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Ontogeny of the digestive tract of thick lipped grey mullet (*Chelon labrosus*) larvae reared in "mesocosms"

Dora Zouiten[a], Ines Ben Khemis[a, *], Raouf Besbes[a] and Chantal Cahu[b]

[a] INSTM (Institut National des Sciences et Technologies de la Mer), Laboratoire Aquaculture, BP 59, 5000 Monastir, Tunisia
[b] Unité Mixte INRA-Ifremer de Nutrition des Poissons, BP-70 Ifremer, 29 280 Plouzané, France

*: Corresponding author : Ben Khemis I., Tel.: +216 71 730 420; fax: +216 71 732 622, email address : ines.benkhmis@instm.rnrt.tn

Abstract:

This work describes the ontogeny of the digestive tract in thick lipped grey mullet (Chelon labrosus) larvae reared until day 36 post-hatching with the semi-extensive technology in mesocosms. Diet was constituted by live preys, rotifers, Artemia and wild zooplankton, then compound diet was added from day 20 (p. h.). Linear growth, weight growth and digestive enzymes specific activities were studied during larval ontogeny. Pancreatic enzymes (trypsin and amylase) and intestinal enzymes (leucine-alanine peptidase "Leu-ala", aminopeptidase N "AN" and alkaline phosphatase "AP") were assayed in larvae sampled throughout the rearing trial to evaluate gastrointestinal maturation along the development.

The trypsin specific activities were very high during the first two weeks and then declined as observed in marine fish species. A following increase in trypsin specific activity from day 20 was attributed notably to ingestion of particle compound diet. In contrast to the pattern generally described in fish larvae, amylase specific activity showed a continuous increase. This could be attributed to the fact that C. labrosus is an omnivorous species and suggests that the fish might be able to use efficiently diets containing higher levels of starch or other carbohydrates since the end of larval development.

Relative expression of intestinal brush border membrane enzymes (AP and AN) and cytosolic enzyme (Leu-ala), showed an abrupt increase of both AP/leu-ala and AN/leu-ala ratios at day 8 (p. h.), indicating that maturation of intestinal tract in C. labrosus larvae is particularly precocious. It is assumed that larvae of C. labrosus might support early co-feeding and weaning strategies, which could reasonably be initiated since mouth opening.

Keywords: *Chelon labrosus*; Mesocosm; Fish larvae; Intestinal enzymes; Pancreatic enzymes

1. Introduction

Mesocosm fish larval rearing appears an interesting and simple technology for mass production of thick lipped grey mullet *Chelon labrosus* juveniles, a target species for aquaculture (Crossetti and Cataudella, 1995) and impounded water seeding programs (Ben Khemis *et al.,* 2006). This hatchery technique is based on an environment, which offers natural food particles during the first critical feeding period of the fish larvae (Naas *et al*. 1991; Divanach and Kentouri, 2000). It has been used for larval rearing of many species with different ecological characteristics (Giannakourou, 1995; Nehr *et al*., 1996; Ben Khemis, 1997; Berg, 1997; Papandroulakis *et al*., 2004, 2005; Zouiten *et al*., 2004; Zaiss *et al*., 2006). These studies pointed out that mesocosms provide an adequate environment for larval development. In addition, authors documented better quality of mesocosm reared larvae and their similarity to wild concerning morphological characteristics (Koumoundouros *et al.,* 1997; Boglione *et al.*, 2001). The "low technology" approach is considered a solution for species of moderate or low market value (Lee and Ostrowski, 2001) or small producers (Shields, 2001).
In mesocosm rearing technique, food from exogenous origin is added in order to avoid over grazing of the natural food chain by the top predator of the system, i.e. the larvae (Divanach and Kentouri, 2000). This added food includes both reared live preys (Georgalas *et al.*, 2007) and compound diet (Ben Khemis *et al.,* 2006) and is essential for improving fish growth and survival. The formulation of a compound diet adapted to the larval requirement of a fish species necessitates the understanding of the development of digestive system of this species. Development of digestive enzyme has been widely studied in many carnivorous fish species: sea bream (Calzada *et al*., 1998); winter flounder *Pleuronectes americanus* (Douglas *et al*., 1999); sea bass (Cahu and Zambonino-infante, 2001; Zambonino-Infante and Cahu, 2001), Atlantic cod *Gadus morhua* and Atlantic halibut *(*Kvåle et al, 2007) or omnivorous fish such as carp *Cyprinus carpio* (Escaffre et al.1997). Some studies reported the effect of the rearing condition or weaning on the development of digestive tract and enzymes (Cahu *et al.,* 1998; Hamza *et al*., 2007). Other works have shown that diet composition affects positively or negatively larval enzymatic development (Zambonino-Infante and Cahu, 1994; Buchet *et al.*, 2000; Tovar *et al.*, 2002; Savoie *et al.* 2006). The maturation process of digestive enzyme can be enhanced, stopped or delayed depending on composition of diet (Zambonino-Infante and Cahu, 2001). However, very few data concerning digestive enzyme development in thick lipped grey mullet are reported in literature. Nevertheless, growth characteristics and first data on phosphatase alkaline activity suggested a specially early development of intestine function in thick lipped grey mullet (Ben Khemis *et al.* 2006). The aim of the present work was to study the development of digestive system of the species, in order to provide data on larvae digestive traits during ontogeny, to verify the hypothesis of precocious digestive tract maturation and to determine whether digestion appears already oriented toward omnivorous feeding since early stages. Trypsin (a protease) and amylase (a glucosidase) were both assayed to study the functional development of exocrine pancreas. For intestinal digestion, leucine alanine peptidase, a cytosolic peptidase and aminopeptidase N and alkaline phosphatase, two brush border membrane enzymes, were assayed to study enterocytes maturation. These enzymes were assayed in *Chelon labrosus* larvae sampled throughout an experimental mesocosm rearing trial.

