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The Morphological and Histological Developmental Study of the Gastrointestinal Tract of Peres Fish (*Osteochilus kappenii*) Larvae

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Abstract

Peres fish (*Osteochilus kappenii*) is a native freshwater fish in Central Aceh, Indonesia. This study aimed to determine the morphology and histology development of the digestive tract of Peres larvae. Understanding the developmental stages is essential to support the success of larval rearing during ontogeny. The parameters observed include the total length (TL) of larvae, daily growth rate, larval morphology, and histology of the digestive tract. Furthermore, the total length of the larvae was measured from 1 to 25 day after hatching (DAH). The larvae sampling was conducted at 1, 2, 3, 4, 8, 10, 15, 20, and 25 DAH. Morphological and histological observations were carried out microscopically, and data were analyzed descriptively. The results showed that the TL of larvae growth at the beginning (1 DAH) and termination (25 DAH) of the study was 4.58 ± 0.24 mm and 9.62 ± 0.79 mm, respectively. Based on the structural development of the body and food sources, the development of percussion larvae was divided into four phases, namely the yolk sac (0-4 DAH), pre-flexion (5-15 DAH), flexion (16-20 DAH), and post-flexion (21- 25 DAH). The yolk was completely absorbed 4 DAH, marking the end of the yolk sac phase, as the Peres fish larvae began to use external feed. At 25 DAH, the larval morphology was fully formed and pigmentation spread throughout the body. The digestive organs and glands are well-developed with differentiation of the midgut and hindgut, increased goblet cells in the intestine, and increased lipid vacuoles in the liver and zymogen in the pancreas to utilize feed optimally.

Keywords: Fish, development, endogenous, exogeneous.

1. Introduction

Peres fish (*Osteochilus kappenii*) is a native freshwater fish in Central Aceh that is spawned and cultivated. This fish is essential for nutritional needs, specifically protein for the people of Central Aceh, situated in the highlands at ± 1000 Meter Above Sea Level and relatively far from the sea (± 100 km to the coastal area of Bireuen Regency) (BPS Aceh Tengah, 2021). The marine fish supply is determined by distributors from coastal areas, although there has been a decline in quality due to poor handling and relatively long distances. Therefore, increasing the production of freshwater fish commodities is necessary, specifically *O. kappenii,* to meet the needs of the residents in Central Aceh District.

O. kappenii experiences several problems at the cultivation stage, specifically high larval mortality. This is due to the lack of information about the functional developmental stages of the larval digestive system, particularly the critical transition from the yolk sac to exogenous feeding during the rearing period. The larval stage is vital to the success of fish farming because it is prone to mortality, specifically during the transition period (Staaterman *et al*., 2012; McCasker *et al*., 2014). Furthermore, survival in the larval stage is influenced by food and environmental factors (Muchlisin *et al*., 2003). The fish undergo morphological changes, and digestive system differentiation becomes more complex during the larval to juvenile stages. In general, each teleost has similar digestive system development, although there were variations in the differentiation period and the functional development during ontogeny (Trevino *et al.,* 2011).

There are several studies on the morphology and histology development of the digestive tract of fish larvae. Putra *et al.* (2012) studied the development of morphology and digestive tract of tomato clownfish (*Amphiprion frenatus*) in a controlled environment. Other studies include Croaker croceine fish, *Pseudosciaena crocea* (Mai *et al.,* 2005), humpback grouper, *Cromileptes altiveles* (Abol-Munafi *et al*., 2011), catfish, *Mystus nemurus* (El Hag *et al*., 2012), striped murrel, *Channa striata* (Paray *et al.,* 2015), ontogeny and morphological development of tilapia (*Oreochromis niloticus*) in Aceh Province, Indonesia (Ismarica *et al*., 2022), yellowtail tetra fish *Astyanax lacustris* (Characiformes: Characidae) (dos Santos *et al*., 2020), *Schizothorax waltoni Regan* and *Percocypris retrodorslis* (Xu *et al*., 2023), orchid

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dottyback fish, *Pseudochromis fridmani* (Chen *et al*., 2022), yellowfin tuna, *Thunnus albacares* larvae (Kwan *et al*., 2019), red sea bream, *Pagrus major* (Khoa *et al*., 2019), Black Amur bream, *Megalobrama terminalis* (Liu *et al*., 2020), *Culter alburnus* (Huang *et al*., 2021), Shi drum, *Umbrina cirrosa* (Karacaoğlan *et al*., 2023), Yellowfin seabream, *Acanthopagrus latus*, Houttuyn 1782 (Morshedi *et al*., 2021), and Arctic fish, *Leptoclinus maculatus* (Pekkoeva *et al*., 2023).

