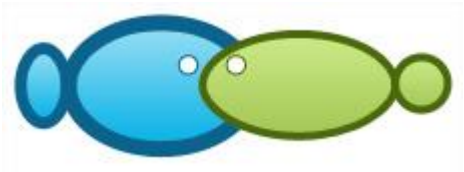

Growth performance, whole-body composition and histological evaluation of the Indian spiny loach *Lepidocephalichthys thermalis* (Valenciennes, 1846) under captive culture conditions

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Abstract. The current research assessed the performance of the Indian spiny loach *Lepidocephalichthys* thermalis in captivity to establish foundational data on growth, nutritional status, and tissue health. The fish were raised on a specially formulated diet, and their growth performance, feed efficiency, body composition, and histological features were evaluated. The formulated diet showed appropriate proximate composition and satisfactory pellet quality. The fish exhibited consistent growth, with a specific growth rate of 1.07±0.29% day^{-1} and a high survival rate of 91.11±3.85%, indicating successful adaptation to the captive environment. The whole-body composition analysis showed high protein and moderate lipid levels, reflecting a favourable nutritional status. Histological examinations of liver, intestine, muscle, and ovary tissues revealed normal cellular structures, including intact hepatocytes, well-formed mucosal folds, dense muscle fibers, and vitellogenic follicles, confirming physiological health and reproductive development in captivity. These findings confirm the feasibility of culturing *L. thermalis* and provide crucial baseline data for future nutritional and domestication research. The study underscores the potential of this native species for diversification and sustainable development in freshwater aquaculture.
Keywords: captive culture, growth performance, histology, *L. thermalis*, proximate composition.

Introduction. A wide ichthyofaunal variety is supported by India's abundant freshwater resources, which include rivers, lakes, streams, reservoirs, and floodplain wetlands. Approximately 2,300 fish species have been reported from Indian waters, of which more than 765 are freshwater species (Ayyappan et al 2006). Among them, small indigenous fishes are important for rural livelihoods and local nutrition because of their high nutritional content, availability, and adaptability (Roos et al 2003; Mohanty et al 2019). However, the expansion of aquaculture has been concentrated on major and exotic carp species, which has led to ecological issues and the loss of biodiversity (Singh & Lakra 2011; Joshi et al 2021). Therefore, aquaculture diversification and biodiversity conservation depend on the development of sustainable culture methods for local fish species.

Loaches are small freshwater fishes distributed throughout Asia, belonging to the order Cypriniformes (Nelson et al 2016). Because of their capacity to adapt to many environments, attractive colouration, and high nutritional content, they are valued as both decorative and food fish. The Cobitidae family includes the Indian spiny loach, *Lepidocephalichthys thermalis* (Valenciennes, 1846), which is native to Sri Lanka and peninsular India (Talwar & Jhingran 1991). Locally known as "Ayirai meen" in Tamil Nadu, it is valued for its flavour and nutritional significance (Velmurugan et al 2025).

In terms of ecology, *L. thermalis* is found in ponds, paddy fields, shallow channels, and slow-moving river edges. It is usually found on substrates that are muddy or sandy (Pethiyagoda 1991). As an omnivorous bottom feeder, it eats organic debris, algae, benthic invertebrates, and detritus (FishBase 2019). The species' nutritional value as a food and

ornamental fish is increased by the high-quality protein, vital fatty acids, vitamins, and minerals that it contains (Mohanty et al 2019; Velmurugan et al 2025).

Aquaculture of *L. thermalis* is still underdeveloped despite its ecological and economic significance, and present demand is mostly satisfied by capture fisheries, which puts pressure on wild populations (Tapkir et al 2017). One of the main obstacles to its domestication is the absence of consistent culture and feeding practices (Renuhadevi et al 2019; Chaudhari et al 2023). Furthermore, wild populations are threatened by overfishing, habitat degradation, and the introduction of exotic species (Tapkir et al 2017; Nobinraja et al 2023). Thus, the development of captive culture methods is crucial to the preservation and long-term use of this native species.

