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

誌名	水産増殖 = The aquiculture
ISSN	03714217
著者	土井, 敏男
巻/号	53巻4号
掲載ページ	p. 425-431
発行年月	2005年12月

Aquaculture Science **53**(4), 425－431 (2005)

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

Toshio Doi[1]

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.

Received June 30, 2005: Accepted October 26, 2005.
[1] Kobe Municipal Suma Aqualife Park, Suma, Kobe, Hyogo 654-0049, Japan.

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 m^3, 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℃. 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 (6μm) 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

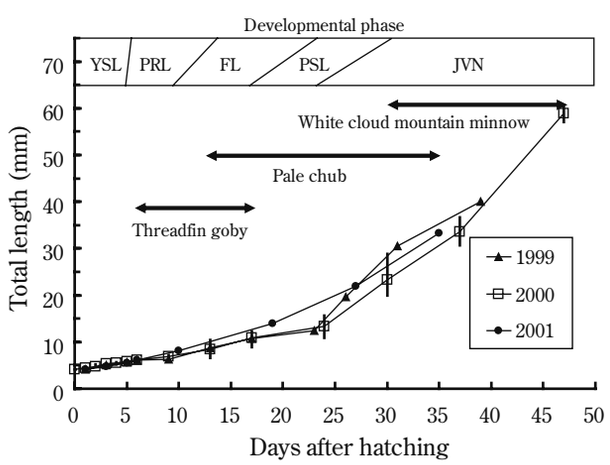

Fig. 1. Growth in total length of mandarinfish *Siniperca chuatsi* used in this study. The vertical bars on the symbols in 2000 indicate the range. Developmental phase is shown at the top of the figure. The feeding schedule is illustrated by arrows. YSL, Yolk sac larva; PRL, Preflexion larva; FL, Flexion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

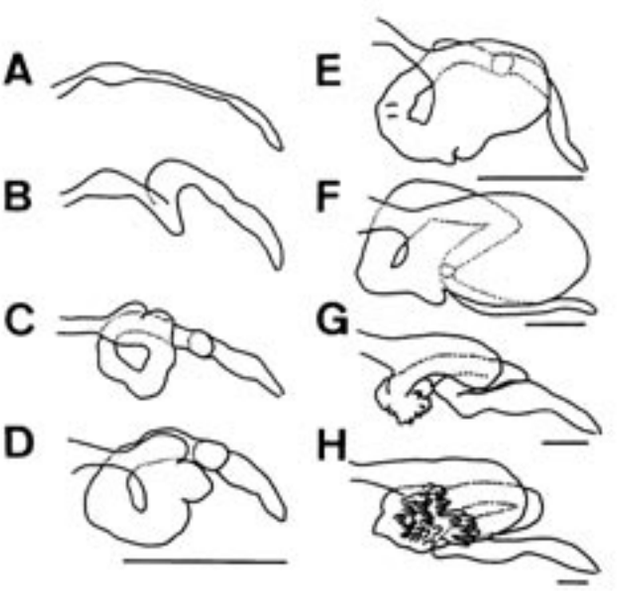

Fig. 2. Morphological development of the digestive system of mandarinfish larvae and juveniles. A: Yolk sac larva, 4.1 mm TL (just hatched); B: Yolk sac larva, 4.8 mm TL [3 days after hatching (DAH)]; C: Yolk sac larva just before feeding, 5.6 mm TL (5 DAH); D: Preflexion larva, 6.1 mm TL (7 DAH); E: 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-flexion 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).

