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Anusandhan Bhawan, Bhimtal-236136
Dist.-Nainital, Uttarakhand, India

COLDWATER FISHERIES SOCIETY OF INDIA

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Coldwater Fisheries Society of India was formed with an objective to implement and expand the research and developmental activities for the benefit of various stakeholders of coldwater fisheries of India. At present the society has registered 114 life members comprising of scientist, academicians coldwater fisheries sector of India entrepreneurs, students and farmers all over the country.

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FOREWORD

First of all, I congratulate Coldwater Fisheries Society of India and its mother institute, Directorate of Coldwater Fisheries Research, Bhimtal for successfully bringing out the first issue of Journal of Coldwater Fisheries.

Coldwater region covers a wide geographical area of the country harbouring 258 species belonging to 21 families and 76 genera. At present, coldwater fisheries sector is experiencing serious threats to both biodiversity and eco-system stability due to habitat destruction/modification, overfishing/over exploitation and climate changes. In order to preserve these threatened eco-system and species for future generations and to support sustainable development, various strategies like ranching, conservation aquaculture, public awareness, habitat restoration with scientific inputs from dedicated researchers are being carried out. And I hope that this Journal of Coldwater Fisheries will provide a forum where data and ideas can be presented facilitating further development of fisheries in coldwaterregion.

In this issue, there are articles based on basic and applied research making new findings; methods which are applicable in practice. Reviews of important aspects are also included in this issue.It is expected that the journal will be highly useful to the researchers, faculty members, students, policy makers and stakeholders in bringing qualitative and quantitative improvement in coldwater fisheries sector.

I wish Coldwater Fisheries Society of India success in future endeavours.

24 September, 2018

Dr. J.K. Jena
DDG (Fy.), ICAR-New Delhi

EDITORIAL

I am glad to present the first issue of the Journal of Coldwater Fisheries. The journal is a product of the dedicated efforts of the editors, reviewers and learned members of the Coldwater Fisheries Society of India. The journal will strive for complete coverage of high quality research articles, critical reviews from a wide range of topics in fisheries resource, aquaculture, fish health, fish nutrition, fish genetics&biotechnology, extension in fisheries sector of coldwater region.

This first issue of the journal contains 15 articles covering diverse aspects of coldwater fisheries. There are articles on diversity of ornamental fishes in Manas River and role of small tributaries in ichthyofaunal diversity of rivers in Uttarakhand. Articles on ecological aspects, such as ecological impacts of dams on the fish diversity of Bhagirathi river and periphyton community structure of Namsang stream, Arunachal Pradesh are also included in this issue.

There are four articles on *Schizothorax* species; one paper deals on effect of spirulina fortified diets on growth performance of *Barilius bendelisis* and *Schizothorax richardsonii*; the other deals with length-weight relationship of *Schizothorax richardsonii* (Gray) and *Schizothorax niger*; the third one is about the reproductive biology of *Schizothorax labiatus* McClelland inhabiting river Jhelum, Kashmir and the fourth discusses about the genetic diversity and divergence of the *Schizothorax progastus* (McClelland 1839) in tributaries of the river, Ganga.

One of the research articles presents the morphological and ultra-structural changes of golden mahseer, *Tor putitora* during early development stages. In one article, nutritional values of fish species *viz.*, *Amblypharyngodon mola, Gagatado lichonema, Garra abhoyai, Glossogobius giuris, Hypsibarbus myitkyinae* and *Puntius sophore* were discussed which will be helpful in promoting fish consumption.

There is one article that discusses about the strategies to enhance aquaculture production in Northeast hill region of India. Other articles on aquaculture aspects include rearing system for larvae of *Labeo dyocheilus* and breeding performance of rainbow trout. There are reviews in this issue discussing about major challenges in coldwater fisheries, mahseer in India, farming of fish in rice fields and development of cell line of coldwater fishes and its application.

We are also sending out a call for papers to all the researchers, students, faculty members and other interested in coldwater fisheries to contribute high quality articles for our next issue. At this point, I should add that we are committed to a speedy review process for every piece of manuscript submitted to us. We endeavour to communicate regarding the papers submitted to us within four weeks with a decision about acceptance or rejection.

Finally, I'd like to thank our Editorial Board for their dedicated effort in bringing out the first issue successfully. In this proud moment of Coldwater Fisheries Society of India, we fondly remember and pay our homage to the stalwarts and predecessors who contributed significantly for the growth of coldwater fisheries sector.

Wish you a pleasant reading

(Debajit Sarma)
Editor in Chief, JCF

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Journal of Coldwater Fisheries 1(1):1-3, 2018

Major challenges through the transforming coldwater fisheries in India

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ABSTRACT

Himalayan River systems in India comprises of the Indus, the Ganges and the Brahmaputra being distinct entities harbouring precious ichthyofaunal diversity. The Schizothoracines are important dominant fishery of these river systems followed by cyprinids while there are numerous small fish species having ornamental value. Unfortunately, the fishery of Himalaya is suffering from anthropogenic stresses, physical barriers in the form of damming and hydropower projects and resulting habitat loss. Since societal development, climate change and ecosystem degradations are stemming into big threats to the natural aquatic resources and their fish germplasm, scientists and policy makers are facing several challenges for sustainable management of the aquatic germplasm resources and aquaculture production enhancement:

Keywords: Fishery resources, Sustainable development, Aquaculture diversification; Recreational fishery; Policy framework

Development of sustainable fishery resources

The subsistence and commercial fisheries of Himalayan states exploit major important species under schizothoracines (*Schizothorax* and *Schizothoraichthys* spp.) and cyprinids (*Tor* spp. and *Labeo spp.*) (Singh *et al.*, 2014). We need to update information on the population status, species diversity and habitat ecology of fish species in selected streams of Indian Himalayan so as the manage the environmental as well as societal needs (Sehgal, 1999; Singh and Akhtar, 2015; Singh and Sarma, 2017; Zaidi *et al.*, 2018). Preparation of database on native and endemic fish species of Central, Eastern and Western Himalayas will prioritize the species for restoration and special concern (Singh *et al.*, 2014; Singh & Akhtar, 2015; Singh & Agrawal, 2017). Our such monitoring will help us in conserving and restoring degraded river stretches, wetlands and other inland water bodies and their important germplasm. For enabling such activities smoothly, institutional governance and community-based management for the open water fisheries will be needed in the Himalayan region. Further, strategic environmental assessment and action towards restoring environmental flow should mandatorily support policy makers. In general, upland aquatic conservation strategies should support sustainable development by protecting biological resources in ways that will preserve habitats and ecosystems. In order to make coldwater fish diversity conservation to be effective, management measures must be broad based and some of the measures in this direction should include ranching of identified locally depleted species, conservation aquaculture to sustain imperiled species, ecological modeling to mitigate the impacts of climate change, ecosystem restoration/mitigation efforts, regulatory measures and public awareness and sensitization (Singh & Akhtar, 2015, Singh & Sarma, 2017; Singh, 2018).

Development of eco-tourism and recreational fishery

Ecotourism is now widely considered as a means to encourage community-based conservation and development through non-consumptive uses and indirect values to the natural biological resources (Singh *et al.*, 2014a,b; Paul *et al.*, 2016). The Indian Himalayas are the abode of developing, ecotourism, fish watches and recreational fishery as there is over 3800 km stretch of river streams which can be beautifully developed for the purpose as angling of mahseer and brown trout is very popular here. The recreational angling community offers a social group that positively support fish conservation as being engaged in various activities such as monitoring, research, management, advocacy and education (Pinder & Raghavan, 2013).

Aquaculture diversification

There is a vast scope and potential for enhancing fish production in hills by bringing natural Himalayan lakes located at different altitudes, newly created and existing upland reservoirs, under scientific management for fishery enhancement. The aquaculture species in hills are trout and carp, which are cultivated in ice-melt cold-water of J&K, Himachal Pradesh, Sikkim, Uttarakhand and Arunachal Pradesh. The diversification of aquaculture has got two perspectives one is the species diversification and the other system diversification. We need to bring in the available technologies such as cage culture, integrated fish farming, RAS, biofloc technology for intensification of aquaculture beside using various local and endemic fish species which have promises to the sector. It should also be attempted to reduce the single crop culture duration or annual culture of two species on rotation. Further live feed and other species-specific feeds should also be developed to adopt more number of culture species into hill aquaculture. In addition new orientation adopting culture of new endemic/ local species under improvised culture system, improved seed and feed, organic farming and application of modern molecular tool will boost up aquaculture production in the sector.

Organic trout farming

Some of the Himalayan states such as Sikkim and to lesser extent Uttarakhand are deemed as Organic states. It is important to develop protocols for organic fish farming including Organic trout farming in these states. This endeavor will attract the buyers and add value to the produce ultimately benefitting the farmers. Due to the increased health consciousness and increased purchasing power of the consumers, demand to ensure mass scale production in future will boost up.

Advanced molecular tools and biotechnology

Traditional breeding has to take advantage of biotechnological tools that are available and being developed in each potential species. Fish genomics has been emphasized recently in order to gather basic knowledge as well as application tools and resources for genetic improvement of cultivable species. Development of genomic resources has to be taken up in each prioritized species through multidisciplinary approach in order to address breeding, nutrition, health, and abiotic stress management. Since skewed sex ration has usually been observed in many important commercial coldwater fish species, milt cryopreservation should be used in gamete exchange programmes, breeding and stock improvement. Further, detailed study for sex determining genes and sex related genes should also be taken up on priority. For growth promotion, characterization of myogenic regulatory transcripts and study on gut microbiome should also be prioritized as there is need to adopt more number of coldwater fish species which are usually slow growing.

In coldwater sector, synthetic peptide based transfection need to be worked out using cell penetrating peptide for developing nanodelivery system. Besides, synthetic neuropeptide hormones viz., Kisspeptin and GnRH analogs should also be worked out for designing bioactive synthetic peptide analogs of kisspeptin and GnRH to improve the reproductive performances of important fish species for expansion of aquaculture. At the same time, synthetic antimicrobial peptides (AMPs) should also be developed to fight out residual effects of anibiotics in fish and inhibiting the growth of fish bacteria.

Health Management in Cold water Fisheries

Health management and disease diagnostics in coldwater fishery is now emerging field requiring greater attention on identification of various pathogens such as parasites, bacteria and viruses including their genomic analysis (Shahi *et al.*, 2018a,b). It is important to develop Risk reduction protocols from pathogenecity of bacteria and viruses that can benefit farmers and aquaculturists from additional science based a deeper understanding of the microbial ecology of coldwater systems and especially the aquaculture practices that influence microbial community diversity and facilitate opportunistic infections. Further, bacteria that utilize sunlight to supplement metabolic activity in mountain region are now being described in a range of ecosystems (Mocali *et al.*, 2017; Wang *et al.*, 2018). While it is likely that phototrophy provides an important competitive advantage, the contribution that these microorganisms make to the bioenergetics of coldwater ecosystems need to be worked out.

Policy framework and institutional support

In coldwater sector, very generalized policy and guidelines are available especially related to closed season, size-limit and bag-limit, lease and farmers welfare in the form of Fishery Acts of State Governments and also the wild life protection act 1972 of Ministry of Environment, Forest and Climate Change since the forest department happens to be the custodian of major aquatic resources in mountains. However, the growing fisheries and aquaculture activities requires more policy frameworks and Institutional support. Some of the aspects requiring immediate attention are: (a) Guidelines and policy for stocking in the river streams, reservoirs and lakes (b) Guidelines on intensification of trout farming practices (c) Best Management Practices (BMPs), protocols and guidelines on cage culture, and species to be cultured (d) transplantation of highly endemic species (e) Preparation of State level action plan to maintain brood stocks for all important native germplasm (e) Collection of wild stocks of ornamental fishes from rivers and other water bodies in the Himalayas and their export need to be strictly curbed. (e) Promotion of Green certification and Eco labeling of fish and fishery products from sustainable fishing/aquaculture practices.

Conclusion

Strategic Research and Development in coldwater fisheries and aquaculture will grossly heighten the potential for lateral as well as vertical expansion if the above pointed actions are implemented. In the hill regions, fish represents an essential, often irreplaceable source of high quality and inexpensive animal protein, crucial for the balanced diet of the marginally food secure communities. Keeping in view the global, national and ecological changes, a technologies based approach is required for enhancing fish production in hills on sustainable basis catering food security and employment.

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Resources, breeding, eco-tourism, conservation, policies and issues of Indian mahseer: A review

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ABSTRACT

Mahseers are distributed in the lacustrine and riverine ecosystems of many Asian countries in the Himalayan belt of Indian subcontinent, from Afghanistan in west to Myanmar in east. They have a reputation of being one of the hardest fighting game fish which attracts anglers from all over the world and manifests higher potential for eco-tourism and ample livelihood opportunities of local communities. However, due to various factors like pollution, environmental degradation, habitat fragmentation by hydroelectric and irrigation projects and indiscriminate fishing, their population in natural water bodies/ habitat had declined sharply. Development of artificial propagation of mahseers helps providing seed for stocking in natural and manmade water bodies, thus promising supplies of the fish for food and sport while assuring the conservation of this magnificent fish. This review article not only aims to summarize the published reports on various aspects related to mahseer taxonomy, resources, breeding, propagation, conservation including recent advances but also enlightens the policies and issues of mahseer fisheries in India.

Keywords: Mahseer; Conservation; Breeding; Captive maturation; Eco-tourism; Ranching

Introduction

Mahseer, an apt name for the long slim creature known to be the toughest fighter amongst freshwater sports fish and is the undisputed king of Indian rivers. It is the largest member of the carp family (Cyprinidae) in the world and reported to a size of 2.75 m (9 ft) in length and 54 kg (118 lb) in weight in their natural eco-systems (Rahman, 1989). Their habitat is characterized with high currents and fast flowing water making this fish an ultimate swimmer. The fish swims upstream and can go through rapids of 20-25 knots (Sarma *et al.,* 2009). Mahseer in the Indian sub-continent encounters in the zone (600-1200 m) of the glacier-fed Himalayan rivers with much more extended distribution to the lower reaches in many river (Sarma *et al.,* 2009). Despite their abundance at one time, the mahseer population has been declining in number and size in natural waters and is considered to be an endangered fish as per IUCN (2014) status. Mahseer is known to be an omnivore fish in its adult stage and have been found to feed on green filamentous algae, insect larvae, small molluscs, and algal coating on rocks (Sarma *et al.,* 2013). Mahseer is an intermittent feeder, besides being mainly herbivorous, is also carnivorous to a lesser extent. However, they prefer carnivorous feed at early stage during larval rearing in hatchery conditions and prefer vegetable matter during adult stages (Sharma *et al.,* 2013). It can be classified as a column and bottom feeder. Mahseer mainly spawn during monsoon i.e. May to August which is the conducive period for the maturation of this fish in natural waters (Bhatt and Pandit, 2015). It has upstream migratory behavior and prefers clean waters for breeding. During the floods, the mahseer ascends to upper reaches of the river, traversing long distances for breeding and spawning and lay eggs in sheltered rock pools, a batch of eggs at a time, repeating the process 3-4 times in a season (Bhatt and Pandit, 2015). The total fecundity of the fish ranges from 3987 to 7320 eggs in the spawners within the size range of 190 to 250 mm total length (Sarma *et al.,* 2016). The eggs of mahseer are demersal and in case there is loose mud on the bed instead of sand or gravel, they can sink and perish (Qasim, 1956a). Considering the importance of mahseers by virtue of their economic, ecological, recreational, heritage, cultural and food values in the Himalayan sub-continent, present review reports the available information on resources, breeding, eco-tourism and conservation as well as discuss on policies and issues for the holistic and sustainable management of mahseer populations.

Taxonomy of mahseer species

Carps with big scales, fleshy lips continuous at the angles of the mouth with an interrupted fold or groove across the lower jaw, two pairs of big barbells, lateral-line scales ranging from 22 to 28, and length of head equal to or greater or less than the depth of body are taxonomically considered as mahseer (Jayaram, 1999). Mahseer belong to three genus namely *Tor* (Gray)*, Neolissochilus* (McClelland) *and Naziritor* (McClelland). However, genus *Tor* constitutes the bulk of mahseer and there are different opinions of the species of mahseer available under different genus. Menon (1989, 2004) described five valid species of genus *Tor* (*Tor putitora*, *T. tor, T. khudree, T. progenius* and *T. kulkarni*) distributed in different parts of India. Desai (2003) opined that the genus *Tor* includes 10 valid species namely *Tor tor* (Deep bodied mahseer), *Tor putitora* (Golden mahseer), *Tor mosal* (Copper mahseer), *Tor khudree* (Deccan mahseer), *Tor mussullah* (Mussullah mahseer), *Tor progeneius* (Jungha mahseer) existing in India while *Tor douronensis* (Malaysian mahseer; Kottelat, 2012), *Tor tambroides* (Thai mahseer), *Tor sinensis* (China), *Tor zhobensis* (Pakistan) are found in other countries. *Tor* species is one of the most threatened groups of freshwater fish in the country and there are no reliable estimates of the number of *Tor* species found in Indian waters, mainly due to the taxonomic uncertainties within this genus (Pinder, 2013; Siraj *et al,* 2007). Out of the present valid species, *Tor khudree*, *T. kulkarni*, *T. malabaricus*, *T. Mussullah* and *T.putitora* are listed as ‘Endangered’ and *T. tor* and *T. progenius* as ‘Near Threatened’ as described in the IUCN Red List of Threatened Species (IUCN, 2014).

Neolissochilus (Rainboth, 1985) is one amongst the mahseers. The name is derived either from its large mouth or scales or from its strength to climb the hill streams like a tiger. These fishes are distributed in India and Southeast Asia and are important game as well as highly esteemed food fish, fetch high market price and are potential candidates for aquaculture. *Neolissochilus* includes more than twenty species in the world. The systematic description of the genus is given below:

The genus includes medium to large carps with barbells of Southern and South-Eastern Asia. The fishes generally migrate upstream to clear, rocky, headwaters, live in deep, slowly moving pools during wet season, and moving downstream at onset of dry season. They attain nearly a meter in length and weigh over 10 kg, but commonly found in the size of 50-60 cm and 2-3 kg (Talwar and Jhingran, 1991). The species of *Neolisochilus* which are available in India are- *Neolissochilus hexagonolepsis, N. hexasticus, N. stracheyi*, *N. paucisquamatus* and *N. stevensonii* (Talwar and Jhingran, 1990; Vishwanath *et al.,* 2009, 2011).

Relationships of genus *Neolissochilus* with *Tor*

Neolissochilus is closely related to *Tor* and occurs over the same range. However, *Tor* is differentiated from *Neolissochilus* in its development of the lower lobe into a fleshy lobe below the mandibular symphysis having a fleshy lobe (Vishwanath, 2014). Gill rakers on ceratobranchial in *Neolissochilus*is 6-9 and in *Tor* 10-16, although *Tor progeneius* may exhibit intermediate, 8-10. The species has no fleshy mandibular lobe and also no cheek tubercles, thus thought to be an intermediate between the two genera. The pharyngeal arches in *Neolissochilus* is relatively shorter and massive than of *Tor*. The articular margin with tooth bearing arm of the pharyngeal bone in *Neolissochilus* is 75^0 while in *Tor*, 45^0. Tooth commences nearer in *Neolissochilus* and farther in *Tor* (Vishwanath, 2014).

Mahseer resources

Mahseer is known to occur from the extreme west in Afghanistan (Darya-i-Kabul; Landai River which rises in northeastern Afghanistan at the base of Kowtal-e Wonay in the Paghman mountain, enters western Pakistan through the

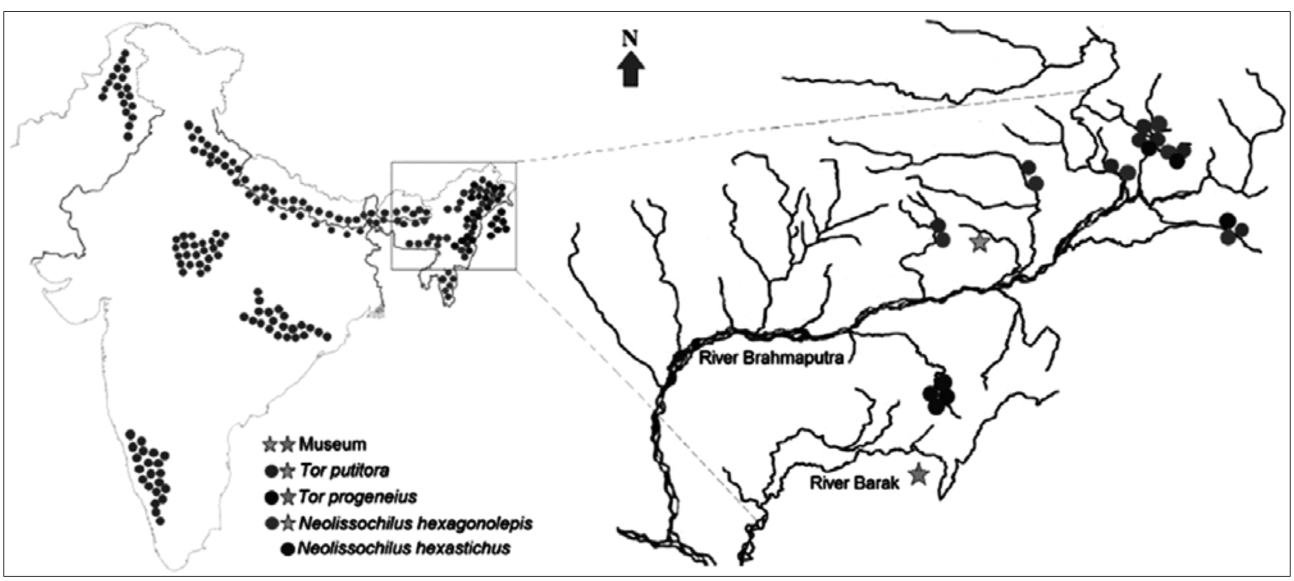

Figure: Distribution of mahseer in India with special emphasis on NE India (Laskar *et al.*, 2013)

Mohmand Hills, the rivers joins the Indus river at Attock) to eastern extremity in the Garo hills, Meghalaya, India suggesting that Mahseer is found in the rivers in and around Himalayas. *Tor putitora* is found in the Indus, Ganga and Brahmaputra river systems draining the Himalayan terrain in Afghanistan, Pakistan, India, Nepal, Bhutan, Sri Lanka and Southeast Asia as far as Malaysia (http: //www.fishbase.org).

Diversity and distribution of mahseer in North-eastern Himalaya

Northeastern region of India harbors 11 mahseer species (5 *Neolissocheilus species* and 6 *Tor species* (Vishwanath *et al.,* 2014). The river Diyung in Dima Hasao district of Assam has been reported to be inhabited by an important species of mahseer, viz., *Neolissochilus hexastichus*. Thus, the *N. hexastichus* is surviving with a small population in the said region which is perhaps its last stronghold although more survey on the occurrence of this species in other locality is attempted. Further, *Tor barakae* described from river Barak in Manipur (Arunkumar and Basudha, 2003) is considered as endemic in the Barak basin. However, until re-evaluated for its taxonomic validity, it seems to be a synonym of *Tor mosal* which was previously recorded from Barak basin and later considered as a Burmese species. In the recent study by Laskar *et al.* (2013) *Tor progeneius* has been designated as a synonym of *Tor putitora* and *N. hexastichus* has been claimed as a valid species and resurrected from synonymy with *Tor tor*. The occurrence of *Tor tor* from Northeast Indian rivers is also not very convincing and need strategic study.

Mahseer in Meghalaya

The distribution of mahseer in Meghalaya has been analyzed in three different categories- (1) Altitudinal distribution (2) Intra drainage distribution and (3) Distribution according to thermal class. In altitudinal distribution pattern species *Neolissochilus hexagonolepis* and *Tor* species have shown wide distributional pattern (Nath *et al.,* 2016). Occurrence of *Neolissochilus hexagonolepis* was common in all rivers and gradient zones ranging from 1491 m to 150 m asl. Though *Tor tor* and *Tor putitora* are widely distributed but their population in the rivers of Meghalaya is very rare. *N hexastichus* has been recorded only from the river Janiaw at Mawsynram located in 1000 m msl (Sarma and Bhuyan, 2007).

Mahseer in Kaladan river, Mizoram

Mahseer are known to occur in the rivers of Mizoram and *Neolissochilus hexagonolepis* has been recorded in river Tuirial (Sarma *et. al*, 2016). A detailed study of the Mahseer fishes has been conducted in river Mat in Mizoram, a river joining the Kaladan drainage system of the Indo-Myanmar region. The Mahseer in river Mat of Kaladan drainage seem to prefer deep pools as one of their favourable habitats in view to provide strong cover to them.

Mahseer of Manas river, Assam

It is reported that upper stretches of Manas river and its tributaries are predominantly inhabited by coldwater fish species specially of masheer (*Tor putitora, Tor tor* & *N hexagonolepis*). However, in due course of time, climate change, natural calamities and anthropogenic activities have brought a vast change in biodiversity of mahseer in Manas river system. It is observed that heavy flow of water during flood seasons has also made prolonged siltation problem in different parts of the river, causing destruction of breeding ground and loss of fish germplasm associated with various manmade factors (Dubey, 1978).

Mahseer in Jatinga river, Assam

River Jatinga has alluvial segment and pool-riffle and braided type of reach. The micro-habitat is dominated by riffle and run with occasional occurrence of trench pools. The river is less entrenched because of V-shaped valley segment and the substrate type is dominated by gravels and cobbles with the occurrence of some boulders and bedrocks; thus, providing suitable feeding and breeding habitat for Mahseer, like *Tor tor* (Ham-Buch), *Tor mosal* (Ham-Buch), *Tor progenius* (McClelland), *Neolissochilus hexagonolepis* (Kar, 2007). In addition to river Jatinga, mahseer have been recorded from others rivers in Barak valley such as *Neolissochilus hexagonolepis* in river Dhaleswari, *Tor mosal* in river Sonai (Mahanta and Sarma, 2009).

Mahseer in Jia- Bharali river, Assam

Presence of *Tor* species is significant for this river encouraging lot of tourists for rafting, fish watching and angling (Borgohain and Bania, 2013). *Tor* species found in Jia- Bharali river is *Tor tor*, *Tor putitora* and *Tor progenius*. The river also harbours *Neolissochilus hexagonolepis,* which fetches a magnificent fishery in the foot hills of Assam and Arunachal Pradesh (Sarma, 2009; Laskar *et al.,* 2013).

Mahseer in Teesta river

The most important indigenous cold water fish species of Teesta river are mahseer/sahar (*Tor* spp.) and katle (*N. hexagonolepis*). Mukherjee *et al.* (2002) recorded 125 fish species from Darjeeling hill area, some of which are found in torrential streams and are remarkably well adopted for clinging to rocks in swift currents. Many feeder streams and rivers joining the Teesta river have spawning grounds of mahseer (Acharjee & Barat, 2013; Bhatt & Pandit, 2015). Spawning beds of the Teesta river located near the Sevok may be selected for Mahseer breeding ground. In the entire stretch of the Teesta river near Sevok Bridge to 1 km upstream and 3 kms downstream are ideal environmental conditions for mahseer spawning: gravel beds, suitable water current, high dissolved oxygen content, good water quality and low turbidity. The changing river conditions affect abundance of Mahseer. However, five river valley project hampering the mahseer migration *vis-a-vis* breeding in the Teesta river system (Menon & Kohli, 2015).

Diversity and distribution of mahseer in Western ghats

Deccan mahseer in Cauvery river

The Deccan mahseer (*Tor khudree*) is known in vernacular Kannada as *Bilimeenu* (white fish) and since long is associated with heritage and belief. It is considered as *Devara meenu* (Divine fish) in some parts of Karnataka and is fed by local people in the sanctuaries and rivers associated with the temples. The mahseer are distributed in the Siwalik Himalaya and lower reaches of the uplands of the Deccan Plateau (FAO, 2003).

Although mahseer occurs at several reaches in the upper stretch of river Cauvery, three reaches are important from the abundance point of view *viz*. Kushalnagar (Walnoor to Kushalnagar), Ramanathapur and Bheemeswari (Shivasamudram falls to Mekedatu) (ZSI, 1982).

Mahseer in Chalakudy river, Kerala

Mahseer inhabits rapid rivers and streams with rocky substrates and attains a maximum size of one meter in length and 23 kg in weight in Chalakudy river (Chandrasekharah *et al.,* 2000). It is one of the common species of the Chalakudy river of Kerela and forms the major fishery for the tribes residing on the banks of the upper reaches of the river in colonies. The species has recorded a decline in the population and a need has arisen to conserve this important resource (Ajithkumar *et al.,* 1999). It may also be noted that there is ample potential for developing a few mahseer angling stations in the Chalakudy river to encourage the sport fisheries and angling tourism (Ajithkumar *et al.,* 1999).

Mahseer in Savitri river

Mahseer, the 'king' of Indian freshwater fish in Raigad is found in the Savitri river (Katwate and Apte, 2014). It is informed by the local people that this is one of the rare species in the Savitri river, locally known as *khadas*.. It is known that a large protected population of mahseer is available at Walan *kond* (Katwate *et al.,* 2014) which is one of the biggest pools in the Savitri watershed, is a notable example of a 'community fish sanctuary' in the northern Sahyadri. The complete absence of mahseer in the tributaries of the Savitri which have dams at numerous sites clearly indicates that the dams have adversely affected the distribution of this species in the river and its tributaries. The mahseer habitats in the upper catchment areas of the Savitri are still free from industrial and urban pollution (Katwate and Apte, 2014).

Deccan mahseer in Indrayani river

Deccan mahseer (*Tor khudree*) is an indigenous fish which was found in the Indrayani river flowing in India's western state of Maharashtra. The fish was last sighted many years ago until it disappeared due to excessive pollution of the waters and urbanization. But due to the efforts of a conservation organization, today the fishes have again made Indrayani their home (Dahanukar *et al.,* 2012).

The Wayanad mahseer of peninsular India

The Wayanad mahseer, *Neolissochilus wynaadensis* (Day, 1873) is one of two species within the genus that occurs in peninsular India; the other being *N. bovanicus*, found in the Bhavani river. Day (1873) described *Barbus* (*Barbodes*) *wynaadensis*, from Vythiri, Wayanad and reported it to be a common species in the larger streams of the region. The species was considered to be endemic to the streams in the Wayanad region of Kerala, until Manimekalan (1998) and subsequently, Yazdani *et al.* (2001) recorded it from Mudumalai, Tamil Nadu. Also Arunachalam *et al.* (2005) recorded it from Abbey Falls near Madikeri, Kodagu, Karnataka. *Neolissochilus wynaadensis* has been considered to be a rare species with several surveys in and around the type locality, failing to collect this species, and very few records available in the recent ichthyological literature which are backed by voucher specimens (Abraham, 2011). Due to its restricted distribution and severe population declines (up to 80%) in the last decade, *N. wynaadensis* has been listed as 'Critically Endangered' in the IUCN Red List of Threatened Species (Abraham, 2011). *Neolissochilus wynaadensis* is endemic to the Western Ghats of India (Dahanukar & Raghavan 2013). In Kodagu, *N. wynaadensis* has also been recorded in Hamyala and Kakkehole. However, no voucher specimens are available. The species is currently known between the altitudinal range of 400–1100 m. The fact that the species has a very restricted distribution, and is confined to only a few tributaries/streams in its range is additionally evident from the studies of Johnson & Arunachalam (2009). *Neolissochilus wynaadensis* prefers fast flowing upland streams and rivers where they occur in both rocky pools (Abraham, 2011) as well as riffles (Kurup *et al.* 2004). Kurup *et al.* (2004) provides information on several microhabitat variables in the locations frequented by *N. wynaadensis*. Abraham (2011) mentioned that the fish also occurs in the Chooralmala and Kanthampara areas in Wayanad (part of the west flowing Chaliyar river system). The species occurs in the Periya Forest Range near Mananthavady, Wyanad (Ali *et al.,* 2014). It is to be noted that, in a comprehensive study of the fishes of Nilgiri Biosphere

Reserve including parts of Wayanad and upper reaches of Chaliyar not a single specimen of *N. wynaadensis* was reported from Chooralmala and Kanthampara suggesting that the species could be restricted to only the east flowing drainages (Easa & Shaji, 1997). Therefore, in the absence of any references to the records, and actual voucher specimens, it is premature to conclude that *N. wynaadensis* occurs in the west flowing drainages as suggested by Abraham (2011). Further studies are, however, required to understand the diversity of this genus especially from the southern regions of the Western Ghats from where many 'nomina nuda' under the genus *Neolissochilus* (Raghavan *et al.,* 2013) as well as previous records (Pillai 1929; Hora & Law 1941) of *N. wynaadensis* are available. There is specifically a need to examine specimens of mahseer identified and catalogued in various museums. The *Neolissochilus* from peninsular India (*N. wynaadensis*) forms a monophyletic grouping with other *Neolissochilus* species from north-eastern India and Southeast Asia. Also, the specimens of *Neolissochilus* found in various locations in Kodagu (Coorg, in Bhagamandala and Mukkodlu), were genetically similar to those that occur in the type locality of the species i.e., Wayanad (Ali *et al.,* 2014). The discontinuous distribution of the genus *Neolissochilus* (Sundaland, Indo Burma, Eastern Himalaya and Western Ghats) could also form the basis for interesting biogeographic hypothesis testing for instance, to check the scenario of 'true disjuncts' (Dahanukar *et al.,* 2013). On the other hand, detailed anatomical and morphological analysis is needed to ascertain if the Western Ghats representatives of the genus are 'false disjuncts' and need a separate generic allocation. Such biogeographic and evolutionary questions should however be validated with larger multi-locus datasets (Ali *et al.,* 2014).

Mahseer resources in Himachal Pradesh

The golden mahseer is an important fauna in the rivers of Himachal Pradesh namely Sutlej, Beas, Chenab. However, in recent years due to their proximity to human intervention, Mahseer stock is threatened with multifaceted dangers posed by construction of series of dams, barrages/ weirs across the rivers on one hand and over-exploitation on the other. While uncontrolled fishing and destructive fishing devices have adversely affected the riverine population, the construction of dams are acting as physical barrier to this migratory species, tending to prevent their access to their usual breeding and feeding grounds. The denial of migration also results in permanent and irrevocable eradication of mahseer stock ranging from depletion to complete extermination (Walia, 2006). The ever-diminishing catches of Mahseer from the river Satluj, Giri, Beas, Chenab and their tributaries clearly bespeaks the effects caused by the construction of Pandoh, Chamera, Pong, Bhakra & Giribata barrages. Regardless of their height, weirs and dams constitute barriers to breeding migration of mahseer in Himachal Pradesh (Butt, 2013). Further, mahseer population is also affected by morphological modifications resulting from completion of river valley projects. These include change in slope, river-bed profile, submersion of gravel zones or riffle section as well as destruction of riparian vegetation and changes in tropic regimes. Most of the negative factors affect upper parts of the streams where lacustrine conditions are superimposed on the river. Downstream, the hydrological conditions get severely altered through reduction of water discharge. The adverse conditions of the flow can extend over many km downstream of the obstruction, so that, fish passages become difficult particularly of mahseer which have a long migratory root (Sehgal *et al.,* 1971; Mahanta and Sarma, 2009)

Indiscriminate hooking, netting, dynamiting and electrocuting have also greatly affected the mahseer availability in the Himachal rivers and streams. In the pursuit of more and more catches, even the declared mahseer sanctuaries have not been spared by the poachers (EMPNRM, 2002). Further, due to reduced availability of large mahseer in the streams, fishing pressure on juveniles is on the increase with the result that streams earlier assuring a bountiful harvest have started giving a dismal picture. Once teeming with thousands of mahseer, streams like Giri, Ashwani, Binwa Neugal, Beas, etc. the returns are sharply declining, raising the number of disgruntled anglers each year (EMPNRM, 2002).

The sharp decline in mahseer catches has also been noticed in Himachal Pradesh reservoirs. Gobind Sagar reservoir- known earlier as store-house of mahseer has recently become a silver carp reservoir. As per available information, mahseer used to constitute as high as 9% of the total catch during 1984-95 which has plummet to a level of 1% during 1999-2000 (Environment Master Plan, 2013). Pong reservoir however has steady catch of mahseer during the last two decades ranging from 60-90 t. The mahseer catches during 1999-2000 were 90 t accounting 20% of the total catch. Further, the average size of mahseer in Pong reservoir has ranged from 1.5 to 1.7 kg during the last 15 years. Against this the average size of mahseer in Gobind Sagar declined from 1.9 to 0.6 kg. during the last 15 years barring the last two years when it has increased to 1.2 kg because of number of management efforts by the fishery department (Petr, 2002). Its migration into the Kangra valley has been completely stopped due to the construction of Pong Dam across the river Beas (Petr, 2002). However, it has established self-reproducing stocks in the fragmented populations The Maharana Pratap Sagar reservoir (Popularly known as Pong Dam) in Kangra with its 42 km length and 2 km breadth, offers a variety of activities and waters are excellent for mahseer fishing. Golden Mahseer with whiskers is available in the dam whilst the silver and black mahseer is also found largely in the tributaries (Petr, 2002).

Mahseer resources of Madhya Pradesh

The Madhya Pradesh Government has declared Mahseer (*Tor tor*) a species of fish found in rivers and reservoirs of the state as state fish. The transformation of fluviatile environment into a lentic one during the impoundment has had an impact on the fish species particularly of mahseer in Gandhisagar (Dubey and Chatterjee, 1976). Mahseer species facing threat of extinction used to be richly found during sixties in the states rivers, including Narmada, Ken, Betva, Tons, Tapti and Chambal. Tawa reservoir has also witnessed extreme decrease in its abundance (Karam Chandani et al,1967). The fish fauna of Jabalpur also included *T. tor* (Malviya, 1961). Mahseer was also reported from Chambal river (Dubey and Mehra, 1959) long back and is regularly fished in Gandhi Sagar reservoir in Madhya Pradesh. Monthly landings of *Tor tor* at Hoshangabad and Shahganj centres, coming from the 48-km stretch of the Narmada during earlier days showed that the fish supported an important fishery all the year round except monsoon months. It was poor during July to September, improved considerably from October onwards, to become outstanding until June. At the Hoshangabad centre during this period the species contributed on an average of 28.0% to total landings and 46.5% to carp landings (Desai, 2003).

Diversity and distribution of mahseer in Central Himalaya

The *Tor* mahseer though reported from entire Himalaya is abundant in the Aravali, Vindhya and Satpura ranges, which can be conveniently called as Central India. The lower reaches of Yamuna, Narmada and Tapi river systems between the plains of Indus, Ganga and Deccan Plateau drain this region. Observations on the size composition of *Tor putitora* in the foothill section of the torrential coldwater Ganga between Rishikesh-Hardwar and its upstream springfed relatively placid-springfed tributaries, the Nayar and Saung and glacierfed torrential of Alaknanda, revealed that only the fingerlings and juveniles (<19 cm) constituted the resident stock in the Nayar and Saung. In the Saung and Nayar, 10-25 cm and 1-22 cm TL accounted for 11-75% and 22-75%, respectively, in the non-migratory and migratory phase (Nautiyal *et al.,* 1997).

It is recorded that the foothill section of the torrential coldwater Ganga between Rishikesh – Hardwar serves as the abode of the juvenile, adolescent and adult component of the Himalayan mahseer stock. The fishes above 15 cm were common in the Ganga. Of these, the fishes measuring 28-64 cm size accounted for 11-50% in the non-migratory phase, while 13-38% in the migratory phase. The percentage of larger fish (>64 cm) was found to be much less, 11-22%. The huge environment of the river is adequate for the fish to attain the large size for which it is well known (Nautiyal *et al.,* 1997).

The migration of *T. putitora* is completed in three phases from mid February to mid September with brooders ascending the breeding grounds, the Nayar and Saung only during peak monsoon (mid July to mid August). The delay in rains may shift the migration to late August. The juveniles move in shoals and so do the adults. Moving in schools is known to save energy (Nautiyal *et al.,* 1997).

Mahseer resources of Jammu and Kashmir

Golden Mahseer population also occurs in the Himalayan range of Jammu & Kashmir. The elegant group of sport fish, Mahseer is considered as the tiger of water in Peril (Joshi, 1987). In Kashmir valley, mahseer was the major fish having a significant socio- economic role. But in recent years it has come under threatened category of fish species and is now rare in catches with dangers posed by construction of series of dams, barrages & weirs across the river in one hand and over exploitation on the other hand. Mahseer (*T. putitora*) of river Tawi, Jammu are facing the biggest threat. The absence of great mahseer (*T. putitora*) in Jhelum is due to presence of the Mangla dam further downstream in Pakistan. This barrage lacks a fish ladder and thus cut the migration route of this anadromous fish which earlier had its spawning grounds in the Kashmir valley (Nyman, 1995). The alarming trend of its decline forced the J&K state fisheries department to establish an exclusive mahseer hatchery at Anji (Distt. Reasi), where successful breeding programme of this species being taken up since 1999.

The imporant streams which support the sizeable mahseer for angling, are Tawi, Naddar, Duddar, Jhajjarkotli, Neeladhab, Ujh, Anji, Behani and Chenab. The mahseer being a migratory fish ascends from plains to the upper reaches of Ravi, Chenab and Jhelum rivers for spawning and descends back every year (Sehgal, 1971). The presence of fry and fingerlings of mahseer in thousands in the spawning streams like Jhajjar- kotli, Duddar, Naddar, Anji, Neeladhab, Behani and Ujh is of importance. They perish when the water level in the streams reaches to a drying point (Sunder and Joshi, 1969).

Breeding and captive management of mahseer

Natural breeding of mahseer

The mahseer are the denizen in the cool water streams in the hills and survive within a narrow range of ecological factors. They mostly remain within 10-2 degree slope (steep to gentle). In the steep region, the substratum is comprised predominantly of boulders, rocks, and cobbles; while, in gentle slope region, the substratum composition becomes cobbles, pebbles, gravels, and sands (Sarma *et al.,* 2016). In the mahseer habitat, the level of dissolved oxygen remains always high due to continuous agitation and bubbling of coldwater. So, the Mahseer fishes are used to live in water having high level of dissolved oxygen, above 6 mg/L (Sarma *et al.,* 2016). Mahseer are also known as seasonal migratory fish, as they migrate upward for reproduction. Golden mahseer is an intermittent breeder and lays eggs at intervals throughout the year, but peak spawning occurs in the monsoon. During June-August, mahseer brooder migrate upwards in the river, sometimes in the tributaries in search of shallow water having pebbles and gravels as substratum and exhibit breeding at the ground conducive for spawning. Thus, there are strict habitat requirements of mahseer which comprise cold water temperature, high level of dissolved oxygen, habitat heterogeneity with occurrence of in-stream pools/ deep storage of water, ground conducive for spawning (Sarma *et al.,* 2016). The fry of mahseer settle in the shallow habitats particularly near the banks of the streams. As soon as the summer retreats, the water in the streams starts immediate recession so that some pits and pockets in the streams get disconnected from the main streams. Unfortunately, thousands of early fry of mahseer which left in such pits and pockets undergo predation and culminate to large scale mortality. The drying up of water in such pockets also causes mortality. This is a serious issue and needs attention for rescuing such fry for rehabilitating them in safe system (Sarma *et al.,* 2016).

Seed production and hatchery technology of golden mahseer

Mahseer is of considerable importance because of its large size. As a food fish, it is highly esteemed and fetches the highest market price in North and Northeast of India. Despite their abundance at one time, the population has been declining in number and size in natural waters. Its population is declining in the natural water bodies because of degradation of the aquatic environment and biological changes in the ecosystem due to urbanization as well as overfishing especially with pressure from illegal catching methods such as electro fishing, poisoning, and dynamiting. As a result, the population has become unsustainable with fish catch from fisher communities being very low in most parts of the country, and the fish is declared endangered (IUCN, 2014). Developments of breeding and culture techniques along with hatchery management practices are the means for conservation, promotion of aquaculture, rehabilitation, sustainability of mahseer population. ICAR-DCFR, Bhimtal has been doing tremendous effort and developed the protocol for seed production and hatchery management of golden mahseer at Bhimtal (Sarma *et al.,* 2009; 2010, 2014, 2016). The water flow is the crucial criteria for the hatchery management of golden mahseer (Sarma *et al.,* 2009, 2010).

Water flow	Rearing capacity
1 litre/minute	Incubation and rearing of 2,000 eggs at 20-25°C.
3-4 litres/minute	Rearing of 2,000 fry (0-3 months) at 20-27°C.
4-6 litres/minute	Rearing of 1,500 fingerlings (4-9 months old).
Flow through hatchery	
Overhead tank	1,000 litres capacity installed at a height of 5 m above ground
Hatchery tanks	Galvanized iron sheets or fibreglass of 200x 60x 30cm size.
Hatching trays	50 x 30 x 10 cm. with synthetic netting cloth 1-2 mm mesh size. 5,000-6,000 eggs can be stocked

There are important possible options for increasing the population of mahseer in natural water bodies through ranching. This involves rearing of brood stock, breeding and propagating their seed on a large scale with due consideration of genetic issues of both brood stock and wild populations, and releasing them to streams, lakes and reservoirs for rehabilitation. The ICAR-DCFR, Bhimtal has standardized the protocol of hatchery technology of golden mahseer and seed has been produced at the mahseer hatchery complex of DCFR regularly for ranching in different parts of India as well for aquaculture purpose (Sarma at al., 2016). Golden Mahseer hatchery and farm has been established at the premises of Eco -Camp ABACA, Nameri by Assam (Bhorelli) Angling & Conservation Association in March 2012 with the technical and financial assistance from ICAR- Directorate of Coldwater Fisheries Research, Bhimtal (Sarma *et al.,* 2016). Brood bank of golden mahseer at Jasingffa Aqua Tourism Resort, Nagaon, Assam in collaboration with DCFR and establishment of mahseer based eco-tourism center is a notable achievement in this area (Sarma *et al.,* 2016).

Breeding and larval development of chocolate mahseer, *Neolissochilus hexagonolepis* in pond environment

Neolissochilus hexagonolepis (McClelland, 1839) commonly known as chocolate mahseer is considered as one of the delicious foods as well as game fish species of the Indian upland region specially of Northeastern Himalayan region. However, the culture of this fish species in large scale is still not common due to lack of adequate supply of seed and knowledge of their feeding and breeding techniques among the farmers. In the natural water bodies, the population of this fish is also decreasing due to various natural and anthropogenic factors (Sarma, 2009). Therefore, the species has been brought to DCFR from Arunachal Pradesh and successfully reared and bred by Sarma *et al.,* (2014) in pond conditions. The larval development of chocolate mahseer has been carried out and it is proven to be an important fish in hill fisheries and aquaculture (Sarma *et al.,*2014). The hatchery produced seed has been stocked in Nongmahir lake Meghalaya during 2014. Also, seed has been reared in Jhora fisheries pond at Kalingpong, Darjeeling, West Bengal along with grass carp (Sarma *et al.,* 2015; Guharoy, 2012). Commendable success also has been achieved at TEC, Lonavala, Maharashtra regarding captive breeding, cage culture & river ranching of pond raised golden & Deccan mahseer (Ogale, 2014; Basavaraja, 2011). Cage culture to grow the fish from fingerling to advanced fingerling size for ranching after imprinting was successfully demonstrated at Walwhan, Lonavala by TPC jointly with CIFE in recent times (Ogale, 2014).

Mahseer in eco-tourism

Mahseer in India is regarded as the king fish for angling sport and mostly liked by anglers all over the world who considered superlative sporting quality of mahseer over the salmon (Thomas, 1881; Lacy and Cretin, 1905). The thrills and sport involved in snaring of mahseer is evident since very past, that has been documented in many famous records like "The rods in India", Thomas (1881), "Anglers Handbook of India" by Lacy and Cretin (1905) and "Circumventing the mahseer and other sporting fish in India and Burma" by McDonald (1948). The amount of fight it exerts during the time of its catching is loved by all the anglers to play with the mahseer (Baruah and Sarma, 2016).

Angling / sport fishing

Angling or sport fishing is one of the most fascinating outdoor physical activities which satisfy diverse taste and pursuits. It is a form of eco tourism promoting sustainable form of resource use contributes to environmental conservation while proving accrued socio-economic benefits to the society through non consumptive use and provides high values to natural biological resources. Tourism is a big business globally accounting for 8 percent of the world's domestic product and 9 percent of the world's total employment as per WTO report (Blanke & Chiesa, 2013).

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 destination 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 (Mahanta et al, 2011). Near home in NE India, the average fishing trip for golden mahseer in Subansiri river of Arunachal Pradesh cost about Rs. 2600 per kg of which market price is only about Rs. 250/- per kg, calculated on an average of 4 fishing trip from Guwahati in 2010-2011 (Batra, 2009).

The essential elements of angling tourism (eco-tourism) may be 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 i.e. "uniting conservation, communities and sustainable travel" for development of an relatively remote inaccessible destination (Harris *et al.,* 2002). It is essentially a tourism activity which minimizes the conflict between resource of 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 the angler like to conserve the nature, the tree line, the riverbed, the clean and clear water bodies and springs with abundance of fish fauna, their spawning ground, the catchment area of the river, the nature and its surrounding as a whole (Eagles, 2002).

Constraints in sport fishing

There are several constraints for which this could not get proper focus among the policy makers as well as the local people in India. These can be classified into the lack of mapping of the sport fishery rivers, lack of awareness among the local people, non-availability of check list of fish and the places with the tour operators etc. People participation in conservation and angling is not in proper order. Difficulties of anglers to commute to remote areas, the redtapism of the local administration, lack of NGO and angling clubs inhibit the westerners. Religious and community ownership of lakes and rivers prohibits angling in some of the most wonderful water bodies (Brown, 2009). Of late, coming up of several hydroelectric projects in different parts of India will definitely wipeout the entire sport fish fauna. Although there is tremendous potentiality of angling tourism in many sites, yet 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 (Brown, 2009).

Mahseer conservation efforts in India

Mahseer is important in the head water stream ecosystem where occupies wide range of food web and is responsible for balancing the ecological pyramids (Laskar, 2012). However, it is well understood that various anthropogenic threats on the aquatic ecosystems, most devastating is the poisoning and dynamiting in the headwaters and the streams therein, have threatened the mahseer population in the natural water bodies and is now considered as an endangered fish (IUCN, 2014). Although such practices of fish harvest have been declared illegal, yet unless mass awareness is generated and fruitful strategy not employed, such practices will continue to aggravate. There is urgent need to conserve and rehabilitate the fish by creating awareness to local stakeholders covering different age groups, and strategic people participation in order to reduce the threats to mahseer fishes in natural water bodies (Mahanta *et al.,* 1994; Mahanta and Sarma, 2009).

Although in India, the Wildlife (Protection) Amendment Act, 2002 redefined the term 'Animal' including fishes, but, existing protected areas are not taking additional protection measure and scientific management for the freshwater fishes. On the backdrop of severe mortality of early fry in the river pockets due to recession of water, there is paramount demand of population rescuing/rehabilitation and restocking of mahseer in safe and suitable water bodies, especially lake and river, for the establishment of germplasm as treasure trove (Sarma *et al.,* 2016). This would augment the conservation effort and allow long run conservation in the way of consequential recruitment in to connecting steams/ rivers through outlet after natural reproduction. In the process of stocking of mahseer in safe zone, the seeds of the indigenous mahseer are to be rescued from nearest streams. Simultaneously, it is the need of the hour to discuss on the issue of creating designated mahseer sanctuaries in India (Sarma *et al.,* 2014).. Mahseer zones are also important breeding sites for recruitment of many other small to large fishes having both food and ornamental value. Virtually, with the aim of mahseer harvest, innumerable biotic components including galaxy of beautiful small fishes are also ruined by the adoption of illegal method of mahseer harvest. Mahseer are meant as key stone species (GRBEMP Interim Report, 2013). Given that, if mahseer are protected and conserved in a fair length of river (covering natural spawning ground), a large number of other small to large endemic fish species would be indirectly protected and conserved and their recruitment in the downstream would be increased.

It may be noted that conservation and establishment of mahseer population in Mehao lake, Arunachal Pradesh was successfully done by ICAR- DCFR in 2009 in collaboration with Rufford Foundation, London (http://www.assamtimes.org/node/12282). The status of Cauvery mahseer (*Tor khudree*) may be categorized as endangered (Adrian & Raghavan, 2013) and several conservation measures are needed to be taken care for increasing its population in the Cauvery water bodies (Adrian & Raghavan, 2013).

The Wildlife Association of South India (WASI), an NGO based in Bangalore, Karnataka, came into existence in 1972 with a mandate 'to conserve and preserve the wildlife. The association also obtained a lease of a 22 km reach of the river Cauvery with the aim to conserve native mahseer populations (Adrian & Rajeev, 2013). The main focus of the WASI effort is to control illegal fishing and replenish wild stocks using captive bred fish (Adrian & Raghavan, 2013). The organization also set up small seasonal fishing camps to promote responsible 'catch and release' mahseer fisheries. The success of WASI encouraged other NGOs such as the Coorg Wildlife Society, private individuals (Jung & Subhan, 2012), and the State Government-owned Jungle Lodges and Resorts (JLR) (www.junglelodges.com) to set up both seasonal and full-time angling camps on the river Cauvery. The income generated from recreational fisheries effectively controlled illegal fishing of mahseer through the establishment of anti-poaching camps, as well as rehabilitation of former poachers as 'Ghillies' or fishing guides, thus providing alternative employment and associated societal benefits (Sehgal, 1999).

Such success was to later capture the attention of international tour operators, and in 2006 a British-based angling tourism specialist, Angling Direct Holidays (ADH), secured an agreement with JLR for a block booking at the Galibore Camp between mid-January and mid- March of each year. Activity during this period has been restricted to a maximum of ten anglers practicing a strict 'catch and release' policy. Catch data from Galibore (number, weight, phenotype notes, etc.) and fishing effort (time) were recorded in daily logs. Preliminary analyses of data collected between 1996 and 2012 demonstrate a dramatic increase in the total number of fish caught over time along with a reducing trend in individual mean weights (Dinesh *et al.,* 2010). Apart from the positive role played by recreational fishing, the success of these efforts also demonstrated the importance of engaging local communities in the conservation of endemic and threatened freshwater fish species. Recreational fishers constitute a social group that offers unique potential to enhance fish conservation. They have a vested interest in preserving or enhancing the resources they depend on and there is ample evidence to demonstrate that anglers work proactively to conserve and where possible enhance aquatic biodiversity (Granek, *et al.,* 2008) as well as motivating others to do so (Parkkila, *et al*, 2010) . In addition, anglers have also been known to participate in developing pro-environmental legislations, and in taking legal action to oppose developments likely to be environmentally damaging (Bate, 2001).

