

Seed Production and farming techniques of Indian Spiny Loach (*L. thermalis*)

Preface

India is rich in flora and fauna and is one of the most biodiverse countries in the world. The country is also blessed with a freshwater fishery with 877 species and 450 small indigenous fish species (SIFS). India is among the most dynamic countries in aquaculture with special emphasis on carp fish farming making India the second largest aquaculture producer. However, despite the abundance of resources mentioned above, Indian fish production is mainly focused on farming a few species, mainly catla, rohu, mrigal, exotic carp, murrel, catfish and a few others. This is due to the problems associated with lack of seed collection techniques and successful breeding methods. The northeastern part of the country has the highest concentration of small indigenous fish species, followed by parts of the central Western Ghats. SIFS play an important role for food and livelihood as well as ornamental fish in aquaculture. Of these, *L. thermalis*, the common Indian spiny loach, has received increasing demand in the domestic market with prices of Rs. 1500-2000/- reported around Tamil Nadu region. This species also has considerable potential on the international market for ornamental fish. The Indian loach is commonly found in clear, slow-flowing streams and adjacent stagnant waters, including rice paddies. The supply of wild-caught specimens of this species primarily meets local demand. However, increasing demand in local markets has led to uncontrolled fishing, resulting in heavy exploitation of small indigenous fishery resources in several regions. Unfortunately, little detailed data is available on the populations of loach species, including *L. thermalis*. The International Union for Conservation of Nature (IUCN) has classified the Indian spiny loach (Ayirai meen) as Least Concern meaning that it is not currently at significant risk of extinction. Nevertheless, the need for sustainable management practices is crucial to preserve these vital resources for future generations. Understanding the ecological and economic roles of species like the Indian spiny loach is essential for promoting biodiversity conservation and responsible aquaculture in India.

The constantly growing world population is contributing to land and water scarcity in many regions of the world. Primarily, the majority of freshwater resources are used for agriculture and industry. However, it is important to recognize that the aquaculture sector is entirely dependent on these water resources, even though it is an emerging industry. Innovative alternatives need to be explored to ensure sustainable production and growth in all sectors. The expansion of the aquaculture sector has led to practices such as intensification of farming, diversification of species and exploitation of water resources.

Commented [BJ1]: •Consider including specific data or comparisons to other biodiverse countries to strengthen this claim and provide a global perspective.
•a few species" but list several. Clarify whether this refers to a limited number of species relative to India's biodiversity or aquaculture's potential.
•Expand on what specific challenges exist in seed collection and breeding. Are these technical, financial, or knowledge-based issues?

By applying advanced breeding and seed production techniques, we can increase the population of different species in their natural ecosystems. In addition, the development of seed production and cultivation techniques can encourage the breeding of these species, ultimately lowering market prices for consumers and allowing farmers to increase their yields. In this context, we have embarked on research focusing on the Indian spiny loach, scientifically known as *L. thermalis*. Our eight years of dedicated efforts have culminated in successful breeding and farming techniques for this species. Drawing on our extensive field experience and knowledge, we developed book chapters that provide comprehensive information on the distribution, collection methods, farming practices, seed production, nutritional quality, and economic aspects of *L. thermalis*.

UNDER PEER REVIEW

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1. Introduction

Lepidocephalus thermalis is widespread in peninsular India and Sri Lanka, and reported that the species population differs in the Indian region. In this species in India, several taxonomic synonymies were used widely as *Cobitiscarnaticus* Jerdon, 1849, *C. mysorensis* Jerdon, 1849, *C. rubripinnis* Jerdon, 1849, it required scrutiny further. *Lepidocephalus thermalis* species are conspecific to the *Cobitis* spp. (from India). The genus *Lepidocephalichthys* is distributed in Asian countries, extending from India to China and Vietnam. Laos, Cambodia and south to Borneo and Java, Bangladesh, Burma, Pakistan, and Thailand (Arunkumar, 2000; Havird & Page, 2010). A *Lepidocephalus thermalis* is a *Cobitidae* family. As the name *Cobitis* is delivered from their suborder of *Cobitoidei* given by Linnaeus, *cobitis* means

“gudgeon” (Acharjee & Barat, 2014; Renuhadevi et al., 2019). The first time they described *Cobitis thermalis* was by Valenciennes in 1846 from the tropical waters of Cania, Sri Lanka. Havird and Page (2010) reported that in the genus *Lepidocephalichthys*, pectoral ray seventh and eighth are modified in the mature male and observed different pectoral fin modifications in Other Cobitids (second ray in Cobitis). He also compared (17) and redescribed (15) valid fish species of *Lepidocephalichthys* as taxonomical identification.

Generally, India is blessed and endowed with rich biodiversity resources compared to other nations. It comprises their genetic resource of around 9456 species. Freshwater fishery resources represent 950 (Paul and Chanda, 2017.), but NBFGR's fish database represents only 877 (Sarkar et al., 2012). Tapkir et al. (2017) reported that more than 2000 freshwater species are known to occur in our country, and many of the new species are recorded only in the Western Ghats. (Paul and Chanda, 2017) mentioned in their study a total of 422 fish species from northeast India and 667 species from the southern region of India. In the Western Ghats, 44 known loach fish species were recorded. Similarly, according to (Acharjee & Barat, 2014), a total of 67 loach species were recorded in North-East India, including the Himalayas and Indo-Burma region and 1043 valid species in 111 valid genera were reported from the suborder of Cobitoidei without mentioning place either globally or nationally by the author. Boron and Juchno (2019) stated that 262 species from 28 genera were recorded from the Cobitidae family, and the genus *Cobitis* comprises 95 species; out of these, 31 species were reckoned to be distributed in European water.

Like some loach, Southeast Asian fish species are primarily used for ornamental trade and aquarium purposes. Because they have good ornamental value based on their small size, bright bands, dots or blotches, colouration, hardiness, peaceful nature and other used for food purposes, and they are sold seasonally in the local market (Acharjee & Barat, 2014; Havird & Page, 2010; Sundarabharathy et al., 2001). (Manoharan et al., 2017) reported that *Lepidocephalus thermalis* is a good source of nutritional components such as protein, lipids, calcium, vitamin A and iron, etc., and some of the local people consider that it known for medicinal value; in this aspect only limited studies are available, and further study is yet to come up. This is a species of subsistence food for many tribal people. It consists of nutraceutical potential with good taste and flavour and is rich in calcium, equal to milk's calcium level. A small indigenous species is an essential ingredient in the diet of rural people living adjacent to the inland waters' resources (Paul & Chanda, 2017).

A small, least conspicuous freshwater fish species provides an immense benefit to both household livelihood and the country's economy, and this is recognized as an essential social benefit as

Commented [BJ2]: •Elaborate on how the population differs—does it pertain to size, distribution, habitat preferences, or other factors? Providing examples would make this statement more informative.
•Clarify the meaning of “conspicuous” here and provide a brief explanation of why this classification is significant.

Commented [BJ3]: The passage provides an extensive overview of India's rich freshwater fish biodiversity but would benefit from greater clarity and consistency. While it highlights different reports on species diversity, discrepancies in data (e.g., 950 vs. 877 species in freshwater resources) need reconciliation or explanation to avoid confusion. Additionally, the mention of specific regions, such as the Western Ghats and Northeast India, adds depth but could be strengthened with examples of key species unique to these areas. The information on Cobitoidei and Cobitidae families is detailed but lacks geographic specificity, which would improve context for readers. Streamlining these details and aligning the figures and sources would enhance the coherence and impact of the content

providing employment and ensuring food security for many rural people (Arthur et al., 2015; Keskar et al., 2017). However, it is challenging to understand inland fisheries resources because of the enormous range of aquatic organisms inhabiting inland water resources. In inland fisheries, there are two types: commercial and subsistence small-scale fisheries; among these two, it is difficult to determine their significant contribution to income or inland production (Welcomme et al., 2010). In many parts of the world, including Asia and Africa, the fisher folk who live near the inland water bodies are mainly involved in harvesting indigenous species through small subsistence fisheries. Similarly, in the northern Western Ghats of India, an indigenous tribal community known as *katkari* is harvesting several native loaches and selling them to the market, and that is subsistence fishery for them as a profitable livelihood option. Due to local market demand and trade, unmanaged fishery results in extreme exploitation of small indigenous fishery resources in several regions. However, there needs to be more knowledge of their population dynamics; in India, population detail is rare for loach fish species. As population exploitation or dynamics are the only details, it is essential to know about the ecological characteristics of particular species. Although, it has helped to execute the conservation strategies of fish species (Prasad et al., 2012). A small Indigenous fish species like *Lepidocephalus thermalis* gradually decreased in population and it went beyond their exploitation rate where the stock is reduced to half its virgin biomass (more than E50) at the western Ghats of the Maharashtra region (Keskar et al., 2017; Kharat & Dahanukar, 2013; Paul & Chanda, 2017).

The environmental parameter influences the growth and health of fish. Temperature and oxygen parameters are considered the most important for every stage of loach fish species, and many fish are sensitive to environmental parameters (Bohlen, 2003). Other essential environmental parameters are highly influenced by anthropogenic activity, such as the expansion of civilization, industrialization, etc. The river is the primary water source for most of the inland ecosystem. Water pollution is caused by the discharge of industrial waste, pesticides, and sewage into the respective aquatic ecosystems. Various types of aquatic pollution, dam construction across the river, and deforestation are significantly intensifying habitat alteration in the freshwater ecosystem, and it has become more vulnerable. Sand mining is causing a severe threat to some freshwater species that naturally inhabit sand, gravel, pebbles, and vegetative areas. In our country, sand mining is an emerging threat to native species (Cooke et al., 2021). A common spiny loach is an inhabitant sand area that involves actively digging the sand bottom to hide and beat high temperatures.

The species *Lepidocephalus thermalis* commonly found in clear, slow streams and their adjacent standing water body, paddy fields, etc.; therefore, this species has more exposure to influence on various

aquatic pollution by anthropogenic activity (S.d & N.b, 1978). Tang et al., 2013 Stated that heavy metals such as copper and zinc are both significantly bioaccumulation in the ova and ovaries of loach fishes. Copper accumulation is more significant than zinc accumulation, but this accumulation depends on exposure concentration and time. (S.d and N.b, 1978) reported that spiny loach is less resistant to the effect of DDT insecticides. This tolerance and resistance may be attributed to age, origin and other factors. In heavy metals, copper usage is higher and occupies the top level (Gosavi et al., 2020). All these metals accumulate in the kidneys, liver, tissue, and gonad, and they are amplified via bioaccumulation and get into the food chain. However, metal accumulation in the gonad causes ultimate failure in the breeding performance of certain aquatic organisms and a chance for biodiversity loss (Tang et al., 2013).

Recently, species diversification has played an essential role in inland fish culture in India. Since farming, carp species alone contribute more fish production to the country, even though, after reporting, much Indigenous food is available nationwide. When farming certain species, that species population can be increased, which helps to conserve them from vulnerability. Ubiquitously reporting that India has a rich biodiversity, our nation is still after China in overall inland fish production. In addition, our nation is mainly dependent on only a few species. Therefore, new species' cultural practices or farming methods can emerge in species diversification and provide varied fish protein for consumer preference. The increasing species spectrum in inland aquaculture has been stressed in recent years, which has realized high yield and farm income (Das et al., 2020). Usually, in maintaining the ecological niche in the pond ecosystem, composite fish farming techniques are followed, and later, several modifications have been tackled several modifications based on demand and input optimization of biomass yield (Das et al., 2019).

In this continuation, *Lepidocephalus thermalis* can be introduced into freshwater aquaculture for farming. Several studies have proven that they have a rich nutritional resource. Mainly, it is rich in calcium compared to other native inland small fishes. As per the IUCN categories, this species is listed under the most minor category due to being recognized as having no evident threat for this species. In many places, this species is used and considered as ornamental value. However, in Tamil Nadu, it is considered a better-tasting inland fish, so it has to fetch a reasonable price all around in the local market. The availability of species loach is very rare in this region due to a lack of cultural practice. Also, systematic study or cultural practices have yet to exist for this species. The loach fish population is decreasing in some regions due to subsistence fisheries, aquatic pollution, climate change, deforestation, construction of a dam across the river, etc. Thus, species stock or population could be rehabilitated through culture propagation. After guaranteeing widespread culture observation among farmers that might prove spiny loach fish a potential candidate species for inland cultivation shortly, culture techniques are required.

Because of the above, this preliminary study is committed to showing the cultural practice in captive conditions with a low-cost input method. For getting primary information, only some attempts have been made to introduce this species into grow-out studies.

2. A comprehensive knowledge on loach identification under the Genus of *Lepidocephalichthys*

Lepidocephalichthys alkaia (Havird and Page, 2010)

Diagnosis of the species:

A mid lateral dark stripe on the caudal fin, which often extends from the base to the fin's terminus and typically spans rays 7–10, sets *L. alkaia* apart from all other *Lepidocephalichthys*. Its rounded or truncated caudal fin, lack of scales on the top of the head, dark stripes or spots on the side, dorsal fin origin immediately behind the pelvic fin origin, 8–10 pre dorsal, 8–10 post dorsal, and dark bars on the dorsum, and moderate size (up to 38 mm SL).



Fig. 1. *Lepidocephalichthys alkaia*

Distribution:

It has been found in Myanmar, kachin Province's Irrawaddy drainage, nan kwe river, oxbow lakes and streams.

Lepidocephalichthys annandalei (Chaudhuri, 1912)

Diagnosis of the species:

The inner rays of the pectoral fin in males come together to produce a vertical osseous crest. However, in females, this does not occur.

The morphology of the osseous crest on the fused seventh and eighth pectoral fin rays can be used to diagnose male *L. annandalei*. The crest resembles a shovel and progressively rises posteriorly from behind the pectoral fin ray origin to reach a



Fig. 2. *Lepidocephalichthys annandalei*

greater elevation. Based on morphometry, colour pattern, and meristic, *L. annandalei* is distinguished by its concavely lunate or notched caudal fin. The eyes of *L. annandalei* are a little swollen.

Distribution:

Chaudhuri documented *L. annandalei* for the first time from the Tista, Jalpaiguri, and Mahananda rivers in Siliguri, North Bengal. Shaw and Shabbeare from the Panchenai River close to Matihara confirmed its existence in Northern Bengal. In his distributional list of the Himalayas, Menon noted that *L. annandalei* was only found in the Brahmaputra drainage. Remarkably, *L. annandalei* is not included in

the most recent list of fishes from North East India, which was created by Vishawanath et al., (2007).

Local name: Annandale loach

3. *Lepidocephalichthys berdmorei* (Blyth, 1860)

Diagnosis of the species:

The depth of the body is identical from head to caudal base. The male's pectoral rays 7-8 united to form a cylindrical structure that is significantly thicker than other rays. The body is yellowish to brown with fine to coarse spots. But, always has a mid-lateral series of huge, irregular blotches. There is a prominent black patch on the upper part of the caudal base.



Distribution:

They can be found throughout Asia, including the Mekong basins (Kottelat, 1992), the peninsula, Thailand, India and Myanmar (Talwar and Jhingran, 1991), and Bangladesh (Rahman, 1989 and 2005). Additionally, they are indigenous to north eastern India, primarily in the drainage systems that drain to the basins of the Manipur Barak and Chindwin Rivers.

Local name: Burmese loach

4. *Lepidocephalichthys goalparensis* (Pillai and Yazdani, 1976)

Local name: Pillai loach

Diagnosis of the species:

The caudal fin is strongly forked, typically has four to five dark, broad, regularly spaced V-shaped bars. It is moderately to large in size up to 4.4 cm SL. The lamina circularis is made up of fused seventh and eighth pectoral rays, which occasionally form small dorsally projecting flanges but never large flanges with serrations.



Distribution:

It has been found in Asia: India, Nepal, Bangladesh, India and Myanmar in the Irrawaddy, Brahmaputra, and Ganges drainages.

5. *Lepidocephalichthys furcatus* (de Beaufort, 1933)

Diagnosis of the species:

The male's pectoral rays 7-8 have a broad, semi-circular plate that is oriented vertically. The dorsal fins origin is above or slightly in front of the pelvic origin. The caudal base has a dark shaped mark, and the base of the branching rays 3-5 has a black spot.



Distribution:

It has been found in Asia; Malay Peninsula, Mekong and Chao Phraya basins.

6. *Lepidocephalichthys guntea* (F. Hamilton, 1822)

Local name: Guntea loach, Scavenger loach and peppered loach.

Diagnosis of the species:

Large size (up to 78 mm SL), caudal fin with dark reticulations, side with dark spots in females and broad dark stripes in males. Rounded or truncated caudal fin, lack of scales on top of head. Dorsal fin origin slightly posterior to or directly above pelvic fin origin. Body depth increasing from snout to

pectoral fin base. Then, remaining constant to caudal fin base, occasionally giving the appearance of an anterior "hunch." There are roughly 65 pre dorsal scales. The top and side of the head are scale-free. The barbels vary in size and the flaps are typically fringed.



