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Development of digestive enzyme activity in spotted rose snapper, *Lutjanus guttatus* (Steindachner, 1869) larvae

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Abstract We describe digestive enzyme activity during the larval development of spotted rose snapper, *Lutjanus guttatus*. Trypsin, chymotrypsin, leucine aminopeptidase, pepsin, amylase, lipase, and acid and alkaline phosphatase activities were evaluated using spectrophotometric techniques from hatching through 30 days. The spotted rose snapper larvae present the same pattern of digestive enzyme activity previously reported for other species in which pancreatic (i.e., trypsin, chymotrypsin, amylase, and lipase) and intestinal (i.e., acid and alkaline phosphatases and leucine aminopeptidase) enzymatic activities are present from hatching allowing the larvae to digest and absorb nutrients in the yolk-sac and live prey by the time of first feeding. The digestive and absorption capacity of the spotted rose snapper increases during the larval development. A significant increase in individual activity of all enzymes occurs at 20 DAH, and around 25 DAH, the juvenile-type of digestion is observed with the appearance of pepsin secreted by the stomach, suggesting that maturation of the digestive function occurs around 20–25 DAH. Our results are in agreement with a previous suggestion that early weaning may be possible from 20 DAH. However, the patterns of enzymatic activities reported in our study should be considered during the formulation of an artificial diet for early weaning of the spotted rose snapper.

Keywords Spotted rose snapper · *Lutjanus guttatus* · Larvae development · Digestive function · Enzyme activity

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Introduction

Successful marine fish larviculture requires an effective feeding schedule based on the nutritional requirements and digestive capabilities of developing larvae. Detailed information regarding the development of the digestive tract and digestive enzyme activities permits estimation of the best time for weaning and provides important information regarding the feed types that can be used as part of larviculture programs (Zambonino-Infante and Cahu 2001). Many studies have examined digestive enzyme activities in different fish larvae, and a general pattern has been reported (Martínez et al. 1999; Cuvier-Péres and Kestemont 2002; Perez-Casanova et al. 2006): following a poor start, enzymatic activity increases with age and can be influenced by the type, quantity, and quality of the feed used (Zambonino-Infante and Cahu 2001; Perez-Casanova et al. 2006). Cytosolic enzyme activities

are high during the first few days of development, followed by a continuous decrease over the following weeks, at which point brush border enzyme activities increase and pepsin activity is first detected in the stomach. This shift in enzymatic activities suggests that digestive function is fully mature by around the third or fourth week of development (Cahu and Zambonino-Infante 1995), marking the end of the larval period and the onset of the juvenile period in terms of digestive function (Peña et al. 2003).

Spotted rose snapper, *Lutjanus guttatus*, is a tropical marine fish commercially important in the northern Pacific coast of Mexico. The species is important both commercially—fetching high prices in domestic and international markets—and for its use in aquaculture. In recent years, several studies have examined a variety of aspects related to the culture of spotted rose snapper, including reproduction (Ibarra-Castro and Duncan 2007; Ibarra-Castro and Álvarez-Lajonchére 2011), larval rearing (Boza-Abarca et al. 2008; Álvarez-Lajonchére et al. 2012), nutrition (García-Ortega 2009), and juvenile production (Álvarez-Lajonchére et al. 2010, 2012). Recently, Galaviz et al. (2012) reported on the development of the digestive tract and trypsin and pepsin activities during the larval period of *L. guttatus*, concluding that the digestive tract was fully functional 25 days after hatching and suggested that weaning could begin on this day. However, an efficient weaning strategy must be based on accurate, detailed information regarding digestive enzyme activity so that the most appropriate type of feed may be selected. Therefore, it is essential that we investigate the development of other pancreatic and intestinal enzymes involved in the digestive process. The objective of the present study was to describe digestive enzyme activity during the larval period of spotted rose snapper, *L. guttatus*. This knowledge will permit a better understanding of larval digestive function and provide detailed information for evaluating the best time to initiate early weaning and the nutritional composition of feed to use with this species.

