www.trjfas.org
ISSN 1303-2712
*Turkish Journal of Fisheries and Aquatic Sciences 10: 123-130 (2010)* DOI: 10.4194/trjfas.2010.0117

# Exocrine Pancreas Development and Trypsin Expression in Cultured European Sea Bass (*Dicentrarchus labrax*) Larvae

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Received 19 January 2009
Accepted 01 December 2009

**Abstract**

The ontogenesis and formation stages of exocrine pancreas in European sea bass (*D. labrax*) larvae were investigated from hatching to 40 days after hatching (DAH). Histological and enzymatical techniques were used to explain the functional development of the pancreas in *D. labrax* with the expression of trypsinogen activity. The incipient pancreas appeared as a lamination of the dorsal wall of the digestive tract. It was observed that the primary visible indication of exocrine cell differentiation was polarization. The first zymogen granules and pancreas with exocrine polyhedral cells appeared on 6 DAH and became abundant as a compact structure located dorsal and slightly posterior to the liver. At the same time, firstly, anus and then mouth were opened, and total lengths of larvae were determined as 3.47±0.26 mm. Until larval metamorphosis, the pancreas became diffuse, spreading throughout the mesentery enclosure, the stomach, the upper intestine and the pyloric caeca. On the other hand, zymogen granules were more numerous and larger, and a greater quantity of material was carried by the ducts, indicating an increased cellular activity. The specific activity of trypsin was determined as early as after hatching (42.54±6.8 mU/mg protein$^{-1}$) at 4.28±0.2 mm total length of larvae and increased immediately during the following days especially after exogenous feeding. The highest tryptic activity was detected on 30 DAH as 122.45±11.76 mU/mg protein$^{-1}$. It is concluded that exocrine pancreas organogenesis is the main critical step of the zymogen granules and trypsin activity is present as early as after hatching and continuously increasing with larval period of *D. labrax*.

*Keywords*: *Dicentrarchus labrax,* exocrine pancreas, histology, trypsin, ontogenesis, growth.

## Kültürü Yapılan Levrek (*Dicentrarchus labrax*) Larvalarında Ekzokrin Pankreas Gelişimi ve Tripsin Aktivitesi

**Özet**

Bu çalışmada açılımdan 40. güne kadar levrek (*D. labrax*) larvalarında ekzokrin pankreasın ontogenik gelişimi ve oluşum aşamaları incelenmiştir. Levrek larvalarında pankreasın fonksiyonel gelişimi tanımlamak için histolojik ve tripsinojen aktivitesinin incelendiği enzimatik teknikler kullanılmıştır. İlk pankreas oluşumu sindirim tüpünün dorsal bölgesinde tabakalaşmış olarak izlenmiştir. Eksokrin hücre farklılaşmasının ilk işaretinin kutuplaşma olduğu gözlenmiştir. 6. günde ilk zimojen granülleri ve ekzokrine polihedarl hücreli pankreas karaciğerin posteiorunda ve dorsal bölgesinde konumlanmış yoğun bir oluşum olarak gözlenmiştir. Aynı zamanda, ilk olarak anüs sonrasında ağız açılmış ve larvaların total boyu 3,47±0,26 mm olarak tespit edilmiştir. Larval metamorfoza kadar, pankreas farklılaşmış ve midenin mesenterik bölgesinde, bağırsağın ve pilorik çekumun üst kısmında yayılmış olarak izlenir. Öte yandan, zimojen granüllerinin sayısının ve hacminin artması ve beraberinde taşınan içerik miktarının artışı hücresel aktivitede meydana gelen artışın açık bir göstergesidir. Tripsin aktivitesi açılımın hemen ardından (42,54±6,8 mU/mg protein$^{-1}$) larva boyu ortalaması 4,28±0,2 mm iken tespit edilmiş ve özellikle eksojen besin alımının başlamasıyla birlikte sonraki günlerde düzenli artış göstermiştir. En yüksek tripsin aktivitesi 30. günde 122,45±11,76 mU/mg protein$^{-1}$ olarak tespit edilmiştir. Sonuçta, levrek larva üretim periyodunda eksokrin pankreas gelişimindeki en kritik aşamanın zimojen granüllerinin oluşumu olduğu ve tripsin aktivitesinin açılımın hemen ardından başlayıp larval gelişime bağlı olarak artmaya devam ettiği bulunmuştur.

*Anahtar Kelimeler*: *Dicentrarchus labrax,* ekzokrin pankreas, histoloji, tripsin, ontogenesis, büyüme.

## Introduction

European sea bass, *Dicentrarchus labrax*, is a major finfish of interest in Mediterranean aquaculture, these hatchery procedures, at their beginning in the 1980s (Barnabe´ and Billard, 1984), had greatly improved during the last two decades. Its growth is relatively rapid and it is a highly valuable commercial fish. Both the weaning and ongrowing techniques of this species are carried out with great profitability at a production level. In aquaculture, high temperatures are applied in order to increase the growth rates in sea bass juveniles.

