

A preliminary study on the growth performance and length-weight relationship of *Lepidocephalichthys thermalis* reared in two different pond conditions

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Abstract

The commercial rearing technique for Indian spiny loach is yet to be standardized. Therefore, a 45-days preliminary study was undertaken in two different pond conditions i.e., lined pond without sand substratum (treatment-1) and lined pond with sand substratum (treatment-2) to evaluate the growth performance and length-weight relation of *Lepidocephalichthys thermalis*. For this, loach fish (2.0 - 2.3 cm and 0.006 – 0.009 g) were collected from natural collection sites and, after proper acclimatization, they were stocked in the prepared experimental ponds. In each pond, 250 number of loach fish were stocked at the early morning and feed with supplementary feed at *ad libitum* level. At the end of trial, significant difference was found in growth performance of fishes reared in two different pond conditions, except survival rate. Significantly higher final weight (0.5253 ± 0.002 g), specific growth rate ($10.80 \pm 0.01\%$ /day), weight gain (0.52 ± 0.002 g), weight gain percentage ($7280.82 \pm 32.12\%$) and gross biomass (76.70 ± 0.81 g) were recorded in the treatment-2 reared fish. Survival rate was in the range of 96 ± 0.67 to $98 \pm 0.67\%$ between two groups. The calculated equation for LWR of loaches as follows, $W = 0.00153 * L^{5.561}$, $r^2 = 0.9806$ and $W = 0.000124 * L^{5.708}$, $r^2 = 0.9823$ for fish reared in treatment-1 and treatment-2, respectively. Between the two treatments, the b value was found to be highest in treatment-2 reared fish. Further, the results of this study showed positive allometric growth in both treatments. The k factor value of was recorded lower (0.57) in treatment-1 group. On the other side, the lined pond provided with sand substratum exhibited higher k factor and it was recorded as 0.75. Overall, the present preliminary study results indicate that *L. thermalis* can be cultured in a controlled environmental pond conditions with sand substratum. However, further studies are needed to improve farming practices with different farming systems and conditions.

Key words: Indian spiny loach; Earthen Pond, Growth performance; Monoculture; Native species.

Introduction

Development of farming techniques for rearing of native fish species is more desirable than introducing of nonnative species for Indian aquaculture culture. In many occasions, introductions of new species had leads to many risky circumstances which includes environmental pollution and threat to native species biodiversity (Lal *et al.*, 2023; Yue *et al.*, 2023). On the other side, certain small indigenous fishes are gradually reaching an extinct phase from the nature environment and very little interest has been placed on their farming compare to culture of major carps by the farmers (Mazumder *et al.*, 2014). Hence, in future aquaculture applications should direct

towards the suitable mitigating measure for sustainable fish production without jeopardizing the environment (Diana *et al.*, 2013).

In general, small indigenous fish are rich in micromineral (calcium, phosphorus) and trace element (copper, zinc, selenium, iodine, magnesium, iron, cobalt and chromium) which plays a significant role in rural folk nutrition. In the past, there is no culture practices for small indigenous fishes and they were considered as weed fish or trash fishes. In present days, they are used in poly culture system for effective ponds fish production (Mohanty *et al.*, 2013; Mandal and Chanda, 2017). Generally, aquaculture required large area and more water

for its production. The population increase subsequently elevated the demand for water and food across the globe. On the other hand, it directly placed a pressure on the aquaculture industry for more production in a sustainable manner. The limitation of land and freshwater availability are heavily affecting the successful expansion of land-based aquaculture industry (Avnimelech *et al.*, 2008). Concurrently, less attention has given to land-based culture of small potential indigenous fishes, despite their rich nutrition, high market price and demand (Bhutia *et al.*, 2021). Additionally, lack of breeding technique is also affecting the culture expansion of small indigenous fishes.