2. Materials and methods

2.1. Broodstock spawning and larval transfer

The study was realized at the marine aquaculture center of INSTM "Institut National des Sciences et Technologies de la Mer" at Monastir (Tunisia). Thick lipped grey mullet fertilized eggs were obtained from captive broodstocks, kept since several years in 10 m^3 concrete tanks at the marine aquaculture center of INSTM. Two females (3.0 and 2.7 kg) received HCG and LHRHa following the protocol described in Besbes *et al,* (2002). The injected females and four running males (mean weight of 1.6 kg) were placed in a spawning tank and spontaneous spawns were obtained. The pooled spawn produced 782 000 eggs and the fertilization rate was 68%. The eggs were transferred in an incubation unit with a flow through of filtered and UV sterilized sea water (T=18.6; pH=8.45; S=37.7). Incubation lasted 5 days and hatching rate was 82%. On day 1 (p. h.),

larvae were distributed into three mesocosms enclosures at a rearing density of 1.5 larvae/liter (30 000 larvae per enclosure of 20 m^3).

2.2. Experimental and rearing conditions

Three cylindrical concrete tanks of 20 m^3 capacity coated with PVC liner were used as mesocosms. These enclosures were protected by a polyester-glass fibre greenhouse and shaded by individual black agricultural curtains fixed horizontally at 2 m over the water surface. Initial filling and subsequent renewal during rearing used natural seawater pumped in the adjacent Laguna of Khniss and filtered on a 360 µm mesh. Before larval distribution, a maturation period of 7 days during which water was maintained stagnant, was applied for plankton development (Divanach and Kentouri, 2000). Water quality was monitored daily for temperature, pH and dissolved oxygen. The rate of water renewal was maintained at 10% per day during the first week of rearing and then it was progressively increased to reach 40% on day 15 (p. h.) and 100% on day 35 (p. h.). It was adjusted according to dissolved oxygen contents of water to guarantee at least 5 mg l^{-1}. Natural and uncontrolled plankton blooms were let to develop within the mesocosms but in absence of any water fertilization to avoid excessive phytoplankton development (Ben Khemis *et al.*, 2006) and related larval death (Ben Khemis, 1997). Cleaning of enclosures by siphoning sedimented wastes was initiated on day 10 (p. h.) and was performed, each other day before weaning. After weaning, cleaning was performed daily to avoid water pollution. Siphoned wastes were filtered and checked for dead fish or excess of uneaten food. Total ammonia nitrogen, which is the main harmful product of fish metabolism and should be maintained below 0.5 ppm, generally does not represent a major problem in the larval unit due to scarce larval biomass (Moretti *et al.,* 1999; 2005). With water renewal, absence of fertilization, siphoning of sedimented wastes and development of phytoplankton, total ammonia nitrogen did not exceed 0.2 ppm in mesocosm enclosures.
The day of filling, water quality parameters were T=18.6 °C, S=37.5 ‰ and pH=8.2. Water temperature remained constant for the 7 days before the stocking of fish larvae. After larval distribution, it decreased gradually to 15±1 °C until day 11 (p. h.) but remained higher than in sea, where natural reproduction takes place in the wild. Then temperature increased progressively to 19.9± 0.6 °C and remained almost constant until the end of experimental trial. Aeration was provided in each enclosure by two air diffusers positioned on the bottom near the tank wall and diametrically opposed. Natural plankton blooms were let to develop within the mesocosms. Phytoplankton was not quantified but densities of wild zooplankton, corresponding to endogenous preys, were estimated daily from 10 l of water siphoned within enclosures and filtrated on a 30µm mesh to a final volume of 50 ml. Three sub-samples of 1 ml from the concentrated sample were then observed *in toto* under a binocular stereomicroscope for counting the zooplanktons.

