Aquaculture 448 (2015) 545–551

Contents lists available at ScienceDirect

Aquaculture

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

Ontogenetic development of digestive enzymes in Sobaity sea bream *Sparidentex hasta* larvae under culture condition

Samira Nazemroaya [a], Razieh Yazdanparast [b,*], Mohammad Ali Nematollahi [a], Hamid Farahmand [a], Qodrat Mirzadeh [c]

[a] Department of Fisheries, Faculty of Natural Resources, University of Tehran, Karaj, Iran
[b] Department of Biochemistry, Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran
[c] Fisheries Organization of Hormozgan, Bandar-e-Abbas, Iran

ARTICLE INFO

Article history:
Received 3 May 2015
Received in revised form 21 June 2015
Accepted 24 June 2015
Available online 30 June 2015

Keywords:
Enzyme activity
Larval development
Ontogeny
Sobaity sea bream
Sparidentex hasta

ABSTRACT

Activities of the main enzymes involved in digestion (pepsin, trypsin, amylase, lipase and alkaline phosphatase) in Sobaity sea bream were studied from hatching up to the juvenile stages (40 days after hatch, DAH). All enzyme activities except for pepsin were detected upon hatching, and their activity subsequently increased with age. Early presence of trypsin and amylase was attributed to a genetically programmed process. Trypsin exhibited fluctuating pattern (with tow peaks at 16 and 30 DAH) triggered by food ingestion and was in response to the diet shifting during the rearing period. Both lipase and amylase activities were enhanced after the onset of the weaning depending upon the microbound diet components. Early presence and incremental trend of alkaline phosphatase from 5th DAH represented early maturation of enterocytes and consequently rapid growth of this species. Pepsin was first detected at 14 DAH and showed a sharp increasing trend up to end of the experiment. Its progressive increase followed by a decrease in trypsin activity after accomplishing weaning exhibited a change in the digestive physiology to reach to an adult-type protein digestion. In conclusion, such early ontogenetic development of the above mentioned enzymes makes it possible to wean Sobaity sea bream larvae earlier than 25 DAH in order to reduce live food usage and improve weaning efficiency.

Statement of relevance: Obtaining knowledge about digestive physiology makes it possible to quantify the capacity of larvae to digest and absorb different types of nutrients from live feed and/or microbound diets and to understand the right time to conduct weaning in Sobaity sea bream larvae earlier than what has been practiced in hatcheries in order to reduce live food usage and improve weaning efficiency.

1. Introduction

The progression of marine fish culture, particularly in the case of a newly introduced species, mainly relies on the production of viable larvae (Alvarez-González et al., 2010). During the early developmental stages, fish are highly sensitive to environmental variables. As illustrated in previous studies, these stages are characterized by low larval survival, high incidence of skeletal deformities, nutritional disorders, extreme sensitivity to handling stress and frequent pathological outbreaks (Bermejo-Nogales et al., 2007; Koumoundouros et al., 2004).

One of the principal factors influencing survival of larvae is adequate nutrition which depends on the effective ingestion, digestion and assimilation of diets containing the required essential nutrients (Lazo et al., 2007). Digestion is a key process in animal metabolism which directly influences the availability of nutrients needed for all biological functions. Since the net efficiency of the whole digestive process mainly depends on the type and function of the digestive enzymes, the study of the digestive physiology has gained global attention. It also is an important tool when studying the nutritional status and adaptation of the organism to dietary change. Especially, beginning of the digestive function and ontogenetic patterns of enzyme activity must be determined in order to quantify the capacity of larvae to digest and absorb different types of nutrients from live feed and/or compound microbound diets (Zambonino-Infante et al., 2008). Moreover, it may be used as an indicator of the type and the level of the main nutrients to comprise in a formulated diet designed to replace the live prey (Alvarez-González et al., 2010; Lazo et al., 2007); and it allows understanding the right time to conduct early weaning using artificial diets (Cahu and Zambonino-Infante, 1994; Cara et al., 2003). On the whole, these information can be used to synchronize feeding practice with larval physiological stages and to clarify adequate age-specific nutritional protocols, thereby improving larval production (Zambonino-Infante et al., 2008). That is why many studies have focused on ontogeny of digestive enzymes during larval development of many species, such as Senegal

