Neolissochilus hexagonolepis (chocolate mahseer): A flagship species for diversification of hill aquaculture in Northeast India

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Mahseers are exceptional game and food fishes found in India, Pakistan, Bangladesh, Afghanistan, Sri Lanka, and Myanmar. They serve as cultural icons with diverse economic, recreational, and conservation value in the rivers of eleven Asian nations. The Mahseer Declaration, held in Kuala Lumpur in 2006, recognised many new species that transcend natural geographical boundaries. Taxonomically, mahseers are classified as carps with large scales, fleshy lips, continuous angles at the mouth with an interrupted fold or groove across the lower jaw, two pairs of large barbells, and lateral-line scales ranging from 22 to 28. The head length is equal to or greater than the depth of the body.

India hosts seven species of mahseer, including *Tor putitora* (golden mahseer/Himalayan mahseer), *T. tor* (tor mahseer), *T. mosal* (copper mahseer), *T. progeneius* (jungha mahseer), *T. khudree* (Deccan mahseer), *Neolissochilus hexagonolepis* (chocolate mahseer), and *T. mussullah* (humpback mahseer). Of these, *T. putitora* and *N. hexagonolepis* are regarded as 'flagship species' in India. While extensive research exists on the former, studies on the latter species are limited and fragmentary.

Neolissochilus hexagonolepis (Mc Clelland), commonly known as chocolate or copper mahseer, holds significant importance as a prominent fish species in Northeast (NE) India. It thrives in nearly all the major rivers and hill-streams of Meghalaya, Assam, Arunachal Pradesh, Sikkim, Manipur, Nagaland, Mizoram, and Tripura – the eight states that comprise North Eastern India. Additionally, this fish species is also present in the Cauvery River, which meanders through the states of Karnataka and Tamil Nadu in South India. Beyond India's borders, the geographical range of *N. hexagonolepis* encompasses Bangladesh, China, Nepal, Myanmar, Thailand, Malaya, Sumatra, and Vietnam.

Distinguished for its remarkable size and robust strength, this fish stands out as a true game fish. It attains an impressive maximum length of 6 feet and can weigh up to 28 kg. Notably, its adaptability to captive environments presents substantial potential for aquaculture, while its exquisite taste contributes to its elevated market value. Revered as a flagship species and an emblem of water ecosystems within NE India, it commands a price ranging from Rs. 400 to 500 per kilogram. The delectable meat of *N. hexagonolepis* is in high demand for its scrumptious flavor and relatively bone-free texture, often surpassing the preference for Indian major carps and Chinese carps. Beyond its culinary appeal, it serves as a bountiful source of high-quality lipids and proteins, making it a valuable asset in diversifying protein sources and promoting a holistic, healthful diet[8].

Neolissochilus hexagonolepis (chocolate mahseer).

Mahseer recipe in the diet of the Khasi tribe, Meghalaya.

Furthermore, this species plays a pivotal role within the unique 'Johra' fishery, an integral practice in terraced ponds nestled amidst the picturesque Darjeeling and Kalimpong hills. This practice not only sustains local communities but also upholds cherished fishing traditions.

Chocolate mahseer also plays a distinct role as a potential ornamental fish during its early life stage, often classified as non-classified within this context. In the beautiful state of Meghalaya, it is known by the local name 'kha saw'. Here, innovative fish farmers engage in the cultivation of the fish within their ponds, utilising them as a resource for generating income through fishing competitions and subsequent sales within the market. However, this endeavor presents challenges, as procuring the fish seeds from local streams, rivers, lakes, etc., involves considerable effort and dedication.

Until recent years, the rivers, lakes, and reservoirs of the region were teeming with the chocolate mahseer. However, its population has suffered a sharp decline due to a combina-

tion of factors. The rampant capturing of both adult brooders and juvenile fish, coupled with habitat degradation, pollution, the construction of barrages obstructing migratory routes, acid mine drainage, the introduction of non-native species (primarily *Cyprinus carpio*), and the absence of strategies to replenish stocks through hatchery-produced seeds and ranching have all contributed to this alarming trend[7]. Consequently, the species has earned a "near-threatened" status according to the International Union for Conservation of Nature (IUCN).