Marine finfish larvae undergo major developmental changes in their digestive functions during the first month of life and it represents the acquisition of an adult mode of digestion (Zambonino Infante and Cahu, 2001). The pancreas and gallbladder primordia could be observed close to or after hatching, while the spleen seems to appear later. A liver, pancreas and gallbladder (between liver and pancreas) were present at hatching, e.g., cod and haddock (Morrison, 1993; Hamlin *et al*., 2000), sea bass, sea bream, *Sparus aurata*, (Elbal *et al*., 2004; Beccaria *et al*., 1991) and wolffishes, *Anarhichas lupus marisalbi*, (Falk-Petersen and Hansen, 2001; Pavlov, 1986). The organogenesis of the pancreas in *D. labrax* was described in detail (Beccaria *et al.*, 1991). It was present as a primordium at hatching, in the form of a dorsal bud on the digestive tract. The first zymogen granules of the exocrine part appear three days after hatch and become abundant at the onset of the trophic phase in Atlantic cod larvae, *Gadus morhua*, (Morrison, 1993). The pancreas with undifferentiated rounded cells became more evident between digestive tract and yolk-sac and then pancreas was clearly distinguishable on 2-3 DAH in common dentex, *Dentex dentex* larvae (Santamaria *et al*., 2004). Additionally, it is further extended during metamorphosis, trypsin and chymotrypsin activity is high and the folded intestine has aminopeptidase-activity (Kjørsvik *et al*., 2004).

Over the past decade, there is an increase in the number of papers, which are devoted to the onset and development of digestive enzyme activities during larval growth of cultured fish species. Physiology and nutrition studies of fish in the early stages of development, as well as the evolution of the digestive enzyme activity are valuable tools to better know the nutritional capabilities of young larvae and establish feeding protocols for optimizing larval mass rearing production (Diaz *et al.*, 1997; Zambonino Infante and Cahu, 2001). The digestive physiology of many marine fish larvae undergoes numerous morphological and functional changes during ontogeny that can substantially influence larval survival under culture conditions. Therefore, the assessment of the presence and level of activity of digestive enzymes may be used as a comparative indicator of the rate of development of the fish larvae, food acceptance, digestive capacity, as well as of their further survival rate (Ueberschär, 1993). It is well known that the secretion of trypsin is known to occur in response to food ingestion and in larval pancreatic tissue, most of the trypsin is present as an enzymatically inactive trypsinogen, whereas most of the trypsin in the intestinal tract is enzymatically active (Ueberschär, 1993; 1995). In addition to this, it is reported that recent results indicate an increasing importance of chymotrypsin relative to trypsin in larval fish, and at times chymotryptic activity may exceed tryptic activity (Lazo *et al*., 2007).

As well described by several studies, digestive protease trypsin is specific of pancreatic protein hydrolysis (Nolting *et al*., 1999; Zambonino Infante and Cahu, 2001). To date, several authors have investigated ontogenic development of main digestive enzymes and also early weaning effects on digestive characteristics larvae of *D. labrax* (Cahu and Zambonino Infante, 1994; Zambonino Infante and Cahu, 1994; Suzer *et al*., 2007c). But a few studies were carried out on histological ontogeny exocrine pancreas which is the main organ enzymatic secretion during larval development (Beccaria *et al*., 1991). Therefore, the objective of this study to describe the ontogenetic development of exocrine pancreas and concurrently digestive protease trypsin activities of *D. labrax* larvae fed on live prey and compound microdiet until end of 40 DAH.

## Materials and Methods

### Larval Rearing

Larval rearing was carried out in 3 closed sea water systems that included 3 cylinder-conical shape 4 $m^3$ tanks per system. The color of the tanks was dark-grey and larvae were stocked at a density of 100 individuals.$L^{-1}$. Water temperature, dissolved oxygen, salinity, pH, ammonia and nitrite levels were monitored daily. Water temperature was maintained between 15 and 20°C (temperature increased day by day from 15 to 16°C between 0 $^{th}$ and 7$^{th}$ days, from 16 to 19°C between day 8 and 21, and from 19 to 20°C between day 22 and 40). During the larval culture period; oxygen, salinity and pH were maintained at >85%, 38‰ and 7.8, respectively. Ammonia and nitrite were kept constant and below 0.01 mg $L^{-1}$. Water exchange rate increased gradually with the age of the larvae. Light was supplied by fluorescent tubes, with a power of 30-100 lux at water surface. Photoperiod was set on light cycle daily until the end of larval rearing period (16 h light : 8 h dark).

