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Ontogeny of the digestive tract and enzymatic activity in white seabass, *Atractoscion nobilis,* larvae

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ABSTRACT

The development of the digestive system and digestive enzyme activity in white seabass larvae, *Atractoscion nobilis*, were analyzed from hatching until 40 days post hatch (dph) using histological and biochemical approaches. The development of the digestive system in *A. nobilis* larvae was similar to that reported for other marine fish species. Larvae at 3 dph (0.55 ± 0.001 mg wet weight and 3.6 ± 0.02 mm total length), cultured at 18 °C in seawater, presented all the structures (i.e. differentiation of the alimentary canal into the buccopharynx, esophagus, anterior and posterior intestines, pancreas with zymogen granules, liver, gall bladder and open mouth) necessary for the digestion and absorption of nutrients such as proteins and lipids (primarily). At this time, the larvae had fully-developed digestive systems that allowed them to digest inert feed and to absorb nutrients throughout the intestine walls. On the other hand, most digestive enzyme activities were detected at the moment of hatching. Trypsin activity was 0.80 ± 0.16 mU/mg protein at 1 dph (0.51 ± 0.001 mg wet weight larvae), and increased gradually during the following days, but most notably after the initial exogenous feeding at 4 dph. The specific activity of chymotrypsin was 7.21 ± 1.29 mU$\times 10^{-4}$/mg protein at 1 dph and reached peak level (15.9 ± 1.02 mU$\times 10^{-4}$/mg protein) at 18 dph (6.6 ± 0.003 mg wet weight larvae). The specific activity of leucine aminopeptidase increased continuously from 1.31 ± 0.05 mU$\times 10^{-3}$/mg protein at 1 dph to 15.91 ± 0.40 mU$\times 10^{-3}$/mg protein at 18 dph. The activity of α-amylase at 1 dph was 1.35 ± 0.09 U/mg protein, increasing to 8.07 ± 0.98 U/mg protein at 16 dph. The activity of pepsin was detected at a very low level (0.71 ± 0.53 U/mg protein) at 10 dph, and a stepwise increase in activity was observed between 16 and 20 dph, reaching maximum level (13.92 ± 0.09 U/mg protein) at 40 dph. These results indicate that the digestive tract develops rapidly in this species and that the stomach becomes functional between 16 and 18 dph. It should, therefore, be possible to start weaning the fish at this young age.

1. Introduction

In southern California and Baja California, the white seabass *Atractoscion nobilis* supports an important commercial and sport fishery (Vojkovich and Reed, 1983). Its wide acceptance and high market value led to over-harvesting the wild stocks in many areas (Drawbridge and Kent, 1997), which in turn led to the development of a comprehensive stock replenishment program that was initiated in southern California in 1983 (Kent et al., 1995). As the production capacity of *A. nobilis* has increased, so too has the interest in applying the technology to commercial farming (Drawbridge and Kent, 2001). Research related to the nutritional requirements of this species is limited; our research group has reported on macronutrient selection and the effects of digestible protein, lipid, and carbohydrate requirements on juveniles and young adults (Durazo et al., 2010; Jirsa et al., 2010; López et al., 2006; 2009).

In typical aquaculture operations, weaning is extended 1 or 2 weeks after the digestive tract is fully developed in order to reduce body malformation and mortality, or performed as early as possible to reduce the cost of live food production. For this reason, knowledge of early ontogeny of the digestive tract and digestive enzyme activity in fish larvae is of value for establishing appropriate feeding and weaning routines for target aquaculture species (Baglole et al., 1997; Zambonino-Infante and Cahu, 2001), as in the case of gilthead sea bream *Sparus aurata* (Sarasquete et al., 1995), red drum *Sciaenops ocellatus* (Lazo et al., 2000), California halibut *Paralichthys californicus* (Alvarez-González et al., 2006; Gisbert et al., 2004), yellow croaker *Pseudosciaena crocea* (Ma et al., 2005; Mai et al., 2005), bullseye puffer

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Sphoeroides annulatus (García-Gasca et al., 2006), and spotted sand bass *Paralabrax maculatofasciatus* (Alvarez-González et al., 2008; Peña et al., 2003), among others.

The objective of this study was to describe the development of the digestive system and the activity of the main digestive enzymes in *A. nobilis* larvae fed live food and a compound microdiet from hatching until 40 days post hatch (dph). This information will be useful for improving current larval rearing practices and feeding protocols, and reducing weaning costs of this fish species.

2. Materials and methods

2.1. Eggs and larval fish rearing

Fertilized eggs of *A. nobilis* were obtained from the Hubbs-SeaWorld Research Institute's marine finfish hatchery in Carlsbad, CA, USA. The eggs were produced by wild-caught brood fish maintained in four separate groups of 50 fish in equal sex ratios. Adult *A. nobilis* were induced to spawn using photothermal control to simulate natural seasonal cycles that were phase-shifted among groups to provide eggs year-round. Adult *A. nobilis* were estimated to weight 15–25 kg and were fed fresh fish and squid 5 days per week with supplementation of vitamins (Jirsa et al., 2010) every other day. The eggs were transported to the Fish Culture and Biotechnology Unit of the School of Marine Science, University of Baja California (UABC) at Ensenada, Mexico. Eggs were treated with 100 ppm formalin for 1 h and stocked at a density of 100 eggs/L in a 1600-L cone bottom tank with 18 °C seawater recirculated at a rate of 1.5–2 L/min through a fluidized bed, UV sterilizer, and foam fractionator.

