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How does weaning anticipation affect gilthead seabream (*Sparus aurata*) larvae digestive capacity, growth and survival?

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ABSTRACT

The gilthead sea bream is one of the most important Mediterranean aquaculture fish species. It is important to study the ability of fish larvae to grow and survive with respect to their feed. The attempt to shorten the period consumed by fish larvae depending on live food would reduce the production cost. Hence, the current study aimed to evaluate the effect of anticipating weaning as early as 27 days old on fish digestive capacity, intestinal histomorphology, growth and survival. Fish larvae were weaned at 27 (W27), 35 (W35), and 43 (W43) days after hatching (DAH), with a co-feeding of 5 days. Samples were collected at the weaning day (T0), 1 week (T1), and 4 weeks after the weaning (T4) to determine their digestive enzymes. Sea bream larvae weaned at different age stages exhibited similar growth and survival rates, aligned with normal intestinal morphology. Regardless of their weaning age, the experimental groups showed identical pattern of digestive enzyme activities variation. W27 fish were able to recover, and at 70 DAH digestive enzymes activities were similar among experimental groups. These results indicate that fish age/size influences the magnitude of digestive enzyme activities. Weaning gilthead sea bream at 27 DAH can have high economic advantage on hatcheries by reducing the production and labour costs of live feeds production.

INTRODUCTION

Gilthead sea bream, *Sparus aurata*, is one of the significant and commercial fish species in the Mediterranean Sea fisheries and aquaculture **(Kallitsis *et al*. 2020)**. **Yúfera *et al*. (2012)** stated that, the sustainable development of aquaculture production depends on the supply of healthy and well-developed juveniles. Shortening live food period has

high economic impacts. **Kolkovski *et al*. (2010)** reported that, in the developed countries hatcheries were industrially important,. The unit efforts reduced the cost ofjuveniles production, though still, the cost of labours represents 40% to 50% of the total costs. Producers highlighted the high cost of live feeds in marine larviculture and recommended the importance of reducing the period of depending on such feed **(Motta *et al*., 2019)**. **Person-Le Ruyet (1989)** stated that the feed costs were significantly reduced when seabass larvae were weaned at 23 DAH instead of 40 DAH. Thus, any cost reduction will substantially improve the hatchery profitability.

During the last decades, several studies conducted on different marine fish species have tested different weaning strategies to replace the live feed by microdiets, among which the age of weaning, the level of live food replacement, the co-feeding period were addressed **(Cahu & Zambonino Infante, 2001; Yúfera *et al*., 2005; Zeytin *et al*., 2016; Eid *et al*., 2018)**. The moment when to start the weaning, the diet palatability and digestibility are the weaning success key factors **(Faulk *et al*., 2007)**. High growth and development rates of larvae depend on feed as a main source of energy and nutrients, but it is important to understand if fish larvae have the digestive capacity to use the feed being provided. In this context, **Zambonino-Infante *et al*., (2008)** noticed that, for optimizing larval production, the larval stages physiological digestive process should be fully comprehended to determine the optimum age-specific nutritional protocols. Nevertheless, diet changes is not the only factor affecting the digestive enzyme activities during ontogeny **(Cahu & Zambonino-Infante, 1994)**. Most marine fish larvae protein digestion relies on pancreatic enzymes during the first 3 to 4 weeks, thus an important milestone regarding digestive physiology is the acquisition of mature intestinal epithelium and the capacity to have an acidic digestion with a fully functional stomach **(Zambonino-Infante *et al*., 2008)**. It was reported that the acid digestion, gastric digestive enzyme production and stomach development show that larvae may be ready for weaning **(Rønnestad *et al*., 2013)**.

Few studies have analyzed weaning strategies for sea bream at different ages, using different microdiets and different co-feeding periods. Overall, weaning at different ages, with different intervals, was analyzed during a period comprised between 20 to 60 DAH **(Kolkovski *et al*., 1997; Pousão-Ferreira, 2009; Soltan *et al*., 2014; Zeytin *et al*., 2016; Eid *et al*., 2018)**. However, those studies were mostly focused on growth and survival. Anticipating the age of weaning for gilthead sea bream may be economically important. Still the information on sea bream digestive enzyme activities at weaning is scattered and needs more understanding to successfully implement early weaning strategies. Therefore, the current study aimed to determine if anticipating the weaning age for gilthead sea bream larvae affects digestive capacity, growth and survival.

MATERIALS AND METHODS

Eggs and larvae rearing

Eggs were obtained from a natural spawn of a *S. aurata* broodstock adapted to captivity at IPMA's Aquaculture Research Station (EPPO; Olhão, Portugal). Newly hatched larvae were reared, according to standard rearing protocols for this species at EPPO **(Pousão-Ferreira, 2009)**. Sea bream larvae were reared until 26 DAH in 1500 L tank at an initial density of 40 larvae/L, fed ad libitum (Figure 1) with rotifers *Brachionus plicatilis* (4 to 20 DAH), *Artemia* sp nauplii (13 to 19 DAH), and *Artemia sp* metanauplii (20 DAH until co-feeding). Rotifers and *Artemia* metanauplii were enriched with RedPepper® (Bernaqua NV, Belgium). Tanks were individually set in a semi-closed system (partial recirculation). Water temperature was controlled and larvae were reared until 70 DAH. The rearing tanks were connected to a recirculation unit equipped with mechanical, biological and UV-filters. Water parameters were monitored daily, with temperature stabilized at 18.6±1.1 °C, salinity at 35±1‰ and dissolved oxygen above 90% of saturation. At 26 DAH, sea bream larvae were transferred to 9 tanks of 300L at an initial density of 10 larvae/L (3000 larvae/tank).