Generally, small indigenous fishes can be cultured in monoculture as well as in polyculture systems. These species are growing very small in size, thus they require less water and area for its culture. A small indigenous fish can grow in a range of 7.5 cm to 25 cm Mohanty *et al.*, 2013; Mandal and Chanda, 2017; Mandal and Nandi, 2018 In this context, the *Lepidocephalichthys thermalis* considered as a remarkable indigenous freshwater fish which is used for food as well as for ornamental purpose. In general, it lives in river, stream and shallow freshwater bodies. Additionally, its endemic to India and Sri Lanka and it has ethnic flavor and rich nutritional value. However, their induced seed production and culture technique are yet to be standardized (Gargotra *et al.*, 2023). This fish

has been categorized under least concern category as per IUCN (Chaudhari *et al.*, 2022), therefore there is a possibility for natural stock enhancement through culture practices. Hence, to explore the aquaculture potential of this species, the present preliminary study investigated the culture possibility of Indian spiny loach under controlled earthen pond system.

Material and methods

Experimental site and fish collection

This study was conducted at Kanyakumari Parakkai Centre for Sustainable Aquaculture, Kanyakumari Dt, Tamil Nadu, India. The *Lepidocephalichthys thermalis* were collected from the various collection sites located in and around the Kanyakumari District (Table 1). The fish were collected by operating the drag net opposite to the water flow of canal or stream. After collection from the wild, immediately, the fish were placed in a separate tank (18 L) filled with oxygenated water for transportation. Prior to transportation, the tank was filled with fine sand (prefer the sand taken from collection site) and water from the wild collection site. In each tank, 200 numbers of loach were stocked and transported under mild aerated condition.

Table 1. The Geographical Location and Area of the Fish Collection Sites.

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"
6	Velladichivillai, Suchindram (KK, Dist.)	8°8'52.332"	77° 27' 27.4032"

In the experimental site, the fish were acclimatized to captive conditions using tanks provided with mild aeration. During the condition, the fish were fed with a

powdered form of feed (groundnut oil cake and cottonseed oil cake mixture). After 30 days of acclimatization, the fish were used for the current study.

Pond preparation and rearing of fish

The study used six numbers of small size lined pond (18 m²) for stocking the fish. Initially, the pond was filled with water up to 20 cm in depth. Prior to water filling, three ponds were filled with riverine fine sand (5 cm) as substrate to mimic the natural ecosystem. Fertilizers were not applied during the pond preparation and the ponds top and bundth were provided with fine nylon net to prevent the entry of birds and another terrestrial animal. The study used riverine water and the water was not exchanged throughout the experiment.

In each pond, 250 number of loach fish (2.0 - 2.3 cm and 0.006 – 0.009 g) were stocked at the early morning. After stocking, based on water colour fertilizers were applied at regular intervals to promote the live feed production. The stocked fish were fed with supplementary feed (GNOC and cottonseed oil cake mixture) up to ad libitum level). The experiment was carried out for 45 days. At regular intervals, water quality parameters were recorded to keep the water quality at optimum level for rearing of loach fish.

Growth sampling

Before stocking into the pond, the fish initial length and weight were recorded by randomly measuring 50 fish individual weight and length. After stoking in experimental ponds, sampling was done at the end of 45th day. A total of 50 fish from each pond were collected and their individual length and weight were recorded. The total length and live weight of fish were measured using of centimeter scale and digital electronic balance, respectively. The estimated following growth parameter were estimated using standard formulas (Mensah and Attipoe, 2013) as mentioned below;

$$\text{Weight gain (g)} = \text{Final weight (g)} - \text{Initial weight (g)}$$

$$\text{Percentage weight gain (\%)} = (\text{Final weight (g)} - \text{Initial weight (g)} / \text{Initial weight (g)}) \times 100$$

$$\text{Specific growth rate (\%/day)} = (\ln \text{ final weight} - \ln \text{ initial weight}) / \text{number of days} \times 100$$

$$\text{Survival rate (\%)} = (\text{Total number of fish harvested} / \text{Total number of fish stocked}) \times 100.$$

$$\text{Gross biomass (g)} = \text{Average final weight (g)} \times \text{Total number of fish harvested}$$

