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Effects of Salinity on Digestive Physiology During Early Ontogeny in Newly Hatched Larvae of European Sea Bass (*D. labrax*)

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Abstract: In this study, the absorption dynamics of endogen food reserves and also specific activities of main pancreatic and intestinal enzymes were investigated in three different salinities (natural sea water, lower salinities as 30 and 25‰) during early ontogeny in larvae of sea bass, *Dicentrarchus labrax*, until mouth opening. Among experimental groups, mouth and anus were completely opened in larvae and also although yolk sac was nearly absorbed in whole larvae, oil globule was not fully depleted at the end of the 120th h after Hatching (HAH). In all experimental groups, trypsin specific activity rapidly rose up during the first 48 HAH then decreased. Also, this activity increased to until end of the experiment. Lipase specific activity slowly increased during the first 48 HAH then rose up to 120 HAH. Amylase specific activity was almost stationary during the first 48 HAH and then gradually increased to the end of the experiment. From this time to the end of the experiment this activity slowly declined. For intestinal enzymes, Alkaline Phosphatase (AP) and leu-ala peptidase activity presented converse profile among the experimental groups. The specific activity of AP demonstrated quite similar pattern for trypsin during the experiment. For leu-ala peptidase activity, opposite pattern was observed for AP activity during the first 24 h, specific activity was relatively higher but after then, it slowly decreased concurrently in all experimental groups. As a result of this study, obtained results clearly summarized that significant differences were recorded in all experimental groups, however, the lower salinity levels (25 and 30‰) in experimental groups demonstrated relatively significant and higher results in terms of endogen reserves absorption, larval growth performance, survival rate and digestive enzyme activities compared to natural sea water experimental group ($p<0.05$).

Key words: Digestive enzymes, salinity, survival, lecithotrophic stage, *Dicentrarchus labrax*

INTRODUCTION

The most common developmental milestones used in the developmental classifications in early life stages of larval fish are hatching and yolk-sac absorption. Also, absorption of endogenous food reserves or more accurately the transition from endogenous to exogenous nutrition is also a critical developmental and ecological transition for fish larvae (Kamler, 2008). The efficiency of yolk-sac absorption gradually decreases during the lecithotrophic stage, since this could be related with more of the yolk nutrients are used for maintenance and energetic purposes as increase in the larval size (Ronnestad *et al*., 1993, 1995; Karlsen *et al*., 1998). Besides, the nutritional quality and components of yolk protein are the biomarker and effective tool for understanding of nutritional capabilities during the embryogenesis. Furthermore, it is well reported that absorption of the endogenous energy sources, yolk-sac and oil globule and also is crucial step for the further organogenetic and developmental stages of marine fish larvae (Kjorsvik *et al*., 2004; Sveinsdottir *et al*., 2006; Kamler, 2008).

Generally, major organogenesis and developmental changes in digestive functions in marine fish larvae have been undergo especially during the 1st month of life until transition to adult mode of digestion. Moreover, during the lecithotrophic stage, the digestive system undergoes further metamorphic developments and the incipient digestive tract becomes dissected into three different anatomical and histological regions: buccopharynx, oesophagus and intestine (Zambonino Infante and Cahu, 2001; Zambonino Infante *et al*., 2008). Additionally,

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many recent literatures have been particularly reported developmental pattern and functional changes in the digestive tract related with variation of pancreatic and intestinal digestive enzymes in larval and weaning stage. In contrast to these, limited studies have been carried out on digestive physiology and enzymatic activity during the yolk-sac stage in cultured marine fish whereas these activities are the main indicator of readiness for the initial feeding of the larvae. Since, activities of digestive proteases could be evaluated better tool for determination of absorption dynamics of yolk-sac and nutritional requirements during embryogenesis of marine fish larvae in early ontogeny.

On the other hand, it is well reported in some cultured species that the newly hatched larvae is vulnerable and very fragile to handling manipulations and alterations in environmental conditions such as water flow (Opstad and Bergh, 1993), light intensity (Karlsen *et al*., 1998; Saka *et al*., 2001; Firat *et al*., 2003) and temperature (Bolla and Holmefjord, 1988; Pittman *et al*., 1989). However, the literature on digestive physiology and external salinity tolerance in yolk-sac larvae of sea bass during lecithotrophic stage is scarce (Ronnestad *et al*., 1998).