Distribution:

Pakistan, northern India, Bangladesh, Nepal, Myanmar, and Thailand are among the Asian countries where it has been detected. In the drainages of the Brahmaputra and Ganges Rivers in north India, Bangladesh, and Nepal. *L. guntea* is common in streams and lakes (sometimes surrounded by dense vegetation). Although reports of the species have come from central India.

7. *Lepidocephalichthys hasselti* (Valenciennes, 1846)

Diagnosis of the species:

It typically has a black ocellated mark at the base of the branched caudal rays 3–4, although it might be replaced with a darker or blacker area. It has fine dots, or blotches and a body with a median longitudinal stripe or a row of adjacent black spots, with an unpigmented stripe above it. Its size can reach 45 mm SL. Caudal fin having a dorsal origin above the pelvic base's posterior extremity and a series (often three to six) of vertical bars.



Distribution:

It has a widespread distribution in Burma, Thailand, Laos, Cambodia, Vietnam, and Yunnan; southward through Peninsular Malaysia to western Indonesia.

Local name: Cobitid loach

8. *Lepidocephalichthys jonklaasi* (Deraniyagala, 1956)

Diagnosis of the species:

The characteristics of *L. jonklaasi* include a truncated or rounded caudal fin, three to six broad, regularly spaced dark bars on the caudal fin, a series of large, vertically elongated black spots on the side that typically form many irregular bars. A dorsal-fin origin anterior to pelvic-fin origin, and moderate or large size (to 45 mm SL).



Fig. 8. *Lepidocephalichthys jonklaasi*

Distribution:

It is an endemic fish species restricted to the wet zone of Sri Lanka, 500m above sea level. There are currently twelve localities in the wet zone where it is known to exist: Beraliya, Dombagaskandha, Madakada, Gilimale, Hiyare, Kottawa, Kanneliya, Weddagala, Nakiyadeniya, Pahiyangala, and Boralugoda.

Local name: Jonklaas loach, spotted loach.

9. *Lepidocephalichthys kranos* (Havird & Page, 2010)

Diagnosis of the species:

Large exposed scales on top of the head, dorsal fin origin just anterior to pelvic fin origin, moderate to large size (to 44 mm SL), a truncated or slightly emarginated caudal fin. A "hunched" body shape (body depth rapidly increasing from snout to pectoral fin origin), and the large muscular nape marking the end of the scaled head. A thin, dark, interrupted stripe along the side of the body present in both sexes are characteristics that set *L. kranos* apart.



Fig. 9. *Lepidocephalichthys kranos*

Distribution:

L. kranos is found in the Chao Phraya drainage in Thailand and the Mekong drainage in Thailand, Laos, and Vietnam. It appears to be widespread there and frequently coexists with *L. hasselti*. Commonly found in environments with dense vegetation, it can be found in ponds, paddy fields, and slow-moving streams.

Local name: Dwarf spined loach

10. *Lepidocephalichthys micropogon* (Blyth, 1860)

Diagnosis of the species:

A strongly forked caudal fin and small barbels (the first rostral pair never reaches the anterior nostril, while the other pairs sporadically reach the orbit) are characteristics that set *L. micropogon* apart. The caudal fin typically has four broad, dark, irregular, V-shaped bars, with the first two alternating between the middle or upper and lower portions of the fin and the last two only on the tips of the caudal lobes. Dorsal fins often have a posterior origin to pelvic fins. A broad dorsally concave flange, occasionally with serrations, is formed by the fusion of the seventh and eighth pectoral rays to form the lamina circular (up to 55 mm SL).



Distribution:

Kottelat and Lim (1992) proposed that the Sittang River basin was the type locality, and the majority are from Myanmar. This species has been obtained from the Brahmaputra drainage in Bangladesh and Assam, India, as well as the Salween, Sittang, and Irrawaddy drainages in Myanmar.

11. *Lepidocephalichthys tomaculum* (Kottelat and K. K. P. Lim, 1992)

Diagnosis of the species:

The characteristics that set *L. tomaculum* apart include a truncated caudal fin, a darkly reticulated caudal fin, no scales on top of the head, a moderate size (up to 39 mm SL). Three to seven pre dorsal and three to six post dorsal thin, transverse dark bars on the dorsum. A deep caudal peduncle that results in a uniform body depth from head to caudal-fin base, and a lamina circularis made up of fused, thickened, and flattened seventh and eighth pectoral rays.

Distribution:

L. tomaculum is mostly found in Peninsular Malaysia, where it has been collected in backwaters with dense vegetation, slow-moving streams, and swamps in the provinces of Selangor, Pahang, Johor, and Terengannu.



12. *Lepidocephalichthys manipurensis* (Arunkumar, 2000)

Diagnosis of the species:

It consists of a caudal fin that is deeply forked, has four to five black W-shaped bars. It has a dorsal fin origin that is just above or just in front of the pelvic fin origin. The head's vertex has no scales. There is a single, dark-black stripe with eight to eleven dots that runs from the tip of the nose to the eye. A tiny, jagged black patch directly above the caudal fin's upper base. From the occiput to the base of the caudal fin, there are eight to nine short, ashy brown bars on the dorsal side. The caudal fin is forked and has four to five W-shaped bands. Stripes or spots on the anal, ventral, and pectoral fins.

Distribution:

The only known locations for *L. manipurensis* are nearby waterbodies in Myanmar and India (Manipur). It is also found in Moreh, Chandel District of Manipur, in the Yu River system, Irrawaddy drainage.

13. *Lepidocephalichthys irrorata* (Hora, 1921)

Diagnosis of the species:

Large, exposed scales on top of the head, dorsal fin origin posterior to pelvic fin origin, moderate to small size (down to 29 mm SL), emarginated caudal fin, caudal reticulations, and caudal lobes with dark, elongated patches along the upper and bottom borders of the fin are all contributing factors. A distinctive rectangular flattened pad emerges right behind the dorsal fin, and the seventh pectoral ray becomes firm and ossified throughout the breeding season and the months leading up to it (May to July). Both the caudal and ventral fins of males have one or more branching fin rays.

Distribution:

L. irrorata has a widespread distribution in India and Bangladesh. It inhabits the Muhuri and Sylhet rivers in Bangladesh as well as lakes and streams in the Brahmaputra drainage in Manipur, India.

Local name: Loktak loach

14. *Lepidocephalichthys coromandelensis* (Menon, 1992)

Diagnosis of the species:

The characteristics of *L. coromandelensis* include a rounded or truncated caudal fin, no scales on the top of the head, a continuous, thin, dark stripe running from the snout to the base of the caudal fin. Dorsal fin origin immediately posterior to pelvic fin origin. A caudal fin with dark reticulations, and moderate to large size (up to 57 mm SL).

Distribution:

It is mainly found in Andhra Pradesh, India.

3. Biology of Indian Spiny Loach

Taxonomic Classification

Kingdom : Animalia

Phylum : Chordata

Class : Actinopterygii

Order : Cypriniformes

Family : Cobitidae

Genus : *Lepidocephalichthys*

Species : *Lepidocephalichthys thermalis* (Valenciennes, 1846)



Fig. 12. *Lepidocephalichthys thermalis*

Species Identification

According to (Paul and Chanda, 2017.) *Lepidocephalus thermalis* identifies with the following morphology characteristics: the body is elongated and slightly compressed anteriorly and strongly posteriorly—there is an inferior mouth and three pairs of barbells. The dorsal fin is inserted somewhat anterior to the pelvic fin, usually nearer the Caudal fin base than the snout-tip. Caudal fin almost squarely truncated. A small patch of scale on the head behind the suborbital spine; on the ventral side of the head, scale extent anterior to the pectoral-fin base but not reaching the isthmus; 30-37 rows of scales between the back of the body and anal fin; scale oval. The colour of the body is grey to delicate grey-green, with 8-10 separate irregular spots along the side of the body. The backside was marbled pale and dark in colour; a small black spot on the upper half-caudal fin dorsal and anal fin with a row spot. Matured *Lepidocephalus thermalis* is smaller when compared to *Lepidocephalus guntia*. Both species do not have difficulty identifying; they vary in colour, size, and body shape proportion from one species to another. *Lepidocephalus guntea* has a distinctive colour pattern and tubular body. (Manoharan et al., 2019) They reported that *Lepidocephalus thermalis* has a pointed “V” shaped head, spots on the entire lateral line, and a dark round spot surrounded with a yellow-white ring near the caudal fin. In contrast, *Lepidocephalus guntea*'s caudal fin is pointed square-edged, and its head is “U” shaped with few scales.

General Distribution

Across the globe and in India, loach fish species are distributed universally, which is endemic to certain places. In many regions, only some species are used as aquarium-grown fish rather than food fish (Acharjee & Barat, 2014). However, in the southern states of Kerala and Tamil Nadu, they use spiny

loach as food fish, which contains ethnic flavour and taste (Manoharan et al., 2017). There are a total of 44 fish species identified in the western ghats, 20 species from the Darjeeling Himalayas, and also a few species from two genera in the West Bengal region (Acharjee & Barat, 2014; Keskar et al., 2015; Paul & Chanda, 2017). The species of *Lepidocephalus thermalis* (Spiny loach) and *Schistura notostigma* (Banded mounted loach) are distributed in Sri Lanka inland waters (Sundharabarathy et al., 2001). Members of the family Balitoridae, nemacheilidae, Cobitidae and Botidae are commonly known as loach. The Cobitidae (Swainson, 1838) family is known as spined or true loaches. Family Cobitidae recorded 29 genera; eight are represented in India (Paul & Chanda, 2017). The author Bleeker, 1863 was described the genus *lepidcephalichthys* from the base species of *Cobitis hasselti* from the genus of *lepidcephalichthys* and recorded 18 species from the world, whereas nine species from our inland water from these genera. The name species of *Lepidocephalus thermalis* was originally called *Cobitis thermalis* (valenciennes.1846) from Kerela. These species are also found in the other states of Tamil Nadu, Maharashtra, Karnataka, and West Bengal, and no research data on the population status, ecology, or threat for this species is available.

Distribution and abundance

The record of fish species distribution helps to understand the geographical history of certain species. Loach is locally called Ayirai meen (vernacular name in Tamil) (Manoharan et al., 2019). Loach fish species are traditionally abundant in almost all kinds of inland water bodies in various districts of Tamil Nadu. Even though this species is widely distributed in small pools, ponds, canals, etc., the present scenario expresses a tragic situation regarding their population because it has decreased tremendously. In a few districts, their population has already become extinct. This has only been experienced in the last few decades. An exception arises in scenarios devoid of anthropogenic influence and the introduction of invasive species, where *Lepidocephalus thermalis* may still be located sustainably within riverine environments and their tributary regions. IUCN categorizes them as minor concerns (LC) (Manoharan et al., 2019).

Generally, the Western Ghats is rich in loach fish diversity (43 species), and invasive species threaten 39 species (Tapkir et al., 2017). They are given above for assessing the resource availability of common spiny loach, two two-stream riverine places for sampling that is namely Pechiparai lower stream (Kanyakumari) and Kalakad riverine (Tirunelveli) area where gentle flow of water and addition to these in the Parakkai region chosen some reservoir outlet as a sampling site. The Tamiraparani River is a perennial river originating from the Western Ghats, and its tributaries are flowing in the southern districts. At last, it joins the Arabian Sea and the Gulf of Mannar. After frequent random sampling, it was found that

significant numbers of loaches were recorded from the chosen as well as adjacent water bodies. This study aims to get the background of biological data on the distribution and their abundance in the altered region. It has been recorded that *Lepidocephalus thermalis* distributed abundantly occurs based on the following criteria: mild running water flow, no turbid water, fined sandy area and no anthropogenic influence. In addition, it states that this species is abundantly distributed in both districts. Specific geographical regions have also been identified for further biological research, focusing on cultivating and diversifying inland species in captivity. These species are found across peninsular India and Sri Lanka.

Habitat of spiny loach

The loach fish inhabit higher and lower altitudes in various parts of the globe (Kottelat, 2012). Hillstream loach fishes are classified under the superfamily of cobitioidea, called true loaches (Keskar et al., 2017). Most of the species adapted to fast-flowing streams, cling to small pebbles, lurk in between stones, and feed on detritus matter. Few specialized in living in the peat swamp forest and cave fishes (Kottelat, 2012). Loaches typically bury themselves in sand or mud in their natural habitat (Kumarai et al., 1979). The species move quickly, concealing under their living substrate, where fine sand, pebbles, gravel, or any other vegetative substrate is present. An act of concealing in Cobitidae fishes could be a fast method, happening at intervals an amount of one or two seconds, whereby the fish itself conceals, though not utterly, the highest of the pinnacle, with eyes and mouth and tail fin alone being exposed. A concealing happens from either a stationary position or once swimming. This hiding purpose is for them to escape from the light, avoid the heat, human disturbance, predators and other biological aspects like reproduction. In the river canal or stream area, this fish occurs near the edge of the shallow running water bodies and has no competition from other fishes. It was also observed to change its colour to blend with the substratum (Kumari et al., 1979). They are not so active and lie on the bottom of the sand unless there is no disturbance.

Feeding habitat

The natural feeding habitat of loaches is detritus (Sundarabarathy et al., 2001) and shows a preference for benthic invertebrates (Havird & Page, 2010). According to Bohlen (2002), the spine loach feed during nightfall in the sand bottom by its specialized filter-feeding mechanism, utilizing sticky areas in its pharyngeal cavity and expelling unwanted sediment through the opercular slit, a method similar to other

Cobitidae species (Caleta et al., 2015). However, the common spiny loach, which is culturing under captivity, is taking feed on during the time with very little quantity, and while feeding, it is coming to the water surface; after taking feed, it rapidly moves down to the bottom within a second. However, it was observed that the feed intake was rapid at nightfall only. Sundarabarathy et al. (2001) reported that spiny loaches perform poor feeding in laboratory conditions compared to wild animals. They reported information on food and feeding habitats, such as acceptance of various foods, especially sinking dried and bottom live foods.

UNDER PEER REVIEW

4. Techniques for the Wild Collection of Indian spiny loach

Loach fish, characterized by their diminutive size, are commonly found in various aquatic environments, including canals, paddy fields, small streams, swamps, and riverine areas. In the southern states of India, particularly Tamil Nadu, there is a significant market demand for loach fish due to their palatable flavour.

Historically, this species has served as a vital subsistence food source for numerous tribal communities. However, due to unsustainable fishing practices, the primary supply of loach fish obtained from wild capture is leading to a decline in their natural populations. Additionally, factors such as subsistence fishing, aquatic pollution, climate change, deforestation, and the construction of dams have further contributed to their decreasing numbers. The absence of effective aquaculture techniques has also hindered the rehabilitation of this species.

In India, there currently needs to be standardized methods for the wild collection of *L. thermalis*, which poses challenges for the successful propagation of loach fish in captivity. This article aims to share insights from our field experiences during wild collection, providing valuable information for researchers, farmers, and students interested in developing breeding or farming techniques for loach fish. The wild collection efforts were conducted to establish a broodstock and to refine the protocols for their captive breeding, utilizing various fish collection nets, including fine-meshed nylon nets, hand nets, and dragnets, across two selected distinct irrigation channels.

Predilection on wild habitation

Loach ecology was studied in the different places of Kanyakumari and Tirunelveli districts, where the availability of loach fish was confirmed (table 1). During our collection expeditions, we observed several general habitats. The distribution of this species is highly dependent on local conditions, as they exhibit sensitivity to disturbances caused by human activities and other aquatic organisms. After detecting such disturbances, loaches tend to swiftly retreat and seek refuge beneath mud, sand, or vegetative cover. This species predominantly inhabits channels characterized by gentle water flow and a depth of less than 25 cm, indicating that shallow waters with mild currents constitute their preferred environment within the natural ecosystem. Furthermore, they are inclined to occupy areas abundant in decaying organic matter and vegetation.



Identification of Collection site

The identification of collection sites was achieved through regular field visits and consultations with local individuals knowledgeable about fish collection. Initially, we pinpointed locations such as small pools, ponds, and canals in the Parakkai region of Kanyakumari district, where loach fish were observed. These sites typically receive water from the Thamirabarani River. Notably, these collection sites are perennial, benefiting from substantial rainfall during the northeast and southwest monsoon seasons, ensuring a continuous water supply throughout the year. Consequently, these perennial water sources serve as habitats for indigenous fish species.

The presence of a white crane serves as a reliable indicator for assessing the availability of loach fish across different collection sites, including canals and small streams. Through our field investigations, we identified two riverine systems as significant resources for loach species: the lower stretch of the Pechiparai reservoir and a small stream within the Kalakad riverine area. We have periodically monitored these collection sites and documented their ecological characteristics. Between the two locations, the Kalakad stream exhibits a narrow and gentle water flow, while the lower stretch of Pechiparai features a broader channel; however, its width diminishes when water release from the reservoir is reduced. Thus, the water discharge from the reservoir can significantly influence the width of the water channel.

