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Breeding of Indian Spiny Loach, *Lepidocephalichthys thermalis* (Valenciennes, 1846) under Captive Conditions

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ABSTRACT

The breeding of Indian spiny loach, *Lepidocephalichthys thermalis* was standardized under laboratory conditions at Madhavarm campus, Tamil Nadu Dr Jayalalithaa Fisheries University, Chennai, Tamil Nadu, India during May–August, 2014. Three different conditions were maintained in the breeding tanks which included gravel bed at the bottom with floating aquatic plants, gravel bed at the bottom without plants and soft sand layer bed at the bottom without plants. Spawning took place in the tanks provided with gravel bed at the bottom with and without plants. The eggs were transparent, adhesive and found attached to the plants and gravels. The incubation period ranged from 20–30 h at a temperature of 29.5°C. The newly hatched larvae were transparent with an average body length of 2 mm when observed microscopically. On day 1, larvae were thread- like with two external gill filaments on either side of the head and a pair of pectoral fin and a rounded caudal fin. On day 5, larvae developed four external gill filaments on either side of the head with prominent eyes, dark pigmentation with an average total length of 3.5 mm. On day 10, larvae had well-developed dorsal fin and a pair of anal fin. On day 10, larvae had an average total length of 13 mm with two pairs of barbels. Four weeks old larvae resembled the adults with an average total length of 20 mm. It is concluded that Indian spiny loach could be successfully bred in captive conditions by providing specialized spawning environment.

KEYWORDS: Spiny loach, *Lepidocephalichthys thermalis*, breeding, spawning, captive condition

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

India is a precious bio-reserve, gifted with vast aquatic resources such as rivers, lakes, streams, beels and derelict water bodies which harbor diverse groups of fishes having immense commercial importance (Jayasankar, 2018; Prasad et al., 2020; Lakra and Gopalakrishnan, 2021). The Indian aquatic environment harbors about 2,358 species of fish with about 877, 113 and 1368 species of freshwater, brackish and marine fishes respectively of which at least 600 have the potential as ornamental fishes (Ayyappan et al., 2006; Tapkir et al., 2017). Among the fish fauna, loaches assume great importance both in ornamental sector due to their small size, bright bands, blotches, coloration, peaceful nature, hardiness, compatibility and which can be reared in aquarium throughout their life span (Mercy et al., 2007; Zaidi, et al., 2018; Goswami et al., 2022) and also food fish industry with demand in domestic as well as export markets. Loaches are the fishes usually lives in shallow freshwater wetland areas such as streams, swamps and paddy fields (Liu and Wang, 2017; Luo et al., 2021; Zang et al., 2022) and they are belonging to the family Balitoridae, Botiidae, Cobitidae, Gastromyzontidae, Nemacheilidae, Serpenticobitidae and Vaillantellidae (Pethiyagoda, 1991; Perdices et al., 2016; Keskar et a., 2018). There are several genera and species of loaches distributed worldwide (Nelson et al., 2016).

Lepidocephalichthys thermalis (Valenciennes 1846) commonly called the Indian spiny loach is an IUCN Least Concern status species of family Cobitidae (Dahanukar et al., 2019). The species is locally referred as *Aiyrai/ Asaree/Pallimeen* (Tamil Nadu), *Asira* (Andhra Pradesh), *Chikani*, *Mura* (Maharashtra); *Jubbi Cowri* (Odisha) and *Bulu* (Bihar) (Daniels Ranjit, 2002). The species is found distributed in India and Sri Lanka (Talwar and Jhingran, 1991; Pethiyagoda, 1991; Ekaratne, 2000). In peninsular India, it is widely found mainly in the states of Maharashtra, Karnataka, Kerala, Telangana and Tamil Nadu (Wagh et al., 2018; Prasad et al., 2020; Kumbar et al., 2021; Thampy et al., 2021; Eldho and Sajeevan, 2022; Nagabhushan, 2022). The demand for common spiny loach is mostly met from the natural collection (Keskar et al., 2017). It is an important species among loaches as they are used both as ornamental and food fish. Nutraceutically, they endow with as an important source of proteins and amino acids, fatty acids (saturated and unsaturated), minerals (calcium, potassium, phosphorus, sodium, magnesium, iron), vitamins A, D, E, K in their edible forms (Manoharan et al., 2019; Mohanty et al., 2019). Non- availability of the required size and quantity, presence of disease infection and parasites are some of the inherent problems in the natural collection of fishes (Evers et al., 2019; Verma et al., 2022; Trujillo-Gonzalez, 2022).

