



Mechanosensory Perception and Food Localisation in *Pangasius nasutus* Larvae: A Developmental Perspective

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Abstract

Patin fish (*Pangasius nasutus*) larvae hatch with immature morphological features, but as the larvae grow, all their sensory organs develop rapidly. The objectives of this study were to observe the behaviour of *P. nasutus* larvae and evaluate the development of their sensory organs. The larvae's behaviour was observed from the time of hatching, while the development of sensory organs was examined through histology and scanning electron microscope (SEM) analysis. This study demonstrated that changes in larval behaviour were influenced by the development of their sensory organs. During the initial stages, at Day 1 after hatching (DAH), most of the sensory organs were not yet fully developed. As a result, the larvae remained at the bottom of the aquarium and swam in a vertical direction. However, as the sensory organs started to develop, their behaviour changed. They became more active and began to swim horizontally throughout the aquarium, exhibiting phototaxis. The increased number of taste buds facilitated easier food detection, while the presence of free neuromasts enabled the larvae to respond to water movement, exhibiting rheotaxis at 3 DAH, the day of their first feeding. Furthermore, the development of other sensory organs, such as larvae's retina, at this stage enhanced their ability to navigate. The study found that larvae primarily use mechanosensory perception to locate food. This reliance is due to the earlier development of taste buds and olfactory organs compared to other sensory organs, with the sensory organ of *P. nasutus* taking 21 days after hatching to fully develop.

Keywords: Patin fish, larval behaviour, sensory organs, Malaysia, freshwater

Introduction

In Malaysia, *Pangasius nasutus*, locally called "Patin Buah", is one of the potential fish to be produced and cultured in a high volume due to its high market demand both domestically and across Southeast Asia, especially in countries like Vietnam (Asdari et al., 2011; Hassan et al., 2011). Consumers prefer *P. nasutus* over other *Pangasius* spp. like silver catfish (*P. micronemus*) and river catfish (*P. hypophthalmus*) (Hassan et al., 2011), even though the price of *P. nasutus* is nearly three times more expensive than both *P. micronemus* and *P. hypophthalmus* as the taste of *P. nasutus* is better (Hassan et al., 2011). In Malaysia, *P. nasutus* can be found predominantly in the rivers of Pahang, mostly

in Maran and Pekan districts (Asdari et al., 2011). People rely on wild stock to supply the fish in the market. Hence, the population of wild *P. nasutus* shows decreasing in number due to overfishing activities, putting it at risk of extinction if there are no actions taken to conserve and preserve the wild population of *P. nasutus* (Hassan et al., 2011; Jaapar et al., 2021). Introducing *P. nasutus* for domestication in commercial aquaculture to reduce dependence on wild populations is of paramount importance (Asdari et al., 2011). However, achieving successful freshwater fish culture, including *P. nasutus*, presents several challenges. Among these are heightened susceptibility to water quality issues and infections, primarily from fungal sources, which severely impact

hatching and survival rates of freshwater fishes (Rahman et al., 2017; Marimuthu et al., 2019). Additionally, the slow growth rate of *P. nasutus* poses a significant obstacle to its successful cultivation (Hassan et al., 2011; Jaafar et al., 2023; Idris et al., 2024). Therefore, comprehensive strategies and technologies need to be developed and implemented to address these obstacles and establish sustainable aquaculture practices.

Sensory organs play a critical role in the survival of fish, enabling them to evade predators and assess food suitability (Mukai et al., 2010). The detection of stimuli from their surroundings triggers a wide range of behaviours in fish, with the behaviour of fish larvae particularly closely linked to their responses (Mukai et al., 1994). Given the heightened sensitivity and fragility of *P. nasutus* compared to other pangasid species, like river catfish (*P. hypophthalmus*), implementing specialised hatchery practices becomes imperative to prevent mortality under controlled conditions.

Consequently, further research is required to collect comprehensive data and develop effective strategies for enhancing the hatching and survival rates of *P. nasutus* in captive breeding programs. Understanding the sensory organs of fish larvae is crucial for gaining insights into their life history in natural habitats and improving larval rearing techniques in hatcheries. Therefore, this study aimed to evaluate the developmental progress of sensory organs, including the eyes, taste buds, olfactory organs, otoliths, and the lateral line, in *P. nasutus* larvae.

Materials and Methods

Ethical approval

The Animal Ethics Committee of Universiti Putra Malaysia gave its consent to this study (approval number R074/2022). All the samples were handled according to the guidelines. The research was carried out at Department of Aquaculture, Faculty of Agriculture and Institute of Bioscience, Universiti Putra Malaysia.

Broodstock

Pangasius nasutus specimens were obtained from a local hatchery, Three Ocean Fish Ponds & Trading Pte Ltd, located in Rawang, Selangor. Six broodstocks, weighing approximately 1 kg to 1.5 kg each, were individually monitored in a tank for several days prior to the breeding process.

