

BEFORE THE NATIONAL GREEN TRIBUNAL
PRINCIPAL BENCH, NEW DELHI
ORIGINAL APPLICATION NO. 1004 OF 2024

IN THE MATTER OF:

Prahlad Roy Goenka & Anr.

...Applicant(s)

Versus

Union of India & Ors.

... Respondent(s)

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FILED BY:**PLACE: New Delhi****DATE: 16.4.2026**
Rakesh Kumar Singh**Advocate On behalf of**

Respondent No. 12

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BRIEF RESPONSE ON BEHALF OF RESPONDENT NO. 12

(ICAR–Central Institute of Coldwater Fisheries Research (ICAR–CICFR), Bhimtal, Nainital, Uttarakhand)

MOST RESPECTFULLY SHOWETH:

1. That the present application raises concerns regarding the decline of Golden Mahseer (*Tor putitora*), an ecologically important fish species of the Himalayan river systems, presently categorized as endangered by the IUCN.
2. That the present response is being filed in compliance with the directions of this Hon'ble Tribunal.
3. That Respondent No. 12, **ICAR–Central Institute of Coldwater Fisheries Research (ICAR–CICFR)** (formerly ICAR–Directorate of Coldwater Fisheries Research), is a research institute under the Indian Council of Agricultural Research (ICAR), Ministry of Agriculture and farmer's Welfare, mandated to undertake research, technology

development, and capacity building for coldwater aquaculture and fisheries in India.

SCIENTIFIC AND TECHNOLOGICAL INTERVENTIONS

4. It is submitted that ICAR–CICFR has undertaken dedicated research, and developed important technologies for the conservation and propagation of Golden Mahseer, as detailed below:

i. Captive Breeding Technology

ICAR–CICFR has taken dedicated research efforts and successfully developed *captive maturation and year-round breeding technology* for Golden Mahseer, enabling assured seed production throughout the year under controlled conditions. The said innovation has been granted a patent

A Copy of Captive Maturation and Breeding of Magnificent Golden Mahseer (*Tor putitora*): A Significant Step Toward Ex Situ Conservation. **(Book chapter);**

A Copy of System for year-round repeated breeding and higher robust fry production of golden mahseer **(Patent No. 432763)**

A Copy of Photo-thermal manipulations induce captive maturation and spawning in endangered golden mahseer (*Tor putitora*): A silver-lining in the strangled conservation efforts of decades **(Research paper)**

is annexed hereto and marked as Annexure- R/1 at page no. 10 to

35

ii. Nutritional Interventions

The institute has developed specialized broodstock and larval feeds, which significantly improve reproductive performance, larval survival, and overall fitness under captive rearing systems enabling quality mahseer brooder development and seed production for stock enhancement/conservation.

A Copy of Comparative assessment of egg and larval quality traits of progeny from wild-collected and captive-matured brooders of endangered golden mahseer, *Tor putitora*: A prelude to quality broodstock development and seed production (Research paper)

A Copy of Tailored broodstock diet fortified with functional nutrients enhances reproductive efficiency and larval quality traits of *Tor putitora*, an endangered cyprinid **(Research paper)** is annexed hereto and marked as Annexure- R/2 at page no. 36 to 62.

iii. Genetic Stock Assessment and Validation

ICAR–CICFR is conducting genetic characterization and stock validation of Mahseer populations in the Himalayan region to support conservation planning and maintain genetic integrity (Annexure 3).

A Copy of De novo assembly and characterization of tissue-specific transcriptome in the endangered golden mahseer, *Tor putitora* **(Research paper)**.

A Copy of Morphometric and Molecular Assessment of *Tor putitora* and *Neolissochilus hexagonolepis* Population in Jia Bhoroli river Ecotone Zone, Assam **(Research paper)**.

A Copy of Phylogenetic relationship and molecular identification of five Indian mahseer species using COI sequence. **(Research paper)** is annexed hereto and marked as Annexure- R/3 at page no.63 to 83.

FIELD-LEVEL CONSERVATION MEASURES

5. In addition to laboratory and hatchery-based interventions, ICAR-CICFR has implemented several field-level conservation measures:

i. Establishment of Hatcheries

Mahseer hatcheries have been established in multiple Himalayan states including Uttarakhand, Sikkim, Arunachal Pradesh, Manipur, Meghalaya, and Nagaland to facilitate seed production and mahseer conservation efforts.

ii. Ranching Programmes

ICAR–CICFR is undertaking regular ranching (stock enhancement) of Golden Mahseer in natural water bodies such as rivers and lakes to restore declining wild populations.

A Copy of Recreational fisheries in Uttarakhand (Technical article)

A Copy of Mahseer in recreational fisheries and ecotourism in India ((Technical article)

A Copy of The endangered mighty mahseer (*Tor putitora*) in the Himalayan waters (**Book chapter**) is annexed hereto and marked as Annexure- R/4 at page no. 84 to 114

iii. Seed Production Facility

A Seed Production Facility has been established at Bhimtal, District Nainital (Uttarakhand), for upscaled seed production and distribution

to State Fisheries Departments for conservation and stock enhancement programmes.

A Copy of Seed production of golden mahseer at ICAR-CICFR (formerly ICAR-DCFR), Bhimtal, Nainital, Uttarakhand **(Few Institute Annual Report Excerpts)** is annexed hereto and marked as Annexure- R/5 at page no. 115 to 120.

CAPACITY BUILDING AND AWARENESS

6. ICAR–CICFR is actively engaged in dissemination and outreach activities:

i. Training and Capacity Building

Regular training programmes are conducted for fisheries officials, researchers, students, and stakeholders on conservation breeding, and mahseer hatchery management.

A Copy of Training and capacity building programmes conducted by ICAR-CICFR **(Few Institute Annual Report Excerpts)** is annexed hereto and marked as Annexure- R/6 at page no. 121 to 123.

ii. Community awareness

Awareness programmes are being organized for local communities to promote conservation ethics and highlight the ecological importance of Golden Mahseer.

A Copy of Awareness programmes on mahseer conducted by the Institute **(Few Institute Annual Report Excerpts)** is annexed hereto and marked as Annexure- R/7 at page no 124 to 130.

SCIENTIFIC POSITION ON CONSERVATION

7. It is respectfully submitted that the decline in Golden Mahseer populations is a **multifactorial issue**, influenced by habitat fragmentation, altered river flow regimes, overfishing, and environmental degradation.
8. ICAR–CICFR, being a scientific institution, has focused on conservation strategies, particularly:
 - Development of breeding technologies
 - Stock enhancement through hatchery-produced seed
 - Studies on stock validation of mahseer species
 - Promotion of mahseer conservation and management
9. The institute continues to provide technical support and advisory services to Central and State agencies for mahseer conservation through training and consultancy.
10. That the answering respondent craves leave of this Hon'ble Tribunal to file a further affidavit(s) if the situation so warrants or this Hon'ble Tribunal so desires.
11. That the supporting affidavit along-with this reply is annexed herewith.

PRAYER

12. In view of the above submissions, it is respectfully prayed that the Hon'ble Tribunal may take note of the scientific and conservation measures undertaken by Respondent No. 12 and may take it on record.
13. It is further respectfully prayed that the Hon'ble Tribunal, may dismiss the Application qua Respondent No.12, and/or may kindly be pleased to pass appropriate orders in the present Application.

FILED BY:



Rakesh Kumar Singh

Advocate On behalf of

Respondent No. 12

ICAR–Central Institute of Coldwater Fisheries Research

Chamber no. 313, New Lawyers Chamber,

Setalvad Building, Supreme Court of India

New Delhi-110001

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Mobile: 917930691

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R.N-50

16-04-2026

AFFIDAVIT

I, Khilawati Rawat, S/o Chandra Singh Sayana, Aged about 58 years, having Office address at ICAR–Central Institute of Coldwater Fisheries Research (ICAR–CICFR), Bhimtal, Nainital, Uttarakhand-263136, Sr. Administrative Officer, On behalf of the Director, ICAR–Central Institute of Coldwater Fisheries Research (ICAR–CICFR), Bhimtal, Nainital, Uttarakhand do hereby solemnly affirm and sincerely state as under: -

1. That I am Authorized Representative on behalf of the Director, ICAR–Central Institute of Coldwater Fisheries Research (ICAR–CICFR), Bhimtal, Nainital, Uttarakhand, Respondent No. 12 and am well conversant with the facts and circumstances of the case and thus competent to swear this affidavit.
2. That the accompanying brief response has been drafted by my Counsel under my instruction based on knowledge and I affirm that

Hemant Dhusia
HEMANT DHUSIA Advocate
Notary Distt H.Q.Nainital
Reg.No.20(10)/2023 16-04-2026

Khilawati
16/4/2026
Khilawati

statement of facts made herein are true and correct to my knowledge.

3. That the contents of the said reply be read as part of this affidavit and same are not repeated here for the sake of brevity.
4. That the annexures filed along with the reply are true copies of their respective originals.
5. That I have read the contents of this reply and I state that nothing material has been concealed thereof and the contents I of this affidavit are true and correct to the best of my knowledge and belief

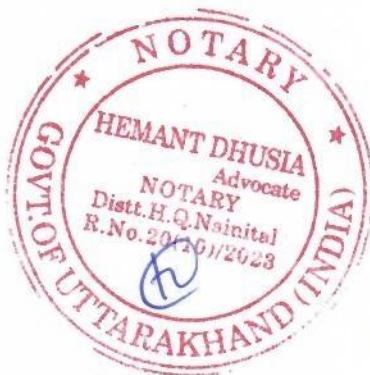
Khilawast
Deponent 16/4/2026

VERIFICATION:

वरिष्ठ प्रशासनिक अधिकारी / Sr. Administrative Officer
भा.कृ.अनु.प.- केन्द्रीय शीतजल मात्स्यिकी अनुसंधान संस्थान
भीमताल - 263136, जिला - नैनीताल, उत्तराखण्ड
ICAR - Central Institute of Coldwater Fisheries Research
Bhimtal - 263136, Distt-Nainital, Uttarakhand

I say that the contents of the above affidavit are true and correct as per my knowledge, no part of it is false and nothing material has been concealed therefrom.

Verified at *Nainital* on *16* this day of April, 2026.



Attested that Sri/smt. *Khilawast* *Ramant*
the Deponent Identified by *Vinay Kumar Singh Nema*
Sworned & Verified the contents of
the Affidavit at *Nainital*
on Date *16.04.2026* ...at *Nainital*

Khilawast
Deponent 16/04/2026

Hemant Dhusia
HEMANT DHUSIA Advocate
Notary Distt H.Q. Nainital
Reg.No.20(10)/2023

वरिष्ठ प्रशासनिक अधिकारी / Sr. Administrative Officer
भा.कृ.अनु.प.- केन्द्रीय शीतजल मात्स्यिकी अनुसंधान संस्थान
भीमताल - 263136, जिला - नैनीताल, उत्तराखण्ड
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16.04.2026



Captive Maturation and Breeding of Magnificent Golden Mahseer (*Tor putitora*): A Significant Step Toward Ex Situ Conservation

4

M. S. Akhtar and A. Ciji

Abstract

The golden mahseer (*Tor putitora*), a culturally significant and endangered freshwater fish species native to the Himalayan region, faces numerous threats due to habitat degradation, overfishing, pollution, and climate change. The conservation of this iconic fish extends beyond biology, encompassing culture, society, and ecology. Ex situ conservation strategies, including captive breeding and hatchery management, are essential to address its multifaceted challenges. This text delves into the importance of golden mahseer in generating livelihoods through sports and recreational fishery, reproductive dysfunction in captive golden mahseer hindering conservation efforts, and necessitating innovative solutions. The advancement of golden mahseer captive maturation and breeding technology contributes to the production of fry for introduction into both natural and artificial aquatic environments, ensuring the preservation of this remarkable fish species. Ex situ conservation offers benefits such as species preservation, genetic diversity maintenance, research and monitoring opportunities, supplementation of wild populations, reduced human disturbance, and enhanced education and awareness. The success of captive maturation and breeding, integrated with in situ conservation, provides hope for the continued existence of the golden mahseer in its natural habitat.

Keywords

Golden mahseer · Ex situ conservation · Reproductive dysfunction · Captive maturation and breeding · Fish-based eco-tourism

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Annexure Document Page No. 1

4.1 Introduction

The golden mahseer (*Tor putitora*), an iconic and endangered (Jha et al. 2018) freshwater fish species (Fig. 4.1) native to the pristine waters of the Himalayan region, is more than just a species of fish; it is a cultural icon, an ecological keystone, and a symbol of nature's majesty. This majestic fish holds recreational value and immense cultural significance (Akhtar et al. 2017). With its striking golden hue, powerful physique, and a legacy entwined with centuries of angling lore, the golden mahseer has captured the imaginations of naturalists, anglers, and conservationists alike (Bhatt and Pandit 2016).

Once teeming in the clear and tumultuous waters of the Himalayan and sub-Himalayan rivers, the golden mahseer faces myriad threats in the modern world. Unabated habitat degradation, overfishing, pollution, dam construction, and climate change have conspired to severely reduce its populations (Nautiyal 1994; Sehgal 1999; Dinesh et al. 2010; Akhtar et al. 2017). Inherent biological traits such as delayed maturity, low fecundity, and slow growth rate have also contributed to its population decline over time (Akhtar et al. 2018). These challenges collectively underscore the urgent need for conservation initiatives, not merely to protect the golden mahseer but to safeguard the entire aquatic ecosystems it represents.

Conservation of the golden mahseer is not just about preserving a single species; it is about conserving entire aquatic ecosystems. These systems, which include diverse fish species, plants, and aquatic invertebrates, provide food and livelihoods for people. Therefore, the conservation of the golden mahseer extends beyond a mere biological imperative; it embodies a cultural, social, and ecological responsibility. It speaks to the need to balance the sustainable use of natural resources with the preservation of the environment for future generations. Hence, the holistic approach to safeguarding the mighty mahseer should combine scientific expertise, community engagement, and ecosystem conservation.

In light of multifaceted challenges, ex situ conservation techniques have become vital in preserving the future of the golden mahseer. In this context, conservation efforts are increasingly focus on strategies like captive breeding, hatchery management, stocking, and ranching. These strategies promise to maintain and restore populations of the golden mahseer while addressing the complex interplay of environmental and socioeconomic factors that threaten its existence.



Fig. 4.1 A golden mahseer female brooder

4.2 Why Is the Mighty Mahseer So Important?

Golden mahseer, the “tiger of the river,” plays a vital role in generating livelihood security through sports and recreational fishery, especially in regions where it is found. Here are several ways in which golden mahseer contributes to this aspect:

1. *Tourism and sports fishing:* Golden mahseer is a prized catch for anglers due to its size, strength, and challenging nature. Anglers from around the world are attracted to regions where this fish is found (Everard and Kataria 2011; Pinder et al. 2019), leading to an influx of tourists seeking the thrill of catching this iconic fish, thereby boosting the local tourism industry. Tourism centered on sports fishing for golden mahseer can create livelihood opportunities for local communities (Bhatt and Pandit 2016; Pinder et al. 2019) through providing guiding services, equipment rentals, and developing fishing lodges and resorts.
2. *Employment opportunities:* The demand for guided fishing trips and equipment rental services creates jobs for local residents, such as fishing guides, boat operators, cooks, and lodge staff. This employment helps improve the livelihoods of people living in areas where golden mahseer is found.
3. *Conservation incentives:* The economic value of golden mahseer for sports and recreational fishing creates a conservation incentive. Local communities and authorities are more likely to protect the fish’s habitat and manage its populations sustainably to ensure a steady supply for anglers. Conservation efforts can lead to better habitat preservation, cleaner rivers, and healthier ecosystems, benefiting the broader environment.
4. *Supporting local economies:* Tourism associated with golden mahseer fishing injects money into local economies. Anglers and tourists spend on accommodations, food, transportation, and other services, boosting the income of local businesses and individuals.
5. *Cultural preservation:* The presence of golden mahseer and the promotion of sports fishing can help preserve local cultural traditions and knowledge related to fishing and river ecosystems. This cultural preservation can create opportunities for cultural tourism, including the sharing of traditional practices and knowledge.
6. *Research and education:* The focus on golden mahseer in sports fishing often goes hand in hand with research and education about the species and its habitat. This contributes to scientific knowledge and educational opportunities in the region, leading to more jobs and knowledge dissemination.
7. *Sustainable management practices:* To ensure the long-term viability of golden mahseer populations for sports fishing, sustainable management practices are often put in place, which includes habitat restoration, stocking programs, and catch-and-release policies, which can create employment opportunities for those involved in conservation efforts.

Hence, golden mahseer has the potential to contribute significantly to livelihood security through sports and recreational fishery by stimulating tourism, creating

jobs, motivating conservation, and preserving local cultures. However, it is crucial to ensure these activities are conducted sustainably to maintain the long-term health of golden mahseer populations and their ecosystems.

4.3 Declining Golden Mahseer: The Underlying Factors

Several factors have contributed to the depletion of golden mahseer populations in the wild (Dudgeon 2011; Grumbine and Pandit 2013; Everard et al. 2018; Pinder et al. 2019). These factors can be categorized into (1) anthropogenic and environmental factors and (2) species-specific factors.

1. **Anthropogenic and environmental factors:** The decline of golden mahseer populations in the wild can be attributed to various anthropogenic (human-induced) factors. These factors include the following:
 - (a) *Habitat loss and fragmentation:* The construction of dams and associated reservoirs can alter the natural flow of rivers, leading to habitat loss and fragmentation. Dams, weirs, and other infrastructure can create barriers to the natural migration and movement of golden mahseer, affecting breeding and genetic diversity. Deforestation and unsustainable land-use practices such as dredging and sand mining contribute to sedimentation and water pollution, degrading the mahseer habitat.
 - (b) *Overfishing:* Overfishing through illegal and unsustainable methods, such as dynamite or poison fishing, can lead to the depletion of golden mahseer populations (Raghavan et al. 2011).
 - (c) *Introduction of invasive species:* The introduction of invasive fish species that compete for resources or prey on golden mahseer can have a detrimental impact on their populations (Gupta and Everard 2019). For example, the introduction of common carp into Nainital lake (Uttarakhand, India) has majorly impacted the golden mahseer population.
 - (d) *Climate change:* Climate change can affect river flow patterns and water temperatures, impacting the survival and reproduction of mahseer (Mats et al. 2009).
 - (e) *Water pollution:* Pollution from industries, agriculture, and urban areas lead to degraded water quality and negatively affect the health of golden mahseer and its breeding ground and food.
 - (f) *Limited enforcement:* In most of the regions, there is inadequate enforcement of fishing regulations, making it challenging to protect golden mahseer from illegal fishing and overexploitation.
 - (g) *Lack of awareness and research:* A lack of comprehensive scientific research and data on golden mahseer is still hindering conservation efforts.
 - (h) *Socioeconomic factors:* In many areas, due to poverty and less availability of livelihood opportunities, local communities are relying on fishing for their livelihoods, which can put additional pressure on golden mahseer populations.

2. **Species-specific factors:** The depletion of golden mahseer populations can be attributed to various species-specific factors, which are unique to this particular fish species. While many of these factors are interconnected with the broader issues mentioned earlier, understanding the species-specific reasons is essential for targeted conservation efforts. Here are some of the species-specific reasons for the depletion of golden mahseer populations:
- (a) *Limited reproductive behavior:* Golden mahseer typically undertake long-distance migrations to spawning grounds (Nautiyal 2014). Dams and other barriers can hinder their ability to access these areas, impacting breeding success.
 - (b) *Slow growth and late maturity:* Golden mahseer have slow growth rates and reach sexual maturity relatively late. This makes them vulnerable to overfishing, as they cannot replenish their populations as quickly as some other fish species.
 - (c) *Specific habitat requirements:* Golden mahseer prefers clear, well-oxygenated waters, and they are highly sensitive to changes in water quality. Habitat degradation and pollution can have a detrimental effect on the reproductive success of this species.
 - (d) *Low fecundity:* The low fecundity (4000–8000 eggs/kg body weight; Joshi et al. 2018) of the golden mahseer has significant implications for the species' population dynamics and might have contributed to its decline in the wild. The limited reproductive output (low fecundity) hinders the ability of the species to rapidly replenish its population.
 - (e) *Longer incubation period and demersal eggs:* The extended incubation (fertilization to hatching) period, usually 75–90 h at a temperature range of 20–23 °C, and the demersal (sinks to the riverbed) nature of the eggs makes them more susceptible to predation. Predators may consume/damage a significant portion of the eggs, further reducing the growth potential.
 - (f) *Skewed sex ratio:* The skewed sex ratio of the golden mahseer in many of its natural habitats is a critical concern. The male-to-female ratio has been notably imbalanced in certain regions, raising concerns about the long-term viability of this iconic species. According to studies, this imbalance is attributed to various factors, including overfishing, habitat degradation, and pollution. It was observed that male mahseer exhibited higher numbers in the immature stock, while females were more prominent in the mature stock (Pathak et al. 2014; Mayank et al. 2016; Joshi et al. 2018). However, in our experimental fishing in Kumaon lakes, a severely skewed sex ratio (dominated by males; 1:5–1:8) has been observed (unpublished data). Hence, skewed sex ratio might also have contributed to the decline of golden mahseer population in the wild. Therefore, efforts to address this issue are crucial for the conservation of golden mahseer, as restoring a more balanced sex ratio is vital for the sustainable reproduction.

4.4 Reproductive Biology of Golden Mahseer

The golden mahseer is a batch spawner, producing eggs intermittently. The ovary displays various size groups of eggs (as shown in Fig. 4.2) throughout its extended breeding season, with the peak of spawning between May and August, mainly during the monsoon season. While *T. putitora* does not exhibit strong sexual dimorphism, a few secondary sexual characteristics can be seen during the breeding season. Males tend to have vibrant coloration and a longer body with rugged pectoral fins, while females typically exhibit more subdued coloration and a deeper body (Arjamand et al. 2013; Akhtar and Ciji 2023).

During the spawning season, the readiness of female mahseer can be assessed by evaluating the softness of the abdomen and the pinkish hue of the vent. They favor clean water with sandy or gravelly substrata for spawning/breeding and undertake potamodromous upstream spawning migrations. These spawning migrations are believed to be triggered by turbid waters and elevated water temperatures (Nautiyal 1994; Bhatt et al. 2004). Consequently, during floods, these fish ascend to the upper reaches of rivers, covering significant distances in search of suitable breeding sites with appropriate substrates. These sites are typically shallow streams (ranging from 0.5 to 3.5 m) containing pebbles, gravel, sand, and silt, as reported by Pathani (1994). In some cases, riverbanks are chosen, where the fish create nests for egg-laying (Nautiyal 1994). The spawning process involves releasing one batch of eggs at a time, and which is repeated many times during the breeding season.

The golden mahseer has relatively low fecundity, with the number of eggs ranging from 7 to 25 per gram of body weight, as indicated by Pathani (1981), Nautiyal and Lal (1985), and Nautiyal (2014). Fertilized eggs take 58–92 h (depending on water temperature) to hatch, and because they are demersal, they can sink and perish on the muddy riverbeds, which makes them vulnerable to predation. The most favorable conditions for egg hatching include high turbidity and temperatures within the range of 16–25 °C (Johnsingh et al. 2006).

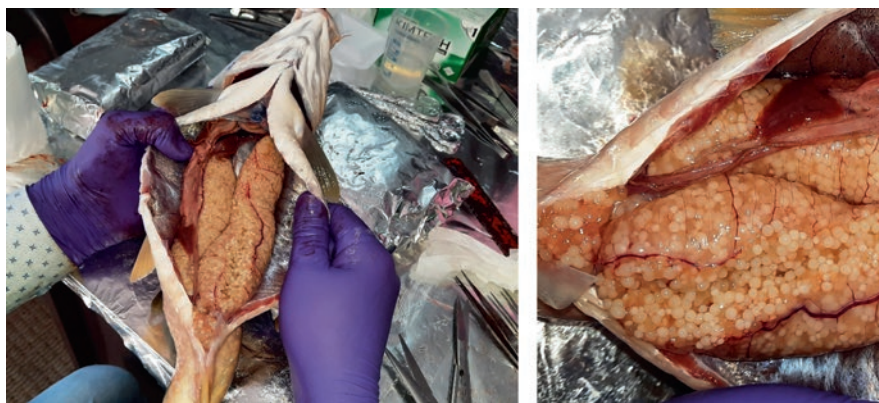


Fig. 4.2 Ovary of *T. putitora* with different egg size. (Reproduced from Akhtar and Ciji 2023)

4.5 Reproductive Dysfunction in Golden Mahseer

Reproductive dysfunction in a fish species can significantly hinder captive breeding and conservation efforts. If a fish species exhibits reproductive dysfunction, such as infertility or the inability to produce viable offspring, it directly reduces the success of captive breeding programs. This can lead to a lack of progress in increasing the population size of the species, which is often the primary goal of conservation efforts. The reproductive dysfunctions observed in captive golden mahseer (Akhtar et al. 2017) prevent them from achieving full ovarian development and maturity. That is why, till recently, the seed production of golden mahseer was done using gravid brooders collected from the wild. This, in turn, greatly impedes the production of a substantial number of golden mahseer offspring for conservation and restoration efforts.

4.6 Development of Captive Maturation and Breeding Technology of Golden Mahseer

Until recently, the production of golden mahseer fry predominantly relied on capturing gravid brooders from the wild, as females in captivity experience stress that hinders their attainment of gonadal maturity (Akhtar et al. 2017). However, the practice of collecting gravid brooders from the wild for stripping and artificial fertilization was found to be unsustainable, as these wild brooders, once gravid, usually would not survive after the stripping process. This method also could not meet the long-term demand for stocking material required for rehabilitation and conservation programs. Despite many attempts by scientists and researchers over the past four decades involving the use of pituitary extract or synthetic hormones, success in the captive breeding of golden mahseer has been limited (Pathani and Das 1979; Pandey et al. 1998; Sehgal 1991). Therefore, the absence of mature female golden mahseer brooders in captivity presented a significant obstacle to large-scale seed production.

The ICAR-Directorate of Coldwater Fisheries Research (ICAR-DCFR) in Bhimtal, India, the premier research institute dedicated to fisheries and aquaculture in the temperate Himalayas, has developed an innovative technology through several years of dedicated research to address this challenge. This indoor technology enables captive maturation and year-round multiple breeding of golden mahseer through the manipulation of various environmental cues (Akhtar et al. 2018, 2020, 2021).

This innovative and patented (Indian Patent No. 432763) technology comprises a SYSTEM and METHOD for year-round multiple breeding of golden mahseer to produce healthy and robust fry (see Akhtar et al. 2021, for more details). It includes using a specialized indoor maturation unit, distinct egg incubators, water quality modifications, and breeding grounds simulation during hatchery operations (Fig. 4.3). These modifications have resulted in increased fertilization rates, enhanced hatching success, and improved survival rates of the fry. More than 72% of female golden mahseer are found to respond to egg stripping using this technology. A single female brooder can be bred minimum of twice and maximum up to four times annually. On an average, each responsive female brooder exhibited an annual relative fecundity of approximately 9000–10,000 eggs/kg of body weight.

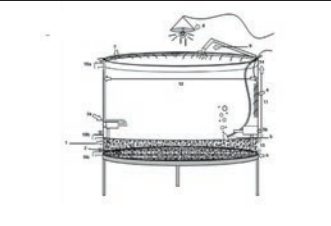
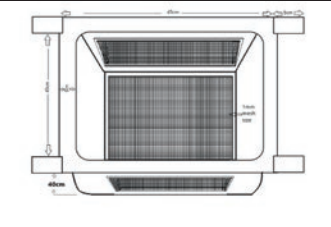
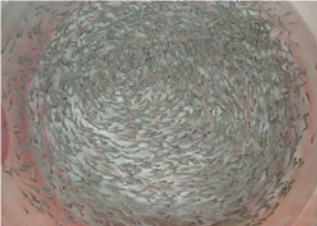
	
<i>Diagram of the 'captive maturation unit'</i>	<i>Brooder being taken out from the captive maturation unit for stripping</i>
	
<i>Golden mahseer brooders</i>	<i>Stripping of captive-matured brooder</i>
	
<i>Golden mahseer egg incubator</i>	<i>Fertilized eggs of golden mahseer matured in captivity</i>
	
<i>Golden mahseer fry produced from captive-matured brooders</i>	

Fig. 4.3 Some essential components of the captive maturation and multiple breeding technology of golden mahseer. (Adapted from Akhtar and Ciji 2023)

As the availability of captive breeding technology is a pivotal tool for ensuring the sustainable ex situ conservation of a vulnerable species, the captive maturation and breeding technology of the golden mahseer will provide an opportunity to revive the species in the long run. Several hatcheries are being established in many Himalayan states using this technology to produce golden mahseer fry/fingerlings for its conservation. Now, the sustainable production of seeds is helping us develop clear plans for releasing captive-bred golden mahseer into their natural habitat. As part of conservation and rehabilitation efforts, advanced fry produced in the hatchery are routinely introduced into open water bodies, particularly the temperate Himalayan lakes and rivers (Fig. 4.4), following a nursery period of 6–7 months.



Fig. 4.4 Release of captive-bred golden mahseer fry into their natural habitat. (Adapted from Akhtar and Ciji 2023)

4.7 Benefits of Ex Situ Conservation

Ex situ conservation, which involves the conservation and management of species outside their natural habitat, can be an effective strategy for the conservation of the golden mahseer for several reasons:

1. *Species preservation*: Ex situ conservation can act as a “genetic safety net” by maintaining a viable population of golden mahseer in controlled environments, reducing the risk of extinction due to habitat loss, pollution, or overfishing in the wild.
2. *Genetic diversity*: It allows for the preservation of genetic diversity within the species. In captivity, controlled breeding programs can be implemented to maintain a diverse gene pool, which is crucial for the long-term health and adaptability of the species.
3. *Research and monitoring*: Ex situ conservation provides opportunities for in-depth research on the biology, behavior, and physiology of the golden mahseer. This information can inform better conservation strategies and management of the species in the wild.
4. *Supplementation of wild populations*: Golden mahseer populations in the wild are fragmented and at risk of decline. Ex situ conservation programs can serve as a source of individuals for reintroduction into the wild, bolstering struggling populations.
5. *Reduced human disturbance*: Human activities such as habitat destruction and overfishing often threaten golden mahseer populations. Ex situ conservation may reduce the direct pressure on wild populations, allowing them to recover more effectively.
6. *Education and awareness*: Ex situ facilities can serve as educational tools, raising awareness about the importance of golden mahseer conservation. This increased awareness can lead to greater public support for conservation initiatives.

4.8 Conclusion

The captive maturation and breeding of golden mahseer is a complex and meticulous process, but it is a vital component of ex-situ conservation efforts. This approach offers hope for preserving this culturally and ecologically important species, ensuring that the golden mahseer continues to thrive in our rivers and streams for generations to come. As we delve deeper into understanding the needs and behavior of this species, the success of captive maturation and breeding will only improve, offering a brighter future for the golden mahseer. However, it is important to note that ex situ conservation should be integrated with in situ (on-site) conservation efforts, such as habitat restoration and sustainable management of wild populations, for a comprehensive and effective conservation strategy. The success of ex situ conservation depends on careful planning, genetic management, and coordination with in situ conservation activities to ensure the long-term survival of the golden mahseer in its natural habitat.

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(Rule 74 of The Patents Rules)

क्रमांक : 011161393
SL No :



पेटेंट सं. / Patent No. : 432763
आवेदन सं. / Application No. : 202111004283
फाइल करने की तारीख / Date of Filing : 01/02/2021
पेटेंटी / Patentee : The Indian Council of Agricultural Research (ICAR)

प्रमाणित किया जाता है कि पेटेंटी को, उपरोक्त आवेदन में यथाप्रकरित SYSTEM FOR YEAR-ROUND REPEATED BREEDING AND HIGHER ROBUST FRY PRODUCTION OF GOLDEN MAHSEER नामक आविष्कार के लिए, पेटेंट अधिनियम, 1970 के उपबंधों के अनुसार आज तारीख फरवरी 2021 के पहले दिन से बीस वर्ष की अवधि के लिए पेटेंट अनुदत्त किया गया है।

It is hereby certified that a patent has been granted to the patentee for an invention entitled SYSTEM FOR YEAR-ROUND REPEATED BREEDING AND HIGHER ROBUST FRY PRODUCTION OF GOLDEN MAHSEER as disclosed in the above mentioned application for the term of 20 years from the 1st day of February 2021 in accordance with the provisions of the Patents Act, 1970.



अनुदान की तारीख : 24/05/2023
Date of Grant :

(Signature)
पेटेंट नियंत्रक
Controller of Patent

टिप्पणी - इस पेटेंट के नवीकरण के लिए फीस, यदि इसे बनाए रखा जाना है, फरवरी 2023 के पहले दिन को और उसके पश्चात प्रत्येक वर्ष में उसी दिन देय होगी।

Note. - The fees for renewal of this patent, if it is to be maintained will fall / has fallen due on 1st day of February 2023 and on the same day in every year thereafter.



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23

सं. \ No. 202111004283

दिनांक \ Dated the 24/05/2023

सेवा मे, \ To :

Address of Service:- L.S DAVAR & COMPANY Globsyn Crystals, Tower 1, 2nd Floor, Block EP, Plot No. 11 & 12, Salt Lake, Sector V, Kolkata-700091, West Bengal. PHONE: 91-33-23571010/ 23571215 FAX: 91-33-23571018/ 23571019 E-MAIL:kolkatapatent@lsdavar.in 7044088580
Email Id:- kolkatapatent@lsdavar.in, docketing@lsdavar.in

विषय :- पेटेंट आवेदन संख्या 202111004283 के संबंध में अधिनियम की धारा 43 के तहत पेटेंट अनुदान तथा पेटेंट रजिस्टर में प्रविष्टि की सूचना
Sub :- Intimation of the grant and recordal of patent under section 43 of the Act in respect of patent application no. 202111004283

महोदय/महोदया,
Sir/Madam,

आपको सूचित किया जाता है कि पेटेंट अधिनियम, 1970 की धारा 12 व 13 तथा उस आधार पर बने नियम के तहत उपर्युक्त पेटेंट आवेदन के परीक्षण [व ----- को हुई सुनवाई] के उपरांत एतद्वारा पेटेंट अनुदान किया जाता है। तथा पेटेंट अनुदान की प्रविष्टि 24/05/2023 को पेटेंट रजिस्टर में कर दी गयी है।

This is to Inform you that following the examination of above mentioned patent application under section 12 and 13 of The Patents Act, 1970 and Rules made thereunder [and hearing held on -----] a patent is hereby granted and recorded in the Register of Patents on the 24/05/2023. The Patent Certificate is enclosed herewith.

पेटेंट संख्या \ Patent No	: 432763
आवेदक का नाम \ Name Of Applicant	: The Indian Council of Agricultural Research (ICAR)
पेटेंट दिनांक \ Date of Patent	: 01/02/2021
पूर्विका तिथि \ Priority Date	: 01/02/2021
परीक्षण हेतु अनुरोध दाखिल करने की तिथि \ Filing date of Request for examination	: 08/02/2021
शीर्षक \ Title	: SYSTEM FOR YEAR-ROUND REPEATED BREEDING AND HIGHER ROBUST FRY PRODUCTION OF GOLDEN MAHSEER
दावों की संख्या \ Number of claims	: 1-9

उपर्युक्त पेटेंट के अनुदान का प्रकाशन अधिनियम की धारा 43 के तहत पेटेंट कार्यालय के आधिकारिक जर्नल में किया जाएगा।

The grant of above mentioned patent will be published in the Official Journal of the patent Office under section 43 of the Act.

पेटेंट अधिनियम 1970 यथा संशोधित पेटेंट (संशोधन) नियम, 2005/ पेटेंट नियम, 2003 यथा संशोधित पेटेंट (संशोधन) नियम, 2016 की धारा 142 की उप-धारा (4) के प्रावधानों के तहत उपरोक्त प्रविष्टि की तिथि से 3 माह के भीतर इस कार्यालय में नवीकरण शुल्क जमा किया जाना चाहिए।

The payment of renewal fee is required to be made at this office within three(3) months from the aforesaid date of recording according to the proviso in sub-section(4) of Section 142 of The Patents Act,1970, as amended by The Patents (Amendment) Act, 2005 / The Patents Rules, 2003 as amended by The Patents (Amendment) Rules, 2016.

Monika Seth

(नियंत्रक पेटेंट)

Controller of Patents

टिप्पणी / Note :

1. संशोधित नवीकरण शुल्क हेतु कृपया महानियंत्रक पेटेंट, अभिकल्प एवं व्यापार चिह्न की आधिकारिक वेबसाइट www.ipindia.gov.in पर उपलब्ध पेटेंट (संशोधन) नियम 2016 की प्रथम अनुसूची (शुल्क) देखें।

For revised renewal fees kindly refer to the First Schedule (fees) of The Patents (Amendment) Rules 2016 available on the official website of Controller General of Patents, Designs and Trade Marks www.ipindia.gov.in

2. कार्यालय द्वारा पेटेंट प्रमाणपत्र की कोई भी कागजी प्रति अलग से जारी नहीं की जाएगी।

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Photo-thermal manipulations induce captive maturation and spawning in endangered golden mahseer (*Tor putitora*): A silver-lining in the strangled conservation efforts of decades

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11-ketotestosterone
Captive maturity
Spawning

ABSTRACT

Present study was undertaken to explore the possibilities of inducing maturity and spawning of *T. putitora* under captivity through manipulation of environmental cues i.e. photoperiod and temperature. In experiment I, adult *T. putitora* were subjected to three photoperiod (8L:16D, 12L:12D and 16L:12D) regimes for 100 days. In females, darker photoperiod (8L:16D) significantly decreased the plasma estradiol levels compared to 12L:12D and 16L:8D photoperiods. Plasma 17 α , 20 β -diOH-P was significantly ($p < .05$) changed both in females as well as males due to different photoperiods. Highest level was observed at 12L:12D in females and 16L:8D in males whereas the lowest level was found in 8L:16D. There was no significant effect of photoperiod on plasma 11-ketotestosterone (11-KT) levels and gonado-somatic index (GSI) of both males and females. In experiment II, effect of temperature (ambient, 21.2 ± 1.4 °C and elevated, 23.7 ± 1.3 °C; Mean $\pm$ SD) on inducing maturity under captive conditions was explored. The level of 17 β -estradiol and 17 α , 20 β -diOH-P was significantly increased in females kept at elevated temperature compared to its ambient counterpart. Correspondingly, GSI of females increased 6-fold at elevated temperature. In fact in the ovary of females reared at elevated temperature, oocytes were in vitellogenic and maturing stage, whereas it was only in cortical alveolar stages in the fishes kept at ambient temperature. On the other hand, there was no marked change found in testicular histology. However, there was presence of some macrophage aggregations on the efferent duct region of testes at elevated temperature. No significant ($p > .05$) effect of temperature was observed on plasma levels of 11-KT of both sexes. Experiment III was meant to explore the possibility of achieving captive spawning by simulating natural spawning ground using a gravel bed bio-filter. Natural spawning occurred after four months of rearing in the simulated facility. Subsequently, fishes further spawned seven times in a period of 4 months on an interval of 15–20 days. Overall, our results suggested that captive maturity and spawning of golden mahseer can be achieved by manipulating the environmental cues. The results solved the prevailing decadal issue of captive maturation of golden mahseer which otherwise has been a major bottleneck for its wide spread rehabilitation and conservation programmes.

1. Introduction

Golden mahseer (*Tor putitora*), is a cyprinid fish which is considered as a flagship species in upland fisheries of Himalayan sub-continent by virtue of its economic, ecological, recreational, heritage, cultural and food values (Nautiyal, 1984; Dinesh et al., 2010; Akhtar et al., 2014). It is 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 (Jayaram, 2010; Akhtar et al., 2013a; Dinesh et al., 2010). It has 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 (Everard and Kataria, 2011). However, due to various factors like pollution, environmental degradation, habitat fragmentation by hydroelectric and irrigation projects and indiscriminate fishing (dynamiting, poisoning etc), its population in natural water bodies/habitat had declined sharply (Sehgal, 1999; Nautiyal, 1994; Oliver et al., 2007; Dinesh et al., 2010). Besides the above mentioned anthropogenic constraints which results in loss of breeding grounds and over-exploitation, the inherent biological factors of this species such as slow growth, delayed onset of maturity, low fecundity and long hatching period are also responsible for the decline of golden

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mahseer population (Nautiyal, 1994; Froese and Pauly, 2011). As golden mahseer has been declared (Jha and Rayamajhi, 2010) as an endangered species (IUCN Red List of Threatened Species), it has become a subject of great concern for biologists, conservationist and policy makers to safeguard and augment the existing stock through the development of seed production technology and habitat conservation.

Over the years, several studies have investigated the reproductive biology/behaviour and protocols for captive propagation of golden mahseer in many of the Trans-Himalayan countries (Nautiyal, 1994; Ingram et al., 2005; Sarma et al., 2010; Ismail et al., 2011; Akhtar et al., 2013b; Sarma et al., 2016b). Knowledge generated over three decades and recent advances resulted in the development of seed production and hatchery technology of golden mahseer at ICAR-Directorate of Coldwater Fisheries Research (ICAR-DCFR), Bhimtal, India and elsewhere (Sehgal and Malik, 1991; ShyamSunder et al., 1993; Sehgal, 1999; Ogale, 2002; Sarma et al., 2016a). However, at present, the breeding and seed production of golden mahseer is being done using wild gravid females collected from lakes and rivers for stripping, artificial fertilization and hatching which is proven as a non-sustainable practice and does not meet the actual demand of stocking material to be used for rehabilitation and conservation programmes (Akhtar et al., 2017). To address the need, development of captive brooders for mass scale seed production of golden mahseer in hatchery is imperative. But, like females of many other economically important fishes (Zohar, 1989), golden mahseer females fail to complete ovarian development and maturation in captive conditions due to endocrine dysfunctions (see Akhtar et al., 2017). Thus, non-availability of golden mahseer matured female brooders in captivity has been a major bottleneck for its mass scale seed production.

To address this, induced breeding using ovaprim has been attempted earlier with very limited success (Tripathi, 1978; Pathani and Das, 1979; Pandey et al., 1998) or no success (Sehgal, 1991). However, manipulations of environmental cues have never been explored to induce maturity in golden mahseer under captive conditions. In the wild, spawning season of golden mahseer coincides with the onset of rains during July to September (Nautiyal, 1984; Pathani, 1983) or during May to September with two (May–June & August–September) peaks (Sehgal, 1987). Pathani (1983) suggested that the fresh warm rainwater coming into the streams may induce the growth and maturation of ova leading to the migration of golden mahseer brooders for spawning. Pathani (1981) also found that golden mahseer at high altitude lakes matures late due to low temperature. From these earlier reports, it is evident that higher temperature and extended day length seem to be the major probable factors responsible for maturation and spawning of golden mahseer in the wild. Reproduction in fish under captive conditions can be controlled by manipulation of environmental cues including photoperiod, temperature or spawning substrate (Mylonas et al., 2010). These stimuli influence the brain by modifying the activities of particular neurohormones and neurotransmitters in order to regulate the secretion of gonadotropins releasing hormone and dopamine from the hypothalamus (reviewed by Redding and Patiño, 1993). Further, the pituitary under hypothalamic control, release gonadotropins (GtH) and at the end of the hypothalamic–pituitary–gonadal

axis, GtH stimulates gametogenesis and steroidogenesis (Lam, 1983). Photoperiod has been shown to be the primary environmental cue that synchronizes reproductive cycles (Lam, 1983; Bromage et al., 2001) while temperature plays a more important role in controlling the specific timing of final oocyte maturation and ovulation (Carrillo et al., 1991; Tveiten and Johnsen, 1999). In cyprinids, temperature has a much greater impact on gametogenesis and rising temperatures have a stimulatory effect at later stages of gonadal development (Bye, 1984). In teleosts, the process of gametogenesis is mainly dependent on sex steroids which are controlled by gonadotropins (Yaron et al., 2003). Sex steroids, such as 17β -estradiol (E_2), 17α , 20β -diOH progesterone and 11-ketotestosterone are known to control different stages of reproduction (Mylonas et al., 2010). External factors such as temperature, photoperiod, rainfall and seasons are reported to elicit production of sex steroids resulting in gonadal development and maturation processes (Lubzens et al., 2010; Schulz et al., 2010). Therefore, for the first time, we attempted to explore the possibility of inducing maturity and spawning of golden mahseer in captive conditions through environmental manipulations.

2. Material and methods

2.1. Experimental design and rearing of fishes

2.1.1. Experiment I: effect of photoperiod on gonadal maturity of golden mahseer in captivity

A total of 36 adult golden mahseer (18♀s and 18♂s; average weight: 655.3 ± 88.47 g and 494.4 ± 83.62 g, mean $\pm$ SD, respectively; which were wild collected and reared in floating cages at lake Bhimtal, Nainital, Uttarakhand for over one year) were collected and brought to the indoor wet lab of ICAR-Directorate of Coldwater Fisheries Research (ICAR-DCFR), Bhimtal and randomly distributed in the three photoperiod groups in duplicates, with equal number of males and females in each grey colour rectangular fibre reinforced plastic (FRP) tanks ($1.8 \text{ m} \times 1.8 \text{ m} \times 0.5 \text{ m}$). The three photoperiod treatment groups were: 8 h light and 16 h dark (8L:16D), 12 h light and 12 h dark (12L:12D) and 16 h dark and 8 h (8L:16D) light (Fig. 1). The intensity of light was maintained at 1400 ± 80 lx using 35 watt compact fluorescent light (CFL) bulbs. Fishes were acclimatized for one week and then experiment was continued for 100 days (March to June 2015). An internal circulation of water in each tank was maintained using submersible pumps (800L/h) placed inside the tank to simulate a stream flow pattern. Continuous aeration using air compressor was provided in each tank to ensure optimum dissolved oxygen level. Each tank was covered with 8 mm mesh nylon net to prevent escape of the experimental fishes and at the same time to allow light to pass into the tanks. The dark condition was maintained by covering the tanks with thick black plastic sheets. Fishes were fed ad libitum twice daily (10.00 h and 17.00 h with ICAR-DCFR's formulated blood diet (Table 1). The broodstock diet was also supplemented with L-tryptophan to ameliorate the stress (Akhtar et al., 2013c) induced by captivity (Akhtar et al., 2017). Every day the tanks were cleaned manually by siphoning out water along with the faecal matters to assure water quality. Fifty

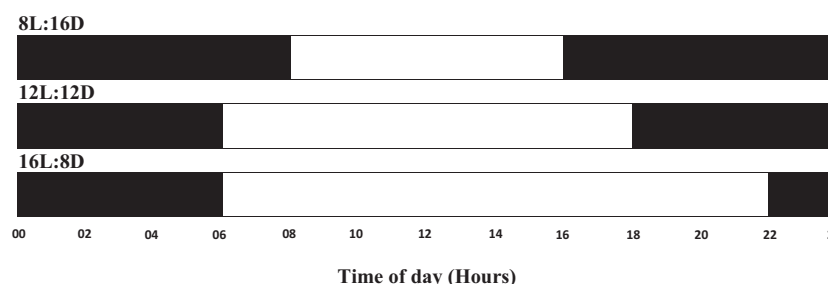


Fig. 1. Schematic diagram of different photoperiod treatments in experiment I. (Black area shows dark phase and white area depicts light phase).

Table 1

Composition and proximate analysis of broodstock diet (% dry matter basis) fed to *Tor putitora* during the experimental periods.

Ingredients	% inclusion in the diet
Fish meal ^a	44.00
Soybean meal ^s	30.00
Wheat flour ^s	14.50
Soy lecithin ^b	0.50
L-tryptophan ^b	0.50
Egg albumin ^b	0.50
Vitamin mix ^c	0.10
Mineral mix ^c	0.50
Vitamin C ^b	0.05
Vitamin E ^b	0.05
Folic acid ^b	0.025
Carboxymethyl cellulose ^b	1.00
Choline chloride ^b	0.10
Yeast extract ^b	0.3
Fish oil ^a	4.00
Soybean oil [#]	4.00
Proximate analyses [% dry matter basis](AOAC, 1995)	
Moisture	6.9 ± 0.12
Crude protein	44.08 ± 0.55
Crude lipid	11.9 ± 0.22
Ash	14.7 ± 0.04

Data expressed as Mean ± SEM, n = 3.

^a Janatha Fishmeal and Oil Products, Udipi, Karnataka, India.

^b Himedia Laboratories, Mumbai, India.

^s Procured from local market.

^c Prepared manually and all components from Himedia Ltd. Mumbai, India.

[#] Fortune Edible Oils and Foods, Adani Wilmar Limited, Ahmedabad, India.

percent water of each tank was exchanged daily before the first feeding of the day. The water quality parameters were monitored periodically (Table 2).

2.1.2. Experiment II: effect of elevated temperature on gonadal maturity of golden mahseer in captivity

To elucidate the effect of temperature on gonadal maturity of golden mahseer in captivity, a second experimental trial was carried out in the Mahseer Hatchery Complex of ICAR-DCFR, Bhimtal. A total of 24 of adult golden mahseer (12♀s and 12♂s; average weight: 814.59 ± 60.44 g and 514.7 ± 47.24 g, mean ± SD respectively) were collected from earthen ponds of ICAR-DCFR, Bhimtal, Uttarakhand, India and randomly distributed into two treatment groups i.e. ambient temperature (21.19 ± 1.4 °C; Mean ± SD) and elevated temperature (23.66 ± 1.3 °C; Mean ± SD) in duplicates with equal number of males and females in each grey colour rectangular FRP tank (1.8 m × 1.8 m × 0.5 m). The temperature in 'elevated temperature group' was maintained using thermostatic water heaters (300 W) submerged in the tank itself (Fig. 2). A photoperiod of 12L:12D (chosen based on the results of experiment I) was maintained using 35 watt CFL bulbs. Fishes were acclimatized for one week and then experiment was continued for 121 days (April–July 2016). An internal circulation of

water in each tank was maintained using submersible pumps (800L/h) placed inside the tank to simulate a stream flow pattern. Continuous aeration using air compressor was provided in each tank to ensure optimum dissolved oxygen level. Each tank was covered with 8 mm mesh nylon net to prevent escape of the experimental fishes and at the same time to allow light to pass into the tanks. During night, the tanks were covered with thick black plastic sheets. Fishes were fed ad libitum twice daily (10.00 h and 17.00 h with ICAR-DCFR's formulated broodstock diet (Table 1). Every day the tanks were cleaned manually by siphoning out water along with the faecal matters to assure water quality. Fifty percent water of each tank was exchanged daily before the first feeding of the day. A flow of 8–10L/h was also maintained in each tank. The water quality parameters were monitored periodically (Table 2).