Its rapid movement and tendency to seek refuge beneath the substrate in its natural habitat characterize *Lepidocephalichthys thermalis*. Consequently, in the lower stretch of Pechiparai, locations with broader water expanses or channels exhibiting increased water flow are deemed unsuitable for collection purposes. Our observations consistently led us to select optimal collection sites characterized by narrow water flows, often accompanied by vegetation or other substrates. In contrast, the Kalakad stream, being small and narrow with a moderate water flow, lacks vegetative production areas, resulting in a diminished yield of loach.

Additionally, we have determined that paddy fields serve as ideal environments for collecting loach brood fish, particularly during the flowering stage of the rice. Effective loach collection sites should possess narrow dimensions, gentle water flow, sandy substrates, and be enriched with vegetation or decomposing organic matter. Furthermore, our research has extended to assessing loach fish populations within the riverine section of the Periyar River in the Theni district, with the distribution of *L. thermalis* documented in Figure 15.

S. No.	Name of collection site	North latitude	East longitude
1.	Kalakad riverine area (Tirunelveli Dist.)	8°30'58"	77° 32' 21"

2.	Pechiparai reservoir (Kanyakumari Dist.)	8°23'12"	77° 17' 50"
3.	Nagercoil, Parakkai region (KK, Dist.)	8°8'30.8112"	77° 27' 30.4416"
4.	Velladichivillai, Suchindram (KK, Dist.)	8°8'52.2276"	77° 27' 26.8272"
5.	Vetha Nagar, Suchindram (KK, Dist.)	8°8'52.08"	77° 27' 26.802"

Table 1: The Geographical Location and Area of the Selected Fish Collection Site



Fig.14. collection of loaches from two distinct locations: a vegetative area and a channel within a paddy field.

Location	Latitude	Longitude
1	9.80265982	77.35203902
2	9.85141	77.380043
3	9.85449	77.381824
4	9.887188	77.385734
5	9.893787	77.395271
6	9.899808	77.399804
7	9.905794	77.398291
8	9.93549	77.475506
9	9.989282	77.459975
10	10.122036	77.541997

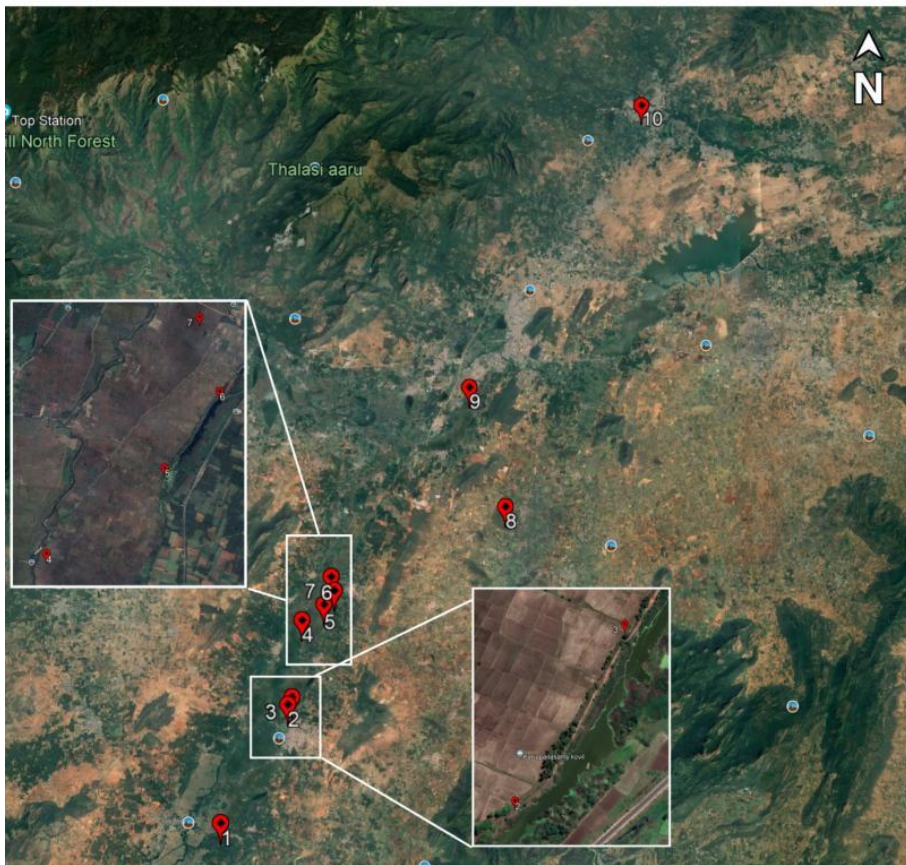


Fig 15: The Mapping of *L. thermalis* available location in Theni Dist.

Collection techniques:

Generally, the optimal times for collecting loach fish are late evening and early morning. Notably, observations indicate that loaches are present throughout the day in the paddy field canal areas. However, collection efforts are hindered during the afternoon, particularly in sunny conditions or during heavy rainfall. This species seeks refuge under shelters to escape temperature variations during sunny periods, while visibility issues during rainy weather significantly complicate the identification of these fish.

Diverse methods have been employed for loach fish collection across various regions of the country. Traditional fishing gear, such as drag nets, has been utilized in certain areas to capture loach species (Keskar et al., 2015). In riverine environments, dip nets have been employed for this purpose. For effective loach collection, it is advisable to use a fine nylon meshed net tailored to the width of the collection site to minimize luminous exposure, as loaches often conceal themselves under rocks or within dense vegetation.

Throughout our field excursions and sampling activities, we utilized a technique involving the closure of one side of a narrow waterway, such as a canal or stream, with a finely meshed nylon net. With the assistance of two individuals, the net was pulled against the current. This action disturbed the substrate in the collection area, facilitating the aggregation of loach fish within the net. Additionally, we employed a hand net as an alternative method for collecting loach fish, which proved quite effective. In this approach, the net was positioned near vegetated regions, and the substrate was disturbed to encourage the loach to enter the hand net. Since loach species are both autochthonous and endemic, the number of fish collected during each sampling trip was relatively low. Furthermore, we refrained from collecting loach fish during periods of heavy rainfall and elevated daytime temperatures at our regular collection sites.

5. Seed Production techniques for Indian spiny loach

Fish breeding plays a crucial role in improving fish production rates. This phenomenon can be linked to the progress made in aquaculture technologies and seed production techniques, as well as the accessibility of fish seeds. Nevertheless, during the fiscal year 2019-20, the overall output of fish seeds reached 52,170.6 million fries, which falls significantly short of the projected target of 22 million tons by 2025 (Rasal, A., et al. 2023). Although established aquaculture and seed production methods are in place, the industry faces various challenges related to climate change, affecting production directly and indirectly. Factors such as extreme monsoon conditions, overfishing, deforestation, and the degradation of aquatic ecosystems contribute to these challenges. Consequently, the country needs to prioritize the development of climate-resilient and sustainable aquaculture practices to achieve its production objectives and meet the growing demand for fish, thereby ensuring nutritional security.

In contemporary times, diversifying fish species is essential for enhancing fish production and making it appealing to consumers. Furthermore, certain species are globally prevalent and play a significant role in overall inland fish production. Additionally, the presence of exotic or invasive species is adversely affecting the populations of native species, leading to ecological changes within the ecosystem. Among small indigenous fish, loach is recognized for its high nutritional value and is remarkably esteemed in the southern districts of Tamil Nadu as one of the most palatable options. This species enjoys considerable market demand and is primarily sourced from natural habitats in regions such as Theni, Madurai, Dindigul, Thirunelveli, and Kanyakumari in Tamil Nadu. The interplay between consumer preferences and supply dynamics contributes to its market demand. The loach is valued for its nutritional benefits and ornamental qualities, characterized by its colouration, banding, and diminutive size, making it a sought-after food fish.

Consequently, it garners significant consumer interest and market demand nationally and internationally. To achieve species diversification and enhance natural stocks, promoting aquaculture practices, including farming and seed production, is imperative, particularly for native species. In this context, the standardization of loach (ayirai meen) seed production techniques is a crucial step towards advancing farming practices within the state.

Captive brood stock development

In a year, it spawns twice during their prolonged spawning activity, and they mainly breed between June to August and October to December. The matured brood stock or juveniles are collected from wild conditions such as canals or riverine areas and are effortlessly acclimatized to captive conditions. In the

case of juvenile fish, water with neutral pH conditions (7.0-7.5) and temperature manipulations (from 26 °C to 22 °C) could be used for faster maturation. On the other hand, brooders require good quality water, mild aeration and diet to attain faster maturity. A Cement or FRP tank with a 3-5 cm height of fined sand bottom will yield better results. Brood fish can be stocked with a density of 150-200 Nos. m⁻² (tank). Feed them with sinking crumble feed twice daily, containing 30-35% protein @ ad-libitum of the biomass for two months of broodstock development. On a fortnight basis, 20% of water exchange is required to maintain good water quality. For natural breeding, males and females should be maintained in separate tanks. Generally, brooders with a group of more than one year are highly preferred for artificially induced breeding.



Fig. 16. Brood Stock Development

If brooders are raised in the cistern or cement tanks, then specific measurements must be taken to raise good-quality brooders. This species grows a maximum size of 7.2 cm and 3g in weight. It lives in shallow water, and mature fish prefer dark places. Maintaining a water depth of 15 cm in height in the cement tank system is preferable, but the resemblance watercolour becomes darks. For that, we need to cover half of the tank, or broken pipes or soil-made bricks or sheets shall facilitate the tanks for fish to get it in their natural comfort. At three-day intervals, 20% of water exchange is essential to maintain clear water and reduce excreted waste. The preferred feeding time is nightfall time, but feed should also be available in the tanks for the fast maturation of fish.

Selection criteria for male and female

The abdomen, which is more prominent in females than males, can easily distinguish male and female (Figure). Males are more petite, slender, and more pigmented than females. Females are more prominent, with bulged abdomen, no modification in the seventh and eighth rays of the pectoral fin, and pale whitish. In contrast, in males, the seventh and eighth pectoral fin has a thin layer (ossified fin rays) with milky whitish. Loach attains sexual maturity at the size of 4.5 cm, 1.24 g (male); 4.5 cm, 1.38 g (female) and minimum size at first maturity is 3.3-3.5 cm, observed spawning type is asynchronous; fecundity ranges between 2000-

Fig. 17. Sexual difference



5000 Nos. The average size of a ripened ovary is 600-768 μ (Kumari & Nair, 1979). The sex ratio of male to female is 1:1 for better breeding performance.

Breeding

The breeding season for *L. Thermalis* is recorded during the southwest and northeast monsoon seasons in different parts of the country. Wild-collected fish show their initial maturity in the figure below.



Fig. 18. Histology Image shows the Maturation of male and female

- The first image shows a mature female, and the second is a mature male. The third picture shows an immature ova (size of fish <3.5cm), confirming the presence of more chromatin and peri nucleolus stages in female fish.
- The presence of more primary vitellogenesis stages (PVS) and secondary vitellogenesis stages (SVS) are highly mature, which indicates a fourth picture. Hence, female fish attain first maturity when the fish size is >3.5cm.

Natural induced breeding

Cement tank:

The naturally induced breeding attempt was conducted at the Theni Centre for Sustainable Aquaculture (then) in the cement tanks system and succeeded with the following protocols. For breeding, water depth shall maintained up to after 15 to 20 cm for entire rearing periods. The matured male above 1.5gm and the female at 2gm were selected for stocking in the breeding tanks. For successful breeding attempts, similar weights of both fishes shall reared into the breeding tanks. The following water quality parameter, like temperature (29-29.6), pH (8-8.5), dissolved oxygen (>5ppm), alkalinity (165-210ppm), and hardness (165-195 ppm), has to be maintained for successful breeding. In addition, the hiding substrate required

is either a broken pipe or pot, etc., and a shower can facilitate reducing the water temperature. In addition, water exchange and frequent disturbance shall be avoided. After two days, fish start to release eggs, and fertilize within the 48hrs. Approximately 800-900 young could be observed on the tenth day in the bottom of tanks. Even though this is an asynchronous spawner, it releases the eggs batch-wise; hence, it shall avoid water exchange; if possible, brooder fish can be shifted to other tanks.

Cistern or tubs system

After selecting matured male and female fish (one pair), they can be reared in 30lit capacity rectangular tubs with 10 cm of water depth. Provide a one or 2-cm layer of sand substrate on the bottom of the tub surface. They maintained the water quality parameters such as water temperature (26-27), pH (6.9-7.5), hardness (60-150 ppm), alkalinity (60-190 ppm) and dissolved oxygen (>5 ppm). Provide any supplementary feed with a vitamin and mineral mixture (feed- ad-libitum). Ensure mild aeration or water flow for better breeding performance. Also, avoid frequent disturbance around the breeding units. Between 21-26 days, fish breeds naturally. The larvae can only be visible after 72 hours of completion. It shows concealing behaviour and swims around on the bottom surface.

Artificial induced breeding techniques:

For any fish, artificially induced breeding requires a successful protocol for proper handling during the breeding process. In loach fish, the following steps have been practised: selection, weighing, and anaesthetizing— injection—recovery.

Pre-injection preparation:

- We selected the matured male and female fish for breeding.
- Fast the fish for at least 24 hours before the injection (this is required to decrease the blood glucose to the baseline level before injection. Tips: Cold water does not raise the glucose level, and anaesthesia will increase it). Nine fish, 10 litres of water.
- For injection, we used 32GS of 1ml insulin syringe.
- The required foam sponges (cut a soft sponge for 70mm height on the flat face, 10-15mm deep) will help hold the fish.
- Weigh the fish before anaesthesia.
- Draw the hormone filling syringe and tube based on the required volume (by the synthetic hormone of WOVAFH 0.02 ml – (1 unit of 1 ml needle) for administration.

Anaesthesia preparation:

- Add 1 drop of clove oil in 1000ml of water at a temperature of 26-27 degrees Celsius and leave over for 1 hr. (get a complete dissolution). (A syringe can used to avoid more drops).
- Observe the fish until gasping stops and operculum movement is prolonged.
- The fish is ready for injection when it does not react to being handled or revived while injecting.
- Gently transfer the fish to the sponges (the sponge should be water-saturated during the injection itself). The fish should be positioned with its abdomen up.



Fig. 19. Application of Anaesthesia

Injection:

- **Injection into the abdominal cavity posterior to the pelvic girdle** or inserting the needle into the middle between the pelvic fins slowly releases the synthetic hormone into the fish body (Fig.).
- The needle should point towards the head and be inserted closer to the pelvic girdle than to the anus.
- For the male below, the dorsal (intra-muscular injection) is suitable, and it should be injected gently. (Dissect fish to get a sense of body wall thickness.)
- After injection, fish are transferred to normal water for recovery. If recovery is slow, swirl the water towards the gill.



Fig. 20. Administration of Synthetic Hormone

Spawning tank

In the tanks, rear aquatic plants hide and maintain the water temperature at 26 °C or below. In general, loaches prefer some substratum for laying eggs. Additionally, the egg needs to be collected after fertilization; therefore, a suitable substratum, such as sand, gravel, and plants, needs to be provided (Figure 5).

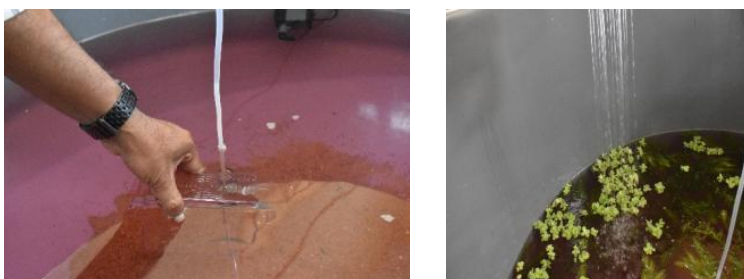


Fig. 21. Spawning tank set-up

Rearing of Early-fry

The early fry is stocked in specially prepared outdoor fibreglass rearing tanks. These tanks have a 2-3 cm thick layer of fine sand on the bottom and a 25 cm water depth. Rice bran or copepod larvae can be given ad libitum as larval food. They reach a size of around 4 cm and are used for grow-out rearing.



Fig. 22. Cultured Live feed

Table: 2. Recommend water quality parameter for rearing of loach species

Water quality parameters	Optimum range
Water temperature (°C)	27-29
Dissolved oxygen (ppm)	>4
pH	7.0 – 7.8
Alkalinity (ppm)	64 - 120
Hardness (ppm)	60 - 125
Ammonia (ppm)	< 0.05

6. Embryonic Development stages

More information is needed regarding the early developmental stages of *L. thermalis*. Therefore, it is essential to conduct comprehensive studies to characterize the various stages of embryonic and larval development in fish, which are crucial for understanding the species' biological clock and developing effective culture techniques. Fish undergo five distinct life stages: the embryonic phase, larval phase, fry phase, ripe phase, and senescent phase. In the context of population dynamics, a thorough understanding of the factors affecting individual survival and reproduction necessitates an examination of mortality schedules, which are closely linked to the organism's life history and reproductive strategies.