In this regard, the goal of the current study was to investigate growth performance, nutritional status, and tissue health in order to determine if *L. thermalis* could be cultured under regulated laboratory settings. Future domestication initiatives and sustainable freshwater aquaculture diversification may be aided by the establishment of baseline culture data.

Material and Method. The goal of the current study was to investigate the growth performance, feed consumption, and viability of captive culture of *L. thermalis* in a controlled laboratory setting at the Zoology department lab at Madras Christian College, Tambaram, Tamil Nadu, India, during the period from December 2024 to June 2025.

Experimental fish and acclimatization. Juvenile *L. thermalis* (Figure 1) were sourced from local fishermen in Vathalagundu, a town panchayat in Dindigul District, Tamil Nadu, India. Prior to research, fish were transported to the laboratory in aerated containers and allowed to acclimate for 20 days under controlled conditions. The Zoological Survey of India (ZSI), Southern Regional Center, Chennai, India, verified taxonomic authenticity and species identification. The authenticated identification report was issued under reference No. F.12-1/92-Tech/194 dated 10 February 2026.

Figure 1. Representative specimen of *L. thermalis* used in the captive culture experiment showing external morphology and total length measurement.

Experimental system and culture conditions. The experimental procedure lasted for 60 days and was conducted in three replicates under a completely randomized design. Each tank contained 15 fish, resulting in a total of 45 fish with an average initial length of 3.38±0.05 cm and an average initial weight of 0.121±0.020 g. The fish were stocked in 20L capacity plastic cylindrical containers (Figure 2) in a static water system. Small stones were placed in each tank as substrate to mimic natural conditions. The photoperiod was kept at 12h light:12h dark, constantly throughout the trial. Tap water was used, which was dechlorinated prior to use and the temperature was maintained between 26.8 and 28.5°C. Water was exchanged once a week to maintain water quality.

Figure 2. Culture setup.

Formulated diet and feeding regime. A formulated sinking pellet diet was prepared in the laboratory as sinking pellets using locally available ingredients (Figure 3). The ingredients were dried, finely ground, and sieved to obtain a uniform particle size before being thoroughly mixed according to the feed formulation. Sunflower oil and warm distilled water were gradually added to form a dough-like consistency. The dough was pelletized using a hand-operated pelletizer, and the pellets were oven-dried at 60°C until a constant weight was achieved. The dried pellets were cooled, packed in airtight polyethylene bags, and stored at 4°C until use. The composition of the formulated diet (g kg^{-1}) is presented in Table 1. The diet was supplemented with chromium oxide (5 g kg^{-1}) as an inert marker as part of standardized feed formulation practice for future digestibility assessment (Davies & Gouveia 2006). Fish were fed at a rate of 5% of body weight per day, offered in two equal meals (morning and evening). Uneaten feed was collected and removed after each feeding session using a siphon.

Figure 3. Preparation of experimental diets for *L. thermalis*

Table 1

Composition of experimental diet (g kg^{-1}) of *L. thermalis*

Diet ingredients	*Inclusion (g kg^{-1})*
Dry fish	400
Soybean	200
Shrimp	50
Wheat flour	150
Rice flour	100
Refined wheat flour (maida)	50
Sunflower oil	30
Chromium oxide	5
Premix[a]	15
Total	1000

