Allometric Growth Patterns of *Leiocassis longirostris* Larvae and their Ecological Significance

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ABSTRACT

In this study, a total of 10 indices were measured from hatching out of the larvae (0 dph; dph for days post hatch) to 19 dph of *Leiocassis longirostris*. The study systematically investigated the early allometric growth patterns of *L. longirostris*, using experimental ecology and a combination of regression equation fitting. It was concluded that *L. longirostris* showed positive allometric growth (P < 0.05) in rostrum length, eye diameter, pectoral length, and post-anal length (0-19 dph) and that the head organs and swimming organs developed preferentially over the rest of the body. Body height showed negative allometric growth (P < 0.05) from 0 to 19 dph. Head height, head length, trunk length, and pre-anal length all showed growth inflection points during the larval period. Prioritizing the growth of the head and swimming organs of *L. longirostris* in the early growth stages enhances the ability to hunt and avoid natural enemies, and helps to provide energy for the growth of the larvae. This growth pattern helps *L. longirostris* larvae adapt to the external environment and improve their survival rate. This study can enrich the basic biological information of the growth process of *L. longirostris* larvae, which is of great significance for *L. longirostris* fry breeding and healthy aquaculture. The practical implications specifically include: optimizing feeding management, improving reproductive efficiency, and disease prevention and health monitoring.

INTRODUCTION

Leiocassis longirostris, belonging to the Siluriformes, Bagridae, is widely distributed in the rivers of the Yellow River, the Yangtze River, the Min River, and the Pearl River of China (Dai *et al.*, 2022). *L. longirostris*, with high nutritional value (Wang *et al.*, 2012), no scales, and intermuscular bones, is one of the excellent fish species in the current aquaculture industry. In the early growth of fish, dramatic physiological and structural changes occur in various functional organs or parts, and preferential growth of those that are most in need of growth to better adapt to the environment (vanSnik *et al.*, 1997). These

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Authors' Contribution

HX: Measured morphological indicators, organized and analyzed data, wrote the paper, as well as proofread changes in formatting and other aspects of the paper. WJ: Participated in the analysis of data and the production of graphs and tables in the thesis. SS: Provided financial and platform support for this project, and advised on the analytical discussion in the thesis as well as the shortcomings in the experimental process.

Key words *Leiocassis longirostri*, Larvae, Allometric growth, Growth inflection point, Early growth stages

body organs or parts will have higher relative growth and differentiation than the rest of the fish body tissues (Gagnat et al., 2016), the phenomenon known as allometric growth. During the early developmental stages, elucidating the relative growth patterns of larvae can characterize the normal growth patterns under specific environmental conditions. This understanding is crucial for the timely optimization of rearing strategies when abnormalities in larval development are found, thereby contributing to fisheries management and aquaculture practices (Peña and Dumas, 2009). In recent studies, the allometric growth of the larvae has been shown in Hemibarbus maculatus Bleeker (Xie et al., 2024), Trachinotus ovatus (Pan et al., 2023), Schizothorax waltoni Regan, Percocypris retrodslis (Xu et al., 2023), Lutjanus peru (Peña et al., 2023) and so on. By measuring and analyzing the allometric growth patterns of the larvae of these species, the results of the studies could improve the aquaculture techniques of these fishes and provide a deeper understanding of their behavior and ecology under captive conditions. This information can be used as a reference for assessing the developmental phenotypes of these fishes and can help to develop feeding strategies that are compatible with their growth patterns,

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ultimately enhancing the efficiency and sustainability of aquaculture. In recent years, studies on L. longirostris have focused on biology (Mou et al., 2022; Pan et al., 2019; Xiao et al., 2020; Xu et al., 2022), nutrition (Pei et al., 2004; Xie et al., 1998; Zhao et al., 2023), and fish fry and adult culture (Bao et al., 2021; Zhang et al., 2021; Zhao et al., 2009), while no systematic studies have been reported for the allometric growth of relevant functional organs or parts in its early growth stages. This study systematically investigates the allometric growth pattern of L. longirostris larvae during the early developmental stages, aiming to understand the prioritization of organs and body parts development as well as the growth rates at various growth stages. This study enriches the foundational studies on the development of L. longirostris larvae and provides a theoretical basis for cultivating seedlings and the healthy aquaculture of this species.