Length and weight relationship calculation

The cube law mostly used to find out the length-weight relationship in fish but this cube law does not fit for small fishes earlier stages. Therefore, the collected data about individual fish length and weight were transformed into log values. Then the log values were fitted in the linear regression curve to find out the slope (b), intercept (a) and coefficient of determination values (R²). Similarly, from the log-transformed length-weight values, correlation coefficient value (r) was calculated using Microsoft Excel (MS-Version, 2016).

$$\log W = \log a + b \log L.$$

Condition factor (K) of fish was derived from the recorded total fish weight (g) and standard length (cm). The K value was calculated using Fulton's condition factor formula (Fulton, 1904);

$$K = 100 W / L^3$$

Where W is the weight of the fish in g, L is the total length of fish cm, the factor 100 used to bring K close to unity.

The relative condition factor Kn calculated by using Le Cren, 1951 formula as below

$$K_n = W / aL^b$$

Where a and b are the exponential form of the intercept and slope, respectively, of the logarithmic length-weight equation. The formula $K = 100 W / L^3$ used for testing the modified condition factor given by Ricker (1975).

Statistical analysis

The length-weight relationship data log transferred to perform the linear regression analysis and graph were prepared using Microsoft office- 2016. Data on growth parameters were analyzed with pair t-test procedure using

IBM -SPSS for windows statistical, version 26. The statistical value $p < 0.05$ was considered to represent the significant levels.

Results

Growth performance

This study performed various growth parameters such as growth rate, weight gain, weight gain percentage, specific growth rate, survival rate and gross production (Table-2). The results of present study found significant

difference ($P < 0.05$) among growth parameters, except for survival rate. Significantly higher final weight (0.5253 ± 0.002 g), specific growth rate ($10.80 \pm 0.01\%$ /day), weight gain (0.52 ± 0.002 g), weight gain percentage ($7280.82 \pm 32.12\%$) and gross biomass (76.70 ± 0.81 g) were recorded in the treatment-1 reared fish. On the other side, significantly lower final weight (0.3294 ± 0.001 g), specific growth rate ($9.61 \pm 0.01\%$ /day), weight gain (0.32 ± 0.001 g), weight gain percentage ($4526.42 \pm 32.21\%$) and gross biomass (45.02 ± 0.51 g) were noticed in the treatment-2 raised fish. Survival rate was in the range of 96 ± 0.67 to $98 \pm 0.67\%$ between two groups.

Table 2. The growth performance of *Lepidocephalichthys thermalis* reared in two different lined pond conditions.

Treatment	Growth parameters						
	Final weight (g)	Growth rate (%)	Specific growth rate (%/day)	Weight gain (g)	Weight gain (%)	Survival rate (%)	Gross biomass (g)
Treatment-1	0.3294 ± 0.001^b	97.84 ± 0.01^b	9.61 ± 0.01^b	0.32 ± 0.001^b	4526.42 ± 32.21^b	96 ± 0.67	45.02 ± 0.51^b
Treatment-2	0.5253 ± 0.002^a	98.64 ± 0.01^a	10.80 ± 0.01^a	0.52 ± 0.002^a	7280.82 ± 32.12^a	98 ± 0.67	76.70 ± 0.81^a

Mean \pm SDs of the loach species in different treatments with a different superscript in a column differ significantly ($p < 0.05$).

Length-weight relationship (LWR)

At the end of forty-five days trial, regression analysis of the LWR of the loaches was carried out and explained in Figures 1 and 2. The length-weight relationship (LWR) parameters of a, b and the correlation coefficient (r), coefficient of determination (r^2) were calculated and presented in Table 3. The calculated equation for LWR of loaches as follows, $W = 0.00153 * L^{5.561}$, $r^2 = 0.9806$

and $W = 0.000124 * L^{5.708}$, $r^2 = 0.9823$ for fish reared in treatment-1 and treatment-2, respectively. Between the two treatments, the b value was found to be highest in treatment-2 reared fish. However, both the treatment b values are higher than isometric ($b=3$; which differ from the ideal cube law of '3') growth value. The correlation coefficient (r) values were recorded in the ranges of 0.9911 and 0.9902 in both the treatments.