Materials and methods

Larval fish rearing and sample collection

Fertilized eggs were obtained from a single, spontaneous spawning of spotted rose snapper, *L. guttatus*, broodstock at the Unidad Piloto de Maricultivos (Mariculture Pilot Unit) at CICIMAR-IPN in La Paz, Baja California Sur, Mexico. Viable, recently fertilized eggs were incubated in two cylinder conical tanks filled with 120 L of mechanically filtered, UV-sterilized seawater at 26 °C, with moderated aeration from the bottom and continuous water flow (500 mL min^{-1}). Around 10 h post-fertilization, three random samples containing 100–150 embryos were placed in three 1-L plastic incubators filled with filtered and UV-sterilized seawater. The plastic incubators were then placed in a water bath at 26 °C with a salinity of 35 psu. At 22 h post-fertilization, yolk-sac larvae were collected from the incubators and anesthetized with 4 % phenoxyethanol. The hatching rate was evaluated using a dissection microscope (Olympus SZ-CTV) and making a distinction between eggs without an embryo (i.e., unfertilized eggs), unhatched eggs, and yolk-sac larvae.

One day after hatching, larvae were transferred from the incubation tanks to six 120-L fiberglass tanks (density of 150 larvae L^{-1}) equipped with a recirculating system. During the study, the water temperature was 26.7 ± 0.21 °C (mean ± std dev), salinity was 36.01 ± 0.60 gL^{-1}, and dissolved oxygen was 4.04 ± 0.38 mg L^{-1}, with a 13:11 (light:dark) artificial photoperiod. Light intensity averaged 1,200 lux at the water surface. Microalgae, *Nanochloropsis oculata*, were added to the tanks as green water at a density of 300,000 cells mL^{-1} from 2 to 18 days after hatching (DAH). Larvae were fed copepod, *Pseudodiaptomus euryhalinus*, nauplii from 2 to 18 DAH (density: two nauplii mL^{-1}), *Brachionus rotundiformis* rotifers enriched with *N. ocullata* from 2 to 7 DAH (density: 3 rotifers mL^{-1}), SELCO™-enriched (Spresso, INVE, Aquaculture Thailand) *B. rotundiformis* rotifers from 8 to 18 DAH (density: 3 rotifers mL^{-1}), *Artemia* nauplii (Artemia Cysts, Vitellus, Bernaqua, Belgium) enriched with SELCO™ (Spresso, INVE, Aquaculture, Thailand) from 14 to 24 DAH (density: 0.5 nauplii mL^{-1}), 10-day-old *Artemia* juveniles from 22 to 24 DAH (density: 3 juveniles mL^{-1}), and 20-day-old *Artemia* adults from 25 to 30 DAH (density: 3 adults mL^{-1}).

Larvae were sampled directly from the rearing tanks using a 100-μm sieve. Samples were taken at hatching and on days 1, 2, 3, 5, 10, 15, 20, 25, and 30 DAH. They were sampled early in the morning, before feeding, to avoid a possible effect over the

enzymatic activity of the larvae due to the presence of prey in the larval digestive tract. On each sampling day, 0.9 g (wet weight) of larvae was taken, placed in 2 mL Eppendorf tubes, and frozen at −80 °C until biochemical analyses were performed. Samples of 15 larvae were taken daily from hatching to 10 DAH and then every 5 days to 30 DAH. They were anesthetized, photographed, and measured for total length with Image Pro Plus v4.5 (Media Cybernetics, MD, USA) digital image analyzer software.

Biochemical analyses

Each sample was homogenized with a tissue grinder in 3.6 mL of distilled water for 1 min and centrifuged at 17,949.49 G for 10 min at 5 °C (Centrifuge Eppendorf 5804R). The supernatant was stored at −70 °C until biochemical analyses were performed. All assays were performed in triplicate using a spectrophotometer (Jenway 6505, UK). The soluble protein concentration was determined following the Bradford (1976) method, using bovine serum albumin as a standard. Activities of all the enzymes are presented as specific and individual activities in relation of soluble protein (Units mg protein^{-1}) and individual larvae, respectively.

Acid (EC 3.1.3.2) and alkaline (EC 3.1.3.1) phosphatases were evaluated by placing 20 μL of the extract in a test tube with 500 μL of buffer at 37 °C for 20 min. We used a solution of *p*-nitrophenyl phosphate as the substrate with 90 mM citrate-HCl with pH 4.8 and 100 mM glycine with pH 10 for acid and alkaline phosphatase, respectively. The reaction was stopped with a sodium hydroxide solution and mixed. The absorbance of the reaction was measured at 410 nm. One unit hydrolyzes 1.0 mM p-nitrophenyl phosphate per min under these conditions (Bergmeyer et al. 1974).