Currently, there are no studies on the development of morphology and histology of the digestive tract of *O. kappenii* larvae. This study on the digestive system development is important to determine the activity of digestive enzymes (Babaei *et al*., 2011), the period of endogenous (occurs during the early developmental stages of fish, primarily in the embryonic and larval stages) and exogenous (begins when fish larvae have absorbed the nutrients from their yolk sac and need to start feeding on external sources for further growth) feeding (Faulk *et al*., 2007), the period of functional changes in the digestive organs (Infante and Cahu, 2001), the process of absorption of food nutrients (Lallès, 2020), and digestive capacity (Gawlicka *et al*., 1995). Therefore, this paper aims to investigate the morphology and histology development of the digestive tract to support the success of rearing *O. kappenii* larvae in the future.

2. Materials and Methods

2.1. O. kappenii peres larvae cultivation

The broodstock of male and female Peres fish was obtained from the Regional Technical Implementation Unit (UPTD) of the Lukup Badak Fish Seed Center (BBI), Pegasing District, Central Aceh, and Indonesia. Furthermore, brood spawning was conducted in a 70 x 45 x 45 cm aquarium, with a male-to-female brooders ratio of 2:1. A semi-artificial spawning technique was employed, and, on completion, the parent was separated from the eggs. The hatched larvae were transferred to a larval rearing tank with aeration until their yolk runs out. In the early phase, plankton was given 3 times a day after the yolk thinned, which was obtained from green water. Furthermore, the larvae were given thawed pellet powder 7 days after hatching.

2.2. Observation of digestive organs and histology

The larvae were sampled on 1, 2, 3, 4, 8, 10, 15, 20, and 25 for morphological and histological analysis. A total of 15 samples were collected from rearing container, and preserved in a sample bottle using a 10% neutral buffer formalin (NBF) solution. For morphological observations, 5 samples of the preserved larvae were randomly selected, then examined using a stereo microscope (Olympus SZX16) and photographs were taken. Meanwhile, 15 samples aged 1 to 7 DAH were used for histological analysis (Putra *et al*. 2012), as well as 5 random samples aged 8 to 25 DAH. The histological preparation process followed methods outlined by Drury and Wallington (1967) Kiernan (1990) and Abdullah-Al Mamun *et al*. (2022). This involved tissue fixation with 10% NBF fixative solution, tissue dehydration with graded alcohol, tissue clarification using xylene, infiltrating the tissue with paraffin, immersing the preparations with paraffin wax, cutting the tissue using a microtome with a thickness of 5 µm, and staining with Haematoxylin and Eosin (H&E). The results were observed under a microscope (Olympus CX21) with digital photos integrated with a computer.

2.3. Data analysis

The total length of the larvae was measured from 1 to 25 DAH with a digital caliper (mm) with an accuracy of ± 0.2 mm. Five samples were collected daily to measure the total body length, then the average was calculated using standard deviation (SD). The length growth pattern of Peres fish larvae was analyzed using Excel software. Measurement of the daily growth rate (DGR) was calculated based on Hopkins (1992): DGR = Lt-Lo/t, where DGR represented the Daily Growth Rate (mm/day), Lt and Lo represented the average total length at the end and the start of the study (mm), respectively. The data obtained were analyzed descriptively.