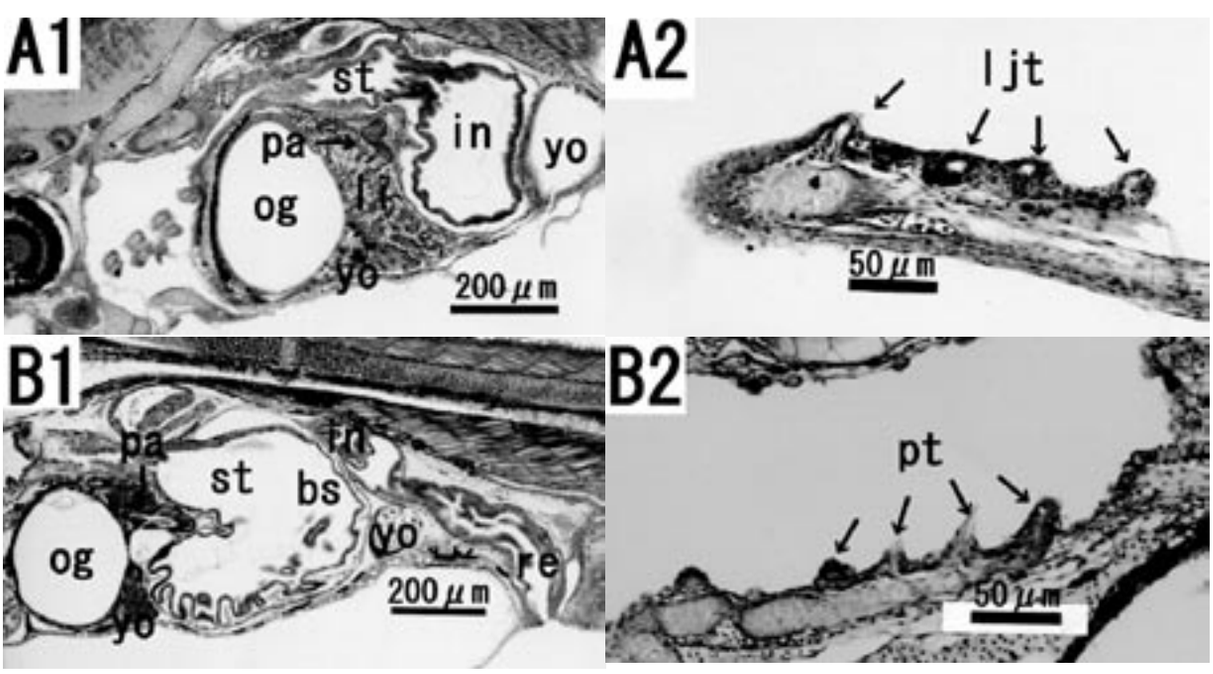

Fig. 3. Photomicrographs of yolk-sac larvae of mandarinfish. Longitudinal sections of the body axis (A1) and lower jaw teeth (A2) of 3 days after hatching (DAH) larva (5.1 mm TL). Longitudinal section of the body axis (B1) and the pharyngeal teeth (B2) of 5 DAH larva (5.7 mm TL). bs, blind sac; in, intestine; li, liver; ljt, lower jaw teeth; og, oil globule; pa, pancreas; re, rectum; st, stomach; yo, yolk.

