Table 2 Morphology development satge of black amur bream

中国水产科学研究院
珠 江 水 产 研 究 所
PEARL RIVER FISHERIES RESEARCH INSTITUTE

Growth and ontogenetic development of digestive functionality in black Amur bream (*Megalobrama terminalis*) (Number: 13827501)

Yaqiu Liu, Weitao Chen , Yuefei Li, Jie Li, Xinhui Li
Pearl River Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou, China

INTRODUCTION

- **Background** In freshwater fish aquaculture, ontogenetic development of digestive enzymes reflects the growth and morphological changes occurring in the larvae at early life stages. It was found that investigating the growth and physiological characteristics during the early fish development stage has helped to elucidate the mechanism of the early life history. Fish larvae exhibit specific digestive physiological properties as their digestive system differentiates and develops, especially variation in the types and activities of digestive enzymes. Accordingly, a comprehensive understanding of variation of digestive enzyme during ontogeny is considerately useful to formulate a reasonable feeding strategy and to improve growth and survival rate.
- **Objective** The function of digestive physiology during ontogenetic development is essential to ensure high survival and growth rates. In order to evaluate the digestive physiological capacity of the black Amur bream (Megalobrama terminalis), changes of morphology and digestive enzyme activity (trypsin, lipase, amylase, pepsin, leucine aminopeptidase and alkaline phosphatase) in larvae were examined from hatching to 40 days after hatching (DAH).

METHOD

- **Larva rearing** Fertilized eggs of *M. terminalis were obtained from the Guangdong* Foshan Dongmin Aquaculture Co., Ltd. The eggs were incubated at a temperature of 22.0 ± 1.3℃ and an oxygen concentration of 7.7 ± 0.3 mg/L in a 600-L tank. The newly hatched larvae were transferred into three separate replicate 300-L cylindroconical tanks, at a rearing density of 1,600 larvae/m3. The water temperature (22.7 ± 0.5℃), oxygen concentration (7.8 ± 0.3 mg/L)and pH (7.4 ± 0.2) were monitored daily. The light regime was 12-hr light: 12-hr dark atan intensity of 3,000 lux per day. Beginning at 5 days after hatching(DAH), the larvae were fed three times a day (07:00, 12:00,17:00), and no food was added to the rearing tank at night.
- **Enzyme assays** The samples were removed from the freezer and placed on ice to thaw. After thawing was complete, the samples were homogenized with an F6/10 Fluko homogenizer at 12,000 × g for 2 min on ice in 0.2 M NaCl (Gawlicka et al., 2000). The resulting homogenate was centrifuged with a cryogenic ultracentrifuge and the supernatant was used to determine the digestive enzyme activities and soluble proteins.

RESULT & DISCUSSION

➢The specific growth rate was 10.82% day^{-1}. During development, the total increase in length was linear, described by the equation y = 0.044x + 2.371(R^2 = 0.991) whereas the total increase in weight was exponential, described by the equation y = 5.068e$^{0.0673x}$ (R^2 = 0.954). As shown in Fig. 1, the black Amur bream grew quickly from 10 DAH with the introduction of Artemia and Chironomidae larvae to its diet (Tab. 1). Morphological development in *M. terminalis* during different developmental stages is shown in Fig. 2.

Table 1 Main nutritional components of diets

Nutritional composition (%)	*Artemia*na uplii	Chironomi daelarvae	Compou nd diet
Crude protein	35.25	6.80	52.6
Crude fat	9.7	0.80	10.3
Crude ash	11.16	0.50	12.7

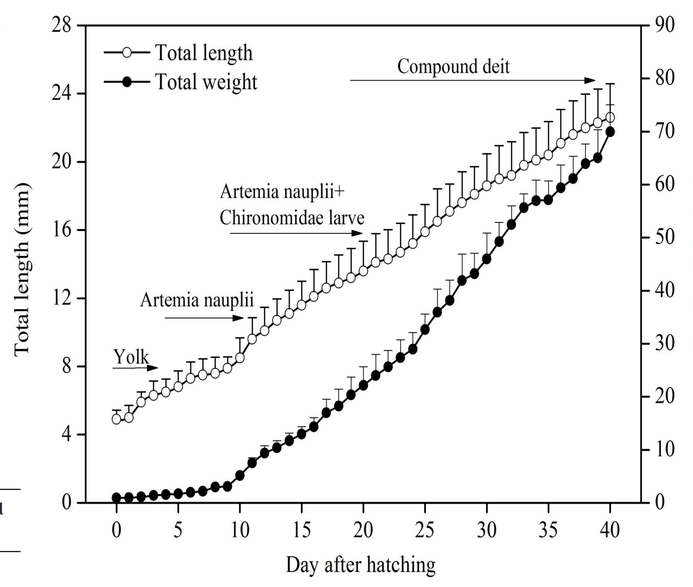

Fig. 1 Total length and weight of black Amur bream larvae and juveniles during the experiment