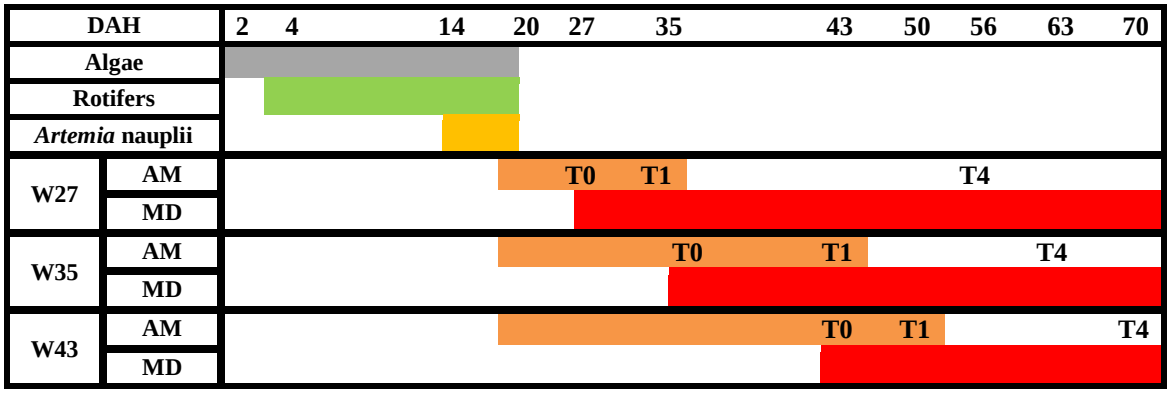

Fig.1. Weaning strategies of gilthead sea bream (*S. aurata*) larvae, co-feeding times treatments and sampling at T0, T1, and T4. AM: *Aremia* metanuplii, MD: Microdiets

Experimental treatments and sampling

The introduction of inert diet was tested at three different ages; namely, at 27 DAH (W27), at 35 DAH (W35), and at 43 DAH (W43). The weaning was consisted of a 5 days co-feeding regime, where the amounts of live feed decreased with increasing amount of microdiets. After this period, fish were fed exclusively on microdiets until 70 DAH. All experimental groups were followed in triplicate. The microdiet used was WinFast (SPAROS, Portugal), and different granulometries were used according to larval developmental stage. The size of microdiets varied from 100–200 µm to 800 µm, respectively for 27 DAH to 70 DAH. Microdiets were offered every hour from 8.00 to 22.00h using automatic feeders; adjusted daily and supplied ad-libitum. Rearing conditions were maintained as previously described. Survival rate was determined at the

end of the trial (70 DAH) by counting all living post larvae. Fish post larvae were sampled for length and dried weight (n=20), at 41, 55 and 70 DAH. For digestive enzyme analysis, fish larvae (n= 50) were sampled when microdiets were introduced (T0), 1 (T1), and 4 (T4) weeks afterwards, for each weaning age, as indicated in Table (1). Groups W27 and W35 were also sampled at the end of the experiment (70 DAH; T4 for W43 treatment). At 70 DAH, 10 post-larvae were collected for histology, fixed wholy in phosphate buffered formalin (4%, pH 7.4) for 24h and then transferred to ethanol (70%) until further process. Sampling was always done before feed distribution.

Table 1. Days post hatching of *Sparus aurata* larvae at the sampling points after diet introduction (T0) for the different experimental groups

	Experimental groups		
Sampling (weeks)	**W27**	**W35**	**W43**
T0	27	35	43
T1	35	43	50
T4	56	63	70

Biometric analysis

Larval total length was determined individually using a binocular microscope (n=20). These larvae were used for dry weight analysis, being first rinsed with distilled water and flash frozen in liquid nitrogen. Dry weight was determined for lyophilized samples (n=20). Survival rate was calculated according to larvae numbers at 26 and 70 DAH.

Enzyme analytical methods

After being sampled, larvae were rinsed with distilled water to remove salts, immediately frozen in liquid nitrogen and then stored at −80 °C until being assayed. Whole body homogenates were used for enzymatic analysis in larvae younger than 36 DAH. Older larvae were dissected in a glass maintained at 0°C to obtain the abdominal cavity. The samples were homogenized in 15 volumes (w/v) of ice-cold distilled water and centrifuged at 3300×g at 4°C for 3 min. For the purification of the brush border membranes, abdominal cavity segments were homogenized in 30 volumes (w/v) of ice-cold Manitol (50 mM), Tris-HCl buffer (2mM), pH 7.0. Purified brush border membranes from the abdominal cavity segment homogenate were obtained following the description of **Guerreiro *et al*. (2010)**. The Enzyme methodologies applied followed the steps described in the study of **Guerreiro *et al*. (2010)**. Amylase (E.C.3.2.1.1) activity was assayed using starch as the substrate dissolved in NaH2PO4 buffer (0.07M), pH 7.4, trypsin (E.C.3.4.21.4) activity was measured at 25°C using BAPNA (Nα-Benzoyl-DL-arginine-p-nitroanilide) as substrate in trizma-CaCl2 buffer (20 mM), pH 8.2. Acid protease (pepsin-like) activity was determined at pH 2 using bovine haemoglobin as substrate dissolved in HCl (1 M). Aminopeptidase N (E.C.3.4.11.2) activity was determined, using L-leucine p-nitroanilide (0.1M) as substrate in buffer phosphate (80