Table 3. The length-weight relationship of *Lepidocephalichthys thermalis* reared in two different lined pond conditions.

Group	Total Length (cm)			The total weight (g)				n	Parameter of the LWR				Growth pattern
	Min	Max	Mean	Min	Max	Mean	a		b	r^2	r		
T1	2	5.7	3.3	0.006	1.06	0.3	50	0.00153	5.561	0.9806	0.9902	+ve	
T2	2	4.5	3.1	0.006	0.39	0.2	50	0.000124	5.708	0.9823	0.9911	+ve	

Min – minimum; ma- maximum; n- number of fish sampled; a – intercept; b is slope value; r^2 –coefficient determination. All given values are pooled data of initial and final length & weight.



Fig: 1 Length-weight relationship of loach reared in lined pond provided with sand substratum



Fig: 2 Length-weight relationship of loach reared in lined pond provided without sand substratum

Condition factor

At the end of study, condition factor (k), relative condition factor (kn), modified condition factor were analyzed to find out the fitness of the loach fish species and well-being of the fishes in the different substrate based environmental conditions, which were shown in Table 4. The k factor value of was recorded lower

(0.57) in treatment-1 group. On the other side, the lined pond provided with sand substratum exhibited higher k factor and it was recorded as 0.75. Similarly, the relative condition factor (Kn) was also recorded higher in the treatment-2 (0.23) reared fish. The relative condition factor was varied in the range of 0.21-0.23 in the present study. The modified condition factor was 0.03 and 0.04 for the treatment-1 and treatment-2 reared fishes, respectively.

Table 4. The values of different condition factors (K, Kn and MCF) f *Lepidocephalichthys thermalis* reared in two different lined pond conditions.

Group	Condition Factor (K)	Relative Condition Factor (Kn)	Modified Condition Factor
Treatment -1 (without sand substratum)	0.57	0.21	0.03
Treatment -2 (with sand substratum)	0.75	0.23	0.04

Discussion

The present study found a significant difference in all growth parameters, except survival rate, thus indicating that the growth of loach fish was varied in the ponds due to the difference in pond bottom. In general, production of fish is mainly influenced by feeding and various abiotic factors such water quality parameters, pond conditions, etc. (Makori et al., 2017). The sand substratum provided in treatment-1 has mimicked their natural habitat, as they inhabit in running waters with sand substratum in natural ecosystem, which might have positively influenced the fish growth performance. SGR is an important parameter which used to assess the growth performance in fish and its values were decreases when the fish grow bigger (Kohla et al., 1992; Ali et al., 2003). In the present study,

significantly higher SGR and biomass were recorded in fish reared in lined pond provided with sand substratum. Loach fish is an omnivore feeder which mainly feeds the detritus food items accumulated in bottom or it grazes the plankton attached in bottom substratum (Sundarabarathy et al., 2001; Keskar et al., 2014; Renuhadevi et al., 2019). Moreover, loach fish can feed such other live food such as diatom, desmids (phytoplankton) and daphnia, ostracods (zooplankton) (Keskar et al., 2014; Renuhadevi et al., 2019). The treatment-1 pond was supported their feeding habitat as they were provided with sand substratum which might helped them to graze the detritus or plankton more efficiently than the lined pond without any substratum.

No significant difference was found between the two treatments in survival rate. Both the study showed

positive allometric growth pattern and closer values of condition factor. This indicates that fishes lived in good environmental conditions supported with optimal water quality parameters for better survival. Additionally, loach has a habit of burrowing under substrate, especially in sand, this may help to avoid or escape the predation of other aquatic and terrestrial animals and to avoid high water temperature risk. They also contain air-breathing organs which supports them to tolerate less dissolved oxygen. Overall, the survival rate of this study revealed that loach fish is a highly environment adoptive species.