[a]Premix: Amino Power tonic containing vitamins, amino acids, and minerals (composition expressed per 100 mL; Growel Agrovet, India): Vitamin A, 80,000 IU; Vitamin B1, 250 mg; Vitamin B2, 25 mg; Vitamin B6, 200 mg; Vitamin B12, 1.5 µg; Vitamin D3, 5,800 IU; Vitamin E, 375 mg; Vitamin K, 50 mg; Pantothenic acid, 150 mg; Choline, 50 mg; Biotin, 0.2 mg; Folic acid, 0.2 mg; Niacinamide, 250 mg; Inositol, 25 mg; Methionine, 750 mg; Lysine, 400 mg; Histidine, 105 mg; Arginine, 156 mg; Aspartic acid, 805 mg; Threonine, 305 mg; Serine, 318 mg; Glutamic acid, 478 mg; Proline, 96 mg; Betaine, 350 mg; Glycine, 712 mg; Peptides, 200 mg; Nucleotides, 200 mg; Alanine, 235 mg; Cysteine, 100 mg; Valine, 215 mg; Leucine, 356 mg; Isoleucine, 225 mg; Tyrosine, 295 mg; Phenylalanine, 211 mg; Tryptophan, 55 mg; Zinc sulphate, 120 mg; Yeast extract, 500 mg; Sodium chloride, 155 mg; Magnesium sulphate, 65 mg; Manganese sulphate, 125 mg; Sodium bicarbonate, 125 mg; Calcium hypophosphate, 40 mg; Copper sulphate, 150 mg; Potassium chloride, 100 mg; Selenium, 50 mg; and Sodium citrate, 100 mg.

Experimental diet physical characteristics. Before initiating the feeding trial, the physical characteristics of the experimental diet were evaluated to determine pellet quality and stability (Khater et al 2014; Rawski et al 2020; Zulhisyam et al 2020). Pellet durability was assessed using the pellet durability index (PDI) method by gently tumbling a known quantity of pellets and determining the percentage of intact pellets after agitation. Bulk density was determined by measuring the weight of pellets occupying a known volume and expressed as g L^{-1}.

Water stability of the experimental feed pellets was evaluated by immersing a known weight of pellets in water for a fixed period, after which the remaining pellets were dried and weighed to calculate percentage stability. Floatability was determined by observing the proportion of pellets remaining afloat after water immersion. Organoleptic properties such as colour, texture, and structural integrity were visually assessed to ensure suitability for feeding under captive conditions.

Water quality parameters. Throughout the feeding trial, the water quality parameters were consistently monitored to maintain appropriate conditions for the experimental fish. Water temperature and pH were monitored daily using a digital thermometer and standard pH indicator strips, respectively.

In accordance with standard water quality evaluation methods (APHA 2017), chemical parameters such as nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+) were examined at 10-day intervals. Additionally, dissolved oxygen (DO) levels were monitored daily to ensure sufficient oxygen availability for the fish

During the experimental phase, the water quality parameters were consistently maintained within the acceptable range for freshwater fish cultivation. Partial water exchange (50%) was performed every 10 days to sustain optimal water quality.

Health monitoring.Fish were monitored daily for abnormal behaviour, external lesions, or signs of disease. Observed mortalities were recorded and removed at once so as to avoid deteriorating the water quality of the affected system.

Growth performance and feed utilization. Growth performance was determined by recording the individual body weight using a scientific electronic weighing balance and total length using a standard measuring scale. The growth and feed efficiency of the *L. thermalis* were assessed after a 60-day feeding experiment using established methods in

aquaculture nutrition research (Cho & Kaushik 1990; Tacon et al 2009; Hua 2021). At the end of the experiment, fish from each tank were collectively weighed after a 24-hour fasting period to reduce the impact of gut contents on their weight. Survival rates were monitored daily for the duration of the experiment. The following growth performance indices were calculated:

Weight Gain (WG):

$$WG = \text{Final mean body weight (g)} - \text{Initial mean body weight (g)}$$

Percentage Weight Gain (% WG):

$$\%WG = \frac{\text{Final body weight (g)} - \text{Initial body weight (g)}}{\text{Initial body weight (g)}} \times 100$$

Specific Growth Rate (SGR):

$$SGR\ (\%\ day^{-1}) = \frac{\ln(\text{Final body weight}) - \ln(\text{Initial body weight})}{\text{Experimental period (days)}} \times 100$$