After the mouth opening, from 6 to 15 DAH the larvae were fed with rotifers (70% *Brachionus rotundiformis* and 30% *Brachionus plicatilis*) cultured with algae and enriched (DHA Protein Selco, Artemia Systems SA, Ghent, Belgium) at a density of 10-15

individuals $ml^{-1}$: from day 10 to day 25 DAH with *Artemia* nauplii (AF480, INVE Aquaculture) at 4-6 individuals $ml^{-1}$ and from 15 DAH until end of the experiment, *Artemia* metanauplii at 2-4 individuals $ml^{-1}$ both enriched with Protein Selco. Extruded microdiet (Caviar, BERNAQUA, Olen, Belgium) was used from 25 DAH until 40 DAH as 8−10% of biomass per day. Larval feeding regime is schematized in Figure 1.

**Sampling**

Growth rate was monitored weekly by sampling groups of larvae from each tank (30 larvae per sample $group^{-1}$) and at the last day of the experiment (40 DAH). Specific growth rate was calculated by formulae SGR = 100 (Ln FBW −Ln IBW)/Δt, with IBW, FBW: initial, final body weight of fish (mg), Δt: time interval (day). At the end of the experiment, larval survival was determined by counting larvae remaining in the tanks. For histological analyses, 10 larvae sampled from each tanks collected daily from 0 to 15 DAH and every third day from 15 to 40 DAH during post-hatching period. Pooled samples of larvae (50−250 individuals, depending on age and size) were collected for enzyme analysis. Sampling days for enzymatic analysis were carried out on 3, 5, 7, 8, 10, 12, 13, 15 DAH and 5-day intervals after this date until 40 DAH. Whole body homogenates were used for enzymatic assays and samples were taken at the same hour, before food distribution.

**Histological Analyses**

For the histological study, 10 larvae were fixed in neutral formalin solution, dehydrated in different alcohol series, embedded in a parafin vax and cut in 3-5 μm-thick sagittal sections using a Leica RM 2125 rotary microtome. The Haematoxylin/Eosin (HE) stain was used for general histological observations to describe the development of the exocrine pancreas. Then, slides with sections were mounted permanently using Entellan. The sections of fish were randomly examined under an Olympus CX31 microscope. Photographs were taken with an Olympus DP20 digital photomicrographic attachment.

**Analytical Procedure of Trypsin**

Samples were collected and 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 x*g*; 30 min at 4°C). Trypsin activity was assayed spectrophotometrically using Nα-Benzoyl-DL-arginine-*p*-nitroanilide (BAPNA) as the substrate (Tseng *et al*., 1982). Absorbance was measured at 253 nm for 5 min. One unit activity of trypsin was defined as 1 μmol of BAPNA hydrolyzed per minute at 25ºC. Enzymatic activities were expressed as specific activity (mU/mg protein) and total activity (mU/larva). Protein was determined by the Bradford method (Bradford, 1976).

**Statistical Analysis**

All measurements were carried out in triplicate. Results are given as mean ± SD. The variance homogeneity of the data was performed using Levene's test. Survival was compared by Fischer's chi-square test and also larval growth enzymatic activity data were compared by one-way ANOVA, followed by Newman-Keul's multiple range test and all significant differences were set at 0.05 level. Statistical analyses were performed by SPSS 15.0 software.

## Results

**Growth**

Growth of *D. labrax* larvae during the study is described in Figure 1. At hatching, total length was measured as 3.85±0.2 mm and mouth and anus closed. Additionally, alimentary tract appeared lying dorsally to the yolk sac. On 6 DAH, firstly anus and then mouth opened, and total lengths of larvae were determined as 4.66±0.29 mm. At this stage, exogenous feeding began and few rotifer and microalgae could be observed in digestive tube. Swimbladder inflation started, and then it begun to elongate under the notochord on 7 DAH and 15 DAH, respectively. Specific growth rate averaged 5.8% $d^{-1}$ and also survival rate was calculated as 42.4%.

**Organogenesis of Pancreas**

On 6 DAH, the mouth was opened and the digestive tract of the *D. labrax* was well differentiated into foregut, mid-gut and hindgut. The pancreas at this stage existed as a distinct and compact organ situated just posterior to the liver (Figure 2A-B). The first zymogen granules and pancreas with exocrine polyhedral cells appeared on 3 DAH. The pancreas with sporadic exocrine polyhedral cells and zymogen granules gradually increased after first feeding from 6 DAH through 15 DAH which mainly depends on size. On 15 DAH, zymogen granules were disposed to clusters in the lumen of tubules and, granules were almost homogeneous and dense. The digestive tract became more complex; however, the post-esophageal swelling still did not exhibit evidence of gastric gland formation between 6 DAH and 20 DAH (Figure 2B-D). The pancreas at these stages was still quite compact and exhibited distinct endocrine islet surrounded by exocrine tissue. By 20 DAH, the pancreas was still found in close association with the liver (Figure 2D). On 25 DAH, the post-esophageal swelling showed histological evidence of true gastric

