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The ontogeny of greater amberjack digestive and antioxidant defence systems under different rearing conditions: A histological and enzymatic approach

José A. Pérez[1] | Ioannis E. Papadakis[2] | Nikos Papandroulakis[2] | Lorenzo Cruces[1] | Efthimia Cotou[3] | Enric Gisbert[4] | Antonio Lorenzo[1] | Constantinos C. Mylonas[2] | Covadonga Rodríguez[1]

[1]Departamento de Biología Animal, Edafología y Geología. Universidad de La Laguna, La Laguna, Tenerife, Spain

[2]Institute of Marine Biology, Biotechnology and Aquaculture., Hellenic Centre for Marine Research, Iraklion, Greece

[3]Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research, Anavyssos, Athens, Greece

[4]Centre de Sant Carles de la Ràpita (IRTA-SCR), Institut de Recerca i Tecnologia Agroalimentaries, Sant Carles de la Ràpita, Spain

***Correspondence**
J.A. Pérez. Departamento de Biología Animal, Edafología y Geología. Facultad de Ciencias, Sección Biología. Universidad de La Laguna. 38206 La Laguna, Tenerife, Spain.
Email: janperez@ull.edu.es

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Abstract

An overall synchronization of morphological and physiological ontogenetic events of the digestive and antioxidant defence systems occurred in greater amberjack (*Seriola dumerili*) larvae reared under intensive (INT) or semi-intensive (MES) conditions for 30 days. The first differentiations of the digestive channel took place at 3–4 days post-hatch (dph) (3.6–3.7 mm). Differentiation of the endocrine and exocrine pancreas begun at 4–5 dph (4.0–4.1 mm), coinciding with a decrease in carbohydrase activity from egg to the onset of exogenous feeding and the maintenance of bile salt-activated lipase and total alkaline proteases. The first gastric glands and pepsin activity were evident at 17–20 dph (5.5–6.2 mm) in both fish groups. The appearance of pyloric caeca had a 5-day delay in the INT compared to the MES larvae (28 versus 23 dph). Antioxidant enzyme activities decreased progressively from 7 to 30 dph in both rearing conditions. However, GPx, SOD at 7 and 18 dph, and GST activities were higher in the INT larvae. Although both larval groups did not globally differ in somatic growth and maturation of digestive function, it seems that the antioxidant defence system of MES larvae had to face less oxidative assaults, which may be attributed to lower stressful and more stable culture conditions.

KEYWORDS

antioxidant enzymes, digestive enzymes, digestive system, greater amberjack larvae, ontogeny

1 | INTRODUCTION

The greater amberjack *Seriola dumerili* is a marine warm-water large teleost fish with rapid growth and excellent flesh quality, which is receiving increasing research attention in the last years due to its great potential for aquaculture diversification (Fakriadis, Lisi, Sigelaki, Papadaki, & Mylonas, 2019; Jerez et al., 2018; Monge-Ortiz et al., 2018; Mylonas et al., 2016; Rodríguez-Barreto et al., 2012, 2017; Sarih et al., 2019; Zupa, Fauvel, et al., 2017; Zupa, Rodríguez, et al., 2017). Although knowledge about the optimal feeding regime for larval rearing is essential for a successful production of fry, available information on greater amberjack is still incomplete (Hamasaki et al., 2009; Mylonas et al., 2016; Papandroulakis, Mylonas, Maingot, & Divanach, 2005). In fact,

Pérez and Papadakis are equally contributed to the study.

larval rearing is considered one of the major bottlenecks for the flourishing culture of this species, due to the low survival rates obtained during this period, which seriously compromise the availability of juveniles for the on-growing stage. Therefore, there is a great need to evolve culture techniques for mass seed production of the greater amberjack. Although there is scattered information about greater amberjack larviculture and larval development, studies evaluating different rearing technologies through physiological parameters, including the morphophysiological development of the digestive function and antioxidant defence, are missing. Moreover, a holistic understanding of the feeding ecology, digestive function and antioxidant response of greater amberjack larvae is crucial for the design of specific dietary regimes, as well as for the adaptation of rearing protocols to meet larval requirements. This knowledge will allow the best presentation of prey and microdiets as well as synchronizing the larval stage of development with rearing processes in order to increase the productivity of this species during the first life stages.

The digestive system enables fish to capture, ingest, digest and finally absorb nutrients from the food, which are transported across the intestinal epithelium to the circulatory system and then to the whole organism (Rønnestad et al., 2013). During the first stages of development until its transformation into a juvenile, numerous changes take place in the digestive system of fish larvae in terms of its morphology and functionality (Gisbert, Nolasco, & Solovyev, 2018; Papadakis, Kentouri, Divanach, & Mylonas, 2013; Papadakis et al., 2009; Przybył, Ostaszeweska, Mazurkiewicz, & Wegner, 2006). Over this period, activity of the digestive enzymes is affected by a number of different factors, and their levels are closely related to the state of maturation of the secreting digestive tissues, which show important variations between species, water temperature and rearing conditions (Koven et al., 2019; Lazo, Darias, & Gisbert, 2011). Therefore, the knowledge of the digestive competence of a fish is essential in order to understand the digestive physiology of larvae and to adjust the feeding protocols to dietary qualitative and quantitative characteristics (Campoverde, Rodriguez, Perez, Gisbert, & Estevez, 2017; Gisbert et al., 2018) contributing to the optimization of diets (Campoverde et al., 2017; Zambonino-Infante et al., 2008) and to the proper understanding of functions and limitations in the processing capacity of the digestive system. In this sense, the combined analysis of the ontogeny of the main digestive tract structures and related digestive enzymes during larval development is of special relevance for proper characterization of the plasticity of digestive processes to deliver nutrients to the rapidly growing larval tissues (Rønnestad et al., 2013) under changeable feeding and environmental conditions.

The antioxidant defence system enables fish to detoxify and balance the production of oxygen reactive chemical intermediates known as reactive oxygen species (ROS), and thus, provides protection from oxidative assaults during larval development and metamorphosis (Dandapat, Chainy, & Rao, 2003). Generally, ROS are continually detoxified and removed in fish cells by the endogenous antioxidant defence mechanisms consisting of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione S-transferase (GST) as well as of non-enzyme antioxidants such as reduced glutathione (GSH) (Wilhelm Filho, 1996). The knowledge of the antioxidant enzyme responses during early life stages is essential for understanding the origin and formation of the protective mechanisms during the life cycle of fish (Diaz et al., 2010; Rudneva, 1999). Antioxidant defence enzymes can be influenced both by inherent factors, such as age, feeding behaviour, food consumption and diet type, and also by external factors, such as toxins present in the water, seasonal and daily changes in dissolved oxygen and water temperature (Bayir et al., 2011; Hegazi, Attia, & Ashour, 2010; Li et al., 2014; Martínez-Álvarez, Morales, & Sanz, 2005; Simčič, Jesenšek, & Brancelj, 2015; Vinagre, Madeira, Narciso, Cabral, & Diniz, 2012). Research performed particularly on antioxidant defence during early life stages in several marine fish showed a fish-species, larvae-age and diet-age dependencies of antioxidant enzymes responses (Fernandez-Diaz, Kopecka, Cañavate, Sarasquete, & Solé, 2006; Kalaimani et al., 2007; Liravi, Salati, Asadi, & Pasha-Zanousi, 2014; Peters & Livingston, 1996; Rudneva, 1999). Thus, the knowledge of these responses during larval development of greater amberjack cultivated under intensive and semi-intensive conditions is of particular interest for optimizing feeding protocols and rearing methodologies for this species.

The present study aims to describe the morphological and physiological ontogeny of the digestive system and the response of the antioxidant defence system of greater amberjack larvae cultured under two different rearing conditions, intensive and semi-intensive. The intensive rearing conditions and the feeding protocol used are an adaptation from the established methods in Mediterranean hatcheries, whereas the semi-intensive methods that allow the use of wild plankton seem to be more appropriate for the culture of this species (Papandroulakis et al., 2005). Although there exist several studies describing larval development in other carangid species (Carton, 2005; Martínez-Montaño, González-Álvarez, Lazo, Audelo-Naranjo, & Vélez-Medel, 2016; Plaza, Leyton, Sayes, Mejias, & Riquelme, 2017; Stuart & Drawbridge, 2011 among others), there is missing information about greater amberjack. In addition, species-specific conclusions cannot be drawn from the above-mentioned studies in order to be applied on greater amberjack larviculture, since each species presents its own developmental patterns and requirements that need to be individually defined. Our results may facilitate the implementation of an adequate feeding strategy adapted to the digestive and antioxidant defence capacities and nutritional needs of greater amberjack during early development, while also addressing options to reduce cannibalism and size dispersion which are of primary importance to boost larval survival and growth. This information will be of great value for synchronizing the larval stage of development with rearing practices, thus improving actual larval rearing protocols for greater amberjack.

2 | MATERIALS AND METHODS

2.1 | Larval rearing

Larval rearing trials were performed in the facilities of the Institute of Marine Biology, Biotechnology and Aquaculture at the Hellenic Centre for Marine Research (HCMR, Crete, Greece). Greater amberjack larvae were reared under two different conditions, the semi-intensive (MES) and the intensive (INT) systems. Eggs used for this study were obtained from induced spawning of wild breeders kept in Argosaronikos SA cage farm (ARGO). After collection, eggs were shipped by air to the hatchery facilities of the HCMR in polystyrene boxes (~12 hr trip). Hatching rate in both systems was estimated to be higher than 95%, an indicator of the initial egg quality.