Feed Conversion Ratio (FCR):

$$FCR = \frac{\text{Total feed intake (g)}}{\text{Total weight gain (g)}}$$

Protein Efficiency Ratio (PER):

$$PER = \frac{\text{Weight gain (g)}}{\text{Protein intake (g)}}$$

Survival Rate (%):

$$\text{Survival rate (\%)} = \frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100$$

Proximate composition analysis. Representative samples of experimental diets and whole fish were analysed for proximate composition following the standard procedures described in the Official Methods of Analysis of AOAC International (AOAC 2016). Moisture content was determined by oven drying at 105°C until constant weight, crude protein was estimated using the Kjeldahl method (N × 6.25), crude lipid was determined by Soxhlet extraction, Crude Fiber by Acid–Alkali Digestion Method and ash content was measured by combustion in a muffle furnace at 550°C. Gross energy content of the experimental diet was determined using an adiabatic bomb calorimeter and expressed on a dry matter basis.

Histological analysis. Three fish from each experimental group were randomly collected at the end of the culture period for histological examination. Fish were euthanized by immersion in ice-cold water, and tissue samples including liver, intestine, muscle, and ovary were carefully dissected and immediately fixed in 10% neutral buffered formalin for 24-48 h (Bancroft & Gamble 2008). The fixed tissues were dehydrated through a graded ethanol series (70%, 80%, 90%, and absolute ethanol), cleared in xylene, and embedded in paraffin wax.

Tissue sections of approximately 5 µm thickness were prepared using a rotary microtome and mounted on glass slides. The sections were stained with hematoxylin and eosin (H&E) following standard histological procedures (Roberts 2012). Prepared slides were examined under a light microscope, and representative photomicrographs were captured for structural observation and documentation.

Statistical analysis. All data were expressed as mean±standard deviation (SD). Statistical calculations were performed using Statistical Package for the Social Sciences (SPSS) version 26.0.

Results. The results of the proximate composition of the experimental diet are presented in Table 2. The composition of the *L. thermalis* diet was examined based on dry matter basis.

Table 2

The proximate composition of the Indian spiny loach diet is shown as a percentage of dry matter basis (%) (n = 3)

Proximate composition	*Value (mean±SD)*
Crude protein (%)	32.33±0.84
Crude lipid (%)	7.71±0.06
Crude fibre (%)	1.25±0.01
Crude ash (%)	17.50±0.40
Moisture (%)	10.81±0.16
Gross energy kcal kg^{-1}	3821

The physical characteristics of the diets, including pellet durability index (PDI), bulk density, water stability, floatability, expansion ratio, and organoleptic acceptability, are presented in Table 3. Organoleptic assessment indicated that the experimental diet exhibited acceptable colour, odour, and texture, with good overall pellet appearance and acceptability.

Table 3

Physical characteristics of Indian spiny loach diet (n = 3)

Physical characteristics	*Value (mean±SD)*
Pellet durability index (PDI) (%)	97.31±1.19
Bulk density (g L^{-1})	114.14±0.91
Water stability (%)	62.67±2.31
Floatability (%)	0.67±1.15
Expansion ratio (%)	1±0.00
Organoleptic acceptability	Good

Values are expressed as mean±SD (n = 3). Organoleptic assessment was based on pellet colour, odour, texture, and overall acceptability.

Water quality parameters such as temperature, pH, dissolved oxygen, ammonium, nitrite, and nitrate were monitored throughout the experimental period and are presented in Table 4. Nitrogenous waste parameters, including ammonium, nitrite, and nitrate, remained within acceptable limits for freshwater fish culture (Boyd & Tucker 1998). Overall, the recorded water quality values indicated stable rearing conditions suitable for *L. thermalis* during the feeding trial.

Table 4

Water quality parameters recorded during the feeding trial of Indian spiny loach (n = 3)

Water parameters	*Value (mean±SD)*
Temperature (°C)	26.8±0.25
pH	7.4±0.2
Dissolved oxygen (DO) (mg L^{-1})	4.00±0.13
Ammonium (NH_4^+) (mg L^{-1})	0.20±0.01
Nitrite (NO_2^-) (mg L^{-1})	0.10±0.00
Nitrate (NO_3^-) (mg L^{-1})	5.09±0.16

The growth performance and feed utilization parameters of *L. thermalis* reared under captive conditions are presented in Table 5. A high survival rate was observed, suggesting successful adaptation of the species to captive rearing conditions.

