all the multiple stakeholders need to come under one frame to sustainably and holistically manage the inland water resources.

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6.42 Morphometric Assessment of *Cyprinus carpio* var. *communis* from Anchar Lake, Kashmir

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Keywords: Anchar lake, Common carp, Coefficient of determination, Morphometry

The environmental changes in the habitats of the fish due to anthropogenic activities results in pollution of the aquatic environment which results morphometric characters to respond to changes in environmental factors. Morphometric study has been proven as useful tool for knowing about fish species, population and races. It is a basic fundamental tool for knowing about development of organisms, systematic, growth, variation and structure thus playing an important role to determine relationship between various parts of the body. The present study was carried out in Anchar lake of Srinagar Kashmir to analyze morphometric measurements of targeted fish (*C. carpio* var. *communis*) during Nov 2021 to April 2022. Total twenty (20) morphometric characters were selected to conduct the present study. Specimen (120) of fish species was collected randomly from different zones of water body. The results of study revealed that total length of fish ranged from 94.93 mm to 199.90 mm with a mean and standard deviation of 142.04 ± 2.00 . The Coefficient of determination (R^2) showed higher value for total length (TL), with maximum for standard length (SL) ($R^2=0.94$) and lowest for snout length (SNL) ($R^2=0.33$). Thus it can be concluded that different level of significance between body features of various variants reveals the possibility of separation among the variants of fish under study as a result of changing environmental conditions or genetic variations, which result from natural selection during long periods of geographical isolation. The findings pertaining to this study provide the baseline information regarding the stock within the water body so that proper strategies and policies could be inculcated for its better management and conservation.

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6.43 Avifaunal Diversity of Selected District (Yamunanagar, Panipat and Faridabad) of Haryana along Yamuna River

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Keywords: Biodiversity, Environment, Avifauna, Scolopacidae, Yamuna river

A post COVID-19 has potential benefits to biodiversity and the environment yet others have suggested both benefits and detriments. The present study was intended to record the Birds biodiversity status of selected stretches of Yamuna river (Yamunanagar, Panipat, Faridabad) Haryana from September 2021 to April 2022. A total of fifty nine (59) Birds species have been recorded from 3 sites (Yamunanagar, Panipat and Faridabad) which belong to 8 orders, 14 families and 59 species. Order Anseriformes were dominant (containing 15 species), followed by the Order Charadriiformes (containing 13 species) and Order Gruiformes (containing 09 species). At the same time, others had only a few bird species. Anatidae was dominant, containing 25 per cent of bird species, followed by Scolopacidae (14%), Ardeidae (13 %) and Charadriidae, Rallidae, and Motacilidae with 7 % of birds species. At the same time, Phalacrocoracidae, Ciconiidae and Laridae had only five % of the total reported bird species. The rest of the families had a few numbers of species diversity, -accounting only two and three per cent. Fifty-nine bird species belonging to 14 families and eight orders have been recorded from Yamuna riverine area in Haryana at selected study sites. Highest 48 species were recorded from site 1 (Yamunanagar area), followed by 44 species from site 3 (Faridabad area) whereas, lowest number of species (41) were observed at site 2 (Panipat area). As per International Union for Conservation of Nature (IUCN) out of 59 bird species, 33 species (56 %) were winter migrants, 16 species (27 %) were residents, and ten species (17 %) were local migrants.

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6.44 Crustacean's Diversity along Mangrove of Sikka Coast, Gulf of Kachchh, Gujarat, India

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Keywords: Mangrove, Crustaceans, Brachyuran crabs, Porcelain crab, Sikka coast

The present study was conducted at two identical sites of Sikka coast namely DCC jetty area and GSFC jetty area. The study was conducted during October 2020 to march 2021. The observations were taken at monthly interval for the abundance of crustaceans species. There are total of 13 species of crustaceans that have been identified. They belong to 13 genera and 11 families, including 9 brachyuran crabs, 2 shrimps, 1 porcelain crab and 1 barnacle. There are nine genera, eight families, and nine species of brachyuran crabs. There are two families, two genera, and two species of shrimp. The mangrove plant *Avicenna marina* is associated with barnacle *Amphibalanus amphitrite*. The GSFC jetty area recorded the highest number of species during the month of December, while the lowest number of species was recorded there in the month of October. At the DCC area, the largest number of species was seen in the month of March and the smallest number in the month of October. During the study period *Metapograpsus thukuhar* and *Austruca irania* (Uca crab) where the most common species belong to the family grapsidae and ocypodidae, respectively.

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6.45 Evaluation of Cyto-Genotoxic Potential of Nickel Oxide Nanoparticles in *Cirrhinus mrigala*

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Keywords: Metal nanoparticles, Erythrocytes, *Cirrhinus mrigala*, Genotoxicity, Cytotoxicity

Nickel oxide nanoparticles (NiO NPs) owing to their unique physical, electrical, chemical, optical, and biological properties have been used extensively in solar cells, light-emitting diodes, biosensors, gas sensors, electrochromic devices, supercapacitors, photocatalytic degradation, antimicrobial agent, anti-corrosive agent, lubricants, catalysts, coatings, ceramics, electronic devices and fuel etc. Use of Nickel oxide nanoparticles (NiO NPs) on such a large scale poses a threat of its release directly or indirectly in the environment. Enhanced use of NiO NPs has necessitated a refined knowledge of their prospective effects on the environment and human health. The objective of this research was to evaluate the cyto-genotoxic effect of NiO NPs on the erythrocytes of *Cirrhinus mrigala*. Exposure of 3.019 mg/L (1/100 of LC₅₀) of NiO NPs was given to the fish for 30 days. For cyto-genotoxic effect, Single Cell Gel Electrophoresis (Comet Assay) and Nucleo-Cellular Abnormality Assay (Micronucleus Assay) was performed. After exposure, percentage increment of 116.6%, 182.9%, 460.8% and 132.5% over control was observed in the length of tail, Tail DNA, tail moment and olive tail moment. Increase in cellular abnormalities was observed to be 471.4% and 1091% for nuclear abnormalities in comparison to control. Common cellular and nuclear abnormalities were micronucleated cell, binucleated cell, eccentric nucleus, nuclear bleb, nucleoid, vacuolated cell, sphaeroocyte, schistocyte, dacrocyte, degmacyte, elliptocyte, ghost cell, helmet cell, cytoplasmic bleb, cytoplasmic notch and cytoplasmic filaments. The above results depict that nickel oxide nanoparticles are a prominent source of genotoxic and cellular damage in fishes and may upset species distribution in polluted water bodies.

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6.46 Ghost Fishing: A Threat to Marine Ecosystem and Efforts to Combat it around the World

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Keywords: Marine ecosystem, Ghost fishing, Ghost gear, Threat, Solutions

The ocean covers 70% of our planet and is home to millions of marine species and creatures. The marine ecosystem contributes significantly to human life by securing livelihood for 10-12% of the world's population and providing a cheap source of nutritional food to nearly 80-85% of people around the globe by supplying 16% of proteins.

The Problem

The self-regulating, self-healing marine ecosystem is under threat of pollution because of untreated domestic sewage, industrial effluents, agricultural runoff, ocean mining, oil spills from accidental discharge, plastic pollution, shipping emissions, radioactivity waste, and noise pollution. Another activity identified as harmful to the marine ecosystem is known as ghost fishing. The fishing activity in which the fishing gear or a portion of fishing gear is abandoned or discharged in the marine environment causes the unintentional trapping of fish or any aquatic animal is known as "ghost fishing", and the gear is called "ghost gear". The estimated figures about ghost gear revealed that thousands of animals in the ocean unintentionally get entangled and die due to these lost gears. Also, these nets damage the aquatic environment by choking the coral reefs, damaging marine habitats, sometimes entangling seabirds, and can wound the propeller of the cruising vessels. At least 6,40,000 tonnes of fishing gear is lost each year, which makes up about 10 percent of all marine debris. Presently, the ghost gear scatters the ocean with the 7,40,000 kilometers of longline, 3,000 square kilometers of gill net, 218 square kilometers of trawl nets, and 75,000 square kilometers of the purse seine. If the current loss rates continue for the next 65 years, there will be enough fishing nets that litter the sea to cover the entire planet.

Solutions

Some researchers have given a solution to reduce the intensity of ghost fishing by avoiding dumping the fishing net into the sea. The FAO and United Nations Environmental Program (UNEP) attributed the ghost fishing to the enhanced scale of global fishing, and the use of highly durable synthetic gear contributed significantly to the problem of ghost nets. The presently used material for fishing, which causes ghost fishing, is better replaced with some alternative material; that will have more strength so that chances of loss can be reduced. In this context, the Central Institute of Fisheries Technology (ICAR-CIFT) is working on producing durable bio-degradable fishing nets. A digital solution includes optimized mobile devices which help the people near the coastal localities to be organized, efficient, and active regarding the identification, collection, and spreading the information about Abandoned, Lost, or Discharged Fishing Gear (ALDFG) and the eOceans e-platform is very useful for collaborative, real-time, and multi-dimensional ocean tracking of ALDFG nets in ocean. The Canadian government has developed the Fishing Gear Reporting System (FGRS) for commercial fish harvesters to report lost gear from any online device. Along with the reporting and monitoring platform, some devices and equipment are developed like Robo-retrieval: Underwater Remotely Operated Vehicles are used to retrieve lost gear from the water. A NetTag is a new technology to locate and recover lost fishing gear in which the small acoustic transponder or tag is attached to the fishing gear. If such gear is lost, the attached tag will automatically send the signals, which can be received by the locator unit on board. There are other techniques have been also advanced like Blue Ocean Gear California has developed Smart Buoys. These buoys continuously monitor and track the fishing gear deployed net and report its location to a mobile phone or website. World Wildlife Fund collaborates with fishers, government agencies, and others all over the world to remove abandoned nets from the sea, establish gear collection and recycling programs, develop tools that enable better tracking and recovery of gear, and approach towards improved gear management and more sustainable fishing practices in small-scale fisheries. A non-profit organization, the Marine Stewardship Council, recognizes and rewards efforts to protect oceans and safeguard seafood supplies for the future. Ghost fishing is a problem all over the globe that affects marine animals and the marine environment. Though many organizations and agencies are working on the issue, more efforts need to be taken towards the awareness of the people to make them realize the threats of ghost fishing and effective measures to reduce the occurrences of the incidence of losing or discharging the fishing gear. Many technological solutions are coming up, but their easy use and affordability to the fishers also need to be considered.

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6.47 Effect of Preservation on Length, Weight and Condition Factor of Snow Trout *Schizothorax esocinus*

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Keywords: Dal lake, *Schizothorax esocinus*, Preservatives, Fixation, Formalin

Formalin and alcohol are important preservatives used in fish studies, and have many effects on morphological and weight indices of fishes. This study aims to quantify the variation in total length and body mass for the snowtrout *Schizothorax esocinus* and to establish total length and body mass correction equations to allow fresh measures to be back-calculated from preserved ones. 45 samples of *S. esocinus* were collected from commercial catches of Dal lake, Jammu and Kashmir. A sequential treatment with 10% formalin and 70% alcohol was carried out to imitate the effects anticipated in normal fish fixation and preservation schedule. Total length (TL) and body weight (W) were measured repeatedly in a sequence for a period of 14 weeks. A significant difference ($p < 0.05$) was observed in the total length relative to fresh specimens after six weeks of alcohol preservation, while as in weight, a significant difference ($p < 0.05$) was reported after two weeks of formalin fixation. These alterations exerted a pronounced effect on condition factor which showed an increment (0.95 to 1.13) in formalin and alcohol.

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6.48 Morphological Differentiation in Wild and Hatchery Stocks of *Cyprinus carpio* var. *communis* from Kashmir

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Keywords: Dal Lake, *Cyprinus carpio* var. *communis*, Morphometrics, Truss network, Principal component analysis

The present study was carried out to determine morphometric differentiation between two populations (hatchery and wild) of *Cyprinus carpio* var. *communis* using conventional morphometric measurements and truss network analysis. A total of 180 samples were collected, 90 individuals from natural habitats (Wild stock: Dal Lake) and 90 from culture environment (Hatchery: National Fish Seed Farm, Manasbal) for the study. Twenty conventional morphometric measurements and 30 truss distances were analysed. Significant differences were observed in 7 of 20 morphometric measurements namely, total length, pre-orbital length, eye diameter, snout length, barble length, caudal fin length and caudal peduncle length. In the present study, 12 point truss network analysis of *C. carpio* var. *communis* was carried out. Principal Component Analysis (PCA) of 30 morphometric measurements extracted from first factor with eigen values >1, explained 88.99% of variance. The component loadings were high mostly in the middle portion of the body depth and head region. The bivariate plots from PC1 & PC2 and PC1 & PC3 extracted from the PCA of *C. carpio* var. *communis* from hatchery and wild indicates more intermixing of the stocks rather than separate stock, while PC3 & PC2 indicates full intermixing of stock which revealed that there are less morphological similarities among the fish from the two populations. The study will not only contribute to biological and ecological clarification of the species but will also help in the development of strategies for natural stock conservation and improving the aquaculture sustainability.

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6.49 Current Status of Fish Fauna in Anchar Lake of Kashmir Valley

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Keywords: Anchar, Common carp, Fish fauna, Kashmir

Aquatic ecosystems include fish species, and any significant changes to the environment in which they exist may have an impact on their productivity, diversity, ecology, and distribution. In order to assess the current status of fish fauna of Anchar lake of Kashmir valley, the investigation was carried out for the period of twelve months from March 2021- February 2022. Monthly samplings were carried out at 5 study sites- Sindh (Inlet), Jenab saab, Center, Skims and Sangam (Outlet). During the study period, a total of 5 species of fishes were recorded and collected from the lake which include *Cyprinus carpio* var. *communis* (Punjaeb Gad), *Cyprinus carpio* var. *specularis* (Punjaeb Gad), *Carassius carassius* (Gang Gad), *Schizothorax niger* (Ale gad), *Schizothorax esocinus* (Churru). Apparently the fishery resources of the Kashmir valley has declined over a period of time indicating many intrinsic and extrinsic disturbances which are altering the health of various lentic and lotic water bodies.