Barring a few tributaries of Teesta river in Sikkim and West Bengal, where the mahseer population may be in good health, the overall status has invariably become endangered. At many places, river courses have changed and spawning beds were destroyed. Keeping this alarming situation in mind, ICAR-DCFR, Bhimtal has transported 2 lakh hatchery produced fingerlings of golden mahseer to stock in Teesta river during 2008-2010. The conservation efforts thus made have positive impact in reestablishing the population of golden mahseer in some of the stretches of Teesta (Sarma *et al.,* 2014).

Walen Kondh in Mahad Taluka of Raigad district in the Western Ghats of Maharashtra is one of the several critical community fish sanctuaries of India that protect the mahseer fish. These sanctuaries have been successful in conserving not only the fish, but also stretches of rivers through their unique actions which find no support from the establishment and limited recognition from the conservation community. In such a scenario, community conserved fish sanctuaries which are scattered across the country are playing a very important role in conserving various species of mahseer as well as stretches of rivers (Dandekar, 2013; Swar, 2002). In Maharashtra, Tilase is one more such small village in Wada Taluka of Thane district which protects mahseer fish in Vaitarna river (Dandekar 2013). Goa too has community conserved fish sanctuaries protecting the mahseer at 'Pistyachi Kon' nesteled between Bhimgad and Mhadei Sanctuaries (Dandekar 2013). In Orissa, along the Mahanadi on the banks of the leaning temple of Huma exist the "Huma Mahseer Sanctuary" (Dandekar 2013). Karnataka has the highest number of community fish sanctuaries in the Western Ghats (Dandekar 2013). Uttarakhand and Himachal Pradesh too have many temple fish sanctuaries, notably the Baijanath Temple complex on the banks of river Gomti in Uttarakhand conserves Golden Mahseer (*Tor putitora*) and supports a small fish sanctuary on its banks (Dandekar 2013). Many isolated fish sanctuaries are also reported from the Ramganaga and Kosi area near Corbett and Kherna. The fishes are also protected at Naldamayanti Tal by the local communities where catching of mahseer is totally banned in the name of religious background (Kumar & Shukla, 2013). In Jogindernagar, a town in Mandi distirct of Himachal Pradesh lies a lake known as Machchiyal, fed by river Uhl. This lake is supposed to be the abode of 'Machendru Devta', the "Fish God". Fishes are fed and worshipped here regularly and fishing is strictly prohibited in the lake. Machchiyal supports a large population of Himalayan Mahseer (Dandekar 2013). In Tura district of Meghalaya, a chocolate mahseer sanctuary has been established by the local community in some river stretch of mighty Samsung ((MSAM, 2014).

It is urged to all concern to document the existing sanctuaries and grant immediate protection to all the existing fish sanctuaries in the country, protecting them from the onslaught of dams and other pressures. These sanctuaries stand testimony to the fact that community conservation is one of the most sustainable and effective ways of protecting ecosystems. It is also well understood that besides contributing to the tourism coffers, angling tourism supports conservation of mahseer. The setting up of anti-poaching camps along the river has curtailed poaching and helped in protection of the river and the aquatic species. Mass media has been so successful in keeping the news about wildlife in front of people, similarly there could be a potential for raising awareness for our often neglected river ecosystems and the species supported by them like mahseer (ADB & ICIMOD, 2006). After all, our riverine ecosystem is as important as our forests. In addition, other riverine species such as the smooth-coated otter, the oriental small-clawed otter and the mahseer (*Tor* and *Neolissochilus sp.*) fish species could have the potential to generate substantial public interest and people love to watch mahseer and play with this magnificent fish (Sarma *et al.,* 2016). There are multiple benefits of promoting and protecting the mahseer species. They are regarded as an apex species in some river systems of India, helping to maintain the overall integrity of the ecosystem. Some scientists have referred to the mahseer as a keystone species, while others suggest promoting the golden mahseer as a flagship fish species for the conservation of Himalayan rivers (Johnsingh, 2006). Promoting Mahseer fish in the mass media can result in tourists visiting hotspots in the rivers for sighting mahseer. This tourism revenue can help the locals a lot and may hopefully lessen the migration of people from rural areas to urban areas (Bhatt & Pandit, 2015).

Issues and strategies required for mahseer fishery development

In central Himalayas the golden mahseer available both in lentic and lotic systems has declined through the years in their size groups and the unit availability in catches by the anglers (Malik, 2011). In Kumaon lakes presently nearly 42% of mahseer are in the length range of 250-400 mm while fishes of above 600 mm in size are usually less than 10%. It is recorded that prior to nineties majority of size groups were in the weight range of 600 –1800 g while earlier to that reports indicate weight range of 4-10 kg with a record of Bhimtal at 28 kg. The percentage of golden mahseer from the Himalayn lotic systems range generally to the tune of 10-20% with low size frequency. Rarely a specimen of > 2kg is noticed barring few deep pools or fast flowing rivers (Bhatt *et al.,* 2000). The reasons for such decline are well documented (Bhatt *et al.,* 2000). In this scenario the conservation through aquaculture of these species becomes the most viable mechanism to propagate these species on one hand and on the other produce the valuable fish protein to provide nutrition security to our people apart from generating economic avenue to our rural population (FAO, 2014).

At present there are some reservations about the suitability of this species as a candidate for economic aquaculture by a farmer. If we are looking at aquaculture practices in Maharashtra , Madhya Pradesh Gujarat, the farmer has choice of species to culture he could club a slow grower and compensate the biomass loss through other fast growing species in his culture combination. But on the other hand, the choice with a farmer in hills is rather limited either he does not have promising combination or if he has, the agro-climatic conditions do not permit him to extend culture period beyond certain months (Meenakumari & Mahanta., 2012; Singh *et al.,* 2014). It is believed from the study that mahseer fish will command a better price in comparison to other carps keeping aside the local preferences but to bring the species to farming status lot of technology development and up-scaling of the existing one is required, some of the aspects of technology gaps are indicated hereunder which require attention at various levels (recommendation of ICAR-DCFR mahseer workshop, 2014):

- Up-scaling of the existing breeding technology especially in Himalayan regions where the low temperatures are acting as constraints at various developmental processes.
- Improvements in nursery management to cut down the losses and adequate the engineering designs for various culture systems.
- Stock improvement especially in hills through better selection of traits or through genetic improvement to develop faster growing strain.
- Critical water requirements both in terms of quality and quantity required to a viable culture system of this fish.
- Production enhancement in a culture system will result in environmental stress to the stocks and cause diseases, this aspect to be addressed with adequate preventive measures.
- Better growth of stocks could also be linked with the balanced diet; the existing diets developed for the species are fundamentally based on the approaches made on other species of carps. There is fundamentally very limited information on the physiology of nutrition in mahseer which should be addressed to answer partly the question of slow rate of growth.

Recent research advances on mahseer

Ontogeny of digestive enzymes in golden mahseer

The objective of the research findings is to get an insight into the development of the larvae digestive functions in terms of different digestive enzymes in order to obtain essential data for the formulation of a compound larval diet. In summary, the activities of digestive enzymes indicate that mahseer larvae are able to digest protein, lipid and carbohydrate at an early stage. In this study, although a sharp increase occurred at the start of exogenous feeding, the specific activity of most digestive enzymes exhibited fluctuations. This variation in enzyme activities during the early stage may be due to the under developed digestive system. The specific activities were gradually increased during the first one week of life and reached higher values during this time, and a decline occurred afterwards. This decrease in specific activities of different enzymes like lipase, protease and amylase on 15 DAH might be due to an increase of body protein (as specific activity is the ratio activity per mg protein) and does not reflect a lowering in digestive capacity. The results of the present study revealed that micro-particulate diets could be formulated for rearing of golden mahseer larvae from 7 DAH onwards, more preferably, from 15 DAH taking into account the digestive capacity of larvae (Akhtar *et al.,* 2013b; Akhtar *et al.,* 2014).

Development and evaluation of a formulated micro diet for larval rearing of golden mahseer

Nutritionally complete, formulated diets are seen as attractive and valuable alternatives to live food. Therefore, numerous attempts have been made to develop formulated diets that effectively replace live food fully or partially. But, formulated diets are used as supplements because when used exclusively, growth and survival are often compromised. Reasons for difficulties in successful rearing the early stages of fish larvae on artificial diets are not specifically known but several factors are thought to play an important role. The main reason is that the digestive systems of larvae are usually not fully developed and may not possess sufficient digestive enzyme activity necessary for effective digestion of artificial diets (Hamlin *et al.,* 2000; Kolkovski, 2001). Researchers speculate that the enzyme manufacturing capacity within the gut is far lower that what is needed and that effective digestion may accomplished through the assistance of exogenous enzymes that originated from the sources of live food (Kolkovski et al, 1997). That is why growth and survival is higher when fish larvae fed with live food due to supplementation effects of exogenous enzymes from them. Cahu and Zambonino (2001) suggest that it will be necessary to formulate diets that are specifically designed to complement the digestive physiology of fish larvae. In this study, an attempt was made to develop a suitable micro diet to substitute live food for larval rearing of golden mahseer considering the ontogeny of digestive enzymes and their functions (Akhtar *et al.,* 2013 a.).

From the study, it is evident that the endogenous enzyme activity in golden mahseer larvae was not sufficient for the digestion of microdiets as indicated by the low growth performance of T-mpd0 group. However, supplementation of dietary digestive enzyme mix in the micro diet positively affected the larval growth. Better growth performance of golden mahseer larvae fed enzyme mix supplemented micro diet in the present study supports the findings of several authors in different species (López-Alvarado, 2015; Patil and Singh, 2014; Kolkovski *et al.,* 1993; Lauff and Hofer, 1984). This may be due to the increase in digestive capacity of larvae because of the ready availability of digestive enzymes along with the feed. Additionally, supplementation of exogenous enzymes may lead to the activation of endogenous enzymes or zymogens in the digestive system and might have resulted in increased digestive capacity and nutrient utilization in larvae. Improved growth and feed utilization was reported in the larvae of *Cyprinus carpio* by exogeneous supplementation of trypsin in its larval diets (Dabrowski *et al.,* 1979). Further, this study revealed that low level incorporation of dietary digestive enzyme mix in the larval diet of golden mahseer resulted in better growth suggesting development of cost effective larval diet.

The study demonstrated that the growth and survival of golden mahseer larvae is highest when fed with live feed *Artemia* nauplii. However, the microparticulate diet supplemented with exogenous dietary digestive enzyme mix (2.0 g Kg^{-1} diet) gives nearly comparable growth with similar survival. Hence, the microparticulate diet supplemented with exogenous dietary digestive enzyme mix at 2.0 g Kg^{-1} diet is an alternative for the feeding of golden mahseer larvae after 15 days of hatching.

Physiological responses of golden mahseer fry to dietary zinc and assessment of its optimum requirement

Minerals are required for the normal life processes of the fish. Many essential micro or trace elements such as zinc, manganese and copper are required for growth and development of fish (Lall, 2002). Like other animals, zinc is a vital micronutrient present in all organs, tissues and body fluids of fish and it act as a stabilizer of membranes and cellular components involved in various metabolic pathways (NRC, 1993). Zinc also function as cofactors of variety enzymes that involved in the biosynthesis and catabolism of carbohydrates, proteins and lipids. Fish can obtain this mineral directly from the water or via their diet; however, dietary intake is the major route of mineral uptake in fish (Wang and Rainbow, 2008). Growth performance of fish can be correlated with the digestive and absorptive capacity (Zhao *et al.,* 2007). Zinc is the cofactor of RNA polymerases involved in protein synthesis (Hayashi *et al.,* 2001). In fish, the content of nucleic acids such as RNA and DNA can be used as a measure of tissue growth and protein deposition (Bastrop *et al.,* 1992).

Despite of its essentiality, studies have also shown that elevated concentrations of zinc can be toxic to fish (Hayashi *et al.,* 2001). Zinc deficiency in fish leads to growth retardation, which is found to be especially pronounced if other minerals are also lacking (Hughes, 1985). Inadequate zinc supply may also result in impaired digestibility of protein and carbohydrate, increased moisture content and lipid concentrations (Satoh *et al.,* 1987). To overcome such deficiency, zinc is usually added in fish feed. A study conducted by Bhagwati *et al.,* (2014) concluded that dietary zinc has significant impact on growth, digestive and zinc related enzyme activities in golden mahseer fry. The optimum dietary zinc requirement of *Tor putitora* fry was found to be in the range of 44.62 to 46.73 mg/kg diet based on growth performance and physiological indices.

Nutritional composition of golden and chocolate mahseer

As a whole freshwater fish is being well recognized for its health promoting characteristics. Freshwater fishes contain high quality protein and various major and minor minerals. It is well known that dietary protein act as replacement of endogenous loss of body protein due to tear, formation of new tissues during growth period and synthesis of blood, hormone, etc. which are protein in nature (Torres 2000, Nurullah *et al.* 2003, Harry 1958, Pedrini *et al.* 1996, Huang *et al.* 2001, Mat Jais 1994, Skonberg *et al.* 2002). Certain amino acids like aspartic acid, glycine and glutamic acid are also known to play a key role in the process of wound healing (Chyun and Griminger 1984, Wahbeh 1997, Zuraini *et al.*2006). Although the nutrient quantity of various freshwater fish species have been characterized, coldwater fishes vary widely in their body composition, amino acids, Fatty acids and major minerals - Na, K, Ca & trace elements - Fe, Mn, Zn, Se in relation to geographical location, seasonal variation and production system (Sarma et al, 2013, 2014, 2015 ; Das *et al.,* 2012; Mohanty et al, 2014). Basic knowledge of nutrient quality is very much essential to deal with the production, processing and marketing of high valued Himalayan golden and chocolate mahseer for human consumption.

Captive maturity and spawning of endangered golden mahseer

Overall results of series of experiments at the ICAR-Directorate of Coldwater Fisheries Research, Bhimtal for decoding the reproductive dysfunction (Akhtar *et al.,* 2017) in captivity suggested that exposure of golden mahseer to 12L:12D could be considered as the physiologically optimum photoperiod for inducing maturity in cultured conditions as evidenced by the elevated levels of 17β-estradiol and 17α, 20β-diOH-P. Temperature was found to be an important and relatively stronger determinant of ovarian maturation in females under captive conditions as compared to photoperiod. Elevated temperature within the physiological limits clearly induced gonadal development and maturation in female golden mahseer as substantially indicated through the analyzed biochemical and phenotypic parameters (Akhtar *et al.,* 2018). However, final oocyte maturation was still unsolved and to address this, we further explored the simulation of spawning substratum (FRP tank installed with gravel bed biofilter system) along with optimum photoperiod and elevated temperature. After four months of rearing the brooders, golden mahseer brooders not only got the captive maturity but also spawned several times in the rearing tank itself. Finally, the study has successfully addressed a longstanding issue of captive maturation and spawning in golden mahseer which otherwise has been a major bottleneck for large scale rehabilitation efforts to conserve this esteemed species in India and Indian sub-continent (Akhtar *et al.,* 2018).

Genetic stock of golden mahseer in India

Genetic diversity is influenced by selection, mutation, migration, population size, and genetic drift and understanding how each of these factors influences the genetic diversity of a population is critical to the conservation of species. In recent years, different morphometric and molecular tools have been successfully used to characterize the natural occurring populations as well as captive resources (Sati *et al.,* 2013; 2014; 2015). Different types of molecular markers like allozymes, microsatellite and rRNA markers (Mahindra *et al.,* 2004; Gopalakrishnan *et al.,* 2008; Singh *et al.,* 2009), mtDNA markers (Sati *et al.,* 2015) as well as their morphometric and meristic characters (Ali *et al.,* 2014; Patiyal *et al.,* 2014) were used to study the variability in the population. Such information is useful for describing the species as well as differentiating the population.

Conclusion

The conservation and sustainable development of mahseer fishery resources require dedicated efforts by integrating capture, culture fisheries and environmental programmes. Environmental laws with strict enforcement mechanisms have to be implemented to achieve the desired objectives to improve the fish habitat and biodiversity conservation. Mahseer is a fish for food, sport and also migratory in behavior therefore, sustainable development of its fishery would need policy and governance intervention from different stake holders apart from fishery researcher. It is essential that fishery research should have linkage and plan intervention strategy in collaboration with the local communities. May be we will have to embed our policy research for mahseer in our research projects right from the start of programme (Vass, 2014).

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Coldwater fish cell line: Development, characterization and applications

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ABSTRACT

Fish cell lines have been used as important *in vitro* tools for carrying out research in different disciplines including toxicology, pathology, biotechnology, developmental biology and biomedical sciences. In recent years, the numbers of fish cell lines have been increasing tremendously covering a wide variety of species and tissues. Fish cell lines developed in the country have been maintained and cryopreserved in National Repositories with the financial assistance from the Department of Biotechnology, Govt. of India, New Delhi. Different projects supported by Department of Biotechnology, Govt. of India have been instrumental in boosting *in vitro* research in India using fish cell lines. Very few cell lines have been developed and characterized from cold water fish species and hence emphasis should be given to develop more cell lines from cold water fish to facilitate *in vitro* research in the area. The development, characterization and application of fish cell lines with special reference to cold water fish are reviewed in this paper.

Keywords: Coldwater fish, Cell line, Applications

Introduction

Cell line has been used as an important *in vitro* tool for carrying out various investigations in physiology, virology, toxicology and biotechnology. The adoption of intensive farming practices, unregulated use of inputs and inbreeding in hatcheries has led to increased disease incidence. There have been several incidences of mass mortality of carps in culture systems suspected to be caused by bacterial and viral diseases. Cell lines from fishes are particularly useful in detecting viruses and studying the molecular and cellular basis of physiological processes and toxicological mechanisms (Fryer & Lannan, 1994; Bols *et al*., 2005). In recent years, cell lines from aquatic animals have attracted considerable attention as a means of expediting disease diagnosis. Several viral diseases in cold water fish species have been reported (Dannevig *et al*., 1995, Crane and Hyatt, 2011). Hence, development of cell line from fish species is indispensable for virus isolation studies and understanding viral pathogenesis. The fish cell lines have been proven to be a valuable, rapid and cost-effective tool in the ecotoxicological assessment of chemicals and environmental samples**.**

The physiology and blood plasma constituents of teleosts are similar with those of terrestrial vertebrates; therefore, the methodology for culture of cells is also similar. Fish cell lines are more advantageous over mammalian cell lines in terms of its maintenance and versatile applications. Because of lower metabolic rates than eurythermic cells, fish cells can be maintained with little care for long periods of time. Thus, permanent fish cell lines, in contrast to the mammalian cells, are easier to maintain and manipulate, and unlike primary cultures, produce highly reproducible results (Wolf & Quimby, 1962). Embryonic and larval cells are the most easy to cultivate being mitotically activated.

The first fish cell line RTG-2 was developed in 1962 using the ovary of a coldwater fish, rainbow trout (Wolf and Quimby, 1962). Since then, an increasing trend in fish cell line development has been observed from a wide variety of tissues representing fish species from both tropical and temperate waters. A comprehensive review by Lakra *et al*. (2011) has reported 283 fish cell lines globally. The latest information enlisting 517 fish cell lines in Cellulosaurus; a knowledge resource on cell lines has been reported by Bairoch (2017). The Department of Biotechnology has been playing a pivotal role in promoting the fish culture related research in India by providing necessary grants to develop and maintain fish cell lines in the country. The first preliminary efforts to develop fish cell culture were made at Central Institute of Freshwater Aquaculture, Bhubaneswar in late eighties of the last century (Kumar, 1987) followed by successful primary cultures from gill tissue of mrigal, *Cirrhinus mrigala* (Sathe *et al*., 1995), kidney of the stinging catfish, *Heteropneustes fossilis* (Singh *et al*., 1995), and caudal fin of *Labeo rohita* (Lakra and Bhonde, 1996) at various laboratories providing a momentum to fish cell and tissue culture research in the country. In India, the research on the development and characterization of fish cell lines is mainly being conducted at several institutions like National Bureau of Fish Genetic Resources (NBFGR), Lucknow; Central Institute of Fisheries Education (CIFE), Mumbai; Central Institute of Freshwater Aquaculture, Bhubaneswar; Central Marine Fisheries Research Institute, Kochi; C. Abdul Hakeem College, Vellore; National Centre for Cell Science, Pune; Fisheries College and Research Institute (FCRI), Tamilnadu Veterinary & Animal Sciences University, Tuticorin; Cochin University of Science and Technology (CUST). Approximately, 50 fish cell lines have been developed by different research groups in the country (Goswami *et al*., 2015). A National Repository of Fish Cell Lines (NRFC) was established during 2010 at ICAR-NBFGR, Lucknow with the financial assistance from DBT, New Delhi for a project National Repository for Conservation and Characterization of Fish Cell Lines at NBFGR, Lucknow (Principal Investigator: Dr. M Goswami). About 50 fish cell lines have been maintained and cryopreserved in the NRFC.

Development and characterization of coldwater fish cell lines in India

Teleost cell lines have been developed from a broad range of tissues such as ovary, fin, swimbladder heart, spleen, liver, eye muscle, vertebrae, brain and skin. A simple and reproducible short-term fish cell culture technique was described for *Tor putitora* by Prassana *et al*., 2000. A TP-1 cell line from cold water fish golden mahseer *Tor putitora* was developed for the first time in India by Lakra et al. 2006. Since then, consistent efforts have been made to develop cell line from cold water fish. The cell line exhibited best growth in the L-15 medium with 20% FBS at 28 ^{0}C.

Three cell culture systems were developed from caudal fin, heart and gill of Tor tor (Kamalendra *et al*., 2010, Kamalendra *et al*., 2011). Subsequently, a permanent cell line TTCF was developed from caudal fin of *Tor tor* by Yadav *et al*., 2012. TTCF cell line was cryopreserved and presently maintained at NRFC with an accession number NRFC003.

The cell culture system from eye, heart, fin and swim bladder of *Puntius (Tor) chelynoides* was developed from eye of *Puntius (Tor) chelynoides* by Goswami *et al*., 2012. The monolayer formed from heart explants exhibited rhythmic heartbeat. The cells were grown in Leibovitz' L-15 media supplemented with 20 % fetal bovine serum (FBS) at 24 °C. The PCE cell line from eye was characterized by DNA barcoding based on amplification of mitochondrial cytochrome oxidase subunit I (COI) & 16S rRNA genes and cytogenetic analysis. The cell line demonstrated expression for GFP reporter gene suggesting that this cell line can be used for transgenic and genetic manipulation studies. Further, genotoxicity assessment of PCE cells illustrated the utility of this cell line as an in vitro model for aquatic toxicological studies. The PCE cell line was successfully cryopreserved and revived at different passage levels. Another PCF cell line was developed from the caudal fin of *Puntius (Tor) chelynoides* by Goswami *et al*., 2014. Immunocytochemistry of PCF cells confirmed its fibroblastic morphological nature. Upon exposure of PCF cells to bacterial extracellular products, significant cytopathic effects were observed which validated the usefulness of the PCF cell line for toxicity assessment as *in vitro* model. The cell line has been maintained in NRFC with an accession number NRFC001.

A SRCF cell line from caudal fin of snowtrout, *Schizothorax richardsonii was developed and characterized* by Goswami *et al*., 2013. The cell line has been maintained in Leibovitz's L-15 medium supplemented with 10% fetal bovine serum (FBS) at 24 ◦C. Transfection of SRCF cells with pEGFP-C1 plasmid showed bright fluorescent signals, suggesting the application of cell line in transgenic and gene expression studies. The cell line has been maiantained in NRFC with an accession number NRFC002.

Applications of fish cell lines

Early work with fish cell lines was initiated with RTG-2, a gonadal cell line derived from rainbow trout (Wolf and Quimby, 1962). Fish cell lines have enormous applications in biomedical research, toxicology, gene regulation, gene expressions and gene transfer (Hightower and Renfro, 1988, Babich *et al*., 1986, Driever *et al*., 1993). The main advantage of cell culture is that cell lines allow higher control of conditions of experiments and at the same time reduces the variability of the in vivo responses that arise due to the responses of fish to stress and environmental influences and to disparate genetic background of farmed fish and shellfish species. Fish cell cultures have been increasingly used in toxicology research for evaluating effects of various chemicals, pesticides and industrial wastes. The effects of different inorganic and organic pollutants on the metabolism of aquatic biological systems have been studied using cell cultures and hence fish cell lines can be used bio-indicators in environmental monitoring. Due to the good correlation found between the *in vitro* data and the *in vivo* fish data, the use of established fish cell lines can represent an alternative tool to acute fish bioassay for toxicity screening of chemicals. RTgill-W1 is an epithelial cell line derived from the gill explants of normal adult rainbow trout (*Oncorhynchus mykiss*) (Bols *et al*., 1994). RTgill-W1 has been used in toxicity testing of industrial effluents (Dayeh *et al*., 2002), including petroleum refinery effluents (Schirmer et al. 2001), polycyclic aromatic hydro-carbons (Schirmer et al.1998) and metals (Dayeh et al.2005) including Cu, Cd, Zn, Fe, and Ni.

In aquatic toxicology, *in vitro* investigations with both freshly isolated cells and permanent cell lines have been used for screening of chemicals or environmental samples (Gagne *et al*., 1996; Mori and Wakabayashi, 2000; Davoren *et al*., 2005; Tan *et al*., 2008). Genotoxicity and cytotoxicity studies have been carried out with many primary fish cells and with different permanent fish cell lines (Brunbeck and Neumuller, 1996; Kamman *et al*., 2000; Abdul *et al*., 2013, Goswami *et al*., 2014; Taju *et al*., 2014; Dubey *et al*., 2015). Proteomic techniques, in particular, offer great potential for insight into chemical modes of toxic action and are useful tools in biomarker discovery (Wetmore and Merrick, 2004; Benninghoff, 2007). Protein expression signatures (PES) of fish cell lines have been developed using 2-DE and image analysis (Wagg and Lee, 2005; Goswami *et al*., 2016).

The utilization of fish cell lines in fish health management focuses on prevention and control of infectious diseases. Cell lines help to understand the pathogenesis of virus, and other intra cellular pathogens like Rickettsia sp. etc. Moreover, cell culture methodologies are useful in diagnosis of viral pathogen by production of cytopathic and syncytial effects and aid in isolation and characterization of virus. The most widely employed application of fish cell lines is the isolation and characterization of viruses. Most commonly used fish cell lines for diagnosis and characterization of intracellular fish pathogens are RTG-2, EPC, FHM, CHSE-214, CCO and BF-2. RTgill-W1 cell line demonstrated its ability to support the growth of a novel paramyxovirus isolated from the gills of disease seawater-reared Atlantic salmon (Kvellestad *et al*., 2003). The complete genome sequence of the virus, dubbed Atlantic salmon paramyxovirus or ASPV, was made possible due to the growth support of RTgill-W1 (Nylund *et al*., 2008). RTgill-W1 cell line could be useful for studies of gill infecting microsporidia such as Loma salmonae (Kent and Speare, 2005), the causative agent for microsporidial gill disease of salmonids affecting aquaculture-raised chinook salmon in Canada (Speare *et al*., 2007). Fish leukocyte cell lines with cells of specific lineage and function like T-cells, B-cells and macrophages developed from catfish, *Ictalurus puntatus*, *Onchorhynchus mykiss* etc. have been employed to generate immunological information useful for disease prevention in farmed fish. Cell lines of gut, skin and gill origin have been used to study the local defense responses. The immuno-response potential of DNA vaccines, recombinant protein and synthetic peptide vaccines and immunostimulants can be studied with the aid of cell lines.

Fish cell lines have useful applications as *in vitro* models for studying the replication and genetics of the viruses, the establishment and maintenance of virus carrier states, effects of antiviral drugs and production of experimental vaccines. *In vitro* fish RBC cultures have been used in investigations of the replication of the viruses and as *in vitro* models of viral induced anemia (Reno and Nicholson, 1980). Fish cell lines have been developed as *in vitro* models for studying various biological processes. The fish cell lines have been used for determining karyotypes and other aspects of cytogenetics including chromosomal polymorphism and speciation, chromosome abnormalities and evolution (Roberts, 1970). Organ cultures from tilapia, eel and trout pituitary glands have been used for studying the production of growth hormone prolactins (Baker and Ingleton, 1975). Tolerance to cold water is important trait which has tremendous economic interest in aquaculture because the success of aquaculture is in many areas restricted by cold winter temperatures (Hinder, 2010). Cell line would be an ideal *in vitro* for facilitating transgenic studies using cold tolerant gene.

Conclusion

The number of fish cell lines has been increased tremendously during the last decade covering wide ranges of tissues in India. Cold water fish cell lines are available in very limited numbers and more cell lines including stem cell should be developed from prioritized cold water fish species to facilitate in vitro genetic and biotechnological research in cold water fisheries and aquaculture. Species specific and tissue specific cell lines are need of the hour for virological and toxicological studies. The fish cell line repositories would be very useful in maintaining the cell lines for in vitro research in the country. This would be the right step for conservation of germplasm and other genetic material of cold water fish species. The cell lines would be valuable complement to whole animal studies for various investigations and thus will resolve many ethical issues associated with biological studies.

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Farming of fishes in ricefields of Northeast India: A review

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ABSTRACT

Rice is the main food crop in North eastern states of India (viz., Assam, Arunachal Pradesh, Nagaland, Meghalaya, Mizoram, Manipur, Tripura and Sikkim) and grown both in uplands and flood plains adapting wide diversity of agro ecosystems. The sources of water for growing rice create rice environments either irrigated or rain fed one. Majority of these wetland areas provide natural habitat, breeding environment and feeding grounds for varieties of aquatic life forms during monsoon season in Northeast India. The wild fishes, prawns, crabs, snails, frogs, tortoises etc., colonize within these flooded fields and thereby their seasonal capture or harvest have become farmer's age old practice in the region. The region due to higher rate of monsoon Precipitation forms diverse wet rice ecologies viz., shallow water rice having water depth always below 50 cm, deepwater rice having water depth above 50 cm or more and stream fed mountain wet rice having regulated water input in addition to need based shallow irrigated rice fields. The farmers' driven changes and innovations have gradually diversified the age old practice of rice field fishery overtime in the region. Many tribal farmers have indigenously adopted water and nutrient management even in mountain valleys and terraces and have been utilizing resources through concurrent farming of rice and fish together. This article is an overview of all such practices from the region highlighting methods of rice-fish integration, livelihood and conservation issues. Hitherto the review on the system is expected to widen its potentiality in other underutilized rice agro-ecosystem of the region.

Keywords: Rice fish culture, Northeast India, Capture fishery, Livelihood, Threat, Biodiversity

Introduction

The northeastern (NE) region of India comprises of eight states viz., Assam, Arunachal Pradesh, Meghalaya, Manipur, Mizoram, Nagaland, Tripura and Sikkim. These states are drained with a huge network of streams and tributaries connected finally with three major rivers respectively forming three great river valleys of Brahmaputra (in Assam), Barrack (both in Assam and Tripura) and Tista (in North Bengal) . As rice is the main staple food for the people, it is grown almost in all the environments of this region of India (Fig. 1). The region is located between latitude 21^0 $57^{//}$ and $29^030^{//}$ N longitude 89^0 $46^{//}$ and 97^0 $30^{//}$ E spreading over the area of 262,190 square kilometer and contribute 8% geographical area of the country. The diversified rice growing environments ranges from its hilly mountain terrains to riverine flood plains constituting both rain-fed and irrigated rice lands (Singh *et al.,* 2006). The agro ecological classification shows that the region encompasses both warm sub humid tropics and cool subtropics with high summer rainfall. The cheapest food source of protein food is fish, harvested generally from the rivers, streams, lakes and reservoirs and additionally from rice fields, irrigation channels, and abundant wastelands etc. Tradionally, the waterlogged rice lands are one of the commonest fishing grounds for the rural people of the region during wet/kharif season (June to November). Biologically, the wet rice fields can be characterized as agronomically managed marshes, which often remain dry from December to April during the year. Physically, the aquatic phase starts from May to November and possesses varying water depth according to it's land topography, local rainfall patterns, water tables, soil quality etc. In aquatic phase, it becomes a rich and productive biological system which cause succession of diversified aquatic plants (aquatic vegetables like *Nymphea*, *Colocasia*, *Ipomea* etc.,), animals (organisms like fish, crab, snails etc.,) and other life forms and provides ecosystem services to people in addition to major food crop of rice (Das *et al.,* 2000). While marsh, pond and stream dwelling organisms colonize in rice fields in this phase, it converts the rice fields into an ecologically stable but a seasonal wetland ecosystem. As the flooded rice fields form the natural habitat for wild fish, so people inhabitating in and around the fields usually used to go for concurrent fishing. Thus, the wet rice fields are being utilized as the common property resources in Northeast India since very old days. Despite, there exists hardly any deliberate culture (intentional stocking of fish seeds and their rearing) of fish in rice field amongst the farmers' in general except the tradition of certain specific tribes (e.g. Apatanis of Arunachal Pradesh and Chakhesang of Nagaland) of the region. In real sense, deliberate fish culture following standard management has still not been popularized in majority of the rice growing areas of the region. However, the fattening of naturally colonized fish stocks or concurrent fishing of auto-stocked fishes from rice fields in monsoon season are the age old practices of the farmers in the region. With the above background, a review on existing methods and practices of rice field fishery, its contribution to people's livelihood along with the future initiative to be undertaken for conserving the systems have been highlighted in this article. Accordingly, the relevant key issues for future propagation of this sustainable farming system have been raised to meet the challenges of food security of the people of the NE Region.

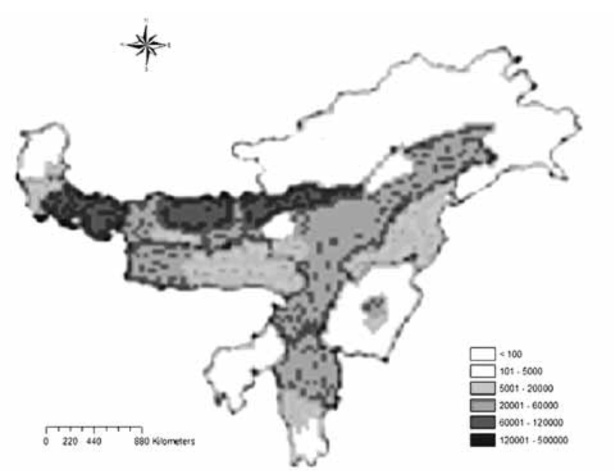

Fig. 1: District map of rice based single cropping area (Yellow colour) in NE region of India
(Source, India rice cropping maps, Govt of India, 1999-2000)

Status and Methods

Fish Farming in Rice fields

The existing practices of fish farming in rice fields of Northeastern (NE) region are primarily concurrent with rice. All the NE states of India lie in a zone of heavy monsoon rainfall (2000 – 5000 mm) during major rice growing season i.e. kharif season (June-November). Therefore, an aquatic phase of 4 - 5 months with varying water depth is maintained naturally in rain fed rice areas of the region mostly. Water gets accumulated in the field in the month of June-July and the recession of floodwater takes place at the end of the season (November -December). Table 1 shows the state wise distribution of such rice areas and areas under rice field fishery within the region. The current status of farming practice among farmers can broadly be categorized into: (a) rice field capture fishery system, (b) wild aquatic cropping system, (c) mountain foot hills and valley rice-fish farming system, and (d) running-water terrace rice-fish farming system (Das, 2002). In former two categories, rice fields are auto stocked i.e. wild fishes/fish seeds take entry into the field alongwith floodwater and thereby forms either purely capture or capture cum culture system where as in later two categories, farmers deliberately stock fish seeds into their flooded fields and rear them intentionally as true culture system. Each system of farming rice and fish together has been emerged out of diversified rice ecologies and the available piscatorial resources of the localities in congruence with farmers driven innovativeness and adoption of the practices as mentioned below:

The rice field capture fishery system

The unmanageable and vast waterlogged rice environments, perennial wet rice lands, oxbow type rice fields or flooded rice fields in river basins when overflow during monsoon and get connected with neighboring watercourses, the naturally occurring gravid female, mature male fishes and young fishlings, adult and juveniles of prawns enter and colonize the field from perennial habitats. They grow concurrently with rice in rice growing seasons for about 4-5 months. This is very common features in the seasonally inundated rice areas of whole Brahmaputra and Barak Valleys of Assam and certain areas of Teesta valley in

Table 1: Potential rice-fish culture areas of the Northeast India

Sl. No.	State	Scope for Cultivation (ha)	Area under cultivation (ha)	£Total area under rice (ha)	Average production (Kg/ha/year)
1.	Arunachal Pradesh	2,650	150	12,000	125
2.	Assam	15,000	NA	2, 48,980	NA
3.	Manipur	1,600	400	15,790	200
4.	Meghalaya	2,200	50	15,790	380 +
5.	Mizoram	4 00	NA	6,810	NA
6.	Nagaland	4 00	120	14,500	250
7.	Tripura	5,000	NA	25,780	300++
8.	Sikkim	NA	NA	4 50	NA
	Total	27,250	720	3, 40,100	251

NA Not Available + Experimental observations (Ghosh, 1981) ++ Experimental observations (Lipton, 1983)
£ Estimated that 10% area is approximately suitable for rice fish farming (Munilkumar and Nandeesha,2007)

Northeastern part of West Bengal. The fishing activities in those area start just after inundation of fields from late June and continue till recession of water level during November-December. In true sense, the area become temporary fishing grounds and the farmers and fishers use those fields for about 5-6 months as the common property resources and capture the fishes mostly by using the gill nets, cast nets and various indigenous fishing traps. The fishing devices are either operated in the rice free spots or fixing the traps (locally called as Chepa, bamboo contraption) at suitable entry and exit points of water in and around the fields. In these fishing methods (capture), the average rate of capture is 3kg/ha/yr (Jhingran, 1983; Hora, 1951). Such practices are highly prevalent in the districts of North Lakhimpur, Dhemaji, Barpeta, Nalbari, Bongaigaon, Dhubri and Cachhar district of Assam and certain districts of Manipur and also within other rice growing flood plains of Northeastern states. The practices of captural fishing are frequently found in deepwater rice (DWR) areas which amount to be more than 0.467 million hectares in the state of Assam only. The district of Lakhimpur (erstwhile) has the highest deepwater rice area (58430ha) followed by Barpeta (47895ha) and Nalbari (29564ha) in the state. In the context of species composition, these flooded fields show enormous diversity of piscine fauna (Table-2). Further, when harvesting of rice starts, after the recession of floodwater at the end of wet season (i.e. in November-December), all the low-lying ditches, marginal swamp and natural depressions in and around the field area are also harvested either by dewatering or by netting operation. The yield of fish ranges from 45.0 to 280.0 kg $ha^{-1}season^{-1}$ in the district (Bayan *et al.,* 1996) where farmers are indigenously involved with such practice (Table-3) for collecting the fishes and other aquatic resources. The tribal folks of lower Assam are also habituated in community fishing from the flooded rice lands and they perform the light fishing with bamboo made kerosene lantern/ or burns bicycle/ rickshaw tyres as stunting device for fish or other aquatic organisms. In addition to fish they used to hunt frogs, crabs and many other aquatic organisms which get allured or stunted because of light followed by hitting with sharp weapons. Besides, the farmers also practice cast netting at the evening hours in rice less patches of open DWR fields or oxbow lake. In this case, at least half an hour before, they apply dough of fish feed or sprinkle certain fish assembling baits in the netting site and there after they cast the net on the spot. In lower Assam, the tribal women are also habituated in collection of wild resources through group fishing in flooded rice fields using an indigenous contraption locally known as *Jakoi*. The group fishing is performed either 2-3 times during the whole rainy season or during the period of water recession at the end of the season. Besides fish, various other aquatic vegetables like *Ipomea, Alternathera, and Nymphaeia* etc. are also harvested for family level consumption.

Table-2: A list of commonly encountered fishes and prawns in rice fields of Northeastern India

Wild fishes		Prawns
Ailia coila (Hamilton, 1822)	*Labeo gonius* (Hamilton, 1822)	*Macrobrachium assamensis* (Tiwari,1952)
Ambhyopharyngodon mola (Hamilton, 1822)	*Labeo rohita* (Hamilton, 1822)	*Macrobrachium birmanicum Schenkel, 1902*
Amphipnous cuchia (Hamilton, 1822)	*Lepidocephalichthys guntea* (Hamilton, 1822)	*Macrobrachium dayanum* (Henderson, 1893)
Anabas oligolepsis Bleeker, 1854	*Macrognathus aculeautus* (Bloch, 1793)	*Macrobrachium lamarrei* (H. Milne Edwards, 1837)
Anabas testudineus (Bloch, 1801)	*Mastacembalus armatus* (Lacepede, 1800)	*Macrobrachium malcolmsonii* (H. Milne Edwards, 1837)
Aorichlhtys aor (Hamilton, 1822)	*Mastacembalus pancalus* Hamilton, 1822	*Macrobrachium rude* (Heller, 1862)
Aplocheilus javanicus Bleeker, 1854	*Monopterus cuchia* (Hamilton, 1822)	*Palaemon styliferus* (H. Milne Edwards, 1837)
Aplocheilus lineatus (Valenciennes, 1846)	*Mystus cavasius* (Hamilton, 1822)	
Aplocheilus panchax (Hamilton, 1822)	*Mystus julio* (Hamilton, 1822)	
Parambassis baculis (Hamilton, 1822)	*Mystus vittatus* (Bloch, 1793)	
Chanda nama (Hamilton, 1822)	*Nandus nandus* (Hamilton, 1822)	
Parambassis ranga (Hamilton, 1822)	*Ompok pabo* (Hamilton, 1822)	
Channa gachua (Hamilton, 1822)	*Pseudeutropicus atherenoides* (Chaudhuri, 1912	
Channa marulias (Hamilton, 1822)	*Systoma sarana* (Hamilton, 1822)	
Channa punctatus (Bloch, 1793)	*Puntius chola* (Hamilton, 1822)	
Channa striatus (Bloch, 1793)	*Puntius conchonius* (Hamilton, 1822)	
Chela cachius (Hamilton, 1822)	*Puntius phutunio* (Hamilton, 1822)	
Laubuka laubuka (Hamilton, 1822)	*Puntius sophore* (Hamilton, 1822)	
Clarias mangur (Hamilton, 1822)	*Pethia ticto* (Hamilton, 1822)	
Colisa labiosus (Day, 1877)	*Rasbora daniconius* (Hamilton, 1822)	
Esomus danricus (Hamilton, 1822)	*Rasbora elanga* (Hamilton, 1822)	
Glossogobius guiris (Hamilton, 1822)	*Rasbosa rasbora* (Hamilton, 1822)	
Heteropneustes fossilis (Bloch, 1793)	*Trichogaster fasciatus* (Bloch & Schneider, 1801)	
Labeo bata (Hamilton, 1822)	*Trichogaster pectoralis* (Regan, 1910)	
Labeo calbasu (Hamilton, 1822)	*Wallago attu* (Bloch & Schneider, 1801)	
	Xenonthodon cancilla (Hamilton, 1822)	

Table-3: Wild fish production from deep water rice field environments of Barpeta district, Assam

Development Blocks	No. of individual and DWR Pockets Surveyed *	Fish production ranges (kg/ha/ season)	Average yield (kg/ha/ season)
1. Barpeta	10	45.0-136.0	98.4
2. Chenga	5	80.0-118.0	97.8
3. Mandia	5	105-215.0	135.5
4. Bhabanipur	12	82.5-280.0	138.0
5. Gobardhana	5	63.5-115.0	75.0
6. Rupahi	4	49.0-120.0	71.0
7. Bajali	5	82.0-160.0	89.8
8. Jalah	4	58.0-116.2	78.0

**Based on farmers interview during the Season (June-December), (Source: Bayan *et al.,* 1996)

Wild aqua-cropping system

The wild aqua-cropping in rice field is a widespread practice in Assam, Manipur and foothill areas of most of the Northeastern states. The system is naturally linked with the typical crop cycle or cropping patterns (Fig. 2) in seasonally flooded rice fields. In this system the farmers or fishers use the whole field as trap and rear those wild fishes that enter the fields till recession of the flood or more period within the ditches or deeper areas. Morphometrically,such fields are either partially/fully impounded rain fed low lands or closed deepwater rice (DWR) lands and are often linked with canal systems of varying sizes and designs either naturally formed or artificially constructed. On an average, canals of 0.6-1.5m depths occupy 8% of the area of such fields (Pillay and Bose, 1957). The rice cultivars grown in these fields are traditional tall type known commonly as *Sali* rice in lower Assam and *Aman* rice in Cachhar district. Elongating or floating *Bao* cultivars are also grown in some closed DWR lands. These plots remain so submerged during monsoon season that water depth may reach above 2.5 meters or more, but dry up again partially or completely during the month of December-January. The rice cultivars are planted often during April-May by direct seeding of rice @ 75 -80 kg seed ha^{-1}and after getting first shower of rain, the seeds are germinated. In some areas, transplanting of 21-35 days old tall seedlings are done in June-July after accumulation of rainwater in the field. These fields are often overflowed in late July/ August and it gets connected with the neighboring watercourses allowing the entry of seeds of various wild fish and prawns. Farmers are habituated to dig up trap ponds inside to provide fish refuge and intentionally facilitate entry of wild fish fry or fingerlings in the field till surrounding open water level goes down from the level of entry points. The situation also prompts other open water wandering fishes to get accumulated in the trap ponds or natural ditches in and around field contour before water recession. These fishes are harvested after dewatering those ditches and canals and the rate of production often varies from 200 to 300 kg ha^{-1}season^{-1}.

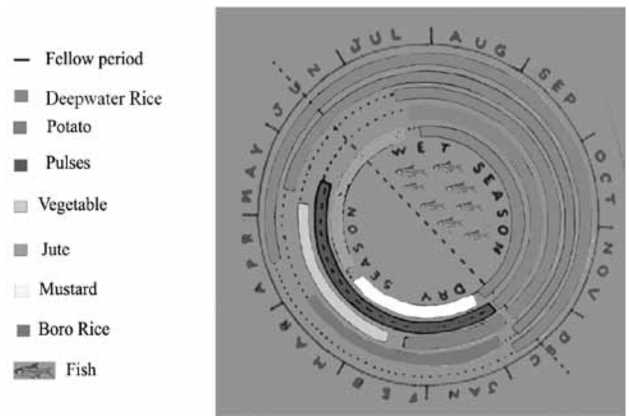

Fig. 2: Cropping pattern of flood plain wet rice lands in Northeast India (Source: After Das, 1991)

In Assam, many of the old fortresses constructed by tribal chief of the state are being used under this type of farming practices. One such fortresses named *Jangal Balahu garh* (37ha) in Nagaon district provided with high perimeter dyke surrounding the entire area, horizontal deep trenches and wooden sluice structures present in one corner of the plot offers a readymade site for rice fish farming (Ghosh, 1992). There are other non-flood prone areas in Goalpara and Dhubri district where farmers/fishers used to practice this system in addition to seasonal capture in flooded rice fields. Under such practices, rice fields are often connected with perennial water bodies by a canal locally called as *'Dong'* in districts of Barpeta, Bongaigaon, Kokrajhar etc., To facilitate the aqua farming, the *Dongs* are finally guarded by bamboo screens for preventing escape of the trapped fishes from the rice field at later phase and harvesting of fishes are performed when *Dongs* are disconnected from the fields. The Bodo and Rajbangshi people of the districts are very well trained in this type of aqua farming. From such harvest, local fishers supply substantial amount of fish to local market during the period. In lowland areas of Barpeta and Nagaon district wild aqua cropping is so popular among the farmers that they often use their jute retting tanks as the trap ponds allowing jute retting in flowing water at roadside channels purposefully. Moreover, in many areas closed rice fields are found to stock with carp seeds deliberately but the land owner hardly follows any standard management (Table-4) of fish culture.

Table 4: Unplanned (deliberate) stocking of fish in rice fields of Barpeta district

Sl. No.	Name of the Development Block	Total area (ha)	Tank area (ha)	Farmers (No.s)
1.	Barpeta	16.33	4.46	21
2.	Chenga	15.73	7.06	39
3.	Mandia	19.00	4.2	19
4.	Bhabanipur	83.25	8.98	47
5.	Gobardhana	4.66	1.6	6
6.	Rupahi	28.8	10.58	19
7.	Bajali	39.13	9.10	45
8.	Jalah	8.13	2.4	13
		207.83	48.33	209

(Source: Survey, DFDO, Barpeta, 1992)

Therefore, farming of fish in flooded rice fields in river valleys of Assam are merely the rearing of auto stocked wild fish with any specific system of management. In fact, these are nothing but 'capture cum culture' (Edwards, 1999) with the natural resources available in the field itself**.** True concurrent system of rice fish culture is rarely practised by the farmer. However, Baruah *et al.,* (1999) described farming methods while evaluating the impact of community management of rice field systems in Assam. The *Banas* (bamboo screens) are erected in waterways, i.e., bridges and culverts, to prevent the escape of farmed fish and the entry of unwanted wild species. Rice is transplanted by mid July. Agricultural lime is applied at the rate of 50 kg ha^{-1}. Cow dung and inorganic fertilizers such as urea are applied at the rate of 10 kg ha^{-1} and single super phosphate at 8 kg ha^{-1}. Stocking is done 15 days after transplantation with early fry of *C. catla, C. mrigala, L. rohita, L. calbasu, Hypothalamichthys molitrix, P. javanicus, C. carpio* in the ratio 14:18:14:9:18:9:18 at 20,000 ha^{-1}. Feed consisting of rice bran and mustard oil cake (1:1) is given at the rate of 2% of biomass. Production level ranges generally from 2,100 to 2,300 kg of rice ha^{-1} and 400 to 450 kg of fish ha^{-1} in such culture practice.

Mountain foot hills and valley farming system

In hilly states of NE India, topographically, certain rice areas are located in almost flat mountain valleys where water accumulates from adjoining hillocks and flows down the valley by gravity. Dwarf varieties of rice are generally grown in such plots integrating mainly with culture of *Cyprinus carpio*. Various small fishes also colonize naturally in those fields during the period of inundation. The states of Arunachal Pradesh, Mizoram, Meghalaya, Nagaland and Tripura hold such rice fields in plenty where farmer intentionally stock young fishes to grow along with their rice crop. These are harvested at the end of rice season either from the irrigation channel or from the specially dug fish refuges within the plots. The production rate from such plots could be raised up to 200 kg $ha^{-1}season^{-1}$. In NE region al the mountain valleys that are cultivated mainly with dwarf varieties of wet rice, may potentially be converted to rice fish cultural plots. The favorable points in supports are: (i) region is an area of high rainfall zone in India (ii) assured irrigation from the streams during monsoon (i.e period of rice cultivation (iii) advantageous climatic conditions of higher altitude (e.g. temperature) for growing fish even in shallow depth. The best example of mountain valley rice fish culture is the practice of Apatani tribe. The Apatani valley is situated at an altitude 1525 m above msl in Arunachal Pradesh. The plots have more or less uniform elevation from each other. Rice plots in mountain valley are gently slopping and are characterized by a 2 & 3-sided dykes constructed around the plots. The productivity obtained from the practices are presented in Table 5. In the valley areas of Northeast, where settled agriculture is noticed such types of plots are in plenty but mostly not utilized for culture fishery. According to Kojeen (2001) the fish farming in rice field has become an additional source of income and important economic avenue among the Apatani farm families of the state. The farmers modified the system (Fig. 3) in such a way that it has become an excellent example of rice fish farming system in hill tracks and also become very intimately related with the socio economy of Apatani tribe in the state. The method known as *Aji gnui assoni*, is a practice of organic hill aquaculture (Saikia and Das, 2004). This is one of the most advanced cultivation practices (Rai, 2005), with the advantage that the land gives sustained yield year after year unlike the Jhum system. The economic and energy efficiency of this agro-ecosystem is exceptionally high. Though capture of wild aquatic resource from rice fields was a practice among Apatanis, there was barely any systematic rice-fish culture until 1956–66. Later the Department of fisheries initiated *paddy-cum-fish culture* in the Lower Subansiri district of Arunachal Pradesh (Saikia and Das, 2004). Since then Apatani system of traditional rice cultivation (using local cultivars and their indigenous agronomy) has been entangled with fish culture. Rice seed bed preparation commences at the end of March to coincide with paddy transplantation in April. Strains of common carp fry (3–5 cm) are stocked at the rate of 2500 ha^{-1} 10 days after transplanting rice in the field and reared for about four months. Farmers harvest 300– 500 kg ha^{-1} of fish and 3,500–4,000 kg of rice ha^{-1} $year^{-1}$ with fish attaining an average size of 500– 700 g after a culture period of five months. The dykes of rice fields are utilized for growing millet in June and are harvested during August-September. *Cyprinus carpio specularis*, *C. carpio communis* and *C. carpio nudus* are cultured in this way. This type of culture relies mainly on organic fertilizer, namely cow dung, poultry droppings, and animal excreta; no chemical or inorganic fertilizer is applied. Heaps of decomposed rice stubble is left on the field before its preparation. Several weeding activities are performed and weeds are kept in piles in the field to allow decomposition and retention of their nutrients. Channels are constructed in the middle of the paddy fields to maintain the water level.

Table 5. Rice & fish productivity from wet-rice fields of Ziro Subdivision, Arunachal Pradesh (2004)

Block	Village / Locality	Area under wet rice	Estimated yield #(dry wt.) [t / ha]	Estimated fish yield (kg/ha)
Ziro I	Hari & Bulla basti	2000 ha	2.0	300.0
	Hiza basti	1500 ha	1.3	250.0
	Hong basti	1500 ha	2.4	230.0
	Michi bami	1000 ha	1.5	250.0
Ziro II	Yajali	1000 ha	1.1	220.0
	Yagloo & Talo	1000 ha	1.2	215.0
Raga	Tamin	10 ha	1.3	150.0
	Total	8010 ha	1.54±0.48	230±45.5

Yield estimated on dry wt basis at 20% moisture
(Where 1 kg wet weight =400 gm dry weight at 19% moisture).
Note: Data collected from the field directly as well as from the information received from Fisheries officer, Ziro, 2004.