MATERIALS AND METHODS

Rearing and maintenance of fish larvae

Eggs of *L. longirostris* were collected from Jinchuanjiang Fisheries Co. Ltd, Dongpo District, Meishan City, Sichuan Province, China (103°45′52″E, 30°08′20″N). The study took about 2,000 newly hatched larvae (0 dph) and placed them into an indoor round-hatching bucket for further rearing. The study adjusted the water inlet valve during the culture period to control the daily water change at 2 m³. The water depth in the hatching bucket was controlled at 0.85 m, the water volume at 0.22 m³, the water temperature at 25 ± 1 °C, and the dissolved oxygen at 5 ± 0.5 mg L⁻¹. Larvae were maintained under a defined photoperiod of 14 h light and 10 h dark.

Larvae were fed enriched rotifers (*Brachionus plicatilis*) at a 15-20 ind mL⁻¹ density from 5 to 12 dph. Rotifers were sourced from outdoor ponds of Jinchuanjiang Fisheries Co. Ltd, Dongpo District, Meishan City, China, and were collected using a plankton net. From 13 to 19 dph, larvae were fed high-protein powdered feed (formulation: crude protein $\geq 68.0\%$; lysine $\geq 2.3\%$; crude fat $\geq 3.0\%$; crude fiber < 6.0%; moisture $\leq 10.0\%$; crude ash $\leq 16.0\%$; calcium $\leq 4.0\%$; and total phosphorus $\leq 13.0\%$), with feeding amounting to 3-5 g each time. Feeding was conducted on a fixed schedule, initiated at 5 dph, with a single feeding session at 12:00 daily.

Data collection

Starting from the time after the fertilized eggs hatched out of the larvae (0 dph), the study randomly took 10 *L*. *longirostris* larvae at 21:00 daily, anesthetized them with tricaine methanesulfonate (MS-222) and observed changes in the external organs of the larvae under a light microscope with an eyepiece graticule. This study calibrated the scale of the eyepiece graticule with a stage graticule. It measured a total of 10 morphometric indices, including total length, rostrum length, eye diameter, head length, head height, pectoral length, body height, trunk length, pre-anal length, and post-anal length (Fig. 1) with an accuracy of 0.01 mm. The study used the 10 % neutral formalin solution to preserve the samples after measurement and keep them in a cool and dry place for checking. When measuring, deformed larvae should be eliminated to avoid errors in the measurement results.





Data analysis

The study organized the experimental data through Excel 2016 for statistics, analyzed the growth relationship between total length and dph using SPSS 27.0 after 7 curve fitting and parameter estimation methods, and selected the best-fitting growth model based on R^2 . The study constructed an allometric growth model for each part of L. longirostris, which was borrowed from the model proposed by Huxley et al. (1993): A power function $y = ax^{b}$ (where y is the character measured, x is the total length, a is the intercept to the y axis, and b is the growth coefficient) was used to do the modeling for log-transformed, and the transformed equation was logy = $\log a + \log x$. When b > 1, it represents positive allometric growth, and the organs or parts of the larvae grow faster than the total length growth of the larvae; when b = 1, it represents isometric growth, and the growth of organs or parts of the larvae is in equal proportion to the total length of the larvae; when b < 1, it represents negative allometric growth, and the organs or parts of the larvae grow slower than the total length growth of the larvae. During the growth process, the body indexes of larvae show a more

complex nonlinear pattern. The power function, due to its form of multiplying variables by powers, can change curves flexibly, which can well simulate and express this nonlinear growth pattern. By fitting the larval growth data to the power function model, we can obtain the equations describing the growth pattern of the larvae, which in turn can be used to predict and understand the characteristics and needs of the larvae at different growth stages. In many statistical methods and model validation techniques, linear relationships are easier to handle and analyze than nonlinear relationships. Power functions can be converted to their linear form by taking logarithms (log-transformed). This allows statistical methods such as linear regression to be applied to estimate model parameters, test the fit of the model, and perform other statistical analyses. In addition, the log transformation can help reduce heteroskedasticity in the data (i.e., the variance of the data is not constant), making the model estimates more accurate and reliable. This modeling treatment is now widely used for allometric growth of larvae such as Saurogobio dabryi Bleeker (Zhang et al., 2018) and Paramisgurnus dabryanus (Zhang et al., 2016).