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THEME VII

Fish Processing, Value Addition, Quality Assurance and Marketing







7.1 Nutritional and Textural Characteristics of Fish Protein Powder and Fiber Enriched Biscuits

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Keywords: Fish protein powder, Fish biscuits, Response surface methodology, Texture analysis

Introduction

Bakery products are popular snack food among all age groups throughout world. Biscuits have its own place among bakery food products as anytime ready to eat snack, however the conventional biscuits possess low nutritional value and contains high energy mainly due to incorporation of ample amount of refined flour and sugar as carbohydrate source. Now day's consumers are health conscious and are demanding healthy food products. Protein and fiber are always considered as healthy nutrients; hence it may be used as functional ingredients to improve the nutritional value of the product. The present study was conducted to evaluate nutritional and textural characteristics of biscuits containing high protein and fiber content using protein powder extracted from muscle of rohu (*Labeo rohita*) fish and ragi (finger millet).

Materials and Methods

Fish protein powder and ragi flour enriched biscuits were prepared through mixing the ingredients as given in Table 1. Fish protein powder was extracted in laboratory from rohu muscle procured from the Instructional cum Research Farm, College of Fisheries, GADVASU, Ludhiana. Optimization of the quantity as in percentage for both ingredients, fish protein powder and ragi flour in biscuits was calculated using Response Surface Methodology (RSM) (Design Expert 8.0.7.1 software). To assess nutritional value of the biscuits, proximate composition was analyzed for moisture, protein, fat, carbohydrate, ash and fiber content as per standard methods (AOAC 2019). All the data were statistically analysed to observe whether there is any significant difference in proximate composition among the different treatments using oneway ANOVA by SPSS 16.00 software. Hardness of biscuit samples was determined in terms of fracturability using 3-point brake test by TA-XT Plus texture analyzer (Stable Micro Systems, England) (Abraha 2018). Colour analysis of baked biscuits was performed using Lovibond Tintometer (Model: RT-300) to obtain L* a* and b* values.

Results and Discussion

Based on quadratic order polynomial model, the software suggested 9 treatment combinations other than control adding fish protein powder and ragi as variable for the formulation of protein and fiber enriched biscuit preparation. The software suggested fish protein powder can be incorporated in the range of 5-15%, ragi 3- 9% replacing refined wheat flour however quantity of other ingredients remain constant in all the treatments (Fig.1). Nutritional value based on proximate composition shows significantly high protein content in biscuits of T4 and T9 ($P \leq 0.05$) which may be due to the incorporation of higher percentage of fish protein powder and ragi flour. Significantly high fiber content recorded in T7 ($P \leq 0.05$), minerals, ash content recorded significantly high ($P \leq 0.05$) in all treatments except Control (C), T1, T3, and T5, whereas significantly high quantity of carbohydrate recorded in control. No significant difference recorded in fat and moisture content of biscuits among all treatment including control (Table 2). T7 showed highest while, T6 showed lowest value for hardness as fracturability in terms of force/kg (Fig 2). Significant variation in values of the colour for L, a and b recorded in treatments (C, T1-T9) ($P \leq 0.05$) (Table 2). Over all among treatment T1-T9 and control, treatment T7 and T9 collectively contains significantly higher levels of protein and fiber.

Table 1 Composition of ingredients (%) for fish protein powder and ragi incorporated biscuits

S. No.	Ingredients (%)	Treatment									
		C	T1	T2	T3	T4	T5	T6	T7	T8	T9
1	Refined wheat flour	60	46	44	52	39	49	47	41	42	36
2	Sugar	16	16	16	16	16	16	16	16	16	16
3	Fat	23	23	23	23	23	23	23	23	23	23
4	Baking powder	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
5	Salt	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
6	Fish protein powder	0	5	10	5	15	5	10	10	15	15
7	Ragi flour	0	9	6	3	6	6	3	9	3	9
8	Vanilla Essence	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

Table 2 Proximate composition (%) and colour attributes of fish protein and ragi fiber incorporated biscuits

Parameters (%)	C	T1	T2	T3	T4	T5	T6	T7	T8	T9
Moisture	0.74±0.02 ^a	0.74±0.02 ^a	0.66±0.03 ^a	0.85±0.03 ^a	0.94±0.05 ^a	0.93±0.02 ^a	1.02±0.72 ^b	0.80±0.04 ^a	0.79±0.06 ^a	0.74±0.03 ^a
Protein	7.54±0.21 ^a	15.74±0.17 ^c	18.04±0.25 ^c	13.67±0.15 ^b	19.57±0.24 ^f	13.24±0.07 ^b	17.29±0.19 ^d	17.93±0.27 ^c	18.37±0.21 ^c	19.91±0.16 ^f
Fat	20.99±2.94 ^a	19.11±0.11 ^a	19.79±0.18 ^a	19.33±0.20 ^a	19.30±0.21 ^a	19.86±0.24 ^a	27.79±8.34 ^a	19.38±0.19 ^a	19.62±0.32 ^a	19.67±0.48 ^a
Ash	1.47±0.03 ^a	2.30±0.04 ^c	3.38±0.07 ^c	1.76±0.06 ^b	3.43±0.07 ^c	2.51±0.04 ^d	3.52±0.05 ^c	3.38±0.05 ^c	3.47±0.05 ^c	3.54±0.07 ^c
Fiber	0.65±0.01 ^a	4.51±0.04 ^f	3.74±0.04 ^c	2.11±0.06 ^b	3.88±0.13 ^c	3.44±0.05 ^d	2.57±0.06 ^c	5.37±0.07 ^b	1.95±0.04 ^b	4.81±0.03 ^e
Carbohydrate	68.61±2.82 ^a	57.6±1.96 ^d	54.39±1.67 ^c	62.34±2.34 ^c	52.88±2.66 ^b	60.02±1.48 ^c	47.17±2.34 ^a	53.14±2.94 ^b	55.8±21.84 ^c	51.33±1.38 ^b
Colour- L	57.27±0.61 ^c	57.01±0.63 ^c	54.68±0.25 ^d	54.67±0.34 ^d	18.75±6.03 ^c	17.61±5.86 ^d	17.74±5.92 ^d	15.19±4.92 ^a	16.02±5.24 ^b	15.62±5.09 ^{ab}
a	10.60±0.16 ^c	7.10±0.33 ^b	9.45±0.18 ^{cd}	10.12±0.16 ^{cd}	8.06±0.26 ^{cd}	7.03±0.14 ^a	8.10±0.29 ^{cd}	8.32±0.36 ^c	8.70±0.50 ^f	8.28±0.33 ^{de}
b	29.96±0.57 ^a	22.41±0.67 ^b	26.83±0.29 ^c	28.24±0.32 ^f	25.54±0.27 ^d	20.54±0.44 ^a	28.37±0.57 ^f	24.19±0.22 ^c	28.93±0.22 ^{fg}	26.39±0.27 ^{de}

* Values with different alphabetical superscripts differ significantly within row (P ≤ 0.05)

Design-Expert® Software
Factor Coding: Actual
Original Scale
(median estimates)
Fish Protein Powder
● Design Points
20.56
8.31
X1 = A: Fish Protein Powder
X2 = B: Ragi
Actual Factor
C: Refined wheat flour = 48.00

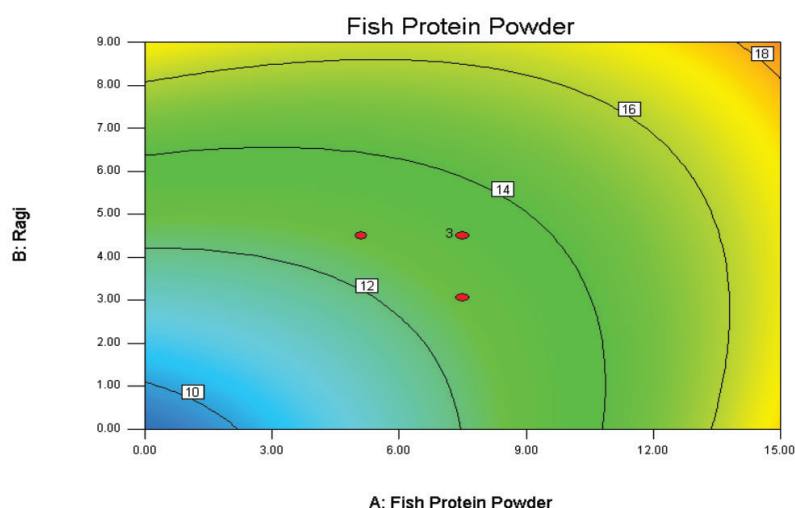


Fig 1. Optimum concentration of fish protein powder, ragi flour replacing refined wheat flour for protein and fiber enriched biscuits

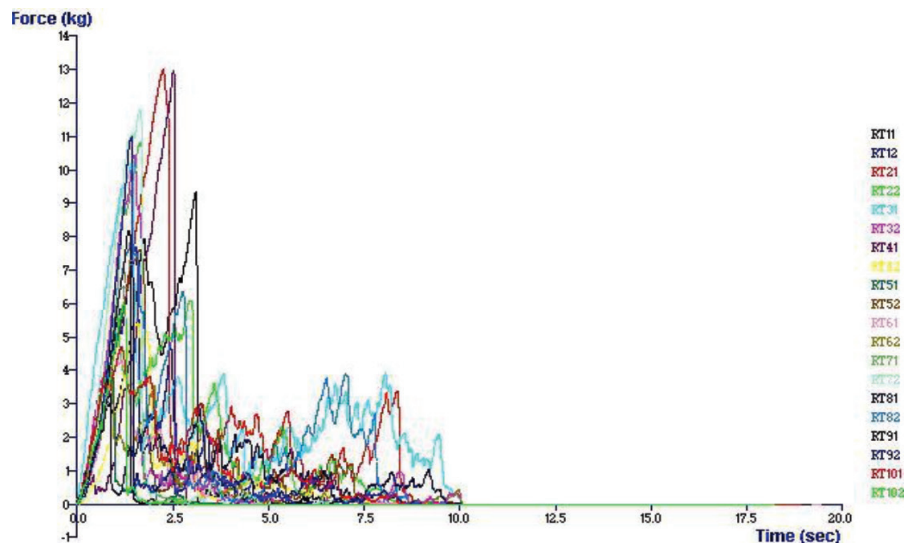


Fig 2. Fracturability of fish protein powder and ragi incorporated biscuits

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7.2 Nutritional and Colour Profile of Carp Protein Powder Incorporated Nutrition Bar

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Keywords: Fish protein powder, Nutrition bar, Proximate composition, Colour profile

Introduction

The confectionary bars offer a readily available convenient food source that requires no preparation, cooking, holds a reasonable extended shelf life at ambient temperature and easy to carry. Hence bars are popular as a convenient snack food and are consumed by the people of all age groups in between meals or even as a substitute for meals. In recent past, demand of nutrition bar has increased tremendous as people became aware about health but most of the existing confectionary bars available in the market are either nutritionally poor or provide carbohydrate in high quantity. Keeping in view the convenient bar along with nutrition, the present work was conducted to assess the acceptability of nutrition bar with added extracted protein powder from carp fish, as an additional option of snack bar for health conscious people.

Materials and Methods

Proteins extraction from carps

To extract fish protein powder, initially fresh carp fillets were minced and treated with food grade acid @ 0.5 % followed by washing with potable water. Further the acid treated fish mince was dried in hot air oven and pulverised to obtain fish protein powder.

Preparation of fish protein powder supplemented nutrition bars

Nutrition bar was prepared mixing Corn flakes, Oats flakes, Peanut, Almond, Cashew, Sesame seed and Salt, ingredients were thoroughly mixed; further to increase protein content in bar, extracted fish protein powder was added at different levels i.e. 2.5 (T1), 5(T2), 7.5(T3), 10(T4) and 12.5 (T5) percent in different treatments (T1-T5), where as in control (C) no fish protein powder was mixed. Jaggery syrup and Dextrin was also added into it as a binder to maintain its shape.

Proximate composition analysis

To assess the nutritional composition of bar (C, T1-T5) prepared by adding fish protein powder; Protein, Fat Ash, Carbohydrate and Moisture content was quantified following the official methodology described by AOAC (2019).

Colour

Colour analysis of nutrition bar was performed using Konica Minolta (Model: Chroma meter CR 400) to obtain colour values as L* a* and b*.

Results and Discussion

Nutrition bar prepared by adding fish protein powder (T1-T5) and control (C) shows average protein content ranged between 16.22 - 27.07% in treatments (T1-T5) whereas in control (C) it was 13.79%, maximum protein recorded in T5 (27.7%), where as maximum fat recorded in control (C) 12.98% (Fig.1). A combination of the colour index, expressed as, L* (degree of lightness), a* (degree of redness) and b* (degree of yellowness) shows that the L*

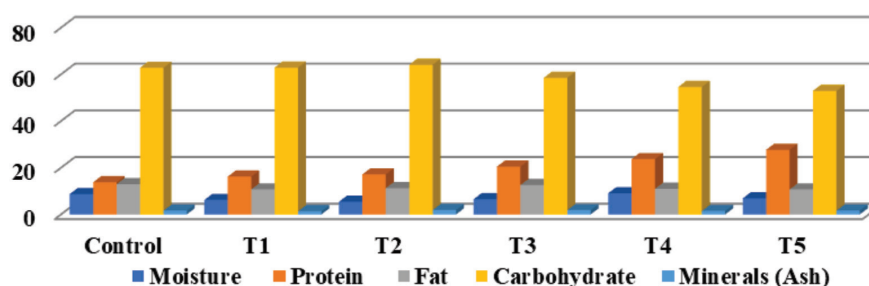


Fig 1. Proximate composition (%) of control and fish protein powder incorporated nutrition bar



value of nutrition bar (C, T1-T5) ranged from 37.89 – 48.05, least value recorded in control (37.89), while the highest value recorded in T2 (48.05). The a* value of nutrition bar (C, T1-T5) ranged from 6.83 – 8.18. The yellowness (b*) value of nutrition bar (C, T1-T5) ranged from 27.78 – 32.35 (Table 1).