2.2 | Semi-intensive system (MES)

A total of one hundred and ten thousand eggs were stocked in an incubator and, after hatching, were transferred to a 40-m^3 indoor tank (2.75 eggs l^{-1}) filled with filtered (5 μm) natural seawater (salinity 37 psu) treated with UV, which was also the water used for subsequent renewal. During the larval rearing, seawater temperature was maintained at 24.0 ± 0.7°C and the pH fluctuated from 8.0 to 8.2. Dissolved oxygen varied from 5.8 to 6.8 mg/L, whereas the rate of water renewal was increased progressively from the initial 15% to 35% of total water volume per day at 17 days post-hatching (dph), to 100% at 22 dph and then to 200% at 30 dph. Aeration was provided at 5 points along the perimeter and in the centre of the tank. A surface skimmer was operational during the appropriate period (5 to 13 dph) to keep the surface free from lipids, a requisite for good swim bladder inflation. The photoperiod was adjusted to constant light from mouth opening to 20 dph and then reduced to 18L:06D for the remaining experimental period. Light intensity varied according to the weather conditions between 500 lux on cloudy days and 1,000 lux on sunny days. During the night when prolonged photophase was applied, light intensity was about 250 lux. The rearing technology employed here is a semi-intensive technique for production of greater amberjack larvae (Papandroulakis et al., 2005).

2.3 | Intensive rearing in closed water recirculation system (INT)

Thirty-six thousand eggs were placed in 0.5-m^3 cylindro-conical tanks (72 eggs l^{-1}) connected to a closed water recirculating system coupled to a biological filter. Tanks were filled with borehole 35 psu water kept at 24.0 ± 0.5°C, pH ranged from 8.0 to 8.2, and the dissolved oxygen was maintained between 6.8 and 7.2 mg/L. Water circulation was achieved through a biological filter during embryogenesis, egg hatching and the autotrophic larval stage with aeration provided at 150–250 ml/min. After first feeding, water circulation was obtained for each tank by means of an airlift pump in order to maintain stable the rearing environment. The water in the biological filter was used for renewal in the larval rearing tanks at a rate of 3% daily until 15 dph and then increased gradually to 50% until 25 dph. A skimmer was installed at the appropriate period (5–15 dph) to keep the surface free from lipids. The photophase was 24L:00D (constant light) from mouth opening until 20 dph and 18L:06D for the remaining experimental period. Light intensity varied between 200 and 800 lux during the day and was ~ 200 lux at night.

2.4 | Feeding protocols

The duration and type of diet for each rearing protocol during the trial are presented in Figure 1. Microalgae (*Chlorella sp*) and rotifers (*Brachionus sp*) enriched with DHA Protein Selco (INVE S.A., Belgium) were daily added in the rearing tanks from 3–4 dph to 23 dph. Rotifers' concentration was kept at 2–3 individuals ml^{-1} in the MES and at 4–5 individuals ml^{-1} in the INT. Instar I *Artemia* AF nauplii (12 to 14 dph) and instar II *Artemia* EG nauplii (14 to 30 dph) enriched with A1 DHA Selco (INVE S.A.) were offered to the larvae at a starting concentration of 0.05 to 0.35 nauplii ml^{-1}. Enrichment in all cases was performed according to manufacturer's instructions. The distribution frequency of zooplankton during the trial was two and two to three times daily, for MES and INT systems, respectively. In both rearing methodologies, microdiets were added progressively according to fish size from 16 dph (MES) to 21 dph (INT) (NRD 2/4, grain size 200–300 μm; NRD 3/5 grain size 300–500 μm, INVE S.A., Dendermonde, Belgium). The distribution of the microdiets was manually at the beginning (a few times daily) and with an automatic feeder afterwards in intervals of two hours for 12 hr daily. In the semi-intensive system, the feeding was supplemented with potential wild prey developed in the tank (mainly harpacticoida copepods), whose densities were not estimated due to their occasional presence on tanks walls. After 20 dph, gilthead sea bream (*Sparus aurata*) eggs (live or frozen) and newly hatched larvae were also delivered to the larvae. The eggs and/or prelarvae were kept in separated incubators and transferred to the larvae tanks once daily, in quantities never exceeding 0.5 ind l^{-1}. The egg/larvae addition was based on observations during rearing of other pelagic species.

2.5 | Sampling procedures

As no tank replication was available for the MES treatment, larvae within the 40-m^3 tank were collected from three different and distant tank areas in order to allow for larval spatial and temporal heterogeneity to attain meaningful significance levels (Gamble, 1990). Once captured, fish larvae were sacrificed with an overdose of anaesthetic phenoxyethanol (Sigma-Aldrich). For the histological study, a total of 10 individuals from both rearing systems (INT and MES) were collected on the following days: 1 day before hatching, 0 (hatching), 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 15, 17, 20, 23, 25, 28 and 30 dph. The total length (TL) of the larvae was determined under

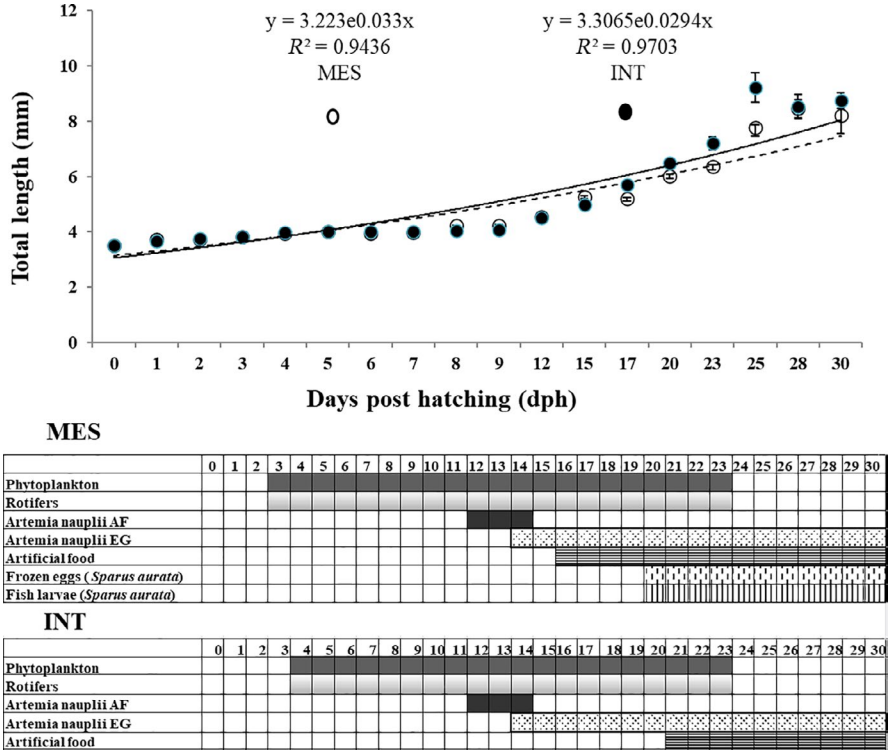

FIGURE 1 Growth performance of greater amberjack larvae (mean ± *SD* of total length) cultured in intensive (INT) and semi-intensive (MES) rearing systems. Below the graph, the rearing protocols used during the rearing procedure are presented, including type and duration of food items provided

graduated stereoscope. Fish samples were preserved in a buffered fixative containing 4% formaldehyde and 1% glutaraldehyde for at least 24 hr (McDowell & Trump, 1976). For the study of main digestive pancreatic and gastric enzymes activities, eggs and larvae were collected by triplicate from the INT tanks before midday and pooled according to their age and size at different ontogenic periods: 0–5, 6–10, 11–15 and 21–30 dph (1500–2000, 380–450, 50–80 and 5–9 larvae, respectively), rinsed in distilled water to remove external salt and immediately frozen at − 80°C until analysis. Additionally, comparisons of enzymatic activities between MES and INT larvae at 12 dph and at the end of the experimental period (30 dph) were also performed. Finally, for the study of the antioxidant enzyme responses, 125 ± 25 mg wet weight of larvae per stage were collected in triplicate at 7 dph (350 larvae), 18 dph (flexion) (60 larvae), 23 dph (8 larvae) and 30 dph (4 larvae). The samples at 23 dph included large (B) and small (S) size larvae (TL 8.52 ± 0.65 mm and 5.95 ± 0.49 mm, respectively).

All animal experiments were approved by the Ethics Committee of the Institute of Marine Research and the relevant veterinary authorities (Ref Number 255,332) and were conducted in certified laboratories (EL91-BIOexp-04) in accordance with legal regulations (EU Directive 2010/63).

2.6 | Histological analyses

Before embedding in methacrylate resin (Technovit 7,100®, Heraeus Kulzer, Germany), larvae were dehydrated in gradually increasing ethanol solutions (70%–96%). Serial sections of 3 μm were obtained with a microtome (Leica, RM 2,245, Germany). Sections were stained with Methylene Blue (Sigma-Aldrich)/Azure II (Sigma-Aldrich)/Basic Fuchsin (Polysciences Inc) according to Bennett, Wyrick, Lee, and McNeil (1976). All the sections were examined using a compound microscope (Nikon Eclipse 50i).

