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Spawning substrate and water velocity preference of Lepidocephalichthys *thermalis* for growth and gonadal maturity in captivity

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Introduction

The Indian Spiny Loach, *L. thermalis,* is a hill stream freshwater fish inhabiting a wide variety of habitats such as annual and perennial ponds, shallow channels and conduits, paddy fields which are subject to inundation during monsoon, shallow edges of streams, and rivers where the current are not strong (Kumari and Nair, 1979). It is indigenous to India and Sri Lanka and is known as the common spiny loach, spotted loach, or Indian spiny loach (Shaji, 2000). Similar species have been reported from India, Indo-China, Myanmar, Nepal, Pakistan, Sri Lanka, Bangladesh, Malay Archipelago, Thailand and Vietnam. It is omnivorous and prefers insect larvae, hence known as a micro-predator (Keskar et al., 2014). It contains high-quality protein and vitamins and is known for its medicinal value (Gargotra et al., 2022). It has an excellent market value in many parts of India and is considered a delicacy.

To diversify a species for culture, the basic understanding of the species biology, its maturation and breeding behaviour, its preferences towards captive rearing conditions and its nutritional requirements are essential. Only limited studies are available on the Indian Spiny Loach. Few works have reported the habitats and distribution of these fishes and explained the maturity stages of female brooders (Kumari and Nair, 1978a, b, 1979). Recently, Gargotra (2023) has documented the effect of dietary vitamins on the captive maturation and spawning of *L. thermalis.* However, the other breeding requirements of *L. thermalis* for successful maturation and spawning are yet to be charted out for standardizing the breeding technology.

In many fish species, spawning success includes behavioural elements that ensure suitable conditions for eggs and young ones after hatching. Selection of an appropriate location through preference for specific spawning structures appears to be a crucial behavioural element (Balon, 1975). The suitability of the selected location for egg and larval development influences the mortality of the offspring, especially in species without parental care (Bohlen, 2003).

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Fish occurrence and distribution in running waters correlate with physical variables, including depth, water velocity, and substrate structure (Senay et al., 2015). Stream hydraulic characteristics, such as channel width, depth, velocity, discharge, and substrate composition, are related to and responsible for hydraulic geometry (Allan and Castillo, 2007). In running waters, water velocity is an important factor shaping fish distribution since, at high velocities, the metabolic cost of a fish holding its position and swimming increases while foraging success decreases (Hill and Grossman, 1993). It was also reported that in running waters, fish occupy and compete for hiding places with low velocity where energy expenditure is reduced (Grabowska et al., 2016; Kukuła et al., 2019). Benthic fish, in particular those with low swimming capacity, occupy shelters like boulders, stones, and vegetation (Prenda et al., 1997) or burrow into soft substrates (Slavik et al., 2000; Jażdżewski et al., 2016). In streams with vertical profiles of water flow, velocity decreases as a logarithmic function of depth, approaching zero at the substrate surface, which enables accelerated sedimentation (Carling, 1996). In this way, velocity plays a significant role in shaping the distribution of fish burrowing into soft bottom substrates (Przybylski et al., 2021).

The spined loach, *Cobitis taenia,* is highly specialized for life in sandy bottoms. During the daytime, the fish burrows in the sand, and at night, it feeds on the sand by a specialized filter-feeding mechanism (Robotham, 1982). Spiny loach remains buried in sand, mud or dense weed growths during the day and is active at night and is solitary mainly (Coad, 2012). Loaches primarily settle in shallow areas of standing or slow-flowing waters where the substrate is fine sediment. However, in the early life stages, the young fish prefer a different microhabitat, as the survival of eggs and young loaches depends on the presence of dense vegetation. It is believed that the selection of loach habitat by loach and their dependence on silty and sandy sediments and sediments rich in organic particles has resulted in their specialized feeding method by shifting the substrate. They feed using a filtration apparatus with sticky zones in the pharyngeal cavity (Caleta et al., 2012).

Bohlen (1990) reported that two Japanese species of Cobitis spawned in very shallow standing water. The spined loach showed a strong preference for dense vegetation as a spawning substrate, indicating this factor has great importance for its reproductive biology. Bohlen (2000) stated that vegetation provides shelter against egg predation and prevents the drifting of the eggs. Ifbsuitable spawning habitat is lacking, the eggs will be more exposed to predation or flushing, in general, to a higher mortality rate (Kamler, 1992).