The inflection point of the growth curve was determined according to vanSnik *et al.* (1997). Two equations were used before and after the inflection point: $logy = a_1 + b_1 logx$, and $logy = a_2 + b_2 logx$. The study performed a t-test on whether b_1 and b_2 were equal to 1 and a t-test on b_1 and b_2 to test whether b_1 and b_2 differed significantly (confidence interval: 95%). The study used the R language ggplot2 package for data visualization and graphing.

RESULTS

Relationship between total length and dph of L. longirostris *larvae*

All seven regression models the study used in the experiment were statistically significant (Table I). Among

them, the cubic regression model had the largest R^2 and F ($R^2 = 0.990$, F = 6169.367) and therefore had the highest reliability of the fitted curves (Fig. 2), with the regression equation: $y = 7.144+ 1.032x - 0.080x^2 + 0.002x^3$. The average total length of 0 dph for *L. longirostris* larvae was 7.19 mm, and their average total length reached 15.03 mm after 20 d of growth, with an average growth rate of 0.39 mm/d. The average growth rate was 0.56 mm d⁻¹ from 0 to 4 dph, 0.22 mm d⁻¹ from 5 to 12 dph, and 0.36 mm d⁻¹ from 13 to 19 dph, with significant differences (P < 0.05) among the 3 stages. It indicated that *L. longirostris* larvae grew fastest when ingesting nutrients from the yolk sacs, had the second fastest growth rate when ingesting high-protein powdered feed, and the slowest growth rate when ingesting rotifers.



Fig. 2. The relationship of increase in length *L. longirostris* larvae during post hatching period (dph).

Allometric growth in head parts

Neither rostrum length nor eye diameter showed a growth inflection point from 0 to 19 dph (Fig. 3A, B), and both showed positive allometric growth, with growth coefficients of b = 1.683 (P < 0.05) and b = 1.291(P < 0.05), respectively. Head height showed a growth

Table I. Parameter analyses of increase in total length L. longirostris larvae during dph.

Model	R ²	F	р	a	Growth coefficient		
					b1	b2	b3
Linear	0.933	2777.089	< 0.001	8.409	0.328		
Quadratic	0.955	2067.260	< 0.001	7.862	0.511	-0.010	
Cubic	0.990	6169.367	< 0.001	7.144	1.032	-0.080	0.002
Compound	0.889	1586.502	< 0.001	8.536	1.030		
Growth	0.889	1586.502	< 0.001	2.144	0.030		
Exponential	0.889	1586.502	< 0.001	8.536	0.030		
Logistic	0.889	1586.502	< 0.001	0.117	0.970		

inflection point at 1 dph (TL = 8.16 mm) (Fig. 3C), with the positive allometric growth of b = 2.282 (P < 0.05) before the inflection point and the negative allometric growth of b = 0.452 (P < 0.05) after the inflection point. Head length showed a growth inflection point at 5 dph (TL = 10.62 mm) (Fig. 3D), with the positive allometric growth of b = 2.432 (P < 0.05) before the inflection point and the isometric growth of b = 1.053 (P > 0.05) after the inflection point.



Fig. 3. Allometric growth of rostrum length (A), eye diameter (B), head height (C) and head length (D) with reference of total length of *L. longirostris* larvae.



Fig. 4. Allometric growth of body height (A), trunk length (B), and pectoral length (C) with reference to total length of *L. longirostris* larvae.

Allometric growth in trunk parts

Body height did not show a growth inflection point from 0 to 19 dph (Fig. 4A), and showed negative allometric growth, with a growth coefficient of b = 0.631 (P < 0.05). Trunk length showed a growth inflection point at 14 dph (TL= 12.66 mm) (Fig. 4B), with the negative allometric growth of b = 0.613 (P < 0.05) before the inflection point and the positive allometric growth of b = 1.328 (P < 0.05) after the inflection point. Pectoral length showed a growth inflection point at 4 dph (TL = 9.97 mm) (Fig. 4C), with the positive allometric growth of b = 3.390 (P < 0.05) before the inflection point and the positive allometric growth of b = 1.501 (P < 0.05) after the inflection point.