Embryonic phase:

The phase that occurs from the fertilization of the egg to the absorption of the yolk is referred to as a cleavage. The incubation period spans from fertilization to the emergence of the organism, culminating in the hatching process. During hatching, the egg capsule softens due to the action of chemicals and enzymes released by ectodermal glands. Additionally, exposure to heat and light enhances the activity

of the embryo, thereby aiding in the hatching process. The embryo typically exits the egg headfirst, followed by the tail. This process can be categorized into two distinct stages: the embryonic stage within the egg and the embryonic stage outside the egg (Langeland and Kimmel., 1997).

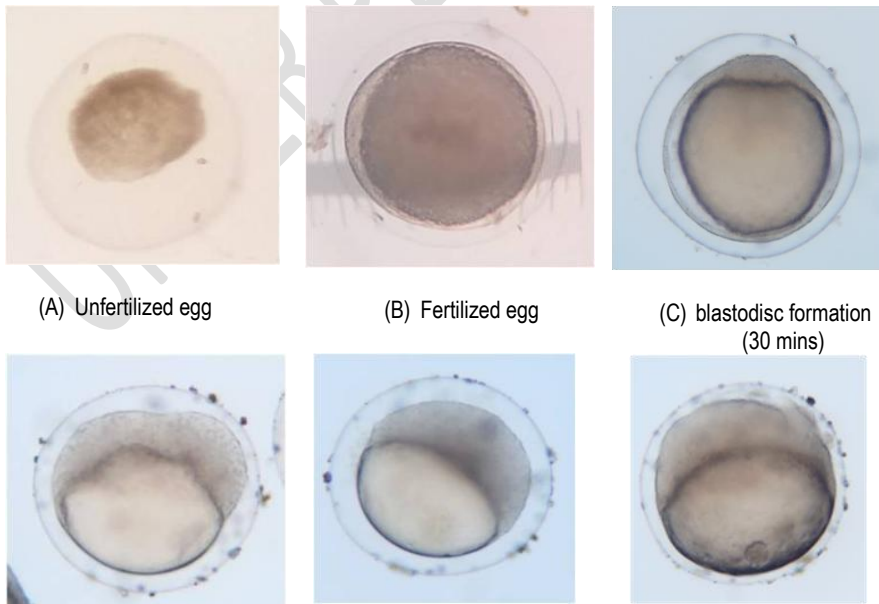
Larval Phase:

Following the hatching process, the larval phase of fish development encompasses several stages, namely pre-larvae, post-larvae, and fry. During the pre-larval stage, fish exhibit a yolk sac, a linear digestive tract, a diminutive head, and eyes that lack pigmentation. They develop pectoral fins as they mature, marking their transition into the fry stage.

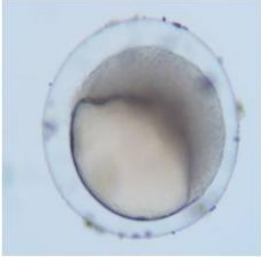
Fry Phase:

The transition from the post-larval phase to the juvenile phase is characterized by the development of scales, with the exception of the Mugilidae family. During this period, scales and the lateral line system emerge, and circulatory and haemoglobin systems begin to form, resulting in a morphology similar to adults, albeit with certain distinctions. Additionally, some species bypass significant metamorphosis and proceed directly to the juvenile stage as nekton.

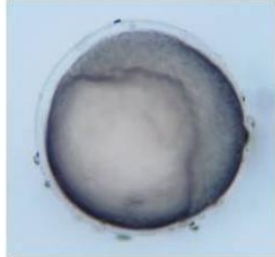
Fig. 23. Embryonic developmental stages of Indian spiny loach



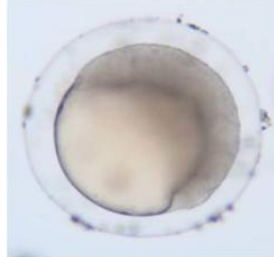
(D) 64-cell stage;
(3 hrs 20 mins)



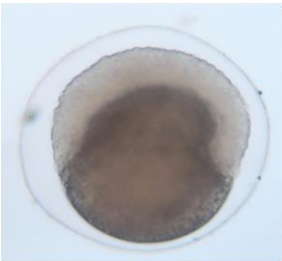
(E) 128-cell stage
(3 hrs 50 mins)



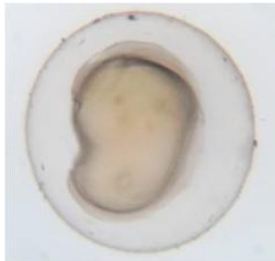
(F) 1k-cell stage
(5 hrs 10 mins)



(G) oblong stage (6 hrs)



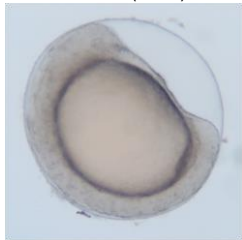
(H) sphere stage (7 hrs)



(I) dome stage (8 hrs)



(J) 50%- epiboly stage
(9 hrs)



(K) germ ring stage
(10 hrs)



(L) 4-somite stage
(18 hrs)



(M) 12-somite stage
(22 hrs)

(N) 20-somite stage
(26 hrs)

(O) 22-somite stage
(27 hrs)



(P) 30-somite stage
(32 hrs)



(Q) 50-somite stage
(48 hrs)



(R) 1stfry (72 hrs)

Fig. 24. Histological microphotography of oocyte maturity stages of Indian spiny loach.

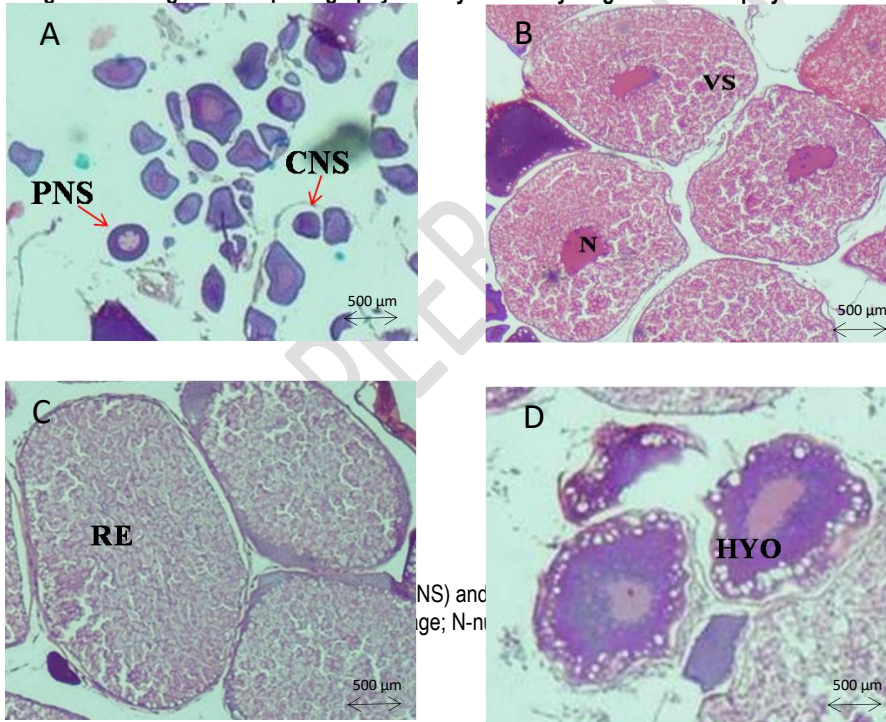


Table.3. Developmental stages of loach fish

Stage name	Time (h)	Characteristics	Fig. No
Unfertilized egg	0 h	Translucent, milky white in colour	1A
Fertilized egg	0 h	Transparent	1B

Blastodisc formation	30 min		1C
64-cell stage	3 h 20 min	Tier formation in a central part of blastomeres	1D
128-cell stage	3 h 50 min		1E
1k-cell stage	5 h 10 min	Formation, mid blastula transition occurs after this stage	1F
Oblong stage	6 h	Formation, ellipsoidal shape	1G
Sphere stage	7 h	Spherical shape	1H
Dome stage	8 h	Beginning of epiboly	1I
50% epiboly stage	9 h	Half coverage of a yolk cell by the blastoderm	1J
Germ ring stage	10 h	Arrest of the epiboly, germ ring and embryonic shield formation	1K
4- somite stage	18 h	Optic primordium, kupffer's vesicle	1L
12- somite stage	22 h	Somites transformation into a chevron shape	1M
20- somite stage	26 h	Optic vesicle, contraction of the yolk cell	1N
22- somite stage	27 h		1O
30- somite stage	32 h	Optic cup and lens placode	1P
50- somite stage	48 h	Early pigmentation in retina and skin	1Q
1 st Fry	72 h	Small pectoral fins and small external gills	1R

7. Farming Techniques

The loach fish population is decreasing in some regions due to subsistence fisheries, aquatic pollution, climate change, deforestation, construction of a dam across the river, etc. Thus, species stock or population could be rehabilitated through culture propagation. Since farming, carp species alone contribute more fish production to the country, even though, after reporting, much indigenous food is available in the nation. Therefore, new species' cultural practices or farming methods can emerge upon species diversification and providing varied fish protein for consumer preference. Spiny loach is a

potential species for inland cultivation shortly, and it is the optional fish species for carp culture. **Farming practices for earthen pond or lined earthen pond**

Site selection:

The Indian spiny loach can be found in an earthen or lined pond. The selection of an appropriate site for fish culture is an essential factor in determining the success of fish farms, and before the construction of fish farms, water, retention and soil fertility have to be taken care of—ecological factors to be considered (soil, water, topography and climate). A need pond criterion such as an even earth surface, without rocks and big trees, etc., is suitable for culturing loach in an earthen pond. The selected sites should have easy access to transport and electricity and low land costs. A mixture of Clay: Sandy (85:15) soil is essential for culturing loach fish, particularly for the earthen system. In many places, the availability of sandy soil is less; in this case, riverine sand mixed with clay that can be used for culture. The more clay soil pond bottom shall be avoided for choosing loach fish culture because more clay or silty substrate would not be favourable for it is concealing the natural behaviour of fish. Inlined earthen ponds do not require a sand substrate.

Pond preparation:

Ponds are categorized into perennial and temporary or seasonal ponds based on water retention capacity. Seasonal ponds can hold water for five to six months. The availability of water duration also differs in fish culture. Therefore, the choice of the Pond should depend on the species or duration of the culture.

The following precaution measures and special attention are required for the precautions existing for loach culture. Before starting or stocking loach culture, a pond bottom shall be sun-dried completely for gas exchange and removal of unwanted fishes. After drying the Pond, the lime shall be applied to correct the soil pH area. The area quantity of lime can decide the application. In addition, one corner of the Pond's bottom-harvesting pit shall be constructed for easy harvest (Fig). While pumping the water from natural resources, certain precautionary measure needs to be taken to prevent the entry of fish larvae, aquatic reptiles, etc., in general,

- Removal of unwanted fishes
- Dyke Maintenance
- The inlet and outlet of the Pond shall be maintained properly
- Correction of pH
- Prior application of fertilization for enhancing plankton production

- If required, use a re-circulatory system
- The following criteria are needed for loach farming practices.
- The size of 15 m² to 40 m² ponds is ideal for loach fish culture.
- The shape of the Pond should be rectangular.
- A dike is entirely built of good soil, and its thickness is 30-40 cm (it should be able to resist water pressure). It should be compact, solid, and leak-free.
- Pond total height is required for up to 1.2 m
- The depth of water volume has to be maintained up to the level of 0.6m – 0.7m. (3 1/2 feet)
- You should always leave the freeboard at 0.3 m (this is essential for avoiding unexpected rain and increasing the water level during sunny periods).
- The pond bottom slope is required for the complete harvesting of loach fish. When constructing the Pond, the dry slope should be deeper than the wet slope (1cm (WS): 3 cm (DS) is preferable).

Naturally, this species has concealing behaviour. The fined sand is filled over the pond bottom as a layer of up to 5 cm thick for favourable fish growth. The bottom of the Pond is constructed with a slope towards the outlet for Easy dewatering water. The outlet needs to be set up at the bottom and constructed prior to dyke construction. The inlet is fixed on top of the Pond, and filling the pond water should take at most hours. In addition to that, predatory or unwanted fish should not enter the Pond, so the inlet and outlet should be adequately knotted with fined mesh.



Figure.25: A schematic diagram for slope construction

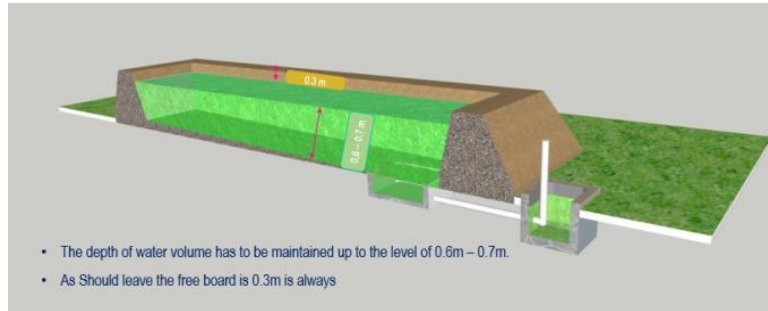


Figure.26. Maintains of required water depth

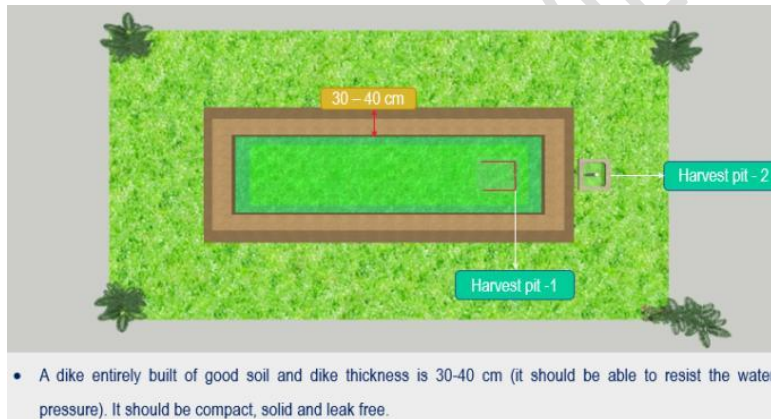


Figure.27. View of full size pond

Pre – Post stocking management:

Application of fertilizer:

A Fertilizer application in fish ponds stimulates the growth of plankton and fish production. These are used in two forms: natural (organic) or synthetic substance (inorganic). The quality of water is very much essential for any fish culture. The application of fertilizer depends on specific water quality characteristics.

(I)Organic fertilizer

Once the pond water is filled, the water quality needs to be analyzed; on the results, the application and usage of lime quantity shall be calculated. The lime application was used to correct the water pH level in Pond. In addition, it releases nutrients into soil and water and balances. Lime helps to shorten the decaying process in the pond bottom because these soil nutrients become enriched. After 15 days of lime application, organic fertilizer has to be applied to the Pond. Organic fertilizer contains organic matter and mineral nutrients produced locally from farm animals. Cow dung manure is mainly used in fish culture ponds. It is the best manure among the animal manure. Raw cow dung (wet) is the best manure for application. Small-scale fish farms mostly rely on these fertilizers because of low cost and local availability.

(ii) Inorganic fertilizer

The application strategies of fertilization depend on the pond substratum, and their optimum level differs from region to region. Moreover, it depends on soil chemistry. The application of inorganic fertilization enhances the primary production of ponds. These fertilizers contain fixed nutrient composition, possess high solubility in water, and cause rapid reaction with pond water after application. It should be used alternatively with organic manure. Inorganic fertilizer is categorized into three categories: nitrogen, phosphorus and potassium. As ammonium sulphate, calcium ammonium nitrate and urea, these fertilizers are used in Pond to enhance the nutrient of nitrogen, and their application should be adhered to based on soil pH. A freshwater-limiting nutrient is phosphorus; therefore, shallow levels occur in ponds. To enhance these nutrients, superphosphates are added, and this fertilization slowly leaches the nutrient into the water and provides phosphorus and calcium nutrients. An application of inorganic fertilizer should be based on the water quality and fish behaviour, and its application dosage level is given in the table below as an example.

Table 4. As Advisable dose of in-organic fertilizer application

S. No	Area	Nitrogen (urea) / kg	Phosphorus (super phosphate) /kg	Potassium (potassium chloride) / kg	Cow dung (kg)
1.	0.25 acre	5.06	5.06	2.55	1012.1

*If needed go for application of inorganic fertilization with the consultation of Aquaculture scientist.

