飼育下におけるケツギョの消化器官および消化酵素活性の初期発達

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Early Development of the Digestive System andDigestive Enzyme Activity of Reared Mandarinfish Siniperca chuatsi
(Perciformes: Sinipercidae)

Toshio Doi

Abstract: Morphological and histological development of the digestive system and changes in digestive enzyme activity were investigated in larvae and juveniles of mandarinfish Siniperca chuatsi reared in an aquarium. Yolk sac larvae (ca. 5.5 mm TL) just before first feeding have a differentiated, functional larval-type digestive system with a large mouth, jaw teeth, pharyngeal teeth and gastric blind sac and they showed activities of both a trypsin-like enzyme and at a relatively low level of a pepsin-like enzyme. Preflexion larvae (ca. 7 – 8 mm TL) preyed on fish larvae using the largely expandable blind sac. Later-phase flexion larvae (ca. 10 mm TL) showed higher pepsin-like enzyme activity than trypsin-like enzyme associated with the appearance of gastric glands. Postflexion larvae (ca. 12 mm TL) established the adult-type digestive system having pyloric caeca. Metamorphosed juveniles (> ca. 20 mm) showed increased gastric glands and pyloric caecal lobules in number, and the mouth and gut in size, and digestive enzyme activities. Early appearance of piscivory of larval mandainfish may be supported by the quantitative development of the oral cavity and gastric blind sac which mainly occurred during the early phase of the larval period (< ca. 10 mm TL), and its acceleration could be done by both the continued quantitative developments and functional development of the adult-type digestive system after the mid phase of the larval period (> ca. 12 mm TL).

Key words: Siniperca chuatsi; Digestive system; Digestive enzyme; Larvae

Mandarinfish Siniperca chuatsi larvae show exclusive piscivory since first feeding stage. They prey on fish larvae of other fish species which are longer than their pre-anal length, but do not eat any zooplankton (Doi and Aoyama 2004). Generally, pelagic larvae of most fish species are plankton feeder (Tanaka 1980; Ikewaki and Sawada 1991), on the other hand a few scombrid fishes, e.g., Japanese Spanish mackerel Scomberomorus niphonius and striped bonito Sarda orientalis, show strong piscivory in the very early larval stage (Shoji et al. 1997; Shoji and Tanaka 2001; Kaji et al. 2002). Some of scombrid species exhibit precocious development of the adult-type digestive system which is well synchronized with the appearance of piscivory far before metamorphosis (Tanaka et al. 1996; Kaji et al. 2002). Though morphology of digestive system in mandarinfish larvae has been reported (Wu 1987; Zheng 1987; Tang and Fan 1993), the functional development has not yet been clarified. Mandarinfish is a valuable food fish in China and has been cultured for a long time, but a stable supply of prey is still under improvement in aquaculture because of their unusual feeding habits (Wu and Hardy 1988; Liang et al. 2001). Therefore it appears to be useful for development of aquacultural techniques as well as early life history research to investigate the functional development of early digestive system of mandarinfish. In this study, the author examined the ontogeny of the digestive system histologically and changes in the digestive enzyme activity for larvae and juveniles of mandarinfish reared in the aquarium.

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Materials and Methods

Fish and rearing method

The broodstock of mandarinfishes were the offspring originated from 40 individuals which were donated from Tianjin City of the Republic of China to Suma Aqualife Park (Municipal Aquarium of Kobe, Japan) in 1987. The eggs were obtained by spontaneous spawning in an exhibition tank (5.6 m³, 2.5 × 1.5 × 1.5 m) of the aquarium in June 1999, 2000, and 2001. Spawning and hatching conditions are as in Doi and Aoyama (2004). The larvae were reared in a 55 l tank with water purified by air lift sponge filter at water temperature of 24–26°C. Threadfin goby Tridentiger obscurus larvae at 5 days after hatching (DAH) were supplied to the rearing tank, additionally pale chub Zacco platypus larvae at 13 DAH and white cloud mountain minnow Tanichthys albonubes larvae at 30 DAH. The growth of larvae and juveniles examined here is shown in Fig. 1.