The females of *Misgurnus anguillicaudatus* scatter their eggs over various substrates (Breder and Rosen, 1966). *Pangio* and *L. thermalis* species spawn in the open water near the surface (Ott, 1995). Kim and Park (1995) considered the different structures of the adhesive membrane of the

oocytes in nine species of Korean cobitid fish to be an adaptation to various spawning substrates. Vasilev et al. (1989) and Bohlen (1990) demonstrated the ecological impact of specialization on a particular spawning habitat.Generally, tolerance of water velocities tends to vary among species and individuals of different size groups, with larger individuals predicted to tolerate greater water velocities (Sagnes and Statzner, 2009). The spined loach distribution in the stream pointed to avoidance of deeper water with higher velocity (Przybylski et al. 2003; Pietraszewski 2015). In contrast, Slavik et al. (2000) did not find strong evidence for water current and depth being key factors shaping spined loach distribution, but it was suggested that other important microhabitat traits, such as a substrate type probably mask the effect of these hydraulic features.

Biology of Indian Spiny Loach

L. thermalis (Valenciennes,1846), commonly called the Indian spiny loach, is a species of the family Cobitidae with the Least Concern status of IUCN (Dahanukar et al., 2019). The species is locally referred to as Aiyrai/ Asaree/ Pallimeen in Tamil Nadu, Asira in Andhra Pradesh, Chikani/ Mura in Maharashtra, Jubbi Cowri in Odisha and Bulu in Bihar (Ranjit, 2002). The species is endemic to India and Sri Lanka (Talwar and Jhingran, 1991; Pethiyagoda, 1991; Ekaratne, 2000). It is prevalent primarily in the states of Maharashtra, Karnataka, Kerala, Telangana, and Tamil Nadu in peninsular India (Wagh et al., 2018; Prasad et al., 2020; Kumbar et al., 2021; Thampy et al., 2021; Eldho and Sajeevan, 2022; Nagabhushan, 2022).

Geographical distribution of loach

Loaches are hillstream freshwater fishes belonging to the order Cypriniformes, and they come under four families: Balitoridae, Botiidae, Cobitidae and Nemacheilidae. Several genera and species of loaches are distributed worldwide (Nelson et al., 2016). They are also found across Asian countries like Bangladesh (Rahman, 1989, 2005), India and Myanmar (Talwar and Jhingran, 1991), peninsular Thailand and the Mekong basins (Kottelat, 1992). In India, it has been reported throughout the Western Ghats ranging from Maharashtra to Kanyakumari (Dahanukar and Raghavan, 2013; Raghavan et al., 2013; Manickam et al., 2014; Keskar et al., 2014, 2015, 2017; Kumkar et al., 2017). The Western Ghats of India hosts about 43 species of loaches belonging to 12 genera, of which 39 species are endemic to this zoogeographical region (Dahanukar and Raghavan, 2013).

Habitat of loach

In general, inhabit annual and perennial ponds, shallow channels and conduits, paddy fields subject to inundation during monsoon, and shallow edges of streams and rivers where the currents

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are not strong (Kumari and Nair, 1978). The loaches prefer slow-moving water and avoid water with high velocity (30 cm/s) (Przybylski et al., 2003; Pietraszewski, 2015). These fishes are also found in substrates of rocks, pebbles, paddy fields and submerged tree roots, etc., although it's sometimes collected from muddy or sandy zones in areas where leaf litter accumulates (Rahman, 2005; Liu and Wang, 2017; Luo et al., 2021; Zang et al., 2022). Generally, loaches are facultative aerobic; hence, it can breathe atmospheric oxygen through a gut located in its hindquarters, which survives outside the water for a short time. It hides in the mud during drought until water is available (Kano et al., 2010). Most of them bury themselves in the sand or gravel of stream bottoms during the day and come out at night for feeding; hence, they are considered nocturnal (Robotham, 1982).

Food and Feeding Habitat

Caleta et al. (2015) explained the spiny loach's feeding habits by stating that the fish swims and feeds at night, whereas they spend most of their day digging into the sand bottom. Hence, they concluded that loaches were predominantly nocturnal animals. The spiny loach feeds mainly in the early morning (Renuhadevi et al., 2019) by exhibiting a preference for benthic invertebrates (Havird and Page, 2010).

Sexual dimorphism

The loaches are small and have elongated body forms with significant variations of colour patterns on the body sides (Vladykov, 1935; Ikeda, 1936). These species are tiny, although some grow to a standard length (SL) of 75 mm (Havird and Page, 2010). Spined loaches are sexually dimorphic fish with a bifid suborbital spine (Nalbant, 2002; Slechtova et al., 2008). As in most other cobitids, mature males in *Lepidocephalichthys* have modified pectoral rays. The innermost (seventh and eighth) pectoral rays fuse and harden to generate this alteration, known as the lamina circularis (Sundarabarathy et al., 2001).

Maturation and breeding of loach

A few investigations on the development of related loach species have also been done (Robotham, 1981; Bohlen, 2003; Kostrzewa et al., 2003; Dey and Barat, 2015). Male *Cobitis spp*. reached maturity at 56 mm total length (TL), whereas females took longer. The average absolute fecundity reported was 2180 eggs at 52 mm TL (Kostrzewa et al., 2003).