* Corresponding author at: Department of Biochemistry, Institute of Biochemistry and Biophysics, University of Tehran, Enghelab St. Tehran, Iran.
E-mail address: ryazdan@ut.ac.ir (R. Yazdanparast).

http://dx.doi.org/10.1016/j.aquaculture.2015.06.038
0044-8486/

sole (Martínez et al., 1999), white sea bream (Cara et al., 2003), red drum (Lazo et al., 2007), sharpsnout sea bream (Suzer et al., 2007a), blackspot sea bream (Ribeiro et al., 2008), spotted sand bass (Alvarez-González et al., 2008; Alvarez-González et al., 2010), common dentex (Gisbert et al., 2009), bay snook (Uscanga-Martínez et al., 2011), white sea bass (Galaviz et al., 2011) and spotted rose snapper (Galaviz et al., 2012).

Sobaity sea bream (*Sparidentex hasta*) is a tropical carnivorous demersal fish distributed in Persian gulf and Indian coasts (Bauchot and Smith, 1984). In the last decades, there has been an increasing interest for Sobaity sea bream production due to its consumer popularity and a new candidate species in marine culture in the southern seashores of Iran and Arabian countries coastlines (Hussain et al., 1981). The total production volume of this species has increased from 11.5 t in 2000 to more than 1300 t in 2012 (RAIS). Like most marine fish, rearing of Sobaity sea bream larvae heavily relies on the use of live food, such as rotifer and *Artemia*. The best survival rate was reported to be around 12.3% for this species at 60 DAH (Teng et al., 1999). However, in recent years, new nutritional programs have been evaluated using artificial diets during the early stages of Sobaity sea bream in order to reduce production cost, to allow a more reliable nutritional food value and to achieve a better larval growth and performance. No study has been carried out on the digestive enzyme activities during larval development of Sobaity sea bream. Thus, to optimize rearing practice and feeding protocols and subsequently to reduce weaning costs, it is essential to have a deeper evaluation on the digestive enzymes activities while Sobaity sea bream is going through the larval development. In this respect, we focused in this study on pepsin as an stomach enzyme, alkaline phosphatase as an intestinal enzyme and some of the pancreatic enzymes such as trypsin, lipase and amylase.

2. Material and methods

2.1. Egg and larval rearing

Captive broodstock of Sobaity sea breams were held in the marine finfish hatchery center of Yaran-e-Darya located in the village of Bandar-e-Moalem, Hormozgan province of Iran. Females (n = 14, average individual mass = 1.36 kg) and males (n = 8, average individual mass = 0.85 kg) were placed together in a 12 m^3 concrete circular tank. Fertilized eggs were obtained by semi natural spawning (without hormonal induction) of domesticated broodstocks following simulation of tidal current by continues increasing/decreasing the water level in the maintenance tank. Floating eggs were collected by water flow in a 150 μm diameter mesh net, counted and transferred to 300-l cylindroconical incubation tanks. Water temperature and salinity during incubation were 20 ± 1.0 °C and 34 ± 0.5 ppt, respectively. No water exchange was performed during incubation and gentle aeration was provided with an air diffuser. After 24 h of incubation, newly hatched larvae were divided into new 4 * 2 * 1.25 m (length_width_height) cement pools with a stock of 50 larvae/l. Larval rearing was done according to previous study (Teng et al., 1999) with some modification. Briefly, microalgae *Nannochloropsis* sp. (10^6 cell/ml) was used for green water culture from the first day up to 20 DAH (days after hatching); larvae were fed rotifers twice a day from 2 DAH to 20 DAH at a density of 5–10 and 10–16 individuals/ml (*Brachionus rotundiformis*, S-type) and (*Brachionus plicatilis*, L-type), respectively, and enriched *Artemia nauplii* with DHA Selco (INVE Aquaculture NV, Belgium) from 16 DAH (2–7 individuals/ml) to 30 DAH (13 individuals/ml). Weaning started at 25 DAH using a microbound diet APEX (200–300 μm) and ended at 30 DAH, when the concentration of *Artemia nauplii* was fully replaced with the artificial diet (300–500 μm) (Coppens Feeds for Aquaculture, Helmond, Netherlands). Thereafter, larvae were fed with the microbound diet from 30 DAH to the end of the study at 40 DAH. No feed was added to the rearing pools at night. Water exchanges started on 10 DAH and the rates of change increased gradually with the age of the larvae. Also, pools were cleaned daily from 10 DAH by siphoning the bottom to remove waste and dead larvae. Rearing tanks were illuminated by overhead artificial fluorescent lights, and larvae were exposed to a 12L:12D photoperiod. Water parameter such as salinity (34 ± 0.5 ppt), pH value (7.7–8.1), dissolved oxygen (6.0 ± 0.5 mg/l), and temperature (21 ± 1 °C) were monitored daily.