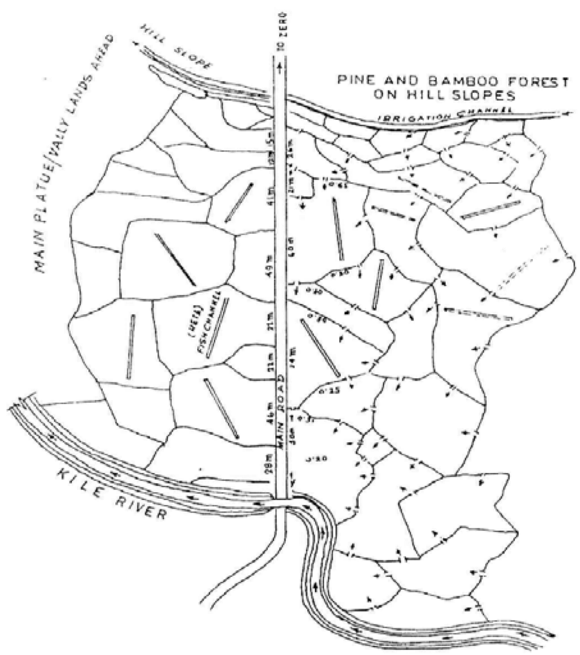

Fig. 3: View of rice fish system developed in Apatani valley, Arunachal Pradesh, India (After Mishra *et al.,* 2004)

Running water terrace farming system

In hilly terrains of Meghalaya, in hilly rice terraces of Apatani valley of Arunachal Pradesh and in certain rice terraces of Nagaland, the fields are laid down in the forms of steps or raised land spaces over mountain slopes. This provides opportunities as in Japan to have running water pisciculture system in rice fields. The terraces are either rain fed or stream irrigated where water trickles down from the plots at higher elevation to lower ones creating a flow through system within the series of interconnected plots. The terrace types of plot are stocked with *Cyprinus carpio* @6000ha^{-1} and fed either with mustard oil cakes 1: rice bran 1 @1kg/ha or simply provided with domestic kitchen waste to get an average production of 186kg/ha/2 months (ICAR Res. Complex leaflet, 1979). In many areas of Arunachal Pradesh particularly in the hills adjoining to Apatani valley, contour terracing has been practiced by the farmers and water is funneled into trench constructed at the edge of each terrace to reduce soil erosion. This seems to have good potential for replication in other hilly rice terraces of NE region where soils have good water retention. A dyke on one side is usually sufficient to retain water in an 800-1000 m^2 trench. An earthen spillway in the dyke is needed to drain off excess water. A one-way bamboo gate across the spillway serves to prevent stocked fish from escaping but also allows wild fish to enter the field. Traditionally simple bamboo gate is being used to prevent stocked fish from escaping from the field. These systems viz., Mountain valley and Running water terrace rice-fish system are being practiced uniquely in Apatani valley of Arunachal Pradesh and certain wet rice terraces of Nagaland. In Nagaland, rice-fish culture is the second major resource for fish production in the state. Presently the total area of paddy field that can be harnessed for integration with fish culture is about 75,700 ha. Dabral (2002) reported that the indigenous farming system of Nagaland is known as *Zabo* originated in Kikuma village of Phek district inhabited by Chakhesang tribe and located at an altitude of 1270 m above msl.The Z*abo* system is being practised in an area of 957.9 ha in the state. Paddy fields are located at lower elevation (Fig. 5). The paddy fields of individual farmer vary from 0.2 to 0.8 ha. The fields are thoroughly rammed at the timing of puddling through treading by human beings, cattle in group and wooden sticks to create a hard pan in order to avoid percolation of water. Seepage losses from shoulder bund are checked with the use of paddy husk. Only one crop (rice) is grown and common variety is "*Tanyekemucah*" (local). The variety matures in about 180 days. The normal seedling rate is 60 kg ha^{-1}. Transplanting is done in June at about 12 cm x 12 cm to 18 cm x 18 cm spacing. Two supplementary irrigations are provided from water harvesting tank. Generally water depth about about 10 cm is maintained in the paddy fields and majority of the farmers practice 'paddy-cum-fish' culture

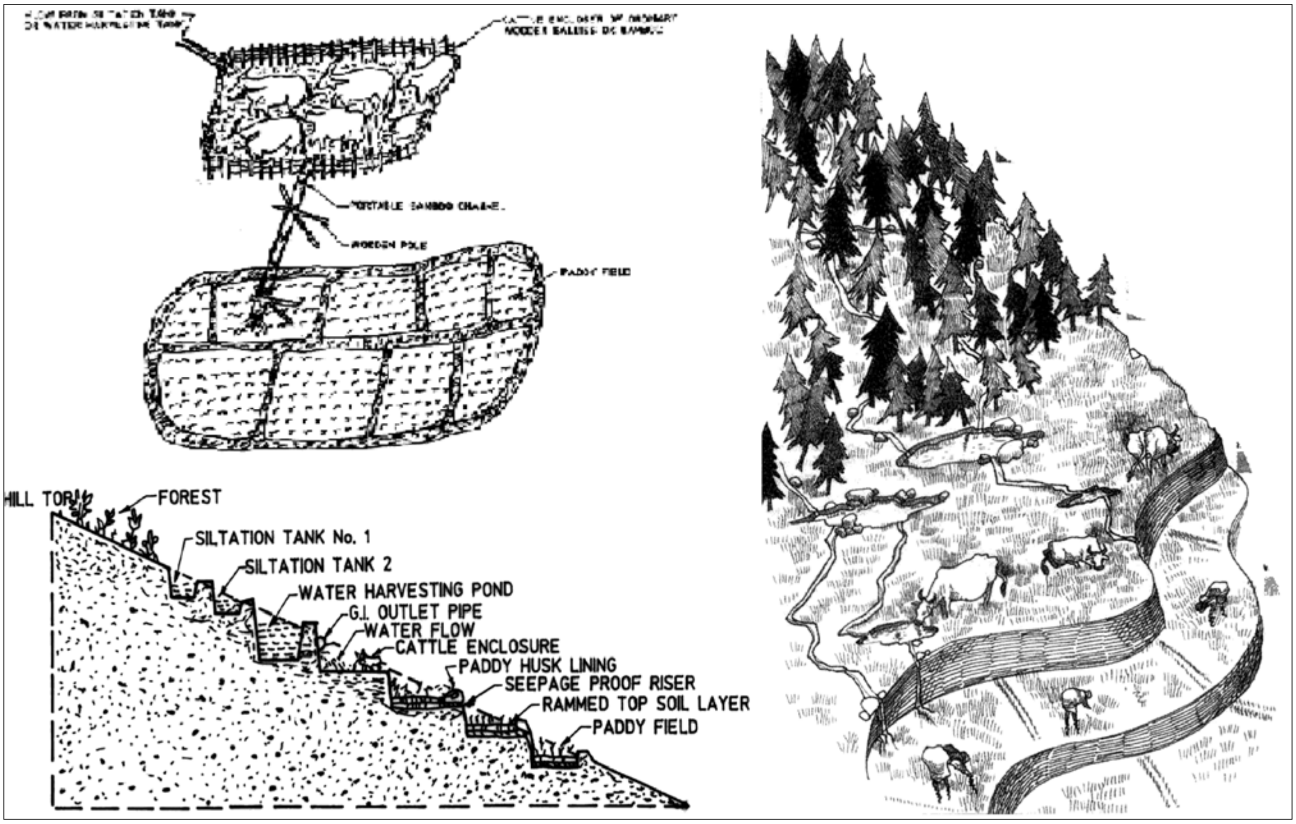

Fig. 4: Field design of Zabosystem of Nagaland. (After Sharma *et al.,* 1994; Dabral, 2002)

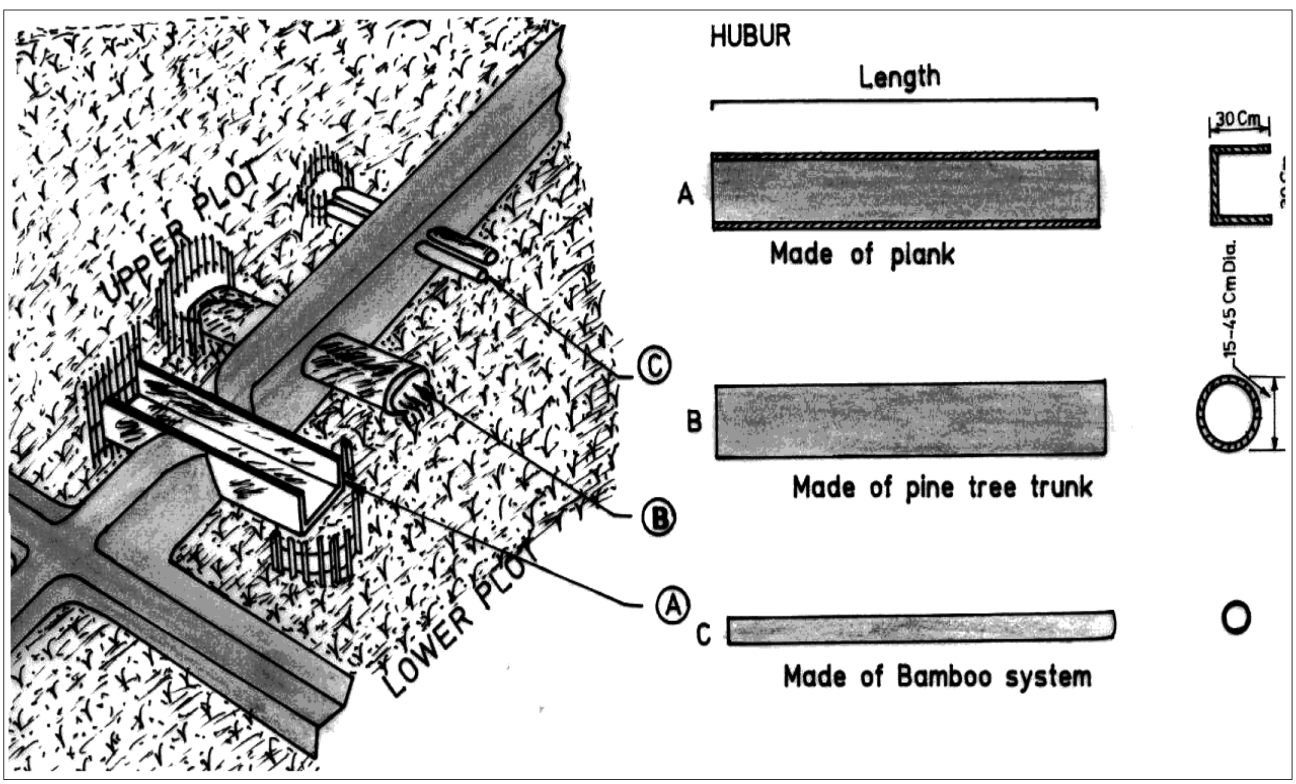

Fig. 5: Design of inlet outlet channel in Apatani ricefish system. (After Dabral, 2002)

with a small refuge pit in the middle of rice field where fish fingerlings are stocked. Before harvesting of paddy crop, water is drained out from the field and fishes accumulates in the pit and the farmers harvest about 50 kg to 60 kg $ha^{-1}$of fish as an additional output along with normal harvest rice ranging from 3.0 t ha^{-1} to 4.0 t ha^{-1}.

Rice-fish culture is also practised in the hills of Manipur especially the Ukhrul disrtict where common carp is mainly cultured.In Mizoram potential area for integration of fish in rice field is available; however there exists hardly any data on ricefish productivity. Further , the rice growing areas of Sikkim though neglible but there are system of Dhan kheti (e.g. Pakhokhet, Shim/Kholyang, Biyansi ete.,) in irrigated rice terraces as well as in watershed based valley rice lands (Sharma *et al.,* 2009) where fish may also be integrated following the models of *Zabo* or *Aji gnui assoni*

Wet rice and resources in wet rice environment

Topographically, the wet rice environments in North east India are available both in flood plains, mountain valleys and terrain hills (Table 1). The rain fed agro ecosystems of monsoon (kharif) season form the characteristic wet rice environments in North eastern states mainly due to high monsoon precipretation. While reviewing the diversity of rice germplasm of the NE region, Hore (2005) described the rice growing ecologies of hilly terrains as well as the indigenous cultivars available in those states. In certain areas, farmers indigenously adopted the system of streams or canal irrigation (Fig. 4&5) based on naturally available water sheds. These systems make judicious use of water in scarcity areas and undulating topographical situations, minimizing soil loss through runoff and maintaining soil health (Dabral, 2002). The use of groundwater irrigation in rice is hardly found except certain flood plain uplands. Among the various types of wet rice lands, the rain fed shallow water (water depth hardly rises above 40 cm), deepwater (water depth remains at or above 50 cm for a month or more) and mountain valley (stream water fed/ rain water fed) rice fields are exploited for rice field fisheries either as captural or as capture cum culture system. Both Brahmaputra and Barrack valley is well known for '*bao*' rice cultivated with traditional deepwater rice (DWR) during kharif season. It forms very conducive habitat (Table- 6a & b) for the aquatic life forms under rice canopy and are rich in plankton, periphyton, macrophytes and other macroorganisms (e.g. crustaceans, insect larvae, annelids, molluscs, and fish). Major phytoplankton belongs to chlorophyceae, cyanophyceae and bacillariophycean algae. Das *et al.* (2007) described the role of attached organisms on submerged rice stem on fish growth under rice fish system of Apatani tribe and designated the system as self-substrating periphyton based aquaculture. There are about more than 150 species of algae reported from various studies (Whitton & Catling, 1980; Catling *et al.,*, 1981; Whitton *et al.,*, 1988a). Many of these algae serve as primary food sources for certain zooplankton and fish population and thereby constitute a complex food web (Fig. 6) in the aquatic environment of DWR (Das, *et al.,*, 1989). Catling (1980) surveyed the pest and general fauna in floating rice fields in Bangladesh paying special attention to insect population on the plant canopy. It is also reported that the fields harbor about 500 species of insects and spiders and over 30 species fish have been found to inhabit the aquatic environment (Choudhury, 1995).

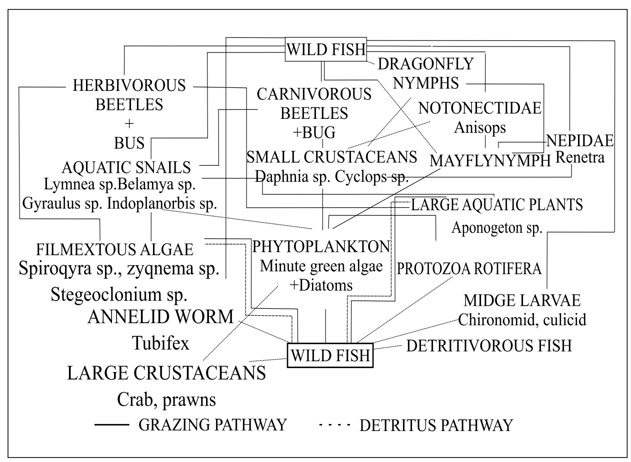

Fig. 6: Food web in wet rice field (After Das, 1991)

Table 6a: Certain habitat parameters of high altitude wet rice environment under rice fish culture (kharif, 2002-03)

Water parameters	Ranges of Mean	Primary productivity parameters	Ranges of Mean
DO(mg/l)	4.2 - 9.6	NPP (mg C/cm^2/hr)	37.54-450.43
Temperature(ºC)	14.0-30.0	GPP (mg C/cm^2/hr)	337.82 -563.04
Conductivity(µmhos/cm)	40.0 - 90.0	Respiration (mg C/cm^2/hr)	37.54 - 480.46
pH	6.11-7.58	**Soil Parameters**	
Turbidity(NTU)	55.6-96.2	pH	5.02 - 5.86
Light intensity(Lux)	760.0-13440.0	Moisture(%)	15.0 - 17.3
CO_2(mg/l)	8.8-15.5	Nitrate nitrogen(ppm)	0.71- 0.25
Total alkalinity(mg/l)	29.0-34.0	Organic Carbon (%)	1.98 - 2.71
Hardness(mg/l)	48.0-62.0	Organic Matter (%)	3.22 - 4.65

(Source: Annual report, 2002-03, UGC sponsored project, Department of Zoology, Arunachal University, Itanagar)

Table 6b: Certain habitat parameters of floodplain *bao* rice environment in Lower Assam ((Kharif,1994 – 97)

Water quality & biological parameters	Range of Mean value	Soil quality	Ranges of Mean value
DO (mg/l)	5.75 – 9.42	Organic Carbon (%)	0.95 -3.12
pH	6.8 -7.8	Phosphorous(g/ha)	99.0- 245.0
Specific conductivity((µmhos/cm)	180.0- 225.0	Potassium(Kg/ha)	500.0-800.0
Temperature (ºC)	24.0- 32.3	Nitrogen (%)	0.09 – 0.21
Water depth(cm)	49.8 -325.4	Specific Conductivity ((µmhos/cm)	210.0 - 432.0
Zooplankton (Unit/l)	325.0 -960.0	pH	6.66 - 7.11
Phyto plankton(Unit/l)	465.0 – 1280.0	Sand (%)	11.4 - 20.4
Periphyton density on glass slide (unit/cm^2/ month)	165.0-352.0	Silt (%)	16.6 - 32.6
Periphyton biomass on rice stem (mg/cm^2 dry weight)	3.4 – 12.6	Clay (%)	42.0 - 70.0

(Source: Das, 1998 Unpublished: Rice fish experimental trials at Bijni and Barpeta district of Assam during 1994-1997)

Puckridge *et al.,* (1988) analyzed the pattern of water depth of the DWR fields. The floodwater is one of the important determining factors for production potential of DWR. The onset of flood, rate of rising water, peak flooding, and average water depth varies in different locations. Catling (1992) reported that the flooding depth is correlated with local rainfall of DWR areas. There occurs fairly rapid rise of floodwater in Vietnam and Burma (5cm/day), often-rapid rise in India (Assam, Bihar, and Uttar Pradesh), Bangladesh, Kampuchea (3.5 – 4.5cm/day) and gradual rise in Thailand, India (West Bengal) and Indonesia (South Kalimatan) (2-4cm/day). The pH of the floodwater is fairly close to neutral (5.9-6.8 in Bangladesh and 6.8-7.9 in West Bengal India) except in acid sulfate soils (Whitton *et al.,* 1988b, Catling, 1992). Setter *et al.,* (1988) described the physico-chemical characteristics of aquatic environment of DWR field in the context of rice cultivation. In relation to fish culture environment, Das *et al.,* (1989) and Mukhopadhyay *et al.,* (1992) advocated that the environment is conducive for rice fish culture except during complete submergence and over flooding leading to the escape of reared fish.

Anthropological perspective

Northeast India despite its multifaceted potential of various resources, the poverty rate in the region is significantly higher than the national average. Agriculture is the mainstay of the economy of northeast India, where more than 85 percent of the total population is rural having sociocultural, ethnic, and linguistic diversity and with more than 200 dominant tribes and many subtribes that reflect the complex social structure. Institutional support, including land tenure, extension services and credit facilities, productive resource base and the distance to the market and service centres are believed to be the major factors influencing agricultural systems in any area of the region (Thapa and Rasul, 2005). In general, the lack of basic infrastructural facilities, communication difficulties with mainland, shortages of employable working skill are major constraint to the promotion of alternative entrepreneurial/ industrial ventures over the resource base of the region. Finally, social unrest and migration of people to urban areas and the anthropogenic impact of development have created a disruptive effect on rural economy and livelihood of the people. Therefore, raising of fish from the rice wetlands though a traditional practice among the people of Brahmaputra and Barak valley but have been affected unfavorably along with other such indigenous and ecofriendly practices recently.

As a source of carbohydrate, rice is the main staple food of the people in Northeastern region. On other hand, fish as the natural food resources from the time immemorial, has been associated with the life of the people who are predominantly tribal. It not only provides protein nutrient but also forms an unbreakable relationship with their culture, religion and tradition. With more than 95% of the population being fish eaters, there is huge gap between supply and demand.Current per capita requirement is about 11kg fish (Munilkumar and Nandeesha, 2007) however availability is much less due to increasing population pressure on natural water bodies as well as declining productivity of the open waters of the region.

In earlier days, wet rice environments in addition to other water bodies of the region were open access resources for wild fishing activities among the common people during monsoon season and huge seasonal capture was possible. Due to abrupt changes in crop agronomy, management practices for high yielding varieties, use of agrochemicals in agriculture and allied sectors, the natural productivity of these fish in open waters in the region declined now a day to the alarming level. Intensification of pond aquaculture using carp species after sixties onwards also caused abrupt damage to small indigenous species (once weed fishes) of fish. Their stocks were abruptly cleaned out from most of the perennial waters for raising the carps only. Wet rice fields, shallow wetlands, seasonal ponds being their gazing and breeding grounds failed to have adequate parental stocks for natural propagation in monsoon seasons. Thus, natural fish productivity of wet rice fields currently declined to such a tragic extent that small indigenous fishes are hard to see in the diet of the rural people even during monsoon months also.

In many hill tribes of these states, rice lands or such resources are either owned, or controlled or managed by clans or village communities. Therefore, management option for those resources remains under strict sociocultural jurisdiction of the tribal community as whole. Due to their ingenuity and skill, certain tribal community has developed many efficient water management systems even in sloppy terraces for growing wet rice (Dabral, 2002; Pulamte, 2008). The Apatanis of Arunachal Pradesh and Chakensansangs of Nagaland are the exemplary tribal communities who adopted the ricefish system based on the available resources and local knowledge in a sustainable manner. Sharma *et al.,* (1994) reported that soils under *Zabo* system of cultivation are fairly rich in organic matter and available nutrients. This makes good yield of rice even in the absence of inorganic fertilizers. In the present context of evergreen revolution, devaluating indigenous knowledge system as "low productive", "primitive", and "old" is no longer a useful attitude (Pulamte, 2008). In the anthropological perspective, the blended technology and innovations of the system of mountain ricefish farming clearly depicts a noble ecological strength which can also be extended to similar such ecologies for boosting up better scope of resource utilization elsewhere in this land locked region.

Contribution of the practices to the livelihood

The social context of rice fish farming in most of Southeast Asian countries is rural poverty and therefore its purpose is to improve incomes, employment scope and distribute wealth using the available accessible resources within the hand of rural mass. This does not preclude support for large commercial and industrial-scale operations as there are mechanisms and models by which these types help in rural economic development. In an overview on rice based small scale aquaculture Demaine and Halwart (2001) mentioned the economic indicators that positively primed the system among the farmers in many of the Southeast Asian countries. The transition from rice monoculture to rice-fish farming is not merely a change in cropping system, more importantly it is a shift towards production of both rice and fishes together - a balanced diet for NE Indians. It is therefore, a critical factor for the health and well-being of farming households of the region. In Brahmaputra and Barak valley, rice field fishing being mostly concurrent activities, farmers have a significantly higher share of fresh fish in their diet. In other aquacultural system, fish farming is a cash crop and thus 80% of the production is sold to local markets while the rest is consumed by the households. Interestingly, rice fish farmers in this region consider fish (mostly small indigenous fishes) as a secondary outcome of rice cultivation and lion's share (more than 60%) of the seasonal fishing or harvest is consumed by the households and rest as surplus are being sent to the local markets. It is a normal trend that farm family prefers to eat small fish which are a valuable source of micronutrients, vitamins and minerals. Rice field fishes have a particular importance for the diets of children and lactating mothers to avoid child blindness and reduce infant mortality. Halwart (2006) stated that the cultivation of most rice crops in irrigated, rainfed and deepwater systems offers a suitable environment for fish and other aquatic organisms. Indeed, it comes as no surprise for those who produce rice that a rich source of biological diversity is to be found in rice-based ecosystems. Wild and gathered foods, from the aquatic habitat, provide important diversity, nutrition and food security as food resources from rice field environments supply essential nutrients that are not adequately found in the diet. Moreover, farmers have experienced that the concurrent culture of fish with rice often increases rice yields. This is reflected in an increase of the net income which is reportedly 7–65% higher on rice-fish farms as compared to rice monoculture farms. Saikia and Das (2008) also opined that rice-based fish farming, though, inevitable as a means of double crop production from the unit land, is often proves as cost-effective practice for marginal and poor farmers. The economic return of the farmers was estimated up to 65.8% per annum from their rice-fish integrated fields. The system of rice-based fish farming by Apatani farmers has, therefore, bears immense potentiality to be recognized as low cost and sustainable farming practice and could be a significant breakthrough for poor and marginal farmers of the rest of the World. A review of projects and programs in several countries and in the region showed three recurring themes common to the progress of this farming, namely: policies and institutional changes in support of poverty-focused development, the driving force of markets, and technology development and adoption by small and poor farmers. The adoption of rice-fish farming in NE region remains rather marginal to date due to socioeconomic, environmental, technological and institutional constraints as the case stated by Nabi (2002) in the context of Bangladesh. Traditionally, wild fish have been harvested from rice fields and was common practice among the people but psychological impact of green revolution slowed down its further development in spite of tremendous potentiality. With the introduction of high yielding varieties (HYV) of rice, the pest control strategy has created a choice among farmers towards chemical pesticides and various other agrochemicals (Berg, 2002; Gupta *et al.,* 2002). Later, IPM approaches though arrived as solution for reducing toxic agrochemicals but the farmers of the region still have been trained adequately to practice the same. Whereas the IPM approach in fish farming in rice fields becoming popularity in many Asian countries, such as China, Philippines, Thailand and Vietnam (Halwart and Gupta, 2004). Weimin (2009) reported that rice-fish culture has achieved significant development in China in the past three decades, in spite of the major socioeconomic changes that have occurred during this period. There are some 1.55 million ha of rice-fish culture in China now, which produces approximately 1.16 million tons of fish products, in addition to about 11 million tons of high quality rice. Fish production from rice fish culture has increased by 13-fold during the last two decades in China. The successful experiences and lessons of rice-fish culture development drawn from China can be a good reference for sustainable rice-fish culture development in the region as well as other parts of the world and thereby it may contribute further to food security and poverty alleviation. He also estimated that the area under rice cultivation in Asia approximates 140.3 million ha, accounting for 89.4% of the world total. It is also evident from various experimental trials that judicious stocking of fish reduce the pest pressure in rice and may provide economic gain to the farmers by reducing the cost of agrochemicals. Sugunan *et al.* (2006) while reviewing livelihood aspect of integrated agricultuture-aquaculture in respect to win-win solution , stated that the integration of capture fisheries and fish culture in rice farming under community management provide further increased production scope from flood prone ecosystem. Intensification of flood plain rice fish system of NE region may also be viable proposition in the light of good governance of community management and thereby the flood prone rice resources will be conserved to advance ecological and economic service to the society. Goswami *et al.* (2004) verified the viability of ricefish farming in NE region at the techno economic aspects on basis of the results obtained from various experimental trials and supported its relevance towards the livelihood option for the rice farmers of the region.

Issues and concern

It has been found from the foregoing discussion and evidences that the rice fish farming practices that exists among the wet rice farmers of the Northeastern region still is very traditional having either low productivity in hilly areas (e.g. culture system) or with declining fishery harvest in flood plain areas (capture or capture cum culture system). The main issues lie with their future improvement and further popularization and extension among the farmers of each and every state having adoption possibilities. The major constraints faced by the farmers are secuirity, apathy, poaching, input shortage, pest diseases and lack of support services (Baruah *et al.,* 2000). On the above back ground generalized but priority issues for augmenting rice fish ecologies may be grouped as follows:

1. The technology packages for culture systems are well standardized and only need is further location specific refinement before its recommendation to the farmers of particular agro climatic zone of the region.
2. Training for improvement of technical as well as farming skill is highly needed in the region towards increased people’s participation and awareness on the system.
3. Flow of financial support from Govt., and Banking sector for promotion of the system require special attention because of increasing cost fish seed, feed, fertiliser and labour.
4. Enhancement of people participation towards conservation and protection of rice wetlands in Northeast India.
5. Promotion of assured Irrigation facilities in rice growing areas that can help to expand rice-fish culture practices through cropping cycle of kharif rice-fish followed by boro rice- fish rotation in the region.
6. Special thrust should be given on R&D initiatives on various aspect of rice fish integration with interdisciplinary and ecosystem approaches for species combination, varietal trials, rice agronomy, pest and disease management, nutrient requirement and uptake, nutrient recycling, greenhouse gas emission, biotechnological interventions, conservation of rice field biodiversity etc. Besides, prevailing systems of the region demand the analysis of their strengths, weaknesses, opportunities and threats (SWOT) on the milieu of system reality for their further promotion and extension.
7. Environmental , socio cultural and economic aspect of the systems should be explored and documented for proper planning and sustainable implementation of the improved technology in the process of integration.
8. Over fishing of the wild piscatorial resources has to be stopped and erection of bunds/barrages on the fish passages connected with perennial waters to be prevented. Wet rice farmer has to be encouraged for providing fish refuge in their rice field towards natural assemblages of wild parental stock entered into flooded field.
9. Priority should of organic approach and avoidances of agrochemicals

10. Practices that evolved on farmers' indigenous knowledge must be documented, validated accordingly.

Major concern for any system improvement deals primarily with environmental impacts, food security and sustainability. Regarding ricefish farming system, these are well proved in many rice growing countries of the world including our neighboring country China. It has already been designated as one of best system of organic farming in our country also. Undoubtedly, the system may be used an important component of further ever green revolution. Bonner (2002) in the context of sustaibility cited a study in Korea which revealed that avoiding pesticides in paddy fields encourages the growth of a certain fish, the muddy loach, *Misgurnus mizolepsis*, which effectively controls mosquitoes that spread malaria and Japanese encephalitis. There are also some studies on rice-fish integrated farming which provide evidence that the introduction of fish into the farming system leads to a decrease in the incidence of malaria, since the mosquito larvae are consumed by the fish. Similar experiences are also cited in the case of integrating ducks into the rice fish farming system (de la Cruz *et al.,*1992).

Threats

The flood plain rice farmers in Northeast India are reluctant to adopt rice-fish farming because of risks of flooding which generally occur due to high precipitation in the region. It has been found that better-off farmers are active in rice-fish farming due to the taking of risk as they describe "there is no gain without risk." Strong and judicious height of the field dykes, erection of pens or placing of fish barricade during culture season may be a viable alternative to prevent fish escape from the culture plots. Adoption of the community co operative system for management of vast ricefield aquaculture, guarding entry and exit points of water channels helps in better retrieval of the fish under rice fish culture. In spite of several constraints, there are enormous opportunities for rice-fish culture development in the region. According to Halwart (2006) any development plans that focus only on increasing yields of rice may possibly give people more rice to eat, but at the same time may take away many of the aquatic animals and plants harvested from and around the rice fields. Many of the aquatic organisms found in rice ecosystems play crucial role biologically as controlling agents of vectors and pests, nutrient recycling etc. Rice fields may also harbour species which are under the threat of extinction (i.e. gradual of loss of aqua-faunal diversity) from the rice field and portray another important threat in front of us. The understanding of the value of aquatic biodiversity from rice-based ecosystems for food and nutrition needs to be well integrated into national agricultural systems that embrace the concepts of an ecosystem approach and the role of agro biodiversity for people and the environment. The lack of adequate knowledge and support to farmers keep them away from the benefits of rice-based fish farming. The novel technique adopted by Apatani farmers in Lower Subansiri district of Arunachal Pradesh, India reduces the knowledge gap to achieve optimum benefit from such farming practice (Saikia and Das, 2004).Gradual shrinkage of rice wet lands in region also pose serious threat for further propagation of the practice. More over, impact of green revolution already kept an impression to the farmers that use of agrochemicals (e.g. chemical fertilizers, pesticides, plant hormones etc.) is mandatory for HYV strains of crop. Besides, sedimentation due to flooding is another threat in the region which gradually deteriorating the flood plain rice ecologies of NE region. The affect of deforestation leading to silting that enters into rice fields with runoff rain water is the primary cause behind such sedimentation.

New initiative to preserve the practices

Harnessing economically viable, socially inclusive and environmentally sustainable livelihood security and growth with upfront management of land, water, crop and social capital is a challenging task. Rice fish farming systems are globally important in terms of food production; and appear to be globally important in terms of three global environment issues: climate change, shared waters, and biodiversity. Therefore, the new initiative for its sustainable development and extention should be under the special national policy frame work for the region under regional programme. With multiple livelihood and ecological values as mentioned above, the traditional rice-fish system is a remarkable model of the biodiversity-enhancing agriculture system. There is tremendous potential to balance the shortcomings of the chemicals-based agriculture and developing ecosystem approaches to managing wetlands and flood plains. A consortia approach for different dimensions of R&D activities would be appropriate for support and collaboration from local communities and also to launch the activities in region. Following initiative will be appropriate for preserving and promoting the practices:

1. Documentation of changing patterns of the traditional rice-fish system and its social contribution
2. Evaluation of impact of policies, institutions and technologies on farmers' practices of the rice-fish systems and identify those policies, institutions and technologies that encourage rice -fish integration
3. Setting up of representative technology/package of practices demonstration sites/villages through people participation (local communities, government and NGO's)

4. Identification and demonstration of successful adaptations to social-economic changes, and explore the multiple values of the rice-fish system in the food safety, eco-agriculture, eco-tourism and ecological conservation
5. Development of networking on conservation and sustainable management of the rice-fish system among communities, local governments, and NGO's

Conclusion

In spite of huge potentiality of wet rice environments, the farmers, in North eastern region are habituated mostly in wild capture of fish from time immemorial and the rarely culture fish along with rice. The concept of intentional rearing or cultural farming evolved far later because of increasing demands of fish in the region. From the study it clear that the rice farmers of the flood plain areas of the region hardly accepted the research and developmental outcome of modern rice fish culture techniques till today. It has happened probably due to majority of the rice farmers are (1) very reluctant to change their traditional working habit (2) less optimistic about the possibilities of gain from intentional culture for the investment risk (3) mainly being in the social caste of rice cultivator, believe fish culture as a caste specific profession (4) often discouraged due to modern agrochemical based extension network of the region. Therefore, it can be said that whatsoever systems of raising fish from rice paddies are in vogue in this part of Indian subcontinent were evolved solely by farmers for meeting their daily protein requirement only . Based on their immediate needs, they probably experimented and innovated less risky adaptive methods of raising wild fish in their waterlogged rice fields to their local context of socio economic condition. It is also noticed that various anthropogenic factors associated with modern agriculture are dwindling the captural resources of the rice field. However, the cultural effort still has remained at subsistence level because of the lack of awareness and extension programmes and farmer's friendly technique for the region. In NE region of India, the climate is no doubt very congenial to improve the status of rice fish culture but it demands interdisciplinary research and development thrust considering compatibility of rice cultivars, fish species and the location specificity of management aspects.

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Mid hill aquaculture: Strategies for enhancing production in Northeast hill region of India

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ABSTRACT

The Northeast region of India comprising the states of Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura are blessed with rich biodiversity and fisheries resources. Fish being one of the cheapest and easily digestible proteins can ensure the nutritional requirements and alleviate malnutrition especially in the hilly states of the region. The altitude in the region varies from almost sea-level to over 7,000 metres (23,000 ft) above MSL. Barring Assam and Tripura, other six states are predominantly in hilly region which are mostly inhabited by different tribes. The fish culture in the lower altitude (less than 400 m above msl) records the highest production while the high altitude aquaculture (>1200 m above msl) produces very little fish. The mid altitude region which cover a large part of NE hill states provide immense potential for aquaculture. However, promoting mid altitude aquaculture (400-1200 m above msl) to farmers in the hill states of the Northeast is a major challenge due to several reasons. The paper discusses the important bottlenecks in promoting mid hill aquaculture and suggests strategies for enhancing fish production to meet the growing demand for fish in the hill region of the Northeast India.

Keywords: Mid hill, Aquaculture, Fish production

Introduction

The NER of India covers an area of 2.62 lakh sq. km. It accounts for 7.9% of total geographical area of the country. The region can be physiographically categorised into the Eastern Himalaya, the Patkai and the Brahmaputra and the Barak valley plains. The region, with mighty Brahmaputra-Barak river systems and their tributaries has predominantly humid sub-tropical climate with hot, humid summers, severe monsoons, and mild winters. Geographically, apart from the Brahmaputra, Barak and Imphal valleys and some flat lands in between the hills of Meghalaya and Tripura, the remaining two-thirds of the area is hilly terrain interspersed with valleys and plains; the altitude varies from almost sea-level to over 7,000 metres (23,000 ft) above MSL. In the mountainous areas of Arunachal Pradesh, the Himalayan ranges in the northern border with India and China experience the lowest temperatures with heavy snow during winter and temperatures that drop below freezing. Areas with altitudes exceeding 2,000 metres (6,562 ft) receive snowfall during winters and have cool summers. Below 2,000 metres (6,562 ft) above sea level, winter temperatures reach up to 15 °C (59 °F) during the day with nights dropping to zero while summers are cool, with a mean maximum of 25 °C (77 °F) and a mean minimum of 15 °C (59 °F) (Dikshit and Dikshit,2014).

With a total population of 4.55 Crores (2011), it accounts for 3.7% of total population of India. In general, aquaculture production is concentrated in tropical & sub-tropical areas and only minor contributions come from temperate areas in the form of high valued fish species. The region produced over 426.59 thousands metric tonnes of fish during 2015-16 with almost 50% coming from aquaculture. With more than 95% of population being fish eaters, there is a huge gap between supply and demand. The current availability of fish in the region is estimated to be around 6.00 kg/person/year, which is lower than the national availability of 9.00 kg/person/year. To meet the growing demand for fish, the region is importing fish from Andhra Pradesh and other Indian states in addition to unaccountable import from the neighboring countries of Bangladesh and Myanmar. Considering the fresh fish requirement @13Kg/per capita, the deficit is estimated at 32.6% (Table 1).

Table 1. Analysis on requirement of fresh Fish in Northeast India

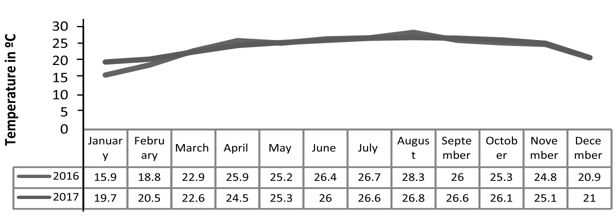
Parameters	Estimated value
Total Population (08 Northeast States)	513.16 lakhs
Fish eating population	95%
Requirement @13Kg/per capita	Approx. 633 Thousand metric tonnes
Current production (2015-16)	426.59 Thousand metric tones
Deficit (Approx)	207 Thousand metric tonnes (32.6%)
NE states contribution to Inland fish production of the country (7162 thousand metric tonnes).	5.95%

Table 2. Water resources of North-East India (Adapted from Goswami *et al.,* 2012)

State	Rivers/streams (km)	Beels/lakes (ha)	Tanks/ponds (ha)	Paddy/fields (ha)	Other suitable water (ha)
Arunachal Pradesh	2000	2500 + cold water	1000	2800	700
Assam	4820	100000	20000	20000	1517
Manipur	2000	40000	5000	40000	10000
Meghalaya	5600	394	1944	5000	3000
Mizoram	1748	32	1800	1560	-
Nagaland	1600	215	2000	10000	-
Sikkim	900	-	-	-	-
Tripura	1200	240	11038	-	-
Total	19868	143381 +110	42752	79360	15217

The region has rivers, streams, flood-locked plain wet land, reservoirs, lakes, ponds (Source: Department of Animal Husbandry, Dairying & Fisheries, Govt of India and Directorate of Fisheries, State Governments). and tanks, paddy fields etc. (Table 2) to support large scale aquaculture activities, which not only can produce fish to meet the regional requirements, but also export the surplus through judicious and efficient use of these water bodies. One of the important bottlenecks is the lack of quality seeds of appropriate fast growing fish species for expansion of mid hill aquaculture.

Further, most farmers possess small to medium size fish ponds where fish growth can be expected only for about 6-8 months in a year under the prevailing climatic condition. Temperature plays an important role in fish growth .The region in recent past has suffered from climatic variability which refers to sudden and discontinuous seasonal or monthly or periodic changes in climate or its components without showing any specific trend of temporal change. An instance of water temperature variability during the years 2016 and 2017 in farm ponds located at 900 m above msl has been presented in Graph 1.The mean monthly water temperature of pond dropped down to 15.9 ^{0}C degree centigrade in 2016 ,while in 2017, it increased to 19.7 ^{0}C for the month of January (Das, 2018). Researchers have predicted an increase in temperature by 2 to 4.5 ^{0}C by the end of the century; best estimate at 3 ^{0}C and very unlikely to be less than 1.5 ^{0}C.



Graph. 1: Monthwise average water temperature in farm ponds located at 900m above MSL during 2016 and 2017

Fish Production

The Northeast contributes about 5.95 % to the total Inland fish production of the country (Table 1). During 2015-16, the total fish production was 426.59 throusands tonnes and among the eight states, Assam registered the highest fish production of 294.2 thousand metric tonnes. The state of Assam contributes 69% of the total fish production of the Northeast India (Fig.2**)** followed by Tripura (16%), Manipur (7%), Meghalaya (3%), Nagaland (2%), Mizoram (2%) , Arunachal Pradesh (1%) and Sikkim (negligible).

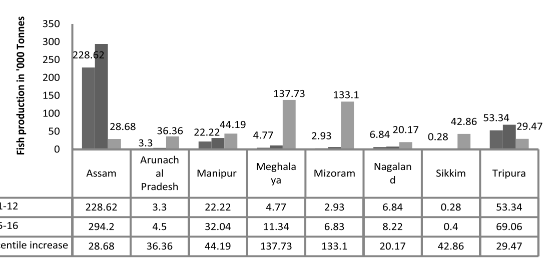


Fig. 1: Percentile increase in fish production of North east states of India (In'000Tonnes) in past three years

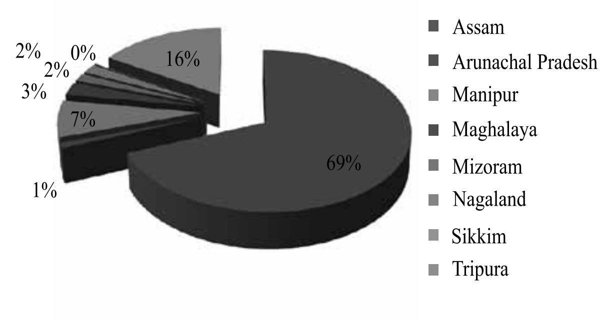


Fig. 2: Statewise percentage contribution of fish production during 2015-16

The annual growth rate of fish production in NE India has registered a positive growth over the years. The percentile increase in fish production as highlighted in Fig. 1**.** shows significant increase in fish production from 2011-12 to 2015-16 in the states of Meghalaya and Mizoram with 137.73 and 133.10 percentage respectively. The average pond productivity especially in the hilly states of northeast region is still low as compared to the national average of more than 2000 kg/ha/year. In order to improve the fish production and productivity in mid hill aquaculture a few strategies are suggested.

Species diversification

The fishery resources of the North eastern region fall within all three types of climate i.e. Tropical, Sub-tropical and Temperate and represent a strong biodiversity. The region harbour several important fish species of both food and ornamental value. In fact the Northeast India is considered as one of the hot spots of fresh water fish biodiversity in the world (Kottelat and Whitten, 1996). The North Eastern Region shares its fish fauna predominantly with that of the Indo-Gangetic fauna and to a small extent with the Burmese and South China fish fauna (Yadav and Chandra, 1994).

Although Sen (2000) described 266 species, Goswami *et al* (2012) listed 422 fish species from northeast India, belonging to 133 genera and 38 families. Since, water temperature in the hills falls below 20°C, the exotic carps (common, grass and silver carps), mahseers and other such coldwater fishes that can grow and survive at lesser temperature than IMCs are more suitable for use in hill aquaculture. Thus, the fish production technologies being practised in plain areas are less viable in the Northeast hill areas primarily due to cold climatic conditions and lack of appropriate infrastructure facilities. Hence, for the sustainability of aquaculture, more species need to be brought into the culture although we all know that each species has a unique set of biological factors that influence its culture potential. Further water quality, growth variations, food conversion rates, predation, and disease problems should also be anticipated.

Aquaculture in the region is basically carp based polyculture. Among all the fish species available in the Northeast, only three Indian major carps (Rohu, Mrigal and Catla) and three exotic carps (Silver, grass and Common carp) are the most common and widely cultured fish species in farmer's ponds. Most of these fishes are warm water species and perform better in plain areas at lower altitude although the Chinese carps withstand relatively colder temperature.

In the Northeast, fish seeds about 11-12 various fish species are commonly produced mostly by the farmer seed producers in the private sectors. In addition, the state of Tripura produces seeds of Pabda, *Ompok pabda*, *Macrobrachium rosenbergii* (Fresh water giant prawn), Manipur and now Meghalaya (ICAR NEH) produce seeds of an endemic minor carp, Pengba, *Osteobrama belangeri* in limited quality. Among all the cultured species; Silver carp, Grass carp and Common carp are reported to perform better in composite culture system in the mid altitude conditions. Common carp plays an important role in augmenting fish production especially in the hill states of the region. However, the local existing stock of common carp has several demerits, resulting in poor growth. To address the problems of early maturation and poor growth of the existing local stock of Common carp (*Cyprinus carpio* var.communis), a maiden effort was made by the ICAR NEH which is located at about 900 meter above mean sea level (MSL) in 2010 to introduce the genetically superior breed/variety of Common carp-Amur (Hungarian strain) in Meghalaya after thorough on-station & on-farm trials and later popularizing it for improving the fish production and productivity from the rural ponds of the tribal farmers in most of the hill states of the Northeast region (Das, 2017a). The fish breeding season in mid hill region is restricted to only few months (June to August), July being the peak. The ICAR NEHR, has been successful in breeding of all the exotic and India major carps. In addition, minor carps, *Labeo gonius*, *Labeo bata and Puntius javanicus* have also been successfully reared and induced bred for promotion of these species in mid hill aquaculture successfully. In recent times one more fish species which is highly esteemed Pengba, *Osteobrama belangeri* (- an endemic & endangered fish species of Manipur) has been introduced in Meghalaya (Das and Singh, 2017). In recent times, the combination of Amur common carp and minor carps is observed to be the best suited for mid hill aquaculture. Thus, there is always a growing demand for the quality seeds of especially Amur common carp and *Labeo gonius* by the farmers of mid hill region. Under mid hill condition both the species attain marketable size within the one year of rearing unlike the Indian major carps.

There is great potential for utilizing indigenous fish species for aquaculture in the mid hill region. The potential candidate species for mid hill aquaculture in North east region are *Tor putitora (*Golden mahseer*), Neolissochilus hexagonolepis* (Chocolate mahseer)*, Amblypharygodon mola,, Chagunius spp, Cirrhinus reba***,** *, L. pangusia, Labeo dyocheilus, Labeo dero, Semiplotus semiplotus etc.* Breeding and culture of these important indigenous fish species shall not only improve the fish production scenario in the hilly states of the region but also shall help in conservation of these valuable fish species. Although there are reports on induced breeding of some theses species, at the moment there are no farmer friendly technologies available for mass-scale production of their seeds to promote aquaculture in ponds, tanks and in other water bodies especially in the mid hill areas. In recent years, the culture of alien fish species such as Thai magur (*Clarias gariepineus*), Tilapia, hybrid Kawoi (*Anabas testeduneus*), Roop chanda (*Colossoma macropomum*), Bighead carp (*Arichthicthys nobilis*) *Pangasius sutchi* etc. pose threat to native fish species and gene pool and may result in ecological imbalance and may lead to loss of bio-diversity. Therefore ,requires adequate measures to eliminate these species from culture system or culture with do's and dont's as prescribed by the ICAR-NBFGR, Lucknow, UP, India for enhancing fish production from confined environment in the mid hill region.

Fish seed production

There is paucity of correct and updated statistics of fish seed production of different fish species in the Northeastern states. Hill states produce very little fish seeds and largely depend on Assam for their requirement. With the advent of the technique of induced breeding of IMC by Chaudhuri and Alikunhi (1957) through hypophysation, it became possible to obtain quality seed of major carps for aquaculture. During 1990's the ready-to-use fish spawning agents like ovaprim (Nandeesha *et al*, 1990, Das *et al*, 1994) revolutionized the fish seed industry in the country. Another Hungarian hormone, Ovopel was also found to be very effective in breeding of carps in Assam (Das, 2004). During late 90's several ready to use hormones for fish breeding were made available in India by Indian pharmaceutical companies. Currently, Ovatide, Gonopro-FH etc are extensively used by the fish seed producers. Thus, with the use of different types of farmer friendly inducing agents and establishment of hatcheries for fish breeding and egg hatching, it has become possible to produce carp fish seeds in large quantity with high survival percentage of larvae. Currently more than 95% of fish seeds in the country are derived from hatcheries located in different parts of the country.

However, nursery rearing of fish spawn to the desired fingerling size has always been a challenging task as mortality is the highest in case of juvenile fish. In absence of suitable nursery ponds, the pond based cage culture system may be one of the alternatives to the rural farmers to increase the survival percentage and growth of fish fry in order to stock their fish ponds with quality fish fingerlings for augmenting fish production in the region. Under the National Agricultural Innovation Project (NAIP), the low cost pond based cage culture was successfully demonstrated for better survival and growth of tender fish in the Garo hills of Meghalaya for production of much needed fish fingerlings for the first time (Das *et al*, 2017).

Integrated small scale aquaculture system

The North East India is recognized as one of regions for promoting organic farming due to negligible usage of chemical fertilizers, pesticides and other chemicals. Therefore, there is great potential for promoting sustainable organic aquaculture especially in the mid hill areas. Most rural tribal farmers in the hill states of the region are poor, however many of them possess livestocks for domestic needs. Since the size of fish ponds in the hill areas are small and principally rain fed and seasonal, the small-scale integrated aquaculture utilizing available on-farm resources has great potential. Further, due to cold climatic conditions, the fish growing period is also short varying between 7-8 months. The systems are highly relevant to poor people and having limited farm area. Therefore, the small-scale integrated aquaculture is an excellent option for food security and economic uplift of the rural farmers of the hill states of the region. Further, water is emerging as international challenge and its most efficient management as well as recycling has been given high priority by almost all countries. However, integrated farming calls for skill in different types of activity such as raising pigs and poultry, crop and vegetable farming, growing grass and aquatic plants and farming of fish (Little & Edwards, 1999). A maiden attempt was made in Assam, India to introduce a farmer participatory small-scale aquaculture research and extension programme in 3 tribal villages of Assam under an innovative scheme of Assam Rural Infrastructure and Agriculture Service Project (World bank) (Das, 2006). The pilot project conducted with a group of resource poor tribal farmers revealed that a production of about 1800 kg/ha/yr could be achieved from small seasonal homestead ponds through integrated use of locally available biological resources (Das, 2006). This implies an excellent opportunity for improving the rural economy through the development of small-scale fish culture enterprises.

The fish based small-scale integrated aquaculture is best suited for resource-poor small and marginal farmers of the hill states of the Northeast region as land holding is small. It reduces the input and increases output and economic efficiency. The ICAR Research complex for NEH region has developed several Integrated fish based farming system suitable for mid hill region (Hazarika *et al*., 2017). The Fisheries division of ICAR NEH at Umiam, Meghalaya standardized two economically and environmentally feasible farming system models under mid hill condition in the region and later popularized among the farmers (Das, 2015; Sharma *et al*., 2017).

In a three year long study at ICAR, Barapani, Meghalaya (900m above msl) Rice–Fish farming in mid altitude yielded a production of 683 kg of common carp per hectare in a rearing period of 237 days without supplementary feeding while in a fish-based poultry cum horticulture integrated system on an average a production of 1500 kg of fish per ha in a rearing period of 11 months could be obtained without supplementary feeding in addition to 2384 eggs from the poultry in 8 months and a production of 41.5 kgs (960 g per sq.mt) of ginger and 25 Kg (396 gms per sq.mt) of turmeric (Das, S.K., 2017b) . The technology may be termed as "0"-input fish farming as no supplementary feeding or external fertilizers were provided for fish farming in this system. The technology of rice-fish farming not only conserves water resources and plant nutrients but also intensifies production of fish protein. In addition, it reduces the operating costs relative to either system in isolation.

Constraints

There are several constraints in development of the aquaculture sector in the hill region. In addition to difficult terrain, non- availability of quality fish seeds of appropriate fish species in time is identified as one of the major constraints in expanding mid hill aquaculture. There is an urgent need to find alternate fish species which can grow in lower water temperature or grow to marketable size within short period of time (7-8 months). Other important constraints to sustainable aquaculture development in the Northeast hill region are i). Non-availability of specific technology, ii). Non-availability of suitable fish food organisms/feeds, iii). Diseases and iv). Non-availability of trained technical manpower. Further, small and seasonal ponds, lack of private entrepreneurship, poor extension machinery in transfer of appropriate technologies, inadequacy in generation of appropriate culture technologies to suit the local demand, under-utilization of aquatic resources and potential low lying areas for fish farming, unscientific management and inadequate infrastructure facilities and financial assistance are some of the important bottlenecks in expansion of mid hill aquaculture.

Conclusion

In general the cold water fish farming has been largely overlooked due to appropriate fish species, lack of suitable technology, poor growth of fish and inadequate infrastructures. The Northeast hill states are predominantly tribal states. It is assumed that knowledge of scientific fish farming is yet to reach majority of fish farmers for expansion and intensification of aquaculture activities. Most tribal farmers of the region are resource poor and possess small to medium sized fish ponds for aquaculture. Therefore there is tremendous scope for increasing productivity through introduction of suitable fast growing fish species appropriate to the region. The Government should take a policy decision to establish brood bank of suitable fish species for quality fish seed production in order to provide quality brood stocks to the selected farmer fish seed producers of the mid hill region.

With large population of domestic animals and huge resources of green foliage in the region, there is ample scope for vertical expansion of aquaculture through use of on-farm resources to meet the demand. Development of site specific farming systems suitable for the terrain based on the elevation and climatic conditions is therefore required for the region. The northeastern states being projected as tourist spots, the potential of development of recreational fisheries and allied activities such as eco-tourism including bait fish culture hold much promise. Lastly, the capacity building and technology demonstration programmes together with easy availability of critical inputs for fish farming in the region will certainly improve the fish production in the coming years.