Allometric growth in other parts of the body

Pre-anal length showed a growth inflection point at 3 dph (TL= 9.48 mm) (Fig. 5A), with the isometric growth of b = 0.989 (P > 0.05) before the inflection point and the negative allometric growth of b= 0.717 (P < 0.05) after the inflection point. Post-anal length did not show an inflection point from 0 to 19 dph (Fig. 5B), and showed positive allometric growth, with a growth coefficient of b= 1.182 (P < 0.05).



Fig. 5. Allometric growth of pre-anal length length (A) and post-anal length (B) with reference to total length of the body of *L. longirostris* larvae.

DISCUSSION

The phenomenon of allometric growth is closely related to the adaptation strategies of the larvae to the complex external environment, which is highlighted by the prioritized growth of important organs or parts during early growth to ensure that the larvae can effectively adapt to the environment. The aquatic environment plays an important role in the early growth of fish (Gisbert et al., 2014; Wisely et al., 2005). Dynamics of preferential development of different organs and parts, adjusted with advancing developmental stages, reflect a refined survival strategy developed over a long period of fish evolution. The results of this study indicate that there are different allometric growth patterns for various organs or parts of the L. longirostris larvae during the early growth stages, which enable the L. longirostris larvae to forage and avoid predators efficiently under different environmental conditions, and thus improve their chances of survival and development.

Relationship between total length and dph

Open feeding is a critical period in the early development of fish, which directly affects the survival

rate and growth rate of larvae (Ma et al., 2007). In the pre-opening period (0-4 dph), the growth of larvae mainly relied on the yolk sac to provide energy, showing a high average growth rate (0.56 mm d⁻¹), during which the internal organs and digestive system of larvae gradually developed and perfected, preparing for the subsequent ingestion of external food. At 5 dph, the study started feeding rotifers. Rotifers are important food organisms in the early stage of the larvae, which are easy to feed, digest, and nutrient-rich. The rotifers provide the larvae with essential proteins, fats, and micronutrients. The average growth rate was 0.22 mm d⁻¹ from 5 to 12 dph, which was a decrease compared to the pre-opening stage. At 13 dph, the study started feeding high protein powder feed (crude protein content \geq 68.0). The high-protein powder feed provides sufficient amino acids and energy to promote the growth and differentiation of tissues in the larval body. The average growth rate was 0.36 mm d⁻¹ from 13 to 19 dph, which was higher compared to the feeding of rotifers. This suggests that the larvae can utilize external nutrients more efficiently to promote self-growth by feeding high protein feeds. Based on the differences in growth rates among these 3 stages, at the beginning of the opening period of L. longirostris larvae, breeders can feed rotifers together with an appropriate amount of high protein powder feed to promote the growth of L. longirostris larvae.

Growth of the head part

Allometric growth of the head part refers to the fact that during fish growth, different parts of the head grow at different rates, thus giving the head part a relative advantage at a particular stage of growth. Rapid head growth is important in early fish growth because it is directly related to feeding, respiration, and nervous system function. The growth of the head part promotes feeding efficiency, the ability to avoid predators, and the adaptation to complex environments. In the long evolutionary history of fishes, most fishes preferentially develop head organs such as the oropharyngeal cavity, gills, and brain. The growth of the oral cavity in larvae is generally expressed in terms of the rostrum length (Hu et al., 2020; Lin et al., 2014; Shan and Dou, 2009; Xie et al., 2023). In this study, the growth of both rostrum length and eye diameter of L. longirostris larvae were in positive allometric growth, which was similar to the findings of Oncorhynchus keta Walbaum (Song et al., 2013), Sinogastromyzon wui (Wen et al., 2024). The rapid growth of the rostrum length improves the feeding efficiency so that the larvae can catch prey earlier and more efficiently to meet the nutritional requirements of their rapid growth. The rapid growth of eye diameter enhances the visual ability of larvae and improves their perception of the water so that they can

better avoid predators and find food. These prioritized developmental characteristics enable the larvae to have stronger survival and adaptive abilities during the early developmental stages.