Morphological and histological observations of digestive system

Several fishes were collected at each developmental stage during the experiment from the larval rearing tank. The specimens in 1999 were fixed in Bouin’s fluid and embedded in paraffin. Serial sections (6 µm) stained with hematoxylin-eosin were observed under a microscope. The specimens in 2001 were fixed in 10% formalin, and dissected to observe the internal organs under a stereomicroscope. Additionally, some specimens cleared by trypsin were used.

Activity of digestive enzyme

The fishes (2–300 individuals) were collected at 1- to 10-day intervals for 47 DAH in 2000. The whole body samples were in yolk sac-flexion phase larvae and the abdomen cut off from body were used in postflexion phase and later fishes. Each sample (ca. 0.1–0.4 g wet weight) was washed and homogenized by a glass homogenizer with distilled water. The homogenate adequately diluted by distilled water was examined as the enzyme solution. Enzyme activities were assayed primarily according to Kawai and Ikeda (1973). The enzyme solution was reacted with the substrate (casein) under pH 8 (trypsin-like enzyme) and pH 2 (pepsin-like enzyme), and then determined the liberated tyrosine with

![Fig. 1](image)

**Fig. 1.** Growth in total length of mandarinfish Siniperca chuatsi used in this study. The vertical bars on the symbols in 2000 indicate the range. Developmental phase is shown at the top of the figure. The feeding schedule is illustrated by arrows. YSL, Yolk sac larva; PRL, Preflexion larva; FL, Flexion larva; PSL, Postflexion larva; JVN, Juvenile.

![Fig. 2](image)

**Fig. 2.** Morphological development of the digestive system of mandarinfish larvae and juveniles. A: Yolk sac larva, 4.1 mm TL (just hatched); B: Yolk sac larva, 4.8 mm TL (3 days after hatching (DAH)); C: Yolk sac larva just before feeding, 5.6 mm TL (5 DAH); D: Preflexion larva, 6.1 mm TL (7 DAH); E: Flexion larva, 8.2 mm TL (10 DAH); F: Postflexion larva, 13.9 mm TL (17 DAH); G: Juvenile, 21.9 mm TL (27 DAH); H: Juvenile, 33.8 mm TL (35 DAH). Scale bar: 1 mm.
Foline’s method. Amylase activity was assayed by determining the liberated glucose from substrate (starch) with the dinitro salicylic acid method. Each enzyme activity was expressed as total activity (mg of tyrosine or glucose liberated /hour/individual).

**Results**

**Development of the digestive system**

_Yolk sac larvae_ Newly hatched larvae ca. 4.1 mm total length (TL) had a large yolk sac with an oil globule and a simple straight primitive gut (Fig. 2A). Three DAH larvae at ca. 4.5 mm TL had the open mouths. The intestine started to rotate (Figs. 2B, 3A1). The jaw teeth were formed (Fig. 3A2). Rudimentary stomach, intestine, liver, and pancreas were already differentiated (Fig. 3A1). Five DAH larvae at ca. 5.5 mm TL, just before first feeding, the intestine completely rotated (Figs. 2C, 3B1), the yolk sac was almost absorbed, but the oil globule remained. Stomach formed a blind sac, and rectum were differentiated (Figs. 2C, 3B1). Zymogen granules were observed in the pancreas (Fig. 3B1). The mouth became larger, and jaw teeth increased in number. Pharyngeal teeth were already formed (Fig. 3B2).

