魚類の消化酵素に関する研究 IV

誌名	日本水産學會誌
ISSN	00215392
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巻/号	39巻8号
掲載ページ	p. 877-881
発行年月	1973年8月

Bulletin of the Japanese Society of Scientific Fisheries 39(8) 877–881 (1973)

Studies on Digestive Enzymes of Fishes—IV Development of the Digestive Enzymes of Carp and Black Sea Bream after Hatching

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(Received March 15, 1973)

The development of protease and amylase activities of carp, *Cyprinus carpio*, and black sea bream, *Acanthopagrus schlegelii*, after hatching was studied. The results obtained are as follows.

Though high tryptic activity occurred in carp eggs and increased with growth after hatching, peptic activity was very weak in the eggs and did not increase along with growth. On the other hand, maltase and amylase activities developed along with growth from 7–10 days after hatching.

Even a carnivorous fish like black sea bream clearly showed amylase activity at 11 days after hatching which increased gradually with growth. Development of the peptic activity in black sea bream coincided with that of differentiation of the gastric gland. The tryptic activity appeared at 3 days after hatching and increased gradually with growth.

In the preceding paper[1], the development of some digestive enzymes of rainbow trout after hatching and the effects of dietary change on the activities of some digestive enzymes in the juvenile stage were reported.

In the present work, development of digestive enzymes of an omnivorous fish, carp, and a carnivorous fish, black sea bream, were studied.

Materials and Methods

Samples Eggs of carp, *Cyprinus carpio*, were supplied by a culturing pond near Maizuru and were hatched in an experimental aquarium at 15°C. After hatching the larvae were fed brine shrimp, *Artemia salina* naupli, for 7 weeks and then fed the commercial pellets for juvenile rainbow trout subsequently. Eggs of black sea bream, *Acanthopagrus schlegelii*, were obtained by fertilizing them artificially from the mature adults at Awajishima, brought to Osaka Prefectural Fisheries Experimental Station and were hatched in a round aquaria (capacity 1 ton) at 20°C with weak aeration. Three to twenty days after hatching, larvae were fed rotifer, *Brachionus plicatilis*, and then *Tigriopus japonicus* or meat of adult black sea bream were fed.

Preparation of enzyme solution and enzymic assay For enzymic assay, all digestive organs were collected from several hundreds of larvae of carp or black sea bream. The methods for enzyme preparation and assay methods were described in the first[2] and

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second[3] papers of this series. The specific activity was expressed as μg of glucose or tyrosine liberated/mg of protein-N/hr.

Results and Discussion

Development of Digestive Enzymes of Carp after Hatching The development of proteolytic activities are shown in Fig. 1. High tryptic activity assayed at pH 9.5 was noticed in the eggs just before hatching but it decreased immediately after hatching. It is suggested that in carp this enzyme may have a role in hatching out, while in rainbow trout a rapid change in the peptic activity before and after hatching was observed as reported in the previous paper.[1]

On the other hand, peptic activity in carp assayed at pH 3.0 was very weak and showed no significant changes before and after hatching, and this activity did not increase along with growth even a few months after hatching. This is because of the lack of stomach and gastric gland in carp. From about a month after hatching, tryptic activity started to increase clearly. The specific activity of this enzyme in juvenile carp, four months after hatching, was one fourth of the adult one.[2]

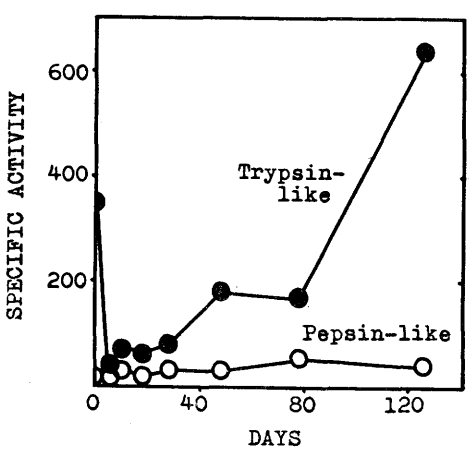

Fig. 1. Development of proteolytic activities of carp after hatching.