3. Results

3.1. Growth of Peres Fish Larvae (O. kappenii)

Length growth pattern of the larvae showed an exponential pattern during rearing (Figure 1). The daily length growth rate from 1 - 25 DAH was 0.201 mm/day. Also, the average total length at 1 DAH was 4.58 ± 0.24 mm TL and reached 9.62 ± 0.79 mm TL at 25 DAH. The total length of the pre-flexion phase from 5–15 DAH was 4.96 ± 0.28 mm TL – 5.84 ± 0.32 mm TL. Growth was rapid at the 16-20 DAH flexion phase, ranging from 5.86 ± 0.53 mm TL – 8.34 ± 0.69 mm TL. The final stage was the post-flexion phase *at* 21-25 DAH, comprising 8.42 ± 0.60 mm TL – 9.62 ± 0.79 mm TL.

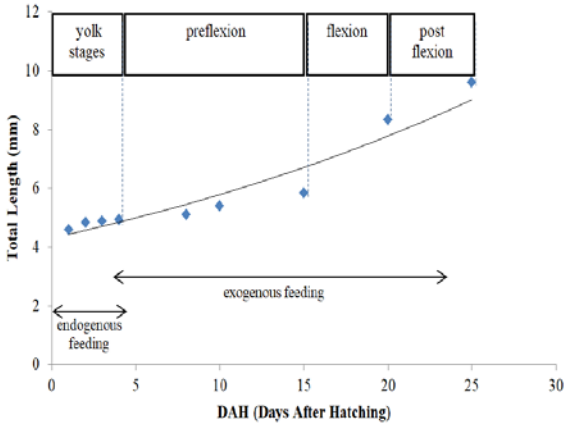

Figure 1. Length growth pattern of Peres (*O. kappenii*) larvae from 1 DAH to 25 DAH.

3.2. Morphology Development of Peres Larvae (O. kappenii)

The stages of morphological development of the Peres fish larvae are divided into 4 based on the structural development of the body and food sources, namely the yolk sac phase, pre-flexion, flexion, and post-flexion (Table 1). Morphological development in the pre-flexion phase occurs 11 days after passing through the yolk sac phase (4 DAH), while the flexion and post-flexion phases have equal durations of 5 days each.

Table 1. Stages of morphological development of Peres fish (*O. kappenii*) larvae

No.	Phase	Characteristics of Morphological Observation	DAH	Food Source
1.	Yolk sac	The mouth is still closed, the eyes are not pigmented, and the body is still transparent.	0-1	Endogenous feeding
		The mouth is starting to open, the eyes are getting pigmented, and the body is still transparent.	2-4	Endogenous-exogenous feeding
2.	Pre-flexion	The yolk has run out, the body still looks transparent, the fins are starting to form but are still simple, the eye organs are starting to enlarge, and the tail bones of the larvae are starting to bend.	5-15	Exogenous feeding
3.	flexion	The fins begin to develop and separate, pigmentation begins to appear on the head, abdomen, and fins.	16-20	Exogenous feeding
4.	Post-flexion	The dorsal fins, pelvic fins, pectoral fins, anal fins, and caudal fins are fully formed, and pigmentation has spread all over the body.	21-25	Exogenous feeding

The results of the morphological development analysis showed fin folds after hatching, located along the body including the dorsal, caudal, anal, and ventral fin folds (2a, b, c, d, e, f, g). However, underdeveloped fins hinder the larvae from swimming actively. The fins are completed at 15-20 DAH and the rays are perfectly formed, specifically on the dorsal and caudal fins (Figures 2 g-h). At 25 DAH, the fish resemble an adult morphologically due to the development of cranial structures including premaxilla, maxilla, dentary, preopercle, and opercle. In addition, the development of the fins is complete at 25 DAH, with the caudal fin structure having urostyle, hypural, parhypural, uroneural, and epural bones (Figure 2i).