Trypsin (EC 3.4.21.4) activity was measured using 100 nM BAPNA as the substrate in dimethyl sulfoxide diluted with 50 mM Tris–HCl buffer with pH 8.2 at 37 °C. After 60 min of incubation, 0.25 mL of 30 % acetic acid was added. The absorbance was measured at 410 nm (Erlanger et al. 1961). Chymotrypsin (EC 3.4.21.1) activity was measured using 0.1 M Tris–HCl with pH 7.8 and 0.5 mM SAAPNA as the substrate. The reaction was incubated for 60 min and the absorbance was measured at 410 nm (Ásgeirsson and Bjarnason 1991). In both cases, a unit is the amount of enzyme required to increase absorbance 0.01 Units at a given wavelength per min.

Lipase (EC 3.1.1.3) activity was quantified by placing 100 μL of 100 mM sodium tauracolate in 1,900 μL of 50 mM Tris–HCl buffer with pH 7.2 and 20 μL of 200 mM β-naphthyl caprylate in a test tube. After 60 min of incubation, 20 μL of 100 mM fast blue BB was added. The reaction was stopped with 200 μL of 0.72 *N*-trichloroacetic acid, and 2.71 mL of ethanol:ethyl acetate was used to clarify the reaction. The reaction mixture was stirred and the absorbance was measured at 540 nm (Versaw et al. 1989). Amylase (EC 3.2.1.1) activity was obtained with 50 mM Tris–HCl buffer with pH 7.5 using 1 % soluble starch as the substrate for 60 min at 37 °C. DNS reagent was added and the mixture was placed in a boiling water bath for 15 min. The absorbance was measured at 550 nm (Vega-Villasante et al. 1993). A unit of activity is the amount of enzyme required to increase absorbance 0.01 Units at a given wavelength per min.

Leucine aminopeptidase (EC 3.4.11.1) was analyzed using leucine p-nitroanilide (0.1 mmol L^{-1} in DMSO) as the substrate and 20 μL of the extract. After 60 min of incubation, the reaction was stopped with 30 % acetic acid and the absorbance was measured at 410 nm (Maraux et al. 1973). One unit of enzyme activity was defined as 1 μg nitroanilide released per minute. Finally, pepsin (EC 3.4.23.1) activity was evaluated using 0.5 % hemoglobin in 0.1 M glycine buffer with pH 2 and incubating for 60 min. The reaction was stopped with 20 % trichloroacetic acid and left for 20 min at 4 °C. Then, it was centrifuged at 15,294.24 G for 5 min. The absorbance was measured at 260 nm (Anson 1938).

Results

Lutjanus guttatus larvae grew exponentially, reaching a maximum standard length of 24.54 ± 3.78 mm (mean ± std dev) at 30 DAH (Fig. 1). Specific and individual larval activities for all enzymes, except pepsin, were recorded from hatching. The yolk sac was totally depleted between 2 and 3 DAH. The specific activities of amylase, lipase, trypsin, and leucine aminopeptidase fluctuated during the yolk-sac stage. After the complete absorption of the yolk sac, specific enzymatic activities increased and fluctuating

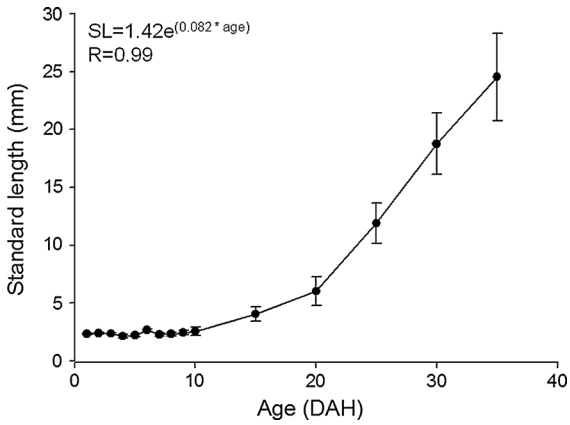

Fig. 1 Standard length (mm) of spotted rose snapper, *Lutjanus guttatus* larvae. Each point represents the mean ($n = 15$), while the *vertical bars* show the standard deviation

levels during larval development were recorded (Figs. 2, 3). On the other hand, the individual larval activity of all the enzymes showed an exponential pattern during larval development. The individual larval activity of acid and alkaline phosphatase, amylase, lipase, trypsin, chymotrypsin, and leucine aminopeptidase showed a significant ($p < 0.05$) increase around days 15 and 20 DAH reaching a significant ($p < 0.05$) maximum activity at 30 DAH (Figs. 2, 3).