Head length showed a growth inflection point at 5 dph. The change from positive allometric growth to isometric growth before and after the inflection point was similar to Lutjanus erythropterus (Cheng et al., 2017), Percocypris pingi (He et al., 2013), and Schizothorax waltoni Regan (Xu et al., 2021). The rapid growth in head length before 5 dph provides more space for the growth of sensory organs and the nervous system, enhancing perception. This trait allows the L. longirostris larvae to effectively avoid natural predators in their early environment. In addition, the positive allometric growth in head length suggests that in the early stages, L. longirostris has a strong competitive advantage in an environment with abundant food resources (Wang et al., 2019). Head length showed isometric growth after 5 dph. It helps the post-opening L. longirostris larvae maintain a coordinated and stable body shape and ensures the synchronized growth of other organs. A longer head helps the larvae survive in the early stages, but in the later stages, optimization of body shape and efficient use of energy are more important. Isometric growth of head length adapts to the ecological demands of later stages and enhances its ability to survive in complex environments. At this stage, more absorbed energy is allocated to the growth and refinement of other important physiological functions, such as improving swimming ability and optimizing overall body shape for a wider range of ecological niches.

Head height showed a growth inflection point at 1 dph. The change from positive to negative allometric growth before and after the inflection point was similar to the findings of L. erythropterus (Cheng et al., 2017) and Sebastes schlegelii (Xi et al., 2014). Head height showed significant positive allometric growth until 1 dph, indicating a faster growth rate during the first 2 days. This growth pattern provides more muscular and skeletal support, resulting in stronger jaw and pharyngeal muscles, which facilitates better ingestion of external food when L. longirostris larvae open for feeding. At the same time, the rapidly growing head height provides more protection and developmental space for the brain and sensory organs to effectively perceive the environment and hunt at an early stage. Head height showed negative allometric growth after 1 dph. The slowdown in head height growth contributes to reduced energy expenditure, lower swimming resistance, and improved athleticism efficiency. This growth pattern is adapted to the ecological demands of later stages, enabling them to maintain efficient athleticism and survival chances under different environmental conditions. The different inflection points of head length and head height are due to the differences in the external morphology of the head in different fish species (He *et al.*, 2012).

Growth of the trunk part

Body height did not show a growth inflection point at 19 dph but rather a negative allometric growth. Body height increases at a slower rate, as evidenced by the fact that the refinement of the internal digestive system is a relatively slow process (Liu et al., 2018). The analysis suggests that this type of growth provides a stable environment for the development of internal organs and ensures that they can develop in a healthy and normal manner. Stable development of internal organs is critical to the overall health and function of the fish, and the slower growth rate in body height allowed sufficient time for the internal organs to be refined. Meanwhile, this negative allometric growth pattern may be closely related to its life habits and ecological adaptation. The relatively slow growth rate of body height implies that L. longirostris tends to be elongated during the growth process, which is consistent with the negative allometric growth of head height after 1 dph. They help the L. longirostris to be more flexible and efficient in catching prey and avoiding predators.

Trunk length showed a growth inflection point at 14 dph. Trunk length showed negative allometric growth with a slower growth rate before 14 dph. The analysis suggests that the differentiation and development of bone and muscle take a secondary role in its early growth process, which is conducive to reducing the distance between the head and the tail and controlling the fish's body length to ensure the balance of the body. This growth pattern makes the athleticism more coordinated (Jiang et al., 2018). The trunk length showed positive allometric growth after 14 dph. It indicates that the nutrient demand of L. longirostris is significantly strengthened, so the digestive tract needs to develop rapidly to adapt to the demand for nutrient absorption, which leads to the lengthening of trunk length (Ma et al., 2007). Meanwhile, the rapid growth of the trunk, as the main part of support and protection, can provide better support and protection, thus improving the survival ability of the larvae.

Pectoral length showed a growth inflection point at 4 dph and showed positive allometric growth before and after it, but the growth rate slowed down after the inflection point, which was similar to the studies of *P. pingi* (He *et al.*, 2013) and *S. waltoni* Regan (Xu *et al.*, 2021). The pectoral length shows significant positive allometric growth before the inflection point, which provides strong athleticism ability and the advantage of predation and avoiding predators. The pectoral length remains positively allometric after the inflection point, reflecting the importance of athleticism and predatory abilities for *L*. *longirostris* larvae. However, the growth rate slows down significantly after 4 dph. The analysis suggests that some energy is allocated to the development of other key organs, such as the digestive system and the sensory organs so that the larvae can better adapt to the new nutrient sources, maintain efficient athleticism ability, and optimize the overall physiological functioning after opening for feeding (Xu *et al.*, 2021).

