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Eggs, Larval Morphology Development and Behavioral Changes of Jade Perch (*Scortum barcoo*) Larvae

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ABSTRACT

This study examined the embryonic development of Jade perch (*Scortum barcoo*) during the early larval stage, emphasizing morphology, sensory organ development, and behavioral changes. Artificial reproduction was conducted using one mature female (1.0 ± 0.0 kg) and two mature males (1.0 ± 0.8 kg) through hormone-induced spawning. The fertilization and hatching rates were recorded as 83.4 ± 0.45% and 86.4 ± 0.78% respectively, and fertilized egg size was 2.73 ± 0.3 mm. Hatching occurred at 20:20 hours after fertilization (hAF) under 27.3 ± 0.7 ℃ and the size of newly hatched larvae was 2.79 ± 0.05 mm, growing up to 4.73 ± 0.07 mm by 90 hours after hatching (hAH). Morphological developments were observed as follows: eye and fin formation at 6 hAH, noticeable pigmentation at 30 hAH, mouth opening at 36 hAH, jaw movement at 42 hAH, and anus opening at 60 hAH. Sensory organs development was observed as follows: lens formation and inner ear formation at 6 hAH, eye pigmentation and olfactory pit formation at 30 hAH. Behavioral changes were observed as follows: onset of vertical swimming at 12 hAH, onset of horizontal swimming at 18 hAH, with larvae showing positive phototaxis and rheotaxis at 30 hAH, and first feeding starting at 72 hAH. This study concludes that Jade perch undergoes normal development, and these primary data can be used to establish a rearing protocol to further enhance the overall growth performance in captivity.

INTRODUCTION

The jade perch (*Scortum barcoo*), also known as Barcoo grunter in Australia, is classified in the order Perciformes, family Terapontidae, genus *Scortum* **(McCulloch & Waite, 1917)**. This species usually grows up to 350mm, but commonly found at approximately 250mm **(Allen, 2002)**. Jade perch is sometimes mistaken by taxonomists for Welch's grunter (*Bidyanus welchi*) and silver perch (*B. bidyanus*), but this species can

be easily identified because of the dark mottled pattern on the side of the fish body. This species inhabits rivers and waterholes with high turbidity and low gradients in central Australia **(Paxton *et al.*, 1989)**. It mainly originates from the Barcoo River, Cooper Creek, Lake Eyre, the Bullo-Bancannia basin in central Australia, and the Gilbert River and its tributaries in northern Queensland **(Queensland Government, 2018)**. This species is known to spawn on the bottom of the water in the wild during the summer floods when water temperatures are above 23°C, with the male protecting the eggs and supplying them with oxygen **(Baensch *et al.*, 1985; Queensland Government, 2018)**. Jade perch flesh is chewy, sweet and juicy, and lacks intermuscular bones **(Jie *et al.*, 2018)**. The fillet can be eaten with the skin on, making it an excellent edible fish compared to other Australian native fish **(Queensland Government, 2018)**. In Hong Kong, the jade perch is eaten raw as sashimi.

Several studies have shown that the jade perch is a highly nutritious fish. Australian Commonwealth Scientific and Industrial Research Organization (CSIRO) study found that the Jade perch had three times more omega-3 fatty acids than the Atlantic salmon (*Salmo salar*) and silver bass (*Morone chrysops*). A study by **Ya *et al.* (2014)** investigated protein (18.4%), lipid (9.00%), and total amino acid (19.99%) content in the jade perch muscle. Jade perch muscle contains many types of amino acids. Among them, glutamic acid accounted for 3.10% and umami amino acids for 7.64% of the total amino acid content. It has been recorded that the jade perch has a low lipid content, but is rich in unsaturated fatty acids, possessing a DHA content of 3.36%, and being rich in calcium, iron, zinc, selenium, **(Ya *et al.*, 2014)**.

Abdelrahman *et al.* (2022) reported in their study that fatty acids were quantified in the edible parts (skin, back, abdomen) and inedible parts (head, bones, visceral fat) of jade perch. The results showed that the head and skin contained significantly more omega-3, 44.96 ± 3.64 mg/g and 40.51 ± 2.07 mg/g, respectively. Bone and skin contained relatively high amounts of omega-6, 24.02 ± 5.53 mg/g and 23.32 ± 5.65 mg/g, respectively. The head contained a large amount of polyunsaturated fatty acids, with a value of 66.6 ± 3.94 mg/g. On the other hand, bones contained a large amount of monounsaturated fatty acids, with a value of 55.54 ± 10.70 mg/g. Saturated fatty acids were most abundant in visceral fat, with a value of 51.69 ± 6.51 mg/g. DHA was the most abundant unsaturated fatty acid with a value of 91.5 ± 0.56 mg/g, followed by EPA with a content of 54.0 ± 0.18 mg/g in the edible and inedible parts. Research has confirmed that Jade perch contains a high number of fatty acids, regardless of whether it is edible or inedible **(Abdelrahman, 2022)**. Hence, Jade perch has been recognized as one of the most promising aquaculture candidate fish species in many parts of the Asian region nowadays.

In order to produce the jade perch in a mass production, further study is needed, particularly to understand its optimal rearing protocol requirement through the understanding of its fundamental biological development. The studies on early life stages such as egg, larval and fry were the foundation on pioneering the production of many

commercially important fish **(Gan *et al.*, 2016)**. Hence, this study will establish comprehensive study in its embryonic and larval development which will be beneficial for Jade perch production to thrive in the aquaculture industry. To provide a better understanding and knowledge of the early life stages for this species, this study looked closely on the morphological, sensory organ and behavioral changes of the jade perch and thus further developing the rearing protocol for this species.