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Nutritional properties of some freshwater fish species of Manipur, India

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ABSTRACT

Proximate composition, total free amino acid polyunsaturated fatty acid, minerals and antioxidant properties of six fresh water fishes, viz., *Amblypharyngodon mola, Gagata dolichonema, Garra abhoyai, Glossogobius giuris, Hypsibarbus myitkyinae* and *Puntius sophore* were determined.were collected from different sites of Manipur. The highest moisture, protein and ash content were recorded in *Puntius sophore.* The lowest moisture content was recorded in *Garraabhoyai* (68.25%). The lowest protein and lipid content was found in *Glossogobius giurius* (9.42% and 2.23% respectively). The lowest ash content was recorded in *Amblypharyngodon mola* (1.39%). The highest similar lipid content (5.83%) was found in two species (*Garra abhoyai* and *Gagatado lichonema*). The total amino acid content was found highest in *Hipsibarbus myitkyinae* (487.00 mg/100g) and lowest in *Puntius sophore* (196.53 mg/100g). The highest docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), arachidonic acid, lenolenic acid and linoleic acid were found in *Amblypharyngodon mola.* The maximum Fe, Cu, Zn and Mn were found in *Amblypharyngodon mola.* The highest Mg, K, Ca and antioxidant activity were recorded in *Puntius sophore.* The fishes studied observed that they are good sources of protein, minerals, antioxidant and PUFA. Thus the consumption of the fishes should be encouraged.

Keywords: Fresh water fishes, Total amino acid, PUFA, Minerals, Antioxidant property.

Introduction

Fish is one of the important sources of animal protein and accepted to be nutritious food for their constituent of desirable component of human diet. Fish has also been an important item in the diet of the people of Manipur since time immemorial. Fish meat has received increased attention as a potential source of animal protein and essential nutrients for human diet and is regarded superior to other meats in having significantly low lipids and high digestibility. Compare to other source of protein, fish protein are known for the excellent source bioactive peptides with valuable nutraceutical and pharmaceutical potentials. The nutritional value of fish meat lies in its protein, lipid, vitamin and mineral contents and also in its caloric value (Higashi, 1962). The analysis of the major constituents (i.e., proximate composition) of fishes is necessary for providing information of the concentrations of protein, lipid, ash and moisture of the particular species (Stansby, 1962).

Beside excellent source of protein they are also an important source of vitamins, antioxidant as well as minerals including Calcium, Iodine, Selenium, Iron, etc. (Decker *et al*. 2001; Sarkar et *al.,* 2013) and also a good source of highly unsaturated fatty acid (HUFA) and polyunsaturated fatty acid (PUFA) especially omega-3 fatty acid viz. eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Huynh *et al.,* 2007). There are abundant evidences manifesting the significance of fish consumption in brain development and learning in child, protect vision and eye health and protection from cardiovascular diseases and some cancer (Sarkar *et al.,* 2013)

In Manipur, any types of big or small fishes are consumed in fresh or process (Sarojnalini, 2010). Small cobitid fish viz., *Syncrossus berdmorei* and *Lipidocephalichthys irrorata* endemic in this area are highly esteem among the people (Sarojnalini and Vishwanath. 1988). There are some reports about the biochemical composition, minerals, antioxidant and PUFA of fishes (Chen and Decker, 1994; Sakuntala *et al*., 1997; Vishwanath *et al.,*1998; Larsen *et al*., 2000; Roos *et al*., 2003; Gokoglu *et al*., 2004; Fatima *et al*., 2007; Sarojnalini, 2010; Sarjubala and Sarojnalini, 2012; Sarojnalini and Sarjubala, 2014;).

So, the aim of the current study was to investigate proximate composition, minerals, antioxidant and PUFA of selected freshwater Fishes of Manipur as an extension to the previous worker on this field and to provide knowledge of nutritional value prior to human consumption.

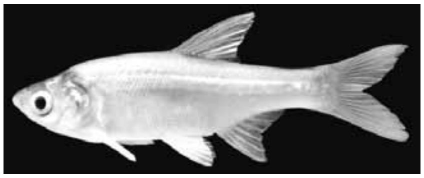

Fig. 1 : *Amblypharyngodon mola*

Fig. 2 : *Gagatado lichonema*

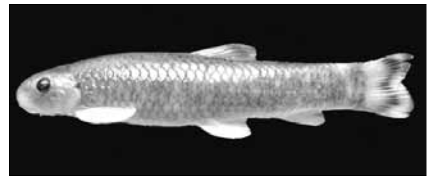

Fig. 3 : *Garra abhoyai*

Fig. 4 : *Glossogobius giuris*

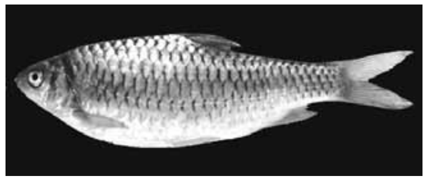

Fig. 5 : *Hypsibarbus myitkyinae*

Fig. 6 : *Puntius sophore*

Materials and Methods

Sample collection

Some fresh water fish species (*Amblypharyngodon mola, Gagata dolichonema, Garra abhoyai, Glossogobius giurius, Hypsibarbus myitkyinae* and *Puntius sophore)* were collected from different sites of Manipur (Fig. 1-6). The respective site of their collection has been shown in Table 1. And the length and weight of the fish species were also shown in Table 2. The fish samples were brought to the Fishery Research Laboratory, Department of Life Sciences, Manipur University, Canchipur with proper caring and were prepared for further analysis.

Table 1: Fish species and their respective collection sites:

Species	Local Name	Collection site
Amblypharyngodon mola	Mukanga	Ima Market
Garra abhoyai	Ngamusangum	Itok river, Yairipok
Gagata dolichonema	Ngarang/Ngayek	Iril river
Glossogobius giuris	Nilonngamu	Moirang Market
Hipsibarbus myitkyinae	Heikaknga	Moreh Market
Puntius sophore	Phabounga	Moreh Market

Table 2: Length-weight of the selected fish species:

Species	Total Length (cm)	Weight (gm)
Amblypharyngodon mola	04.46-4.90	01.46-01.51
Gagata dolichonema	07.90-09.80	05.31-07.00
Garra abhoyai	06.01-07.52	08.21-10.04
Glossogobius giuris	13.20-14.60	20.35-24.38
Hipsibarbus myitkyinae	12.15-13.53	22.87-24.32
Puntius sophore	04.80-5.90	03.48-05.55

Proximate compositions

Proximate compositions (moisture, protein, lipid and ash) were determined by using the methods of AOAC, 1995. Total protein values were obtained by multiplying the nitrogen value with 6.25 (Osborne and Voogt, 1978).

Total Free amino acids

Total amino acid were determined by the method described by Moore and Stein (1948). Calculation of the amount of total free amino acids was done by using standard curve prepared from leucine by pipetting out 0.1-1.0 ml (10-100 µg range) of working standard solution. Results were expressed in percentage equivalent of leucine.

Polyunsaturated fatty acids (PUFA)

The polyunsaturated fatty acids content were determined following the method of AOAC, 1995. 2ml of the sample solution was transferred to a reaction tube and the solvent was evaporated. Absolute ethanol and KOH glycol reagent are added and mixed thoroughly. The air in the tube was removed by heating at 180^0C. A tube with only reagent and ethanol, which serves as a blank, was run with each set of samples.

Mineral Analysis

Mineral (Fe, Zn, Ca, Cu, Mg, Mn, K and Na) analysis was done following the method of Perkin-Elmer, 1996. Ash of respective sample was digested in HNO_3 making carbon free and make up to the volume of 50 ml distilled water and subjected to analysed the dissolved metal content.

Determination of Antioxidant activity

The antioxidant activity of the fishes extract was examined by comparing it to the activity of known antioxidants such as ascorbic acid by scavenging of DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity. The free radical scavenging capacity of the extracts was determined using DPPH (Cuendet *et al*. 1997). Ascorbic acid was used as reference antioxidants.

Statistical analysis

The data were analysed using one-way analysis of variance (ANOVA) and the significant differences between means of experiments were determined by post hoc Duncan's multiple range test. A significance level of 0.05 was chosen. Data were analysed using SPSS package (Version 17.0). Differences were considered significant at P<0.05 (Sokal and Rohlf, 1974).

Results

The Proximate composition for the above selected fish samples were shown in Table 3. The moisture content was found to be highest in *Puntius sophore* (76.35±0.09%) and lowest in *Garra abhoyai* (68.25±0.39%). The crude protein content ranged from 9.42±0.47% in *Glossogobius giurius* to 20.50±0.08% in *P. sophore*. The relative lipid content varied from one fish species to another. The highest lipid content was recorded in *G. abhoyai* and *Gagata dolichonema* (5.83±0.15%) and lowest in *G.giurius* (2.23±0.15%). The ash content was found high in all the fish species except in *Amblypharyngodon mola* (1.39±0.01%). The highest ash content was recorded in *P. sophore* (4.38±0.03%).

The Total amino acid content of the fish species were shown in Fig. 7. The highest total amino acid was recorded in *Hipsibarbus myitkyinae* (487.00±0.2mg/100g) and lowest was in *Puntius sophore* (196.53±0.14mg/100g).

Further, polyunsaturated fatty acid (PUFA), minerals and antioxidant property were analysed for two species (viz., *Puntius sophore* and *Amblypharyngodon mola*). The polyunsaturated fatty acid (PUFA) consists of Docosahexaenoic acid (DHA), Eicosapentaenoic acid (EPA), Arachidonic acid (AA), lenolenic acid and lenoleic acid. The polyunsaturated fatty acid content of fresh *Puntius sophore* and *Amblypharyngodon mola* are shown in Table 4. The highest DHA (2.9mg/100g), EPA (0.12mg/100g), lenolenic acid (1.4mg/100g) and linoleic acid (2.3mg/100g) were found in *A. mola*.

The mineral element content of *Amblypharyngodon mola and Puntius sophore* is shown in Fig. 8. The highest Fe (482mg/100g), Cu (4.8mg/100g), Zn (109mg/100g) and Mn (35mg/100g) were found in *A. mola.* Whereas, the highest Mg (228mg/100g), K (208mg/100g), Na (82mg/100g) and Ca (902mg/100g) were recorded in *P. sophore.*

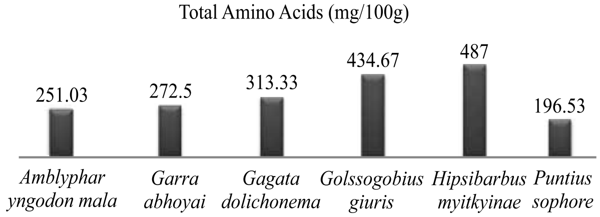

Fig. 7: Graph showing the Total amino acid content of the fish species.

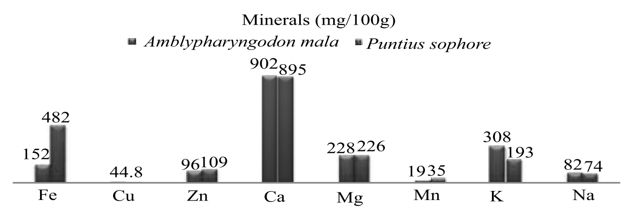

Fig. 8: Mineral content of *Amblypharyngodon mola* and *Puntius sophore*(mg/100g)

Table 3: Proximate composition of the selected fresh water fish species.

Species	Moisture %	Protein %	Lipid %	Ash %
Amblypharyngodon mola	74.69±0.00	14.11±0.00	5.82±0.00	1.39±0.01
Gagata dolichonema	72.52±1.27	12.42±0.84	5.83±0.15	3.73±0.74
Garra abhoyai	68.25±0.39	10.56±0.21	5.83±0.15	3.35±0.11
Glossogobius giurius	76.21±1.11	9.42±0.47	2.23±0.15	3.77±0.03
Hipsibarbus myitkyinae	74.12±1.78	14.16±0.57	5.00±0.62	3.49±0.13
Puntius sophore	76.35±0.09	20.50±0.08	2.46±0.14	4.38±0.03

The results are mean±S.D. of the samples taken in triplets.

Table 4. Polyunsaturated fatty acid content of fresh *Puntius sophore* (mg/100g).

Fish species	DHA	EPA	AA	Lenolenic Acid	Lenoleic Acid
A.mola	2.91±0.00^{d}	0.12±0.00^{a}	0.13±0.00^{b}	1.4±0.00^{b}	2.3±0.00^{d}
P.sophore	0.75±0.00^{b}	0.04±0.00^{a}	0.06±0.00^{a}	0.91±0.00^{a}	2.19±0.00^{d}

DHA=Docosahexaenoic acid; EPA=Eicosapentaenoic acid; AA=Arachidonic acid
Values are shown as mean±standard error of triplicates.
Values within the same row have different superscripts are significantly differences *(P<0.05)*.

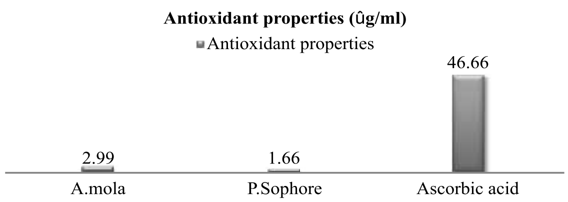

Fig. 9: Antioxidant properties of *Amblypharyngodon mola*and *Puntius sophore* (µg/ml).

The antioxidant activity of two fishes was shown in Fig. 9. The inhibition at 50% (IC_{50}) value of reference ascorbic acid was 46.66µg/ml and higher the IC_{50} value lesser the antioxidant properties. The antioxidant activity was highest in *Puntius sophore*(1.66µg/ml) and lowest in *Amblypharyngodon mola* (2.99µg/ml).

Discussion

Proximate composition

The moisture content in the present study is comparable with the values as reported by Sarojnalini and Vishwanath (1988). They reported that moisture content was 71.00 to 80.00% in some fresh water fishes of Manipur. The relative lipid content varied from one fish to another. This might be due to their ecological niche and feeding habits. In many species, there is a build-up of fat during feeding season and its proportion decreases substantially after spawning (Nair and Mathew, 2000). The protein content in the present work was found in moderate to high percentage. The relatively high to moderate percentage of protein could be attributed to the fact that fishes are good. sources of pure protein, but the differences observed in the present values might be due to the fishes' consumption or absorption capability and conversion potential of nutrients from their diet or local environment in to such biochemical attribute needed by the organisms body (Burgress, 1975). The higher ash content in the fresh water fish species might be due to its higher bony consistency and high scaly nature. Such fish offer minerals in their edible forms more abundantly than large-sized fish do (Higashi, 1962). And the total amino acid content is related with the increased or decreased in the availability of foods.

Polyunsaturated fatty acid content

Fish lipids are mostly distributed among liver, muscle and mesenteric fat. The results show the polyunsaturated fatty acid content of these two fishes. The present study shows that the total PUFA level varies among the species. In two samples 5 fatty acids were identified. Of the polyunsaturated fatty acids viz., DHA, EPA, AA, linolenic and linoleic acid were found varied. The most significant ($p<0.05$) highest content of DHA, EPA, AA, linolenic and linoleic acid were observed in *Amblypharyngodon mola.* The EPA value was found lowered in the two fishes than Tra catfish (0.76mg/100g) and Atlantic salmon (61.12mg/100g). This might be due to the species and also a number of factors can influence in fatty acid composition, such as water temperature, time of capture, salinity and feed type etc (Fatima *et al.,* 2007). The polyunsaturated fatty acids composition may vary among species of fish, even among fresh water and marine fish. The compositions of fatty acids are influenced by season, species, age, food availability and life cycle (AbdRahman *et al*., 1995). The changes in amounts of EPA and DHA observed in the results were caused by changes in amounts of water and lipid content. The changes in lipid extractability had also some influence on the amount of measured EPA and DHA, what in turn directly affected percentages and composition of fatty acids (Chen and Zhang, 2006 and Mottram, 1998).

Mineral content

The Ca contents of two fishes ranged from 895-902mg/100g. This value is higher than that reported by other authors Gokoglu *et al*., 2004 and Lall, 1995. The Magnesium (Mg) contents of the fish ranged from 226-228mg/100g. This value is higher than that reported by other author (Zamil *et al*., 1992). The Fe contents of the fish ranged from 152-482mg/100g. This value is higher than that reported by Hoffman *et al.,* 1994 and Gokoglu *et al*., 2004. The Cu contents of the fishes were between 4-4.8mgand100g. This value is higher than that reported by other authors (Hoffman et al., 1994, Rosa et al., 2007 and Gokoglu *et al*., 2004). The Zn contents of the fish ranged from 96-109mg/100g. This value is higher than that reported by Zamil *et al*., 1992 in Flat needle fish (0.41mg/100g). The high levels of Zn might be due to contamination (Zamil *et al*., 1992). The Na content of the raw fish was 74-82mg/100g. This value is higher than that reported by Zamil *et al*., 1992 in Flat needle fish (72.8mg/100g). The Mn contents of the raw fishes ranged from 19-35mg/100g. This value is higher than that reported by Zamil *et al*., 1992. The K content of the raw fish ranged between 193-208mg/100g. This value is higher than that reported by other author (Beyza and Ozeren, 2009). However the present value was lower than that of Zamil *et al*., 1992. Data from the literature indicate that the contents of macro- and microelements in fish depends on species and feeding type (Gladyshev *et al*., 2001).The bioaccumulation of minerals (Zn, Cu, Mn, Fe) also depends on fish weight and body length (Anan *et al*. 2005) and age (Farkas *et al*., 2003). Windom *et al*., 1987 attributed such variations to the chemical forms of the elements and their concentration in the environment. The mineral content of fish makes fish unavoidable in the diet as it is a good source of different minerals that contribute greatly to good health.

The minerals were recorded variation in their concentration of sample (Eyo, 2001).

Antioxidant properties

The antioxidant properties of the raw fishes ranged from 1.66-2.99µg/ml. These values are lower than the ascorbic acid. Many proteins have been shown to have antioxidative activity against peroxidation of lipids or fatty acids. Kawashima *et al*., 1979 investigated the effects of many synthetic peptides on lipid oxidation and found that some peptides having branched-chain amino acids (valine, leucine and Isoleucine) showed antioxidative activity. The peptides containing basic amino acids are electron acceptors that take electrons from radicals formed during the oxidation of unsaturated fatty acids (Chen and Decker, 1994). Shailaja *et al*., 2012 reported the seer fish protein has exhibits the antioxidant activity. Amino acids are also suggested to have antioxidant properties as reaction products with carbonyls from oxidizing lipids. Various studies have shown that reactions between oxidized lipids and amino acids produce many non-enzyme browning reaction compounds, which exert antioxidative properties (Alaiz *et al*., 1995; Alzaiz *et al*., 1996 and Ishtiaque *et al*., 1996).

Conclusion

The results suggest that the studied fresh water fishes have high protein, low lipid, high mineral content, antioxidant activity and high PUFA. The Fatty acids like DHA, EPA have beneficial effects on diseases such as coronary heart disease and may also contribute to various health benefit, growth and development for human. It also provides the importance of Fresh water fishes for their high nutritive value ahead of consumption. And in many low-income states like Manipur, fish is important for livelihoods, income and as food for the rural poor who suffer from malnutrition, including micronutrient deficiencies. So, it can play role in nutritional security for the rural people of low-income groups. Further works are still undergoing in the nutritional importance of the fresh water fishes and its contribution to human health and economy of the state.

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Sub-speciation tendencies, genetic diversity and divergence of the *Schizothorax progastus* (McClelland 1839) in tributaries of the Ganga river in Indian Himalayas

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ABSTRACT

In the Ganga river *Schizothorax progastus* is widely spread all along the course including its tributaries Bhagirathi and Alaknanda, each about 200 Km in length. Frequent exchanges occur close to the confluence in lower stretch of these tributaries however, there was hardly any chance of mixing 100 to 140 Km upstream of the confluence, especially after the construction of Maneri dam in 1984 on the Bhagirathi and Srinagar Dam in 2014 on the Alaknanda. The present investigation determines and compares morphomeristics and molecular characteristics of *S. progastus*, in the Bhagirathi and Alaknanda. The sample size consisted of 36 specimens of *S. progastus* from the Bhagirathi and 34 specimens from the Alaknanda for morphometric analysis, while 3 samples from each river for molecular characterization. In both the rivers, most of the body parameters in relation to Total Length (TL) and Head Length (HL) were genetically controlled. Mitochondrial DNA cytochrome oxidase I (COI) gene analysis revealed no significant genetic divergence between the samples of *S. progastus* from Bhagirathi and Alaknanda. The study indicated no intra-specific sequence divergence in *S. progastus* within the Ganga river. This study also confirmed the significance of COI gene in such studies and supports the possible genetic changes based on morphometeric data.

Keywords: *S. progastus,* Ganga River, Cytochrome oxidase I, Molecular characterization.

Introduction

Schizothoraicine fishes are widespread in the Himalayan rivers. *Schizothorax* (snow trout) endemic to the Himalayas true to its name occurs in the glaciers, snow-fed streams and lakes. Many species are endemic to various parts of the Himalaya, such as Kashmir, Nepal. The Indus river basin lies insulated from other Himalayan watersheds such as the Ganga and Brahmaputra. Similarly, the latter two meet in the plains but the exchange of coldwater fish fauna cannot take place because snow trout inhabit the ice-cold–oxygen rich waters, available only in the Himalayan domain. Similarly, many rivers of the Ganga basin that descend from the Himalaya form confluence in the Gangetic Plains and offer no opportunities for the exchange of Schizothoraicids inhabiting these rivers leading to isolation, important for morphological and genetic variability and hence speciation.

Molecular characterization of fishes under Schizothoracinae has been undertaken over last few years (Wu, 1984; Wu and Tan, 1991; Chen, 1998; Chandra, *et al*., 2012; Barat *et al*., 2012). Phylogenetic relationships among genera and species under Schizothoracinae have been investigated based on morphological characters, RAPD analysis by Chen and Chen, 2000; 2001; and mitochondrial cytochrome b gene sequence analysis by Dekui *et al.,* 2004; and Qi *et al.,* 2005; and Barat *et al.,* 2012.

This study was hence designed to examine the sub-speciation tendency and molecular diversity (nucleotide divergence) of the *Schizothorax progastus* stocks within source- rivers of Ganga basin, isolated from each other in the Indian Himalaya.

Materials and Methods

Study area

The Yamuna, Ganga and Kali are major river systems of Ganga river basin in the Indian Himalaya. The Yamuna and the Kali meet the Ganga far down in the mid-gangetic plains. However, some of their tributaries form confluences in the Himalayan domain, some closer to source and some far away from the source. The Alaknanda and Bhagirathi are two such rivers that are isolated from each other for nearly 200 Km from their source. The exchange in the snow trout fauna is possible only through the confluence, but the likelihood of this exchange gets reduced for individuals inhabiting the river stretch close to respective source. In order to ensure the isolation of stocks among the rivers forming confluence, the snow trout samples were collected far above their

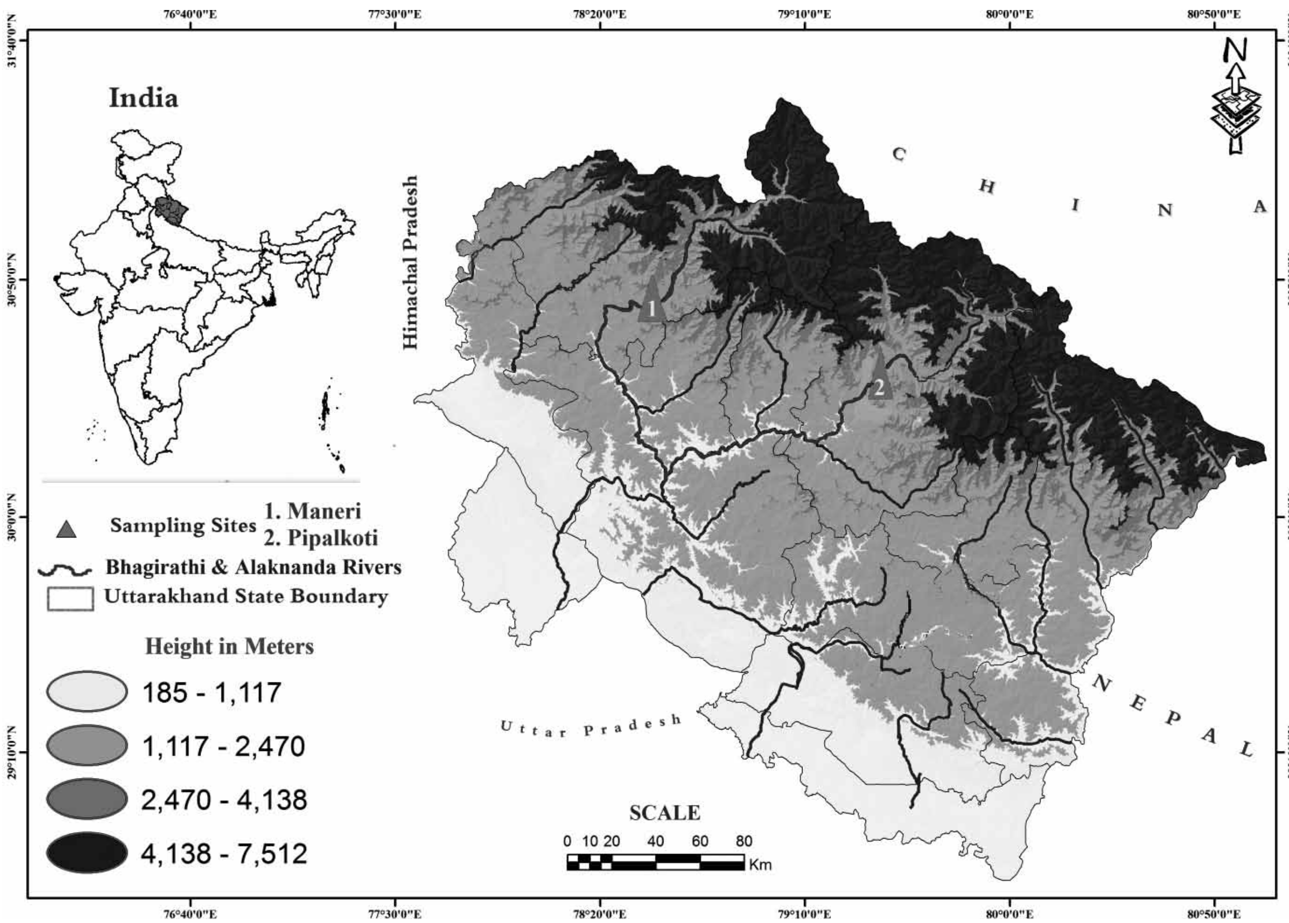

Fig. 1: Map of Ganga river basin showing the sample collection site.

confluence. The Alaknanda and Bhagirathi were sampled at Birahi and Maneri, 140 Km and 100 Km respectively upstream from their confluence at Devprayag (Fig. 1). These locations fall in the 1200-1500 m asl altitude range. Their exchange is further hampered by human interventions for hydropower generation; Maneri (1984), Tehri (2006) and Koteshwar Dams (2011) on the Bhagirathi while Srinagar (2014) and Vishnuprayag Dams on the Alaknanda. Hence, the fish stocks are severely fragmented. *S. progastus* was sampled from the Bhagirathi above Maneri Dam while the Alaknanda between Srinagar and Vishnuprayag Dams for the study.

Morphological analysis

The snow trout samples were obtained at monthly intervals from April 2014 to April 2015 from the rivers selected for the study. For morphometric analysis there were 36 specimens from the Bhagirathi and 34 specimens from the Alaknanda, while 3 samples from each river for molecular analysis were studied. The snow trout catch was obtained from local fishermen who fish for their livelihoods.

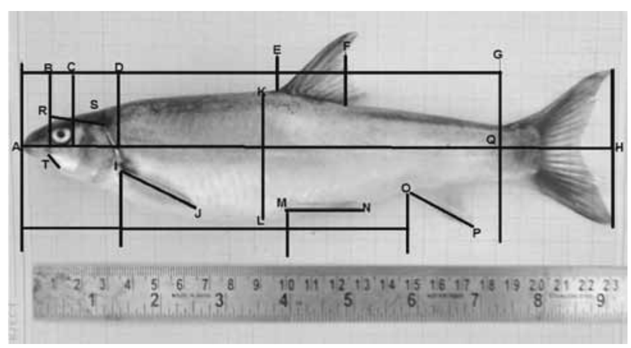

Fig. 2: Outline diagram showing morphometric features of *Schizothorax progastus* selected for the study.

AH=Total length(TL); AG=Standard Length (SL); AD=Head Length (HL); AE= Pre Dorsal distance(PreDD); FG= Post Dorsal distance (PostDD); KL= Body depth (BD); IJ= Pectoral Fin length (PFL); MN=Ventral Fin Length (VFL); OP= Anal Fin Length (AFL); EF= Depth of Dorsal fin (DOD); QH= Caudal Fin Length (CFL); AO= Pre Anal Length (PreAL); AM= Pre Ventral Length (PreVL); AI= Pre Pectoral Length (PrePL); IM= Pectoral ventral Distance (PecvenDist); MO= Ventral anal distance (Ven Anal Dist) and in relation to Head length, BC= Eye diameter (ED); AB= Pre orbital Distance (PreOD); CD= Post orbital Distance (PostOD); T= Length of barbel (LOB) and RS= Head width (HW).

Since, the fish obtained from them were already dead and consisted mostly of various species of *Schizothorax*, they were preserved in 10% formalin and brought to the laboratory for identifying and segregating *Schizothorax* species.The morphometric measurements were made on the basis of description provided by Jayaram, 2010; (Fig. 2).

The selected 15 and 5 characters in total and head length respectively (Fig. 2) have been studied:in proportion to Total Length (TL) and Head Length (HL). Body proportions were tabulated to obtain the range. The various morphometric characters were then classified on the basis of range difference into genetically (<10 %), intermediate (10-15 *%)* and environmentally (>15 %) controlled characters Johal *et al.,* 1994.

Isolation of Genomic DNA, Amplification and Sequencing

The muscle sample from the base of dorsal fin was used for genomic DNA isolation following Phenol: Chloroform: Isoamyl alcohol method of Sambrook *et al.,* 2001. Quality of DNA extracted from samples was checked by 0.8% agarose gel electrophoresis. The quantity of DNA was estimated using Spectrophotometry at 260nm. DNA isolate was stored at 4°C for future use.

The mitochondrial cytochrome c oxidase subunit I (COI) was amplified using primers FishF1 5'-TCAACCAACCACAAAGACATTGGCAC-3' and FishR1 5'- TAGACTTCTGGGTGGCCAAAGAATCA-3' Ward *et al.,* 2005; in a 50µl volume with 100ng template DNA, 10pmole of each specific primer, 250µM of each dNTPs, 1.0 U of Taq DNA polymerase and 1XTaq buffer containing 1.5mM $MgCl_2$. The PCR conditions consisted

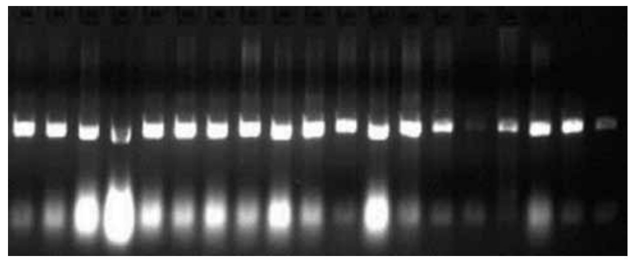

Fig.3: Agarose Gel electrophoresis image for amplified mitochondrial COI region.

Table1: Comparison of range differences in body parts of *Schizothorax progastus* between river Bhagirathi and Alaknanda.

Nature of characters	Body Parameters	Bhagirathi		Alaknanda	
In proportion to Total Length		Range	Range Diff.	Range	Range Diff.
Genetically Controlled	SL	0.94-1.83	1.39	0.75-1.34	1.05
	HL	4.42-8.48	6.45	5.29-6.75	6.02
	PreDD	1.92-3.61	2.76	2.51-2.69	2.6
	PostDD	2.51-5.15	3.83	3.18-3.44	3.31
	BD	4.29-8	6.14	163-5.14	4.89
	PFL	6.34-11.9	9.15	6-6.59	6.29
	VFL	6.63-12.5	9.58	6.81-7.53	7.17
	AFL	6.95-12.1	9.57	5.84-6.59	6.22
	DOD			9.3-10.4	9.9
	CFL	3.47-6.26	4.86	4.61-5.41	5.01
	PreAL	1.14-2.28	1.71	1.57-1.72	1.65
	PreVL	1.50-3.24	2.37	2.30-2.51	2.4
	PrePL	4.05-8.48	6.26	5.24-5.86	5.55
	PecPelDist	2.56-5.39	3.97	4-4.39	4.19
	Pel Anal Dist	3.74-8.92	6.33	4.23-4.68	4.46
Intermediate	DOD	7.3-14.6	10.95		
In proportion to Head Length					
Genetically Controlled	ED	6-8.5	7.25	5.33-7	6.16
	PreOD	2.21-2.61	2.41	2.43-2.75	2.59
	PostOD	1.94-2.30	2.12	1.52-2.66	2.09
	L of Barbel	2.06-2.46	2.26	1.77-2.41	2.09
	HW	5.16-12.33	8.75	6.33-7.33	6.83

of initial denaturation at 94°C for 3min, followed by 35 cycles of 94°C for 40s, 58°C for 40s, 72°C for 60s and a final extension at 72°C for 10min. The PCR products were visualized using 1.2% agarose gel (Fig. 3) and the amplicons were purified and sequenced in both directions commercially in automated genetic analyzer ABI 3730 (Applied Biosystem, Carlsbad, CA) following Bigdye terminator v.1.1 cycle Sequencing Kit (Applied Biosystem, Carlsbad, CA). Simplicity and un-ambiguity were observed among the sequences of mitochondrial region.

Phylogenetic analysis

Sequences were aligned using Clustal W. Length differences were resolved by inserting alignment gaps and positions that could not be aligned unambiguously were excluded. Base composition between samples was estimated from aligned sequences. All the sequences obtained were grouped according to their sampling location i.e. river basins and sequence divergence was calculated as the number of base substitutions per site from averaging over all sequence pairs within each group using the Kimura 2-parameter model (1980). The evolutionary history was inferred using the Neighbor-Joining method based on the Tamura-Nei model (1993) with 1000 bootstrap replications. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method (1980) and are in the units of the number of base substitutions per site. All analyses were performed in MEGA 7.0.

Results

Morphometric analysis

The present study illustrates that in the river Bhagirathi among the 14 of the 15 selected variables of *S. progastus* in relation to TL, except DOD (an intermediate character) were found to be genetically controlled. In relation to HL all characters were also found genetically controlled. On other hand in the river Alaknanda all the 15 selected variables in relation to Total Length (TL), and 5 in relation to Head Length (HL) were found to be genetically controlled (Table 1).

Molecular analysis

The COI sequence analysis across species revealed average nucleotide frequencies for *S. progastus* as T=27.6%, C=27.5%, A=26.5%, G=18.5% in Bhagirathi and as T=27.9%, C=27.5%, A=26.5%, G=18.1% in Alaknanda (Table 2). The average percentage divergence (K2P) distance for *S. progastus* was 0.0009% (Table 3).

Table 2: Nucleotide composition of cytochrome oxidase I gene of *S.progastus* in the rivers Bhagirathi and Alaknanda.

S. progastus	T%	C%	A%	G%	A+T%	C+G%
Bhagirathi	27.6	27.5	26.5	18.5	54.1	46
Alaknanda	27.9	27.5	26.5	18.1	54.4	45.6

Table 3: Estimates of average evolutionary divergence over sequence pairs within species.

S. progastus	Evolutionary Divergence	% Evolutionary Divergence
Bhagirathi	0.0009	0.00094%
Alaknanda	0.00091	0.00097%

The nucleotide sequences of COI gene were aligned in order to determine the phylogenetic relationships between the two samples of *S. progastus*. The phylogenetic tree, generated using three methods (Neighbour-joining, Minimum evolution, Maximum parsimony) was similar. The Neighbour-joining (NJ) tree (Fig. 4) of COI gene sequences showed that the two populations of the river Bhagirathi and Alaknanda form a monophyletic group.

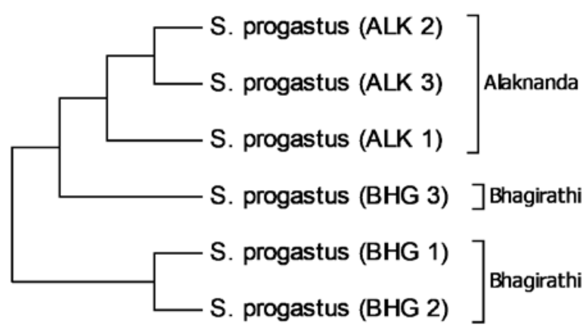

Fig. 4: Phylogenetic relationships of taxa of the two populations by Neighbour-joining method.

The low nucleotide diversity (T = 0.3%, G= 0.4%, C and A = 0%; combine, C+G = 0.6% and A+T= 0.3%) and genetic distance was observed in the *S. progastus* of Alaknanda and Bhagirathi.

Discussion

Morphometric analysis

Vladykov (1934) maintains that in fish species showing restricted distribution, the majority of morphometric characters show a narrow range and are genetically controlled. On the contrary, in species that have a wide range of geographical distribution, most of the characters are strongly influenced by the environment. Many species of snow trout are most abundant and widely distributed in the streams/ rivers of the Himalayas. In India geographical distribution of *S. progastus* is Jammu & Kashmir valley, Ganga river in Uttar Pradesh and Brahmaputra river in Assam (Talwar and Jhingran, 1991).

Based on this criterion *Schizothorax progastus* has restricted geographical distribution because the majority of the 20 morphometric characters examined show narrow range differences and are genetically controlled in relation to TL and HL respectively. There were no environmentally controlled characters. Thus both stocks despite isolation from each other by virtue of distant location do not differ in morphometeric features, showing lack of sub-speciation tendency. Similar results were obtained for *S. richardsonii* in Kamal and Yamuna rivers in Uttarkashi (Negi and Negi, 2010).

Earlier, Bhatt *et al.* 1998; observed no tendency of sub-speciation in *Tor putitora* from the foothill section of the Ganga (>50% characters genetically controlled) in contrast to Johal *et al*., 1994.whofound sub speciation tendency in *Tor putitora* from Govindsagar as >50% characters were intermediate and environmentally controlled. Negi and Nautiyal, (2002) compared *Barilius bendelisis* and *B. vagra* from spring fed river Khanda and observed relatively greater tendency of sub-speciation in *B. bendelisis* (54.5%) than *B. vagra* (63.9%). Johal and Kaur (2005) reported the moderate tendency in *Barilius bendelisis* to form the taxonomic categories below species level such as subspecies from some streams of Himachal Pradesh. In *Crossocheilus latius latius* from Ranjit Sagar Wetland, Onkar and Saima, 2015; observed that 13 of 18in TL were genetically controlled. Contrary it in *Garra gotyla gotyla* from Ranjit Sagar Wetland have been studied in percentage of only3 of 18 characters were genetically controlled, 13 were intermediate and 2 were environmentally controlled (Brraich and Akhter, 2015).

Molecular analysis

The efficacy of COI gene in identification and phylogenetic relationship of the fish species with designated barcodes have been proved by many authors (Lara *et al*. 2010; Ward *et al.* 2009). The universal primers amplified the target region in all samples generating the COI barcodes. Sequence alignment resulted in 582bp per taxon after exclusion of the primer sequence and equal length alignment. No insertions, deletions or stop codons were observed in any of the sequence and supporting the hypothesis that all the amplified sequences derive from a functional mitochondrial COI gene.

The results show that there was low intra-specific sequence divergence in the selected rivers. Chandra *et al.,* (2012) however, observed the average percentage divergence distance of 0.0012% for *S. progastus* from the Khanda and Dugadda Gad and suggested that there was lower intra-specific sequence divergence for *Schizothorax* species in the Garhwal region. Dekui *et al.,* 2004; Barat *et al*, 2012; Ambili *et al*., 2014; also observed that close relationship among the species that were distributed in the same drainage system. Though small sample size was used in the present study for the morphometeric and moleculer analysis, the investigation indicates lack of tendency of sub-speciation, at both phenotypic as well as genetic level.

More extensive study is required on the phylogenetic relationships of this fish based on morphomeristics and different molecular markers, as the recent development in these techniques can be very useful for establishing taxonomical and phylogenetic relationships. It may also increase the knowledge of the ichthyologists in planning conservation and management strategies for these important fish species along with their natural habitat.

Conclusion

The study revealed lack of sub-speciation tendency and intra-specific genetic variability in *Schizothorax progastus* inhabiting the Alaknanda and Bhagirathi. COI gene sequences showed that two populations in the river Bhagirathi and Alaknanda form a monophyletic group supporting the conclusion of morphometric analysis.

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Optimum stocking density and rearing system for larvae of *Labeo dyocheilus* in coldwater conditions

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ABSTRACT

Labeo dyocheilus, an indigenous minor carp is a potent candidate species for polyculture of carp in mid altitudinal area (1000-2000 m msl) of Himalayan region. Attempt has been taken to standardize the best suitable larval rearing system for *L. dyocheilus* in mid altitudinal region by evaluating the growth performance and survival of the larvae in different systems i.e. FRP tank, polylined tank, earthen pond and cemented tank and to standardize stocking density. The highest length gain was found to be 36.4 ± 2.2 mm in polytanks which is significantly ($P < 0.05$) higher than the rest of the treatments, specific growth rate was found to be highest 3.54 ± 0.05% shown by the larvae reared in polytanks, which was significantly ($P < 0.05$) higher compared to other systems. The survival rates were found to be 75±2.29%, 72±2.05%, 37±2.4%, 25±2.6% respectively in FRP tanks, polytanks, earthen pond and cemented tanks. Stocking density of 10 larvae per litre is optimum in respect of other treatments with average weight gain of 0.442±.041 and survival rate of 48% in 120 days of rearing in polytank nursery ponds Hence, Rearing of *Labeo dyocheilus* fry in polytank nursery pond with stocking density of 10 larvae per litre water volume is an economic and technical feasible practice.

Keywords: Candidate species, Polytank, Stocking density, Minor carp

Introduction

L. dyocheilus commonly known as Kali, are highly valued species of Kumaon Himalayan region that inhabiting upland streams and rivers at an elevation of 400-800 msl. Over the last 10 years its wild population of this species undergone steady decline in distribution and abundance due to anthropogenic activities and it has been categorized as threatened (Desai, 1994) and vulnerable (Dubey, 1994, Prasad, 1994). Mixed culture of carp species is relevant aquaculture practice for mid altitudinal area (1000-2000 m msl). As per the consumer preference and limited existing candidate species, this species may be used as a candidate species in the mixed carp farming system in mid altitudinal area. For this purpose, seed of this species is basic requirement. In natural condition, this is an important species of fish biodiversity in the cold water resources but it occurrence and population is being affected regularly due to anthropogenic activities. Hence, advanced sized seed of this species is preferably required for the wild stock augmentation of this species in natural resources. Sarkar *et al*., 2001 succeed in breeding of this species in natural environment. Normal ovarian development of *L. dyocheilus* under captivity in Tarai region of Uttarakhand, indicates the possibility of the breeding (Singh *et al*., 2008). The technology for the induced breeding in cold water condition of this species is available (Pandey *et al*., 2011). Further, successful larval rearing for the mass seed production is required to be developed.

Stocking density is an important factor that affect on growth, efficiency and reproductive performance in fish. Specific stocking density can have positive and negative effect on fish growth (Merino *et al*., 2007). Variation in stocking density of fish may change growth and survival rate (Miao, 1992). Fish larvae have slow growing and low survival rate at high stocking density (Huang and Chiu, 1997). Therefore, it is necessary for maximum utilization of resource (aquaculture ponds) to predetermine and standardize the optimum stocking density for each species in order to obtain the best possible output.

Several studies have investigated the effects of stocking density on growth of different farmed species including Gold fish (*Carassius auratus*) (Niazie *et al*., 2013), rainbow trout (*O. mykiss*) (Gholipour *et al*., 2007), common carp (*Cyprinus carpio*) (Imanpoor *et al*., 2009), endangered mahseer (*Tor putitora*) (Aminur *et al*., 2005) and Thai climbing perch (*Anabas testudineus*) (Khatune *et al*., 2012). Giant gourami (*Osphronemus goramy*) showed that potential density had no significant effect on the growth indices of fish (Ebrahimi *et al*., 2010). Currently, information of optimum stocking density of larvae of this species and suitable system for rearing is lacking. Growing period of cold water fish is March to October. As the breeding period of this species in cold water condition was found 3rd week of July to end of August (Pandey *et al*., 2011), larval rearing period start from September and these winter months are the lean period for the growth of fish due to low water temperature.

Keeping the above facts in mind, the present study was undertaken to standardized the best suitable larval rearing system with optimal stocking density for *L. dyocheilus* in mid altitudinal region by evaluating the growth performance and survival of the larvae in different systems i.e. FRP tank, polylined tank, earthen pond and cemented tank and to standardized stocking density. Survival of larvae and growth related data like length gain, weight gain, specific growth rate (SGR) and percent weight gain were studied to find out the suitable nursery system for successful larval rearing of this species in mid altitudinal region.

Material and Methods

Experimental site and procurement of fish larvae

Experiment was conducted in three phases at DCFR farm, Bhimtal (29.21'N and 79^0 34'E, Altitute 1370 m msl). For the procurement of seed, induced breeding was conducted during third week of July according to the method described by Pandey *et al*., 2012 by using ovaprim as inducing agent. 4 days old spawn were acclimatized and used for the first phase of the experiment to standardize the stocking density.

Experiment 1

The first experiment was conducted for two months (60 days) from 21th July to 21th September. Larvae were stocked having average length of 3.6 ± 0.24 mm and weight of 0.006 ± 0.01 g respectively after the yolk sac absorption. Rearing of larvae after yolk sac absorption was done in four different systems i.e. FRP tanks, polylined tanks, earthen pond and cemented tanks with three replications. The larvae stocked under the different systems were fed with 30% protein level comprising mixture of groundnut oil cake, fish meal and rice bran in equal proportions by weight administered two times daily at 9 am and 5 am at the rate of 25% body weight for the initial 15days after that feeding rate gradually reduced to 10% with increase in size. Sampling was done at every 7 days of interval. Ten larvae were randomly collected from each tank to take the length and weight data. The weight (mg) and length (mm) were measured by using an electric balance and graph paper, respectively. Sampling was done before the application of feed to avoid the biasness of weight due to presence of excessive feed.

Experiment 2

The second experiment of larval rearing was run for four months from 22nd September to 22nd December to standardize the optimum stocking density in polylined tank. Larvae were stocked having average length of 4.3 ± 0.10 mm and weight of 0.007 ± 0.01 g respectively with four stocking density treatment with three replicates i.e. 5, 10, 15, 20 larvae per liter.

Spawn were provided with supplementary diet of 30% protein level comprising mixture of groundnut oil cake, fish meal and rice bran in equal proportions by weight. Feeding was done two times daily at 9am and 5 am at the rate of 25% body weight for the initial 15days after that feeding rate gradually reduced to 10% with increase in size . Sampling was done at every 7 days interval. Ten larvae were randomly collected from each tank to take the length and weight data to estimate the best growth and survival for optimizing stocking density.

Following formulae were used to determine the different growth parameters in each experiment.

1) Length gain of larvae (mm) = Average final length of larvae – average initial length of larvae.

2) Weight gain of larvae (g) = Average final weight of larvae – average initial weight of larvae.

3) Percent gain in length = Average final length of larvae – average initial length of larvae/ Average initial weight × 100

4) Percent gain in weight = Average final weight – average initial weight /Average initial weight × 100

5) Specific growth rate (SGR) = InW2 - InW1/ T2 - T1 × 100

Where, W2 = Final live body weight (g) at time T2

W1 = Initial live body weight (g) at time T1

6) The survival rate = No. of larvae alive /Total number of stocked×100

The gain in weight and length, specific growth rate of the fry and survival rate of the larvae were all tested using one-way analysis of variance (ANOVA). During the experimental period following water quality parameters like temperature (^{o}C), pH and dissolved oxygen (ppm) content of rearing water were recorded weekly by Celsius thermometer, digital DO (Dissolved Oxygen) meter and portable digital pH meter.

Result and Discussion

The initial average length and weight of the larvae were 3.60±0.24 mm and 0.006±0.01 g, respectively for all treatments. The final average length of the larvae reared in FRP tank, polytank, earthen tank and cemented tank of were 12.32±1.3 mm, 17.32±1 mm, 16.77±1.5 mm and 13.90±1.2 mm while the final average weight were 0.022±0.05 g, 0.033±0.02 g, 0.030±0.04 g and 0.023±0.03 g, respectively.

Table 1. Growth performance of *Labeo dyocheilus* larvae of different systems after 60 days of rearing, (mean ± SD)

Parameters	Polylined Tank	Earthen pond	Cemented tank	FRP Tank
Initial length (mm)	3.60±0.24	3.60±0.24	3.60±0.24	3.60±0.24
Initial weight (g)	0.006±0.01	0.006±0.01	0.006±0.01	0.006±0.01
Final length (mm)	17.32±1.1 ^{a}	16.77±1.5 ^{a}	13.90±1.2 ^{b}	12.32±1.3 ^{c}
Final weight (g)	0.033±0.02 ^{a}	0.030±0.04 ^{a}	0.023±0.03 ^{b}	0.022±0.05 ^{b}
Length gain (mm)	13.72±1.2 ^{a}	13.17±1.5 ^{a}	10.13±1.3 ^{b}	8.72±1.6 ^{c}
Weight gain(g)	0.027±0.0 ^{a}	0.024±.041 ^{a}	0.017±.05 ^{b}	0.013±.027 ^{c}
Percent length gain	381.11±4.32 ^{a}	365.83±5.2 ^{ab}	286.11±5.9 ^{b}	242.22±3.3 ^{c}
Percent weight gain	450±9.23 ^{a}	400±12.22 ^{a}	283.33±14.40 ^{b}	216.66±11.90 ^{c}
Specific growth rate (% per day)	2.68±0.06 ^{a}	2.56±0.04 ^{a}	2.23±0.05 ^{b}	2.16±0.06 ^{c}
Survival (%)	79±2.5 ^{a}	35±2.2 ^{c}	44±2.4 ^{b}	78±3.4 ^{a}

* Values with different superscripts in a row are significantly different (one way ANOVA P < 0.05).

The highest length gain was found to be 36.4 ± 2.2 mm in polytanks which is significantly (P < 0.05) higher than the rest of the treatments. Similarly, the highest gain in weights of the larvae was 0.027±0.0 g was also found in polytanks which is significantly (P < 0.05) higher than those of the other three treatments followed by 0.024±.041 g in earthen ponds, 0.017±.05 g in cemented tanks and 0.013±.027 g in FRP tanks. The highest percent length gain was 381.11±4.3 and percent weight gain was 450±9.23 respectively observed in the larvae reared in poly tanks. After completion of the experiment, the highest specific growth rate as 2.68±0.06 % shown by the larvae reared in polytanks, which was significantly (P < 0.05) higher compared to other systems. The survival rates were found to be 79±2.5 %, 35±2.2 %, 44±2.4 %, 78±3.4 % respectively in FRP tanks, polytanks, earthen pond and cemented tanks (Table 1).

To standardize the stocking density suitable for best growth performance and survival of larvae, four different stocking densities in polytanks were applied for the rearing of 120 days. The initial average length and weight of the larvae were 4.10±0.02 mm and 0.068±0.01 g, respectively for all treatments with stocking density of 5,10,15 and 20 larvae per litre water volume. At the end of the experiment, it was revealed that stocking density of 10 larvae per litre is optimum in respect of other treatments with average weight gain of 0.442±.041 and survival rate of 48% in 120 days of rearing in polytank nursery ponds (Table-2). Though the growth and survival at stocking density of 5 larvae per litre is little higher than the optimum density of 10 larvae per litre, but is non- significant different (P < 0.05).

Table 2. Growth performance of *Labeo dyocheilus* larvae of different stocking density after 120 days of rearing, (mean ± SD)

Parameters	20	5	10	15
Initial length (mm)	4.1±0.02	4.1±0.02	4.1±0.02	4.1±0.02
Initial weigth (g)	0.068±0.01	0.068±0.01	0.068±0.01	0.068±0.01
Final length (mm)	20.6±1.3 ^{c}	40.5±1.3 ^{a}	38.2±2.0 ^{b}	24.2±1.0 ^{bc}
Final weigth (g)	0.26±0.03 ^{c}	0.51±0.05 ^{a}	0.42±0.03 ^{b}	0.27±0.03 ^{bc}
Length gain (mm)	16.5±1.2 ^{c}	32.3±2.2 ^{a}	34.1±1.7 ^{b}	20.1±1.8 ^{bc}
Weight gain(g)	0.192±0.03 ^{c}	0.442±.041 ^{a}	0.352±.05 ^{ab}	0.202±.027 ^{b}
Percent length gain	376.74±5.32 ^{c}	846.51±6.2 ^{a}	786.04±5.5 ^{b}	439.53±4.3 ^{bc}
Percent weight gain	3471±23.44 ^{c}	7042±26.50 ^{a}	6042±32.49 ^{b}	3900±26.42 ^{bc}
Specific growth rate(% per day)	2.94±0.06 ^{c}	3.52±0.04 ^{a}	3.38±0.05 ^{ab}	3.03±0.06 ^{b}
Survival (%)	27±2.6 ^{c}	49±2.29 ^{a}	48±2.05 ^{a}	32±2.4 ^{b}

* Values with different superscripts in a row are significantly different (one way ANOVA P < 0.05).

Table 3. Average water quality parameters of different system

	Dissolved Oxygen(mgl-1)	pH	Temperature(^{0}C)
FRP tanks	6.7±0.2	8.1±0.3	20.2±1.2
Polytanks	6.4±0.1	8.5±0.1	23.4±0.9
Earthen pond	7.1±0.3	8.0±0.2	18.0±1.7
Cemented tank	7.0±0.25	8.6±0.2	22.0±1.5

* Value presented in the table is the mean value of four observation of every month.