Furthermore, sea bass is the one of the most produced and common species in the Mediterranean aquaculture and also recent larviculture techniques have been focused on quality fry production. Especially, in order to enhance of formation of functional swimbladder in larvae, relatively lower salinity levels (30 and 25‰) than natural sea water are used during the early ontogeny by adjusting fresh water addition into natural sea water. Therefore, some different salinity levels are used in sea bass larviculture both lecithotrophic and exotrophic stages. In contrast to this, natural sea water is commonly used in case of lack of fresh water during larval development. Therefore, this study is designed to determine whether different salinity levels could affect growth performance, absorption of endogen food reserves and also attempts to effects on digestive enzyme activities in *D. labrax* larvae during the early ontogeny.

MATERIALS AND METHODS

Egg incubation and larval rearing: The broodstock fish, 16 females (2.85 kg mean weight) and 16 males (2.62 kg mean weight) were subjected to natural photoperiod (14 h light: 10 h dark) of natural rearing seasons (January to March) and the sea water temperatures varied throughout the experimental period between 14.5-16.0°C. After spawning, eggs were incubated in 50 L incubators at an initial density of 2500 eggs L^{-1} with a gentle flow (2 L min^{-1}) of sea water of 15.0±0.5°C.

After hatching, larvae were stocked at density of 100 ind L^{-1} in (three tanks for each salinity level) nine 200 L cylindrical-conical tanks for three experimental groups. The color of the tanks was dark grey. Water flow rate and aeration was adjusted to 5% of the total volume per hour and 40 mL min^{-1}, respectively. During larval culture period, temperature, oxygen and pH were maintained at 15.5±0.5°C >85% and 7.9, respectively. Ammonia and nitrite were kept constant always below 0.01 mg L^{-1}.

Experimental design and sampling: All experiments were conducted in triplicate. In order to evaluate its effects, salinity was decreased gradually in experimental tested levels until end of experiment. In group A and B, salinity adjusted as 25 and 30‰ during the lecithotrophic stage, respectively whereas natural sea water salinity (38.2±0.3‰) was applied in group C and evaluated as control. For monitoring of growth, the total length of the larvae, the length and width of the yolk-sac and the diameter of the oil globule for 50 individuals from each tank were measured every 8 h. The total length, the length of two axes of the spheroid yolk-sac (L, major axis; H, minor axis) as well as the diameter (d) of the spherical oil globule was measured to the nearest 0.01 mm from the digital photographs by using TpsDig Software (Version 1.37). The yolk-sac (Vys) and oil globule (Vog) volumes were calculated using the formulae produced by Blaxter and Hempel (1966) and also Cetta and Capuzzo (1982) as follows:

$$Vys = 4/3\pi \times (L/2) \times (H/2)^2$$

And:

$$Vog = 4/3\pi \times (d/2)^3$$

Moreover, pooled samples of larvae (50 per sample) were collected 8 h intervals for enzymatic analysis. Whole body homogenates were used for enzymatic assays and samples were taken at the same hours. The experiments were ended when the first mouth openings were observed in larvae in all experimental groups. At the end of the experiment, survival rates were determined by counting the number of live specimens in the experimental tanks.

Enzymatic assays: Samples were collected and whole body homogenized in 5 volumes v/w of ice-cold distilled water. Extracts utilized for enzyme assays were obtained after homogenization of larvae (35 mg mL^{-1}) in cold 50 mM Tris-HCl buffer, pH 8.0 followed by centrifugation (13.500×g; 30 min at 4°C). Trypsin and amylase activity were analyzed according to Tseng *et al*. (1982) using Nα-Benzoyl-DL-Arginine-P-Nitroanilide (BAPNA) and starch substrate, respectively. Lipase

activity was conducted by method by Mckellar and Cholette (1986) as modified by Versaw *et al.* (1989) using β-naphtyl caprylate as substrate. Assays of Alkaline Phosphatase (AP) and leucine-alanine peptidase (leu-ala) activity were measured according to Bessey *et al.* (1946) and Nicholson and Kim (1975) methods by using p-Nitrophenylphosphate (pNPP) $MgCl_2$ and leucine-alanine as the substrate, respectively. Enzyme activities were calculated as micromoles of substrate hydrolyzed per minute (i.e., U) at 37°C for AP and leu-ala and 25°C for trypsin. Enzyme activities were expressed as specific activities, i.e., U/mg soluble protein. Protein was determined by the Bradford procedure (Bradford, 1976).