Breeding methodology

Matured broodstocks underwent a 24 h fasting period before capture and handling. To induce ovulation and enhance hatchability success, female broodstocks received an injection of Ovaprim (Syndel, Canada), a commercial mixture of GnRH and domperidone, at a

dose of 0.6 mL.kg⁻¹ of female body weight (Chakraborty, 2021). A second hormone injection was administered to females 12 h after the initial injection to further support ovulation. Similarly, males were given a single Ovaprim injection at a dose of 0.4 mL.kg⁻¹ at the same time as the female Ovaprim injection, with the aim of increasing the quantity of collected semen and reducing its viscosity. Both males and females received an intramuscular injection below the dorsal fin. Following the hormone injection, broodstocks were anaesthetised with MS-222 (100 ppm) and both males and females were hand-stripped to collect eggs and sperm. The collected eggs and sperm were then dry-mixed, and water was added to facilitate fertilisation. Subsequently, the eggs were allowed to hatch after a 36 h incubation period.

Larval rearing

The unhatched eggs were removed from the tank to maintain water quality and prevent fungus or bacterial infection. After the larvae started to feed exogenously, they were fed with a commercial larval feed (FS Feed™ Ornamental, F&S Aquatech Pte. Ltd. Malaysia) from 3 days after hatching (DAH) onwards and were gradually fed with a commercial fish pellet from Ocean Star International Inc., USA, from 8 to 9 DAH onwards. Starting at 15 DAH, the larvae were administered with commercial fish pellets only (Saki Hikari®Kyorin, Japan). One hour after feeding, the uneaten feed and the larvae fry excrement were removed with a siphon. Throughout the larviculture period, the water quality was monitored. Water exchange was carried out every day, 30 % per day, to maintain the water parameters at the optimal level for larval growth and survival. The water quality was measured through its salinity, water temperature, pH, and dissolved oxygen (DO) using a multi-parameter (556 MPS, YSI, USA), while ammonia was monitored using an ammonia test kit (HI96700, Hanna, Italy).

Monitoring and behaviour study

Larval behaviour, encompassing swimming patterns, cannibalism tendencies, phototaxis, and rheotaxis responses, was meticulously observed daily within a controlled environment of a 120 L transparent glass aquarium with gentle aeration. For this purpose, 100 hatched larvae were separated and placed into a 5 L glass aquarium in three replicates for observation. To specifically investigate fish phototaxis, a carefully designed experiment was conducted using a small electric light that could be positioned at various points around the aquarium to create localised light zones. This setup enabled the assessment of whether the larvae displayed a tendency to cluster around the light source or avoid it, indicating their phototactic response. The intensity and duration of the light exposure were varied systematically to observe changes in larval behaviour under different light conditions.

In addition, a customised setup involving a gentle, adjustable water flow system was implemented within

the same environment to study rheotaxis. This system allowed for the observation of larvae's orientation and swimming behaviour in response to varying flow directions and speeds, simulating conditions that would be encountered in their natural habitats.

During the observation period, particular attention was given to tracking swimming behaviours, any instances of cannibalism among the larvae, and their responses to both light (phototaxis) and water flow (rheotaxis). The transparent glass aquarium provided an optimal setting for comprehensive visual analysis of the larvae's behaviours, enabling the collection of valuable data essential for a deeper understanding of their developmental processes and responses to environmental cues.

Histology

Pangasius nasutus larvae were fixed in Bouin's solution (Carleton et al., 1967) within 12 h to soften the tissues and prevent samples from autolysis or putrefaction using standard techniques (Humason, 1979). The samples were then immersed in low to higher concentrations of alcohol (70–100 %) to dehydrate the samples and replaced it with a wax block. This wax block was then hardened to create a tissue block for the sectioning procedure. The samples underwent clearing process in xylene solvent and were finally soaked in paraffin wax (Leica Biosystems, Germany) at 60 °C for 2 h. Later, the samples were cut into 6 µm by using rotatory microtome and floated in 38 °C water baths before placed on glass slide for staining procedure. Subsequently, the slides were stained with haematoxylin-eosin (Richard-Allan Scientific, UK) to enhance the coloration of the tissue and muscle (Slaoui and Fiette, 2011). To preserve the samples on glass slides from damage and long-term degradation, they were covered with DPX mounting medium. The prepared slides were then examined using a compound microscope (DM750, Leica, Germany) at a magnification of 25×, aided by eye piece camera (AM7023, DinoEye, Taiwan), to facilitate accurate and detailed observation and analysis of the larval structures and tissues.