The specific activity of acid phosphatase showed a maximum peak at hatching ($3.63 \times 10^{-2} \pm 7.12 \times 10^{-4}$), before significantly decreasing ($p < 0.05$) at 5 DAH. A second peak of activity was recorded at 10 DAH ($0.02 \pm 1.94 \times 10^{-4}$), followed by a decrease and then a steady increase up to 30 DAH ($0.02 \pm 2.82 \times 10^{-4}$) (Fig. 2a). Alkaline phosphatase showed a relatively constant level of specific activity up to 15 DAH and then began to increase, with a maximum peak at 30 DAH ($0.01 \pm 5.84 \times 10^{-5}$) (Fig. 2b). Amylase presented two peaks of specific activity, the first one at the onset of exogenous feeding (2 DAH, $11.05 \pm 2.32 \times 10^{-2}$) and the second one at 5 DAH

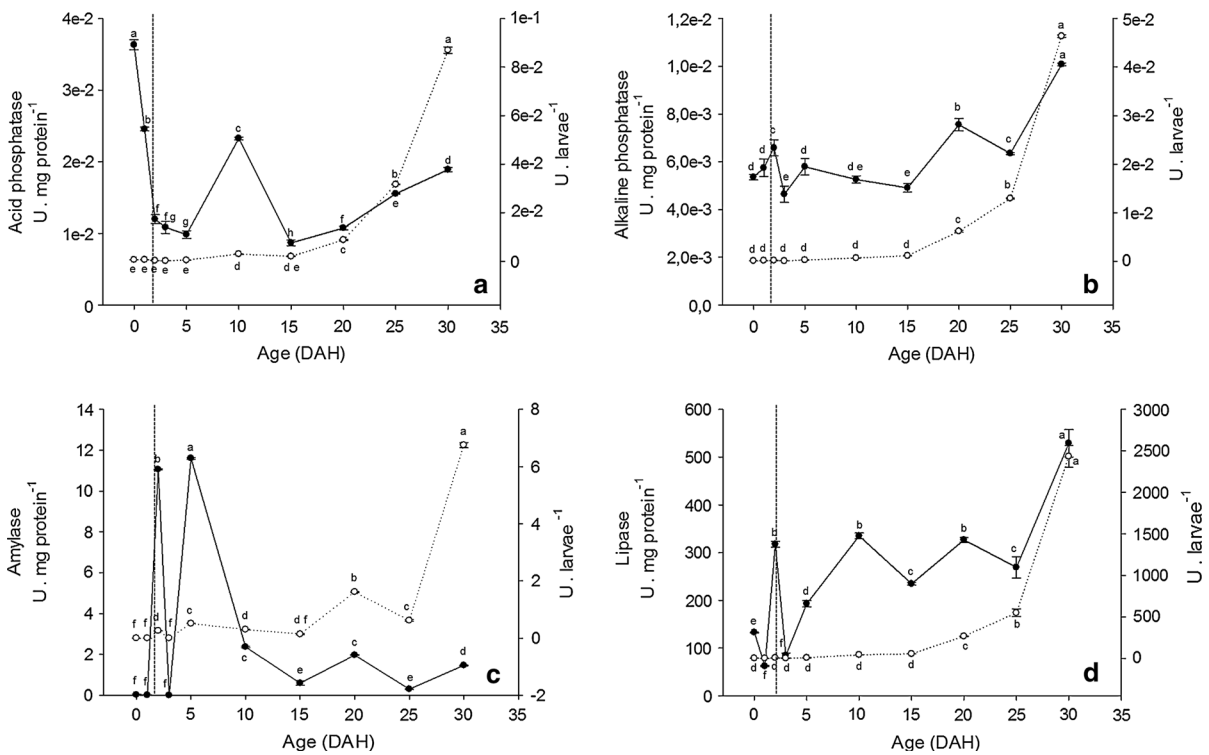

Fig. 2 Specific (*filled circle*) (Units mg protein^{-1}) and individual larval (*open circle*) (Units larvae^{-1}) enzymatic activities of the spotted rose snapper *Lutjanus guttatus* during the larvae culture. **a** Acid phosphatase activity, **b** alkaline phosphatase activity, **c** amylase activity, **d** lipase activity. *Dotted line* indicates the onset of exogenous feeding. *Different letters* indicate significant difference ($p < 0.05$) between the days of development. Each point represent the mean ($n = 3$ pooled larvae) and the *vertical bars* the standard deviation