2.1.3. Experiment III: a pilot experiment combining elevated temperature and optimum photoperiod in a FRP tank installed with gravel bed-biofilter

In the previous experiments (I&II), we found that optimal photoperiod and elevated temperature distinctly imparted a positive effect on gonadal maturity of golden mahseer under captive conditions. However, we could not achieve captive spawning under the said conditions of these two experiments. This was probably due to fact that golden mahseer being a lithophil and prefers to spawn on water beds with a proportionate mixture of gravels and sandstones in the wild by making nesting pits (Nautiyal, 1984). Hence, considering the prior knowledge, a pilot experiment was conducted to test the possibility of captive spawning in a FRP tank installed with gravel bed-biofilter and provided with optimum photoperiod and temperature. For this purpose, a circular navy blue coloured FRP tank (0.8 m height, 1.45 m diameter, and ≈1.3 m³ capacity) was used (Fig. 3B). For the bed-filter, a plumbing design was made (Fig. 3A and B) by connecting a series of parallel PVC pipes (~25 mm diameter) using elbows and T joints. A vertical pipe connected the plumbing arrangement to a power head (3200–6000L/h capacity). The above PVC plumbing design was laid on the bottom of the circular tank and mixture of river gravels and sandstones (3.0–12 mm) were uniformly spread over it to a thickness of 13–15 cm. The plumbing design was meant to enable the suctioning of water through gravels uniformly across from the tank bottom area which reduced dead zones while enhancing nitrifying bacterial growth throughout the gravel surfaces and trapping faecal matter and uneaten feed pellets. In this way, gravels served as both mechanical as well as biological filter. The depth of water column was maintained at 50–55 cm. The initial turnover rate of water in the tank was 4 times/h and later increased up to 6 times/h which resulted in more crystal clear water. Thus gravel bed bio-filter simulated natural spawning bed and at the same time helped in maintaining water quality comparable to their pristine natural habitats (Table 2). Moreover, we tested the gravel bed biofilter efficiency using fish less cycling (i.e. after the inoculation of biofilter with the addition of a mug of gravels from a matured gravel bed filter) by adding 3 ppm of total ammonia nitrogen (TAN; in the form of NH₄Cl) and measured the dynamic changes of TAN, nitrite and nitrate. Fig. 3C shows that first addition of ammonia required 6 days for

Table 2

Physico-chemical parameters of rearing water in different treatment groups under different photoperiods (experiment I) and temperatures (experiment II).

Physico-chemical parameters	Photoperiod (Exp. I)			Temperature (Exp. II)		Exp. III with bed biofilter
	8L:16D	12L:12D	16L:8D	Ambient	Elevated	
Temperature (°C)	16.4 ± 3.1	16.7 ± 2.8	16.5 ± 2.7	21.19 ± 1.4	23.66 ± 1.3	25.36 ± 0.69 °C
Dissolved oxygen (mg/L)	6.8 ± 0.9	6.5 ± 0.64	6.62 ± 0.56	6.68 ± 0.9	6.43 ± 0.73	6.55 ± 0.24
pH	7.9 ± 0.48	8.1 ± 0.34	8.1 ± 0.49	7.9 ± 0.41	8.1 ± 0.53	7.69 ± 0.32
Alkalinity (mg/L)	120.0 ± 5.7	116.2 ± 8.9	118.7 ± 4.7	127.0 ± 5.7	119.2 ± 6.2	72.2 ± 2.52
Total ammonia-N (mg/L)	0.076 ± 0.006	0.083 ± 0.04	0.073 ± 0.03	0.089 ± 0.025	0.091 ± 0.022	< 0.001
Nitrite (mg/L)	0.094 ± 0.02	0.083 ± 0.01	0.084 ± 0.01	0.097 ± 0.041	0.113 ± 0.05	0.05 ± 0.002
Nitrate (mg/L)	0.71 ± 0.23	0.74 ± 0.19	0.87 ± 0.24	0.76 ± 0.18	0.81 ± 0.21	10.43 ± 1.13
CO ₂ (mg/L)	16.1 ± 1.7	15.3 ± 2.1	15.8 ± 1.9	16.4 ± 2.2	16.8 ± 1.8	4.0 ± 0.52

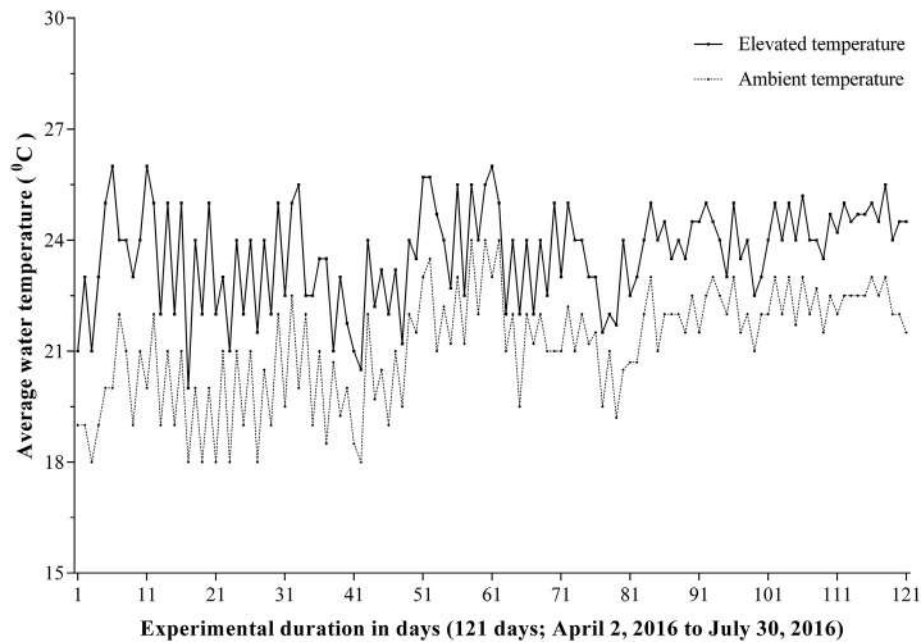


Fig. 2. Average daily water temperature profile of the treatment groups in Experiment II.

complete ammonia conversion and 7 days for complete nitrite reduction, whereas at 3rd addition, the TAN and NO_2 levels were reduced to zero within a day and this point was considered as the maturation of biofilter. A total of 5 adult golden mahseer (3♀s and 2♂s; average weight: 1.1 ± 0.12 and 0.82 ± 0.08 kg, mean $\pm$ SD respectively) were collected from cement cistern ponds of ICAR-DCFR, Bhimtal (which were wild collected and reared in these cemented ponds for over one year) and stocked into the experimental FRP tank. The water temperature was maintained at $25.36 \pm 0.69^\circ\text{C}$ using two thermostatic water heaters (300 W) submerged in the tank itself. Continuous aeration using air compressor as well as venturi system aeration of power heads was provided in the tank to ensure optimum dissolved oxygen level and the tank was covered with 8.0 mm mesh nylon net to prevent jumping out of fishes. An egg collection tray was provided using airlifting mechanism to indicate any spawning event. The tank was kept in the wet lab of ICAR-DCFR and a photoperiod of 12L:12D again was maintained using a 35 watt CFL bulb. Fishes were fed ad libitum twice daily (10.00 and 17.00 h) with the same ICAR-DCFR's formulated broodstock diet (Table 1). A water flow of 6–7L/h was also maintained in the tank to further ensure the quality of water. Approximately 60% water was exchanged through siphoning of bottom once every 15 days to remove organic matter, nitrate and maintain alkalinity. This pilot experiment was continued from May 2017 to January 2018.

2.2. Water quality analysis

Temperature was measured using digital thermo meter (MEXTECH-DT9, India) which was calibrated to mercury thermometer; dissolved oxygen was measured using Winkler's method (Himedia, Laboratories, Pvt. Ltd. India); Alkalinity and CO_2 using American Public Health Association (APHA) methodology (Himedia, Laboratories, Pvt. Ltd. India); pH was measured using HANNA pH meter (HI 98130, Mauritius); TAN, nitrite, nitrite were measured adopting microplate methodology. TAN was measured using salicylate method (Verdouw et al., 1977); nitrite was measured using Griess reagents; nitrate was estimated by reducing the nitrate to nitrite using vanadium chloride (Miranda et al., 2001).

2.3. Blood sampling

At the end of the experiments (experiment I and II), four females and four males from each treatment group were anesthetized using clove oil (50 $\mu\text{L/L}$ water). Body surface of the sedated fish (both males and females) were wiped with blotting paper. Blood was drawn by puncturing the caudal vein with 5 mL 22-gauge disposable medical syringe (HMD Ltd., New Delhi, India), previously rinsed with 2.7% EDTA solution as anticoagulant. The collected blood was gently shaken to prevent haemolysis and transferred to 1.5 mL centrifuge tubes. The tubes were maintained on ice and then centrifuged at 4000 rpm for 10 min at 4°C . The resultant plasma was collected in several aliquots and stored at -20°C until further analysis of various parameters.

2.4. Gonado-somatic index and biochemical assays

Gonado-somatic index (GSI) was calculated as per the equation: $\text{GSI} = [\text{gonad weight} / \text{total body weight}] \times 100\%$. Assessment of different hormones in the plasma samples (17 β -estradiol and 11-keto testosterone) was performed using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Cayman Chemical Company, Michigan, USA). Plasma 17 α , 20 β -dihydroxyprogesterone was analyzed using commercial ELISA Kit from MyBioSource, Inc. (San Diego, USA). All the assays were performed following the assay kit manufacturer's procedures. A standard curve was run for each ELISA plate and all the samples (biological replicates, $n = 4$) and standards were analyzed in duplicates (i.e. technical replicates, $n = 2$). All the absorbance was measured by using Eon-C multimode microplate spectrophotometer (Biotek, USA). The intra-assay coefficients of variance for all assays were $< 10\%$.

2.5. Gonadal histology

At the end of experiment II, tissue samples of ovary and testes were collected for histology. The ovary samples were fixed in bouin's fixative while testes samples were fixed in 10% neutral buffered formalin (NBF) solution. The samples for histological analysis were further processed in series of alcohols (70, 95, and 100%), xylene and paraffin and finally embedded in paraffin blocks as described by Sharma and Patino (2013). The samples were sectioned in cross section at 4 μm thickness and

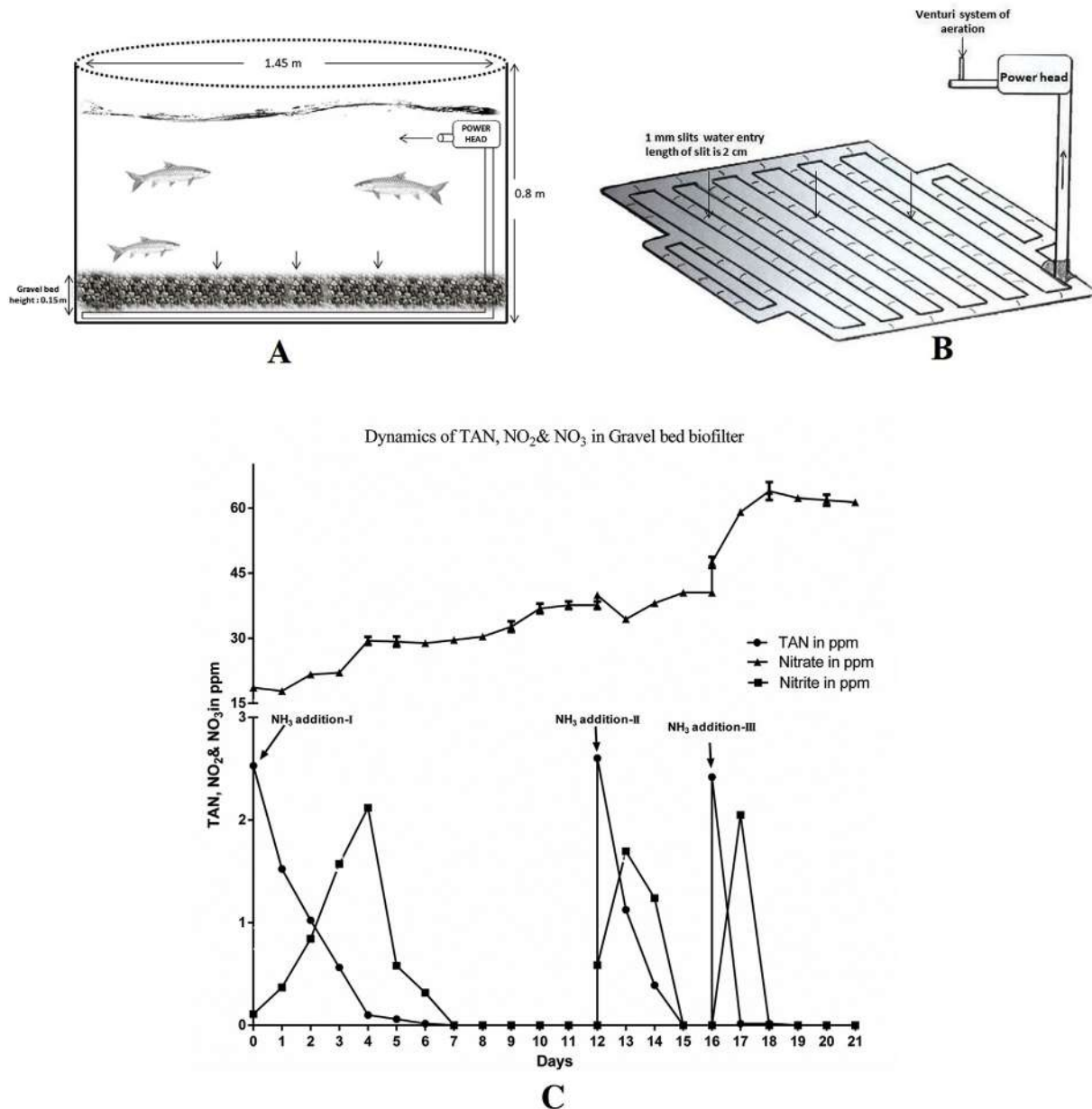


Fig. 3. A–B. (A) Gravel bed-biofilter installed circular FRP tank used in experiment III. (B) The schematic diagram of the bio-filter made of PVC pipes. Slits of 1.0 mm width and 2.0 cm length were made for entry of water. The 'arrows' indicate the direction of water flow through the gravel bed and pump. The distance between two parallel pipes was kept at 15 cm. (C) Efficiency of gravel bed biofilter (after an inoculation by adding a mug of gravel from a matured gravel biofilter) and dynamics of TAN, NO₂ and NO₃ in a fish less cycling. (11.59 g of NH₄Cl $\approx$ to 3 ppm of TAN in 1 m³ of water).

stained with hematoxylin and eosin. Microscopical observations were made with a compound microscope (Leica DM 2000) and photomicrographs were taken with a Leica digital camera (MC, 170 HD; Leica Microsystems, Wetzlar, Germany).

2.6. Statistical analysis

The values of all the measured parameters from individual fish (both males and females) of experiment I and II were subjected to two-way analysis of variance (2-WAY ANOVA) to study the difference between treatments groups in both sexes. Tukey's multiple comparisons were used to determine the significant differences between the means. Comparisons were made at 5% probability level. All the data were analyzed using statistical package SPSS (Version 19). Data are expressed as mean $\pm$ standard error of mean (SEM); $n = 4$.

3. Results

3.1. Experiment I: effect of photoperiod on gonadal maturity of golden mahseer in captivity

There was a significant ($p < .05$) difference in plasma 17 β -estradiol (E₂) and 17 α , 20 β -dihydroxy progesterone levels under different photoperiod regimes (Fig. 4A, B and Table 3). In females, darker photoperiod (8L:16D) significantly decreased the estradiol levels compared to 12L:12D and 16L:8D photoperiods. However, longer than 12 h illumination (16L:8D) had no effect on the levels of E₂. Males also registered the presence of E₂ (10–12% that of females) but did not change upon different photoperiod treatments. The level of plasma 17 α , 20 β -dihydroxy progesterone was significantly ($p < .05$) changed both in females as well as males due to different photoperiods. The highest level was observed at 12L:12D in females and 16L:8D in males, whereas the

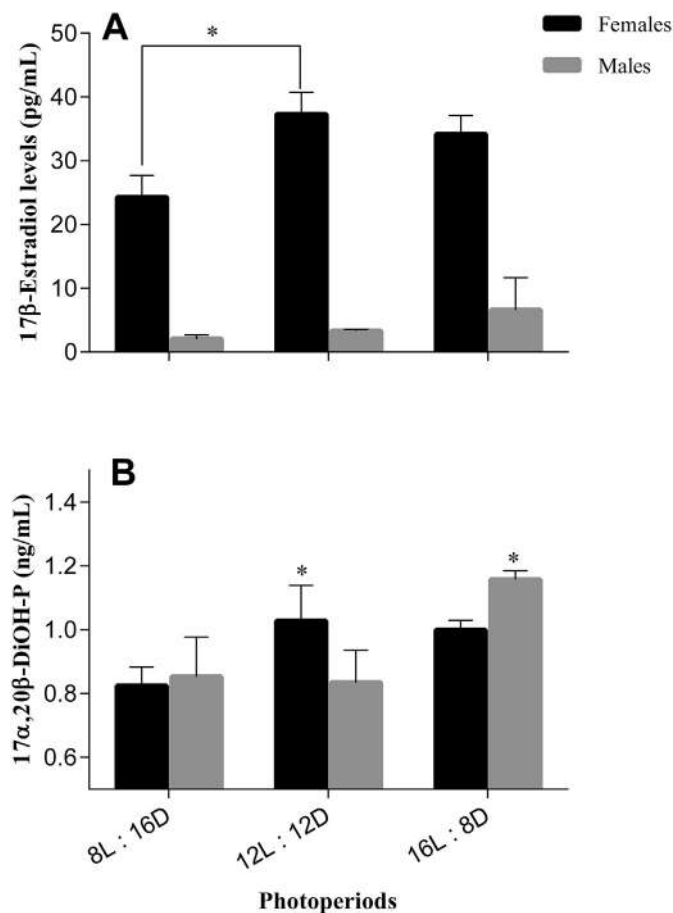


Fig. 4. Effect of different photoperiods on plasma (A) 17β-estradiol levels and (B) 17α, 20β-dihydroxy progesterone levels of golden mahseer (Experiment I). Data are expressed as mean $\pm$ standard error of mean; $n = 4$. Presence of asterisk (*) shows data are significantly ($p < .05$) different between treatments.

lowest level was found in darker photoperiod (8L:16D). Different photoperiodic treatments had no significant ($p > .05$) effect on 11-ketotestosterone and gonado-somatic index (GSI) both in females and males (Fig. 5A, B and Table 3).

3.2. Experiment II: effect of elevated temperature on gonadal maturity of golden mahseer in captivity

The level of 17β-estradiol in females was significantly ($p < .05$)

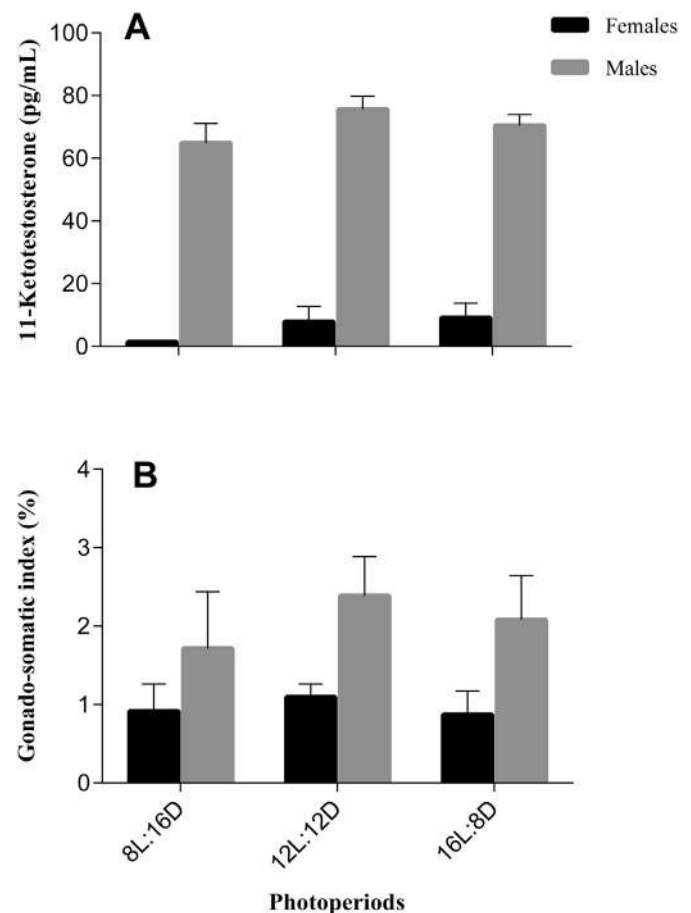


Fig. 5. Effect of different photoperiods on plasma (A) 11-ketotestosterone levels and (B) gonado-somatic index (GSI) of golden mahseer (Experiment I). Data are expressed as mean $\pm$ standard error of mean; $n = 4$.

increased ($\sim 95\%$) in elevated temperature group compared to its ambient counterpart (Fig. 6A and Table 3). But, there was no significant change in the levels of E_2 in males due to temperature. In females, the level of plasma 17α, 20β-dihydroxy progesterone was significantly ($p < .05$) increased ($\sim 135\%$) due to elevated temperature (Fig. 6B and Table 3). Males did not register any significant change in 17α, 20β-dihydroxy progesterone levels due to temperature treatment. No significant ($p > .05$) effect of temperature was observed on the plasma levels of 11-ketotestosterone of both males and females (Fig. 7A and Table 3). Importantly, gonado-somatic index of females significantly

Table 3

Two-way ANOVA analysis of various reproductive parameters estimated in golden mahseer, *T. punitora* broodstock reared under different photoperiods (experiment I) and different temperatures (experiment II).

Parameters	Two-way ANOVA analysis							
	Interactions (Exp. I)				Interactions (Exp. II)			
	Effect of photoperiod	Effect of sex	Photoperiod x Sex		Effect of temperature	Effect of sex	Temperature x Sex	
			Photo. x ♂	Photo. x ♀			Temp. x ♂	Temp. x ♀
17β-Estradiol	*	****	ns	*	****	**	ns	**
11-Ketotestosterone	ns	****	ns	ns	ns	****	ns	ns
17α,20β-DiOH-P	*	ns	*	*	**	ns	ns	**
Gonado-somatic index	ns	*	ns	ns	**	ns	ns	**

non significant-ns.

**** $p < .001$.

** $p < .05$.

* $p > .05$

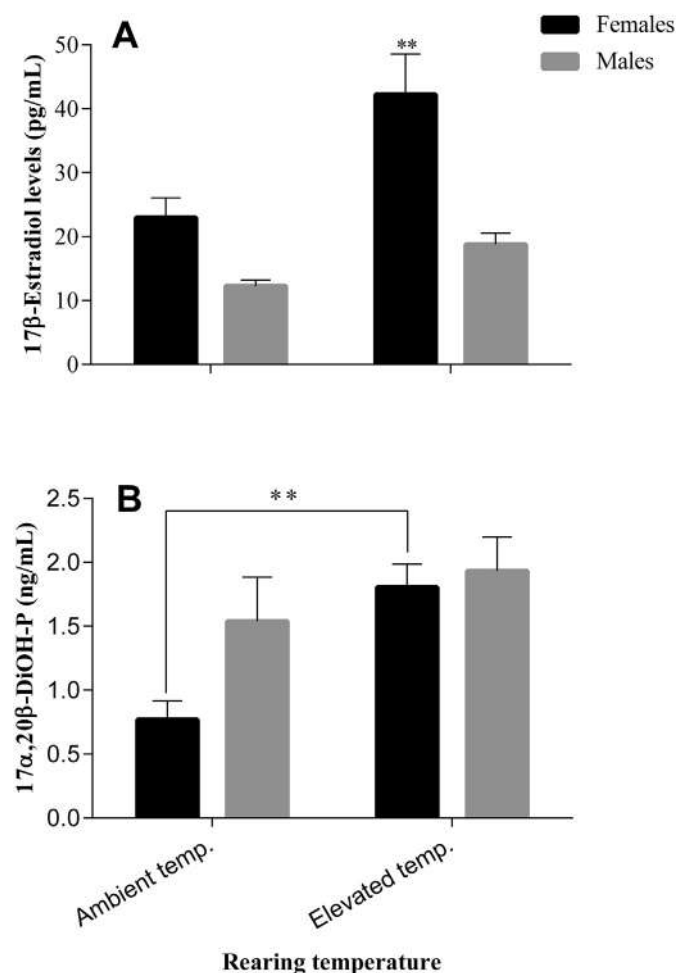


Fig. 6. Effect of different temperatures on plasma (A) 17β-estradiol levels and (B) 17α, 20β-dihydroxy progesterone levels of golden mahseer (Experiment II). Data are expressed as mean $\pm$ standard error of mean; $n = 4$. Presence of asterisk (*/**) shows data are significantly different between treatments.

increased (6-fold $\uparrow$) at elevated temperature compared to its ambient counterpart whereas GSI of males remained unchanged (Fig. 7B and Table 3).

Ovarian histology revealed that temperature had a profound effect on gonadal maturity of females in captive conditions. Histological photomicrographs of the ovaries of fishes reared in ambient temperature showed oocytes only up to cortical alveolar (CAO) stages while in ovaries of fishes reared at elevated temperature had vitellogenic (VO) and maturing oocytes (MO) where germinal vesicle was found migrating off the centre in addition to CAOs and perinucleolar oocytes (Fig. 8). However, we did not observe any evidence of germinal vesicle breakdown. On the other hand, there was no marked change found in testicular histology of fishes reared in either ambient temperature or at elevated temperature. The lobular region of testes at both temperatures was found to be with full of spermatozoa. However, there was presence of some macrophage aggregations (MA) on the efferent duct region of testes (Fig. 9) from fishes reared at elevated temperature.

3.3. Observations from a pilot experiment combining elevated temperature and optimum photoperiod in a tank installed with gravel bed-biofilter

As mentioned in Section 2.1.3, this pilot experiment using gravel bed biofilter and applying the optimum results of the earlier experiments (12L:12D photoperiod and elevated temperature) was meant to explore the possibility of achieving captive spawning by creating a

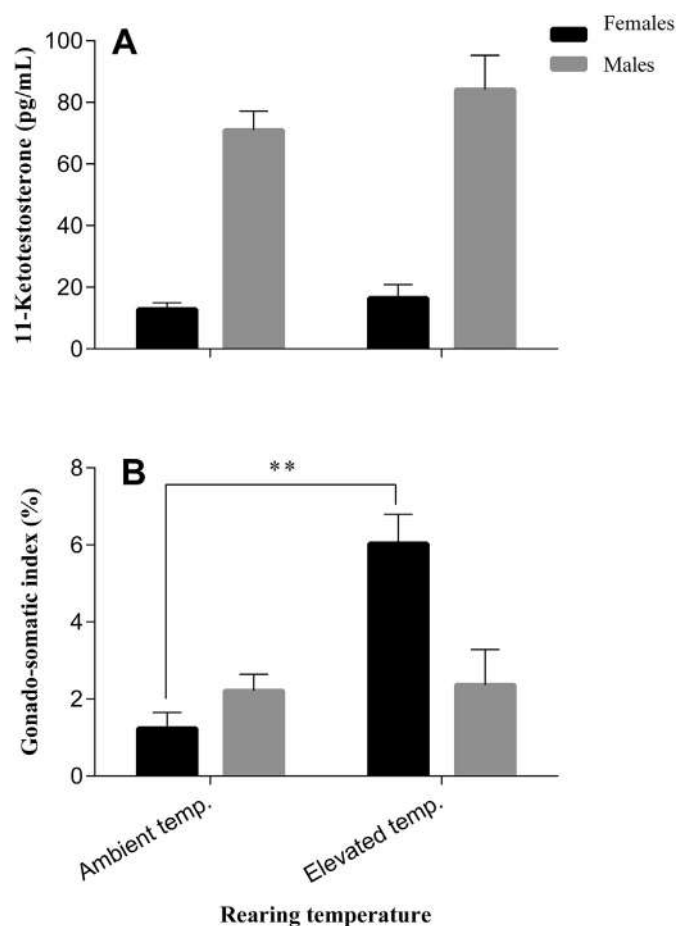


Fig. 7. Effect of different temperatures on plasma (A) 11-ketotestosterone levels and (B) gonado-somatic index (GSI) of golden mahseer (Experiment II). Data are expressed as mean $\pm$ standard error of mean; $n = 4$. Presence of asterisk (**) shows data are significantly different between treatments.

simulated spawning ground. On 19th September 2017 (after four months of rearing), we were able to retrieve 113 fry of golden mahseer from the tank. The fry were advanced and probably the spontaneous spawning might have taken place in the first week of September 2017 itself. We could not observe the first spontaneous spawning probably because the eggs were demersal and settled down at bottom and after fertilization became transparent making them difficult to see in the crystal clear water of the tank. The retrieved fry were kept in a glass aquarium having temperature similar to the tank from where they were retrieved and fed with exogenous chicken egg yolk twice daily and growth was monitored. On 19th September 2017, we injected ovotide (a GnRH analog) @ 0.5 mL/kg body weight to females and @ 0.15 mL/kg body weight to males just to see whether it responds to induced spawning or not. Twelve hours after injection, the brooders spawned. Some eggs were collected and incubated at 25.21 ± 0.55 °C and we studied the developmental events. Further, on 30th September 2017, we retrieved 132 fry and kept them in a thermoregulated aquarium for monitoring their growth. The incubation period was found to be 68–74 h and average length at hatching was 11.36 ± 0.54 mm (Mean $\pm$ SEM, $n = 6$). Yolk-sac absorption was completed 7–8 days after hatching. A linear trend ($Y = 3.2694 \times + 5.8565$; $R^2 = 0.9494$) of growth in length and a steep growth in weight was observed (Fig. 10). After 15 days of second (induced) spawning, brooders again spawned on 6th October 2017 without any hormone administration. The occurrence of further spontaneous spawning by the fishes was observed on 14th November, 3rd & 21st December 2017 and 7th January 2018. So far brooders spawned seven times spontaneously within a period of

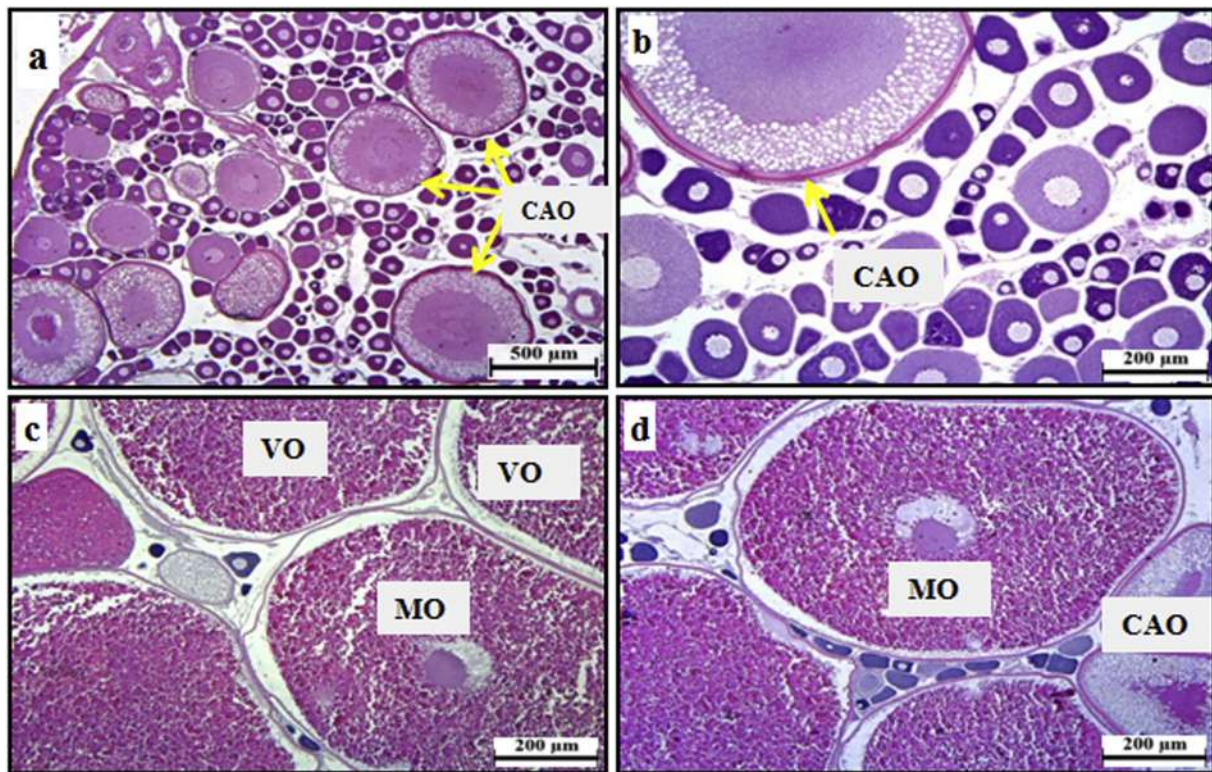


Fig. 8. Ovarian histology of golden mahseer: (a & b) and elevated temperature (c and d) (Experiment II). [CAO - cortical alveolar oocyte; VO – vitellogenic oocyte; MO – maturing oocyte].

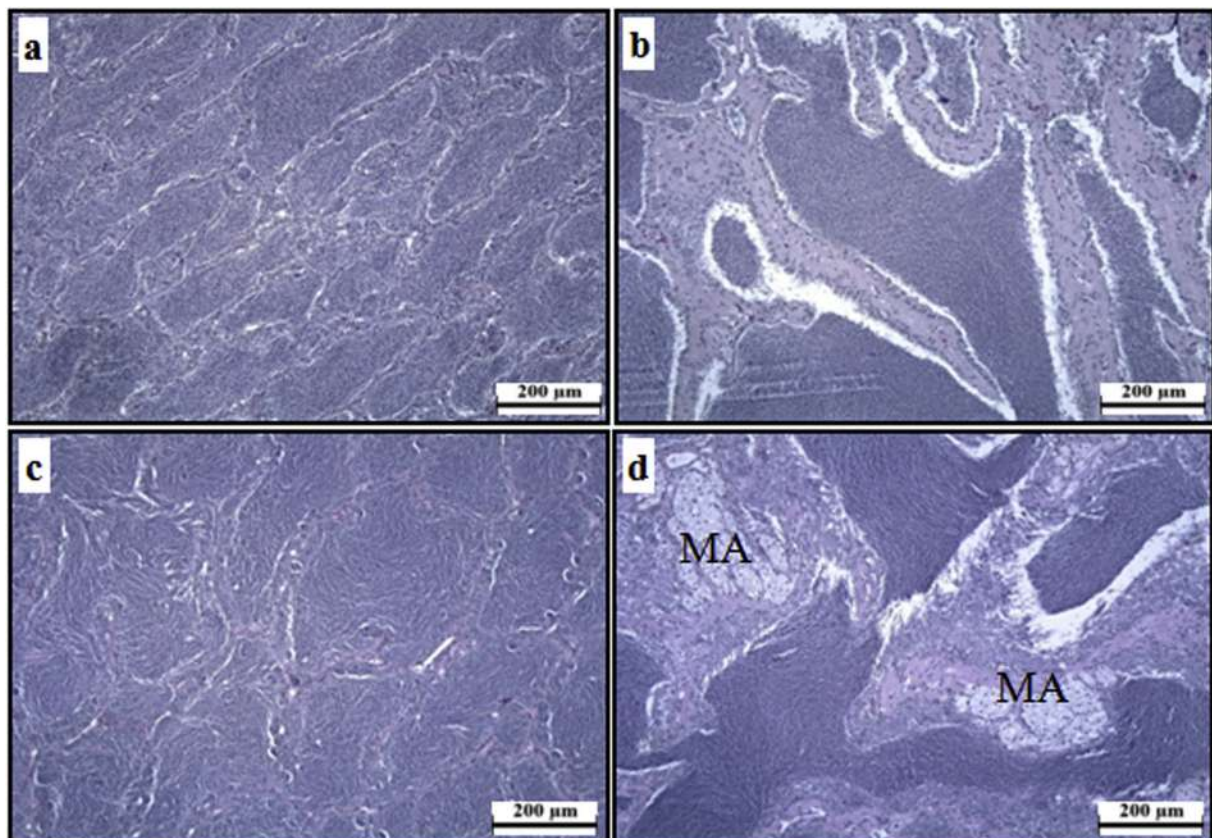


Fig. 9. Testicular histology of golden mahseer (Experiment II): Testes from fishes reared ambient temperature [lobular (a) and efferent duct region (b)] and elevated temperature [(c) lobular region and (d) efferent duct region; MA – macrophage aggregate].

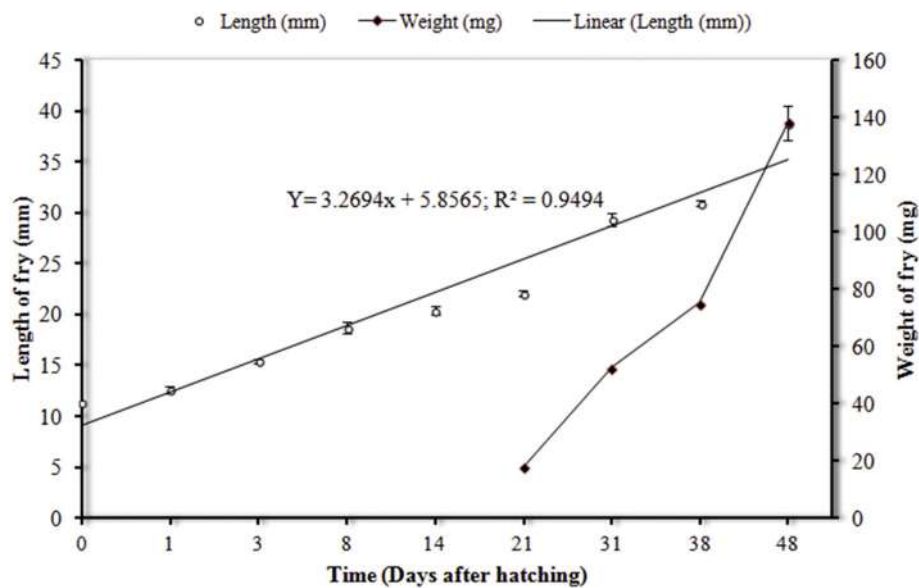


Fig. 10. Graphical representation of growth of captive spawned golden mahseer larvae/fry until 48 days after hatching when reared at $\sim 25^\circ\text{C}$. Data expressed as Mean $\pm$ SEM, $n = 6$ (Experiment III).

4 months on an interval of 15–20 days.

4. Discussion

Golden mahseer, *Tor putitora*, is an endangered cyprinid which has generated a lot of attention in the Trans-Himalayan countries not only due to its economic and recreational significance but also due to the alarming decline of its population. Its rehabilitation through stock enhancement/ranching has been advocated by various national and international bodies (FAO, 2006) besides other conservation measures. However, to make rehabilitation programmes to be wide-spread and effective, large scale seed production of golden mahseer using captive brooders is the immediate need. Despite several efforts made during the last four decades (Sehgal and Kumar, 1977; Tripathi, 1978; Pathani and Das, 1978; Sehgal, 1991; Pandey et al., 1998), the issue of achieving maturity and spawning using captive brooders of golden mahseer remained unsolved. The cited studies were mainly focused on achieving induced spawning either by hormonal administration or hypophysectomy and did not explore the reasons/factors responsible for reproductive dysfunction in captivity which probably would have been the first prerequisite to its captive maturation and breeding programme. In this direction, firstly, we investigated and reported the probable factors manifesting reproductive dysfunction of female golden mahseer in captive conditions (Akhtar et al., 2017). Following that, through the present series of experiments, we have attempted to explore the possibility of inducing maturity and spawning of golden mahseer in captive conditions through environmental manipulations.

As per our hypothesis that environmental cues such as temperature and photoperiod could influence gonadal development and maturation processes in golden mahseer, the reproductive indices analyzed in the present study were found to be modulated by photo-thermal manipulations. In females, darker photoperiod (8L:16D) significantly decreased E_2 levels compared to 12L:12D photoperiod, but > 12 h illumination (16L:8D) had no positive effect on the levels of E_2 (Fig. 4A; Experiment I). Corroborating our findings, shorter photoperiod has been reported to have suppressive /inhibitory effect on estradiol levels in many fish species (Ciereszko et al., 1997; Biswas et al., 2005; Dindoj and Macgregor, 1981) resulting in poor gonadal development or spawning response. Low levels of plasma E_2 have also been measured during the regressed stages of the sexual cycle due to shorter day length in *Mugil cephalus* (Dindoj and Macgregor, 1981), *Tilapia aurea*

(Terkatin-Shimony and Yaron, 1978), *Salmo trutta* (Billard et al., 1978), *Salmo gairdneri* (Van Bohemen and Lambert, 1981) and *Salmo salar* (Taranger et al., 1999). On the other hand, longer photoperiod or longer day length was found to enhance E_2 levels in hatchery-produced meagre, *Argyrosomus regius* in captivity (Mylonas et al., 2013a; Schiavone et al., 2012), in female yellow perch, *Perca flavescens* (Ciereszko et al., 1997) and in Eurasian perch *Perca fluviatilis* (Migaud et al., 2004), which we could not observe in the case of golden mahseer. In our second experiment, the plasma levels of E_2 in female brooders were significantly ($p < .05$) increased due to increase in temperature (Fig. 6A). Similar to our findings, E_2 levels were found in higher concentrations in *Argyrosomus regius* during warmer months compared to colder months leading to the development of the gonads under captive conditions (Mylonas et al., 2013). The elevated levels of E_2 in female golden mahseer reared at higher temperature suggested a higher degree of maturational competence. But it is to be noted that, higher temperature beyond the physiological limits is reported to be inhibitory on sex steroids production in many fish species (Van Der Kraak and Pankhurst, 1996; King and Pankhurst, 2007; King et al., 2003). Estradiol was also detected in males but in low concentration manifesting its role in spermatogenesis as reported in many teleosts (Miura and Miura, 2003; Chaves-Pozo et al., 2005). However, neither photoperiod nor temperature had any significant impact on E_2 levels of male golden mahseer.

The 17α , 20β diOH-progesterone (17α , 20β diOH-P) secreted by the ovarian follicles (Schulz et al., 2010) acts as a maturation inducing hormone in many fish species (Scott and Canario, 1992) and mediates final oocyte maturation (Vazirzadeh et al., 2014) in females. In males, 17α , 20β diOH-P promotes testicular hydration and spermiogenesis (Nagahama, 1994). Besides, 17α , 20β diOH-P also plays a significant role in the attainment of sperm motility in several teleosts (Nagahama, 1994). The secretion of 17α , 20β diOH-P in female golden mahseer was reported to be 2-fold lower in captivity than in wild and it was identified as one of the probable factor leading to its reproductive dysfunction in captive conditions (Akhtar et al., 2017). In the present study, highest plasma level of 17α , 20β diOH-P was observed at 12L:12D in females and at 16L:8D in males, whereas the lowest level was found in shorter photoperiod (8L:16D) (Fig. 4B, Experiment I). On the other hand, in females, the level of plasma 17α , 20β diOH-P was significantly ($p < .05$) increased ($\sim 135\%$) due to elevated temperature (Fig. 6B, Experiment II). Males did not register any significant

change in 17α , 20β diOH-P levels due to temperature difference. Corroborating the present results, longer photoperiod and higher temperatures were found to elevate 17α , 20β diOH-P concentration in many fish species (Van Der Kraak and Pankhurst, 1996; Wang et al., 2006; Wang et al., 2010; Mylonas et al., 2013) and resulted in advancement of gonadal development and maturation process.

Neither photoperiod nor temperature had any significant effect on the plasma 11-ketotestosterone (Fig. 5A & Fig. 7A) levels of golden mahseer males and females. But, 11-KT concentration in males were several fold higher than in females underlining the fact that 11-KT is the main reproductive hormone in males which regulates the process of spermatogenesis (Schulz et al., 2010), stimulates sertoli cell function, promotes spermatogonial proliferation (Miura and Miura, 2003) and underlies secondary sexual characteristics in males (Lintelmann et al., 2003). In females, 11-KT reportedly has positive effect on growth and development of pre-vitellogenic oocytes in Atlantic cod (Kortner et al., 2009), short finned eel (Lokman et al., 2007) and coho salmon (Forsgren and Young, 2012). Under our culture conditions, the male golden mahseer easily matures in captive conditions (Akhtar et al., 2017) and are found spermiating round the year (personal observation).

Nevertheless, in the present study (in experiment II), the gonadosomatic index (% GSI) of females increased by 6-fold when reared at elevated temperature while the GSI of males remained unchanged (Fig. 7B). This phenotype (highest GSI observed in females maintained at elevated temperature) was apparently due to both oogonial proliferation and accumulation of yolk in many oocytes (Yaron, 1995) as evidenced in our histological observations. Further, this corresponds to the fact that the GSI of golden mahseer in their natural habitat was found highest during the months of June–August when day length and water temperature were higher than the other months (Nautiyal, 1984).

In fish, several distinct stages of development occur during the complete maturation of the oocyte. Oocytes undergo pre-vitellogenic and vitellogenic growth phases when the oocytes grow in size, develop follicular layers and accumulate yolk (Goetz, 1976). Within the range of physiologically optimum temperature, higher temperatures usually speed up the process of gonadal development and oocyte maturation without any adverse effects (Mylonas et al., 2010). In the present study (Experiment II), increase in temperature had profound effect on the development of ovary in females, thus imparting maturity in captive conditions which was also evidenced by the 6-fold increase in GSI. Histological analysis of ovaries of fishes reared in ambient temperature revealed the presence of oocytes only up to cortical alveolar stage while the ovaries of fishes reared at elevated temperature had vitellogenic and maturing oocytes in addition to cortico- alveolar and perinucleolar oocytes (Fig. 8). Vitellogenic phase of oocytes is followed by the migration of the germinal vesicle which is considered as the indicator of maturation process (Goetz, 1976). In the present study, germinal vesicles of many oocytes were found migrating off the centre confirming that elevated temperature induced maturity in female golden mahseer. However, we did not observe any evidence of germinal vesicle breakdown which is otherwise the sign of final oocyte maturation in fish (Mylonas et al., 2010; Yaron et al., 2003) and occurs when there is LH surge (Nagahama, 1994; Gen et al., 2003). As a result, we did not observe any ovulation/spontaneous spawning at elevated temperature (in experiment II) and the lack of LH surge could have been the probable reason behind constraints in attaining the final oocyte maturation. On the other hand, there was no marked change found in testicular histology of fishes either reared in ambient temperature or at elevated temperature. The lobular region of testes at both temperatures were found with full of spermatozoa. However, there was presence of some macrophage aggregations on the efferent duct region of testes (Fig. 9) from fishes reared at elevated temperature. Macrophages migrate to the infection sites and phagocytose the pathogens besides, modulation of immune response for optimum organ functioning and regeneration. Residual spermatozoa are resorbed in the testis itself where they are phagocytosed by sertoli cells and by some macrophages (Billard, 1986;

Billard et al., 1982). Although males of golden mahseer mature well in captivity and remain in oozing condition throughout the year (personal observation), the elevated temperature in the present study probably have accelerated the process of spermatogenesis. The presence of macrophage aggregates at elevated temperature suggests that the higher amount of residual spermatozoa, as a result of accelerated spermatogenesis, was resorbed by these macrophage aggregates. However, the exact role of macrophage aggregates in male golden mahseer needs further investigation. In other fishes, temperature is reported to accelerate spermatogenesis and spermiation in fish (Gillet et al., 1981; Vilela et al., 2003; Lacerda et al., 2006).

With an optimum photoperiod of 12L:12D (inferred from experiment I), increase in temperature (experiment II) successfully induced maturity in golden mahseer especially in females under captive conditions. However, final oocyte maturation/spontaneous spawning did not occur in the prevailing conditions of the experiment II. It is reported that golden mahseer is a lithophil and in wild (streams, lakes and rivers), preferably spawns on shallow and marginal grounds of gravels and sandstones (Kulkarni and Ogale, 1978; Nautiyal, 1984; personal observation). Moreover, rise in temperatures along with the presence of suitable spawning substratum is reported to stimulate final oocyte maturation and ovulation in cyprinids (Stacey, 1984). Therefore, based on the findings of earlier experiments (I and II) and their natural preference, we explored the possibility of captive spawning of golden mahseer using a FRP tank installed with gravel bed-biofilter (experiment III). The gravel bed bio-filter served as a simulated spawning substratum and supplied good quality water with controlled water temperature. After four months of raising the brooders in the FRP tank with gravel bed-biofilter, 113 fry of golden mahseer were retrieved from the tank, probably 2 or 3 weeks after the spontaneous spawning which happened unnoticed. Following which, we intramuscularly injected ovotide (a GnRH analogue) to females and males @ 0.5 and 0.15 mL/kg body weight respectively. Again, the brooders spawned after 12 h of injection. This suggested that induced spawning through hormonal injection could be successful after the brooders attain maturity through photothermal manipulations under conducive captive conditions. After 15 days of second (induced) spawning, brooders again spawned spontaneously without any hormone administration. Subsequently, spontaneous spawning occurred seven times within four months on an interval of 15–20 days. The embryonic development, incubation period and growth of fry (Fig. 10) were faster at $25.21 \pm 0.55^\circ\text{C}$ compared to growth and development at ambient temperature (Sharma et al., 2016; Sarma et al., 2016a, 2016b). Yolk-sac absorption was also faster and completed 7–8 days after hatching than 11–12 days at ambient ($22\text{--}23^\circ\text{C}$) temperature (Sharma et al., 2016). The fecundity, fertilization rate and hatching rate are not reported here as it was not possible to know how many females participated in these spontaneous spawning events. Moreover, all the fertilized eggs in the tank remained hidden in the inter-crevices of the gravels at the bottom due to its demersal nature and suction pressure through gravel due to power head pump. After fertilization and water hardening, eggs became transparent making it invisible in the water of the tank and it was difficult to retrieve all of them. Hence, we are unable to present the true picture of fecundity, hatching rate, survival rate etc. in this report. Based on these positive observations, we plan to conduct further systematic breeding trials with scope to observe the spawning events under water.

