Aquaculture 302 (2010) 112–123

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Ontogeny of the digestive tract in yellow catfish *Pelteobagrus fulvidraco* larvae

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ARTICLE INFO

Article history:
Received 10 March 2009
Received in revised form 18 July 2009
Accepted 16 February 2010

Keywords:
Pelteobagrus fulvidraco
Ontogeny
Larvae
Digestive tract
Histology
Ultrastructure

ABSTRACT

The study on histological and ultrastructural characteristics of the digestive tract of yellow catfish (*Pelteobagrus fulvidraco*) was carried out from hatching (0 day after hatching, DAH) until 35 DAH. Larvae for this study were maintained in the laboratory conditions (water temperature ranged from 23 °C to 25 °C). They were fed with zooplankton from 3 DAH to 17 DAH, with zoobenthos added from 10 DAH, and only zoobenthos from 18 DAH to 35 DAH. Development of the digestive tract in *P. fulvidraco* followed the general pattern described for other fish species with some peculiar findings. At hatching, it consisted of an undifferentiated straight tube laying over the yolk sac. The digestive tract was differentiated into buccopharynx, esophagus, primary stomach and intestine by 2 DAH. The liver and pancreas also appeared at this time. The intestine became differentiated into anterior and posterior regions separated by the intestine bend at 3 DAH. Gastric gland appeared in cardiac stomach at 3 DAH, the earliest appearance time among fishes studied to date. Oxynticopeptic cell contained pepsinogenic granules and abundant tubulovesicular systems at 3 DAH. As larvae grew, more pepsinogenic granules but less tubulovesicular systems were found in oxynticopeptic cell. The abundant visible tubulovesicular systems suggested that oxynticopeptic cell was still in rest phase with little hydrogen chloride (HCl) secreted at the first appearance time. The ultrastructure of oxynticopeptic cell indicated the asynchronous development of acid-secreting and pepsinogen-secreting function. The epithelial absorptive cell of the anterior and posterior intestinal segments showed electron-opaque lipid droplets and heavy pinocytosis, respectively at 3 DAH. Heavy pinocytosis could be observed in the posterior intestine until 25 DAH. Lipid vacuole accumulation appeared in liver at 13 DAH, the same time as the storage of abundant glycogen. These results suggested that the development of the digestive tract of *P. fulvidraco* larvae was functional rapidly, however it was still incomplete at 3 DAH. The functions of digestive tract and accessory glands were developed gradually until 25 DAH.

1. Introduction

Fish larvae rearing is considered as a major bottleneck in aquaculture. There exists a transitional period from endogenous to mixture feeding and to exogenous feeding in the ontogenesis of the digestive system. The survival rate is low specially during weaning to artificial feed. The underlying reason is that the digestive tract at this time is largely undifferentiated with incomplete function (Govoni et al., 1986). Histological and biochemical description of the ontogeny of digestive system may provide important information for establishing sound rearing methods to improve commercial larval rearing (Zaiss et al., 2006). Many studies were focused on important commercial marine species such as summer flounder *Paralichthys dentatus* (Bisbal and Bengtson, 1995), spotted sand bass *Paralabrax maculatofasciatus* (Pena et al., 2003), gilthead sea bream *Sparus aurata* (Elbal et al., 2004), large yellow croaker *Pseudosciaena crocea* (Mai et al., 2005), yellowtail kingfish *Seriola lalandi* (Chen et al., 2006), and shi drum *Umbrina cirrosa* (Zaiss et al., 2006). These studies helped the weaning practices of these species. However, little research has been done in freshwater aquaculture species in recent years.

Yellow catfish (*Pelteobagrus fulvidraco*) is an important commercial freshwater species in China. It has a promising market potential in China, Japan, South Korea, East and South Asia. Due to its high market value, the culture of this species has increased rapidly in recent years (Lu et al., 2008; Pan et al., 2008). However, larvae rearing became a major bottleneck because of its high mortality which caused by uncorrected culture feeding strategies simply derived from the traditional carp culture. Although a few studies were carried out to focus mainly on its formulated diets (Wang et al., 2005), effect of diets on digestive enzyme activity and gene expression (Wang et al., 2006), taste buds distribution in the barbell (Zhang et al., 2006) and effect of diet items on foregut and liver histological changes (Lu et al., 2008), little is known on the early life stages of *P. fulvidraco*, especially on their morphological and internal development relating to functional capabilities. To date, no study has been documented on ontogeny of the digestive system in *P. fulvidraco*, thus correct feeding strategy related to morphological development is unknown.

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doi:10.1016/j.aquaculture.2010.02.020

In order to enhance the success of larvae rearing of *P. fulvidraco*, we need to know the ontogeny of its digestive system thoroughly. The purpose of this study was to understand the morphological structure and the ultrastructure of digestive tract during the ontogeny of *P. fulvidraco* from hatching to 35 days after hatching (DAH). We hope that this information would provide fundamental knowledge for larvae rearing management for this species.

2. Materials and methods

2.1. Eggs and larvae rearing

Fertilized eggs of the same batch of broodstock *P. fulvidraco* were obtained from the hatchery of the National centre for *P. fulvidraco* in Jingzhou, China, and transported to laboratory in the Fisheries College, Huazhong Agriculture University (Wuhan, China). Upon arrival, all eggs were hatched in $4\times4\times1$ m cement incubators at 24 ± 1 °C. After hatching, the larvae were stocked in 300 L fiberglass rearing tanks at the density of 5 fish/L. All 10 rearing tanks were connected with a recirculation system. Water volume replacement of the system accounted for 10% per day. The water exchange rate through fiberglass rearing tanks varied from 10% to 300% per day according to the activity of larvae. During the rearing period, water temperature, dissolved oxygen and pH value maintained 24 ± 1 °C, 7–9 mg/L and 6.8–7.6, respectively. Total ammonia–nitrogen ($[NH_4^+ + NH_3]$–N) was less than 0.2 mg/L and nitrite level was below 0.09 mg/L. Fish were held under natural photoperiod condition throughout the trial.