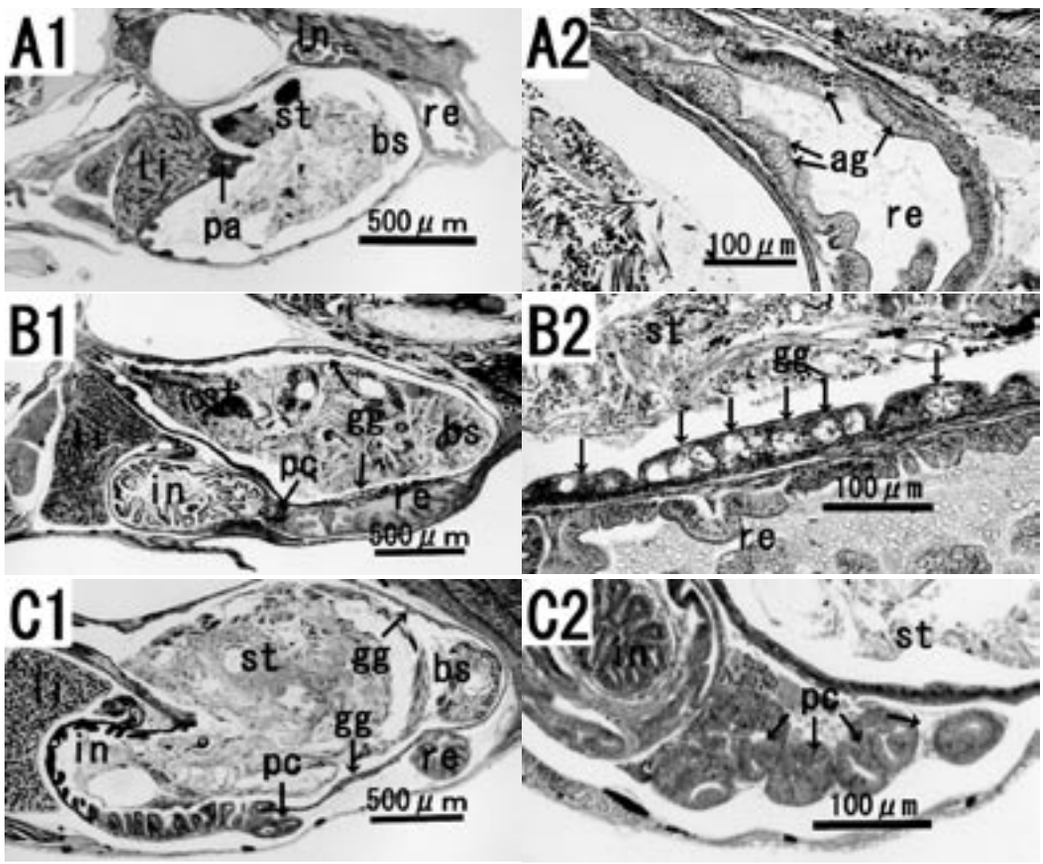

Fig. 4. Photomicrographs of larvae of mandarinfish. Longitudinal section of the body axis (A1) and rectum (A2) of 9 days after hatching (DAH) preflexion larva (6.3 mm TL). Longitudinal section of the body axis (B1) and the stomach wall (B2) of 17 DAH flexion larva (10.8 mm TL). Longitudinal section of the body axis (C1) and cross section of the pyloric caeca (C2) of 23 DAH postflexion larva (12.5 mm TL), ag, acidophilic granule; bs, blind sac; gg, gastric gland; in, intestine; li, liver; pa, pancreas; pc, pyloric caeca; re, rectum; st, stomach.

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

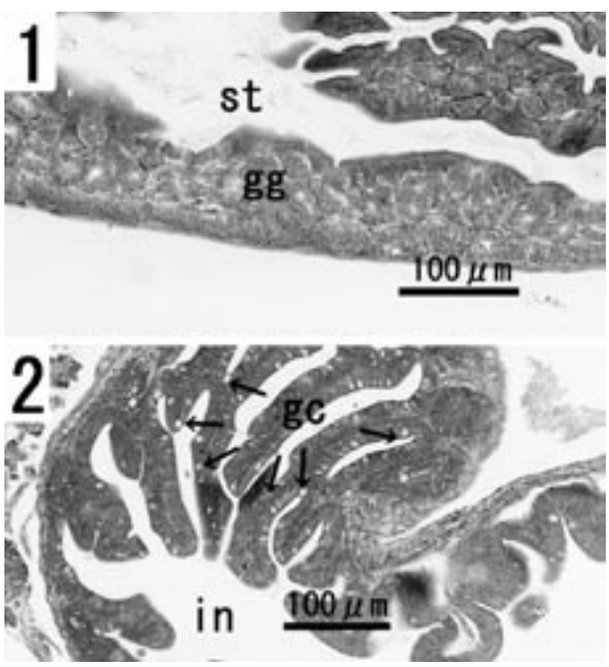

Fig. 5. Photomicrographs of juveniles 26 days after hatching (20.1 mm TL) of mandarinfish. Longitudinal section of the stomach wall (1) and intestine (2). gc, goblet cell; gg, gastric gland; in, intestine; st, stomach.