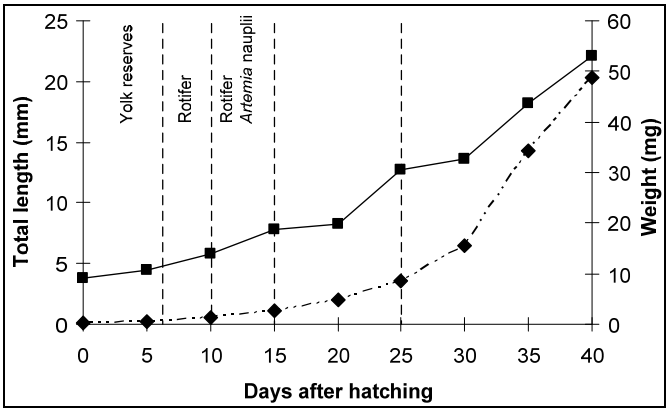


**Figure 1.** Growth of *D. labrax* larvae: total length (■); weight (◆). Each mean±SD is a pool of 30 larvae. Feeding regime was summarized by dashed lines.

gland formation indicating that it could be defined as a functional stomach (Figure 2E). The pancreas at this stage presented a diffuse distribution interspersed throughout the mesentery surrounding the intestine whereas the liver remained as a compact organ situated anterior and ventral to the digestive tract. Also, appearance of zymogen granules altered and spreading along the lumen of the tubules on 25 DAH. On 40 DAH, the digestive system, with the exception of the pyloric caeca, started the formation (Figure 2F). Until metamorphosis, the pancreas became enlarged, spreading throughout the mesentery enclosure, the stomach and the upper intestine. On the other hand, zymogen granules were numerous and larger, and a greater quantity of material was removed by the ducts, indicating increased cellular activity (Figure 2 and Figure 3).

### Trypsin Activity

The specific activity of trypsin was determined on day 3 (42.54±6.8 mU/mg protein$^{-1}$) at 4.28±0.2 mm. Total length of larvae and sharply increased immediately during the following days especially after exogenous feeding. As expected, from 6 DAH (mouth opening) specific tryptic activity continuously increased depending on larval age and size until 30 DAH. After that, approximately 1/3 rate a sharp decline was measured in this activity until 35 DAH ($P<0.05$). Then, this decline continued on specific activity of trypsin until the end of experiment ($P>0.05$). The highest tryptic activity was detected on 30 DAH as 122.45±11.76 mU/mg protein$^{-1}$ (Figure 4).

Generally, the developmental pattern of the total activity of trypsin was similar to that of specific activity on 30 DAH as exponential increase with larval age and development. After detection at hatching, it sharply increased and fluctuated up to 25 DAH ($P<0.05$). From this date, slight decreases were measured until end of the experiment ($P>0.05$). The peak of total activity was measured on 23 DAH as 9.22±0.61 mU/larva (Figure 4).

## Discussion

The digestive tract of many marine fish larvae undergoes numerous morphological and functional changes during ontogeny that can substantially influence larval survival under culture conditions. Increasing our knowledge on the digestive capacity and nutritional requirements of the larvae of cultured species for aquaculture will help in the development of optimal feeding protocols and greatly improve production under hatchery conditions. In this study, exocrine pancreas organogenesis and pancreatic protease, trypsin activity were investigated in the *D. labrax* larva until 40 DAH.

As a similar evolution as described for the other cultured species, histological determinations suggest that the development of the digestive tract, involving the presence of functional liver, pancreas and gall bladder, enabled early sea bass larvae to ingest, digest and assimilate the first exogenous food even before endogenous reserves were completely resorbed (Micale *et al*., 2006, 2008; Santamaria *et al*., 2004; Sarasquete *et al*., 1995). The first zymogen granules appeared 3 days after hatching and became abundant at the commencement of the trophic phase (Beccaria *et al*., 1991).

Exogenous feeding was onset with rotifers after 6 DAH. At mouth opening (6 DAH), the gall bladder, liver and pancreas were already differentiated and connected to the intestine by the bile and main pancreatic duct, respectively. On 3 DAH, the first zymogen granules were observed into exocrine pancreas. *D. labrax* larvae are able to digest carbohydrates, and then on 5-6 DAH first lipid and protein absorption was discernible in vacuolar texture of midgut and hindgut. It was suggested that activity of some pancreatic enzymes, i.e., protease, amylase could be defined related with morphological development of zymogen granules according to other authors (Caruso *et al.*, 2001; Micale *et al.*, 2006; Suzer *et al*., 2007c). Additionally, it is reported that ontogenetic expression digestive proteolytic enzymes,