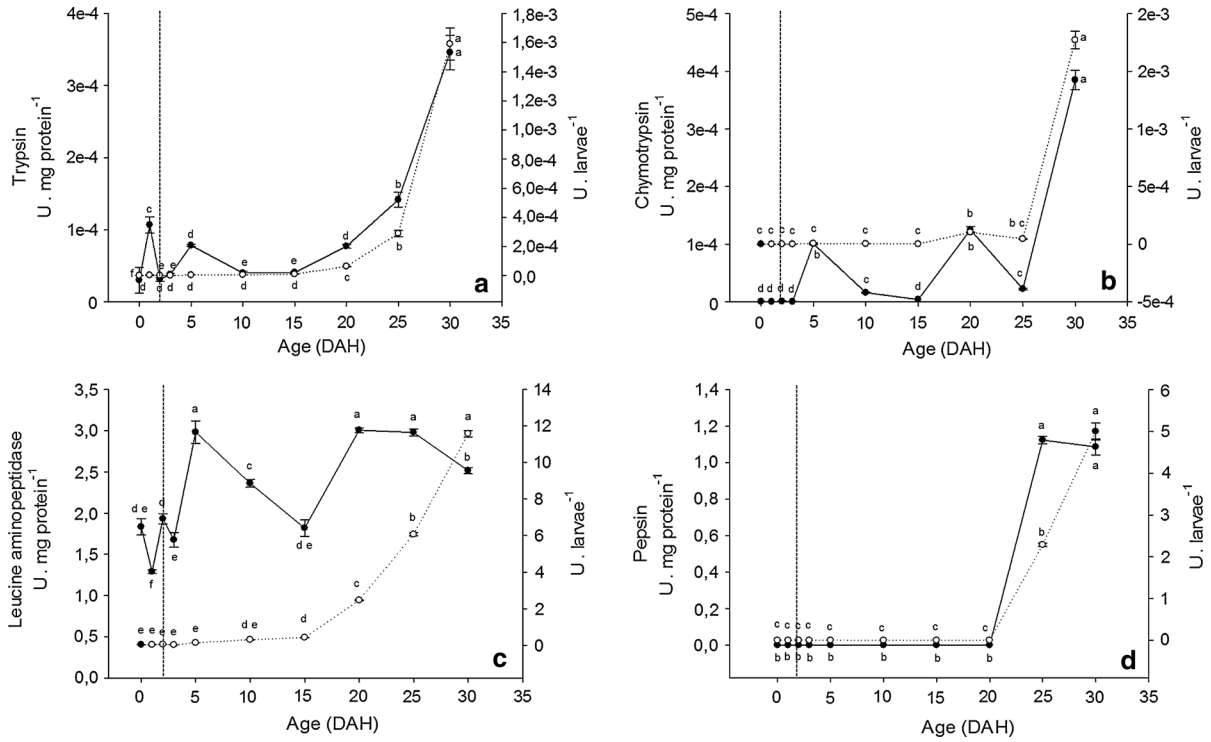

Fig. 3 Specific (*filled circle*) (Units mg protein^{-1}) and individual larval (*open circle*) (Units larvae^{-1}) enzymatic activities of the spotted rose snapper *Lutjanus guttatus* during the larvae culture. **a** Trypsin activity, **b** chymotrypsin activity, **c** leucine aminopeptidase activity, **d** pepsin activity. *Dotted line* indicates the onset of exogenous feeding. *Different letters* indicate significant difference ($p < 0.05$) between the days of development. Each *point* represents the mean ($n = 3$ pooled larvae) and the *vertical bars* the standard deviation

(11.60 ± 0.04) followed by a major and significant ($p < 0.05$) decrease, and then fluctuating levels until 30 DAH (Fig. 2c). Lipase-specific activity significantly decreased ($p < 0.05$) after first feeding, then significantly increased ($p < 0.05$) at 5 DAH, and then the specific activity varied continuously during larval development with an increasing trend reaching a peak at 30 DAH (Fig. 2d).

Trypsin fluctuated during the first few days of development up to 10 DAH; afterward the specific activity increased, reaching a maximum at 30 DAH ($3.45 \times 10^{-4} \pm 0.2.41 \times 10^{-5}$ Units mg protein^{-1}) (Fig. 3a). Chymotrypsin showed low levels of specific activity in yolk-sac larvae and then increased with continuous fluctuations from 5 to 25 DAH, reaching a maximum at 30 DAH ($3.84 \times 10^{-4} \pm 1.68 \times 10^{-5}$ Units mg protein^{-1}) (Fig. 3b).