mM), pH 7.0, alkaline phosphatase (E.C.3.1.3.1) activity was assayed using pNPP 5 mM(p-nitrophenylphosphate) as substrate in a solution of carbonate buffer (30 mM), pH 9.8, acid phosphatase (E.C.3.1.3.2) activity was determined using pNPP 5.5 mM (p-nitrophenylphosphate) as substrate made in a solution of citrate buffer 0.1 M (citric acid and sodium citrate), pH 4.8. Enzymes activities were calculated as micromoles of substrate hydrolysed per minute (i.e., U), at 25°C for trypsin and 37°C for alkaline phosphatase and aminopeptidase. Amylase activity was presented as the equivalent enzyme activity required to hydrolyze 1 mg of starch in 30 min at 37°C. Pepsin activity was presented as specific activity with 1 U, representing 1.0 mM equivalent of tyrosine liberated per minute per mg of protein at 37°C. Protein was evaluated according to Bradford method **(Bradford, 1976)**. Enzyme activities were presented as specific activities, i.e., U/mg protein or mU/mg protein, and as segmental activities, i.e., total activity per larvae segment.

Histologic processing and evaluation

Histology samples stored in ethanol were processed and sectioned using standard histological techniques. Briefly, the visceral mass was gently excised from the visceral cavity and placed in a labelled casset suitable for processing. Samples were processed in a tissue processor (Model Citadel 2000, Thermo Scientific, Nanjing, China) and sectioned with a microtome (Model Jung RM 2035, Leica Instruments GmbH, Wetzlar, Germany). Slides were stained with haematoxylin and eosin using an automatic slide stainer (Model Shandon Varistain 24-4, Thermo Scientific, Nanjing, China). Slides containing the anterior, mid, and distal sections of the intestine were chosen for observation. The intestinal tissue was evaluated with particular attention given to structure of mucosal folds, width and cellularity of the lamina propria and submucosa, number of intraepithelial lymphocytes, number of eosinophilic granular cells and nucleus position and supranuclear vacuolization within the enterocytes. Images were acquired with Zen software (Blue edition; Zeiss, Jena, Germany).

Statistical analysis

Data obtained in this study was presented as means ± standard deviation (SD). At 70 DAH, digestive activities for different experimental groups were compared by one-way ANOVA, after checking normality and variance homogeneity assumptions. For growth and digestive enzyme activities, a two-way analysis of variance (ANOVA) at a 95% confidence limit was used with factors of age at weaning, time after weaning and the interaction of these factors, and means were compared by Duncan's test ($P < 0.05$). Statistical analysis was performed using SPSS software (SPSS for Windows 16; SPSS Inc., Chicago, IL, USA).

RESULTS

Larvae length, weight growth and survival

The 41, 55, and 70 DAH total length, 70 DAH standard length and wet weight growth and 70 DAH survival of *S. aurata* juveniles weaned at different ages recorded no considerable ($p > 0.05$) differences (Table 2).

Table 2. The effect of age of weaning on length, weight growth, and survival rate of gilthead seabream larvae

Parameter	DAH	W27			W35			W43		
Total length (cm)	41	1.33	±	0.15	1.32	±	0.15	1.34	±	0.17
	55	1.86	±	0.29	1.88	±	0.24	1.88	±	0.28
	70	3.08	±	0.62	3.07	±	0.49	2.96	±	0.59
Standard length (cm)	70	2.84	±	0.59	2.83	±	0.47	2.73	±	0.56
Final wet weight (g)	70	0.38	±	0.26	0.35	±	0.17	0.32	±	0.21
Survival rate%	70	48	±	6.47	45	±	4.91	47	±	4.06

Digestive enzymes of larvae

The digestive enzyme activities of *S. aurata* larvae weaned at different ages exhibited significant ($p<0.05$) increase with time. W43 was significantly ($p<0.05$) higher than W27 and W35 for most of the enzymes studied regardless of time post weaning. Specific and total activities of trypsin, amylase, pepsin, alkaline phosphatase, aminopeptidase, alkaline phosphatase brush border; also, at T4, the acid phosphatase and aminopeptidase brush border total activities were considerably ($p < 0.05$) greater than T1 and T0. At T4, specific activity of acid phosphatase was suggestively ($p<0.05$) more than T0, while T1 was not significant ($p>0.05$). Whereas, aminopeptidase brush border specific activity showed no significant differences ($p>0.05$) between T4, T1, and T0 (Figs. 2, 3& 4).