Table 5

The growth performance and feed utilization of the Indian spiny loach during the experimental period (n = 3)

Parameter	*Value (mean±SD)*
Weight gain (WG) (g)	0.11±0.04
Length gain (LG) (cm)	0.11±0.08
Specific growth rate (SGR) (% day^{-1})	1.07±0.29
Percentage weight gain (PWG)	97.52±33.78
Feed conversion ratio (FCR)	5.39±4.88
Protein efficiency ratio (PER) (%)	1.19±0.41
Survival rate (SR) (%)	91.11±3.85

The whole-body proximate composition of *L. thermalis* following the culture period is presented in Table 6. The values obtained indicate a satisfactory nutritional condition of fish maintained under captive culture conditions.

Table 6

Whole-body proximate composition (%) of Indian spiny loach under captive culture conditions (n = 3)

Proximate composition	*Value (mean±SD)*
Crude protein (%)	60.79±0.31
Crude lipid (%)	11.52±0.23
Crude ash (%)	23.16±0.42
Moisture (%)	78.51±0.22

Histological examination of selected organs of *L. thermalis* reared under captive conditions revealed normal tissue organization (Figure 4).

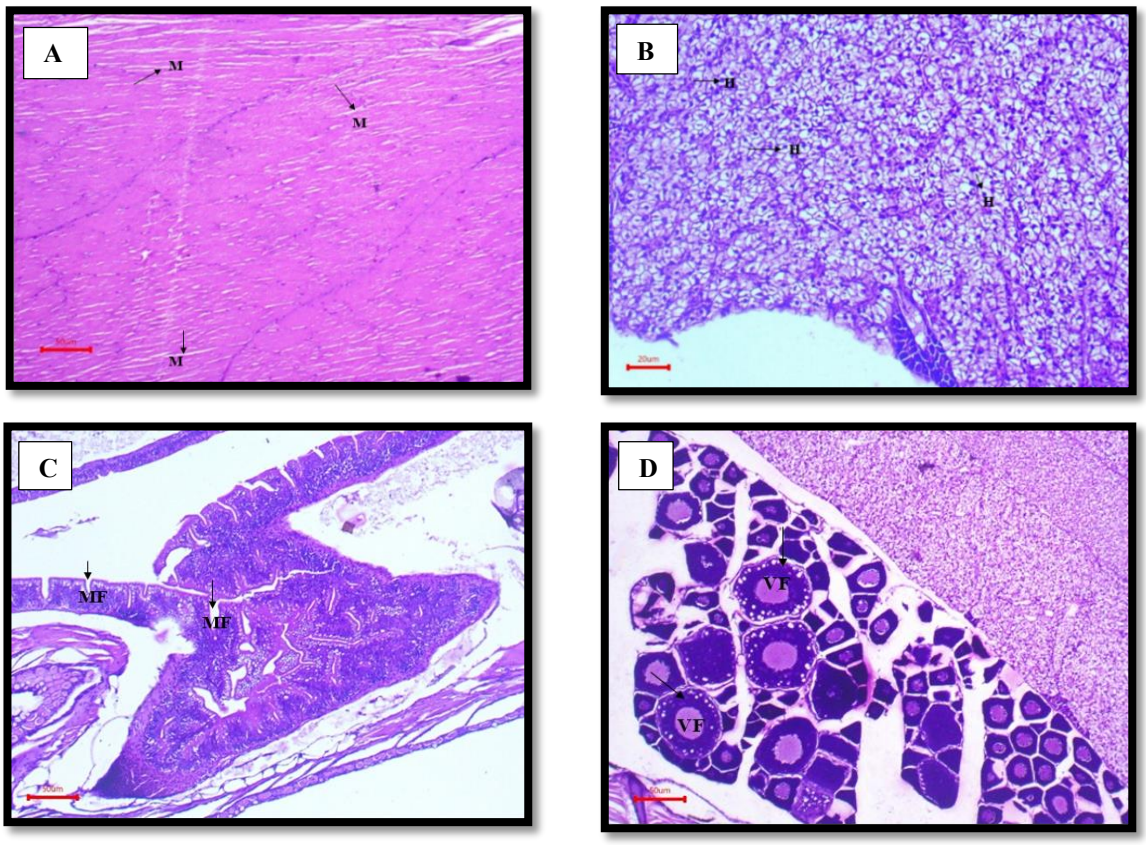

Figure 4. Histological sections of experimental fish tissues stained with hematoxylin and eosin (H&E): (A) Muscle tissue showing normal muscle fibers (M); (B) Liver tissue showing hepatocytes (H) and sinusoidal spaces; (C) Intestinal section showing mucosal folds (MF); (D) Ovary showing developing oocytes and vitellogenic follicles (VF). Scale bars: A = 50 µm; B = 20 µm; C = 50 µm; D = 50 µm.

Liver sections showed well-arranged hepatocytes with centrally located nuclei and no cellular degeneration, indicating a normal metabolic condition. Intestinal tissues exhibited intact mucosal folds and epithelial lining, suggesting proper digestive structure and adaptation to the formulated diet. Muscle fibres appeared compact and uniformly arranged without signs of structural damage. Ovarian sections displayed well-developed vitellogenic follicles, indicating normal gonadal development and reproductive health under captive culture conditions. Overall, histological observations confirmed that the captive rearing environment maintained the fish's normal physiological condition.