2.3. Larval and juvenile feeding and rearing

The live preys included both wild zooplankton naturally developing in the enclosures, and reared *Brachionus* and *Artemia*. Reared preys were enriched, according to the instructions provided by the manufacturer, with DHA-Protein Selco (INVE) and DC-Super Selco (INVE) respectively. Adjunction of reared preys was performed once daily in the morning after zooplankton sampling. Until beginning of weaning (day 20 p. h.), the density of potential preys, considered as the sum of endogenous and added zooplankton, was maintained over 0.3 individual ml^{-1}. The wild zooplankton was dominated by copepods, mainly nauplii and copepodite stages, and wild rotifers as *Synchaeta*. Daily mean preys densities of the enclosures are given in Fig. 1. Consumption of newly hatched *Artemia* type AF (INVE) was tested since day 10 (p.h) but distribution in rearing enclosures was initiated on day 12 (p. h.) after having observed its effective capture by the larvae. Between day 16 and day 20, small size *Artemia* (type AF - INVE) was progressively shifted to one day enriched large size *Artemia* (type EG - INVE). The delivery of the commercial formulated diet Replace II from Rich SA (100-300 µm) began on day 20 (p. h.), after development of intestinal adult mode of digestion according to our preliminary study (Ben Khemis *et al.*, 2006). Formulated feed proximal composition was 58% proteins, 18% lipids, 8% carbohydrates. The complete weaning onto artificial diet was achieved on day 33 (p. h.) after a co-feeding period of 16 days, including three days of drastic reduction of *Artemia* rations. Daily rations of formulated feed were 1, 5, 7 and 20 g per enclosure from day 20 to day 23, from day 24 to day 28, from day 29 to day 31 and from day 32 to day

36, respectively. These rations, based on previous results and observation of both behavior and feed ingestion, were subdivided into 12 to 20 fractions and distributed manually. During co-feeding, at least four meals were given prior any live preys distribution (Ben Khemis *et al.,* 2006).