At T0, trypsin, amylase, pepsin, alkaline phosphatase, aminopeptidase, alkaline phosphatase brush border total activities of W43 and W35 were considerably ($p<0.05$) greater than W27. Pepsin, acid phosphatase, alkaline phosphatase specific and aminopeptidase total activities of W43 were knowingly ($p<0.05$) developed compared to those of W27 and W35. Trypsin specific activities of W43 were meaningfully ($p<0.05$) advanced compared to those of W35, which were pointedly ($p<0.05$) greater than W27. Aminopeptidase specific activities of W43 were expressively ($p<0.05$) developed than W27, which were considerably ($p<0.05$) developed than W35. Alkaline phosphatase brush border specific activity of W27 was ominously ($p<0.05$) greater than W35 and W43.

At T1, acid phosphatase, alkaline phosphatase total and specific, aminopeptidase specific activities of W43 were meaningfully ($p>0.05$) greater than W35 and W27. Alkaline phosphatase brush border, trypsin specific, and alkaline phosphatase brush

border total activities W35 were noticeably ($p<0.05$) higher than W43 and W27. Amylase specific and total activities W43 were noticeably ($p<0.05$) higher than W27, which were significantly ($p<0.05$) higher than W35. Pepsin specific and total activities W43 were considerably ($p<0.05$) higher than W35 which were significantly ($p<0.05$) higher than W27. Trypsin and aminopeptidase total and aminopeptidase brush border specific and total activities were not significant ($p>0.05$) between W27, W35, and W43.

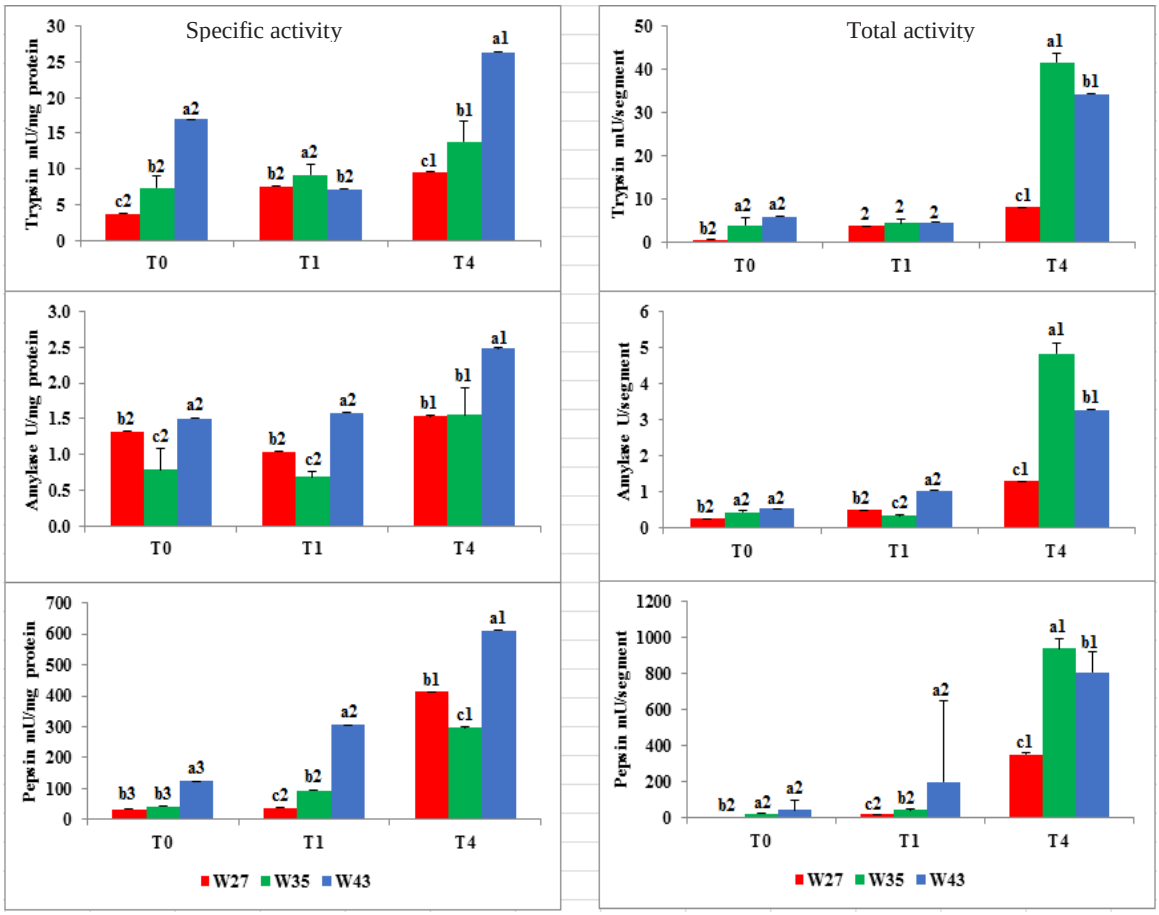

Fig. 2. The pancreatic and stomach digestive enzymes and specific and total activities of *Sparus aurata* larvae, weaned at different ages (W27, W35, and W43) at the starting, after one, and four weeks of weaning (T0, T1, and T4)

Letters refer to significant differences ($P < 0.05$) among ages at weaning at the different weeks, whereas numbers indicate significant differences ($P < 0.05$) within each experimental group at different weeks