As there is no standard breeding methodology reported for this species which has a good market demand, this study was carried out with an objective to standardize the breeding methodology for *L. thermalis* in controlled lab conditions in aquaria which may which may contribute to provide some information with conservation of this natural resource and help the freshwater ornamental fish sector.

2. MATERIALS AND METHODS

A simple method for breeding of Indian spiny loach, *L. thermalis* was standardized under laboratory conditions at Madhavarm campus, Tamil Nadu Dr Jayalalithaa Fisheries University, Chennai, Tamilndu, India during May - August, 2014.

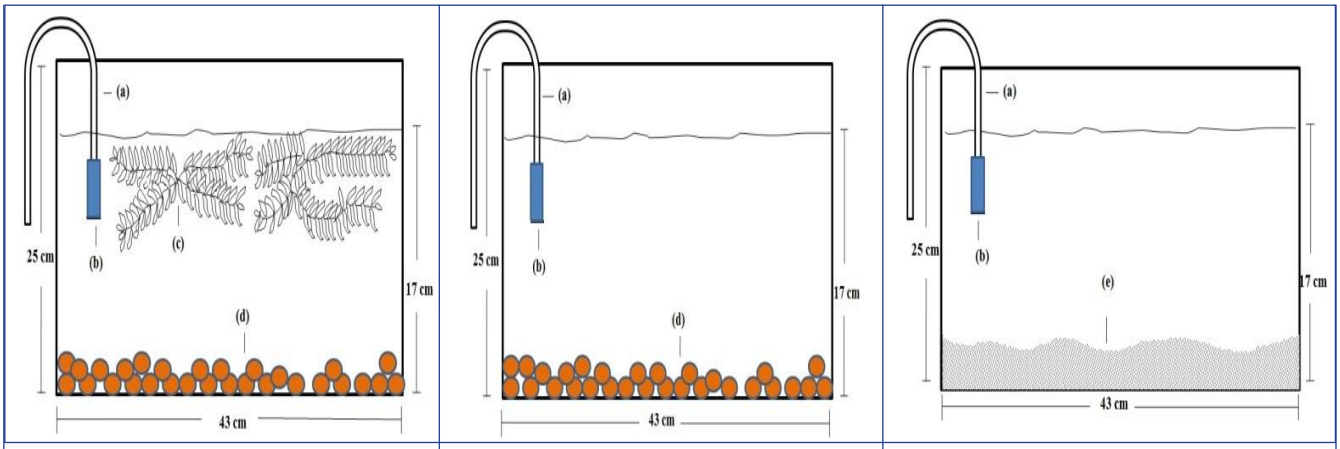
2.1. Experimental design

Brood fishes (above 50 mm in length) of *L. thermalis* were collected from lakes and small water bodies in and around Chennai, Tamil Nadu (Figure 1). The fishes were brought to the laboratory in live conditions and acclimated in aquarium tanks. The breeding experiment was carried out in triplicates in glass aquarium tanks measuring, 43×26×25 cm^3. Three different breeding conditions were provided in the breeding tanks which included a) gravel bed at the bottom and floating aquatic plant (*Ceratophyllum demersum*), b) gravel bed at the bottom without plants and c) only soft sand layer bed at the bottom (Figure 2).

Sex identification was carried out based on the presence of ossified spine on inner side of the pectoral fin in males (Sundarabarathy et al., 2001) and based on the genitals. Two pairs of matured adult male and female (1:1) spiny loaches were placed in each breeding tank. The physico-chemical parameters and the levels at which they were maintained during the experiment were, pH -8.0 to 8.2; water temperature -27 to 29 °C; total hardness-150 mg l^{-1}; Total alkalinity- 190 mg l^{-1} and ammonia-N below 0.1 mg l^{-1}. Water exchange was done at weekly intervals at 50% level. The brooders were fed with live food like tubifex worm, blood worm, Daphnia and commercial feed (30% crude protein).