Growth of other parts of the body

The pre-anal length showed a growth inflection point at 3 dph and changed from isometric growth to negative allometric growth before and after it, which was similar to the findings of *P. pingi* (He *et al.*, 2013). It indicates that both the consumption of yolk sacs and the energy gained from ingesting external nutrients of *L. longirostris* larvae are mainly used for the differentiation and development of organs without focusing on the growth (Ma *et al.*, 2007), which also supports the argument that *L. longirostris* larvae showed positive allometric growth in organs such as rostrum length, eye diameter, and pectoral length from 0 to 19 dph.

Post-anal length showed positive allometric growth without a growth inflection point from 0 to 19 dph, which was closely related to the swimming ability of fish. Rapid growth in post-anal length increases the amplitude and strength of tail athleticism in fish, thus improving swimming speed and flexibility. They help *L. longirostris* larvae show higher survivability in feeding, avoiding natural enemies, and searching for habitats. They are essential for enhancing their adaptability and chances of survival in complex environments (Ma *et al.*, 2007). This growth pattern has been preserved during the evolutionary process so that *L. longirostris* larvae contribute to the survival rate when exposed to complex environments.

Application of these allometric growth pattern findings to aquaculture practices

In aquaculture practice, it is of great significance to clarify the allometric growth pattern of *L. longirostris* larvae. The rapid growth patterns of the rostrum length, eye diameter, and post-anal length of *L. longirostris* larvae, can help the breeders to adjust the nutrients in the feeds, especially to appropriately increase those growth-promoting ingredients, such as proteins. This can better meet the nutritional requirements of the larvae during the critical growth period and promote their healthy growth. In addition, the rapid growth of these organs or parts of *L. longirostris* larvae may be related to their adaptation to the environment. Therefore, clarifying the growth patterns also helps breeders to optimize the parameters of the culture environment, such as appropriately increasing the

water temperature and light intensity, which may help to further optimize the growth conditions of the larvae (Wen *et al.*, 2024). Finally, clarifying the positive allometric growth patterns of these organs or parts of *L. longirostris* could also help breeders to more accurately determine the growth stage and health status of the fish. This can help develop more targeted culture management measures, such as timely feed feeding, disease prevention, and treatment. The body height of *L. longirostris* larvae showed negative allometric growth. Determining this slow growth pattern can help breeders rationalize the breeding density and design breeding facilities that are more suitable for what they need for growth, such as the depth and width of the hatching bucket, to promote the healthy growth of the larvae.

Determining the growth inflection points of these organs or parts of L. longirostris larvae is also of great importance in aquaculture practice. In the study, head height, head length, trunk length, and pre-anal length all showed growth inflection points during the larval period. By determining the growth inflection point, breeders can more accurately select larvae with excellent growth potential for breeding, thus producing better-quality species. In addition, before and after the growth inflection point, the nutritional and environmental requirements of the larvae may change. Understanding these changes, breeders can make timely adjustments to feed formulations, feeding strategies, and water quality management to ensure that the fish are provided with an optimal environment for growth (Cheng et al., 2017). Finally, growth inflection points are often accompanied by changes in the physiology and metabolism of the fish, which may make them more susceptible to disease. Therefore, understanding growth inflection points can help to develop targeted disease prevention and control measures to improve the survival of larvae.

CONCLUSION

The study analyzed the external physical characteristics of *L. longirostris* larvae by biostatistical methods in detail, and the results showed that *L. longirostris* larvae showed allometric growth in the head part (rostrum length, eye diameter, head height, head length), trunk part (body height, trunk length, pectoral length), and other parts of the body (pre-anal length, post-anal length), which fully reflected the ability of *L. longirostris* larvae's adaptation to the growth environment in the early stage of growth. The growth of head organs and swimming organs or parts such as rostrum length, eye diameter, pectoral length, and post-anal length were prioritized in the developmental stage of *L. longirostris* larvae to enhance their ability to hunt and avoid natural enemies, which helped to provide energy for the development of the larvae, adapt to the external environment and improve the survival rate. The study results are of great significance in guiding the artificial cultivation of fry and the healthy breeding of *L. longirostris* larvae.

DECLARATIONS

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IRB approval

All experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Southwest University.

Ethical statement

All state and institutional guidelines for the care and use of animals were followed in this research.

Statement of conflict of interest

The authors have declared no conflict of interest.

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