Stocking:

Based on the preliminary and farmers' trail studies, the following stocking densities were determined for loach culture in earthen ponds. Even though it can breed naturally, there is a possibility for variation

in the final biomass harvest from Pond to Pond. The preferable stocking rate is 70-72 gm (0.4 -0.5g/fish wt.) of loach in one square meter. At the end of the three-month culture duration, we could harvest approx. 225 – 230 gm/ square meter. While harvesting, the partial harvest method is recommended to avoid animal shortage for stocking. In addition, the stocking rate of 2kg is considered in 22m³ for a lined earthen Pond or bio floc tank system. At the end of 3-4 months of the culture period, we could harvest approximately 10-11 kg by adopting the following protocols.

- Once the water is filled to the desired depth, one litre of probiotics must be added to the culture pond.
- After that, fish can stocked and start feeding.
- Weekly, 750 ml of panchakarma should be applied in the culture. (The preparation of panchakarma is as follows: cow dung 10kg, cow urine 10kg, cow milk and curd every 2 litres, ghee 1kg, sugarcane or molasses 3 litres, ripped banana 12 Nos, yeast 100gm, respectively. Add the entire item and ferment it anaerobically for 15-20 days.)
- Once exchanged, 20% of the water from the total quantity is used. Feeding twice a day with 10% of body weight.

Feeding:

Loach fish is an omnivorous. In a natural system, it mainly feeds on detritus, plankton, insects, etc. If it is adopted in captivity, it can also be fed a supplementary diet. If fishes are cultured with more stocking density, then supplementary feeding is necessary to meet the feed demands of the animal. In any culture system, supplementary feed should be provided twice daily, containing 6-7 % of the fish's body weight. If fish weight reaches beyond 1.5 gm, then 10-12% of fish body weight is preferable because natural breeding would take place to enhance the stock.

Significant fish growth shall achieved through best-feed management practices. The supplementary feed should contain a mixture of all macro and micronutrient compositions for better fish growth, especially for loach fish; 35% of protein is recommended, and it significantly contributes to fish growth. Our trials recommended Groundnut oil cake (GNOC) and Cotton seed cake (CSOC) with a 60:40 ratio; this feed composition helps to attain quick maturity of fish and breed naturally in the culture system. The supplement shall be fed enriched with lactobacillus as probiotics to attain better growth. **Harvesting:**

After 3-5 months, these species are ready to harvest from the culture pond. The suitable size for harvest is above 5 cm or 1-1.5gm of weight. Loach fish breed naturally in the Pond; hence, the partial harvest and partial stocking have to be done in the culture pond. Otherwise, it is better to maintain an additional fish pond required for stocking. So that the seed crisis can be managed, harvesting can be done after the culture pond has been dewatered completely.



Indian spiny Loach is a suitable candidate species for the scarcity of water and land prevailing area. In addition, they are almost considered hardy. It is also suitable for poly-culture with carp fry. It has more consumer preference and fetches a high market price. However, the market price is higher when fish are sold in live conditions. It is an alternative species for carp and other inland fish cultures. It might be contributing to species diversification and future candidates in aquaculture industries.

Grow-out culture of loach in modified tanks:

The ever-increasing global population necessitates access to clean water, land, and nutritious food. With 4% of the world's freshwater resources, India is in a critical situation as it is categorised as a water-stressed country. The aquaculture industry plays a significant role in meeting the nutritional demands of the expanding global population. Projections indicate that by 2050, this industry will provide nutritious food to support nine million people. However, most water resources are directed towards the agricultural sector, resulting in a shared water resource scenario with aquaculture. This shared usage limits the availability of water for aquaculture.

In light of this situation, the aquaculture industry must double its production through sustainable expansion, all while minimising water and land usage to mitigate the impact on these resources. Apart from intensification, diversifying the cultivation of native species proves beneficial in efficiently utilising water resources, thereby enhancing water security. Fish species like *L. thermalis* (Indian spiny loach)

hold market value and medicinal significance in the local market. Notably, this species can survive several hours out of water and endure adverse environmental conditions. Considering these factors, a preliminary grow-out study was conducted at the field level to promote the future cultivation of this species. The study took place in indigenously designed poly-lined iron frame tanks.

Fabrication methods for poly-lined

Tank material

Various dimensions of rust-resistant iron material were used to fabricate a rectangular tank for loach fish culture. The selection of iron material was specifically based on its ability to provide sufficient strength to contain the tank's required volume of water. The tank components include an iron-meshed frame and a low-density polyethylene sheet.

Tank construction

The construction design involved creating a rectangular tank with dimensions of 2 m² (1 m width x 2 m length). A 3 mm thick iron material, including corner legs, was employed to construct the box frame. The total height of the four corner legs was 50 cm, with the box frame fixed 15 cm above the bottom. The mesh size of the iron frame was 3 cm. A low-density polyethylene sheet (200 microns) measuring 3.75 m² (1.5 m x 2.5 m) was laid over the iron mesh frame (Figures 1-8). 2.3 Location: The tank is portable and can be moved to any required location. However, it is essential to place it on an even surface. These tanks can be positioned indoors for optimal water quality maintenance during the grow-out phase. It is advisable to avoid waterlogged areas to prevent rusting of the tank frames. Ensure proper fixing of the poly sheet to eliminate contact between water and the iron frame box. The tank has a capacity of 700 litres of water with 5-6 kg of riverine sand.

Grow-out culture

Pre-stocking management

Before filling the water, a thin layer of sand (up to 5 cm) should be spread over the tank bottom. The sand must be washed thoroughly and sun-dried at least three times. Following this, water should be filled up to a height of 25-30 cm, creating a minimum culture water volume of 400 litres. The freeboard area should be 10-20 cm. Fish can be stocked three days after water filling. Floating weeds should cover at least 20% of the water surface, and a few stones can be added as substrate to mimic the natural environment.

Stocking of fishes

Typically, indigenous farmers practice a one-year grow-out period for this fish, depending on water availability. Stocking density is a crucial factor influencing growth, survival, and production. Although no stocking density study is reported for this species, based on our preliminary studies, this species can be stocked at a rate of 800 individuals/m² (average length: 3.5 cm, weight: 0.24 g). A total of 1,600 individuals/2 m² were stocked.

Food and feeding

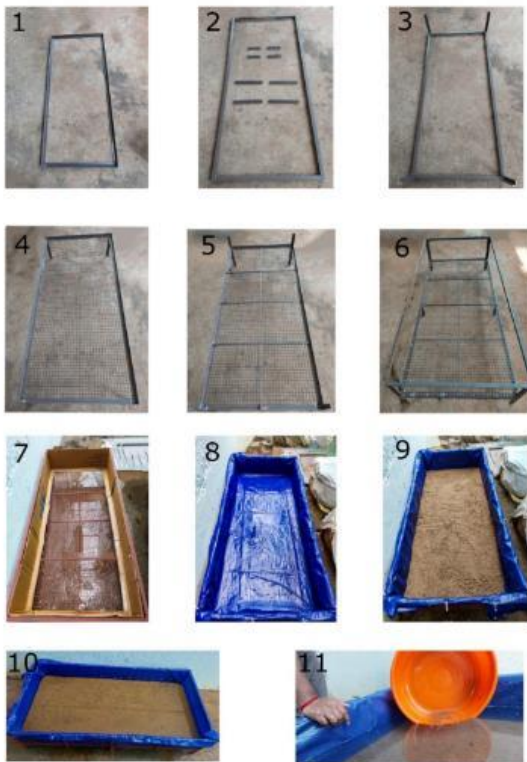
Loach's natural feeding habitat is detritus, and they exhibit nocturnal feeding behaviour. They feed on algae, detritus, insect larvae, and copepods using their specialised filter-feeding mechanism. A supplementary feed mixture of GNOC and cottonseed oil cake (60:40) can be provided at an ad-libitum level throughout the culture period. Other sinking feeds can also be used, as the species is highly adaptable to various feeding situations. During feeding, the species comes to the surface, takes feed, and returns to the bottom immediately. At the end of the 90-day culture period, the study calculated an FCR value of 1.2.

Harvest and yield

In nature, loaches hide in the sand substrate, making harvesting possible only by completely draining the tank water. While draining, the sand substrate is collected to harvest the hiding fish, and then the sand particles are placed back in the tank. In the 90-day grow-out culture, the total harvest was 1,005 g (average length: 4.9 cm, weight: 0.67 g), contributing to a net yield of 621 g using 400 litres of water. The study found that by increasing culture duration and altering feed composition and feeding rate, a net production level of 1 kg can be easily achieved in 400 litres of water while maintaining favourable water conditions. The fish can be sold in live condition at the local market at a reasonable price, earning a profit of Rs. 1,300 from a single tank. Increasing the number of tanks and using vertical platforms in a smaller area may further boost the profit of this fish culture.

Fig. 29. Fabrication of Modified Cages

Fig A: 1-3: Base structure fabrication; 4-6 tank bottom and side covered with iron square mesh size (3 cm); 7: tank side covered with duplex carton; 8: tank covered with polyethylene sheet; 9: substrate provided; 10: water filled; 11: stocking of loach.



8. Food and Feeding

The quality of feed plays a crucial role in the part of successful farming activities. The supplementary feed is one of the essential components in the semi-intensive and intensive system to achieve animal growth performance in specific periods. Intensive aquaculture is mainly reliant on feeding inputs for its fish production. Hence, the feed cost expense is considered the most expensive operational cost in fish farming compared to other operational costs. Because feed ingredient sources are mainly used as by-products of agro-industries. Hence, the ingredient cost is reflected in the overall feed cost. In order to maintain and use the feed efficiently, the feeding methods are essential for fish production.

Nutrition and Feeding practices

Generally, loach fishes are omnivorous; in the wild, it is preferable to consume small insects, plankton, copepods, etc. However, for captivity rearing, specialised feeds are required for their culture. If fish is cultured with more stocking density, then supplementary feeding is necessary to meet the feed demands of the animal. In any culture system, supplementary feed should be provided twice daily, containing 6-7 % of the fish's body weight. If fish weight reaches beyond 1.5 gm, then 2-3% of fish body weight is preferable. However, the application of feeding daily can be decided through frequent growth sampling because this animal takes natural breeding while culture. Significant fish growth can be achieved through best-feed management practices. The supplementary feed should contain a mixture of all macro and micronutrient compositions for better fish growth, especially for loach fish >35% of protein is recommended, and it contributes significantly to fish growth. Farmer trials recommend Groundnut oil

cake (GNOC) and Cotton seed cake (CSOC) with a 60:40 ratio; this feed composition helps to attain quick maturity of fish and breed naturally in the culture system. Hence, the following primary feed ingredients are required to formulate suitable loaches feeds.

Table 5. List of feed ingredients

S. No.	Ingredients	Quantity
1.	Ground nut oil cake (GNOC)	700 gm
2.	Cotton Seed oil cake (CSOC)	200 gm
3.	Vitamin and mineral mixture	10gm
4.	Fish oil	10ml
5.	Fish meal or prawn meal	60 gm
6.	Wheat bran or rice bran	20gm

Proper nutrition profile of feed is crucial for fish health, growth, and welfare. Use species-specific feed that meets all nutritional requirements, including essential amino acids, fatty acids, vitamins, and minerals. Functional ingredients like immunostimulants and probiotics should also be added to enhance health and stress resistance. In addition, feed quality should be regularly checked through proximate and stability tests.

Method of Feeding:

Sufficient feeding is required for significant growth; hence, feeding shall be applied two times (morning and evening) in the culture system. Loaches live in the pond bottom as groups. Thus, one group does not mix up with other groups; therefore, feeding its spread in different places in the pond enables it to receive feed from all fish. The feed must be in crumbled form, with the sinking type most preferable. Avoid feeding during rainy and cloudy times.

Live feed:

The cultivation of live feed and the provision of supplementary diets can establish a balanced feeding ecosystem. Live feed, which includes both phytoplankton and zooplankton, serves as a critical resource in aquaculture. Key freshwater zooplankton species include rotifers, daphnia, and *Moina. L. thermalis* engages in natural breeding upon reaching maturity. Therefore, the availability of live feed is crucial for ensuring the health of fish larvae, as artificial diets alone do not fulfil all necessary nutritional requirements.

Live feed is rich in essential nutrients such as proteins, lipids, carbohydrates, vitamins, minerals, amino acids, and fatty acids. The larval rearing stage represents one of the most precarious

Commented [BJ4]: more specific details on the nutritional components of live feed that make it superior to artificial diets, such as its role in enhancing growth rates or immunity in larvae

phases in fish culture; however, it can also be highly profitable if larvae are adequately nourished with live feed, thereby mitigating mortality risks. Newly hatched larvae begin exogenous feeding once their yolk sac is fully absorbed and their mouth size is less than 0.1 mm. Additionally, they possess a primitive digestive system with low digestibility, factors that are not adequately addressed through artificial diets. Consequently, the enhancement of live feed within the culture pond is paramount.

The application of either inorganic or organic fertilisers can significantly enhance the presence of live feed within pond ecosystems. The balanced application of nitrogen, phosphorus, and potassium (N: P: K) is crucial for the growth of phytoplankton, which serve as primary producers, while zooplankton act as primary consumers. An increase in phytoplankton populations can lead to a rise in zooplankton production. Fertilisation based on the nutrient levels present in the pond is applied to promote plankton



growth effectively. However, excessive application of fertilisers can result in eutrophication, posing severe challenges to the aquatic culture ecosystem. Applying fertilisers every ten days, using organic or inorganic forms, is advisable. Organic fertilisers require time for nutrient dissolution and leaching into the water, whereas inorganic fertilisers release nutrients immediately upon application. Single super phosphate and urea are examples of inorganic fertilisers, while cow dung is an organic fertiliser source.

Fig. 30. Rotifer Culture

Use of Probiotic

After the 21st century, organic farming or sustainable fish production has attracted consumers and producers recently. Nowadays, its techniques are emerging in agriculture and the allied sector. Even in the country, many farmers adopted eco-friendly farming methods by applying fermentative products into the pond to boost fish production. Anaerobic fermentation produces lactic acid bacteria, enhancing the digestibility of available water nutrition and promoting a balanced environment. The probiotic application started almost four decades ago by Kozasa, who made the first empirical application of probiotics in aquaculture—probiotic means used to name microorganisms associated with the

beneficial effects for the host. Evidence shows that probiotics can improve nutrient digestibility, increase stress tolerance, and encourage reproduction. The research of probiotics for aquatic animals is increasing with the demand for environment-friendly aquaculture.

Fermentation:

To maintain the healthy water ecosystem of *L. thermalis*, the fermentation products are applied in the loach culture pond every week. Tamil Nadu inland fish farmers widely adopt and follow the methods of fermentation and application in fish ponds. The following ingredients are used for anaerobic fermentation (table 6).

Table 6. List of ingredient for making fermentation

S. No.	Ingredients	Quantity
1.	COW Urine	10 lit.
2.	Cow dung	10 kg
3.	Jaggery	2 kg
4.	Ground nut oil cake	2 kg
5.	Soil	2 kg

Preparation and use:

The ingredients above and their specified quantities shall combine within the 100-litre cylindrical FRP tanks. It is essential to maintain a shaded area for the fermentation tank. Subsequently, the container must be airtight and mixed twice daily: once in a clockwise direction in the morning and once in an anti-clockwise direction in the evening, continuing this process for 15 to 20 days. Initially, the odour may be unpleasant; however, if fermentation proceeds correctly, the undesirable smell will transform into a pleasant aroma. Once fermentation is complete, the mixture shall be transferred to a container and sealed tightly for future use. An application rate of 1 litre per 20 m³ or 3.5% of total volume is recommended fortnightly for direct use in fish ponds.

9. Water Quality Management

Hill stream fishes of the superfamily Cobitioidea, generally called loaches, are known by more than a thousand species. However, the scientific knowledge of this group is mainly restricted to taxonomy and biology. Very little information exists on any demographical aspects of this group. However, studies attempt to standardise suitable water quality parameters for its culture under captivity. Because water quality is a critical determinant of fish health and productivity within aquaculture systems, each fish species has a specific optimal range of environmental conditions essential for maximising their performance.

Consequently, aquaculture practitioners must maintain water's physical and chemical parameters as closely as possible to these optimal conditions. Deviations from these ideal ranges can lead to suboptimal growth, erratic behaviour, and increased susceptibility to diseases or parasites. In severe instances, prolonged exposure to unfavourable conditions may result in fish mortality.

Pond water comprises two primary categories of substances: suspended particles, which include non-living materials, as well as microscopic flora and fauna, known as plankton, and dissolved substances, which consist of gases, minerals, and organic compounds. The composition of pond water is subject to continuous variation influenced by climatic conditions, seasonal changes, and the specific usage of the pond. Effective management practices aim to regulate this composition to create the most favourable environment for fish. To achieve optimal water quality, fish farmers must thoroughly understand the physical and chemical factors influencing water quality. The success of aquaculture operations is intrinsically linked to water quality management; thus, fish farmers should be well-versed in the principles of water quality management in ponds. Loach fish, in particular, can thrive in slightly acidic,

neutral, and mildly alkaline conditions. The following water quality parameter is suitable for rearing loaches in captivity (table 7).