2.4. Larval sampling and analysis

Larvae were always sampled before morning food distribution. For growth study, 15 larvae were sampled in each enclosure daily from day 1 to day 10 and then on day 14, 20, 28 and 36. They were anesthetized with ice-cold seawater, then fixed with glutaraldhyde (2.5% in phosphate buffered solution pH 7.4) and kept refrigerated until length and weight measurement. Photographs of the fish were taken using a digital camera (Nikon Coolpix 4500) mounted on a trin-ocular stereomicroscope (Hund Wetzlar) and length measurements were carried out using image analysis software ImageJ 1.29. An object micrometer was photographed with each set of photos to avoid errors due to the auto-focus of the camera. Drained weights of fixed larvae were measured immediately after photographing. Larvae were weighed individually, except for specimens of less than 5mg (i.e. larvae less then 20 days old) for which pooled weights were taken.
For enzymatic assay, larvae were sampled on day 0, 4, 8, 14, 20, 28 and 36. Samples corresponded to 120, 70, 60, 40, 30, 20, 15 larvae collected from each enclosure, respectively. They were immediately stored at -80°C pending assay. Before homogenizing in ice cold distilled water, larvae were vortexed in 500 µl ice cold distilled water during 30 sec to obtain released enzyme (supernatant S1). This supernatant contained the secreted pancreatic enzymes, i.e. trypsin and amylase (Ma *et al.,* 2005). The larvae were then homogenized in 1 to 2 ml ice cold distilled water, depending of the weight of the sample, with a homogenizer (Polytron, PT-MR 2100) during 30 sec then centrifuged at 3300×g for 3min. This supernatant (S2), was used to analyze unreleased pancreatic enzymes (trypsin and amylase) and intestinal enzymes (alkaline phosphatase [AP], aminopeptidase N [AN] and leucine-alanine peptidase [Leu-ala]). Amylase and trypsin activities were assayed according to Métais and Bieth (1968) and Holm *et al.* (1988), respectively. The brush border membrane enzymes, alkaline phosphatase and amminopeptidase N were assayed according to Bessey *et al.* (1946) and Maroux *et al.* (1973), respectively. Assay of the cytosolic leucine-alanine peptidase was performed using the method of Nicholson and Kim (1975). Activities were measured as µmoles of substrate hydrolysed min/mg protein, at 37°C for AP, Leu-ala and AN, and at 25°C for trypsin (Zambonino-Infante and Cahu, 1994). Amylase activity represented the equivalent enzyme activity required for hydrolyzing 1 mg of starch in 30 min at 37°C (Zambonino-Infante and Cahu, 1994). Enzyme activities were expressed as segmental activity (sum of S1 and S2 mU or U/larvae) and specific activity (mU or U/mg protein). Protein was determined by the Bradford procedure (Bradford, 1976).
The result are given as means ± S.D (n=3). The variance homogeneity of the data was checked using Levene's test. Ratios of segmental enzymes activities were $\arcsin(x^{1/2})$ transformed. Results were compared by analysis of variance (ANOVA) followed by the Tukey's test when differences were found at $\alpha=0.05$ (Zar, 1999). Statistics were performed using Statistica 5.5 (StatSoft, Inc.).

3. Results

The larvae measured 3.41±0.48 mm in total length at day 1 (p. h.) and 3.84±0.31 mm at day 5 (p. h.) when mouth opened. During all the rearing period, no significant differences of sizes or weights were observed between the larvae of the different enclosures. The results were hence analyzed globally after pooling. The linear growth of the larvae could be divided into two distinct phases. From hatching to day 14 (p. h.) larval growths was remarkably slow. At that stage, the larvae measured 4.05±0.50 mm. Afterward, growth accelerated sharply and the larvae reached 11.02±1.72 mm on day 29 (p. h.) and 14.12± 2.67 mm on day 36 (p. h.). Initial body weight of larvae was 0.62±0.02 mg. It reached 28.53± 9.15 mg at the end of the rearing trial. Both linear growth (Fig. 2A) and weight growth (Fig. 2B) presented similar patterns.
Segmental activities of pancreatic (Fig. 3A) and intestinal (Fig. 3B) enzymes, which reflect enzyme content per larva, showed an increase comparable to larvae growth. Trypsin and amylase activities were detected from

hatching, both in supernatant (S1) and in pancreatic tissues (S2). We choose to present specific activity of secreted pancreatic enzymes as this fraction (in S1) corresponds to the active enzyme in the intestinal lumen. Trypsin specific activity increased at mouth opening and remained high the following days (Fig. 4A). It decreased sharply at day 14 (p. h.) and afterwards it showed a progressive increase until day 28 (p. h.) and remained at high level until the end of the experiment. Amylase specific activity showed a regular increase from hatching to day 20 (p. h.) and reached a plateau (Fig. 4B).
Specific activities of Leu-ala peptidase (table 1) decreased progressively between hatching and day 8 (p.h.) while specific activities of aminopeptidase N and phosphatase alkaline increased with larval age from mouth opening until day 14 (p. h). Then, aminopeptidase specific activity maintained at a same level while alkaline phosphatase specific activity decreased.
Both ratios of intestinal enzymes AN/Leu-ala and AP/Leu-ala showed a sharp increase at day 8 (p. h.) (Fig 5). Afterwards, AN/Leu-ala ratio varied slightly until the end of the experiment while AP/Leu-ala ratio decreased progressively.