5. Conclusion

Overall, the results of the present study suggest that 12L:12D could be considered as the physiologically optimum photoperiod as evidenced by the elevated levels of 17β -estradiol and 17α , 20β -diOH-P. Maturity enhancing effect of longer photoperiod than 12L:12D is ruled out while shorter photoperiod is proven to be unfavourable. Moreover, 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. It is further concluded that the gonadal maturity and spontaneous spawning of golden mahseer in captivity can be achieved by photothermal manipulations coupled with the provision of substratum and clear water conditions as in the case of experiment III. Further studies are needed to resolve several issues such as poor egg retrieval, repeated spawning with short intervals and amenability to stripping procedures to maximize the availability of viable offsprings. Finally, the present four-year 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. Further, this study have the potential of making the golden mahseer seed production technology sustainable, viable and cost-effective by avoiding dependence on wild brooders as well as the potential of reversing the population status of golden mahseer from 'endangered' to 'abundance' in future.

Conflict of interest

There is no conflict of interest of authors to declare.

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Project: AQ-21	Coldwater fish nutrition and feed development
Sub project 2:	Nutritional intervention for improving reproductive competence and larval quality traits of golden mahseer, <i>Tor putitora</i> in captivity
Period:	April 2020 – March 2023
Personnel:	Ciji, A. (PI), M.S. Akhtar, B.S. Kamalam, Rajesh, M., P. Sharma
Funding Support:	Institutional, ICAR-DCFR

The technology for mass scale seed production of endangered golden mahseer using captive broodstock has recently been developed at ICAR-DCFR, Bhimtal. However, the reproductive competence in terms of reproductive output (fertilization, incubation time, and hatching rates), egg quality (egg size increase during hardening, biochemical composition, carotenoid content, and ovarian fluid characteristics) and larval fitness traits (growth, micro-anatomical development, survival, starvation response, and critical thermal tolerance) of progeny from captive-matured golden mahseer brooders is relatively less in comparison with wild-collected brooders, which may be linked to broodstock nutrition as reported in several other species. In this milieu, an improved broodstock diet has been formulated and prepared by incorporating some nutrients/additives to the existing broodstock diet to evaluate the reproductive performance and larval quality.

were fed with a control and a test diet in captive maturation units (FRP tanks) installed with gravel bed-biofilter at the mahseer hatchery complex. Feeding of test diet notably improved fertilization and hatching rates, egg and sperm quality as well as larval survival. Further, plasma, gametes (egg and milt), and larval samples have been collected and various biochemical and gene expression analyses are in progress to elucidate the effects of supplements on brooder's reproductive competence and larval fitness.



Fig.: Experimental Setup

For that, triplicate groups of golden mahseer adults (♀: 0.9 – 1.2 kg; ♂: 0.3 – 0.7 kg body weight)



Fig.: Eggs of brooders fed with control diet



Fig.: Eggs of brooders fed with test diet



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Tailored broodstock diet fortified with functional nutrients enhances reproductive efficiency and larval quality traits of *Tor putitora*, an endangered cyprinid

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ABSTRACT

The effects of a tailored broodstock diet (containing certain amino acids, vitamins, fish oil, immunostimulant and pigment) on golden mahseer (*Tor putitora*) reproductive efficiency, gamete quality, and offspring fitness were investigated. For that, triplicate groups of golden mahseer adults (female: 0.8–1.3 kg, $n = 6$; male: 0.5–1.1 kg, $n = 3$) were fed with either of two diets - control and test diet. Broodstocks fed the test diet showed improved reproductive outcomes, such as a marginal increase in fecundity and a significantly greater fertilization rate, in comparison to control diet. The incubation period and hatching were unaffected by the broodstock diet; however, eggs from test diet-fed broodstocks had a shorter hatching window. Survival of larvae from these broodstocks was significantly higher. Egg quality assessment showed that broodstocks fed test diet produced significantly larger eggs with higher carotenoid content. Male broodfish fed the test diet showed significant improvement in milt quality parameters, viz. milt volume, sperm count, spermatocrit, and sperm viability, with decreased sperm abnormalities. Although there were no significant changes in seminal plasma protein, glucose, or triglyceride levels, the total antioxidant capacity was significantly higher, and thiobarbituric acid reactive substances (TBARS) were lower in the test diet group. In females that were fed the test diet, plasma 17 β -estradiol and vitellogenin were significantly elevated. At 75 days after hatching, larvae from broodstocks fed with the test diet showed improved growth and survival and elevated critical thermal maximum. These results underscore the promising benefits of tailored broodstock nutrition in enhancing reproductive success and offspring performance in golden mahseer.

1. Introduction

Golden mahseer, *Tor putitora* (Hamilton, 1822), often referred as the 'king of game fish' is a flagship coldwater fish species of the Trans-Himalayan region and a cultural icon of Asia with significant recreational, commercial, and conservational values (Dinesh et al., 2010; Akhtar et al., 2014; Nautiyal, 1984). Unfortunately, in the recent past, this species has suffered a steep population decline,

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mostly driven by anthropogenic activities, climatic variables, and certain inherent biological traits. Consequently, the golden mahseer has been categorised as 'Endangered' in the IUCN (International Union for Conservation of Nature) Red List of Threatened Species (Jha and Rayamajhi, 2010). Hence, considering its precarious status, concerted efforts by researchers, academicians, and policymakers primarily centred on its conservation, with strategies focusing on artificial breeding, seed rearing and subsequent ranching into its natural habitats. The efficacy of such conservation programmes largely depends on the hatchery production and availability of high-quality juveniles. In this direction, ICAR-Central Institute of Coldwater Fisheries Research, Bhimtal, India, has recently made commendable advancements in the captive maturation, breeding and seed rearing technologies of golden mahseer through environmental manipulations, warding off the major reproductive dysfunctional constraints in females under captivity (Akhtar et al., 2017, 2018, 2021a, 2022).

Despite these advancements, the reproductive and hatchery output of captive-matured golden mahseer broodstocks remain comparatively lower than those of wild-caught broodstocks (Ciji et al., 2022), a trend usually noticed across several other species as well. More specifically, spawning performance (fertilization and hatching success), egg quality (size, biochemical composition, carotenoid content, incubation time), and larval performance (growth, micro-anatomical development, survival, fitness, and tolerance to stress) were noticeably lower in captive-matured broodstocks than their wild counterparts (Ciji et al., 2022). Factors such as inadequate broodstock nutrition and confinement-induced stress are likely to contribute to reduced reproductive performance in captivity.

Undoubtedly, broodstock conditioning/preparation through high-quality diets has been found to improve reproductive efficiency, hatchery output, and offspring performance while reducing age at first maturity in numerous fishes (Engdaw and Geremew, 2024; Izquierdo et al., 2001). Several nutrition-related aspects, such as feeding duration and ration, nutrient levels and composition, have been shown to influence various reproductive parameters (Izquierdo et al., 2001; Watanabe and Vassallo-Agius, 2003; Hachero-Cruzado et al., 2009). Different nutrients and additives, such as proteins and amino acids (Rønnestad et al., 2003; Moran et al., 2007; Izquierdo et al., 2001), long chain fatty acids and phospholipids (Kottmann et al., 2020; Fernandez-Palacios et al., 1995; Luo et al., 2017), vitamins (Blom and Dabrowski, 1995; Furuita et al., 2003; 2009; Izquierdo et al., 2001; Santiago and Gonzal, 2000), carotenoids (Watanabe and Vassallo-Agius, 2003), immunostimulants and probiotics (Akhtar et al., 2021b; Ciji et al., 2024; Nargesi et al., 2020; Nargesi and Falahatkar, 2023) have been linked to several reproduction-associated processes, including fecundity and gonadal maturation, gamete quality, breeding performances, hatchability, and offspring quality (Sangsawangchote et al., 2010).

Further, the nutrients present in the yolk-sac, which are transferred from broodstock to the embryos and larvae, are shown to influence the quality of the progeny by supporting their development during the endogenous feeding phase, and hence the hatchery output is invariably influenced by the broodstock diet (Kerrigan, 1997; McCormick, 2003). The nutrient reserves within the yolk-sac play a central role in fish such as golden mahseer, which undergo prolonged pre-hatch development and extended endogenous feeding phases (Ciji et al., 2022). The composition and richness of the yolk content greatly determine their development, early growth, survival and fitness. Nevertheless, the quality of the embryo and larvae plays a crucial role in shaping their growth and fitness in later life stages (Kjørsvik et al., 2003). In sterlet sturgeon (*Acipenser ruthenus*), larval growth was found to be significantly influenced by broodstock diet (Saramah et al., 2019), although studies on the broader effects of broodstock nutritional history on offspring performance in fish remain limited.

In this study, we hypothesise that the reduced reproductive performance of golden mahseer in captivity may be partially attributed to sub-optimal broodstock nutrition. Further, being a batch spawner that spawns multiple times in a year (Akhtar et al., 2018, 2021a), nutritional intervention could be an effective approach to address the current issues with the captive breeding performance of golden mahseer. The present study aimed to develop and evaluate a broodstock diet enriched with a blend of nutrients and functional additives to enhance the reproductive output traits and progeny quality. This study was also intended to understand the effect of maternal dietary background on the overall performance of the offspring.

2. Materials and methods

2.1. Ethical statement

The fish handling and experimentation procedures were carried out in accordance with all applicable international, national and the institutional guidelines. The Animal Care and Use Committee of the ICAR-CICFR approved the protocols for fish maintenance and handling during experiments (Approval No. DCFR/IACUC/12/06/2022/2).

2.2. Broodstock management and experimental set-up

Fifty-four adults of golden mahseer were collected from the earthen ponds and randomly distributed into six captive maturation units (two dietary treatment groups in triplicates; nine fish (six females and three males) in each replicate tank with a total of twenty seven fish (18 females and 9 males) per treatment) made of round fibre reinforced plastic (FRP) tanks (1000 L capacity) at the mahseer hatchery of ICAR-Central Institute of Coldwater Fisheries Research (ICAR-CICFR), Bhimtal. Every replicate tank was stocked with six females (0.8 – 1.3 kg body weight) and three males (0.5 – 1.1 kg) of golden mahseer. A gravel bed-biofilter was installed in each maturation unit/tank, and continuous aeration was ensured. The desired water temperature (25°C) for maturity induction and breeding (Akhtar et al., 2018; 2021a) was maintained using a submersible thermostatic water heater (1000 W). A photoperiod of 12 L:12D (1200 ± 80 lux) was maintained using LED bulbs (Empire Electrotech, Ahmadabad, Gujarat, India). The tanks were cleaned and partial water exchanges were performed periodically to ensure optimum water quality, and the physico-chemical characteristics of

water were checked (Multiparameter probes, YSI, USA) at regular intervals. The source of water for the experiment was from borewell (ground water) and a flow rate of ~300 mL/minute was maintained in each tank. To prevent the fish from escaping, a nylon net was placed over the tanks. The fish were acclimatized in the unit for 15 days, and then the experiment was continued for 180 days. Rearing of the broodstocks was done following the standardized captive maturation protocols (Akhtar et al., 2018; 2021a).

2.3. Experimental diets and feeding

Golden mahseer broodstocks were fed two distinct experimental diets: a control (basal) diet and a test diet enriched with a specialized nutritional supplement blend, incorporating the basal diet plus selected amino acids, essential vitamins, fish oil, immunostimulant, and natural pigment. The ingredients and proximate composition of the experimental diets (6 mm pellet) are given in Table 1. Fish were hand-fed the respective diets *ad libitum* twice a day (09:30 and 17:00 h) for 180 days.

2.4. Captive breeding

Once the broodstocks were matured (usually after 90 days in the maturation system), male broodfish were separated to avoid natural spawning. The female broodfish were periodically monitored, with particular attention given to the abdomen to assess the presence of ripened eggs. Gravid broodfish with ripened eggs were removed from the maturation tank, and the eggs were subsequently collected through dry stripping. Six breeding sets from each dietary treatment group, each consisting of 12 golden mahseer broodfish (six female and six males), were selected for the comparative evaluation of egg quality, reproductive efficiency and larval/offspring performance.

2.5. Fertilization and early development of eggs

Before stripping, the urogenital area of the fish was cleaned with tissue paper to prevent gamete contamination. For egg stripping, one person held the female brood fish with its body arched towards the dorsal side, and another person gently pressed the belly with thumb and index fingers, moving from the anterior side towards the vent. This allowed the ripened eggs to be easily released into a

Table 1

Ingredients and proximate composition of broodstock diets (% dry weight basis) fed to golden mahseer brooders during the experimental trial.

Ingredients	% inclusion	
	Control	Test
Fish meal ^a	41.50	41.50
Fish protein hydrolysate	1.50	1.50
Soybean meal	6.00	6.00
Fermented Soybean meal	10.00	10.00
Rice distillers dried grains	5.00	5.00
Dry acetes	5.00	5.00
Wheat flour	23.40	22.08
Fish oil	2.00	4.00
Vegetable oil	2.00	0
Lecithin	2.50	2.50
Vitamin pre-mix ^b	0.20	0.20
Mineral pre-mix ^b	0.20	0.20
Preservative	0.20	0.20
Binder	0.50	0.50
Supplement mix^c		
Vitamin C	0	0.05
α-tocopherol acetate	0	0.05
Retinyl acetate	0	0.002
Folic acid	0	0.025
L-tryptophan	0	0.50
DL-methionine	0	0.30
Thiamine hydrochloride	0	0.025
Pyridoxine HCl	0	0.025
Yeast extract	0	0.3
Astaxanthin (Carophyll pink)	0	0.05
Proximate analyses (%)		
Moisture	6.30	6.45
Dry matter	93.70	93.55
Crude protein	42.25	42.03
Crude lipid	10.09	10.16

^a Janatha Fish Meal and Oil Products, Karnataka, India.

^b ICAR-CICFR and Growel Feeds Pvt. Ltd., India.

^c Himedia, India.

bowl. Egg fertilization was then carried out by directly pouring milt from an oozing male from the same dietary treatment onto the eggs (2 mL of milt into 100 mL of eggs) by putting light pressure on the male brood fish's abdomen. The mix of eggs and milt was gently swirled and allowed to rest for ten minutes. Subsequently, the excess milt was removed by washing the eggs (3–4 times). After adding fresh tap water to keep all the eggs submersed, the bowl was covered and left in the shade (30 min) for water hardening of eggs. Subsequently, they were shifted into egg incubators (kept in a flow-through FRP trough) for further development. The incubation of eggs was carried out at 23.0 ± 0.5 °C. The fertilized eggs from each breeding set were maintained separately. Different performance indicators such as fertilization rate (%), incubation time (hour), hatching rate (%), hatching window (time interval between hatching initiation to its completion in hours), and survival rate till completion of yolk sac absorption (%) were noted.

2.6. Egg size (diameter) measurements and biochemical analysis

The diameter of the unfertilized and water-hardened eggs at different intervals was measured using a stereozoom microscope (Leica S9i, Germany). The egg diameter during water hardening at 30, 60, and 120 min was determined by taking egg sub-samples ($n = 15$) from the incubator onto a petri dish and then measuring their size under a stereozoom microscope.

Following the manufacturer's instructions, commercial kits (Span Diagnostics, India) were used to measure the glucose and protein content of unfertilised eggs. For that, the egg (~ 50 numbers) was homogenized in distilled water. The total carotenoid content of eggs was extracted following the [Torrissen and Naevdal \(1984\)](#) method using acetone as the solvent and then computed following [Oliveira et al. \(2010\)](#).

2.7. Stripping of golden mahseer male broodfish for sperm quality assessment

Six males from each dietary treatment ($n = 6$) were used for milt collection at the end of the experiment. To prevent contamination of the milt, the fish's urogenital area was cleaned with tissue paper. Then the milt from each broodfish was collected into two sets of 1.5 mL centrifuge tubes by putting light pressure on the broodfish's abdomen. After stripping, the fish were placed back in their tanks. Once milt was collected, the tubes were put on ice and immediately taken to the laboratory for seminal plasma collection and evaluation of sperm quality. Following a 10-minute centrifugation of milt samples (4 °C at 8000 $\times$ g), the resultant supernatant (seminal plasma) was collected in fresh centrifuge tubes and stored at -20 °C until analysis.

A hemocytometer was used to count the number of sperm. First the sperm cells were fixed in a solution of formol-citrate (1:2000; milt: fixative) for 10 min. The formol-citrate was made using trisodium citrate dihydrate (2.9 g) and formaldehyde (35 % v/v; 4 mL) in distilled water to 100 mL. Afterwards, the formol-citrate preserved sperm (10 μ L) was placed into the hemocytometer (in triplicates/fish), and were counted under a light microscope (40X). Sperm cells in ten Neubauer chambers of the hemocytometer were counted. The sperm viability was determined according to [Viveiros et al. \(2012\)](#). In short, 40 μ L of a dye solution containing eosin (5 %) was carefully mixed with fresh milt (10 μ L), and smear slides were made in triplicate and examined under a light microscope. Red or pink-stained sperm cells were regarded as dead, whilst unstained cells were counted as alive. From each slide, one hundred spermatozoa were counted, and viability % was calculated. To assess the sperm abnormality index (sperm morphology), the formol-citrate fixed sperm (15 μ L) was mixed with 0.5 μ L of Rose Bengal (3 %) stain on a glass slide (in triplicates), and the resultant mixture was gently dispersed with a pipette tip to prevent mechanical damage. After being allowed to air-dry, the slides were examined under a microscope (100X). From each slide, one hundred spermatozoa were examined, and the number of abnormal spermatozoa was counted, and the % abnormality was determined. Abnormal sperm were identified based on [Maria et al. \(2012\)](#).

2.8. Analysis of seminal plasma

Seminal plasma glucose, protein and triglyceride were measured using commercial kits (Span Diagnostics, India), following the manufacturer's instructions. Seminal plasma antioxidant capacity (measured as μ M Trolox equivalents) and thiobarbituric acid reactive substances (TBARS; measured as μ M Malondialdehyde-MDA) were assessed using analytical kits from HiMedia Laboratories, India, as per the manufacturer's procedures.

2.9. Measurement of plasma sex steroids and vitellogenin

Six males and six females ($n = 6$) per treatment group were collected randomly and sedated using clove oil (100 μ L clove oil per liter of water). Instantly, blood was drawn from the caudal vein (24-gauge needle rinsed with 2.7 % EDTA), transferred to centrifuge tubes (1.5 mL) and then centrifuged at 4000 rpm (10 min, 4 °C). The resulting plasma was collected and stored at -20 °C for sex steroid and vitellogenin measurement. Plasma 17 β -estradiol (from female fish only) and 11-keto testosterone (11-KT; from male fish only) were quantified using commercial enzyme-linked immunosorbent assay (ELISA) kits from Cayman (Michigan, USA), while 17 α , 20 β -dihydroxyprogesterone (17 α , 20 β DHP; from both male and female), and vitellogenin (from female only) were measured using ELISA kits from MyBioSource Inc. (San Diego, USA). All the assay were performed following manufacturer's guidelines, and undiluted plasma samples were used. For every microplate, a standard curve was generated, and all the samples ($n = 6$) and standards were analyzed in duplicates in an Eon-C (Biotek, USA) multimode spectrophotometer. The intra and inter-assay coefficients of variation were less than 10 %.

2.10. Larval/offspring performance (growth, survival and thermal tolerance)

The larvae produced from broodstocks of different dietary treatment groups were raised separately in rectangular FRP tanks (220.0 cm×50.0 cm×40.0 cm), and growth was checked by weighing the larvae in batches (each batch contained 15 larvae). After yolk sac absorption, the larvae were initially fed macerated goat liver (30 days), followed by a formulated diet (45 % crude protein and 12 % lipid). In the larval rearing tanks, a gentle water flow (250–300 mL/minute; sourced from borewell) was maintained, and aerators were provided to maintain optimum dissolved oxygen. Water quality was monitored periodically and was found to be within the safe range for cyprinids. A multi-parameter water testing apparatus (HQ40d, Hach, USA) was used to measure temperature, dissolved oxygen and pH, while commercially available kits (HiMedia, India) were used to measure alkalinity, ammonia and nitrite. The water quality parameters were in the following range: dissolved oxygen 5.6–6.5 mg/L; temperature 21.6–22.4 °C; pH 6.6–7.1; alkalinity 70–80 mg/L; ammonia < 0.01 mg/L, and nitrite < 0.1 mg/L.

The thermal tolerance (critical temperature minimum – CT_{min} and critical temperature maximum - CT_{max}) of golden mahseer fry produced from broodstocks under different dietary treatment groups were measured following the standard critical thermal methodology (Beitinger et al., 2000; Ford and Beitinger, 2005). Thirty fry (75 dph) each from different dietary treatments ($n = 15$ fry each for CT_{min} and CT_{max}) were randomly selected. For CT_{min} determination, 15 fish were placed in a circular basin (35 L) attached to an

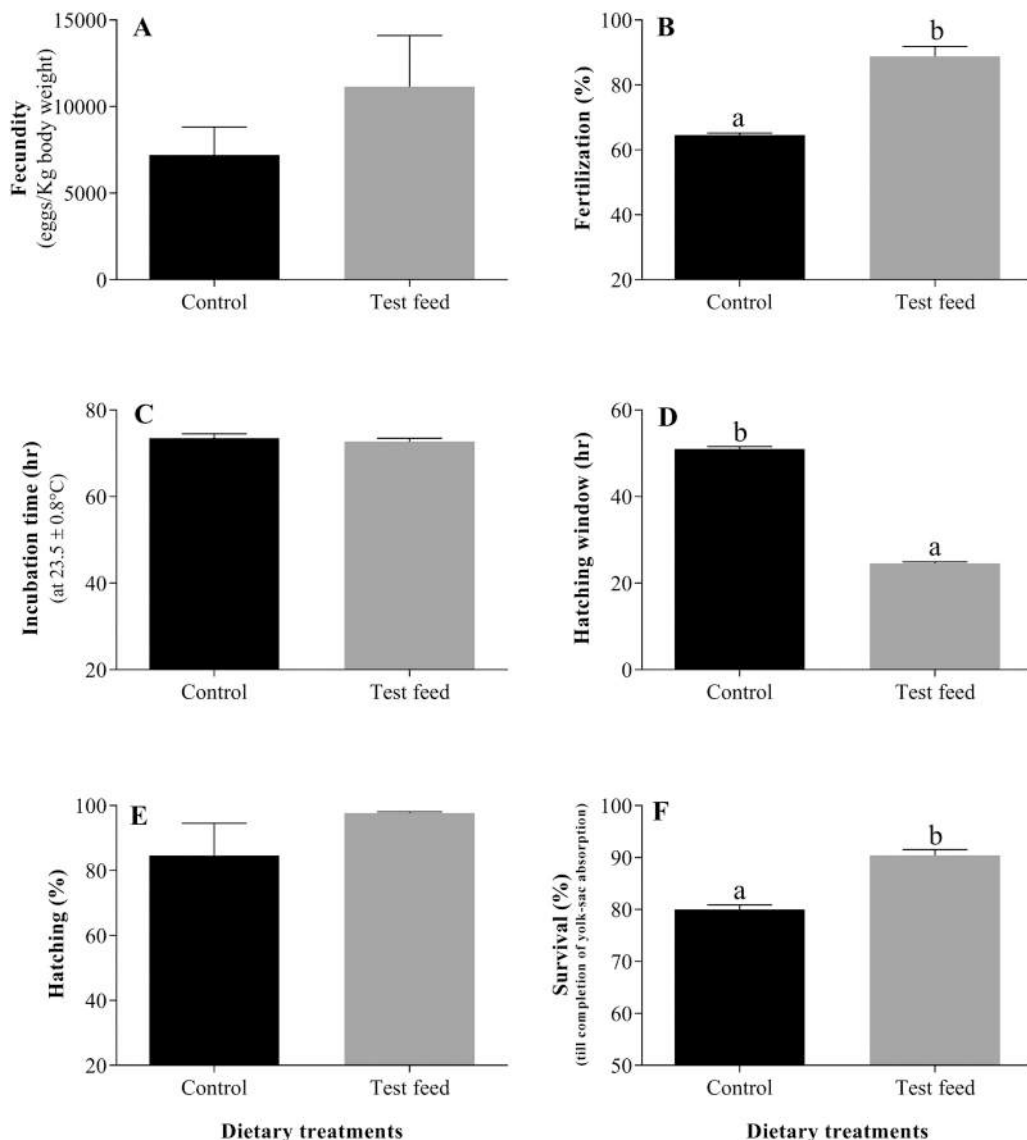


Fig. 1. Reproductive performance - fecundity (A); fertilization rate (B); hatching rate (C); incubation time (D); hatching window (E) and survival at the end of endogenous feeding phase (F) of golden mahseer brooders fed with control and test feed (Data expressed as Mean ± SEM, $n = 6$; Different superscripts (a, b) above the bars in each panel, if any, indicate significant difference, $P < 0.05$, Student's t -test).

aquarium chiller. For CT_{max} , the other 15 fish were placed in a water bath (10 L) with a heating element and thermostat (LabTech, UK). Water circulation was provided in both units to maintain uniform temperature. A digital thermometer (accuracy ± 0.1 °C) along with a multi-parameter probe (HQ40d, Hach, USA) were placed in both the unit to measure water temperature and dissolved oxygen levels. In both units, the initial temperature was set at 22°C, which was the average temperature during larval rearing. The experimental fish was then subjected to steady and constant linear temperature increase or decrease (0.3 °C per minute), until a near-lethal endpoint was reached. During this trial, the fish behaviour and water temperature were carefully observed to determine the exact temperature at which thermal end points are reached. Loss of equilibrium and righting response was taken as the CT_{max} end point, while no response to prodding marked the CT_{min} end point (Ford and Beiting, 2005). When the experimental fish reached the thermal end points, the corresponding temperature was recorded. The thermal scope was calculated as the difference between CT_{max} and CT_{min} values.

2.11. Statistical analysis

The normality and homogeneity of variances of data within each experimental group were confirmed ($p > 0.05$) using the Shapiro–Wilk test and Levene’s test, respectively. All the data were then subjected to Student’s *t*-test using SPSS (Version 19). Statistical significance was defined as a probability value of less than or equal to 0.05. The results are expressed as Mean $\pm$ SEM (standard error of mean). The graphs were plotted in Prism v.8.0 (GraphPad, La Jolla, CA, USA). All the correlation analyses were performed in MS Excel.

3. Results

3.1. Reproductive performance

Data pertaining to reproductive performance, such as fecundity, fertilization, hatching rate, incubation time, hatching window as well as survival at the end of endogenous feeding phase are shown in Fig. 1. Overall, the reproductive performance of golden mahseer broodstocks was improved by tailored broodstock nutrition. While the fecundity of *T. putitora* broodfish fed with the test diet was higher compared to those fed with basal/control diet, the difference was not significant. The fertilization rate (%) showed a marked improvement in broodstocks fed with the test diet. Notably, the hatching success and incubation period were not affected by the test diet. Nevertheless, the hatching window (duration between the start and completion of hatching) was shortened significantly in egg batches from test diet-fed broodstocks. Furthermore, larvae obtained from the test diet fed broodfish had a significantly higher survival rate at the end of the endogenous feeding phase. Exogenous feeding commenced almost simultaneously in larvae from both the broodstock dietary treatment groups.

3.2. Egg quality assessment

The mean diameter of unfertilized eggs of broodstocks fed with the test diet was noticeably higher (Table 2). Similarly, the diameter of water-hardened eggs obtained from test diet-fed broodfish during all the time points (30, 60 and 120 min) was significantly larger as compared to those from basal diet-fed fish. The cumulative (0–120 min) increase in egg diameter attributed to the higher water uptake rate was marginally (~ 2.3 %) greater in the eggs from the broodstock fed the test diet compared to those from the control group. Glucose and protein levels were similar in eggs produced from both the dietary treatment groups (Fig. 2A & B). Eggs produced from the test diet-fed broodstocks had significantly higher total carotenoid content than those from the control group (Fig. 2C), and this was visually evident by differences in the egg colours (pale whitish in the basal diet versus reddish orange in test diet-fed individuals). We observed a positive correlation between egg total carotenoid and larval survival rate at the end of the endogenous feeding phase ($r^2 = 0.76$).

3.3. Sperm quality assessment

Broodstock nutrition influenced various sperm quality indicators such as sperm count (Fig. 3A), spermatocrit (Fig. 3B), sperm viability (Fig. 3C), sperm abnormality index (Fig. 3D), and milt volume (4 A). The sperm count ($28.19 \pm 2.83 \times 10^9 \text{ mL}^{-1}$ in test versus $25.82 \pm 5.56 \times 10^9 \text{ mL}^{-1}$ in control), spermatocrit (62.55 % in test versus 57.73 % in control), and milt volume (5.7 mL in test versus

Table 2

Comparative assessment of egg diameter changes in golden mahseer brooders fed with control and test feed during water hardening. (Data expressed as Mean $\pm$ SEM, $n = 15$; Data bearing different superscripts under each column (a, b) are statistically significant, $P < 0.05$, Student’s *t*-test).

Hardening duration	Control		Test feed	
	Egg diameter (mm)	% increase in diameter	Egg diameter (mm)	% increase in diameter
Unfertilized egg	$2.56^a \pm 0.03$	-	$2.74^b \pm 0.03$	-
Hardened for 30 min	$2.98^a \pm 0.09$	16.72 ± 1.22	$3.25^b \pm 0.03$	18.66 ± 1.23
Hardened for 60 min	$3.06^a \pm 0.02$	20.34 ± 1.19	$3.36^b \pm 0.04$	22.82 ± 1.52
Hardened for 120 min	$3.11^a \pm 0.01$	22.16 ± 1.51	$3.41^b \pm 0.02$	24.48 ± 1.14
Cumulative change (0–120 min)	$0.56^a \pm 0.03$	22.16 ± 1.51	$0.67^b \pm 0.03$	24.48 ± 1.14

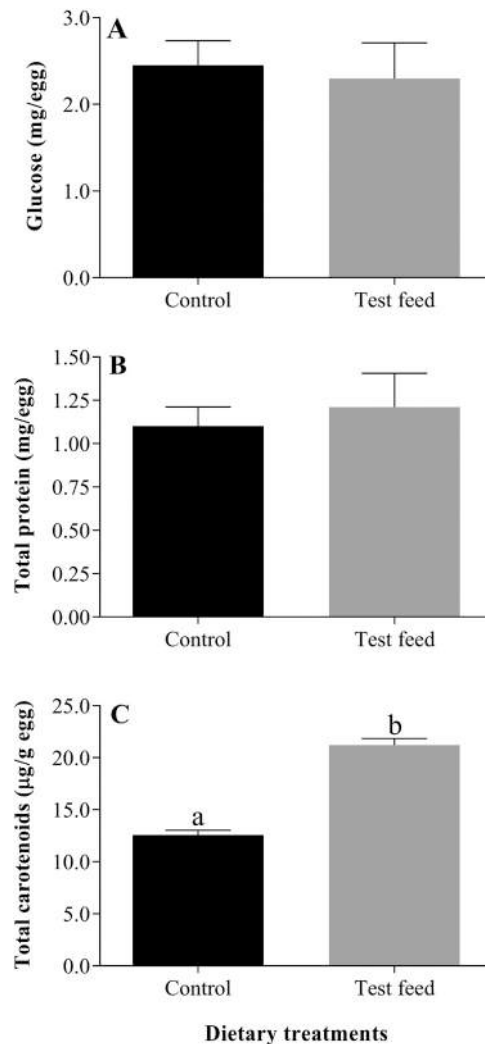


Fig. 2. Egg biochemical composition (glucose, total protein and total carotenoids) of golden mahseer brooders fed with control and test feed (Data expressed as Mean $\pm$ SEM, $n = 6$; Different superscripts (a, b) above the bars in each panel, if any, indicate significant difference, $P < 0.05$, Student's t -test).

4.8 mL in control) were substantially higher in test diet-fed individuals. Further, broodstocks fed with the test diet exhibited significantly higher sperm viability ($77.85 \pm 3.42\%$) with fewer morphological abnormalities ($3.43 \pm 0.57\%$). Sperm count and spermatocrit were statistically correlated with fertilization rate ($r^2 = 0.75$, $r^2 = 0.93$, respectively). Notably, no significant differences were seen in the seminal plasma energy reserves such as glucose, total protein, and triglycerides (Fig. 4B - D). The seminal plasma of test diet-fed individuals, however, displayed significantly higher antioxidant capacity (Fig. 4E) with lower TBARS values (Fig. 4F). The sperm abnormality was positively correlated ($r^2 = 0.74$) with plasma TBARS levels.

3.4. Reproductive hormones

Plasma 17β estradiol and vitellogenin were significantly higher in test diet-fed female broodfish (Fig. 5A & B). In contrast, plasma maturation inducing hormone (17α , 20β -dihydroxy progesterone) showed no difference between control and test diet-fed female broodfish (Fig. 5C). Similarly, plasma levels of 11-keo testosterone and 17α , 20β -dihydroxy progesterone in male broodfish did not differ between the two dietary treatment groups (Fig. 5C & D).

3.5. Larval/offspring performance and thermal tolerance

After 75 dph, golden mahseer larvae obtained from broodstocks fed with the test diet showed significantly higher growth/weight gain and survival (Fig. 6A). The CT_{max} was significantly higher ($\sim 0.6^\circ\text{C}$) in offspring of test diet-fed broodstocks (Fig. 6B). On the other

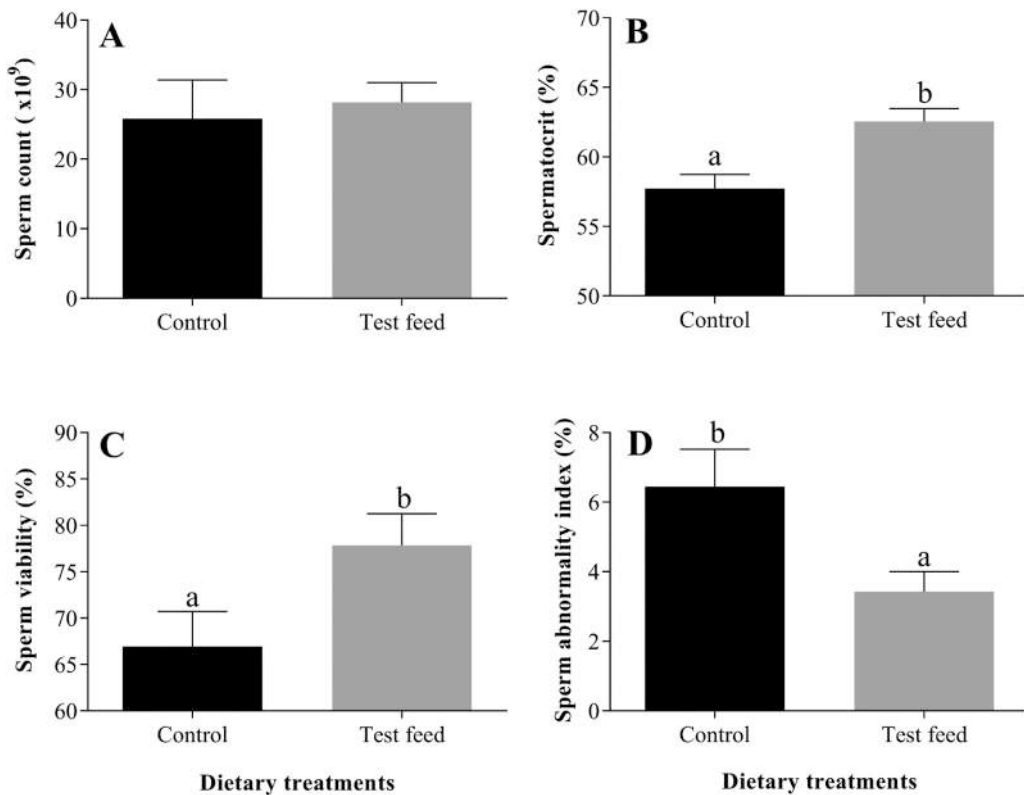


Fig. 3. Sperm count (A); spermatocrit (B); sperm viability (C); sperm abnormality index (D) of golden mahseer brooders fed with control and test feed (Data expressed as Mean $\pm$ SEM, $n = 6$; Different superscripts (a, b) above the bars in each panel, if any, indicate significant difference, $P < 0.05$, Student's t -test).

hand, no significant difference was noticed in CT_{min} and thermal scope between progenies obtained from broodstocks under different dietary conditions (Fig. 6C & D).

4. Discussion

It is widely recognized that several parental/broodstock nutrition-related factors greatly influence reproductive success, efficiency and offspring health in all vertebrates, including fish. The nutrients required to initiate embryonic development must be incorporated into the egg when it is an oocyte within the ovary (Tata, 1986). Several independent and discrete studies in teleosts documented the influence of different nutrients on reproduction (Watanabe et al., 1984a, b; Sahu et al., 2021; Fontagne-Dicharry et al., 2017, 2010; Akhtar et al., 2021b; Ciji et al., 2024). The present study investigated how improved broodstock nutrition through a cocktail of nutrients, additives/pigment, and immunostimulant affects the reproductive performance, gamete and offspring quality in an endangered cyprinid, the golden mahseer, in order to develop high-quality broodstock for breeding programmes.

Fecundity and egg diameter/size are often used as important markers for the assessment of reproductive performance in females (Ciji et al., 2022; Torsabo et al., 2022). The nutritional state of the female during ovarian recrudescence has been shown to influence egg size (Bromage et al., 1990; Tyler et al., 1994). In the current study, the egg size/diameter increase during water hardening was significantly higher in eggs from test diet fed broodstocks, indicating a higher water influx. The egg weight increase during water hardening is considered a reliable indicator of egg quality (Lahnsteiner et al., 2001; Ciji et al., 2022). Previous studies in cyprinids demonstrated that fertilization rate and egg viability were linked to water uptake rate during hardening (Lahnsteiner et al., 2001). In the current study, the broodfish fed the test diet recorded a marginal increase in fecundity and hatching rate, but the difference was not statistically significant. Although the relationship between egg size and hatching rate remains ambiguous, larger eggs are likely to have a higher fertilization and hatching success (Brooks et al., 1997). Further, the fertilization rate is highly influenced by the quality of both male and female gametes (egg and sperm). In the present study, the substantially higher fertilization rate recorded in test diet-fed broodfish indicates improved gamete quality. The enhanced reproductive/spawning performance of golden mahseer broodstocks fed with the test diet may be attributed to higher content of dietary essential fatty acids especially n-3 PUFAs, essential amino acids, vitamins, antioxidants, and carotenoids as previously suggested by several authors (Izquierdo et al., 2001; Migaud et al., 2013; Xu et al., 2017). Supplementation of broodstock diets with various nutrients and additives viz., essential fatty acids enhanced fecundity and spawning frequency in tilapia, *Oreochromis niloticus* (Izquierdo et al., 2001; Engdaw and Geremew, 2024); and astaxanthin

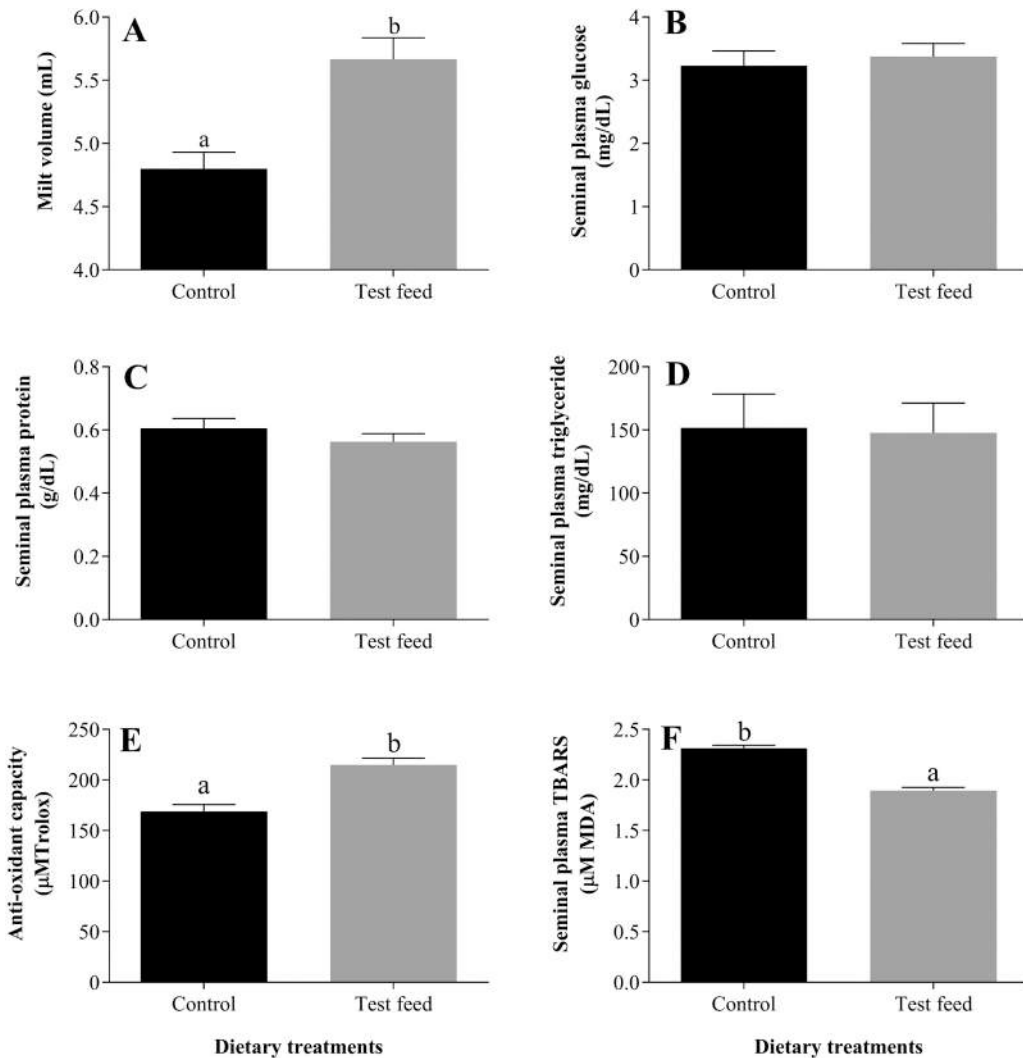


Fig. 4. Milt volume (A), seminal plasma glucose (B); total protein (C); triglyceride (D); antioxidant capacity (E) and TBARS (F) of golden mahseer brooders fed with control and test feed (Data expressed as Mean $\pm$ SEM, $n = 6$; Different superscripts (a, b) above the bars in each panel, if any, indicate significant difference, $P < 0.05$, Student's t -test).

improved fertilization rate and fecundity in goldfish, *Carassius auratus* and Atlantic cod, *Gadus morhua* (Tizkar et al., 2015; Sawanboonchun et al., 2008; Hansen et al., 2016). In our recent study, 0.5 % dietary β -glucan in broodstock diet improved fertilization and hatching rates in golden mahseer (Ciji et al., 2024). Earlier studies in red seabream demonstrated that feeding commercial diets supplemented with phosphatidyl choline, astaxanthin and vitamin E shortly before spawning resulted in eggs with significantly higher viability and hatching rates (Watanabe et al., 1991a, b). Broodstock dietary fortification with vitamins A, E and C has been shown to influence egg quality, fertilization success, hatching rates, embryonic/larval survival in several fish species (Furuita et al., 2003; 2009; Eskelinen, 1989; Blom and Dabrowski, 1995; Takeuchi et al., 1981). Based on literature survey, no single study evaluated the reproductive performance of fish in response to a supplement mixture to corroborate our findings. Furthermore, the significantly higher survival of hatchlings produced from broodstocks fed test diet at the end of the endogenous feeding phase indicates a strong effect of parental nutrition on the developmental competence of embryos and larvae of golden mahseer. Bobe and Labbé (2010) defined egg quality or oocyte developmental competence as the ability of the egg to be fertilized and subsequently develop into a normal embryo. It has been well recognized that during endogenous feeding phase, fish larvae rely on oocyte-derived nutrients for survival and embryonic development is supported by maternal factors incorporated into the eggs (Brooks et al., 1997; Bobe, 2015). We did not notice any significant difference in the glucose and protein content of eggs from both groups. Likewise, few studies reported no significant correlation between egg composition and egg quality (Faulk and Holt, 2008; Lahnsteiner et al., 1999). While, a positive relation of carotenoids on egg quality and viability has been documented in fish (Salze et al., 2005; Alijanpour et al., 2015), as observed in the present study. We also found that adding astaxanthin to the diet significantly increased the carotenoid content of golden mahseer eggs, which is in line with previous studies where the authors reported that the carotenoid in eggs is maternally transferred (Salze

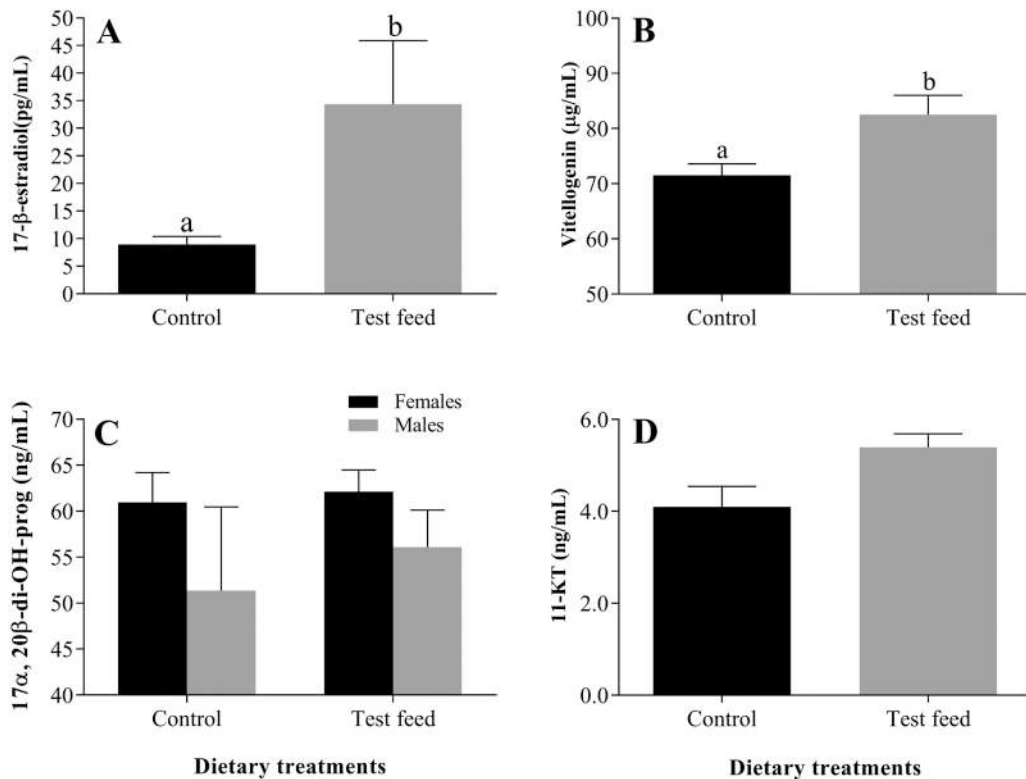


Fig. 5. Plasma 17β estradiol (A; only in female); vitellogenin (B; only in female); 17α, 20β-dihydroxy progesterone (C; both in male and female) and 11-keo testosterone (D; only in male) of golden mahseer brooders fed with control and test feed (Data expressed as Mean ± SEM, $n = 6$; Different superscripts (a, b) above the bars in each panel, if any, indicate significant difference, $P < 0.05$, Student's t -test).

et al., 2005; Sawanboonchun et al., 2008) and its content in the broodstock diet would be reflected in their eggs (Youngson et al., 1997; Pickova et al., 1999). Although higher carotenoid content is observed in eggs produced from broodfish fed the test diet, the level reported in eggs from wild-collected broodfish is much higher (Ciji et al., 2022), indicating scope for further enhancement.

Studies documented that broodstock dietary composition can affect the spawning performance by altering plasma sex steroid levels (Navas et al., 1998). The improved reproductive performance of golden mahseer fed with the test diet compared to the control group can be associated with their better endocrine profiles. More precisely, plasma 17β-estradiol and vitellogenin were higher in broodfish fed the test diet. Vitellogenin is the key nutrient source for the developing embryos, and hence, the higher vitellogenin might also have contributed to their superior egg quality as reported earlier in other aquatic species (King et al., 2003; Pamungkas et al., 2020). Similar to our observations, administration of carotenoid-rich feed to broodstock improved estradiol, egg number and egg weight in fishes (El-Gamal et al., 2020; Meng-Umphun, 2009). Further, as observed in the present study, reduced fecundity, egg quality and survival to hatching were observed in sea bass broodstock with low plasma estradiol (Cerdas et al., 1994; 1995).