Scanning electron microscopy (SEM)

The samples were fixed in 4 % glutaraldehyde for 24 h, followed by three consecutive 10 min washes with 0.1 M sodium cacodylate buffer to remove the fixative. Subsequently, a post-fixation step was performed using 1 percent buffered osmium tetroxide for 2 h at 4 °C to enhance sample contrast for improved observation. The samples were then washed again with 0.1 M sodium cacodylate buffer. For dehydration, the samples were sequentially immersed in acetone at concentrations of 30, 50, 75, 95, and 100 percent, with each concentration step lasting 15 min. Next, the samples were placed in a critical point drying (CPD) basket and subjected to dehydration using liquid carbon dioxide (CO₂) at a pressure of 800 PSI for 30 min

(CPD 030, Leica, Germany). Following dehydration, the dried samples were affixed to stubs using double-sided tape and mounted on gold-coated plates. The materials were then examined using an electron microscope (JSM-IT100, JEOL, Japan). The larval development was documented using photographs captured with a combination of light and scanning electron microscopy techniques.

Results

The morphological, behavioural, and sensory changes in *P. nasutus* larvae are summarised in Table 1. At 1 day after hatching (DAH), the larvae were observed to remain near the bottom and corners of the aquarium. They exhibited vertical swimming behaviour, aligning themselves with the water flow. Each lower and upper jaw of the 1 DAH larvae possessed a single pair of barbels. Phototaxis was evident as the larvae swam towards the light source emitted by a torch. By 2 DAH, the larvae transitioned to horizontal swimming, following the water flow. The barbels of the larvae had elongated compared to the previous day. At 3 DAH, the larvae began actively feeding on *Artemia* nauplii and cannibalism among the larvae was not observed. Additionally, the larvae demonstrated clear phototaxis and rheotaxis as they swam against the water flow. At 4 DAH, the larvae consistently swam in the middle layer of the aquarium and actively searched for food. The comprehensive illustration of *P. nasutus* fry physical development is shown in Figure 1.

Development of sensory organs

Eyes

Pangasius nasutus larvae hatched with undeveloped eyes, although the eye vesicles were visibly present. On 1 DAH, the larvae exhibited pigmented eyes, and the retina began to differentiate into distinct layers. Notably, at 3 DAH, the layers of the retina started to develop (Fig. 2b), with a significant increase in the number of cone cells. At 4 DAH, the eyes of *P. nasutus* larvae appeared morphologically complete, showing recognisable layers within the retina. However, the 4-day-old larvae, as depicted in Figure 2c, did not exhibit rod cells, making them an exception. The emergence of rod cells was estimated to occur at 15-days-old, although they were not observable under a light microscope. The visual cell nuclei, primarily located in the outer nuclear layer, were abundant and surpassed the number of cone cells.

Olfactory organ

In newly hatched larvae, small nasal pits devoid of indentations were observed. As the larvae grew, the nasal pits expanded, and within 1 DAH, their outline became distinctly visible. By the 2 DAH, numerous cilia were evident in the sensory epithelium. Several ciliated receptor cells and abundant ciliated neurosensory cells were also observed. The sensory

Table 1. Chronology of morphological, sensory, and behavioural development of *Pangasius nasutus* larvae. The table details the progression of body length (BL $\pm$ SD mm), morphological features, behaviours, and sensory organ developments from 1 day after hatching (DAH) to 21 DAH. Newly hatched larvae (BL $\pm$ SD mm; 4.26 ± 0.3) had large yolk sacs, and their mouths and anuses were not yet open.

Day after hatching BL (mm)	Morphology	Behaviour	Sense organs
1 4.2 $\pm$ 0.3	- Large yolk sac - Mouth and anus not open	- Stay at the bottom and corners of the aquarium - Start to swim in vertical directions - Swam in short distance	- Unpigmented eyes - Cilia in nasal pits lack depressions - One pair of auditory vesicles - One pair of free neuromasts on the head
2 4.7 $\pm$ 0.3	- Barbels on upper and lower jaw - Notochord flexed - Mouth open - Anus open - Primordia of pectoral fins appeared	- Avoided a transparent pipette - Swam actively in vertical directions - Showed clear phototaxis	- Eyes were slightly pigmented - Taste buds appeared - Small neuromasts appeared
3 6.3 $\pm$ 0.5	- Barbels on upper and lower jaw longer - Yolk sac degrade	- Commenced feeding on <i>Artemia</i> nauplii - Swam actively in horizontal directions - Showed clear phototaxis and rheotaxis - Showed cannibalism	- Many cilia in nasal pits - Many taste buds on the skin surface, in the buccal cavity, and on the gills semicircular canal, utricle, and saccule - Free neuromasts developed - Lagena, and three otoliths at 3 days old
4 7.04 $\pm$ 1.3	- Notochord is fully flexed, and fin rays are visible on both the caudal and anal fins - Pigments on head and trunk surface	- Swam continuously in the middle layer of the aquarium - Cannibalism	Number of taste buds increased
5 7.88 $\pm$ 0.2	Pectoral fin appeared	Swim against water current	15 % of the free neuromasts were observed to be positioned sunk on the head
10 9.48 $\pm$ 0.5	Fin rays on dorsal fin		- Number of taste buds and free neuromasts increased 35 % of the free neuromasts were observed to be positioned sunk on the head
15 13.7 $\pm$ 1.8		Swam freely through the aquarium	
21 21.8 $\pm$ 1.1	Deeply pigmented	Swam in a school	- Four lamellae in nasal pit - Free neuromast sunk on the head

Mean $\pm$ standard deviation of body length; n = 15.

epithelium of the pits continued to enlarge, exhibiting folding patterns on the third day after hatching (Figs. 3, 4). Subsequently, on the fifth day after hatching, the epithelium descended beneath the larvae's skin. By the tenth day after hatching, the larvae's olfactory pits had divided into two distinct portions, the anterior and posterior segments (Fig. 4).