2.2. Sampling

To examine the activities of the digestive enzymes in Sobaity sea bream larvae, samples were randomly collected from rearing pools in triplicate at 0, 2, 5, 10, 14, 17, 20, 25, 30, 35, and 40 DAH. The sampling was performed in the morning prior to larval feeding to reduce the potential effects of exogenous enzymes from undigested live food in fish gut (Kolkovski, 2001). Variable numbers of larvae (60 to 100, depending on their size) were filtered using a 200 μm mesh net, subsequently anaesthetized with Tricaine Methanesulfonate (MS-222, Sigma), rinsed with distilled water to eliminate salt from larval mass, and after getting the excess water with a soft tissue, the samples were immediately frozen in liquid nitrogen and stored at −70 °C until further analysis.

To analyze the larval growth, additional pool samples of 30 larvae each were taken at the same sampling days and fixed with buffered phosphate formalin solution (4%). Total length was measured on the taken photographs by a stereomicroscope (Laica, Germany) equipped with a versatile digital microscope (Dino-Lite), followed by dinocapture 2.0 software (version 1.4.5.B) using an image analyzing system (Image J software, version 1.29, USA) with 0.001 mm precision (Schneider et al., 2012). The individual wet weight (mg) of further 20 larvae was also recorded with a digital balance (Sartorius, Gottingen, Germany; precision of 0.01 mg) after removing excess water with soft tissue. Specific growth rate (SGR) was calculated using the followed formulae: SGR = 100 (Ln FBW − Ln IBW) / Δt, with IBW, FBW: initial, final body weight of fish (mg), Δt: time interval (day). The survival rate was determined by summing up the number of live fish at the end of the experiment and the number of sampled larvae divided by the total number of stocked larvae multiplied by 100.

2.3. Extract preparation and the enzymes assays

For larvae greater than 25 DAH, the digestive organs were dissected by cutting out the head, tail and dorsal side of the fish. Dissection was done on a prechilled glass plate maintained around 0 °C. Larvae younger than 25 DAH were too small to dissect, so the intact larvae homogenized. Samples were homogenized in 10 volumes (v/w) of 0.15 M NaCl, centrifuged at 15000 ×*g* for 15 min at 4 °C. Each supernatant was aliquoted into different volumes depending on the assay, and then stored at −70 °C.

Pepsin (E.C.3.4.23.1) activity was measured by using Casein as the substrate (Rungruangsak-Torrissen et al., 2006). Briefly, 200 μl of casein (1% in 60 mM HCl) was added to 200 μl of the crude enzyme. The mixture was incubated at 37 °C for 30 min. One ml of 5% TCA was added to stop the reaction. The mixture was kept at room temperature for 30 min, and centrifuged at 5000 ×*g* for 20 min. Then, 1 ml of 0.5 M NaOH was added to 500 μl of the digested supernatant and 300 μl of Folin-Ciocalteu reagent (1:3 dilution), and kept for 10 min at room temperature. Then, the absorbance of the mixture was measured at 720 nm and compared with L-tyrosine standard curve. One unit of enzyme activity was defined as 1 μmol of tyrosine liberated per min per ml supernatant.

Trypsin (E.C.3.4.21.4) activity was measured by incubating 100 μl of the crude extract with 700 μl of 1.25 mM benzoyl-L-arginine p-nitroanilide as the substrate in 0.2 M Tris–HCl buffer pH 8.4, at 50 °C for 10 min. The reaction was terminated by the addition of 800 μl acetic acid (30%). The production of *p*-nitroaniline was measured at 410 nm, and compared with the *p*-nitroaniline standard curve (Torrissen et al.,