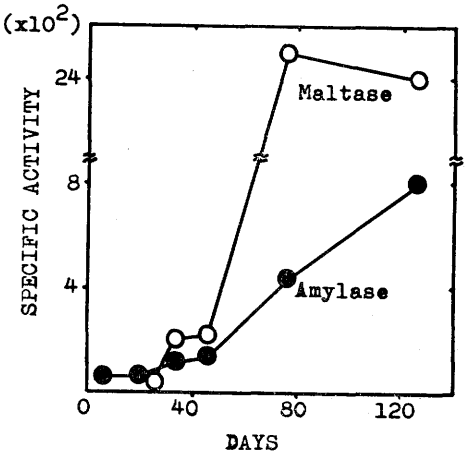

Fig. 2. Development of maltase and amylase activities of carp after hatching.

On the other hand, maltase and amylase activities were detected from 7–10 days after hatching as shown in Fig. 2. These carbohydrases clearly increased from about a month after hatching similarly to the tryptic activity. The specific activities of the carbohydrases in juvenile carp, four months after hatching, were one half of the adult one.[2] The slow increase in digestive enzyme activities of carp as compared with that of rainbow trout may be due to the difference in tissue differentiation in the two fish after hatching and in the amount of diet fed. Moreover, the diet composition also

may have affected on the rate of increase of these enzymes in fish.

Development of Digestive Enzymes of Black Sea Bream after Hatching In a previous paper[2], the carbohydrase activities of a carnivorous fish, red sea bream, *Pagrus major*, were studied and this fish showed fairly high maltase and amylase activities in the digestive tracts.

In this experiment, the enzyme development of a similar carnivorous fish, black sea bream, was studied.

Fig. 3 shows the development of amylase activity and the increase of total length as an index of growth after hatching. Amylase activity was noticed distinctly 11 days after hatching and it increased along with growth. The activity level in the larval stage was similar to that of carp. The significance of the finding that a carnivorous fish such as black sea bream shows a fairly high amylase activity from the larval stage is not clear. Ayu, *Plecoglossus altivelis*, larvae fed only zooplankton also showed amylase activity in the period of 25–30 mm in total length, and this activity also increased with growth.[4]

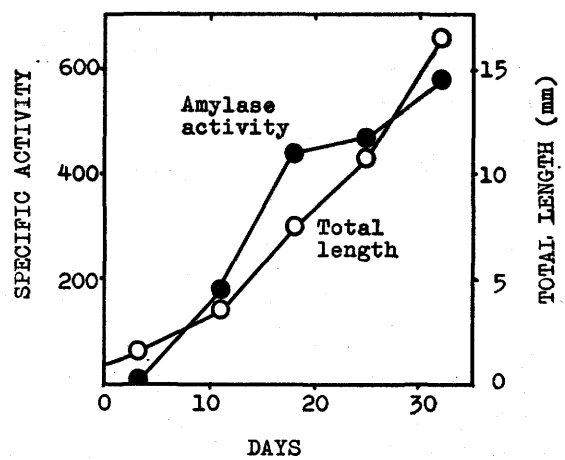

Fig. 3. Development of amylase activity and total length of black sea bream after hatching.

From these findings, amylase activity of fish, both carnivorous and herbivorous, seem to evolve along with growth, and the diet composition is not always a dominant factor for the appearance of this enzyme. It is very interesting and important to inquire into this problem in order to know the biochemical mechanisms of fish especially in the larval stage, and also to exploit the artificial diets for this fish.

Development of proteolytic activities of black sea bream after hatching is shown in Fig. 4. Before hatching, a considerable high peptic activity was detected and it decreased rapidly after hatching. The activity began to increase again about three weeks after hatching when the gastric gland had differentiated, and the development of this enzyme was remarkably prompt. In this period, therefore, the fish seems to change their diets from small zooplanktons to large ones such as copepoda, or fish meat.