Zooplanktons (mainly *Moina*) were offered to larvae from first-feeding time (3 days after hatching, DAH) to 17 DAH. From 10 DAH on, the zoobenthos (mainly *Limnodrilus hoffmeisteri*) were also added to the rearing tanks. After 18 DAH, only zoobenthos were given. Zooplankton and/or zoobenthos were fed to satiation 2 times (0800 h and 1800 h) per day. Excess feed and feces were removed before feeding.

2.2. Fish sampling and growth measurements

Fish larvae were randomly sampled daily from hatching to 18 DAH, and then samples were collected on 20, 25, 30, 35 DAH, respectively. Total length (L_T) of 30 specimens was individually measured to the nearest 0.1 mm using an ocular micrometer with the aid of a dissected microscopy and a vernier caliper when the larvae were 10 mm. Wet weight was weighed by microbalance (0.0001 g). Ten specimens were used to describe the morphology of digestive tract. Ten specimens for histological observation were fixed with 5% phosphate buffered formalin (pH 7.4). Five samples for ultrastructure observation were fixed with 2.5% glutaraldehyde adjusted to pH 7.4 with 0.1 M phosphate buffer at 4 °C. Specimens from hatching to 4 days were processed for whole, while from this age onwards they were dissected. Sample was cut into approximately 1 mm^3 in volume.

2.3. Histological analysis

Ten fixed specimens of each sampling day were dehydrated in graded series of ethanol and embedded in paraffin individually. A series of sagittal and cross sections (5–7 μm) were cut from each paraffin block, mounted on glass slides, air dried, stained with haematoxylin and eosin (H&E). The sections were examined and photographed under a Nikon ECLIPSE 80i microscope (Nikon Corporation, Kanagawa, Japan) equipped with a Nikon Digital Sight DS-U2 camera (Nikon Corporation, Japan).

2.4. Ultrastructural analysis

Pre-fixed samples were post-fixed in 2% OsO_4 with the 0.1 M phosphate buffer for 2 h, and then dehydrated and embedded in epoxy resin and SPI-812, respectively. Ultrathin sections obtained with a Leica UC6 ultramicrotome were stained with uranyl acetate and subsequently with lead citrate. The observations and recording of images were performed and recording of images were performed with a HITACHI H-7650 transmission electron microscope (HITACHI Corporation, Japan) at 80 KV and a Gatan 832 CCD camera (Gatan, Pleasanton, USA).

3. Results

3.1. Fish growth

Total length of larval fish and wet weight averaged 7.1 ± 0.3 mm and 3.3 ± 0.6 mg, respectively at 2 DAH, and increased to 33.6 ± 0.6 mm and 481.0 ± 39.4 mg at 35 DAH (Fig. 1).

3.2. Buccopharynx

At hatching, the head kept close to the large yolk sac. The buccopharynx was not formed. It was still separated into anterior and posterior cavity by membrane (Fig. 2-1) at 1 DAH and opened until 2 DAH, lined by a single layer of squamous epithelium, and contained differentiated oral valves lined by squamous epithelium (Fig. 2-2). Taste buds and goblet cells were visible at 3 DAH when larvae started exogenous feeding (Fig. 2-3). Rudimentary jaw teeth penetrated the premaxillary epithelium at 5 DAH, and numerically increased at 8 DAH (Fig. 2-4). The number of taste buds and goblet cells in the folds of the buccopharyngeal epithelium became more numerous as larvae grew. The folds of the buccopharynx increased continuously with age.

3.3. Esophagus

At hatching, the esophagus could not be distinguished. At 1 DAH, esophagus appeared as a narrow and short lumen with a simple squamous epithelium in front of incipient stomach (Fig. 2-1). At

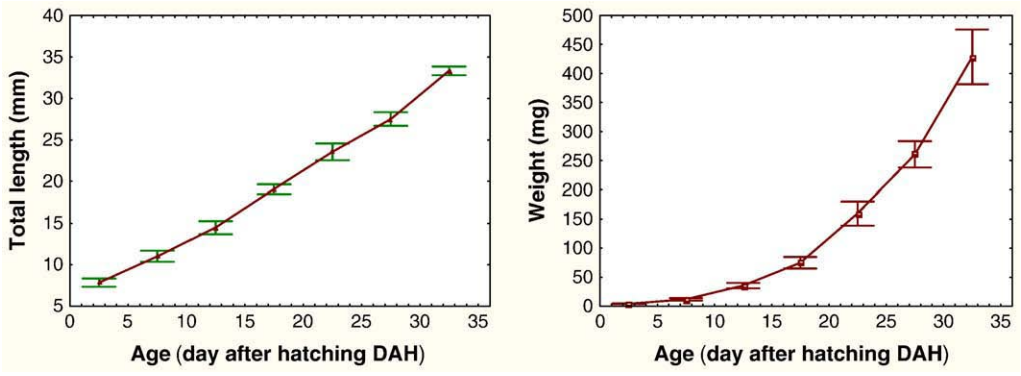

Fig. 1. Growth in total length and weight of *P. fulvidraco* larvae from 2 DAH to 35 DAH.