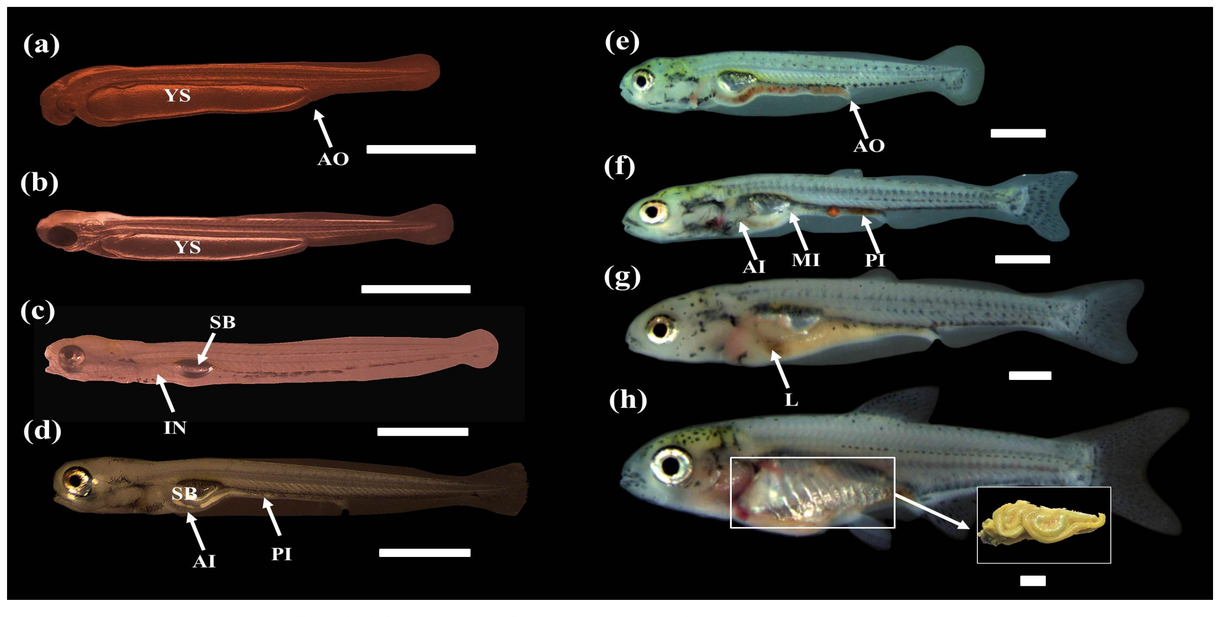

Fig. 2 Morphology Change of black Amur bream larvae and juveniles.

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Main stage	Time	Description	Figure
Larva Stage	New hatching	Digestive tract of newly hatched larvae was a simple straight narrow tube on the dorsal side of the yolk sac	Fig 2a
	2 DAH	Mouth is open, digestive tract is not penetrated; eyes become pigmented	Fig 2b
	4 DAH	Yolk sac was almost absorbed; eyes obvious pigmented	Fig 2c
	6 DAH	Anterior intestine tract was enlarged and curved	Fig 2d
	9 DAH	intestinal mucosa folds increased significantly	Fig 2e
Juvenile Stage	15 DAH	An obvious curvature formed in the anterior intestine, while the second bladder compartment was formed	Fig 2f
	20 DAH	the upper and lower jaw is well developed, and hepatopancreatic was formed completely	Fig 2g
	35 DAH	Scales were well covered, while the digestive system was developmental and 8-10 curls were found on intestine tract	Fig 2h

➢The specific activity of trypsin peaked at 5 DAH and then decreased dramatically, and it increased significantly again from 8 to 10 DAH, reaching a stable level after 20 DAH. Pepsin activity was first detected in black amur bream at 15 DAH and gradually increased towards the end of the experiment. The specific activity of lipase displayed obvious peaks at 5 and 20 DAH.

➢The specific activity of amylase peaked at 8 DAH, and then decreased sharply, becoming stable after 20 DAH. The specific activity of alkaline phosphatase increased significantly from hatching to 5 DAH, and tended to be stable after 15 DAH.

➢The secretion of pepsin on 15 DAH suggests the readiness of digestive system and the timing for inert diet introduction. *M. terminalis* is an omnivorous fish, and the relatively low specific activity of amylase reflects the partial carnivorous nature during its juvenile stage.