MATERIALS AND METHODS

1. Broadstock management

Broodstock management was performed on adult jade perch. A total of twenty jade perch bloodstocks with an approximate body weight of 800- 1000g were reared in an outdoor 9-t circular fiberglass tank. Water quality was maintained at a temperature of 26.0 - 29.0℃, a pH of 7.5 – 8.5, and a dissolved oxygen level of 4.0 – 7.0mg/ L. The broodstock were fully fed once a day with a marine fish compound feed **(Leong Hup Aqua, Malaysia)**.

During broodstock selection, the selected jade perch were moved to a separate tank and anesthetized with an appropriate amount of Transmore (NIKA Trading co., Malaysia). Once sufficiently anesthetized, they were cannulated, and the reproductive papillae were observed to select suitable broodstock. One female and two males were selected from the broodstock tank. In this experiment, the female broodstock was injected with hormones twice and the male broodstocks were injected once. The female broodstock was given the first injection of 400 IU of human chorionic gonadotropin (HCG) per kg of body weight, and the second injection of 1600 IU of HCG after an interval of 8:30 hours. 16:30 hours after the second injection, eggs were collected by stripping. The male broodstocks were injected with 1000 IU of HCG per kg of body weight, and 25:00 hours after injection, sperm was collected by stripping. Injections were administered on the back of the broodstock, and a wet towel was used to move the fish to reduce shock during the injection. After the hormone injection, a black cover was placed over the top of the circular aquarium to prevent light and shadows from causing stress to the broodstock.

2. Stripping and artificial fertilization

When the female broodstock was ready, the eggs and sperm were collected using the stripping method described by **Ch'ng and Senoo (2008)**. Once the female broodstock was confirmed to have matured, it was taken out of the tank and placed gently on a table. Then, eggs were stripped from the genital pore and collected using a plastic bowl. Once the male broodstock was confirmed to be matured, it was taken out of the water in the same way as the female broodstock. The sperm that oozed out from the genital pore was collected

using a self-made milt collector. This method involved creating a negative pressure inside the sperm collector by sucking with the mouth and then inserting the reproductive organ into the sperm collector to collect sperm. Once the sperm and eggs were collected, culture water was added to the egg management tank to activate the sperm and fertilize the eggs. At this time, the mixture was stirred gently using a brush. After fertilization, the eggs were stored in a 10-L bucket and a 500-L circular tank.

3. Incubation of egg and observation

During the incubation period and egg development phase, water temperature, pH, and dissolved oxygen (DO) levels were maintained at 27.0 – 29.0℃, 7.00 – 8.00, and 4.0 – 7.0mg/ L, respectively. Embryonic observations began at the blastopore closure stage, which occurred at 0 hAF. Eggs were sampled regularly from the incubation tank and observed and measured under compound microscope (Nikon, Eclipse E600) and a profile projector (Mitutoyo, PJ-A3000). The following formulas were used to determine the observation parameters **(Gan *et al.*, 2016)**.

$$\text{Fertilization rate (\%)} = \frac{\text{Total number of fertilized eggs}}{\text{Total numbers of eggs}} \times 100$$

$$\text{Development rate (\%)} = \frac{\text{Total number of survived eggs}}{\text{Total numbers of fertilized eggs}} \times 100$$

$$\text{Hatching rate (\%)} = \frac{\text{Total hatched larvae}}{\text{Total fertilized eggs}} \times 100$$

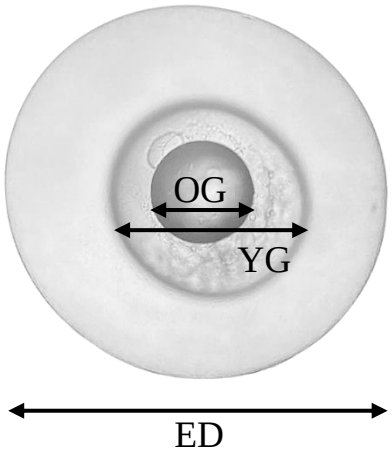

Fig. 1. The measurement of egg diameter (ED), yolk globule diameter (YG), and oil globule (OG) diameter of Jade perch

4. Larval rearing and morphological observation

In this experiment, the morphological development of the jade perch larvae was observed every 6h from hatching (0 hAH) until 90 hAH. Total sample of larvae was 5 in each observation. The hatched larvae remained in the incubation tank with the addition of *Nannochloropsis* spp at 500,000 cells/mL. The water quality was monitored using a multiparameter probe (HI 9828, Hanna Instruments Inc., USA), and the parameters of DO (7.9 ± 0.3 mg/L), pH (7.6 ± 0.4), temperature (27.3 ± 0.7 ℃) were maintained. Body morphometric measurements were taken on the newly hatched larvae, including their total length (TL), the length (L) and height (H) of their yolk sacs, and the diameter (D) of their oil globules (Fig. 1) **(Bagarinao, 1986)**: The larvae were fed using rotifers (5 – 10 ind./ml) from 48 hAH. Larvae were fed four times per day until satiation. Sampling and observation were conducted every 6 hours form 0 until 90 hAH using digital camera. Throughout the experimental period, rotifers were provided as the initial feed for the larvae from 40 to 90 hAH due to their appropriate size and rich nutritional content, especially essential fatty acids and proteins important for early larval development.