Antioxidants play an important role in protecting gametes (egg and sperm) from reactive oxygen species (ROS) or oxidative stress. Owing to their high polyunsaturated fatty acid (PUFAs) content, spermatozoa are particularly vulnerable to oxidative stress, which represents a major cause for cellular damage (Shiva et al., 2011; Slowinska et al., 2013). The seminal plasma plays a pivotal role in protecting the sperm from the harmful effects of ROS (Shiva et al., 2011). Dietary administration of antioxidants has been shown to improve egg and sperm quality as well as spawning/fertilization performance in several fish (Tizkar et al., 2015; Sawanboonchun et al., 2008). Further, Lahnsteiner (2009) documented a positive effect of methionine on sperm quality traits in common carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*), by virtue of its role in antioxidant defence. In the current study, various antioxidants (vitamin E, vitamin C, and astaxanthin) and methionine incorporated in the test diet might have protected spermatozoa from lipid peroxidation, resulting in increased sperm viability with lower abnormality/structural damage. Feeding the test diet reduced the abnormal spermatozoa population by approximately 46 %. This may be attributed to the higher total antioxidant capacity and lower TBARS values noticed in the seminal plasma of test diet-fed broodstocks, as antioxidants are known to reduce sperm DNA and structural/membrane damage, thereby reducing abnormalities (Castro et al., 2019). Previous studies demonstrated that dietary supplementation of antioxidants has been shown to increase the antioxidant potential of seminal plasma and their resistance to lipid peroxidation, consequently improving sperm oxidative stability and sperm quality in fish (Akhtar et al., 2021b; Mansour et al., 2006). Further, supplementation of broodstock diets with astaxanthin (150 mg/kg) and β-glucan (0.5 %) improved sperm quality and concentration in goldfish (Tizkar et al., 2015) and golden mahseer (Akhtar et al., 2021b), respectively. Engdaw and Geremew (2024) opined that fertilization of eggs is highly influenced by sperm count and sperm quality, which in turn is known to be affected by

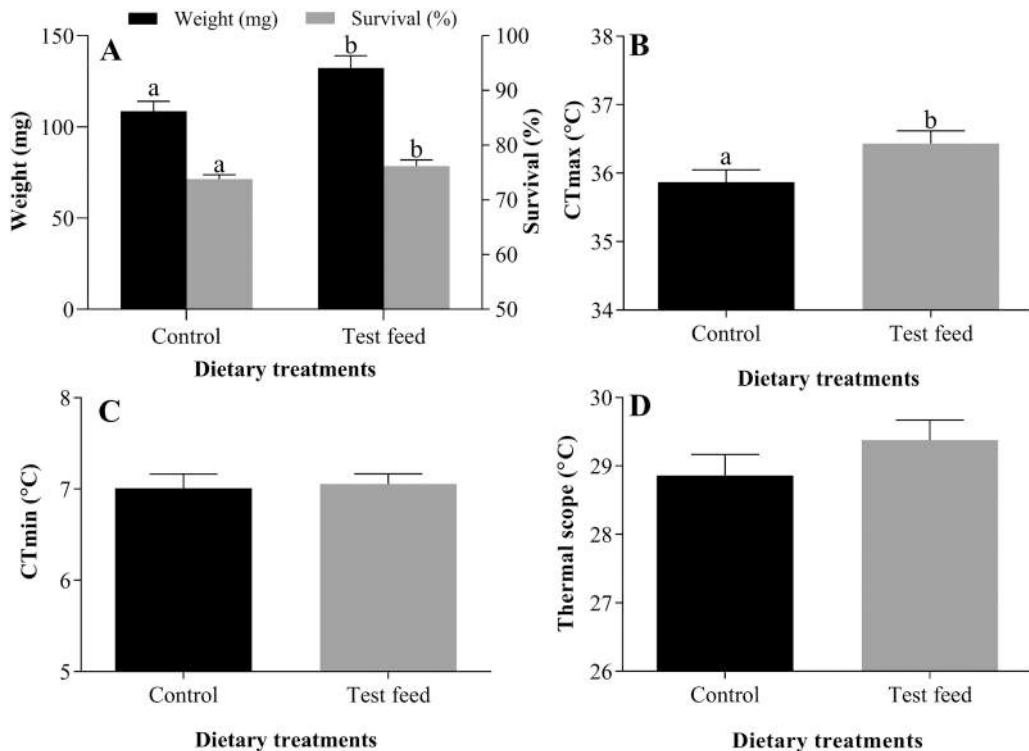


Fig. 6. Growth & survival (A); CT_{max} (B); CT_{min} (C) and thermal scope (D) of larvae (75 DPH) obtained from golden mahseer brooders fed with control and test feed (Data expressed as Mean $\pm$ SEM, $n = 6$ for growth (bulk weighed in batches, each batch with 15 individuals), $n = 3$ for survival (3 replicate tanks, each tank containing ~ 500 larvae); and $n = 15$ for CT_{max} , CT_{min} & thermal scope). Different superscripts (a, b) above the bars in each panel indicate a significant difference ($P < 0.05$, Student's t -test).

broodstock nutrition. In the present study, test diet-fed broodstocks exhibited a 19 % increase in milt volume over the control diet-fed groups. The significantly higher milt volume and spermatocrit in the present study suggest a strong influence of dietary components on the quantity of milt produced.

Several recent studies have investigated broodstock dietary intervention as a strategy to improve offspring performance, as it is known to affect larval survival, growth rates and stress resistance (Akhtar et al., 2021c; Zambonino-Infante et al., 2019; Turkmen et al., 2017; Saramah et al., 2019). In the present study, golden mahseer fry (75 dph) obtained from broodfish fed with the test diet recorded significantly higher growth/weight, survival and CT_{max} . Supplementing methionine in broodstock diets improved offspring growth in rainbow trout (Fontagne-Dicharry et al., 2017; Cleveland et al., 2020). The significantly higher CT_{max} and the relatively broad thermal scope noticed in mahseer fry obtained from test diet-fed broodstocks indicate their elasticity to cope with higher/varying environmental temperatures. Enhanced survival and thermal tolerance in the offspring (75 dph) of test diet-fed broodfish could contribute to greater ranching success of golden mahseer, owing to improved resilience to temperature variability and limited food availability in the wild.

5. Conclusion

Higher fertilization rates, improved egg and sperm quality, and higher larval survival at hatching indicate significant improvement in the reproductive success of golden mahseer with the use of tailored broodstock nutrition. The positive correlation between egg carotenoids and larval fitness underscores the vital role of maternal nutrition in the success of early developmental stages. Improved sperm viability and reduced sperm abnormalities, attributed to elevated antioxidant capacity, further strengthen the practical need for dietary interventions in golden mahseer broodstock management. The higher larval fitness, as evidenced by improved survival rates, growth, and thermal tolerance, underlines the enduring benefits of optimized broodstock nutrition on larval quality. These results further substantiate the utility of broodstock dietary manipulation as an effective tool for improving seed production in golden mahseer hatcheries. By ensuring the sustainable production of high-quality seed, refined nutritional protocols will not only boost hatchery success but also contribute to the conservation and restoration of golden mahseer. Future studies should focus on identifying the optimum levels of added micronutrients and additives in the broodstock diets of golden mahseer.

CRediT authorship contribution statement

Alexander Ciji: Conceptualization, Methodology, Feed formulation, Experiment execution, Data curation, Formal analysis, Investigation, Visualization, Writing – original draft. **Md. Shahbaz Akhtar:** Methodology, Experiment execution, Data curation, Formal analysis, Investigation, Visualization, Writing – review & editing. **Biju Sam Kamalam:** Feed formulation and feed logistics, Visualization. **Manchi Rajesh:** Methodology, Writing – review & editing. **Prakash Sharma:** Methodology, Writing – review & editing.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

All data generated or analysed in this study are included in the article, and no additional data set has been generated.

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Comparative assessment of egg and larval quality traits of progeny from wild-collected and captive-matured brooders of endangered golden mahseer, *Tor putitora*: A prelude to quality broodstock development and seed production

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ABSTRACT

In this study, we assessed the differences in reproductive outputs (fertilization and hatching rates); egg quality (egg size increase during hardening, biochemical composition, carotenoid content, and characteristics of ovarian fluid); and larval performance traits (growth, microanatomical development, survival, starvation response, and critical thermal tolerance) of progeny from captive-matured and wild-collected golden mahseer brooders. Fertilization and hatching rates were significantly higher in wild-collected brooders than in captive broodstock. Captive brooder eggs were significantly larger in size and weight but had significantly lower energy/nutrient (total protein, glucose, and triglyceride) reserves and total carotenoids. On the contrary, ovarian fluid from captive females had significantly higher levels of total protein, glucose, and triglycerides, possibly indicating weaker egg membrane and nutrient leakage due to membrane damage or lysis. During water hardening, the cumulative weight increase due to a higher water uptake rate was significantly higher in the wild origin eggs than captive brooder eggs. CT_{max} of larvae obtained from captive-matured brooders was lower during the early developmental stages (15 dph), but became similar to the CT_{max} of wild brooder's larvae at 35 dph. On the other hand, CT_{min} of captive origin larvae was higher than their wild counterparts at all examined stages. There were no differences in starvation tolerance of larvae between the two brooder types. Histological observations revealed that the intestinal nutrient absorption and hepatic nutrient accumulating efficiency during the early developmental stages was relatively lower in captive origin larvae, corresponding to the comparative growth metrics observed until 35 dph. Also, the survival percentage at 90 dph was lower in the larvae of captive brooders than their wild counterparts. Collectively, these findings indicate potential areas for improvement in the captive broodstock management of the endangered cyprinid, golden mahseer.

1. Introduction

Golden mahseer (*T. putitora*), the 'king of game fish' is an important cyprinid fish in Asian countries due to its varied economic, recreational, and conservational importance (Nautiyal, 1984; Dinesh et al., 2010; Akhtar et al., 2013; Akhtar et al., 2014). However, in recent years, the population of golden mahseer in their natural habitat has declined sharply due to anthropogenic activities (such as habitat fragmentation, environmental degradation, pollution, over-exploitation, and indiscriminate fishing) together with the inherent biological factors (such as

delayed maturity, low fecundity, and slow growth rate) (Nautiyal, 1994; Sehgal, 1999; Dinesh et al., 2010). Consequently, it is listed as 'endangered' in the International Union for Conservation of Nature (IUCN) red list (Jha and Rayamajhi, 2010). Therefore, the development of artificial propagation protocol and ranching/stocking of golden mahseer juveniles has been identified as the most immediate solution for reviving its natural population. However, till recent past, seed production of golden mahseer was achieved using gravid brooders collected from the wild (ShyamSunder et al., 1993; Sehgal, 1999; Ogale, 2002) as females failed to mature in captivity (Akhtar et al., 2017). Very recently, ICAR-DCFR

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has standardized the technology for captive maturation and year-round breeding of golden mahseer through environmental (temperature and photoperiod along with spawning substratum) manipulations (Akhtar et al., 2018), reducing the dependency on wild brooders for its seed production. Nevertheless, the success of the ranching program begins with the quality of progeny released, and progeny fitness could be a deciding factor for their survival in the natural habitat (Le Vay et al., 2007). Accumulating evidence suggests that hatchery-reared fish have lower fitness in their natural environments, and several studies in different teleosts unambiguously reported poor egg quality and post-hatchling (larval) performance of captive/farmed broodstocks as compared to their counterparts in the wild (Salze et al., 2005; Araki and Schmid, 2010; Lanes et al., 2012; Bardon-Albaret and Saillant, 2017). Therefore, this study was aimed to compare the quality traits of eggs and larvae produced from wild-collected and captive-matured brooders of golden mahseer.

Earlier studies demonstrated that egg quality and its nutrient composition could have a major role on embryonic and larval development (Wickins et al., 1995; Palacios et al., 2001) as well as the fitness of larvae and fry (Kjørsvik et al., 1990; Bromage et al., 1994). Numerous indicators, including fertilization and hatching rates, egg size (reviewed by Bobe and Labbé, 2010), yolk protein/egg composition (Olin and Vonderdecken, 1990), and free amino acids (Rønnestad et al., 1992), have been used to assess egg quality in several teleosts. In cyprinids, egg fertilization rates were correlated with ovarian fluid parameters, egg size increase during water hardening, and egg composition (Lahnsteiner et al., 2001). During ontogeny, free amino acids are released by the proteolysis of yolk proteins for meeting the osmotic and nutritional requirements of the early stages of development (Finn and Fyhn, 2010). On the other hand, egg carotenoid levels were also proven to enhance the larval quality of several fish species (Salze et al., 2005; Alijanpour et al., 2015).

Furthermore, the development of the digestive system and accessory glands influences the developmental compatibility of larvae after the onset of exogenous feeding (Sharma et al., 2016). The survival activity index (SAI), an indicator of larval tolerance to starvation, has been routinely used as the practical measure of larval activity and viability (Mushiakhe et al., 1993). The thermal tolerance limits of hatchery-produced golden mahseer fry can be considered as another fitness criterion for their post-release survival in the wild. The captive maturation and breeding of golden mahseer have been achieved by rearing the females at higher temperatures (4 to 5 °C higher than the ambient temperature). Few studies in aquatic animals reported that higher temperature exposure of parents enhances the thermal tolerance of their offspring (Donelson et al., 2012; Salinas and Munch, 2012; Wang et al., 2015). On the other hand, in the wild, fishes experience widely fluctuating thermal environments in contrast to the relatively uniform thermal regimes in controlled laboratory/pond conditions contributing to higher thermal tolerance of wild fish (Brown and Feldmeth, 1971; Holland et al., 1974; Feminella and Matthews, 1984). In this context, in the present study, reproductive outputs (fertilization and hatching rates); egg quality (egg size increase during hardening, biochemical composition, carotenoid content and characteristics of ovarian fluid); and larval performance traits (growth, microanatomical development, survival, starvation response, and critical thermal tolerance) were compared between wild and captive brooders of golden mahseer to understand the egg and larval quality differences so as to devise further management strategies to improve performance of captive-bred young ones.

2. Materials and methods

2.1. Maintenance of brooders and captive spawning

Adult golden mahseer (♀: 0.9–1.2 kg; ♂: 0.6–1.0 kg body weight) from the earthen pond was brought to four captive maturation units made of round FRP tanks (1000 L water volume) installed with gravel

bed-biofilter at the mahseer hatchery complex of ICAR-Directorate of Coldwater Fisheries Research (ICAR-DCFR), Bhimtal. Each captive maturation unit accommodated six females and three males. Rearing of the brooders was done as per the protocols described by Akhtar et al. (2018). Fishes were fed *ad libitum* twice daily with a broodstock diet (Akhtar et al., 2018). After the brooders matured (usually three months from stocking), male brooders were segregated to avoid natural/auto spawning. Fish were stocked in the maturation unit in the month of March so that captive breeding could coincide with their natural spawning season (May to July). The female brooders were watched after the males were separated, and observations were made to their bellies to determine the presence of ripened eggs. Ready-to-spawn females were identified by their behavioural changes, such as the making of spawning pits, fanning, and aggressive water splashing. The gravid brooders possessing ripened eggs from the rearing tank were taken out, and subsequently, stripping of eggs was done. Gravid wild fish (both male and female) were collected during the natural spawning season (May to July) as per the methodology described by Akhtar et al. (2017). Fish were taken to the Mahseer hatchery Complex of ICAR-DCFR, Bhimtal, and selected for stripping following the methods described above for the captive-matured brooders. A total of 12 female brooders (six wild and six captive-matured) of golden mahseer were used for a comparative assessment of egg quality and larval performance.

2.2. Fertilization, incubation and hatching of eggs

For stripping of eggs (from both captive and wild), the brooders were held by one person arching the body of the fish towards the dorsal side, and another person applied gentle pressure to the belly with thumb and index fingers running from the anterior side towards the vent. The ripened eggs were easily released into a bowl as a result of this. A certain amount of milt (2 mL/100 mL of eggs) from an oozing male was put directly onto the eggs, gently swirled with a feather, and left for 10 min without adding water. Following this, the eggs were washed three to four times to remove any excess milt. Subsequently, a fresh spell of water was added to the bowl to keep all of the eggs submerged, and the bowl was kept in a shadowy place for 30 min to allow water hardening of eggs. After that, the eggs were placed in golden mahseer egg incubators for hatching.

2.3. Measurement of egg size (weight and diameter) and size increase during water hardening

The weight of the unfertilized, non-hardened, and water-hardened eggs (during different time points) was determined with an analytical balance. The weight of eggs during water hardening in each time point (0, 15, 30, 60, 90, and 120 min) was determined by transferring subsamples of eggs ($N = 10$) from the incubator onto the petri plate kept on the pan of the balance. Before weighing, the water (ovarian fluid in the case of unfertilized and non-hardened eggs) on the egg's surface was removed by wiping with tissue paper. Attention was given to avoid egg damage while transferring and wiping. The diameter of the eggs was measured with the help of graph paper.

2.4. Collection and analysis of ovarian fluid

When the females were stripped, two aliquots of ovarian fluid were immediately collected from each female with the help of a pipette. They were centrifuged at 4000 xg for 10 min to remove any contaminating blood cells and tissue debris. The pH of the supernatant was measured within 60 min (after collection) using a pH meter (Hach, USA). Subsamples of ovarian fluid were stored at -20 °C for biochemical (glucose, total protein, and triglyceride) assays.

2.5. Biochemical analysis of egg and total carotenoid estimation

Glucose, total protein, and triglyceride content of eggs (unfertilized) and ovarian fluid were estimated using commercial kits (Span Diagnostics, India) following the manufacturer's instructions. The egg samples (approximately 50 eggs) were homogenized in distilled water for glucose and total protein, while in a mixture of methanol–chloroform–water (2:2:1.8 v/v) for triglyceride estimation. Free amino acid (FAA) was first extracted in trichloroacetic acid (TCA). Briefly, six subsamples (each containing approximately 50 eggs) were taken and extracted in 500 µL of 6% TCA in cryotubes by homogenizing. The mixture was then centrifuged (6000 xg for 20 min at 4 °C). The supernatant was collected, and FAA was then estimated using ninhydrin reagent. The total carotenoid content of the egg was extracted following [Torrisen and Nævdal \(1984\)](#) method using acetone as the extracting solvent and then calculated according to [Oliveira et al. \(2010\)](#).

2.6. Early larval performance and larval viability (starvation tolerance test and thermal tolerance study)

The larvae from captive and wild broodstocks were reared separately in fibre reinforced plastic (FRP) tanks, and growth performance was monitored at regular intervals by bulk weighing the larvae (weighed in three batches, each batch containing 15 individuals). The larvae were fed with macerated goat liver for 30 days after yolk sac absorption, followed by crumbled formulated diet containing 45% crude protein and 12% lipid. A gentle water flow (7–10 L/h) was provided in the larval rearing set-up, and dissolved oxygen was maintained using aerators. Water quality parameters were monitored regularly and were found to be within the acceptable range for cyprinids (dissolved oxygen: 5.5–6.3 mg/L; temperature: 21.5–22.6 °C; pH: 6.6–7.0; alkalinity: 70–80 mg/L; ammonia: <0.01 mg/L and nitrite: <0.1 mg/L). Temperature, dissolved oxygen and pH were monitored with a multi-parameter water testing apparatus (HQ40d, Hach, USA). Alkalinity, ammonia and nitrite were measured using commercially available kits as per the manufacturer's protocols (HiMedia, India).

The comparative larval viability or starvation tolerance was assessed in terms of the Survival Activity Index (SAI) following [Shimma and Tsujigado \(1981\)](#). For this, 45 fry (30 dph) produced from three wild-collected (15 fry from each brooder) and 45 fry (from three captive brooders-15 from each) were kept separately in six beakers (5 L capacity), and continuous aeration was provided throughout the SAI test. The daily cumulative mortality was recorded until all the fry were dead, and SAI was calculated from the number of surviving larvae and the number of days they survived, as follows:

$$SAI = 1 / N \sum_{i=1}^k (N - hi) \times i$$

Where N is the total number of larvae, hi is the cumulated mortality by ith day, k is the number of days elapsed until total larval mortality under starvation.

At 15 and 35 dph, the critical temperature maximum (CT_{max}) and critical temperature minimum (CT_{min}) of golden mahseer fry produced from wild and captive broodstocks were determined following the standard critical thermal methodology ([Beitinger et al., 2000](#); [Ford and Beitinger, 2005](#)). For this, a total of twenty fry each from wild and captive brooders (N = 10 fry each for CT_{max} and CT_{min} tests) were randomly selected at different developmental time points (15 and 35 dph; N = 10 fry/test in each developmental period). For CT_{max} determination, all the selected fish (ten) were placed in a laboratory water bath (10L) with a built-in heating element and thermostat (LabTech, UK). For determining CT_{min}, the other ten fish were transferred into a circular plastic tub (35 L) connected to an aquarium chiller. Water circulation was provided in both units to ensure uniform temperature. The initial temperature in both units was set at 22 °C, which corresponded to

Table 1

Comparative assessment of reproductive output traits (at 22 ± 0.5 °C) of wild-collected and captive-matured golden mahseer brooders.

Reproductive output traits	Wild	Captive
Fertilization rate (%)	91.33* ± 0.88	81.67 ± 1.61
Hatching rate (%)	85.50* ± 1.41	79.00 ± 1.81
Incubation time (hr)	88.33 ± 1.89	118.33* ± 1.45
Hatching window (hr) [§]	35.50 ± 1.26	44.67* ± 1.26
Exogenous feeding (dph)	12–13	10–12
Survival % (till 90 dph)	91.33* ± 1.09	83.83 ± 1.92

[§] Time interval between the start of hatching till completion; Data are expressed as Mean ± SE (N = 6); values bearing asterisk (*) are significantly different between wild and captive brooders, P < 0.05, Student's t-test.

the average temperature during nursery rearing. The procedural steps were performed as per our previous study ([Ciji et al., 2021](#)). The thermal scope was calculated as the difference between CT_{max} and CT_{min} measurements.

2.7. Larval histology

The larvae (N = 10) collected at each time point (1, 5, 10, 15, 20 dph) were fixed in Bouin's solution following the sedation. The pickled samples in Bouin's solution were rinsed with tap water overnight before being processed in a series of alcohols (70, 95, and 100%), xylene, and paraffin, and then finally embedded in paraffin blocks. The whole larvae were sectioned in different orientations at a thickness of 5 µm; some were sectioned longitudinally from the ventral side, while others were sectioned transversely at various lengths of the body. Finally, they were stained with a combination of alcian blue (pH -2.5), hematoxylin, and eosin, then viewed with a compound microscope (Leica DM 2000) and photomicrographed with a Leica digital camera (MC, 170HD; Leica Microsystems, Wetzlar, Germany). Histological identification of organs and their physiological conditions for comparing the developmental rates in larvae of two families were made following [Sharma et al. \(2016\)](#) and [Kumari et al. \(2021\)](#).

2.8. Statistical analysis

All the comparative assessment data were subjected to Student's test using the statistical tool SPSS (Version 19). One-way ANOVA analyzed the thermal tolerance data at different larval stages. Statistical significance was defined as a probability value of less than 0.05. The results are expressed as mean ± standard error (SE). The graphs were plotted in Prism v.5.0 (GraphPad, La Jolla, CA, USA).

2.9. Ethical statement

The Institutional Animal Ethics Committee at the ICAR-DCFR, Bhimtal, Nainital, Uttarakhand, India, approved all animal handling and experimentation procedures.

3. Results

3.1. Fertilization, incubation and hatching of eggs

The wild stock had higher fertilization and hatching success than the captive-matured stock. The fertilization rates of egg batches from captive-matured fish were significantly lower than those from wild fish ([Table 1](#)). Likewise, the hatching rates of eggs from captive females were noticeably lower than those from wild females ([Table 1](#)). The incubation period (at 22 ± 0.5 °C) of captive eggs is longer (118.33 ± 1.45 h) in contrast to shorter time (88.33 ± 1.89 h) in wild origin eggs ([Table 1](#)). Besides, the hatching window (time interval between the start of hatching till its completion-100% hatching) is relatively longer in the case of eggs produced from captive brooders ([Table 1](#)). In the case of

Table 2

Comparative assessment of absolute and relative increase in weight and diameter of eggs from wild-collected and captive-matured golden mahseer brooders during water hardening.

Egg weight (mg)	Wild	Captive	Wild	Captive
	Absolute measure		Relative increase (%)	
Unfertilized	6.62 ± 0.06	8.52* ± 0.12		
Non-hardened	6.92 ± 0.08	8.65* ± 0.13		
Hardened for 15 min	8.98 ± 0.06	10.25* ± 0.07	29.77* ± 2.57	18.48 ± 2.94
Hardened for 30 min	11.72 ± 0.17	11.72 ± 0.13	30.51* ± 3.18	14.34 ± 2.85
Hardened for 60 min	12.52 ± 0.05	12.87 ± 0.18	6.83 ± 1.21	9.81* ± 1.42
Hardened for 90 min	12.60 ± 0.06	12.98 ± 0.10	0.64 ± 0.23	0.85 ± 0.31
Hardened for 120 min	13.12 ± 0.07	13.20 ± 0.07	4.13* ± 0.54	1.69 ± 0.32
Cumulative increase (0–120 min)	–	–	70.86* ± 1.08	52.26 ± 1.53
Egg diameter (mm)				
Unfertilized	2.40 ± 0.04	2.68* ± 0.07		
Non-hardened	2.57 ± 0.07	2.72 ± 0.06		
Hardened for 15 min	2.87 ± 0.05	3.0 ± 0.03	11.37 ± 3.25	11.96 ± 2.55
Hardened for 30 min	3.43 ± 0.04	3.58 ± 0.07	19.77 ± 1.11	18.17 ± 2.03
Hardened for 60 min	3.72 ± 0.06	3.86 ± 0.06	8.35 ± 1.46	7.92 ± 1.69
Hardened for 90 min	3.78 ± 0.04	3.97 ± 0.03	1.85 ± 0.94	2.92 ± 1.70
Hardened for 120 min	3.88 ± 0.03	4.07 ± 0.03	2.67 ± 0.69	2.49 ± 0.88
Cumulative increase (0–120 min)	–	–	47.20 ± 1.44	42.35 ± 3.09

Data are expressed as Mean ± SE (N = 10); values bearing asterisk (*) are significantly different between wild and captive brooders, $P < 0.05$, Student's *t*-test. The percentage of relative increase is calculated from the preceding time point (*i.e.*, 0–15, 15–30, 30–60, 60–90 and 90–120 min).

Table 3

Biochemical composition of eggs (unfertilized) and ovarian fluid samples from wild-collected and captive-matured golden mahseer brooders.

Biochemical indicators	Eggs*		Ovarian fluid#	
	Wild	Captive	Wild	Captive
Total protein	191.65 ^b ± 8.09	141.96 ^a ± 14.41	1.73 ^a ± 0.34	41.28 ^b ± 4.88
Glucose	11.82 ^b ± 0.37	8.31 ^a ± 0.53	11.67 ^a ± 0.28	28.00 ^b ± 0.23
Triglyceride	84.59 ^b ± 13.92	24.27 ^a ± 5.42	14.88 ^a ± 3.68	45.82 ^b ± 4.03

* mg/g eggs (wet weight).

mg/dL ovarian fluid; Data are expressed as Mean ± SE (N = 6); under each category (eggs and ovarian fluid), values bearing different superscripts (a and b) are significantly different between wild and captive brooders, $P < 0.05$, Student's *t*-test.

wild origin larvae, exogenous feeding began 12–13 days after hatching in contrast to 10–12 days in captive origin larvae.

3.2. Egg size (weight and diameter) and size increase during water hardening

The mean weight and diameter of unfertilized eggs of captive brooders were significantly higher (Table 2). Likewise, the mean weight of non-water-hardened and 15 min water-hardened eggs from the

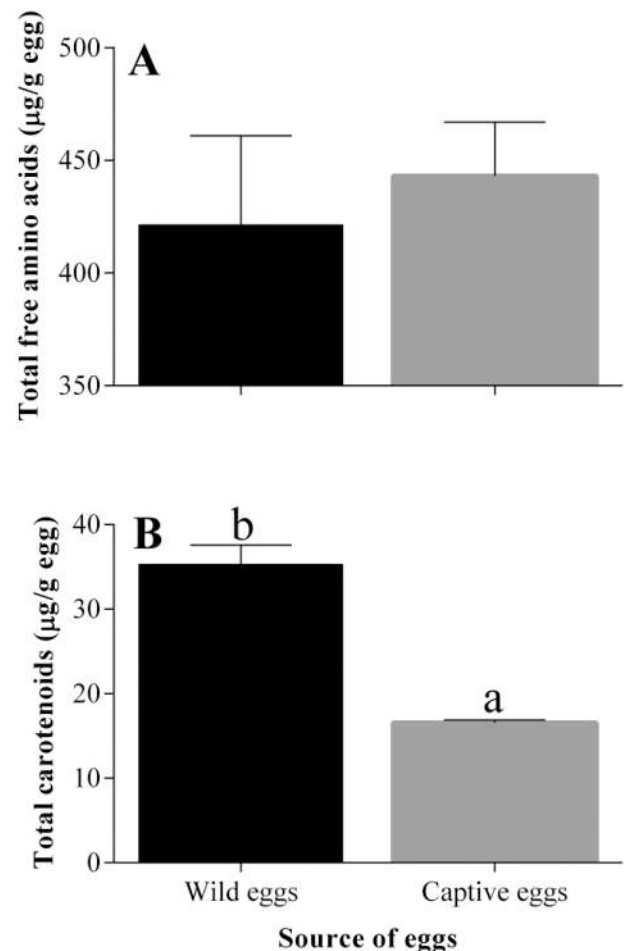


Fig. 1. Total free amino acids (A) and total carotenoids (B) in the eggs of wild-collected and captive-matured golden mahseer brooders. Data are expressed as Mean ± SE, N = 6; Bars bearing different superscripts (a, and b) indicate statistically significant differences ($P < 0.05$, Student's *t*-test).

captive females was also considerably higher than those from wild brooders. However, the mean weight recorded during other time points of water hardening (30, 60, 90, and 120 min) was not different between eggs from captive and wild females. The size (both weight and diameter) of captive and wild origin eggs was statistically non-significant after 120 min of water hardening. The cumulative increase (%) in weight (0–120 min) due to a higher water uptake rate was significantly higher in the wild origin eggs, as compared to captive brooder eggs.

3.3. Biochemical composition and total carotenoid content of eggs

Total protein, glucose, and triglyceride content were significantly lower in eggs produced from captive-matured female brooders (Table 3). Wild origin eggs contained approximately 1.4 fold higher total protein and glucose and 3.5 fold higher triglyceride levels than captive origin eggs. On the other hand, the free amino acid level did not differ significantly, although eggs from wild brooders displayed higher mean values (Fig. 1A). Eggs produced from captive brooders had significantly lower total carotenoid content than wild origin eggs (Fig. 1B). This was also visibly evident by the pale-whitish color of captive eggs in contrast to golden yellow colored eggs from wild females.

3.4. Characteristics of ovarian fluid

Statistically significant differences were noticed in ovarian fluid composition with respect to total protein, glucose, and triglycerides

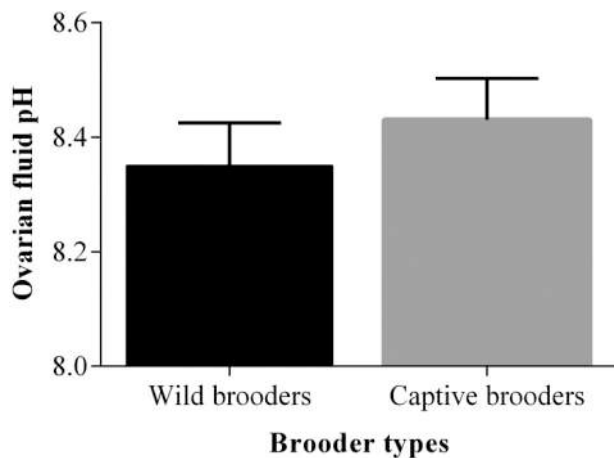


Fig. 2. pH of ovarian fluid taken from wild-collected and captive-matured and golden mahseer females. Data are expressed as Mean $\pm$ SE, $N = 6$.

between wild and captive-matured brooders (Table 3). The ovarian fluid from captive females recorded significantly higher total protein, glucose, and triglyceride content (Table 3). However, the ovarian fluid pH was similar in captive-matured and wild-collected females (Fig. 2).

3.5. Early larval performance and larval viability

During the first 35 dph (days post-hatching), the length and weight were relatively higher in larvae produced from wild broodstock than those from captive brooders (Fig. 3). However, at 50 dph, the comparative growth performance was statistically non-significant and, after a while, the growth performance of fry produced from captive brooders outperformed the growth of fry produced from wild brooders (Fig. 3). The survival rate (first three months from hatching; 90 dph) was significantly higher in wild origin larvae (Table 1).

Based on the starvation tolerance test, the survival activity index (SAI) of wild and captive-bred fry was not statistically significant (Fig. 4A). On the other hand, results of the thermal tolerance study revealed that the CT_{max} of golden mahseer fry produced from captive-matured broodstock was significantly lower during initial development stages (15 dph) but became comparable or equal to the CT_{max} of wild fry on 35 dph (Fig. 4B). However, the CT_{min} of captive-bred golden mahseer fry was found to be significantly higher (by 0.5 °C) than those

from wild brooders until 35 dph (Fig. 4C). Similarly, the thermal scope was markedly lower in captive-bred golden mahseer fry at all examined developmental stages (Fig. 4D).

3.6. Larval histology

Digestive system development and differentiation, namely buccal cavity, esophagus, fore, mid, and hindgut in larvae of both captive and wild parents, occurred at the same time of development. However, the liver and the pancreas appeared to be less developed in larvae of the wild brooder, at 5 dph (Fig. 5, 6, and 7). The progressive structural development of the midgut at 5 and 10 dph are shown in Fig. 5. At 5 dph, in larvae of captive brooders, zymogen granules were visible, although scanty (Fig. 6a); but, the same in wild brooders larvae, were completely absent (Fig. 6b). However, in other time points, the granules were equally abundant in both groups (Fig. 6). But the hepatocyte's size dynamics from 5 to 20 dph (Fig. 7) were different depending on their parents. In captive brooders' larvae, hepatocyte size appeared to decrease gradually from 5 to 15 dph (Fig. 7a, c, and e) and reached a minimum size at 15 dph (Fig. 7e). Gradual size regains, in them, was seen at 20 dph. In larvae of wild brooders, at 5 dph, hepatocytes were significantly smaller than those of captive. But the same at 10 dph were bigger. Maximum size reduction of hepatocytes in larvae of wild parents, as well, was observed at 15 dph. But the hepatocytes size regain in juveniles of wild parents, observed at 20 dph, was more prominent than those of wild (Fig. 7g and h, for comparison). In short, the post hatched development of liver, after 5 dph, in the wild brooders' larvae appeared to be significantly faster. The size of the hepatocytes at 10 and 20 dph was also bigger with more nutrient load than the same in captive brooders' larvae. By 15 dph significant progression in structural/anatomical and functional development of the digestive system took place, and the larval nutrition completely shifted from endogenous to exogenous feeding. At 5 dph, the intestine appeared to be a thin tube with a narrow lumen lined by mucosa but without folds. Mucosal goblet cells were absent at that time point in larvae of both parental types. At 10 dph, folds were visible, cross-sectional area of the intestine, and its luminal distension had increased equally in larvae of both families.

At 15 dph, interesting functional differences in mid and hindgut were observed between the larvae from captive and wild brooders in terms of fat and protein absorption (Fig. 8). Protein absorption, indicated by supranuclear vesicles with eosinophilic content in the apical part of the hindgut, was prominent in larvae of wild brooders (Fig. 8f and g) compared to those of captive (Fig. 8b and c) brooders. Similarly, fat

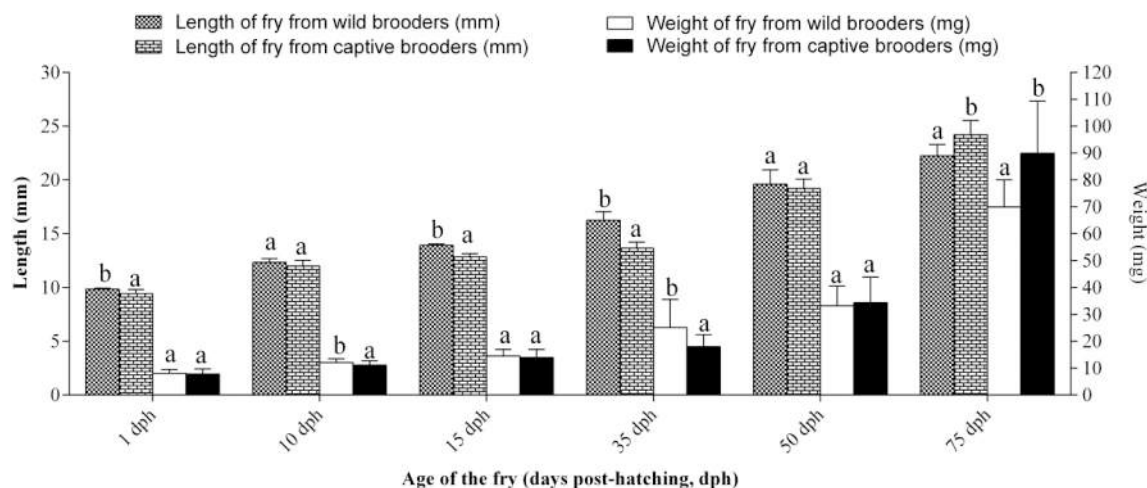


Fig. 3. Growth performance of larvae obtained from wild-collected and captive-matured golden mahseer brooders. Data are expressed as Mean $\pm$ SE, $N = 45$ (bulk weighed in 3 batches, each batch with 15 individuals); Bars with different superscripts under each time point indicate statistically significant differences ($P < 0.05$, Student's t-test).

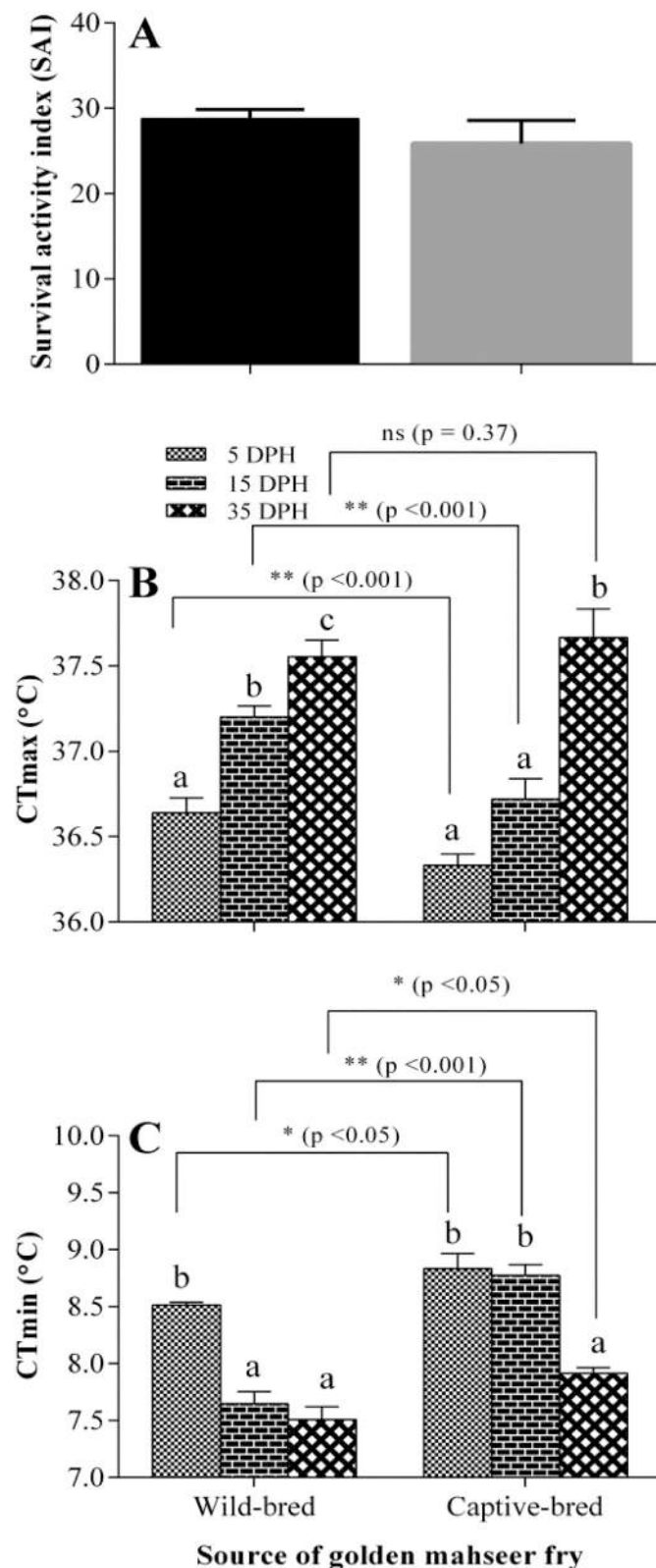


Fig. 4. Starvation tolerance (A), critical thermal tolerance limits (B and C) and thermal scope (D) of larvae obtained from captive-matured and wild-collected golden mahseer brooders. Data are expressed as Mean $\pm$ SE, $N = 10$; Bars bearing asterisk (*) indicate statistically significant differences between wild and captive brooder offspring ($P < 0.05$, Student's t-test). Thermal tolerance (CT_{max} and CT_{min}) and thermal scope ($CT_{max} - CT_{min}$) bars within each group (wild or captive) bearing different superscripts (a and b) are statistically significant ($p < 0.05$, One way ANOVA).

absorption, indicated by clear vacuoles in the apical part of mucosa in the midgut, was significantly less (in terms of their size and abundance) in larvae of captive brooders than those of wild counterparts (Fig. 8d and h, for comparison). In both larval types, liver and pancreas were visible at 5 dph, not on the day of hatching.

4. Discussion

The egg size (weight and diameter) and biochemical composition are often used as important markers for evaluating egg quality and reproductive performance. The nutrients in the yolk sac reserves of the egg fulfil the nutritional requirement of larvae until first feeding. Therefore, eggs should contain sufficient quantities of all essential nutrients necessary for embryo development and early larval growth (Rønnestad et al., 1999). The significantly higher amounts of total protein, glucose, and triglycerides recorded in wild origin eggs of golden mahseer indicate the availability of sufficient nutrient/energy reserves for meeting the catabolic and anabolic demands of developing embryos and larvae. Numerous studies have already been demonstrated higher energy reserves in viable eggs versus non-viable eggs and wild versus captive origin eggs of several teleosts (Srivastava and Brown, 1991; Nguyen et al., 2012). On the other hand, few studies reported no significant correlation between egg composition and egg quality (Faulk and Holt, 2008). For instance, in lake trout, the triglycerides and proteins in eggs were not related to their viability (Lahnsteiner et al., 1999). The positive influence of carotenoids on egg viability has already been demonstrated in fish (Salze et al., 2005; Alijanpour et al., 2015). The significantly lower total carotenoid levels observed in eggs from captive golden mahseer females than those from wild broodstock agree with earlier studies in other fish species (Craik, 1985; Salze et al., 2005). This variation in total carotenoids observed between wild and captive eggs might result from the absence of any carotenoid sources in the broodstock diet fed to mahseer brooders matured in captivity. In contrast, in the wild, they obtain carotenoids from phytoplankton and algae. Earlier studies also documented that the carotenoid content of the broodstock diet will be reflected in their eggs (Youngson et al., 1997; Pickova et al., 1999).

Further, the diameter and weight of captive golden mahseer eggs were significantly higher than wild origin eggs indicating potential quality differences. Previous studies in several fishes also reported higher egg weight and diameter as indicators of poor quality eggs, and the water influx into the eggs during their retention in the coelomic cavity (post-ovulation time) had been proposed as the reason for the bigger size of those eggs (Aegerter and Jalabert, 2004; Lahnsteiner, 2000; Mansour et al., 2008). Conversely, larger egg size was reported as an essential criterion of viable eggs in several fishes, and many authors correlated larger egg size with higher survival and larval viability (Kamler et al., 1982; Srivastava and Brown, 1991; Marteinsdottir and Steinarnsson, 1998; Kjørsvik et al., 2008; Nguyen et al., 2012). The egg weight increase during water hardening is considered a reliable indicator of egg quality. It is associated with the influx of water depending on the amount of osmolytes released from the cortical vesicles (Alderice, 1988; Lahnsteiner et al., 2001; Mansour et al., 2008). In cyprinids, fertilization rate and egg viability were known to be linked with water uptake rate during hardening along with the weight of water-hardened eggs (Lahnsteiner et al., 2001). Although the mean weight and diameter of unfertilized wild origin eggs of golden mahseer were significantly lower in comparison to captive eggs, their relative weight/diameter increase (due to higher water uptake rate) during different time points of water hardening were either higher or comparable to captive eggs indicating a strong cortical reaction and superior quality of wild origin eggs. The egg weight increase during water hardening can also be positively correlated with osmotically active components such as glucose level in wild eggs ($y = -0.180x + 14.14$, $R^2 = 0.863$). Therefore, the low levels of osmolytes viz., glucose, and free amino acids possibly contributed to the captive egg's lower water uptake rate during hardening.

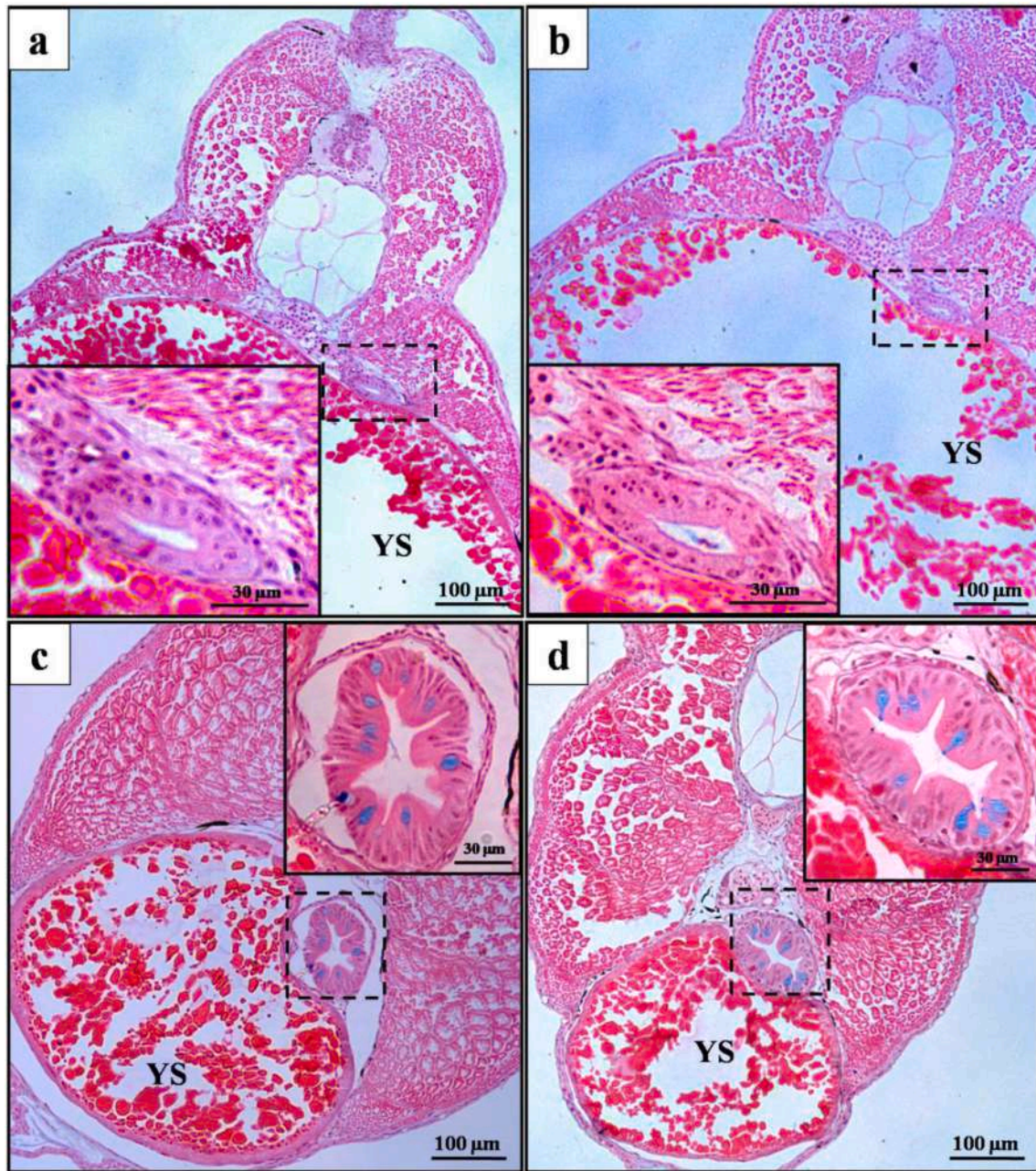


Fig. 5. Photomicrographs showing the sections of midgut of golden mahseer larvae obtained from captive matured (a and c) and wild collected (b and d) brooders at 5 dph (a and b) and 10 dph (c and d). Insets indicate the midgut cross-section. YS-yolk sac.

In cyprinids, the ovarian fluid is produced by the epithelium of the ovarian cavity and eggs will remain immersed in it until spawning/stripping (Lahnsteiner et al., 1997). Hence, it has been demonstrated that the pH and protein levels of the ovarian fluid significantly influenced egg viability and fertilization rate in several teleosts (Aegerter and Jalabert, 2004; Lahnsteiner et al., 1999; Mansour et al., 2008; Jia et al., 2015). For instance, pH of ovarian fluid below 8.0 (in rainbow trout) and 8.4 (in lake trout) is associated with poor egg quality (Aegerter and Jalabert, 2004; Lahnsteiner et al., 1999). Moreover, higher ovarian fluid pH (≥ 8.0 ; alkaline pH) was reported to improve sperm motility and sperm motility duration in rainbow trout (Yoshida and Nomura, 1972; Wojtczak et al., 2007). In the present study, both wild and captive females of golden mahseer displayed similar ovarian pH values (~ 8.4). The higher amounts of glucose, proteins, and triglycerides recorded in the ovarian fluid of captive females of golden mahseer possibly indicate

weaker egg membrane and nutrient leakage due to membrane damage or egg lysis in the ovaries, as reported earlier in several fishes (Lahnsteiner et al., 2001). Also, the glucose, protein, and triglyceride levels in ovarian fluid and eggs of both wild and captive brooders were inversely related. Females of lake trout, *Salmo trutta lacustris*, and Arctic charr, *Salvelinus alpinus* with higher quantities of ovarian fluid protein displayed poor egg quality and low fertilization rate (Lahnsteiner et al., 1999; Mansour et al., 2008). Likewise, the lower ovarian fluid protein levels ($0.54 \pm 0.01 \text{ mg mL}^{-1}$) observed during mid spawning season in turbot is associated with a higher fertilization rate (Jia et al., 2015). We also observed a comparatively lower fertilization rate in captive golden mahseer females with higher ovarian fluid protein levels. Based on the literature survey, the relation between glucose and triglyceride content of ovarian fluid and reproductive performance has not been studied in fish.