4. Discussion

Growth of *Chelon labrosus* larvae (size and weight) in the present work exhibited a similar pattern of changes compared to the previous descriptions of Cataudella *et al*. (1988) and Boglione *et al*. (1992) or our earlier work (Ben Khemis *et al*., 2006). It seems hence that the lower initial rearing temperatures did not affect larvae development in a major way. It comes out that the two first weeks of life of *C. labrosus* larvae are characterized by a remarkably slow growth. We previously suggested that this extended low initial growth is characteristic of early larval development of mullets and proposed that it could be due to allocation of available energy and building materials in priority to physiological changes (among which digestive functions) rather than in size growth. The study of digestive development is consistent with this hypothesis. During the present experiment, larvae received live prey, until day 32 (p. h.). This diet can be considered as natural food allowing proper development in mullet like in other marine fish larvae species, as pointed out by Ma *et al.* (2005) in the yellow croaker (*Pseudosciaena crocea*). The ontogenetic changes of the intestinal tract of thick lipped grey mullet observed in this work should represent theorically the normal development pattern, i.e. the pattern which should be observed in the wild, at the same temperature, when larvae can eat *ad libitum*.
It has been demonstrated that digestive tract and digestion process undergo major developmental changes during the first weeks of life in fish (Walford and Lam, 1993; Sarasquete *et al.*, 1995; Zambonino-Infante and Cahu, 1994, 2001). Pancreas secretion function constitutes the first step of maturation process of digestive function, and the second is the onset of brush border membrane enzymes in the intestine (Ma *et al.*, 2005). Larvae of *Chelon labrosus* exhibited high trypsin activities during endogenous feeding (before mouth opening) and the first feeding days, indicating a large potential to synthesize enzymes, as generally observed in fish larvae (Cahu and Zambonino-Infante, 2001). The observed decline in trypsin specific activity which reached the minimum at 14 (p. h.) should be attributed to larvae growth, and thus to an increase of body protein. In fact, specific activity is the ratio activity per mg protein and does not reflect a lowering in digestive capacity (Ma *et al.*, 2005), as demonstrated by the continuous increase of segmental trypsin activity. So, the initial pattern of trypsin enzyme activities during thick lipped grey mullet development was similar to that already described in other species, seabass, sole and red drum (Zambonino-Infante and Cahu, 2001). The increase of trypsin specific activity observed from day 20 onwards could be attributed to two factors. In one hand, growth sharply increased from day 20 and trypsin has been shown to be a growth indicator in fish larvae (Rungruangsak-Torrissen *et al.,* 2006). On the other hand, enriched *Artemia* and compound diet were introduced since day 16 and day 20 (p. h.), respectively. Hence, the food quantity considerably increased in mesocosms. It was demonstrated in fish larvae that trypsin activity responds to an increase in dietary rations (Zambonino-infante *et al.*, 1996; Pedersen *et al*., 2003). Furthermore, the compound diet incorporates a high protein level, 58%, and trypsin activity should be related to dietary protein concentration as reported by Péres *et al.* (1998) in sea bass larvae.
Amylase specific activity has been shown to be high during young larval stages and generally decrease during the development of the larvae (Cahu and Zambonino-Infante, 2001). In this study, amylase specific activity

showed a continuous increase during the first 20 days of larval development and was maintained at a high level onwards. This continuous increase and elevated plateau should not be induced by an augmentation of dietary carbohydrates as observed by Ma et al (2005) in yellow croaker larvae. Indeed, the compound diet was introduced on day 20 (p. h.), i.e. at the end of amylase specific activity increasing period. In addition, it contained 8% carbohydrates, which is not different of carbohydrate content of copepods and *Artemia* (Ma *et al.*, 2005). Thus, this capacity to synthesize amylase at developed larval stages can be attributed to *C. labrosus* digestive specificity. Comparative studies of digestive enzymes in fish with different nutritional habits have demonstrated that amylase activity is greater in herbivorous and omnivorous fish than in carnivorous fish (Hidalgo *et al.*, 1999; Fernández *et al.*, (2001). Information on digestive enzymes of young mullets is scarce (Das *et al.*, 1987; Barman *et al.*, 2005). But it is known that adult thick lipped grey mullets feed mainly on benthic diatoms, epiphytic algae, small invertebrates and detritus (Ben-Tuvia, 1986; Crossetti and Cataudella, 1994). The capacity of synthesizing amylase and thus to feed on vegetal resources seems to be expressed since early larval development stages in *C. labrosus*. The existence of well developed amylase synthesis capacity may be an interesting feature from the perspective of feed formulation (Fernández *et al.*, 2001). The young thick lipped grey mullets would be able to use efficiently diets containing high levels of starch or other similar low cost amylolitic energetic compounds. The utilization of carbohydrates can help achieve the development of diets with minimized cost as it is an inexpensive source of energy (Lazo *et al.,* 2007).