The LWR are carried out in stock assessment and to study the population characteristics of aquatic species. The LWR pattern helps to know the relationship between body size and shape. Through LWR and K_n , fish production, stocking density, maturity, spawning can be calculated (Ama-Abasi, 2007; Keskar *et al.*, 2015; Gohain and Deka, 2017; Biswas *et al.*, 2018). The present study recorded 'b' values in the range of 5.5 – 5.7 which is greater than the theoretical 'b' value 3 (cube law). If 'b' value $b < 3$ and $b > 3$ fish which exhibits the negative and positive allometric growth pattern in fish, respectively. And if the $b = 3$ it shows the isometric growth pattern in fish. The present study b value indicates the positive allometric growth pattern in stocked fish which further dictate heavier weight compared to length (Zubia *et al.*, 2014). In earlier studies, 'b' values of *L. guntea* female fish found to be positive allometric growth whereas male found to be negative allometric growth (Biswas *et al.*, 2018). Similarly, Gohain and Deka, (2017) and Rahman *et al.*, (1997) found highest value of b in females (3.76) compare to male fish (2.66) in *Leptocephalus guntea* but the same study reported a positive allometric pattern (3.02) when they pooled the sex (male and female). Contradictory to this, in *Lepidocephalichthys thermalis*, both male and female showed negative allometric growth (2.4987) (Balaganesan *et al.*, 2018). Interestingly, fish raised in lined pond with sand substratum displayed a breeding behavior which further confirms that substrate-based environment not only favored the growth, it also positively influenced the maturation and breeding. On the other side, fish raised in lined without sand substratum did not exhibit any breeding behavior.

Condition factor is widely used to compare the fitness and condition of fishes. The k factor equals to 1 or greater than 1 is considered as animal in good condition. If the same value is lesser than 1, it suggests fish not in good physiological condition (Famoofo and Abdul, 2020). In this study, the recorded k factor was lesser than 1, but close to the ideal level (1). In general, K factor depends on the fish length and weight and it can be affected by several biotic and abiotic factors. Relative condition factor used to determine the deviation from theoretically expected weight at a specific length. K_n factor +1 or close to 1 always indicates that fish in good condition but lesser than this value considered as fish in poor condition (Jisr *et al.*, 2018; Zubia M, *et al.*, 2014). Similarly, Singh and Nautiyal, (2017) reported the lesser value of K_n in other hill stream fishes which is much closer to our findings. Better feeding conditions may significantly increase the condition factor in fishes within the controlled condition (Sundarabarathy *et al.*, 2001).

Conclusion

In India, the pond-based aquaculture systems are mainly relying on the carp culture compare to other species. Therefore, in India overall inland fish production comes from carp and the country is called as carp country. India has huge potential in indigenous fishery resources but only very few species have been cultured and their breeding techniques have been standardized. Thus, paving the way for diversification of species in Indian aquaculture production, especially with small indigenous fishes having rich nutritional profile. In this context, *L. thermalis* is most suitable freshwater fish found in Tamil Nadu, which has better taste, rich in nutrition and has good market potential. Besides that, this species has minimal wastage during post-harvest cleaning compare to other fish, as this fish can be consumed as whole fish after cooking. Moreover, most of the indigenous fishes are categorized under vulnerable phase because of their demands are meet out only from the wild capture. Considering the above points, a preliminary study undertaken to investigate the grow out farming aspects of *L. thermalis* under two controlled pond systems. The results of this study revealed that loach fish culture is possible in controlled lined pond system when provided

with sand as substrate. Further, the culture requires lesser use of water, land, feed and other management. Therefore, it can be said that loach is a promising suitable candidate species for fish farming practices. However, further studies are required to understand the production of *L. thermalis* in different farming systems.

Conflict of Interest: The authors have no conflict of interest to declare.

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