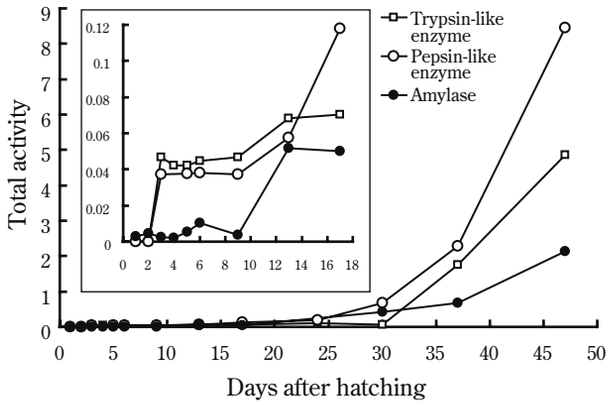

Fig. 6. Change in activities of the digestive enzymes of mandarinfish larvae and juveniles. Total activities are expressed as tyrosine (for trypsin and pepsin-like enzymes) or glucose (for amylase) mg liberated/hour/individual. Inserted figure shows the detail of total activity from 0 to 18 day after hatching.

TL). The peptic activity rapidly increased in 17 DAH larvae (10－11 mm TL) and it was higher than the tryptic 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 tryptin-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 planktivores 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 larvae 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. 1mm 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.

Acknowledgments

I express my gratitude to the Tainjin Aquatic Products Institute for providing the parental fishes and the staff of Suma Aqualife Park for the opportunity of this study. I am very grateful to Dr. M. Tanaka (Field Science Education and Research Center, Kyoto University) for his critical reading of the manuscript and much valuable advice.

References

Anand, S., T. Kurokawa and T. Suzuki (2002) mRNA expression of pancreatic enzyme precursors and estimation of protein digestibility in first feeding larvae of the Japanese flounder, *Paralichthys olivaceus*. *Comp. Biochem. Physiol. Part A*, **132**, 629-635.

Chiang, L. (1959) On the biology of Mandarinfish, *Siniperca chautsi* of Liangtze Lake. *Acta Hydrobiol. Sin.*, **3**, 365-385. (In Chinese with English abstract).

Doi, T. and S. Aoyama (2004) Exclusive piscivory of reared Mandarinfish *Siniperca chuatsi* larvae during the first feeding stage. *Suisanzoshoku*, **52**, 221-229 (in Japanese with English summary).

Doi, T., S. Aoyama and I. Kinoshita (2004) Ontogeny of Mandarinfish, *Siniperca chuatsi*, reared in an aquarium. *Icthyol. Res*. **51**, 337-342.

Higuchi, M. and Y. Oshima (1974) Preliminary studies on the biology of Spanish mackerel and possibility of seedling release in Seto Inland Sea. *Saibai-giken*, **3**, 43-60 (in Japanese).

Ikewaki, Y. and K. Sawada (1991) Food habit of marine fish larvae. In "*Early Development in Fishes*" (ed. by M. Tanaka). Koseisha-koseikaku, Tokyo, pp. 86-104 (in Japanese).

Kaji, T., M. Kodama, H. Arai, M. Tagawa and M. Tanaka (2002) Precocious development of the digestive system in relation to early appearance of piscivory in striped bonito *Sarda orientalis* larvae. *Fish. Sci*. **68**,