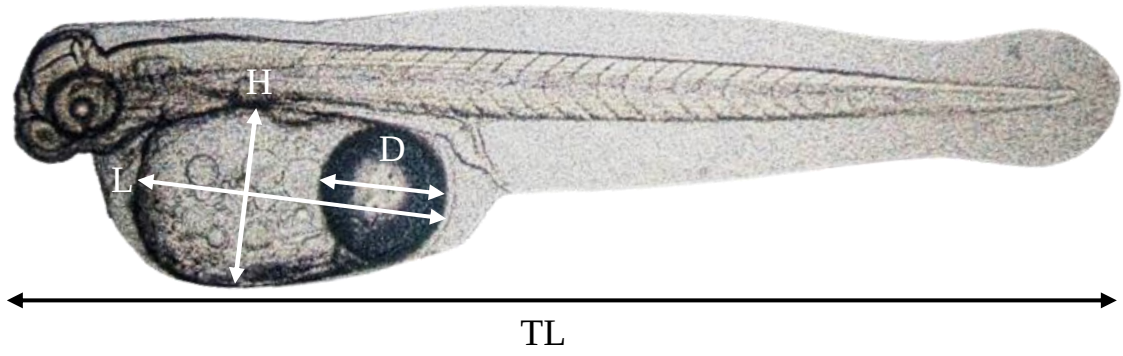

Fig. 2. The measurement of total length (TL), yolk sac length (L), yolk sac height (H), and oil globule diameter (D) of Jade perch

$$\text{Yolk sac volume} = \frac{\pi}{6} \times L \times H^2$$

$$\text{Oil globule volume} = \frac{\pi}{6} \times D^3$$

5. Behavioral change observation

In this experiment, the basic behavior of the jade perch, such as their taxis and swimming behavior, was observed, and attention was given to phototaxis, rheotaxis, and swimming behavior, with the naked eye. Phototactic and rheotactic responses were assessed as either positive or negative, while swimming behavior was evaluated based on the larvae's swimming style.

For phototaxis and rheotaxis, samples were taken and observed every 6 hours from immediately after hatching (0 hAH) to 90 hAH, as well as for morphological development, as similarly described by **Rahmah *et al.* (2012)**. For phototaxis, 50 larvae were placed in a 7 L plastic tank with breeding water. The tank was placed in a dark room, and 30–50 lumens of light (Google Pixel 7) was irradiated from one side, and the reaction was observed 5 minutes later. For rheotaxis, 20–30 larvae were placed in a 1000ml glass beaker with breeding water. After that, a glass rod was used to stir the water clockwise 10 times to create a weak water current, and the larvae's reaction to the current was observed. For the observation of swimming behavior, 50 larvae were placed in a 7L plastic tank together with breeding water. After placement, the tank was left still for 5 minutes, and the larvae's swimming style was observed to be floating, vertical, or horizontal.

6. Larval sampling for sensory organ observation

Samples were taken every 6 hours, from 0 to 90 hAH. During each interval, 10 larvae were collected. According to a previous method by **Jackson *et al.* (2006)**, the histological procedures included fixation, dehydration, clearing, paraffin, embedding, sectioning, staining, mouting, and glass slide observation. All the sampled larvae preserved in 10% formalin were in Davidson’s solution for 24 hours. Davidson’s solution was prepared with the combination of 37% paraformaldehyde, ethyl alcohol, and glacial acetic acid. The samples were further dehydrated using 70% ethanol, then 80%, 90%, 95%, and 100% ethanol. For each ethanol replacement, the dehydration process took an hour. The specimen was immersed in xylene twice for 30 minutes each. The samples were subsequently put into the crucible. After that, the specimens were fixed into a paper holder with paraffin wax. The samples were kept overnight until they solidified. The embedded specimens are the mounted to a cube of paper. The embedded specimens were then trimmed into cubic form and followed by section cutting and staining process.

RESULTS AND DISCUSSION

1. Egg characteristic, development, and hatching

The newly stripped jade perch eggs were transparent, spherical, and measured 1.03 ± 0.02 mm in diameter, each containing a single oil globule. A total of 84.6 g of stripped eggs were collected, estimated at 1,500 eggs/g, resulting in approximately 127,000 eggs. The fertilization rate was 83.4%. After fertilization, the eggs absorbed water and swelled to 2.73 ± 0.30 mm in diameter. Fertilized eggs initially sank slowly but became suspended in the water column with gentle aeration.

Developmental progression was documented in detail. Cleavage stages began with the 1-cell stage at 0:28 hAF and reached the multi-cell stage by 1:26 hAF. Blastula stages followed between 1:32 and 4:00 hAF, then gastrulation occurred from 5:09 to 6:19 hAF. Neurulation proceeded from 6:46 to 9:09 hAF, marked by blastopore closure and embryoid body formation. Organogenesis began with tail bud formation at 10:55 hAF and continued through optic vesicle development and muscular contraction by 14:36 hAF. Hatching was first observed at 22:20 hAF (30.1%), increasing to 73.6% at 23:50 hAF, and reaching 86.4±5.6 % by 25:10 hAF. The full sequence of developmental stages is summarized in Table (1) and Fig. (1.).