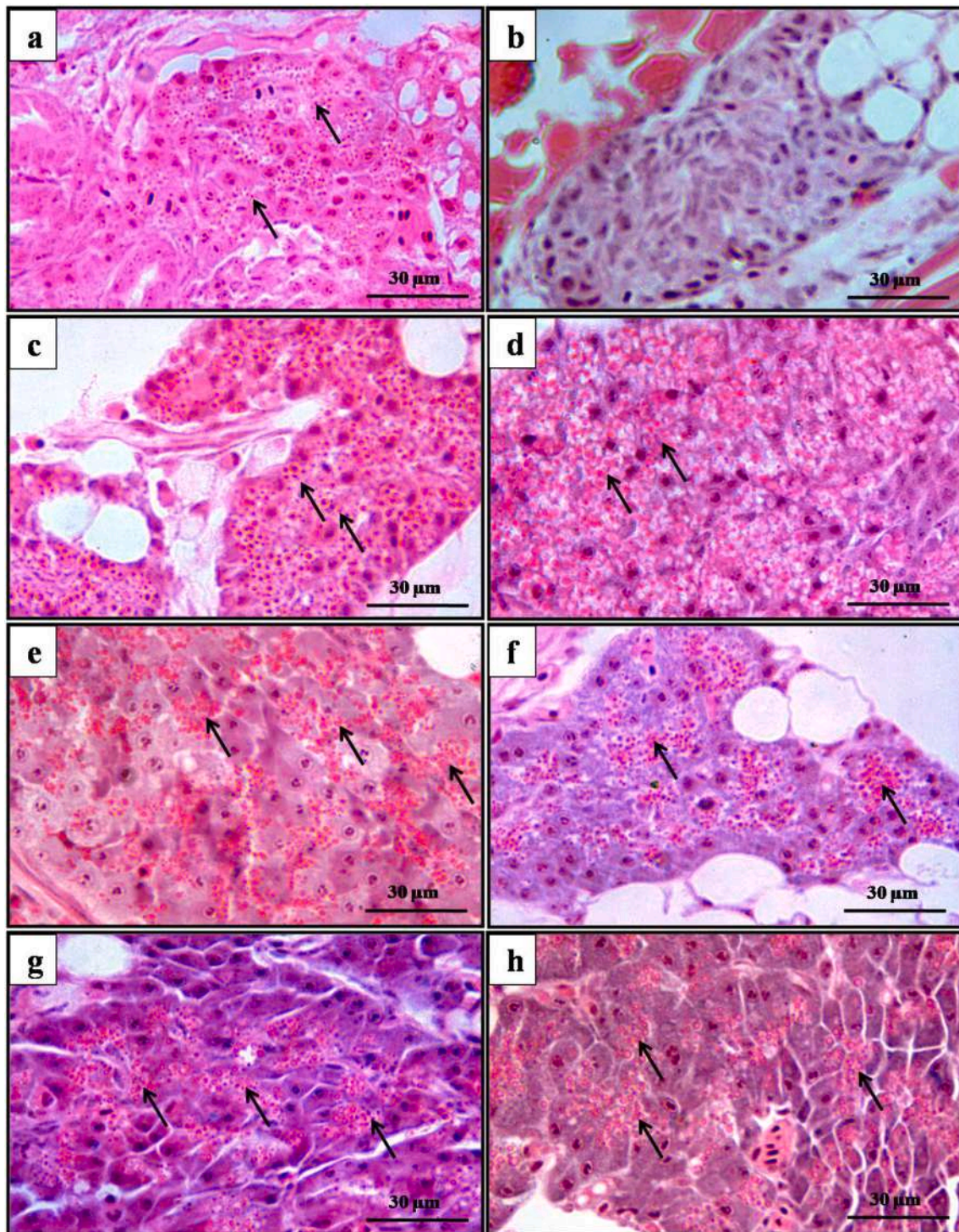


Fig. 6. Photomicrographs showing the pancreatic zymogen granule (indicated by arrows) abundance dynamics in golden mahseer larvae obtained from captive matured (left panel; a, c, e and g) and wild collected (right panel; b, d, f and h) brooders at 5 (a and b), 10 (c and d), 15 (e and f) and 20 (g and h) days post hatching. The pattern of zymogen granule abundance dynamics is similar between the two groups, except at 5 dph; where, zymogen granules are scanty visible (a) in captive brood larvae, but absent in the larvae of wild brooders (b).

Rapid growth is the primary indicator of overall development and the vital outcome of closely linked physiological processes, including the efficiency of digestion and absorption of nutrients, metabolism and growth of all body cells, etc. It was reported that golden mahseer larvae possess a developmentally equipped digestive system at first feeding (Sharma et al., 2016). Still, the growth was not promising until 45 dph as more energy was invested in metamorphosis, and histological studies

revealed that golden mahseer larvae are expected to equip with a fully functional digestive system around 45 dph onwards (Sharma et al., 2016). In the present study, from that point, the growth of captive and wild origin larvae was similar and later became significantly higher in larvae from captive brooders. A similar growth pattern has been observed in Eurasian perch larvae obtained from domesticated spawners compared to those from the wild spawners (Palinska-Zarska et al.,

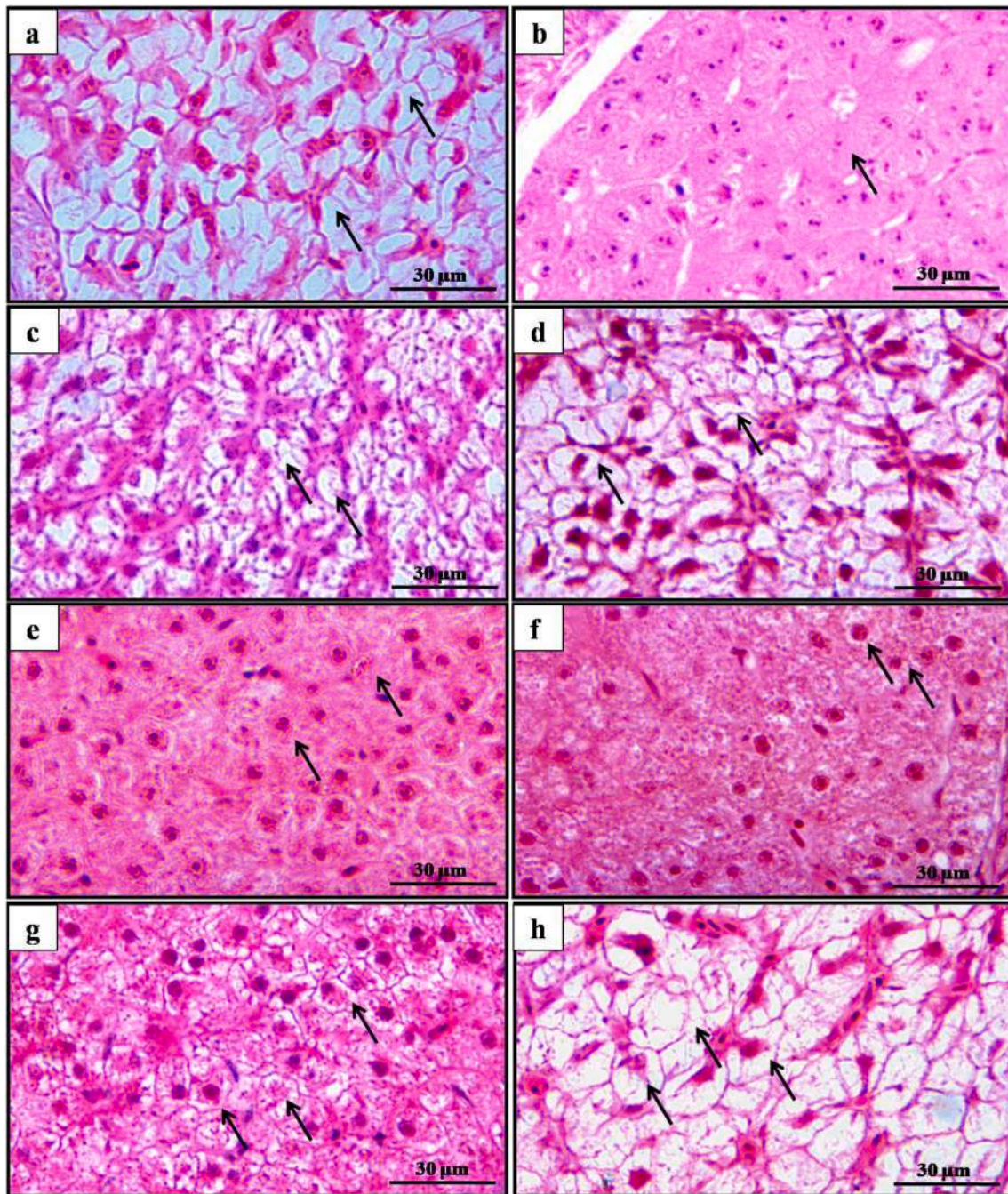


Fig. 7. Photomicrographs showing the hepatocytes (indicated by arrows) size (indicator of hepatic nutritional load) dynamics of golden mahseer larvae obtained from captive matured (left panel; a, c, e and g) and wild collected (right panel; b, d, f and h) brooders at 5 (a and b), 10 (c and d), 15 (e and f) and 20 (g and h) days post hatching. The pattern of hepatocytes size dynamics is not same for larvae/ juveniles of captive and wild brooders. In progenies of captive parents, hepatocyte size decreased gradually from 5 to 15 dph (see a, c, and e), and reached minimum at 15 dph, and started regaining at 20 dph. The hepatocyte size change was more dynamic (looks faster) in wild counterparts than the captive ones, based on rate of change in size. Especially, hepatocyte size of wild offspring at 10 dph (d > c) and 20 dph (h > g) was bigger than that of captive brood larvae. Size depletion at 15 dph, was common in larvae of both the brooders.

2020). The pancreas and liver of larvae from captive brooders, at 5 dph, appeared more developed compared to those from the wild origin, and this was due to their long incubation period. However, the post hatched development of the digestive system of wild brooders' larvae, after 5 dph, appeared to be comparatively faster. The gain in nutritional fitness of wild brooders' progeny from 5 to 20 dph was significantly faster than captive ones. Prominent histo-physiological differences observed between two types of progeny were the elevated capacity of intestinal lipid and protein absorption and hepatic nutrient regaining in larvae of wild brooders, coinciding with their faster growth (until 35 dph) and

survival. This may be due to dense nutrient reserves (glucose, total protein, and triglyceride) in wild origin eggs compared to captive eggs.

The non-significant difference in starvation tolerance measured as the survival activity index (SAI) of fry from wild and captive broodstock of golden mahseer is in agreement with earlier studies in Atlantic cod, wherein the authors noticed similar SAI between larvae produced from wild and captive broodstocks (Lanes et al., 2012). Similarly, the starvation tolerance test performed in high and low-quality egg batches of common dentex and red porgy reported similar SAI (Giménez et al., 2006; Aristizabal et al., 2009). Regarding thermal tolerance, although

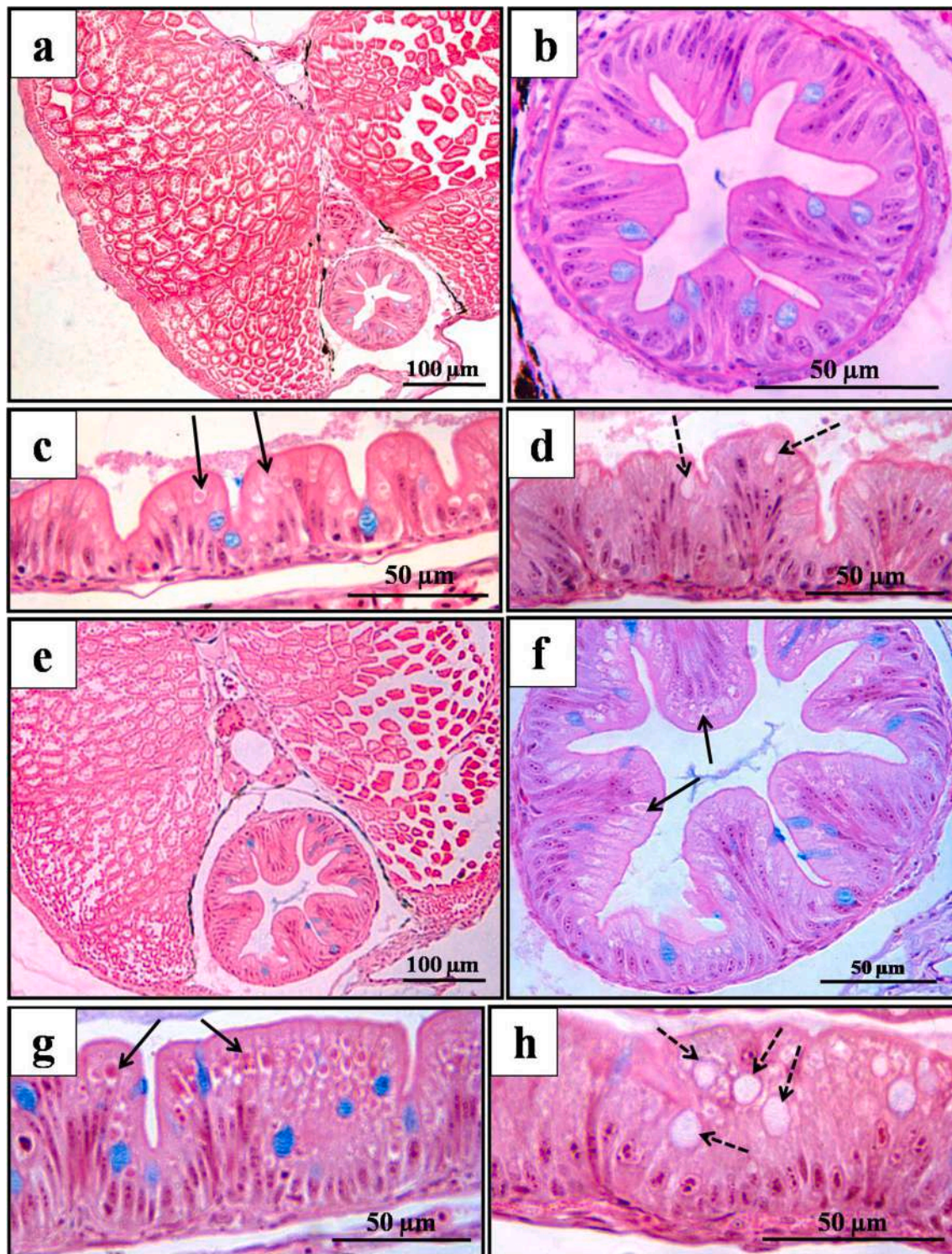


Fig. 8. Photomicrographs showing the sections of mid and hindguts of golden mahseer larvae from captive matured (a, b, c, and d) and wild collected (e, f, g, and h) brooders at 15 dph. Images 'b and f' from hindgut (transverse section) are the magnified versions of 'a and e', respectively. Images 'c and g' are the sagittal sections of hindgut showing the supranuclear vesicles (indicated by solid arrows). Images 'd and h' are the sagittal sections of midgut showing lipid absorption (indicated by broken arrows).

the critical thermal maxima (CT_{max}) of captive-bred golden mahseer fry were lower at initial stages (15 dph), they became comparable during later developmental stages indicating their fitness to cope with the weather/climate extremes similar to wild origin fry. On the contrary, CT_{max} of hatchery-reared brown trout, rainbow trout, and brook trout were lower than their wild strains (Vincent, 1960; Carline and Machung,

2001), while Morgan et al. (2019) reported higher CT_{max} in laboratory populations of zebrafish. On the other hand, the critical thermal minima (CT_{min}) of captive-bred mahseer fry remained higher ($\sim 0.5^{\circ}C$), suggesting a significantly lower cold tolerance. So far, no studies have evaluated the comparative CT_{min} of wild and captive-bred fish to substantiate our findings. A recent study in amphibians showed no

differences between CT_{min} of wild and captive-bred tadpoles of *Aga-lychnis spurrelli* (Pintanel et al., 2020). The relatively broad thermal scope observed in wild origin fry (29.5 °C and 30.05 °C at 15 and 35 dph respectively) indicates their higher flexibility to cope with environmental temperatures, particularly at early developmental stages (15 dph) as compared to captive ones (27.95 °C and 29.75 °C at 15 and 35 dph respectively).

5. Conclusion

The comparative assessment in the present study elucidated that the reproductive output of captive-matured brooders, their egg quality, and larval fitness are lesser than their wild counterparts. Moreover, the study results provide comprehensive leads for interventions in the captive maturation and hatchery management of golden mahseer. Notably, the egg quality traits and larval histological observations are suggestive of nutritional interventions to develop captive broodstock of higher reproductive competence for the production of golden mahseer larvae with higher fitness. Hence, the present investigation is a prelude to quality broodstock development and seed production of endangered golden mahseer for achieving a higher success rate of restoration/rehabilitation programs.

Author's contributions

Conceptualization, design & execution of the experiments, data analysis, and original draft of the manuscript were done by AC and MSA, whereas AP and PHT performed thermal tolerance trials. MKD processed histological samples, and PS executed histological data interpretations and reviewed the manuscript. BSK and MR performed data analysis and reviewed the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Author statement

Alexander Ciji: Conceptualization, design & execution of the experiments, data analysis, and original draft of the manuscript.

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Manees Kumar Dubey: Processed histological samples.

Anupam Pandey: Executed thermal tolerance trials.

Priyanka H. Tripathi: Executed thermal tolerance trials.

Manchi Rajesh: Data analysis and manuscript reviewing.

Biju Sam Kamalam: Data analysis and manuscript reviewing.

Prakash Sharma: Executed histological data interpretations and reviewed the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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De novo assembly and characterization of tissue-specific transcriptome in the endangered golden mahseer, *Tor putitora*



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ABSTRACT

The golden mahseer (*Tor putitora*) graces most of the Himalayan Rivers of India and neighboring South Asian countries. Despite its several importance as a research model, as food, and in sport fishing, knowledge on transcriptome database is nil. Therefore, it was targeted to develop reference transcriptome databases of the species using next-generation sequencing. In the present study, 100,540,130 high-quality paired-end reads were obtained from six cDNA libraries of spleen, liver, gill, kidney, muscle, and brain with 28.4 GB data using Illumina paired-end sequencing technology. Tissue-specific transcriptomes as well as complete transcriptome assembly were analyzed for concise representation of the study. In brief, the *de novo* assembly of individual tissue resulted in an average of 31,829 (18,512–46,348) contigs per sample, while combined transcriptome comprised 77,907 unique transcript fragments (unigenes) assembled from reads of six tissues. Approximately 75,407 (96.8%) unigenes could be annotated according to their homology matches in the nr, SwissProt, GO, or KEGG databases. Comparative analysis showed that 84% of the unigenes have significant similarity to zebra fish RefSeq proteins. Tissue-specific-dominated genes were also identified to hypothesize their localization and expression in individual tissue. In addition, 2485 simple sequence repeats (SSRs) were detected from 77,907 transcripts in the combined transcriptome of the golden mahseer. This study has generated organ-specific transcriptome profiles, which will be helpful to understand the local adaptation, genome evolution, and also future functional studies on immune system of the golden mahseer.

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1. Introduction

The golden mahseer is one of the most important coldwater fish species. It has great importance as a research model, as food, and in sport fishing. However, its slow growth and reproductive dysfunction in captive condition is a major concern for domestication and aquaculture practice. The population in the wild is also reported to be declining due to several human and environmental factors and therefore, declared as an endangered fish (<http://www.iucnredlist.org/>). Due to its ecological and economic importance particularly in the Himalayan regions, several attempts were made to propagate and rehabilitate the species in rivers and lakes by artificial breeding using sexually matured fishes from wild (Pandey et al., 1998). Assessment of genetic vigor and population genetic variability was carried out using mitochondrial gene and microsatellite markers in geographically isolated populations from the Ganga, the Indus, and the Brahmaputra basins (Sati et al., 2015). To study the genetic profiles, polymorphic microsatellite markers from a partial genomic library were identified (Sahoo et al., 2013). Complete mitochondrial genome of this species has also been

reported (Sati et al., 2014) for the proper phylogeny of the species in the family Cyprinidae.

The information on genetic profiles is still not enough to study the complete genome evolution, environmental challenges that species is facing, the immunology, the developmental biology, etc., of this species. The emergence of next-generation sequencing technologies has made possible to generate significant gene/genetic resources through transcriptome profiling of non-model species. The availability of annotated gene collections provide the baseline for future functional genomics applications. Hence, the major objective of this study was to create a reference set of transcriptome using multiple organs that would be helpful to understand the local adaptation, genome evolution, and also future functional studies on immune system of the golden mahseer.

2. Materials and method

2.1. Fish samples

All protocols were performed in accordance with Animal welfare Act approved by ICAR-DCFR Ethical body. Live fishes were collected from the River Kosi (Ramnagar, 29.40°N 79.12°E) using cast net and transported to wet laboratory. The fishes were acclimatized for

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1 month with proper feeding and aeration. After acclimatization, 5 individuals (size range 50–100 cm) were sacrificed and different organs (spleen, liver, gill, kidney, muscle, and brain) were collected in RNAlater® (Ambion Inc., Austin, Texas) and stored at -80°C .

2.2. Isolation and qualitative and quantitative analyses of total RNA

Total RNA was isolated from 6 tissues (spleen, liver, gill, kidney, muscle, and brain) from 5 individuals each using solution based RaFlex Total RNA isolation Kit (GeNei, Bangalore, India) as per manufacturer's recommended protocol. RNA was DNase treated at 30°C for 30 min to remove genomic DNA contamination. The quality of total RNA was checked on 1.2% denatured agarose gel for the presence of intact 28S and 18S rRNA bands. Total RNA was quantified using Qubit®3.0 fluorometer (Thermo Fisher Scientific, Waltham, USA).

2.3. Illumina MiSeq 2X150 PE library preparation

The RNA samples of each tissue from 5 individuals were pooled separately to minimize the variation among individual fish. Library preparation was started with mRNA isolation and enrichment using TruSeq RNA Library Preparation Kit v2 (Illumina, San Diego, USA) as per manufacturer's recommended protocol. The mRNA is fragmented enzymatically. The fragments were then subjected to first and second strand cDNA conversion, followed by end repair, adapter ligation, and finally ended by index PCR amplification of adaptor-ligated library. Quantitative and qualitative estimation of library was performed using HT DNA High Sensitivity Assay Kit (Caliper Life Sciences, Hopkinton, Massachusetts, USA) on Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, USA).

2.4. Cluster generation and sequencing run

After obtaining the Qubit concentration for the library and the mean peak size, 10pM of library was loaded into the reagent cartridge of MiSeq reagent kits v2 (Illumina, San Diego, USA) 300 cycles PE. Cluster generation was carried out by hybridization of template molecules onto the oligonucleotide-coated surface of the flow cell. Immobilized template copies were amplified by bridge amplification to generate clonal clusters. The kit reagents were used in binding of samples to complementary adapter oligos on paired-end flow cell. The adapters were designed to allow selective cleavage of the forward strands after re-synthesis of the reverse strand during sequencing. The copied reverse strand was then used to sequence from the opposite end of the fragment. The libraries were sequenced on Illumina Miseq 500 platform using 2×150 PE chemistry at Xcelris Genomics Ltd., Ahmedabad.

2.5. Sequence filtering and de novo assembly

The raw reads generated from each 6 samples were subjected to quality check using parameters like per sequence analysis, per base analysis, and overrepresentation analysis using Fastx toolkit and CLC Genomics Workbench v.7.5.2 (CLC Bio, Aarhus, Denmark). Further, the reads were separately trimmed for ambiguity, low quality, and PCR duplicates. The *de novo* assembly of the clean reads of all 6 organs was carried out separately using Trinity v2.0.5 (Grabherr et al., 2011) with default parameters. The assembled transcript contigs were validated using CLC Genomics workbench v.7.5.2 by mapping high-quality reads back to the assembled transcript contigs with the settings mismatch cost of 2, an insert cost of 3, a minimum contig length of 200 bp, a similarity of 0.8, and a trimming quality score of 0.05. The high-quality data of all six samples were also concatenated into single file as a total transcriptome of *Tor putitora* using Trinity v2.0.5.

2.6. CDS prediction, functional annotation, and simple sequence repeat (SSRs) identification

The transcript contigs were used to identify the coding region using TransDecoder on default parameters to identify the coding sequences from assembled transcript contig of 6 organs, i.e., spleen, liver, gill, kidney, muscle, and brain separately (Supplementary File 1, CDS length distribution). The functional annotation of CDS generated from each sample and concatenated transcripts of all six organs (total transcriptome) were performed separately by aligning the contigs to non-redundant database of NCBI using BLASTx with an e-value threshold of $1e-06$. Gene ontology (GO) annotation is used by Blast2Go Pro software suite v3.0. The GO sequence distribution was analyzed for all the three GO domains, i.e., biological processes, molecular functions, and cellular components. Each annotated sequence was assigned to detailed GO terms. KEGG pathway analysis was performed using KEGG automatic annotation server (KAAS) (www.genome.jp/tools/kaas/). All the CDS were compared against the KEGG database using BLASTx with threshold bit-score value of 60 (default). Transcripts exclusive to each organ and those common in between organs were identified using In-house Pearl Script. We also identified simple sequence repeat (SSR) from the CDS of total transcriptome (merged CDS of all six tissues) using pearl script MISA (<http://pgrc.ipk-gatersleben.de/misa/>). The parameters were adjusted for identification of perfect di-, tri-, tetra-, and penta-nucleotide motifs with a minimum of 8, 5, 5, and 5 repeats, respectively. Only microsatellite sequences with flanking sequences longer than 50 bp on both sides were collected for primer designing.

3. Results and discussion

3.1. Sequence and assembly

The transcriptome sequencing using 2×150 bp chemistry on the Illumina Miseq500 generated a total of 100,540,130 high-quality reads containing 28,705,168,638 bases across six organs (spleen, liver, gill, kidney, muscle, and brain) yielding 28.4 GB data. The raw data of each sample were deposited separately at the NCBI Sequence Read Archive (SRA) under the study accession number as shown in Table 1. The reads statistics of six organs are also shown in Table 1.

De novo assemblies of each library resulted in an average of 31,829 (18,512–46,348) contigs per sample. Brain tissue had the highest contig count, totaling approximately 60.3 Mbp. The lowest contig count was observed in muscle tissue; with approximately 42.4 Mbp. The longest contig length was 11,907 bp, and the contig N50 was 1206 bp. The assembly statistics of each tissue was shown in Table 1.

3.2. CDS prediction, functional annotation, and SSR identification

The contigs were used to identify the coding regions using TransDecoder of all six tissues. The CDS predictions were 23,832 in spleen, 26,454 in liver, 25,489 in gill, 28,937 in kidney, 25,790 in muscle, and 22,819 in brain, respectively. BLASTx hit resulted in the annotation of 22,989, 25,639, 24,581, 28,116, 24,668, and 22,151 CDS in spleen, liver, gill, kidney, muscle, and brain, respectively. The CDS length distributions among six tissues were shown in Supplementary File 1, CDS length distribution. When the CDS of all six tissues were concatenated, it was observed that 257 transcripts were between the smallest group of 200 to 300 bp, and the highest numbers of 27,624 transcripts were ≥ 1000 bp in length. The next highest 11,507 transcripts were between 500 and 600 bp (Fig. 1).

A *de novo* assembly was also prepared from all six tissues to create a reference transcriptome that resulted in 77,907 transcripts. Functional annotation was performed by aligning them to non-redundant database of NCBI using BLASTx with an e-value threshold of $1e-06$. The BLASTx hit resulted in the annotation of 75,407 CDS and 2500 CDS of no BLAST hit. Majority of the sequences showed matches with the eukaryotes, i.e.,

Table 1
Read and assembly statistics of golden mahseer's transcriptome

Description	Spleen	Liver	Gill	Kidney	Muscle	Brain
NCBI SRA accession no.	SRX767362	SRX767364	SRX768559	SRX768561	SRX770050	SRX800776
Sample ID	SAMN03196595	SAMN03196596	SAMN03196597	SAMN03196598	SAMN03196599	SAMN03196600
No. of high-quality reads	18,456,821	15,355,597	20,875,681	17,011,668	14,847,540	13,992,823
Number of contigs	25,733	38,343	36,683	25,357	46,348	18,512
Total transcriptome length (in base pairs)	48,421,544	47,919,997	49,432,894	59,609,973	42,439,395	60,329,006
Max transcript size (in base pairs)	9129	9694	11,455	16,876	76,237	14,184
N50 value	1878	1493	1989	2342	894	3249

mammals, teleost fishes, and few prokaryotes. Among the teleost fishes, most sequences showed similarity match with *Danio rerio* (84%), *Oreochromis niloticus* (2%), and *Cyprinus carpio* (1%), followed by other fishes (5%). A gene ontology (GO) sequence distribution helps in specifying all the annotated nodes comprising of GO functional groups. CDS associated with similar functions are assigned to same GO functional group. The term within themselves could be assigned to one or more transcripts in the transcriptome. The GO annotations resulted a total of 190,679 GO term assignment to the annotated transcriptome comprising 69,372 (36.38%) assignments from biological process, 70,391 (36.92%) from molecular function, and 50,916 (26.7%) from cellular components ontology (Supplementary File 1, GO distribution). There was not much difference in the expression profiles in association with GO classification when compared to entire transcriptome as well as six individual tissues respectively (Supplementary File 1, GO distribution). In GO distribution, the transcripts are involved with only 22 biological process terms, 12 molecular function terms, and 8 cellular component terms. The mapped CDS fall in to six broad categories such as metabolism, genetic information processing, environmental information processing, cellular processes, organismal system, and diseases (Supplementary File 1, pathway distribution).

In case of individual tissues, the highest assignments of GO terms mapped were cellular and metabolic process in association with biological process, binding and catalytic activity associated with molecular function, and cell and organelle in association with cellular component process (Fig. 2). When we compared the GO distribution among six tissues, the apparent contribution of liver in metabolic process (20.46% of its total biological process) and brain in cellular process (23%) was found. Further, the involvement of muscle in response to stimuli (3.7%) suggested more exposure of muscle to external environment than other organs, particularly gills (3.0%). Two GO terms, i.e., binding activity (48.16%) and catalytic activity (38.3%), in the molecular

function process were significantly overexpressed in the kidney than other organs. The highest assignments in case of cellular component category were cell (61.4%) and synapse (1.2%) in brain and organelle (26.0%) in liver (Supplementary File 1, GO distribution).

The transcripts involved in KEGG pathway were distributed under six categories, metabolism, genetic information processing, environmental information processing, cellular process, organismal system, and diseases. Tissue-specific total numbers of CDS were mapped in KEGG pathway: 9696 CDS (42.17%) in spleen; 13,002 (50.71%) in liver; 11,289 (45.9%) in gill; 13,745 (48.88%) in kidney; 11,305 (45.8%) in muscle; and 10,442 (47.1%) in brain. It was observed that liver is the major site of metabolism, with 2743 CDS involved in the mechanism and 1571 CDS involved in genetic information processing. It was also observed that lipid metabolism (425 CDS) was the major pathways under the metabolism category in liver (Supplementary File 1, KEGG annotation). In kidney, 2082 CDS were annotated to be involved in environmental information processing, 1845 CDS in cellular process, 3127 CDS in organismal system, and 2933 CDS in diseases (Supplementary File 1, KEGG annotation). The associations of 1533 CDS in signal transduction under environmental information processing pathway, 783 CDS in immune system under organismal system, and 1177 CDS in infectious diseases under diseases pathway in the kidney tissue were also significant. Comparatively, most of the transcripts were associated at least in four major KEGG pathways in the kidney tissue than other tissues.

3.3. Tissue-specific transcripts' profiles

The functionally annotated transcripts were considered for common and exclusive transcript study. The hit accessions of all annotated CDS from all six tissues were extracted and were analyzed as unique using in-house Perl script. Concisely, 1377 unique sequences from spleen,

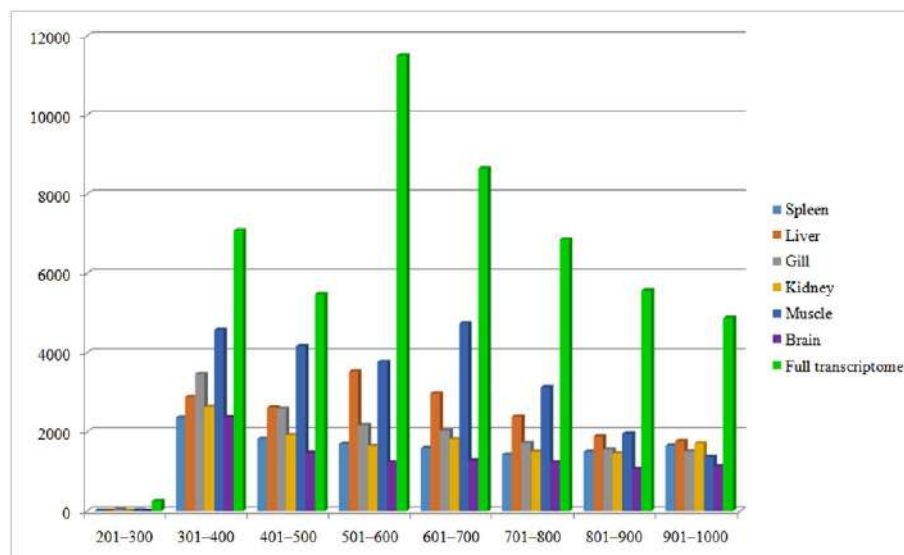


Fig. 1. CDS length distribution of tissue-specific as well as concatenated transcriptome of golden mahseer.

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1175 from liver, 981 from gill, 837 from kidney, 2011 from muscle, and 412 from brain were identified (Supplementary File 2). We observed different interleukins, toll like receptors, immunoglobulins, MHC class 1 antigen, semaphorins, and plexins among most dominant transcripts (2.1%) in the spleen that are mostly involved in antigens processing and immune system process. Spleen is the organ that shows its efficiency in trapping and processing antigens for blood filtering in fishes (Zapata et al. 1996, Zapata et al., 2006). Therefore, the expression of genes related to immune system and antigen processing was evident in spleen. However, semaphorins act as axon guidance factors for neural development, but it was also observed to regulate both immune cell interactions and immune cell trafficking during physiological and pathological immune response (Takamatsu et al., 2010).

Apolipoproteins (0.17%) and molecular chaperones like *Dnajc* and *HSP 27* (0.51%) overexpressed in liver. Apolipoproteins in liver compensate the metabolic demand through lipid mobilization and transport (Huth and Place, 2013) and may be the possible reason of its overexpression. The KEGG pathway mapping of liver's transcripts also showed the association of highest number of transcripts in metabolism. The *Dnaj* proteins also known as HSP 40 are common heat shock proteins that assist in folding or unfolding of other proteins or macromolecule structures in cells (Ellis, 1987). They are found to be induced under several stress conditions i.e., thermal stress, hypoxia and diseases (Song et al., 2014). However, it was also suggested by Roberts et al. (2010) that they play major role in immune responses. Therefore, the overexpression of these HSPs in liver might be an act of normal defense mechanism.

Out of 981 exclusive transcripts in gill tissue, transcripts like actins (0.71%) and claudin (0.81%) were highly expressed. Actin 2 and actin-related protein 10 were overexpressed among actin group of transcripts. The similar results have been obtained by Cui et al. (2014) in the transcriptome analysis of gills of *Takigugu rubripes*. Cui et al. (2014) stated that actin forms microfilaments which contribute in the processes such as intracellular transport of vesicles and organelles. Eight different isoforms of claudins were also found to be overexpressed in the present study. Krause et al. (2008) reviewed the structure and functions of claudins as a transmembrane protein of tight junctions, which enable the vital functions of epithelial organs. Cui et al. (2014) also observed six highly expressed claudin genes in gill transcriptome.

In the exclusive transcript analysis of kidney tissue, 837 transcripts were identified, out of which 21 (2.5%) transcripts were dominated as different solute carrier family genes (Supplementary File 2). These solute carrier families include zinc transporter, neurotransmitter transporter, sodium-glucose transporter, anion exchanger, acetyl CoA transporter, etc., which play a major role in renal organic anion transport. The kidney functions to filter and resorb the inorganic and organic ions, amino acids, bicarbonate, carbohydrates, lipids, and sometimes water itself (Dantzer, 1988) to minimize the ion loss. Therefore, it is evident the overexpression of solute carrier family genes in kidney to maintain the physiological balance in the aquatic environment.

Among 2011 exclusive transcripts in muscle, eleven (0.54%) transcripts were annotated to myosin genes (myosin IIIa, myosin VIIa, myosin IG, myosin heavy chain, myosin binding protein, tropomyosin alpha-1 chain, etc.) and four (0.19%) integrin genes. Fishes remain most active in their aquatic environment, and they need stronger muscle function. The myosin is the primary regulator of muscle strength and contractility (Rooij et al., 2009). Garcia de la serrana et al. (2012) also observed some isoforms of myosin heavy chain, myosin light chain, and myosin binding protein in the muscle transcriptome of the Gilthead seabream (*Sparus aurata*).

The transcriptome of brain tissue comprised 412 exclusive transcripts: two synapsin IIa, three neural adhesion molecules, two potassium voltage-gated channel, and six protocadherin, and its isoforms were dominant among the exclusive transcripts. Synapsins are involved in the regulation of neurotransmitter release at synapses (Ferreira and Rapoport, 2002). The neural adhesion molecule observed in the present

study, normally expresses on the surface of neurons and has a role in cell–cell adhesion, neurite outgrowth, synaptic plasticity, etc. (Senkov et al., 2006). The potassium voltage gate channel Zerg24 (*kcnh2*) plays important role in ion channel and found to be expressed in the brain of zebra fish also (Leong et al., 2010). Frank and Kemler (2002) reviewed the role of protocadherins in tissue morphogenesis, the formation of neuronal circuits, the modulation of synaptic transmission, and the generation of specific synaptic connection. Chen et al. (2008) observed that the transcripts involved in protection and repair of neural tissue were highly represented in brain tissue transcriptome of *D. mawsoni*.

3.4. Identification of simple sequence repeats

A total of 2485 microsatellites were identified from 77,907 unique sequences of merged transcriptome using perl script MISA (<http://pgrc.ipk-gatersleben.de/misa/>), and primers were designed using Primer3 v.2.3.6. Out of these 2485 sequences, 360 contained di-nucleotide, 2104 tri-nucleotide, 19 tetra-nucleotide, and 2 penta-nucleotide repeats. There are about 32 microsatellite containing transcripts for which the primer could be designed successfully (Supplementary File 1, SSR markers). These microsatellites markers in the transcriptome of *Tor putitora* are linked with protein coding genes and would be useful for genetic linkage map.

4. Conclusion

As far as we are aware, this is the first non-model fish species of Himalayan Rivers, in which the transcriptome profile of different organs and a reference data bases of full transcriptome is studied. We have generated the transcriptomes of six organs (23,832, 26,454, 25,489, 28,937, 25,790, and 22,819 CDS in spleen, liver, gill, kidney, muscle, and brain, respectively) and a concatenated full transcriptome containing 77,907 contigs. We also identified organ-specific transcripts and 2485 CDS-associated microsatellites. The information generated in the present study using GO annotation, KEGG pathway, comparative study on organ-specific genes, and gene-associated microsatellites would be helpful for evolutionary studies, mitigation of environmental, and climate challenges and rehabilitation of this species in the Himalayan water bodies. These data are also to be used as reference transcriptome to study other coldwater fishes.

Conflict of interest

The authors report no conflict of interest on content of the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.mgene.2015.11.001>.

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Morphometric and Molecular Assessment of *Tor putitora* and *Neolissochilus hexagonolepis* Population in Jia Bhoroli river Ecotone Zone, Assam

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ABSTRACT

Background: Ecotones are areas of overlap between different ecosystems, referred to as 'junction zones or tension belts', that harbor a distinct collection of organisms. *Tor putitora* and *Neolissochilus hexagonolepis* are two threatened Mahseer species which requires urgent conservation attention. Bhalukpong area in the River Jia Bhoroli is an ideal example of an ecotone, as its ecosystem transforms from resembling a foothill river ecosystem to a river floodplain ecosystem. However, studies on threatened fish populations in ecotone zones, which could be used as conservation sites, are lacking. Therefore, the present study aims to generate firsthand information on the relationship between the populations of these threatened fish and their foothill-floodplain river interface ecotone in the Jia Bhoroli River.

Methods: The data on water quality, topography was collected for a period of one year from January to December, 2021 along with fish samples. The mahseer species were identified and catch per unit effort (CPUE) was also calculated.

Result: Identification of the collected mahseer fish as *T. putitora* and *N. hexagonolepis* were confirmed using both morphometric as well as molecular tools. CPUE was found to be 4.3-12.6 for *N. hexagonolepis* and 4.1-10.3 for *T. putitora*. The ecotone zone was found to have a thriving mahseer population and ecotones are believed to play a crucial role as a diverse food source for the fish. Conserving Bhalukpong as a designated ecotone site in the Jia Bhoroli River as a fish sanctuary can help to protect their populations. Further research that encompasses all aspects of ecology is recommended for the future.

Key words: Conservation, Ecotone, Jia bhoroli, Mahseer, Population.

INTRODUCTION

The concept of an ecotone was first introduced by Clements in 1905 as a way to describe the boundary between two different biological communities. An ecotone is defined as "a zone of transition between adjacent ecological systems, with a set of characteristics determined by the strength of interaction between the adjacent ecological systems" (Holland, 1988). It represents a region characterized by a relatively swift transition, giving rise to a narrow ecological region that separates two distinct and relatively uniform community types (van der Maarel, 1990). The River Jia Bhoroli originates in the upper Himalayan range of Arunachal Pradesh at an elevation of 5400 m msl where it is known as Kameng, It then flows through the Sonitpur district of Assam before joining the mighty River Brahmaputra at Tezpur (elevation 69 m msl). The middle reaches of the Jia Bharali, from Bhalukpong to Balipara in Sonitpur district, provide excellent opportunities for sport fishing and rafting. Bhalukpong area in the River Jia Bhoroli is an ideal example of an ecotone, as it shifts from resembling a foothill river ecosystem to a river floodplain ecosystem. This transition results in a high level of fish diversity, due to the unique combination of the two contrasting ecosystems (Naiman *et al.*, 2005).

Mahseer, a globally renowned sport and table fish, is considered as the National Heritage of India (Nautiyal, 2014).

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They are flow-loving cyprinids (Order: Cypriniformes, Family: Cyprinidae) characterized by large scales and can grow to be large-bodied carps with a maximum recorded weight of 54 kg. They are taxonomically partitioned into the genera *Naziritor*, *Neolissochilus* and *Tor* (Kottelat, 2013; Froese and Pauly, 2018; Eschmeyer *et al.*, 2017). Only species of the genus *Tor* are considered as true mahseer (Desai, 2003) and this genus includes 16 valid species. These species

are considered highly potamodromous, with upstream spawning migrations often over considerable distances being necessary for successful reproduction (Nautiyal *et al.*, 2001 and 2008).

Golden Mahseer, *Tor putitora*, is a species of fish that is widely distributed in the Himalayan region and across South and Southeast Asia, from Afghanistan, Pakistan, India, Nepal, Bangladesh, Bhutan, Myanmar, Sri Lanka, Western Iran to Thailand. This fish is restricted to montane and sub-montane streams and rivers in the Indus, Ganga and Brahmaputra river basins and is found at an elevation of 70-1891 m above sea level and latitude of 8°N to 36°N. The species has been declared Endangered (EN) by the International Union for Conservation of Nature (IUCN) Red List due to severe threat from overfishing, loss of habitat, decline in quality of habitat resulting in loss of breeding grounds and from other anthropogenic effects such as construction of dams leading to blocking their migrations and affecting their breeding (Jha *et al.*, 2018).

Chocolate mahseer, *Neolissochilus hexagonolepis*, is a freshwater fish that inhabits hill streams and rivers in India, Bangladesh, Nepal, Myanmar, Indonesia and China. In India, it is found in water bodies located at 50-2000 m above sea level in states such as Arunachal Pradesh, Assam, Bihar, Jharkhand, Meghalaya, Nagaland, Uttar Pradesh and West Bengal. It is an omnivorous, opportunistic feeder that feeds on green filamentous algae, aquatic insects, small fish, weeds and mollusks (Majhi *et al.*, 2013). The species has been declared as Near Threatened (NT) as per IUCN Red List due to factors such as habitat loss, pollution and increasing water temperature in its inhabiting area (Arunachalam, 2010).

Previous researchers (Samways and Stewart, 1997; Attril and Rundle, 2002) have emphasized the function of ecotones in determining the biotic makeup of ecosystems, but there is a dearth of information regarding the role of ecotones in conserving threatened species. The lack of specific studies on Mahseer populations especially for *T. putitora* and *N. hexagonolepis* in ecotone zones makes the current study important and first of its kind. It aims to generate new information regarding the relationship between this fishes populations and their foothill-floodplain river interfaces ecotone environment in the Jia Bhoroli river. The results of this study will contribute to a better understanding of the ecology of this species and facilitate the development of informed conservation and management decisions.

MATERIALS AND METHODS

Study area and sampling

During the present study, fish samples were collected from the wild stocks in Bhalukpong area (27°0'56"N 92°38'46"E) of the Jia Bhoroli river which falls near the Nameri National Park (Fig 1). The samples were collected with the help of local fishermen using various fishing methods (gill nets, angling and cast nets) from January to December 2021, following the guidelines of the National Biodiversity Authority

of India (Biological Diversity Act, 2002). The fish specimens were identified using standard taxonomic keys (Vishwanath and Nebeshwar, 2009; Kottelat, 2013). Fish that weighed less than 140 g were counted, photographed and then released back into the water, while the remaining samples were brought to the laboratory for further morphometric, meristic and molecular analysis. These samples were deposited in the Fish Museum of the Department of Aquatic Environment Management, College of Fisheries, Assam Agricultural University, Raha, Nagaon, Assam (COFAAUMU 01-55). The population abundance was calculated as:

Total abundance or Catch Per Unit Effort (CPUE) =

$$\frac{\text{Total catch}}{\text{Sum of effort, Sum of the effort remains 10}}$$

(Khajiria *et al.*, 2014)

Water quality parameters

Water samples were collected from Bhalukpong of River Jia Bhoroli from January to December, 2021. Some of the physical parameters like depth and surface water temperature (Mercury thermometer) were determined on the spot. Other parameters like, dissolved oxygen, pH, total alkalinity, total hardness of the water samples was analyzed in the laboratory following APHA (2017).

DNA extraction and barcoding

Further, for molecular analysis, fin and muscle tissue samples were dissected from the mahseer specimens and stored in 100% ethanol. The extraction of total DNA from the ethanol-preserved tissue was carried out according to the standard DNA barcoding methods for fish as described by (Ward *et al.*, 2005). A 655 bp region from the 5' end of the COI gene was amplified using the primers (Table 1) outlined by Ward *et al.* (2005) through a Polymerase Chain Reaction (PCR) with a total volume of 50 µl. The thermal cycling conditions consisted of an initial step of 2 minutes at 95°C followed by 35 cycles of denaturing (94°C, 30 seconds), annealing (54°C, 30 seconds) and extension (72°C, 1 minute) with a final extension of 10 minutes at 72°C. The PCR products were screened for success on a 1.0% agarose gel. The sequencing of the PCR products was outsourced to Eurofin India, Pvt. Ltd. The sequences were then manually edited, aligned and proofread using Clustal W in MEGA 10.0 software and submitted to GenBank (NCBI).

Statistical analysis

All the analysis was performed in Microsoft Excel 2016. The results are displayed either in range or Mean±SD based on applicability. Correlation analysis was done between Total Length and other external features.

RESULTS AND DISCUSSION

Odum and Barrett, (1971) defined an ecotone as an area where two or more different ecosystems overlap and interact. They described it as a transition zone between communities, such as between a forest and grassland or between a soft

bottom and hard bottom marine community. Odum also referred to the ecotone as a "junction zone or tension belt" that contains a unique set of organisms from the overlapping communities, as well as organisms that are specific to and often restricted to the ecotone itself. Mahseer, a globally renowned sport and table fish, is considered the national heritage of India (Nautiyal, 2014). *T. putitora* and *N. hexagonolepis* are two threatened Mahseer fishes which need to be conserved (Jha *et al.*, 2018; Arunachalam, 2010). Even though there are multiple reports of assessment of these species in different rivers and other ecosystems. But no specific study has been conducted in an ecotone zone of a river. Thus, the current study is the first of its kind to study of two important threatened in a foothill-floodplain river interfaces ecotone zone.

Water quality parameters of River Jia Bhorali at Bhalukpong during the sampling period are given in Table 2. The study site is a flowing stream characterized by a moderate current and an average width of 60 m and depth of 1.5 m. The riverbed is composed of boulders (5%), cobbles (35%), coarse gravels (40%) and fine gravels (20%) according to the Indian Standard Soil Classification System (Ranjan and Rao, 2000). The riparian zone is made up of shrubs, bushes and small trees like *Ziziphus mauritiana* in

the terrestrial part and pools and riffles in the aquatic part. The banks of the river are covered by the dense forest of Nameri National Park.