Eggs hatched approximately 48 h after fertilization. Yolk-sac larvae were stocked at a density of 30 individuals/L in nine 100-L experimental tanks. Beginning at 4 dph, larvae were fed three times per day (08:00, 12:00, and 17:00 h) exclusively with *Artemia* metanauplii (Salt Creek Inc., Salt Lake City, UT, USA) enriched with lipid emulsion (Bio-Marine Algamac 3050™) at a concentration of 0.6 g/L. The *Artemia* were supplied at a concentration of 5 nauplii/mL only up to 15 dph. At 16 dph, the amount of live food was decreased and a combination of enriched *Artemia* metanauplii and formulated diet (Otohime Japanese Marine Weaning Diet, Red Mariculture; protein 52.11%, lipid 16.3%, ash 11.2%, particle size 200–1410 μm) was supplied. The weaning period was complete at 24 dph, when live food was no longer supplied. Larvae were fed the microdiet from 24 to 40 dph (end of the trial).

2.2. Sampling

Depending on size, between 20 and 150 larvae were collected using a 200 μm dip net, 1 h after the first daily feeding. Samples were collected daily starting at 0 dph (free embryos) through to 6 dph, every 2 days from 8 dph until 20 dph, and every 4 days thereafter until 40 dph. After sampling, larvae were anesthetized with tricaine methanesulfonate (MS 222), rinsed with distilled water, freeze-dried, and stored at −80 °C until analysis.

Supplementary samples were collected from the rearing tanks each day after hatching until 6 dph and on 8, 12, 15, 18, 24, 36, and 40 dph. These samples were used to measure total length and wet weight of larvae. Additionally, an average total length (measured to the nearest 0.1 mm) was calculated for each sampling day by measuring the larvae under a dissecting microscope using a digitizing camera and PAXcam2 (PAX-it version 6, MIS Inc., USA) software. Wet weight (measured to the nearest 0.1 mg) was calculated by weighing the subsample of larvae using an analytical balance (Sartorius Gottingen, Germany; precision of 0.1 mg), then counting the larvae contained in the subsample. Individual larval weight was determined by dividing the subsample weight by the number of larvae in the sample.

2.3. Enzymatic activity

For each sampling time, three samples of 150 larvae until 10 dph, 50 larvae until 20 dph, and 20 larvae until 40 dph were used for enzymatic analyses. Because of the difficulties associated with dissecting and removing the digestive tract of small larvae, whole body homogenates were used for enzymatic analyses in larvae younger than 16 dph. After this age, the larvae digestive system was dissected on a glass slide supported on a frozen mini-table.

Each sample was homogenized with a tissue grinder in 1 mL of distilled water chilled to 4 °C. A subsample of the homogenate was stored at −80 °C until later analysis of leucine aminopeptidase activity. The remaining sample was centrifuged at 14,000*g* for 30 min at 4 °C, and the supernatants were stored at −80 °C until analysis.

2.3.1. Proteases (endopeptidases)

The level of soluble protein in pooled samples was determined using the method described by Bradford (1976). Trypsin activity was assayed according to Erlanger et al. (1961), using BAPNA (N-α-Benzoyl-DL-arginine *p*-nitroanilide) as substrate. The mixtures were incubated at 37 °C and the absorbance of the reaction products was measured at 410 nm. Chymotrypsin activity was measured by the method of Hummel (1959), as modified by Applebaum et al. (2001), using BTEE (N-Benzoyl-L-tyrosine ethyl ester) as substrate. The mixtures were incubated at 37 °C and the absorbance of the reaction products was measured at 256 nm. The reaction of trypsin was stopped by adding 30% acetic acid. One unit of enzyme activity was defined as 1 μg nitroanilide released per minute, using a molar extinction coefficient of 8.8 for trypsin and of 964 for chymotrypsin.

Leucine aminopeptidase was measured at 37 °C as suggested by Appel (1974), using leucine P-nitroanilide as substrate. The reaction of leucine aminopeptidase was stopped by adding 30% acetic acid. The mixtures were incubated at 37 °C and the absorbance of the reaction products was measured at 410 nm. One unit of enzyme activity was defined as 1 μg nitroanilide released per minute, using a molar extinction coefficient of 8.2.

Acid proteinase (pepsin) activity was evaluated as described by Sarath et al. (1989), using 2% hemoglobin as substrate. The enzyme crude extracts and the substrate were incubated at 37 °C and the absorbance of the reaction products measured at 280 nm. One unit of enzyme activity was defined as 1 μg tyrosine released per minute, using the molar extinction coefficient of 0.005.

2.3.2. Alpha-amylase

The α-amylase assay was performed according to Vega-Villasante et al. (1993), using soluble starch (1%) as substrate. One unit corresponded to the amount of enzyme required to increase by 0.01 units the absorbance at 540 nm per minute.

Specific and total enzyme activities in digestive extracts were determined using the following equations:

(1) Units/mL = (Δabs reaction final volume (mL))/(MEC·time (min) extract volume (mL));
(2) Units/mg protein = Units per mL/mg of soluble protein

Δabs represents the increased absorbance at a determined wavelength and MEC represents the molar extinction coefficient for the product of the reaction (mL/μg/cm). The results are presented using Eqs.(1) and (2), and all assays were carried out in triplicate.

2.4. Histology

Conventional histology and hematoxylin–eosin (H&E) staining were performed on larvae fixed in 2% paraformaldehyde for 24 h at 4 °C, before being washed, dehydrated, cleared, and embedded in paraffin. Sagittal sections (5 μm) were obtained with a conventional microtome, placed on gelatin-coated slides, re-hydrated, and H&E-