Statistical analysis: Data are presented as mean±SD. The variance homogeneity of the data were analyzed using Levene's test when necessary, the data were log transformed (specific activity of alkaline phosphatase). Survival data were compared by Fischer's χ^2-test and also larval growth, absorption of endogen food reserves (oil globule and yolk-sac) and enzymatic activity data were compared by two-way ANOVA (salinity*time) followed by Newman-Keul's multiple range test when comparisons were found at the 5% level. Obtained data were statistically analyzed by SPSS 15.0 Software.

RESULTS AND DISCUSSION

Larval growth and survival: Growth of newly hatched larvae of *D. labrax* in all experimental groups during the 120 HAH period of study is presented in (Fig. 1). Initial larval size was 3.23±0.1 mm in all experimental tanks. At the end of the experiment, total lengths were measured as 5.36±0.15, 5.32±0.12 and 5.12±0.11 mm for group A, B and C, respectively. Also, survival rates were calculated as 89.1±2.6, 87.2±3.7 and 76.2±1.6% for group A, B and C, respectively. It is clearly recorded that both total length development and survival rates of A and B groups were significantly higher compared to control group ($p<0.05$).

Absorption of endogenous food reserves: In all groups, absorption of endogenous food reserves was observed in decline exponentially as expected (Fig. 2). Yolk-sac reserves almost depleted and remained 15.17±0.2, 14.62±0.1 and 12.92±0.1% volumetrically in group A, B and C, respectively with respect to the initial volume of yolk-sac. At the end of the experiments, final yolk-sac volumes were calculated as 0.054±0.019, 0.052±0.016 and 0.046±0.018 mm^3 for group A, B and C, respectively.

Besides, oil globule reserves were not fully depleted and remained 42.19±0.4, 40.48±0.3 and 34.72±0.3% volumetrically in group A, B and C, respectively for initial volume (Fig. 3). Final oil globule volumes were estimated as 0.0051±0.0012, 0.0049±0.0014 and 0.0042±0.0013 mm^3 for group A, B and C, respectively at the end of the experiments. Clearly, absorption of endogenous food reserves both yolk-sac and oil globule were significantly different among groups ($p<0.05$).

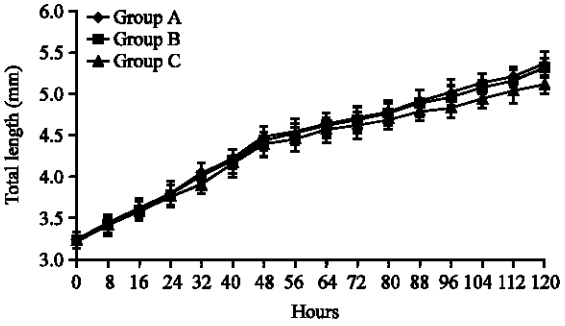

Fig. 1: Total length development of newly hatched larvae of *D. labrax* in experimental groups. Each mean±SD is a pool of 30 larvae

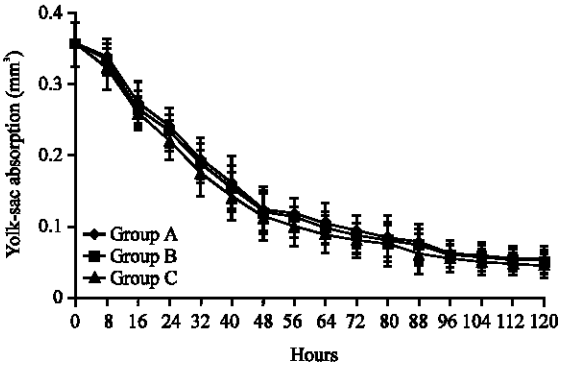

Fig. 2: Absorption of yolk-sac in newly hatched larvae of *D. labrax* in experimental groups. Each mean±SD is a pool of 30 larvae