_Preflexion-postflexion larvae_ Preflexion-postflexion larvae after initiation of feeding at 6 – 8 mm TL (6 – 13 DAH). The digestive system increased in volume with the same shape to the primitive stage (Fig. 2D), however the blind sac was expanded when fish were preyed on (Figs. 2E, 4A1). The gastric glands have not yet differentiated. The liver was expanded gradually. Acidophilic granules appeared in the epithelium of the rectum (Fig. 4A2). Flexion larvae ca. 10 mm TL (13 – 17 DAH). The blind sac was enlarged posteriorly further by the large volume of feed materials ingested, and primitive pyloric caeca began to differentiate (Fig. 4B1). Gastric glands first appeared in the stomach mucosa (Fig. 4B1, B2). Acidophilic granules disappeared in the rectal epithelium (Fig. 4B2). Postflexion larvae ca. 12 mm TL (17 – 24 DAH). The gastric glands increased in number, and the pyloric caeca differentiated (Figs. 2F, 4C1, C2).

![Fig. 3. Photomicrographs of yolk-sac larvae of mandarinfish. Longitudinal sections of the body axis (A1) and lower jaw teeth (A2) of 3 days after hatching (DAH) larva (5.1 mm TL). Longitudinal section of the body axis (B1) and the pharyngeal teeth (B2) of 5 DAH larva (5.7 mm TL). bs, blind sac; in, intestine; li, liver; lj, lower jaw teeth; og, oil globule; pa, pancreas; re, rectum; st, stomach; yo, yolk.](image-url)
Juveniles  
Juveniles over ca. 20 mm TL (> 26 DAH). Both gastric glands and pyloric caecal lobules increased further in number (Figs. 2G, H, 5-1). Many goblet cells were present on the intestinal epithelium (Fig. 5-2).

Change in the digestive enzyme activity

Changes in activities of trypsin-like enzyme, pepsin-like enzyme and amylase during early stage of the mandarinfish are shown in Fig. 6. Yolk sac larvae younger than 3 DAH did not show any enzyme activities. The activity of trypsin-like enzyme and, at a low level, pepsin-like enzyme first appeared at 3 DHA larvae. Both of the enzymes remained nearly constant activities from 3 DAH larvae to 9 DAH flexion larvae (7 – 8 mm TL), during which the first feeding stage occurred. Very low level of amylase activity appeared at just after the first feeding larval stage (6 DAH). All of these enzyme activities increased at 13 DAH larvae (ca. 9 mm TL).
Digestive System and Enzyme of Siniperca chuatsi

The peptic activity rapidly increased in 17 DAH larvae (10 – 11 mm TL) and it was higher than the trypsic activity. Over the postflexion larval stage (ca. 12 mm TL, 24 DAH), each of the enzyme activities increased as development proceeded, especially the pepsin-like enzyme showed higher activity and followed by trypsin-like enzyme.

Discussion

The growth rate of this rearing experiment was several days behind some previous studies. The authors reported that the number of days from hatching to juvenile stage (ca. 15 mm TL) were 17 – 19 days (Zheng 1987; Luo et al. 1992; Doi et al. 2004), while they were 19 – 25 days in the present experiment. This time lag may be caused by absence of suitable size prey fish between threadfin goby and pale chub. On the other hand, the total length (or the developmental phase) at which each digestive organ differentiated in this experiment corresponds with the results in the following reports. For example, expanded stomach and rotated intestine appeared at ca. 5.5 mm TL (Tang and Fan 1993), primitive pyloric caeca was observed in larvae at 9.4 mm TL (Tang and Fan 1993) and 9.74 mm TL (Zheng 1987), and differentiated pyloric caeca were observed at ca. 12.5 mm TL juvenile (Chiang 1959; Wu 1987). Therefore, it seems to be reasonable that the digestive system develops along with growth of both total length or developmental phase rather than age after hatching.

