Development of Digestive System and Digestive Enzyme Activities of Larval and Juvenile Bluefin Tuna, *Thunnus thynnus*, Reared in the Laboratory

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Abstract: Development of digestive system and changes in the activity of trypsin-like enzyme, pepsin-like enzyme and amylase were studied in larval and juvenile bluefin tuna, *Thunnus thynnus* reared in the laboratory. Liver, gall bladder, pancreas, and the demarcating region between intestine and rectum formed within 36 h after hatching. After feeding commenced trypsin-like enzyme and amylase activities increased as the larvae grew. During the preflexion phase (within 10 days after hatching), revolution of the intestine concluded; and pharyngeal teeth and mucous cells of esophagus differentiated. During the flexion phase (11 to 17 days after hatching), functional jaw teeth were found and blind sac, gastric glands and pyloric caeca begin to form. Pepsin-like enzyme activity increased and functions of stomach and pyloric caeca developed from the postflexion phase to the transitional period to the juvenile (17 to 25 days after hatching). The rate of percentage of preanal length to standard length was constant (around 40%) until 11 days after hatching, then increased to 65% at day 26, and did not change from 26 days to 30 days. These results suggest that the developments of the digestive system until 11 days (preflexion phase) are mainly qualitative and those from then to 26 days (flexion phase to postflexion phase) are both qualitative and quantitative. This quantitative and qualitative development of the digestive system might contribute to the rapid growth in the juvenile stage.

Key words: *Thunnus thynnus*; Digestive system; Digestive enzyme; Larvae

The world and Pacific Ocean catches of bluefin tuna are much less than those of skipjack tuna, *Euthynnus pelamis*, yellowfin tuna, *Thunnus albacares*, bigeye tuna, *Thunnus obesus*, or albacore, *Thunnus alalunga*, but the fishery is still of considerable economic value.1) Harada et al. 2-6) have succeeded in long-term rearing of young bluefin tuna captured by troll in a net-cage. However, few studies on the rearing larvae from the eggs of bluefin tuna have been reported7). Thus, following a previous study8, here we investigated the development of the digestive system and changes in activity of the digestive enzymes during the larval and juvenile stages in bluefin tuna reared in the laboratory.

Materials and Methods

Fish and rearing methods

The fertilized eggs that 7-year-old broodstock spawned naturally were collected with plankton nets from net-cages (30 × 30 × 15 m) near the Oshima station of Fisheries Laboratory, Kinki University, in July 1994. The eggs were transported to concrete tanks (20-60 m³) on land and reared for 30 days after hatching. The feeding schedule and changes in the total length of fish used for the histological study are shown in Fig. 1.

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Histological study

Ten to twenty fish were sampled daily from the concrete tanks (20 m³) for 30 days after hatching. For light microscopy, the fish were fixed in Bouin’s fluid and embedded in paraffin. Serial sections (5-7 μm) were stained with hematoxylin-eosin.

Digestive enzyme assays

The fish were collected at 0.5, 2, 4, 7, 10, 14, 19 and 29 days after hatching. The samples weighted 0.5-1.0 g (wet base). Each day the sample was taken from 4 lots of 2 stations because of the survival rate of each lot was very low. The sampling lot number, time, water temperature and foods are shown in Table 1. The three digestive enzyme activities (trypsin-like enzyme, pepsin-like enzyme and amylase) were assayed according to the method of Kawai and Ikeda (1973)⁸,⁹) (Table 2). The enzyme activities are expressed as μg of tyrosine or glucose liberated/30 min/mg body weight or individual.

Results

Development of the digestive system

Twelve hours after hatching (average standard length: SL, 3.60 mm): The posterior part of the fore-gut and the mit-gut were slightly open, but the epithelia were not differentiated (Fig. 2-A).

Thirty-six hours after hatching (SL, 3.77 mm): The digestive tract opened completely from the mouth to the anus, and the liver, pancreas and gall bladder had been formed. The intestine and rectum had become clearly differentiated with the development of a sphincter between mit-gut and hind-gut (Fig. 2-B).