Table 1. Colour profile of control and fish protein powder incorporated nutrition bar

Parameters	l*	a*	b*
Control	37.896	7.37	27.78
T1	47.566	6.88	29.59
T2	48.054	8.186	32.352
T3	45.79	6.838	30.108
T4	40.304	8.026	29.726
T5	40.046	7.406	29.89

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AOAC 2019. *Official Methods of Analysis of the Association of Official Analytical Chemists*, AOAC International, Washington, D. C.

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7.3 Application of Fish Waste Dispersion to Enhance the Shelf-life of Fish Fillets under Chilled Storage

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Keywords: Fish waste, Dispersion, Fish fillets, Chilled storage

Introduction

Commercial processing (beheading, de-shelling, degutting, removal of fin and scales, filleting of fish) results in a huge amount of solid waste, offal or by-products. Fish filleting processing resulted in 75% of the total fish weight as waste. These wastes when discarded in open environment create pollution as well as become the source of many infectious diseases. Hence, present study was done to enhance the shelf-life of basa fish (*Pangasius bocourti*) fillets using dispersion developed from the myofibrillar protein obtained from basa fish waste using acetic acid gelation.

Materials and Methods

Fish waste mince (100 g) was taken and washed with chilled water (1:4, w/v) followed by centrifugation at 10000 g for 15 min at 4 °C. The obtained pellet was washed with NaCl (50 mM) (1:4, w/v) followed by centrifugation at 10000 g for 15 min. at 4 °C. The washing step with 50 mM NaCl was done twice. The obtained pellet was lyophilized at -50 °C for 24 h under vacuum to obtain dry powder. The protein powder (1%) was suspended in 2% acetic acid solution for 12 h at 20 °C to obtain the dispersion. Glycerol concentration used was 50% of protein powder. The basa fish fillets were dipped in obtained solution for 5 min. followed by drying under fan. The samples were packed in polypropylene package and stored under chilled condition. The microbiology and total volatile basic nitrogen (TVBN) were estimated during storage.

Results and Discussion

The protein content of obtained powder was found to be 87.6%. The fish fillets dipped in dispersion were safe up to 8 days with acceptable microbial load (Table 1) and TVBN value (Table 2), whereas control samples were spoiled microbiologically on day 2 of storage (Table 1). The appearance of control and coated basa fish fillets with storage is shown in Fig. 1. Hence, dispersion developed from fish waste helped in enhancing the shelf life of fillets to a great extent with good quality as well as utilization of fish waste which will be helpful in reducing environmental pollution.

Table 1 Total aerobic count of control and coated fish fillets

Total aerobic count (Log CFUg ⁻¹)					
Sample	Day 0	Day 2	Day 4	Day 6	Day 8
Control	6.59 ± 0.04 ^d	8.97 ± 0.07 ^e	Bad	Bad	Bad
Coated	4.35 ± 0.04 ^l	4.55 ± 0.04 ^b	4.65 ± 0.04 ^c	4.63 ± 0.03 ^c	4.66 ± 0.01 ^c

The results shown are mean ± standard deviation, n=6. Different letters in the row/column indicate significant differences (p < 0.05). Bad- Not done as samples were spoiled

Table 2 Total volatile basic Nitrogen of control and coated fish fillets

TVBN (mgN / 100 g sample)					
Sample	Day 0	Day 2	Day 3	Day 6	Day 8
Control	11.2 ± 2.8 ^b	Bad	Bad	Bad	Bad
Coated	9.3 ± 3.2 ^a	14.93 ± 3.5 ^c	16.8 ± 2.8 ^d	18.67 ± 4.5 ^e	22.4 ± 5.6 ^f

The results shown are mean ± standard deviation, n=6. Different letters in the row/column indicate significant differences (p < 0.05). Bad- Not done as samples were spoiled



Control, Day 0



Coated, Day 0



Control, Day 2



Coated, Day 8

Fig. 1 Appearance of control and coated basa fish fillets

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7.4 Screening of Some Seaweeds for Antibacterial and Antifungal Activities Against Pathogenic Bacteria

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Keywords: Seaweeds, Antibacterial, Antifungal, Solvents

Introduction

The marine environment has a rich biological and chemical diversity. This diversity of marine organisms and habitat offers a wide variety of natural marine compounds. Several oceanic compounds show pharmacological activities and are also helpful in the discovery of novel bioactive compounds, primarily for deadly diseases like Cancer, Acquired immuno-Deficiency Syndrome (AIDS), Arthritis, etc (Pham-Huy and Huy 2022). Many other compounds have been developed as analgesics or to treat inflammation etc. The present work studied the antibacterial and antifungal activity of four different solvents extracts of five seaweeds collected from Tuticorin coast, Tamilnadu.

Materials and Methods

Collection of some seaweeds and preparation

Fresh seaweed samples of *Caulerpa scalpelliformis*, *Ulva lactuca*, *Padina tetrastromatica*, *Stoechospermum marginatum*, and *Acanthophora spicifera* were handpicked from Hare Island, Tuticorin. The shade-dried samples were then powdered for the extraction of antimicrobial compounds.

Preparation of organic seaweed extracts

The organic seaweeds extracts were prepared by following the method of El Shafay et al (2016). Five-gram powdered algal samples were soaked in 50 mL analytical grade petroleum ether, benzene, chloroform and methanol for three days. Extracts were filtered and concentrated in a rotary evaporator at 35°C. The residual water was then removed with a vacuum pump. The weighted crude extracts were suspended in the dimethyl sulfoxide (DMSO) to a final concentration of 50 mg/mL and stored in a refrigerator.

Bioassay

For bioassay, fungal pathogen *Candida albicans* ATCC 90028 and five gram-negative bacterial pathogens namely *Salmonella typhi* ATCC 35640, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 33420, *Klebsiella pneumoniae* ATCC 700603, *Escherichia coli* ATCC 25922 and one pathogenic gram-positive bacteria namely *Staphylococcus aureus* ATCC 25923 were used. A nutrient broth was maintained as control without inoculating the test organisms. Amikacin-AK30-30mcg/disc, Cephalexin-CN30-30mcg/disc, Ciprofloxacin-CIP5-5mcg/disc and Fluconazole-FLC25-25mcg/disc were used as control medicine. The agar diffusion method was followed for the assay.

Results and Discussion

Among five different seaweeds collected from the seawater of the Hare Island, Tuticorin, the extracts of some of the seaweeds were found to show specific activity against the pathogens studied. The antibacterial (Table 1) and antifungal (Table 2) activities of different extracts of marine algae against the pathogens have been tabulated. The study showed the antifungal activity of methanol extract of *Ulva lactuca* against *Candida albicans*. Further, the petroleum ether extract of *Caulerpa scalpelliformis*, *Ulva lactuca*, and *Padina tetrastromatica*; the benzene extract of *Stoechospermum marginatum* were active against *Candida albicans*. None of the extracts of the red alga *Acanthophora spicifera* were active against *Candida albicans*. The zone of inhibition indicates that these extract exhibit activities quite comparable with the commercial antifungal agent Fluconazole. The petroleum ether extract of *Caulerpa scalpelliformis* was more effective (32mm) than the commercial antifungal agent Fluconazole (27mm). The petroleum ether extract of *Acanthophora spicifera* was not found to possess required sensitivity against *Salmonella typhi*, but the methanol extract of *Stoechospermum marginatum* was active against *Salmonella typhi*. The methanol extract of a *Padina tetrastromatica* and the benzene extract of *Stoechospermum marginatum* showed Ciprofloxacin-like and Amikacin-like activity (14 mm) against *Pseudomonas aeruginosa*. The petroleum ether extracts of *Padina tetrastromatica* was more effective (17 mm) than the commercial antibiotics. The chloroform extract of *Ulva lactuca* and *Acanthophora spicifera* and the benzene extract of *Acanthophora spicifera* showed lesser activity than needed against *Klebsiella pneumoniae*. The methanol extract of *Stoechospermum marginatum* and *Acanthophora spicifera* were active against *Escherichia coli*. All the extracts studied were inactive against the *Staphylococcus aureus*.

Table 1. Antibacterial activities of sea weeds extracts from Hare Island of Tuticorin coast

SL No.	Name of the bacterial pathogens	Crude Algal Extract and Commercial medicine used																				
		Control medicine			Caulerpa scalpelliformis			Ulva lactuca			Acanthophora spicifera			Padina tetrastromatica			Stoechospermum marginatum					
		AK30	CN30	CIP5	PE	BZ	CF	MN	PE	BZ	CF	MN	PE	BZ	CF	MN	PE	BZ	CF	MN		
1.	<i>Salmonella typhi</i> ATCC 35640	16	20	21	-	-	-	-	-	-	-	-	15	-	-	-	-	-	-	-	16	
2.	<i>Pseudomonas aeruginosa</i> ATCC 27853	14	15	14	-	-	-	-	-	-	-	-	-	-	-	14	17	-	-	14	-	-
3.	<i>Proteus vulgaris</i> ATCC 33420	16	20	21	-	-	-	-	16	-	-	-	-	-	-	-	-	-	-	15	-	-
4.	<i>Klebsiella pneumoniae</i> ATCC700603	26	29	30	-	-	-	-	-	-	23	-	-	20	24	-	-	-	-	-	-	-
5.	<i>Escherichia coli</i> ATCC 25922	16	20	22	-	-	-	-	-	-	-	-	-	-	-	16	-	-	-	-	-	16

*PE-Petroleum ether; BZ-Benzene; CF-Chloroform; MN-Methanol; FLC25-Fluconazole; AK30-Amikacin; CN30-Cephalexin; CIP5-Ciprofloxacin

*Zone in mm indicates the distance from the border of the disc to the edge of the clear zone.

Table 2. Antifungal activities of sea weeds extracts from Hare Island of Tuticorin coast

SL No.	Name of the fungal pathogens	Crude Algal Extract and Commercial Medicine Used																					
		Control medicine			Caulerpa scalpelliformis			Ulva lactuca			Acanthophora spicifera			Padina tetrastromatica			Stoechospermum marginatum						
		FLC25	PE	BZ	CF	MN	PE	BZ	CF	MN	PE	BZ	CF	MN	PE	BZ	CF	MN	PE	BZ	CF	MN	
1	<i>Candida albicans</i> ATCC 90028	27	32	-	-	-	24	-	-	-	-	-	-	-	22	-	-	-	-	-	22	-	-

*PE-Petroleum ether; BZ-Benzene; CF-Chloroform; MN-Methanol; FLC25-Fluconazole

*Zone in mm indicates the distance from the border of the disc to the edge of the clear zone



The present study provided the information about the antibacterial and antifungal activities of selected algal extracts and thereby emphasized the critical role of traditional medicine and created a quest for the discovery of bioactive compounds from the natural sources.

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7.5 Quality Attributes of Biscuits Supplemented with Protein Isolates from Pangas Processing Waste

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Keywords: Fish protein isolates, Biscuits, Quality, Properties, Acceptability

Introduction

Pangas fish processing has been increasing day by day in Indian fish markets and it is going to be one of the majorly consumed fish in the near future. Processing of pangas fish generates considerable amounts of waste, which sometimes accounts for more than 50% of the total processed fish. Recovering proteins from these wastes for edible purposes would help in better management and utilization of these large quantities of wastes (Surasani et al 2019). Biscuits have been one of the important snack foods of Indians and the market share of biscuits has been steadily increasing. Moreover, biscuits would serve as a good vector for supplementing fish proteins due to its stability and acceptability. The present work assessed the suitability of supplementing biscuits with protein isolates obtained from pangas processing waste.

Materials and Methods

Preparation of pangas fish protein isolates

Fresh pangas fillet frames were ground and added with 3 volumes of chilled potable water followed by its homogenization, pH-shift aided solubilization (pH 13.0) and precipitation. The obtained precipitate was neutralized followed by drying at 45 °C in a hot air oven and pulverization to fine powder.

Preparation of protein isolate supplemented biscuits

For preparing fish protein isolate supplemented biscuits, wheat flour in the dough was replaced with 2.5, 5.0, 7.5 and 10.0% fish protein isolates followed by the mixing and preparation of biscuits. The ingredients used in the preparation were Wheat flour (100 g), Sugar (30 g), Margarine (28 g), Salt (1 g), Baking powder (0.9 g), Vanilla (1.5 g) and Water (20 g). After the dough making, it was shaped and baked at 175 °C for 10-12 min, followed by cooling, packing and storage.

Quality evaluation

The protein isolate supplemented biscuits were analyzed for its physical properties (Diameter, thickness, spread ratio and top grain score), color characteristics, texture and sensory quality (Appearance, odor, texture, flavor and overall acceptability) as per the methods described by Abraha et al (2018).