Taste buds

The taste buds cannot be found on newly hatched larvae but can be found at 1 DAH larvae's barbels. The number of taste buds in 2 DAH larvae were increased greatly around the larvae's mouth, barbels, and in the buccal cavity. The taste buds can be detected on the lips of 3 DAH larvae (Fig. 5a). The number of taste buds

in the head area and the buccal cavity increased as the fish grew larger (Fig. 5b).

Free neuromasts

On a newly hatched larvae's head, one pair of free neuromasts was found and the number of the neuromasts increased gradually along the fish's growth. In 1 DAH, there were eight free neuromasts found on the head and three free neuromasts on the flank (one side of the body) while the developed free neuromasts were found on the head and the flank of 2-days-old larvae (Fig. 6a). The developed free neuromasts were distributed in two lines. 10-days-old larvae had 22 free neuromasts on the head and 61 free neuromasts on the flank of the body. Some of the free

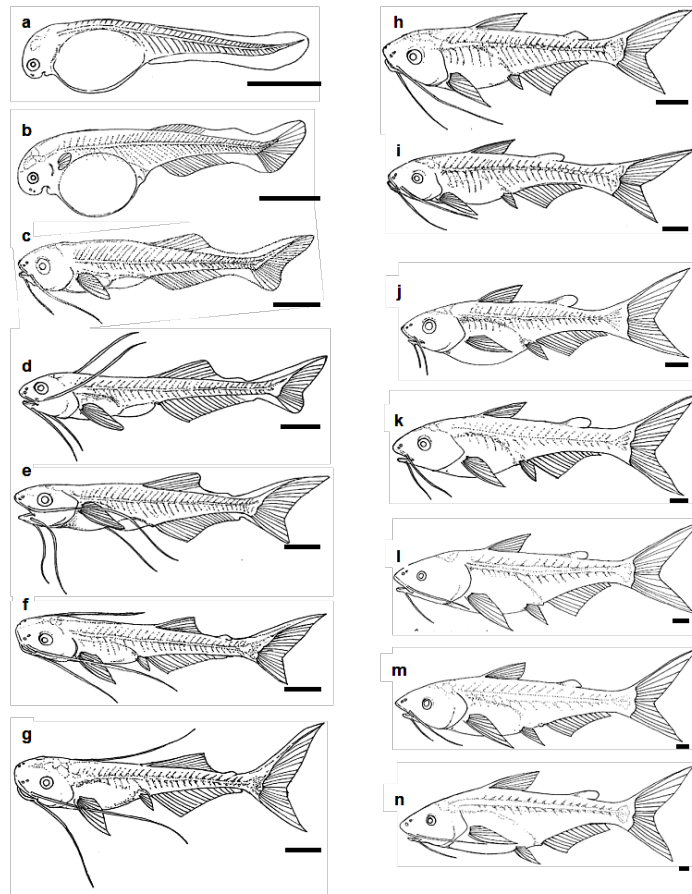


Fig. 1. The changes in the morphological characteristics of *Pangasius nasutus* larvae at different developmental stages. The images depict the larvae at the following days after hatching (DAH): (a) 1 DAH, (b) 2 DAH, (c) 3 DAH, (d) 4 DAH, (e) 5 DAH, (f) 6 DAH, (g) 8 DAH, (h) 10 DAH, (i) 12 DAH, (j) 14 DAH, (k) 16 DAH, (l) 18 DAH, (m) 20 DAH, and (n) 21 DAH. Each scale bar in the images represents 1 mm.