Table 1. Sampling conditions of bluefin tuna used for determining the activity of digestive enzymes

<table>
<thead>
<tr>
<th>Lots No.</th>
<th>Days after hatching</th>
<th>Sampling time</th>
<th>Water temperature (°C)</th>
<th>Foods</th>
</tr>
</thead>
<tbody>
<tr>
<td>FL-1*¹</td>
<td>0.5</td>
<td>10:00</td>
<td>27.5</td>
<td>--</td>
</tr>
<tr>
<td>FL-1</td>
<td>2</td>
<td>13:30</td>
<td>27.1</td>
<td>Rotifer</td>
</tr>
<tr>
<td>FL-1</td>
<td>4</td>
<td>13:30</td>
<td>27.5</td>
<td>Rotifer</td>
</tr>
<tr>
<td>NC-1*²</td>
<td>7</td>
<td>18:00</td>
<td>27.8</td>
<td>Rotifer</td>
</tr>
<tr>
<td>FL-2</td>
<td>10</td>
<td>14:00</td>
<td>27.7</td>
<td>Rotifer, Brine shrimp, Copepoda</td>
</tr>
<tr>
<td>NC-1</td>
<td>14</td>
<td>11:00</td>
<td>28.8</td>
<td>Rotifer, Brine shrimp</td>
</tr>
<tr>
<td>NC-2</td>
<td>19</td>
<td>10:00</td>
<td>25.0</td>
<td>Rotifer, Brine shrimp, Larval fish</td>
</tr>
<tr>
<td>NC-2</td>
<td>29</td>
<td>16:00</td>
<td>25.0</td>
<td>Rotifer, Larval fish, Artificial diets</td>
</tr>
</tbody>
</table>

*¹ FL: Fisheries Laboratory of Kinki University, Shirahama.
*² NC: Kinki University Fish Nursery Center, Shirahama.

Table 2. Assay methods for the activity of digestive enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>Buffer</th>
<th>Assay method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypsin-like enzyme</td>
<td>Milk casein</td>
<td>0.1M NH₄Cl-NH₄Cl</td>
<td>Folin's method</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(pH 9.5)</td>
<td></td>
</tr>
<tr>
<td>Pepsin-like enzyme</td>
<td>Acid denatured hemoglobin</td>
<td>0.25M CH₃COONa-HCl</td>
<td>Anson's method</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(pH 2.0)</td>
<td></td>
</tr>
<tr>
<td>Amylase</td>
<td>Soluble starch</td>
<td>0.1M NaH₂PO₄-Na₂HPO₄</td>
<td>Somogyi-Nelson's method</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(pH 7.0)</td>
<td></td>
</tr>
</tbody>
</table>
Sixty hours after hatching (SL, 3.81 mm): The fish had begun feeding. The eosinophilic granules measuring $6.2 \pm 0.3 \, \mu m$ in diameter appeared in the rectum epithelium (Fig. 3-A).

Three days after hatching (SL, 3.71 mm): The yolk sac was absorbed mostly, but small oil globule remained. The eosinophilic granules in the rectum increased in number (Fig. 3-B).

Five days after hatching (SL, 4.32 mm): The intestine was completed to coil (Fig. 4).

Seven days after hatching (SL, 4.82 mm): Many mucous cells were observed in the esophagus (Fig. 5-A).

Eight days after hatching (SL, 4.98 mm): The pharyngeal teeth were formed (Fig. 5-B).

Ten days after hatching (SL, 5.58 mm): The blind sac was formed and gastric glands began to appear in the stomach (Fig. 6-A, B).

 Twelve days after hatching (SL, 6.06 mm): The upper jaw teeth were formed.

Fourteen days after hatching (SL, 7.06 mm): The lower jaw teeth were formed (Fig. 7-A).

Fifteen days after hatching (SL, 7.48 mm): The blind sac was enlarged and the gastric glands are increased in number (Fig. 7-B). The pyloric caeca appears in the pyloric portion (Fig. 8-A).

Sixteen days after hatching (SL, 7.75 mm): Many goblet cells were observed in the intestine epithelium near the pyloric portion (Fig. 8-B).

Twenty days after hatching (SL, 9.34 mm): The blind sac enlarged remarkably and gastric glands were distributed throughout most of the wall in the stomach (Fig. 9).

Twenty-five days after hatching (SL, 14.21 mm): The pyloric caeca developed and showed complex structure (Fig. 10).