At T4, amylase, aminopeptidase, alkaline phosphatase, and alkaline phosphatase brush border specific, acid phosphatase and alkaline phosphatase brush border total activities of W43 were meaningfully ($p>0.05$) greater than W35 and W27. Trypsin, aminopeptidase and pepsin total activities of W35 were noticeably ($p<0.05$) higher than W43, which were considerably ($p>0.05$) developed than W27. Trypsin specific activity of W43 were noticeably ($p<0.05$) greater than W35, which were knowingly ($p>0.05$) higher than W27. Pepsin specific activity of W43 were noticeably ($p<0.05$) greater than W27,

which were expressively (p>0.05) higher than W35. Acid phosphatase specific activity of W27 and W43 were noticeably (p<0.05) greater than W35. Alkaline phosphatase total activity of W35 and W43 were noticeably (p<0.05) higher than W27. Aminopeptidase brush border total activity of W35 were noticeably (p<0.05) greater than W27, which were significantly (p>0.05) advanced than W43. Aminopeptidase brush border specific activity were not significant (p>0.05) between W27, W35 and W43 (Figs. 2, 3& 4).

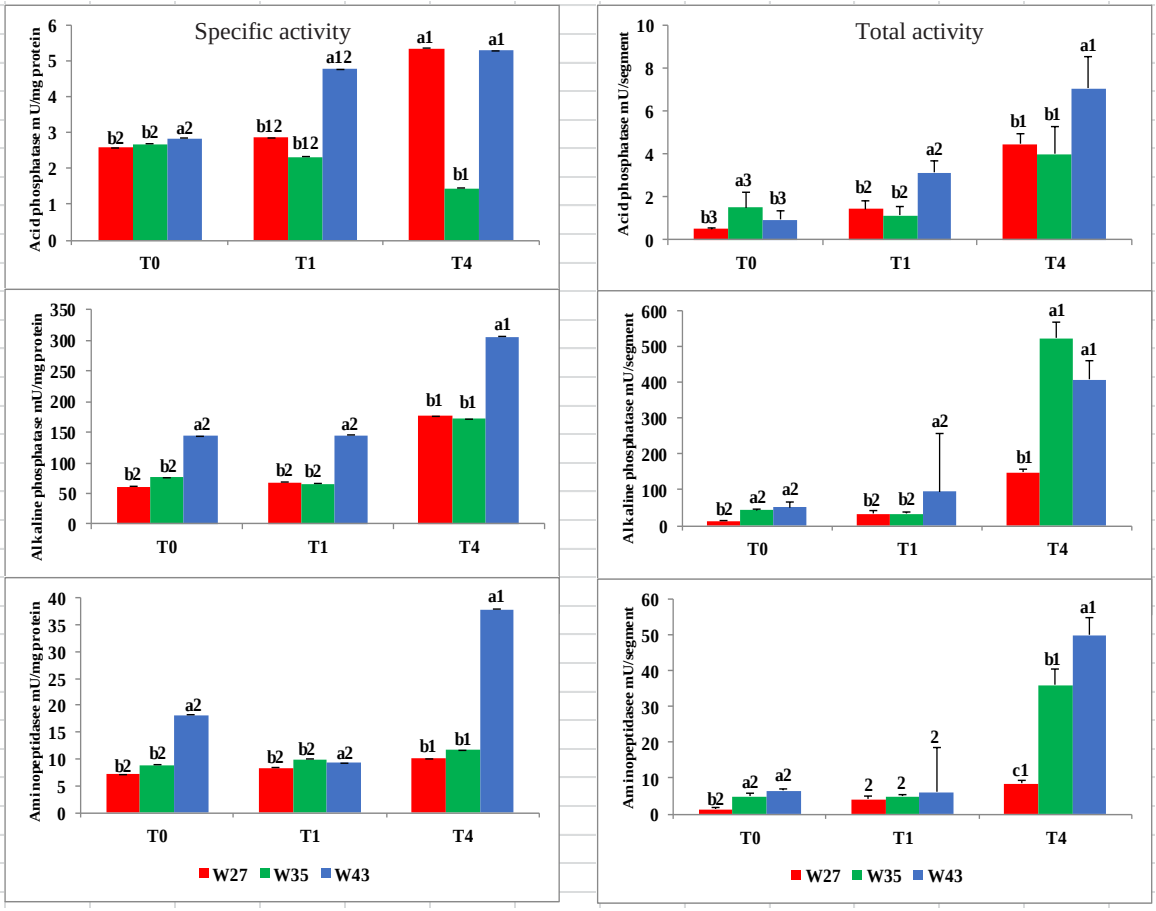

Fig. 3. The intestinal digestive enzymes specific and total activity of *S. aurata* larvae weaned at different ages (W27, W35, and W43) in the start, after one, and four weeks of weaning (T0, T1, and T4)

Letters refer to significant differences (P < 0.05) among ages at weaning at the different weeks, whereas numbers indicate significant differences (P < 0.05) within each experimental group at the different weeks

At 70 DAH, the digestive enzyme activities of *S. aurata* larvae weaned at different weaning ages (Table 3) were similar (p > 0.05) except for trypsin and amylase total activities. These activities of amylase were higher for fish from W43 group when compared to fish from W35 group (p < 0.05). Whereas fish from W27 and W43 exhibited higher trypsin values than W35 (p < 0.05).