According to Lingen, 1989, it is empirical that stocking density has direct effect on food supply, space for living and water quality. Jena *et al*., 1998 found similar results in *C. catla, L. rohita* and *C. mrigala.* Havey (1980) also found that a significant inverse linear relationship between stocking rate and growth. Retardation of growth under high stocking densities has been explained by several workers. Forselius (1973) linked such depression in growth to unavailability of proper space. Physiological stress may also cause poor growth performance under high stocking densities as observed in *Salmo gairdneri* (Wedemeyor, 1976) and *Oncorhynchus kisutch* (Fagerlund *et al.,* 1981), whereas Smart (1981) attributes low growth due to poor water quality. In the present study water quality parameters in all the treatments were giving almost similar trend without significant variations ($P<0.05$) which clear cut indicated that even the highest stocking density in the present study (20 larvaeL^{-1}) did not impose any environmental stress. Similar finding were reported by Jena *et al*., 1998 in IMCs fry and Zalina *et al*., 2011 in *Anabas testudinius.* Results of Field experimentation reflected that rearing of *Labeo dyocheilus* fry in polytank nursery pond with stocking density of 10 larvae per litre water volume is an economic and technical feasible practice.

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Morphological changes during early development of endangered golden mahseer *Tor putitora*

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ABSTRACT

Embryonic development of golden mahseer eggs were completed within 94-120 hours after fertilization at an ambient temperature of 20^{o} C ± 2^{o} C. The first cleavage occurs about 3hours post fertilization, with cleavage interval of about 35 minutes. The blastula period last from 6h 45m to 12h 10m post fertilization. During this period; the embryo enters midblastula transition (MBT), the yolk syncytial layer (YSL) forms, and epiboly begins, which continues till gastrulation period. The gastrulation period generally lasts from 16h 30m to 36h. Embryonic development was completed within 94-120 hours post-fertilization at 20º C ± 2º C. The present study provides preliminary insight about the early development progression in Golden mahseer, which can help in developing better rearing strategies for early larval rearing to achieve better survival and eventually leading to population enhancement in natural environment.

Keywords: Embryo, Larvae, Development, Golden mahseer

Introduction

Golden mahseer (*Tor putitora*) is one of the well-known large freshwater game fish of Mountain Rivers and lakes of most Trans-Himalayan countries. It is one of the most-sought after species providing the main fishery in the uplands all along the Himalayan belt extending from Kashmir in the north-west to Sadiya in the north-east. The fish is also known as Greyhound or the thick-lipped mahseer and has been observed to attain the weight of 70-80 kg. (Misra, 1962). Anglers regard golden mahseer as one of the finest sport-fish and it is a source of recreation to innumerable sportsmen both Indian & foreigners. Thomas, 1897 in his famous book "The Rod in India" stated that pound for pound mahseer is far superior to 'lordly salmon' in sporting qualities. To the local fishermen too, mahseers have been of considerable importance because of their large size, hardy texture, high commercial value and longer shelf life.

An important reason of teleost evolutionary success is their reproductive system, which has been functionally acclimated in all aquatic environmental conditions (Koc, 2010). Reproduction represents one of the most important aspects of the different species biology and the maintenance of viable populations depending on its success (Suzuki and Agostinho, 1997). Different fish species live in special ecological conditions; therefore, they have unique reproductive strategy, with special anatomical, behavioural, physiological, and energetic adaptations (Moyle and Cech, 2004). The envelope of teleost fish's egg has a key role in this reproductive success. Teleosts develop mature eggs enclosed in a hard and complex eggshell (chorion), which is formed by protein components organized into a complex structure. This structure plays an essential role in control of the relation between the external and the internal egg environments. It allows for respiratory gas diffusion, provides mechanical protection and thermal insulation, and permits sperm entry (Hamodrakas, 1992).

Ultrastructural characteristics of the chorion and the micropyle of teleost eggs differ in different species, and have recently been considered as a criterion for identification of eggs (Ohta *et al.*, 1983; Chen et al., 2007). Shape, number and size of micropyle and also reinforcement type of the micropyle canal; the number and length of the longest and shortest ridge in the micropyle region; the diameter, number, and arrangement of the accessory openings; the adhesive structures of egg and mode of them to the substrate; ornamentation and the thickness of the membrane have been used for taxonomic purposes (Hirai and Yamamoto, 1986; Riehl, 1993; Giulianini et al., 1994; Chen et al., 1999; Li et al., 2000; Bless and Riehl, 2002; Esmaeili and Johal, 2005; Huysentruyt and Adriaens, 2005; Chen et al., 2007; Costa and Leal, 2009).

Some characters such as shape of the external surface of the egg membrane, number and shape of micropyles, the branching pattern of the tubules in the primary membrane, the width of the membrane and the degree of egg adhesiveness are species typical, although they represent a convergent similarity in some species (Vorobyeva and Markov, 1999; Huysentruyt and Adriaens, 2005; Costa and Leal, 2009).

The chorion surface has also been analysed using scanning electron microscopy (Ohta et al., 1983; Johnson and Werner, 1986; Costa and Leal, 2009; Koç, 2010). Johnson and Werner (1986) described the external morphology of the chorion of five freshwater fishes and concluded that scanning electron microscopy was a powerful tool for identifying fish eggs. On the other hand, the micropyle and its microstructures in unfertilized eggs are important characters in gamete recognition and fish egg identification, therefore its morphology may be species specific (Ginsburg, 1968; Kobayashi and Yamamoto, 1981; Chen *et al.*, 2007). The microstructure of micropyles and surface ornamentations, however, may change during fertilization.

Materials and Methods

Healthy running male (average total length 30 ± 3.0 cm and average weight 550 ± 10 g; n=6) and gravid female (average total length 35 ± 4.0 cm and average weight 750 ± 15 g; n=6) broodstock of *T. putitora* were collected during spawning season (May, 2016) from Bhimtal Lake Uttarakhand, India. Brooders were stripped for eggs and semen and brought to ICAR-DCFR hatchery. Eggs were fertilized by gently mixing them with semen/sperm cells. Fertilized eggs were kept in hatching tray under flowing water condition.

The incubation and hatching unit comprised a set of meshed trays (50 ×30 ×10 cm with mesh size of 1.0mm) placed on fibreglass-reinforced plastic troughs (200 ×60×30 cm) having a water flow of 1.0-1.2 litre per minute. The eggs were uniformly spread in a monolayer on the meshed trays at a densityof 4000 - 5000 eggs per tray. Water quality parameters were monitored daily and maintained constant over the entire incubation and hatching period (temperature: 20^{o}C ± 2^{o}C, pH: 7.2 ± 0.5, ammonia: 0.007 ±0 .002 mg L^{-1}, nitrite: 0.02 ± 0.001 mg L^{-1}, dissolved oxygen: 7.3 ± 0.5 mg L^{-1}). From each developmental stage, random samples were taken from different trays for further analysis. A particular development stage was determined when more than 50% of all specimens reached that stage.

Morphological development of embryos was followed with the use of Olympus stereomicroscope with bottom and top lighting to enable clear observation of organogenesis and to determine the time sequence of different developmental stages. For S.E.M. examination of the embryos, samples of each stage of development were fixed in a solution of 23% glutaraldehyde in 0.1 M sodium cacodylate buffer pH 7.4 and stored at 4” C until processing for S.E.M. (Glauert, 1980). Egg samples were also preserved in buffered formalin for measuring size at a later time using profile projector.

Results

A brief description of the characteristics and time sequence of the stages of embryonic development in *Tor putitora* follows:

Zygote period (0 - 3h)

The newly fertilized egg can be considered to be in the zygote period until the first cleavage occurs, about 3 hours 15minutes post fertilization. The egg and yolk is about 2.6 ± 0.1 mm and 2.3 ± 0.1 mm respectively in diameter at the time of fertilization.

Stages during the zygote period

One-cell stage (0-3 h): The chorion swells and lifts away from the newly fertilized egg (Fig. 1 Fertilization also activates cytoplasmic movements, easily evident within about 2 hrs. Nonyolky cytoplasm begins to stream toward the animal pole, segregating the blastodisc from the clearer yolk granule-rich vegetal cytoplasm (Fig. 2 & 3). This segregation continues during early cleavage stages.

Cleavage period (3h 15m – 8h)

After the first cleavage the cells, or blastomeres, divide at about 35 mins intervals (Figs. 4-10). The cytoplasmic divisions are meroblastic; they only incompletely undercut the blastodisc, and the blastomeres, or a specific subset of them according to the stage (Kimme1 and Law, 1985a), remain interconnected by cytoplasmic bridges. The six cleavages that comprise this period frequently occur at

Fig 1: 2 hrs, Zygote Period

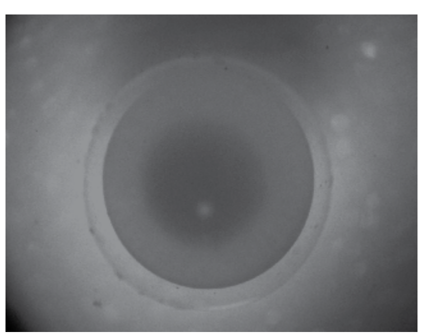


Fig 2: 2 hrs 30 mins, Zygote Period

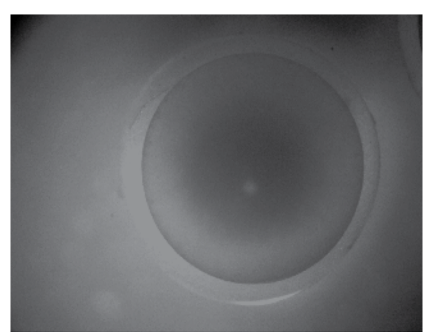


Fig 3: 3 hrs, Zygote Period

regular orientations (Figs. 4-10) so that one can see how many blastomeres are present are by their arrangement; counting them is unneccessary.

Stages during the cleavage period

Two-cell stage (3h 15m): The first cleavage furrow, ending the first zygotic cell cycle, is vertically oriented, as is usual until the 32-cell stage. The furrow arises near the animal pole and progresses rapidly toward the vegetal pole, passing through only the blastodisc and not the yolky region of the egg (Fig. 4). Near the bottom of the blastodisc the furrow changes to a horizontal orientation to undercut the blastodisc.

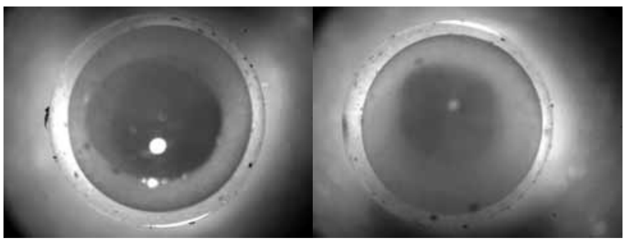


Fig. 4: 3hrs 15 min, Two-cell stage

Four-cell stage (3 h 50m): The two blastomeres cleave incompletely (Fig. 5) and in a single plane that passes through the animal pole at right angles to the plane of the first cleavage. Hence, cycle 3 begins with four blastomeres in a 2 x 2 array. A view from the animal pole reveals that the blastodisc is ellipsoidal in shape. The second cleavage plane is oriented along the longer axis.

Eight-cell stage (4 h 25m): Cleavages ending cycle 3, still incomplete, occur in two separate planes, parallel to the first one, and on either side of it. They cut the blastodisc into a 2 x 4 array of blastomeres. As the dechorionated embryo usually lies in a dish, the four-cell aspect, rather than the two-cell aspect, faces the observer. This “face” view is along the odd-numbered cleavage planes (furrows 1 and 3 are visible; Fig. 6). The dechorionated embryo tends to lie in the same orientation through late blastula stages.

16-cell stage (5 h): The fourth set of cleavages also occurs along two planes, parallel to and on either side of the second one, and produces a 4 x 4 array of cells. Use care to distinguish this stage from the eight cell stage, because they look similar in face view (Fig. 7).

32-cell stage (5 h 35m): The cleavages ending cycle 5 often occur along four parallel planes, rather than two, lying between those of the first and third cycles. However, oblique orientations of the furrows are now common. Frequently the 32 blastomeres of this stage are present in a 4 x 8 array, but other regular patterns, as well as irregular ones involving one or more of the blastomeres, also occur (Kimmel and Law, 198513). In a side view one usually sees two tiers, or horizontal rows, of blastomeres between the margin and the animal pole (Fig. 8). This is because the plane of the blastodisc is curved; marginal cells are more vegetal, and they lie partly in front of the nonmarginal ones positioned closer to the animal pole.

64-cell stage (6 h 10m): Cleavages ending the sixth cycle pass horizontally, so that in an animal polar view the blastomere array may look similar to the 32-cell stage, although the cells entering cycle 7 are smaller. From the side the cell mound looks distinctly higher (Fig.10). For the first time some of the blastomeres completely cover other ones.

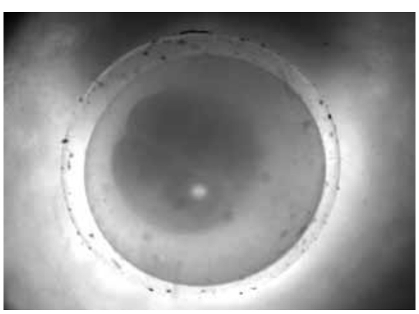


Fig. 5: 3 hrs 50min, 4 cell stage

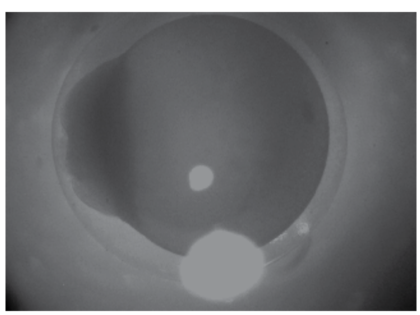


Fig. 6: 4 hrs 25min, 8 cell stage

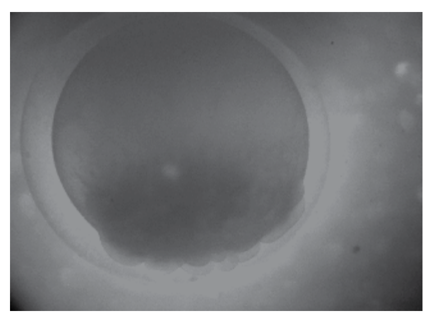


Fig. 7: 5 hrs, 16 cell stage

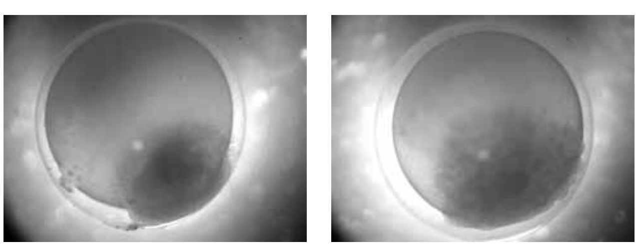


Fig. 8: 5 hrs 35 min, 32 cell stage

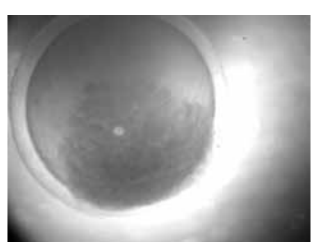


Fig. 9: 6 hrs

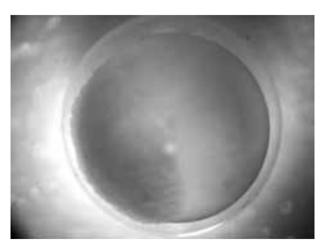


Fig. 10: 6 hrs 10min, 64 cell stage

The buried cells, or deep cells, each arise as one of the two daughters of the four central blastomeres that were present at the 32-cell stage.

The other daughter remains superficial, in the topmost tier of what is now the enveloping layer (EVL) of the blastodisc. During the same cleavage the horizontal divisions of marginal blastomeres present at cycle 6 produce two EVL sister cells, and in a face view of the 64-cell stage one sees three tiers of EVL cells.

Blastula period (6h 45m – 12h 10m)

We use the term blastula to refer to the period when the blastodisc begins to look ball-like, at the 128-cell stage, or eighth zygotic cell cycle, and until the time of onset of gastrulation (cycle 14). Important processes occur during this blastula period; the embryo enters midblastula transition (MBT), the yolk syncytial layer (YSL) forms, and epiboly begins. Epiboly continues during the gastrulation period.

Stages during the blastula period

128-cell stage (6 h 45m): The eighth cycle begins with 128 blastomeres arranged as a high mound of cells, a solid half ball perched on the yolk cell. The oblong shape of the blastodisc remains as described for the four-cell stage. As seen from a face view, the EVL cells line up in about five irregular tiers between the animal pole and the margin. (Fig. 11)

256-cell stage (7 h 20m): A face view at the end of the eighth set of cleavages reveals the EVL cells in about seven irregular tiers. The EVL cells thin out considerably during the interphase of this ninth cycle.

512-cell stage (7 h 55m): Here begins midblastula transition; cell cycles lengthen gradually during the next several divisions. In face view, about nine somewhat irregular tiers of EVL blastomeres occur between the margin and animal pole. During the last part of this stage and particularly as they enter the tenth mitosis, the marginal blastomeres (the first-tier EVL cells) begin to lose their lower borders where they join the yolk cell (Fig.).

High stage (10 h:. This stage marks the end of the period during which the blastodisc perches "high" upon the yolk cell (Fig.12). There is still a pinched ring, a constriction, where the marginal cells meet the YSL. One distinguishes the high stage from earlier ones by the appearance and numbers of both blastodisc cells and YSL nuclei. Most blastodisc cells complete zygotic cell cycle 12, whereas a few complete cycle 13 during this stage.

Oblong stage (11 h): The animal-vegetal axis of the blastula shortens, with the blastodisc compressing down upon the yolk cell, as one could imagine to result from a uniform increase in tension at the surface. The constriction at the blastodisc margin that has been present since the elevation of nonyolky cytoplasm during the one-cell stage diminishes (Fig.13) and then disappears. Eventually the blastula acquires a smoothly outlined ellipsoidal shape, as viewed from the side, and the stage is named for this oblong shape.

Sphere stage (12 h): Continued shortening along the animal-vegetal axis generates a late blastula of smooth and approximately spherical shape. The overall shape then changes little the next several hours, well into the period of gastrulation, but cell rearrangements that begin now seem to occur more rapidly than at any other time in development. One distinguishes this stage from the dome stage that comes next by the appearance, at a very deep plane of focus, of the face between the lower part of the blastodisc and the upper part of the yolk cell, the I-YSL. At sphere stage specifically this interface is flat, or nearly flat (Fig.14).

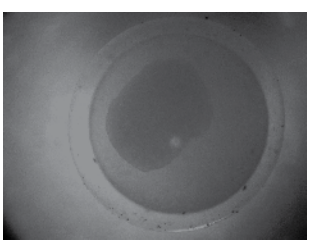


Fig. 11: 6 hrs 45 mins, 128 cell stage

Fig. 12: 10 hrs, high stage

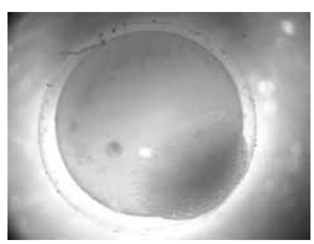


Fig. 13: 11 hrs, oblong stage

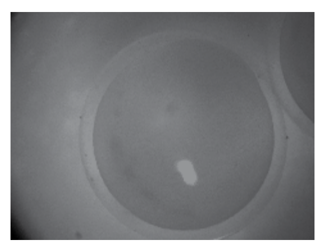


Fig. 14: 12 hrs, sphere stage

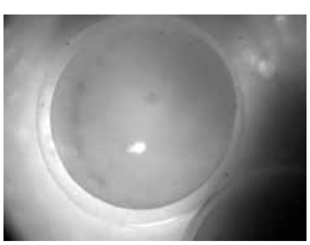
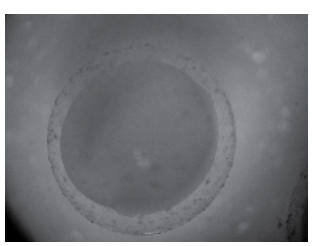


Fig. 15: 13 hrs, Dome stage



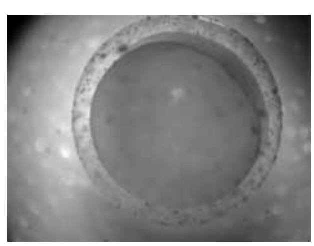


Fig. 16: 13hrs 30 mins

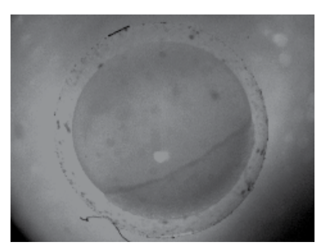


Fig. 17: 14 hrs, 30% epiboly

Dome stage (13 h): Deep to the blastodisc the I-YSL surface begins to dome toward the animal pole (Fig.15). This prominent and rapidly occurring change in the interface between the yolk cell and the blastodisc represents the first sure sign that epiboly is beginning. EVL cells are in a long cycle 13, and many deep cells enter cycle 14.

30%-epiboly stage (14 h): Epiboly, including doming of the yolk cell, produces a blastoderm, as we may now call it, of nearly uniform thickness (Fig.17).

Percent-epiboly means the fraction of the yolk cell that the blastoderm covers; percent-coverage would be a more precise term for what we mean to say, but percent-epiboly immediately focuses on the process and is in common usage. Hence, at 30%-epiboly the blastoderm margin is at 30% of the entire distance between the animal and vegetal poles, as one estimates along the animal-vegetal axis.

The blastoderm thickness is not exactly uniform in many embryos at this stage. Observing the marginal region from a side view, as the blastula is rotated about the animal-vegetal axis will reveal one region along the margin that is noticeably thinner and flatter than elsewhere. This particular region will become the dorsal side of the embryo (Schmitz and Campos-Ortega, 1994).

Gastrula Period (16 h 30m – 36 h)

Epiboly continues, and in addition, the morphogenetic cell movements of involution, convergence, and extension occur, producing the primary germ layers and the embryonic axis.

Stages during the gastrula period

50%-epiboly stage (16 h 30m): Epiboly displaces theblastoderm margin to 50% of the distance between the animal and vegetal pole. The margin remains at aboutthis same position for about 1 hour (Fig.18), the duration of this stage and the next two. Hence, it is important to distinguish these early gastrula stages by examining the thickness at and along the marginal region of the blastoderm. At this 50%-epiboly stage radial intercalations have produced a blastoderm that isvery uniform in thickness (Fig.18).

Germ-ring stage (17 h 5m): The germ ring forms as a thickened annulus at the blastoderm margin, as onecan discern in an animal-polar view (Fig.19). The germ ring appears nearly uniform in structure around the entire circumference of the blastoderm. (cycle 14)

Shield stage (20 h): An animal polar view most easily reveals the embryonic shield, as well as the germ ring. Both the epiblastand hypoblast are locally thickened at the shield (Fig.20). Epiboly remainsat 50% until late shield stage,when the yolk cell can be judged to be more than halfcovered by the blastoderm. Many DEL cells are beginning cycle 15.

75%-epiboly stage (24 h): As epibolycontinues the shape of the embryo itself becomes more along the animal-vegetal axis(Fig. 21) The embryonic shield becomes less distinctive, as compared to shield stage, as its cells repack to elongatethe shield along the AP axis and narrow it mediolaterally.

90%-epiboly stage (30 h): The bit of uncovered yolk cell protruding from the neighbourhood of the vegetal pole may now be considered a yolk plug (Fig. 22a).The dorsal side of the blastoderm is very distinctivelythicker than the ventral side (Fig. 22b).

Bud stage (36 h): Epiboly comes to a close as theblastoderm completely covers the yolk plug, defining 100%-epiboly. Convergence and extension movementshave spread the blastoderm across the yolk cell fasteron the dorsal side than on the ventral side, and because of this asymmetry, the pointat which the yolk plug disappears is not at the vegetal pole of the yolk cell, butsomewhat ventral to it. Just dorsal to the site of yolk plug closure, and usuallywithin 10-15 minutes of the closure, the posterior or caudal end of the embryonic axis develops a distinctswelling, thetail bud, for which can be named as the bud stage (Fig. 23).

Segmentation period (36- 70 h)

A variety of morphogenetic movements now occur, the somites develop, the rudiments of the primary organs become visible, the tail bud becomesmore prominent and the embryo elongates (Fig. 24-27). The AP and DV axes are unambiguous. The first cellsdifferentiate morphologically, and the first body movements appear.

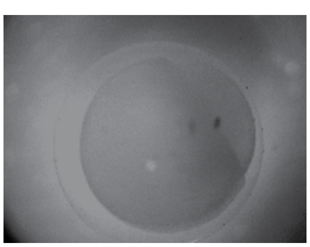


Fig. 18: 16 hrs 30 mins, 50 % epiboly

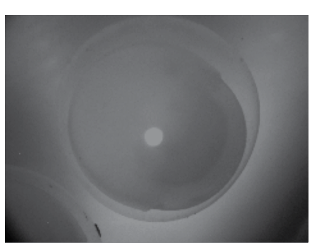


Fig. 19: 17 hrs 5 mins, Germ ring

Fig. 20: 20 hrs, Shield stage

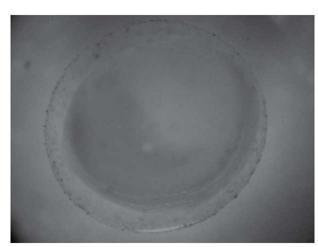


Fig. 21: 24 hrs, 75% epiboly

Fig 22a: 30 hrs

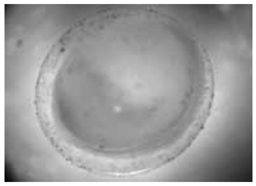


Fig 22b: 32 hrs

Fig. 23: 36 hrs, bud

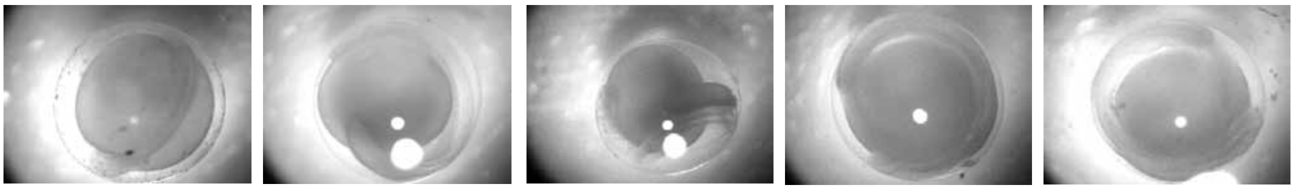


Fig. 24: 40 hrs

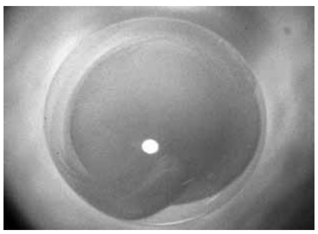


Fig. 25: 45 hrs

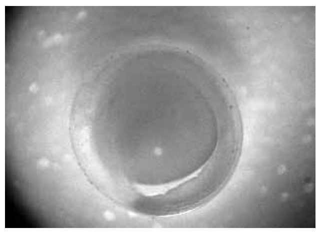


Fig. 26: 50 hrs

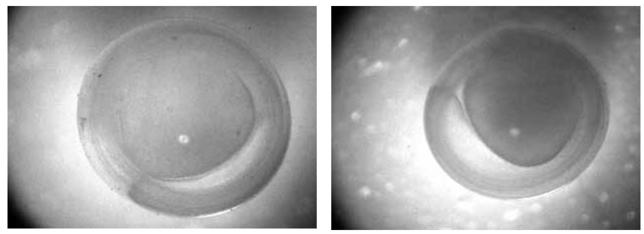


Fig. 27: 63 hrs

Pharyngula period (70-100 h)

Ballard (1981) coined the term "pharyngula" to referto the embryo that has developed to the phyolotypicstage, when it possesses the classic vertebrate bauplan.According to von Baer's famous laws this is the time of development when onecan most readily compare the morphologies of embryos of diverse vertebrates, and for the golden mahseer we approximate the period as the 70-100 h of embryonic development (Fig. 28-30). The embryo is most evidently nowabilaterallyorganizedcreature,enteringthepharyngula period with a well-developed notochord, and a newly completed set of somites that extend to the end of a long post-anal tail. The nervous system is hollow and expanded anteriorly. With the rapid cerebellar morphogenesis of the metencephalon, just preceeding the pharyngula period, the brain is now sculptured into five lobes.

Hatching period (94-120 h)

Whether or not an embryo has hatched, its development progresses, hour by hour, and generally individuals that have spontaneously hatched arenot more developmentally advanced than ones remaining in their chorions. We arbitrarily call the creatures"embryos" until the end of the 4th day, and afterward, "larvae," whether they have hatched or not (Fig. 31-37**).**

During the hatching period the embryo continues togrow at about the same rate as earlier**.** Morphogenesis of many of the organ rudiments is nowrather complete and slows down considerably, withsome notable exceptions including the gut and its associated organs. However, these endodermal structuresare difficult to visualize in the living embryo because of their deep positions, and we do

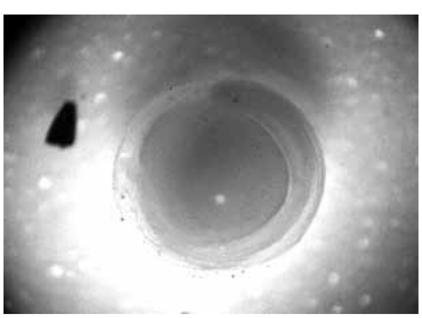


Fig 28: 74 hrs

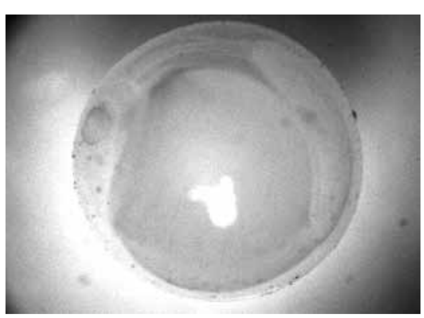


Fig 29: 98 hrs

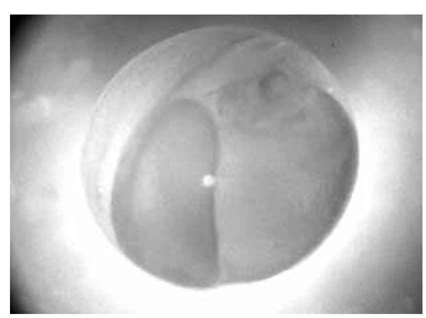


Fig 30: 98 hrs

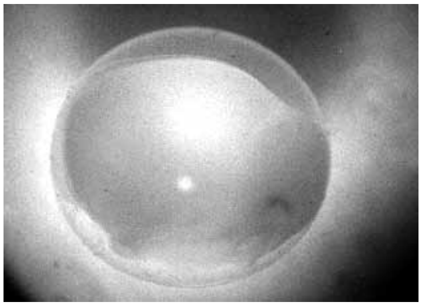


Fig. 31: 104 hrs

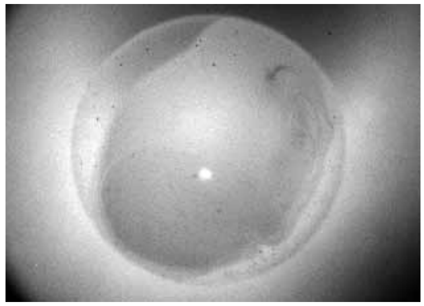


Fig. 32: 104 hrs

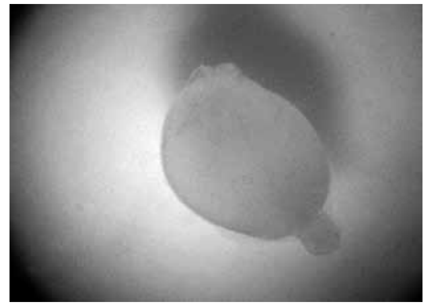


Fig. 33: 110 hrs

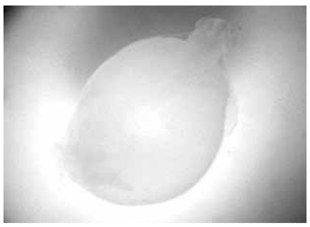


Fig. 34: 110 hrs

Fig. 35: 110 hrs

Fig. 36: 110 hrs

Fig. 37: 110 hrs

not consider them completely here. Much easier to see are the rapidly developing rudiments of the pectoral fins, the jaws, and thegills. Pectoral fin development continues to be a usefulfeature for staging, especially during the early part ofthe hatching period.

At the onset of the period thepaired fin rudiments are elongated buds, each already containing centrally located mesenchymal condensations that will form the girdle cartilages. The distal epithelial fold capping the bud, which developed from the apical ectodermal ridge, nowexpands intotheblade ofthefinproper,andstrengtheningactinotrichia appear. At the same time a circulatory channel appears as a continuous loop at the base (Fig. 30).

Discussion

The findings of the study exhibit that *Tor putitora* have a longer early developmental period than other cyprinids. For example, the morula stage in *T. putitora* begins approximately three hours after fertilization. Similarly, the morula stage in the Deccan mahseer, *Tor khudree* (Sykes 1839) begins approximately five hours post-fertilization at water temperatures of 20-23°C (Sangma & Basavaraja, 2010). During the morula stage, a dome-shaped blastoderm is formed in *T. putitora* by aggregation of embryonic cells in layers, as observed in *Pseudoplatystoma coruscans* (Spix & Agassiz 1829) (Marques *et al*., 2008). In *Pseudoplatystoma* spp. hybrids, the morula stage was distinguished by its “half-berry”-like structure (Faustino *et al*., 2010). At approximately six hours post-fertilization in *T. putitora*, two layers of cells were observed in the centre of the blastodisc with its outer enveloping layer having individual nuclei, mitotic figures, yolk syncytial layer and yolk globules. However, in embryos of *P. maculatus*, the blastodisc is characterized by having different nuclei, mitotic figures, YSL and the penetration of yolk globules as fragments into the YSL, which occur within two hours post-fertilization at a water temperature of 25-29°C (Buzollo *et al*., 2011).

In the present investigation, the gastrula stage in *T. putitora*wasobserved16hourspost-fertilizationatanambient water temperature of 20º C ± 2º C. However, In *T. khudree*, the gastrula stage is observed approximately 18 hours post-fertilization at a water temperature of 20-23°C (Sangma & Basavaraja, 2010). In this stage epiboly continues, and in addition, the morphogenetic cell movements of involution, convergence, and extension occur, producing the primary germ layers and the embryonic axis.

In epibolic stage, blastoderm cells move to cover the yolk mass and, during migration, the internal blastoderm cells move dorsally and converge to form the embryo axis (Leme dos Santos & Azoubel, 1996). The epibolic movement in *T. putitora* ends with the closure of the blastopore by the blastoderm. The yolk fraction that remains uncovered by the blastoderm when cell movements cease then change into a plug-like structure called the ‘yolk plug’, as also observed in *Pseudoplatystoma* spp. hybrids (Faustino *et al*., 2010).

At the segmemtation stage (approximately 36 hours post-fertilization), a variety of morphogenetic movements now occur, the somites develop, the rudiments of the primary organs become visible, the tail bud becomes more prominent and the embryo elongates. In *Tor khudree*, a pea-shaped embryo was observed at about 48 hours post-fertilization at 20-23°C (Sangma & Basavaraja, 2010).

In pharyngula period the embryo is most evidently now a bilaterally organized creature, entering the pharyngula period with a well-developed notochord, and a newly completed set of somites that extend to the end of a long post-anal tail. The nervous system is hollow and expanded anteriorly. With the rapid cerebellar morphogenesis of the metencephalon, just preceding the pharyngula period, the brain is now sculptured into five lobes.

In the present study, hatching was observed at approximately 94-120 hours of post-fertilization. In *T. khudree*, hatching occurs at approximately 110-122 and 65-70 hours post-fertilization at water temperatures of 20-23°C and 26-27°C, respectively (Nandeesha *et al*., 1993). Hatching time of two indigenous Malaysian mahseers, *Tor tambroides* (Bleeker 1854) and *Tor douronensis* (Valenciennes 1842), is reported to be 68-82 and 69-90 hours respectively after fertilization at 26-30ºC (Ingram *et al*., 2005). At the onset of the period thepaired fin rudiments are elongated buds, each already containing centrally located mesenchymal condensations that will form the girdle cartilages. The distal epithelial fold capping the bud,which developed from the apical ectodermal ridge, now expands into the blade of the fin proper, and strengthening actinotrichia appear. At the same time a circulatory channel appears as a continuous loop at the base Similar types of vitelline arteries arising from the yolk sac and entering into the pharyngeal region have been reported previously (Moitra *et al*., 1987). These vitelline arteries are not only involved in gas exchange but also supply nutrients from the yolk sac region (Moitra *et al*., 1987).

In conclusion, *T. putitora* has a longer early developmental period than other cyprinids under similar rearing temperatures. The first cleavage occurs about 3hours post fertilization, with cleavage interval of about 35 minutes. The blastula period last from 6h 45m to 12h 10m post fertilization. During this period; the embryo enters midblastula transition (MBT), the yolk syncytial layer (YSL) forms, and epiboly begins, which continues till gastrulation period. The gastrulation period generally lasts from 16h 30m to 36h. Embryonic development was completed within 94-120 hours post-fertilization at 20º C ± 2º **C.** The present study provides preliminary insight about the early development progression in Golden mahseer, which can help in developing better rearing strategies for early larval rearing to achieve better survival and eventually leading to population enhancement in natural environment.

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Conflict of interest

The authors report no conflict of interest.

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Ecological impacts of dams on the fish diversity of Bhagirathi river in central Himalaya (India)

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ABSTRACT

Bhagirathi river in Garhwal region of northern India is dammed at four locations (Maneri Bhali Phase I, Phase II, Tehri Hydroelectric Dam and Koteshwar Dam) for hydro power generation resulting in the fragmentation of riverine habitat. Substantial part of river is forced through tunnels where the river has lost identity leading to discontinuity in its original course while, considerable segment of river has also been converted into deep impoundments. These Dams have affected seasonal cycles of floods, natural flow regime, and has caused increased sedimentation in impounded section of river affecting habitat quality, life-history stages and population dynamics of the biota. These effects have been found to get augmented in lower stretch due to construction of dams in cascades. Consequently, the composition and structure of fish assemblages in the river has been affected. Most affected species have been found *Schizothorax, Glyptothorax, Pseudecheneis,Garra,Labeo*, *Crossocheilus, Noemacheilus, Barilius, Psilorhynchus, Clupisoma, Mastacembelus,* and migratory *Tor,* spp., which are indigenous rheophilic species requiring distinct habitats to fulfil their life cycle stages. Populations of fast flowing riverine species have been collapsed or even disappeared from fragmented sections of the river system.

Key words: Himalaya, Dams, Fish diversity, Habitat ecology, River fragmentation.

Introduction

Dams constitute obstacles for longitudinal exchanges along fluvial systems of the river. The construction of a dam results in “discontinuities” in the River Continuum (Ward and Stanford, 1983). The number and size of reservoirs/dams are increasing continuously due to increasing world demand for energy and water. Large number of dams have been built in hill states of India for harnessing the hydro energy besides water supply for drinking, household purposes and irrigation. These dams have been built over the rivers for many reasons including water supply, irrigation, power generation and recreational purposes which influence the natural river hydrograph and characteristics of the water. A regulated-river hydrograph may show a decrease in the median annual flow or changes to the timing and magnitude of high and low flows (King *et al*. 2003). The ‘discontinuities’ in the river continuum and irreversible alteration in the natural hydrological regime of rivers affects the habitat quality and the dynamics of the biota. The flow and water quality of river also gets altered, which have the potential of detrimental effects on river ecosystems (Poff *et al*., 1997; Richter *et al*., 2003). However, the river ecosystems recover from these effects after travelling a long distance downstream to dam due to resilience.

The obliteration of river ecosystem has direct impact on the aquatic biodiversity. The floral and faunal communities that inhabit the river and river margins have evolved to adapt to their rivers own peculiar pattern of flood and drought, slow and fast current. Due to such alteration in the ecology and fragmentation of the rivers, the inhabited communities get influenced which either disappear or move to other places (Rawat and Agarwal, 2015).

A three-level hierarchy of effects from dams is postulated by Petts (1984). First-order effects include changes to water quality, sediment load and flow regime. Second-order effects include changes to channel cross-section, bed-sediment movement and primary production, as a consequence of first order modifications. Third-order effects, such as changes to macro invertebrate communities and other biota, arise as a consequence of both first- and second-order effects. But with hardly any assessment of these impacts on river ecosystem, the dam construction is being planned and carried on continuously in various rivers of the world. Sufficient literature is available regarding the effects of dams and other regulating structures on the aquatic biodiversity (Walker, 1985; Reid and Brooks, 2000; Growns and Growns, 2001; Lloyd *et al*., 2003; Payne *et al*., 2004; Ayoade *et al*., 2009; Agarwal *et al*., 2011, 2018a and b, Ayoade and Agarwal, 2012) but informations are still scanty on the effects of serial impoundments of rivers including the Bhagirathi river on indigenous fish diversity (Agarwal *et al*., 2014).

Materials and Methods

Physiography of study area

The Bhagirathi is a large glacial fed and turbulent Himalayan river in India. It emerges from Gangotri glacier (Gaumukh), Latitude: 30° 56'N; Longitude: 79° 03'E at an elevation of 3812 m above sea level. Traditionally it is also considered to be the source of the mighty river Ganga. It flows for about 217 km before merging with river Alaknanda at Devprayag (474 m asl). The terrain between Gaumukh to Gangotri (3037m asl) is essentially devoid of biota due to hostile conditions. The morphometry of river channel varies tremendously throughout its length. Initially the river has a north western course and has carved out many deep gorges. Low volume in the upper stretch of river increases downwards due to joining of a number of tributaries. The river banks too vary in nature. In the uppermost reaches they are gorge like and rocky or full of huge boulders, and partially or fully matured boulders were found in the middle course. While pebbles, cobbles, sand, and silt were observed in lower stretch besides fully mature boulders of varying size. Rocky substrate dominates at Devprayag.

The river is joined by a number of small important tributaries on its left and right banks. These tributaries are Mandakini, Kheera, Kakora, Jalandhari, Pilang, Kumalti, Assiganga, Indrawati, Varuna, Ranu-ki-gad, Kharmola gad and Bhilangna. The Assiganga and Bhilangana are its major tributaries. The stretch between Gangotri (Latitude: 30° 59'56.2"N; Longitude: 78° 54' 56.5"E; Elevation 3037m asl) to Gangnani (Latitude: 30° 55'15.4"N; Longitude: 78° 40' 43.2"E, Elevation 1945 m asl) is apparently insignificantly influenced by human interventions except road construction and small human settlements. The river between Gangnani (1945 m asl) to Devprayag (474 m asl) is fragmented due to the construction of dams (Maneri Bhali Dam Project I and II, Tehri and Koteshwer dams). Due to these four dams in cascade, the considerable stretch of river Bhagirathi from Maneri to Koteshwar has been altered in term of its natural flow. Either the main river course has become semi stagnant reservoir or it has almost dried up due to channelizing/river diversion into tunnels. Wherever river flows in its natural course (between Gangnani to Devprayag), riffles are the major habitat type followed by rapids and pools. In such segment of river, the fiducial limits of water temperature range between 6.0- 18.5°C with moderate velocity (0.68 -1.52 m/s). Besides the above mentioned four existing dams, it is also proposed that the Bhagirathi river system shall further be dammed at 15 more sites; nine of which are under construction (Fig. 1).

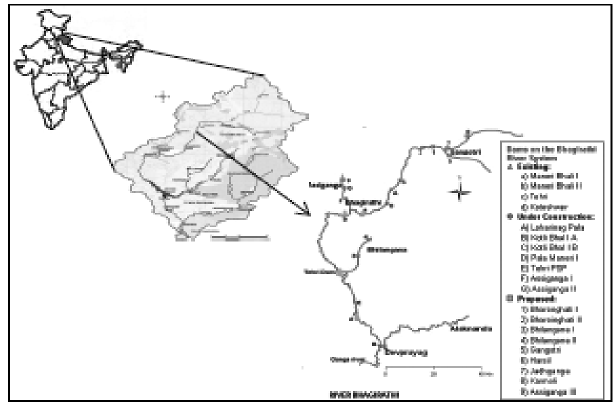

Fig. 1. Study area showing location of dams on the river Bhagirathi in different stages of planning and implementation.

Sampling, water analysis and fish diversity

The stretch of 217 km of river Bhagirathi from its origin (Gaumukh) to its confluence with Alaknanda at Devprayag was surveyed to study the extent of physiographic alteration in river caused by hydro power projects (HPPs). Depending on the accessibility to reservoir/river, numbers of random sites were selected for collection of fish samples and sampling of physico-chemical attributes. Experimental fishing was carried out during daytime (06:00-17:00 hrs) with the help of local fishermen. Fishes were collected with cast nets (dia. 2.0 m, mesh size 1.8 x 1.8 cm), gill nets (mesh size 1.2 x 1.2 cm, L x B = 12 x 1.5 m) and 'phans' (indigenous nets, using several fine nylon loop knotted over a long nylon cord of 5-8 m length, rope is spread on the bottom of stream cross section with stones tied in few loops).Indigenous fish traps viz. 'goda', 'pot trap' were also used (Singh and Agarwal, 2014a). At each sampling site, cast net were used at least twenty times during each sampling occasion along the river length. Fish samples were also collected from nearby market and lending centre associated with the river system. Identification of fish samples was done with the help of Day (1878), Talwar and Jhingran (1991), Badola (2009) and Jayaram (2010). Taxonomic discrepancies at species level were resolved using the latest database. After preparation of recent data base of fish fauna of Bhagirathi River, it was compared with earlier works on the fish diversity of Bhagirathi river system by Badola (1979); Singh *et al*. (1987).

Habitat ecology and substratum type of river was characterised after following Armantrout (1999). Hydrological attributes of the Bhagirathi river (temperature, velocity, pH, turbidity, dissolved oxygen, free CO_2 and total alkalinity) were analysed following APHA (2005).

Results and Discussion

Dams constructed on the river Bhagirathi

The Bhagirathi river system has been dammed at four locations (Table 1). It is first impounded for the Maneri Bhali Phase 1 dam (reservoir area 2 km) at Maneri. Here, the river is diverted through a tunnel (of 12 km length) substantially reducing water in its natural course of 13 km length. The river regains its original shape at Tiloth (1 km before Uttarkashi) when the water from the tail race tunnel of the Maneri Bhali Phase I Hydro Power Project joins in the natural river course. River hardly flows for about 2 km before it is again impounded and channelized into tunnel (16 km length) for Maneri Dam Phase II at Joshiyara-Gyansu downstream to Uttarkashi town, leaving 22 km river stretch with very low water discharge up to Dharasu. Thereafter, from Dharasu to Chinayalisaur (3 Km) it flows naturally.

From Chinayalisaur to Tehri, a 44 km river stretch has been converted into semi stagnant water body due to its impoundment for Tehri Dam. Downstream of Tehri Dam, 19.5 km river length again has been impounded for Tehri PSP stage and Koteshwar dam. Due to construction of these dams in series, the physiography and flow pattern of river is altered in the different segments from Maneri to Devprayag which has ultimately affected the occurrence of indigenous fish species in this highly regulated river stretch (Table 2).

Table 1. Dams on the river Bhagirathi and extent of river fragmentation

Hydropower projects running on the Bhagirathi River system	Length of original river course impounded	Length of river course with very low water discharge due to its diversion in to tunnel	Major Impact
Maneri Bhali Phase I Dam (30^o 44'N; 78^o 31'E Elevation 1297 m)	2 km (Upstream Maneri) Surface area 1.8 Km^2	13 km Maneri to Tiloth	• Flow pattern and temperature regime of river changed. • Flooding pattern changed. • Downstream of river is starved with nutrient.
Maneri Bhali Phase II Dam (30^o 44'N; 78^o 26'E Elevation 1122 m)	~3 km (Upstream Joshiyara)	22 km (Joshiyara to Dharasu)	
Tehri Dam (30^o 23'N; 78^o 28'E Elevation 830 m)	44 km (Chinyalisour to Tehri)	1.5 km (width of Dam at river bed 1.2 km)	• Density and Diversity of fish fauna is highly affected.
Koteshwar Dam (30^o 15'N; 78^o 29'E Elevation 612.5 m)	19.5 km (downstream Tehri to Koteshwer) Surface area 29 Km^2	-	
Total Length of river = 217 km	68.5 km	36.5 km	48.4% of riverine habitat is lost/ altered

Table 2. Salient features of the river Bhagirathi along with fish assemblage in different stretches from Gaumukh to Devprayag

Salient features of the river Bhagirathi in various parts from its journey Gaumukh to Devprayag		Fish Species found
1	Gaumukh to Gangotri (19 km stretch) Represents natural and pristine state, Steep bed with rocks and boulders, cool, clean water with low primary producers, no human intervention.	No fish
2	Gangotri to Harsil (21 km) Represents natural and pristine condition, low primary producers, narrowest channel, Deep Gorges, steep bed with rocks and boulders.	No fish
3	Harsil to Jhala (07 km) Pristine natural condition, three tributaries joins, wide flood plain, Flat bed with pebbles and sand, Meandering and bifurcating into several channels with dynamic system of riffles and pools.	*Schizothorax richardsonii*, Tor putitora
4	Jhala to U/S Maneri Bhali stage I (MB-I) Power Project (~36 km) Pristine natural condition, Steep and narrow river bed intermingled with large boulders, pebbles and sand, high velocity.	S.richardsonii, Glyptothorax pectinopterus, Pseudecheneis sulcatus
5	MB stage I U/S to M B stage I Barrage (02 km impoundment) River impounded, Slow moving water body, increase sedimentation, Dam has fragmented/ discontinued Riverine environment, River diverted to 14 km long tunnel for Tiloth power House.	S. richardsonii, S. plagiostomus T. putitora

	Salient features of the river Bhagirathi in various parts from its journey Gaumukh to Devprayag	Fish Species found
6	Maneri Bhali stage I Barrage to MB-1 Tail waters (Tiloth) (13 km) Flow regime disrupted, highly regulated reduced flow. Some time river bed may exposed due to very low water discharge. Deep pools provide shelter to fishes at this time. Fish fauna (number and size) is affected before joining of Asiganga to Bhagirathi at Gangori. *The exotic fish *Salmo* commonly found in Assiganga (a major tributary of Bhagirathi river), take route to Bhagirathi R. near its confluence thus shows presence in the fish catch from Bhagirathi near Gangori. *Common carp (*Cyprinus carpio*) are bred in Gangori hatchery located on the right bank of river Bhagirathi. During rainy season rearing pond got flooded and the common carp seed washed away into the Bhagirathi river. Now this species is also showing its presence in the fish catch.	*S. richardsonii, S. plagiostomus, Schizothoraichthys progastus, Salmo trutta fario, Cyprinus carpio, Barilius bendelisis, B. barna*
7	MB-1 Tail waters Tiloth to Maneri Bhali stage 2 U/S(01 km) Uttarkashi Modified fluctuating flows depending upon Power turbine operation at Tiloth power house (MB stage-1HPP), water from Assiganga give additional flow, River bed consisting of large and small sized stones and pebbles with moderate current provide breeding ground to the fishes. Riffles and pools are the characteristics of this segment.	*S. richardsonii, S. plagiostomus, S. progastus, T. putitora, Salmo trutta fario, Cyprinus carpio, Garra gotyla gotyla, G. pectinopterus, P. sulcatus*
8	Maneri Bhali Stage-2 U/S to MB-2 Barrage (03 km) (Uttarkashi to Joshiyara) River impounded for Maneri Bhali stage 2 HPP, Flow reduced. Increase sedimentation, River fragmented 2nd time, Reservoir provide habitat for Mahseer fish sp. Breeding ground of snowtrout and mahseer are disturbed.	*S. richardsonii, S. plagiostomus, S. progastus, T. putitora, T. chilinoides, Cyprinus carpio*
9	MB-2 Barrage to MB-2 tail water (~22 km) (Joshiyara to Dharasu U/S) Flow regime disrupted, reduced flow. Some time river bed may exposed due to very less water discharge. Deep pools provide shelter to fishes at the time of less discharge. Water from three small streams (Varuna gad, Ranu-ki-gad, Kharmola gad) on the right bank between Joshiyara to Dharasu keep alive the Bhagirathi river in this stretch. Fishes are mostly of small size.	*S. richardsonii, S. plagiostomus, Barilius bendelisis, B. barna, Noemacheilus rupicola, N. montanus*
10	MB-2 tail water to Tehri Dam reservoir U/S (~03 km) Modified fluctuating flows depending upon Power generation at MB stage-2 HPP), River bed consisting of large and small sized stones and pebbles. Riffles and pools are the characteristics of this segment.	*S. richardsonii, S. plagiostomus, S. progastus, Cyprinus carpio T. putitora, T. chilinoides, Garra gotyla gotyla G. pectinopterus, P. sulcatus*
11	Tehri U/S to Tehri Dam (44 km river length Impoundment) River impounded for Tehri HPP, Flow reduced. Increase sedimentation, increased depth, fluctuating littoral zone with steeper banks, Potential for release of green house gases due to decomposition of submerged biomass. River fragmented 3rd time. Breeding and feeding ground of bottom feeder indigenous *Schizothorax, Garra, Glyptothorax and Pseudecheneis sp.* get destroyed. Reservoir provide good habitat for Mahseer (*Tor* spp.) culture. Exotic common carp introduced, thrive well posing significant threat to native species.	*Cyprinus carpio, Tor putitora, S. progastus*
12	Tehri Dam D/S to Koteshwer Dam (22 km) River impounded 4th time for Koteshwar Dam and Tehri PHP This reservoir face daily high fluctuation in water level and water flow depending on the Power generation in Tehri power Plant. Reservoir bank has steep slope. Flow regime disrupted.	*S. richardsonii, S. plagiostomus, S. progastus, Cyprinus carpio, T. putitora, Labeo dyocheilus*
13	Koteshwer Tail water to Devprayag (24 km) Frequent fluctuation in flow depends upon power generation at Tehri and Koteshwer Power Plant. River bed consists of big and small stones and pebbles, combination of riffles and pools. River has less discharge some times. River starved with nutrient. Flooding cycle disrupted from seasonal to daily. Thus breeding and feeding biology of fishes is adversely affected.	*S. richardsonii, S. plagiostomus, S. progastus, T. putitora, T. tor, Garra gotyla gotyla G. pectinopterus, P. sulcatus, B.bendelisis, L. dyocheilus, Botia dario*

Effects of dams on physiography of the river

The physiography of river has been altered drastically from being swiftly flowing (due to steep gradient) into semi stagnant water with characteristic flat gradient and large volumes of water. In the 'run of river valley power projects' swiftly flowing water of river has been forced through tunnels creating discontinuity in original river course. Narrow valley with regular waterfalls has been converted into flat water surface. This has resulted into high silt deposition and trapping of nutrient in the impounded area. High silt deposition in the reservoir area has covered rocky substratum with enormous sand and silt. This has caused destruction of feeding and breeding ground of the indigenous bottom feeder, lithophil spawner fish species. However, due to diversion of river into hydro power tunnels at several locations, river became discontinuous or dried up, leaving water only in isolated pools. This situation becomes more prominent during winter months (October to March) as discharge from barrage is very low in order to keep reservoir level up (river discharge is also minimum during winter months upstream of the reservoir). Due to increased sedimentation and trapping of silt in the impounded area, river gets starved of nutrients in the downstream to the dam. Reduction in sediment load in downstream water further causes increased erosion of river banks and beds. This physiographic degradation of Bhagirathi river is brought about by intensified dam construction activities that have severely affected the indigenous fish fauna.