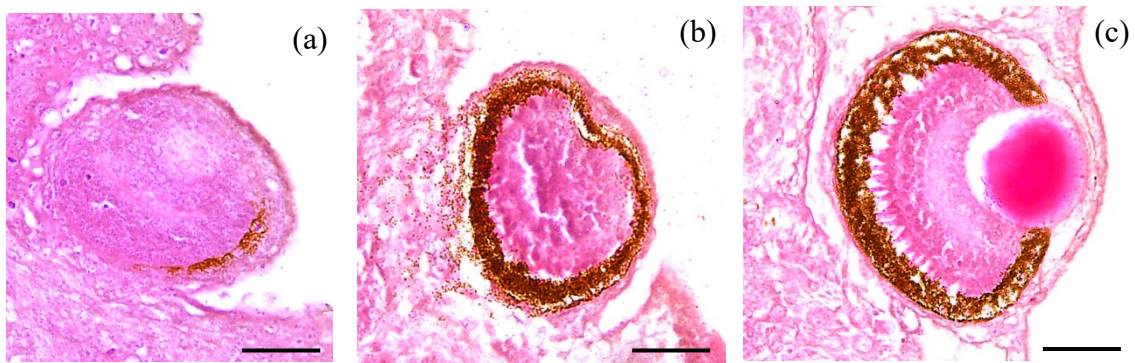


Fig. 2. The developmental stages of the retina in *Pangasius nasutus* larvae. (a) At 2 days after hatching (DAH), the eyes exhibited pigmented epithelium. (b) By 3 DAH, the number of cone cells increases rapidly, and the layers of the retina began to form. (c) By 4 DAH, all layers of the retina were identifiable, and the eyes were almost morphologically complete, except for the absence of rod cells. The scale bar in each image represents 100 µm.

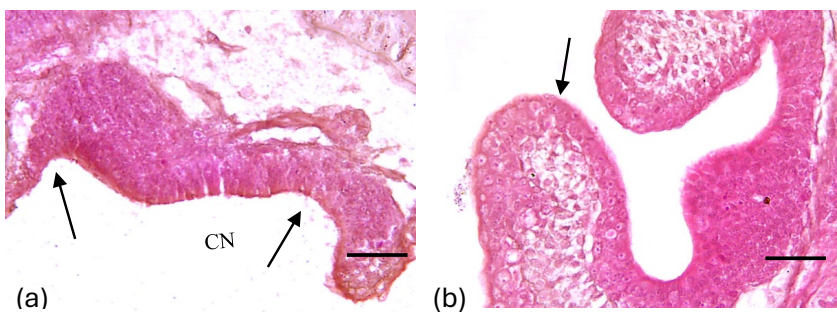


Fig. 3. The histological images of the nasal pits of *Pangasius nasutus* larvae at different stages of development. (a) The arrow indicates the expansion of the nasal pits at 3 days after hatching (DAH), which contain ciliated nonsensory cells. (b) By 4 DAH, the nasal pits have folded, also indicated by the arrow. The scale bar in each image represents 100 µm.