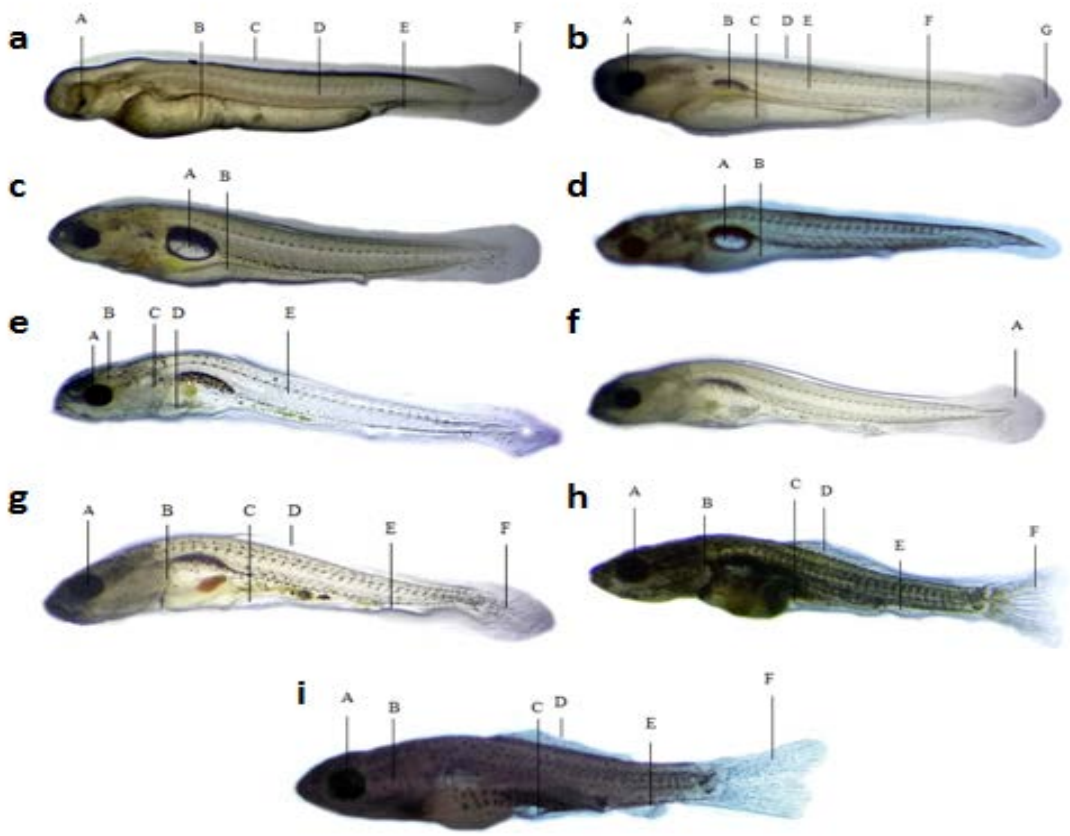

Figure 2. Morphological development of Peres fish (*O. kappenii*) larvae. **(a)** 1 DAH (4.58 ± 0.24 mm TL) A, eye; B, egg yolk (yolk sac); C, dorsal fin folds; D, notochord; E, anal fin folds; F, caudal fin fold; **(b)** 2 DAH (4.82 ± 0.30 mm TL) A, eye; B, swim bladder; C, egg yolk (yolk sac); D, dorsal fin folds; E, notochord; F, anal fin folds; G, caudal fin fold; **(c)** 3 DAH (4.88 ± 0.21 mm TL). A, swim bladder; B, egg yolk (yolk sac); **(d)** 4 DAH (4.94 ± 0.31 mm TL). A, swim bladder; B, egg yolk (yolk sac); **(e)** 8 DAH (5.1 ± 0.12 mm TL). A, eyes; B, head; C, pectoral fins; D, abdomen; E, notochord; **(f)** 10 DAH (5.4 ± 0.29 mm TL). A, caudal fin; **(g)** 15 DAH (5.84 ± 0.32 mm TL). A, eyes; B, pectoral fins; C, pelvic fins; D, dorsal fin; E, anal fin; F, inclined coccyx; **(h)** 20 DAH (8.34 ± 0.69 mm TL). A, eyes; B, pectoral fins; C, developed pelvic fins; D, developed dorsal fin; E, developing anal fin; F, developed caudal fin; **(i)** at 25 DAH (9.62 ± 0.79 mm TL). A, eyes; B, pectoral fins; C, pelvic fins; D, dorsal fin; E, anal fin; F, caudal fin.