Discussion. The survival rate of 91.11±3.85% recorded in the present study demonstrates that *L. thermalis* can successfully adapt to captive laboratory conditions, which is a prerequisite for developing any culture protocol for this species. Survival is a primary indicator of culture suitability, reflecting the combined influence of environmental stability, stress tolerance, and dietary acceptance (Boyd 2015). The water quality parameters recorded during the study, including temperature, pH, dissolved oxygen, ammonium, nitrite, and nitrate, remained within acceptable ranges for freshwater fish culture (Boyd & Tucker 1998), thereby supporting the health and survival of the experimental fish. Velmurugan et al (2024) reported comparatively higher survival rates of 96-98% in pond-based systems, and the marginal difference from the present study is understandable given that static laboratory units lack the larger water volume and substrate availability of pond environments. Nonetheless, survival exceeding 91% in a confined static system firmly establishes the species resilience under captive management, an observation further supported by Nguyen & Tran (2025), who recorded 91.11-93.33% survival in loach fish under formulated diet trials.

Growth performance, expressed as an SGR of 1.07±0.29% day^{-1} and a PWG of 97.52±33.78%, is consistent with the inherently slow growth biology of benthic loach species that allocate energy conservatively due to their bottom-dwelling ecology (Jobling 1994). Velmurugan et al (2024) recorded a weight increase from 0.24 to 0.67 g over 90 days in *L. thermalis* grow-out trials, which is broadly comparable to the present growth trajectory when accounting for the longer culture duration and higher temperature (28°C) in that study. Gargotra et al (2024) further demonstrated that selenium supplementation at 0.35 mg kg^{-1} significantly improved weight gain to 0.39±0.08 g in *L. thermalis* over 60 days, suggesting that micronutrient enrichment of the present diet could further enhance growth responses in future trials.

The FCR of 5.39±4.88 and PER of 1.19±0.41 are characteristic of wild-sourced native fish during early domestication, where behavioural adaptation to artificial feeds constrains feed intake and digestion efficiency (De Silva & Anderson 1995). Renuhadevi et al (2019) identified 35% crude protein as the optimum level for *L. thermalis*, yielding the best FCR and growth responses; the present diet contained 32.33% protein, slightly below this threshold, which likely contributed to the elevated FCR. Velmurugan et al (2024) reported a more favourable FCR of 1.2 using a supplementary diet over 90 days, reflecting the combined benefits of a longer acclimation period, larger fish size, and higher culture temperature. These comparisons indicate that optimizing dietary protein toward 35% would meaningfully improve feed utilization in future studies.