Results and Discussion

Biscuits that were supplemented with pangas protein isolates had diameter of 6.5-6.9 cm, thickness 0.6-0.8 cm and spread ratio of 8.37-11.5, being highest for biscuits supplemented with 2.5% protein isolates. The top grain score of 1.0 was observed for biscuits from all the lots. No significant difference was observed in the physical properties of biscuits supplemented with different levels of fish protein concentrate (Abraha et al 2018). The insignificant difference in physical properties of biscuits with protein isolates might be due to low concentration of fish protein isolates in the mixture that could not cause considerable changes in the quality during processing. No significant difference was observed in the whiteness of the biscuits, while hardness was maximum for biscuits from control group (18.63 ± 2.30 Force. Kg). No significant difference was observed any of the sensory attributes among the biscuits supplemented with different levels of pangas protein isolates. The average acceptability scores ranged between 8.0 and 9.0 for biscuits from different treatments. Abraha et al (2018) reported that biscuits produced using fish protein concentrate has higher level of acceptance compared to the control group. However, biscuit prepared from wheat flour alone had significantly higher scores for its flavour compared to other lots (9.0). All the sensory attributes were insignificantly different ($p \geq 0.05$) compared to control group biscuit as well as among inclusion levels. These non-significant differences in sensory attributes are attributed to the non-fishy flavour and odor of the protein powders, that did not affect the sensory quality and acceptability of the biscuits.

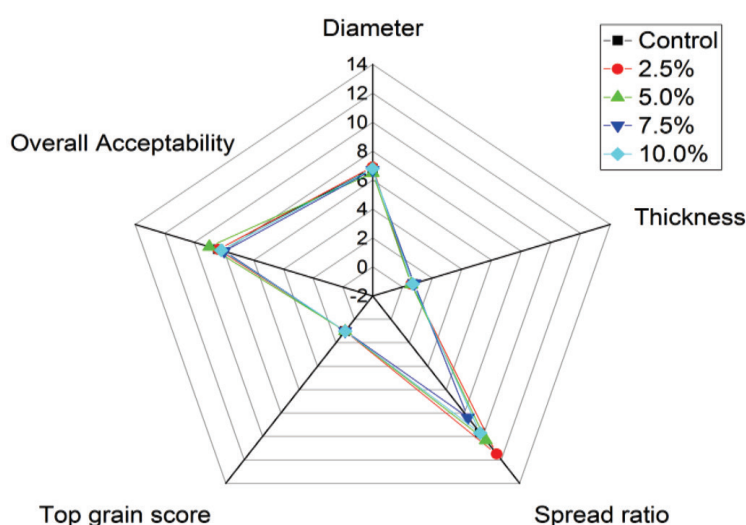


Figure 1. Physical properties of biscuits incorporated with different levels of fish protein isolates

Table 1. Color and texture values of biscuits incorporated with different levels of fish protein isolates

Treatment	L* value	a* value	b* value	Whiteness	Hardness (Force.Kg)
Control	55.56 ± 2.21 ^a	13.90 ± 0.64 ^a	33.78 ± 1.37 ^a	42.43 ± 1.09 ^a	18.63 ± 2.30 ^a
2.5% Isolate biscuits	55.01 ± 0.71 ^a	13.91 ± 0.29 ^a	33.18 ± 1.69 ^a	42.37 ± 1.04 ^a	12.10 ± 1.20 ^b
5.0% Isolate biscuits	55.39 ± 1.12 ^a	14.01 ± 0.48 ^a	31.23 ± 1.00 ^{ab}	43.76 ± 1.15 ^a	13.44 ± 1.25 ^b
7.5% Isolate biscuits	51.09 ± 0.12 ^b	12.46 ± 1.38 ^a	25.78 ± 2.15 ^c	43.29 ± 1.36 ^a	10.70 ± 1.78 ^b
10.0% Isolate biscuits	54.25 ± 1.09 ^a	12.64 ± 1.21 ^a	30.16 ± 1.36 ^b	43.75 ± 1.21 ^a	11.62 ± 1.67 ^b

Values in the table represent average values of three readings, presented as Mean ± SD (N = 3). Values in the same column with different superscripts are significantly different ($p < 0.05$). L*-Lightness, a*- Red/Greenness, b*- Blue/Yellowness

Table 2. Sensorial quality of biscuits incorporated with different levels of fish protein isolates

Treatment	Appearance	Odor	Texture	Flavor	Overall acceptability
Control	8.8 ± 0.44 ^a	8.4 ± 0.89 ^a	8.2 ± 0.83 ^a	8.4 ± 0.89 ^a	8.4 ± 0.89 ^a
2.5% Isolate biscuits	8.6 ± 0.89 ^a	8.0 ± 1.41 ^a	8.2 ± 0.83 ^a	8.4 ± 0.89 ^a	8.4 ± 0.89 ^a
5.0% Isolate biscuits	8.8 ± 0.44 ^a	8.6 ± 0.54 ^a	8.6 ± 0.54 ^a	8.4 ± 0.54 ^a	9.0 ± 0.1 ^a
7.5% Isolate biscuits	7.8 ± 0.83 ^a	7.8 ± 1.09 ^a	8.0 ± 0.70 ^a	7.8 ± 1.3 ^a	8.0 ± 1.0 ^a
10.0% Isolate biscuits	7.6 ± 0.89 ^a	7.6 ± 1.34 ^a	8.4 ± 0.54 ^a	7.8 ± 1.30 ^a	8.2 ± 0.83 ^a

Values in the table represent average values of three readings, presented as Mean ± SD (N = 3). Values in the same column with different superscripts are significantly different ($p < 0.05$).

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7.6 Chitosan and Epigallocatechin Gallate Grafted Chitosan-based Composite Films: Antioxidant and Antimicrobial Activities, and Its Application for Shelf-life Extension of Refrigerated Asian seabass (*Lates calcarifer*) Slices

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Keywords: Asian sea bass, Chitosan-epigallocatechin gallate, Composite film, Shelf-life, Vacuum packaging

Chitosan (CS) has been touted as a promising choice for the development of bioactive films or packaging; nevertheless, it is soluble in aqueous acid, resulting in strong odor and flavor in foods. To overcome this, CS-epigallocatechin gallate (EGCG) conjugate (CEC) possess with higher water solubility and bioactivities was incorporated in CS film solution to develop CS/CEC composite (CS/CEC-COMP) film. CS/CEC-COMP film was prepared to enhance the bioactivities of CS based films and its application for shelf-life extension of Asian seabass slices (ASBS) stored in air or under vacuum packaging at 4 °C. CS (1%, w/v) and CEC (1%, w/v) were dissolved in acetic acid (1%, v/v) and water, respectively. Both solutions were mixed in ratio of 8:2 (v/v) to prepare CS/CEC-COMP film. CS film (without CEC) was act as control. Antioxidant (AO) and antimicrobial (AM) activities of the resultant CS and CS/CEC-COMP films were determined. ASBS were packed in CS and CS/CEC-COMP films for shelf-life extension, in which microbiological, chemical, and sensory properties were monitored during storage at 4 °C for 15 days. CS/CEC-COMP film had higher total phenolic content (18.69 mg EGCG equivalent/g sample) and AO activities than CS film ($p < 0.05$). CS/CEC-COMP film showed enhanced growth zone inhibition toward *Pseudomonas aeruginosa* (18.3 mm) and *Listeria monocytogenes* (21.3 mm) ($p < 0.05$). The SEM images of tested bacteria confirmed the antimicrobial activity of CS/CEC-COMP film. ASBS wrapped with CS/CEC-COMP film and packed under vacuum (ASBS-COMP-VP) conditions resulted in the lower microbial loads ($< 10^6$ CFU/g) than other samples ($p < 0.05$) up to 15 days. ASBS-COMP-VP possessed the lower oxidation of polyunsaturated fatty acids, which was mainly caused by free radical scavenging and antimicrobial activities of CS/CEC-COMP film.

CS/CEC-COMP film had higher bioactivities than CS film. Furthermore, shelf-life of ASBS with sensory acceptability prolonged for at least 12 days at 4 °C.

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7.7 Inhibitory Effect of Curry (*Murraya koenigii*) and Moringa (*Moringa oleifera*) Leaves Extracts Conjugation with Copper Sulfide Nanoparticles on Quality Changes and Melanosis of Pacific White Shrimp (*Litopenaeus vannamei*) during Chilled Storage

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Keywords: Copper sulfide nanoparticles, Curry leaves extract, Moringa leaves extract, Melanosis, Pacific white shrimp, Quality changes

This study aimed at the evaluation of the treatment of curry leaves extract (CLE) and moringa leaves extract (MLE) conjugated with copper sulfide nanoparticles (CuNPs) on melanosis and improving the quality of chilled stored Pacific white shrimp for a period of 15 days. In this study, the antioxidant activity of the extract by DPPH, ferric reducing antioxidant power (FRAP), total phenolic content (TPC), total antioxidant capacity (TAC), and GC-MS was used to identify the bioactive compound. The FRAP, TPC, TAC value of CLE (208.76±0.246 mgE Fe (II)/g, 25.126±0.5986 mg Eq GAE/g, 82.226±1.32 mgE Asc/g) were significantly higher as compared to MLE (168.22±0.981 mgE Fe (II)/g, 18.025±0.547 mg Eq GAE/g, 73.044±0.474 mgEAsc/g). The treatment with CLE+MLE+CuSNPs sample had relatively lower values of biochemical indices and microbial count than the other treatment. This biopreservation techniques noted in this study may offer a new alternative to preservation techniques.

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7.8 Fish Dressing cum Waste Collection System- A Step towards Hygienic Primary Processing, *Swachhata* in Fish Markets and Wastes to Wealth

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Keywords: Descaling, Fish dressing, Steaking, Primary processing, Waste management

Primary processing is an essential post-harvest unit operation of finfish. Finfish dressing for fish retailers, small-scale fish processors, caterers, and fish handlers is a most cumbersome activity, and unhygienic handling, steaking, and waste disposal practices are commonly seen in the fish markets which in turn makes the fish market a dirty place for consumers to visit. To ease this operation, to provide a solution for hygienic handling, to ensure food hygiene and safety with proper product display, and to ease appropriate waste disposal, ICAR-CIPHET developed a fish dressing cum waste collection system that can be operated easily and for a long time with less drudgery by the fishers. It consists of i) a dressing table (100x48x120 cm), ii) a round cutter composed of stainless steel (SS, 25.4 cm dia.), iii) a water spraying system for cleaning, iv) a motor (0.1 HP, 9400 rpm) and v) a PVC pulley (20 cm dia.); vi) a foot rotor pulley (53 cm dia.) and vii) liquid waste disposal systems. The machine can be used to steak and descale fish @ 2-3 kg fish (carps)/minute. It collects liquid and solid wastes in separate covered containers. It also has a fly protection cover, continuous rinsing system, and waste-water filtration system. This machine is expected to contribute immensely towards *Swachh* Bharat Mission and Blue Revolution in India and ensure food safety for fish consumers. Further, the systematically collected solid wastes and liquid waste can facilitate the utilization of wastes for promoting waste to wealth.

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7.9 Effect of Protein Hydrolysate from Splendid Squid (*Loligo formosana*) Fins on Textural Properties, Acceptability and Volatile Profile of Gel from Threadfin Bream Surimi

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Keywords: Squid fin, Protein hydrolysate, Flavorant, Surimi, Gel properties

Splendid squid fins (*Loligo formosana*) protein hydrolysates (SF-PH) prepared using alcalase at various degrees of hydrolysis (DH) (10-40%) were characterized and determined for antioxidant activities and functional properties. Moreover, the effects of the selected PH sample on the gel properties, volatile profile, and acceptability of threadfin bream surimi gel were also elucidated. When DH was increased to 40% from 10%, the highest yield was obtained ($p < 0.05$). However, lightness was reduced with augmenting DH, which was supported by the increasing redness and yellowness ($p < 0.05$). In general, antioxidant activities upsurged with increasing DH, except metal chelating activity and DPPH radical scavenging activity, which were decreased at higher DHs. Among all samples, SF-PH having DH 40% (SF-PH-40) showed the highest relative solubility, foaming capacity, and foaming stability. SF-PH-40 was rich in essential and hydrophobic amino acids. Moreover, SF-PH-40 possessed a higher proportion of smaller molecular weight (MW) peptides. SF-PH-40 was rich in aspartic acid/asparagine and glutamic acid/glutamine. SF-PH-40 contained a high number of volatile compounds associated with squid flavor, which could be used as alternative squid flavorant. Therefore, SF-PH-40 was added to threadfin surimi gels at various concentrations (0, 1, 2, 3, and 4%; w/w) to produce squid-flavored gels. It was noticed that with increasing levels of SF-PH, breaking force and deformation were decreased as compared to the control gel (CON; without SF-PH) ($p < 0.05$). However, among the gel added with SF-PH, the gel containing 1 and 2% SF-PH had the highest breaking force ($p < 0.05$). The whiteness of all samples was slightly decreased with the upsurging amount of SF-PH, which was supported by the increasing redness and yellowness of the SF-PH powder. The water holding capacity was increased with augmenting levels of SF-PH ($p < 0.05$). Sensory analysis revealed that gel added with 2% SF-PH had a higher squid odor and flavor likeness score than CON ($p < 0.05$). The storage modulus (G') of SF-PH added samples was decreased as compared to the CON sample. The CON gel had a finer and more compact network as compared to the gel added with 2 and 4% SF-PH. Alcohols, carboxylic acids, ketones, and ether were the major volatile compounds present in both gel samples as indicated by the volatile profile. In addition, thiophene, 3-methyl-, contributing to squid flavor, was found in a gel containing 2% SF-PH. Overall, SF-PH with high antioxidant activities and rich in essential amino acids could be used as a squid flavorant in the threadfin surimi gel without a markedly negative impact on gelling and textural properties.