3.3. Development of Digestive Tract Histology of Peres Larvae (O. kappenii)

A day after hatching, the digestive tract appeared as a small straight undifferentiated tube covered by a system of simple columnar epithelium, attached dorsally to the yolk. The yolk, acting as a food reserve, appears large and is surrounded by simple squamous epithelium (Figure 3), and the food source is endogenous feeding. Gastrointestinal tract differentiation occurs at 4 DAH during the transition phase to exogenous feeding where the esophagus, stomach, and intestines differentiate and the mouth, pharynx, and anus have completely opened (Figure 4a). The intestinal lumen volume (intestine) expands at 4 DAH as well as the opening of the mouth, indicating that they are prepared to receive food from outside.

The organs, digestive glands, liver (food secretion), and pancreas (enzyme secretion) start differentiating in 2-3 DAH, though they are unwell developed. At 4 DAH, the liver and pancreas are visibly growing, indicating that digestion is commencing. The exogenous feeding has been started at 4 DAH based on the development of digestive organs and glands.

Figure 4b showed that the intestine separated into 2 parts, the midgut and hindgut, at 15 DAH. The length increases with development and intestinal folds become more complex. Additionally, goblet cells are observed in the folds 15 DAH.

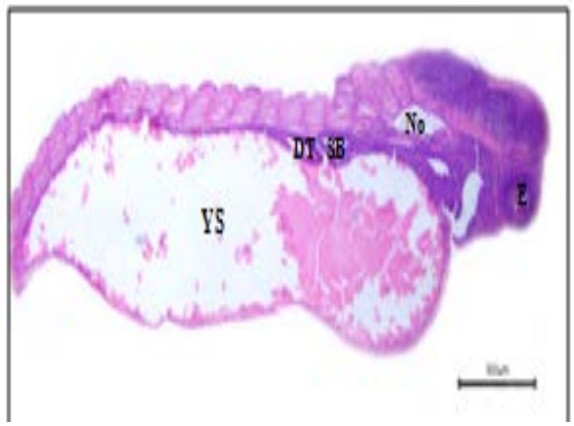

Figure 3. Cross sections of Peres fish (*O. kappenii*) larvae at 1 DAH (HE, x 4). YS, yolk sac; DT, digestive tract; SB, swim bladder; No, notochord; E, eye.

The intestinal volume increases at 20 DAH, accompanied by an increased villi length in the midgut and hindgut. In addition, the liver was enlarged, followed by a rapid increase in vacuolar lipid granules and hepatocytes (Figure 4c). The liver lobes were enlarged and elongated, with increased hepatocytes and lipid vacuoles, which were clearly visible. At 24 DAH, the midgut and hindgut were fully differentiated, with a complex villi complex, and increased goblet cells. In the liver, enlargement continues, and it appears to be filled with vacuolated lipid granules. The amount of zymogen in the pancreas increased greatly (Figure 4d). Figure 2f showed that the tail fin develops at 10 DAH. The caudal fin separates from the dorsal fin (Figure 2g) at the end of the pre-flexion phase.

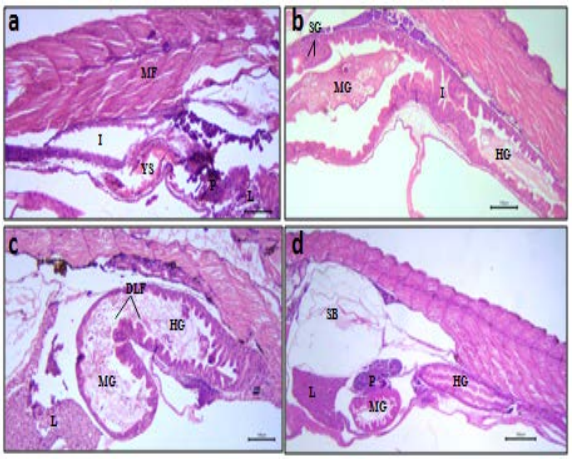

Figure 4. Cross-sectional micrograph of the digestive tract of Peres (*O. kappenii*) larvae. **(a)** 4 DAH (HE, x 10) YS, yolk sac; I, intestines; L, liver; MF, muscular fiber; P, pancreas; **(b)** 15 DAH (HE, x 10). MG, midgut; HG, hindgut; I, intestines; SG, goblet cells; **(c)** 20 DAH (HE, x 10). MG, midgut; HG, hindgut; L, liver; DLF, digested live feed; **(d)** 25 DAH (HE, x 4).