1212-1218.
Kawai, S. and S. Ikeda (1973) Studies on digestive enzymes of fishes-Ⅲ. Development of digestive enzyme of rainbow trout after hatching and the effect of dietary change on the activities of digestive enzyme in the juvenile stage. *Bull. Jpn. Soc. Sci. Fish.*, **39**, 819-827.
Kurokawa, T. and T. Suzuki (1996) Formation of the diffuse pancreas and the development on dijestive enzyme synthesis in larvae of the Japanese flounder *Paralichthys olivaceus*. *Aquaculture*, **141**, 267-276.
Kurokawa, T. and T. Suzuki (1998) Development of intestinal brush border aminopeptidase in the larval Japanese flounder *Paralichthys olivaceus*. *Aquaculture*, **141**, 113-124.
Liang, X., H. Oku, H. Ogata and X. He (2001) Weaning Chinese perch *Siniperca chuatsi* (Basilewsky) onto artificial diets based upon its specific sensory modality in feeding. *Aquacult. Res.*, **32**, 76-82.
Luo, X., T. Xu, Z. Wu and Z. Wang (1992) Observation on development of embryo, larva and juvenile of mandarinfish, *Siniperca chuatsi*. *Fish. Sci. Technol. Inf.*, **19**, 155-168.
Shoji, J., T. Kishida and M. Tanaka (1997) Piscivorous habits of Spanish mackerel *Scomberomorus niphonius* (Scombridae) larvae in the Seto Inland Sea. *Fish. Sci.*, **63**, 388-392.
Shoji, J. and M. Tanaka (2001) Strong piscivory of Japanese Spanish mackerel *Scomberomorus niphonius* (Scombridae) larvae from their first feeding. *J. Fish Biol.*, **59**, 1682-1685.
Tanaka, M. (1969) Studies on the structure and function of the digestive system in teleost larvae-II. Characteristics of the digestive system in larvae at the stage of first feeding. *Jpn. J. Ichthyol.*, **16**, 41-49.
Tanaka, M. (1971) Studies on the structure and function of the digestive system in teleost larvae-III. Development of the digestive system during postlarval stage. *Jpn. J. Ichthyol.*, **18**, 164-174 (in Japanese with English summery).
Tanaka, M. (1972) Studies on the structure and function of the digestive system in teleost larvae-Ⅳ Epithelial change in the posterior-gut and protein ingestion. *Jpn. J. Ichthyol.*, **19**, 172-180 (in Japanese with English summery).
Tanaka, M. (1980) Feeding and survival in marine fish larvae-I. Food organisms of wild larvae. *Aquabiology*, **2**, 440-447 (in Japanese with English summary).
Tanaka, M., T. Kaji, Y. Nakamura and Y. Takahashi (1996) Developmental strategy of scombrid larvae: high growth potential related to food habits and precocious digestive system development. In "*Survival Strategy in Early Life Stages of Marine Resources*" (eds. by Y. Watanabe, Y. Yamashita and Y. Oozeki), Balkema, Rotterdam, pp. 125-139.
Tang, Y. and E. Fan (1993) A study on the development of digestive organs and feeding habit of *Siniperca chuatsi* (Basilewsky). *Acta Hydrobiol. Sin.*, **17**, 229-336 (in Chinese with English summary).
Watanabe, Y. (1981) Ingestion of horseradish peroxidase by the intestinal cells in larvae or juveniles of some teleosts. *Bull. Jpn. Soc. Sci. Fish.*, **47**, 1299-1307.
Watanabe, Y. (1982) Intracellular digestion of horseradish peroxidase by the intestinal cells of teleost larvae and juveniles. *Bull. Jpn. Soc. Sci. Fish.*, **48**, 37-42.
Wu, Z. (1987) Development of the digestive organs and feeding behavior of larval *Siniperca chuatsi*. *Irrig. Fish.*, **1987**, 39-43 (in Chinese).
Wu, Z. and R. Hardy (1988) A preliminary ethological analysis on the feeding behavior of mandarin fish. *Freshwater Fish*, **5**, 18-21 (in Chinese).
Zheng, S. (1987) Observation on development and growth of larval and juvenile mandarin fish. *Fish Culture*, **1987(3)**, 1-4 (in Chinese).

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

土井敏男

飼育下におけるケツギョ *Siniperca chuatsi* 仔稚魚の，消化器官の形態的，組織学的発達と消化酵素活性を調べた。摂餌開始直前の卵黄仔魚（全長約5.5 mm）は，大きな口，顎歯，咽頭歯，胃盲嚢とともに仔魚型消化器官を形成した。また，この段階の仔魚は，トリプシン様酵素活性とやや低いペプシン様酵素活性を示した。上屈期仔魚（全長約７－８mm）は，他種の仔魚を捕食し始め，胃盲嚢は餌により拡張した。全長約10 mm上屈期仔魚には，トリプシン様酵素より高いペプシン様酵素活性を示し，胃腺があらわれた。全長約12 mmの上屈後期仔魚は，幽門垂を含む成魚型消化器官を備える。その後，変態した稚魚（全長約20 mm以上）は，胃腺と幽門垂の数を増やすとともに，口，消化器官の大きさ，消化酵素活性をそれぞれ，さらに増大させた。ケツギョ仔稚魚の初期からの魚食性への適応は，仔魚期前半（全長約10 mm以下）では口腔や胃盲嚢の量的発達により，仔魚期後半（全長約12 mm以上）では引き続いて起こる量的発達とともに，胃腺や幽門垂を備えた成魚型消化器官の形成による機能的発達によると考えられる。