This fish's value extends beyond its ecological importance; it holds significance for food security, nutrition, recreational sport fishing, and ecotourism. Acknowledging this, three states within NE India, namely Meghalaya, Nagaland, and Sikkim, have designated *N. hexagonolepis* as their official "State fish." This proactive measure aims to raise public awareness and galvanise conservation efforts. Among these states, Meghalaya stands out with its robust commitment to preserving aquatic biodiversity. Boasting 79 fish sanctuaries that provide a haven for various fish species, including the chocolate mahseer, the state has taken a proactive stance through its Meghalaya State Aquaculture Mission (MSAM), 2012-17, which has been actively engaged in conservation activities since 2012[1].

Taxonomy of *Neolissochilus hexagonolepis*

The taxonomy of mahseer fish has been a subject of confusion due to the varying morphological traits they exhibit. Challenges arise from incomplete morphometric information in original descriptions, scarcity of original holotypes of mahseer species, and disagreements over the recognition of specific morphological characteristics. This has led to a somewhat disorderly taxonomy, with certain mahseer species being described multiple times.

Traditionally, the differentiation of the two mahseer genera has been based on the presence of the labial groove, which is discontinuous in *Neolissochilus* and continuous in *Tor*, as well as the disparity in the number of gill rakers on the first arm of the gill arch (8-9 in *Neolissochilus* and 11-13 in *Tor*). Identifying chocolate mahseer in their early life stages has proven challenging. However, regular field observations have facilitated the development of key indicators for identifying *N. hexagonolepis*. For instance, young fish measuring 2-5 cm exhibit a distinctive black blotch at the base of their caudal fin, contrasting with their silvery body color. This attribute differentiates *N. hexagonolepis* from other genera like *Barilius*, *Danio*, *Garra*, and *Puntius*, which often coexist within the same habitat.

A mature chocolate mahseer boasts an elongated, slightly flattened body profile that is not excessively deep. The dorsal and ventral profiles demonstrate nearly symmetrical arches; the head is relatively compact and terminates in a blunt rounded shape. Rows of sturdy horny tubercles are evident on the snout's sides, positioned in front and beneath the eyes. The lips are robust and encircle the mouth's angle, although the labial groove is significantly interrupted in the center. The lower jaw is coated with a sharp horny covering. A pair of barbells exists on the maxillary, with the maxillary pair longer than the rostral pair. The fish exhibits captivating coloration,

Chocolate mahseer is the state fish of Meghalaya.

featuring olive-green hues on its upper regions and silvery-white tones below, with a prominent dark yellowish-green lateral band positioned above the lateral line. The sides of the head are adorned with golden accents. In larger individuals, the back, head, fin bases, and scales assume a blackish-grey tint, while opercular plates, fins, and scales are tipped with yellow.

Sexual dimorphism and growth pattern

Sexual dimorphism is evident in *N. hexagonolepis*, as males demonstrate a faster growth rate compared to females. Despite this, females ultimately attain a larger size than their male counterparts. Within the same age cohort, a noticeable disparity in size exists between females and males; the sex ratio stands at approximately 1 female to 1.26 males.

The ICAR Research Complex for NEH Region, Meghalaya conducted a comprehensive study on the length-weight relationship and condition factor of this fish species in the Umngot River, a vital breeding and spawning ground in Northeast India[2]. Over the course of January to December 2022, a collection of 467 specimens was obtained from various sections of the river, utilising gill nets and cast nets. These specimens exhibited a size range of 26 to 162 mm in length and a weight range of 0.23 to 155.0 g.

Examination of fish population parameters unveiled that the growth pattern of *N. hexagonolepis* within the river environment follows a trend that is either negative allometric or almost isometric. Specifically, the growth coefficient 'b' was calculated as 2.88 during the pre-monsoon season, 2.74 during the monsoon season, and 2.46 during the post-monsoon season. These calculations were accompanied by high regression coefficients (r^2), ranging between 0.95 and 0.98. Furthermore, the condition factor (Fulton's condition factor) exhibited variations, with a value of 0.95 (ranging from 0.49 to 1.8) during the pre-monsoon period, 0.87 (ranging from 0.5 to 1.3) during the monsoon period, and 0.80 (ranging from 0.24 to 1.25) during the post-monsoon period.

Captivating colouration in chocolate mahseer.

Breeding

In their natural habitat, *N. hexagonolepis* engages in spawning activities in distinct batches throughout specific time periods. These reproductive events typically occur during January-February, May-June, and July-September. Males generally achieve their first maturity milestone at around 9 months of age, while females reach this stage slightly later, at around 11 months of age. Female fish achieve their first maturity at a minimal length of 17.8 cm and a weight of 70 g. In comparison, males reach maturity at an average length of 17.3 cm and a weight of 50 g. In ripe females, the gonado-somatic index (GSI) measures 16.2, offering an indicator of the reproductive activity in relation to the overall body weight. Additionally, the average fecundity factor, which quantifies the number of eggs produced by a female, is reported to be 3,000 to 3,500 per kg body weight.