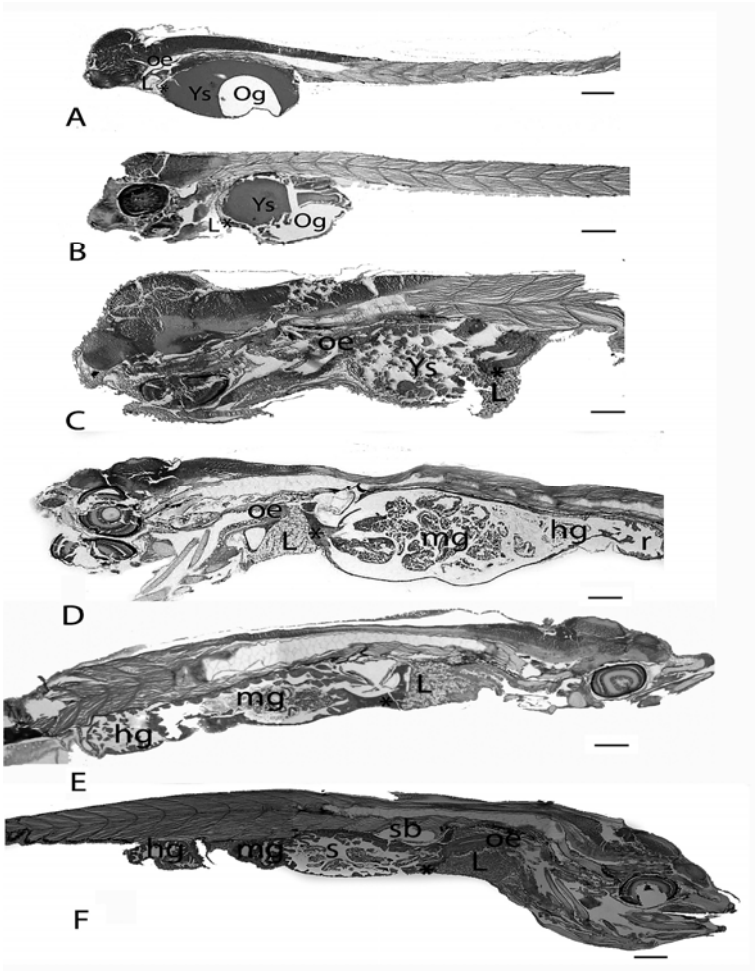


**Figure 2.** Organogenesis of the exocrine pancreas and associated structures during larval ontogeny of *D. labrax*. (A) 1 DAH, (B) 3 DAH, (C) 6 DAH, (D) 10 DAH (E) 25 DAH and (F) 30 DAH. Stars; indicate the pancreas, L; liver, og; oil globule, ys; yolksac, oe; oesophagus, mg; midgut, hg; hidgut, r; rectum, s; stomach, sb; swim bladder. Scale bar = 200 µm.

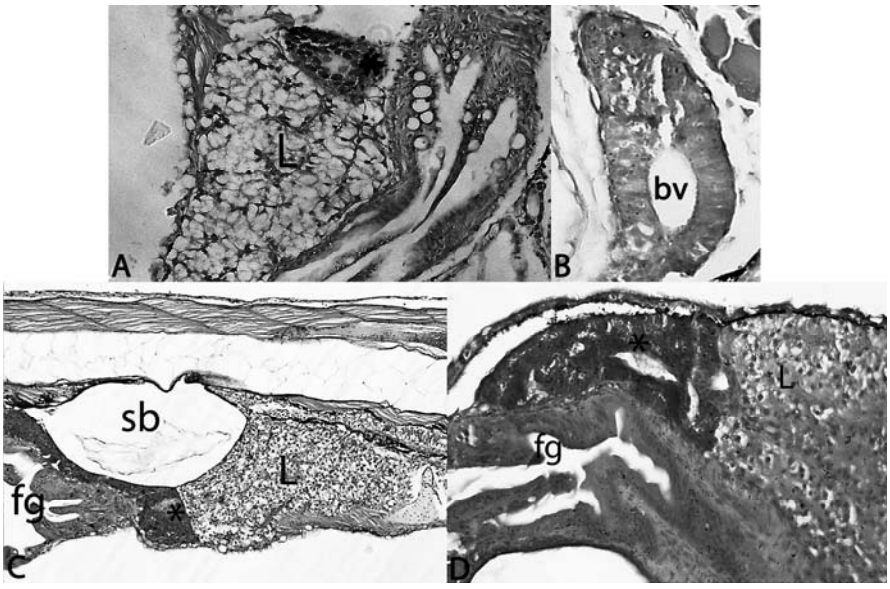


**Figure 3.** Light micrographs of the differentiation of pancreas during ontogenesis in larval sea bass. Stars; indicate the pancreas, L; liver, fg; foregut, bv; blood vessel, sb; swim bladder.