Leucine aminopeptidase fluctuated constantly throughout larval development. The lowest ($p < 0.05$) specific activity was recorded at 1 DAH (1.29 ± 0.02 Units mg protein^{-1}). Two peaks of activity were evident, at 5 DAH (2.98 ± 0.13 Units mg protein^{-1}) and at 20 DAH (3.00 ± 0.02 Units mg protein^{-1}), after which a decrease in activity was recorded through 30 DAH (Fig. 3c). The specific activity of pepsin was first detected at 25 DAH and remained relatively constant up to 30 DAH (1.12 ± 0.02 and 1.08 ± 0.04 Units mg protein^{-1}, respectively) while the individual activity of pepsin increased significantly ($p < 0.05$) from 25 to 30 DAH (Fig. 3d).

Discussion

The studies of the digestive enzymatic activity are an important aspect in marine fish larviculture since they contribute to develop feeding schedules and to infer the best moment to initiate weaning based on the digestive capabilities of the larvae. Our results showed that the digestive enzymatic activity in the spotted rose

snapper is present throughout the larval development. Similar findings have been reported for several other species (Martínez et al. 1999; Kim et al. 2001; Álvarez-González et al. 2008; Shan et al. 2009). There are, however, differences in terms of the intensity and time of appearance of certain enzymes, which may be explained either as a response to changes in rearing conditions, in the composition and type of the diet, or as a consequence of the growth and development of new organs and tissues, as well as the tropical or template species' affinity (Martínez et al. 1999; Uscanga-Martínez et al. 2011).

With the exception of pepsin, all enzyme activities were detected from hatching, which means that early in development, the spotted rose snapper larvae have the capacity to digest proteins, lipids, and carbohydrates found in the yolk sac and oil globule. The presence of digestive enzymes at hatching and before exogenous feeding has been reported, for example, in *Perca fluviatilis* (Cuvier-Péres and Kestemont 2002), *Paralichthys californicus* (Álvarez-González et al. 2006), *Anarhichas minor* (Savoie et al. 2008), *Dentex dentex* (Gisbert et al. 2009), and *Sparus aurata* (Naz, 2009). Some reports support the hypothesis that the digestive enzymes used to hydrolyze macronutrients in the yolk sac and oil globule are genetically programmed to become active during the first days of development, prior to mouth opening and first feeding (Carnevali et al. 2001; Cuvier-Péres and Kestemont 2002).

Alkaline and acid phosphatases are hydrolases involved in functions like the hydrolysis of inorganic phosphate for energy production and nutrient transport across the cell membrane, and their presence in the digestive tract may indicate the development of enterocyte brush border (Martínez et al. 1999; Ribeiro et al. 1999; Shan et al. 2009). In the spotted rose snapper larvae, the activity of both phosphatases was detected from hatching. In species like *Paralabrax maculatofasciatus* (Peña et al. 2009) and *Acipencer baeri* (Gisbert et al. 1999), the activity of these enzymes was detected in the brush border of the enterocytes using enzyme-histochemistry also from hatching. A similar pattern of specific activity has been reported in the larvae of species like haddock and Atlantic cod (Pérez-Casanova et al. 2006), *Paralabrax maculatofasciatus* (Álvarez-González et al. 2008), Senegal sole (Ribeiro et al. 1999), and turbot (Cousin et al. 1987). An increment in the enzymatic activity of these enzymes during larval development, as the one observed in the spotted rose snapper at 20 DAH, has been associated with an increment in the capacity for nutrient absorption in the intestines of larval teleosts (Segner et al. 1989).

Amylase activity was high during the first few days of development, while a notable decrease was observed during the rest of larval development. High amylase activity early in development has also been reported in sea bass (Cahu and Zambonino-Infante 1994) and sea bream (Moyano et al. 1996), and the sharp decrease in the activity was reported for *Solea senegalensis* (Martínez et al. 1999) and *Paralabrax maculatofasciatus* (Álvarez-González et al. 2008) larvae.

This pattern in the specific amylase activity suggests that during the early days of development, the larvae have the ability to use carbohydrates. Péres et al. (1996) suggested that the amylase activity during the early days of development is genetically programmed. The fluctuating levels of specific activity of amylase during larval development have been reported in other species like *Paralichthys californicus* (Alvarez-González et al. 2006), *Cichlasoma urophthalmus* (López-Ramírez et al. 2011), and in every case, low levels of this enzyme are reported, which may be indicative of the carnivorous habits of the species and may reflect the low nutritional requirements of these species for carbohydrates at later stages of development (Zambonino-Infante and Cahu 2001). However, further research is necessary to establish these requirements in spotted rose snapper.