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7.10 Comparative Study on Nutritional and Microbial Quality of Fresh, Dried and Half Burned Smoked Snakehead (*Channa punctatus*) and Tilapia (*Oreochromis mossambicus*)

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Keywords: Nutritional, Microbial, Dried fish, Half burned smoked fish

Fresh, dried and half burned smoked *Channa punctatus* and *Oreochromis mossambicus* fish were procured from fish market, Kawardha, Chhattisgarh and divided into two parts, one for nutritional composition and other for microbial study. The microbial load of fresh, half burned smoked and dried *C. punctatus* were found 3.7×10^3 cfu/g, 1.7×10^3 cfu/g, 6.3×10^4 cfu/g respectively. The microbial load of fresh, half burned and dried *O. mossambicus* were 1.8×10^3 cfu/g, 1.0×10^3 cfu/g and 8.6×10^3 cfu/g respectively. In both the fish species, dried fish showed the highest bacterial count and half burned smoked fish showed least bacterial count. The proximate analysis showed that dried tilapia had highest fat content (9.58%) compare to fresh (6.29%) and half burned smoked (7.71%). Similarly, fresh *C. punctatus* showed lowest (3.79 %) and dried highest (7.15%). The high microbial load was found in dried fish procured from market can be attributed to improper handling and drying process adopted by local processor.

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7.11 Development and Quality Evaluation of Functional Protein Enriched Multigrain Bread Incorporated with Chicken Meat Powder and Fish Protein Isolate

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Keywords: Functional meat bread, Multigrain, Fish protein isolate, Chicken meat powder, Protein enrichment

Baked products are the most widely consumed foods in the world and bread is one of the most cherished products. It is rich in carbohydrates but lacks essential-quality protein. The present study was planned to enrich the pre standardized formulation of bread with chicken meat protein and fish protein isolate for improvement in its quality characteristics, which are crucial for a balance healthy existence for consumers. Three different levels of fish protein isolate and chicken meat powder were used, replacing flour in formulation, for development of functional product. The optimization of levels were carried out on the basis of physico-chemical (pH, texture and colour profiles), compositional (moisture, fat, protein, ash content) and sensory properties (8- point descriptive scale). In comparison to whole multigrain bread, the protein content of the bread samples with the inclusion of fish protein isolate and chicken meat powder were enhanced significantly. The colour profile was comparable to control product and improvement in textural attributes was observed. It was concluded that incorporation of both protein sources improved nutritional profile and textural attributes of multigrain bread and can be used as functional additives in industries.

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7.12 Physiological Characterization of Fish Scale Degrading Bacteria from the Marine Environment

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Keywords: Scale, Bio-degradation, Waste management, Bio-economy

Fish scales, which weigh typically between 1-2% of the body weight of fish, are an important component of fish waste generated post-processing. Due to their slow degradation and negative environmental impact, fish scales offer a formidable challenge for their disposal. The biological method of degrading the scales is an effective and environment-friendly method of remediating fish waste. Thus, this study aimed to identify and characterize fish scale degrading bacteria from the marine environment and employ them to hydrolyze fish scales in a laboratory scale. Fifteen isolates used in this study were identified by sequencing of partial 16SrRNA gene. Preliminary experiments using Rohu and mixed carp scales revealed that all 15 isolates could degrade the scales at varying levels in Luria Bertani (LB) broth and in simple nutrient broth containing peptone or tryptone and salt. Following this, the degradation experiments were carried out for 5, 10, 15, and 20 days using different media prepared in seawater. The moisture, protein, ash, and fat contents of degraded scale products were 15.4%, 6.02%, 44.1%, and 1.02%, respectively. Of all the bacterial strains tested in this study, PF-A9 showed the highest scale degradation efficiency of 68% in LB broth prepared in seawater after five days of incubation. Other isolates also exhibited scale degrading abilities in the range of 40-60% over varying incubation periods. Some isolates such as MC-A5, SE-A7, BS-A8, BS-A12, SM-A13, VS-KT02, and LF exhibited efficient scale degrading abilities in all the media tested in this study. This study shows that bacteria of marine origin can be potentially valuable for remediating fish scale and reducing environmental impact. Further, the degradation of scales can be achieved using fish waste as the growth medium which would make the process economical and ensure a circular and bio-economy towards utilizing all forms of fish waste generated in fish processing industries.

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7.13 Effect of Brine Treatment on the Microplastic Abundance in Salt-cured Indian Mackerel (*R. kanagurta*)

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Keywords: Brine, Brine salting, Microplastics, Contamination

Microplastics are considered ubiquitous global pollutants. An expanding body of research on fish illustrates the significance of microplastic contamination in marine environments. The presence of microplastics in various foods including seafood has been reported, however the route of the microplastic contamination is still remaining uncovered. The present study investigates the potentiality of unrefined curing salt as the major cause of contamination in brine-salted fish products. For this study, three concentrations of brine solution – 5 %, 15 %, and 26 % were prepared from the curing salt. The fishes were subjected to brining process for 24 h to evaluate the microplastic transfer from salt to fish (n=30). The samples (edible flesh part) were taken from each treatment at every 4 h interval for examining the microplastic abundance. The microplastic abundance range in 5 %, 15 %, and 26 % brine salted mackerel after every 4 h till 24 h brine treatment was found to be 2.16 ± 0.76 to 7.33 ± 1.25 , 3.33 ± 0.76 to 8.8 ± 0.76 , 3.66 ± 0.76 to 10.16 ± 0.76 respectively. The comparison between the three treatment groups 5 %, 15 %, and 26 % revealed that the mean abundance of microplastics at 26 % were significantly higher than 5 % at all the time intervals except for the initial 4 h, but the mean abundance of microplastics in 15 % were significantly similar to both the 5 % and 26 % brine salted mackerel ($p > 0.05$) at all the time intervals throughout the salting period. The major characteristics of microplastics such as size, color, and morphology were $< 100 \mu\text{m}$, black, and fibres respectively. The presence of microplastics was ascertained by the application of Nile red, a fluorescent dye followed by observation under the fluorescence microscope.

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7.14 Enzymatic Activity and Characteristics of Trypsin Immobilized Alginate-Chitosan Beads as Influenced by Drying Methods and Trehalose/Glycerol

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Keywords: Alginate-chitosan beads, Enzyme encapsulation, Trypsin, Protein digestion

Digestive enzymes play an important role in digestion of foods. Various digestive enzymes such as lipase, proteases and amylase are excreted into the gastrointestinal tract for hydrolysis of fats, proteins and carbohydrates, respectively. Proteases, especially from microorganisms, have been widely employed for digestive enzyme supplementations. Despite their availability, high price is still a main concern for its application. Thus, the alternative and cheap proteases are of interest, where the process cost can be lowered. Fish viscera are inedible portion generated in huge amounts, turn into a wastage and create the pollution. These wastes are identified as a promising source for digestive enzymes, particularly trypsin. Skipjack tuna trypsin loaded beads were obtained by chitosan and alginate ionotropic gelation. Impact of drying methods (air-drying and freeze-drying) on characteristic and relative trypsin activity of skipjack tuna trypsin loaded beads were studied. Freeze-drying method had more homogeneous, spherical and smoother surface with larger size of beads than those of air-drying. When comparing between freeze-drying and air-drying methods, the higher relative activity was obtained in freeze-drying method, especially after simulated gastric digestion. Sugar such as trehalose and/or sugar alcohol like glycerol have been used in encapsulating materials to stabilize the enzyme, particularly during drying. Trehalose and/or glycerol at different concentrations (2.5 and 5%, w/v) were added in encapsulating materials and proteolytic activity of entrapped trypsin after freeze-drying and after simulated gastric digestion was monitored. Freeze-dried trypsin loaded beads with 5% glycerol showed highest relative activity after gastric exposure. Moreover, highest degradation was attained when fish mince and sodium caseinate were hydrolyzed with freeze-dried trypsin loaded beads containing 5% glycerol at a level of 100% of proteins tested in *in vitro* simulated gastrointestinal tract. This was indicated by highest degree of hydrolysis and drastic protein degradation. Therefore, freeze-dried trypsin loaded beads containing 5% glycerol had better physical characteristics, which could withstand acidic condition and retain trypsin activity, thus favoring proteolysis when delivered to intestinal tract. Consequently, hydrolysis of proteins was enhanced. This could be beneficial for aiding the patients with maldigestion such as elderly, etc.

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THEME VIII

Capacity Building and Socio-Economic Empowerment







8.1 Employment Scope for Fisheries Professionals in India and Chhattisgarh: A Case Study

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Keywords: Fisheries professional, Scope, Opportunities, Fisheries sector

Introduction

India is the sixth largest producer of fish and second largest producer of inland fisheries in the world. The fisheries sector provides employment to about 14 million people in fishing and related activities directly or indirectly. For each person employed in capture fisheries and aquaculture production, many jobs are created in the allied activities including fish harvest, post-harvest, pharmaceuticals, bio fuel production, aqua-clinics, organic manure etc. During the past five decades, fish production has increased at an annual rate of 4.1% though the manpower requirements in fisheries sector is the major bottleneck. In India, requirement of fisheries graduates is more than 15,457 by 2025 against a supply of only 4,570 students per annum (Agrawal et al 2016).

Materials and Methods

The survey was conducted of existed various public and private sector are involved for fisheries development in the state directly or indirectly such as state fisheries department/colleges/Institution/Federation/Center/Company Group/Farmer etc. The study was based on secondary data. The secondary data were collected from various reports, journal and annual reports of the government department as well as venture sources of related individual information.

Results and Discussion

Fish seed hatchery of capacity 1.0 to 5.0 crore fry would need 8-10 fisheries graduates and 12-24 technicians. In addition, for genetic integrity conservation and management additional 4-5 professionals with post graduate and 2-3 with doctorate are needed. In fish feed industry, A major unit of 10,000 tonnes per annum employs about 100 fisheries graduates and they would require an additional 250 by 2025. The fisheries departments of Chhattisgarh have projected an additional need for 2,838 posts by 2025 taking the total to 7,684 by that year. The study was revealed that predicted requirement of fisheries professionals were average 8131/year in state and 7598/year in out of state during 2021-2025 (DoF, 2021). Chhattisgarh state has only one College of Fisheries situated under Dau Shri Vasudev Chandrakar Kamdhenu Vishwavidyalaya at Kawardha (Dist. Kabirdham) with 100 outturn capacity of fisheries professionals (B.F.Sc). In addition, recently started Fisheries Polytechnic could produce only 30 fisheries technicians against the huge requirement in the sector. Need to increase the number of institution with required staff for excellent development of fisheries and optimum utilization of natural reassures in Chhattisgarh.

Table 1 Average intake and outturn of students in fisheries sciences

Undergraduate (UG)	Postgraduate (PG)			
	Intake	Outturn	Intake	Outturn
College of Fisheries	100	100	6	6
Fisheries Polytechnic	30	30	-	-

Table 2 Requirement of Fisheries Professionals in fisheries sub-sectors 2021-2025

Sector	Expected Requirement	Increased Requirement/ Year	2021	2022	2023	2024	2025
Aqua Farms	29x20=580	10%	580	638	702	772	849
	59x10=590		590	649	714	785	864
	126x5=630		630	693	762	839	922
Fish Seed Hatcheries	69x10=690	10%	690	759	835	918	1010
	09x5=45		45	50	54	60	66
Fish Feed Industries	4x150=600	13%	600	678	766	866	978



Fish Processing Industries	1000 for 5% of total production	20%	1000	1200	1440	1728	2074
Fish Meal Industries	300 for 5% of trash fish	10%	300	330	363	399	439
Ice Plant	4x40=160	10%	160	176	194	213	234
Fishing Gear Industries	150	10%	150	165	182	200	220
Total			4745	5338	6012	6780	7656
Research, Development and Extension							
State Government							
Department of Fisheries	1846	3%	1846	1901	1958	2017	2078
Krishi Vigyan Kendra	26	3%	26	27	28	28	29
Assistant Professor	35	3%	35	36	37	38	39
Total			1907	1964	2023	2083	2146
National Level							
MPEDA	79	5%	79	83	87	91	96
Krishi Vigyan Kendra	400	3%	400	412	424	437	450
Assistant Professors	682	3%	682	702	724	745	768
Financial Institutes	5000	2.5%	5000	5125	5253	5384	5519
Entrepreneurship	1000	5%	1000	1050	1103	1158	1216
Total			7161	7372	7591	7815	8049
Grand Total			13813	14674	15626	16678	17851

Fisheries sector was quickly developed area in Chhattisgarh. In the state requirement of fisheries professionals in fisheries will be increased. The supply of fisheries professionals in both private and public sector were less as compared to demand in Chhattisgarh. The study was revealed that predicted requirement of fisheries professionals were average 8131/year in state and 7598/year in out of state during 2021-2025.

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8.2 Constraints in Capacity Building of Field Veterinarians through ICT Tools in Punjab

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Keywords: Constraints, Capacity building, Field Veterinarians, ICT

Introduction

Punjab is ranked at the top of major Indian states in terms of agriculture and livestock sector. Being an integral part of it, livestock plays an important role in uplifting rural economy with contribution of about 1/10th of the gross state domestic product of Punjab. All of this was possible with the help of programmes implemented by the Department of Animal Husbandry, Punjab, throughout the years promoted via posters, pamphlets, news and social media. The pace at which our growth and development is accelerating it is hard to keep up and share our work and progress through print media. There is a dire need of inclusion of Information and Communication Technology (ICT), for instant outreach of information to the society. The advantages of ICT are endless, from instant communication to global availability of information in seconds and educating field veterinarians. No doubt, ICT is the best tool which one can use to reach out, still very few of the field veterinarians are well acquainted with it. Therefore, an effort has been made to study the constraints in capacity building of field veterinarians through ICT tools in Punjab and suggestions to improve it.