Effect of dam on the physico-chemical profile of river Bhagirathi

Physical and chemical attributes of the river Bhagirathi after its impoundment for Tehri dam reservoir were analysed seasonally and were compared with secondary data (Table 3). Comparison of both data showed a statistically significant difference between pre and post impoundment. Water temperature showed high variation in both lotic and lentic environment. Increase in mean annual water temperature was recorded from lotic to lentic environments in all the seasons. Prior to impoundment it was 14.7 ±1.61°C which rose to 21.7 ±2.67°C after impoundment in summer season. Each season also recorded an increase in temperature. Increase was significantly high in monsoon and autumn while low in winter season (Table 3). Rise in mean temperature may have been due to heat up of water, as in large reservoir, water surface is exposed to the sun for longer periods. Water velocity of river ranged between (2.4-0.60 m^{-s}) which was observed almost nil in all the seasons after impoundment. Dissolved oxygen also increased in all seasons after impoundment of river. An increase by 1 mg^{-1} in summer while by 0.9, 0.7 and 0.2 mg^{-1} in monsoon, autumn and winter seasons respectively. This increase of dissolved oxygen could be due to high planktonic growth in reservoir

Table 3. Physico-chemical characteristics of the Bhagirathi river: the pre and post -impoundment stages in the submergence zone of Tehri hydro power project

Parameters	Seasons	Water temp.(°C)	Water velocity (m/s)	DO (mg/l)	FreeCO_2 (mg/l)	Total alkalinity (mg/l)	pH	Turbidity (NTU)
Pre Impoundment Agarwal et al. 2003	**S**	14.7±1.61	1.0±0.2	9.0±0.59	2.4±0.21	9.7±2.44	8.1±0.2	181±139
	M	16.6± 0.48	2.4 ±0.5	8.4±0.27	2.8±0.29	4.5±0.91	7.7±0.3	404±121
	A	13.4±2.01	1.3±0.5	9.3±0.26	2.4±0.25	5.2±0.88	8.0±0.1	76±52
	W	10.1±1.9	0.6±0.1	10.5±0.25	1.7±0.25	10.3±2.26	8.3±0.1	24±4
Post Impoundment (2007-08)	S	21.7±2.67	-	10.0±0.31	-	37.0±4.37	7.3±0.1	4±3
	M	27.5±1.08	-	9.3±0.35	-	58.0±14.9	7.2±0.1	27±15
	A	24.5±3.28	-	10.0±0.46	-	85.4±6.9	7.3±0.1	17±6
	W	12.6±3.00	-	10.7±0.45	-	50.0±2.93	7.6±0.0	2±1

S= Summer, M= Monsoon, A= Autumn, W= Winter area. Ayoade *et al*. (2009) and Agarwal *et al.* (2018a and b) found that there is high plankton growth in reservoir. Free CO_2 concentration in flowing river ranged between 2.8-1.7 mg^{-1} in monsoon and winter seasons respectively. In lentic portion it was found completely absent. The increase in dissolved oxygen and absence of free CO_2 in the lentic water maybe due to increased photosynthesis by high phyto-planktonic growth.

Total alkalinity of lotic environment was very low, but increased significantly in all seasons after its conversion into lentic environment. According to Moyle (1946) water body with total alkalinity above 50 mg/l can be considered productive due to high level of phosphorus and other elements. Thus it may be safely stated that river impoundment is more productive than the riverine section due to high total alkalinity. pH of lentic environment has been reduced in all seasons after alterations in the riverine environment, which may be due to increase in photosynthetic activities in the reservoir area. The pH of water is affected as photosynthetic activity removes carbon dioxide from water and shifts the carbonate-bicarbonate equilibrium (Ayoade *et al*. 2009). The river water was highly turbid, while its impoundment has drastically reduced the turbidity of water. The river with high flow erodes huge sediments from the riparian zone attributing to its high turbidity. In the reservoir area the water is standing and thus sediments get deposited on the bottom leading to low turbidity.

Impact of serial dam construction on ichthyofauna of the Bhagirathi river

Fish species distribution in the hill streams depends on many factors viz. flow rate, nature of substratum, water temperature and food availability. Up and downstream of dams, marked changes in fish populations of Bhagirathi river were observed as a consequence of blockage of migration routes, disconnection of the river and floodplain, changes in flow regime, change in physiochemical attributes (e.g. temperature, turbidity and dissolved oxygen), primary productivity and channel morphology. These changes may benefit some of the exotic species but generally have an adverse effect on the majority of native species as observed in the Bhagirathi river impoundment for Tehri Dam where common carp (*Cyprinus carpio*) has registered its significance presence while native *Schizothorax* spp. have been extirpated. Agarwal *et al.* (2011) and Singh and Agarwal (2017) have reported significant changes in distribution of fish species in the Bhilangana River brought out by the construction of dam.

The existence and survival of hill stream rheophilic fish species of Bhagirathi river system has also been drastically affected by the construction of dams in cascade. Present study reported altogether 24 species from Bhagirathi river system (Table 4). The Cyprinidae was major dominant family along with other families like Cobitidae and Sisoridae. The fish community structure in Bhagirathi river was characterized by a few specialized cyprinids types, specifically the snow trout (*Schizothorax* and *Schizothoraichthys* spp.), the mahseer (*Tor* spp.), the lesser barils (*Barilius* spp.) the stone sucker (*Garra* spp.), true hill stream loaches (*Noemacheilus spp.)* and sisorid torrent cat fishes (*Glyptothorax*, *Pseudecheneis* and *Botia* spp).

Prior to the emergence of dam, a total of 41 fish species (Badola, 1979) and 39 fish species (Singh *et al*., 1987) were reported from the river Bhagirathi. Overall composition was contributed by *Schizothorax, Schizothoraichthys, Tor, Labeo, Crossocheilus, Garra, Barilius, Noemacheilus, Glyptothorax, Pseudecheneis* and some other like *Botia, Psilorhynchus, Clupisoma, Mastacembelus and Salmo* species. Present study has revealed the decline in the fish composition of Bhagirathi river (Table 4). Similar decline in density and diversity of fish spp. has also been reported by the construction of dam on Niger River (Lae, 1995) and construction of Petit saut Dam in French Guiana (Merona and Albert 1999). Marchetti and Moyle (2001) opine that changes in flow volume and patterns can have adverse impact on the structure, distribution and composition of fish community. Transformation of riverine habitat to lentic habitat has resulted in the extirpation of resident riverine species. Following changes seem to be acting as a limiting factor for fish diversity of the Bhagirathi river-

Habitat loss

Of the 217 km long stretch of Bhagirathi river, ~68.5 km of river stretch is impounded and 36.5 km long river stretch is forced through tunnels, reducing the river discharge in its natural/original course leading to dried up portions for the significant period of the year (Table 1). Thus a significant area (48.4%) of the riverine fish habitat has been altered or lost which has resulted into the decline in fish diversity and abundance of the Bhagirathi river as most of hill stream fishes are habitat specific. The fish distribution of any river is related with habitat composition (Probst *et al*. 1984; McClendon and Rabeni, 1987).

Blockage in the migration route of fishes

Dams or any construction across rivers is always a barrier for fishes which move from one part of stream/ river to another as part of its life cycle. The erection of Koteshwer dam, Tehri dam, Maneri hydro power projects I and II have blocked the migration routes. On the free flowing Bhagirathi river, 260.5 m high Tehri dam has no fish pass. Thus it has completely blocked the free movement of fishes upstream and downstream of Tehri dam resulting into isolation/fragmentation of fish gene pool of Bhagirathi river into Tehri upstream and Tehri downstream population.

Table 4. Fish fauna of Bhagirathi river system before and after the river fragmentation

Scientific name with order and family	Prior to emergence of dam		After dams construction
	Badola (1979)	Singh et al. (1987)	
ORDER CYPRINIFORMES			
1. Family Cyprinidae			
Barilius barila	P	p	ab
B. barna	p	p	p
B. bendelisis	p	p	p
B. shacra	p	ab	ab
B. vagra	p	p	p
Chagunius chagunio	p	ab	ab
Crossocheilus latius latius	p	p	ab
Cyprinus carpio	ab	ab	p
Garra lamta	p	p	ab
G. prashadi	ab	p	ab
G. gotyla gotyla	p	p	p
Labeo dero	p	p	ab
L. dyocheilus	p	p	p
Raiamas bola	p	p	p
Schizothorax richardsonii	p	p	p
S. sinuatus	ab	p	p
S. plagiostomus	ab	p	p
Schizothoraichthys curvifrons	ab	p	p
S. esocinus	ab	p	ab
S. intermedius	ab	p	ab
S. micropogon	ab	p	ab
S. niger	p	p	ab
S. planifrons	p	ab	ab
S. progastus	ab	p	p
Tor chilinoides	p	p	p
T. hexasticus	p	ab	ab
T. putitora	p	p	p
T. tor	p	p	p
2. Family Cobitidae			
Botia almorhae	P	ab	ab
B. dario	p	p	p
Noemacheilus bevani	p	p	p
N. denisonii	p	ab	ab
N. montanus	p	p	p
N. multifasciatus	p	p	p
N. rupicola	p	p	p
N. savona	p	p	p
N. zonatus	p	p	ab
3. Family Psilorhynchidae			
Psilorhynchus balitora	P	ab	Ab
ORDER SILURIFORMES			
1. Family Schilbeidae			
Clupisoma garua	P	p	Ab
2. Family Sisoiridae			
Euchiloganius hodgarti	P	ab	ab
Glyptohorax brevipinnis	p	p	ab
G. cavia	p	p	ab
G. conirostris	p	p	ab
G. kashmirensis	p	ab	ab
G. madraspatanum	p	p	ab
G. pectinopterus	p	p	p

Scientific name with order and family	Prior to emergence of dam		After dams construction
	Badola (1979)	Singh et al. (1987)	
G. telchitta	p	ab	ab
G. trilineatus	p	p	ab
Pseudecheneis sulcatus	p	p	p
ORDER MASTACEMBELIFORMES			
1.Family Mastacembelidae			
Mastacembelus armatus	Ab	p	Ab
ORDER SALMONIFORMES			
1.Family Salmonidae			
Salmo trutta fario	P	ab	P
TOTAL SPECIES REPORTED	41	39	24

p- present, ab- absent

Tor species (mahseer) which earlier freely move up and down in the stream for the completion of their life cycle get affected seriously. However, Tehri dam authorities have taken initiative to stock mahseer seed in the Tehri reservoir as a mitigation measure to support mahseer population upstream of the Dam.

Destruction of breeding grounds by sedimentation

Blocking of river with dams and barrages of hydro power projects has resulted into the deposition of material eroded by rivers on the reservoir substratum which has destroyed the breeding grounds of hill stream fishes. Many hill stream fishes viz. *Schizothorax*, *Tor*, *Garra, Glyptothorax, Pseudecheneis,* require shallow stony substratum on the river banks for breeding (Singh *et al*. 1985; Agarwal, 2001). But construction of dam has reduced the flow of river in impounded area resulting into increase sedimentation/silt deposition leading to destruction of natural breeding ground of indigenous fish species.

Destruction of feeding grounds

Indigenous *Schizothorax, Garra, Glyptothorax and Pseudecheneis* spp. are bottom feeders and have special adaptive features for scraping periphyton/algae/benthic invertebrates as their food items from the pebble, cobble, stones and boulders of the fast flowing Bhagirathi river. According to Power (1973) the presence of bottom cover in the form of boulders and large stones greatly enhances the holding capacity of hill streams for fish. The construction of dam/barrages has resulted into reduced flow, rise in water column and deposition of high silt. Even a few centimetres of sediment layer over the natural substrata is enough to have negative effect on the foraging and spawning of fish. High silt deposition in the upstream of dam upto the tail end of submerged zone has lead to phenomenal change in the characteristics of substrate composition. Such changed habitat (disappearance of stony substratum along with food items), compel these fish species to move upstream in search of their preferred substratum and food for survival. This has caused the complete disappearance of bottom feeding native fish species especially snow trouts and Himalayan loaches from the impounded stretches of the river Bhagirathi.

Changes in river flow patterns

In case of Bhagirathi River, flow pattern has been changed as 1). from fast flowing river to very slow moving or almost stand still water body (upstream to dams), 2). Reduced or no water flow in the dry zone (after diversion of river in to tunnels) and 3). Fluctuations in water flow downstream to dam (release of water through power turbines). The species like *Schizothorax, Garra, Glyptothorax*, *Pseudecheneis* adapted for fast turbulent current with adhesive apparatus, are unable to survive in slow moving high water column (Agarwal and Singh, 2009, Singh and Agarwal 2014b). Downstream fish population have also been adversely affected by the unnatural rapid fluctuations due to dam operation in peak and low hours of power demand. Fish and other organisms are adapted to the monthly, seasonal, annual and inter-annual variability in flow for their survival and reproduction (Thompson and Larsen 2004). The natural flooding in rivers provide important environmental cues to the snow trout and other hill stream fishes for maturation of gonads, spawning or maintaining annual life history stages (Singh and Agarwal 1986, Agarwal, 2001, 2008). In the absence of natural flooding as environmental cue, reproduction in these fishes has been hampered. Furthermore daily pulse release from power turbine also caused significant lateral and vertical fluctuation of river water affecting the nursery and foraging areas of fish and acts as a limiting factor for aquatic ecosystem and its biodiversity. Most common attribute of downstream flow regulation is a decrease in the magnitude of flood peaks and an increase in low flows (McCartney 2009).

Change in the thermal regime

Water temperature influences many important ecological processes and it is an important factor affecting growth in freshwater fish, through feeding behaviour, food assimilation and the production of food organisms. The study on pre and post impoundment of Bhagirathi River for Tehri dam reservoir revealed that an overall significant rise in sub-surface water temperature in all the seasons of the year (Table 3). The relatively large mass of standing water in reservoirs allows heat storage and produced a characteristics seasonal pattern of thermal behaviour. Depending on geographical location, water retained in deep reservoirs has become stratified. Release of cold water from the hypolimnion (i.e. the deep cold layer) layer of reservoir, is the greatest "non-natural" consequence of stratification. Even without thermal stratification, water released from reservoirs is often thermally out of phase with the natural regime of the river (McCartney, 2009). The leisurely moving comparatively warmer water of the impounded area of Bhagirathi river has favoured the exotic common carp which has registered their overriding presence over the native species (snow trout and mahseer) in the recent years as also observed for the Bhilangana river impoundment (Agarwal *et al*. 2011, Singh and Agarwal 2014b).

Changes in the nutrient flow

A total of ~68.5 km long stretch of the entire Bhagirathi River has been impounded by the dams. This submerged zone of the river is acting as a nutrient trap. The changes in the nutrient flow have adversely affected the downstream fish population. However, few species like common carp and mahseer may have been benefitted in the submergence zone of Tehri dam but these has affected the fish composition in this stretch. Sivakumar (2008) has also observed that nutrient availability is an important ecological determinant for the fish species composition in the Himalayan River.

Conclusion

The lotic environment of Bhagirathi was comprised of 41 fish taxa (Badola, 1979) and 39 fish taxa (Singh *et al.,* 1987) prior to emergence of dams on it. This was indicative of good health of the Bhagirathi river ecosystem and diverse habitats conducive for breeding and nursery grounds of hill stream fishes. As a consequence of inclusive alteration of riverine habitat into lentic habitat by the four dams in cascade, the spatio-temporal distribution of these fish taxa is severely influenced with existence of only 24 fish species for the whole stretch of the river. This decrease in fish diversity seems directly linked to the loss of critically important fish habitats. Therefore, an effort should be made to conserve these important hill stream fish species, the most important component of aquatic biodiversity in the headwater streams of Central Himalaya (Garhwal) India.

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Effect of spirulina (*Spirulina platensis*) fortified diets on growth performance of two important cold water fishes viz. *Barilius bendelisis* and *Schizothorax richardsonii*

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ABSTRACT

Two important coldwater species viz. *Barilius bendelisis* and *Schizothorax richardsonii* of almost similar length and weights were collected from local streams and reared in laboratory condition for sixty days in triplicate to study the effect of spirulina (*Spirulina platensis*) fortified diets (0, 3, 5, 7 & 10 %) on their growth performance. Both the species are important coldwater species and very much considered as food and ornamental value. Supplementing 7-10% spirulina in the diets of both the fishes resulted increased weight gain (WG- 224& 226 %, respectively), specific growth rate (SGR-2.0 for both the species) and decreased food conversion ratio (FCR-1.2 for both the species) in comparison to control (T0) group (WG 88 %; SGR 1.0 % and FCR 3.4). Results revealed that supplementing 7-10% of spirulina in fish diet results higher growth rate, decreased FCR with higher survival rate, leads to higher return from the fisheries business.

Keywords: Coldwater fishes, Spirulina, Growth, Survival, FCR.

Introduction

The Indian hill trout (*Barilius bendelisis*) and Snow trout (*Schizothorax richardsonii*) are small indigenous fish endemic to Himalaya, thrives well in coldwater streams, lakes and rivers (Jha et al., 2010). Both are having commercial importance due to their ornamental and food value hence widely cultured in hilly regions of Himalaya (Jha *et al*., 2012).

The food value of fish is determined with the quality and quantity of protein and other nutrients present in the muscle while the ornamental value is associated to the coloration due to carotenoids or pigment bearing substance in the tissue (Halten *et al*., 1997). Inadequate pigmentation, retarded growth with degraded muscle composition is always noticed in cultured fishes due to lack of carotenoid and other nutrient content in their artificial diets (Jha *et al*., 2010). In wild, fishes are getting quality food required for their proper growth, pigmentation and nutrient profile. But, in captive condition, lack of nutrients and pigment bearing substance, results in retarded growth, faded coloration and degraded nutrient profile of the fish.

In an aquaculture system, natural food is not sufficient to sustain optimum production. Hence it is vital to provide a nutritionally balanced diet for optimum aquaculture productivity. Nutritionally balanced diet contains carbohydrate, fat, proteins, vitamins, minerals and carotenoids for sufficient growth and pigmentations (Jha *et al*., 2010).In aquaculture industry, many feed additives like carrot, beet root, red pepper, marigold flower, rose petals, China rose, chestnut flowers, spirulina, crustacean wastes, yeast, synthetic astaxanthin, vitamin C and vitamin E have been utilized since long to achieve desired flesh quality of various fish species (Chapmann, 2000; Gocer *et al*., 2006; Ezhil *et al*., 2008; Jha *et al*., 2012).

As feed additive, dried algae improve growth, feed efficiency, carcass quality and physiological response to stress and disease in several species (Mustafa and Nakagawa, 1995).Amongst various algae, spirulina is considered a rich source of protein having 65-70% protein (Lornz, 1999), vitamins, minerals, essential amino acids, fatty acids (gama-linolenic acid (GLA), antioxidant and pigment such as carotenoids (Belay *et al*., 1996) in addition, it is also effective as an immune-modulator (Takeuchi *et al*., 2002). Spirulina is the only microalgae additive, which demonstrates benefits to growers that offset the initial cost and provide a significant cost/performance ratio. Spirulina has been studied over the globe by the scientists as a feed supplement for various fishes and found to significantly improve growth, survival and feed utilization (Belay *et* *al.*, 1996; Takeuchi, 2002). Therefore, the purpose of this study was to investigate the effect of spirulina (*Spirulina platensis*) fortified diets on growth performance of above mentioned two important coldwater fishes in order to increase their production.

Materials and Methods

Experimental animal

Fingerlings of *B. bendelisis* and *S. richardsonii* of the average weight of about 5.6g were collected from a local stream of Nainital (Uttarakhand), transported in a circular container (500 L) with sufficient aeration, to the experimental site at hatchery complex of Directorate of Coldwater Fisheries Research (ICAR), Bhimtal and were acclimatized to the experimental rearing conditions for one week. During acclimation, fish were fed with control diet. After acclimatization, fishes were transferred to 15 uniform size experimental fiberglass tanks (separately for both the fishes) of 100L capacity and reared for 60 days.

Experimental design and feeding

Five iso-nitrogenous (35.25±0.9% crude protein) diets were prepared with graded level of (0, 3, 5, 7 and 10% of diet) spirulina (SP) meal. Both the fishes (150 nos. of each) were randomly distributed in 5 experimental groups, separately, in triplicate following a completely randomized design (CRD). There were 5 treatment groups, viz.T0 (control), T1 (3% SP), T2 (5% SP), T3 (7% SP) and T4 (10% SP). The control (T0) was common for both the fishes, while treatments were divided into eight group viz. for *Barilius bendelisis* it was BS-1 (fed on T1), BS-2 (fed on T2), BS-3 (fed on T3) and BS-4 (fed on T4) and for *Schizothorax richardsonii* it was SS-1 (fed on T1), SS-2 (fed on T2), SS-3 (fed on T3) and SS-4 (fed on T4). The physicochemical parameters of water were analyzed by APHA (1995) methodology, and were within the optimum range (dissolved oxygen: 6.0–8.5 mg/l, pH: 7.3–8.2 and temperature: 18–20 °C) throughout the experimental period. All the groups were fed their respective diets. Feeding was done at 5% of the body weight. Daily ration was divided into 2 split doses: about 2/3rd of total ration was given at 09:00 h and the rest at 18:00 h. The fecal matters were removed by siphoning and a constant water flow (2-3 L/M) was maintained by providing inlet at one and outlet at the other end to ensure optimum dissolved oxygen throughout the experimental period. A very few accidental mortality (10%) was observed during the 60 day experimental feeding trial. The composition of the diets are given in the Table-1.

Table1: Diet composition (%)

Ingredients	Diets				
	T_0	T_1	T_2	T_3	T_4
Fish meal	20.27	20.27	20.27	20.27	20.27
Soybean meal	20.27	20.27	20.27	20.27	20.27
Rice bran	29.73	26.73	24.73	22.73	19.73
Wheat bran	21.73	21.73	21.73	21.73	21.73
Vitamin-Mineral Mix[1]	2.00	2.00	2.00	2.00	2.00
Vegetable oil[2]	2.00	2.00	2.00	2.00	2.00
Fish oil[3]	2.00	2.00	2.00	2.00	2.00
Sodium alginate[4]	2.00	2.00	2.00	2.00	2.00
Spirulina meal	**0**	**3.00**	**5.00**	**7.00**	**10.00**
Total	100	100	100	100	100

[1]Agrimin Forte (Virbac Animal Health India Pvt. Ltd., Mumbai-59, India). Each kg contains- Vitamin A-7, 00,000 I.U., Vitamin D3-70, 000 I.U., Vitamin E-250 mg, Nicotinamide-1000mg, Cobalt-150 mg, Copper-1200 mg, Iodine-325 mg, Iron-1500 mg, Magnesium-6000 mg, Manganese-1500 mg, Potassium-100 mg, Selenium-10 mg, Sodium-5.9 mg, Sulphur-0.72%, Zinc-9600 mg, Calcium-25.5%, Phosphorus-12.75%. [2]Ruchi Soya Industries Ltd., Raigad, India. [3]Procured from local market; [4]Himedia Ltd. India

Growth study

Growth rate of fish was measured in terms of weight gain (WG %), specific growth rate (SGR %) and feed conversion ratio (FCR) using the following equations.

SGR (%) = [(Log final weight – Log initial weight) / number of experimental days] × 100

WG (%) = [(final mean weight – initial mean weight) / initial mean weight] × 100

FCR= feed given (dry weight) / body weight gain (wet weight)

Statistical analysis

Mean values of all the parameters were subjected to one way ANOVA to study the treatment effect, and Tukey's test (HSD) were used to determine the significant differences between two means. Comparisons were made at 5% probability level. All the data were analyzed using statistical package SPSS (Version 12.01).

Results and Discussion

The proximate composition of the diets used in this study is given in the Table-2. The proximate composition of the diets did not varied significantly.

Table 2: Proximate composition of different diets (% dry matter basis)

Particulars	Diets				
	T_0	T_1	T_2	T_3	T_4
Moisture	13.28±0.02	13.40±0.03	13.15±0.01	13.34±0.05	13.30±0.02
Crude protein	35.21±0.08	35.32±0.05	35.10±0.02	35.20±0.01	35.09±0.02
Ether extract	7.65±0.02	7.80±0.01	7.25±0.02	7.97±0.03	7.45±0.02
Ash	10.85±0.05	11.01±0.02	10.90±0.04	11.00±0.03	11.08±0.05

Data expressed as Mean±SE, n=3

The growth parameters of *Barilius bendelisis* and *Schizothorax richardsonii* in experimental groups at the end of feeding trial are shown in Table 3 and SGR-FCR are compared in Fig. 1. Supplementation of 10 % dietary spirulina meal (T4) significantly (P<0.05) increased specific growth rate (SGR %) and reduced feed conversion ratio (FCR) compared to control group (T0) in case of *B. bendelisis*, while in case of S. richardsonii7 % dietary spirulina meal (T3) reported significant (P<0.05) in terms of specific growth rate (SGR %) and reduced feed conversion ratio (FCR) compared to control group (T0). Improved growth rate and decreased FCR was noticed with the diet containing higher quantity of carotenoids in the form of spirulina meal and an inverse relation between SGR and FCR was also noticed in the present study (Fig. 1). This is due to the fact that utilization of carotenoids leads to improved growth and decreased FCR (Jha *et al*., 2013). Sarma *et al*. (2010) also observed the similar trend and reported that with increase in spirulina content in the fish diet as protein source, FCR decreases, the similar results were also confirmed by both the species in the present study.

Table 3: Growth performance of *Barilius bendelisis* and *Schizothorax richardsonii* fingerlings fed diets with graded level of spirulina meal, mean±SD, n=3 (10 fish per replicate).

Treatments	WG (%) Mean±SD	SGR (%) Mean±SD	FCR (Mean±SD)
Control	88±16[a]	1±0.1[a]	3.4±0.9[c]
BS-1	148±6[b]	1.5±0[b]	1.8±0.1[b]
BS-2	174±37[b]	1.7±0.2[c]	1.6±0.4[b]
BS-3	203±24[b]	1.8±0.1[c]	1.3±0.2[a]
BS-4	224±15[c]	2±0.1[c]	1.2±0.1[a]
SS-1	166±7[b]	1.6±0[b]	1.6±0.1[a]
SS-2	217±31[c]	1.9±0.1[c]	1.3±0.2[a]
SS-3	226±6[c]	2±0.1[c]	1.2±0.1[a]
SS-4	226±25[c]	2±0[c]	1.2±0[a]

Values in a column with different superscripts differ significantly ($p<0.05$).

In resemblance with the present observation, Christiansen *et al*. (1995) used astaxanthin as a source of carotenoid and observed lower FCR in Atlantic salmon juveniles, similarly, the growth of fish was achieved higher when carotenoids from different sources were added in their diets (Jha *et al*., 2012) and a linear relationship between dietary supplementation of carotenoids (spirulina as source) and growth of fish was reported by Sarma and Jha (2010). Spirulina contains carotenoids and carotenoids are known to have a positive role in metabolism in fish (Tacon, 1981).

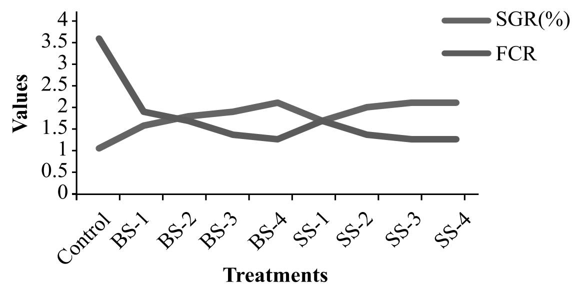

Fig. 1: Comparison of SGR and FCR of *B. bendelisis* and *S. richardsonii* fingerlings fed diets with graded levels of spirulina meal.

It can be concluded from the above observations that supplementation of 7-10% of spirulina in fish diet results higher growth rate, decreased FCR with higher survival rate, leads to higher return from the fisheries business.

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Role of small tributaries in ichthyofaunal diversity of rivers in Uttarakhand

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ABSTRACT

An attempt has been made to assess the ichthyofaunal diversity of the major rivers of Uttarakhand state with main focus for subsidiary tributaries. The estimated total length of river network is 10927.9 kms including streams, tributaries and rivulets, which support occurrence of 83 fish species belonging to 39 genera. Conservation and restoration efforts on large rivers often focus on the main streams, but not for subsidiary tributaries, which provide unique habitat and serve as spawning sites and nursery grounds. Despite their abundance on the landscape and important role in ecosystem, tributaries are ignored in commonly used cartographic depictions, but play an important role in the ichthyofaunal diversity.

Keywords: Tributaries, Ichthyofaunal diversity, Spawning sites, Nursery ground

Introduction

Uttarakhand is located between latitude $28^0 40'$-$31^0 29'$N and longitude $77^0 35'$-$81^0 5'$E covering an area of 53,566 km² in North West Himalayan region. The state has unique ecosystem and bestowed with vast and varied water resources in the form of rivers, rivulets, streams, streamlets, lakes. Biodiversity is essential for stabilization of ecosystems (Ehrlich and Wilson, 1991). Himalayan ecosystem is unique in its biodiversity and it is the origin point of two mightiest river systems in Uttarakhand i.e. Ganga river system from Gangotri glacier and Yamuna river from Yamunotri glacier having so many rivers like Alaknanda, Bhagirathi, W-Ramganga, Kosi, Yamuna, Saryu, Mandakini and Sharda along with many small streams and tributaries.

Fish is an indicator of habitat suitability and health of any aquatic system (Goreman and Karr, 1978) however; thermal regime is an important limiting factor affecting distribution pattern and abundance of fish. Various abiotic and biotic factors are the key factors for determining the fish diversity, community structure and species assemblages in the stream and rivers (Minns, 1980). Suitable spawning ground, shelters and feeding ground play important role in the life cycle of fish and consequently in the sustainability of fish population. Scanty information is available on the aquatic faunal diversity and density for the study area as majority of the earlier studies were focused on the main river course and its major tributaries. In the Himalayan streams, the distribution of fish depends upon the flow rate, type of substratum, water temperature, and availability of food and the hydrographical features of the basin (Sehgal, 1988). Sunder *et al.* (1999) enlisted total of 218 fish species for the entire Himalayan region. Himalayan fish fauna was classified under subsistence and commercially important fish groups *i.e.* Carps (*Labeo* and *Tor* spp.), lesser barils (*Barilius* spp.), schizothoracines (*Schizothorax* & *Schizothoraichthys* spp.), garrids (*Garra* spp.) and sisorids (*Glyptothorax* & *Glyptosternum* spp.) (Vass, 2005). Coldwater species inhabiting in the Himalayan rivers belong principally to 5 different families, Salmonidae, Cyprinidae, Cobitidae, Sisoridae and Mastacembelidae; out of which fishes comprising major commercial fishery belong to Cyprinidae represented by 3 sub-families, i) Cyprininae-Mahseers and minor carps, ii) Rasborinae-Indian trouts, and iii) Schizothoracinae-Snow trouts (Moza, 2002). The exotic brown trout (*Salmo trutta*) is an introduced fish (Sehgal, 1999) which has been established in some uplands streams of the state. Owing to complex microclimatic conditions coupled with thermal variables, the capture fishery in the state is still at very low pace. Though the ecological information and ichthyofaunal diversity of the main rivers are available, but fish diversity in small tributaries and their defined role in biodiversity conservation is still lacking in the sustainable fishery of the state. Hence, an attempt has been done to describe the role of small streams and tributaries in the ichthyofaunal diversity of the aquatic ecosystem of the state.

Study area and methods

The entire drainage of State including Ganga basin, Yamuna and Tons basin and Kali-Sharda along with their tributaries has been covered for resource mapping. The rivers in these areas have been digitized in GIS platform by using ArcGIS v.10.1 (The Environmental System Research Institute, USA). The ichthyofaunal diversity of the different tributaries was worked out by field survey; however, available literature was also used for the major rivers and streams. Sampling for fish fauna was carried out using cast net and drag net in the sample sites to represent wide range of habitat conditions with the study area. The density of species wise fish fauna were recorded wherever fish occurred. The biomass of the fish species was calculated based on the total fish catch. A minimum of 15 netting was made for the period of 2 hours in the sampling area to collect the available fish specimen. The percentage catch composition was calculated based on the total weight.

$$\% \text{ Biomass} = \frac{\text{Total weight of the fish species (gm)}}{\text{Total fish catch (gm)}} \times 100$$

Catch per unit effort (CPUE) is the number or weight of fish taken during defined period of effort. The CPUE was calculated as;

$$\text{CPUE} = \frac{\text{Total weight of the fish (kg)}}{\text{Time (hr)}}$$

Fish including their spawns, fry and fingerlings caught from the tributaries were identified up to genera/species level with the help of keys given in Jayaram (1999), Menon (1987) and Talwar and Jhingran (1997). Samples of periphyton were collected by scraping of 3cm^2 area of the boulders and preserved in 1ml of Lugol's solution. The keys of Ward and Whipple (1959) and Trivedy and Goel (1984) were used for identifying the algae. Horizontal and vertical hauling was carried out by 0.25mm pore size plankton net to assess the species richness of phytoplankton and zooplanktons. Quantitative and density estimation of plankton were made using plankton net and preserved in Lugol's solution for identification using keys given by Pennak (1989); Fitter and Manuel (1986) and Edmondson (1992). Enumeration of plankton samples were done following methods given in APHA (1998). Density of phytoplankton was assessed by counting in Sedgwick Rafter cell. Benthic macro-invertebrates were collected from the designated sampling sites using Surber's Square Foot Sampler device or Eckman dredge depending on bottom types through random sampling (Welch, 2003). Simpson's Diversity Index (D) and Shannon-Weaver index (Shannon-Weaver, 1949) was calculated following the standard procedure.

Results and discussion

The state has a dense network of glacier fed, snow fed and spring fed tributaries of varying magnitudes from snowline to foothills, thus providing habitats defined by ice-cold and normal temperature regimes (Nautiyal 2001). The estimated total length of the main river courses in the state is about 2057.1 kms, while it is 10927.9 kms along subsidiary tributaries (Table-1).

Table 1. Length of Rivers, streams and tributaries of Uttarakhand state

River	Length of main rivers (km)	length of subsidiary tributaries (km)
Alaknanda	191	1648.2
Dhauliganga	91.9	293.8
Pindar	106.6	242
Mandakini	91	1143
Bhagirathi	184	1039.2
Bhilangna	74.1	281.1
Nayar	69.5	252.9
Yamuna	183	149.2
Tons	209	302.8
Kosi	183	408
Ramganga -W	168.5	420
Kali	233	213.7
Dhauliganga	42.9	86.5
Goriganga	97.7	322
Ramganga-E	99.3	423.2
Gomti	32.6	108.8
Other drainage network		1536.4
Total River length (km)	2057.1	8870.8
Overall Length (km)	10927.9	

Ichthyofaunal diversity and distributional pattern

About 64 fish species have been reported from Garhwal region, while Kumaon region is reported to have 31 fish species (Kumar, 2002). The dominant species reported are *Schizothorax richardsoni* and *Tor putitora*. Badola and Pant (1973) reported 18 species of fish from District Uttarkashi. Singh *et al.,* (1987) published a geographical and distributional list of ichthyofauna and reported a total of 68 species from Garhwal Himalaya. The present review and field survey reveals that Uttarakhand possess 83 species belonging to 39 genera, 14 families out of which 40 species have food value, 8 species are of exclusively ornamental value and 5 species are of sport fishery including world famous sport fish, Golden Mahseer (Table-2).

The Ganga river basin is one of the largest inland river basin of India draining a catchment area of 0.86 million km^2. The Ganga rises as Bhagirathi in Himalayas at 'Gaumukh' at the height of 3892 m. Most of the tributaries are glacial or spring origin, while a few have catchments in the high reaches without snow. In Upper stretch, some of the important tributaries include Bhagirthi, Bhilangna,

Table 2. Fish Species recorded in Rivers and their tributaries of Uttarakhand state.

Sr. No.	Scientific Name	IUCN Red List Status	Importance
Family : Cyprinidae			
1	*Barilius barna* (Ham.)	Least Concern	Ornamental/food value
2	*Barilius bendelisis* (Ham.)	Least Concern	Ornamental/food value
3	*Barilius barila* (Ham.)	Least Concern	Ornamental
4	***Barilius vagra* (Ham.)	Vulnerable	Ornamental/food value
5	*Barilius shacra* (Ham.)	Least Concern	Ornamental
6	****Raiamas bola* (Ham.)	Endangered	Ornamental
7	*Danio aequipinnatus* (McClelland)	Least Concern	Ornamental
8	*Devario devario* (Ham.)	Least Concern	Ornamental
9	*Danio rerio* (Ham.)	Least Concern	Ornamental
10	*Chagunius chagunio* (Ham.)	Least Concern	Food value/ornamental
11	*Cirrhinus mrigala* (Ham.)	Not Evaluated	Food value
12	*Garra prashadi* (Ham.)	Least Concern	Ornamental
13	*Garra lamta* (Ham.)	Least Concern	Ornamental
14	***Garra gotyla gotyla* (Gray)	Vulnerable	Ornamental
15	***Schizothorax richardsonii* (Gray)	Vulnerable	Ornamental
16	*Schizothorax progastus* (McClelland)	Least Concern	Food value
17	*Schizothoraichthys labiatus* (McClelland)	Not Evaluated	Food value
18	*Schizothoraichthys esocinus* (Heckel)	Not Evaluated	Food value
19	*Schizothorax sinuatus* (Heckel,)	Not Evaluated	Food value
20	*Schizothorax plagiostomus* (Heckel)	Not Evaluated	Food value
21	*Schizothorax curvifrons* (Heckel,)	Not Evaluated	Food value
22	*Schizothorax niger* (Heckel,)	Not Evaluated	Food value
23	*Schizothorax intermedius* (McClelland)	Not Evaluated	Food value
24	**Schizothorax micropogon* (Regan)	Near Threatened	Food value
25	*Bangana dero* (Ham.)	Least Concern	Food value
26	*Labeo dyocheilus* (McClelland)	Least Concern	Food value
27	*Labeo boga* (Ham.)	Least Concern	Food value
28	*Labeo rohita* (Ham.)	Least Concern	Food value
29	*Labeo calbasu* (Ham.)	Least Concern	Food value
30	*Labeo gonius* (Ham.)	Least Concern	Food value
31	*Cyprinus carpio* var. communis	Not Evaluated	Food value
32	*Oxygaster bacaila* (Ham.)	Least Concern	Food value
33	*Puntius chola* (Ham.)	Least Concern	Food value/ornamental
34	*Puntius ticto* (Ham.)	Least Concern	Food value/ornamental
35	*Puntius conchonius* (Ham.)	Least Concern	Food value/ornamental
36	*Puntius sarana* (Ham.)	Least Concern	Food value/ornamental
37	*Puntius phutunio* (Ham.)	Least Concern	Food value/ornamental
38	*Puntius sophore* (Ham.)	Least Concern	Food value/ornamental
39	**Tor tor* (Ham.)	Near Threatened	Food value/sports
40	****Tor putitora* (Ham.)	Endangered	Food value/sports
41	***Naziritor chilinoides* (McClelland)	Vulnerable	Food value/sports
42	*Rasbora daniconius* (Ham.)	Least Concern	Ornamental

Sr. No.	Scientific Name	IUCN Red List Status	Importance
43	*Esomus danrica* (Ham.)	Least Concern	Ornamental
44	*Crossocheilus latius latius* (Ham.)	Least Concern	Ornamental
Family : Cobitidae			
45	*Botia dario* (Hamilton, 1822)	Least Concern	Ornamental
46	*Lepidocephalichthys guntea* (Ham.)	Least Concern	Ornamental
47	*Nemacheilus beavani* (Günther)	Least Concern	Ornamental
48	*Nemacheilus corica* (Ham.)	Least Concern	Ornamental
49	*Nemacheilus botia* (Ham.)	Least Concern	Ornamental
50	Nemacheilus denisoni (Ham.)	Least Concern	Ornamental
51	*Schistura rupecula* (McClelland)	Least Concern	Ornamental
52	*Schistura savona* (Ham.)	Least Concern	Ornamental
53	*Schistura multifasciata* (Day)	Least Concern	Ornamental
54	*Schistura scaturigina* (McClelland)	Least Concern	Ornamental
55	*Acanthocobitis botia* (Ham.)	Least Concern	Ornamental
56	*Paraschistura montana* (McClelland)	Not Evaluated	Ornamental
57	**Balitora brucei* (Gray)	Near Threatened	Ornamental
Family : Amblycipitidae			
58	*Amblyceps mangois* (Ham.)	Least Concern	Ornamental
Family : Sisoridae			
59	*Glyptothorax cavia* (Ham.)	Least Concern	Ornamental
60	*Glyptothorax pectinopterus* (McClelland)	Least Concern	Ornamental
61	****Glyptothorax madraspatanus* (Day)	Endangered	Ornamental
62	*Glyptothorax trilineatus* (Blyth)	Least Concern	Ornamental
63	*Glyptothorax telchitta*	Least Concern	Ornamental
64	*Glyptothorax brevipinnis*	Data deficient	Ornamental
65	*Glyptothorax conirostris* (Steindachner)	Data deficient	Ornamental
66	*Pseudecheneis sulcata* (McClelland)	Least Concern	Ornamental
67	**Bagarius bagarius* (Ham.)	Near Threatened	Food value
Family : Bagridae			
68	*Mystus vittatus* (Bloch)	Least Concern	Food value
69	*Mystus bleekeris* (Day)	Least Concern	Food value
70	*Mystus seenghala* (Sykes)	Least Concern	Food value
71	*Mystus tengra* (Ham.)	Least Concern	Food value
Family : Belonidae			
72	*Xenentodon cancila* (Ham.)	Least Concern	Food value
Family : Claridae			
73	*Clarius batrachus* (Linn.)	Not Evaluated	Food value
Family : Mastacembilidae			
74	*Mastacembelus armatus* (Lacepède)	Least Concern	Food value/ ornamental
75	*Mastacembelus punctatus* (Ham.)	Least Concern	Food value/ ornamental
Family : Channidae			
76	*Channa punctatus* (Bloch)	Least Concern	Food value/ ornamental
77	*Channa gachua* (Ham.)	Least Concern	Food value/ ornamental

Sr. No.	Scientific Name	IUCN Red List Status	Importance
Family : Anabantidae			
78	*Colisa fasiatus* (Bloch)	Least Concern	Food value/ ornamental
Family : Nandidae			
79	*Nandus nandus* (Ham.)	Least Concern	Ornamental
Family : Schilbedae			
80	*Clupisoma garua* (Ham.)	Least Concern	Food value/ ornamental
Family : Siluridae			
81	**Ompok bimaculatus* (Bloch)	Near Threatened	Food value
Family : Salmonidae			
82	*Salmo trutta fario* (Linn.)	Least Concern	Food value/ sports
83	*Oncorhynchus mykiss* (Wal.)	Least Concern	Food value

*Near Threatened, ** Vulnerable, ***Endangered

Alaknanda, Pindar and Nandakini. Habitat structure in these tributaries have substrata consisting of boulders, stones and sands mixed with pebbles in the upper reaches and mixture of mud and sand in lower reaches. 52 species were recorded in the five rivers and their tributaries of Ganga river system, of which *Schizothorax richardsonii, Tor putitora, Tor chelynoides, L. dyocheilus, Bangana dero, Barilius bendelisis, Schizothoraichthys progastus and S. plagiostomus* are important food fishes while others though smaller in size and of low economic value are significant for biodiversity. As per the drainage wise distribution in the sampling, 52 species in Alaknanda, 32 in Bhagirthi, 29 in Bhilangna 48 in Pinder and 21 in Nandakini were recorded with richness in the subsidiary tributaries of these rivers. Snow trout, *Schizothorax* spp. (80-98%) and Mahseer, *Tor* spp. (1-3%) are the dominant species observed in the main river course and in different tributaries along with minor fishes. These species in the normal course of its life cycle migrate within the stream/river from higher elevation to lower elevation during winter months and *vice-versa* during summer.

Fishery resources of river Yamuna and Tons are not well known and most of the studies reported on the fish diversity and density in the foot hills of Garhwal Himalaya (Nath *et al*., 1994; Moza *et al.,* 2005). As mentioned, upper Himalayan stretch of the river Yamuna has not been explored in detail and according to Sehgal (1992) Yamuna was known to hold fish mostly in the middle and lower segment which have been evaluated by CIFRI and documented by Jhingran (1975), Mishra and Moza (2001) and Moza and Mishra (2001). A total of 26 fish species were mentioned in the river Yamuna at the foot hills of Garhwal Himalaya by Mallik (2011). Ishaq and Khan (2013) have reported a total of 24 species belonging to 6 families from Kalsi to Asan in the river Yamuna. Similarly river Tons has also not been explored above Kalsi, the confluence point of river Yamuna and Tons. In the lower stretch of river Tons a total of 19 species were reported by Negi and Mamgain (2013). Cypriniformes comprises the dominant group represented by 19 species belonging to 9 genera in Yamuna and Tons. *Schizothorax* spp., *Barilius* spp. and *Tor* spp., was the common species. However, the population of these species is fragmented but the occurrence of juveniles of all the species reflects the environment conducive for the breeding activities to sustain the population. The places having steep slopes and fast water current have been observed with the presence of *Garra gotyla* gotyla and *Glyptothorax* sp. such as Bhadri gad. The density of the fish occurrence was observed increasing in the downstream of Yamuna and Tons. Snow trout (Schizothoracins) and barils prefer the snow fed streams where suitable water temperature supports their life cycle. These species are available throughout the Yamuna basin mainly confined in the side tributaries of the main river course and adjoining places of the tributaries. The juveniles of *Schizothorax* and *Barilius* spp. were recorded in most of the side tributaries in the shallow areas and also where the water temperature was observed in the range between 9-21°C.

W. Ramganga and its tributaries were recorded for the presence of 32 fish species belonging 7 families. Barils, snow trout, minor carp and loaches were the major contributory groups of fish in the catch composition. Near Kheeda presence of good number of mahseer was observed. This location might be serving as shelter ground and possibly breeding ground for mahseer. Near Bhikayasen, minor carps mainly *Bangana dero* and *Labeo dyocheilus* are found in abundance. *Raiamas bola* is also found however, the occurrence was occasional. Near Masi, Mahseer is found in abundance in different size ranges (75-200 g). Based on the sampling it was found that the diversity is low in upper and middle stretches of the main river courses and Shannon index indicated towards the moderately stress condition of the

Table 3. Rivers, streams and their tributaries of Uttarakhand state

Rivers	Tributaries
Alaknanda River (1839.2 kms)	Madhyamaheshwar, Kali Ganga, Vasuki Ganga, Byung Gad, Baram Gad, Nauna Gad, Ata Gad, Pindar & Mandakini.
Bhagirathi River (1223.2 kms)	Hunuman Ganga, Bingsi Gad, Ganwan Gad, Pilang Gad, Kola Gad, Kundl Gad, Lod Gad, Bhela River, Son Gad , Syalam Gad & Rishi Ganga.
W-Ramganga River (588.5 kms)	Ram Ganga River , Bhelichin Nala, Bagri Gad, Bhojpatri Gad, Mehar gad, Jhiniya Ga, Lathiya Gad, Dokanna Nala, Kalapani Gad, Hari Gad, Sauron Gad & Tanar Ki Gad.
Kosi River (591 kms)	Lamgada Gad, Ban Gad, Dhaula Gad , Nariye Gad, Kali Gad, Ghatt Gad, Swal Nadi, Sakuni Gad, Ghat Gad, Kaluwa Gad, Taklari Gad, Gaunchhil Gadhera, Baurar Gadhera & Khalgari Gad.
Yamuna & Tons River (844 kms)	Supin , Pabber River, Khaneda Gad, Pali Gad, Badyar Gad, Rikhnar gad, Badri Gad, Aglar Nadi, Asan, Patar, Dogra Khad, Dogra Khad & Bajhetu Nala.
Saryu River (619.1 kms)	Gomati River, Khir Ganga, Revti Ganga,Gason Gad, Lahor Nadi, Galipatal Gad, Belang Gad, Chhira Gad, Galasar Canal, Kaplani Gad, Saran Gadhera, Gainar Gad, Lamtara Gad, Tuspati Gad & Bhadrapati Nala.
Mandakini River(1234 kms)	Madhyamaheshwar River, Kali Ganga,Vasuki Ganga, Byung Gad & Sone Ganga.
Sharda River (1608.5 kms)	E –Ramganga, Goriganga, Dhauliganga, Sarju River, Jakula river, Bagri Gad, Bhojpatri Gad, Jhiniya Gad, Sil Gad, Titar Gad, Mangarh Gad, Shilang Gad & Kalapani Gad.

Table 4. Simpson's Diversity Index (D) and Shannon-Weiner index in major streams and tributaries of Uttarakhand state

Index	Bhagirathi	Bhilangna	Alaknanda	Ganga	Yamuna & Tons	tributaries of Alaknanda	tributaries of Yamuna
Shannon_H	1.532	1.486	1.564	2.502	2.180	2.354	2.468
Simpson_1-D	0.734	0.7223	0.7743	0.8730	0.6754	0.8790	0.8980

main rivers. However, both the index reflects comparatively better diversity and low stress condition in the subsidiary tributaries of these rivers (Table-4).

Catch per unit effort (CPUE)

Catch per unit effort (CPUE) is proportional to the average density of the location fished. CPUE was calculated during different months/season at different locations in main rivers & their tributaries during the survey. The data of CPUE indicated decrease in trend with increasing altitude in the basin; indicative of low fish occurrence/density in the middle and higher reaches of the river basin. CPUE data also revealed that fish catch is higher during post-monsoon to pre-monsoon. Monsoon season has minimum catch record. The CPUE recorded in the range of 0.02 kg/hr - to 0.38 kg/hr in the middle zone of Ganga & Yamuna basin, while it ranged from 1.12 kg/hr to 5.24 kg/hr in the lower zone of these rivers. In side tributaries of upper zone of these rivers, the CPUE was recorded in the range of 0.04kg/hr to 2.70 kg/hr. Earlier studies (Moza *et al.* (2005) also indicate that fish biomass range between nil-10 kg/day (pre-monsoon) and nil-20 kg/ day (winter) in Yamuna.

Major breeding/spawning sites and spawning season

The presence of larvae, juvenile and adult of different species indicated the suitability of the sites for fish spawning, nursery for larval development, growth and survival of fish species. The larvae/juvenile of indigenous species spotted at different places in Yamuna and Tons & its tributaries (*e.g*. Near Paligad, Sari gad, Barni gad, Aglar khad, Bhadri gad, Gadu gad, Kedar Ganga and near Rupin) indicated its wide presence at different altitudes and its breeding in the streams. The juveniles of *Schizothorax* spp. was in abundance at many locations particularly at confluence points of Alaknanda, Yamuna and Tons with the favorable conditions prevailing in the area in terms of water flow, food availability, less turbidity and suitable shelters available for growth and survival. The presence of gravid female fish at few locations (*e.g.* Kedar Ganga) also confirmed its natural breeding in the stream. Certain isolated pools created at meandering of the river also provide the spawning habitat such as breeding pools of minor carp near Bhikiyasen. About 30 spawning grounds were observed in tributaries of Alaknanda basin such as the Birahi Ganga, Bal Ganga (Bal khila), Nandakini and Pinder Rivers.

Food and feeding ground

The Benthic macro-biota in highland streams play important role in distribution of fishes and other aquatic organisms. The population density of micro biota is in the range of nil-1.26 million units/cm^2. Bacillariophyceae (52.9-87.0%) is the dominant group of the algae followed by green algae, Chlorophyceae (1.0-8.2%) and blue green algae, Cynophyceae (0.2-15.5%). In periphyton community, *Gomphonema* contributes more than 60% of the total periphytic population. In fast flowing streams, phytoplanktons are present in dominance with least occurrence of zooplankton. The density of the plankton is in the range of 0-422 unit/L in upper reaches of the rivers to 116-14320 units/L in middle and lower reaches. Bacillariophyceae (12.4-89%) is the dominant group of phytoplankton followed by green algae and blue green algae. The decreasing temperature supports higher percentage of the Oligochaete. Occurrence of dipterans as dominant group is present in the middle reaches of the rivers. Trichoptera are well represented in all river basins. The wet biomass of these invertebrates ranged between 0.432-34.0 g/m^3 (23-656 nos./m^2) with higher occurrence of juveniles of Plecoptera in the upper reaches of the rivers.

Conclusion:

Habitat features such as suitable water quality, migration routes, spawning grounds, feeding sites, resting sites and shelter from enemies and adverse weather are determining factors for the propagation and survival of riverine fish species in uplands. In this context, small streams and side tributaries, which occur across or in sides of the main rivers and remain differ from the main steams for temperature gradient, water chemistry, hydrological regime, substrate type, food resources and riparian pools, all of which support the abundance and diversity of the biota. The confluences of these resources serve a source of colonists, providing spawning sites and rearing area having rich food sources. These small water sources also contribute in environmental flow in the downstream of the dams. Tributaries also provide shelters and protected areas to the local migratory species during natural calamity and high turbidity in the main river course. These resources are important for ichthyofaunal diversity and sustainability of aquatic ecosystem.