When spawning occurred in the night, the eggs could be easily seen floating or attached to the floating aquatic plants and gravel bed. Parent fishes were removed from the

Figure 1: Brood fish of *L. thermalis*



Aquaria with Gravel bed at the bottom and floating aquatic plants	Gravel bed at the bottom without plants	Only soft sand layer bed at the bottom

Figure 2: Schematic drawing of the setup maintained in breeding aquaria for spiny loach. The aquaria were equipped with an aeration tube (a); air stone (b); floating aquatic plants (c); gravel bed (d) and soft sand bottom (e)

aquaria after spawning. Eggs were incubated at temperatures between 29 and 30 ˚C under weak indirect illumination. The developmental stages were observed periodically (1, 3, 5, 7, 10 and 17 days) under the microscope (4x and 10x) and documented. When all the larvae attained free swimming stage, they were transferred to aquaria (30 L capacity) with aeration and 100% water exchange was provided every 2 weeks. Exogenous food was given until the larvae reached 5– 10 mm size and when the larvae reached 15-20 mm size, they were transferred to larger aquaria.

3. RESULTS AND DISCUSSION

3.1. Breeding conditions

The present study, Indian spiny loach showed preference to specific substrate for breeding, i.e. gravel bed at the bottom with and without plants. No spawning response was observed in the breeding aquaria provided with sand layer bed at the bottom. This indicates a preference for vegetation and gravel bed in water medium by the spawning fishes, as such substrate provide shelter and prevents egg predation and drifting of eggs due to water movement and associated high mortality. Since the mortality rate during very early development is understood as critical for recruitment (Kamler, 1992), absence of suitable spawning environment may seriously affect population growth in fishes.

Observations on the spawning substrate preference by various species of loaches have been documented by researchers. The females of *Misgurnusanguilli caudatus* scatter the eggs over various substrates (Breder and Rosen, 1996). The spined loach, *Cobitis taenia* prefers dense vegetation as a spawning substrate (Bohlen, 2003). Almora loach, *Botia almorhae* prefers to spawn in clean and transparent water with sand pebbles at the bottom and few aquatic weeds (*Potamogeton* sp., *Myriophylum* sp., *Hydrilla* sp., *Azolla* sp., etc. (Joshi and Pathani, 2009). Eggs of *Sabanejewia sp.* are small, sticky and scatter across all kinds of substrate, and postulated that the spawning strategy of *Sabanejewia sp.* is different from that of *Cobitis* sp (Bohlen, 2004).

3.2. Developmental stages

The fertilized eggs were transparent, adhesive and found attached to the floating plants and gravels. At a temperature range of 29.5- 30˚C the embryos hatched after 20-30 h. Observation of eggs, embryo and developmental stages of larvae are presented in Table 1. The various developmental stages of *L. thermaphilis* is shown in Figure 3. Present study, *L. thermalis* eggs were observed to be adhesive and transparent similar to the eggs of *Lefua echigonia* (Aoyama and Doi, 2011), *C. taenia* (Bohlen, 2003), *Tiaroga cobitis* (Stephen and Minckley, 1990) and *C. takatsuensis* (Takaaki et al., 1998). The study revealed that the incubation period of *L. thermalis* was 20-30 h at 29.5^{0}C. It is reported that *C. taenia* embryo hatch out after 50-60 h at 21 ^{0}C (Bohlen, 2003). In this study, newly hatched larvae were 2 mm in average total length, with two external gill filaments on either side, a pair of pectoral fin and a rounded caudal fin similar to the larvae of *C. takatsuensis* (Takaaki et al., 1998) and *L. echigonia* (Aoyama and Doi, 2011).