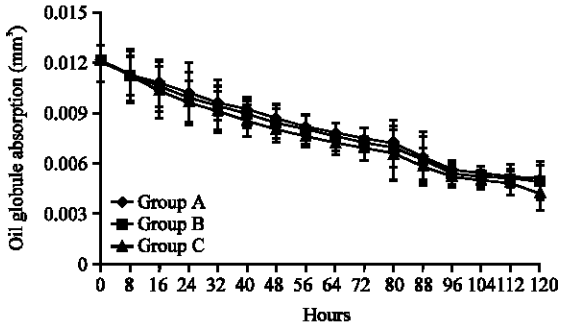

Fig. 3: Absorption of oil globule in newly hatched larvae of *D. labrax* in experimental groups. Each mean±SD is a pool of 30 larvae

Table 1: Specific activities (mU/mg/protein) of pancreatic and intestinal assayed in whole body homogenate of newly hatched larvae *D. labrax* during the lecithotrophic stage with 24 h intervals

	Hours					
Enzymes	0	24	48	72	96	120
A						
Trypsin	5.68±0.8^{a}	14.57±0.900^{b}	19.94±1.800^{b}	10.37±0.80^{a}	15.46±1.20^{b}	36.80±5.20^{c}
Lipase	22.56±2.3^{a}	34.35±2.800^{a}	39.43±4.300^{a}	78.43±9.40^{b}	121.75±15.8^{c}	129.52±14.8^{c}
Amylase	76.43±7.9^{a}	85.23±9.600^{a}	86.74±11.30^{a}	254.36±28.9^{b}	394.82±48.6^{c}	686.43±86.4^{d}
AP	24.84±2.9^{a}	44.87±3.100^{b}	86.23±5.600^{c}	68.45±7.70^{bc}	89.61±9.60^{c}	244.24±29.5^{d}
Leu-ala	745.2±62.8^{a}	1452.8±185.70^{b}	1154.80±184.5^{c}	824.90±88.1^{a}	733.60±92.4^{a}	627.1±96.20^{a}
B						
Trypsin	5.68±0.8^{a}	11.24±0.900^{b}	18.23±1.600^{c}	9.89±0.90^{b}	14.23±1.30^{c}	32.60±4.90^{d}
Lipase	22.56±2.3^{a}	32.76±2.400^{a}	37.82±4.400^{a}	74.83±8.80^{b}	113.42±14.6^{c}	112.27±15.3^{c}
Amylase	76.43±7.9^{a}	82.84±9.700^{a}	85.37±15.30^{a}	223.89±22.5^{b}	388.42±38.9^{c}	624.32±78.6^{d}
AP	24.84±2.9^{a}	42.66±3.600^{b}	78.46±6.300^{c}	67.23±7.20^{bc}	86.28±9.40^{c}	234.84±28.5^{d}
Leu-ala	745.2±62.8^{a}	1328.30±164.9^{b}	1128.40±192.7^{c}	784.80±62.7^{a}	694.30±95.7^{a}	618.9±82.70^{a}
C						
Trypsin	5.68±0.8^{a}	10.28±1.100^{a}	12.74±1.800^{ab}	8.52±1.20^{a}	10.77±1.60^{a}	23.70±4.80^{c}
Lipase	22.56±2.3^{a}	31.20±2.600^{a}	34.53±4.100^{a}	68.52±6.80^{b}	101.85±11.3^{c}	102.12±12.6^{c}
Amylase	76.43±7.9^{a}	83.60±8.600^{a}	82.30±12.50^{a}	204.63±24.6^{b}	365.75±33.4^{c}	608.47±79.5^{d}
AP	24.84±2.9^{a}	34.76±4.200^{a}	66.81±7.400^{b}	61.42±6.60^{b}	78.25±9.30^{b}	202.25±26.9^{c}
Leu-ala	745.2^{a}±62.8	1284.30±158.2^{b}	1068.30±148.7^{c}	724.80±96.7^{a}	628.30±45.2^{a}	582.9±74.70^{a}

Results are presented as means±SD (n = 3). Superscript letters in a row are significantly different ($p<0.05$)

Enzyme activity: Trypsin specific activity exhibited an exponentially increase more than approximately 2 and 3 fold in all experimental groups for the first 48 HAH. Then, this activity slightly declined until 80 HAH but it started to increase significantly and continued until end of the experiment. The highest specific activity of trypsin was found in group A as 36.8±5.2 mU/mg/protein (Table 1). Specific activities of trypsin were significantly different in all experimental groups ($p<0.05$).