The presence of lipase activity before the onset of exogenous feeding has been widely reported in marine fish larvae (Martínez et al. 1999; Chen et al. 2006; Gisbert et al. 2009; Moguel-Hernández et al. 2009; Shan, et al. 2009). In the case of the spotted rose snapper, lipase-specific activity was present from hatching and increased at 2 DAH and decreased dramatically at 3 DAH and then gradually increased until 10 DAH and a significant decrease was detected at 15 DAH. After that, a fluctuating increase was observed until reaching a maximum activity at 30 DAH. A similar pattern of activity was reported for larval *Seriola lalandi* (Chen et al. 2006), *Paralichthys californicus* (Álvarez-González et al. 2006), *Paralabrax maculatofasciatus* (Álvarez-González et al. 2008) and *Miichthys miiuy* (Shan et al. 2009). The high level of lipase activity during the endogenous

feeding period of *L. guttatus* is presumably due to the action of this enzyme on the triacylglycerides present in the oil globule. However, it is widely accepted that lipid digestion during larval development depends on the activity of two types of bile salt-dependent lipases: the first type is activated during the embryonic development to digest the lipids in the yolk sac and oil globule, and the second type is secreted by pancreas to digest the lipids in the food (Bouchet et al. 2000; Díaz et al. 2002). In our study, we used a short-chain fatty acid substrate to evaluate lipase activity, Álvarez-González et al. (2006) used the same substrate to evaluate lipase activity of larval *Paralabrax maculatofasciatus* and suggested that a more accurate picture of the lipase activity could be achieved by the use of more suitable substrates. Additionally, it has been reported that a significant part of lipase activity comes from other larval tissues and not just from the digestive tract, particularly when whole body homogenates are used, like in our study, which can result on an overestimation of the digestive capacity of the larvae (Oozeki and Bailey 1995; Martínez et al. 1999; Perez-Casanova et al. 2006).

Protein digestion in most marine fish larvae generally begins in the alkaline environment of the intestines with contributions of pancreatic enzymes like trypsin and chymotrypsin (Cuvier-Péres and Kestemont 2002; García-Gasca et al. 2006; Álvarez-González et al. 2008; Shan et al. 2009). In the case of *L. guttatus*, both specific and individual activities of trypsin were evident from hatching and increased continuously during larval development. The same pattern was observed by Galaviz et al. (2012) in this species, although the reported values were slightly different, possibly due to variation in feeding schedule, food type or culture conditions. Contrary to this pattern, several studies have reported fluctuating levels of trypsin activity during development with a decrease during the third or fourth week of development (Martínez et al. 1999; Cuvier-Péres and Kestemont 2002; Chen et al. 2006; Álvarez-González et al. 2008; Shan et al. 2009). These fluctuations in the pattern of activity of trypsin have been related to a response of changes in the amount and composition of the live food and changes in the expression of the enzymes as a response to changes in larval metabolism (Zambonino-Infante and Cahu 2001). The specific activity of chymotrypsin fluctuated during the development of the spotted rose snapper. The first peak in chymotrypsin activity was registered at 5 DAH. Similar results were reported in larval red drum *Sciaenops ocellatus* suggesting that it is associated with the onset exogenous feeding and that the presence of this enzyme as early as hatching contributes to protein digestion in larvae by compensating the absence of a functional stomach and pepsin activity (Applebaum et al. 2001). Leucine aminopeptidase was present from hatching, and gradually increased up to 20 DAH, at which point a decrease was observed through 30 DAH. This pattern was also observed in bay snook, *Petenia splendida* larvae (Uscanga-Martínez et al. 2011) and in *Paralabrax maculatofasciatus* (Álvarez-González et al. 2008). The increase in the activity of leucine aminopeptidase has been associated with the presence of food in the digestive tract and this general pattern of activity with a decrease after the appearance of pepsin activity resembles the activity of other brush border enzymes, which are indicative of a more efficient absorption process in the enterocytes (Zambonino-Infante and Cahu 2001).