Table 1. Egg development stages from fertilization to hatch of jade perch

Stage	Developmental stage	hAH
Fertilize	Fertilize	00:00
	Blastodisc Formation (1 Cell)	00:28
	2 cells	00:34
Clevage	4 cells	00:44
	8 cells	00:58
	16 cells	01:10
	Multi cells	01:26
	High Blastula	01:32
Blastula	Mid Blastula	03:32
	Low Blastula	04:00
	Early Grastula	05:09
Glastula	Mid Grastula	05:35
	Late Grastula	06:19
Neurula	Blastopore closure	06:46
	Early Neurula	07:12

	Embryoid Body formation	09:09
	Tail Bud	10:55
Organogenesis	Organogenesis and Saarcomere Emergence	11:10
	Optic Vesicle Formation	12:09
	Muscular Contraction	14:36
Hatching	Hatching	20:20-25:10

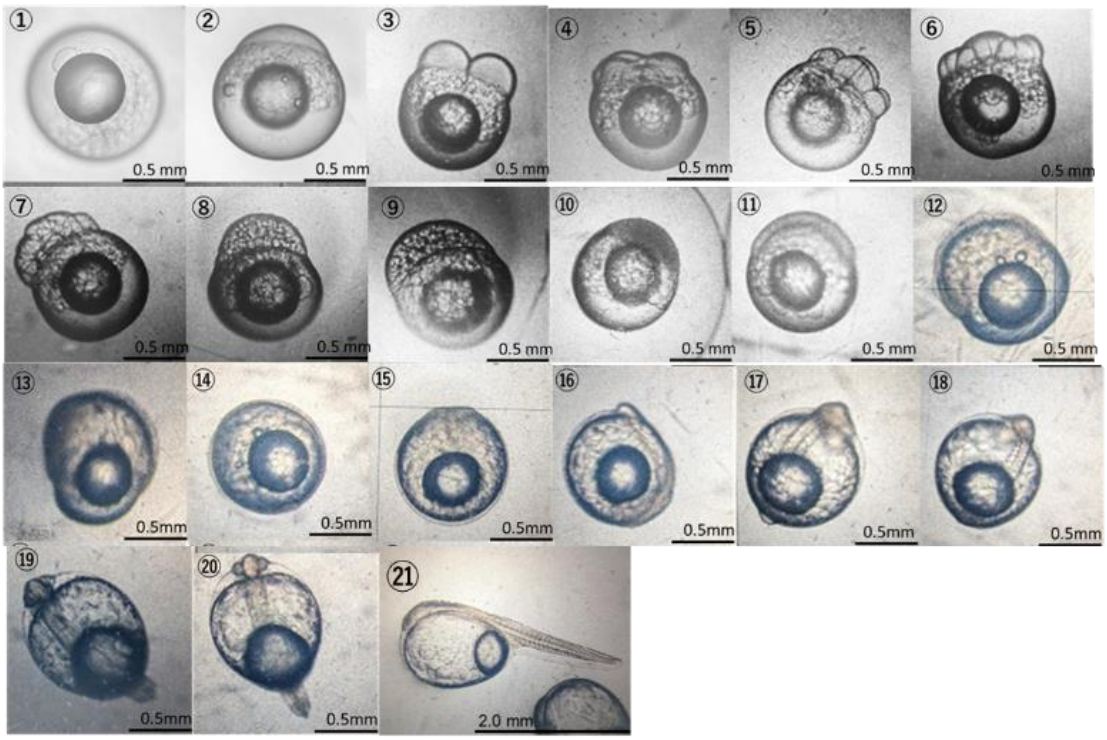

Fig. 3. Egg development of jade perch:)1) fertilized, 2) blastodisc formation, 3) 2-cell, 4) 4-cell, 5) 8-cell, 6) 16-cell, 7) multi-cell, 8) high blastula, 9) mid blastula, 10) low blastula, 11) early gastrula, 12) mid gastrula, 13) late gastrula, 14) blastopore closure, 15) early neurula, 16) embryoid body, 17) tail bud, 18) organogenesis & sarcomere emergence, 19) optic vesicle, 20) muscular contraction, 21) hatching

Fertilization and hatching rates are key indicators of early larval viability in aquaculture **(Ching *et al.*, 2018)**. In this study, HCG alone at specific dosages and intervals achieved a high hatching rate of 86.4±5.6 %, comparable to or exceeding results from other hormone protocols.

Unlike **Jie *et al.* (2018)** and the **Singapore Food Agency (2019)**, which used hormone combinations, this study achieved successful hatching using HCG alone, indicating its sufficiency for inducing spawning in the jade perch while maintaining high reproductive efficiency.

This study differed from **Jie *et al.* (2018)** by extending the injection interval to 8.5 hours and collecting eggs 16.5 hours after the second injection, aligning with species-specific ovulation timing **(Mylonas *et al.*, 2009)**. These findings highlight the importance of synchronizing hormone administration with ovulation to optimize egg quality and hatching success.

Furthermore, the method of spawning induction differed across studies. **Jie *et al.* (2018)** allowed natural spawning by pairing males and females (1:1) after hormone administration, while the **Singapore Food Agency (2019)** performed artificial stripping 26–28 hours post-injection. In contrast, this study determined an optimal egg collection time of 16.5 hours post-second injection, demonstrating the importance of precise timing in controlled spawning procedures.

These findings show that HCG alone, with optimized timing, effectively induces spawning in the jade perch, providing a simpler and cost-effective method. Further research should investigate environmental factors and hormone dosages to enhance the protocol and larval survival.

Unfertilized eggs measured 1.03 ± 0.02 mm and expanded to 2.73 ± 0.30 mm after fertilization, similar to sizes reported by **Jie *et al.* (2018)** and **SFA (2019)**. Embryonic development and hatching time are influenced by factors such as water temperature, which affects metabolic rates and developmental progress **(Aydin & Dilek, 2004; Sapkale *et al.*, 2011)**.