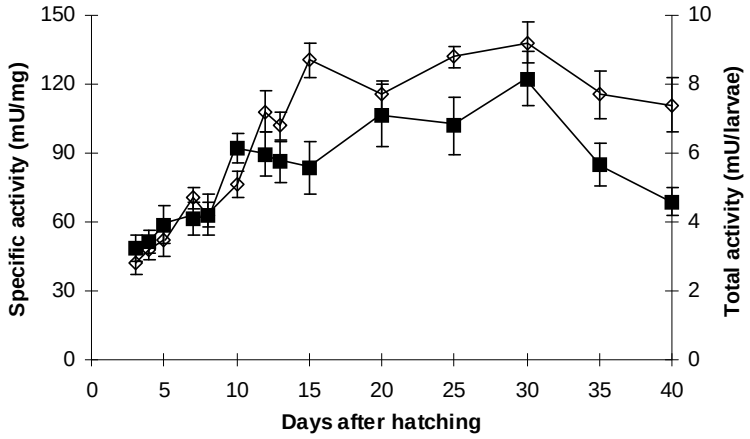


**Figure 4.** Specific (mU/mg protein) and total (mU/larva) activities of trypsin during larval development of *D. labrax* up to day 40. Results are expressed as means ±SD (n=5).

trypsin and chymotrypsin, and also amylase were detected concurrently with the formation of zymogen granules in some cultured species such as common pandora, *Pagellus erythrinus* (Suzer *et al*., 2006), red porgy, *Pagrus pagrus* (Suzer *et al*., 2007b), Senegale sole, *Solea senegalensis* (Ribeiro *et al*., 1999), and yellowtail kingfish, *Seriola lalandi* (Chen *et al*., 2006). Furthermore, as in some Sparid larvae, the cells of the liver and pancreas of *D. labrax* are similar to each other and liver appeared compact basophilic tissue next to the yolk-sac (Sarasquete *et al*., 1995; Guyot *et al*., 1995; Santamaria *et al*., 2004).

It is well known that the secretion of trypsin is known to occur in response to food ingestion and in larval pancreatic tissue most of the trypsin is present as an enzymatically inactive trypsinogen, whereas most of the trypsin in the intestinal tract is enzymatically active (Ueberschär, 1993; 1995). Also, it is well recorded that trypsin and chymotrypsin are specific of pancreatic protein hydrolysis (Nolting *et al*., 1999; Zambonino Infante and Cahu, 2001). Furthermore, onset and changes of development patterns of trypsin and chymotrypsin in larval fish could be genetically controlled and increased by age and size (Zambonino Infante and Cahu, 2001). A similar pattern about activities of these protease increases in early stages and then relative reduction is reported in some Sparids such as *P. erythrinus* (Suzer *et al*., 2006), *P. pagrus* (Suzer *et al*., 2007b), sharpsnout seabream, *Diplodus puntazzo* (Suzer *et al*., 2007a) and other marine fish larvae such as herring larvae, *Clupea harengus*, (Pedersen and Andersen, 1992) and tilapia larvae, *Oreochromis niloticus* (Drossou *et al*., 2006). The above stated decline in specific enzyme activities of these digestive proteases during larval ontogeny of *D. labrax* could be basically explained by the normal increase of tissue proteins in growing larvae, which reflects anatomical and physiological changes in fish larvae, and does not correspond to a lowering in the amount of digestive enzymes or dietary shifts (Zambonino Infante and Cahu 2001). In this study, trypsin activity was firstly detected on day 3 before mouth opening. In other papers, it was recorded that trypsin activity observed before mouth opening on day 3-4, and also this result paralleled with previous studies of *D. labrax* larvae (Cahu and Zambonino Infante, 1994; Zambonino Infante and Cahu, 1994; Zambonino Infante and Cahu, 2001). Moreover, it is reported that dietary shifting and modulations are strongly affected tryptic activity in marine fish larvae (Nolting *et al*., 1999; Kolkovski, 2001; Zambonino Infante and Cahu, 2001). As described by several authors, substitution of live food by microdiet in *D. labrax* larvae especially caused to fluctuations in trypsin activity due to dietary protein content of food (Cahu and Zambonino Infante, 1994; Zambonino Infante and Cahu, 1994; Nolting *et al*., 1999). In this study, it was observed that specific activity of trypsin changed by live food and microdiet introduction.

It is reported that the fluctuations in specific enzyme activities covered the period of morphological differentiation in the digestive tract and associated glands (Zambonino Infante and Cahu, 2001). After the formation of gastric glands, the digestive system became functional and the specific activities of these digestive enzymes remained constant, while the total enzyme activities increased gradually with age. As reported in some fish larvae, trypsin specific activity could be decreased with age and size after gastric gland formation (Cahu and Zambonino Infante, 2001; Kolkovski, 2001; Zambonino Infante and Cahu, 2001). Similar pattern for tryptic activity as an important decrease was found in *P. erythrinus*, *P. pagrus*, *S. senegalanesis, S. aurata* and *D. labrax* larvae (Moyano *et al*., 1996; Ribeiro *et al*., 1999; Zambonino Infante and Cahu, 2001; Suzer *et al*., 2006; 2007c). The second

phenomenon supported for this decline in tryptic activity could be considered that ontogenic development of brush border membrane and enterocyte mainly occurred in the same time. In previuos studies, it could be noted that specific activity of alkaline phosphatase and aminonpeptidase N were gradually increased with the decline of tryptic activity and formation of gastric glands (Cahu and Zambonino Infante, 1994; Zambonino Infante and Cahu, 1994; Zambonino Infante and Cahu, 2001).