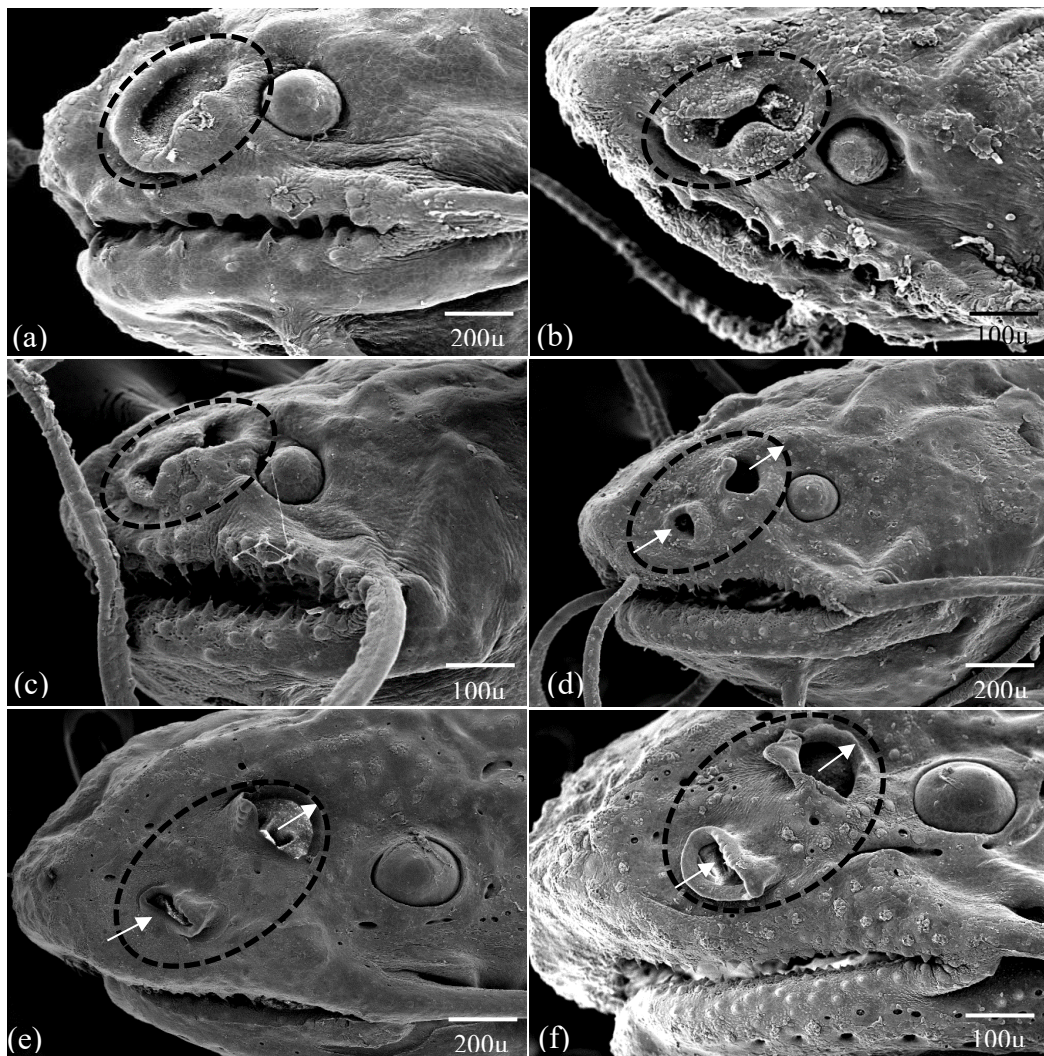


Fig. 4. Scanning electron micrographs showing the development of the olfactory organs in *Pangasius nasutus* larvae at different days after hatching (DAH). (a) At 3 DAH, the nasal pits are extended. (b) By 4 DAH, the nasal pits start to fold. (c) At 5 DAH, the folding of the nasal pits continues, forming a pit. (d) By 9 DAH, a fully developed pit is visible. (e) At 17 DAH, the nasal pits merge and extend further. (f) At 21 DAH, the nasal pits are fully developed. The arrow above indicates the direction of water flow into the nasal pits.

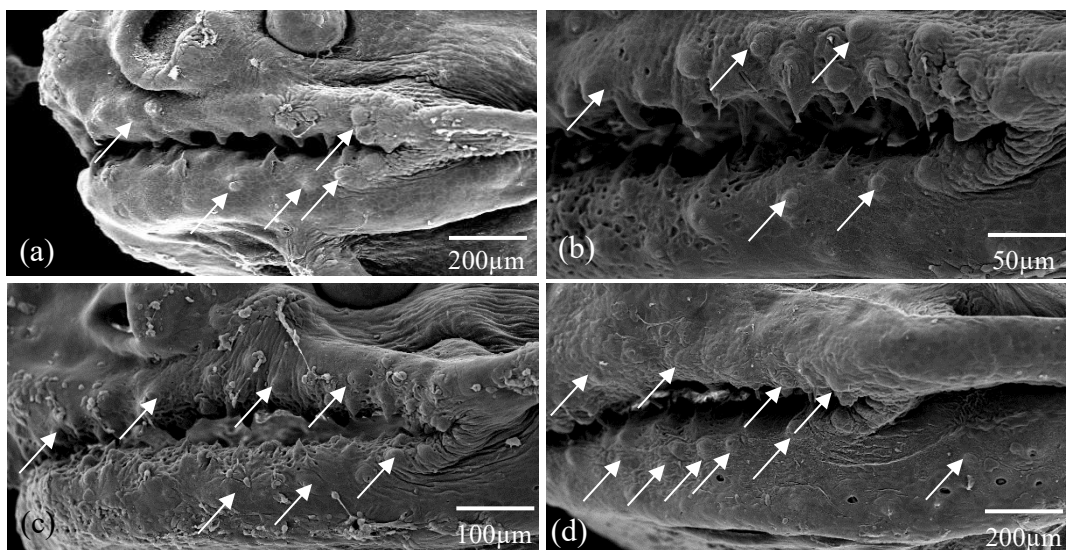


Fig. 5. Scanning electron microscope images of *Pangasius nasutus* larvae, focusing on the development of taste buds at various days after hatching (DAH). (a) At 3 DAH, numerous taste buds are present on the mouth. (b) By 5 DAH, (c) 9 DAH, and (d) 13 DAH, the number of taste buds on the surface of the mouth and within the buccal cavity gradually increases as the larvae grow.