2.7 | Determination of digestive enzymes activities

Determinations of pancreatic (α-amylase, bile salt-activated lipase, total alkaline proteases) and gastric (pepsin) enzyme activities were based on methods previously performed and described by Gisbert, Gimenéz, Fernández, Kotzamanis, and Estevez (2009) and processed as recommended by Solovyev and Gisbert (2016) in order to prevent sample deterioration. Briefly, samples were completely homogenized (Ultra-Turrax T8, IKA©-Werke) in 5 volumes (v/w) of ice-cold Milli-Q water and centrifuged at 3,300 × *g* for 3 min at 4°C, and 1 ml aliquots of supernatant was kept at −80°C until their analysis for enzyme quantification.

Larval digestive capacities during early life stages before stomach development and acidic digestion were evaluated by measuring the activity of total alkaline proteases according to the method of García-Careño and Haard (1993). Alkaline proteases activity was determined at room temperature using azocasein (0.5%) as substrate in Tris-HCl 50 nmol/l (pH 9) for 10 min. Reaction was stopped with 20% TCA (trichloroacetic acid), samples were centrifuged at 10,000 × *g* for 5 min, and absorbance of the supernatant was measured at λ = 366 nm. One unit (U) of alkaline proteases activity was defined as 1 μmol of azo dye released per min and per ml of homogenate.

Alpha-amylase (E.C. 3.2.1.1) was quantified according to Métais and Bieth (1968) using 0.3% soluble starch dissolved in Na_2HPO_4

buffer (pH 7.4) as substrate. The reaction was stopped with 1 N HCl and, after the addition of 2 ml of N/3000 iodine solution (Merck, Darmstadt), the absorbance was read at λ = 580 nm. Alpha-amylase activity (U) was defined as the amount of starch (mg) hydrolysed during 30 min per ml of homogenate at 37°C.

Bile salt-activated lipase (BAL, E.C. 3.1.1) activity was assayed for 30 min at 30°C using *p*-nitrophenyl myristate as substrate dissolved in 0.25 mM Tris-HCl, pH 9.0, 0.25 mM 2-methoxyethanol and 5 mM sodium cholate buffer. The reaction was stopped with a mixture of acetone:n-heptane (5:2), the extract centrifuged at 6,000 × *g*, and the increase in absorbance of the supernatant determined at λ = 405 nm. BAL activity (U) corresponded to the μmol of substrate hydrolysed per min per ml of enzyme extract (Iijima, Tanaka, & Ota, 1998).

Finally, pepsin (E.C. 3.4.23.1) was quantified by mixing the enzymatic extract with the substrate (2% haemoglobin solution in 1 N HCl at pH 2.0) and incubated for 10 min at 37°C. The reaction was stopped with 5% TCA and the extract centrifuged at 4,000 × *g* for 6 min at 4°C. The absorbance of the supernatant was read at λ = 280 nm. One unit of activity (U) was defined as 1 μmol of tyrosine liberated per min and ml of homogenate (Worthington Biochemical Corporation, 1972).

Enzymatic activities are expressed as the total activity defined as units per larva (U/larva) and specific activity as units per mg protein (U/mg protein). Soluble protein of crude enzyme extracts was quantified as described by Bradford (1976), using bovine serum albumin as standard. All the assays were made in triplicate from each pool of larvae and absorbance read using a spectrophotometer (Beckman Coulter DU800).

2.8 | Determination of GSH content and antioxidant enzymes activities

To assess the antioxidant defence ability of the developing greater amberjack larvae, the concentration of total GSH and the enzymes' activities of total SOD, GPx, GR and GST was determined. Supernatants were prepared and analysed for enzyme activities in a manner similar to that described in Hamre, Penglase, Rasinger, Skjærven, and Olsvik (2014) with minor modifications. In brief, 25 mg (for GSH) and 100 mg (for SOD, GPx, GR and GST) of frozen samples from each biological sample were placed in Eppendorf tubes (1.5 ml). A 12 × volume of ice-cold homogenization buffer was added in each sample, homogenized with a pellet pestle (cordless motor, Sigma-Aldrich) and centrifuged at 3,000 × *g* for 10 min or at 10,000 × *g* for 20 min, for GSH or for all the other enzymes, respectively. Homogenization buffer for GSH was a 5% (w/v) metaphosphoric acid and 0.6% sulfosalicylic acid (w/v) mixture, while for all the other enzymes, a 0.1 M phosphate buffer pH 7.4 containing 0.15 M KCl, 1 mM dithiothreitol (DTT), 1 mM EDTA and 0.1 M phenylmethylsulfonylfluoride (PMSF) was used. Supernatants were collected and stored at −80°C until analysis. The GSH was analysed according to Rahman, Kode, and Biswas (2006) and the GPx, GR and GST according to McFarland et al. (1999). The SOD activity was analysed with a commercial kit (706,002, Cayman Chemical Co., MI) according to the manufacturer's instructions. Total protein concentrations were measured with a Coomassie Brilliant Blue reagent (Sigma-Aldrich) according to Bradford (1976).

2.9 | Statistical analysis

Data are presented as mean ± *SEM* unless otherwise stated. For the description of growth performance as total length (TL) as a function of time, an exponential equation was used. Prior to the statistical analysis of the activities of digestive and antioxidant enzymes, the data were checked for normality with the one-sample Kolmogorov–Smirnov test and for homogeneity of variances with the Levene's test. A one-way analysis of variance (ANOVA) followed by a Tukey *post hoc* test or a Games–Howell test was performed to determine significant differences among developmental stages. A Student *t* test was applied for comparisons between both rearing technologies (MES versus INT). The level of significance was established at $p < .05$. All statistical comparisons were conducted using SPSS for Windows 21.0 (IBM-SPSS Inc.).

3 | RESULTS

3.1 | Growth performance

No differences in growth of greater amberjack larvae were observed between both culture systems during the experimental period ($p > .05$). Growth performance adjusted exponentially to the equation: $y = 3.223\, e^{0.033x}$, $R^2 = 0.9436$, for the MES and $y = 3.3065\, e^{0.0294x}$, $R^2 = 0.9703$, for the INT rearing systems (Figure 1), where variable *y* indicates larval TL and variable *x* the time in dph.

3.2 | Ontogeny of the digestive system

Overall, there was a synchronization of the ontogenetic events occurring in greater amberjack larvae from both rearing protocols during the first life stages (Figure 2). Moreover, the activity of the digestive enzymes activities of greater amberjack larvae along the experimental period at each sampling period in the INT system is plot in Figure 3.

3.3 | Period 1:0–5 dph (MES, 3.5–4.0 mm TL; INT, 3.5–4.0 mm TL)

From hatching (3.5 ± 0.04 mm TL) until 2 dph (3.7 ± 0.05 mm TL), the digestive tract appeared as a closed straight tube located dorsally to the yolk sac (Figure 4a) and it consisted of a single-layer epithelium of cuboidal and columnar cells. The liver developed rapidly. The early hepatic cells appeared at 2–3 dph (3.8 ± 0.1 mm TL) and were initially located behind the yolk sac under the anterior intestine and

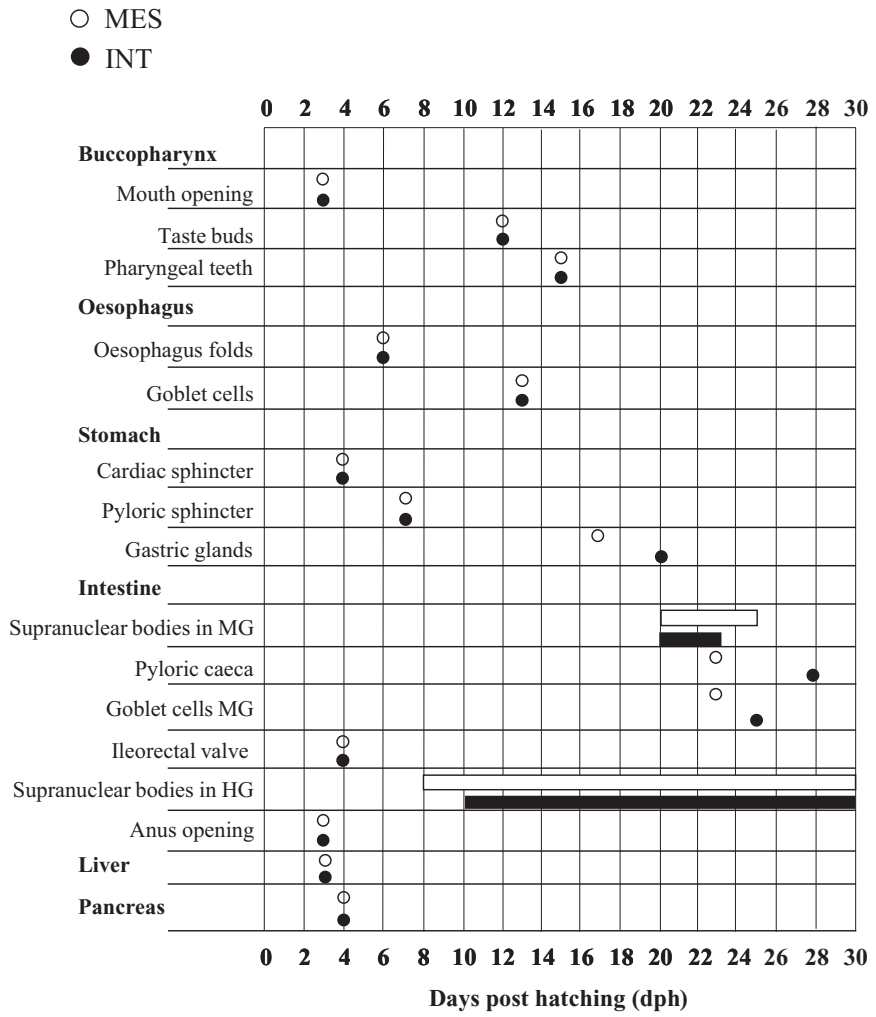

FIGURE 2 Schematic representation of the appearance (open solid circles indicate the MES, black solid circles indicate the INT system) of the main developmental structures examined in greater amberjack larval digestive system, as a function of days after hatching (dph, horizontal axis). Horizontal bars (white MES and black INT) indicate the period that supranuclear bodies (vacuoles) were present in the anterior-median intestine (midgut, MG) and hindgut (HG). Below, mean values of the total length of greater amberjack larvae for each sampling day and rearing system are presented