Materials and Methods

The constraints in capacity building of field veterinarians through ICT tools in Punjab were studied through pre-designed and pre-tested questionnaire. Fifty-four field veterinarians were selected randomly from all over Punjab. The information regarding constraints in capacity building of field veterinarians through ICT tools were collected through personal interview methods. The data was analyzed by using the software package SPSS version 18.0 and results were tabulated to know the constraints in capacity building of field veterinarians through ICT tools and suggestions to improve it, thereof.

Results and Discussion

Among the constraints in capacity building of field veterinarians through ICT tools, the majority of the respondents (94.4%) admitted that improper infrastructure for ICT is the major hassle (Rank I) in utilization of ICT by field veterinarians (Table 1). This was followed by inadequate number of ICTs in veterinary hospitals/dispensaries (83.3%), lack of training in ICTs (83.3%) and non-availability of audio-visual aids in veterinary hospitals/dispensaries (77.8%). Further, internet connectivity (72.2%, Rank IV), interrupted power supply (72.2%, Rank IV), lack of computers (70.3%, Rank V) and lack of knowledge in using ICT tools (66.6% Rank VI) were also some comparatively less important constraints among field vets towards capacity building through ICT tools.

By a brief discussion with the field veterinarians, it was suggested that proper infrastructure facilities (89.3%), regular training and workshops on ICT utilization (78.3%) in addition to the provision of ICT tools (74.6%) like laptops, tablets, computers etc. along with a supply of high-speed internet connection (66.5%) and power supply (62.0%) should be provided for smooth functioning and better communication. ICT usage should be promoted by improving social participation of veterinarians by making formal groups on various social media apps (55.8%).

Table 1 Constraints in capacity building of field veterinarians through ICT

Sr. No.	Perceived Constraint	Frequency	Percentage	Ranking
1.	Lack of infrastructure facility for ICTs	51	94.4	I
2.	Inadequate number of ICTs in V.H/V.Ds	45	83.3	II
3.	Lack of training in ICTs	45	83.3	II
4.	Non availability of audio visual aids in V.H/V.Ds	42	77.8	III
5.	Internet connectivity problems	39	72.2	IV
6.	Interrupted power supply	39	72.2	IV
7.	Lack of computers	38	70.3	V
8.	Lack of knowledge in using ICT tools	36	66.6	VI

V.H.: Veterinary hospitals; V.D.: Veterinary dispensaries





Lastly, the provision of audio-visual aids (projectors, televisions, and computers) should be given priority (71.8%) to encourage and promote the ICT utilization among field veterinarians of Punjab. These results are in conformity with the findings of (Sireesha et al 2014, Baig et al 2016).

The study conclusively revealed that improper infrastructure, inadequate number of ICTs, insufficient number of training programmes, and non-availability of audio-visual aids were the major constraints that need to be addressed to maximize the development and capacity building of field veterinarians through ICT tools in Punjab.

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8.3 Hurdles in Capacity Building and Adoption in Fish Production in Punjab

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Keywords: Hurdles, Capacity Building, Adoption and Fish Production

Introduction

Fish is a reasonably priced and important resource of animal protein which contains vitamins, minerals and oils with low level of cholesterol. Gradual increases in human populace, revenue and demography have brought about a rise in fish consumption due to its nutritional significance over the years. Therefore, to ensure we are food secured, we need to increase the production of protein-based foods for this ever-increasing population through sustainable fish farming. The State of Punjab witnessed a rapid growth in the aquaculture sector in the last two decades. Aquaculture production per ha in Punjab is more than double the national average production of 2600 kg/ha/year. Farmers are gradually moving from the traditional practice to advanced and improved methods of fish cultivation with a focus on fish feed as a means of increasing output. Despite rapid growth in the aquaculture sector, there are still hurdles faced by farmers in capacity building and adoption of fish production like lack of awareness, feed adulteration, low water quality, high input cost and non-availability of irrigation water. Viewing these facts, the present study designed to investigate the hurdles faced by farmers in capacity building and adoption of fish production in Punjab.

Materials and Methods

The present study was conducted in 2022 to study the hurdles faced by farmers in capacity building and adoption of fish production in Punjab. Forty farmers were randomly selected and interviewed with a pre-tested questionnaire. A set of 13 items containing the hurdles faced by farmers in capacity building and adoption of fish production were presented to the respondents. The information about independent variables viz. age, and education was collected with the help of structured schedule and scales. The data were analysed by using the software package SPSS version 18.0 and results were prepared to know the hurdles as well as suggestions to overcome it.

Results and Discussion

The distribution of age of the selected farmers in Punjab revealed that the respondents of all age groups were involved. However, young farmers (less than 30 years) were less interested (15%) in aquaculture compared to old age groups. It is interesting to note that about 35% of the farmers belong to the above 50 years age group and the majority of them belong to middle age (30-50), constituting around 50%. For any occupation, knowledge is necessary as it helps to improve technical efficiency and maximise production and profit in a sustainable manner. Lack of education was one of the major hurdles in capacity building as education plays a vital role in the adoption process because it is easy to understand and get required information by educated persons than the illiterate ones. Our survey revealed that 22.5% of the respondents were illiterate. Among the literate respondents, only 15% had primary level education, while 27.5% and 35% of the respondents were up to secondary and higher secondary, respectively. High cost of infrastructure (75%) and feed (70%), lack and expensive laboratories (67.5%) were the major hurdles faced by farmers in the adoption of fish production along with high cost of disease-free certified fish seed stock (67.5%). Lack of access to processing and marketing facilities (65%) and delay in the release of subsidies (47.5%) followed by lack of skilled labour (47.5%), lack of cooperatives and FPOs (40%) further discourages farmers to adopt fish production. Lastly, lack of knowledge about extension programs (30%), government schemes (32.5%), management practices (30%) and disease prevention (30%) proved to be hurdles in capacity building. The findings are in agreement with the results reported by Omitoyin & Osakuade, (2021) and Singh et al. (2015).

Development of fish farming clusters, promoting fish farmers producer organizations (FFPOs), cooperatives and contractual farming models for fish farming are the potential ways to overcome existing limitations through collective efforts. Regional extension camps can create awareness, and interest and helps in capacity building for fish production. Timely release of subsidies can immensely benefit the farmers and encourages them to adopt fish production. Ensured low-cost high-quality supply of certified fish seed from registered hatcheries through an agency in the government or private sector is required. Lastly, identifying clusters of farmers involved in aqua-culture and establishing government-funded labs for easy access and affordable testing can benefit the farmer in the long run.



Table 1 Frequency, percentage and ranking of hurdles

Hurdles	Frequency (n=40)	Percentage	Ranking
Lack of education	9	22.5%	IX
Lack of knowledge about government schemes	13	32.5%	VII
High cost of infrastructure	30	75%	I
High cost of feed	28	70%	II
Lack/high cost of disease-free/certified (SPF) fish seed source	27	67.5%	III
Lack of skilled labour	19	47.5%	V
Lack of awareness about extension programs	12	30%	VIII
Lack of fish FPOs/cooperatives/contractual farming	16	40%	VI
Inadequate knowledge about management practices	12	30%	VIII
Lack and expensive laboratories for seed and water testing	27	67.5%	III
Lack of access to processing and marketing facilities	26	65%	IV
Lack of knowledge about diseases and its prevention	12	30%	VIII
Problems in subsidy release/delay in subsidy release	19	47.5%	V

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8.4 Constraints in Empowerment of Rural Women for Self-Sustainability

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Keywords: Constraints, Empowerment, Rural women, Self-sustainability

Introduction

Indian rural economy is run by either agriculture or livestock sector and woman plays a vital role in both the sectors, as they are largely a household enterprise. Nearly 78% of women are engaged in agriculture as compared to 63% of all economically active men. Approximately 50% of agricultural and livestock workers are women, whereas in rural India the percentage is as high as 84% (Kumari et al 2016). Although, women play a significant role in agriculture and allied sectors but still their contributions are ignored since ages. Even women have less access than men to agricultural related assets, inputs and services. Only 11% women have access to land holdings, that too, mostly as small and marginal farmers. Farm produce is marketed commonly by men and that gives them complete control over household finance. Only 5% of women farmer are benefited from extension services (Tiwari 2010). Despite their eagerness they have often not been able to take advantage of opportunities from new technologies, innovations and markets. Viewing these facts, the present study was designed to investigate the constraints in empowerment of rural women as its specific objective.

Materials and Methods

The present study was conducted in 2022 to study the constraints in empowerment of rural women in Punjab. Sixty rural women were selected randomly and interviewed with a pre-tested questionnaire. A set of 12 items, containing information on constraints in empowerment of rural women in Punjab were presented to the respondents. The information about independent variables viz. age, marital status and education was collected with the help of structured schedule and scales. The data was analysed by using the software package SPSS version 18.0 (SPSS, 2009) and results were compiled to know the constraints as well as suggestions to improve it.

Results and Discussion

Average age of respondents who participated in this study was 38.8 years and most of them (66.7%) belonged to 30-55 years age group. Out of total respondents about 58.3% were under matric, 20% were graduates and 16.7% were without any formal education. About 80% women were married whereas rest of women were single parent (15%) or unmarried (5%).

Among the constraints in empowerment of rural women, majority of the respondents (86.7%) admitted that the dual role at farm and home is the main hurdle (Rank I) due to increase in the drudgery workload of women (Table 1). This was followed by lack of extension services (80%, Rank II) especially for the women and lack of awareness about Women Empowerment Schemes (73.3%, Rank III). Male dominance, lack of credit (70% each, Rank IV) as well as lack of improved tools specific for women (66.7%) were the other major constraints in women empowerment. Lack of land ownership (61.7%), lack of decision making (56.7%), lack of technological innovations for women (55%) and lack of knowledge and skills (45%) further impeded the growth of women. Lack of ICTs especially mobile phones (35%) and lack of education (41.7) also played a role in impeding the process of empowerment of rural women. The findings are in agreement with the results reported by Kumari et al (2016). The results highlight that there is dire need to empower rural women by specifying their role in farming. The development of improved tools, technological innovations and awareness towards government schemes, improving knowledge level, women oriented extension services, access to ICT tools, land ownership and credit facility are the potential ways to make them self-sustainable.

Table 1 Constraints in empowerment of rural women for self-sustainability

Constraints		Frequency (n=60)	Percentage	Ranking
Lack of education		25	41.7	X
Dual role at farm and home		52	86.7%	I
Lack of land owners		37	61.7%	VI
Lack of credit	No loans	42	70%	IV
	Loans procured	18	30%	



	Used by women	9	50%	
Male dominance		42	70%	IV
Lack of decision making		34	56.7%	VII
Lack of improved tools specific for women		40	66.7%	V
Lack of knowledge and skill		27	45%	IX
Lack of extension services especially for women		48	80%	II
Lack of awareness about women Empowerment Schemes		44	73.3%	III
Lack of access to ICTs	Mobile	21	35%	XI
Lack of technological innovations for women		33	55%	VIII

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8.5 Geospatial Analysis for Sustainable Aquaculture Expansion: A Case Study on Water spread area Mapping and Fish Production Potential in Dimbhe Reservoir, Maharashtra

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Keywords: Normalized difference water index, Water spread dynamics, Water presence frequency, Bathymetry, Fish culture

Introduction

Indian reservoirs with an area of 3.91 million ha possess a lot of potential for fisheries that offer economic and social security, especially in rural areas. Poor stocking compliance & lack of information about seasonal water availability for aquaculture activities are mainly causing low productivity in Indian reservoirs. By FY 2024–25, the Government of India aims to increase the current fish production in reservoirs through cage culture from 2.44 lakh metric tonnes to 6.29 lakh metric tonnes. But planning aquaculture and fisheries activities in a reservoir require knowledge of water spread dynamics and potential fish culture areas. Geospatial technologies in inland water resource management and decision-making for fish culture have been widely used around the world. The current study used Sentinel II Multispectral Instrument (MSI) images (2019 to 22) to examine the water spread dynamics and define potential locations for intensive fish culture in Dimbhe reservoir, Maharashtra, India.

Materials and Methods

Mapping of the spatial extent of the water spread area (WSA) for identifying the potential sites, area for stocking, & fish culture in the reservoir has been done using Normalized Difference Water Index (NDWI) and Water Presence Frequency (WPF) of Sentinel II MSI (2019-22) images for the month of Feb (Rabi season) which is considered as water available for at least 8 months, and May (Zaid / summer season) which shows the minimum water extent which is available in a waterbody for at least 11 months, and total average fingerlings requirement, fish culture yield potential was calculated. The WPF was generated and mapped using seasonal and perennial water spread area in the 2019-22 monsoon season. Bathymetry study was also performed using the Digital Elevation Model and kriging interpolation to determine suitable locations for fish culture in the reservoir, which is critical.