**Changes in the digestive enzyme activities**

The changes in activity of the trypsin-like enzyme, pepsin-like enzyme and amylase ($\mu g$ of tyrosine or glucose liberated/$30 \, min$/mg body weight) of larvae and juvenile bluefin tuna after hatching out are shown in Fig. 11. The

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**Fig. 2.** Longitudinal sections of larvae aged 12 h (A) and 36 h (B). yo, yolk sac; og, oil globule; pa, pancreas; gb, gall bladder; li, liver.

**Fig. 3.** Longitudinal sections of larvae aged 60 h (A) and 3 days (B). it, intestine; re, rectum; rst, rudimentary stomach.
Fig. 4. Longitudinal serial sections of larvae aged 5 days. The photomicrographs (1-4) show coiling of the intestine.

Fig. 5. Longitudinal sections of larvae aged 7 days (A) and 8 days (B). Arrow shows mucous cells of esophagus. ab, air bladder; no, notochord; pt, pharyngeal teeth.

Fig. 6. Longitudinal section (A) and cross section (B) of larvae aged 10 days. gg, gastric gland.
Fig. 7. Longitudinal sections of larvae aged 14 days (A) and 15 days (B). ljt, lower jaw teeth.

Fig. 8. Longitudinal sections of larvae aged 15 days (A) and 16 days (B). pc, pyloric caeca; gc, goblet cells.

Fig. 9. Longitudinal sections of larva aged 20 days.

Fig. 10. Longitudinal sections of juvenile aged 25 days.
trypsin-like enzyme activity increased along with growth after hatching and showed a peak at day 14 after hatching. The pepsin-like activity was low until 10 days after hatching, but increased rapidly after that. Amylase activity increased along with growth after hatching and showed a peak at day 7 after hatching. The changes in activity of the trypsin-like enzyme, pepsin-like enzyme and amylase (μg of tyrosine or glucose liberated/30 min/individual) of larvae and juvenile bluefin tuna after hatching out are shown in Fig. 12. The total activities of the trypsin-like and pepsin-like enzymes increased after 10 days old. Amylase activity increased slowly along with growth and its value was the lowest among three enzymes at day 29 after hatching. At day 10 after hatching, both the trypsin-like enzyme and amylase showed low levels of activity. Epitheliocystis-like cysts\textsuperscript{10,11} were found in the dorsal fins of the fish in this lot aged 11 days.

Discussion

Relationship between differentiation age of the characters of digestive system and the developmental stages of bluefin tuna are shown in Fig. 13. Liver, gall bladder, pancreas, and the demarcating region between the intestine and rectum formed within 36 h after hatching. Trypsin-like enzyme and amylase activities increased as the larvae grew after commencement of feeding. During the preflexion phase (within 10 days after hatching), coiling of the intestine was concluded, the pharyngeal teeth formed and mucous cells of the esophagus differentiated. During the flexion phase, functional jaw teeth were found and the blind sac of the stomach, gastric glands and pyloric caeca began to form. All the organs were differentiated at this period, though they had not fully developed as those of adults. The pepsin-like enzyme activity increased and functions of the stomach and pyloric caeca developed from the postflexion phase to the transitional phase and to the juvenile. The differentiation ages of the characters of digestive system are almost the same as the data of Kaji et al.\textsuperscript{7}.

In many species, the major differences of digestive system between larvae and adults are the absence of a stomach and pyloric caeca at the early stage of their life time. In Heterosometa\textsuperscript{12-14}, gastric glands are formed during the metamorphosis phase. Tanaka\textsuperscript{15} reported that in spite of day-wise variations, the gastric glands are always formed at about the same phase viz. at about two thirds of the postlarval stage in four species; red sea bream,
Fig. 12. Changes in activity of the digestive enzymes (μg of tyrosine or glucose liberated/30 min/individual) from larval and juvenile bluefin tuna after hatching out.