	0	1	2	3	4	5	6	7	8	9	12	15	17	20	23	25	28	30
MES	3.52	3.67	3.75	3.83	3.99	4.05	4.03	4.02	4.05	4.09	4.53	5.00	5.69	6.49	7.2	9.23	8.54	8.74
INT	3.52	3.72	3.73	3.81	3.96	4.01	3.94	3.98	4.23	4.23	4.55	5.26	5.19	6.01	6.35	7.77	8.47	8.20

Total length (mm)

later surrounding the anterior part of the intestine (Figure 4b). The pancreas appeared as an undifferentiated tissue at 2 dph, but differentiation in endocrine and exocrine regions begun between 4 and 5 dph (4.0 ± 0.1 mm TL) (Figure 4c).

Regardless of the rearing protocol, the first differentiation events of the digestive system took place at around 3–4 dph. During this period, the mouth and the anus opened and the separation of the digestive tract into distinct regions occurred (Figure 4d). The ileo-rectal valve that separates the midgut from the hindgut also appeared at this stage (Figure 4e).

Regarding digestive enzymes, α-amylase, BAL and total alkaline protease activities were detected at this stage, even prior to hatching and to the onset of exogenous feeding (3 dph; 3.8 ± 0.1 mm TL). The specific activity of α-amylase (U/mg protein) significantly decreased from the egg to 5 dph larvae (Figure 3; ANOVA, $p < .05$), while that of BAL and total alkaline proteases remained stable (ANOVA, $p > .05$). Pepsin activity was not detected during this developmental stage.

3.4 | Period 2:6–10 dph (MES, 4.0–4.2 mm TL; INT, 3.9–4.3 mm TL)

Within this period, both feeding regimes were based mainly on rotifers. At 6 dph (3.9–4.0 mm TL), folding of the oesophageal mucosa occurred (Figure 4f), whereas the pyloric and cardiac sphincters at the intestine indicated the area where the stomach will start developing (Figure 4g). Supranuclear vacuoles were present in the larval hindgut at 8 dph in the MES (4.1 ± 0.1 mm TL) and at 10 dph in the INT (4.3 ± 0.1 mm TL) (Figure 4h), being visible up to 30 dph in both rearing conditions.

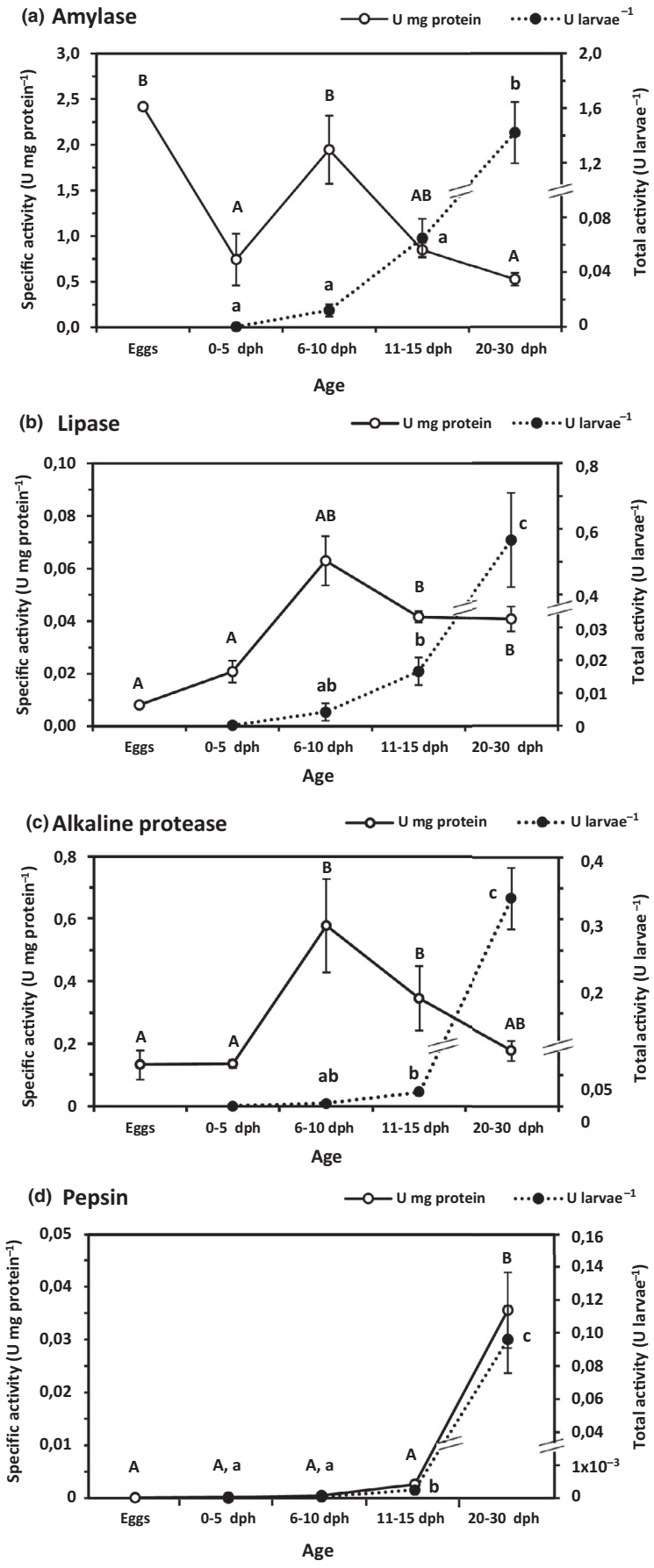

FIGURE 3 Specific activity (U mg protein^{-1}, white circles) and total activity (U larvae^{-1}, black circles) of digestive enzymes during the ontogeny of the digestive tract of greater amberjack larvae cultured under intensive conditions. Different letters indicate significant differences ($p < .05$) between larval ages