Water quality in the study area was found to be intermediate between that of foothill river water and floodplain river water when compared to previous reports of this river (Khound *et al.*, 2012; Singh *et al.*, 2020). The continuous input of organic materials, such as plant litter, into the river may be a primary factor in this classification and the river continuum concept provides a suitable framework for its description. However, the riparian flora and fauna require further study.

The current study thoroughly examined and evaluated each collected specimen. The general body shape of identified *T. putitora* and *N. hexagonolepis* can be seen in Fig 2 (a and b). The body colour of fresh specimens *T. putitora* were greenish and silvery on the side of the body, but turns reddish yellow or golden on the anal and pectoral fins. Mouth with lower jaw slightly shorter than the upper jaw, caudal fin deeply forked. The dorsal fin ray of the specimens found to be 11 (2/9); pectoral fin ray was 14-15, anal fin ray 7, pelvic fin ray count 8-9 while caudal fin ray count was 20. Lateral line scale found to vary between 25-27; scales above lateral line 4.5 and below lateral line 2.5 (Table 3 and 4). Based on

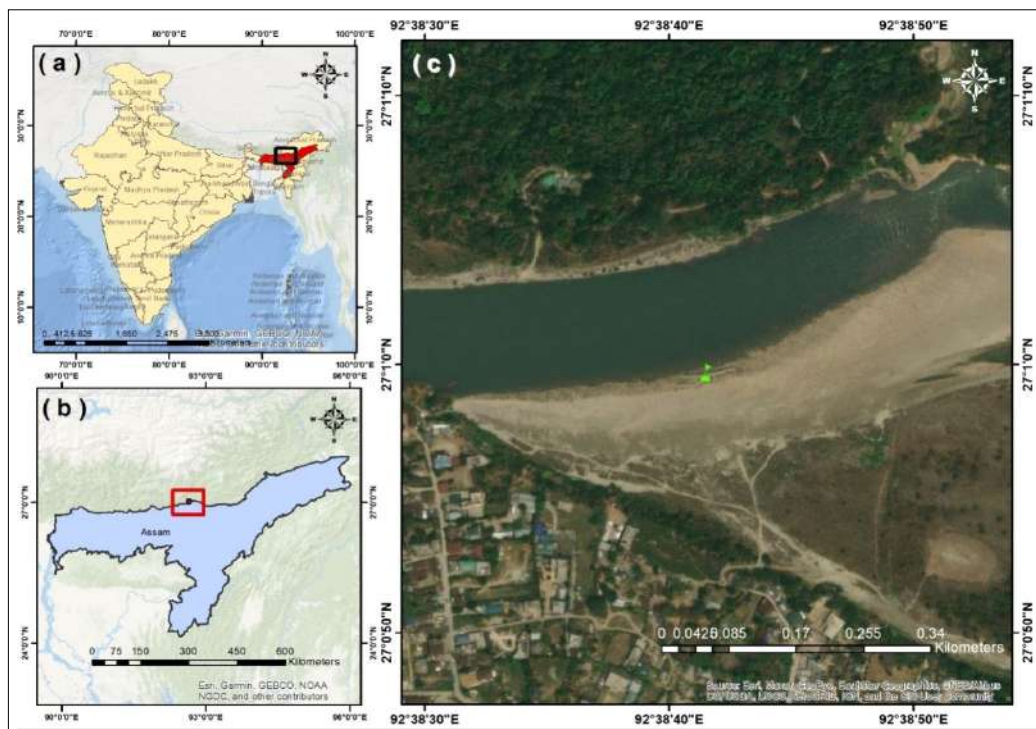


Fig 1: Map showing sample collection area in Bhalukpong ecotone zone of River Jia Bhoroli.

Table 1: Primers used during the study.

Gene	Primer sequence (5' to 3')	Product size (bp)	Reference
COIF1	TCAACCAACCACAAAGACATTGGCAC	655 bp	Ward <i>et al.</i> , 2005
COIR1	TAGACTTCTGGGTGGCCAAAGAATCA		

this, the derived fin formula of *T. putitora* is D11(2/9)P14-15V8-9A7(2/7)LLS25-27. The standard length (SL) of *T. putitora* ranges 234-219 mm. A positive correlation was observed between total length and all the external body parts in *T. putitora* (Table 5). In relation to TL, the highly correlated body parameters were FL (0.983), SL (0.932), VFL (0.906) and GL (0.905).

The scales of *N. hexagonolepis* were hexagonal in shape as its name derived from it, snout length was less

than that of the body depth and body color was dark brown in freshly collected specimens. The specimens were found to have 26-32 lateral line scales, 3.5-4.5 scales above lateral line and 2.5 scales below lateral line. The dorsal fin ray of the specimens found to be 11 (2/9); pectoral fin ray was 14-15, anal fin ray 7, pelvic fin ray count 8-9 while caudal fin ray count was 18-21. Lateral line scale found to be 25; scales above lateral line 3.5 and below lateral line 2.5 (Table 3 and 4). Based on this, the derived fin formula of *N. hexagonolepis* is D11(2/9)P14-15V8-9A7(2/7)LLS25. The standard length (SL) of *N. hexagonolepis* ranged from 211-256 mm. Positive correlation was observed between total length and the external body parts of *N. hexagonolepis* except ED, LJL, GL, GW, DFH and DoM. The highly correlated body parameters in relation to TL were VFL (0.910), DaE (0.898), HD (0.895) and PFL (0.895).

The morphometric as well as meristic counts of *T. putitora* and *N. hexagonolepis* specimens recorded during the present study were found to be similar as described by previous

Table 2: Water quality parameters of River Jia Bhoroli at Bhalukpong.

Physico-chemical parameters	Range
Temperature	13-29°C
pH	7.2-8.2
Dissolved oxygen	4.9-7.7 mg/L
Free carbon dioxide	0-13.3 mg/L
Total alkalinity	88.5-119.6 mg/L
Total hardness	74.3-96.5 mg/L

Table 3: Morphometric count of two important Mahseer i.e. *Tor putitora* and *Neolissochilus hexagonolepis*.

Morphometric count	<i>Tor putitora</i>			<i>Neolissochilus hexagonolepis</i>		
	Mean	Median	SD	Mean	Median	SD
Weight (gm)	164.636	166.5	11.713	187.455	190	20.124
Total length (TL) (mm)	268.818	270	8.168	287.182	286	14.058
Fork length (FL) (mm)	212.455	213	9.122	252.303	251	22.099
Standard length (SL) (mm)	223.682	223.500	4.357	236.182	236	15.220
Head length (HL) (mm)	52.666	52.705	1.641	53.834	53.580	2.623
Pre-dorsal length (PdL) (mm)	93.470	93.245	1.100	83.175	93.980	30.571
Snout length (SnL) (mm)	21.075	20.210	2.148	21.937	21.900	1.970
Inter-orbital width (IoW) (mm)	24.188	21.695	6.253	23.573	23.650	2.542
Post-orbital length (PoL) (mm)	27.429	26.275	3.359	28.634	27.980	4.801
Eye diameter (ED) (mm)	17.306	15.955	3.679	13.686	13.270	2.730
Upper jaw length (UJL) (mm)	15.357	13.830	4.226	14.779	14.920	1.367
Lower jaw length (LJL) (mm)	18.023	17.305	2.151	16.131	15.760	2.617
Anal length (AL) (mm)	146.177	143.170	6.681	156.505	157.870	35.476
Girth length (GL) (mm)	146.334	145.435	3.420	149.385	163.800	36.696
Body depth (BD) (mm)	50.666	49.580	3.393	63.147	65.480	5.716
Head depth (HD) (mm)	36.290	35.330	2.537	40.250	39.730	4.631
Gape width (GW) (mm)	18.948	18.210	2.090	15.265	15.110	2.942
Dorsal fin height (DFH) (mm)	58.855	58.305	2.358	60.084	58.800	6.077
Dorsal fin base (DFB) (mm)	23.155	22.785	1.521	28.053	26.540	5.796
Pectoral fin length (PFL) (mm)	38.921	37.910	2.725	43.092	43.420	5.313
Pectoral fin base (PFB) (mm)	9.625	9.130	1.481	13.286	11.920	3.608
Anal fin length (AFL) (mm)	40.200	39.215	2.604	43.656	43.480	2.910
Anal fin base (AFB) (mm)	15.483	14.905	2.520	15.774	15.120	2.522
Ventral fin length (VFL) (mm)	32.998	32.335	2.612	37.777	37.900	3.924
Ventral fin base (VFB) (mm)	15.631	14.360	2.699	12.697	12.120	1.973
Depth of mouth (DoM) (mm)	10.246	9.485	2.226	10.013	10.180	2.869
Depth of eye (DaE) (mm)	26.915	26.030	2.602	31.410	31.960	3.944
Depth at dorsal fin (DaDF) (mm)	49.265	48.845	1.726	58.739	58.300	6.474
Depth at pectoral fins (DaPF) (mm)	40.854	39.975	2.663	46.341	47.570	4.567
Depth at anus (DaA) (mm)	35.870	34.505	2.811	39.700	40.940	4.751
Caudal fin height (CFH) (mm)	28.704	27.930	2.612	43.211	33.240	16.039
Length of the caudal peduncle (LoCP) (mm)	24.255	23.680	2.324	29.979	26.710	7.593

authors (Vishwanath *et al.*, 2011; Langer *et al.*, 2013 and Laskar *et al.*, 2013). The positive correlation between total length and other external morphological features observed during the present study indicates isometric growth pattern

Table 4: Morphometric relationship table of *Tor putitora* and *Neolissochilus hexagonolepis*.

Co-relation	<i>Tor putitora</i>	<i>Neolissochilus hexagonolepis</i>
TL/FL (mm)	0.983	0.646
TL/SL (mm)	0.932	0.867
TL/HL (mm)	0.839	0.712
TL/PdL (mm)	0.745	0.709
TL/SnL (mm)	0.886	0.691
TL/IoW (mm)	0.845	0.739
TL/PoL (mm)	0.858	0.506
TL/ ED (mm)	0.742	-0.503
TL/UJL (mm)	0.862	0.793
TL/LJL (mm)	0.844	-0.519
TL/AL (mm)	0.876	0.392
TL/GL (mm)	0.905	-0.242
TL/BD (mm)	0.856	0.747
TL/HD (mm)	0.831	0.895
TL/GW (mm)	0.850	-0.406
TL/DFH (mm)	0.855	-0.457
TL/DFB (mm)	0.879	0.873
TL/PFL (mm)	0.884	0.895
TL/PFB (mm)	0.869	0.608
TL/AFL (mm)	0.801	0.772
TL/AFB(mm)	0.871	0.593
TL/VFL (mm)	0.906	0.910
TL/VFB (mm)	0.878	0.256
TL/DoM (mm)	0.865	-0.034
TL/ DaE (mm)	0.868	0.898
TL/DaDF (mm)	0.847	0.848
TL/DaPF (mm)	0.804	0.862
TL/DaA (mm)	0.832	0.846
TL/CFH (mm)	0.818	0.885
TL/LoCP(mm)	0.846	0.810

of *T. putitora* in natural condition. Langer *et al.* (2013) also reported similar morphometric relationship in Golden Mahseer from Jhajjar stream, J and K, India.

DNA barcoding

Identification of the Mahseer species were confirmed through DNA barcoding, the generated mitogenome sequences were submitted to NCBI and accession numbers were obtained (Table 5). The construction of a phylogenetic tree using maximum likelihood and neighbor-joining algorithms showed that the COI gene sequences of the specimens were closely related to those of *N. hexagonolepis* and *T. putitora* from other parts of the world (Fig 3). The overall genetic distance calculated within the species was 0.002 and 0.01 for *T. putitora* and *N. hexagonolepis*, respectively.

The COI gene sequence of *T. putitora* and *N. hexagonolepis* was compared to other sequences from various locations around the world available in the NCBI GenBank database. The blast alignment showed that the specimen was 98% identical to the other sequences of *T. putitora* and *N. hexagonolepis*, which, according to Ward *et al.* (2005) confirms their identity as the species with 98-99% resemblance. The mitogenome sequences of the Mahseer species generated from the River Jia Bhoroli, Brahmaputra drainage is the first such kind of information. Phylogenetic analysis supports the close evolutionary relationship between the species, with a highest bootstrap value of 100. Despite the geographical distance, the COI gene sequences of the compared species are genetically similar, suggesting conservation throughout the evolutionary process. Based on both morphological and phylogenetic analyses from COI gene sequences, it is concluded that the barb species are *T. putitora* and *N. hexagonolepis*.

Population

The survey carried out over the course of one year studied a total of 1026 nos. of *N. hexagonolepis* and 937nos. of *T. putitora* in the Bhalukpong region of Jia Bhoroli river with the density of *N. hexagonolepis* (with a CPUE of 4.3-12.6) higher in the study area compared to *T. putitora* (with a CPUE of 4.1-10.3). Both species showed the highest density during March and the lowest in January. The current study found a high

Table 5: DNA barcoding details of Mahseer species from River Jia Bhoroli generated during the study.

Species	River	Primer	Identified	Barcode submitted	Accession no.
<i>Neolissochilus hexagonolepis</i>	Jia Bhoroli	(COX1)	Yes	Yes	MZ540413
<i>Neolissochilus hexagonolepis</i>	Jia Bhoroli	(COX1)	Yes	Yes	MZ540411
<i>Neolissochilus hexagonolepis</i>	Jia Bhoroli	(COX1)	Yes	Yes	MZ540314
<i>Neolissochilus hexagonolepis</i>	Jia Bhoroli	(COX1)	Yes	Yes	MZ520586
<i>Neolissochilus hexagonolepis</i>	Jia Bhoroli	(COX1)	Yes	Yes	MZ664315
<i>Neolissochilus hexagonolepis</i>	Jia Bhoroli	(COX1)	Yes	Yes	MZ664305
<i>Neolissochilus hexagonolepis</i>	Jia Bhoroli	(COX1)	Yes	Yes	MZ664303
<i>Neolissochilus hexagonolepis</i>	Jia Bhoroli	(COX1)	Yes	Yes	OM060678
<i>Neolissochilus hexagonolepis</i>	Jia Bhoroli	(COX1)	Yes	Yes	OM060688
<i>Tor putitora</i>	Jia Bhoroli	(COX1)	Yes	Yes	OM060681
<i>Tor putitora</i>	Jia Bhoroli	(COX1)	Yes	Yes	OM060682
<i>Tor putitora</i>	Jia Bhoroli	(COX1)	Yes	Yes	OM060687

abundance of adult as well as young individuals of both fish species in this area. The riffles and pools provide excellent habitats for young and small fish (de Moraris *et al.*, 1995). The reproductive success of these populations is highly sensitive to any modifications of this ecotone zone (Schiemer and Zalewski, 1992). The fish fauna in this ecotone zone was found to be rich and diverse and these ecotones likely play an important role as a diversified food source for the fish (Khajuria *et al.*, 2014). The higher abundance of fish in the

study area indicates favorable conditions for these fish in this ecotone.

According to the IUCN, *T. putitora* and *N. hexagonolepis* are classified as endangered and near threatened, respectively (Jha *et al.*, 2018; Arunachalam, 2010). The major reasons of this classification are rise in water temperature, overfishing, loss of habitat, decline in quality of habitat resulting in loss of breeding grounds and from other anthropogenic effects (Sarma *et al.*, 2022). Hence, urgent efforts for conservation of these fish species are need

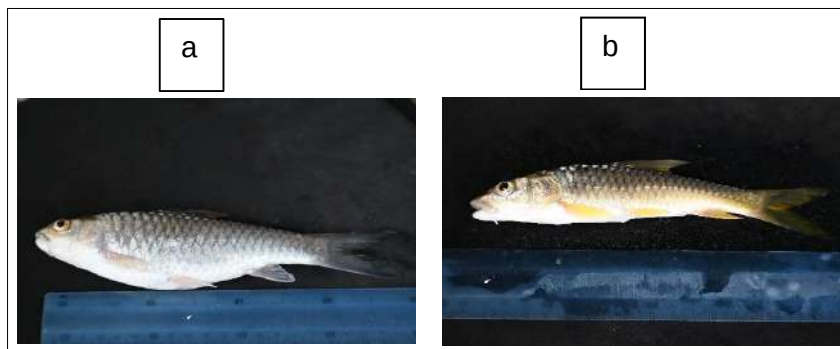


Fig 2: Preserved specimen of (a) *Neolissochilus hexagonolepis* (b) *Tor putitora*.

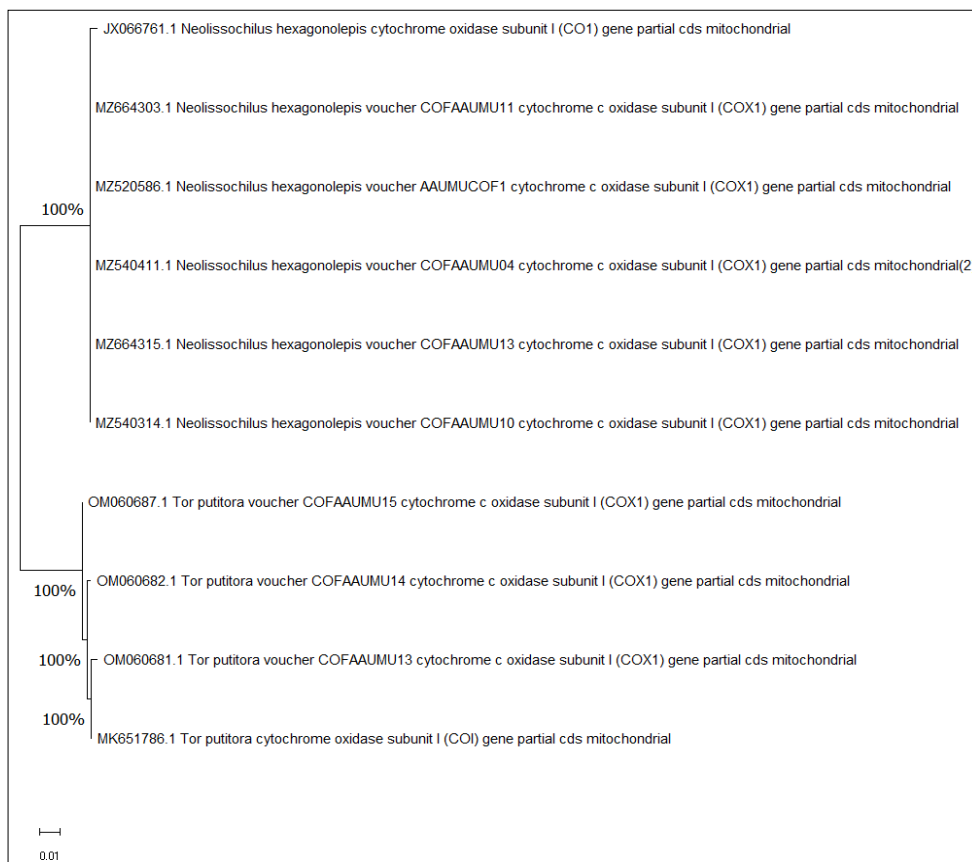


Fig 3: Neighbour-joining tree based on mtDNA COI gene sequence of *Tor putitora* and *Neolissochilus hexagonolepis* generated during the present study.

of the hour. Past studies suggested that ecotones may act as hotspots for speciation, making them valuable areas for conservation investment as they can serve as centers for biodiversity. Chapman *et al.* (1996) claimed ecotones as refugia for endangered fishes as populations in ecotones are often pre-adapted to changing environments and may be more resilient to environmental changes, such as climate change and invasions by non-native species. As ecotones are small in size yet rich in biodiversity, conserving these areas as *in-situ* conservation may be a cost-effective strategy for maintaining biodiversity (Karl, 2017).

CONCLUSION

In conclusion, the findings of this study suggest that the ecotone zones of the Jia Bhoroli river are a refuge for threatened Mahseer population as it provides food and shelter. Both *T. putitora* and *N. hexagonolepis* are in need of conservation efforts as their populations are declining due to various anthropogenic activities. Conserving Bhalukpong as designated ecotone sites in Jia Bhoroli river as fish sanctuaries can give a big boost to restoration efforts of these threatened fish species. Further research with all the other factors of ecology should be conducted for better understanding of importance of ecotones for conservation of threatened fish species.

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Conflict of interest

All the authors declare no potential conflict of interest.

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Phylogenetic relationship and molecular identification of five Indian Mahseer species using *COI* sequence

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Abstract

This study examined the phylogenetic relationship and identification of five Mahseer species (*Tor putitora*, *Tor tor*, *Tor khudree*, *Tor chelynoideis* and *Neolissochilus hexagonolopis*) using partial sequencing of a Cytochrome Oxidase I (*COI*) DNA barcodes. The sequence analysis data showed that 134 (21.61%) sites out of 628 sites were variable without insertion or deletion. Rate of transition (70.5%) were higher than transversion (29.41%). There was a high inter-species divergence (range 4.1% to 12.2%) in Mahseer species as compared to intra-specific sequence divergence (1.7% for *T. putitora*, 1.2% for *T. tor*, 1.4% for *T. khudree*, 3.0% for *T. chelynoideis*, 0.26 % for *N. hexagonolopis*). The phylogenetic tree, constructed by maximum parsimony, maximum likelihood and unweighted pair group average methods revealed similar results suggesting that *T. putitora*, *T. tor* and *T. khudree* had a close relationship to each other while maximum divergence was observed in *T. chelynoideis*, which was also confirmed by the genetic distance data. The results indicate that *COI* sequencing or bar-coding is useful in unravelling phylogenetic relationship and identification of Mahseer species.

Key words

Bar-coding, Cytochrome Oxidase I, Genetic distance, Mahseer, Phylogenetic relationship

Introduction

Mahseer is the common name used for genera *Tor*, *Neolissochilus* and *Naziritor* belongs to family Cyprinidae. At present 46 Mahseer species are known of which 23 species belong of genus *Tor*, 22 species to genus *Neolissochilus* and one specie to genus *Naziritor* respectively (Eschmeyer *et al.*, 2004). Mahseers are important food as well as game fishes of the Asian region. They are endemic to Asia and are distributed across a number of countries such as Nepal, Pakistan, India, Sri Lanka, Malaysia and some South-east countries of Asia (Shrestha, 1990; Nguyen *et al.*, 2008). However, species characterization using morphology and anatomical characters sometimes cause errors in proper identification of closely related species. Because of these issues, molecular markers have been used as a complementary tool for taxonomic identification (Hebert *et al.*, 2003). Very little information is available regarding

species characterization using molecular tools in Mahseer species. A comprehensive literature survey revealed that Nguyen *et al.* (2008) studied Mahseer species using molecular tools and examined the phylogenetic relationships using mitochondrial gene. Mitochondrial DNA (mtDNA) is one of the widely used molecular markers for studying intra-specific and interspecies variation in animals (Avice, 1986; Billington and Hebert, 1988). The mtDNA data has provided new perspectives on taxonomically debatable taxa and confusing questions of phylogeny in which choice of gene is also of great significance (Simon *et al.*, 1994; Groves and Shields, 1996; Lunt *et al.*, 1996). Hebert *et al.* (2003) proposed that the analysis of sequence diversity in Cytochrome Oxidase I (*COI*) gene can provide an effective tool for species diagnosis. They argued that sequence diversity in this gene could be use to create a “barcoding” system that would enable the identification of all animal life. *COI* gene has a faster evolutionary rate (Simon *et al.*, 1994) and is

thus capable of providing a better resolution at the interspecific level. Hence, the present study aims to infer the species identification and phylogenetic relationships of different Mahseer species using *COI* sequences.

Materials and Methods

Sample collection: A total of 22 individuals of 5 species belonging to two genera *Tor* (*Tor putitora*, *Tor tor*, *Tor khudree* and *Tor chelynooides*) and *Neolissochilus* (*Neolissochilus hexagonolopis*) were collected from different geographically isolated location of India (Fig. 1, Table 1). Caudal fin samples of each fish were cut and placed in 2 ml vials containing 75% ethanol and voucher fish specimens immediately fixed in 4% formalin. The fin samples were kept at -20°C until DNA extraction.

Genomic DNA isolation: Genomic DNA was isolated from alcohol preserved 50mg fin tissue samples using proteinase k and phenol chloroform method (Sambrook et al., 1989). Isolated genomic DNA was precipitated with 2-2.5 volume of chilled ethanol. The DNA pellet was washed twice with

70% ethanol, air dried and resuspended in 1X TE (10mM Tris-HCl, pH 8.0 and 1mM ethylene diaminetetraacetic acid disodium salt) buffer and kept at -20°C till further use. The quality of DNA was checked by 0.8% agarose gel electrophoresis and the concentration of DNA was estimated in UV-VIS spectrophotometer (Thermo Scientific, England) at 260nm and 280nm absorbance.

PCR amplification and sequencing of PCR product: Partial sequence of Cytochrome Oxidase I (*COI*) gene was amplified by PCR (Eppendorf, Mastercycler gradient) using universal primers; CIF: 5'-AGTATAAGCGTCTGGGTAGTC-3' and COIA(L): 5'-CCTGCAGGAGGAGGAGAYCC-3' (Palumbi et al., 1991). Amplification was conducted in 50µl reaction volume containing 5µl of 10x PCR buffer (100mM Tris, pH 9.0, 500mM KCl, 15 mM MgCl₂, 0.1% Gelatin) (B-Genei, India) and 1 unit of Taq DNA polymerase (B-Genei, India), 200 µM of each dNTPs (dATPs, dCTPs, dGTP, dTTPs) (B-Genei, India), 25 pmol of each primers and 100ng of genomic DNA. The thermal profile used to amplify *COI* gene consisted of an initial denaturation of 95°C for 2 min; followed by 35 cycle of 94°C for 1 min, 54°C for 50 sec, 72°C for 50 sec and

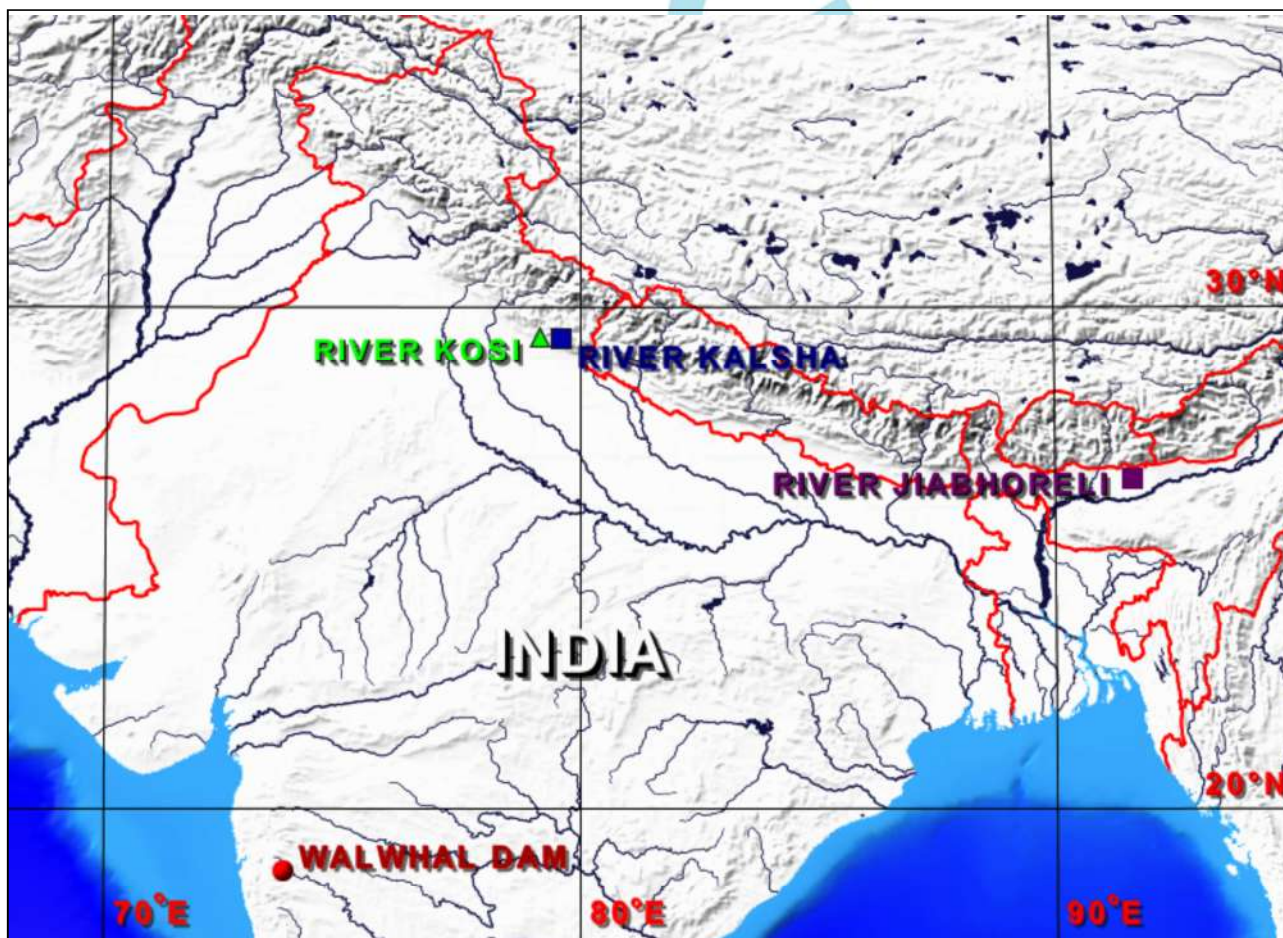


Fig. 1 : Sites of Mahseer species collection

Table 1 : Species, drainages, collection sites, number of haplotype and GenBank Accession numbers of specimens for this study

Species	No. of specimen	Drainages and collection sites	Coordinate location	Genbank accession numbers
<i>Tor putitora</i>	5	River Jia bhoreli, Bhalukpong (Assam-Arunachal Pradesh)	27°02'N; 92°35'E	JX204443-JX204447
<i>Tor putitora</i>	1	River Kosi, Ramnagar (Uttarakhand)	29°24'N; 79°07'E	JX204448
<i>Tor tor</i>	3	River Kosi, Ramnagar (Uttarakhand)	29°24'N; 79°07'E	JX204431-JX204433
<i>Tor khudree</i>	4	Walwhan dam, Lonavla (Maharashtra)	18°45'N; 73°45'E	JX204439-JX204442
<i>Neolissochilus hexagonolopis</i>	5	River Jia bhoreli, Bhalukpong (Assam-Arunachal Pradesh)	27°02'N; 92°35'E	JX204434-JX204438
<i>Tor chelynoides</i>	4	River Kalsa, Chanfi (Uttarakhand)	29°22'N; 79°34'E	JX204427-JX204430

a final extension at 72°C for 5 min. PCR products were checked in 1.2% agarose gel in 1X TBE (Tris-HCl, boric acid, EDTA, pH 8.0) buffer and visualized with ethidium bromide (Sambrook *et al.*, 2001) under UV-Gel-Documentation system (Alpha Imager 3400, Alpha Innotech Corporation, USA). Molecular weights were determined using 100 bp DNA marker (Fermentas, Canada).

The amplicons were purified before sequencing with Qiaquick columns (Qiagen, USA) as per manufacturer's instructions. Sequencing was performed in ABI Prism 3100 automated sequencer (Applied Biosystems, USA) using Bigdye terminator with same primers used for amplification of the target gene.

Sequence data analysis: The CHROMAS (Version 1.45) program was used to display the fluorescence based DNA sequencing analysis. The multiple sequence alignments were done using the CLUSTAL X program version 1.81 (Thompson *et al.*, 1997). Numbers of invariable, variable, singleton variable, parsimoniously informative sites and number of haplotypes were calculated using software DNAsp version 4.5 (Rozas *et al.*, 2003). Sequence divergence within and between the species were calculated using DNA STAR. The MEGA version 4.0 software (Tamura *et al.*, 2007) was used to construct the phylogenetic relationship among five species of Mahseer based on UPMGA, maximum-parsimony (MP) method. CLC genomics work Bench (version 5.1) was used to construct the maximum likelihood (ML) tree. Bootstraps support was calculated using 1000 replication.

Results and Discussion

The mtDNA *COI* gene of 628 bp length was successfully amplified and sequenced for 22 individuals in this study. The sequences obtained were aligned and compared with other GeneBank *COI* sequences. All the sequences representing *COI* gene were submitted to the GenBank with accession numbers given in Table 1. Empirical base frequency were A=27.2%, C= 23.9%, G= 18.0% and T=30.9%. Nucleotide sequences of *COI* gene in Mahseer species were A+T rich (58.1%) with anti-G bias of 18.0%, a

characteristics of the mitochondrial genome (Cantatore *et al.*, 1994), which were also reported in many fishes (Johns and Avise, 1998; Luhariya *et al.*, 2012).

The nucleotide sequences of five species of Mahseer were aligned to determine the variable sites (Table 2). The alignment data showed that 134 sites (21.61%) out of 628 were variable without any insertion or deletions. Among these 134 variable sites, 108 sites (80.59%) were parsimony information polymorphic while 26 sites (19.40%) were singleton variable sites. Most of the variable sites (98 sites, 73.13%) were in the first codon. It indicated several year of evolution involved in the genetic evolution of different cyprinid species (Springer and Douzery, 1996; Wang *et al.*, 2002; Barat *et al.*, 2012). A total of 153 mutations were observed in 134 sites. Rate of transition (108, 70.5%) were higher than transversion (45, 29.41%), which include all three codon position. A high transition bias is well known in vertebrate mtDNA (Meyer, 1993).

So far, 20 haplotypes were observed within these 5 species; 4, 3, 4, 6 and 3 haplotype in *T. khudree*, *T. chelynoides*, *T. putitora*, *T. tor* and *Neolissochilus hexagonolopis* respectively and no haplotype were shared by the five species. These haplotypes were used for further phylogenetic analysis.

The sequence divergence between the species (Table 3) ranged from 4.1% to 12.2%, showed close relationship between *T. tor* and *T. khudree* while maximum divergence were observed for *T. chelynoides*. Furthermore, average percentage divergence of individual species of *T. putitora* was 1.7%, *T. tor* was 1.2%, *T. khudree* was 1.4%, *T. chelynoides* was 3.0% and *N. hexagonolopis* was 0.26%. There was high inter-species sequence divergence for Mahseer species as compared to intra-specific sequence divergence. According to Hebert *et al.* (2003) intra-species divergence value were typically <3%.

The nucleotide sequences of *COI* gene were aligned in order to determine the phylogenetic relationships among five species of Mahseer. The topology of the ML, UPMGA and MP tree estimated were identical (Fig. 2). The

Table 2 : The variable sites of Cytochrome Oxidase I (COI) gene fragment of five species of Mahseer

Nucleotide position →	1 1 1 1 1 1 1 1																																														
	4	5	6	7	8	9	1	1	2	2	2	2	2	2	3	4	4	5	5	5	5	6	6	7	7	7	7	8	8	8	8	9	9	9	0	0	1	2	2	3	3						
Species↓	0	3	6	0	4	6	7	8	9	1	0	1	3	0	1	4	6	8	5	7	0	3	4	6	8	0	2	5	8	9	1	4	7	3	9	8	1	4	6	3	7						
<i>Tor khudree</i> 1	T	G	C	A	G	A	A	G	T	C	T	A	A	T	C	T	T	G	A	C	G	A	T	A	C	C	T	C	C	G	T	T	A	T	T	C	C	C	G	G	A	C					
<i>Tor khudree</i> 2	.	.	.	.	.	.	.	.	C	.	T	.	.	.	T	.	C	.	.	.	.	T	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	.	.	.	.				
<i>Tor khudree</i> 3	.	.	.	C	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.				
<i>Tor khudree</i> 4	.	.	.	C	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.				
<i>Neolissochilus hexagonolopis</i> 1	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	T	.	C	.	C	.	.	A	.	.	.	.	.	.	.					
<i>Neolissochilus hexagonolopis</i> 2	.	.	.	.	.	.	.	.	C	.	T	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	T	.	.	C	.	.	.	A	.	.	.	.	.	.	.					
<i>Neolissochilus hexagonolopis</i> 3	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	T	.	.	C	.	.	.	A	.	.	.	.	.	.	.					
<i>Neolissochilus hexagonolopis</i> 4	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	T	.	.	C	.	.	.	A	.	.	.	.	.	.	.					
<i>Neolissochilus hexagonolopis</i> 5	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	T	.	.	C	.	.	.	A	.	.	.	.	.	.	.					
<i>Tor chelynoides</i> 1	.	.	.	.	.	.	.	.	C	.	.	G	.	G	.	.	.	.	C	.	.	.	.	.	.	.	C	T	.	A	.	.	G	.	C	T	T	T	A	.	.	.					
<i>Tor chelynoides</i> 2	.	.	.	.	G	.	C	.	.	G	.	G	.	G	.	.	.	.	C	.	A	.	C	T	.	.	.	A	.	A	.	G	.	C	T	T	T	A	A	T	.	.					
<i>Tor chelynoides</i> 3	C	.	.	.	.	.	.	.	C	.	.	G	.	G	.	.	.	.	C	.	.	.	.	.	.	.	C	T	.	A	.	G	T	A	.	.	G	.	C	T	T	T	A	.			
<i>Tor chelynoides</i> 4	.	.	.	.	.	.	.	.	C	.	.	G	.	G	.	.	.	.	C	.	.	.	.	.	.	.	C	T	.	A	.	.	A	.	G	.	T	T	T	A	.	.	.				
<i>Tor putitora</i> 1	.	T	.	.	.	.	.	.	C	.	.	G	.	C	.	.	.	.	G	.	.	.	.	.	.	.	T	.	T	.	T	A	.	.	.	.	A	.	.	.	.	.	.				
<i>Tor putitora</i> 2	G	C	A	G	.	.	.	.	C	.	.	G	.	C	.	.	.	.	T	G	.	.	C	.	T	.	T	.	T	A	.	.	.	.	.	A	.	.	.	.	.	.					
<i>Tor putitora</i> 3	.	C	G	.	A	G	.	.	C	.	.	G	.	C	.	.	.	.	T	.	G	.	.	.	.	.	.	T	T	T	.	A	.	.	.	.	A	.	.	.	.	.					
<i>Tor putitora</i> 4	G	T	.	.	.	.	.	.	C	.	.	G	.	C	.	.	.	.	G	.	.	.	.	.	.	.	.	T	T	T	.	T	A	.	.	.	.	A	.	.	.	.	.				
<i>Tor putitora</i> 5	G	T	.	.	.	.	.	.	C	.	.	G	.	C	.	.	.	.	T	.	G	.	.	C	.	T	.	T	.	T	A	.	.	.	.	A	.	.	.	.	.	.					
<i>Tor putitora</i> 6	.	T	.	.	.	.	.	.	C	A	.	C	G	C	C	T	.	.	.	C	C	.	G	.	.	.	T	T	T	C	.	T	A	A	.	.	.	A	.	.	.	.					
<i>Tor tor</i> 1	G	T	.	.	.	.	.	.	C	.	.	A	.	.	C	.	.	.	T	G	.	.	C	T	.	C	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.					
<i>Tor tor</i> 2	G	T	.	.	.	.	.	.	G	.	.	A	.	.	C	.	.	.	G	.	.	.	C	T	.	C	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.				
<i>Tor tor</i> 3	G	T	G	.	.	.	.	.	G	.	.	A	.	.	.	.	.	.	G	G	.	.	.	C	T	T	C	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.				
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	4	4	5	5	5	6	6	7	9	9	0	0	1	1	2	2	2	3	3	5	5	5	7	9	0	1	2	2	3	4	5	6	6	7	7	7	8	9	9	0	0	1	1	2	3		
	3	5	2	4	7	0	3	8	0	3	2	5	1	4	7	0	3	9	2	8	3	8	9	1	8	8	6	2	5	8	4	6	2	1	7	0	3	9	8	1	5	6	9	2	8	7	0
<i>Tor khudree</i> 1	G	T	G	A	G	T	A	T	T	C	A	C	T	G	C	T	T	G	T	T	T	C	T	T	A	C	T	C	G	G	T	C	A	T	A	C	T	G	A	G	C	T	C	A	C	C	
<i>Tor khudree</i> 2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
<i>Tor khudree</i> 3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
<i>Tor khudree</i> 4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
<i>Neolissochilus hexagonolopis</i> 1	.	.	.	C	.	.	.	.	T	.	A	.	C	.	.	.	.	.	G	.	.	.	.	.	.	.	.	A	.	G	.	G	.	C	.	G	.	.	.	G	.	T	T	.	.		
<i>Neolissochilus hexagonolopis</i> 2	.	.	.	C	.	.	.	.	T	.	A	.	C	.	.	.	.	.	G	.	.	.	.	.	.	.	.	A	.	G	C	G	.	C	.	G	.	.	.	G	.	T	T	.	.		
<i>Neolissochilus hexagonolopis</i> 3	.	.	.	C	.	.	.	.	T	.	A	.	C	.	.	.	.	.	T	.	G	.	.	.	.	.	.	A	.	G	C	G	.	.	G	.	.	.	G	.	T	T	.	.			
<i>Neolissochilus hexagonolopis</i> 4	.	.	.	C	.	.	.	.	T	.	A	.	C	.	.	.	.	.	T	.	G	.	.	.	.	.	.	A	.	G	C	G	.	.	G	.	.	.	G	.	T	T	.	.			
<i>Neolissochilus hexagonolopis</i> 5	.	.	.	C	.	.	.	.	T	.	A	.	C	.	.	.	.	.	G	.	.	.	.	.	.	.	.	A	.	G	C	G	.	C	.	G	.	.	.	G	.	T	T	.	.		
<i>Tor chelynoides</i> 1	.	C	.	G	.	.	G	C	G	T	G	.	C	A	.	C	.	A	A	C	C	.	.	C	T	A	A	.	T	.	C	.	T	.	A	C	.	.	C	T	.	.	.	.			
<i>Tor chelynoides</i> 2	.	.	G	.	.	.	G	C	G	T	G	.	C	A	.	C	.	A	A	C	C	.	.	C	T	A	A	.	T	.	C	.	T	.	A	C	.	.	C	T	.	.	.	.			
<i>Tor chelynoides</i> 3	.	C	.	G	.	.	G	C	G	T	G	.	C	A	.	C	.	A	A	C	C	.	.	C	T	A	A	.	T	.	C	.	T	.	A	C	.	.	C	T	.	.	.	.			
<i>Tor chelynoides</i> 4	.	.	G	.	G	.	G	T	G	.	C	A	.	C	.	A	.	A	A	C	C	.	.	C	.	A	.	.	T	.	C	.	T	.	A	C	.	.	C	T	.	.	.	.			
<i>Tor putitora</i> 1	.	.	.	A	.	.	.	.	.	.	.	.	T	.	.	.	.	.	C	.	.	.	.	.	.	.	.	A	A	C	.	.	.	.	.	.	G	.	C	.	G	A	.	.	.	T	
<i>Tor putitora</i> 2	.	.	.	A	C	.	.	.	.	.	.	.	T	.	.	.	.	.	C	.	.	.	.	.	.	.	.	A	A	C	.	.	.	.	.	.	G	.	C	.	G	A	.	.	.	T	
<i>Tor putitora</i> 3	.	.	.	A	.	.	.	.	.	.	.	.	T	.	.	.	.	.	C	.	.	.	.	.	.	.	.	A	A	C	.	.	.	.	.	.	G	.	C	.	G	A	.	.	.	T	
<i>Tor putitora</i> 4	.	.	.	A	C	.	.	.	.	.	.	.	T	.	.	.	.	.	C	.	.	.	.	.	.	.	.	A	A	C	.	.	.	.	.	.	G	.	C	.	G	A	.	.	.	T	
<i>Tor putitora</i> 5	.	.	.	A	.	.	.	.	.	.	.	.	T	.	.	.	.	.	C	.	.	.	.	.	.	.	.	A	A	C	.	.	.	.	.	.	G	.	C	.	G	A	.	.	.	T	
<i>Tor putitora</i> 6	.	.	.	A	C	.	.	.	.	.	.	.	T	.	.	.	.	.	C	.	.	.	.	.	.	.	.	A	A	C	.	.	.	.	.	.	G	.	C	.	G	A	.	.	.	T	
<i>Tor tor</i> 1	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	A	G	.	.	.	.	.	T			
<i>Tor tor</i> 2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	A	G	.	.	.	.	.	T			
<i>Tor tor</i> 3	T	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	G	.	.	.	.	.	T			

Cont...

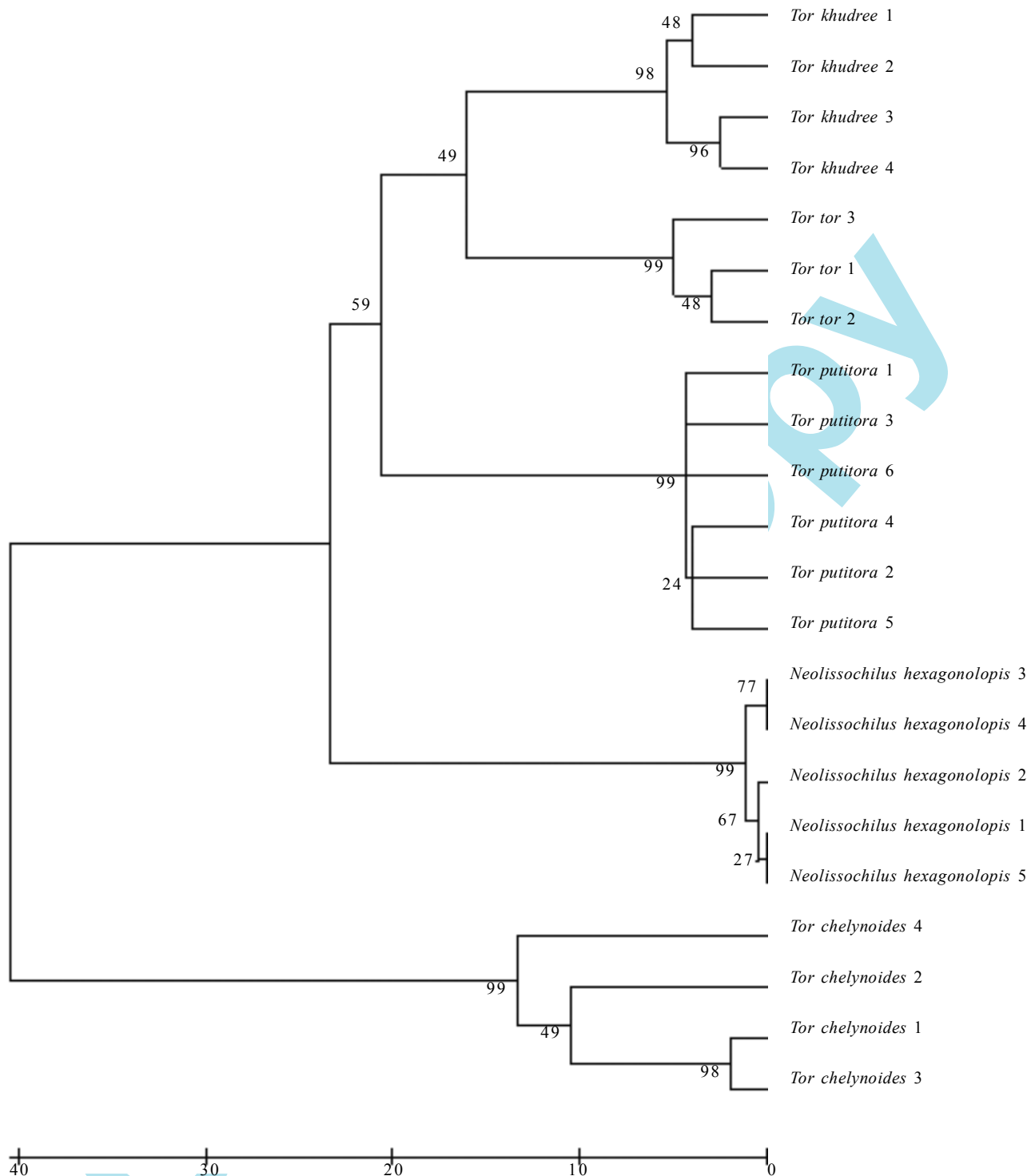


Fig. 2 : Phylogenetic tree (MP tree) based on 628 bp mitochondrial Cytochrome Oxidase I DNA sequences for five species of Mahseer

the relationship of *T. chelynooides* with other *Tor* species. Analysis based on Cytochrome Oxidase I gene are capable of discriminating Mahseer species with high accuracy, each species has a characteristics *COI* sequences that offers the

prospect to identify typical Mahseer species. Further studies are required to investigate the phylogenetic relationship of *Tor cheiloides* with other *Tor* species based on more mtDNA genes. This study should provide a

benchmark data for other studies.

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Recreational fisheries in Uttarakhand

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The hill state of Uttarakhand in the western Indian Himalayan Region encompasses a landmass of 53,483 km² area, comprised of around 86% hills and 14% plains. Uttarakhand State is administratively set into two divisions, Kumaun and Garhwal, with a total of 13 districts. The unique landscape and terrain of the state gives rise to enormous natural aquatic resources in the form of 2,686 km of rivers and streams, 132 upland lakes (covering 297 ha) and 14,120 ha of man-made reservoirs^{1,2}. Improving utilisation of these water resources for angling and recreational fisheries is an obvious possibility for increasing employment opportunities and generating income for the livelihoods of hill people. The development of sport fisheries or recreational fisheries will equally help in conserving the aquatic flora and fauna by increasing understanding their mode of propagation and sustainability. At present, opportunities for angling and recreational fisheries in

the state are limited to certain pockets and a wider preferment of sport fisheries with passable investment and awareness campaigns in potential areas is imperative. Application of geo-informatics for mapping potential natural resources in the state can be of immense help in decision support for policy makers, aiding the suitable utilisation and management of water bodies for sport fisheries.