Results and Discussion

The results show that the WSA declined to 30% between the two time periods, with 70.44% (1445.39 ha) of the area covered with water for 8 months and 39.60% (812.45 ha) retaining water for 11 months, respectively. Estimated average number of fingerlings required to utilize the available water in Dimbhe reservoir is 0.56 million with a production potential of 112.88 metric tonnes. It was found that the perennial water spread area available in the Dimbhe reservoir is 551.22 ha, comprising 26.9% of the total reservoir which is most suitable for permanent cage culture practices. More than 66% water availability is shown in the perennial area, with maximum depth areas (20-33 meters) suited for cage culture and lower depth regions also suitable for pen culture due to water availability and shallow depth throughout the year. Based on the water dynamics and depth of Dimbhe reservoir, a culture-species matrix has been developed to provide an overall concept of the feasible culture methods and candidate species. In

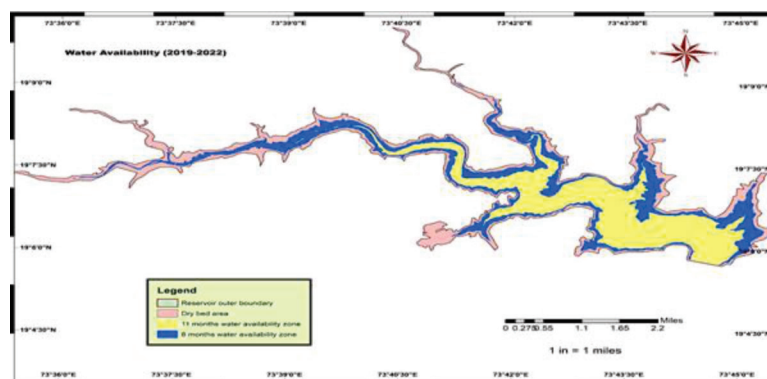


Figure 1 Dynamics of the watershed in Dimbhe reservoir

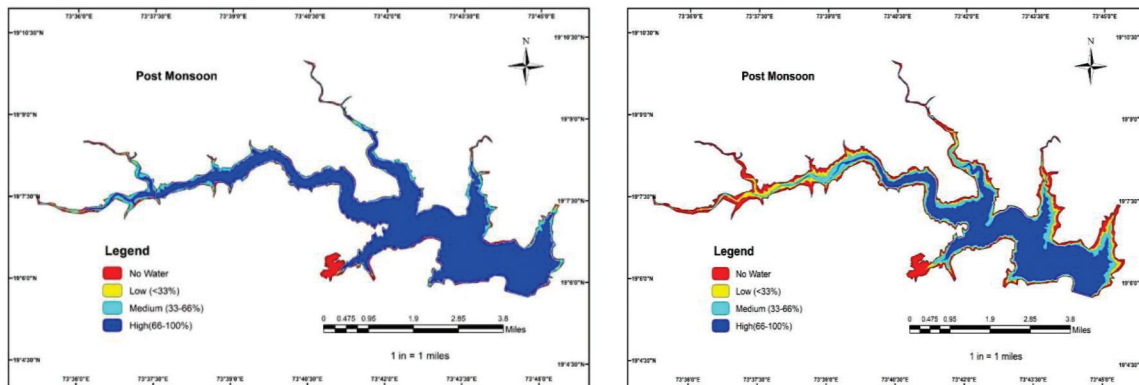


Figure 2 Comparison between post monsoon and pre monsoon water dynamics

2022, the stocking density is 9 lakh, which is significantly lower than the predicted stocking density (0.56 million) & proposed to be increased in future in Dimbhe reservoir. The selection of ideal culture methods and prospective species based on location would aid in the optimal usage of the previously underutilized reservoir. This research can be useful in planning scientific ranching and the advancement of cage culture in areas, which can be replicated in other reservoirs for tapping the fish culture potential and to plan appropriate interventions for reducing their yield gap.

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8.6 Status of KVKs and Fisheries in Coldwater Regions of India

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Keywords: KVK, SMS, Training needs, Constraints, Coldwater regions

Introduction

KVKs provide farm extension services at the district level through regular On-Farm Trials (OFTs), Frontline Demonstrations (FLDs), and capacity development of farmers and extension personnel. KVKs play a vital role in enhancing the agricultural production. Though Coldwater fisheries contribute relatively less to the total inland fish production, huge potential exist in some regions where KVKs can play a catalytic role. This study assesses the present status of KVK-led fisheries extension services in Jammu and Kashmir, Himachal Pradesh and Uttarakhand, as well as SMS training needs and constraints in J&K.

Materials and Methods

The study was conducted during May, 2022 and relied on secondary data sourced from the annual reports of ATARI Zone I, Ludhiana, ICAR KVK portal, individual KVK websites and reports. A general profile of these KVKs in Punjab, Himachal Pradesh, Uttarakhand and Jammu Kashmir was prepared and compared. SMS responses for training and information need as well as constraint analysis was only taken from U.T. of Jammu & Kashmir using a structured online survey. Responses were collected from Subject Matter Specialists (SMSs) of each KVK except Doda. Out of 76 SMS, 40 responded (53%) to the online survey. Few responses had to be collected using a telephonic interview. A simple percentage analysis was done to bring out the salient aspects of present status. For need assessment and constraint analysis, responses were taken on 3 point and 5 point Likert-like scale respectively. Weighted average method was used for ranking the needs and constraints in order of importance. Weighted average for each constraint was calculated by multiplying frequency of each constraint with respective weight.

Results and Discussion

The results revealed that majority of KVKs are under SAUs in all the three regions. Most of the KVKs in J&K were established during 2005-2014 while in Himachal Pradesh and Uttarakhand majority were established during 1995-2004. Among the SMS, only 4% were fisheries SMS in J&K, 2% in Uttarakhand (UK) while there was none in Himachal Pradesh (HP). Digital presence of KVKs was modest with only half the KVKs in J&K having own the websites. During 2017-2021, only 1% of the total OFTs/FLDs/trainings conducted by KVKs were related to fisheries in J&K and UK while no fisheries related activities were reported for HP. Vacancy levels in KVKs were found to be high with 37.5% of SMS positions in J&K, 25.9% in HP and 46.1% in UK lying vacant. The average age of SMS in J&K was 42 years with 90% being men. SMS spent 25 % of their time on extension and advisory services, on an average. The most felt fisheries related training needs were trout culture and Best Management Practices in fisheries / aquaculture. Formation of producer organization, identifying training needs of farmers, and ICT application were the top extension related needs perceived by them. Lack of fish rearing unit, poor staff strength, work load and lack of fisheries experts were the reported constraints. Thus, it is recommended that the KVKs in areas with potential water resource for fisheries should focus on fisheries extension also and should have Fisheries SMS positions. The existing vacancies in KVKs should be filled with skilled professionals at the earliest. Demonstration units (fish rearing units) may be established in identified districts with underutilised potential for fisheries. Websites of all the KVKs, along with extension literature, need to be updated regularly in both English and the state language. Trainings should be given to SMS based on the identified thematic areas.

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8.7 Performance Evaluation of Fisheries Cooperative Societies in Selected Districts of Uttar Pradesh

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Keywords: Fisheries co-operative societies, Level of performance, Organizational atmosphere, Lucknow, Gorakhpur

Introduction

Different studies indicated that more than half of the aquatic area in Uttar Pradesh (U.P.) is underutilization and if managed and utilized efficiently it will help to increase the fish production of the state. According to rough estimate of the Directorate of Fisheries (DoF) U.P., about 26,000 people are engaged full time in fishing-related activities. In Uttar Pradesh Fisheries cooperatives are suffering from acute sickness. Most of these co-operatives are now functioning as pocket organizations of few strongholds that operate and run these organizations without consulting and involving its members in planning and decision-making process. Lack of transparency and trust deficit among members makes cooperative weak also. Further, there is very less research work carried out on the functioning, working pattern and organizational atmosphere of fisheries co-operative in U.P. Keeping in view of the above problems, the present study was planned to investigate the performance and factors influencing the functionality of fisheries co-operatives in the selected districts of Uttar Pradesh.

Materials and Methods

The study was conducted in purposively selected Lucknow and Gorakhpur divisions because of the highest number of active fishery co-operatives i.e. 138 and 113 respectively. Further from Lucknow division Hardoi (29 fishery cooperatives) and Sitapur (26 fishery cooperatives) and from Gorakhpur division, Gorakhpur (45 fishery cooperative) and Deoria (31 fishery cooperatives) districts were purposively selected because of highest number of fishery co-operatives. Further 13 fishery co-operatives from each district and 5 numbers of respondents from each co-operative were selected by using random sampling method. Altogether 52 co-operatives and 260 respondents were selected from 4 districts. To track performance of cooperative societies, Cooperative Performance Index Tool was used. Organizational Atmosphere and Organizational Commitment were the two factors used for measuring the achievement of fishery cooperatives. Mean frequency and standard deviation was used to interpretate the data. Performance and achievement of cooperative society was categorized into Low, Medium and High.

Results and Discussion

The social profile of the respondents of all 4 selected districts revealed that out of 260 respondents 104 respondents (40%) were of young (up-to 35 years) age group, study also revealed that 174 respondents (66.92%) had schooling from middle school to college level and more than half of the respondents (59.62%) were from Other Backward Category who engaged in fisheries activity. One of the major finding of this study is that majority (92.69%) of the respondents did not had their own pond.

Table 1. Performance of Fishery Cooperative Societies

(N=52)

Level of Performance	Deoria M=87.85 SD=8.07		Gorakhpur M=75 SD=7.29		Hardoi M=83.46 SD=7.04		Sitapur M=80 SD=9.02		Pooled
	F	P	F	P	F	P	F	P	
Low ($<\bar{x} - SD$)	2	15.38	3	23.08	1	7.69	2	15.38	8
Medium ($<\bar{x} \pm SD$)	9	69.23	9	69.23	10	76.93	9	69.23	37
High ($<\bar{x} + SD$)	2	15.38	1	7.69	2	15.38	2	15.38	7
Total	13	100	13	100	13	100	13	100	52

F= Frequency, P=Percentage, M=Mean, SD= Standard Deviation

As per the CPI tool and data of table 1 revealed that in both of the divisions i.e., Gorakhpur and Lucknow, the performance of the fishery cooperative societies were of medium level.



Table 2 Organizational Atmosphere of Cooperative Societies for members (N=260)

Level of Atmosphere	Deoria M=61.02 SD=9.93		Gorakhpur M=60.62 SD=9.41		Hardoi M=58.12 SD=9.66		Sitapur M=50.52 SD=8.81		Pooled
	F	P	F	P	F	P	F	P	
Low (< \bar{x} -SD>) Unfavorable	12	18.46	14	21.54	14	21.54	11	16.92	51
Medium (< \bar{x} ±SD>) Moderate	41	63.08	40	61.54	39	60	45	69.23	165
High (< \bar{x} +SD>) Favorable	12	18.46	11	16.92	12	18.46	9	13.85	44
Total	65	100	65	100	65	100	65	100	260

Organizational Commitment of Cooperative Societies for members (N=260)									
Level of Commitment	Deoria M=49.37 SD=9.70		Gorakhpur M=49.06 SD=8.97		Hardoi M=48.20 SD=9.15		Sitapur M=52.29 SD=7.09		Pooled
	F	P	F	P	F	P	F	P	
Low (< \bar{x} -SD>)	7	10.77	9	13.85	8	12.31	12	18.46	36
Medium (< \bar{x} ±SD>)	47	72.31	44	67.69	46	70.77	42	64.62	179
High (< \bar{x} +SD>)	11	16.92	12	18.46	11	16.92	11	16.92	45
Total	65	100	65	100	65	100	65	100	260

It is clear from the table 2 that the organization atmosphere of cooperative societies were of moderate level for majority (63.46%) of respondents from all 4 districts. We can say that it is the perception of the respondents which makes the atmosphere good or bad for work and majority (68.85%) of the respondents were moderately committed for their cooperative society. The above results showed that the performance, organizational atmosphere and organizational commitment, all were of medium level. Further moderate level of organizational atmosphere and organizational commitment shows that there is correlation between both of them and these two variables also affects the performance of cooperative societies which was also found of medium level.

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8.8 Study of Factors Influencing Fish Consumption Pattern in Punjab (India)

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Keywords: Constraints, Fish consumption, Health benefits, Pattern, Punjab

Introduction

Fish is considered as the cheapest source of animal protein and along with that, flesh of fish is generally called as white meat which has agreeable taste and flavour. As far as nutritive value is concerned, fish is comparatively superior than other animal flesh consumed by human beings, in respect to its protein content/quality, easy digestibility, omega-3 Fatty Acids, minerals and vitamins. Therefore, it is advocated that fish should be part of human diet for amazing health benefits.

Worldwide, consumption of aquatic foods has reached about 20.2 Kg per capita in 2020 – more than double of the average consumption as compared to that of 1960s (9.9 Kg per capita) (FAO, 2022). India is the second largest aquaculture producer in the world. However, fish-consuming population in India is estimated to be around 55%, with an average annual per capita fish consumption of about 9kg. Among Indian states, Tripura tops in terms of per capita fish consumption (29kg) (Handbook on Fisheries Statistics, 2020). However, per capita fish consumption is very less in the north-western region of the country including Punjab (0.4 kg). Keeping the above facts in view, an attempt was made to assess the factors influencing fish consumption patterns in Punjab.

Materials and Methods

The study was carried out in the Punjab (India) through a structured questionnaire to collect information about fish consumption patterns in the state from 2021-22. A Google form was created in English as well as Punjabi and circulated through e-mails, whatsapp groups & various social media platforms. The questionnaire included the respondents' personal details and different parameters of fish consumption pattern. The total sample size for consumer survey was 636, including the respondents from urban and rural areas. The collected information was compiled in the form of tabulating frequencies, percentages and graphs (pie and bar graphs) for efficient representation and interpretation of data.