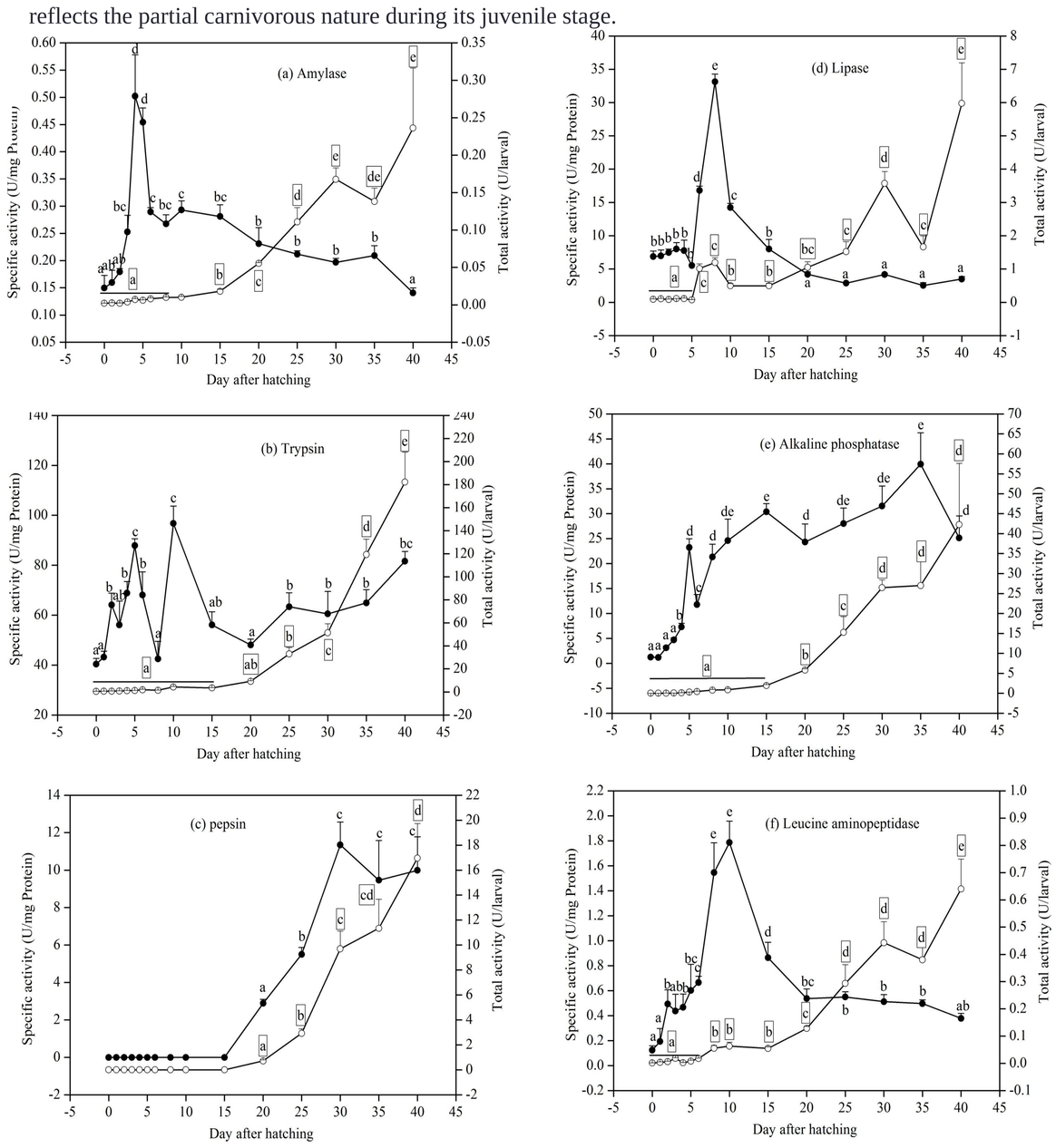

Fig. 3 Fluctuations in the total (○, U/larvae) and specific (●, U/mg protein) activities of digestive enzymes in the black Amur bream larvae and juveniles during the experiment

CONCLUSION

- **The black Amur bream belongs to the fast-growing species as the durationof digestive ontogeny of the larvae is much shorter than other slow-growing species. The specific activities of digestive enzymesin *M. terminalis* changed constantly from 3 to 20 DAH, whereas *the* digestive enzymes of the juveniles were relatively stable after 20 DAH to the end of the experiment (40 DAH). Our findings on the development of the digestive system in black Amur bream provide effective information for the ontogeny of fish larvae, which is useful to improve theseedling cultivation and the technology of healthy breeding.**