Water temperature ranged from 27 to 29°C, similar to previous studies. **Jie *et al.* (2018)** reported hatching times of 21–26 hours, while this study's optimal egg collection was 16.5 hours post-fertilization, indicating individual variation. **Luo *et al.* (2006)** found smaller eggs (0.87–0.92 mm) with high hatching rates at 26.7–27.4°C, suggesting that hatching success depends on temperature, egg size, and broodstock condition.

Fish gamete quality depends on factors like age, broodstock management, feeding, and water quality, all affecting embryo and larval survival **(Ivan *et al.*, 2013)**. Stress from poor hatchery conditions harms gamete quality and survival. Thus, optimizing broodstock care and maintaining stable environments post-fertilization are vital for consistent egg quality and reproductive success.

Fertilized fish eggs swell from water absorption, so measurement timing must be standardized when comparing diameters. This study confirmed hatching at 16.5 hours post-fertilization, while **Jie *et al.* (2018)** and **SFA (2019)** reported different times. Variations

may reflect species differences, hatching enzyme activity, or environmental factors like water flow.

This study observed embryos moving inside intact egg membranes, suggesting that the timing of membrane rupture and hatching is influenced by both internal biochemical factors and external stimuli like water flow and aeration. Water flow likely stimulates hatching enzymes, aiding membrane breakage. **Tanaka *et al.* (1982)** showed that varying aeration and flow improved hatching rates in the grass carp (*Ctenopharyngodon idellus*) and the silver carp (*Hypophthalmichthys molitrix*), highlighting the importance of water movement. Optimizing water flow and aeration in hatcheries is therefore crucial to enhancing the jade perch hatching success.

To better understand the jade perch egg development, future studies should use more broodstock and examine how water quality factors like temperature and salinity affect water absorption. Given the variability in timing between organogenesis and hatching (14–21 hours), detailed analysis is needed to achieve uniform hatching for stable mass production. Research should focus on optimizing management practices to improve hatching rates and synchronize development. Additionally, the effects of rearing conditions, water flow, and aeration on embryo development and hatching efficiency warrant further investigation, especially the role of controlled water flow and aeration systems in optimizing hatching.

2. Yolk sac and oil globule absorption

The yolk sac volume in newly hatched larvae was initially measured at 0.462 ± 0.079 mm^3 and showed a steady decline over time, decreasing to 0.035 ± 0.008 mm^3 by 90 hours after hatching (hAH). Despite this reduction, the yolk sac was not completely absorbed by 90 hAH, indicating ongoing utilization of yolk reserves during early larval development. In contrast, the oil globule volume remained relatively stable throughout the period, with a maximum size of 0.097 ± 0.066 mm^3 observed at 60 hAH and a minimum of 0.038 ± 0.009 mm^3 at 90 hAH, suggesting limited metabolic use compared to the yolk sac (Fig. 2).

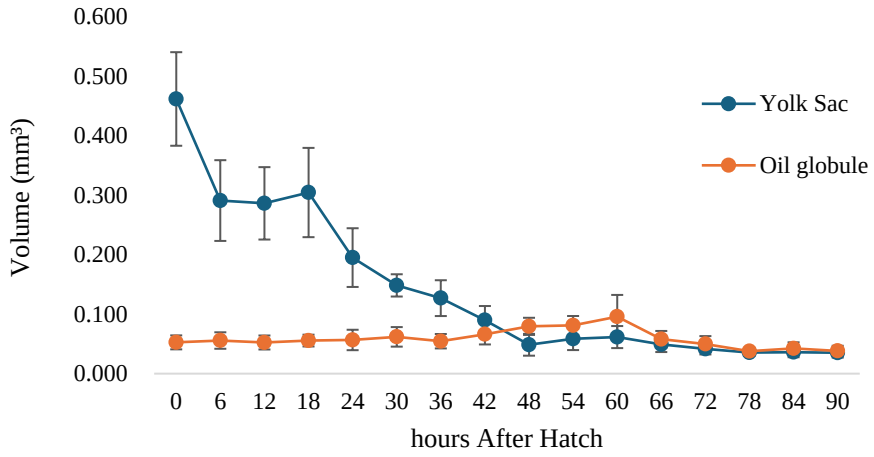

Fig. 4. Yolk sac and oil globule volumes in jade perch larvae from 0 to 90 hours after hatching (hAH)

Fish yolk is rich in proteins, lipids, carbohydrates, vitamins, and minerals, supplying essential energy for egg development and newly hatched larvae. Generally, during the period when the yolk remains, external feeding is unnecessary, and energy supply continues until larval development progresses. **(Kamler, 2008)**. The size of the yolk in fish larvae and the duration required for yolk absorption after hatching vary significantly among fish species **(Saburo, 1957; Shiraishi *et al.*, 1957; Sari *et al.*, 2023)**

In this study, yolk sac and oil globule absorption in the jade perch larvae was tracked from hatching to 90 hAH. The yolk sac volume decreased from 0.462 ± 0.079 mm³ at hatching to 0.049 ± 0.018 mm³ by 48 hAH. Most yolk was absorbed within 48 hours, but a small amount (0.035 ± 0.008 mm³) remained at 90 hAH, indicating complete absorption takes longer. These results align with **Luo *et al.* (2021)**, who reported absorption mostly by 48 hAH but complete uptake after 72 hours.

The oil globule volume was 0.097 ± 0.066 mm³ at hatching and decreased to 0.038 ± 0.009 mm³ by 90 hAH, with no significant change. **Luo *et al.* (2010)** reported that oil globules persist longer than the yolk sac, likely serving as an energy source until 4 dAH.