It could be concluded that the ontogenic development of the digestive system of *D. labrax* larvae followed the same general pattern that most cultured species described to date. Also, it is commonly known that exocrine pancreas organogenesis is the main critical stage of the trypsin expression concurrently with the onset of the larval feeding. This developmental pattern suggests that trypsin contributes to protein digestion in *D. labrax* larvae by synchronously compensating with trypsin for the absence of pepsin until formation of functional stomach. Moreover, it can be determined that the patterns of activity of the primary digestive enzymes involved in the digestive processes of larval *D. labrax* indicate early functional development of this system.

## Acknowledgements

The authors would like to express their sincere gratitude to Assoc. Prof. Dr. Hakan POSTACI for the excellent cooperation for histological analysis and also the staff of the Kılıç Sea Products Inc., Oren, Milas, Muğla, TURKEY where the experiments were conducted with their most efficient technical assistance.

## References

Barnabe´, G. and Billard, R. 1984. L'aquaculture du bar et des sparide´s. Ed. INRA Publ. Paris, 542 pp.

Beccaria, C., Diaz, J.P., Connes, R. and Chatain, B. 1991. Organogenesis of the exocrine pancreas in the sea bass, *Dicentrarchus labrax* L. reared extensively and intensively. Aquaculture, 99: 339-354.

Bradford, M.M. 1976. A rapid sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72: 248-254.

Cahu, C.L. and Zambonino Infante, J.L. 1994. Early weaning of sea bass (*Dicentrarchus labrax*) larvae with a compound diet: effect on digestive enzymes. Comparative Biochemistry and Physiology, 109: 213–222.

Cahu, C.L. and Zambonino Infante, J.L. 2001. Substitution of live food by formulated diets in marine fish larvae. Aquaculture, 200: 161–180.

Caruso, G., Genovese, L., Micale, V., Spedicato, M.T. and Mancuso, M. 2001. Preliminary investigation of the digestive enzymes in *Pagellus erythrinus* (Linneo 1758) larvae. Mar. Freshw. Behav. Physiol., 34: 265– 268.

Chen, B.N., Qin, J.G., Kumar, S.M., Hutchinson, W.G. and Clarke, S.M. 2006. Ontogenetic development of digestive enzymes in yellowtail kingfish *Seriola lalandi* larvae. Aquaculture, 260: 264–271.

Diaz, M., Moyano, F.J., Garcia-Carreno, F.L., Alarcon, F.J. and Sarasquete, M.C. 1997. Substrate SDS-PAGE determination of protease activity through larval development in sea bream. Aquaculture International, 5: 461–471.

Drossou, A., Ueberschär, B., Rosenthal, H. and Herzig, K. 2006. Ontogenetic development of the proteolytic digestion activities in larvae of *Oreochromis niloticus* fed with different diets. Aquaculture, 256: 479–488.

Elbal, M.T., Garcia Hernandez, M.P., Lozano, M.T. and Agulleiro, B. 2004. Development of the digestive tract of gilthead sea bream (*Sparus aurata* L.). Light and electron microscopic studies. Aquaculture, 234: 215-238.

Falk-Petersen, I.B. and Hansen, T.K. 2001. Organ differentiation in newly hatched common wolffish. Journal of Fish Biology, 59:1465-1482.

Guyot, E., Dı´az, J.P. and Connes, R. 1995. Organogenesis of the liver in sea bream *Sparus aurata*. Journal of Fish Biology, 47: 427– 437.

Hamlin, H.J., Hunt von Herbing, I. and Kling, L.J. 2000. Histological and morphological evaluations of the digestive tract and associated organs of haddock throughout post-hatching ontogeny. Journal of Fish Biology, 57: 716-732.

Kjørsvik, E., Pittman, K. and Pavlov, D. 2004. From fertilization to the end of metamorphosis - functional development. In: E. Moksness, E. Kjørsvik and Y. Olsen (Eds.), Culture of cold-water marine fish. Blackwell Publishing Ltd., Oxford: 204-269.

Kolkovski, S. 2001. Digestive enzymes in fish larvae and juveniles-implications and application to formulated diets. Aquaculture, 200: 181–201.