4. Discussion

The results showed that the average total length of the larvae in the early phase of the yolk sac was quite lower, ranging from 4.58 ± 0.24 mm – 4.94 ± 0.31 mm TL. This is because the larvae utilize the yolk for the ontogeny development of the body, specifically the cranial and appendicular structures (Woltering *et al*., 2018). According to Sari *et al.* (2015), the slow growth is due to the energy intake from endogenous feeding to perfect the immature organs, whereas excess energy is used for growth in length and weight at the end of the larval phase.

Figures 2a, b, c, and d illustrated that the yolk sac phase showed incomplete morphology. The larvae rely on endogenous feeding, obtaining nutrients from the egg yolk at 1-3 DAH. However, there is a transition from endogenous to exogenous feeding (food from outside) at the end of this phase (4-5 DAH). The food in the pre-flexion to post-flexion phases originates from exogenous feeding. At 4 DAH, the larvae have functioning eyes and mouth, enabling exogenous feeding activities. The yolk was still visible; hence, the larvae were not fully dependent on external feed. There are differences regarding the yolk expiration in other species, including Snakehead, *Channa striata* (Paray *et al.*, 2014), and Spotted murrel, *Channa punctatus* (Haniffa, 2003), where the yolk was completely absorbed in 3 DAH. In Caspian cutus, *Rutilus frisii cutum* (Jafari *et al.*, 2009), the yolk sac was completely absorbed 20 DAH and the mouth opens 3 DAH allowing external feeding.

The pre-flexion stage begins when the yolk is depleted (4-5 DAH) and continues until the development of larval urostyle bones in the caudal fin (15 DAH). This urostyle bone supports the epural, uroneural, hypural, parhypural, and fin bones (Senevirathne *et al*., 2020). The bending of the tailbone (notochord) and the maturation of the caudal fin of the tomato clownfish, *Amphiprion frenatus*, was found at 4 DAH indicating a transitional phase from pre-flexion to post-flexion (Putra *et al.*, 2012; Boglione *et al*., 2013). However, the development of the urostyel bone structure in the Rainbow kurumoi fish, *Melanotaenia*

parva, occurs at the age of 16-20 DAH (Kadarini *et al*., 2013).

At 8 DAH, *O. kappenii* larvae showed more signs of development. Although they remained simple and their bodies retained its transparency, the larvae progressed into developing bigger eyes and pigmented cranium and abdomen (Figure 2e). Similar results on the Striped snakehead, *Channa striatus*, were reported by Marimuthu and Haniffa (2007).

The flexion stage started from 16-20 DAH, where pigmentation began to appear on the cranium, abdomen, and fins. Furthermore, the fins began to develop fully and the dorsal, caudal, anal, and ventral fins were separated to enable the larvae to swim at 20 DAH (Figure 2h). The post-flexion stage began at 21-25 DAH, showing the formation of the dorsal, ventral, pectoral, anal, and caudal fins to resemble adult fish and the pigmentation spread throughout the body (Figure 2i). After 25 DAH, no significant morphological development was observed. The changes that occurred involved an increase in body length and weight, indicating the end of this phase. Furthermore, the larvae enter the final stage, the juvenile phase. Previous studies on fish from the same family (Cyprinidae), such as *Chalcaburnus tarichi*, revealed that the larval period ended at 35 DAH (Unal *et al*., 2001), while that of in *Schizothorax zarudnyi* Nikolskii, 1987 *(Actinopterigii: Cyprinidae)* ended at 21 DAH (Moghadam *et al*., 2014).