Table. 7. Suitable water quality for rearing of loaches in captivity

S. No.	Parameters	Favorable Level
1.	Temperature	27 – 29 degree Celsius
2.	Dissolved oxygen	> 4 ppm
3.	pH	6.5 – 8.5
4.	Hardness	165 ppm
5.	Ammonia	0.01 ppm
6.	Nitrate	< 5 ppm
7.	Nitrite	< 0.05 ppm

Water depth

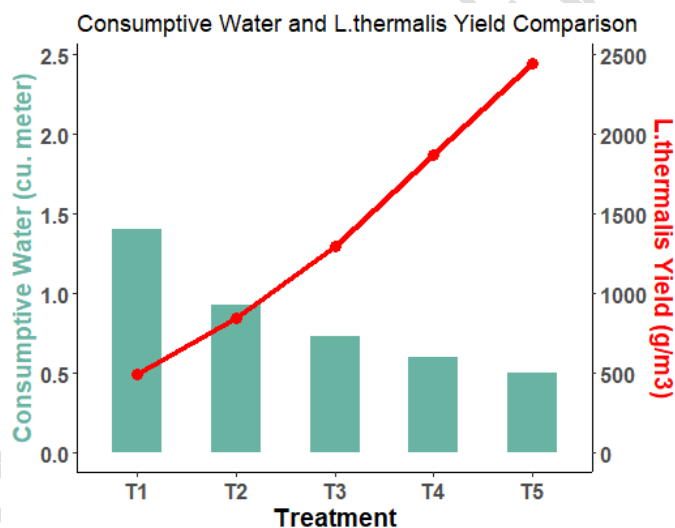
The water depth is considered an essential physical factor in aquaculture because most water variables and primary productivity depend on the water depth. Suppose water depth is maintained low, which may facilitate the formation of a thermocline layer over the surface water and oxygen depletion. In addition, there is a chance of increasing the algal density at the bottom of the pond. Hence, depth plays a vital role in the culture and production of aquatic animals in order to maintain various variables in the pond. For the culture of *L. thermalis* in captivity, the ideal water depth of 0.7m is required during the culture periods under captivity. However, it is significantly less when compared with other freshwater fish cultures. Due to this, during the sunny period, there is a chance for increasing the surface water temperature. To avoid the water temperature hike in the loach culture pond, the floating aquatic weeds can be introduced into the cultured pond; it provides favourable environmental conditions for the fish and helps reduce excess available nutrients. Otherwise, 70% of the shade net can cover the pond with the 8ft. height to avoid direct sunlight penetration.

Water quality deterioration:

L. thermalis lives on the pond bottom and has the habitat of nibbling or feeding continuously before it engulfs. It has been observed that the release and deposition of faecal matter is more concentrated in the pond. The faecal deposition can double at the bottom of the pond if there is more stocking. These faecal and uneaten feeds can highly deteriorate water quality in the loach-reared ponds. Hence, 20% of water exchange is recommended to maintain optimal water quality.

Water budget

The scarcity of water resources poses a significant obstacle to the advancement of aquaculture and the sustainable production of fish (Ferosekhan et al., 2024). Moreover, the unplanned and inefficient water utilisation within the aquaculture sector further restricts its growth potential. Intensive aquaculture practices must prioritise maximising production while minimising water consumption (Mohanty et al., 2014), as fish farming is inherently more water-intensive than traditional agricultural practices (Sharma et al., 2013). Ultimately, the profitability of aquaculture operations is determined by survival rates and growth metrics, which are reflected in production yields. Consequently, the overall net production is closely linked to the stocking density employed. To evaluate the water requirements for *L. thermalis*, a study was conducted over 90 days in 250-litre capacity FRP tanks, utilising various stocking densities of *L. thermalis*. The findings indicated higher stocking densities were associated with reduced water consumption (Fig.31).



During the study, the 20% of water exchange carried over. The water exchange be halted in favour of an appropriate alternative; it is possible to further reduce the consumptive water use associated with the production of *L. thermalis*. Nevertheless, additional comprehensive studies are necessary to explore various culture systems to optimize the

Fig. 31. Consumptive water use for loach production

During the study, 20% of water exchange carried over. If the water exchange is halted in favour of an appropriate alternative, it is possible to further reduce the consumptive water use associated

with the production of *L. thermalis*. Nevertheless, additional, comprehensive studies are necessary to explore various culture systems to optimise the water budget for this species.

Water velocity

The species *L. thermalis* is capable of inhabiting both lotic and lentic environments. Nevertheless, it is more abundantly found in areas characterised by gentle water flow, such as creeks or channels, rather than regions with high-velocity currents. This species demonstrates significant growth potential regardless of water velocity or circulation. In the context of aquaculture, particularly in raceways or recirculating aquaculture systems commonly utilised for cultivating riverine fish, the importance of optimal water velocity cannot be overstated. Our research indicates that among various water velocities tested, a flow rate of 20 cm/s resulted in the highest gonad somatic index (GSI) of 11.24 ± 0.42 , absolute fecundity of 1286.33 ± 96.64 , relative fecundity of 1928.46 ± 35.87 , and an ova diameter of $653.43 \mu\text{m}$. Therefore, a water velocity of 20 cm/s is deemed ideal to achieve optimal maturation and growth performance.

Water quality for breeding

The standards for water quality can differ based on the specific culture and breeding practices employed. *L. thermalis* can reproduce through naturally induced breeding, which can be achieved by adjusting the water temperature. A rapid reduction in water temperature from 29°C to 26°C can trigger the spawning process of *L. thermalis* within a rearing system; however, the optimal water temperature range for this species is between 24°C and 26°C. Additionally, it is essential to maintain a pH level between 7.5 and 8, with alkalinity ranging from 165 ppm to 210 ppm, hardness between 165 ppm and 195 ppm, and concentrations of ammonia, nitrite, and nitrate kept below 0.001 ppm for better breeding performance.

Monitoring and managing water quality is a crucial key to the effective operation of aquaculture enterprises. A fundamental understanding of the necessary water quality parameters is a prerequisite for the successful, sustainable, and profitable implementation of aquaculture practices, as these parameters directly influence the success of the specific culture.

Commented [BJ5]: Explain how these water quality standards compare to those of other freshwater species to highlight *L. thermalis*'s unique requirements. The statement about naturally induced breeding through temperature adjustment is intriguing but could be supported with details about the duration or timing of the temperature shift. Including information on how deviations from these parameters impact breeding success would also enhance the usefulness of the guidelines for practitioners.

10. Packing and Transportation

The transportation of seeds or broodstock from either wild sources or hatcheries to rearing farms represents a crucial phase in intensive aquaculture practices. Historically, individuals utilized earthen pots, known as Hundies, for this purpose. However, the failure to adhere to established scientific protocols has led to significant mortality rates during transport. Young fish exhibit high levels of activity, which can rapidly deplete the available oxygen within the transport containers. Consequently, anaesthetization has been adopted in recent decades to mitigate their activity and maintain their health during transit. Nevertheless, before applying anaesthetics, it is essential to conduct studies to determine the optimal dosage for specific species, as these requirements can differ based on species, size, and the duration of transport.

Parameters to be considered before transportation

Oxygen Requirements

The oxygen demand of spawn, measured in milligrams per gram of body weight, is tenfold that of fry and fingerlings. The tolerance ratio is determined by elevating the oxygen concentration and assessing the carbon dioxide levels present. The oxygen consumption rate is directly related to the

organism's size or body weight. Specifically, if two specimens have the same weight but differ in length, their oxygen consumption rates will remain equivalent.

Ammonia, Oxygen Consumption Rate and Temperature

Spawns can endure a concentration of 2.5 ppm of dissolved free ammonia and 15 ppm of dissolved ammonia in the form of inorganic salts. An increase in ammonia levels leads to reduced oxygen and increased carbon dioxide levels within the bloodstream. Metabolic processes are directly influenced by temperature, with higher temperatures correlating to increased metabolic activity.

Causes of Mortality

During transport, the dissolved oxygen levels in the water decrease while carbon dioxide levels rise. Fish metabolic activities contribute to the accumulation of ammonia, urea, and uric acid in the water, resulting in stress for the fish. High-density transport can exacerbate this stress, potentially leading to mortality. Additionally, using inappropriate vessels may cause physical harm to the fish.

Transportation Methods:

In the country, two primary methods of fish seed transportation are utilized: the open method and the closed method. The open method involves using slings to transport containers along narrow pathways, during which water is continuously splashed or agitated to enhance aeration. This process, however, can elevate stress levels among the seeds, potentially resulting in increased mortality rates. Stressed seeds may exhibit lethargy, rendering them more susceptible to predation and injury. Commonly used containers in this method include earthen pots and aluminium pots reinforced with coir mesh.

Conversely, the closed method utilizes fibreglass containers or plastic bags for transportation. Before collection, the seeds undergo conditioning and oxygenation facilitated by gas cylinders. The containers are filled with a mixture of two-thirds oxygen and one-third water. Food is held to prepare the fish, and they are placed in crowded environments. Subsequently, the plastic bags are secured within light tin containers or cardboard cartons for extended transport via road or rail.

Care before transporting the seeds

- Stop feeding 24 hours before packing.
- First, the seeds are bathed in either potassium permanganate (2-3 ppm) or common salt (0.3%) for a few hours.
- Conditioning to reduce the mortality rate during transportation
- Seeds should not be handled with bare hands.
- Late evening is the best time for transportation (excellent hours). Pack the fish in cool, clean water.
- When transporting the seeds, avoid direct sunlight falling into the packaging bags.
- Check the plastic bags for any leakage.
- Put pieces of newspaper between the bags, the wall, and the bottom.
- To prepare the packing, fill them with water sourced from the location where the seeds were collected. The seeds should be placed in a plastic bag that is one-third filled with water and two-thirds filled with oxygen, then securely tied with string to ensure proper containment.
- Loach seeds with a size of 2-3 cm can be transported in plastic bags with oxygen under pressure, with 1200 - 1500 pieces in a bag with 6-6.3 l of water and 14 l of oxygen under pressure for more than 12 hours of transportation. However, the loach species is a facultative air-breathing fish, so it can tolerate less oxygen for a long duration of transport.



Fig. 32. Packing of seeds

Determination of quantity of fish:

Quantity of fish seeds for transportation depends on their size, mode duration of transportation, salinity of the medium and the ambient temperature. Formula to find the number of fish seeds:

$$N = (DO-2) \times V / C \times h$$

Where.

DO= dissolved oxygen in ambient water in mg/l

V= volume of water in litres.

C= rate of oxygen consumption by the individual fish (mg/hr).

h= duration of transportation (hr).

Anaesthetizing:

Anaesthetics play a crucial role in aquaculture during fish transportation, reducing stress caused by handling and travel. Stress in fish can lead to immune suppression and physical injuries. Anaesthetics are used to lower metabolic activity for transportation in aquaculture, requiring rapid induction and recovery to minimize stress. Various anaesthetics like MS222, Quinaldine, benzocaine, 2-Phenoxyethanol, Aqui-Stm, metomidate, carbon-di-oxide, and clove oil are used, where dosage and exposure duration determine the achieved stage. Fish may initially become hyperactive during induction and recover to a normal state. Quick induction and minimal handling reduce recovery times. Recovery duration varies based on species and drug used, ranging from minutes to hours. Fish are typically immersed in anaesthetic baths for absorption through the gills, and water quality is crucial for handling large numbers. Larger fish need higher anaesthetic concentrations, and some drugs may have prolonged effects, especially in larger or gravid fish. Weakened or diseased animals are more sensitive to anaesthetic treatment. Since there are no studies available for *L. thermalis*. Hence, the experiment was conducted with different dosages, sizes, and temperatures to determine the sedation to the normal state of *L. thermalis*. Clove oil is used as an anaesthesia agent in these studies because it is inexpensive. Various behaviour changes are recorded in seconds (table. 9).

Table.8. phase of physiological response caused by anaesthesia (Hamackova et al., 2004).

	Phase	Characteristics
Anaesthesia induction	0	Physiological position, normal locomotor activity

	I	Physiological position, increased locomotor activity
	II a	Decreased locomotor activity, slight tilting on the flank
	II b	Flank position; immobilization
	III	Breathing stopped
Recovery from anaesthesia	II b	Flank position; immobilization
	II a	Uncoordinated locomotion, signs of physiological position
	I	physiological position, decreased locomotor activity
	0	Physiological position, normal locomotor activity

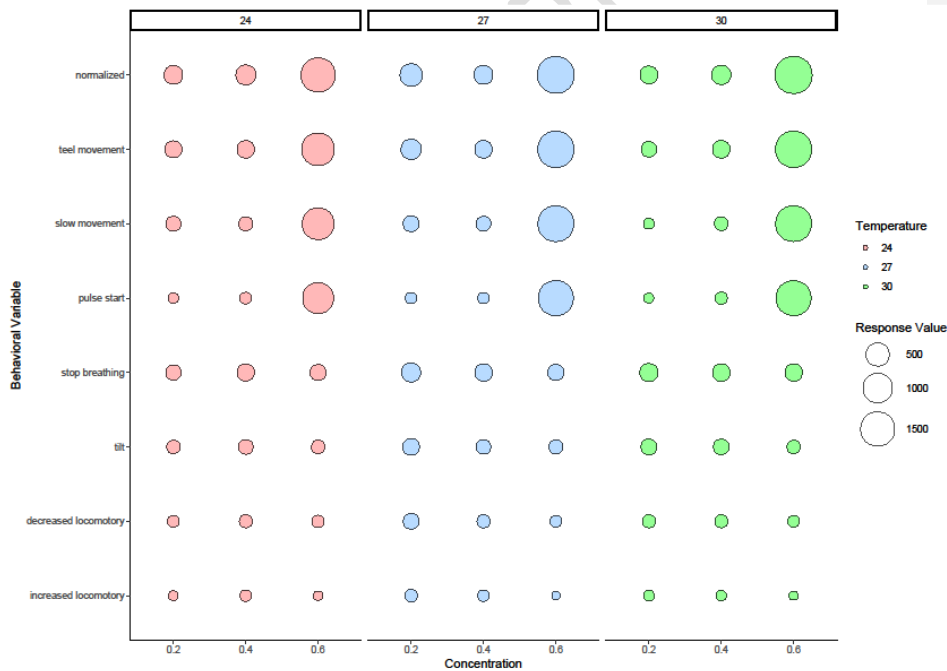


Fig. 33. Effect of various concentration of anesthetic against 2.5 -3 cm size of *L. thermalis*

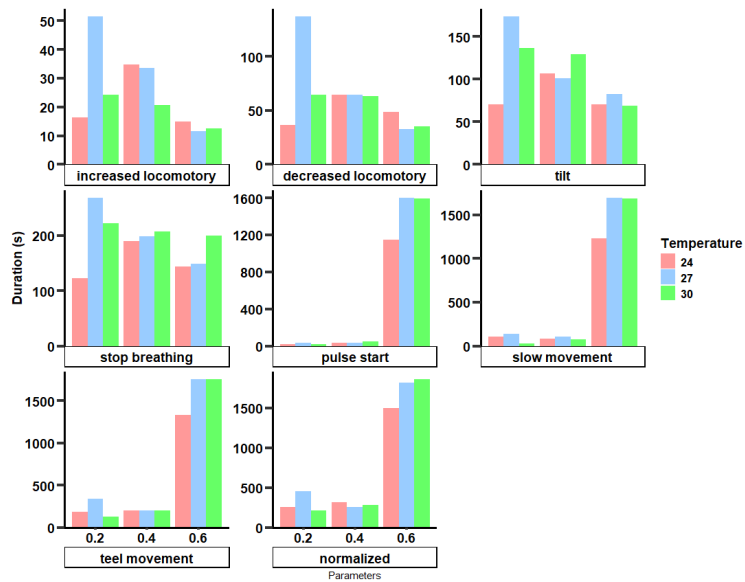


Fig.34. Effect of various concentration of anesthetize against behaviour changes

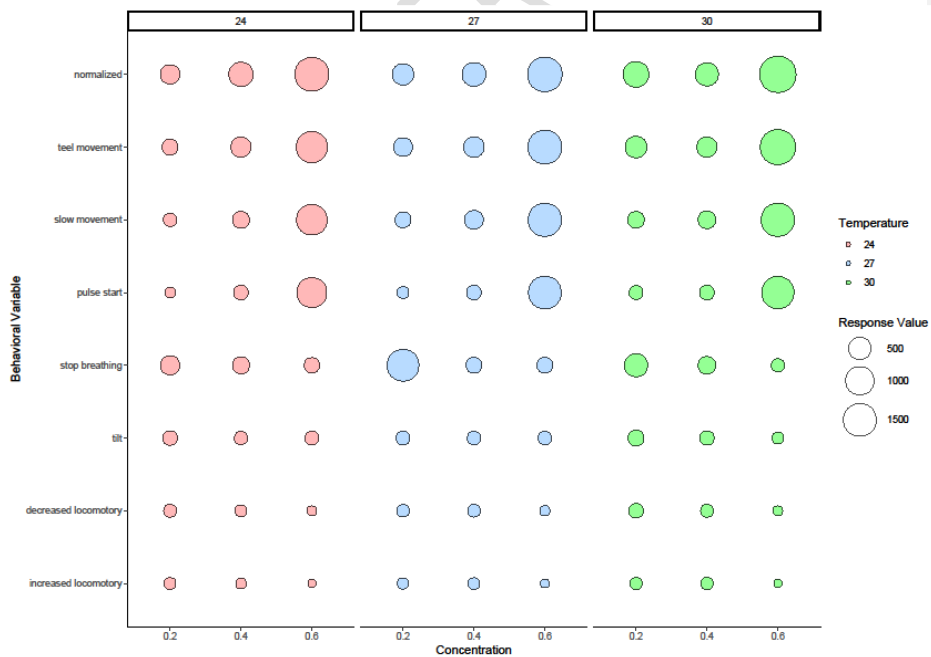


Fig.35. Effect of various concentration of anesthetize against 3-5 cm size of *L. thermalis*