Fig. 13. Relationship between differentiation age of the characters of digestive system and the developmental stages of larval and juvenile bluefin tuna. Developmental stages: , Yolksac; , Transition; , Preflexion; , Flexion; , Postflexion; , Transition; , Juvenile.
*Pagrus major*, black sea bream, *Acanthopagrus schlegeli*, scorpion fish, *Sebastiscus marmoratus*, and ayu, *Plecoglossus altivelis*. In addition, gastric glands are formed during the later half of the postlarval stage in the larvae such as yellowtail, *Seriola quinqueradiata*[^16], Japanese whiting, *Sillago japonica*[^17], milkfish, *Chanos chanos*[^18], freshwater goby, *Chaenogobius annularis*[^19]. Tanaka[^15] also reported that the differentiation time of the pyloric caecum corresponds with the respective transitional period from larvae to juvenile in the four species mentioned above. On the other hand, both gastric glands and blind sac were formed 10 days after hatching and 15 days before the juvenile stage in bluefin tuna. Pyloric caecum was also differentiated in the early stage (15 days after hatching). In the skipjack tuna[^20] and yellowfin tuna[^21], both the blind sac of the stomach and pyloric caeca were observed in the larva of 4.1 mm in standard length and 4.5 mm in total length, respectively. Thus, these characters of skipjack tuna and yellowfin tuna are formed earlier than those of bluefin tuna. In Spanish mackerel[^3], *Scomberomorus niphonius*, the larvae is already equipped with gastric glands and the blind sac of the stomach at the time of feeding commencement, and pyloric caeca begin to form at 7-8 days after hatching. It seems to be a characteristic of Scombriform that the digestive system is formed in the early stages. Either way, the bluefin tuna larvae in which the digestive system is differentiated at relatively early stages, may consume the energy heavily in the larval stage. Adult of bluefin tuna have a well developed pyloric caeca and the number of caeca are over 20,000. In the larvae, pyloric caeca were developed rapidly from flexion to juvenile stages. In addition, the ratio of preanal length to standard length was constant (around 40%) until 11 days after hatching, and then it increased to 65% until 26 days. The rate was not changed from 26 days to 30 days (Fig. 14)[^2]. These results suggest that development of the digestive system until 12 days was qualitative and that from 12 days to 26 days it was quantitative. This quantitative and qualitative development of the digestive system might contribute to the rapid growth (Fig. 1)[^2] in the juvenile stage.

In many species[^8,9,14,22-26], the trypsin-like enzyme and/or amylase activities are detected at the time of feeding commencement. Both trypsin-like and amylase activities were found in bluefin tuna larvae aged 2 days after hatching when the feeding commenced, and they increased along with the growth. The trypsin-like and amylase activities per mg of body weight decreased 15 days and 10 days after hatching, respectively, although the decrease rate of amylase activity was higher than that of the trypsin-like enzyme. The pepsin-like enzyme activity rapidly increased after differentiation of the gastric glands and activity per mg of body weight was maintained at high value until the juvenile stage (29 days). These results might suggest that changes in the food habit might occur with alterations in the external and digestive systematical morphology and/or metabolism patterns. Thus we would like to further investigate the nutritional requirements and characteristics of the metabolic pattern in larval and juvenile bluefin tuna.

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References


人工孵化クロマグロ仔稚魚の消化系の
発達と消化酵素活性

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山本真司・熊井英水

クロマグロの稚苗生産に関する基礎的知見を得ることを目的として、人工孵化クロマグロの孵化後の成長に伴う消化系の発達および消化酵素活性について調べ、次の結果を得た。孵化後36時間目までに、肝臓、胆囊および臓臓の形成され、トリプシン様酵素およびアミラーゼ活性は孵化開始後成長に伴い増加した。Preflexion 期（孵化後10日まで）に腸の回転が完了し、咽頭部および食道の粘液細胞が分化した。Flexion 期（孵化後11〜17日）に機能的な腸がみられ、胃盲囊、腺腎および幽門帯が形成さり始めた。ペプシン様酵素活性は胃腺の形成に伴って増加し、Postflexion 期から稚魚への移行期（孵化後17〜25日）にかけて胃の機能および幽門帯が著しく発達した。標準体長に対する肛門前長の比は孵化後11日目までは約40％で一定であったが、26日目にかけて約65％まで増加し、26日目から30日目まではほとんど変化しなかった。

以上の結果から、クロマグロの消化系は孵化後11日目まで（Preflexion 期）は主として質的に発達し、それ以降26日目まで（Flexion 期から Postflexion 期）は量的な発達が伴うことが示唆され、このような量的および質的な消化系の発達が稚魚期の急速な成長に貢献するものと推察した。