On the other hand, the tryptic activity was detected 3 days after hatching, during which period the larva had consumed its yolk and started to feed, and the activity increased in parallel to the growth of this fish. These results suggest that the protein digestion is carried out by the trypsin-like enzyme from 3 days to about 20 days after

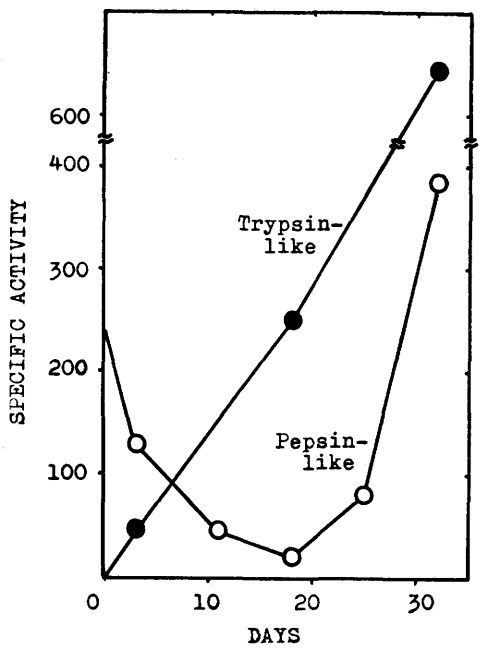

Fig. 4. Development of proteolytic activities of black sea bream after hatching.

hatching, and after this period, the pepsin-like enzyme becomes dominant for the protein digestion.

Similar results were reported in Ayu larvae.[4] Thus in Ayu the total peptic activity at the stage of 40 mm larva was about 500-fold higher than that of 25–30 mm larva. This is due to the differentiation of gastric gland after the stage of 35 mm larva; histological observations supported this explanation.

Fig. 5 shows the activity changes of proteolytic enzymes of rainbow trout, carp and black sea bream just before and after hatching out. Tryptic activity in a stomachless fish like carp changed remarkably during the experimental period, while peptic activity in rainbow trout and black sea bream changed rapidly in this period. These results indicate that the enzymes playing roles in hatching are different depending on fish species.

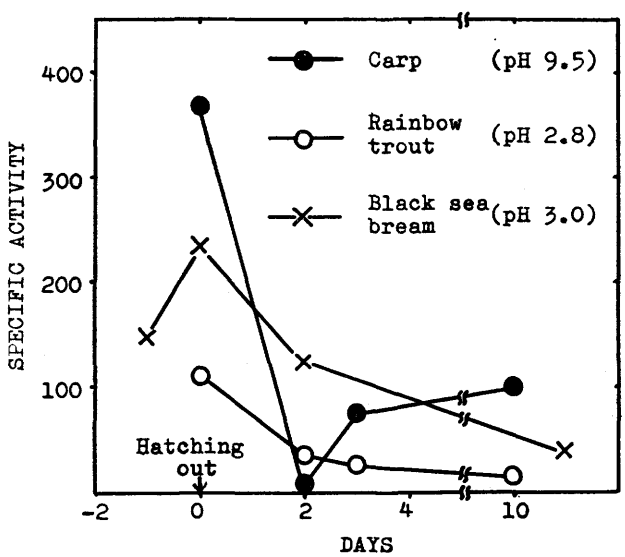

Fig. 5. Proteolytic activities of rainbow trout, carp and black sea bream before and after hatching.

Hatching enzymes secreted from hatching gland are generally considered to be trypsin-like and the enzymes of salmon showed the highest activity at 20°C and pH 9.5.[5] We conclude from the findings reported here that this is not always the case in all species. It has recently been suggested that the hatching enzymes consist of several kinds of hydrolases including proteolytic and lipolytic enzymes.[6]

Acknowledgement

Grateful acknowledgement is made to the staffs of Osaka Prefectural Fisheries Experimental Station for supplying the larva of black sea bream and discussing the results.

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