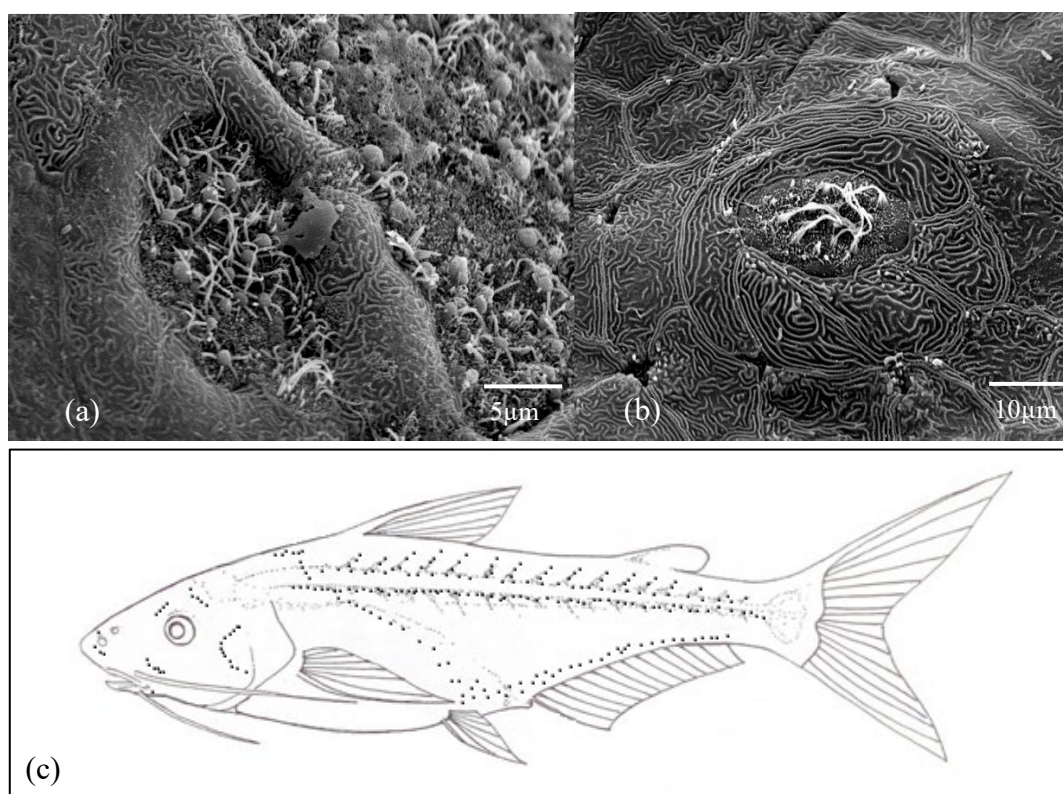


Fig. 6. Scanning electron microscope images of free neuromasts in *Pangasius nasutus* larvae at various developmental stages. (a) At 2 days after hatching (DAH), developed free neuromasts are observed on the head. (b) By 3 DAH, the number of developed free neuromasts on the head has increased significantly. (c) At 21 DAH, the distribution of free neuromasts is visible across the entire body of the larvae.

neuromasts had already sunk or started to sink under the skin on the larvae's head.

Inner ears

In newly hatched larvae, round auditory vesicles were identified (Fig. 7a), while in 2 days old larvae, there were a few cavities observed at the auditory vesicles and three semicircular canals were also found (Fig. 7b). The canals are utricle, and saccule, each of which is equipped with the macula. In the inner ear of 3-days-old larvae's inner ears, three pairs of otoliths and a lagena were identified.

Discussion

Pangasius nasutus exhibits a rapid development of sensory organs, a pattern observed in other catfish species such as the African catfish (Mukai et al., 2008) and sutchi catfish (Mukai et al., 2010). Enhancing rearing practices to improve survival rates is crucial as sensory organ development is closely linked to behavioural changes.

This study presents a practical method to determine the initiation of first feeding by observing behavioural shifts, such as a transition from a fixed position to

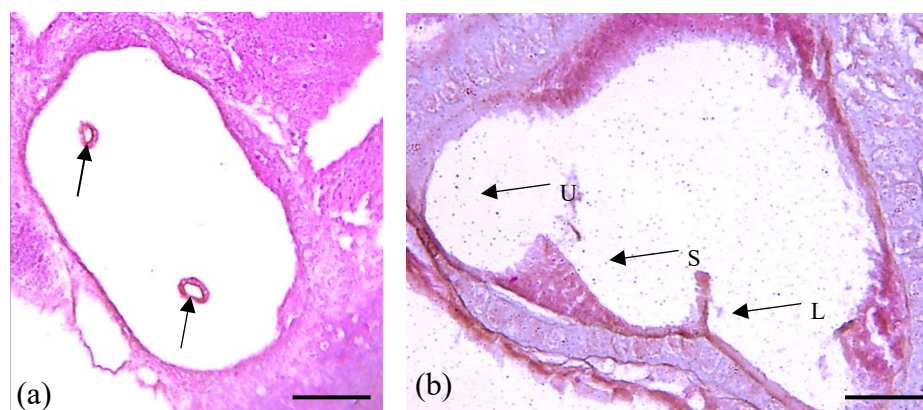


Fig. 7. The development of the auditory vesicles in *Pangasius nasutus* larvae. (a) At 2 days after hatching (DAH), round auditory vesicles with several cavities are observed. (b) By 3 DAH, the three semicircular canals begin to develop, along with the utricle (U), saccule (S), and lagena (L). Each scale bar in the images represents 100 μ m.

swimming at the bottom of the tank, coinciding with the development of sensory organs and specific morphological characteristics while the yolk reserves are still being partially absorbed. Previous research has highlighted the importance of timely first feeding, as delaying it can negatively impact survival (Gisbert et al., 2004; Zhang et al., 2009; Garcia et al., 2020), growth (Shan et al., 2008), deformity risk (Dou et al., 2005; Peña and Dumas, 2005), and increase the likelihood of starvation, which can lead to cannibalism during early larval stages.