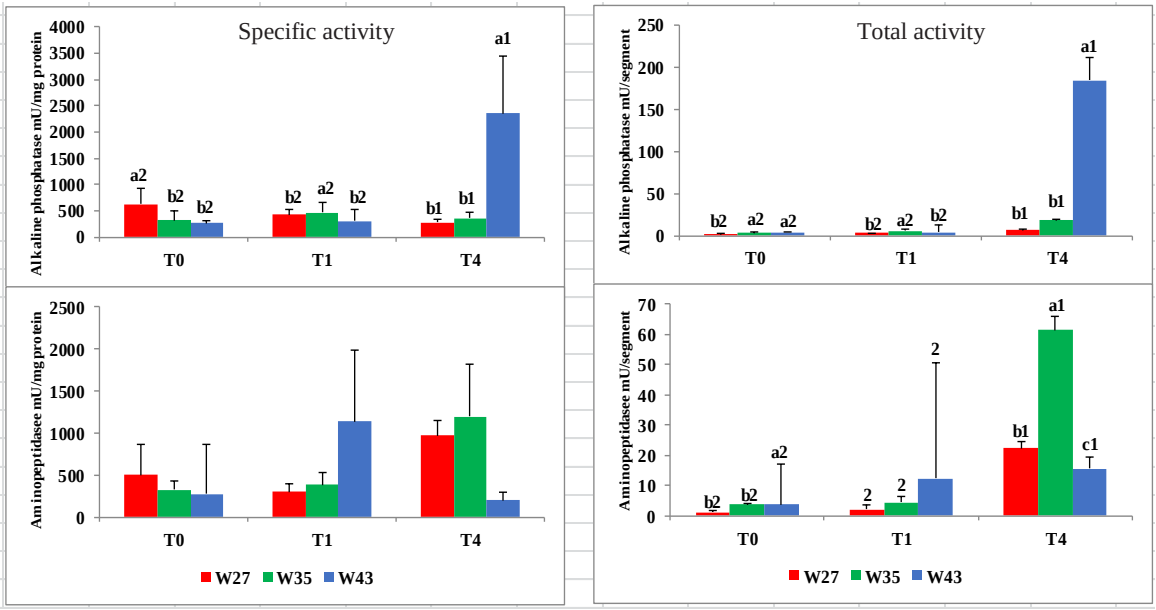

Fig. 4. The brush border digestive enzymes specific and total activity of *S. aurata* larvae weaned at different ages (W27, W35, and W43) in the start, after one, and four weeks of weaning (T0, T1, and T4)

Letters refer to significant differences ($P < 0.05$) among ages at weaning at the different weeks, whereas numbers indicate significant differences ($P < 0.05$) within each experimental group at the different weeks

Table 3. Enzymes of 70 DAH *S. aurata* larvae weaned at different weaning ages

Treatment	W27	W35	W43
Trypsin mU/mg protein	21.39 ± 5.14	17.63 ± 4.94	26.35 ± 6.79
Trypsin mU/segment	30.07 ± 3.17^{a}	22.57 ± 1.77^{b}	34.24 ± 3.02^{a}
Amylase U/mg protein	2.06 ± 0.36	2.01 ± 0.38	2.48 ± 0.35
Amylase U/segment	2.91 ± 0.19^{ab}	2.61 ± 0.28^{b}	3.27 ± 0.16^{a}
pepsin mU/mg protein	677.33 ± 51.74	561.79 ± 25.83	611.84 ± 105.35
pepsin mU/segment	967.13 ± 88.53	745.17 ± 146.66	808.80 ± 110.32
Acid phosphatase mU/mg protein	5.14 ± 1.63	3.69 ± 1.46	5.29 ± 0.92
Acid phosphatase mU/segment	7.37 ± 2.34	4.70 ± 1.48	7.06 ± 1.46
Alkaline phosphatase mU/mg protein	294.99 ± 59.54	252.01 ± 16.67	278.17 ± 20.21
Alkaline phosphatase mU/segment	418.61 ± 67.10	332.47 ± 55.22	407.24 ± 53.55
Aminopeptidase mU/mg protein	34.52 ± 8.09	29.73 ± 2.10	37.81 ± 4.83
Aminopeptidase mU/segment	49.13 ± 10.49	39.12 ± 5.52	50.01 ± 4.70
Brush border			
Alkaline phosphatase mU/mg protein	3295.59 ± 1653.16	3370.24 ± 1230.12	2346.73 ± 1098.46
Alkaline phosphatase mU/segment	179.46 ± 3.55	128.25 ± 49.80	184.65 ± 27.33
Aminopeptidase mU/mg protein	303.02 ± 158.26	256.11 ± 17.52	197.76 ± 98.25
Aminopeptidase mU/segment	16.70 ± 3.70	10.11 ± 3.77	15.44 ± 4.00

Different letters in the same row mean significant ($P < 0.05$) differences

Intestinal epithelium histology

Overall, the intestinal tissue from gilthead sea bream of the different experimental groups presented the normal architecture, with well-developed mucosal folds, regardless of the age at weaning. The lamina propria and submucosa were detected with focal inflammatory changes, intraepithelial leukocytes, and eosinophilic granular cells numbers

increased (Fig. 5B). The distal section of the intestine showed well- developed vacuolization, with focal hyper vacuolization, displacing the enterocyte nucleus. Accumulation of amorphous eosinophilic material, probably of proteic nature, was observed within the DI vacuoles.