These results suggest the nutritional transition period (NTP) in the jade perch occurs around 2 dAH, marking the optimal start for feeding. Since oil globules persist until 4 dAH, gradually introducing feed may improve survival and growth. Further research is needed to optimize feed types and methods.

3. Larval growth

The total length of Jade perch larvae increased steadily with age (Fig. 3). From an initial 2.78 ± 0.05 mm at hatching, it grew to 4.57 ± 0.10 mm by 48 hAH. Growth slowed thereafter, reaching 4.73 ± 0.07 mm by 90 hAH.

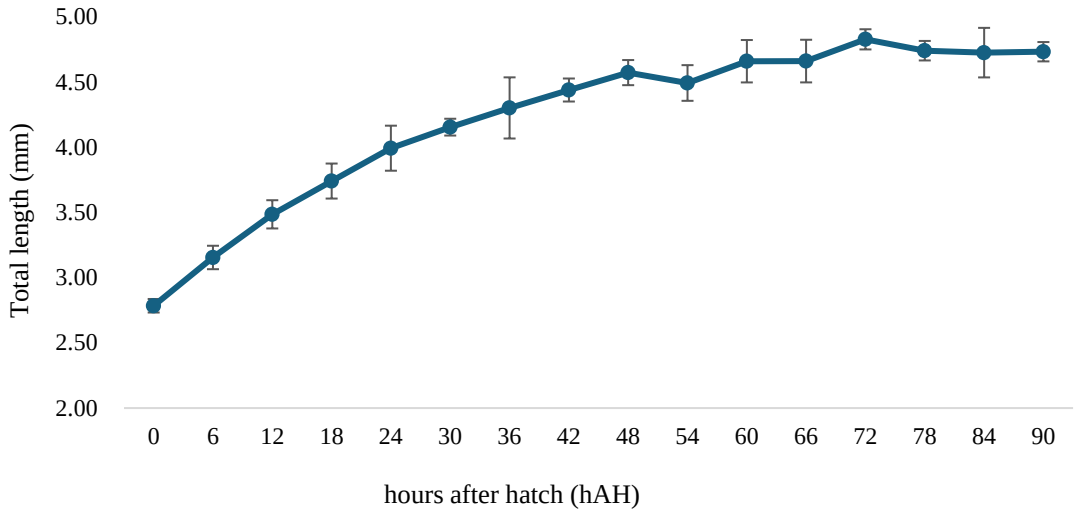

Fig. 5. Incremental changes in total length (mm) of jade perch larvae from 0 to 90 hours after hatching (hAH)

In this study, Jade perch larvae showed rapid growth within the first 24 hAH, increasing from 2.78 ± 0.05 mm at hatching to 3.49 ± 0.11 mm by 12 hAH. Growth continued more gradually thereafter, reaching 4.30 ± 0.23 mm at 36 hAH and 4.57 ± 0.10 mm at 48 hAH.

Published data **(Luo *et al.*, 2006)** showed that jade perch larvae had a total length of 2.65 ± 0.19 mm at hatching, increasing to 3.35 ± 0.15 mm at 6 hAH, 3.69 ± 0.17 mm at 12 hAH, and 4.09 ± 0.12 mm at 18 hAH, indicating rapid early growth. By 48 hAH (day 2), length reached 4.84 ± 0.21 mm and peaked at 5.04 ± 0.13 mm on day 3 (72 hAH), before slightly decreasing to 4.98 ± 0.47 mm at 96 hAH. This minor decline may result from temporary growth stagnation, limited sample size, or individual variation.

Compared to our findings, both studies show similar early growth trends. However, in our study, growth slowed after 24 hAH, while the published data showed a continued increase until 72 hAH. These differences may be due to variations in larval batches or rearing conditions.

Water temperature and rearing conditions strongly affect growth **(Aritaki *et al*., 2004; Yoseda *et al*., 2006)**. Since the published study did not specify environmental conditions, differences from this research are possible. Food quality, quantity, and broodstock health may also impact growth rates.

Comparing this study with published research confirms common the jade perch growth trends, influenced mainly by environment and rearing conditions. Future studies should explore these factors further to develop stable growth management practices.

4. Larval morphology and sensory organ development and behavioral changes

Larval morphology, sensory organ development, and behavioral changes in Jade perch larvae from 0 to 90 hAH are summarized in Fig. (4). The development of the eye, olfront pits and inner ear are shown in Figs. (5, 6 and 7), respectively. At 0 hAH, enlargement of the fin, floating, formation of lens and inner ear were confirmed. At 12 hAH, the larvae were observed swimming vertically, repeatedly going back and forth between the water surface and the bottom of the beaker. At 18 hAH, horizontal swimming of the larvae was observed, and at 24 hAH, the formation of the tail was confirmed. At 30 hAH, the following were observed: positive phototaxis and rheotaxis, pigmentation of the eyes, and formation of olfactory pits. At 36 hAH, mouth opening and at 42hAH, jaw movement was observed. Feeding began at 48 hAH, swimming ability improved at 54 hAH, and anus opening was confirmed at 60 hAH. At 72 hAH, teeth formation, intestinal tract moving and feeding was confirmed. At 78 hAH oil globule shrink was observed.