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Study on gonadal maturation of *Schizothorax labiatus* McClelland inhabiting river Jhelum, Kashmir

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ABSTRACT

The present study was conducted on *Schizothorax labiatus* McClelland fish collected randomly from river Jhelum for a period of 12 months for determination of different phases of gonadal maturation and spawning. The mean monthly GSI in the male fish fluctuated from 1.38±0.11 (August) to 6.19±0.84 (April) while as in females GSI fluctuated from 1.80±0.35 (August) to 11.12±2.09 (April). From September to April mean GSI values showed an increasing trend reaching peak in April then decreasing gradually during the following months, therefore the fish was found an annual breeder and matures in the month of April and spawns in May. Annual gonadal cycle in both the sexes consisted of six stages *viz.,* (i) Immature or resting phase (ii) Preparatory or Developing phase (iii) Maturing phase (iv) Mature phase (v) Spawning phase (vi) Spent phase. Histological studies showed follicular atresia both during pre-spawning and post-spawning period. The study provides an understanding about maturity, gonadal development, environmental influence on gonad activity and thus forms a tool for the development and proper management of *S. labiatus* McClelland fishery in Kashmir.

Keywords: Annual, Jhelum, Gonadal cycle, *Schizothorax labiatus.*

Introduction

High altitude water bodies of Kashmir valley harbors the indigenous Cyprinids like Schizothoracids (snow trouts), among other snow trouts *Schizothorax labiatus* McClelland locally known as 'Chush' is an important food fish inhabiting Jhelum River System. Most fish species inhabiting the Himalayan region are small in size and their size, growth and distribution depends on environmental conditions such as water temperature, velocity of water current, nature of substratum, availability of food and their feeding habits (Yousuf *et al.,* 2003; Bhat *et al*., 2010). The ability to reproduce successfully in a fluctuating environment, determines the success of the fish, as reproduction is a vital component in sustenance, replenishment and progeny maintenance of each and every living organism (Moyle & Czech Jr., 2000). For proper formulation of capture fisheries management policies, reproductive characteristics such as gonadal development, duration of spawning season and associated endocrinal changes which are unique to each fish species are used (Macer, 1974; Johannes, 1978 and Kathirvelu *et al*., 2003). Studies related to reproduction of many species indicates that the reproductive cycle of fishes are closely associated to the environmental changes particularly temperature, day length and food supply influencing gonadal development initiation and fecundity. Reproductive parameters like size at first maturity, fecundity, sex ratio etc. are important in formulation of management measures (Bal and Rao, 1984). Thus this study on GSI, histology of gonads in *S. labiatus* was conducted for understanding reproductive biology required for proper fishery management, since information on these aspects of gonadal maturation and spawning are scarce in this coldwater fish i.e. *S. labiatus*.

Material and Methods

A total of 310 specimens of *Schizothorax*, McClelland of all available sizes, were collected randomly from River Jhelum over a period of twelve (12) months and timely brought to Fishery Resource Management (FRM) Laboratory of Faculty of Fisheries, Rangil, Ganderbal, and fishes were categorized into different size groups.

The fish specimens (both male and female) were dissected open and their gonads were collected to record their length and weight. Various maturity stages of gonads of both the sexes were determined based on microscopic observation in preserved and macroscopic observations (morphological appearance) in fresh samples following the standards laid down by Lovern and Wood (1937).

The Gonadosomatic index (GSI) was calculated for both the sexes using the formula given by Qasim (1973).

$$GSI = \frac{\text{Weight of gonad}}{\text{Weight of fish}} \times 100$$

After GSI recordings, gonads were immediately fixed in Bouin's fluid for a period of 24 hours and later washed in 70 per cent alcohol in various grades till the yellow colour of picric acid fades away. Further, the gonads were dehydrated in ascending grades of ethanol, cleared in xylene and embedded in paraffin wax. Sections were cut and stretched on albumenized slides and fixed overnight and later deparaffinised in xylene and rehydrated in descending grades of alcohol to distilled water. Sections were stained in haematoxylin, differentiated in alcohol. After washing, sections were stained with eosin and then dehydrated and cleaned. They were then mounted in DPX (Luna, 1968) and stages and changes in gonadal development were recorded through microphotography.

Results and Discussion

Gonadosomatic Index (GSI)

Besides being useful for determination of fish reproductive period, GSI is good indicator of gonadal development (Rheman *et al.*, 2002). The mean monthly mean GSI during the present study fluctuated between 1.38±0.11 (August) and 6.19±0.84 (April) in males while as in females the values fluctuated between 1.80±0.35 (August) and 11.12±2.09 (April). The minimum and maximum values observed in case of males and females are recorded in Table 1. The mean GSI values were recorded higher in females compared to that of males (Fig. 1). During the period between September to April mean GSI values showed an increasing trend reaching peak in April then decreasing gradually during the following months and reaching a minimum value in August (Fig. 2).

Table 1: Mean (±) GSI values of *Schizothorax labiatus* McClelland

	Minimum	Maximum	Mean± SEM
Male	0.87	14.72	3.80±0.39
Female	0.83	23.12	5.18±0.14

In *S. labiatus* McClelland like most of the other teleosts the reproductive glands (testes in males and ovaries in females) are typically paired structures and their reproductive cycles exhibit various phases varying with seasons.

Ovaries

The ovaries in *S. labiatus* McClelland are similar to those of other teleosts and go through a series of stages which are identified visually by size, colour, transparent or opaque, thus the ovarian cyclicity (Table 2) in *S. labiatus* McClelland during the present study has been observed as:

1) Stage I: Immature or Resting phase (Plate 1 A)
2) Stage II: Developing or Preparatory phase (Plate 1 B & C)
3) Stage III: Maturing phase (Plate 1 D)
4) Stage IV: Mature or Pre spawning phase (Plate 2 A)
5) Stage V: Spawning phase (Plate 2 B & C)
6) Stage VI: Spent phase (Plate 2 D)

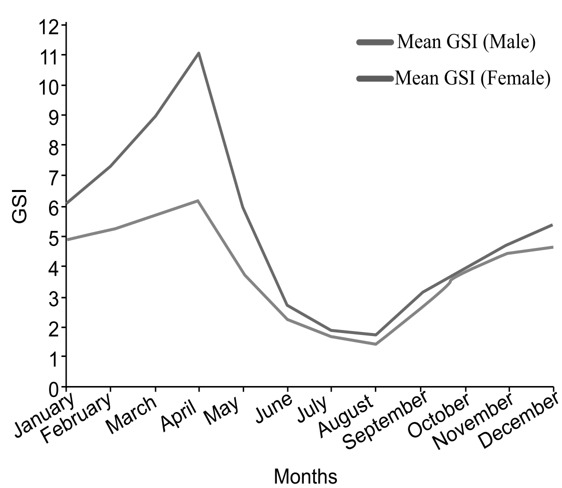

Fig. 1: Comparative analysis of monthly variation in mean GSI of male and female *S.labiatus*

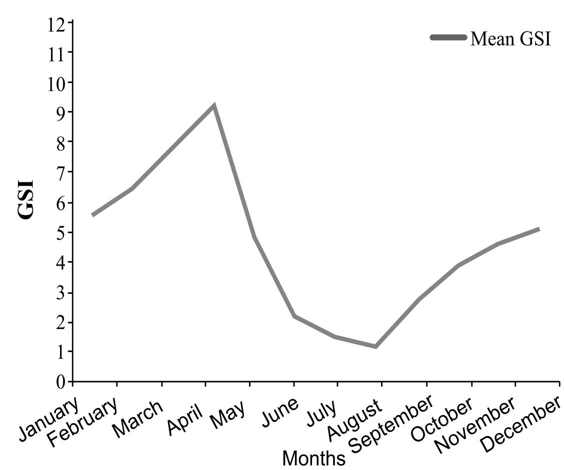

Fig. 2: Monthly variation in GSI values of *S. labiatus*

Table 2: Annual stages of maturation in ovaries of *Schizothorax labiatus*

STAGE		DURATION	GSI value	DESCRIPTION
I	Immature or Resting phase.	Sep - Oct	3.08 & 4.25	Thin, thread like and pale in color ,little vascularization ,less than 1/3 of the body cavity ova in this phase are transparent ,invisible to the naked eye.
II	Developing or Preparatory phase.	Nov - Dec	4.77 & 5.36	Marked increase in size and weight, occupying greater part of the body cavity ,opaque and light yellow in color beginning of maturation, characterized by the presence of yolk vesicle in peripheral ooplasm .
III	Maturing phase.	Jan - Feb	6.10 & 7.32	Increase in size turning yellow in color invaded with blood vessels occupying ¾th of the body cavity. Ova visible to the naked eye.
IV	Mature or Pre spawning phase	Mar - Apr	9.05 & 11.12	Increase in weight and volume, deep yellow in color, attaining maximum weight and were highly vascularized.
V	Spawning phase	May - June	5.90 & 2.61	Large, turgid, deep yellow to orange in color filling the body cavity. The oocytes are large, loose and almost separate from each other in the ovary, a slight pressure on the abdomen evacuates eggs from the belly.
VI	Spent phase.	Jul - Aug	2.01 & 1.80	Reduction in size appearing flaccid and dull in color. Unspawned ova & number of small ova were seen.

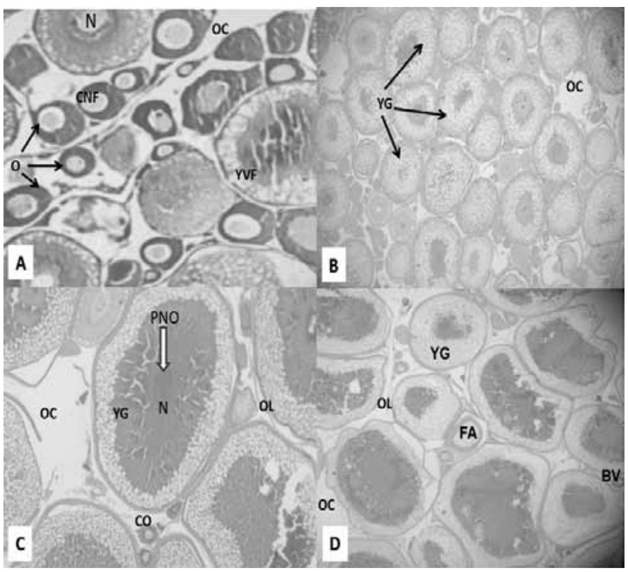

Plate 1. Photomicrographs (50X) of T.S of Ovary of *S. labiatus* showing different stages, A (Stage I), B (Stage II), C (Stage II at 100X) and D (Stage III)

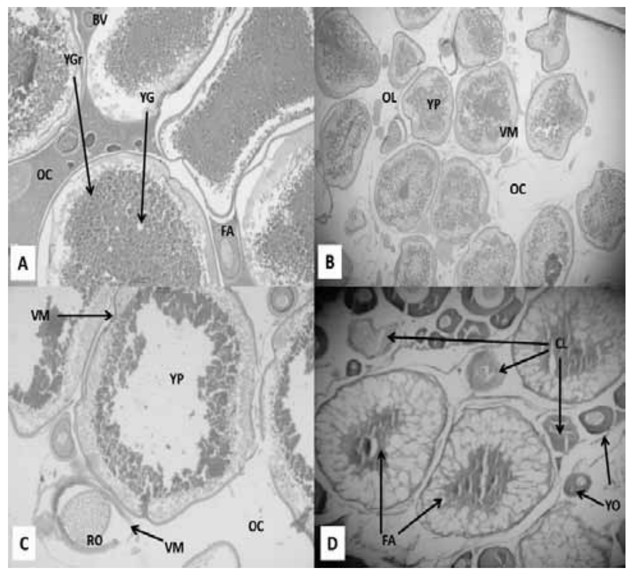

Plate 2. Photomicrographs (100X) of T.S of Ovary of *S. labiatus* showing stages, different A (Stage IV), B (Stage V; 50 X), C (Stage V) and D (Stage VI)

O = Oocyte, YG = Yolk Globules, BV = Blood vessel, FA = Follicle Atresia, CNF = Chromatin nuclear follicle,YVF = Yolk Vesicle Follicle, N = Nucleus, PNO = Perinuclear oocyte, OC = Ovarian cavity, CO = Chromatin nuclear oocyte, OL = Ovarian lamellae, YGr = Yolk Granules, CL = Corpus luteum, VM = Vitelline membrane, OL = Ovarian lamellae, YP = Yolk Plate, YO = Young oocyte, RO = Releasing oocyte.

Testes

S. labiatus McClelland exhibited the following cyclic changes in testes and various phases in annual cycle are described in Table 3.

1) Stage I: Immature or Resting phase (Plate 3 A)
2) Stage II: Developing or Preparatory phase (Plate 3 B)
3) Stage III: Maturing phase (Plate 3 C)
4) Stage IV: Mature or Pre spawning phase (Plate 3 D)
5) Stage V: Spawning phase (Plate 4 A)
6) Stage VI: Spent phase (Plate 4 B)

Table 3: Annual stages of maturation in testes of *Schizothorax labiatus*

	STAGE	DURATION	GSI value	DESCRIPTION
I	Immature or Resting phase	Sep - Oct	2.71 & 3.94	thin, thread like translucent pale in colour
II	Developing or Preparatory phase	Nov - Dec	4.41 & 4.57	The testes appear thin ribbon like transparent and occupy very small portion of the body cavity.
III	Maturing phase	Jan - Feb	4.86 & 5.16	Moderately thick and convoluted , grey-whitish extending up to ½ of the body cavity and visible primary and secondary spermatocytes.
IV	Mature or Pre spawning phase	Mar - Apr	5.65 & 6.19	increase in weight and volume ,deep yellow in color , attaining maximum weight and were highly vascularized.
V	Spawning phase	May - June	3.69 & 2.63	Moderately thick and convoluted, grey-whitish extending up to ½ of the body cavity and visible primary and secondary spermatocytes.
VI	Spent phase	Jul - Aug	1.68 & 1.38	The weight and volume of testes is reduced due to the discharge of sperms making testes look slightly flaccid and flabby.

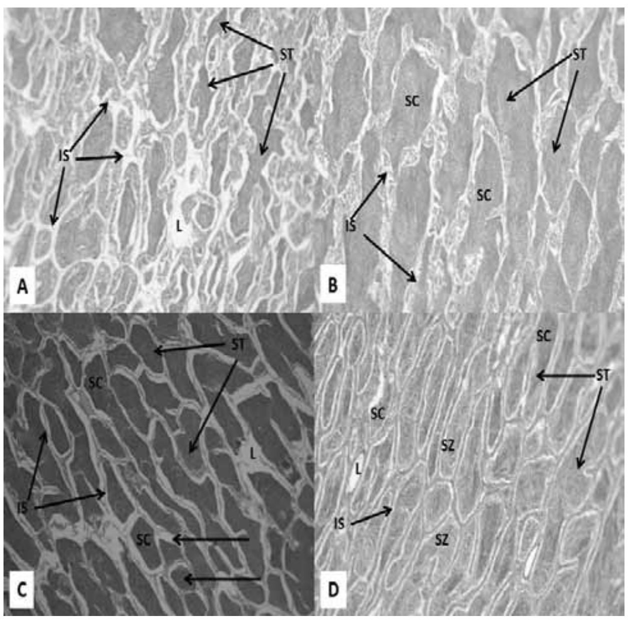

Plate 3: Photomicrographs (50X) of T.S of testes of *S. labiatus* showing different stages, A (Stage I), B (Stage II), C (Stage III) and D (Stage IV)

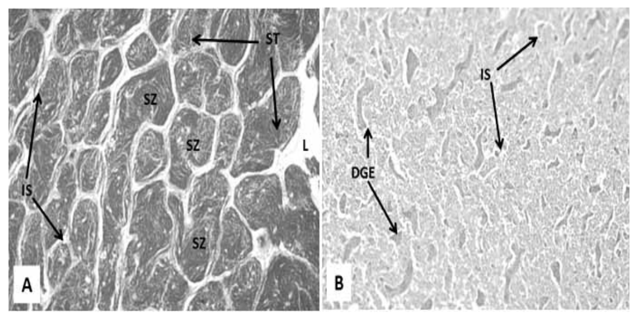

Plate 4: Photomicrographs (50X) of T.S of testes of *S. labiatus* showing, different stages A (Stage V), B (Stage VI)

IS = Interstitial space, **ST** = Seminiferous tubule, **L** = Lumen, **SC** = Spermatocytes, **SZ** = Spermatozoa **DGE** = Discontinuous germinal epithelium

In the present study highest GSI as observed during April indicates that the fish matures in this month and spawning starts in the month of May when the GSI abruptly decreases, however, higher value of GSI were reported from females as compared to males. The gonadosomatic index or maturity index is an indirect method for estimating spawning season of a species (Biswas, 1993). Shafi (2012) observed the Gonadosomatic index (GSI) of *C. carassius* and reported higher values in case of females than in males. Hussain (2014) while studying breeding biology and fecundity of *S. niger* from Dal lake revealed the GSI highest during March i.e peak maturity period of the fish, then it decreased gradually upto June attaining its lowest value in July. Snowtrout, *S. niger* exhibits spawning from mid April to May end (Malhotra, 1966). Shafi (2013 a) while studying breeding biology of *Schizothorax niger* from Dal lake revealed that the GSI recorded was highest during February. The gonadosomatic index increase as maturation progresses and reaches a peak at maturity and decrease abruptly when the fish becomes spent. Generally females exhibit comparatively higher GSI values than males (Khan, 1945; Ganpatti and Chako, 1954; Pathak and Jhingran, 1977; Piska and Devi, 1993). Similar observations were recorded during the current study in *S. labiatus* as well which showed the maximum GSI in the month of April (6.19 in males and 11.12 in females) and minimum GSI in the month of August (1.3 for males and 1.8 for females).

The reproductive glands (testes of males and ovaries of females) of *S. labiatus* McClelland are typically paired structures. Many studies have been performed on histological and morphological changes of ovary in fishes (Biswas, 1993). Cyclic changes in development are seen in mature gonads (Fouche *et al.,* 1985). *S. labiatus* McClelland clearly exhibited cyclic changes in the gonads and various phases in annual cycle. The testes of *S. labiatus* McClelland were thin, thread/ribbon like translucent in immature and preparatory, opaque in maturing phase, grey whitish in mature phase and creamish in spawning phase when they reach to their maximum development and look slightly flaccid and flabby because of the discharge of sperms during spent phase. In different species, the spermatogenic activity starts at different times of the year (Rai, 1965; Nair, 1966; Shrestha and Khanna, 1976; Nautiyal, 1983). Such variations are probably due to the local physico - chemical and environmental factors (Ahsan, 1966). The ovaries in *S. labiatus* McClelland were thin, thread like pale to light yellow in colour in immature and preparatory phase, yellow in maturing phase, deep yellow in mature phase and deep yellow to orange in spawning phase, flaccid and dull in spent phase. In *S. labiatus* McClelland, atretic oocytes were seen both during the pre spawning and during the post-spawning period. Small atretic previttogenic oocytes are relatively of rare occurrence. Atresia is accompanied by the shrinkage of follicles, resulting in disorganization of nuclear and ooplasmic component. The occurrence of large cortical alveoli is the most characteristic feature of the teleost egg (Donato *et al.*, 1980; Takahashi, 1981), dog fishes and other cartilaginous fishes (Guraya, 1982). Various investigators have termed the cortical alveoli as cortical vacuoles, intra vacuolar yolk, intravesicular yolk, carbohydrate yolk, yolk vesicle etc. In teleosts atretic oocytes may be present throughout the year (Braeckevelt and McMillan, 1967) or only during the post-spawning period (Rajalakshmi, 1966). Several factors have been found responsible for follicular atresia, mostly hormonal of intra-ovarian and extra-ovarian sources (Saidapur, 1978) and unfavourable environmental conditions i.e. photoperiod (Saxena and Anand, 1977), overcrowding, temperature and inadequate feed supply (Lam, 1983). In addition, increasing water pollution by insecticides, pesticides and industrial effluents have also been reported to affect the metabolism of fish ovary resulting in the increased incidence of follicular atresia (Saxena and Bhatia, 1983; Mani and Saxena, 1985). All such factors seem to be responsible for higher follicular atresia in *S. labiatus* McClelland which leads to less spawning/or release of mature ovum from the ovary, which ultimately means less recruitment of fishes. If the aquatic environments of Kashmir valley are not managed properly, it is certain that with the every passing year the new spawn of the fish is going to decrease and the production of fish from natural waters will also decrease.

The fish spawned during the short period of time with peaks during April and May which reveales that the fish is an annual breeder. The gonads of both the sexes of *S. labiatus* McClelland were found to pass through the six phases in the annual cycle. Atretic oocytes were seen during the pre-spawning and post-spawning period of the fish in good numbers. *S. labiatus* McClelland still exists in good conditions and good numbers in river Jehlum. The less number of females as compared to males and the more number of follicular atresia suggest that all is not well with *S. labiatus* in Jehlum River System but if proper fishery management steps are taken at an earliest, the *S. labiatus* fishery can be enhanced for fish production.

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Diversity, distribution and conservational approach of hillstream ornamental fishes in Manas river, India: An eastern hotspot region

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ABSTRACT

Assessment on ornamental fish diversity and its distribution was made in the snow-fed origin Manas River of Eastern Hotspot region, India. Fish data and water quality parameters were collected between August, 2016 and July, 2018. A total of 105 species belonging to 71 genera and 33 families were recorded from the entire length of river. The calculated value of Shannon-Wiener diversity index showed diversification in the distribution of fish species with overall index value of 3.86. However, the distributional pattern of the fish species indicate significant difference from upstream to downstream with 52.50 % species distributed uniformly throughout the course of the river. The study also reports that 12.50 % of the collected fish species to be vulnerable and threatened according to IUCN, 2015 status. Jacquard Index has been employed to evaluate the similarities and dissimilarities among the collected fish fauna from different sampling sites. Maximum diversity variance (470.25) was observed in the retreating monsoon season. For conservation and management strategies, measures like halting of siltation, regulation on open cast sand mining, scientific fishing, control of water pollution etc., should be taken into consideration.

Keywords: Fish diversity, Diversity Indices, River Manas, India

Introduction

The North Eastern (NE) region is considered as global hotspots of freshwater fish biodiversity in the world (Kottelat and Whitten 1996). Due to its unique topographical and hydro biological conditions is a paradise for a variety of ornamental fish species. The River Manas, which forms one of the most important snows fed originated river of Assam has a total length of 376 kilometers (flows about 272 kilometers in Bhutan and then 104 kilometers through Assam) before it joins the mighty Brahmaputra River at Jogighopa (90°34'18"E and 26°11'57.4"N). In Assam, the river is bifurcated at Mothanguri (90°57'41.9"E and 26°46'90.2"N) to give rise a tributary named Beki which passes through the Manas National Park (26°35' to 26°50'N and 90°45' to 91°15'E. Boulders, cobbles, pebbles, gravels and sands are the major characteristic feature of the River Manas at its upstream providing suitable habitat for spawning and rearing of coldwater fishes. On the contrary, the downstream is bedded with mud and sand providing habitat for plain dwellers fishes.

Ornamental fishes are attractive colorful fishes having a wide diversity of colors and color patterns with peculiar mode of movement and food intake. Now a days, selection of fishes not only depends on the coloration of fish but also taking into consideration certain other remarkable morphological characteristics such as bizarre shape, specific spots or marks, fin structure, prolonged tail etc., (Das and Biswas, 2009). The valuation in commercial terms has been increasing tremendously with the passage of time. The entire supply of Indian ornamental fish is primarily dependent on wild catch (85%) and few on artificially bred varieties of exotic fishes (Mahapatra *et al*., 2006). Due to this continuous exploitation of the wild stock of fishes, many ornamental fishes have become highly endangered.

Prior to the present study, few works had been carried out on the population and distribution of ornamental fish fauna of the Manas River system (Dey and Sarma, 1967; Dubey, 1978; Basistha, 2006). Mahapatra *et al* (2004) has recorded 187 species of ornamental fish from Assam. Das and Biswas (2009) recorded 62 ornamental fish species in the floodplain wetlands of upper Brahmaputra basin. Sarma and Dutta (2000) had conducted studies on the conservation aspect of two coldwater fish species, *Schizothorax molesworthii* and *Schizothorax richardsonii* of Beki and Manas River. However, nothing has been reported on the diversity and distribution of ornamental fish species from the river system with respect to its habitat ecology and present status. Therefore, in the present investigation an effort has been made towards that end throughout the length of the river flowing within the state.

Materials and Methods

Study Area

A total of 104 km of the river from Mothanguri (26°46'90.2" N and 90°57'41.9" E) to the lower stretch (26°11'57.4"N and 90°34'18"E) has been taken into consideration and were shown diagrammatically in Fig 1. Six study sites were selected along the entire length of River Manas viz., Mothanguri (S_1) (90°57'41.9" E and 26°46'90.2"N) with elevation 287 MSL; Narayanguri (S_2) (90°55'16.7" E and 26°29'71.1" N) with elevation 186 MSL; Bekipar (S_3) (90°43'15.9" E and 26°21'24.7" E) with elevation 134 MSL; Mora Manas (S_4) (90°44'15.3" E and 26°22'26.3" N) with elevation 118 MSL; Kalgachia (S_5) (90°45'17.3" E and 26°23' 31" N) with elevation 98 MSL and Jogighopa (S_6) (90°34'18" E and 26°11'57.4" N) with elevation 44 MSL. The study was carried out during August, 2012 to July, 2014. The distance from one sampling site to another was about 35-40 km and the locations of sampling sites were documented using global positioning system.

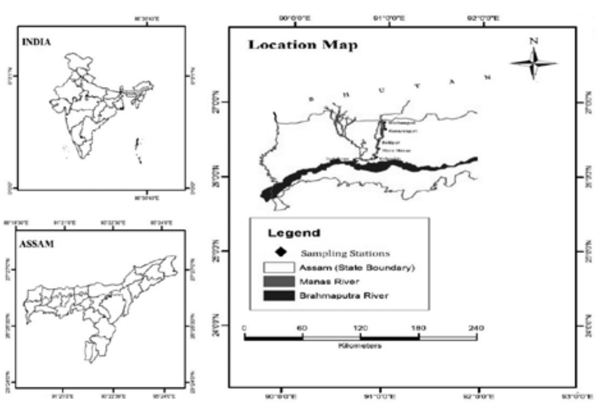

Fig. 1: Map showing the sampling sites of Manas River, India (Mothanguri, Narayanguri, Bekipar, Kalgachia and Kalgachia). Map scale 1: 50000.

Physico-chemical parameter analysis

For physical and chemical parameters analysis, water samples were collected from all the selected sites twice in a season. In NE India, seasons were classified by Barthakur (1986) as pre-monsoon (March-May), monsoon (June-August), retreating monsoon (September- November) and winter (December to February). Parameters like Dissolved Oxygen [DO (mg l^{-1})], Free Carbon dioxide [FCO_2 (mg l^{-1})], Hardness as $CaCo_3$ (mg l^{-1}) Alkalinity as $CaCO_3$ (mg l^{-1}) and Chloride (mg l^{-1}) were estimated titrimatically adopting the method of APHA (2005). Water Temperature (°C) was measured by Mercury Thermometer, Turbidity (NTU), Conductivity, Total Dissolve Solid [TDS (ppm)] and pH were measured by Nephelometer (Systronics), Conductivity meter (Systronics), TDS meter (Systronics) and pH meter (Systronics) respectively.

Fish collection and identification

Fishes were collected from landing centre as well as directly by visiting the area where maximum fishing practices were carried out. In each sampling site, experimental fishing were also carried out with the help of local fishers through cast net (90′, 1″; 9′, 1/2″) and gill net (75x1.3 m, 2″). The collected fish specimens were identified following Jhingran (1991); Talwar and Jhingran (1991); Jayaram (1999) and Vishwanath *et al.* (2014). Identification of fishes was also validated with the help of online facilities viz., www.calacademy.org/research/ichthyology/catalog of fishes. The river microhabitat has been classified followed after Armantrout (1999); Arunachalam (1999); Johal *et al.* (2002); Manoj kumar and Kurup (2002) to determine its relation with fish species richness.

Statistical analysis

All the statistical analysis for species diversity, abundance and fish distribution in the river system was made using the computer programs, MS Excel and MEGA software. Rarefaction curves, species distribution plot and abundance plot (rank) distribution for the collected fish species has been prepared using Bio Diversity Pro software. The relative abundance (percentage of catch) of fish species across these six different sampling stations was carried out by the following formula.

Number of samples of particular fish species x 100 / Total number of samples

The fish diversity indices were calculated as per the standard method (Shannon-Weiner 1963): **H' = - Σ *pi* log (*pi*)**, where **H'** = The Shannon-Weiner Diversity Index and ***pi*** = the relative abundance of each group of organisms. Similarity of the species was calculated using Jacquard's index (JI): **Sj =j/(x+y-j)**, where Sj is the similarity between any two sites X and Y, j the number of species common to both the sites X and Y, x the total number of species in site X and y total number of species in site Y.

Results and Discussion

Physico-chemical parameters

Seasonal fluctuations of the physico-chemical parameter of each site are shown in Table 1. Parameters like pH, DO, FCO_2, Hardness, Alkalinity, Chloride and Turbidity were found fluctuating from one sampling site to another throughout the period of investigation. The pH value was estimated neutral at S_1 and S_2 (winter season), whereas in all other sites it was slightly alkaline and its values ranged from 6.55 to 8.6. DO values ranged from 7.55 mg l^{-1} to 11.3 mg l^{-1}, however, at S_5 the levels of DO was

Table 1. Mean physico-chemical variables of six sampling sites (S_1, S_2, S_3, S_4, S_5 and S_6) of Manas River (2016-2018), NE India (n=4).

Sl. No.	Variables	S_1	S_2	S_3	S_4	S_5	S_6
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
1	Water Temp. (°C)	20.75 ± 2.99	22.0 ± 3.37	21.5 ± 3.32	22.0 ± 3.16	22.75 ± 2.87	23.25 ± 3.77
2	pH	7.76 ± 0.84	7.55 ± 0.65	7.78 ± 0.35	7.86 ± 0.27	8.0 ± 0.42	8.05 ± 0.41
3	FCO_2 (mg l^{-1})	6.6 ± 1.80	4.4 ± 2.11	4.4 ± 1.65	8.8 ± 3.11	4.4 ± 1.27	6.6 ± 2.77
4	DO (mg l^{-1})	8.77 ± 0.58	9.2 ± 0.57	8.4 ± 0.41	9.4 ± 0.96	9.1 ± 1.60	9.5 ± 0.26
5	Hardness (mg l^{-1})	86.25 ± 13.48	78.75 ± 9.64	105.5 ± 54.04	128.25 ± 54.84	152.75 ± 84.31	169.5 ± 70.74
6	Alkalinity (mg l^{-1})	140 ± 50.66	158.75 ± 47.68	140 ± 29.72	171.25 ± 31.98	177.5 ± 47.35	186.25 ± 40.29
7	Chloride (mg l^{-1})	15.64 ± 3.23	14.42 ± 4.20	15.27 ± 4.63	15.4 ± 3.06	20.15 ± 2.14	21.65 ± 2.32
8	Turbidity (NTU)	20.92 ± 10.84	20.39 ± 11.03	22.59 ± 12.20	30.61 ± 14.66	22.45 ± 14.93	26.73 ± 12.69
9	TDS (ppm)	38.98 ± 20.59	45.38 ± 17.20	41.4 ± 17.83	49.43 ± 15.56	62.63 ± 10.94	83.73 ± 8.45
10	Conductivity (mhos cm^{-1})	46.25 ± 18.53	44.1 ± 13.62	46.08 ± 18.53	48.8 ± 13.64	49.6 ± 14.78	50.6 ± 12.86

observed both minimum and maximum in pre-monsoon and monsoon season. The FCO_2 level was observed minimum at S_3 (winter) and maximum at S_6 (monsoon) and its value ranged between 3.3 mg l^{-1} and 8.8 mg l^{-1} in all the sampling sites. The estimated values of alkalinity ranged from 105 mg l^{-1} to 235 mg l^{-1} throughout the study period with highest at S_5 (winter) and lowest at S_1 (monsoon). Similarly, the values of hardness and chloride in the entire sampling site ranged from 61 mg l^{-1} to 272 mg l^{-1} and 10.11 mg l^{-1} to 24.52 mg l^{-1} respectively. Turbidity was observed moderate in all the study sites during the period of investigation. The level of TDS was ranged from 24.2 ppm to 92.6 ppm, whereas the conductivity values ranged between 32.7 mhos cm^{-1} and 73.7 mhos cm^{-1}.

The relationship (Pearson correlation coefficient) between the hydrological parameters of water sample was analyzed statistically which are presented in Table 2. Correlation values were considered significant at P≥0.5 and both positive and negative correlation-ship among the obtained values of physico-chemical variables had been observed. Water Temperature, pH, FCO_2, DO and hardness were positively correlated with each other whereas, negative correlation was observed between water temperature and conductivity; water temperature and turbidity; pH and alkalinity; DO and alkalinity; DO and turbidity; alkalinity and turbidity respectively.

Table 2. Correlation coefficient matrix between different physico-chemical parameters of Manas River, India

Parameters	Water Temp.	pH	FCO_2	DO	Hardness	Alkalinity	Chloride	Cond.	TDS	Salinity	Turbidity
Water Temp.	**1**	0.014	0.360	0.164	0.265	0.312	0.175	-0.500	-0.495	0.131	-0.043
pH		**1**	0.406	0.128	0.088	-0.203	-0.391	0.256	0.163	-0.655	0.724
FCO_2			1	0.427	0.375	0.101	0.093	0.076	0.043	-0.251	0.291
DO				**1**	0.203	-0.054	0.248	0.194	0.177	-0.042	-0.041
Hardness					**1**	0.690	0.456	-0.063	-0.069	0.136	0.080
Alkalinity						**1**	0.434	-0.369	-0.373	0.098	-0.282
Chloride							**1**	-0.062	-0.034	0.371	-0.283
Cond.								**1**	0.992	-0.295	0.294
TDS									**1**	-0.190	0.220
Salinity										**1**	-0.401
Turbidity											1

Species diversity, abundance and distribution

During the period of investigation, a total of 105 species under 71 genera and 33 families were recorded (Table 3), among which, 36.54 % species belong to the inhabitants' of coldwater origin, 63.46 % species of plain water origin and 52.50 % species having uniform distribution in both upstream and downstream of the river. From the present investigation, it has been recorded that about 1.92 % of fish species is in endangered category; 3.85 % in vulnerable category; 6.73 % in near threatened category; 77.88 % least concerned, 7.69 % not assessed and about 1.92 % are data deficient species according to IUCN, 2018 (ver. 3.1).

Table 3. Fish faunal diversity of river Manas with IUCN status, 2018 (ver. 3.1)

Sl. No.	Scientific Names	Family	IUCN Status	Relative Abundance (R.A.) in %					
				S_1	S_2	S_3	S_4	S_5	S_6
1	*Acantopsis multistigmatus*	Cobitidae	NT	0.05	0.00	0.05	0.00	0.00	0.00
2	*Ailia coila*	Schilbeidae	NT	0.00	0.00	0.36	0.05	0.00	0.10
3	*Amblypharyngodon mola*	Cyprinidae	LC	0.00	0.05	5.76	0.67	0.57	1.60
4	*Anabas testudineus*	Anabantidae	DD	0.05	0.00	0.10	0.36	0.31	0.36
5	*Aplocheilus panchax*	Aplocheilidae	LC	0.05	0.00	0.00	0.00	0.05	0.00
6	*Badis badis*	Badidae	LC	0.00	0.00	0.36	0.00	0.46	0.21
7	*Balitora edsii*	Balitoridae	LC	0.00	0.10	0.00	0.00	0.00	0.00
8	*Barilius bendelisis*	Cyprinidae	LC	0.00	0.21	0.51	0.00	0.00	0.00
9	*Barilius shacra*	Cyprinidae	LC	0.05	0.05	2.16	0.00	0.00	0.00
10	*Barilius vagra*	Cyprinidae	LC	0.00	0.00	0.05	0.00	0.00	0.00
11	*Batasio tengana*	Bagridae	LC	0.00	0.00	1.29	0.15	0.00	0.05
12	*Botia almorhae*	Cobitidae	LC	0.00	0.00	0.05	0.00	0.00	0.00
13	*Botia dario*	Cobitidae	LC	0.00	0.00	0.00	0.10	0.00	0.46
14	*Botia histrionica*	Cobitidae	LC	0.05	0.05	0.05	0.00	0.05	0.00
15	*Cabdio morar*	Cyprinidae	LC	0.26	0.31	3.19	0.77	0.31	0.46
16	*Cantophrys gongota*	Cobitidae	NA	0.00	0.00	0.10	0.00	0.05	0.00
17	*Chaca chaca*	Chacidae	LC	0.05	0.00	0.05	0.00	0.05	0.00
18	*Chagunius nicholsi*	Cyprinidae	LC	0.10	0.00	0.05	0.10	0.05	0.00
19	*Chanda nama*	Ambassidae	LC	0.00	0.26	0.77	0.31	0.77	0.41
20	*Channa gachua*	Channidae	LC	0.00	0.10	0.05	0.36	0.31	0.36
21	*Channa punctata*	Channidae	LC	0.00	0.00	0.41	0.10	0.26	0.36
22	*Channa stewartti*	Channidae	LC	0.05	0.05	0.00	0.00	0.10	0.00
23	*Channa striata*	Channidae	LC	0.00	0.00	0.21	0.00	0.10	0.00
25	*Cirrhinus reba*	Cyprinidae	LC	0.05	0.00	0.21	0.05	0.00	0.26
26	*Clarias magur*	Clariidae	EN	0.00	0.00	0.00	0.00	0.21	0.00
27	*Ctenopharyngodon idella*	Cyprinidae	NA	0.00	0.00	0.05	0.00	0.00	0.00
28	*Cyprinus carpio*	Cyprinidae	VU	0.00	0.05	0.00	0.00	0.00	0.00
29	*Danio rerio*	Cyprinidae	LC	0.05	0.00	0.31	0.00	0.05	0.00
30	*Devario acuticephala*	Cyprinidae	VU	0.10	0.00	1.80	0.00	0.00	2.06
31	*Devario aequipinnatus*	Cyprinidae	LC	0.00	0.00	0.00	0.00	0.00	0.05
32	*Devario assamensis*	Cyprinidae	VU	0.00	0.00	0.82	0.00	0.15	0.00
33	*Devario devario*	Cyprinidae	LC	0.00	0.00	0.10	0.00	0.00	0.10
34	*Esomus danrica*	Cyprinidae	LC	0.10	0.00	2.47	0.05	1.44	4.37
35	*Eutropichthys murius*	Schilbeidae	LC	0.00	0.00	0.05	0.05	0.00	0.00
36	*Eutropichthys vacha*	Schilbeidae	LC	0.05	0.00	0.05	0.00	0.00	0.00
37	*Gagata cenia*	Sisoridae	LC	0.00	0.05	2.57	0.05	0.00	0.00
38	*Garra gotyla*	Cyprinidae	LC	0.10	0.00	0.21	0.00	0.00	0.00
39	*Garra kempi*	Cyprinidae	LC	0.00	0.00	0.15	0.00	0.00	0.00
40	*Garra lamta*	Cyprinidae	LC	0.10	0.10	0.00	0.00	0.00	0.00
41	*Glossogobius giuris*	Gobiidae	LC	0.00	0.10	0.62	0.00	0.05	0.15
42	*Glossogobius gutum*	Gobiidae	NA	0.00	0.00	0.41	0.00	0.15	0.00
43	*Glyptothorax botius*	Sisoridae	LC	0.00	0.00	0.10	0.00	0.00	0.00

Sl. No.	Scientific Names	Family	IUCN Status	Relative Abundance (R.A.) in %					
				S_1	S_2	S_3	S_4	S_5	S_6
44	*Glyptothorax cavia*	Sisoridae	LC	0.10	0.00	0.21	0.00	0.00	0.05
45	*Glyptothorax indicus*	Sisoridae	LC	0.10	0.15	0.21	0.00	0.00	0.00
46	*Glyptothorax telchitta*	Sisoridae	LC	0.05	0.05	0.36	0.00	0.00	0.00
47	*Tariqilabeo latius*	Cyprinidae	LC	0.00	0.26	0.67	0.05	0.05	0.31
48	*Gudusia chapra*	Clupeidae	LC	0.00	0.00	0.00	0.05	0.00	0.00
49	*Hara hara*	Erethistidae	LC	0.05	0.05	0.15	0.00	0.00	0.00
50	*Heteropneustes fossilis*	Heteropneustidae	LC	0.05	0.00	0.00	0.00	0.36	0.15
51	*Hypostomus plecostomus*	Loricariidae	NA	0.00	0.00	0.00	0.00	0.10	0.00
52	*Johnius coitor*	Sciaenidae	LC	0.00	0.00	0.00	0.00	0.00	0.05
53	*Labeo bata*	Cyprinidae	LC	0.00	0.00	0.05	0.00	0.00	0.05
54	*Labeo calbasu*	Cyprinidae	LC	0.00	0.00	0.00	0.00	0.10	0.31
55	*Labeo catla*	Cyprinidae	LC	0.00	0.00	0.00	0.10	0.10	0.10
56	*Labeo dyocheilus*	Cyprinidae	LC	0.05	0.05	0.00	0.00	0.00	0.00
57	*Laubuca laubuca*	Cyprinidae	LC	0.00	0.00	0.05	0.05	0.00	0.05
58	*Leiodon cutcutia*	Tetraodontidae	LC	0.05	0.00	0.21	0.15	0.00	0.15
59	*Lepidocephalichthys goalparensis*	Cobitidae	LC	0.00	0.00	0.21	0.05	0.62	0.00
60	*Lepidocephalichthys guntea*	Cobitidae	LC	0.10	0.05	0.62	0.10	0.72	0.15
61	*Macrognathus aral*	Mastacembelidae	LC	0.05	0.00	0.21	0.00	0.41	0.00
62	*Macrognathus pancalus*	Mastacembelidae	LC	0.10	0.00	0.51	0.05	0.51	0.41
63	*Mastacembelus armatus*	Mastacembelidae	LC	0.00	0.00	0.31	0.00	0.05	0.05
64	*Monopterus cuchia*	Synbranchidae	LC	0.00	0.00	0.00	0.00	0.10	0.00
65	*Mystus cavasius*	Bagridae	LC	0.00	0.00	0.31	0.21	0.05	0.31
66	*Mystus sp.*	Bagridae	LC	0.00	0.00	0.05	0.00	0.00	0.00
67	*Mystus tengara*	Bagridae	LC	0.10	0.00	0.77	0.31	1.44	0.21
68	*Mystus vittatus*	Bagridae	LC	0.10	0.05	0.05	0.26	0.26	0.21
69	*Nandus nandus*	Nandidae	LC	0.00	0.00	0.41	0.00	0.10	0.15
70	*Neolissochilus hexagonolepis*	Cyprinidae	LC	0.00	0.00	0.00	0.00	0.05	0.00
71	*Notopterus notopterus*	Notopteridae	LC	0.00	0.00	0.00	0.00	0.21	0.00
72	*Olyra kempi*	Olyridae	LC	0.00	0.21	0.05	0.00	0.00	0.00
73	*Ompok pabda*	Siluridae	NT	0.10	0.00	0.00	0.00	0.00	0.05
74	*Opsarius barna*	Cyprinidae	LC	0.05	0.15	0.00	0.10	0.05	0.00
75	*Opsarius tileo*	Cyprinidae	LC	0.00	0.15	0.00	0.21	0.10	0.00
76	*Oreochromis mossambicus*	Cichlidae	NT	0.10	0.05	0.00	0.00	0.05	2.57
77	*Osteobrama cotio*	Cyprinidae	LC	0.00	0.00	0.10	0.05	0.00	0.05
78	*Pachypterus atherinoides*	Schilbeidae	LC	0.10	0.00	0.67	0.00	0.00	0.41
79	*Paracanthocobitis botia*	Balitoridae	LC	0.05	0.15	0.93	0.00	0.05	0.10
80	*Parambassis lala*	Ambassidae	NT	0.00	0.00	0.31	0.00	0.21	0.62
81	*Parambassis ranga*	Ambassidae	LC	0.00	0.00	1.96	0.10	0.51	0.67
82	*Pethia conchonius*	Cyprinidae	LC	0.10	0.10	0.31	0.00	0.00	0.05
83	*Pethia ticto*	Cyprinidae	LC	0.05	0.05	0.15	0.10	0.15	0.26
84	*Pseudolaguvia foveolata*	Erethistidae	DD	0.05	0.10	0.00	0.10	0.00	0.00
85	*Psilorhynchus sacatio*	Psilorhynchidae	LC	0.00	0.10	0.05	0.05	0.00	0.00
86	*Puntius chola*	Cyprinidae	LC	0.10	0.21	0.57	0.67	0.93	0.87
87	*Puntius sophore*	Cyprinidae	LC	0.10	0.26	0.77	0.31	0.72	3.86
88	*Puntius terio*	Cyprinidae	LC	0.10	0.10	1.13	0.21	0.46	0.26
89	*Pygocentrus nattereri*	Serrasalmidae	NA	0.00	0.00	0.00	0.00	0.05	0.00
90	*Raiamas bola*	Cyprinidae	LC	0.00	0.00	0.21	0.00	0.15	0.10
91	*Rhinomugil corsula*	Mugilidae	LC	0.00	0.00	0.36	0.00	0.00	0.00
92	*Salmostoma bacaila*	Cyprinidae	LC	0.00	0.00	0.15	0.00	0.00	0.10
93	*Salmophasia phulo*	Cyprinidae	LC	0.00	0.00	1.90	0.10	0.98	0.57

Sl. No.	Scientific Names	Family	IUCN Status	Relative Abundance (R.A.) in %					
				S_1	S_2	S_3	S_4	S_5	S_6
94	*Schistura fasciata*	Balitoridae	NA	0.05	0.05	0.10	0.05	0.00	0.00
95	*Schizothorax labiatus*	Cyprinidae	NA	0.05	0.10	0.21	0.00	0.00	0.00
96	*Schizothorax richardsonii*	Cyprinidae	VU	0.05	0.15	0.05	0.15	0.00	0.00
97	*Semiplotus semiplotus*	Cyprinidae	NA	0.10	0.05	0.10	0.00	0.05	0.00
98	*Setipnna phasa*	Engraulidae	LC	0.00	0.00	0.10	0.00	0.05	0.15
99	*Systomus sarana*	Cyprinidae	LC	0.00	0.05	0.00	0.00	0.00	0.00
100	*Tor putitora*	Cyprinidae	EN	0.00	0.05	0.00	0.00	0.00	0.00
101	*Tor sp.*	Cyprinidae	NT	0.00	0.10	0.15	0.00	0.00	0.00
102	*Tor tor*	Cyprinidae	NT	0.00	0.21	0.05	0.00	0.00	0.05
103	*Trichogaster fasciata*	Osphronemidae	LC	0.15	0.21	0.93	0.21	0.41	0.93
104	*Trichogaster labiosus*	Osphronemidae	LC	0.00	0.00	0.00	0.00	0.26	0.00
105	*Xenentodon cancila*	Belonidae	LC	0.05	0.00	0.31	0.00	0.21	0.15

Abbreviation: R.A., Relative Abundance; EN, Endangered; VU, Vulnerable; LC, Least Concern; NT, Near Threatened; NA, Not Accessed and DD, Data Deficient.

Among the plain water fish species, the relative abundance of *Amblypharyngodon mola* of Cyprinidae family was relatively higher (8.65 %), followed by *Esomus danrica* and *Puntius terio* (both from Cyprinidae family) with 8.44 % and 6.02 % respectively. Similarly, the relative abundance of hills-stream fish species viz., *Barilius shacra* (Cyprinidae) was observed relatively higher (2.26 %), followed by *Lepidocephalichthys guntea* (Cobitidae) and *Tariqilabeo latius* (Cyprinidae) with 1.75 % and 1.34 % respectively. The study records three exotic fish species: *Ctenopharyngodon idella*, *Cyprinus carpio* and *Oreochromis mossambicus* with relative abundance of 0.05 %, 0.05 % and 2.82 % respectively. It is worth mentioning that *Pygocentrus nattererii*, which is a ornamental marine fish species, had been captured from S_5 site with only single species.

Highest recorded species were from Cyprinidae family contributing 43 (41.35 %) species followed by Cobitidae with 7 (6.73 %) species, Bagridae and Sisoridae family [each with 5 (4.81 %) species] respectively (Fig 2). The members of Cyprinidae family were found distributed uniformly throughout the entire stretches of the river. The Shannon–Weiner diversity index of the collected fish species showed diversification in the distribution of fish species with overall index value of 3.86. The highest fish diversity was recorded during retreating-monsoon season, whereas lowest diversity was recorded during monsoon season. Maximum fish diversity was recorded in S_3 and S_4 followed S_5 & S_5, whereas minimum fish diversity was recorded in the upper stretches i.e., S_1 and $S_{2.}$

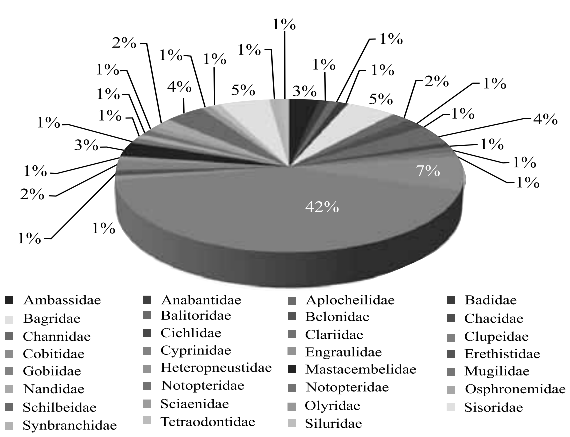

Fig. 2: Family wise percentage of collected fish species of Manas river, India

The rarefaction curve (Fig. 3) indicates the maximum species richness in retreating monsoon followed by pre-monsoon, winter and monsoon season. Fig. 4 reveals the wide variation of species in all the seasons. It has also reflects the fish biodiversity richness of the river. The abundance plot of the samples has reflected the same result of biodiversity richness in the river (Fig. 5). The JI values between site S_4 and S_6 were calculated highest while it

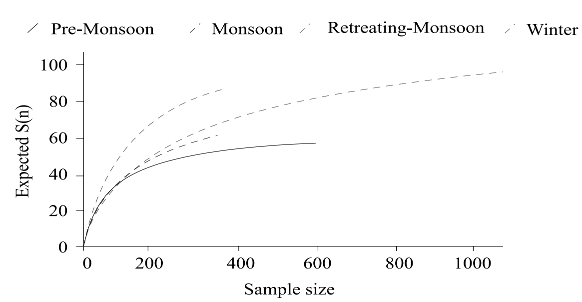

Fig. 3: Rarefaction curves for each season, where X axis shows individual actually sampled and Y axis shows the estimation of the expected number of species in the Manas River, India

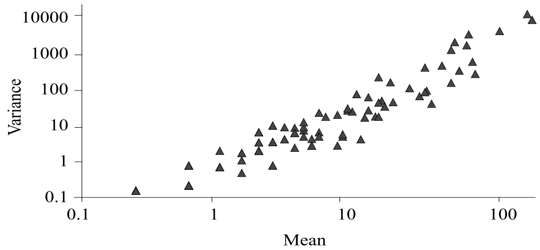

Fig. 4: Species distribution plot for whole fish community sampled in the Manas River, India

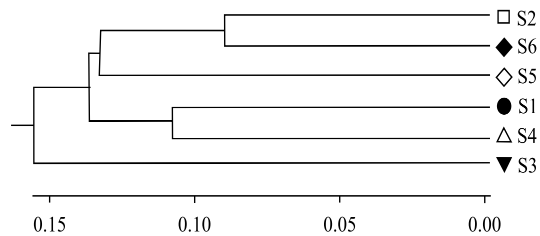

Fig. 6: Dendogram showing similarity in species composition across six sampling stations of Manas River, India

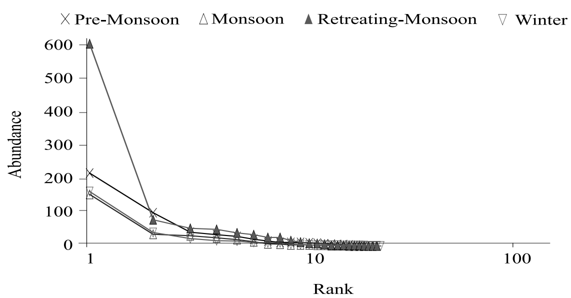

Fig. 5: Abundance plot (Rank) of fish diversity sampled in four seasons in Manas River, India

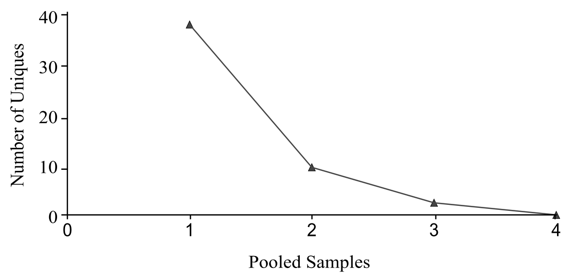

Fig. 7: Species richness in each season with number of unique fish species sampled from Manas River, India

was calculated lowest in site S_3 and S_6. The similarity in fish species composition across the River has been shown as a dendogram (Fig. 6); obtain from the JI coefficients of similarity using the average linkage method. The number of unique species in each season for pooled data is shown in the Fig. 7.

Details of fish diversity of the Manas River are shown in Table 4. In the retreating monsoon season, maximum diversity variance was found as 470.25 with SD ± 21.69 for 84 species with 942 individuals. Lowest variance (16.36) was calculated in the winter season with SD ± 4.05 with 319 individuals with 76 species. The fish species richness between different sites showed significant difference. Maximum richness was recorded at S_3 followed by S_5, S_6, S_4, S_2 and S_1 respectively. Statistical correlations between collected fish species of the river with selected physico-chemical parameters (pH, dissolved oxygen, alkalinity and turbidity) indicate positive correlation on the total number of fish species throughout the year (Table 5).