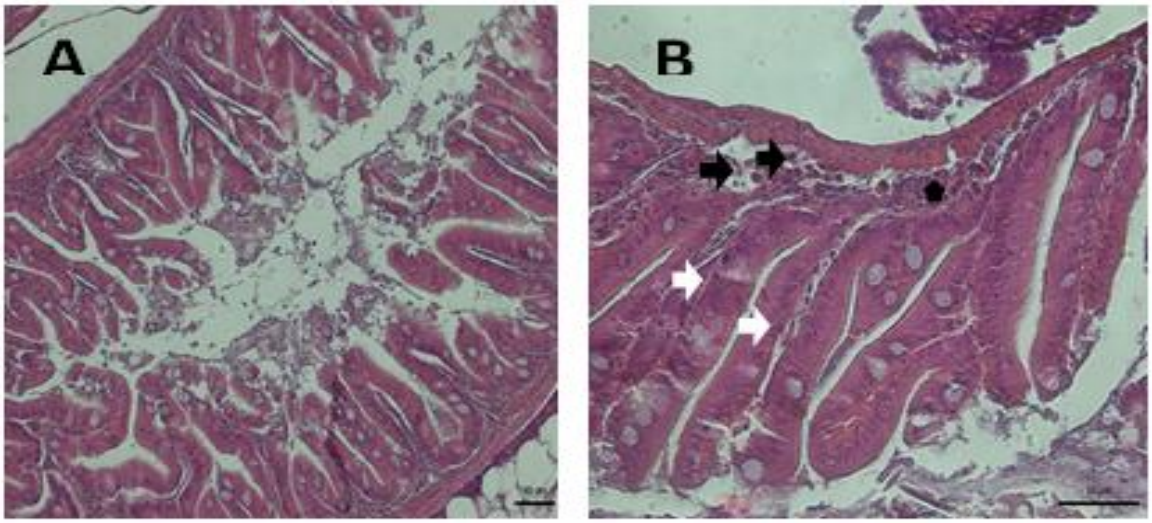

Fig. 5. A. Intestinal architecture of sea bream larvae at 70DAH; **B.** Inflammatory changes observed in the intestine with increased submucosa width (star) and infiltration of eosinophilic granular cells (black arrow) and leukocytes (white arrow).

DISCUSSION

Anticipating the weaning in two weeks for gilhead sea bream, 27 DAH instead of 43 DAH, resulted in similar indices of performance (growth and survival) and digestive physiology. The adequate age for weaning gilhead sea bream was the objective of different authors using different weaning strategies **(Soltan *et al*., 2014; Zeytin *et al*., 2016; Eid *et al*., 2018)**. They analyzed the impact of weaning on growth and survival, but no clear result was obtained for the suitable interval to wean gilthead sea bream. In a study analyzing ages every five days; between 35 and 60 DAH, the author reported that weight gain was higher at 55 and 60 DAH, whereas length and survival were higher at 60 DAH **(Eid *et al*., 2018).** In another study by **Soltan *et al*. (2014),** gilthead sea bream was weaned at 25, 27, 29, 33, 36, and 39 DAH**,** and the higher weight, length, and survival were obtained by fish weaned at 36 or 39 DAH. However, no noticeable differences were reported for gilthead sea bream length weaned at different ages (21, 26, 34, and 44 DAH) **(Zeytin *et al*., 2016)**. These results are similar to the current study, where despite the size and developmental differences at the moment of weaning (27, 35, and 43 DAH), at the end of the study at 70 DAH no noticeable impacts were witnessed for length, weight, growth, and survival at the end of the study; at 70 DAH. This indicates that it is possible

to anticipate weaning as early as 27 DAH, resulting in potentially and economically advantages **(Kolkovski *et al*., 2010)**.

The larvae weaning success depends not only on the diet composition, but also on the larvae digestive capacity. **Yúfera *et al*. (2004)** deduced that gilthead sea bream attains a fully developed stomach with acidic digestion around 100 DAH. The full development of stomach with an acid mode of digestion indicates that larvae may be ready for weaning **(Cahu & Zambonino Infante, 2001; Rønnestad *et al*., 2013)**. When weaning starts before the full stomach development, protein digestion depends on the pancreas to produce alkaline proteolytic enzymes, as described for European sea bass larvae **(Cahu & Zambonino Infante, 2001)**. The increase in amylase and lipase specific activity for early weaned white sea bream larvae reflected the microdiet introduction, an adaptation to carbohydrates and lipids existing in the diet, as was already defined for other *Sparidae* species **(Suzer *et al*., 2007; Ribeiro *et al*., 2008; Guerreiro *et al*., 2010)**. Based on this information, and since in the present study, the older weaning age tested was 43 DAH, gilthead sea bream larvae relayed on pancreatic enzymes for digestion. However, it was notorious that digestive capacity increased with fish development since at 43 DAH higher specific activities were observed for amylase and trypsin, when compared to W27 and W35. This reflected an enhanced digestion of dietary protein and carbohydrates, which was maintained higher during the 4 weeks (T4) after diet introduction for the different weaning ages studied. The trypsin enzyme activity was considerably lower in sea bream and Senegalese sole larvae consuming rotifers paralleled to larvae consuming *Artemia* and microdiet **(Gamboa-Delgado *et al*., 2011; Zeytin *et al*., 2016)**. Larvae species, age, and feeding regime stimulate efficient gut digestion of feed **(Lauff & Hofer, 1984; Kolkovski, 2001)**. The similarity of trypsin activity in larvae fed *Artemia* and microdiet at 44 DAH **(Zeytin *et al*., 2016)**. **Guerreiro *et al*. (2010)** reported that white sea bream larvae, weaned at 20 or 27 DAH, exhibited similar trypsin specific activities after 3 weeks. The pepsin activity followed a similar pattern to pancreatic enzymes with weaning, exhibiting higher values with larval development. This observation suggests that, although stomach was not yet fully developed at 43 DAH, the number of gastric glands in stomach produced levels of pepsin already quantifiable by the analytical method used **(Anson, 1938)**. The pH used for the *in vitro* determination of pepsin activity was pH 2, whereas **Yúfera *et al*. (2004)** determined *in vivo* that, only around 1g seabream larvae exhibit an acidic digestion but with pH values around 4. These observations indicate that pepsin activity of sea bream larvae might be lower under rearing conditions, and that the type of protein used for diet formulations must consider this aspect of digestive physiology.