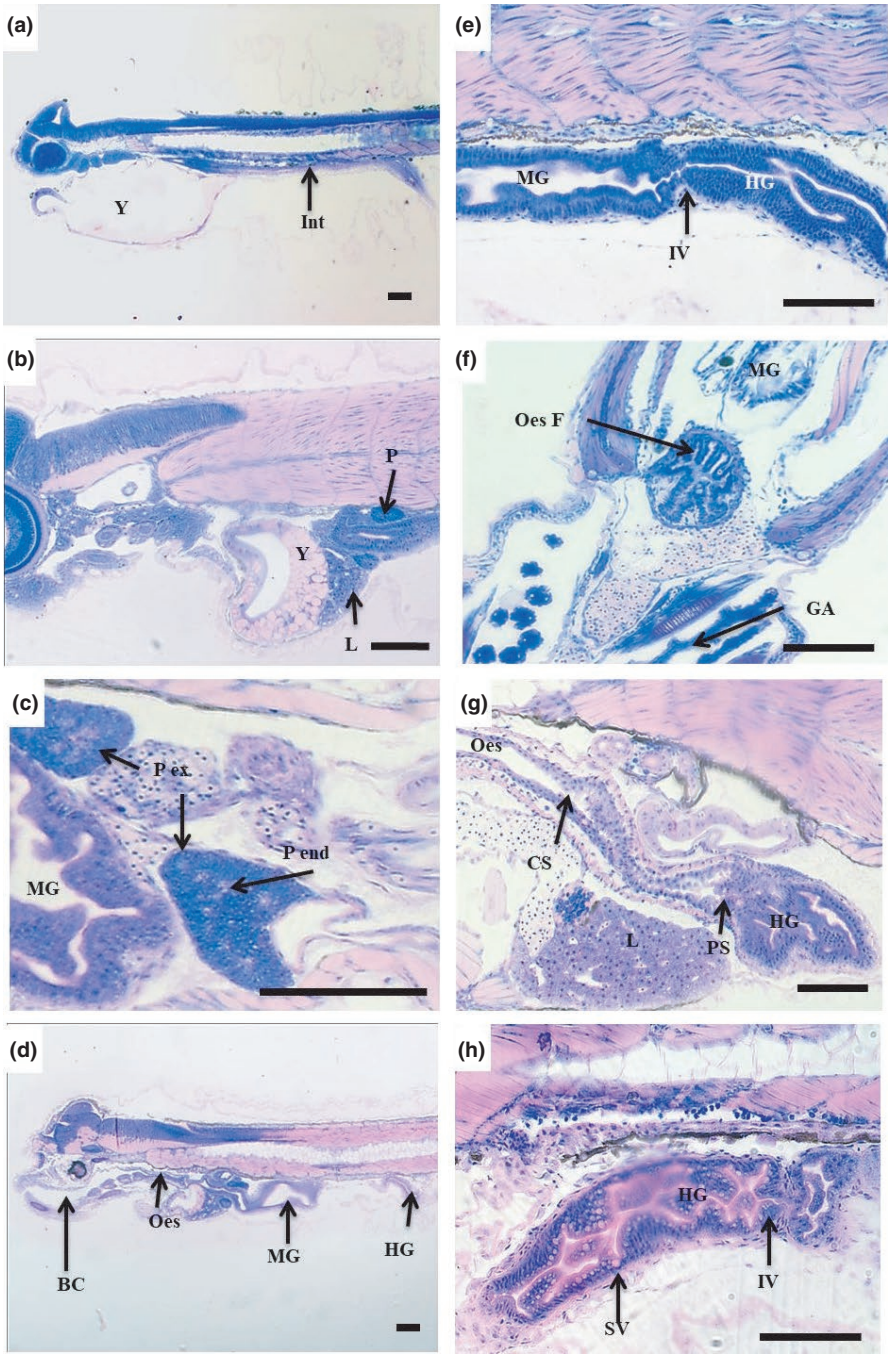

FIGURE 4 Microphotographs of histological sections from greater amberjack larvae at different developmental stages. (a) At 1 dph showing digestive tract as a closed straight tube located dorsal to the yolk sac. (b) At 2 dph when the liver and pancreas appeared. (c) At 4 dph when the pancreas differentiated to endocrine and exocrine parts. (d) At 4 dph showing the different characteristic areas of the digestive system. (e) At 4 dph when the ileo-rectal valve appeared. (f) At 6 dph showing the formation of folds at the oesophagus. (g) At 6 dph showing the formation of the stomach area. (h) At 8 dph showing the supranuclear vacuoles at the hindgut. BC = buccopharynx; CS = cardiac sphincter; GA = gill arches; HG = hindgut; Int = intestine; IV = ileo-rectal valve; L = liver; MG = midgut; Oes = oesophagus; Oes *F* = oesophageal folds; P = pancreas; P end = endocrine pancreas; P ex = exocrine pancreas; PS = pyloric sphincter; SV = supranuclear vacuoles; Y = yolk. Scale bars represent 100 μm

In addition, pepsin remained undetected, whereas BAL activity was maintained unvariable and that of amylase and alkaline proteases increased with respect to the previous period when data are expressed as U/mg protein (ANOVA, $p < .05$) but remained constant when reported as U/larva (Figure 3; ANOVA, $p > .05$). No differences in the enzymatic activities between both larval groups existed at 7 dph (data not shown).

3.5 | Period 3:11–15 dph (MES, 4.5–5.0 mm TL; INT, 4.5–5.3 mm TL)

The first taste buds were formed along the buccopharyngeal epithelium at 12 dph (4.5 mm TL) (Figure 5a), whereas goblet cells appeared at the oesophagus at 15 dph (INT = 5.3 ± 0.2 mm; MES = 5.0 ± 0.2 mm), increasing their number over time (Figure 5b). At this age, the first pharyngeal teeth also appeared at the posterior part of the buccopharynx area (Figure 5c).

During this period, the activity of pancreatic enzymes (α-amylase, BAL and total alkaline proteases) remained stable compared to the previous stage (ANOVA, $p > .05$), whereas pepsin was firstly detected in both rearing systems (Figure 3). When comparing the activity of larval digestive enzymes between treatments at 12 dph, α-amylase was higher in intensive- than in semi-intensively reared larvae (Figure 6; *t* test, $p < .05$), BAL and total alkaline proteases presented the opposite trend ($p < .05$), and pepsin did not show any significant variation (Figure 6; *t* test, $p < .05$).

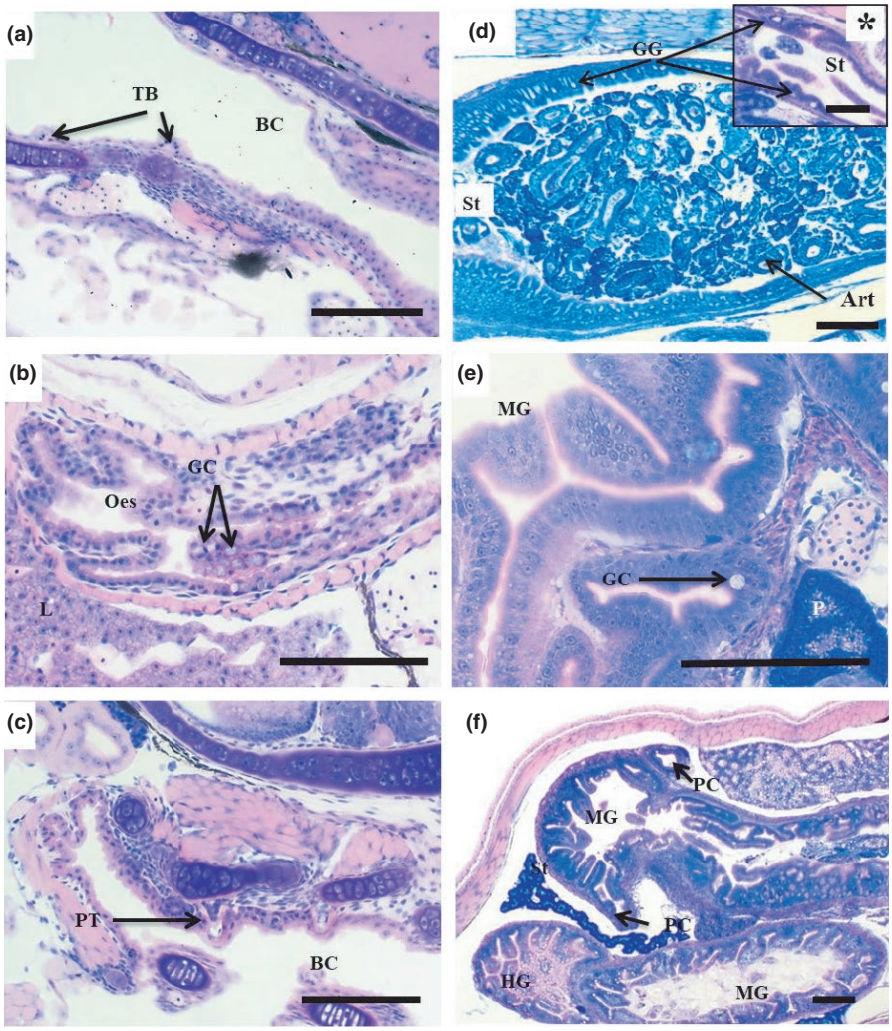

FIGURE 5 Microphotographs of histological sections of greater amberjack larvae at different developmental stages. (a) At 12 dph when the taste buds appeared. (b) At 15 dph when the goblet cells appeared at the oesophagus. (c) At 15 dph showing the pharyngeal teeth at the buccopharynx. (d) At 17–20 dph, the picture with an asterisk (smaller in size) presents the first appearance of gastric glands on the stomach area whereas the large picture presents the proliferation of gastric glands at 30 dph. (e) At 23–25 dph showing the goblet cells at the midgut and (f) at 23–28 dph showing the formation of the pyloric caeca. Art = *Artemia* nauplii; BC = buccopharynx; GC = goblet cells; GG = gastric glands; HG = hindgut; IV = ileo-rectal valve; L = liver; MG = midgut, Oes = oesophagus; PT = pharyngeal teeth; PC = pyloric caeca; St = stomach; TB = taste buds. Scale bars represent 100 μm

3.6 | Period 4:20–30 dph (MES, 6.5–8.7 mm TL; INT, 6.0–8.2 mm TL)

The first gastric glands at the pyloric portion of the stomach were evident at 20 dph (6.0 ± 0.2 mm TL) in the INT group and at 17 dph (5.7 ± 0.4 mm TL) in the MES group (Figure 5d).

Supranuclear bodies in the midgut appeared between 20–23 and 20–25 dph in INT and MES larvae, respectively (Figure 2). The first goblet cells were evident in the midgut at 23 dph (7.2 ± 0.7 mm TL) in the MES group and at 25 dph (7.8 ± 1.0 mm TL) in the INT one (Figure 5e). Moreover, the appearance of pyloric caeca had a 5-day delay in larvae reared under intensive conditions compared to those of the semi-intensive group (28 versus 23 dph) where mean fish length was 8.5 ± 1.2 and 7.2 ± 0.7 mm TL, respectively (Figure 5f).

As it is shown in Figure 3, pancreatic enzyme activities were similar to those of the previous stage (ANOVA, $p > .05$) when given as U/mg protein, but dramatically increased when reported per individual larvae. Regardless of the units considered, pepsin significantly increased in comparison with previous stages (ANOVA, $p < .05$). At 30 dph, the activity of pepsin was *ca.* 2.5 times higher in intensively reared larvae than in MES larvae ($p < .05$) whereas alkaline proteases were higher in the INT group only when expressed as U/mg protein (Figure 6).