The early and rapid development of the digestive system of mandarinfish observed here was different from those of both planktovores common to most of marine teleost larvae and piscivores which are observed in some scombrid larvae as an extreme case. General planktivorous larvae have a primitive larval-type digestive system at first feeding stage, and then establish an adult-type digestive system which is characterized by a functional stomach with gastric glands and pyloric caeca right before transformation to juvenile period (Tanaka 1969, 1971). This development of the digestive system found in common fishes seems to be similar to that of mandarinfish, because the gastric glands and pyloric caeca differentiate at a later larval phase. However mandarinfish larvae already have well differentiated jaw teeth, pharyngeal teeth and gastric blind sac just before the first feeding stage, even though the digestive system is basically the larval-type (Fig. 3). The larvae, moreover, had a large head [Head length (HL) = ca. 24 – 27% of Standard length (SL)], developed mouth [Upper jaw length (UJL) = ca. 15 – 19% of SL] and elongated abdomen [Pre-anal length (PAL) = ca. 44 – 46% of SL] (Doi et al. 2004). Thus they can prey on other fish larvae by the developed mouth and teeth, and store them in an expandable blind sac, at the preflexion and flexion larval stages.

On the other hand, scombrids undergo differentiation the adult-type digestive system before the mid phase of larval period (Tanaka et al. 1996). The timing of transformation to the adult-type digestive system, varies with species, and generally corresponds with the appearance of piscivory (Tanaka et al. 1996). Spanish mackerel Scomberomorus niphonius which is a typical piscivore since onset of feeding same as mandarinfish has a blind sac, gastric glands, pyloric caeca and jaw teeth at the first feeding stage (Tanaka et al. 1996; Shoji et al. 1997). It is a point in common between the two species to develop a blind sac which is possible to expand.
to allow the digestion of large prey in the first feeding stage. However mandarinfish does not have gastric glands and pyloric caeca till the later flexion stage (Fig. 4). Before the stomach and gastric glands are differentiated, general planktivorous larva digest food items by pancreatic and intestinal enzymes (Kurokawa and Suzuki 1996, 1998; Anand et al. 2002), and by intercellular digestion in the posterior gut (Tanaka 1972; Watanabe 1981, 1982). After feeding initiation, early larvae of mandarinfish were observed to have low pepsin-like enzyme activities (Fig. 6) and the presence of zymogen granules in pancreas (Fig. 4A1) and acidophilic granules in rectal epithelial cells (Fig. 4A2). Therefore, the digestive physiology of early larval mandarinfish was the same as that of common fishes. The increase of liver in volume and amylase activity during this phase would suggest relatively active metabolism of carbohydrate. At the later flexion stage (over ca. 10 mm TL), high pepsin-like enzyme activity with gastric glands was observed, and acidophilic granules disappeared (Figs. 4B2, 6). These could indicate that the intracellular digestion was reduced and the adult-type gastric digestion was activated during this period. Subsequently, mandarinfish increased the number of gastric glands and pepsin-like enzyme activity, and enlarged the pyloric caeca (Figs. 2G, H, 4C1, C2, 5-1, 6). The head, mouth and abdomen also developed larger (HL = 35 – 45% SL, UJL = 15 – 28% SL, PAL = 50 – 72% SL) from postflexion larvae to juvenile phase (Doi et al. 2004). These developments could enhance the efficiency capture, digestion and ingestion.

In conclusion, early appearance of piscivory in mandarinfish larvae may be supported by the quantitative development of the oral cavity and gastric blind sac which mainly occurred during early phase of the larval period (under ca. 10 mm TL), and its acceleration could be realized by both the continued quantitative development and the functional development of the adult-type digestive system after mid phase of the larval period (over ca. 12 mm TL). Although the early larvae did not develop adult-type digestive system, they showed rapid growth the same as Spanish mackerel (ca. 1 mm TL / day) (Higuchi and Oshima 1974; Doi and Aoyama 2004). Therefore, it is suggested that the early stage larvae might have a high ability of digestion and ingestion adapted for piscivory by both the pancreas intestine and rectum except for enzymatically a fully functional stomach. To clarify the ontogenetic development of digestion, it will be necessary that the functions of these organs are examined in detail. Additionally, the development of swimming and feeding organs would be important for predation of fish larvae.

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