The availability of popular game fishes adapted to different thermal regimes of this hill state has given the region an enviable reputation in angling and recreation. Anglers come from different corners of the country as well as from abroad. Anglers are provided with a Fishing License for rod and line fishing with a nominal fee of INR 120 for foreign nationals and INR 80 for Indian nationals by the Uttarakhand Department of Fisheries on the day of their arrival. Research institutes



Tor putitora (photo credit: Gagan Bakshi).

2015



Tehri dam is the highest in India.

such as ICAR-DCFR at Bhimtal have been instrumental in undertaking research programmes in species validation for mahseers and other cold water fishes of the Indian Himalayan Region using taxonomic tools such as morphometric identification, mitochondrial DNA markers, environmental DNA (eDNA) and DNA barcoding. Furthermore, concerted effort has been made by the scientists of the institute with the officers of the Uttarakhand Department of Fisheries, anglers, and fishing guides in identifying key angling sites to promote recreational fisheries in the state.

The sport fishes of Uttarakhand

Mahseer

The golden mahseer or Himalayan mahseer (*Tor putitora*) is the most popular game fish amongst anglers in India for its superlative fighting quality, known as the 'tiger in water'. Since angling was a favourite pursuit of the British, the lakes of the Kumaon hills were stocked with fish by Sir H. Ramsay during 1858 for sport fishing³. This fish is an endangered species of cyprinid and inhabits rapid streams, riverine pools, lakes and temple tanks in the Himalayan region. Mahseer belong to three genera, namely *Tor*, *Neolissochilus* and *Naziritor*. However, *Tor* constitutes the bulk of mahseer species. Efforts have been made to augment the population of mahseer in natural and manmade waters bodies through captive breeding and seed production. Altogether, three hatcheries for mahseer have been established in Uttarakhand, one operated by ICAR-DCFR Bhimtal, and two more at Satpuli and Tehri / Koteshwar under the aegis of the Uttarakhand Department of Fisheries.

Goonch angling

The group of *goonch* (in Hindi), *baghair* (in Bengali) and *baghmas* or *gorua* (in Assamese) are basically represented by two species namely *Bagarius yarrelli* and *B. bagarius*. These fishes belong to the order Siluriformes and family Sisoridae. At present they are considered to be 'vulnerable' on the IUCN Red List due to their over exploitation in the natural systems. The muscular build of the fish has given rise to the name 'giant devil catfish' and it is considered an ideal quest for an angler interested in a big catch. The home of the fish is the Western Ramganga River and Kali River in Uttarakhand, which flow through Nainital and Champawat districts. Goonch is less frequently observed on the baits of the anglers which



The goonch (photo credit: Camp the Himalaya).



Trout seed production at Bairangana.

may be attributed to a lack of information on their preferred habitat, food and feeding, migration pattern, and diurnal behaviour in the wild.

Brown trout angling

Enumerating the river resources showed a total combined length of 10,928 km of river network in the state of which 3,151 km has the potential to sustain suitable habitat for brown trout based on the criteria of elevation and the existing thermal conditions⁴. These rivers are within a temperature range below 16°C, are clear and well-oxygenated (6.0-9.5 mg/l), as well as being in sparsely populated areas. Some of the important stretches of river and their upstream areas harbouring the brown trout *Salmo trutta fario* have been reported as Nandakini, Pranmati Gad, Dhaul Ganga, Laisar Gad, Bhilangna River, Har Ki Dun, Pinder⁵; Asiganga, lake Dodi tal, Balkhila Gad, and Madhu Ganga⁴. Applying the tools of GIS in the present communication showed that the Nandakini, Dhaul Ganga West, Laisar Gad, Bhagirathi, Bhilangna, Asiganga, Pranmati Gad, Bal Ganga, Badiyar Gad, Gomati, Balasuti, and Pinder rivers situated in the northern part of Uttarakhand can provide potential ground for brown trout⁶. These river stretches may further be considered for establishing angling beats which can offer potential fishing spots for brown trout using catch and release practice. Although seed production of brown trout in the state is very small, attempts have been made at the trout hatchery of Bairangana to produce seed from 200 brown trout broodstock in recent years.

Other fishes

Altogether, 64 fish species have been reported from the Garhwal region and 31 from the Kumaon region of Uttarakhand by Kumar (2002). This data was updated to a total of 132 species of fishes belonging to 67 genera, 27 families and 8 orders by Uniyal and Uniyal (2021). Apart from the fish groups mentioned above, the other game fishes encountered are cyprinids viz., *Raiamas bola*, *Labeo dyocheilus*, *Bangana dero*, *Barilius* spp., Schizothoracines and a few catfishes. These groups of fish are occasional catches that may be encountered while in search of the major game fishes.

Recreational hotspots in Uttarakhand

One of the best sites in the world for angling of golden mahseer is at Pancheshwar, at the confluence of the Saryu and Mahakali rivers. The Mahakali River demarcates the boundary between Nepal and India. Pancheshwar is located 35 km from Lohaghat town in Champawat District of Uttarakhand. Angling fests and competitions are organised every year in joint collaborations among angling camps, Kumaon Mandal Vikash Nigam (KMVN), and the Department of Tourism. Hatchery produced mahseer seed from ICAR-DCFR Bhimtal are also released at Pancheshwar from time to time to enhance the stock of endangered species. Many angling camps such as The Himalayan Outback, Camp the Himalaya, Pancheshwar Fishing Retreat and The Golden Cast are active in the region to provide logistical support and accommodation during the angling season. The best time to go

for fishing at Pancheshwar is before the arrival of monsoon and before onset of winter. Fish guides namely Mr Sanjeev Paroriya, Mr Shyam Gurung, Mr Roshan and Mr Hoshiyar Singh are true adventurers in the region who support visitors in their quest for mahseer and goonch. Other important angling sites for golden mahseer in Uttarakhand are Vanghat / Marchula along the Western Ramganga River; Vyasghat at the confluence of Ganga and Nayar rivers; and Ramnagar and Almora along Kosi River.

Vanghat is an excellent mahseer beat, located in Nainital district of Uttarakhand. This promising angling spot lies along the cascading Western Ramganga River that meanders through the verdant forest of Jim Corbett National Park. The mighty golden mahseer and giant devil catfish goonch have drawn avid anglers around the globe to Vanghat as witnessed by angler Mr Gagan Bakshi. Many eco-camps and resorts have been established in the vicinity to provide accommodation and fishing trips for visitors. September to May is the best time for fishing in the Western Ramganga River. Vanghat has a rich biodiversity in the form of exotic birds, butterflies, mammals, reptiles and other animals as the area is located within the national park. Angling can be done with the permission of Divisional Forest Officer in those locations where human threat is minimal.

Vyasghat can be another excellent option for angling golden mahseer. The search for mahseer starts from Devprayag, the confluence point of the Bhagirathi and Alaknanda rivers



Indian trout barb Raiamas bola.

situated 14 km upstream of Vyasghat. The route follows downstream for a stretch of 36 km over the Ganges. Similarly, 4-5 km of river is covered over the Nayar River from the confluence point.

Bhimtal based ICAR-DCFR collaborated with the Department of Fisheries, Department of Tourism and District Administration of Pauri Garhwal, Government of Uttarakhand in organising a three day interactive meet and angling competition among the anglers, fishing guides, farmers, officers, and scientists during 20-22 November 2020 with an objective to highlight Vyasghat as an important site for promoting mahseer angling on the global map, as well as to raise awareness on generating revenue and rural employment



Snow trout are an anglers' delight.

among the local people through eco-tourism. The programme also stressed undertaking other ancillary tourism activities such as rafting down the Ganges, trekking, bird watching, camping and sight-seeing in the hills, which have positive direct and indirect consequences towards the conservation of mahseer⁹. Banyan by the Ganges, a resort along the riverside with a picturesque background invites global tourists to spend a relaxing vacation where one can enjoy the thrill of taming the 'tiger of water'. September to November is the ideal time for fishing at Vyasghat as stated by the proprietor, Mr Manishankar Ghosh.

Ramnagar and Almora are two important mahseer angling locations along the Kosi River. Ramnagar is situated south of Jim Corbett National Park. The Kosi River forms the eastern boundary of the park down from Mohan Township (near Marchula) to Ramnagar. These two sites on the Kosi River are important habitats for the golden mahseer and other hill stream fishes such as *Naziritor chelynooides*, *Labeo dyocheilus*, and *Bangana dero*. One can also witness the catch of mahseer and other hill stream fishes at the Ramnagar market. Almora is a populated hill station of Uttarakhand and is known for its wildlife, culture, and cuisines. Anglers of Almora in association with ICAR-DCFR have made attempts to replenish the declining fish stock in the Kosi River with hatchery produced golden mahseer fingerlings. Both the areas are yet not fully organised for inviting avid anglers worldwide and the fishing is done on traditional lines. Many resorts and home stays are in the vicinity to provide accommodation and healthy food to the visitors here. People fish almost round the year except the peak monsoon season, as spoken by angler Mr Mohan Rayal.

The lakes of Uttarakhand namely Nainital, Bhimtal, Sattal, Naukuchiatal and Naldamayanti Tal are stocked with mahseer, and the fish is a major attraction for tourists. People offer the fish puffed rice, baked bread and biscuits, generating additional income for local vendors. The Lake Development Authority here takes care of the recreational amenities in the lakes for the benefit of tourists. Uttarakhand State is also known as Dev Bhoomi, as people believe the existence of God and Goddesses in every particle and henceforth make the holy land here a deity. This gives rise to many temple fish sanctuaries in the region. One of the exemplary examples is the Baijanath temple on the banks of the Gomti River in Bageshwar District where golden mahseer is conserved. Many such secluded mahseer sanctuaries can be witnessed on the Kosi River in the Kherna region and Ramganga River near Jim Corbett National Park. ICAR-DCFR has made a concerted effort in stocking these waters with young mahseer as a conservation measure and to support natural propagation of the fish. In addition, the confluence of rivers has also religious meaning in the Hindu tradition and is locally called *prayag*. Devotees across the country visit prayags with believe that a dip at the confluence cleanses the heart, mind and soul of a person and advances them closer to liberation or *mukti* (salvation). The panch (five) prayags are Vishnuprayag at the confluence of the Vishnu Ganga and Dhauliganga rivers; Nandaprayag at the confluence of the Alaknanda and Nandakini rivers; Karnaprayag at the confluence of the Alaknanda and Mandakini rivers; and Devprayag at the confluence of the Alaknanda and Bhagirathi rivers. All these prayags have a temple at the peak of the adjoining hills. The fish are not caught or disturbed in these confluences adjacent to the temples, which serve as natural reserves of fish and other aquatic biodiversity. These temple reserves are



Ranching of young mahseer at Pancheshwar.



A catch of golden mahseer at Vyasghat.



Participants of the interactive meet at Vyasghat.

not declared fish sanctuaries and are managed by the temple priest and devotees together through community participation. Such community-based conservation measures deserve recognition and concerted efforts need to be made to ensure the protection of fish species. These sanctuaries along the river stretches harbour healthy riverine fish populations and are an excellent way to prevent extinction of highly threatened fish species such as mahseer.



A winner receiving an award during the angling competition at Vyasghat.



Distribution of ICAR-DCFR hatchery produced mahseer young for ranching in the Kosi River at Almora in association with anglers.



A haul of hill stream fishes at Ramnagar market.

A school of golden mahseer conserved in tanks of Baijnath temple.

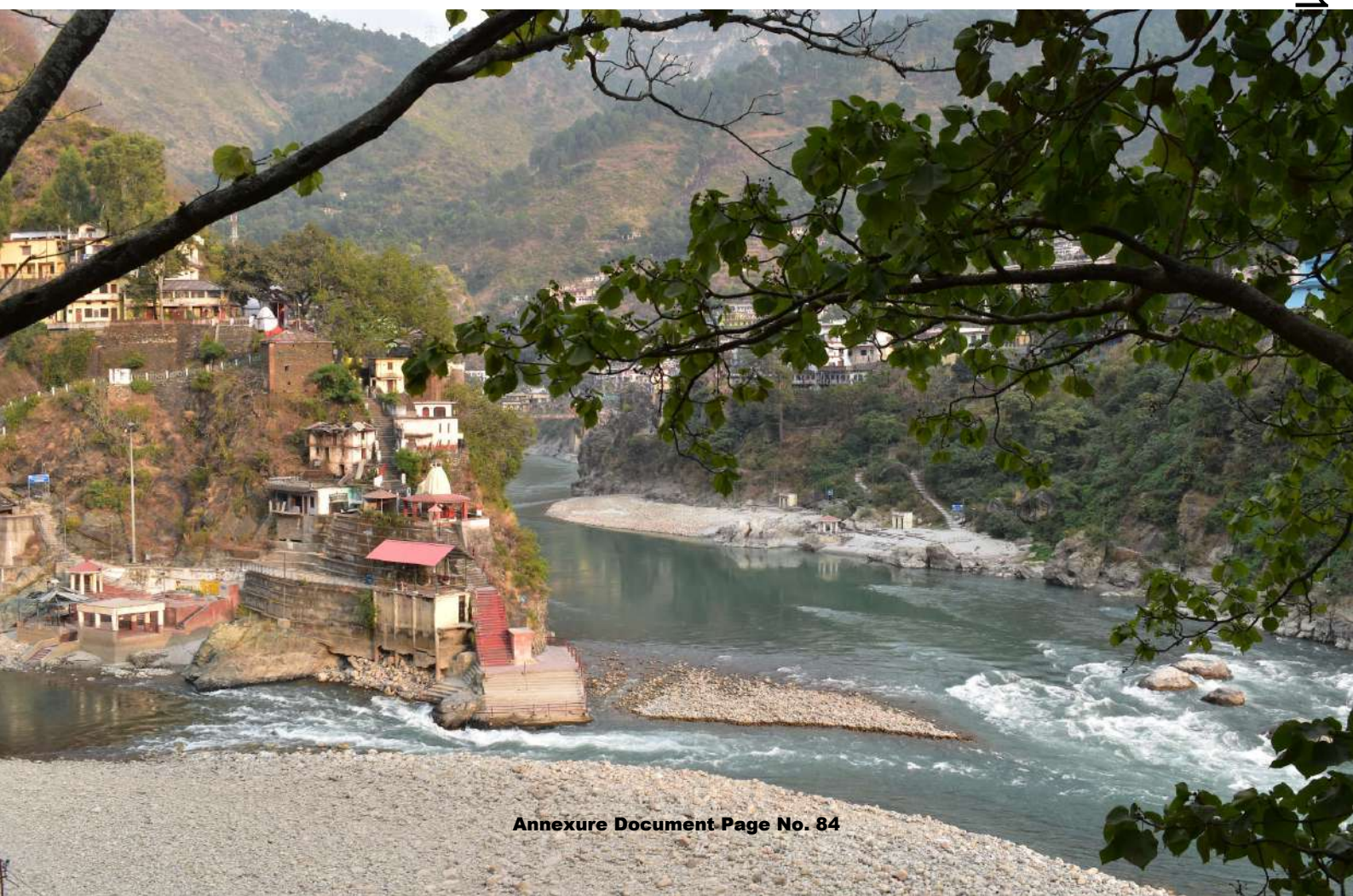
Conclusion

Recreational fishing is fishing for pleasure or competition is a popular leisure activity in the country. Today, recreational fishing is considered as an ecotourism activity, which is defined as "responsible travel to natural areas that conserves the environment, sustains the well-being of the local people, and involves interpretation and education". Ecotourism can be a viable option in Uttarakhand as the state has numerous pristine destinations where fauna and cultural heritage are the primary attractions, and henceforth can provide viable income-generating options for economic development for local communities. Angling for mahseer, goonch, hill trout





Above: Origin of Ganga River at Devprayag. Below: Temples at river confluence point at Rudraprayag.



and other fish along with associated recreational avenues depends on healthy aquatic ecosystems. The ever-increasing population in the country imposes a significant pressure upon these inland water ecosystems including through destructive and illegal methods of fishing. However, the fish guides, resort and home stay owners are vigilant these days towards the poaching of fish on their respective beats as they are aware of the endangered status of the game fishes. Furthermore, active, and concerted efforts by the various public sector organisations have generated mass awareness at the rural front in conserving game fishes in their natural habitat for economic benefit. Commercial catch of these important game fishes by fishermen for consumption have been stopped and fishing is practiced solely on a catch and release basis by anglers. Fishing for recreation in these parts of Uttarakhand will enable future generations to experience destinations relatively untouched by human intervention, thus making them more passionate towards nature.

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Mahseer in recreational fisheries and ecotourism in India

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A catch of 11.5 kg golden mahseer by Mr. Dorjee Sona from Yamne River in East Siang District during March 2016.

Mahseer, a large bodied potamodromous freshwater fish endemic to Asian rivers is a flagship species of considerable economic and recreational interest. Mahseer is the most popular game fish amongst anglers in India as this fish is the toughest fighter amongst freshwater sports fish and is therefore known as the undisputed king of Indian freshwaters. The existence of such a mighty game fish in Indian water is depicted in Vedic times¹ in the writings of King Someswara (1127 AD) in his *Matsya Vinoda* on account of angling of mahseer with rod and line². Angling of mahseer in the country was further pursued by the British, attracting the attention of anglers Beevan (1877), Thomas (1873, 1897), Dhu (1906, 1918, 1923) and Nevil (1915). The lakes of Kumaon hills were reportedly stocked with mahseer by Sir H. Ramsay during 1858 for sport fishing³. Codrington (1946) and McDonald (1948) wrote about its natural history and on special traits of fish from an angler's point of view. The adventures of the English in search of mahseer across the country was described by Ghorpade (1978). Kulkarni and Ogale (1979) who considered mahseer the noblest sport fish of India, a great favourite of anglers, both from India and overseas. Belonging to the carps, family Cyprinidae, India is home to 15

species out of 47 species of mahseer that exist in the world⁴. Mahseer belong to three genus namely *Tor* (Gray), *Neolis-sochilus* (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 genus⁵.

Recreational fishing or sport fishing is one of the most sought after adventure tourism activities and has a booming international business opportunity. The most common form of recreational fishing is done with a rod, reel, line, hooks and any one of a wide range of baits. The three common methods for capturing mahseer are fly fishing, spoon fishing and bait fishing, although recent innovations are reported. Fly fishing or fly casting method employs artificial fly to hook the fish with the help of rod and line. In spoon fishing, spoon like artificial metallic shining lures are used basically to fish 'heavier' waters where mahseer occurs. Bait fishing or bait casting is performed using both natural and artificial lures. The natural baits are earthworms, minnows and insects.

Today, recreational fishing is an intrinsic part of ecotourism which involves travel to destinations where pristine fauna and cultural heritage are the primary attractions and can provide viable income-generating options for economic development for local communities and opportunity of education and activism among travelers, making them more passionate towards nature and its resources.

In this article, we have made an attempt to compile information on recreational mahseer fisheries in India. The Indian aquatic resources in the form of rivers, streams and lakes and the importance of the mighty mahseer as a game fish through the perspectives of active anglers and vision of stakeholders and researchers is highlighted herewith. In this context, the river systems of the Indian Himalayan Region are worth mentioning which offer some of the most remote, pristine, wild and untapped resources harbouring mahseer and providing opportunity for fishing and recreation in the country. The river conditions are varied with different terrains of the Himalayas influencing fishing techniques⁶. Undoubtedly, one of the most successful ways to hook mahseer in these torrential rivers is also by floating on rafts downstream over a period of time enabling to access the remotest angling spots.

Arunachal Pradesh

Subansiri River: This river of Arunachal Pradesh originates in the Himalayas and enters into India via Taksing of Upper Subansiri District, flowing down along the towns of Daporijo and Ziro, to enter into the vast wilderness of the lower valleys and finally terminating to meet the mighty Brahmaputra. Anglers among the sparse human habitation travel along the

river Subansiri as adventure enthusiasts in these picturesque mountains of Arunachal Pradesh, with crystal clear post monsoon waters coursing through them and home to the big fighting fishes *Tor putitora* (golden mahseer), *Neolissochilus* spp. (chocolate mahseer) and the *Bagarius bagarius* (goonch) as shown.

The Siang River belt: This river flows for 294 km through the hills before joining the Brahmaputra River in the plains. The Siang River belt is mostly inhabited by the Adi tribe and sub-tribes *Minyongs*, *Padams*, *Shimongs*, *Milangs*, *Pasis*, *Karkos*, *Ashings*, *Pangis*, *Tangmas* and *Boris*. Their life is closely mingled with nature where hunting and angling (*gekar* in Adi language) are some of their oldest traditional practices. Mahseer (locally *Ngocho*) is a favorite species for the anglers of the belt. A few of the preferred sites along the banks of river Siang are Bodak, Sigar, Raling, Kyiet, Berung, Sika-bamin for angling due to the abundance of mahseer along with an accessible topography and water movement. Attention has been paid recently towards developing ecotourism in the Siang belt by arranging rafts, fish rods, tents and other basic amenities and in turn inviting innumerable tourists from different parts of the country.

The Menchukha region is another angler's paradise, situated in West Siang District of Arunachal Pradesh, 180 kilometres northwest of headquarters Aalo and is only 29 kilometres away from Indo-China border. The Siyom River (locally known as Yargyap Chu) flowing through Menchukha township is home for the exotic and Indian trouts. Mr Dorjee Sona, an active angler from Menchukha, encountered golden and chocolate mahseer abundantly in the Yamne River, which flows from Upper Siang District and meets the mighty Siang River



A golden mahseer of 12.6 kg weight from the Subansiri River in November 2014.



A chocolate mahseer (*Neolissochilus stracheyi*) caught by diving in the Basar area of the Siang drainage.

near Ponging village. Anglers of the region have developed a community based anglers association "Arunachal Anglers" with an objective to strengthen the protection measures for the endangered group of fish – mahseer.

Lohit River: Angling and fishing camps in the Lohit are famous for mahseer (*Tor putitora*, *Tor tor*, *Neolissochilus* spp.) and catfish (*Bagarius bagarius*) fishing and adventure sports. One of the major angling and fishing camps along the Lohit is Tezu situated at 27°55'N latitude and 96°10'E longitude. The angling and fishing season over the Lohit starts from October to April whereof the maximum temperature remains 20° C during the day.

Pare River: This river flows through Papum Pare District along the small township of Sagalee situated at an altitude of 162 m asl between 27°14'N latitude and 93°61'E longitude, and located 29 km east of the state capital Itanagar. The Pare River harbours the golden mahseer and chocolate mahseer, says a young avid angler Mr Ngurang Neega from Sagalee town. The angler arranges angling expeditions for the visitors from neighbouring states Nagaland, Meghalaya, Sikkim, Assam and tourist from countries like Nepal and Bhutan. The competitions are done on the principle of catch and release and the fish are not harmed. The angling competitions have remained successful to associate the local community in promoting eco-tourism in an unrevealed place of Arunachal Pradesh.

Kameng River: The river Kameng in Arunachal Pradesh originates in Tawang District from a glacial lake below snow-capped Gori Chen mountain at an elevation of 6,300 metres asl. The river is known to harbour the mahseer in the upper and lower reaches as witnessed in the hooks of the anglers of the area. Catch and release angling competitions are organised on regular basis at places like Seppa, Nameri, Bhalukpong with an objective to promote conservative angling with rod and reel and to spread awareness for protection of the river system along with its natural habitat specially

the local species, which due to illegal poaching, chemical poisoning, electrocution and dynamiting process has caused much damage in the recent era as reported by an angler Mr Biplab Chakraborty.

Assam

Jia Boreli River: This river is one of the major south flowing tributaries of the Brahmaputra. The river harbours the legendry mahseers *Tor tor* (locally lao pithia), *Tor putitora* (locally sonali pithia), *Tor mosal* (locally lobura), *Tor progenius* (locally jungha pithia) and *Neolissochilus hexagonolepis* (locally boka pithia) which create a magnificent fishery in the foot hills of Assam and Arunachal Pradesh³.

The Assam Bhoreli Angling and Conservation Association (ABACA), situated at the banks of the Jia Bhoreli River at Nameri, Sonitpur District is one of those eco-camps set up for popularising adventure sports among the rural youth, angling on catch and release and create awareness on conservation of fish⁷. The camp presently sustains on organising angling, camping, rafting, trekking, bird watching, heritage and culture. The camp is also bestowed with breeding and seed production facilities for propagation of golden mahseer. Mr Gautam Tamuli, a life member of ABACA and once an active angler in the region for the search of mahseer during 1987-1995 was much appreciated in those days for his record catch of golden mahseer (*Tor putitora*) weighing 12 kg and 20 kg at Nameri Mukh, 17.4 kg at 17 Mile near Bhalukpong and a 12 kg golden mahseer at Old Nameri Mukh. His record catches can be witnessed today from the preserved teeth of some of those mahseer restored in his residence.

Karbi Anglong and West Karbi Anglong: These are the hills districts of Assam. The district with dense tropical forest and numerous tributaries of the mighty Brahmaputra has sultry temperature of 23° to 32° Celsius in summer and 6° to 12° Celsius in winter. The rivers are reported by Mr Chatra Pator

(I/c SDFDO) to harbour many species of mahseer viz., *Tor tor*, *Tor putitora* and *Neolissochilus* spp. creating an immense potential for angling in these hilly districts of Assam. Some of those potential angling sites are (a) Koka at the banks of river Kopili with beautiful waterfalls (b) Amreng at the banks of river Amreng, provided with a tourist lodge and a heritage old British made bridge across the river (c) The Amtereng Dam site of Karbi Langpi Hydro project over river Karbi Langpi with the attraction of the reservoir (d) Siloni, a river island over the river Longnit attracting the anglers for day out.

Mr Rupsing Hanse, a prominent angler of village Harlongjove, Hamren sharing his experiences recalled his prize winning catch of a chocolate mahseer weighing 4.2 kg during 1996-97. The angler further added that with these bountiful of resources, both the hill districts of Assam has an immense potential for recreational fisheries and eco-tourism, provided certain policies are framed and implemented based on the present issues with the concerted effort of the state departments, research organizations and local community.

Manas River: This river is a transboundary river in the Himalayan foothills between southern Bhutan and India situated 26°10' to 26°50'N Latitudes and 90°00' to 91°00'E Longitude. It is one of the biggest river systems meeting the river Brahmaputra. It is reported that upper stretches of river Manas and its tributaries are predominantly inhabited by mahseer species viz., *Tor putitora*, *Tor tor* and *N. hexagonolepis*. Mr Pranab Bora, once an active angler, mentioned to have a record catch of a golden mahseer weighing 12.5 kg in 1981 with a landing time of 35 minutes. Mahseer of size 2.0-2.5 kg was a usual catch during those days in 1970-71 at river Manas. He further spoke about Late Sanjay Deb Roy, the then a senior forest official to hook a 20 kg mahseer at Manas River.

Nagaland

Nagaland is endowed with abundant lakes, rivers, reservoirs, ponds and offers numerous sport fishes like golden and chocolate mahseer and other indigenous species to catch. The kaleidoscope diverse topography, culture and rich biodiversity of the state attracts tourist from all over the world. Some of the important mahseer endowed rivers in the districts of Nagaland are Dhansiri and Intangki (Dimapur), Milak and Dikhu (Mokokchung), Doyang (Wokha), Arachu, Lanyi, Seidzu, Tesuru and Tizu (Phek), Zungki and Likhimro (Kiphire). To promote tourism through sport fishing and to create awareness to conserve the indigenous and endemic fish species of the State, an Anglers Association Nagaland (AAN) organises angling festival, angling competitions and special days like World Fish Migration Day in lakes, large ponds and reservoirs belonging to local communities and individuals of the area.

Sikkim

Sikkim with its vast river systems is an anglers' delight where the mighty Teesta River and Rangeet provide ample scope for mahseer. Some of the important confluences of major rivers within Sikkim which forms the hotspot to hook the mighty mahseer as reported by an angler Mr Ian Christopher are (i) Singtam Town - river Teesta and Rani River (ii) Rorathang Town - Rorathang River and Rani River (iii) Rangpo Town - Rorathang River and Teesta River (iv) Jorhang Town - Rangeet River and Rambang River (v) Teesta Town - Teesta River and Rangeet River. Angling is mostly done during the

Table 1: Specification of fishing equipment used by few representative mahseer anglers in India.

State	River	Angler (s)	Major species of mahseer	Biggest catch sizes (kg)
Arunachal Pradesh	Subansiri	Mr. Naushad Ali	<i>Tor putitora</i> , and <i>Neolissochilus</i> spp.	5.7-18.0
	Siang and tributaries	Mr. Dorjee Sona	<i>Tor putitora</i> , and <i>Neolissochilus</i> spp.	8.3-11.5
	Pare	Mr. Ngurang Neega	<i>Tor putitora</i> , and <i>Neolissochilus</i> spp.	10.0-12.0
Assam	Kameng	Mr. Biplab Chakraborty	<i>Tor putitora</i> , and <i>Neolissochilus</i> spp.	1.6-13.0
	Jia Borelli	Mr. Gautam Tamuli; Mr. Naushad Ali	<i>Tor tor</i> , <i>Tor putitora</i> , <i>Tor mosal</i> , <i>Tor progenius</i> and <i>Neolissochilus hexagonolepis</i>	20.0
	Manas	Mr. Pranab Bora	<i>Tor putitora</i> , <i>Tor tor</i> and <i>N. hexagonolepis</i>	2.0-20.0
Nagaland	Dhansiri, Intangki, Milak, Dikhu, Doyang, Arachu, Lanyi, Seidzu, Tesuru, Tizu, Zungki, Likhimro	Anglers Association Nagaland	<i>Tor putitora</i> , and <i>Neolissochilus</i> spp.	Not determined
Sikkim	Teesta and Rangeet	Mr. Ian Christopher	<i>Tor putitora</i> , and <i>Neolissochilus</i> spp.	3.0-24.0
Meghalaya	Pools and sanctuaries	Mr. Bhutto Marak	<i>Neolissochilus</i> spp.	1.0-5.0
Mizoram	Rivers of Kolodyne drainage	Local residents	<i>Neolissochilus</i> spp.	Not determined
Uttarakhand	Mahakali, Saryu	Mr. Mohan Rayal	<i>Tor putitora</i>	15.42 kg
Peninsular India	Narmada, Cauvery	Mr. Derek Dsouza	<i>Tor khudree</i>	Data not available
			<i>Neolissochilus</i> spp.	



Mr Ian Christopher angling mahseer in the Rangeet River, Sikkim.

pre and post monsoon seasons and the major mahseer encountered in these rivers are the golden mahseer and chocolate mahseer.

Meghalaya

Fee fishing: Meghalaya is literally known as the 'Abode of clouds' with the fame of having the highest rainfall on earth and this makes the state full of aquatic resources. The state is predominantly inhabited by the Khasis, Jaintias and Garos who are fascinated for angling as their common traditional fishing practice for recreation. Rich in chocolate mahseers namely *Neolissochilus hexagonolepis* and *N. hexastichus*, local entrepreneurs from areas Myllem, Smit, Mairang, Umsning and Laitlyngkot of Khasi hills have ventured into fee fishing for anglers allowing fishing in their own ponds and tanks on payment basis. Fishing competitions are also being organized once or twice in a year in these areas.

Mizoram

Angling, as a whole is an age old practice for the people of Mizoram and is performed on traditional means by simple hook and line for catching the locally available carps, catfishes, murrels etc till date. Md Mintul Ali, Scientist of KVK Mamit identifies some of the potential larger water bodies for recreational fisheries as Palak, Tamdil, Rungdil and Rengdil lakes and Serlui reservoir. Recent study has shown that the rivers of the state viz., Chhimtuipui, Mat, Serlui and Tuirial harbour a variety of mahseer species viz. *Tor tor* and *Neolissocheilus hexagonolepis* as stated by the Department of Fisheries, Govt. of Mizoram.

Uttarakhand

Fishing in Uttarakhand has been an awesome experience for those who try for mahseer fishing. Some of the important locations known for sport fishing and eco-tourism are given below.

River Mahakali: The confluence of river Mahakali and Saryu at Pancheshwar along the Indo-Nepal border is one of the least fished but the best known mahseer (*Tor* spp.) rivers in Kumaon region of Uttarakhand. Rafters can indulge in adventurous white water river rafting with fishing in this water.

River Kosi: River Kosi in Corbett National Park is witnessed with bountiful of golden and chocolate mahseers. Betalghat and Chhara are the best angling sites in Kosi River.

River Ramganga: The best places to locate golden mahseer for angling along the Ramganga River are Machula bridge and Van Ghat pools.

Himachal Pradesh

Himachal Pradesh is endowed with numbers of fast flowing rivers and streams in the name of Beas, Sutlej, Ravi, Tirthan, Sainj, Uhl, Baspa, Pabar, Lambadug, Giri, Rana, Nugal Gai, Baner, Bata etc., originating from glaciers, rumbling and swirling along the rugged mountain passing through awesome gorges, canyons, alternating with pools and fiery rapids. Mahseer is one of the major fish available in these streams along with exotic and indigenous trout and an array of catfishes. Each year a large number of anglers both from home and abroad visit the state in the pursuit of age-old art of angling. Fishing in these streams is regularized under the State Fisheries Act, where the anglers are provided with a fishing license and are charged with nominal fees for angling. A few of the lucrative mahseer grounds in Himachal Pradesh are at Kangra valley, Sari Marog (confluence of the Binwas tributary with the river Beas), Mandh-Khad confluence, Lambagaon pool, Neogal confluence, Chamba pattan, Kuru pool, Dehra and Pong dam reservoir and Gaura.

Jammu and Kashmir

In Kashmir valley, mahseer has been the major fish having socio-economic and recreational significance. Efforts are being made for the development of mahseer sport fisheries by the Department of Fisheries, Govt. of J&K in areas of Tawi River (Jammu), Ujh stream, Ravi River (Kathua), Tawi River (Udhampur), Poonch River (Poonch), Rajouri Wali Tawi (Rajouri), Salal reservoir, Nallah Rud, Ans, Chenab, Pouni Nallah (Reasi).

Peninsular India

Anglers on the lookout for the mahseer can also try their luck in the rivers of the Indian peninsula – Godavari, Cauvery, Krishna and Mahanadi – almost throughout the year (except



Lures for mahseer.

during the monsoons). In Karnataka, the lure of the Cauvery proves difficult to resist for anglers – it is probably the best river for sport-fishing in peninsular India.

Cauvery River: In Karnataka, Galibore, Bheemeshwari and Doddamakali are amongst the important fishing camps along the Cauvery. Anglers from all over the world pay their annual visit to these exclusively reserved stretches to pit their wits against the mahseer while non-anglers revel in nature's bounty. The major species of mahseer that can be spotted at the angling sites of the Cauvery is *Tor khudree*. Much effort has been made by Wildlife Association of South India (WASI) and Coorg Wildlife Society (CWS) for the protection of the habitat of mahseer and the adjoining pristine stretches of the river. Licenses are issued to anglers for fishing strictly on 'catch-and-release' as told by angler Mr Derek Dsouza.

Chalakudy River: This river, the 5th longest river in Kerala, harbours *Tor tor* as one of its important fish species⁸. The river with its importance for its reservoirs and adjoining townships provides ample scope for development of mahseer angling⁹. The Wayanad mahseer, *Neolissochilus wynaadensis* is another species in the larger streams of the region^{10,11}.

Fish sanctuaries

Fish sanctuaries are under implementation in Meghalaya under Meghalaya State Aquaculture Mission (MSAM) with an objective to conserve and enhance the aquatic bio-diversity and to promote untapped and underprivileged places as tourist spots to benefit the local rural people. A few of these functional fish sanctuaries of the state are (i) Amlayee mahseer fish sanctuary at Nongbareh (West Jaintia Hills) over the Amlayee River where 1.5 km of its stretch is protected for chocolate mahseer (*Neolissochilus hexagonolepis*) and at the same time inviting visitors for active participation in sport fishing on the concept of 'catch and release' angling, (ii) Asim Bibra fish sanctuary over Chibok River (East Garo Hills) where community fishing event is celebrated every year with an objective to conserve the God gifted aquatic hotspots for development of livelihood ways, (iii) Deku Dobagre fish sanctuary established over Bugai River (South Garo Hills) based on the richness of chocolate mahseer, (iv) Wachi Wari fish sanctuary (West Garo Hills) over Simsang River providing an ethnic beauty for an ideal fish watching platform for chocolate mahseer by feeding them, (v) Songkal Wari fish sanctuary (West Garo Hills) on Simsang River between two main towns Tura and Williamnagar, where any visitor can view thousands of mahseers swimming in shoal based on the concept of in-situ aquatic life conservation. Seeing the encouraging results, 'wari' at Rombagre, Selbalgre were established and within a span of five years, this concept has



An angler with a chocolate mahseer caught in the Pare River.



An angler with a 7 kg golden mahseer caught from the Kameng River in October 2015.

reached to more than hundred in the area. This has led to the population of mahseer on the rise in Meghalaya following the practice of catch and release by the anglers. Females are not too far behind in the state and undoubtedly have the most number of female anglers anywhere in India.

Mahseer watching

Fish watching platforms have gained much momentum in the recent times as this practice is considered to have multiple positive benefits to human health. Keeping fish in tanks and ponds also facilitates in-situ conservation of these endangered fish - mahseer. Many of the upland lakes, religious and protected tanks, stretches of rivers and streams, pools are examples of fish watching spots. Mahseer can be best watched in the upland Kumaon lakes viz., Nainital, Bhimtal, Naukuchiatal and Sattal and tanks such as Nal Damayanti. Similarly, the districts of Tawang, Lower Dibang valley, Papumpare and Upper Siang in Arunachal Pradesh are best known for bestowing numerous numbers of lakes having multidimensional importance for angling, scenic beauty, bird watching and hill trekking etc. Ward's lake, Jarain pitcher plant lake and Thadlaskein Lake at Meghalaya, Dighalipukhuri at Guwahati, Machchhiyal in Himachal Pradesh are others which offer excellent sites for development of fish watching spots together with other recreational amenities like boating, children's park, food courts etc.

Jasingfaa Aqua Tourism Centre: This centre, situated in Assam, is a leading example to set up a fish based adventure tourism activities in the country bliss with the amenities to satisfy the passionate anglers, bird and fish watching. Of late,

the highly priced sport fish golden mahseer was introduced with the aegis of ICAR-DCFR, Bhimtal and reared specially for brood banking and fish watching with a motto "*Help golden mahseer to save biodiversity for humanity*" and with an objective to protect the fish under controlled conditions so that generation to come will have the opportunity to know and enjoy encountering this majestic river fish. Angling festival and special workshops on angling tips are being arranged regularly in the centre.

Conclusion

India has tremendous potentiality for mahseer recreational fisheries in many states, provided the tourism sector is developed to the expected level starting from identifying the potential areas followed with formulation of effective policies and guidelines for entrepreneurship development and supported with ancillary services. The type of ecotourism developed in the country on fish angling and fish watch has both as outlined has positive indirect and direct consequences towards the conservation of mahseer. In this context, mahseer has been recognised as the 'state fish' in 7 states of India and has all the qualities to become a 'national freshwater fish' because of its fame for its magnificent colour, fighting skills and sizes¹².

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A prized catch of golden mahseer from the Mahakali River at Pancheswar in 2016.

Pramod Kumar Pandey
Nityanand Pandey
Md. Shahbaz Akhtar *Editors*

Fisheries and Aquaculture of the Temperate Himalayas



The Endangered Mighty Mahseer (*Tor putitora*) in the Himalayan Waters

6

Md. Shahbaz Akhtar and Alexander Ciji

Abstract

Golden mahseer (*Tor putitora*), also called “mighty mahseer,” is a prime fish species in the temperate Himalayas. It plays an important role in the socio-economic upliftment of the local people in the hill region through fish-based ecotourism and sport fishery. However, due to indiscriminate exploitation and other anthropogenic pressure, the wild stocks of golden mahseer have dwindled significantly in the past, and eventually, it has been declared an “Endangered Species” by the International Union for Conservation of Nature. Therefore, there is an urgent need to conserve and rehabilitate this prime and priced fish species of the temperate Himalayas through different strategies and action plans. The development of breeding technology of golden mahseer helps to provide seeds for stocking in natural and man-made water bodies assuring the conservation of this magnificent fish. The present book chapter briefly describes golden mahseer status, and conservation approaches, emphasizing its captive breeding and seed production.

Keywords

Golden mahseer · Ranching · Angling · Captive breeding · Fish-based ecotourism

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6.1 Introduction

The *Tor* spp., the most magnificent fish group found in many rivers and streams of the Himalayas, is collectively called “mahseer.” Taxonomically, mahseer is an iconic group of game fishes belonging to the family Cyprinidae, mostly to the genus *Tor*, but also includes certain species of the genera *Neolissochilus* (e.g., chocolate mahseer, *Neolissochilus hexagonolepis*) and *Naziritor* (e.g., dark mahseer, *Naziritor chelynoides*). Despite some phenotypic similarities, only members of the genus *Tor* are regarded as “true mahseers” (Desai 2003; Nguyen et al. 2008), and this chapter mainly focuses on the *Tor putitora* (Hamilton, 1822), a “flagship” species of the Indian subcontinent. Golden mahseer, king mahseer, mighty mahseer, the tiger of waters, and the king of game fishes (Fig. 6.1) are all common names for *Tor putitora*. The big size, appealing golden color, culinary quality, and fighting abilities of the fish have piqued the interest of the human population, fishermen, and researchers (Bhatt and Pandit 2016). Mahseer has always offered fishermen better sports than salmon (Thomas 1897). There have been reports of 275-cm-long and 54 kg fish in Indian lacustrine waters (Everard and Kataria 2011; Nautiyal et al. 2008). Numerous vernacular names, such as mahseer (big head), mahasaul (huge body), and mahasalka (large scale), are derived from these characteristics (Nautiyal 2014). In addition, mahseers have been revered as “God’s fish” for ages over their entire geographic range. Sacred and masculine depictions of fish similar to mahseers may be found in Hindu holy scriptures, symbols, motifs, sculptures, and ancient literature (Jadhav 2009; Nautiyal 2014). At several temple ponds, there is a substantial population of mahseer, and these “temple sanctuaries” or “temple pools” are protected because of the social ideals and feelings of devotees, in addition to the involvement of communities and temple officials (Pinder et al. 2019).



Fig. 6.1 A golden mahseer (*T. putitora*)

6.2 Geographical Distribution

T. putitora (golden mahseer) is found in many Southeast Asian countries including India, Afghanistan, Bangladesh, Pakistan, Nepal, Bhutan, and Myanmar. This species is naturally distributed (Fig. 6.2) throughout the rivers of the South Himalayan drainage (specifically the Indus, Ganges, and Brahmaputra) from Pakistan in the west, all the way through India, Nepal, Bhutan, and Myanmar, with its range extending throughout the Eastern Brahmaputra catchments comprising the north-eastern states of India and Bangladesh (Rahman 1989). In India, golden mahseer primarily inhabits the Himalayan rivers in the foothills, and its range extends from Hindukush-Kabul-Kohistan in the Northwest Himalaya to Sadiya (Brahmaputra) in the Northeast Himalaya. Golden mahseer is a rheophilic species that enjoys rocky/stony/sandy substrates in hill streams (Nautiyal 2014). It also thrives well in semi-lacustrine waters of the Himalayas (Bhatt and Pandit 2016). The mighty mahseer's cylindrical body, muscular tail, and hypertrophied lips enable it to survive in rapidly flowing streams. The temperature range of 15–25 °C is considered optimal for golden mahseer. The species is potamodromous and performs extensive upstream spawning migrations (Bhatt and Pandit 2016).

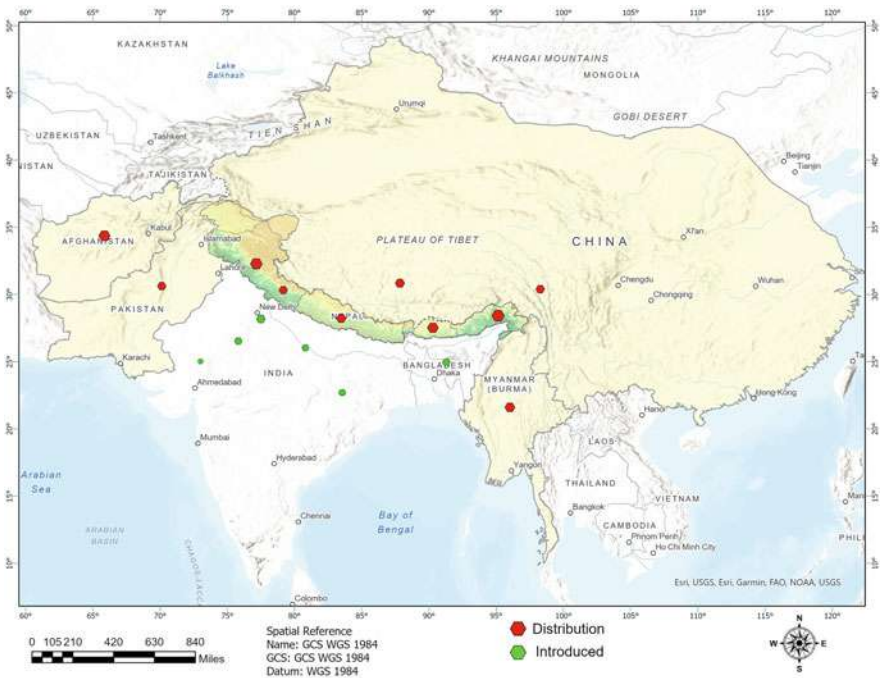


Fig. 6.2 Distribution of golden mahseer (*T. putitora*) across the temperate Himalaya (Map courtesy: Dr. Ravindra Posti)

6.3 Major Threats and Population Status of Golden Mahseer

Mahseer, *T. putitora*, formed a substantial natural fishery in the major riverine and lake ecosystems of the Indian Himalayas. The mahseer population in natural waters has decreased in size and quantity despite once being abundant. The unprecedented population pressure and decline are attributed to anthropogenic activities such as habitat fragmentation, environmental degradation and declining water quality, drainage basin alteration and deforestation, pollution, overexploitation, invasive species, and indiscriminate and destructive fishing (Nautiyal 1994; Sehgal 1999; Dinesh et al. 2010; Akhtar et al. 2017). Inherent biological traits such as delayed maturity, low fecundity, and slow growth rate have also contributed for its population decline over time (Akhtar et al. 2018). Consequently, it is now classified as an endangered species (IUCN 2014) due to the decrease in *T. putitora* populations in several Asian regions (Hussain and Mazid 2001). Females do not attain sexual maturity until they reach 4 years or older (Nautiyal 1994). However, sexually mature males are usually spotted even at 2+ years of their age. The age classes of *T. putitora* most frequently harvested are 2+ to 4+ years. Consequently, catching a significant number of fish before they reach sexual maturity reduces population size (Bhatt and Pandit 2016). Furthermore, *T. putitora*, threatened by overfishing, habitat loss, and degradation, has led to the destruction of its reproductive habitats across the water bodies. It is anticipated that the current proliferation of dams in the Himalayan region, either already built or scheduled to be built, will have a domino effect on the breeding migrations of the species. Because of its economic, sporting, and cultural significance, the golden mahseer has been designated as the “state fish” of four Indian Himalayan states (Arunachal Pradesh, Himachal Pradesh, Jammu and Kashmir, and Uttarakhand). Numerous organizations are working to prevent its further decline (Bhatt and Pandit 2016) through various conservation strategies.

6.4 Food and Feeding Habits

Due to the large mouth opening and size of the golden mahseer, it was once believed that it was a carnivorous fish. Later, studies indicated that food preference varies with age and size (Nautiyal and Lal 1984). For example, hatchlings prefer planktonic food that is abundant in their natural environments, while as fish grows larger, their feeding habit shifts toward carnivory. However, they are opportunists who feed on a variety of plant and animal items. Mahseer has been reported to consume green filamentous algae, small mollusks, insect larvae, and algal coatings on rocks. It was observed that the diet of mahseer fingerlings in the wild comprised of insect matter (81.4%), plant matter (15.9%), and other foods such as fish (1.6%) (Nautiyal and Lal 1984). Hence, the eating preferences of golden mahseer are highly dependent on availability of food. Consequently, several studies classified the species as insectivore, carnivore, herbi-omnivore, and carni-omnivore (Nautiyal and Lal 1984; Das and Pathani 1978; Badola and Singh 1980). In general, the mahseer is a carnivorous fish during its early stage and an omni-herbivore during its adult phase.

6.5 Reproductive Biology

The golden mahseer is a batch spawner, producing eggs intermittently (different size groups of eggs can be seen in the ovary; Fig. 6.3), during its extended breeding season, with the majority of spawning occurring between May and August (monsoon season). No strong sexual dimorphic character is evident in *T. putitora*, although a few secondary sexual characteristics have been documented in hatchery stocks (Fig. 6.4) during the breeding season. Males have bright coloration and an elongated body with rough pectoral fins, whereas females have a dull coloration and a deep body (Arjamand et al. 2013). During spawning season, the ripeness of the female mahseer can be noted by feeling the softness of the abdomen and the pink coloration of the vent. It has a preference for clean water with sandy or gravelly bottoms for breeding and performs potamodromous upstream spawning migrations. It is believed that spawning migration is triggered by turbid waters and elevated



Fig. 6.3 Ovary of golden mahseer containing eggs of different sizes



Fig. 6.4 Captive-reared female (upper) and male (lower) brooders of golden mahseer

water temperatures (Nautiyal 1994; Bhatt et al. 2004). Consequently, during floods, the mahseer ascends to the river's higher reaches, travelling great distances in search of suitable breeding grounds or substratum. They typically lay their eggs in shallow (0.5–3.5 m) streams, containing pebbles, gravel, sand, and silt (Pathani 1994). Suitable spawning environments include riverbanks, where fishes dig nests to lay eggs (Nautiyal 1994). One batch of eggs is released at a time, and the process is repeated numerous times during the breeding season. The fecundity of golden mahseer is very low, ranging from 7 to 25 eggs per gram of body weight (Pathani 1981; Nautiyal and Lal 1985; Nautiyal 2014). The fertilized eggs require 58–192 h to hatch, and because they are demersal, they may sink and die on the muddy riverbeds (Johnsingh et al. 2006). Additionally, their extended developmental period leaves them susceptible to predation. The optimal conditions for egg hatching are high turbidity and temperatures between 16 °C and 25 °C (Johnsingh et al. 2006). In the Indian subcontinent, the predominant spawning grounds of *T. putitora* are distributed throughout the Himalayan river systems (Joshi 1988; Bhatt et al. 2004; Atkore et al. 2011; Shrestha 2002; Dasgupta 1991a, b; Zafar et al. 2001).