Lazo, J.P., Mendoza, R., Holt, G.J., Aguilera, C. and Arnold, C.R. 2007. Characterization of digestive enzymes during larval development of red drum (*Sciaenops ocellatus*). Aquaculture, 265: 194–205.

Micale, V., Garaffo, M., Genovese, L., Spedicato, M.T. and Muglia, U. 2006. The ontogeny of the alimentary tract during larval development in common pandora *Pagellus erythrinus* L. Aquaculture, 251: 354-365.

Micale, V., Di Giancamillo, A., Domeneghini, C., Mylonas, C.C., Nomikos, N., Papadakis, I.E. and Muglia, U. 2008. Ontogeny of the digestive tract in sharpsnout sea bream *Diplodus puntazzo* (Cetti, 1777). Histology and Histopathology, 23: 1077–1091.

Morrison, M. 1993. Histology of the Atlantic cod, *Gadus morhua*: an atlas. Part 4. Eleutheroembryo and larva. Canadian Special Publication of Fisheries and Aquatic Sciences, 119C: 496.

Moyano, F.J., Diaz, M., Alarcon, F.J. and Sarasquete, M.C. 1996. Characterization of digestive enzyme activity during larval development of gilthead seabream (*Sparus aurata*). Fish Physiology and Biochemistry, 15: 121–130.

Nolting, M., Ueberschär, B. and Rosenthal, H. 1999. Trypsin activity and physiological aspects in larval rearing of European sea bass *(Dicentrarchus labrax)* using live prey and compound diets. Journal of Applied Ichthyology, 15: 138–142.

Pavlov, D.A. 1986. Developing the biotechnology of culturing White Sea wolffish, *Anarhichas lupus marisalbi*. II. Ecomorphological peculiarities of early ontogeny. Journal of Ichthyology, 26(6):156-169.

Pedersen, B.H. and Andersen, K.P. 1992. Induction of trypsinogen secretion in herring larvae (*Clupea harengus*). Marine Biology, 112: 559–565.

Ribeiro, L., Sarasquete, C. and Dinis, M.T. 1999. Histological and histochemical development of the digestive system of *Solea senegalensis* (Kaup, 1858) larvae. Aquaculture, 171: 293– 308.

Sarasquete, C., Polo, A. and Yu´fera, M. 1995. Histology and histochemistry of the development of the digestive system of larval gilthead seabream *Sparus aurata* L. Aquaculture, 130: 79–92.

Santamaria, C.A., Marin de Mateo, M., Traveset, R., Sala, R., Grau, A., Pastor, E., Sarasquete, C. and Crespo, S. 2004. Larval organogenesis in common dentex *Dentex dentex* L. (Sparidae): histological and histochemical aspects. Aquaculture, 237: 207–228.

Suzer, C., Fırat, K. and Saka, Ş. 2006. Ontogenic development of the digestive enzymes in common pandora, *Pagellus erythrinus*, L. larvae. Aquaculture Research, 37: 1565–1571.

Suzer, C., Aktülün, S., Çoban, D., Kamacı, H.O., Saka, Ş., Fırat, K. and Alpbaz, A. 2007a. Digestive enzyme activities in sharpsnout seabream (*Diplodus puntazzo*) larvae. Comparative Biochemistry and Physiology, 148: 470–477.

Suzer, C., Kamacı, H.O., Çoban, D., Saka, Ş., Fırat, K., Ozkara, B. and Ozkara, A. 2007b. Digestive enzyme activity of the red porgy (*Pagrus pagrus,* L.) during larval development under culture conditions. Aquaculture Research, 38: 1178–1785.

Suzer, C., Firat, K., Saka, S. and Karacaoglan, A. 2007c. Effects of early weaning on growth and digestive enzyme activity in larvae of Sea Bass (*Dicentrarchus labrax* L.). The Israeli Journal of Aquaculture–Bamidgeh, 59(2): 81-90.

Tseng, H.C., Grendell, J.H. and Rothman, S.S. 1982. Food, deodenal extracts, and enzyme secretion by the pancreas. American Journal of Physiology, 243: 304–312.

Ueberschär, B. 1993. Measurement of proteolytic enzyme activity: significance and application in larval fish research. In: B.T. Walther and H.J. Fhyn (Eds.), Physiological and biochemical aspects of fish development, University of Bergen, Norway: 233–239.

Ueberschär, B. 1995. The use of tryptic enzyme activity measurement as a nutritional condition index: laboratory calibration data and field application. ICES Marine Science Symposium, 201: 119–129.

Zambonino Infante, J.L. and Cahu, C.L. 1994. Development and response to a diet change of some digestive enzymes in sea bass (*Dicentrarchus labrax*) larvae. Fish Physiology Biochemistry, 12: 399–408.

Zambonino Infante, J.L. and Cahu. C.L. 2001. Ontogeny of the gastrointestinal tract of marine fish larvae. Comparative Biochemistry and Physiology, 130: 477–487.