The intestinal enzymes activities assayed in *C. labrosus* larvae, exhibited inverse evolutions between cytosolic and brush border membrane enzymes. Leucine alanine peptidase specific activity decreased from hatching until a minimum reached at day 8 (p.h.) while both phosphatase alkaline and aminopeptidase N increased. This pattern is typical of intestinal maturation, indicating that brush border enzymes relayed cytosolic enzyme for digestion as described in Zambonino-Infante and Cahu (2001).

The ratio of a brush border membrane enzyme activity to a cytosolic enzyme activity is considered as an indicator of the development of intestinal digestion (Zambonino-Infante and Cahu (2001). Indeed, maturation of intestine epithelium of larvae is initiated few days after hatching with the differentiation of the brush border membrane (BBM) of enterocytes (Vu, 1983). Then, the maturation process is characterized by a sharp increase in BBM enzymes (Kvåle *et al.*, 2007) associated in some species with a coexisting decrease in cytosolic enzymes (Zambonino-Infante and Cahu, 2001). The maturation process of enterocytes has been extensively described in several fish species such as common carp *Cyprinus caprio* (Escaffre *et al.,* 1997), sea bream *Sparus aurata* (Calzada *et al*., 1998), red drum *Sciaenops ocellatus* (Buchet *et al*., 2000), yellow croaker *Pseudosciaena crocea* (Ma *et al*., 2005; Mai *et al*., 2005). A proper achievement of this process determines larval survival according to Cahu and Zambonino-Infante (1995).

In sea bass larvae, the maturation of intestine, which characterizes the adult mode of intestinal digestion, ends approximately in the fourth week of post-hatching development (Cahu and Zambonino-Infante, 1995). In cold water species such as cod (*Gadus morhua*) and halibut (*Hippoglossus hippoglossus*) the maturation occurs later, at approximately 40 to 50 days post first feeding (Kvåle *et al.*, 2007) while in subtropical species such as red drum (*Scianops ocellatus*), it occurs around day 18 (Buchet *et al.*, 2000). In our study, both AP/leu–ala and AN/leu–ala ratios increased sharply at day 8 (p. h.) indicating that maturation of intestinal tract of thick lipped grey mullet is particularly precocious. These results suggest that thick lipped grey mullet larvae could be weaned at very early stages, just few days after mouth opening. In fact, the intestinal maturation was associated to the acquisition of a capacity of marine fish larvae to digest compound diet, i.e to be weaned successfully; yet good weaning results depend on a diet that fulfils the larval requirement and takes into account the digestive features (Kvåle *et al.*, 2007).

After intestinal maturation, AP/leu–ala ratio showed significant decreases in young *C. labrosus*, contrarily to the pattern generally observed in fish larvae. It could be attributed to early acquisition of digestive features of the species feeding regime. German *et al.* (2004) observed a decrease in alkaline phosphatase activities in two herbivorous prickleback species (*Cebidichthys violaceus* and *Xiphister mucosus*) as the fish shifted from a carnivore diet toward an algal diet during ontogeny. Furthermore, these authors showed that digestive enzyme activity is genetically programmed to match ontogenetic shifts in diet.

5. Conclusion

This study presents the first data on digestive enzymes quantification in *C. labrosus* larvae. The results point out that the capacity of synthesizing amylase and thus to feed efficiently on vegetal sources seems to be expressed since early larval development stages in *C. labrosus.* Hence a diet with higher levels of glycolytic compounds could be introduced into larval feeding sequence.
The results also indicate that maturation of intestinal tract is particularly precocious, suggesting that these larvae could be weaned at very early stages, just few days after mouth opening. Using last generations of high performance microdiets, we would reasonably suggest applying co-feeding strategies since mouth opening.