3.7 | Antioxidant defence system

Regarding the antioxidant response and the levels of the antioxidant enzymes, GPx, SOD and GST activities tended to significantly decrease in every developmental stage compared to 7 dph (Figure 7; ANOVA, $p < .05$). In addition, significantly lower activities of GPx, SOD and GST were also evident in MES larvae compared to INT larvae in most developmental stages (Figure 7; *t* test, $p < .05$). On the other hand, GSH content was significantly higher at 18 dph than at 7 dph for both rearing strategies (Figure 7; *t* test, $p < .05$), following a trend to a progressive reduction of the enzyme content with larval age. A gradual decrease in GR activities was also recorded with age which was, however, not significant between culture conditions, with the exception of those measured at 7 dph which were lower for the MES group (Figure 7; *t* test, $p < .05$).

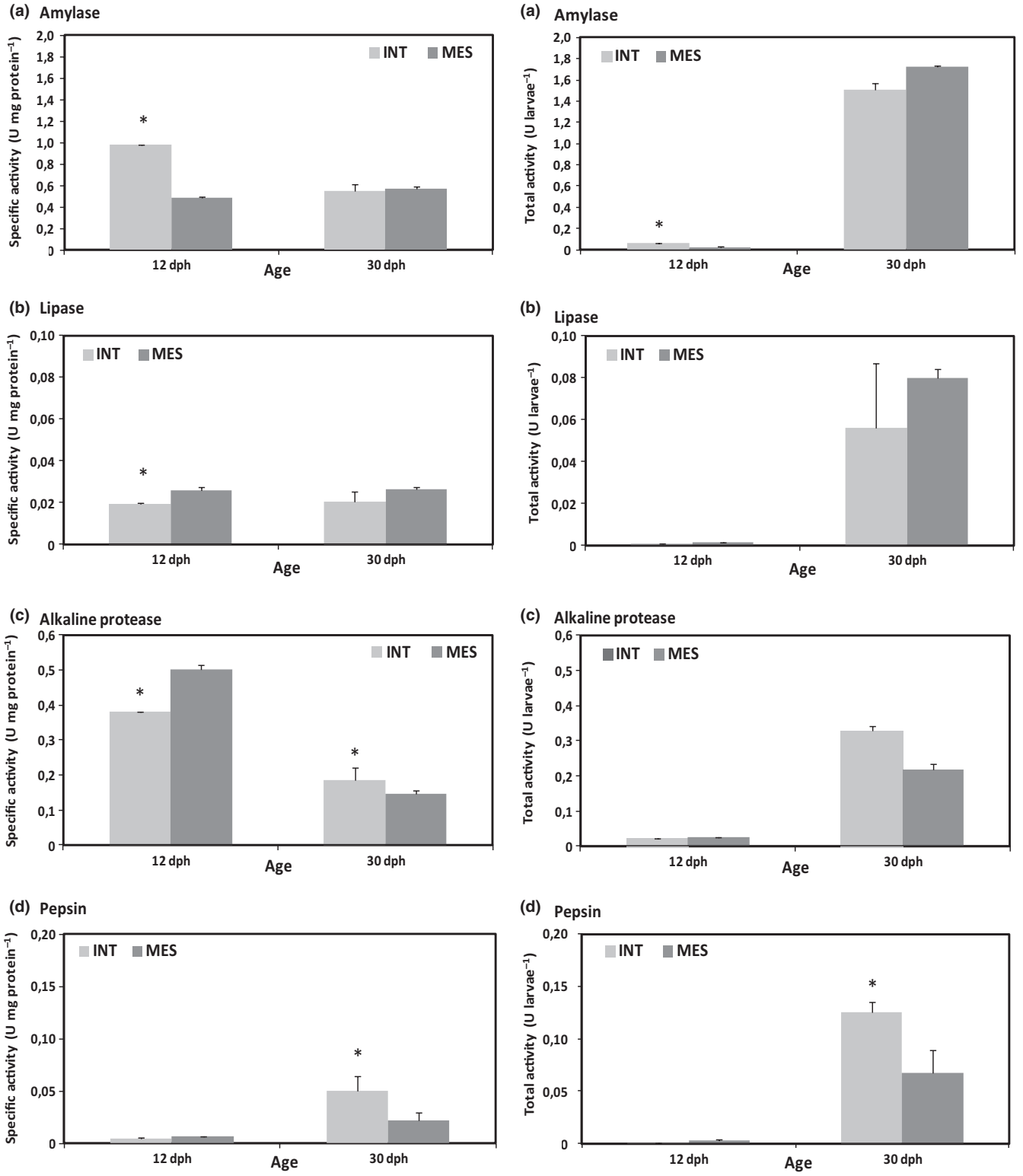

FIGURE 6 Comparison of specific activity (U mg protein^{-1}) and total activity (U larvae^{-1}) of digestive enzymes of greater amberjack larvae between intensive and semi-intensive rearing systems at 12 and 30 dph. Asterisks (*) indicate significant differences ($p < .05$) between INT and MES rearing systems for a particular enzyme and larval age

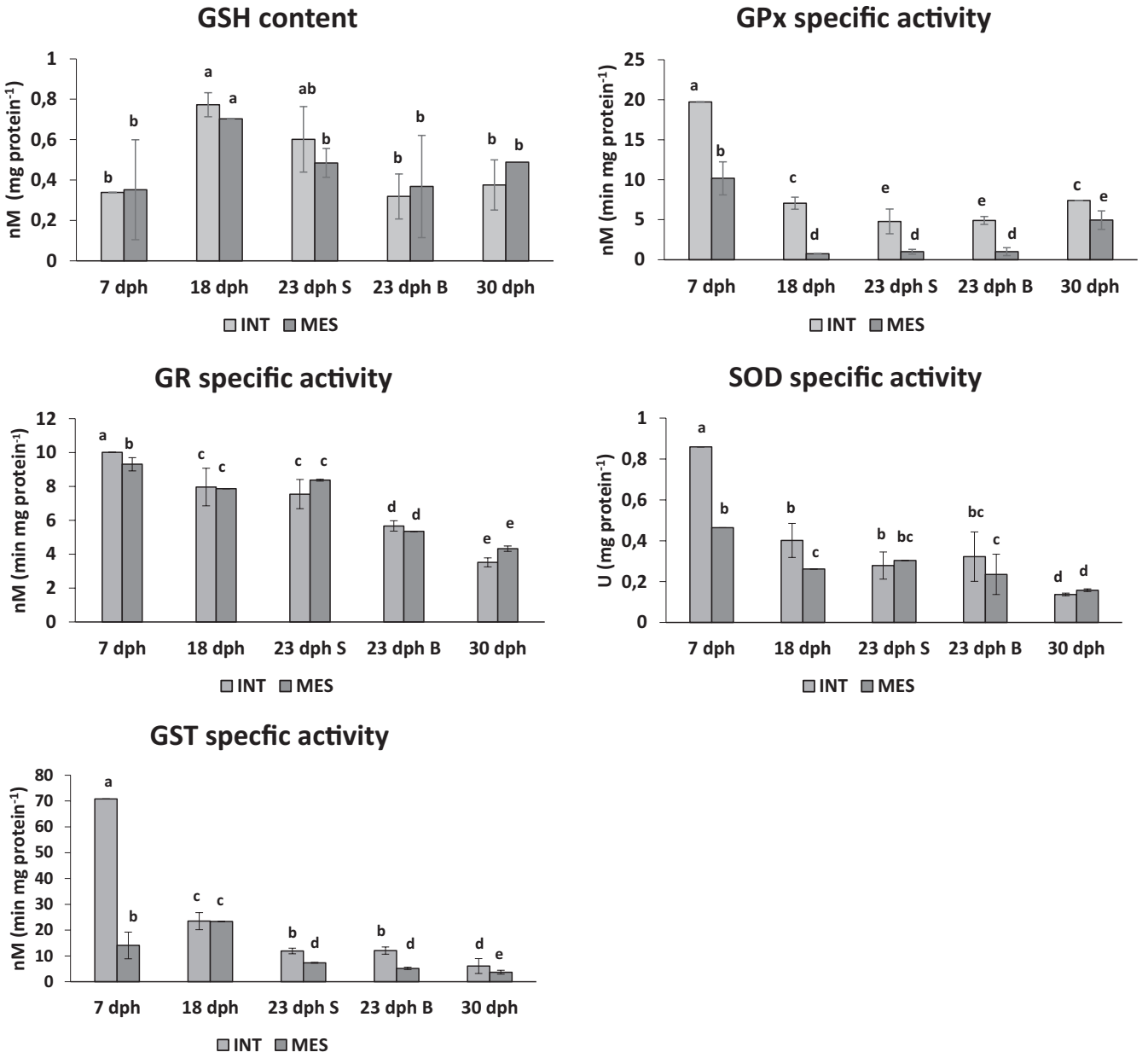

FIGURE 7 aChanges in antioxidant defence system (GSH content and GPx-, GR-, SOD- and GST-specific activities) of developmental larval stages of *Seriola dumerili* cultured in intensive and semi-intensive rearing systems (S and B refer to small and large larvae, respectively). Different letters indicate significant differences ($p < .05$) between larval ages

4 | DISCUSSION

4.1 |

The ontogeny of the digestive system of greater amberjack can be considered as a rapid process, similarly to other carangid species such as *S. lalandi* (Chen, Qin, Kumar, Hatchinson, & Clarke, 2006) and *S. rivoliana* (Teles et al., 2017). The development of the digestive capacity is controlled by endogenous factors, and generally, it is genetically programmed, which results in similar developmental patterns between teleost fish larvae (Rønnestad et al., 2013). However, the time of appearance of the digestive structures and their functionality can be influenced by a number of factors of which temperature and feeding regime are among the most critical issues (Kamler, 2002). As developmental rates are correlated with the larval TL, rearing protocols have also to be synchronized with larval size. Generally, in marine fish species, the feeding protocol has to include firstly rotifers, whereas *Artemia* nauplii have to be offered at larger sizes. However, these general principles have to be tailored for each fish species in order to adapt rearing conditions to larval ontogeny. In this sense, the histological description of the main digestive organs, the ontogenetic profile of digestive enzymes and their dietary adaptation may be used as reliable indicators of larval development, food acceptance, digestive capacity and their further larval performance (Rønnestad et al., 2013; Ueberschär, 1993). It is of special relevance

to merge data in the same study from two types of approaches, that is morphological (histological analysis) and functional (assessment of the activity of digestive enzymes) development information on the digestive system; as in some cases, the presence of a morphologically distinct organ (*i.e*. stomach) does not match with its functionality (Solovyev et al., 2016).