During the spawning season, distinguishing between mature male and female *N. hexagonolepis* is achievable through distinct visual cues. A mature male can be identified by the release of milt upon gentle pressure applied to the belly area. In contrast, mature females exhibit a fully distended and soft abdomen, along with a slightly swollen pinkish vent and anal fin.

For controlled breeding purposes, the broodstock of males (weighing 50-100 g) and females (weighing 200-300 g) can be cultivated in concrete tanks at a temperature range of 18-22°C. The recommended stocking density is 2-3 individuals per square meter, with meticulous attention to water quality ensured through effective filtration systems. Clear, pristine water conditions, coupled with a balanced diet containing 35-40% protein, contribute to optimal health. Regular health assessments are integral to this process. When well-managed conditions are maintained, the species can naturally undergo multiple spawning events in captivity without the need for synthetic hormones. In cases where natural spawning doesn't occur, a single dose of hormones such as Ovafish or Gonopro FH can be administered intramuscularly. The recommended dosages are 0.2-0.3 ml/kg body weight for males and 0.6 ml/kg body weight for females, while maintaining a ratio of 1 female to 2 males[5].

The timing of stripping operations is typically synchronised with the female's signs of ovulation, which typically manifest about 10-15 hours after hormone injection. During this process, the female's eggs are gently extracted through repeated stripping, often performed in a dry tray. This is followed by a similar procedure for males to obtain milt, which is subsequently mixed with the eggs using a feather for approximately 1-2 minutes. Gradual addition of water facilitates fertilisation, and the mixture is rinsed several times with clean water. The resultant fertilised eggs are demersal, displaying a white coloration and possessing a diameter of 2-2.5 mm. After fertilisation, the eggs of *N. hexagonolepis* can be successfully incubated in perforated hatching trays, which are placed in elongated tubs with a continuous flow of oxygen-rich water during the entire incubation phase. While multiple breeding cycles are achievable in captivity using the stripping technique, the most responsive period tends to be August. The species produces non-adhesive eggs, though they exhibit a mild stickiness prior to water hardening. Fertilisation rates generally range between 80 to 85%. The incubation duration varies, spanning from 80 to 95 hours, and hatching success rates range from 75 to 80%. Larvae complete yolk-sac absorption around 6-7 days after hatching, provided optimal water quality is maintained. To progress from spawning to rearing, newly hatched fry can be cultivated in glass aquaria, where they exhibit an average survival rate of 80-85% within 15-20 days of rearing. During this timeframe, the fry achieve an average length of 2 to 2.5 cm and a weight of 0.2 to 0.25 g. Subsequent to this phase, the fry can be transferred to cement cisterns at a density of 1 individual per litre. On average, a survival rate of 80-90% can be attained after 2-3 months of rearing. Throughout this period, the fish undergo a growth spurt, with an average length of 4-5 cm and a weight of 1-2 g being achieved.

Culture aspects

The growth performance of *N. hexagonolepis* was studied in earthen ponds and a polyhouse setting, each spanning 200m^2, at the fish farm complex of ICAR NEH in Meghalaya over a 6-month period. Fish seeds were collected from the Umngot River and stocked at a rate of one fish per litre in each setting. They were fed a locally prepared diet consisting

Umngot River, a potential breeding and spawning ground for chocolate mahseer.

of rice polish, dry fish, mustard oil cake, and a vitamin-mineral mixture, with a protein content of 36.2%. The feeding rate was 5% of their body weight during the initial two months, followed by 4% in the subsequent two months, and then 3% in the final two months.

The average water temperature was 18.8°C (ranging from 8.6 to 23.2°C) in pond conditions, while it was 21.2°C (ranging from 13.2 to 28.8°C) in polyhouse conditions. The average dissolved oxygen content was 7.85 ± 0.85 ppm in the pond and 7.75 ± 1.55 ppm in the polyhouse. The average pH was 7.8 ± 0.2 in the pond and 7.5 ± 0.3 in the polyhouse. The average total dissolved solids were 86 ± 22 ppm in the pond and 82.5 ± 29.5 ppm in the polyhouse. The average total alkalinity was 58.62 ± 4.8 ppm in the pond and 62.63 ± 5.6 ppm in the polyhouse. The average ammonia content was 0.65 ± 0.25 ppm in the pond and 0.50 ± 0.35 ppm in the polyhouse.