Lipase specific activity showed similar pattern for trypsin, constant increase was observed significantly to 88 h followed by slight decline to 104 HAH. After this hour, it slowly increased again until end of the experiment. The peak of lipase activity was detected in group A at 88 HAH as 143.62±16.9 mU/mg/protein (Table 1). There were significant differences in specific activities of lipase in among groups ($p<0.05$).

Specific activities of amylase were almost stationary during the first 48 HAH in all groups and then slowly increased until end of experiment. The highest specific activity of this enzyme was established at 120 HAH in group A as 686.43±86.4 mU/mg/protein (Table 1). Significant differences were calculated in all experimental groups ($p<0.05$).

Specific activities AP and leu-ala peptidase presented contrast profile among the experimental groups. The specific activity of AP demonstrated quite similar pattern for trypsin during the experiment. It was fluctuated to 88 HAH and then it suddenly increased and maintained until end of the experiment. The maximum value for AP specific activity was determined in group A as 244.24±29.5 mU/mg/protein (Table 1). Specific activities of AP were significantly different in all experimental groups ($p<0.05$).

On the contrary to AP activity in the initial measurement of leu-ala peptidase activity were relatively higher during the first 24 HAH. Then, it presented slow decrease consecutively in all experimental groups. The peak of leu-ala peptidase activity was analyzed in group A at 24 h as 1452.8±185.7 mU/mg/protein (Table 1). At the end of the experiment, significant differences were recorded among groups fpr leu-ala activities ($p<0.05$).

Over the past decade, an increasing number of researches are devoted to the onset and identification of the developmental pattern of digestive enzyme activities during larval maturation of cultured fish species due to better tool for description of nutritional capabilities in different developmental stages of larval fish. Moreover, the most crucial developmental stages during the early ontogeny of larval fish are both hatching and absorption of endogen food reserves and also transition from endogenous to exogenous nutrition under culture conditions (Kamler, 2008). In this study, influences of different salinity levels (natural sea water as 38.2, 30 and 25‰) on absorption of endogenous food reserves (oil globule and yolk-sac) some digestive enzyme activities (proteases, amylase and lipase) and animal husbandry (mainly growth and survival) were investigated in newly hatched larvae of *D. labrax* during the lecithotrophic stage.

Among the numerous abiotical factors that affect fish larval feeding and locomotors activity, salinity plays a major role in inland and marine aquaculture and also salinity levels is important for fish and larvae which must be cultured in a specific salinity ranges, depending on the developmental stage. In present study three different salinity levels (natural sea water, 30 and 25‰) were examined due to common salinity levels used in larval

rearing of this species in Mediterranean aquaculture. Larval development was satisfactory under lower salinity levels (in group A and B) and also these conditions exhibited significant increases in husbandry performance of larvae except in group C treated natural sea water. Moreover, larval survival rates usually indicate success of larval rearing process. In similar with larval growth, survival rates were determined relatively satisfactory higher under lower salinity levels (group A and B) also significant differences were noted for natural sea water treatment (group C). Similar results were reported by Saillant *et al.* (2003) that the researchers estimated that the lower salinity levels presented faster development in cultured larvae until their initial feeding and also noted that this better situation was only observed at 15°C in this species. These findings are agreed with the current results which better results were detected at lower salinity levels (group A and B) at 15°C and also it is thought that this is closely related with the temperature-salinity interaction in cultured fish larvae as reported by Alliot *et al.* (1983) in this species whereas sea bass juveniles presented relatively higher growth performance in lower salinity (25‰) at same temperature (15°C). In addition, similar results were recorded in yolk-sac larvae of Atlantic halibut (*Hippoglossus hippoglossus*) which are cultured in different salinities (between 27 and 42‰) and the highest survival rates were recorded between 27 and 32‰ levels (Lein *et al.*, 1997). There are close agreements in these findings with present study. Furthermore, similar phenomenon was noted by Barman *et al.* (2005) in grey mullet and also it is thought that populations of live food (rotifera and Artemia) were higher at high salinities, however, growth was presented relatively lower profile under culture conditions.