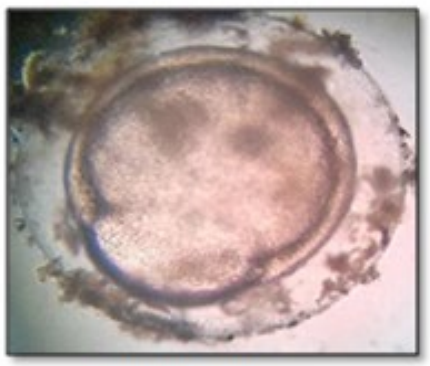
It was observed that newly hatched larvae had two external gill filament on either side similar to observations recorded (Bohlen, 2000) in *C. takatsuensis* (Bhimalchar and David, 1945; Aoyama and Doi, 2011); *M. caudatus* (Lei et al., 2014). Similar structure have been found to be present in other fishes like little skates, *Raja erinacea* 25-30 days after spawning (Plester and Bemis, 1992) and Atlantic sharpnose shark, *Rhizoprionodon terraenovae* (Hamlett et al., 1985).

On the day five, four such gill filament were noticed on each

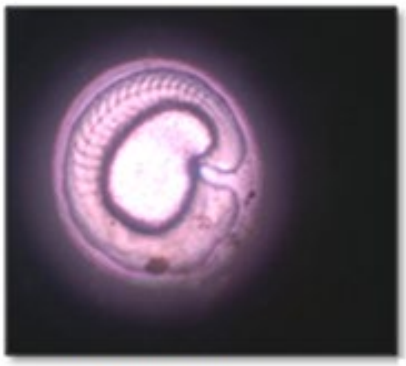
Table 1: Observations on the egg, embryo and larvae of *L. thermalis*

Stage	Duration after hatching	Observations
Egg	0 h	Transparent and adhesive
Embryo	18 h	18 somites stage, sticky, embryo attached to leaf of plants and gravels
Newly hatched larvae	Day 1	ATL*- 2 mm; dorso-ventrally flattened; External gill filaments (2 nos.) on either side of the head; a pair of pectoral fin, rounded caudal fin and ben-thic nature
Larvae	Day 3	ATL*- 2.7mm; Two nos. of well-developed gill filaments on either side of the head; well-developed eyes; a pair of well-developed pectoral fin
Larvae	Day 5	ATL*- 3.5mm ; 4 nos. of external gill filaments on either side of the head; discontinuous pigmentation on body
Free swimming larvae	Day 7	ATL*- 5 mm; last pair of gill filaments is branched
Larvae	Day 10	ATL*-8 mm; well-developed dorsal fin and a pair of anal fin
Larvae	Day 13	ATL*-10 mm; well-developed dorsal fin, anal fin; barbels start to develop
Larvae	Day 17	ATL*-13 mm with 2 pairs of well-developed barbels.
Larvae	Day 30	ATL*-20 mm; 3 pairs of barbels; resembled the adults

ATL*: Average total length



Fertilized egg



Embryo (18h)

Newly hatched larva (Day 1)

Larval stage (Day 3)

Larval stage (Day 5)

Larval stage (Day 7)

Figure 3: Continue...