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Figures

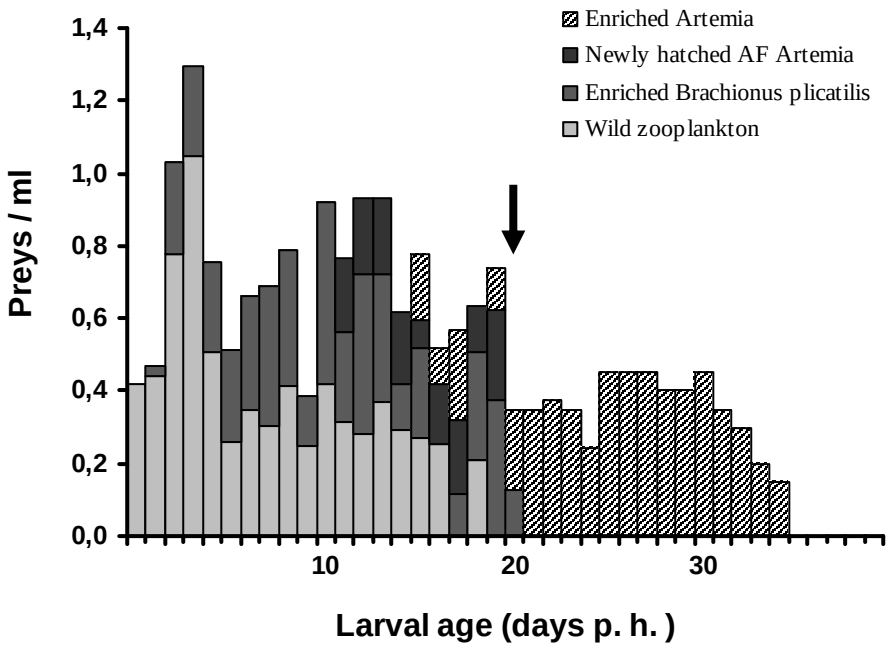

Fig. 1: Mean daily estimation of endogenous and exogenous live prey densities in rearing enclosures. Arrow indicates beginning of compound diet delivery.

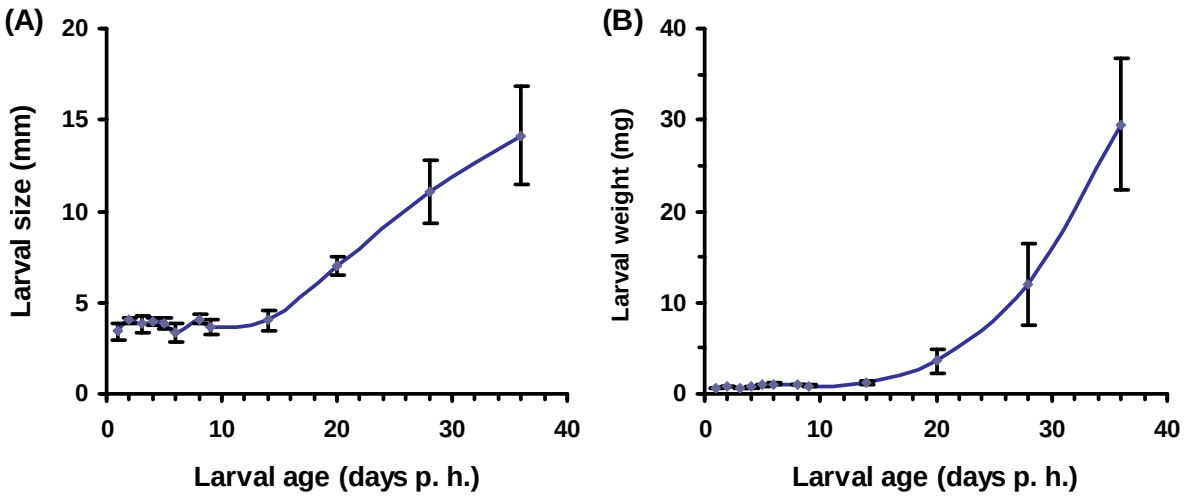

Fig. 2: Growth of *Chelon labrosus* larvae in mesocosm. Data are given in mean ± SD and are expressed in mm for total body length (A) and in mg for weight (B).