Results and Discussion

Total 636 responses were recorded through online survey from both rural and urban areas with a contribution of 53% and 47%, respectively covering the 22 districts of Punjab. In respect to gender wise participation in survey, 56.9 % were male respondents while 43.1 % were female. On the basis of responses received, 42.8 % respondents were classified as fish eaters while the rest 57.2 % as non-fish eaters. During the survey, people from Punjab prefer to eat spineless fish like - *Wallago attu* (Mallhi), *Sperata seenghala* (Seenghara) *Pangasius pangasius* (Pangas) etc, in the form of curry or fried fish. The frequency of eating fish / fish products is lower in Punjab as majority of the respondents include the fish/ fish products in their diet, once in a month. The major reason behind less fish consumption in the state may be attributed to the consumer preference for chicken, mutton, pork etc. by non-vegetarian population of Punjab (<40%) and most of the fish-eating population prefers to eat spineless fish. The non-fish eating / vegan respondents (62.9 %) have chosen the options of religious restraints and / or lesser preference towards smell and taste to fish/ fish products for not consuming fish. The fish consumption patterns (Table 1) and knowledge of fish consumers about the benefits of fish consumption (Table 2) is summarized as below-

Table 1 Fish consumption pattern in the Punjab state

S. No.	Parameter	Response	% of consumers
1.	Preference of fish or fish products over other animal meat	Due to nutritional value	60.7
		Due to taste	37.5
2.	Form of fish consumption	Both fresh fish and fish products	46.0
3.	Frequency of eating fresh or frozen fish	Once in a month	55.5
4.	Frequency of eating fish products		60.3
5.	Preferred fish product	Fried fish	80.9



6.	Criteria for selecting fish product	Quality & taste	54.4
7.	Preferred fish species	Catfish species	41.2
8.	Source of buying fish	Local market	75.4
9.	Any problem encountered with fish consumption	No	80.9

Table 2. Knowledge of consumers about fish nutritional value

S. No.	Parameter	Response	% of consumers
1.	Parameters to decide quality and fresh fish	Smell, color, skin texture, gills color	60.7
2.	Fish as a good source of Omega-3	Yes	91.2
3.	Benefits of eating fish for human health	Yes	86.4
4.	Reason for spoilage of fish	Improper storage (Temperature & microorganisms)	57.7

It may be concluded that people of Punjab prefer spineless fish and fish products depending upon their taste. The responses recorded during this online survey about the fish consumption patterns in different districts of Punjab, represented the lower per capita fish consumption which further provokes the need to create awareness about the nutritional benefits of eating fish and promote consumption of fish among Punjabi folks.

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8.9 Analysis of Fish Markets of Khagaria District, Bihar

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Keywords: Fish, Market, Gini coefficient, Marketing channel, Marketing efficiency

Introduction

A market is a place where market-determining forces operate to establish the equilibrium price and quantity. However, the market is not the same as marketing. Marketing is a term used as a managerial process by which individuals or groups interact with each other to fulfil their needs by creating utility and exchange of products with certain terms and conditions. The fisheries sector in India has focused mainly on production-oriented efforts to increase production rather than being market-oriented to increase the producer's share in the consumer's rupee. An efficient marketing system not only ensures a greater share to the producers in the consumer's rupee but also decreases the marketing cost which may positively affect producer as well as consumer surplus. In changing scenario of today's world, the market is an important component which can really bring socio-economic empowerment for the fish farmers/ fishers. Market structure, conduct and performance are interrelated to each other. The term market structure refers to those characteristics of the market which affect the trader's behavior and performance. Market conduct refers to the patterns of behavior of firms especially in relation to pricing and their practices in adapting and adjusting to the market. Market performance refers to the economic results that flow from the industry as each firm pursues its particular line of conduct (Acharya 2004). With this background, present study was conducted to observe the structure, conduct and performance of fish markets in Khagaria district of Bihar. The district is quite familiar for captured fisheries based traditional fish markets with remarkable fluctuations in fish catch and price.

Materials and Methods

A survey was conducted using structured pre-tested interview schedule and a sample of sample size 110 was collected from fish wholesalers, retailers and producers in Khagaria district, Bihar. Analysis was done using descriptive statistics, Gini coefficient, Coefficient of Variance (CV), marketing channel, market margin, price spread and marketing efficiency methods to assess the market structure, conduct and performance of fish markets.

Result and Discussion

Fish markets in the district were found to be traditional type and price determination in the market was done through negotiation. The results of the Gini index for wholesalers, retailers and producers were estimated as 0.49, 0.49 and 0.50 respectively representing the existence of inequality among market participants. The results of the coefficient of variation of wholesaler, retailer and producer were found to be 3.70, 3.78 and 15.21 respectively. The net margin for wholesalers was found to be far greater than retailers as well as producers. The gross total market margin (GTMM) was found to be 70.16% for the wholesalers. Among the three marketing channels, channel 3 where producers directly sell produce to the consumers was found to be the most efficient channel. Therefore, the study concluded that in order to make the fish markets efficient, state marketing board and or Dept. of Fisheries, Bihar may intervene to raise the infrastructure facilities, has to bring reforms in the conventional marketing system and promote market-led extension for fish farmers/ fishers. It will directly reflect an increase in the producer's share in the consumer's rupee which in turn uplift the socioeconomic condition of the fish farmers. The development of an efficient market information system with the involvement of Department of fisheries, Bihar may help in better flow of market information among various marketing participants. This study assures policymakers in making market-oriented policies that are necessary for the socioeconomic empowerment of fish farmers/ fishers.



Table 1. Gini coefficient for the wholesalers of Khagaria district

Categories (In Kg)	Proportion of wholesaler (X)	Cumulative %	Monthly Sale	Proportion	Cumulative % (Y)	XY	G
1500 -3000	0.1	0.1	2250	0.03	0.03	0.003	0.486
3001- 6000	0.2	0.3	9000	0.12	0.15	0.03	
6001- 9000	0.4	0.7	30000	0.4	0.54	0.022	
9001-12000	0.2	0.9	21000	0.28	0.82	0.16	
12001-15000	0.1	1	13500	0.18	1	0.1	
Coefficient of variation for wholesaler, retailers and producers of Khagaria district							
Particular	Selling Price (Mean)		Standard deviation		CV		
Wholesaler	108		4		3.704		
Retailer	165.4		6.25		3.779		
Producers	101.3		14.59		15.21		

Table 2 Price spread for different marketing channels of Khagaria district

Marketing Channel	Producer's share in consumer's rupees	Price Spread
Channel -1	55.80 %	123.05
Channel- 2	65.29 %	106.49
Channel- 3	100 %	00.00
Marketing Efficiency		
S. No.	Particulars	Market Efficiency
1.	Marketing Channel -1	0.75
2.	Marketing Channel – 2	1.01
3.	Marketing channel – 3	1.58

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8.10 Biofloc Fish Farming in Kerala: Economic Viability and Resource Efficiency

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Keywords: Aquaculture, Allocative efficiency, Biofloc fish farming, Economic viability, Resource efficiency

Fish is a staple food source in Kerala because it is a coastal state. The fishing industry is crucial to the state's overall financial improvement. The Maximum Sustainable Yield (MSY) limit has been exceeded, meaning there is very little room for new fish yield from maritime fisheries. In order to increase productivity and thereby meet the growing demand for fish, the government of Kerala thus implemented new innovative aquaculture techniques including biofloc fish farming. The use of biofloc in fish farming offers tremendous possibilities for producing fish from a relatively small space. Biofloc is a relatively new method of aquaculture in Kerala and as such, is not yet very well-known. This study aims to evaluate the economic viability and resource use effectiveness of biofloc fish farming in Kerala and, in turn, evaluate its potential to be implemented as a primary aquaculture method in Kerala that contributes to meeting the state's rising demand for fish. This study, which will be based on the primary data collected from biofloc fish farmers across the state, utilizes economic indicators such as net profit, operating ratio, net benefit earnings ratio, cost benefit ratio and return on investment for measuring the economic performance of the biofloc farms. Further this study will analyse the resource use efficiency and allocative efficiency of biofloc fish farms.

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8.11 Nature's Superfood Fish- A Remedy in Reducing Jharkhand's Malnutrition

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Keywords: Essential amino acids, Omega-3 fatty acids, Malnutrition, Mitigate

Fishes are considered a nutritionally valuable part of the human diet. It contains all the essential amino acids in desirable potency for human consumption as well as superabundant in long chain omega-3 fatty acids (PUFA) which are health-friendly. Fish protein is the cheapest source of animal protein and its affordability and availability is much better than the other animal protein sources. Nutrient profiling of fishes shows that it is nature's superfood rich in proteins, lipids, fats, vitamins and minerals. Furthermore, the fisheries is an important vibrant sector in India which contributes around 1% to the country's Gross Added Value (GVA) and over 5.37% to the agricultural GVA as a whole, according to Fisheries Statistics 2018. India is the second largest fish producing country in the world after China accounting for about 6.56% of global fish production. Further as per, the Global Hunger Index (GHI) 2016, India ranks 97 out of 116 countries, it would be imperative that special focus is directed towards malnutrition especially among its children and more so for the state of Jharkhand which is one of the poorest states of the country. According to National Multidimensional Poverty Index (2015-2016) around 47.99% of the population of Jharkhand is nutritionally deprived leading towards the cases of hunger and malnutrition. Thus, towards this goal a reviewed study has been done for Jharkhand state wherein different species of fish along with its nutritional content have been undertaken which would go a long way to mitigate the nutritional issues amongst the population of the state especially children.

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8.12 Farm-ponds of Plastic Line: Potential Use for Aquaculture in Ahmednagar District of Maharashtra

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Keywords: Aquaculture, Farm-ponds, Ahmednagar, Polyline

Farm-ponds are artificial structures constructed to store surface runoff water or rainwater for the use of irrigation in semi-arid regions. Recently, Government of India has initiated several schemes to boost up the construction of farm-ponds through various subsidies, encourage farmers to reduce the risk of agricultural production, of which a large proportion is used to irrigate horticultural crops, and meet farmers' household and livestock needs. National Horticulture Mission scheme in 2005-06 has been introduced by the Government to enhance the production of horticulture crops, under which 2,039 farm-ponds were constructed in Ahmednagar district. Government of Maharashtra has launched the 'Magel tyala Shet-tale' [Polylined Farm-ponds on Demand] scheme which proves vital for the development of lakhs of farm-ponds. Out of the total farm-ponds, the highest number of 17,021 farm-ponds were built in Ahmednagar district. Aquaculture plays a vital role in global efforts to eliminate hunger and malnutrition by supplying fish and other aquatic products rich in protein, essential fatty acids, vitamins and minerals. However, farm-ponds can be used judiciously for aquaculture and can generate supplementary income for farmers. The present study highlights the potential of farm ponds for aquaculture. The investigation of this study was done through primary data collected on ground level and secondary data derived from state department, and the data was analysed using statistical tools. The data reveals that the average farm pond area was about 0.49 acres, which translated to a total farm-pond area of 10,290 acres in Ahmednagar. Most of the farmers were male between 36 and 50 years of age, were educated till secondary education level, and derived their primary income from agriculture. Thus, the socio-economic status of the farm-pond owners seemed to be suitable for fish culture. Further, farmers had 11 months of water in their farm-ponds, which is convenient for fish farming as one crop of fish culture can be done easily during that period. The biggest problem with the reported farm-ponds was its depth (average 9.46m) which restricts fish farming, as the recommended depth of fish pond lies between 1.5-2m. Hence, there is a need to perform a detailed scientific study on farm ponds with respect to fish farming and demonstrate suitable practices of fish rearing in such farm-ponds. Therefore, incisive analysis of largely untapped private farm pond water resources and exploring it for subsidiary fish farming is the need of the hour. In fact, diversified farming minimizes the risk of climate change and is a way forward for sustainable income, which aids in attaining the goals of doubling farmers' income (DFI) and 'more crop per drop' in the country.

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1.24 Effect of Papaya (*Carica papaya*) Leaf Meal on Growth of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) Fingerlings

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Keywords: Nile tilapia, Fish, Papaya leaf meal, *Carica papaya*, Feed ingredients

The present experiment was conducted for 60 days to assess the effect of Papaya (*Carica papaya*) leaf meal on growth, proximate composition and digestibility of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) fingerlings. This experiment was conducted between July and November 2022. Experiment diets were prepared by supplementing the basal diet with papaya leaf meal at different five levels i.e. control –T₀ (without papaya leaf meal) and treatments: T₁ (2%), T₂ (4%), T₃ (6%), T₄ (8%) replacing equal amount of basal diet. The fishes were fed once each day at 3% of their body weight. The parameters of water quality during the study were not negatively impacted by the diet supplemented with papaya leaf meal, and only slight variations in the parameters of water quality were observed in different treatments. However, the treatments significantly outperformed the control group in terms of weight increase, gain in % SGR, GCE and FCR. According to the findings, T₄ was recorded with the highest weight gain (38.81 gm), highest percentage weight gain (130.94 %), highest SGR (1.394), highest GCE (0.476) and best FCR (2.097). It is advised to utilise Papaya Leaf meal (8%, 8 g/kg diet) as an alternative fish feed ingredient because of the greater development rate.

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1.25 Growth Performance of Pacific White Shrimp, *Litopenaeus vannamei* (Boone, 1931) at Selected Locations in Rajasthan and Punjab in Relation to Water Quality Parameters

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Keywords: Shrimp, *Litopenaeus vannamei*, Production, Water quality, Underground saline water

The production of shrimp has experienced the highest economic growth, globally. Due to significant advancements in brackish water aquaculture, India ranks top in shrimp exports. In inland saltwater, shrimp aquaculture has also been actively growing. The present study was carried out at three selected aquaculture shrimp farms at locations *viz.* Partappura (Sri Ganganagar), Taranagar (Churu) in Rajasthan and Shamkhera (Sri Muktsar Sahib) in Punjab during the year 2022 to assess the production methodology and to document the water quality parameters in shrimp culture ponds. Two ponds from each selected locations were randomly selected. For collecting shrimp growth data and water samples, all of these selected shrimp farm sites were visited at interval of fifteen days from March to July 2022. Information about shrimp stocking rate, feed, weight gain, survival rate *etc* was obtained. Assessments were carried on the water quality parameters, such as water temperature, transparency, pH, salinity, electrical conductivity, total dissolved solids, dissolved oxygen, ammonia, nitrate, sodium, potassium, magnesium and ortho-phosphate, FCR and SGR.

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