The pattern of development of digestive enzyme activities in *S. dumerili* is similar to that reported to other temperate and warm-water marine species (Rønnestad et al., 2013), and especially to that of *S. lalandi* (Chen et al., 2006). In brief, the activity of the main carbohydrases, proteolytic and lipolytic enzymes was detected before and just after hatching. Regardless of detecting BAL activity during the embryonic and at hatching stages in greater amberjack, it does not mean that embryos and/or newly hatched larvae digest lipids contained in their yolk sac reserves by means of this lipolytic enzyme produced by the exocrine pancreas. In fact, these results indicate that the spectrophotometric method for assessing this enzyme, whose activity is enhanced by means of bile salts (sodium cholate), is not specific (Nolasco-Soria et al., 2018), and it may also detect lipases hydrolysing triglycerides and wax esters contained in the yolk (Heming & Buddington, 1988). Yolk protein serves two primary functions: it provides amino acids for tissue growth and supplies energy via catabolic processes (Heming & Buddington, 1988). However, the detection of alkaline protease activities before hatching may be mainly attributed to the presence of chorionase, an alkaline proteolytic enzyme involved in hatching (Hagenmaier, 1974; Yamagami, 1988) rather than to yolk protein digestion. These results would be in agreement to those reported by Segner, Rosch, Schmidht, and Von Poeppinghausen (1989), who stated that trypsin, the main alkaline pancreatic protease, activity was not found in the yolk syncytium of fish larvae. In this sense, yolk amino acids and proteins may be obtained by non-selective bulk endocytosis (Heming & Buddington, 1988) and receptor-mediated pinocytosis (Rønnestad & Fihn, 1993), respectively. Amylolytic activity has been reported by several authors in the yolk of fish (Gawlicka et al., 2000; Naz, 2009, present study) and poultry embryos (Ikeno & Ikeno, 1991), although this activity may not be correlated to pancreatic α-amylase, since the exocrine pancreas was not fully differentiated during the embryo stage. Amylolytic activity during embryonic development and during the lecithotrophic stage may be attributed to carbohydrate (glycoproteins) utilization contained in yolk reserves as source of energy and structural components (Cetta & Capuzzo, 1982; Kamler, 2002; Whyte, Clarke, Ginther, & Jensen, 1993).

After hatching, the activity of the above-mentioned enzymes tended to increase in parallel to the development of the exocrine pancreas as it has been reported in other fish species (see review by Rønnestad et al., 2013). Similar to most fish species described so far, the increment in activity of pancreatic enzymes in *S. dumerili* would be correlated to the morphogenesis of the exocrine pancreas (4–5 dph; 4.0 ± 0.1 mm TL) and the accumulation of zymogen granules in pancreocytes, and to the complete resorption of the yolk sac and transition to exogenous feeding, as our histological data were indicated. These results are in agreement with those reported in another carangid species such as the golden pompano *Trachinotus ovatus* (Ma et al., 2014) and *S. rivoliana* (Teles et al., 2017). The decrease in activity of α-amylase from hatching to the onset of exogenous feeding in comparison with values found in embryos may be genetically programmed rather than dietarily induced as it has been reported in most carnivorous fish larvae, even though the magnitude of the above-mentioned ontogenic decrease in activity is species-specific (Govoni, Boehlert, & Watanabe, 1986; Rønnestad et al., 2013; Zambonino-Infante & Cahu, 2001). In addition, the higher activity of α-amylase found in *S. dumerilii* larvae aged 12 dph reared under INT conditions may be attributed to a delay in the maturation of the digestive function (Zouiten, Ben Khemis, Slaheddin Masmoudi, Huelvan, & Cahu, 2011), as it has been previously reported when data of α-amylase activity have been used as marker of the functionality of the digestive system and accessory glands (Zambonino-Infante & Cahu, 2001, 2007). However, the above-mentioned delay in digestive function maturation in *S. dumerilii* larvae was compensated within a few days for intensively reared specimens as data from larvae sampled at 30 dph from both rearing methodologies indicated. After the onset of exogenous feeding, total activity of the three assayed pancreatic enzymes, when expressed as U/larvae, sharply increased until the end of the study at 30 dph (8.2–8.7 mm TL). Similarly to other species, total alkaline proteases and BAL were the main pancreatic enzymes during the first days after first feeding in *S. dumerili*, indicating that peptides and proteins, as well as lipids (triglycerides), are the principal sources of energy for sustaining larval growth and development. In particular, alkaline proteolytic enzymes, especially trypsin and chymotrypsin, the main pancreatic alkaline proteases, are generally regarded as being particularly significant in the early life stages of fish larvae because of the absence of a functional stomach with its acid protease, pepsin (Rønnestad et al., 2013). Under present rearing standard conditions and in agreement with other studies assessing the changes in alkaline protease-specific activities along larval development in carnivorous fast-growing species (Chen et al., 2006; Ma et al., 2014; Solovyev et al., 2016; among others), major changes in the activity of total alkaline proteases were found between the onset of exogenous feeding until the early juvenile stage at 30 dph. However, the large deviation values in alkaline protease-specific activities found in different sampling points (6–10, 11–15 and 20–30 dph) denoted a heterogeneous larval population, which probably masked somehow the above-mentioned activity peaks associated with pancreas differentiation and changes in food items. Under the current experimental conditions, alkaline proteolytic specific activity was slightly different among the rearing system (MES versus INT) and tended to decrease at late larval stages (21–30 dph), concomitantly to the sharp increase in pepsin activity observed during this period. However, when expressed per larvae, the pancreatic protease activity was still high at day 30 post-hatching. These results suggest that even though the stomach was functional at early ages (17 dph, 5.7 ± 0.4 mm TL for MES), and peptic activity appeared and increased due to the

presence of abundant gastric glands as histological data revealed, the complete transition to a juvenile digestion pattern based on acid digestion was not achieved in *S. dumerili* specimens aged 30 dph and measuring 8.2–8.7 mm in TL. The presence of eosinophilic supranuclear bodies in the hindgut also further supports this observation (Ma et al., 2005; Teles et al., 2017). It is really feasible that this process occurred at later stages (Zambonino-Infante & Cahu, 2001), but current experimental design and sampling schedule did not allow to validate this hypothesis, and further, longer studies (>30 dph) are needed to elucidate this concern. Regardless of this fact, present results are in agreement with those reported for *T. ovatus*, where pepsin activity was detected at higher body sizes of 5 mm in TL and then progressively increased at older ages (Ma et al., 2014). When comparing pepsin activity between both rearing protocols, it becomes apparent that regardless of the late appearance of gastric glands in larvae from the INT group in comparison with those larvae reared in MES (Figure 2), pepsin activity was higher in *S. dumerili* from the INT group at 30 dph. Such differences in pepsin activity between both systems may be attributed to the feeding protocol and could be considered as dietarily induced rather than differences in the maturation of the stomach, since larvae from the INT were mainly fed on enriched *Artemia* and a compound diet, whereas those from the MES treatment were offered a more diverse diet (*Artemia*, copepods, compound diet, and eggs and fish larvae). Thus, differences in the level of protein, peptide and free amino acid contents between food items may explain such differences in acid protease activity (Zambonino-Infante & Cahu, 2007).