The appearance of pepsin activity signals the onset of acid digestion of proteins in the stomach after the appearance of the gastric glands and has been considered as a major change toward an adult-type of digestion since the digestive tract become fully developed (Peña et al. 2003). In our study, pepsin activity was first detected at 25 DAH. In a previous study in *L. guttatus* larvae, Galaviz et al. (2012) reported the presence of pepsin from 20 DAH, coinciding with the differentiation of the gastric glands. This slight difference in the time of detection of pepsin activity between both studies may reflect differences due to culture conditions, feeding schedule, prey type, prey quantity, and larval growth. The presence of pepsin activity in the digestive tract varies with species, for example, has been reported at 12 DAH in *Paralabrax maculatofasciatus* (Álvarez-González et al. 2008), at 8 DAH in *Cichlasoma urophthalmus* (López-Ramírez et al. 2011), at 24 DAH in *Dicentrarchus labrax* (Zambonino-Infante and Cahu 1994), at 22 DAH in *Sciaenops ocellatus* (Lazo et al. 2007), and at 28 DAH in *Pagrus pagrus* (Suzer et al. 2007). Some authors have reported a decrease of trypsin and chymotrypsin activities after the appearance of pepsin in the digestive tract (Walford and Lam 1993; Suzer et al. 2006; Galaviz et al. 2011). However, this is not the case of the spotted rose snapper larvae (Galaviz et al. 2012, this study)

where the activity of these enzymes increased after the appearance of pepsin activity suggesting the importance of alkaline protein digestion in this species.

The decrease in the specific activity of some enzymes observed during the larval development has been related with the increase in the protein content of the larvae and not to the decrease of the digestive capacity of the larvae (Zambonino-Infante and Cahu 2001). On the other hand, the individual enzymatic activity of the spotted rose snapper showed a constant increase for all the enzymes tested, indicating that the larvae increase their digestive capacity during development. It is interesting to note that most of the individual enzymatic activities showed a significant increase around 20 DAH. It is widely accepted that the presence of pepsin in the digestive tract triggers a more effective mechanism of extracellular protein digestion of the larvae and combined with an increase in brush border enzyme activities (i.e., alkaline phosphatase and leucine aminopeptidase) and a decrease in cytosolic enzyme activities indicates the maturation of the digestive function of fish larvae (Zambonino-Infante and Cahu 2001). This pattern has been reported for most marine fish larvae although the time to maturation varies by species. In the case of *L. guttatus* larvae, a previous study by Galaviz et al. (2012) showed that pepsin activity started at 20 DAH, which considering the results of this study suggests that maturation of the digestive (pepsin, amylase, trypsin, chymotrypsin, lipase activities) and absorptive (acid and alkaline phosphatases, leucine aminopeptidase activities) functions of the spotted rose snapper larvae initiates around 20–25 DAH.

One aspect that has been reported in other studies is the effect of the exogenous enzymes from live prey in the digestive capacity of fish larvae and the results may vary depending on the species. For example, in larval haddock and cod, Pérez-Casanova et al. (2006) reported no amylase activity; rather, rotifers were responsible for 100 % of the amylase activity in the larvae of both species. Similar results were reported by Gawlicka et al. (2000) for Atlantic halibut larvae, where enriched *Artemia* nauplii contributed 52.3 % of the enzyme's activity. In the case of protease activity, authors agree that the activity from the prey is not high and that their contribution was limited to an autolytic process in the prey, and it may be neutralized by the alkaline contents of the larval gut (Díaz et al. 1997; Bolasina et al. 2006). In our study, larvae were sampled in the morning before feeding to reduce the presence of prey in the digestive tract. However, enzymatic activity in the different prey should be evaluated in order to establish their possible role in the digestive capacity of the spotted rose snapper larvae.

In conclusion, spotted rose snapper larvae present the same pattern of digestive enzyme activity previously reported for other species in which pancreatic (i.e., trypsin, chymotrypsin, amylase, and lipase) and intestinal (i.e., acid and alkaline phosphatases and leucine aminopeptidase) enzymatic activities are present from hatching allowing the larvae to digest and absorb nutrients in the yolk-sac and live prey by the time of first feeding. The digestive and absorption capacity of the spotted rose snapper increases during the larval development. A significant increase in individual activity of all enzymes occurs at 20 DAH, and around 25 DAH, the juvenile-type of digestion is observed with the appearance of pepsin secreted by the stomach, suggesting that maturation of the digestive function occurs around 20–25 DAH. Our results support previous findings by Galaviz et al. (2012) regarding trypsin and pepsin activities during the larval development of the spotted rose snapper, and considering the general pattern of the other enzymatic activities, we agree with their suggestion that early weaning may be possible from 20 DAH. However, the patterns of activities reported in our study should be considered during the formulation of an artificial diet that can be used for early weaning of the spotted rose snapper.

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