According to obtained results, although endogen food reserves were nearly depleted in all experimental groups, salinity deeply affected the absorption of these reserves. As expected, larvae stocked in the natural sea water presented relatively faster absorption for their yolk-sac and oil globule reserves than the larvae exposed in lower salinity levels ($p<0.05$). Besides, it is thought that these ontogenic patterns of absorptions closely related the growth parameters and also could be accepted better tool for understanding the animal performance to evaluate endogen food reserves during the lecithotrophic stage. Although, relatively faster absorption rate for both yolk-sac and oil globule reserves was observed in natural sea water conditions, it has still undepleted reserves at the mouth opening. Ronnestad *et al.* (1998) reported that yolk-sac was nearly consumed (95%) at 100 HAH and but the oil globule was still present (about 30%) at the presumed onset of exogenous feeding at natural sea water salinity.

It is well known that the trypsin is the key digestive protease which hydrolyzes peptide bonds for digestion of protein and also evaluated as indicator for description of nutritional requirements and conditions of cultured fish larvae. Besides, it is generally detected shortly before and/or concurrently with the mouth opening of larval fish (Ueberschar, 1995; Zambonino Infante and Cahu, 2001; Zambonino Infante *et al.*, 2008). In all groups, trypsin specific activity presented gradually ~2 and 3 fold increase for the first 2 days and also salinity levels affected tryptic activity in experimental groups. The profile of trypsin activity in sea bass larvae is agreement with the results reported by Hartling and Kunkel (1999) and Sveinsdottir *et al.* (2006). These researchers suggested that biochemical digestion of yolk-sac proteins in winter flounder (*Pleuronectes americanus*) and Atlantic cod (*Gadus morhua*) was slower during the early ontogeny but exhibited relatively faster profile between 8 and 12 days after hatching. Besides, it is recorded that the involvements of serine proteases, especially trypsin, play key roles in in the embryogenesis and the absorption of yolk nutrients in fish larvae (Hiramatsu *et al.*, 2002). Additionally, similar profile of trypsin activity was recorded in *H. hippoglosssus* larvae in the yolk-sac stage (Gawlicka *et al.*, 2000). The researchers pointed out that the highest trypsin activity was measured at 230 degree days (dd) whereas before mouth opening this activity continued to decline until 276 dd. As described by Moutou *et al.* (2004) it is also thought that the activation of zymogen granules could be influenced by salinity variations due to existence at outside the cell boundaries and also these variations could be triggered mechanism of the zymogen activations in the intestinal lumen during the lecithotrophic stage. In similar, fluctuations in activity of digestive proteases caused salinity effect in the juveniles of both *S. aurata* and grey mullet (*Mugil cephalus*) were recorded. As reported by Barman *et al.* (2005) intestinal protease activities was affected from the salinity fluctuations in which the highest specific activities were measured in the relatively lower salinities (10‰) in the *M. cephalus* juveniles (1.48±0.15 g). Also, it is recorded that both activity of total acid proteases in the stomach and trypsin in the intestine were found significantly higher in the lower salinity group (20‰) compared to higher salinity (33‰) however, in the intestine, the total activity of alkaline proteases and chymotrypsin were significantly lower in the lower salinity group in *S. aurata* juveniles (26.5±0.9 and 24.1±1.5 g, respectively) (Moutou *et al.*, 2004).

Pancreatic lipase usually hydrolyses the triglycerides and also plays a relatively minor role in lipid digestion in fishes. Besides, specific activity of lipase is usually

related oil and FAA profile of larval diet (both live food and extruded microdiet) however, information about abiotical factors effecting lipase activity during lecithotrophic stage is scarce in cultured fish species (Zambonino Infante and Cahu, 2001; Zambonino Infante *et al*., 2008). Moreover, it is clearly reported that endogen food reserves, yolk-sac is consist of basically lipoproteins and phosphoproteins and also oil globule is composed mainly triglycerides in teleost larva (Kamler, 2008). Additionally, according to the results, researchers hypothesized that there could be close correlations between speed of oil globule absorption and profile of lipase activity. In this study, oil globule was absorbed (>60%) relatively faster for the first 88 h while specific activity of lipase constantly increased until this hour in all experimental groups ($p<0.005$). After this, depletion of oil globule was very slowly and also not fully absorbed (>40%) in all experimental groups at the end of the experiment. Therefore, it strongly correlated that lipase activity decreased and slowly fluctuated after from 88 h to the end of experiments. Concisely, it is thought that there could be close relations between duration of oil globule depletion and specific activity of lipase due to triglyceride content of oil globule. Moreover, similar lipolytic activity was determined in *H. hippoglosssus* larvae during the lecithotrophic stage however decline in specific activity relatively earlier hours, 179 dd (Gawlicka *et al*., 2000). This difference on profile of lipase could be originated from relatively longer lecithotrophic stage and genetically programmed absorption period in this species.