Table 2. Morphology development, behavioral changes and sensory organs development of jade perch larvae from 0 hAH to 90 hAH

Hours after hatch (hAH)	Morphology development	Behavioral changes	Sensory organs development
0		Floating	
6	Fins spread		Lens formation inner ear formation
12		Vertical swimming	
18		Horizontal swimming	
24	Tail constricted		
30		Confirmation of phototaxis and rheotaxis	Eye pigmentation olfactory pits formation
36	Mouth open		
42	Jaw moving		
48		Feeding start	
54		Improving swimming ability	
60	Anus open		
72	Intestinal tract moving	Confirmation of feeding	
78 - 90	Oil Globule Shrink		

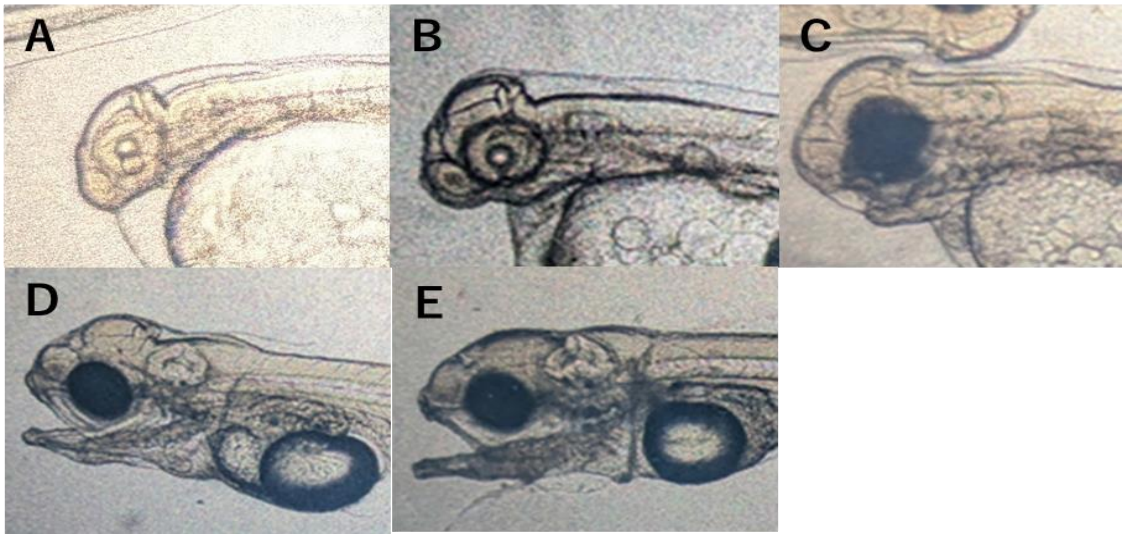

Fig. 4. Eye development of jade perch larvae. A – E profile projector observation of eye. A: 0 hAH, B: 18 hAH, C: 30 hAH, D: 48 hAH, E: 90 hAH

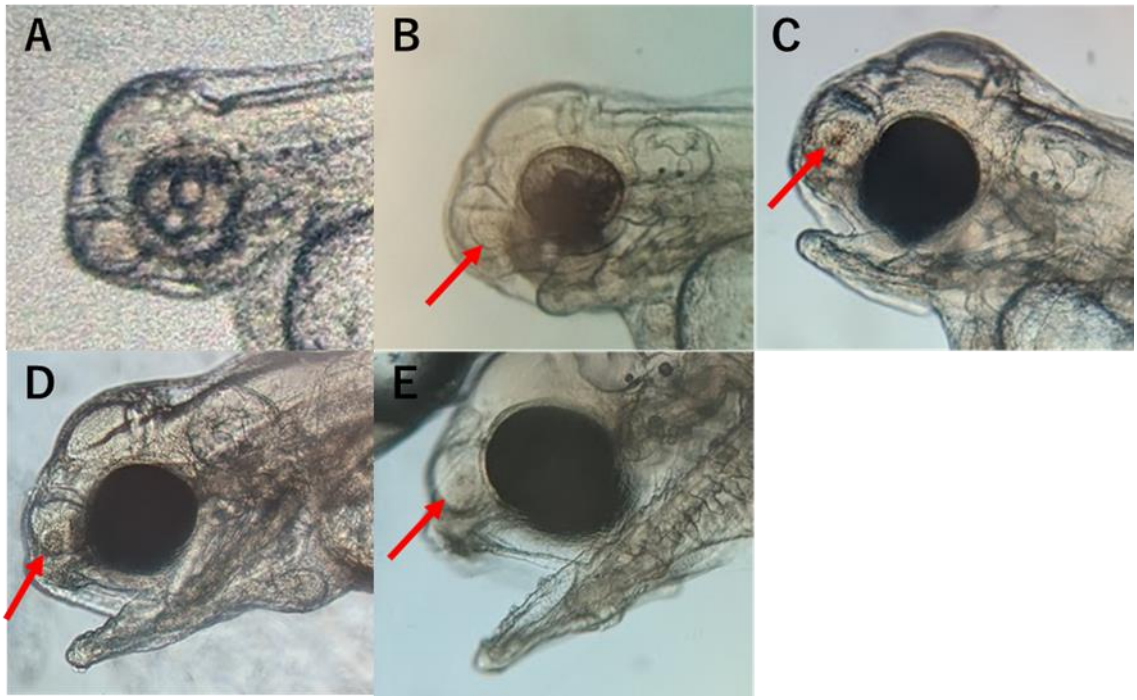

Fig. 5. A: Microscopic observation of olfront pits 24 hAH. **B – E:** Light microscope observation of olfront pits. **B:** 30 hAH, **C:** 36 hAH, **D:** 42 hAH, **E:** 72 hAH