At the beginning, the fish had an average weight of 2.03 ± 0.32 g. By the end of the culture period, in pond conditions, the fish reached an average weight of 44.1 ± 5.7 g, while in the polyhouse conditions, they achieved an average weight of 42.1 ± 4.5 g. The specific growth rates were 1.71 ± 0.15% per day in the pond and 1.69 ± 0.13% per day in the polyhouse. Survival rates were 91% in the pond and slightly higher at 92.5% in the polyhouse. The total fish biomass yield was 8,015.3 g in the pond and 7,790.4 g in the polyhouse.

Based on these findings, it can be concluded that pond environments typically provide a wider range of natural food sources, including microorganisms, zooplankton, and phytoplankton, contributing to enhanced growth rates. On the other hand, the slightly lower growth rate in the polyhouse condition can be attributed to the more controlled and potentially restricted environment. While polyhouses offer advantages such as protection from external predators and weather fluctuations, they may provide a more limited range of food sources, which could potentially affect growth rates. The higher survival rates in the polyhouse can be attributed to the controlled environment that minimises external stressors and predation risks, ensuring a stable habitat for the fish throughout the culture period.

The proximate composition of fish under two rearing settings was also studied. The moisture content averaged 75.66% in the pond and slightly higher at 76.08% in the polyhouse, reflecting potential water-related environmental differences. Crude protein content stood at 16.25% in the pond and slightly elevated at 16.52% in the polyhouse, indicative of varied dietary sources. Crude lipid content was 5.83% in the pond and marginally lower at 5.62% in the polyhouse, suggesting possible diet-driven distinctions. Ash content averaged 1.12% in the pond and slightly higher at 1.15% in the polyhouse, potentially related to environmental mineral availability. These variations underscore the impact of rearing

Fish samples collected from the Umngot river for population parameter studies.

environment and diet on chocolate mahseer's nutritional composition, crucial for effective aquaculture management tailored to specific conditions and growth objectives.

Food and feeding habits

The fish is a column-to-bottom dweller and occasionally rises near the water's surface to nibble on flowing food items. Zooplankton is the dominant food in their fry stage, while phytoplankton is most dominant in their fingerling/juvenile phase. A study in Meghalaya indicated that, in nature, vegetable matter, algae, and insects form the basic food items of the fish, and there is a rise and fall in the feeding intensity of the fish during the breeding season, with the feeding intensity increasing after the spawning season[3].

The fish is found to be a voracious feeder not only in riverine water but also in pond conditions[4]. In captivity, the larvae can be maintained on a planktonic diet for a period of one month. Subsequently, they can be raised with a mixture of rice bran and mustard oil cake (1:1) with daily feeding at 10% of the total fish biomass (the amount may be split into 2-3 times). The protein requirement for the fish varies from 35 to 40%. Fish meal can be incorporated into their diets at a 15% level. While slaughterhouse wastes and soybean meal can be supplemented in their diets, research evidence is required on this aspect.

For pond culture, the fish can be offered a diet containing mustard oilcake, wheat bran, wheat middlings, rice polish, and rice bran. For seed raising, diets can be formulated using soybean meal, silkworm pupae, rice/wheat starch, casein, gelatin, and cod liver oil fortified with vitamins and minerals at appropriate levels, containing about 40 to 45% crude protein. A study conducted at the fish farm complex of ICAR Research Complex for NEH Region in Meghalaya indicated that a feed containing rice bran, mustard oilcake, silkworm pupae, fish meal, and a local herb (*Gynura crepidoides*) delivers satisfactory growth in chocolate mahseer.

A diet prepared using deoiled silkworm pupae (50%), rice bran (22%), mustard oilcake (10%), tapioca flour (17%), and a vitamin-mineral mixture (1%) and has a proximate composition of moisture: 6.14%, crude protein: 40.04%, crude lipid: 5.69%, NFE (nitrogen-free extract): 14.6%, crude fibre: 10.43%, and ash: 14.60% is also effective for rearing chocolate mahseer. The incorporation of 17-alpha methyl-testosterone at 7.5 mg/kg in a formulated diet has a positive effect on the growth and survival of mahseer.

Water quality requirement

Effective chocolate mahseer farming necessitates precise control of water quality parameters due to their significant impact on fish health, growth, and overall aquaculture

Tail rot.