Furthermore, as reported by several studies, specific activities of amylase has been presented increased profile due to using of glucides during the 1st week of life in young teleost larvae and then it usually exhibited lower activities but closely related with starch content of extruded microdiet throughout weaning stage (Peres *et al*., 1996; Zambonino Infante and Cahu, 2001; Zambonino Infante *et al*., 2008). In this study, obtained results supported this phenomenon that similar developmental pattern for this enzyme were measured during the experiment however, salinity variations significantly affected the amylase activity. Moreover, similar findings were recorded that specific activity of amylase exhibited significantly higher values as similar as tryptic activity in the digestive tract of the grey mullet (*M. cephalus)* juveniles maintained at 10‰ (Barman *et al*., 2005) and fat snook (*C. parallelus*) juveniles stocked at 15 and 35‰ salinity levels (Tsuzuki *et al*., 2007). It is possible to estimate that specific activity of amylase in the juvenile and older stages in fish could be related carbohydrate contents of the diet. In contrast to these, it is clearly estimated that amylase activities were detected only in the 230 and 276 dd stages (not detected 161 and 179 dd) at statistically insignificant levels in *H. hippoglossus* larvae (Gawlicka *et al*., 2000).

Intestinal AP plays multiple roles for nutrient absorption but it is usually presents lower activities in newly hatched larval fish due to not commencement of intestinal maturation during the lecithotrophic stage. Also, after the fully depletion of yolk sac, glycogen being reconstituted during the transition period from endogenous to exogenous feeding however, glycogen is mainly accumulated in the liver of the larval fish during the lecithotrophic stage. Moreover, it is well reported that differences between species in hepatic glycogen contents during the endogenous feeding stage might possibly be related to the yolk lipid content and larval energy metabolism at this stage (Hoehne-Reitan and Kjorsvik, 2004). As observed for trypsin, salinity variations were significantly affected AP activity in all experimental groups. In addition, as observed in this study in earlier stages (161 and 179 dd) of Atlantic halibut (*Hippoglossus hippoglossus*) larvae, AP activity was relatively lower and then slowly increased in further stages (230 and 276 dd) (Gawlicka *et al*., 2000). As described by Moutou *et al*. (2004) similar pattern for AP were recorded in sea bream juveniles whereas relatively higher specific activities of AP was detected at lower salinity level (20‰) than high salinity (33‰) level. The researchers suggested that variations in water salinity could be effected zymogen activation since it takes place outside the cell boundaries in the intestinal lumen.

In contrast to AP activity, leu-ala peptidase, a cytosolic enzyme is generally exhibited higher profile for the first week of life in marine fish larvae. Besides, appearance of this enzyme at early larval stages involved that this was a genetically programmed process that would allow the absorption of yolk nutrients and also sequence of intestinal development (Zambonino Infante and Cahu, 2001; Zambonino Infante *et al*., 2008). During the lecithotrophic stage, specific activity of leu-ala peptidase was presented opposite pattern with AP activity in all experimental groups. Additionally, lower salinity levels (25 and 30‰) significantly affected this enzymatic activity however opposite pattern by AP activity was measured during the lecithotrophic stage. Also, there is lack of information about its activity during this stage however, these results were in agreement with the study by Zambonino Infante and Cahu (2001) and Zambonino Infante *et al*. (2008) which indicated that decrease in leu-ala peptidase is a common feature during larval ontogeny, since cytosolic enterocyte enzyme activities progressively decrease with larval growth.

CONCLUSION

This is the first study to examine the influence of different salinity levels on husbandry parameters and main digestive enzyme activities in newly hatched larvae of *D. labrax*. It is clearly estimated that lower salinity levels than natural sea water, especially 25‰ were an effective for both absorption of endogenous food reserves (oil globule and yolk-sac) and activities of digestive enzymes in *D. labrax* larvae. As a result, it is thought that more intense endogen food reserves of larvae under lower salinity levels caused to better growth performance and absorption parameters and also higher digestive enzyme activities during the lecithotrophic stage in this species. Further, studies should be conducted under other abiotical parameters such as temperature, light intensity and dissolved oxygen levels, since it could be possible to increase growth parameters and digestive enzyme activities by the external factors variations.

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