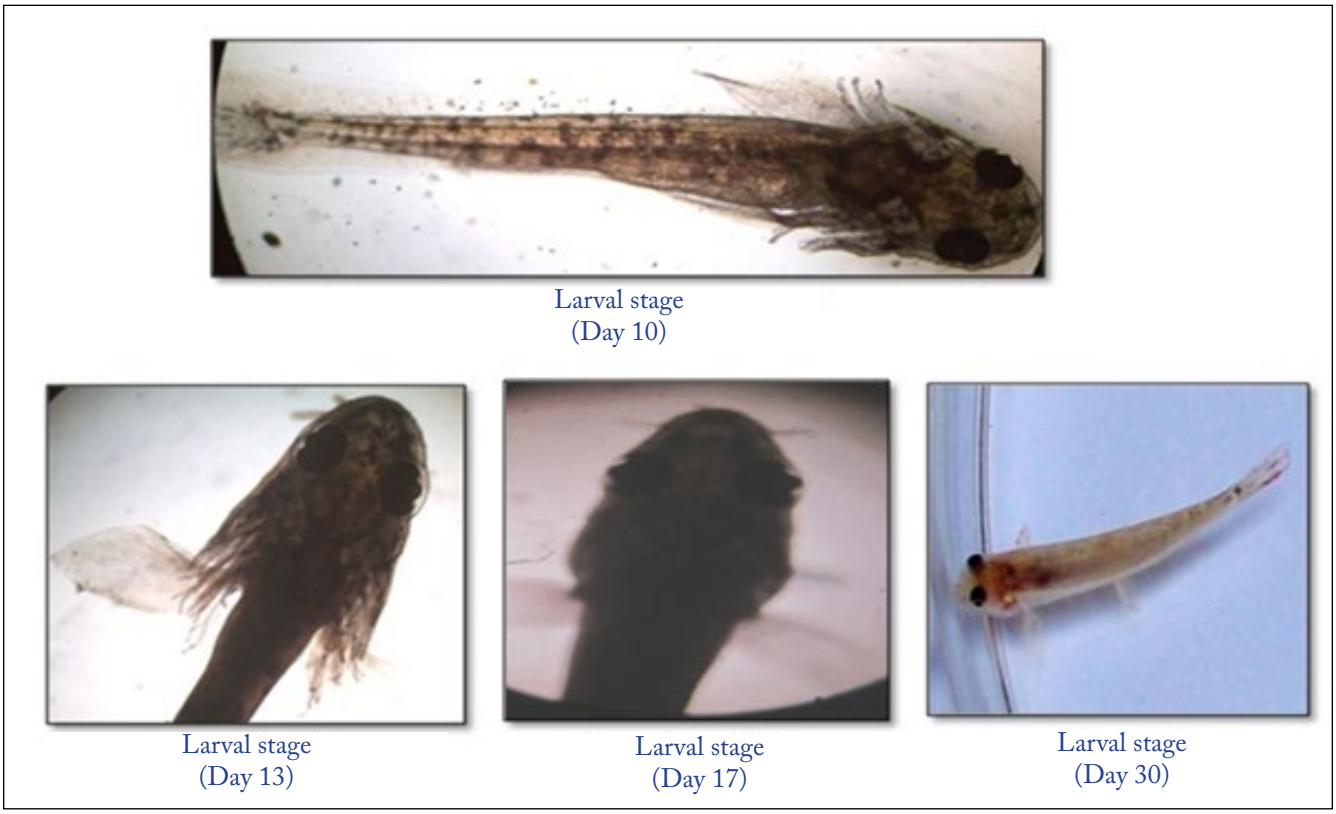


Figure 3: Developmental stages of *L. thermalis*

side and on day seven the last gill filament was branched. They show constant movement under the opercular flaps. Later, these gill filaments were gradually absorbed giving place to normal internal gills. The development of external gills is probably intended to provide a great respiratory surface in adverse conditions.

Just hatched larvae *of L. thermalis* were benthic in nature. The larvae and juveniles of *L. thermalis* were observed to swim with their abdomen facing towards substrate and ventral glass wall of rearing tank. The breeding season of *L. thermalis* has been reported to be mainly from April to October (Rita Kumari and Balkrishnan Nair, 1978; Kumari and Balkrishnan Nair, 1979) which is before and during the rainy season. The breeding trials were carried out in this study during the months of May-June.

3.3. Feeding

In the present study, the larvae accepted exogenous feed 4 to 5 days after hatching. Larvae of *Misgurnus anguillicaudatus* were began to feed exogenously after day 3 of hatching and the larvae fed with live daphnia *Moina micrura* (Wang et al., 2008). Microalgae and micro-particulated powder (56% crude protein) diet were accepted by the larvae upto 15 days. The weaning of *M. anguillicaudatus* with enriched cladocerans supplemented with formulated feed; after 30 days, gradually eliminate the live food over 7-10 days; then switch completely to formulated feed improves the growth and the survival of larvae (Wang et al., 2009). There after the larvae accepted the floating feed along with the micro-particulate feed. The optimum protein requirement for the better growth of Indian spiny loach (*L. thermalis*) was 35% (Renuhadevi et al., 2019).

4. CONCLUSION

Indian spiny loach required aquatic vegetation and gravels rather than the sand substrate for breeding. Providing such suitable spawning conditions would help to improve the seed production and conservation of the Indian spiny loaches which is a commercially important ornamental and a food fish.

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