Whole-body proximate composition revealed a crude protein content of 60.79± 0.31% and crude lipid of 11.52±0.23%, indicating satisfactory nutritional status and effective protein deposition from the formulated diet. These values are consistent with published body composition data for *L. thermalis*, which report approximately 44% crude protein and 11% crude fat on a fresh weight basis (Velmurugan et al 2025; Manoharan et al 2019). The absence of excessive lipid accumulation confirms that the 7.71% dietary lipid level was nutritionally appropriate without inducing adiposity (De Silva & Anderson 1995), while the moderate ash content of 23.16% reflects adequate skeletal mineralization under the present dietary regime (NRC 2011).

Histological examination of all sampled tissues confirmed normal cellular architecture and physiological health throughout the culture period. Liver sections showed well-arranged hepatocytes with centrally positioned nuclei and no evidence of vacuolation or necrosis, confirming normal metabolic function and dietary adequacy (Roberts 2012).

Intestinal tissues displayed intact mucosal folds with a well-developed epithelial lining, indicating successful digestive adaptation to the formulated diet and efficient nutrient absorption capacity, consistent with observations in fish maintained on nutritionally balanced feeds (Buddington et al 1997). Compact and uniformly arranged muscle fibres further confirmed normal somatic growth during the culture period.

Ovarian sections containing well-developed vitellogenic follicles confirmed that active gonadal development was maintained throughout captive rearing, which is a significant finding for future breeding programme development. Gargotra et al (2024) documented multiple oocyte development stages in captive *L. thermalis* and showed that dietary selenium supplementation maximized gonadosomatic index (24.17±3.40%) and absolute fecundity (4827.7 eggs/g body weight), highlighting the influence of broodstock nutrition on reproductive outcomes. Velmurugan et al (2025) further demonstrated that temperature reduction from 26 to 22°C significantly enhanced final gamete maturation rate to 63.24±4.26% in captive *L. thermalis*, confirming the species' reproductive responsiveness under controlled manipulation. The presence of vitellogenic oocytes in the present study, without any hormonal or environmental induction, confirms that captive rearing under standard laboratory conditions did not suppress the normal progression of oogenesis in this species.

Overall, the results of the present study are in close agreement with published findings on *L. thermalis* captive culture and confirm that this indigenous species can be successfully reared on a formulated sinking pellet diet under laboratory conditions. The survival rate, body composition, and normal tissue histology collectively validate the physiological suitability of the captive environment, while growth and feed utilization data identify dietary protein optimization and extended culture duration as primary targets for improvement in future studies. These baseline findings provide an important foundation for advancing the nutritional standardization, domestication, and sustainable aquaculture development of *L. thermalis* in India.

Conclusions. The current research offers foundational data on the performance of *Lepidocephalichthys* thermalis in captivity under controlled laboratory settings. The species demonstrated high survival rates, consistent growth, and effective feed utilization, indicating successful adaptation to captive conditions and acceptance of formulated diets. The whole-body proximate composition showed a satisfactory nutritional status, while histological analysis of liver, intestine, muscle, and ovarian tissues confirmed normal structural integrity and physiological health. The presence of vitellogenic follicles further indicates that the captive environment supported normal reproductive development. Overall, the results underscore the feasibility of culturing *Lepidocephalichthys thermalis* and provide crucial reference data for future studies on nutrition, digestibility, and domestication. The findings suggest that this native species holds potential for diversification and sustainable development in freshwater aquaculture.

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Conflicts of Interest. The authors declare that there is no conflict of interest.

Data Availability. The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethical Approval. Ethical approval was not required for this study as it involved routine aquaculture husbandry practices and observational analyses.

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