The histological analysis showed that *O. kappenii larvae* at 1 DAH exhibited an undifferentiated, small straight tube-shaped digestive tract, located at the top and connected to the yolk (Figure 3). Furthermore, the digestive tract is lined by a network of simple columnar epithelium and the food sources are endogenous. This result was similar in the Hamun mahi, *Schizothorax zarudnyi* (Moghadam *et al*., 2014); Malaysian river catfish, *Mystus nemurus* (El Hag *et al*., 2012), Stripped murrel, *Channa striatus* (Paray *et al.*, 2015), and Large yellow croaker, *Pseudoscianea crocea* (Mai *et al.*, 2005).

Gastrointestinal tract differentiation of *O. kappenii* commenced at 4 DAH during the transition phase to exogenous feeding, where the esophagus, stomach, and intestine begin to differentiate and the mouth, pharynx, and anus have completely opened (Figure 4a). The volume of the intestinal lumen (intestine) increases at 4 DAH as well as the opening of the mouth, indicating the readiness to consume external food. Although the early phase (yolk sac) of the larval exhibit intestinal functionality, it is structurally and functionally less complex than the late larval phase (post-flexion) (El Hag *et al.*, 2012). In other species of the Cyprinidae family, namely *Chalcaburnus tarichi*, the digestive tract differentiate at 5 DAH and exogenous feeding begins at 6 DAH (Unal *et al*., 2001), while *Schizothorax zarudnyi* at 3-8 DAH (Moghadam *et al.*, 2014).

The digestive gland organs of *O. kappenii*, such as the liver start differentiating at 2-3 DAH and are unwell developed (Figure 4). Andriyanto and Marzuqi (2012) reported similar results on *Cromileptes altivelis* fish, Mai *et al*. (2005) on *Pseudosciaena crocea* fish, and Paray *et al*. (2015) on *Channa striata* fish. The liver and pancreas grow and become clearly visible at 4 DAH, indicating the initiation of the food digestion process. Based on the development of digestive organs and glands, the larvae utilize external feed 4 DAH.

The histological results showed that the intestine was clearly separated into 2 parts, the midgut (middle intestine) and hindgut at 15 DAH (Fig. 4b). However, previous studies reported that they were separated at 2 DAH in *Amphiprion frenatus* (Putra *et al.*, 2012) and 6-7 DAH in *Cromileptes altiveles* fish (Abol-Munafi *et al*., 2011; Andriyanto and Muzaki, 2013). The histological results also showed an increase in intestinal length and the complexity of intestinal folds as development progressed. Elongated and complex intestinal folds facilitate food absorption in the intestine (Andriyanto and Muzaki, 2013). At 15 DAH, the microvilli network in the intestine developed, expanding the surface to enable efficient absorption (Merrifield *et al*., 2009; Firdus *et al*., 2020a; Firdus *et al*., 2020b). The intestinal volume increased at 20 DAH and the villi lengthened in the midgut and hindgut. Furthermore, the liver was enlarged at 20 DAH, accompanied by a rapid increase in vacuolar lipid granules and hepatocytes (Figure 4c).

The midgut and hindgut were differentiated and formed at 25 DAH. Furthermore, the villi were complex and the goblet cells increased. The liver became larger and was filled with vacuolated lipid granules, while the zymogen in the pancreas increased (Figure 4d). The pancreas developed around the intestines, corresponding to the complexity and quantity of digested food (Andriyanto and Muzaki, 2013). The development of digestive tract organs and glands at 25 DAH indicated the final larval phase. The well-developed functional digestion at this stage enables the larvae to utilize feed optimally.

5. Conclusions

The end of the yolk sac phase in Peres fish is characterized by the complete absorption of the yolk at 4 DAH, which marks the transition from the endogenous to the exogenous feeding phase. Furthermore, the development of digestive organs and glands showed that the larvae were able to feed externally at 4 DAH. At 25 DAH, the developed larvae resemble adult fish and the pigmentation spread throughout their bodies, signaling the final phase of the larvae. The development of the well-developed digestive tract and glands at 25 DAH allowed the larvae to efficiently utilize feed. Future studies on enzymatic activity within the digestive system is suggested to obtain more comprehensive information regarding to the larvae developmental of Peres fish.

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Conflict of Interest

None.

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