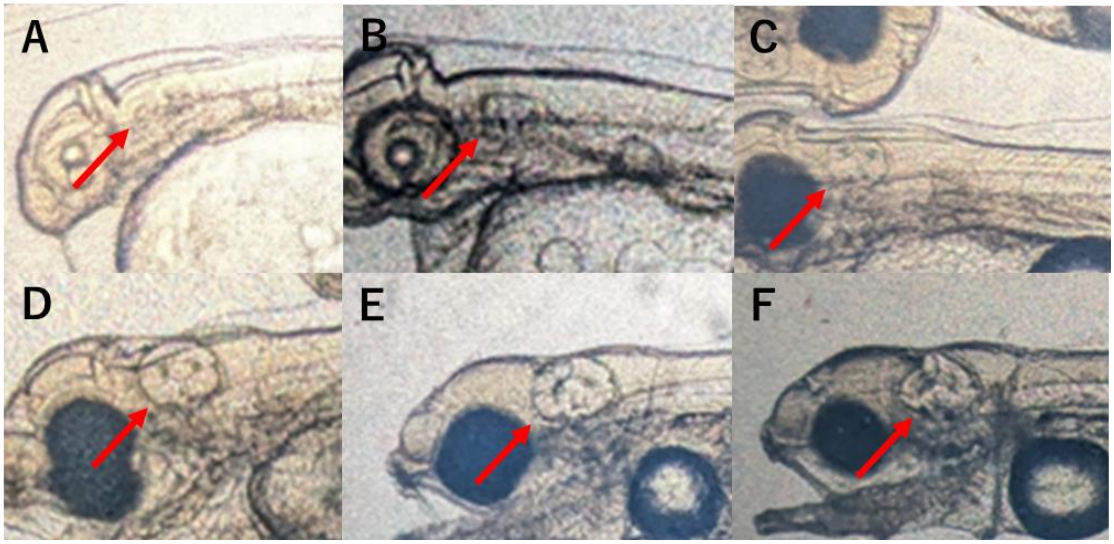

Fig. 6. Microscopic observation of inner ear. Red arrow indicates the inner ear **A:** 6 hAH, 18 hAH, 30 hAH, 56 hAH, 78 hAH, 90 hAH

Larval growth and development are significantly influenced by environmental factors such as water temperature and salinity, which can result in intra-species variation **(Houde, 1974; Polo *et al.*, 1991; Bridget *et al.*, 2004; Martell *et al.*, 2005)**. This study examined the developmental progression and feeding behavior of the jade perch larvae. By integrating findings from **Luo *et al.* (2010)** and **SFA (2019)**, we obtained key insights into the timing of first feeding, prey selection, and the onset of competition and cannibalism

In the study by **Luo *et al.* (2010)**, it was reported that rotifers (*Brachionus plicatilis*) were provided from day 3 to day 7, moina (*Moina* sp.) from day 4 to day 14, and artificial feed from day 8 to 9, with cannibalism observed from day 7 onward. Furthermore, Jade perch larvae were fed with *Artemia* (*Artemia* sp.) as the optimal feed for 3-day-old larvae. **Cladocera** and *Artemia* (*Artemia* sp.) were recommended for 8-day-old larvae. Similarly, other research also used rotifers (*Brachionus plicatilis*) from day 3 to day 7, moina (*Moina* sp.) from day 4 to day 14, and observed cannibalism starting at day 7. These data suggest that food supply quantity and method significantly affect feeding behavior and cannibalism in the jade perch. They emphasize the critical role of feeding timing and food selection in the growth of larvae.

In contrast, in the present study, the jade perch larvae began feeding at 48 hAH and showed healthy growth thereafter. This feeding initiation timing differs from that observed in the **Luo *et al.* (2010)** and other research studies, where feeding began earlier. However, starting feeding at 48 hAH in this study may have prevented cannibalism, as no cannibalism was observed during the growth process. The development of sensory organs (eyes, olfactory pits, inner ear) in the larvae was closely linked to feeding behavior, and the ability to begin feeding at 48 hAH appears to be an appropriate timing for optimizing feeding behavior. Notably, visual and olfactory development at 30 hAH likely played a significant role in the larvae's feeding initiation.

Luo *et al.* (2010) identified *Artemia* (*Artemia* sp.) as the optimal feed for 3-day-old larvae, and this aligns with the importance of food selection in the growth of the jade perch larvae. In this study, larvae began feeding at 48 hAH, which could be attributed to the maturation of sensory systems that enabled better food detection and consumption. This suggests that visual and olfactory cues are crucial for feeding initiation, and their development is essential for ensuring the larvae begin feeding efficiently. Cannibalism in Jade perch larvae, as observed in the studies by **Luo *et al.* (2010)** and other research, is likely triggered by a lack of sufficient food supply. **Luo *et al.* (2010)** noted cannibalism from day 7, likely due to inadequate food provision. In contrast, the present study avoided cannibalism by ensuring appropriate feeding initiation at 48 hAH, supporting the idea that well-timed feeding initiation helps prevent such behaviors and promotes healthy growth.

CONCLUSION

By integrating the findings of **Luo *et al.* (2010)** and other relevant research with the current study, we conclude that feeding behavior and growth observed in the jade perch larvae are significantly influenced by environmental factors such as water temperature, food supply timing, and sensory organ development. These findings suggest that optimizing the timing of feeding initiation and ensuring the right food selection are essential for the efficient cultivation of the jade perch larvae. Further studies on the effects of environmental factors and feeding strategies on feeding behavior and growth will be crucial for improving aquaculture practices for this species.

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