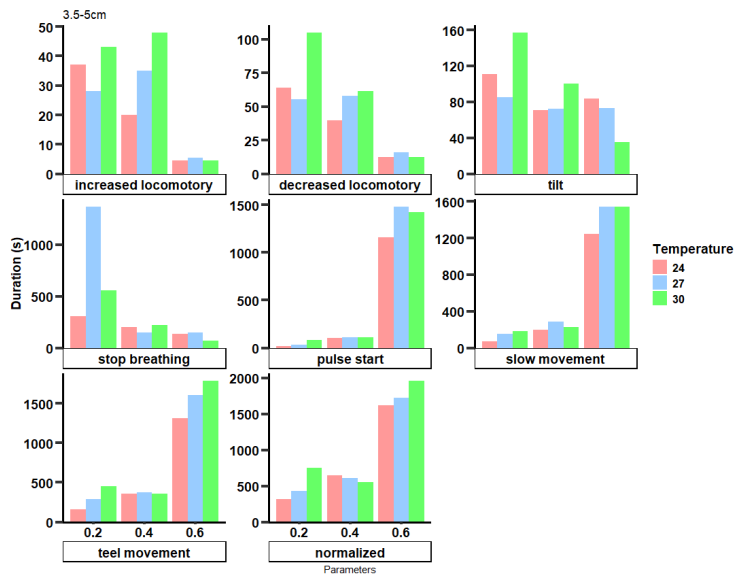


Fig. 36. Effect of various concentration of anesthetize against behaviour changes

Table 9. Anesthetize effect on Loach species with different concentration, size and temperature of clove oil.

Main effect	Sedation				Recovery			
	Increased Locomotion	Decreased Locomotion	Tilt Movement	Stop Breathing	Pulse Start	Slow Movement	Tilt Movement	Normalized
I. Dose (conc.)								
0.2 ml	25.48 ± 2.6 ^b	56.95 ± 2.26 ^b	94.85 ± 6.19 ^b	405.60 ± 106.9 ^a	25.63 ± 22.34 ^c	86.51 ± 29.9 ^c	191.9 ± 27.02 ^c	304.0 ± 25.5 ^c
0.4 ml	31.98 ± 2.6 ^a	58.65 ± 2.26 ^a	96.75 ± 6.19 ^a	195.40 ± 106.9 ^b	73.91 ± 22.34 ^b	161.35 ± 29.9 ^b	282.2 ± 27.02 ^b	443.35 ± 25.5 ^b
0.6 ml	8.9 ± 2.6 ^c	26.16 ± 2.26 ^c	69.13 ± 6.19 ^c	140.0 ± 106.9 ^c	1400.8 ± 22.34 ^a	1491.2 ± 29.9 ^a	1590.0 ± 27.02 ^a	1745.0 ± 25.5 ^a
II. Temperature								
24 °C	21.25 ± 2.6	44.33 ± 2.6	85.55 ± 6.1	183.6 ± 106.9	414.87 ± 22.3 ^c	488.78 ± 29.9 ^c	590.65 ± 27.02 ^c	774.20 ± 25.5 ^c
27°C	23.21 ± 2.6	49.26 ± 2.6	82.7 ± 6.1	357.9 ± 106.9	546.53 ± 22.3 ^a	641.8 ± 29.9 ^a	731.95 ± 27.02 ^b	844.35 ± 25.5 ^{ab}
30°C	21.9 ± 2.6	48.16 ± 2.6	91.48 ± 6.1	199.50 ± 106.9	538.95 ± 22.3 ^b	608.48 ± 29.9 ^b	741.70 ± 27.02 ^a	874.30 ± 25.5 ^a
III. Size								
2 - 3.5cm	21.58 ± 2.1	53.12 ± 1.8 ^a	94.80 ± 5.06 ^a	174.23 ± 87.3	500.86 ± 18.2	564.88 ± 24.4	658.33 ± 22.06	746.60 ± 20.8 ^b
3.5 -5cm	22.66 ± 2.1	41.38 ± 1.8 ^b	79.02 ± 5.06 ^b	319.76 ± 87.3	499.86 ± 18.2	594.48 ± 24.4	717.86 ± 22.06	915.30 ± 20.8 ^a
Interaction effect	P Value				P Value			
Dose x temperature	0.778	0.137	0.092	0.479	<0.001	0.004	<0.001	0.002
Dose x size	0.132	0.003	0.343	0.168	0.054	0.034	0.045	0.005
Size x temperature	0.605	0.980	0.052	0.368	0.712	0.987	0.712	0.926
Temp x dose x size	0.035	0.002	0.246	0.426	0.688	0.641	0.583	0.274

Values expressed as mean ± SEM. Means with the superscript letter in the columns are significantly different (P < 0.05).

In these results (table 9), we determined the effect of clove oil concentration, temperature and size for the sedation and recovery of loach fishes. As clove oil, concentration increased sedation time decreased and recovery time increased. In addition, recovery time showed increased with increase of dose and temperature. Due to less sedation time and recovery time has recorded in 0.2 ml in 24°C for the both size 2-3.5 and 3 -5 cm of loach, hence it is the ideal concentration for handling of loach species. Between Temperature, size and dose does not have any statistical effect but dose with size and temperature (dose x temperature; dose x size) had a significant effect on the treatments.

UNDER PEER REVIEW

11. Nutritional Quality

Understanding the nutrient composition of major food items is a valuable tool for examining the linkage between food production, nutrient admission, and nutrient consumption, as well as for designing initiatives, policies and programs, such as the development of improved production technologies. Besides, fish serves as a good source of protein, omega fatty acids and vitamins & minerals, which are available at affordable prices for human consumption. In general, marine fish have high amounts of EPA and DHA rather than freshwater fish. In addition, the nutrient profile may vary from natural to culture and raw or cooked meat. Hence, knowing the nutrient profile for different prospects of certain fishes are very much essential. *L. thermalis* Indian spiny loach grows very small, it is categorized under IUCN least concern but it has more nutritional and subsistence food fishes in some parts of Tamil Nadu. Large-size fish have less edible mineral nutrients than small fishes. However, rural people mostly consume this kind of small fish. Despite low meat yield, many consumers do not prefer to consume small tiny fishes.

Commented [BJ6]: The statement about rural consumption patterns is valuable but could be supported with examples or statistics to illustrate the socioeconomic factors influencing these preferences

The nutritional composition of *L. thermalis* raw and cooked was studied (Manoharan et al. 2017) and reported that 33.09% (saturated) 32.86% (monounsaturated), 30.49% (polyunsaturated fatty acids), glycine (715 mg/100 g), histidine (375 mg/100 g), alanine (237 mg/100 g) and arginine (130 mg/100 g). Minerals are calcium (924 mg/100 g), zinc (0.14 mg/100 g), iron (1.86 mg/100 g). Boiled fish had reduced levels of glycine (410 mg/100 g) and alanine (124 mg/100 g) further this author stated that no difference in the fatty acids between boiled and raw fish.

The *L. thermalis* culture technology standardized in our institute for the first time in the country. In addition, we analysed fatty acid profile and proximate composition of cultured *L. thermalis* and the results depicted in table (10 & 12).

Table 10. Proximate analysis of cultured (captivity) of *L. thermalis* (weight of 0.4gm).

S. No.	Variable	Cultured fish (0.4gm)
Proximate composition (%)		
1.	Moisture	74.49 ± 0.353
2.	Fat	2.67 ± 0.049
3.	Protein	20.56 ± 0.219
4.	Ash	1.29 ± 0.035

This proximate composition is lesser when comparable with wild collected Indian spiny loach because (Manoharan et al. 2017) reported 44.15 ± 0.45 of crude protein in raw wild caught *L. thermalis* with size of 0.75gm and this species collected from the vaigai river region of Sholavandhan, (10.0167N, 77.9667E) (table 11). Sex, age, nutrition availability, catch season etc. might be influence the varying of protein content of fishes.

Table. 11. Morphometric variables and proximate analysis in raw and boiled *L. thermalis* (Manoharan et al., 2017)

S. No.	Variable	Raw fish	Boiled fish
Morphometry			
1.	Length (cm)	5.82 ± 1.63	5.69 ± 1.47
2.	Width (cm)	2.11 ± 1.02	1.98 ± 0.09
3.	Weight (g)	0.75 ± 0.23	0.67 ± 0.19
Proximate composition (%)			
1.	Moisture	72.18 ± 0.28	70.95 ± 0.56
2.	Crude Fat	11.13 ± 0.10	10.42 ± 0.12
3.	Crude Protein	44.15 ± 0.45	45.24 ± 0.30
4.	Carbohydrate	32.54 ± 1.25	31.56 ± 1.17
5.	Ash	12.18 ± 0.7	11.78 ± 0.75
6.	Energy value (kcal/100g)	~ 406.93	~ 404.98

Table. 12. Macro and micro mineral composition of *L. thermalis* (Manoharan et al., 2017)

S. No.	Minerals (mg/100 g of fish)	Raw fish	Boiled fish
1.	Calcium	924.99 ± 0.01	911.03 ± 0.07
2.	Phosphorus	44.52 ± 0.07	43.21 ± 0.01
3.	Sodium	42.25 ± 0.02	39.20 ± 0.01
4.	Potassium	221.03 ± 0.17	220.96 ± 0.03
5.	Iron	1.86 ± 0.01	1.69 ± 0.07
6.	Magnesium	96.0 ± 0.04	93.79 ± 0.01
7.	Sulphur	1.1 ± 0.01	0.79 ± 0.04
8.	Zinc	0.14 ± 0.07	0.12 ± 0.14
9.	Selenium	0.004 ± 0.04	0.003 ± 0.02
10.	Copper	0.07 ± 0.02	0.03 ± 0.04
11.	Manganese	0.15 ± 0.28	0.16 ± 0.57

Table. 13. Fatty acid profile of cultured *L. thermalis*.

S. No.	Fatty acid (g/100g of lipid)	
Saturated fatty acid		
1.	Lauric acid (C12:0)	1.2
2.	Myristic acid (C14:0)	3.6
3.	Pentadecanoic acid (C15:0)	1.2
4.	Palmitic acid (C16:0)	29.2
5.	Heptadecanoic acid (C17:0)	1.2
6.	Stearic acid (C18:0)	7.2
7.	Heneicosanoic acid (C21:0)	0.08
8.	Lignoceric acid (C24:0)	1.6
Mono Unsaturated Fatty acids		
9.	Myristoleic acid (C14:1 n-5)	0.8
10.	Palmitoleic acid (C16:1)	13.6
11.	Heptadecanoic acid (C17:1)	0.8
12.	Oleic acid (C18:1 n9c)	24
13.	Eicosenoic acid(C20:1n9)	1.6
Poly Unsaturated Fatty Acids		
14.	Linoleic acid (C18:2n6c)	2.8
15.	Alpha linolenic acid (C18:3 n3)	1.2
16.	Eicosadienoic acid (C20:2)	0.4
17.	dihomo-gamma-linolenic acid (C20:3n6)	0.8
18.	Eicosatetraenoic acid (C20:4 n3)	2
19.	Eicosatrienoic acid (C20:3n3)	0.4
20.	Eicosapentaenoic acid (C20:5 n3)	1.6
21.	Docosahexaenoic acid(C22:6 n3)	2
Fatty Acid Groups and Ratios		
	Σ SFA	46
	Σ MUFA	40.8
	Σ PUFA	11.2
	Σn3 PUFA	7.2
	Σn6 PUFA	4
	n3/n6	1.8
	SFA/PUFA	4.11
	SFA/MUFA	1.13

The fatty acid composition of cultured *L. thermalis* was analysed and the values are shown for the first time in Table (14). The fatty acid composition can be influenced by various environmental factors, such as the type of feed available for fish consumption and also by environmental factors. This is also evident in the earlier reports of (Manoharan et al., 2017) because the recorded fatty

acids contents more in the wild caught than culture condition. This small indigenous fish has a certain amount of SFA, MUFA and PUFA. Fatty acids are essential for human daily life and fatty acid groups can potentially reduce the risk of cardiovascular and nervous diseases. This small native fish serves as a nutrient-rich food for people who eat it regularly.

Commented [BJ7]: •it could be improved by clarifying and expanding key points. For example, specify the exact levels or types of SFA, MUFA, and PUFA found in cultured *L. thermalis* compared to wild-caught fish to emphasize the nutritional value and differences.
•the inclusion of Table 14 should be accompanied by a brief summary of its key findings for better integration into the text.

UNDER PEER REVIEW

12. Economics in loach fish culture

The economic aspects of aquaculture can be analyzed at both micro and macro levels, providing a comprehensive understanding of its impact on the industry and society. Microeconomics delves into the management strategies and operational factors that influence efficiency at the farm level. This includes examining how individual farmers make decisions regarding resource allocation, production techniques, and cost management to maximize their output and profitability. Key elements such as the choice of species to cultivate, feeding practices, and disease management are critical in determining the operational success of aquaculture farms.

On the other hand, macroeconomics takes a broader view, evaluating the social benefits and costs associated with aquaculture projects. This perspective considers how aquaculture contributes to food security, job creation, and economic development at the national or regional level. It also assesses aquaculture practices' environmental impacts and sustainability, which can have far-reaching implications for communities and ecosystems. In cases where aquaculture demonstrates significant social advantages—such as improving nutrition and providing livelihoods—but struggles to attract private investment, public support mechanisms become essential. These mechanisms may include credit assistance to help farmers access necessary capital, marketing initiatives to promote aquaculture products, extension services to disseminate knowledge and best practices, training programs to enhance skills, and research funding to drive innovation and improve production techniques. Such support is particularly crucial during the initial phases of development when risks are higher, and returns on investment may not be immediately apparent.

The significance of economic analysis in aquaculture cannot be overstated, as it serves as a foundation for decision-making by individual farmers and the formulation of effective aquaculture policies. Policymakers rely on robust economic data to understand the sector's dynamics, identify challenges, and design interventions that promote sustainable growth. Consequently, there is a pressing need to enhance the quality and availability of economic data for analysis. This includes collecting comprehensive information on production costs, market prices, and the economic contributions of aquaculture to local and national economies.

Fisheries occupy a distinctive position within a nation's economic framework and livelihoods. Fish is a high-value commodity that plays a vital role in food systems. They provide diverse income sources for individuals, communities, nations, producers, and processors alike. However, a systematic approach to assessing fish profitability is often lacking, hindering the sector's growth and

Commented [BJ8]: illustrating how specific choices, such as cultivating a particular species or adopting advanced feeding practices, have improved profitability for individual farmers would make the argument more tangible.

sustainability. Applying scientific methods and effective management practices can lead to substantial profitability in fish farming, enabling farmers to optimize their operations and achieve better financial outcomes.

Fish culture involves essential cost elements that require meticulous management to achieve profitability. These elements consist of both fixed costs and variable inputs. A determination of profitability or failure in this farming practice can only be made through an evaluation of net returns. Therefore, conducting an economic assessment is imperative for cultivating any fish species. *L. thermalis* was raised and cultivated in lined earthen ponds over four months in this context. The descriptive economic analysis of this species has been conducted and presented in the accompanying table.