In the present study, fish larvae digestive response was different, depending on larval size/stage of development, but larvae intestinal maturation was not affected by weaning. Younger larvae showed decreased activities one week after the weaning, whereas at later stages, digestive enzyme levels were similar or even higher, resulting that

after 4 weeks fish exhibited similar digestive capacity. Juveniles were able to adapt to the new diet, and enzyme activities were identical when they reached the development age (70 DAH), regardless of the time of co-feeding start (W27, W35, and W43). **Guerreiro *et al*. (2010)** stated that, the intestinal maturation was not affected by weaning in the white sea bream larvae. Although the digestive enzyme activities decreased in younger larvae after weaning (W20), fish digestion was recovered. **Guerreiro *et al*. (2010)** assessed that acid phosphatase specific activity reached its peak at 20 DAH. While, **Cara *et al*. (2003)** recorded that it peaked around 22 DAH. **Piattelli *et al*. (1997)** indicated that alkaline phosphatase and acid phosphatase enzymes had important roles on the digestive process and on bone mineralization, formation, and resorption, respectively. Similarly, the acid phosphatase specific activity of juveniles in the present result reached its peak at T4. Late weaning (W43), alkaline phosphatase and aminopeptidase specific activity increased significantly at T4 (70 DAH), indicating intestine digestive capacity improvement related to larval development. In addition, the white sea bream (*D. sargus*) larvae aminopeptidase and alkaline phosphatase increased at late weaning (W27); at 48 DAH (T3) **(Guerreiro *et al*. 2010)**. The intestinal enzyme activities witnessed a gradual increase till 50 DAH for *D. Puntazzo* **(Suzer *et al*., 2007)**. While, for *D. dentex,* the alkaline phosphatase and aminopeptidase were high at 6 DAH **(Gisbert *et al*. 2009)**. Whereas, alkaline phosphatase of *D. sargus* were high at 9 DAH **(Cara *et al*., 2003)**. Different metabolic and physiological events cause noticeable larvae digestive enzymes and specific activity fluctuations **(Zambonino-Infante *et al*., 2008)**.

Guerreiro *et al*. (2010) noted that, white sea bream (*D. sargus*) larvae, until 33 DAH, alkaline phosphatase and aminopeptidase brush border increased considerably for W20 and W27, but W27 had higher activities than W20. Similarly, the present results concerning the alkaline phosphatase and aminopeptidase total activity brush border of fish juveniles substantially increased by time and completed after weaning.The present study recorded that enzymes specific and total activities increased by the time significantly, regardless of the time of weaning, which indicates that juveniles digestive capacity enhanced timely also. The larvae started co-feeding late (W43) and the juveniles recorded higher noticeable enzyme activities than at earlier stages (W27 and W35), but without noting any considerable effect on juveniles growth or survival because juveniles had recovered. The same fidings were observed in the study of **Zeytin *et al*. (2016)** conducted on sea bream, *S. aurata*. Other studies noticed identical observations with regard to *D. puntazzo*, *S. senegalensis*, *P. bogaraveo*, *D. sargus* and *A. regius*, respectively **(Ribeiro *et al*., 1999; Suzer *et al*., 2007; Ribeiro *et al*., 2008; Guerreiro *et al*., 2010; Martins *et al*., 2019)**.

CONCLUSION

This study has successfully documented the baseline data for gilthead sea bream, S. *aurata*, weaning critical stages. Although at W43 and W35, juveniles showed higher enzyme activities when compared to W27, yet at 70 DAH, the younger larvae were able to recover and exhibited zootechmical performance and digestive capacity, similar to fish weaned later. Therefore, gilthead sea bream larvae can be weaned as early as 27 DAH with 5 days co-feeding, without considerable affecting juveniles' growth performance or survival rates. Weaning gilthead sea bream at 27 DAH contributes to save live feed production, including time consumed and labour efforts, as well costs of live feeds production in an attempt to enrich it. Overall, this is considered high economic advantage without noticeable growth or survival impacts.

DECLARATION OF COMPETING INTEREST

Authors declare no conflict of interest, and the experimental animals were handled as prescribed by international conventions in the use of animal as research subjects.

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