6.6 Captive Breeding Technology and Seed Production

After the inclusion of the golden mahseer in the *IUCN Red Data Book* as an endangered species (Jha and Rayamajhi 2010), biologists, conservationists, and policymakers became very concerned about preserving and increasing the existing stock through technological solutions and other conservation methods. Artificial propagation and seed production have been regarded as the most viable strategy for rehabilitating this valuable fish. The production of large quantities of seeds from captive broodstocks, followed by the release of healthy fry into their natural habitat (stock enhancement) has been promoted as one of the possible and pragmatic ways for stock replenishment and conservation (Akhtar et al. 2020). Numerous studies have investigated the reproductive biology/behavior and approach for captive propagation of golden mahseer in numerous Trans-Himalayan nations over the years (Ingram et al. 2005; Sarma et al. 2010; Ismail et al. 2011; Akhtar et al. 2013; Sharma et al. 2016). Unfortunately, golden mahseer fails to complete ovarian development and maturity in captivity due to oxidative stress induced endocrine dysfunctions (Akhtar et al. 2017). Therefore, gravid female brooders, collected from the wild, were exploited in the development of golden mahseer seed production and hatchery technologies in the recent past (Sehgal and Malik 1991; Sunder et al. 1993; Sehgal 1999; Ogale 2002; Sarma et al. 2016). This practice of wild collection of gravid brooders for stripping and artificial fertilization has been demonstrated to be non-sustainable, as gravid wild brooders, collected through gill nets die after stripping (Fig. 6.5). Furthermore, the huge demand of stocking material cannot be achieved from wild brooders. Consequently, the paucity of mature golden mahseer brooders in captivity significantly hindered its large-scale seed production for conservation and rehabilitation.



Fig. 6.5 A trapped golden mahseer brooder in a gillnet (destructive fishing)

Numerous scientists and researchers have attempted captive breeding of golden mahseer using pituitary extract or synthetic hormones during the past four decades with minimal success (Tripathi 1978; Pathani and Das 1979; Pandey et al. 1998; Sehgal 1991). Thus, the lack of mature female golden mahseer brooders in captivity has been a significant barrier to producing seeds on a large scale. ICAR-Directorate of Coldwater Fisheries Research (ICAR-DCFR), Bhimtal, India, the only premier research institute engaged in the research and development of fisheries and aquaculture in the temperate Himalayas, has developed an indoor technology for captive maturation and year-round multiple breeding of golden mahseer (Fig. 6.6) by manipulating different environmental cues (Akhtar et al. 2018, 2020, 2021b). This invention includes a system and method for year-round multiple breeding of golden mahseer to produce fry (Akhtar et al. 2021a, c). Using a unique indoor maturation unit, separate egg incubators, modifying water quality, and simulating breeding and grazing grounds during hatchery operation increased the fertilization, hatching, and fry survival. More than 72% of golden mahseer females were found to respond to egg stripping with the current technology, and a single female brooder was found to breed a maximum of four times and a minimum of two times annually. The average annual relative fecundity of each responding female brooder was calculated to be 9680 ± 811 eggs/kg body weight. As a conservation and rehabilitation measure, hatchery-produced advance fry are now routinely ranched into open water bodies (the temperate Himalayan lakes and rivers), following a 6- to 7-month raising period in nursery tanks.

6.7 Mahseer Conservation Strategies

The population of golden mahseer in India is expected to have decreased by more than 50% in the past, and it may reduce by as much as 80% in the future, primarily as a result of the construction of dams across the Himalayan rivers (IUCN 2015;



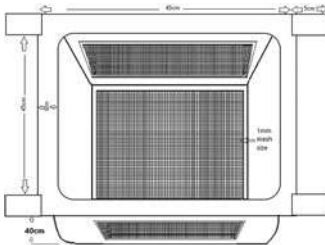
Captive maturation unit



Golden mahseer brooders



Stripping of captive-matured brooder



Golden mahseer egg incubator



Fertilized eggs of golden mahseer matured in captivity



Golden mahseer fry produced from captive-matured brooders

Fig. 6.6 Some important components of the captive maturation and multiple breeding technology of golden mahseer

Sharma et al. 2015). Therefore, the conservation of golden mahseer in the rivers of the Himalayas presents a big challenge for those who work in the conservation field and those who formulate public policy and manage fisheries. To begin the process of recovering golden mahseer, the first thing that needs to be done is to use this species as a “flagship” species to educate people about its positive effects on the environment and the ecosystem (Laskar et al. 2013; Gupta et al. 2014). For the purpose of mounting a mahseer conservation effort that is both effective and focused on achieving particular objectives, recent years have seen the launch of programs like “Project Mahseer,” which is analogous to “Project Tiger” (Nautiyal 2011; WWF 2013). Without stringent protection measures such as the restoration of river connectivity to enable spawning migration, habitat restoration, strict enforcement of rules and regulations (complete fishing ban during monsoon, mesh/equipment size restriction, and penalty for catching/landing small size groups), the establishment of protected areas, etc., conservation and rehabilitation of mahseer may fail. It is possible for various stakeholders, including recreational fisheries groups, to play an important part in the protection of mahseer by practicing catch-and-release fishing. This recreational fishing helps to protect mahseer while also providing employment opportunities and economic benefits to the local fishermen (Bhatt and Pandit 2016; Pinder et al. 2019). It is also recommended that the key sections of the Himalayan rivers be designated as important habitats for the golden mahseer so that it can be protected and brought back to its former glory.

The establishment of hatcheries for ex situ conservation of mahseer is a technique that would be effective over the long run (Bhatt and Pandit 2016). Despite this, there is less number of operational hatcheries in India, dedicated to *T. putitora*. Flow-through mahseer hatcheries have been developed by the ICAR-Directorate of Coldwater Fisheries Research at the Iduli fish farm in Roing, Arunachal Pradesh; the Eco-camp, ABACA, Nameri National Park in Tezpur, Assam; and the Bagua fish farm in Sikkim. If the environment in which we release the fry is fragmented, it is imperative that we adhere to the recommendations made by the IUCN regarding the release, site selection, and longitudinal connectivity (IUCN/SSC 2013). Only then we will be able to successfully disperse hatchery-produced fry into natural water bodies. Ranching operations, in which hatchery-produced healthy juveniles are reintroduced into various temperate Himalayan lakes and rivers, are being carried out on a regular basis by the ICAR-DCFR, Bhimtal, a leading research institute in cold-water fisheries. These ranching operations are being carried out as a step toward mahseer restoration (Fig. 6.7) and rehabilitation.

6.8 Recreational Fishing and Ecotourism Prospects

Ecotourism is a sustainable form of resource use that contributes to the preservation of the natural environment and offers socioeconomic benefits to the people who participate in it. These benefits come from the ecotourism industry’s utilization of natural or biological resources in ways that do not involve direct consumption. Fishery-based ecotourism is growing and has the potential to create new jobs.



In Naukuchiatal Lake, Nainital, Uttarakhand



Kosi river at Ramnagar, Nainital, Uttarakhand

Nainital Lake, Nainital, Uttarakhand

Fig. 6.7 Some glimpses of golden mahseer ranching programs taken up by ICAR-DCFR

Anglers from all over the world travel to the temperate Himalayan rivers to catch the mighty mahseer since sport fishing is quite popular in the Indian Himalayas (Fig. 6.8). About 3800 km lengths of river and stream stretch in the Indian uplands hold sizeable mahseer for angling.

The revenues generated through the sale of fishing licenses help manage fish and wildlife, while the money spent by anglers and tourists on recreational fishing and other logistics generates employment for the locals. Among the Himalayan streams and rivers, river Beas and its tributaries in the foothill region and the river Giri (Himachal Pradesh); Yamuna between Tajwala (Haryana) to Dhak Pathar; Ganga between Rishikesh to Tehri and its tributaries; the Kali, Saryu, East and Western Ramganga, East and Western Nayar, Song, Kosi (Uttarakhand), river Chenab and its tributaries, and rivers Jia-bhoroli, Dibang, Subansiri and Manas (North Eastern Himalayan region) are important fishing sites for mahseer. Apart from these, the lakes of Kumaon (Bhimtal, Naukuchiatal, Nal Damyanti Tal, and Sattal) contribute substantially to mahseer fishery and provide ample scope for fish sports. Besides natural water bodies, the suitable ponds and pools available along the scenic valleys, mountains, or riverbanks in the region could be used to develop mahseer-based sport fishery units with the development of infrastructural facilities. This will help in the overall conservation and management of golden mahseer in the Himalayan waters.



Fig. 6.8 Golden mahseer angling in the North Eastern Himalayan region. (Photo courtesy: Mr. Nausad Ali)

6.9 Conclusion

Golden mahseer has been one of the prides of the temperate Himalayan waters. Therefore, it is the moral duty of all the stakeholders, from researchers to policymakers, to safeguard its population. Dedicated efforts are required to conserve mahseer fishing resources and promote their sustainable development successfully. The conservation initiatives must integrate its capture and culture fisheries with environmental protection programs. Environmental regulations with stringent enforcement mechanisms must be implemented to manage mahseer fisheries effectively. The sustainable development of the golden mahseer fishery would require the intervention of policy and governance from various stakeholders and fisheries researchers. Perhaps, we may have to incorporate our policy research for mahseer into our research initiatives as soon as the program is initiated. There are many identified potential areas in the Himalayan states which can be developed for mahseer-based ecotourism/eco-parks.

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8. Coldwater Fish breeding and Seed Production

8.1 Seed production of golden mahseer (*Tor putitora*)

A cornerstone of ICAR-DCFR is the breeding and seed production of golden mahseer to support conservation and rehabilitation initiatives. In 2024, the Directorate undertook extensive hatchery operations, successfully implementing advanced photo-thermal manipulations to induce captive maturation in golden mahseer broodstock. During the breeding cycle, a total of 221000 eggs were stripped, achieving a fertilization success rate of 60 % to 63%, with hatching rates ranging from 62% to 65%. Following three to four months of nursery rearing in FRP tanks, the fry were transferred to the nursery pond at the Mahseer Hatchery Complex for further

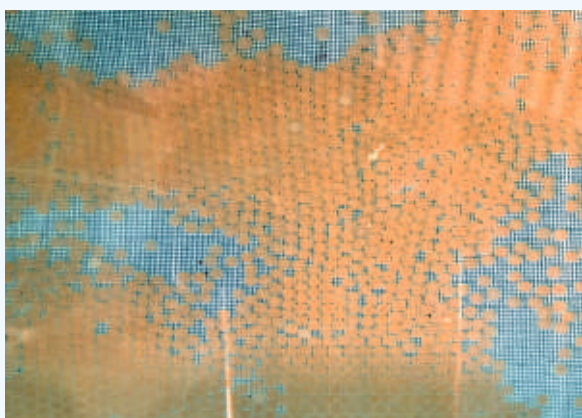
growth into fingerlings. The Directorate also played a crucial role in stock enhancement programs by supplying 10,000 advanced fry to the Department of Fisheries, Govt. of Uttarakhand (Pauri District) and directly ranching 10000 advance fry in the NEHU lake and Umtrew lake, Meghalaya. Approximately 25,000 eggs and fry were utilized for scientific experiments and student research. Apart from seed production, managed broodstock and nursery rearing. During the year, Rs. 2.0 lakh was generated as revenue to the Directorate by selling mahseer seeds. Dr. M. S. Akhtar, Senior Scientist and In-charge of Mahseer Hatchery, and Dr. Ciji Alexander, Senior Scientist coordinated all hatchery activities. [Report by: Dr. M.S. Akhtar, Sr. Scientist, ICAR-DCFR]



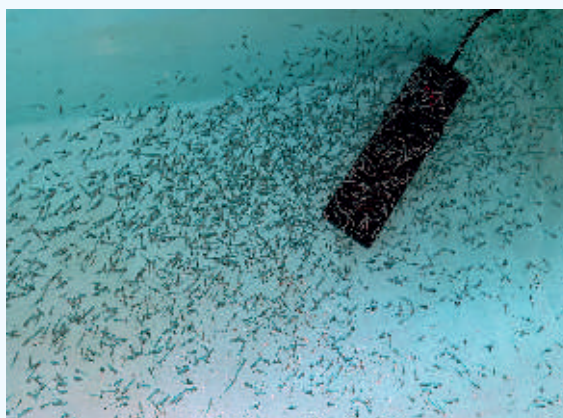
Fig. Brooders under maturation unit



Fig. Mixing of milt onto eggs



Eggs under incubation



Golden mahseer early fry



8. Coldwater Fish breeding and Seed Production

8.1 Seed production of golden mahseer (*Tor putitora*)

One of the central activities of the Directorate is breeding and production of golden mahseer seeds to conserve and rehabilitate the species. In 2023, we carried out breeding and seed production activities throughout the year. Using photo-thermal manipulations, we matured golden mahseer brooders in captivity and stripped 293150 eggs. The fertilization rate ranged from 71% to 87%, the hatching rate was observed between 70% and 82%, and the survival rate was between 72% and 83% until 60 days post-hatching. After three to four months of nursery

rearing in the FRP tanks, the fry were transferred to the nursery pond at the Mahseer Hatchery Complex for further development into fingerlings. Fifteen thousand advanced fry were supplied to Tehri Garhwal and ten thousand advanced fry to Pauri of the Department of Fisheries, Govt. of Uttarakhand for ranching purpose. We also supplied 20 thousand advanced fry, 100 yearlings and 10 brooders to UJVN Mahseer Hatchery. Approximately 30,000 eggs and fry were utilized for experiments and student research. Apart from seed production, managed broodstock and nursery rearing. During the year, Rs.9.1 lakh was generated as revenue to the Directorate by selling mahseer seeds. Dr. M.S. Akhtar, Senior Scientist



Fig. Brooders under maturation unit



Fig. Mixing of milt onto eggs

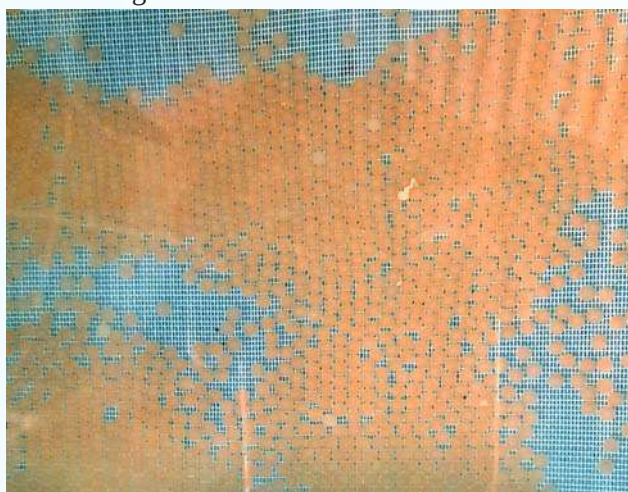


Fig. Eggs under incubation

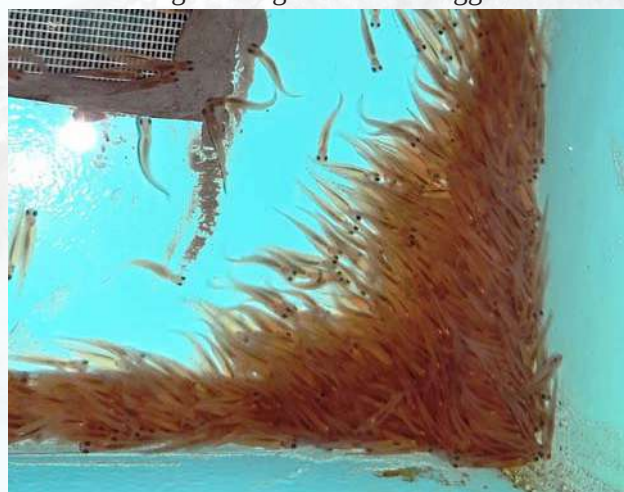


Fig. Golden mahseer early fry

8. Coldwater Fish breeding and Seed Production

8.1 Seed production of golden mahseer

Breeding and seed production of golden mahseer for its conservation and rehabilitation is a core activity of the Directorate. During the year 2021, multiple breeding and seed production of golden mahseer, matured in captive conditions through photo-thermal manipulations, were done throughout the year and striped a total of 18,4200 eggs. We observed a fertilization rate of 82-88%, hatching rate of 83-87% and 80-86% survival rate. The nursery rearing was done in the flow-through hatchery. After three months of nursery rearing, the fry were stocked into the nursery pond of the Mahseer Hatchery Complex of the Directorate to raise them into yearlings/yearlings. Ranching of seven thousand fingerlings was done into Naukuchiatal lake and Suyal river, Almora. Around 16000 eggs and fry were used for different research purposes in the Directorate. The entire activities were coordinated by Dr M.S. Akhtar.

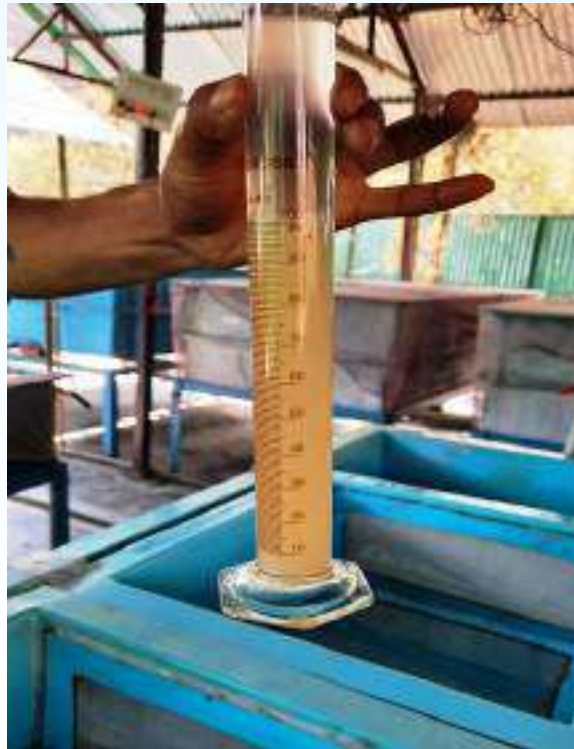
[Report by: Dr M.S. Akhtar, Sr. Scientist, ICAR-DCFR]



Stripping of eggs from a golden mahseer brooder matured in captivity



Golden mahseer fry produced in captivity



Fertilized eggs to be kept for incubation

8.2 Breeding and seed production of Rainbow trout at Experimental Fish Farm, Champawat

Breeding, seed production and culture of rainbow trout is an important activity at the Experimental Fish Farm, ICAR-DCFR, Champawat. At present, 3000 adult (1.2-4.8 kg), and 1500 yearlings (15-200 g) are being maintained at the farm. The farm raised rainbow trout brooders were used for the breeding and seed production during Dec, 2019-Feb, 2020. Eight lakh eyed ova and 6000 fingerlings of Rainbow trout were produced at EFF, Champawat using 780 kg female brooders and 690 kg male brooders. Recorded fertilization rate was about 80-85%. With the use of a new low-cost RAS system for incubation of Rainbow trout eggs designed and developed by a team of scientists (A.K. Giri, Rajesh M, Biju S, Garima and Kishor Kunal) at ICAR-DCFR the survival rate of 70% (green egg to hatching) could be achieved, which was earlier around 20-25% in the old flow through system at EFF, Champawat. The activities of seed production and rearing of rainbow trout were coordinated by Mr. Kishor Kunal and Ms Garima at EFF, Champawat.

[Report by: Dr K. Kunal and Mrs. Garima, Scientists, at Experimental Field Centre, ICAR-DCFR, Champawat]

8

Coldwater Fish Breeding and Seed Production

8.1 Captive maturation and multiple breeding of golden mahseer

Until recently, the breeding and seed production of endangered golden mahseer (*Tor putitora*) was being done using wild gravid brooders collected from natural water bodies which have been a destructive and non-sustainable practice. This dependence on wild brooders was because golden mahseer females fail to complete ovarian development and maturation in captive conditions due to endocrine dysfunctions. Therefore, to address this issue on priority basis, ICAR-Directorate of Coldwater Fisheries Research, Bhimtal, through

institutional projects AQ16a and AQ16d, embarked upon conducting series of experiments for over last seven years and finally achieved captive maturity and multiple breeding through photo-thermal manipulations. This technology of captive maturity and multiple breeding of endangered golden mahseer have resulted in producing a substantial number of fry of golden mahseer round the year on sustainable basis which otherwise has been a major bottleneck for large scale rehabilitation efforts to conserve this esteemed species.

[Report by: Dr. M.S. Akhter, Scientist, ICAR-DCFR]



Captive maturation system



Stripping of captive matured brooder



Fertilized eggs of golden mahseer matured in captivity



Golden mahseer fry produced from captive matured brooders

8.2 Seed production of chocolate mahseer

Chocolate mahseer is widely recognized as sport fish and food fish in coldwater rivers and streams of India, Bangladesh, Bhutan, Nepal and Myanmar. The population of chocolate mahseer in the natural water bodies had declined alarmingly due to the over-exploitation and habitat lost over the past years in most of its range. Breeding and seed production of chocolate mahseer is not standardized in captivity. Chocolate mahseer adults were procured from Meghalaya, India and maintained in captivity in mahseer hatchery of ICAR-Directorate of Coldwater Fisheries Research, Bhimtal. Broodstock were reared in flow-through rectangular tanks provided with the environmental conditioning. A gravel based tray constituting 1/3rd of the tank was provided in flow through tank. Fish were stripped for the seed production.

[Report by: Ms. P. Dash, Scientist, ICAR-DCFR]

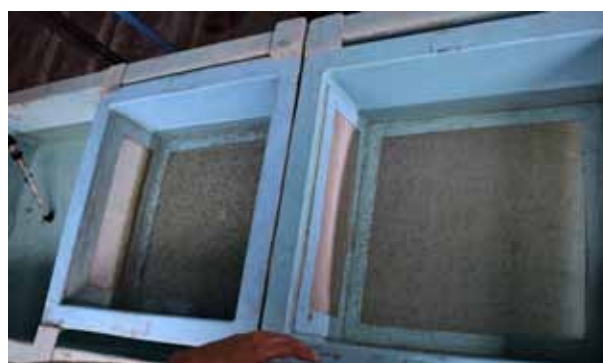


Fig.: Breeding and seed production of chocolate mahseer at ICAR-DCFR

8.3 Seed production of improved common carp

Common carp (*Cyprinus carpio*) is a very important candidate fish in mid Himalayan region. It is being cultured widely either alone or in Polyculture system in the central and lesser Himalayan region in cemented as well as earthen tanks, ponds etc owing to its growth potential and minimal maintenance. In order to obtain higher fish productivity in uplands, two improved Hungarian strains of common carp 'Ropsha scaly' and 'Felsosomogy mirror carp' were introduced at Champawat experimental farm in the year 2007. These species were found more suitable for hill farming due to their fast growth and wide temperature tolerance (5-32°C). The species spawn twice during a year, i.e. March-May and July-

August. Breeding and seed production of improved common carp was carried out at EFF, ICAR-DCFR, Champawat following old conventional hapa breeding method without hormone administration. Eggs were collected using plastic twines extracted from unused plastic sacks. About 150-200g of twines per kg of female were used for collection of eggs. Sex ratio was kept 1.0-1.5:1.0 (Male: Female) by weight. Fertilized eggs were adhesive, pale yellowish in colour and have diameter ranging from 1.2-2.1 mm. Recorded fecundity was about 0.5-1.0 lakh eggs/kg body weight. Hatching took place after 84-112 hrs post fertilization and yolk absorption within 72-90 hrs at 18-22°C. The survival percentage of egg to spawn and spawn to fry was found 40-45% and 30-35%, respectively. A total of 5 lakh fry (15dph) were

8. Coldwater Fish breeding and Seed Production

8.1 Seed production of golden mahseer (*Tor putitora*)

A key focus of the Directorate's work is the breeding and seed production of golden mahseer for the sake of conserving and rehabilitating the species. In the year 2022, numbers of breeding and seed production activities of golden mahseer were carried out throughout the year. The golden mahseer were brought up to maturity in captivity by the use of photo-thermal manipulations. In all, we could strip 225300 eggs. We observed the fertilisation rate in the range of 72 to and 82%, the hatching rate was between 75 and 85%, and the survival rate was between 75 and 90 % till 60 dph. Following a period of nursery rearing (3 months), the fry were placed in the nursery pond at the Mahseer Hatchery Complex of the Directorate so that they could be raised into fingerlings. Ten thousand advanced fry were ranched into Nainital lake. In all, about 30000 eggs and fry were used for various experiments and student research work. During the year, 8000 advanced fry were sold to Fisheries Department, Pauri Garhwal, Uttarakhand and generated a revenue of Rs. 80,000.00 (Rupees eighty thousand only). Dr M. S. Akhtar, Senior Scientist and In-charge, Mahseer Hatchery coordinated all the hatchery activities.

[Report by: Dr M.S. Akhtar, Sr. Scientist, ICAR-DCFR]



Stripping of eggs from a golden mahseer brooder matured in captivity



Eggs under incubation



Brooders under maturation unit



Newly hatched golden mahseer fry

training was regularly attended by 74 participants (50 men and 24 women) which included academic faculty and students from fisheries colleges and agriculture universities, spread across 19 states of India and fisheries officials from Bhutan. With four sessions per day, this online training had 20 lectures and case studies covering aspects such as climate change and aquatic ecosystems / biodiversity, impact of climate change on coldwater fisheries and hill aquaculture, vulnerability and risk assessment in aquaculture, environmental factors governing rainbow trout farming in India, stress coping strategies of aquatic organisms, climate-resilient aquaculture production systems, nutritional / husbandry interventions and biotechnological / genetic tools to mitigate climate change impacts, role of conservation aquaculture and live gene banking, integrated farming systems, disease incidences, biosecurity and health management, and key role of citizen-scientist programs. The training was coordinated by Dr. Biju Sam Kamalam from ICAR-DCFR, and Dr. Krishna Reddy and Ms. Kiranmayi Dhenuvakonda from MANAGE.

12.6 Training on “Seed Production and Hatchery Management of Golden Mahseer”

A five-day training programme on “Seed production and hatchery management of golden mahseer” was organised at ICAR-Directorate of Coldwater Fisheries Research from 10-14 June 2024. During the training period, different aspects of golden mahseer and its resources, environmental impact on mahseer, bio-filter system for maturation and spawning of mahseer in captive condition, health management, angling and ecotourism prospects of mahseer, ex-situ and in-situ conservation of mahseer, ranching and its model guidelines were deliberated and discussed. Trainees also got the opportunity to learn DPR preparation for establishing a mahseer hatchery. Training equipped trainees with the knowledge, skills and best practices necessary for successful seed production, fostering the growth and sustainability of golden mahseer populations. Training was coordinated by Dr. M.S. Akhtar, Dr. Ciji Alexander and Dr. Renu Jethi.



12.7 Exposure Visit and Training on “Feed and Disease Management”

ICAR-DCFR organized a two-day exposure visit and training on ‘Feed and disease management’ for rainbow trout farmers from Himachal Pradesh during 31st July to 1st

August 2024, at Bhimtal. During this program, lectures on best feed and health management practices in trout farming and control of common diseases in trout farms was delivered to the farmers, followed by question-and-answer sessions. The farmers were also demonstrated feed mill operation,



reproductive physiology and larval development of coldwater fishes, an outlook of genomics and transcriptomics approaches and applications in fisheries etc. Students got the opportunity to visit river Gauri located in Champawat, Sattal & Naukuchiatal Lakes in Bhimtal and Nainital Lake for water & soil sampling and plankton sample collection. This training was coordinated by Dr. Biju Sam Kamalam, Dr. Rajesh M, Dr. Renu Jethi, Dr. Prakash Sharma and Dr. Kishor Kunal.

12.12 Training on “Captive breeding and hatchery management of golden mahseer”

Five-day training programme on “Captive breeding and hatchery management of golden mahseer” was conducted at ICAR-Directorate of Coldwater Fisheries Research during 10-14 July 2023. The training was attended by 15 participants, including entrepreneurs and students from College of Fisheries Science, HAU, Hisar. During the training period, different aspects of golden mahseer and its resources, ecological significance, conservation challenges, mahseer-based angling and ecotourism prospects and the



Fig. Hatchery demonstration and group photo of the trainees

crucial role of captive breeding and hatchery management for its conservation were deliberated and discussed. There were theoretical sessions and practical demonstrations about the various aspects of golden mahseer seed production, such as captive maturation and breeding, hatchery management, water quality analysis, fabrication of bio-filters, brood stock nutrition, larval nutrition and nursery rearing, etc. Training was coordinated by Dr. Ciji Alexander, Dr. M.S. Akhtar, Dr. Prakash Sharma, and Dr. Renu Jethi.

12.13 In-plant Training on “Broodstock rearing, breeding and hatchery operation of golden mahseer”

A twelve-day in-plant training on "Broodstock rearing, breeding and hatchery operation of golden mahseer" was conducted during 24 September to 05 October, 2023 for the personnel from UJVN Ltd. Dehradun at ICAR-DCFR, Bhimtal. The training was coordinated by Dr. M. S. Akhtar, Dr. Ciji Alexander and Dr. Renu Jethi. The main objective of the training was to impart *learning by doing* different activities related to golden mahseer maturation & breeding, broodstock management, feeding management, water quality monitoring, data storing, fish handling, nursery rearing, netting operation etc. so that the trainees would operate and manage the newly constructed mahseer hatchery at Juddo, UJVN HEP site, Dehradun.



Fig. Netting for the assessment of broodstock and stripping of brooder by trainees

12.14 Training on “Basic technique in solid peptide synthesis (SPSS)”

A seven-day training programme on “Basic technique in solid peptide synthesis (SPSS)” was



Fig. RAS training and field exposure visit at Ranchi, Jharkhand

12.6 Hands-on-training on hatchery management and seed production of golden mahseer

ICAR-DCFR, Bhimtal organized a three-day training programme on "Hands-on-training on hatchery management and seed production of golden mahseer" during 29th September-01st October, 2022. State Fisheries Department officials, entrepreneurs and students attended the training, which was coordinated by Dr M.S. Akhtar (Course Director), Dr Ciji Alexander, and Dr Renu Jethi (Co-course Directors). There were both theoretical sessions and practical demonstrations about the various aspects of golden mahseer seed production, such as captive maturation and breeding, hatchery management, water quality analysis, fabrication of bio-filters, brood-stock nutrition, larval nutrition and nursery rearing, etc. The training concluded with distribution of certificates to the participants by the Director.



Fig. Demonstration of mahseer seed rearing to participants

12.7 Five days of hands-on training on "Recirculating Aquaculture System for Intensive Farming of Fish"

ICAR-Directorate of Coldwater Fisheries Research, Bhimtal, conducted five days hands-

on training on "Re-circulating Aquaculture System for Intensive Farming of Fish" from 5-9th December 2022. Twenty trainees attended the training, including state fisheries department officials, farmers, entrepreneurs, aquaculture /fisheries start-ups, and marketing executives. The training program included a lecture and practical demonstration of all the aspects of RAS. Dr Rajesh M. (Scientist) delivered the lecture on "Recirculating Aquaculture System: Concept, Design and Components, Water quality monitoring in Recirculating Aquaculture Systems: theory and estimation, Bio-planning, product quality management and techno-commercial feasibility of RAS, water flow and biofilter design and practical demonstration of different RAS models of the Directorate. Ms Nahida Rashid and Mr Junaid (Research Scholar) conducted the practical demonstration of various water quality assays required for fish farming in RAS. Dr Renu Jethi (Senior Scientist) delivered a lecture on "Support from the state, central government, NABARD, NFDB, NCDB for fisheries and aquaculture development", and Dr Biju Sam Kamalam (Scientist) deliberated on the topic "Standard operating procedures and risk management in RAS and Rainbow trout: biology, nutrition, breeding seed production and culture". Two guest lectures were also organised on the topic of "Feed for Recirculating Aquaculture System: Feeding the fish or System?" and "Importance of RAS in sustainable fish production: a global perspective" by Dr Arjen Roem (Strategic Marketing Director, Skretting) and Dr Antony Jesu Prabhu Philip (Scientist, Institute of Marine Research, Bergen, Norway), respectively. On the fourth day, Dr Suresh Chandra (Principal Scientist) deliberated on the "Disease management aspect of RAS". A

11.4 First Angling Competition cum Interactive Workshop on Mahseer organized by ICAR-DCFR held at Vyasghat, Pauri Garhwal (Uttarakhand)

A three days angling competition cum Interactive workshop was successfully conducted by ICAR-Directorate of Coldwater Fisheries Research in collaboration with Department of Fisheries, Govt. of Uttarakhand at Vyasghat of Pauri Garhwal district (Uttarakhand) during 20-22 November, 2020. The angling competition was participated by 21 avid anglers from Marchula, Almora, Pancheswar, Pauri, Dehradun of Uttarakhand and other neighbouring states and witnessed by 50 personnel representing scientists, departmental officials, district administration and farmers. During the competition, the anglers could hook golden mahseer *Tor putitora* (Hamilton, 1822) and Indian hill trout *Raiamas bola* (Hamilton 1822) as the major catch in rivers Nayar and Ganga. The largest catch weighed 13 pounds in the hooks of Mr.



Fig.: Big catch of Golden mahseer by Mr. Satpal from Himachal Pradesh



Fig.: Prize distribution by the Hon'ble MLA, Pauri

Satpal from Himachal Pradesh, followed with a 10 pounds mahseer in the hooks of Mohd. Ali Khan and 5 pounds by Mr. Tegbir Mann from Dehradun (Uttarakhand). The maximum numbers of fishes were caught by Mr. Sanjeev Paroriya from Marchula (Uttarakhand) followed by Mr. Mohd. Ali Ghazi from Rampur (Uttar Pradesh) and Mr. Shyam Gurung from Marchula (Uttarakhand). The outcomes of the programme are: Recognition of Vyasghat as a major mahseer angling destination for the tourist on the world map; Promotion of the SC village (Baggi) to earn revenue through mahseer based recreational fisheries and eco-tourism; Conservation of mahseer in the river systems through mahseer angling and eco-tourism. Dr. Deepjyoti Baruah, Ex. Sr. Scientist; Dr. R. S. Halder, Chief Technical Officer coordinated the programme.

11.5 Socioeconomic upliftment of Scheduled Caste population of Chamoli district, Uttarakhand through Rainbow trout farming intervention

A collaborative programme was undertaken by ICAR-DCFR with Department of Fisheries, Chamoli district, Govt. of Uttarakhand on the title "Socioeconomic upliftment of Scheduled Caste population of Chamoli district through Rainbow trout farming intervention under SCSP project, 2019-20" at Chamoli district, Uttarakhand. Altogether 18 SC fish growers were benefited. The programme witnessed the following outcomes: (i) Completed repairing and renovation of concrete



raceways for trout culture in Chamoli district. (ii) Advanced sized trout fingerlings were stocked in the raceways. (iii) High quality trout feeds were given to the 18 beneficiaries. (iv) Fish nets and fish disinfectants were distributed



- A recirculatory aquaculture system (RAS) hatchery was provided to the State Fisheries Department, Government of Arunachal Pradesh.

10.5 Golden mahseer ranching and awareness programmes

Golden mahseer holds significant economic, recreational, and cultural value in the rivers and lakes across eleven Asian nations, including India. Despite its past abundance in the waters of Himalaya, the golden mahseer population has drastically reduced in recent years, calling for urgent conservation measures. ICAR-Directorate of Coldwater Fisheries Research, Bhimtal, the Department of Fisheries and the Zoology Department of the North Eastern Hill University (NEHU) has launched a joint initiative for conservation of the endangered golden mahseer in the State of Meghalaya under NEH activity. On this occasion, a seed

ranching programme was conducted on 10 September 2024 at the NEHU lake and Umtrew lake, Meghalaya to conserve and restore the declining population of the species in the natural waters of the region. The event was graced by Prof. P.S. Shukla, Vice Chancellor, NEHU, Shillong along with officers of Fisheries Department, Meghalaya, and scientists of ICAR-DCFR, Bhimtal. Prof. Shukla expressed his commitment to conserving the golden mahseer in the natural ecosystems of Meghalaya. He highlighted that the joint initiative will bolster the population of the species and conserves its genetic diversity. As part of the event, 10,000 hatchery reared fingerlings of golden mahseer from ICAR-DCFR were released into these two lakes of Meghalaya. Over 100 participants actively took part in the program, marking a significant step toward the conservation of this vital species.



Fig. Glimpses of ranching programme

The program coordinators, including Dr. S.K. Mallik, Scientist, Dr. Suresh Chandra, Principal Scientist, Dr. M.S. Akhtar, Senior Scientist, and Dr. R.S. Halder, Chief Technical Officer emphasized the critical need for community participation in mahseer conservation and called for safeguarding the fish breeding grounds as a crucial step toward reviving its population.

10.6 Training programme on 'Entrepreneurship development through aquaponics'

A 3-day training programme on 'Entrepreneurship development through aquaponics' was conducted from 17-19 December 2024 aimed at fostering entrepreneurial skills through innovative and

7.1.3 Seed stocking in farmers' pond

Due to COVID pandemic, many fish farmers of the area could not able to get the desired quality of seed. The most favourable growth period for carp species in hills is from March -April to September-October months. With an initiative to provide technical help and critical inputs like fish seed; 16 fish famers' ponds of Saladi (Bhimtal) area were stocked with cage reared one year old stunted fingerlings of grass carp, common carp and silver carp in the month of May, 2020. Dr S. Chandra, coordinated the distribution program.



Fig.: ICAR-DCFR initiative to provide critical inputs (carp seed) during COVID-19 to farmers of Bhimtal

7.1.4 ICAR-Directorate of Coldwater Fisheries Research takes initiative for conservation and rehabilitation of endangered golden mahseer (*Tor putitora*) in Kumaon lakes

Mahseer is a cultural icon of diverse economic, recreational and conservational value in rivers and lakes of eleven Asian nations (FAO) and has been declared as 'State Fish' of seven states of India. Despite their abundance at one time in Kumaun rivers, mahseer population has been declining in natural water bodies. In this context, ICAR-Directorate of

Coldwater Fisheries Research (DFCR), Bhimtal successfully organized a seed ranching programme of golden mahseer in three prime lakes of Kumaon region viz. Bhimtal lake, Naukuchiatal lake and Sattal Lake on 4th November, 2020 as an attempt to save and increase the population of golden mahseer in the respective lakes. Sh. Deepak Chanotiya, Chairman, Bhimtal Nagar Panchayat and Sh. Surendra Surya, Gram Pradhan, Sattal graced the occasion and emphasized upon the need of people's participation in the conservation of mahseer. They also felt that necessary measures to be taken to save the fish by protecting its breeding grounds.

Dr. Debajit Sarma, Director (Acting), DCFR, Bhimtal expressed his concern to save golden mahseer in the natural water bodies of Kumaun region. The Directorate has taken up several steps for seed production of golden mahseer in the hatchery complex and release of the seed in the different streams/rivers/lakes of Kumaun region to increase the population of this fish in the natural habitat and also to conserve the germplasm. On this occasion, thirty thousand fingerlings (10 thousand fingerlings in each lake), which were produced from the captive matured brooders in the DCFR's Mahseer hatchery, were ranched/stocked in all the three Kumaon lakes. More than 30 participants including scientists such



Fig.: Releasing golden mahseer fingerlings into Naukuchiatal lake

as Dr. Suresh Chandra, Principal Scientist; Dr. Siva C., Scientist; Dr. M. S. Akhtar, Scientist, and staffs of DCFR, staffs from the Fisheries Department, Bhimtal and local people participated in the event. The programme was coordinated by Dr. M. S. Akhtar.



Fig.: Releasing golden mahseer fingerlings into Bhimtal lake



Fig.: Golden mahseer fingerlings for ranching in river Kosi, Almora during 17th July 2020

7.1.5 Collaborative work in PPP mode with ICICI Foundation

ICAR-DCFR is undertaken collaborative work in PPP mode with ICICI Foundation for upliftment of rural livelihood security of hill fish farmers in different villages of Nainital and Almora districts of Uttarakhand. Under this two days training was

organized to the selected farmers to know-how of the scientific fish farming. In collaboration with the ICICI Foundation polythene lining, fish seed, fish feed etc. were also provided to the selected farmers.

Two day training workshop on “Development of Hill Fish Farming for upliftment of Rural Economy in Uttarakhand” was organized among the selected fish farmers of Ramnagar Block (Nainital District) and Molekhal Block (Almora District) of Uttarakhand during 27-28 November, 2020 in collaboration with the ICICI Foundation for Better Growth. First day 15 selected fish farmers were present from Jassa Ganja, Madaiya, Thari, Puchhdi, Kandla, Narayanpur Muliya, Lachhampur Theree etc. villages of Ramnagar Block (Nainital District). The training was organized at National Rural Livelihood Mission (NRLM) Meeting Hall at Ramnagar on 27th November 2020 for upliftment of livelihood to the poor peoples of the area. Mr. N. D. Bhatt, Incharge, Block Development Officer (BDO) of Ramnagar Block graced the occasion as Chief Guest and Dr. Shiv Kumar Srivastava, Block Mission Manager (BMM) as Guest of Honour and Mr. Rahul Saxena, Development Officer, ICICI Foundation was also present during the training programme.

Second day the training was organized at Govt. Senior Secondary School, Painsia, Salt, Molekhal (Almora). Mr. Ram Avatar Tewari, Principal Incharge, Govt. Senior Secondary School, Painsia, Salt graced the occasion as Chief Guest. Some of the senior faculty members of the Institute were also present during the training programme. Another 20 selected fish farmers from Jhimar, Kupi, Simli Heet, Bhitakote, Masaniya Banj etc. villages of Molekhal Block were participated in the training on 28th November 2020. During the occasion the trainees were trained about the primary requirements for fish farming, construction of different type of ponds, eradication of aquatic weeds, cleaning of unwanted fishes from culture ponds, production of natural food for fish, required water quality management, stocking of fish ponds with quality seed, enhancement of productivity of pond, feeding of fish, integrated fish farming with hen, poultry, goat, cow etc., overall management of fish ponds, common diseases of fish and their control, harvesting of fish and marketing etc. and briefed

- Dr Basava Purushottam (IAS) Secretary, Animal Husbandry, Cooperatives, Dairy Development, Agriculture, Horticulture · Government of Uttarakhand visited at Experimental Fish Farm, Champawat on 26th December 2022.



Fig. Dr Basava Purushottam (IAS) Secretary, Animal Husbandry, Cooperatives, Dairy Development, Agriculture, Horticulture · Government of Uttarakhand visited EFF, Champawat

7.5 Consultancy & Other Services

7.5.1 Conservation of endangered golden mahseer

Mighty mahseer, *Tor putitora* has been the icon of recreational and sports fisheries in India and Indian-subcontinent. It is one of the sought-after game fishes that attracts anglers worldwide and has immense potential for fish-based eco-tourism generating sufficient employment opportunities for local inhabitants. Once applauded as the 'tiger of the rivers', *T. putitora*

is in perils due to anthropogenic activities and its population has been declining in natural water bodies. Therefore, rehabilitation and conservation of golden mahseer is imperative for its sustainability.

In this context, ICAR-DFCR has consistently been carrying out rehabilitation and conservation efforts for several years through ranching of hatchery-bred fry and fingerlings into rivers, lakes and reservoirs of Himalayan region. In continuation of the bonafide conservation efforts, the Directorate in collaboration with the Nainital district administration successfully organized a seed ranching programme of golden mahseer in Nainital lake, Nainital on 23 July, 2022. During this event, ten thousand fingerlings were stocked.

Shri Dhiraj Singh Garbiyal, District Magistrate and Shri Sandeep Tiwari, Chief Development Officer graced the occasion and emphasized upon the need of conservation of mahseer in the lakes of Kumaon. Dr Pramod Kumar Pandey, Director, ICAR-DCFR, Bhimtal interacted with media and expressed his concerns to save golden mahseer in the natural water bodies of Kumaon region. More than 50 participants including scientists, district administration officials, media persons and local people participated in the event. The programmes were coordinated by Dr R. S. Patiyl, Principal Scientist and Dr M. S. Akhtar, Senior Scientist.



Fig. Ranching programme in Nainital Lake, Nainital

7.1.10 Slogan writing competition on Antimicrobial resistance in fish

A slogan writing competition was organised on

24th November, 2021 for the staff of the Directorate. About 60 participants were present in the competition.



Slogan writing competition on Antimicrobial resistance in fish

7.1.11 Group discussion on use of chemicals and drugs in Hill aquaculture with state fisheries officials of Uttarakhand

A group discussion was held with the fisheries officials of DOF Uttarakhand at Bhimtal on 24th November, 2021 to make them aware of the banned antibiotics and chemicals in aquaculture. They are advised to encourage the Hill farmers to adopt best management practices for fish production and timely report about the incidence of disease for proper medication and treatment. The Department officials were informed that high levels of AMR already seen

in the world today are the result of overuse and misuse of antibiotics and other antimicrobials in humans, animals (including farmed fish), and crops. As per the reports, every year globally, almost 700,000 lives are lost. More than 58,000 children die every year in India alone from antibiotic-resistant infections. Thus we should take utmost care to prevent the misuse of antibiotics and chemicals in aquaculture for achieving the goal of one health approach. Seven state department officials were present.



Group discussion on use of chemicals and drugs in Hill aquaculture with state fisheries officials

On this occasion, Dr Pramod Kumar Pandey, Director, ICAR-DCFR, Bhimtal expressed his concern over injudicious use of drugs, chemicals and antibiotics in aquaculture, which may lead to develop antimicrobial resistance in fish. He emphasized on prudent use of any aquadugs for production and health management in fish farming practices. He also encouraged the scientists of the Directorate to conduct more awareness programmes on AMR for the general public, fish farmers, fisheries officials and staff in near future. Along with Dr S. K. Mallik, Scientist, Nodal officer, National Campaign on AMR, all the above said activities under the campaign were jointly coordinated by Dr Suresh Chandra, Principal scientist, Fish Pathology, Dr Neetu Shahi, Senior scientist, Animal Biotechnology, and Dr R.S. Tandel, Scientist, Fish Pathology & Microbiology and Dr Raja Aadil H. Bhat Fish

Pathology & Microbiology and Mr Partha Das, Technical assistant, ICAR-DCFR.

7.1.12 Rehabilitation and conservation of endangered golden mahseer

Mighty mahseer especially *Tor putitora* has been the icon of recreational and sports fisheries in India and Indian-subcontinent. It is one of the sought-after game fishes that attracts anglers worldwide and has immense potential for fish-based eco-tourism generating sufficient employment opportunities for local inhabitants. Once applauded as the 'tiger of the rivers', *T. putitora* is in perils due to anthropogenic activities and its population has been declining in natural water bodies. Therefore, rehabilitation and conservation of golden mahseer is imperative for its sustainability.

In this context, ICAR-Directorate of Coldwater Fisheries Research (DCFR), Bhimtal has consistently been doing rehabilitation and conservation efforts for several years through ranching of hatchery-bred fry and fingerlings into rivers, lakes and reservoirs of Himalayan region. In continuation of the bonafide conservation efforts, the Directorate successfully organized a seed ranching programme of golden mahseer in Naukuchiatal lake, Nainital and in Suyal river (at Vishwanath ghat), Almora during 17th and 23rd February, 2021 respectively. In these events, five thousand fingerlings in Naukuchiatal lake and two thousand fingerlings/yearlings in Suyal river were stocked.

Dr Dilip Kumar, Former Director, ICAR-CIFE, Mumbai, Dr S.N. Ogale, Lonawala, and Sh. Anil Chanotiya, Member, Bhimtal Nagar Panchayat graced the occasion (at Naukuchiatal lake) and

emphasized upon the need of people's participation in the conservation of mahseer. An awareness programme was also organized at Vishwanath ghat (Suyal river) to take necessary measures to save golden mahseer by protecting its breeding grounds. On this occasion, aquaculture inputs such as feed and aqua-medicine were also distributed among farmers. Dr Debajit Sarma, then Director (Acting), ICAR-DCFR, Bhimtal expressed his concerns to save golden mahseer in the natural water bodies of Kumaun region.

More than 30 (at Naukuchiatal) and >100 participants (at Suyal river) including scientists, fisheries department officials, farmers and local people participated in these events. Both the programmes were coordinated by Dr M. S. Akhtar, Senior Scientist and Dr Suresh Chandra, Principal Scientist.



Ranching of golden mahseer fingerlings in Naukuchiatal lake and River Suyal at Vishwanath Ghat, Almora, Uttarakhand



Interaction by Dr D. Sarma, Principal Scientist, ICAR-DCFR

7.1.13 Scientific and technical support to fish farms in Ramgarh

To promote aquaculture initiatives in Nainital district, scientific guidance and input support were provided for upcoming fish farmers in Ramgarh, namely Mr. Prithvi Singh, Mr. Kamlesh Harnwal, Mr. Abhishek Harnwal and Mr. Naveen Lal Shah. This includes farm visits, guidance in the construction of

new raceways, water quality analyses, stocking and biomass monitoring, feed management and farm operation. Efforts are also being made to support and reinstate rainbow trout farming in places affected by the flash floods in October 2021. This activity was coordinated by Dr Rajesh, M., Dr Biju Sam Kamalam, Dr R.S. Haldar and Dr Debajit Sarma.



Reply of R-12 in OA No-1004/2024

1 message

Rakesh Singh <rakeyshsingh@gmail.com>
To: Ankur Sood <ankursoodoffice@gmail.com>

Thu, 16 Apr 2026 at 19:14

Sir,
Kindly Be in the Receipt of Response/Reply of Respondent No. 12.pdf in OA No-1004/2024 bearing cause title as Prahlad Roy Goenka vs MOEFCC & ORS..

Thanks & Regards,
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 **OA 1004 of 2024 Reply to NGT Respondent No. 12-1.pdf**
16.1 MB