Economic analysis

Based on the results obtained in the following culture, ~~economic evaluation (operational) of the lined earthen pond were projected for the intensive culture of *L. thermalis* by using the calculation of (Sontakke and Haridas, the economic evaluation (operational) of the lined earthen pond was projected for the intensive culture of *L. thermalis* using the calculation of Sontakke and Haridas (2018).~~

- Total production (in kg) = Number of animals X Average weight ÷ 1000
- Feed requirement = Total production (in Kg) X FCR
- Total profit = Total production (in kg) x Cost of fish (in Rs.)
- Net profit = Total profit – Expenditure

Table.14. Economic evaluation was done by projecting the values obtained in the Experiment

S. No.	Title	Description
1.	Species cultured	Indian spiny loach (<i>L. thermalis</i>)
2.	Water spread area or pond size	40 m ²
3.	Avg. depth	06.-0.7 m
4.	Advisable Stocking	102 gm/ m ²
5.	Culture period / crop duration	3-5 months
6.	Cost of seed	1 kg/1500
7.	Cost of feed	Rs. 60/kg
8.	Expected Biomass harvest.	600-800 gm/ m ² in over of 5 months
9.	Selling price/ market price	Rs. 1500-2000 /kg

Table.15. Cost estimation for total area of one pond (40m²)

S. No.	Particulars	Total Amount (Rs.)
A. Capital cost		
1.	Earth work excavation and construction of bund	5000.00
2.	Pond liner per sq.ft Rs.6/- (435 sq.ft.)	2610.00
3.	Miscellaneous	2000.00
Subtotal (A)		9610.00
B. Operational cost		
5.	Probiotic 20 lit used (own made) Rs.12.5/lit	250.00
6.	Feed cost @ Rs. 60/kg for 42 kg	2520.0
7.	Seed cost @ Rs. 50 paisa/seed for 4kg	3000.00
8.	Manpower @ Rs. 8000/Month for managing four ponds, the economic is performed here single pond it may taken 2000/ Month for 5 months	10000.00
9.	Miscellaneous charge	2500.00
Subtotal (B)		18270.00
C. Total Expenditure (A+B)		27880.0
D. Production @ 24 kg (Selling price @1500/kg)		36000.00
E. Net profit (D-C)		8120.00

(* Based on field ~~finding, there might be a possibility for the increase of total production with extend of culture duration~~-findings, there might be a possibility of increasing total production with an extended culture duration.)

Economics for grow-out in modified tanks:

The main assumption used in the development of ~~budget for 125 poly-lined cages in 50m²(each 25 row/column) model for loach farming in the~~ budget for 125 poly-lined cages in a 50m²(each 25 row/column) model for loach farming in the micro-pond system for one production cycle. Cost and price information is given in Indian rupee and the values are as of February 2024.

Table.16. Component wise cost of loach farming

S. No.	Particulars	Amounts (in Rs.)
A. Fixed cost		
1.	Cage fabrication cost* (125 cages)#	625000

2.	Poly lined sheet*	100000
	Total fixed cost	725000
B. Variable cost		
i	Loach fingerlings cost – Rs. 0.50 paisa/seed)*	100000
ii	Feed cost (approx. Rs. 80/kg)*	7500
iii	Labor charge one person @8000/month	24000
iv	Harvesting and miscellaneous charges*	5000
	Subtotal operational cost	136500
C.	Total operational cost	861500
D.	Break – even price	5 years

(*Based on author experience as conducted experiment from one cage; # incase if we use low cost material or bag used for cage fabrication, then the total fixed cost can reduced up to 90%).

Table.17. Economics of loach farming (cage system – 50 m²)

Profitability measurements	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6
Total cost	861500	685100	508700	332300	155900	146000
Yield* (kg/62cages)	248 kg	248 kg	248 kg	248 kg	248 kg	248 kg
Cost of production (Rs./kg)	3473.7	2762.5	2051.2	1339.9	628.6	588.7
Average price	1300	1300	1300	1300	1300	1300
Gross income	322400	322400	322400	322400	322400	322400
Net income	-539100	-362700	-186300	-9900	166500	176400
Monthly income	0	0	0	0	13875	14700

(*the loach initially stocked 500g per cage (125 cages), produced 1kg/cage and from the harvest half of the net yield (500g) used for rearing in next production cycle, so that investment for seeds cost can be removed (partial harvest and partial stock). Therefore, the yield calculated for only 62 cages).

Conclusion:

The rapidly growing aquaculture industry faces numerous challenges that will likely impact future fish production. Simultaneously, the industry must provide solutions, employing upcoming technologies to meet the demands of the growing population. These challenges include the need to address nutritional

requirements, double farmer income, efficiently utilise water resources, and conserve and diversify aquatic species, the economics of which are discussed in the economics chapter.

The Indian spiny or true loach emerges as a promising candidate for future aquaculture, given its various advantages. It requires less water and land, involves lower investment with higher profitability and increased production, demands minimal cultural management, aligns with regional and local market preferences, boasts a high nutritional profile, lacks strong spines, has a shorter culture duration, and is highly suitable for vertical farming. Consequently, this single species addresses the pressing challenges the aquaculture industry faces. This foundational study serves as a stepping-stone for farmers in our country to initiate pilot farming of this species. Moreover, it paves the way for adopting diverse cultural practices for this fish. The developed modified tank-based rearing system for loach proves highly beneficial for loach culture in hilly regions. It is particularly suitable for areas with limitations in land and water resources.

Being an indigenous ornament and food fish, the Indian spiny loach has a more outstanding market demand in the local Indian market. However, the demand is met only through the wild collection of these fish from natural resources. Wild collection, coupled with burgeoning anthropogenic threats to natural ecosystems, especially aquatic ecosystems, significantly threatens the biodiversity of this fish in wild conditions, placing immense pressure on the captive breeding and seed production of this species. Additionally, fish farmers have a huge demand for this species to carry out commercial farming practices. Therefore, it is the right time to explore the breeding and seed production of *Lepidocephalichthys thermalis* under captive conditions. The KKPCeSA has succeeded for the first time in testing both artificial and naturally induced breeding and their rearing techniques in the Indian spiny loach. However, further detailed studies on the standardization of different breeding methods are required for commercial seed production to meet the farmers' demands.

13. Reference

1. Acharjee, M.L., Barat, S., 2014. Loaches of Darjeeling Himalaya and Adjoining areas of West Bengal: their Prospects as Ornamental Fish and Constraints 7.
2. Arthur, R., Friend, R., Béné, C., 2015. Social benefits from inland fisheries: implications for a people-centred response to management and governance challenges, in: Craig, J.F. (Ed.), *Freshwater Fisheries Ecology*. John Wiley & Sons, Ltd, Chichester, UK, pp. 500–512. <https://doi.org/10.1002/9781118394380.ch39>.

Commented [BJ9]: the passage effectively highlights the potential of the Indian spiny loach as a sustainable and profitable candidate for aquaculture while addressing critical industry challenges. However, the discussion would benefit from more specific examples of the technologies and methods used in the modified tank-based rearing system and their scalability. Additionally, while the economic and environmental advantages of farming the loach are well-presented, a clearer linkage between these benefits and broader aquaculture goals, such as doubling farmer income or water conservation, could strengthen the argument. The mention of KKPCeSA's success in breeding techniques is promising but would be more impactful with detailed results or data to support its effectiveness. Lastly, emphasizing how these efforts align with conservation goals to mitigate biodiversity threats from wild collection would make the narrative more cohesive and compelling.

3. Arunkumar, L., 2000. Loaches of the genus *Lepidocephalichthys*, from Manipur, with description of a new species. *Journal of Fish Biology* 57, 1093–1104. <https://doi.org/10.1111/j.1095-8649.2000.tb00473.x>.
4. Bohlen, J., 2003. Temperature and oxygen requirements of early life stages of the endangered spined loach, *Cobitis taenia* L. (Teleostei, Cobitidae) with implications for the management of natural populations. *Archiv für Hydrobiologie* 195–212. <https://doi.org/10.1127/0003-9136/2003/0157-0195>.
5. Boron, A., Juchno, D., 2019. Loaches of *Cobitis* - fish with high potential for hybridization and subsequent polyploidization. How to determine the “potential for hybridization”? Does it exist? *Front. Mar. Sci.* 6. <https://doi.org/10.3389/conf.fmars.2019.07.00029>.
6. Caleta, M., Buj, I., Mrakovcic, M., Mustafic, P., Zanella, D., Marcic, Z., and Katavic, I., (2015). Endemic of Croatia. *Communities, Cyprinidae. Bijdragen tot de Dierkunde*, 33-35.
7. Cooke, S.J., Twardek, W.M., Lynch, A.J., Cowx, I.G., Olden, J.D., Funge-Smith, S., Lorenzen, K., Arlinghaus, R., Chen, Y., Weyl, O.L.F., Nyboer, E.A., Pompeu, P.S., Carlson, S.M., Koehn, J.D., Pinder, A.C., Raghavan, R., Phang, S., Koning, A.A., Taylor, W.W., Bartley, D., Britton, J.R., 2021. A global perspective on the influence of the COVID-19 pandemic on freshwater fish biodiversity. *Biological Conservation* 253, 108932. <https://doi.org/10.1016/j.biocon.2020.108932>.
8. Das, P.C., Kamble, S.P., Velmurugan, P., Pradhan, D., 2019. Evaluation of minor carps intercropping in carp polyculture vis-à-vis other grow-out cropping patterns of carp farming. *Aquac Res* 50, 1574–1584. <https://doi.org/10.1111/are.14034>.
9. Das, P.C., Sahoo, P.K., Kamble, S.P., Murmu, K., Basudha, C., 2020. Compatibility of pengba, *Osteobrama belangeri* (Valenciennes) with Indian major carps and evaluation of its ideal incorporation level in carp polyculture system in planes of India. *Aquaculture* 518, 734845. <https://doi.org/10.1016/j.aquaculture.2019.734845>.
10. Ferosekhan, S., Velmurugan, P., Radhakrishnan, K., Kamble, S., Bairwa, M. K., Sahoo, S.K. & Das, P. C., (2024). Water budgeting for production of hatchlings, fry and fingerlings of Asian catfish, *Clarias magur*. *Aquaculture*. 583. 740620. [10.1016/j.aquaculture.2024.740620](https://doi.org/10.1016/j.aquaculture.2024.740620).
11. Gosavi, S.M., Tapkir, S.D., Kumkar, P., Verma, C.R., Kharat, S.S., 2020. Act now before its too late: Copper exposure drives chemo-ecology of predator-prey dynamics of freshwater common spiny loach, *Lepidocephalichthys thermalis* (Valenciennes, 1846). *Environmental Research* 186, 109509. <https://doi.org/10.1016/j.envres.2020.109509>.
12. Hamackova, J., Lepicova, A., Kozák, P., Stupka, Z., Kouril, J. L. P., (2004). The efficacy of various anaesthetics in tench (*Tinca tinca* L.) related to water temperature. *Czech Journal of Veterinary Medicine*. 49. 467-472. [10.17221/5741-VETMED](https://doi.org/10.17221/5741-VETMED).
13. Havird, J.C., Page, L.M., 2010. A Revision of *Lepidocephalichthys* (Teleostei: Cobitidae) with Descriptions of Two New Species from Thailand, Laos, Vietnam, and Myanmar. *COPE* 2010, 137–159. <https://doi.org/10.1643/CI-08-240>.

14. Keskar, A., Kumkar, P., Paingankar, M.S., Padhye, A., Dahanukar, N., 2015. Length-weight and length-length relationships of seven loach species (Teleostei: Cypriniformes) from five localities in northern Western Ghats, India. *J. Threat. Taxa* 7, 8025–8220. <https://doi.org/10.11609/jott.2462.7.15.8025-8220>.
15. Keskar, A., Raghavan, R., Kumkar, P., Padhye, A., Dahanukar, N., 2017. Assessing the sustainability of subsistence fisheries of small indigenous fish species: fishing mortality and exploitation of hill stream loaches in India. *Aquat. Living Resour.* 30, 13. <https://doi.org/10.1051/alr/2016036>.
16. Kharat, S.S., Dahanukar, N., 2013. Population dynamics of the Hill Stream Loach *Acanthocobitis moored* (Sykes, 1839) (Cypriniformes: Nemacheilidae) from northern Western Ghats of India. *J. Threat. Taxa* 5, 4562–4568. <https://doi.org/10.11609/JoTT.o3301.4562-8>.
17. Kottelat, M., 2012. *Conspectus cobitidum: An inventory of the loaches of the world* (Teleostei: Cypriniformes: Cobitoidei). *The Raffles Bulletin of Zoology Suppl.* 26, 1–199.
18. Langeland, J. and C. B. Kimmel. 1997. The embryology of fish. In S. F. Gilbert and A. M. Raunio (eds.), *Embryology: Constructing the Organism*. Sinauer Associates, Sunderland, MA, 383–407.
19. Manoharan, S., Kuppu, R., Uthandakalaipandian, R., 2017. Bioprospecting the Anti-microbial Properties of *Lepidocephalus thermalis* (V.). *Journal of Biologically Active Products from Nature* 7, 270–277. <https://doi.org/10.1080/22311866.2017.1351890>.
20. Manoharan, S., Kuppu, R., Uthandakalaipandian, R., 2019. Deciphering the morphological and molecular characteristics of fresh water fish *Lepidocephalus thermalis* (V.) — DNA barcode approach. *INDIAN J EXP BIOL* 7.
21. Mohanty, R., Kumar, A., A.Mishra, Panda, D., Patil, D., 2014. *Water Budgeting and Management: Enhancing Aquacultural Water Productivity*.
22. Paul, B., Chanda, A., 2017. A study on small indigenous freshwater fish under family Cobitidae Swainson, 1838 from Paschim Medinipur, West Bengal, India 6.
23. Prasad, G., Ali, A., Harikrishnan, M., Raghavan, R., 2012. Population dynamics of an endemic and threatened Yellow Catfish *Horabagrus brachysoma* (Günther) from Periyar River, southern Western Ghats, India. *Journal of Threatened Taxa* 2333–2342.
24. Rasal, A., Patnaik, m., Murmu, K., Mohanty, R.K., Sundaray, J.K., Swain, j.k., Mahapatra, K.D., (2023). Climate smart carp hatchery: Bringing resilience to sustainable fish seed production. *Aquaculture*. 571. 739476. [10.1016/j.aquaculture.2023.739476](https://doi.org/10.1016/j.aquaculture.2023.739476).
25. Renuhadevi, M., Ahilan, B., Cbt, R., Padmavathy, P., Jeevagan, I., Prabu, E., 2019. Evaluation of Optimum Protein Requirement for Indian Spiny Loach (*Lepidocephalus thermalis*). *International Journal of Current Microbiology and Applied Sciences* 8, 1650–1657. <https://doi.org/10.20546/ijcmas.2019.807.196>.

26. Rita Kumari SD and Balakrishnan Nair N (1979) Oogenesis in a tropical loach *Lepidocephalus thermalis* (Cuv. & Val.). *J. Anim. Sci.* 88:45-54.
27. S.d, R.K., N.b, N., 1978. Toxicity of some insecticides to *Lepidocephalus thermalis* (Cuv. and Val). *Proceedings of the Indian National Science Academy.*
28. Sharma, K.K., Mohapatra, B.C., Das, P.C., Sarkar, B., Chand, S., 2013. Water budgets for freshwater aquaculture ponds with reference to effluent volume. *AS* 04, 353–359. <https://doi.org/10.4236/as.2013.48051>.
29. Sontakke, R., & Haridas, H., (2018). Economic Viability of Biofloc Based System for the Nursery Rearing of Milkfish (*Chanos chanos*). *International Journal of Current Microbiology and Applied Sciences.* 7. 2960-2970. 10.20546/ijcmas.2018.708.314.
30. Sundarabarathy, T.V., Edirisinghe, U., Dematawewa, C.M.B., Nandasena, K.G., 2001. JVIorphology and Some Biological Aspects of Common Spiny or Lesser Loach (*Lepidocephalichthys thermalis*) and Banded Mountain or Spotted Loach (*Schistura notostigma*) of Sri Lanka. *trop agriculture research* 13, 413–420.
31. Tang, J.-X., Li, J.-R., Liu, Z.-L., Zhao, H., Tao, X.-M., 2013. Effects of Zn²⁺ and Cu²⁺ on loach ovaries and ova development. *Zoological Research* 34, 5.
32. Tapkir, S.D., Kharat, S.S., Kumkar, P., Gosavi, S.M., 2017. Effects of the invasive Tilapia on the Common Spiny Loach (Cypriniformes: Cobitidae: *Lepidocephalichthys thermalis*) - implications for conservation. *J. Threat. Taxa* 9, 10642–10648. <https://doi.org/10.11609/jott.2220.9.9.10642-10648>.
33. Welcomme, R.L., Cowx, I.G., Coates, D., Béné, C., Funge-Smith, S., Halls, A., Lorenzen, K., 2010. Inland capture fisheries. *Philosophical Transactions of the Royal Society B: Biological Sciences* 365, 2881–2896. <https://doi.org/10.1098/rstb.2010.0168>.