Table 4. Description of fish diversity observed in the Manas river during each season

Season	Mean Individuals	Variance	SD	SE	Total Individuals	Total Species	Max.	Mean CI
Pre-Monsoon	4.84	99.42	± 9.97	0.96	523	50	61	18.75
Monsoon	2.85	40.43	± 6.36	0.61	308	54	37	7.62
Retreating-Monsoon	8.72	470.26	± 21.69	2.09	942	84	138	88.69
Winter	2.95	16.36	± 4.05	0.39	319	76	20	3.09

Note: SD - Standard deviation; SE - Standard Error; Max. - Maximum; Mean CI - Mean confidence interval

Table 5. Correlation between fish diversity and few important physico-chemical attributes of Manas river

Parameters	Seasons			
	Winter	Pre-Monsoon	Monsoon	Retreating-Monsoon
pH	0.61	0.53	0.43	0.48
DO	0.28	0.56	0.47	0.53
Alkalinity	0.30	0.34	0.51	0.46
Turbidity	0.49	0.65	0.69	0.33

The species diversity of Manas River is so peculiar that it harbours both coldwater and plain water fish species most of which has ornamental value. About 60–70% fishes of the Manas River system are ornamental fishes (Dey, 1989). Basistha (2006) reported 79 fish species from the Manas-Beki river system. Thus, the occurrence of 105 fish species from the entire river stretch including 35 coldwater species exceeds the earlier records. Ornamental fish species belonging to almost all the major families were found in this river system and the presence of coldwater fishes indicates that the river still remains the habitat for coldwater species. Although the number of fish species observed was satisfactory, however, due to lack of previous information on the quantification of fish species relative abundance, it could not be possible to compare the present fish diversity status with the earlier records. Yet as per the information gathered from the local stake holders, it can be summarized that the abundance of all the fish species including coldwater species reduced drastically in last couple of years.

The present investigation also recorded a decreasing trend in the relative abundance of hill stream ornamental variety viz., *Acantopsis multistigmatus*, *Aplocheilus panchax*, *Barilius vagra*, *Botia almorhae*, *Danio rerio*, *Glyptothorax botius*, *Labeo dyocheilus*, *Neolissochilus hexagonolepis* and *Tor tor*. This might have resulted due to the seasonal variations in the level of habitat attributes like Water Temperature, pH, DO, FCO_2 etc. across different sampling sites of the river which is in conformity with the findings of Sarma and Dutta (2009). Water temperature was always considered as a limiting factor which influences geographical and local occurrence of species within one water system (Sehgal, 1999).

The presence of exotic fish species viz., *Ctenopharyngodon idella*, *Cyprinus carpio* and *Oreochromis mossambicus* in the river system indicates the alternation of river habitat ecology and heavy influx of water during monsoon seasons. Flooded water not only originate prolonged siltation problem in different parts of the river, but also caused destruction of breeding ground and declining the diversity of indigenous fish species. Moreover, the presence of these exotic fish species may cause serious threat to other native fish species of the river ecosystem in the near future. Bhaumik *et al*. (2006) reported that harvesting of *Oreochromis mossambica* for commercialization at increased level in self maintained reservoirs, may get released into the nearby river system during flood season. The exotic fishes are a competitor to the native indigenous fish species for food and habitat (Singh *et al*., 2013). They may prey upon native fishes; introduce new diseases and parasites, resulting in the degradation of the physicochemical nature of aquatic ecosystems (Singh *et al*., 2013). All this will subsequently lead to loss of biodiversity (Singh and Lakra, 2011). The occurrence of marine fish species, *Pygocentrus nattererii* (aquarium species) at site S_5 may be due to its released into the river or may be due to migration factor from River Brahmaputra which links up to the Bay of Bengal Sea.

Species occurrence in any ecosystem is directly related to the abiotic factor than that of species interaction (Peres-Neto, 2004). Present investigation showed that the variations of physico-chemical parameters are having direct impact in fish species richness. Fish communities in river system typically follow a pattern of increasing species richness, diversity and abundance from upstream to downstream (Welcomme, 1985; Bayley and Li, 1994; Granado, 2000). Species diversity and species richness were higher at sites S_3, S_6 and S_5 in comparison to sites S_4, S_2 and S_1. This may be because of many tributaries in the middle and lower stretches than the upper stretches. The analysis of the trophic structure of fish diversity during the period of investigation indicates dominancy of omnivorous fishes in all sites followed by carnivorous, herbivorous and planktivorous fish. The variations in the habitat attributes like Water Temperature, pH, DO, FCO_2, etc., across different sites was attributed to differences in habitat degradation which might be responsible for variation of fish species diversity and their distribution in the river ecosystem.

The Shannon–Wiener diversity index (3.86) has indicated high species richness of the river. Minimum fish diversity was recorded in S_1 & S_2 because both the sites are located inside the protected area of Manas National Park (where fishing was only allowed to the investigating team only for few days with prior permission). The different biodiversity indices (Rarefaction plot, species distribution plot, abundance plot etc.) accessed in the present study reveals diverse nature of fish faunal in the Manas River. The calculated values of Jaccard similarity indices showed high values for sites S_3, S_4, S_5 and S_6 while calculated low for sites S_1 and S_2 (Specht and Paller, 2004). The present investigation also made an observed that even 1°C rise in water temperature have direct impact in decreasing dissolved oxygen level. Similar observation had been made by Sarma and Dutta (2009) which reported that due to 2°C to 3°C rise in annual average water temperature there was a decreasing trend in dissolved oxygen content from 7.5 mg l^{-1} to 6.5 mg l^{-1} in certain areas of the river system. This might be one of important reason for degrading the habitat ecology and suitability of the existing cold water fish fauna of the river ecosystem.

Specific habitat requirements of aquatic organisms may be characterized by many factors, including water depth, flow velocity, temperature and substrate. Natural flow regimes and hydrological variability (quantity, timing and duration of flows and floods) are considered essential for maintaining biodiversity and fisheries of any seasonal river systems (Poff *et al.,* 1997). It was also evident in the present study that due to various ongoing natural (flood, erosion, etc.) and other anthropogenic threats in the river system, the annual catching percentage of both plain water as well as coldwater fishes has been reducing drastically. Maintaining this annual flood pulse and the variable patterns of flows (erratic flow regimes) should be the first priority in water management of the Manas River system. Mitigation measures should include the restoration of hydrological and sediment dynamics, riparian vegetation, river habitat diversity and floodplain connectivity (Tockner and Stanford, 2002). The practice of unscientific and overfishing in many parts of the river which caused few fish species of the river to be in endangered category should be checked at the earliest.

From the study, it can be summarized that Manas River still provide suitable habitat for rich aquatic species though the rivers are subjected to varied pressures (both anthropogenic and natural). Therefore, for conservation and management issues of the existing ornamental fish species (both plain and hills stream), diversified strategies is the prime need of the hour. These strategies may include halting of siltation, open cast sand mining, scientific fishing and control of water pollution. The harnessing, development and management of Manas River and its natural resources would contribute not only to economic development for rural society of the region, but also balancing of ecological integrity of the river system. More research is required to understand basin-wide threat mechanisms, interactions and scales of response. More investment is required in monitoring and evaluation of the river ecosystem to determine the success of such efforts.

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Periphyton community structure of Namsang stream, Arunachal Pradesh

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ABSTRACT

The periphyton community structure of Namsang stream, a perennial stream situated in Eastern part of Tirap district, Arunachal Pradesh was studied during 2012-13. During the study period, 47 different genera of periphyton were recorded of which 45% belong to Bacillariophyceae followed by 30% Chlorophyceae, 13% Myxophyceae and 12% Animal community. Periphyton biomass of Namsang stream found to range between 1400 and 18,100 ucm^{-2} throughout the different seasons. Retreating monsoon found to be the peak season for periphyton growth during the present investigation while the least counts were observed during monsoon months. CCA revealed that none of the environmental variables except water depth showed noticeable impact over the growth and proliferation of the periphyton population of Namsang stream. Diatom genus like *Navicula*, *Frustulia*, *Melosira*, *Gyrosigma*, *Pinnularia*, *Synedra*, *Nitzschia*, *Ceratonies*, *Cocconies*; green algae like *Spirogyra*, *Ankistrodesmus*, *Chlorella, Chaetophora*; myxophyceae like *Lyngbya*, *Anabaena* and *Oscillatoria* as well as animal community belong to Copepoda found to be the most commonly occurring periphyton organisms of Namsang stream.

Keywords: Periphyton, Namsang stream, Arunachal Pradesh

Introduction

Periphyton plays an important role in flowing waters because it is virtually the only primary producer in the ecosystem. Contribution of periphyton to carbon fixation and nutrient cycling can be significant, particularly in lotic systems (Wetzel 2005). Periphyton in streams and rivers are an important component of aquatic ecosystems, providing food for invertebrates, and thus fish, in local and downstream ecosystems (Finlay *et al.,*., 2002). Periphyton growth can be light-limited (Quinn *et al.,*., 1997a, b; Kiffney and Bull, 2000) or nutrient-limited (Cascallar *et al.,*. 2003; McCormick and Stevenson, 1998), or both, and is influenced by temperature (Francoeur *et al.,*., 1999; Morin *et al.,*., 1999; Robinson and Minshall, 1998; Weckstroem and Korhola, 2001). Although this community is frequently subjected to adverse physical conditions such as high stream velocities or high turbidity levels, it is characterized by a very rapid recovery. Because of its ubiquitousness and rapid turnover it provides both food and shelter for the benthic fauna of a stream. Since the organisms involved are not equipped with a means of procuring the essential elements from the stream bed, the production of this community is also closely related to the characteristics of the water mass flowing by. Consequently, an evaluation of the periphyton community has long been recognized as a means of evaluating stream bio dynamics. Periphyton assemblages can be spatially complex and temporally variable depending on a wide range of environmental and biological factors (Biggs and Kilroy 2004; Peterson 2007). In streams, light (DeNicola *et al.,*. 1992), nutrients (Biggs and Close 1989), temperature (DeNicola 1996), current velocity, physical disturbance (Biggs and Close 1989), substrate type (Murdock and Dodds 2007), competition (Stevenson *et al.,*.1991) and invertebrate grazing (Peterson *et al.,*. 2001) can all influence periphyton assemblages. Periphyton community structure, species composition, and succession respond to environmental conditions and thus can be used to classify waterways (Denicola *et al.,*. 2004; Wargo and Holt, 2004). In addition, these algal communities can and have been used as biotic indicators of ecological condition and change in condition in response to human and natural disturbance (Cascallar *et al.,*., 2003; Chessman *et al.,*., 1999; Denicola *et al.,*., 2004; Hamsher and Vis, 2003; Komulaynen, 2002; McCormick and Stevenson, 1998; Stevenson, 1998). Periphyton forms the primary producer in the hill streams; they play an integral part of aquatic food chain particularly in hill streams where number of plankton is comparatively low due to fast water current, steep gradient and low nutrient content. Information on periphyton in Indian rivers are meagre in contrast to lakes and reservoirs (Jha, 1979; Laal *et al.,*. 1986; Sugunan and Pathak, 1986; Krishna Rao, 1990; Kumar, 1995; Sukumaran and Karthikeyan, 1999, Gurumayum *et al.,*., 2000; Singh *et al.,*. 2002; Daimari *et al.,*., 2005 and Singh and Das, 2009).

The present communication is an effort to throw some light on periphyton communities of Namsang stream, a perennial stream of Tirap district of Arunachal Pradesh, India and to understand the effect of different environmental variables on it.

Materials and Methods

Study area

Namsang stream, also known as "Chathju" originates from Jankhrum (1211 metres m.s.l) in Eastern part of Tirap district, Arunachal Pradesh at a longitude of $95^{0}39^{/}$ E and latitude of $27^{0}6^{/}$ N. (Fig.1). The stream traverses a meander course of C.50km in Tirap district of Arunachal Pradesh before it debouches into the plains near Jaipur Reserve Forest (120 metres m.s.l) to join the river Buri Dihing in Assam.

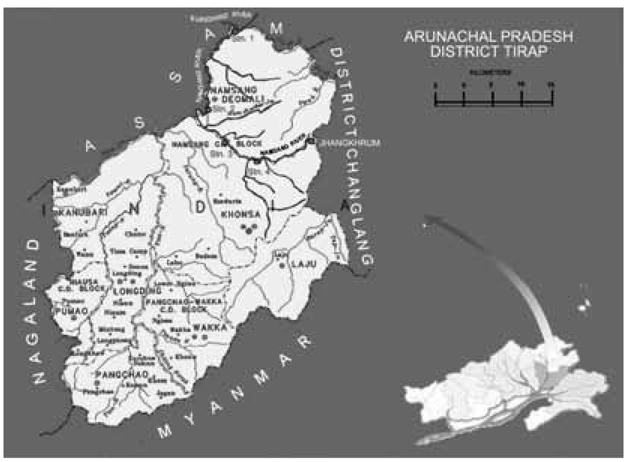

Fig.1: Map of Tirap district showing the study areas

Sampling protocol

The present study was conducted for a period of 2 years from 2012-2013. During this period, water and periphyton samples were collected from four different stations of the stream selected based on their altitude in four different seasons viz. premonsoon (March to May), monsoon (June to September), retreating monsoon (October and November) and winter (December and February) after Barthakur (1986).

Station 1: Station 1 was the confluence point of Namsang stream with the river Buri Dihing located at a longitude of $95^{0}30^{/}$ E at an altitude 120 m m.s.l.

Station 2: Station 2 was located at a distance of 10 km from station 1 (Longitude 95^{0} $25^{/}$ E, Latitude 27^{0} $14^{/}$ N, altitude 151 metres m.s.l.

Station 3: Station 3 was at a distance of 5 km from station 2 located at a Longitude of 95^{0} $28^{/}$ E, Latitude 27^{0} $13^{/}$ N, altitude 300 metres m.s.l.

Station 4: The fourth station was fixed at a distance of about 6km from Station 3 at a longitude of $95^{0}29^{/}$ E at an altitude of 333 metres m.s.l.

The samples were collected during early morning hours between 7:00 a.m. and 9:00 a.m. Parameters like temperature, pH and dissolved oxygen were recorded at the spot. Water samples were collected in plastic bottles with specific precautions and brought to the laboratory for the analysis of other parameters viz. free carbon dioxide, alkalinity, total dissolved solids, total hardness, calcium, chloride, magnesium, silicate, total iron, nitrate, phosphate and dissolved organic matter were analysed as per the standard methods (Welch, 1948; Welcher, 1963; Jhingran *et al.,*., 1969 and APHA, 2005).

Periphyton samples were collected by scrapping one to two square centimetre surface areas of stone/ boulders, wood and old logs that were submerged in the running water. The collected samples were preserved in 4-5% formalin in separate tubes. In the laboratory from the known volume of the sample, counting was done using Sedgwick rafter cell (Sharma and Saini, 2005). The periphytic forms were identified up to generic level with the help of standard books like Edmondson (1959), Needham and Needham (1966) and ICAR monograph series on algae (Ramanathan, 1964; Philipose, 1967).

The periphyton ucm^{-2} was obtained using the equation

$$ucm^{-2} = \frac{Nxn}{a}$$

Where

N = No. of periphyton per ml

n = Total vol. of periphyton sample

a = Area of the collected periphyton sample

Data analysis

The Species Richness (S), Simpson's index (D) were determined following Gleason (1922) and Shannon-Weiner index ($H^{/}$) of plankton samples was calculated as per Shannon (1948). A canonical correspondence analysis (CCA) was performed to relate different physico-chemical parameters to periphyton population (McCune and Mefford, 1999). Pseudo ANOVA was performed based on 1000 permutations to assess the statistical significance.

Results and Discussion

Water quality parameters

The common features in respect of water quality of Namsang stream were water temperature (15.0-25.0°C), average water depth (0.7-5.0 m), water velocity (0.46-2.33

msec^{-1}), slightly acidic to slightly alkaline pH (6.0-7.7), medium alkalinity range (24-60 mgl^{-1}), soft to moderately hard water (26-76 mgl^{-1}), rich DO (4.4-18.28 mgl^{-1}), high silicate (4.0-10.8 mgl^{-1}), high DOM (10-31 mgl^{-1}), high CO_2 (0.88-13.2 mgl^{-1}), moderate to rich calcium (12-34 mgl^{-1}), low specific conductivity (40-92 µmhos cm^{-2}), high values of magnesium (10-50 mgl^{-1}), TDS (35-116 mgl^{-1}) , iron (0.04-15.0 mgl^{-1}) and poor values of phosphate (0.01-0.4 mgl^{-1}), nitrate (nil-0.84 mgl^{-1}) and chloride (2.0-24.0 mgl^{-1}) (Table 1). Physico-chemical parameters were mostly found to be in the favourable ranges for the aquatic organisms.

Table 1: Physico-chemical parameters of Namsang stream during the study period

Sl. No.	Parameter	Range
1.	Water temperature (^oC)	15-25
2.	Water Depth (m)	0.7-5.0
3.	Water velocity (msec^{-1})	0.46-2.33
4.	pH	6.0-7.7
5.	Total Alkalinity (mgl^{-1})	24.0-60.0
6.	Total Hardness (mgl^{-1})	26.0-76.0
7.	Dissolved Oxygen (mgl^{-1})	4.4-18.28
8.	Free CO_2 (mgl^{-1})	0.88-13.2
9.	Specific Conductivity (µmhos cm^{-2})	40.0-92.0
10.	TDS (mgl^{-1})	35.0-116.0
11.	PO_4-P (mgl^{-1})	0.01-0.4
12.	NO_3-N (mgl^{-1})	Nil -0.84
13.	DOM (mgl^{-1})	10.0-31.0
14.	Silicate (mgl^{-1})	4.0-10.8
15.	Ca (mgl^{-1})	12.0-34.0
16.	Mg (mgl^{-1})	10.0-50.0
17.	Fe (mgl^{-1})	0.04-15.0
18.	Cl (mgl^{-1})	2.0-24.0

Periphyton composition

Duringthepresentstudy,47differentgeneraofperiphyton were recorded belonging to 8 classes: Bacillariophyceae, Chlorophyceae, Myxophyceae, Rhizopoda, Nematoda, Zooflagellate, Tardigrada and Copepoda. The study showed that, Bacillariophyceae dominated the periphyton biomass of Namsang stream (45%) followed by Chlorophyceae

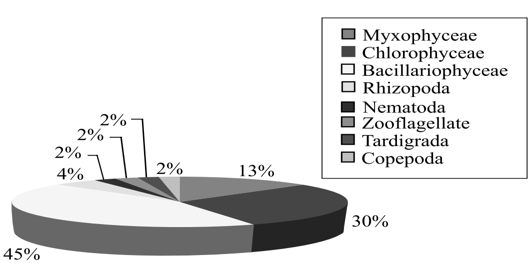

Fig.2: Percentage contribution of different periphyton groups in Namsang stream

(30%), Myxophyceae (13%) and Animal community (12%) (Fig. 2). Periphyton composition of Namsang stream is presented in Table 2. Laal *et al.,*. (1986), Sukumaran *et al.,*. (1996), Kawosa (2001), Liang and Li (2008) and Singh and Das (2009) also reported Bacillariophyceae dominance in periphyton biomass of water bodies. Station 1 was found to be the richest (44 genera) in terms of richness of periphyton followed by station 2 (39 genera), station 3 (28 genera) and station 4 (21 genera).

Table 2: Periphyton composition of Namsang stream

Periphyton Groups	Genera
Bacillariophyceae	*Amphora, Anomonies, Calonies, Ceratonies, Cocconies, Cymbella, Diatomella, Eucocconies, Frustulia, Gomphonema, Gomphonies, Gyrosigma, Liemorpha, Melosira, Navicula, Nitzschia, Pinnularia, Rhabdonema, Surirrela, Synedra, Thalosiothrix*
Chlorophyceae	*Ankistrodesmus, Cladophora, Cosmarium, Closterium, Chlorella, Chaetophora, Coelestrum, Microspora, Mougeotia, Oedogonium, Scendesmus, Spirogyra, Ulothrix , Uronema.*
Myxophyceae	*Anabaena, Oscillatoria, Merismopodia, Phromidium, Rivularia, Lyngbya.*
Rhizopoda	Euglypha, Arcella
Nematoda	*Criconema*
Zooflagellate	*Oicomonas*
Tardigrada	*Hypsibus*
Copepoda	*Cyclops*

Periphyton biomass

Periphyton density of Namsang stream showed a seasonal variation of (1400-18,100 ucm^{-2}) during the study period (Fig. 3). The Periphyton abundance observed by earlier workers on reservoirs, pond and lake were within the range of 2,200-5,264 ucm^{-2} (Srivastava and Desai, 1985), 1,907-4,782 ucm^{-2} (Kumar, 1995) and a much lower value (1441 ucm^{-2}) was observed by Laal *et al.,*. (1986); from different stretches of river Ganga. Gurumayum *et al.,*. (2000) observed much higher value of periphyton population from the hill streams of Arunachal Pradesh (15000 to 71,001ucm^{-2}). A higher range of periphyton population (16, 520- 85,000 ucm^{-2}) was also reported by Daimari (2003) from the hill streams of Arunachal Pradesh. This may be due to the fact that Periphyton are more in the lentic sector of hill streams, the least disturbed zone of ecosystem that could provide wider area of stones/boulders, old logs for its attachment. In the present investigation it was found that retreating monsoon represents the peak season for periphyton growth in all the stations during both the years (except station 1 during 2nd year). This finding is in agreement with the findings of Srivastava and Desai (1985), Kumar (1995) (periphyton of Ravisankar Sagar reservoir), Sukumaran and Kartikeyan (1999) in Markonahali reservoir, Karnataka and Daimari (2003) in Kameng, Dikrang, Subansiri and Ranga rivers of Arunachal Pradesh. The lowest count of periphyton was recorded during monsoon season in all the stations during both the years. This is in agreement with the findings of Daimari (2003) in rivers of Arunachal Pradesh. Bacillariophyceae was the most dominant group of periphyton in all the season and in all the stations. Laal *et al.,.* (1982), Sukumaran *et al.,.* (1996), Gurumayum *et al.,.* (2000), Kawosa (2001), Daimari (2003), Liang and Li (2008) and Singh and Das (2009) also reported Bacillariophyceae dominance in periphyton count. According to Hynes (1970) and Whitton (1975), the factors that influence the growth of periphyton population are light, temperature, water current, substrate, scoring effects of floods, water chemistry and grazing. The influence of light on periphyton assemblages can be substantial. Green algae are usually associated with high light level and Hynes (1970) reported a red algae (Batrachospermum) to be found exclusively in shade area. In contrast many diatoms seem to be unaffected by seasonal changes in light. An ambitious study of McIntire and Phenny (1965) studied the production and metabolism of periphyton communities that developed under two light regimes. The light adapted assemblage consisted of 46% diatoms, 42% Cyanobacteria and 12% green algae and under low light levels were 67% diatoms, 26% Cyanobacteria and 7% green algae. In reference to this finding, in the present study it has been observed that during retreating monsoon when the river water is very clear and light intensity reached up to the river bed due to shallow depth Bacillariophyceae population in the four stations ranged 42.62 to 60.78 %, Chlorophyceae 15.68 to 25.96% and Myxophyceae 6.89 to 19.35%; during winter, Bacillariophyceae were 46.15 to 73.33%, Chlorophyceae 13.33 to 23.52% and Myxophyceae 12.82 to 30.76% and during pre-monsoon, Bacillariophyceae were 40 to 60.52%, Chlorophyceae 18.42 to 30.76% and Myxophyceae 3.94 to 23.33%. While during the monsoon season the turbidity of river water increased due to monsoon rain and flood and hence the light

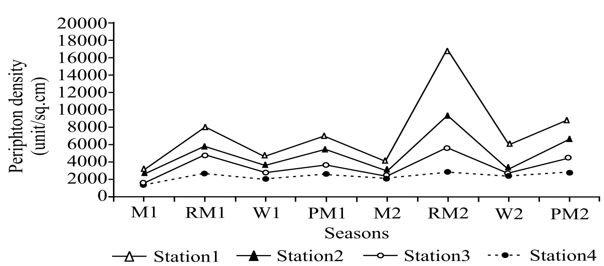

Fig.3: Seasonal variation of periphyton biomass during the study period

intensity does not reach the river bed. Myxophyceae was not recorded from station 4 during the 1st year monsoon and in other four stations the ranges of different periphyton groups were Bacillariophyceae 46.87 to 70.58%, Chlorophyceae 13.95 to 28.57% and Myxophyceae 3.57 to 12.5%. It has also been noticed that diatoms population was unaffected by neither high nor low light due to seasonal changes.

Decreased Myxophyceae and Chlorophyceae contribution in periphyton population is a common phenomena noticed by many workers (Daimari, 2003; Singh and Das, 2009). Low nutrient value in the physico-chemical properties of the water may probably result to their low contribution. Many workers reported positive and negative correlation between silicate and periphyton population from different habitat (Hutchinson, 1967; Sukumaran and Karthikeyan., 1999 and Kawosa, 2001). Earlier studies reported a silicate range of 4.0 to 7.8 mgl^{-1} from rivers of Arunachal Pradesh (Gurumayum *et al.,* 2000; Daimari, 2003). According to Lund (1954), silicate contents should be above 0.5 mgl^{-1} for rich growth of diatom. During the present study, Silicate values were in the range of 4.0 to 10.8 mgl^{-1}, which explained the dominance of diatoms in all the seasons.

Current influences substrate characteristics, which in turn affect site stability for attachment and growth. In addition flow results in the continuous renewal of gases and nutrients, and so current speed affects diffusion rates of needed materials into the cells. McIntire and Phenny (1965) observed that diatoms especially *Synedra*, dominated under faster current. In the present study also all the stations with fast water current dominated by diatoms. Substrate influence periphyton population in addition to providing stability against high flows. In the present observation also the periphyton community was found attached to the rocks surface that submerged in water. Again it has been observed that during monsoon the peripyton population was lowest which may be due to the fact that the surface destruction by monsoon rain or flood which deposited clay, sand and silt particles on the river bed. Animal community under periphyton was reported by Gurumayum *et al.* (2000) from Subansiri river of Arunachal Pradesh and Daimari (2003) from Kameng, Dikrang, Ranga and Subansiri rivers of Arunachal Pradesh. During the present investigation also, animal communities belonging to Zooflagellates, Nematoda, Copepoda, Tardigrada and Rhizopoda were observed. During the winter season (both the years), animal community found to be absent in all the stations which may be attributed to low temperature.

Periphyton richness and diversity

Periphyton richness and diversity of Namsang stream with the help of diversity indices is shown in Table 3.

Table 3. Diversity indices for periphyton genus of Namsang stream

Year	Season	Station	Index			
			H	Simpson	Simpson Inverse	S
First	Monsoon	1	2.5154	0.9102	11.1304	14
		2	2.1966	0.8801	8.3404	10
		3	2.0685	0.8581	7.0488	9
		4	1.6731	0.7959	4.9000	6
	Pre-monsoon	1	3.0286	0.9467	18.7532	23
		2	2.7744	0.9322	14.7541	18
		3	2.3568	0.8955	9.5660	12
		4	2.1391	0.8775	8.1650	9
	Retreating monsoon	1	3.3663	0.9629	26.9359	32
		2	2.9850	0.9428	17.4846	23
		3	2.6027	0.9158	11.8767	16
		4	2.1181	0.8704	7.7156	9
	Winter	1	2.6858	0.9263	13.5650	16
		2	2.4235	0.9060	10.6364	12
		3	2.0979	0.8644	7.3770	9
		4	1.7717	0.8264	5.7619	6
Second	Monsoon	1	2.4671	0.9075	10.8129	13
		2	2.2555	0.8906	9.1429	10
		3	2.0471	0.8672	7.5301	8
		4	1.9793	0.8507	6.6962	8
	Pre-monsoon	1	3.2332	0.9544	21.9429	30
		2	2.9034	0.9386	16.2966	21
		3	2.4587	0.9002	10.0174	14
		4	2.1367	0.8756	8.0357	9
	Retreating monsoon	1	3.6220	0.9687	31.9620	43
		2	3.1502	0.9540	21.7505	25
		3	2.6984	0.9207	12.6136	18
		4	2.2910	0.8907	9.1524	11
	Winter	1	2.6997	0.9242	13.1860	17
		2	2.3111	0.8945	9.4754	11
		3	1.9630	0.8419	6.3233	8
		4	1.8513	0.8284	5.8276	7

During the present investigation, species richness found to be maximum at Station 1 during Retreating monsoon both the years followed by Premonsoon, Winter and Monsoon. Similar trend was observed for Stations 2, 3 and 4. Shannon's and Simpson's diversity index also showed similar trend for all the stations. Species evenness of periphyton was higher in station 2 during all the seasons of both the years except first year Pre-monsoon and Retreating monsoon. The rarefaction estimate for expected species richness showed near similarity among all the stations of study.

Effect of Environmental variables on periphyton community

Canonical Correspondence analysis (CCA) was applied to evaluate the role of physico-chemical factors over the growth and distribution of the periphyton population. From the result it becomes clear that none of the environmental variables except water depth showed noticeable impact over the growth and proliferation of the periphyton population of Namsang stream. From the ordination diagram it becomes clear that commonly occurring periphyton genera of the stream occupies the central position of the ordination graph and the periphyton with lesser occurrence were seen at the positions distant from the centre. Diatom genus like *Navicula*, *Frustulia*, *Melosira*, *Gyrosigma*, *Pinnularia*, *Synedra*, *Nitzschia*, *Ceratonies*, *Cocconies*; green algae like *Spirogyra*, *Ankistrodesmus*, *Chlorella, Chaetophora*; myxophyceae like *Lyngbya*, *Anabaena* and *Oscillatoria* as well as animal community belong to Copepoda occupies the central position of the ordination plot (Fig. 4).

Regarding the impact of physico-chemical parameters over the growth and distribution of periphyton population of Namsang stream CCA indicates the relationship of *Navicula*, *Anomonies*, *Closterium* and *Eucocconies* with water depth, but the position of these periphyton in the ordination also suggest that their growth starts retarding after a certain depth. Diatom *Surirela* also grows along with the arrow indicating direct impact of this factor over its proliferation. Periphyton genus like *Cocconies*, *Rivularia*, *Thalassiothrix*, *Oedogonia*, *Oicomonas*, *Amphora* and *Scendesmus* showed weak positive correlation with water depth. On the other hand periphyton genus like *Spirogyra*, *Synedra*, *Anabaena*, *Nitzschia*, *Oscillatoria*, *Cholrella* and *Calonies* showed negative correlation with water depth. The abundance of the rest of the periphyton genus does not show any effect or influence of the change in physico-chemical factors analysed for the stream (Fig. 4).

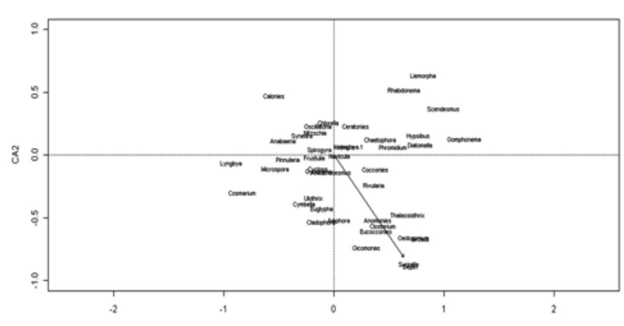

Fig.4: Spatial ordination resulting from CCA of periphyton population of Namsang stream with respect to physico-chemical parameters

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Length weight relationship and condition factor of *Schizothorax richardsonii* (Gray) and *Schizothorax niger*

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ABSTRACT

The aim of the study is to know the length-weight relationship and condition factor of two different snow trout species (*Schizothorax richardsonii* and *Schizothorax niger*). A total of 90 samples of each specimen were collected with help of cast net. There was a significant correlation between length and weight, the overall regression length-weight relationship equation was y=2.572x-1.543 and R^2=0.846 for *S. niger* and y=0.327x-2.163 and R^2 = 0.891 for *S. richardsonii*. While the condition factor computed for *Schizothorax niger* were 0.79469 ± 0.014 and for *Schizothorax richardsonii* was 0.76740±0.024 which indicated more isometric growth pattern of *S. richardsonii*. The "b" value for *Schizothorax niger* 2.572044 and *S. richardsonii* 3.027319 indicated better condition of *S. richardsonii* and physiological stress for *Schizothorax niger* in natural habitat.

Keywords: Length-weight relationship, Condition Factor, *S. niger, S.richardsonii, Himalayan region*

Introduction

Length-weight relationship studies of fishes are considered as an important tool for understanding of fish. Length is a linear measure (in centimeter) and the weight of a fish (in gram) is approximately equal to its volume (cubic centimeter). Hence, weight of a fish is a function of length. The relationship can be expressed by the hypothetical law $W= aL^b$. Length-weight relationship establishes the mathematical relationship between length and weight of fish (Bayer, 1987). The relationship also provides information on the changes in the well being of the fishes that happens during their life cycle. This relationship can be used in setting up of yield equations, estimate the number of fishes landed and for comparing the population over space and time (Beverton and Holt, 1957). The mathematical relationship between length and weight of fishes is a practical index suitable to understand their growth profile and general fitness, to compare the morphology between species or the same species across different geographical regions (Santos *et al.,* 2002).

Apart from length-weight relationship, the condition factor (K) is equally important in fisheries. Study of the length-weight relationship (LWR) of fishes are very important in fisheries, because it allows the estimation of the average weight of the fish of a given length group by establishing a mathematical relation between them (Sarkar *et al.*, 2008; Mir *et al.,* 2012). In fish, the condition factor (K) reflects, through its variations and information on the physiological state of the fish in relation to its welfare and is used to indicate suitability of an environment (Lizama *et al.,* 2002). Recent studies dealing with condition factor and length–weight relationships of important species are well documented in many species of fishes, some of those by Serajuddin, 2005, Prasad and Ali, 2007 and Gupta *et al.,* 2011. Schizothoracines contribute an economically significant group of hill stream fishes all along the Himalayan belt especially in Shivalik Himalaya.

Most fish species inhabiting the Himalayan region are small in size. Their distribution depends on environmental conditions such as velocity of water current, nature of substratum, and the availability of food. *Scizothorax niger* being a truely lacustrine fish does not show any spawning migration. In Shivalik Himalayas, *Scizothorax richardsonii*, locally known as 'Asela' has fishery importance and constitutes a principal component of subsistence food fishery of the region. This species is less abundant in Ladakh region of Himalayas, in comparison to other regions (Sivakumar, 2008).

Thus, the present study aimed to study the length-weight relationship and condition factor of two Snow trout species namely, *Schizothorax richardsonii and Schizothorax niger.*

Material and Methods

The length frequency data of combined sex of fresh samples of *Schizothorax richardsonii* were collected from the nearby streams of kumaun region of Uttarkhand and *S. niger* from Dal lake, Jammu & Kashmir respectively with the help of cast net and drag net. A total of 90 specimens of both fish species were collected and samples were cleaned and surface moisture was removed using tissue paper. Then the total length which was measured from the tip of snout to the caudal fin end with the help of centimeter scale and weight was taken in electronic weighing machine in grams.

The determination of length-weight relationship was made by applying the log transformation equation $W = aL^b$ to the data (Hile, 1936) Where,

W = Total weight of the fish in grams.

L = Total length of the fish in centimeters.

a = Initial growth constant and.

b = Regression co-efficient.

Index of well-being or condition factor is another way of expressing the relationship between length and weight of a particular fish. The relative condition factor (Kn) was calculated by the formula:

Kn = w/Wl,

Where w is the calculated weight and WI is the observed weight.

Result and Discussion

In order to determine the length weight relationship of *Schizothorax niger and schizothorax richardsonii*. Based on the scatter diagrams plotted for weight against length the y=2.572x-1.543 and R^2=0.846 for *S. niger* and y=0.327x-2.163 and R^2 = 0.891 for *S. richardsonii*. While

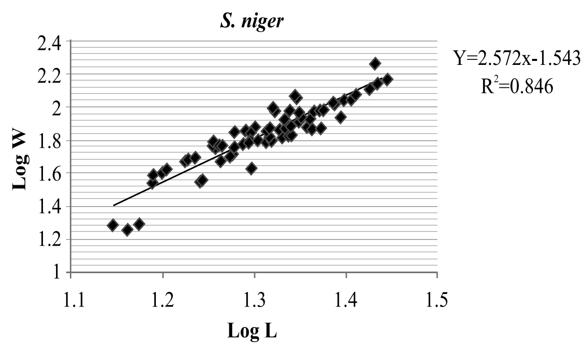

Fig.1: Scatter diagram showing length weight relationship of *Schizothorax niger*

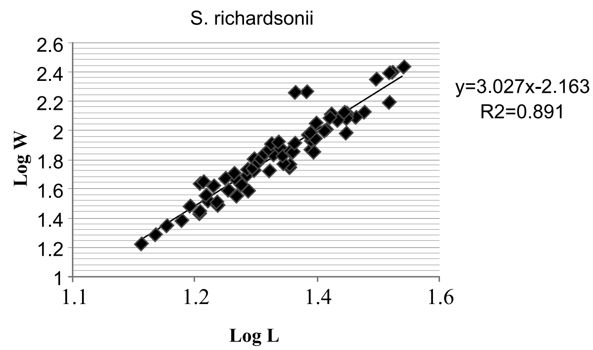

Fig.2: Scatter diagram showing length weight relationship of *Schizothorax richardsonii*

the condition factor computed for *Schizothorax niger* were 0.79469 ± 0.014 and for *Schizothorax richardsonii* were 0.76740 ±0.024 The "b" value for *Schizothorax niger* 2.572044 and *S. richardsonii* 3.027319 which indicated better condition of *S. richardsonii* and physiological stress for *Schizothorax niger* in natural habitat. All results are shown in Table1 and in Fig. 1 and 2.

The relationship between fish length and weight is considered to be of prime importance. This relationship is often used to compute the standing stock biomass (Smith, 1996 and Taskavak & Bilecenoglu, 2001) and also to estimate fish weight by knowing its length.

In general and despite many variations in fish forms between species, "*b"* is close to 3, indicating that fish grow isometrically; values significantly different from 3.0 indicate allometric growth (Tesch, 1971). Pauly (1984) has suggested that "b' value less than 3 indicated that fish becomes more slender as it increases in length and with a value greater than 3 denotes stoutness indicating allometric growth.

Qadri *et al*., (1983) recorded the value of 'b' to be equal to 2.448 in case of *S. richardsonii* of Sindh Nallah**.** Mir *et al.* (2012) reported the different values of 'b' (<3) in different months in *S.*

curvifrons from River Jhelum. Khan and Sabah (2013) reported the exponential *value* of 'b' equal to 2.69 for *S. curvifrons.* The present study is in conformity of the previous work and indicated isomeric growth pattern in the

Table 1: Length-weight parameters and measurements for *Schizothorax niger and richardsonii*

Fish Species	Mean Length (L) ± SE Mean	Mean Weight (W) ± SE Mean	Initial growth constant (a)	Regression co-efficient (b)	r^2	K±SE
S. niger	20.918	80.489	0.028619	2.572044	0.846	0.79469 ± 0.014
S. richardsonii	20.859	74.194	0.006861	3.027319	0.891	0.76740 ±0 .024

wild population of *S. richardsonii*. However, *Schizothorax niger* showed comparatively less value of 'b'.

The length-weight relationship of fish have significant importance in studying the growth, gonadal development and general well-being of fish population (LeCren,1951: Pauly,1993 and Nagesh *et al.,* 2004) and for comparing life history of fish from different localities (Petrakis and Stergion, 1995). The empirical relationship between length and weight of the fish enhance the knowledge of natural history of commercially important fish species thus making conservation possible (Yousuf *et al*., 2003; Mir *et al*., 2012). Condition factor (Kn) is also a useful index for monitoring of feeding intensity, age and growth rates of fish (Yousuf *et al*., 2006; Kumolu-Johnson and Ndimele, 2010). Kn values of different length- groups exhibited variations which ranged from 0.94 to 1.14 during different months, with an average value of 0.7 which indicated that fish is under physiological stress in this water body. (Shaheena and Yousuf , 2012). Thus, similar results were found in present study with an average Kn value of 0.79469± 0.014 and 0.76740±0.024 for *S. niger* and *S. richardsonii* respectively. (Indu and Dhanze, 2010) studied the value of condition factor 'Kn', which ranges from 0.87 to 1.10 which is at higher side as compared to present study.

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Study on age dependent breeding performance of rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792)

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ABSTRACT

Breeding and large scale seed production of important fish species in uplands hatcheries intensely get influenced by biological and physicochemical parameters. Identification of major limiting factors and their appropriate management in varying field conditions have been found beneficial in augmenting seed production. An attempt has been made in present study to corroborate effect of the brooder age on breeding performance, fecundity and egg survival. Study was conducted for a period of three years in 3+(B1), 4+(B2) and 5+(B3) year old farmed female rainbow trout (*Oncorhynchus mykiss*) brooders at Champawat farm in Uttarakhand to assess their suitable breeding age. Effect of brooders age on relative egg fecundity, egg quality, fertilization rate and survival during seed production operations was evaluated. Brooders were in weight range of 612.86±6.15, 679.90±8.36 and 1013.70±35.96g in B1, B2 & B3 age group respectively. Among three age groups, significantly highest ($p<0.05$) relative fecundity of 1997.73±65.28 eggs/kg of body weight in B2 and lowest 1575.74±54.15 eggs/kg in B3 was observed. Trout brooders in B1 group those attended first maturity at the age of 3+ in raceways, demonstrated uneven eggs size (27%) with a relative fecundity of 1988.10 ± 59.30 eggs/kg of body weight confirming stages of maturation. Size and individual weight of egg increased with consequent increase in weight and age of fish. Although, average initial fertilization rate after one hour was similar in all age groups, however uniformity of egg size was found highest (90%) in B2 group. Higher survival of hatchling observed in B1 and B2 groups compared to B3 group.

Keywords: Rainbow trout, Brooder age, Fecundity, Fertilization, Survival

Introduction

Among cultivable fishes, rainbow trout, *Oncorhynchus mykiss* contributes substantially in upland aquaculture production in the country. Superior growth in lower water thermal regime, hardy nature, simple breeding protocol, optimum artificial feed uptake, wide temperature tolerance and high market price are some of the useful attributes owing to which rainbow trout is widely preferred in upland aquaculture systems (Vass, 2002). Easy accessibility to quality fish seed is an essential requirement for obtaining good yield from a fish farm. Adoption of suitable brooder husbandry practices has been identified as crucial factor in production of large scale quality seed under changing environmental conditions. For getting sustained quality seed production, use of better quality female gametes obtained from farmed broodstock is of utmost importance (Beacham and Murray, 1985). It has been observed that besides maintenance of physicochemical parameters like improving water quality supply of hatchery, cleanness maintenance in egg rearing troughs through regular removal of dead and decayed eggs, shells and other substrates help in reducing egg loss, however, age of brooders also determine the egg fertilization rate, hatching and their survival percentage in hatcheries.

Several studies on reproductive performance of trout and salmon of different age and size group have been investigated (Pitman, 1979; Gall, 1974; Lahnsteiner, 2000;; Shanspour, *et al*., 2009; Aliniya *et al.,* 2013 and Leblanc, *et al.,* 2014). Researchers have also documented intra and inter specific variation in the size of eggs which also effect fertilation and survival of eggs (Aliniya *et al.,* 2013). Further, studies have demonstrated that the female brooders age significantly influence the quality of gamete which in turn influence the fertilization and survival efficiency (Springate, 1990; Springate and Bromage, 1985). With gradual increase of brooder age and size, consequent alteration in ovarian fluid composition and fecundity takes place Presumably, these physiological changes influence the egg quality, fertilization, hatching. Older and bulkier females produce larger eggs than younger and smaller fish in hatchery reared trout (Gall, 1974).

To determine relationship between female brooders age and reproductive performance in rainbow trout, present field study was designed and carried out for a period of three years in farmed female rainbow trout (Oncorhynchus mykiss) brooders to assess their suitability for stripping and large scale seed production based on relative egg fecundity, egg quality, percentage fertilization rate and survival during the hatchery operations independent of other external and internal rearing parameters.

Material and Methods

Study was carried out at Champawat trout farm of Directorate of Coldwater Fisheries Research, located between 29° 5” and 29° 30” North and 79° 59”and 80° 3”East with an altitude of 1620 msl in Uttarakhand state of India Farm raised rainbow trout brooders were reared in 150 and 30 sq m raceways with a stocking density of 3-6nos (3.0 - 4.0 kg) /sq m receiving natural stream water from Chhirapani @ 120-200 L/min. Pelleted feed @ 4-5% of fish body weight was fed twice in a day. To maintain optimum hygienic conditions fourth nightly cleaning of raceways and disinfection of fishes was carried out. Management measures were maintained identical in all brooder raceways during the study period. Segregation of male and females brooders before stripping was undertaken. Fecundity, egg size, fertilization rate and survival were studied in randomly selected 21 nos of female brooders each year. After assessing the maturity status of both male and female, brooders were transferred to hatchery and manually eggs were stripped and placed in clean and dry trays. Over the eggs, milt collected from the males was mixed gently with the help of a bird feather for adequate fertilization of eggs. Maximum dark conditions were maintained inside the hatchery to avoid exposure of eggs to direct light during stripping, fertilization and incubation operations. Individual trout brooder weight, total stripped un-fertilized egg weight, fertilization rate after one hour and final larval survival were recorded. 70-100 nos. of eggs from each fish were collected to estimate individual egg weight and repeated in 10 trout. Dry fertilization method adopted and eggs were left in trays for 15 min, washed thoroughly to remove unfertilized eggs and debris matter from the trays in slow running water and then transferred to hatching flow through troughs for further incubation @ 2500±250 per tray. The eggs which turned half or full white colored after one hour were siphoned out from the trays and counted and percentage fertilization was calculated. Relative fecundity was determined by total stripped eggs divided by average weight of individual eggs. Physicochemical parameters of incoming water and rearing facilities were analyzed and monitored on regular basis. Final larval survival was determined by total numbers of eggs incubated minus survived larvae of three age groups of fishes.

Statistical analysis

Mean value of parameter were subjected to one-way analysis of variance (ANOVA) to study the treatment effect and Duncan’s Multiple Range Tests (DMRT) were used to determine the significant differences between the mean value. Comparisons were made at 5% probability level. All the data were analyzed using statistical package SPSS (Version 16) (SPSS Inc., Chicago, IL, USA)

Results

No significant difference was observed in mean brooder weight in B1 and B2 groups of fishes, but mean brooder weight significantly differed in the brooders of B3. Weight wise, total numbers of eggs stripped in B2 and B3year class brooders were same while in B1 groups, it was significantly less. The number of stripped eggs per kg of

Table 1: Effect of female brooders age on fecundity egg quality and survival in *Oncorrhyncus mykiss*

Age of Weight	brooder weight	stripped egg weight (g)	Nos of eggs/kg fish	Mean Egg Weight mg	Fertilization rate	Mean Survival
B1 (3+Year)	612.86_c ± 6.15	73.33_b ±2.43	1988.10_a ±59.30	60.00_b ±1.32	78.20_b ±0.86	66.5_b ±1.44
B2 (4+ Year)	679.90_b ± 8.36	81.50_b ±2.13	1997.73_a ±65.28	60.00_b ±0.90	81.00_{ab} ±1.16	78.2_a ±1.12
B3 (5+ Year)	1013.70_a ± 35.96	102.24_a ± 3.35	1575.74_b ±54.15	65.50_a ±0.74	82.8_a ±1.04	55.4_c ±1.11

Table 2: Physiochemical Parameters of raceways

Year	*Water Temp*. (°c)	pH	DO (mg/l)	Hardness (ppm)	D. Iron (ppm)	Nitrate (ppm)	Chloride (ppm)
2012	2.5-22.5	7.0-7.8	5.8-7.8	25-75	0.0-0.5	0.01-0.03	10-110
2013	2.8-23.2	6.5-7.6	5.2-8.0	10-80	0.1-0.6	0.01-0.03	10-80
2014	2.0-23.5	7.0-7.6	6.0-8.4	25-75	0.1-0.3	0.02-0.05	10-30

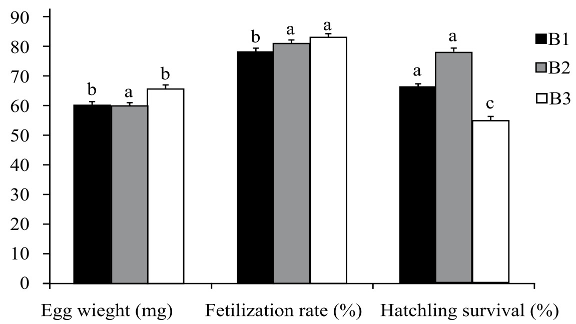

Fig. 1: Effect of female brooders age on egg weight (mg), fertilization rate (%) and hatchling survival (%) in *Oncorrhyncus mykiss.* Bars with different superscript (a,b,c,) on the top differ significantly (P<0.05). Values are expressed as mean ± SE, n=10.
B1 indicates 3+ year old female *O. mykiss* brooder
B2 indicates 4+ year old female *O. mykiss* brooder
B3 indicates 5+ year old female *O. mykiss* brooder

brooders in rainbow trout differed significantly in three age groups of fishes. Significantly un-swollen egg weight was higher in B 3 group compared to B1 and B2 and fertilization rate significantly differed in B1 and B3. The survival after hatching was highest in B2 followed by B1 and B 3 group of rainbow trout. The water parameters in trout raceways and in hatchery remained under normal limits.

Discussions

Although, relative fecundity in terms of eggs /g of fish body weight differed in all the three age groups in farm raised female rainbow trout brooders, however, there was no significant (p>0.05) difference in relative fecundity between 3+ year age group (B1) and 4+ year age group (B2) of rainbow trout. Relative fecundity in 5+ (B3) age group brooders was significantly (p<0.05) lower than B1 and B2. In B1, presence of uneven egg (27%) with varying weight of eggs showed that when female fish get first maturity, number of maturing eggs is also high which affected fertilization and further development of eggs. Initially for 1 hour there was no difference in fertilization rate however, with advancement of egg development stages, higher egg damage was observed in B1 indicating less developed eggs, while normal development of B2, could be attributed to better uniform maturation of stripped eggs. In the beginning of female brooder maturity as observed in in B1 group, fecundity was higher but all size of eggs was not uniform and therefore fertilization rate got affected. Our finding is with agreement of studies conducted by Pitman, 1979, and Shamspour *et al.,* 2009 on *Oncorhynchus mykiss* and *Salmo trutta macrostigma* respectively. Increases of fecundity with age have been reported by several researchers in Atlantic salmon (Brannas *et al.*, 1985, Bromage and cumaranntunga, 1998, Bromage *et al*., 1992 and Heinimaa & Heinimaa, 2004). Highest fecundity, fertilization and larval survival of B2 group probably may be due to uniform maturation, optimum stripping, smaller egg size compared to B3 and also availability of suitable temperature regime in advance egg incubation phases.

In the present study over ripening of eggs before normal breeding period with transparent coelomic fluid observed in B3 may be a reason of lowered fertilization rate. In rainbow trout, second year onwards, brooder attains higher growth and reaches up to 1.1-1.8 kg under normal rearing density and conditions. Rainbow trout in B3 age group had significantly lower relative fecundity with bigger egg size (65.5 mg) as compared to B1 and B2 groups (60 mg). Our findings are corroborated with the previous reports where fecundity declined with age (Siraj *et al*., 1983, Ridha and Cruz, 1989).The study conducted by Leblanc *et al.,* 2014 on Arctic charr **(***Salvelinus alpines) observed that* there is no direct correlation between egg size, percent hatching and survival which is similar to the results obtained in present study. Weight of all three age groups brooder differs significantly. Physicochemical parameters in rearing raceways were normal except increase in temperature during June every year. Inherent quality of egg also helps in better survival during incubation with increasing input and maintenance cost of livestock and higher demand of trout seed, the importance of rearing good quality brooders stock is being realized widely for obtaining quality seed. In this context it is important to rear suitable age group of female rainbow trout brooders, as bigger size old brooders require higher maintenance producing lesser number of eggs. Ridha

And Cruz (1989) also reported that compared to 2 and 5 year old Nile tilapia, 1 year old brooders had higher number of eggs. Although egg size and total fecundity increases with age but lower fecundity has been reported by various researchers (Springate *et al*., 1984; Bromage and Cumaranatunga, 1988).The average egg weight in B3 age group trout was higher than B1 and B2. Similar results have been shown by Gall, 1974. The incubation period vary with temperature and lower the temperature longer the incubation period with chances of egg infections. In our study at water temperature range of 3.0 – 7.0^{0}C, longest incubation period of 48 days was taken for hatching of trout eggs in B3 while in B1 and B2 total 42 and 38 days were taken at the water temperature range of 4.7- 8.7^{0}C and 5.0 - 9.1^{0}C respectively.

Conclusions

Overall results of present study demonstrated that under prevailing environmental and management conditions, maximum egg realization was achieved in B2 (4+ year old) female rainbow trout with average weight of 679.90 ± 8.36. Hence, it is suggested that 4+ year old brooders are more suitable for large scale seed production compared to new and old brooders.

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JOURNAL OF COLDWATER FISHERIES

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All sections of the typescript should be of A4 size paper, double-spaced and with sufficient margins (2.5 cm). All pages should be numbered. **Title page** should bear the title of the paper, name(s) and complete postal address of author(s) including present address of author(s) if applicable, telephone, fax numbers and e-mail address of the corresponding author. A concise running title of not more than 40 characters inclusive of spaces should also be given in this page. Full papers should generally be arranged in the following sequence: Abstract, Keywords, Introduction, Materials and methods, Results and Discussion, Conclusion (optional), Acknowledgements, References, Figure legends, Tables, Figures.

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For Books: Campbell, I. 2009. The challenges for Mekong River management. In: Campbell, I. (Ed.), The Mekong: biophysical environment of an international river basin. Elsevier, Amsterdam, p. 403-419.

For edited symposia/special issues:

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For technical papers/reports:

Cheshire, K. J. M., Ye, Q., Wilson, P. and Bucater, L. 2012. From drought to flood: Annual variation in larval fish assemblages in larval fish assemblages in a heavily regulated lowland temperate river. Technical Report Series No.12/6, Goyder Institute for Water Research, Adelaide, 62 pp.

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