Red spots.

success. The optimal temperature range of 18-22°C is essential as it influences metabolic rates and enzymatic activity, affecting growth and physiological functions. Additionally, a study in Meghalaya on temperature quotient (Q_{10}) and standard metabolic rate has revealed a preferred range of 23-27°C, with the growth optimum at 25°C[6]. This preference is tied to the fish's thermal tolerance zone (41.68°C[2]) and high active respiration rates (0.5-0.54), underlining the importance of maintaining appropriate thermal conditions.

Maintaining a pH range of 7.5-8.5 is crucial as it impacts nutrient availability, metabolic processes, and fish behavior. Dissolved oxygen levels exceeding 5 mg/L are essential to prevent hypoxia and maintain optimal respiration rates. Controlling ammonia and nitrite concentrations is vital due to their potential toxicity, particularly on fish gills and overall health. Adequate water hardness of 50-150 mg/L ensures proper osmoregulation and overall well-being.

Clear water with minimal turbidity supports feeding and breeding behaviors by enhancing visual cues and light penetration. Adequate water flow prevents stagnant conditions, aiding nutrient dispersion and waste removal. The incorporation of natural habitat elements, such as substrate and vegetation, simulates the fish's ecological preferences, promoting natural behaviors and well-being.

Disease

The information pertaining to disease and health management of *N. hexagonolepis* is limited and fragmented. There is a dearth of data on microbial and nutritional diseases affecting the fish. Occasionally, instances of fungal infections, predominantly *Saprolegnia* infections, have been observed in mahseer housed in confinements like ponds or tanks with restricted water exchange. Another occasional observation includes body darkening and skeletal deformities due to

Fish with no scales remaining on the body.

nutritional deficiencies. Notably, reports exist of corneal hypertrophy, a condition marked by corneal damage and shrunken eyes during advanced infection stages.

In our recent study in Meghalaya, a number of illnesses were recorded in fish, including tail rot, red spot, cotton wool disease, and a condition where fish with no scales remained on the body were seen. The understanding of stress and its management in chocolate mahseer is still lacking. Stress, whether triggered by environmental or physical factors, significantly impairs breeding performance. Stress in brood fish can lead to non-spawning or partial spawning in hatcheries, impacting overall reproductive competence.

Given the limited insights into disease, health, and stress management in the fish, dedicated research is essential to formulate effective strategies for maintaining their well-being and optimising aquaculture practices.

Socio-economic and technical aspects

Chocolate mahseer fisheries hold a significant yet seasonal livelihood opportunity for numerous fisher communities in NE India, particularly in the hills. This species is harvested using simple methods such as long lines, pole and line fishing in natural water bodies, and drag nets in ponds. The accessibility of this fishery, requiring minimal capital, equipment, and skill, makes it accessible to individuals of various backgrounds. The fish commands a premium price, approximately Rs 400-500/kg in Meghalaya, indicating its economic viability. Its delectable taste and consumer preference contribute to its demand.

Processing is usually minimal, as the fish is favored fresh. In Meghalaya and other hill states, fishermen obtain licenses for seasonal mahseer capture. However, due to its endangered status, efforts are shifting towards stock enhancement and aquaculture to counter declining catches. Although chocolate mahseer aquaculture is sporadic, there's potential for co-culture with Indian major carps. Several state governments have established farms and hatcheries, recognising the species' economic potential.

As focus intensifies on conservation and sustainable practices, chocolate mahseer farming offers income generation and employment prospects for local hill communities. It represents a balance between species recovery, economic benefits, and community livelihoods.

Points to ponder and way forward

Several factors can drive the growth of chocolate mahseer farming in hilly regions. One key aspect is ensuring a consistent supply of high-quality seeds to farmers, which can be achieved through well-functioning hatcheries and seed production facilities. Developing cost-effective feeds with optimal Feed Conversion Ratios (FCR) is crucial, as it directly impacts production costs and profitability. Efficient marketing channels and accessible transportation facilities are essential to connect farmers with markets effectively. Guaranteeing the availability of clean and suitable water sources is vital for maintaining the health and growth of the fish. Implementing disease management strategies and addressing predation risks contribute to sustained farming success.

Given the species' slow growth rate, genetic improvement through selective breeding or biotechnological tools holds promise for enhancing growth potential. Environmental manipulation and the formulation of appropriate supplementary feeds offer avenues for growth enhancement. These measures can collectively create opportunities for increased fish production and income for hillside farmers. By addressing these multifaceted aspects, chocolate mahseer farming can become a sustainable and profitable venture, benefiting both the farmers and the conservation of this endangered species.

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