BAL is an important enzyme for the hydrolysis of a wide range of lipids such as glycerophospholipids, cholesterol esters and lipid-soluble vitamins (Rønnestad et al., 2013). In *S. dumerili*, BAL total activity sharply increased with the transition to exogenous feeding and remained stable afterwards, whereas there were not clear differences in activity regarding the larval rearing method considered. In *T. ovatus*¸ lipase showed two ontogenic activity peaks, one coinciding with first feeding as in the present study and a second one when golden pompano were fed with *Artemia* nauplii (Ma et al., 2014). In contrast, in *S. dumerili* and *S. lalandi* this peak in activity associated with a shift in the type of live prey (rotifer versus. *Artemia* nauplii) (11–15 dph) was not detected, which may be associated with the larval sampling procedure used in the present work where individuals were pooled by periods of 5–10 days, whereas in other similar studies larvae were sampled more often. Independently of the rearing methodology considered, histological data revealed that the percentage of area covered by lipid vacuoles in the liver decreased between 11 and 15 dph in comparison with younger ages. These results differed from those observed in *S. rivoliana* (Teles et al., 2017), differences that may be attributed to different larval rearing and *Artemia* enrichment procedures. Under present experimental conditions, the above-mentioned decrease in the level of accumulation of fat stores in the hepatic parenchyma in *S. dumerili* larvae may be correlated to a change in food items (*Artemia* AF versus. EG nauplii) coupled with a higher energy demand to support the higher somatic larval growth observed during this period. In addition, the former results were also correlated with a decrease in GSH and in the activity of GR, GPX and GST enzymes, which is in agreement with the above-mentioned hypothesis.

The comparison between MES and INT revealed that there was a large conservation in the ontogenic differentiation of digestive structures during the first developmental stages, independently of the rearing procedure applied as it is shown in Figure 2. The only remarkable variations were found in the final differentiation of the digestive system and, in particular, related to the appearance of gastric glands and pyloric caeca. These two digestive structures were detected in *S. dumerili* larvae at smaller sizes in larvae reared in MES (5.7 and 7.2 mm in TL, respectively) in comparison with those from the INT system (6.0 and 8.5 mm in TL, respectively). This pattern was also observed in European sea bass (*Dicentrarchus labrax*) (Zouiten et al., 2011) as well as in meagre (*Argyrosomus regius*) (Papadakis et al., 2013; Solovyev et al., 2016) when comparing these two rearing strategies. The two major differences between both systems are the larvae density, which is more than one order of magnitude lower in the MES, and the type of preys that larvae may encounter. While in the INT rearing, the prey items are limited to rotifers and *Artemia* provided on a continues base, during the MES rearing, a greater variety of prey items are available to the larvae, including rotifers and *Artemia* together with copepods and also icthyoplankton (eggs and prelarvae). Hence, MES larvae have a greater prey variety to select from, expressing thus their preference and balancing better their requirements. Although there are very few studies evaluating the impact of these two different rearing procedures on larval development and quality, the former authors suggested that the proliferation of wild zooplankton on semi-intensive tanks besides the presence of enriched live preys (rotifers and *Artemia* nauplii) had a key effect on promoting larval development, although this process was compensated within a few days for the intensively reared larvae (Zouiten et al., 2011). Deviations from these values obtained under standard rearing conditions may be indicative of problems in larval quality, development and/or rearing conditions (Zambonino-Infante & Cahu, 2001).

Antioxidant endogenous defence plays an important role in detoxifying ROS and providing cell protection from oxidative assaults during larval development (Dandapat et al., 2003), a period highly demanding in energy and oxygen uptake. Antioxidant defence system can be influenced by both intrinsic factors including age, diet type, feeding behaviour and food consumption as well as by extrinsic factors such as seasonal and daily changes in dissolved oxygen, water temperature, toxins present in the water and stocking density (Bayir et al., 2011; Hegazi et al., 2010; Li et al., 2014; Liu, Jia, Han, Huang, & Lei, 2016; Simčič et al., 2015; Vinagre et al., 2012). In this sense, there are a number of studies on the antioxidant defence system in fish, particularly in relation to specific antioxidant enzymes activities and also in relation to larval age and development (Dorval, Leblond, & Hontela, 2003; Hamre et al., 2014; Kalaimani et al., 2007; Liravi et al., 2014; Peters & Livingstone, 1996; Skjærven, Penglase, Olsvik, & Hamre, 2013).

Generally, the endogenous antioxidant system includes some antioxidant enzymes which catalyse the reaction of ROS degradation. Therefore, SOD protects against oxidative damage by catalysing the reaction of dismutation of the superoxide anion (O_2^-) to H_2O_2, GRx reduces both hydrogen peroxides and organic hydroperoxides, GR catalyses the reaction to form GSH (glutathione) and maintain a ratio GSH/GSSG (oxidized GSH), and GST detoxifies some of the secondary ROS produced by reaction with cellular constituents (Rudneva, 2013). However, there have not been relevant studies specifically investigating the activity of the antioxidant enzyme systems in developmental larval stages of the greater amberjack. The present study has shown that readily measurable specific activities of most important antioxidant enzymes were present in the first life stages of greater amberjack larvae (from 7 dph to 30 dph) in both rearing technologies. Overall, the results indicated that the activities of GPx, SOD, GR and GST tended to decrease at 18 dph, after the flexion stage, while this reduction was significantly lower in the semi-intensive (MES) than in the intensive (INT) rearing methodology. According to literature, a similar progressive reduction in antioxidant enzyme activities with regard, however, to different dph fish-dependent stages of larval development has been found in Atlantic cod (*Gadus morhua*) for GPx, but not for SOD (Hamre et al., 2014), in turbot (*Scophthalmus maximas*) for SOD but not for GR and GPx (Peters & Livingstone, 1996), in *Dentex dentex* for SOD (Mourente, Tocher, Diaz, Grau, & Paster, 1999) and in silver carp (*Hypophthalmichthys molitrix*) for GPx and SOD (Liravi et al., 2014). Conversely, a progressive induction for GPx has been indicated in the fast-growing Asian seabass (*Lates calcarifer*) (Kalaimani et al., 2007) and for SOD in *Salmo iridaeus* (Aceto et al., 1994). Depletion in GSH content has been attributed either to higher level of ROS production that converts more reduced GSH to its oxidized form (GSSG) (Ou, Nourooz-Zadeh, Tritschler, & Wolff, 1996) or to a decreased activity of GR (Costagliola, 1991). Thus, the depletion in GSH content observed in 7 dph greater amberjack larvae could be explained by a higher level of ROS production as the activity of GR was significantly higher at this stage compared to all the other stages. The overall patterns of all antioxidant enzymes observed during the developmental stages of *S. dumerili* larvae might suggest specific compensatory mechanisms of antioxidant defence to compensate ROS production/removal (neutralize ROS) and to eliminate oxidation assaults due to a high metabolic rate (Solé, Potrykus, Fernández-Díaz, & Blasco, 2004). Present results also revealed that oxidative assaults appeared to be more severe in INT than in MES rearing system as the enzymes activities were higher in the former larvae. Reduced antioxidant enzyme activities in the semi-intensive system could have been related to a lower oxidative assaults and more stable rearing conditions than in the intensive system that may be related to the lower larvae density in MES compared to INT. Thus, we assumed that the reduction/induction of antioxidant enzymes activities indicated during larval stages might be related to the changes in the levels of ROS. It has been reported that oxidative stress in aquatic organisms is more profound during nutritional deficiency (Hidalgo, Expósito, Palma, & de la Higuera, 2002; Morales, Pérez-Jiménez, Hidalgo, Abellán, & Cardenete, 2004; Mourente et al., 1999; Tocher et al., 2003), elevated temperature (Hwang & Lu, 2002), hypoxia (Kolkovski et al., 2000), exposure to xenobiotics (Dandapat et al., 2003; Pedrajas, Peinado, & López-Barea, 1995; Peña-Llopis, Ferrando, & Peña, 2003; Rudneva & Zalevskaya, 2004) and stocking density (Liu et al., 2016). Taken into account the two rearing protocols, we hypothesize that either the lower larval density and/or the prey variety (Figure 1) might have influenced the lower antioxidant enzymes activities obtained in MES compared to INT system. Besides, the increase in antioxidant and digestive enzymes activities at similar larval stages (*i.e.* 7 dph and 6–10 dph, respectively), as well as the progressive decrease in both antioxidant and digestive enzymes activities after the 7 dph stage revealed that both digestive and antioxidant enzymes followed the same trend regardless of the physiological process considered. Overall, various aspects of intensive aquaculture stimulate stress responses in fish larvae because artificial systems are frequently exposed to a range of "unnatural" stressors, which are related to rearing practices (Zouiten et al., 2011). Thus, antioxidant enzymes play an important role in inactivation of ROS and thereby control oxidative assaults as well as redox signalling. Both processes change across the life span of the organism and thus modulate its sensitivity and resistance against free radical damage.

5 | CONCLUSIONS

This study is the first one describing the morphoanatomical and functional development of the digestive and antioxidant defence systems in *S. dumerili* and assessing the impact of two different larval rearing methodologies on the larval digestive and antioxidant functions. Coupling histological and biochemical data, we were able to show that *S. dumerili* larvae had a functional digestive system at the onset of exogenous feeding when the digestive process was basically alkaline, regardless of the rearing technology considered. The morphogenesis of the stomach and pyloric caeca was affected by the rearing protocol, appearing earlier in fish cultured in semi-intensive in comparison with those reared in intensive conditions. However, the above-mentioned differences were compensated within a few days for the intensively reared larvae as data on pepsin activity indicated. Although both rearing strategies did not differ in larval performance in terms of somatic growth and maturation of the digestive function, present results showed that larvae reared in the intensive system experienced elevated oxidative assaults in comparison with larvae reared in the semi-intensive system, which may be attributed to more stable and favourable rearing conditions in the semi-intensive technology.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

José A. Pérez https://orcid.org/0000-0002-5662-7246
Enric Gisbert https://orcid.org/0000-0002-7457-8468
Covadonga Rodríguez https://orcid.org/0000-0002-2805-0048

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