First feeding was observed in this study at 72 h after hatching (hAH), consistent with the findings of Mukai et al. (2010) for the sutchi catfish, *P. hypophthalmus*. At this stage, feeding activity was frequently observed at the bottom of the tank, and the presence of *Artemia* was detected in the intestine. The initial signs of cannibalistic behaviour in *P. nasutus* were observed at 72 hAH, characterised by pursuit and biting of the rear body region (although consumption of the entire prey was rare). This behaviour coincided with the emergence of teeth and an increase in mouth size, highlighting the importance of early initiation of feeding during the larval stages for survival. This behaviour occurred earlier than the timing reported by Rahmah et al. (2011) for the sutchi catfish at 54 hAH.

Interestingly, despite the absence of rod cells for superior night vision, cannibalistic behaviour was frequently observed throughout the day. This behaviour could be facilitated by the presence of taste buds that develop on the barbels, lips, palate, and gill arches, enhancing the fish's foraging sensitivity and aligning with its nocturnal behaviour. Although it is common practice to feed *P. nasutus* during the day, this feeding pattern may result in starvation at night, increasing the risk of cannibalism. Adjusting photoperiods (day and night lengths) and feeding patterns (constant, daytime, or night-time feeding) should be considered to enhance larval survival by reducing cannibalistic behaviour. Additionally, the development of quantitative methods for analysing taste preferences in fish, such as the use of fluorescent labelled artificial foods, can provide further insights into their feeding behaviours and preferences (Okada, 2015; Okomoda et al., 2019).

Based on the behavioural observations conducted in this experiment, the larvae of *P. nasutus* tend to aggregate in the bottom corner of the tank during the day but exhibit active swimming behaviour at night. Faucher et al. (2010) proposed a connection between this aggregation behaviour and the distribution of free neuromasts on the head and trunk. Our study further supports this notion by demonstrating that free neuromasts influence larval aggregation, as previously noted in marine teleosts that efficiently respond to sensory stimulation even in dark conditions (Blaxter and Fuiman, 1989). However, the current understanding of free neuromasts in *P. nasutus* larvae remains fragmented and based on random and

inconsistent observations.

Even before hatching, the presence and size of neuromasts suggest their crucial role in the larvae's sensory awareness, particularly in species lacking functional eyes. Since most species do not feed in the dark, it is doubtful that neuromasts play a significant role in feeding. In the context of cannibalism in African catfish, research suggests that group behaviour may increase physical contact between individuals (Applebaum and Kamler, 2000). Therefore, maintaining an appropriate larval density that focuses on providing sufficient resting space at the bottom surface area should reduce the likelihood of cannibalism. Additionally, exploring the potential of utilising the three-dimensional space of the entire tank to offer shelter could be further investigated for the same purpose.

The development of sensory organs, including eyes, olfactory organs, inner ears, lateral lines, and taste buds, is crucial for *P. nasutus* larvae, particularly for locating food during the transition to exogenous feeding and to protect themselves from predators. Insufficiently developed sensory organs can impede the larvae's ability to find food, leading to growth issues and even starvation. Additionally, inadequate sensory organ development renders the larvae vulnerable to predators, which can significantly impact the species' survival and population stability in their natural habitats.

Understanding the development of sensory organs in *P. nasutus* larvae holds significant importance in expanding our knowledge of these fish at their early stages of life. This valuable research can provide insights into identifying the most suitable feeding strategies for *P. nasutus* larvae, considering factors such as their age, size, and dietary preferences, especially during their initial larval phase. The provision of appropriate and timely nutrition is crucial to ensure optimal growth, prevent growth stunting, and promote healthy growth rates in the larvae. By implementing these findings, fish farmers can effectively breed and cultivate *P. nasutus* in controlled environments. Consequently, this reduces the dependency on wild-caught *P. nasutus* and contributes to the conservation and preservation of the species, helping to safeguard against the risk of extinction. Ultimately, the insights gained from studying sensory organ development and feeding preferences in *P. nasutus* larvae can significantly enhance the survival rates and overall productivity of the species in aquaculture settings.

Conclusion

In summary, the behaviour of *P. nasutus* larvae is intricately linked to the development of their morphology and sensory organs. Through careful behavioural observations, valuable insights were gained to identify the specific needs and requirements of the larvae. This knowledge is instrumental in devising and implementing effective rearing methods

that aim to optimise the survival and well-being of *P. nasutus* larvae in future aquaculture practices. By understanding the impact of morphological and sensory development on larval behaviour, researchers and fish farmers can tailor their approaches to create favourable rearing conditions and provide appropriate stimuli to promote healthy growth and development. This in-depth knowledge of larval behaviour and its relationship to physiological changes can significantly contribute to the success of *P. nasutus* aquaculture, ultimately leading to improved production outcomes and sustainable practices.

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