Robotham (1981) reported that male spiny loach *C. taenia* (L.) reached maturity at 1+ year when they reached 40 mm length, whilst females reached maturity a year later. In contradiction to this, Marconato and Rasotto (2009) stated that both males and females of *C. taenia* reached sexual maturity in the second spring following hatching, with an average male-to-female ratio of 1:1.34

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and females predominating the population. Similarly, Ekmekç and Erk'akan (2003) said that both males and females of *C. simplicispina* reached maturity at the age of 2 years.

Substrate preference of loach

Substrates may serve different functional purposes according to the fish species or even within the same species. Many African cichlids use substrate during the breeding season for nest building (Fryer and Iles, 1972). As some cichlid species have substrate-related feeding habits (Fryer and Iles, 1972), it is also possible that different substrate types impact foraging differently. Webster and Hart (2004) showed that three sticklebacks prefer complex over simple substrates for foraging purposes. Horstkotte and Plath (2008) related different diets to substrate preference in pupfish. Hoglund et al. (2005) demonstrated the role of substrate in crucian carp as a relevant anti-predator feature of the environment in their study of monoamines and avoidance behaviour.

Effect of substrate in reproductive biology

Several studies suggest that round gobies prefer structurally complex, hard, rocky habitats with ample shelter for breeding (Miller, 1986; Vanderploeg et al., 2002). The stone loach, *N. barbatulus* (L., 1758), the kissing loach, *Leptoboti curta* (Temminck and Schlegel, 1846), the small striated spined loach, *Cobitis* sp., and the middle striated spined loach, *Cobitis sp*., are known to use spaces among submerged plants for their spawning site (Aoyama and Doi, 2006). Similarly, another study on stone loach (*B. barbatula*), indicated a strong preference for shallow microhabitats with depositional substrate, sometimes combined with some cover (Prenda et al., 1997). In accordance, Welton et al. (1983) found that, in the Mill Stream (England), stone loach had a clear preference for macrophyte areas with a substratum of silt. These fishes also preferentially spawn over substrates with the highest velocity, indicating that the preferred habitat for spawning would probably be riffles or runs. These habitats would provide the eggs with well-oxygenated water and minimal fine sediments. Additionally, spawning in locations with minimal flow indicated that the appropriate substrate size is more critical than water velocity. Increased sedimentation delayed the onset of spawning and reduced the reproductive success of stream fishes, leading to a decline in fish abundance (Berkman and Rabeni, 1987; Burkhead and Jelks, 2001).

Effect of substrate on feeding, growth and survival

The type of substrate has a significant influence on the growth and survival of offspring (Keshavanath et al., 2001; Galhardo et al., 2009; Nakamura et al.,2009; Snickars et al., 2010; Chen et al., 2015; Wu et al., 2018). The growth and development of farmed fish are closely associated with specific habitat features such as substrate type and brightness (Garpe and Marcus, 2003). Keshavanath et al. (2001) found that Deccan mahseer (*Tor khudree*) growth was significantly

affected by substrate type, and individuals grew best in tanks with bamboo substrate. Similarly, goldfish (*Carassius auratus*) grew significantly better on mud sediment than on sand substrate (Su et al., 2011).

Water velocity preference of loach

Flow velocity describes the energy flux of water moving down the river channel, and flowing water plays a crucial role in transporting and exchanging nutrients and oxygen. Fish occurrence and distribution in running waters correlate with physical variables, including depth, water velocity, and substrate structure (Poff and Allan, 1995; Rosenfeld, 2003; Senay et al., 2015). In running waters, fishes occupy and compete for refuges, i.e. hiding places with low velocity where energy expenditure is reduced (Harding et al., 1998; Van Kessel et al., 2011; Kakareko et al., 2016; Grabowska et al., 2016; Kukuła et al., 2019). Benthic fish, in particular those with low swimming capacity, occupy shelters like boulders, stones, and vegetation (Prenda et al., 1997) or burrow into soft substrates (Slavik et al., 2000; Jażdżewski et al., 2016).

Conclusion

The overall maturation performance of fish reared with different substrates, it was found that the maximum GSI, absolute fecundity, relative fecundity and ova diameter were observed in the fishes reared in. The highest levels of cortisol and progesterone were recorded in fishes. The histological sections of *L. thermalis* exhibited oocytes at various developmental stages, indicating the asynchronous spawning behavior of fish and the presence of a relatively higher proportion of the secondary vitellogenin stages was observed in fishes. The periphyton growth was noticed only on the substrates. wooden substrate with a water velocity significantly increased the maturation and growth performance of *L. thermalis.*

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