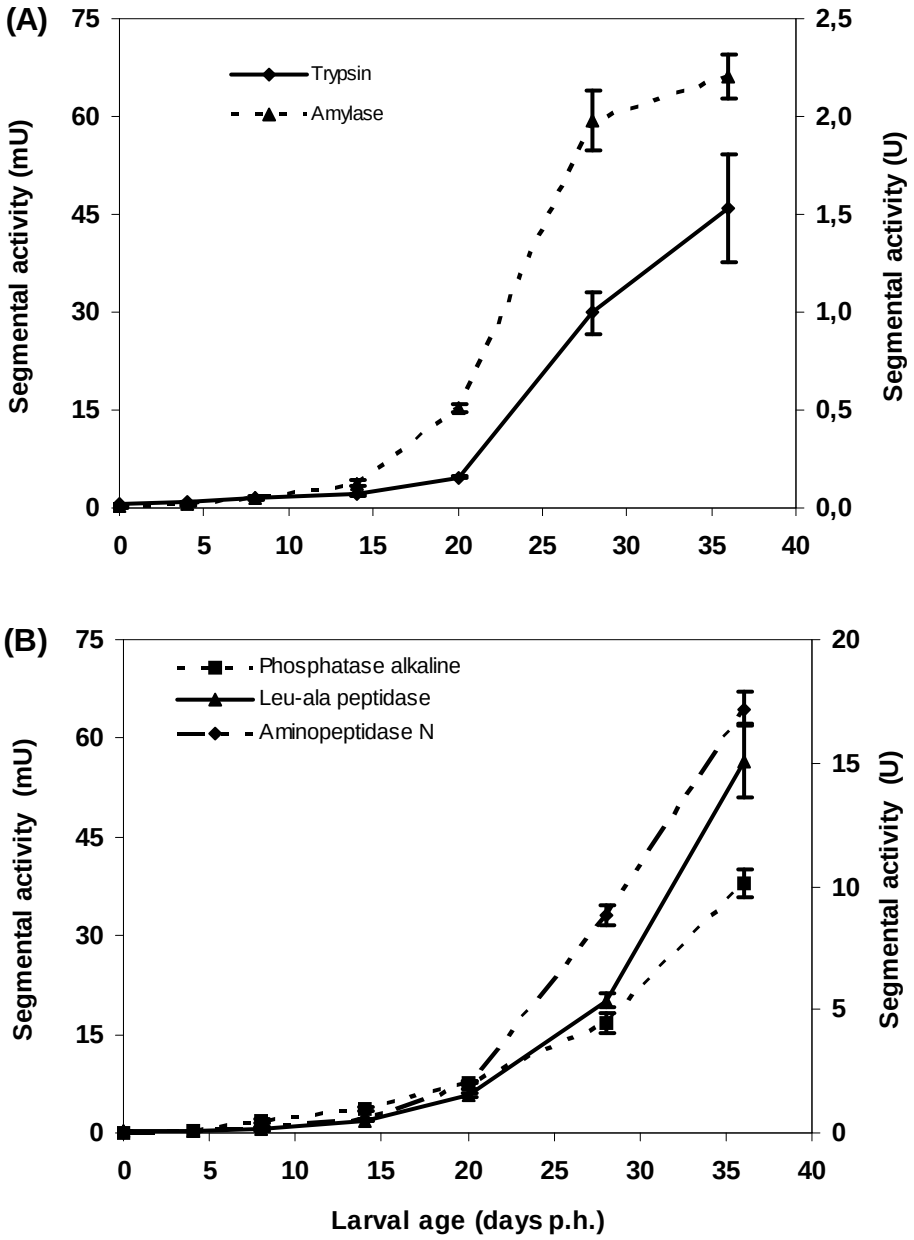

Fig. 3: Segmental pancreatic (A) and intestinal (B) enzyme activities in *C. labrosus* larvae. Data are given in mean ± SD (n=3). They are expressed in mU for trypsin, aminopeptidase N and phosphatase alkaline and in U for amylase and leucine-alanine peptidase.

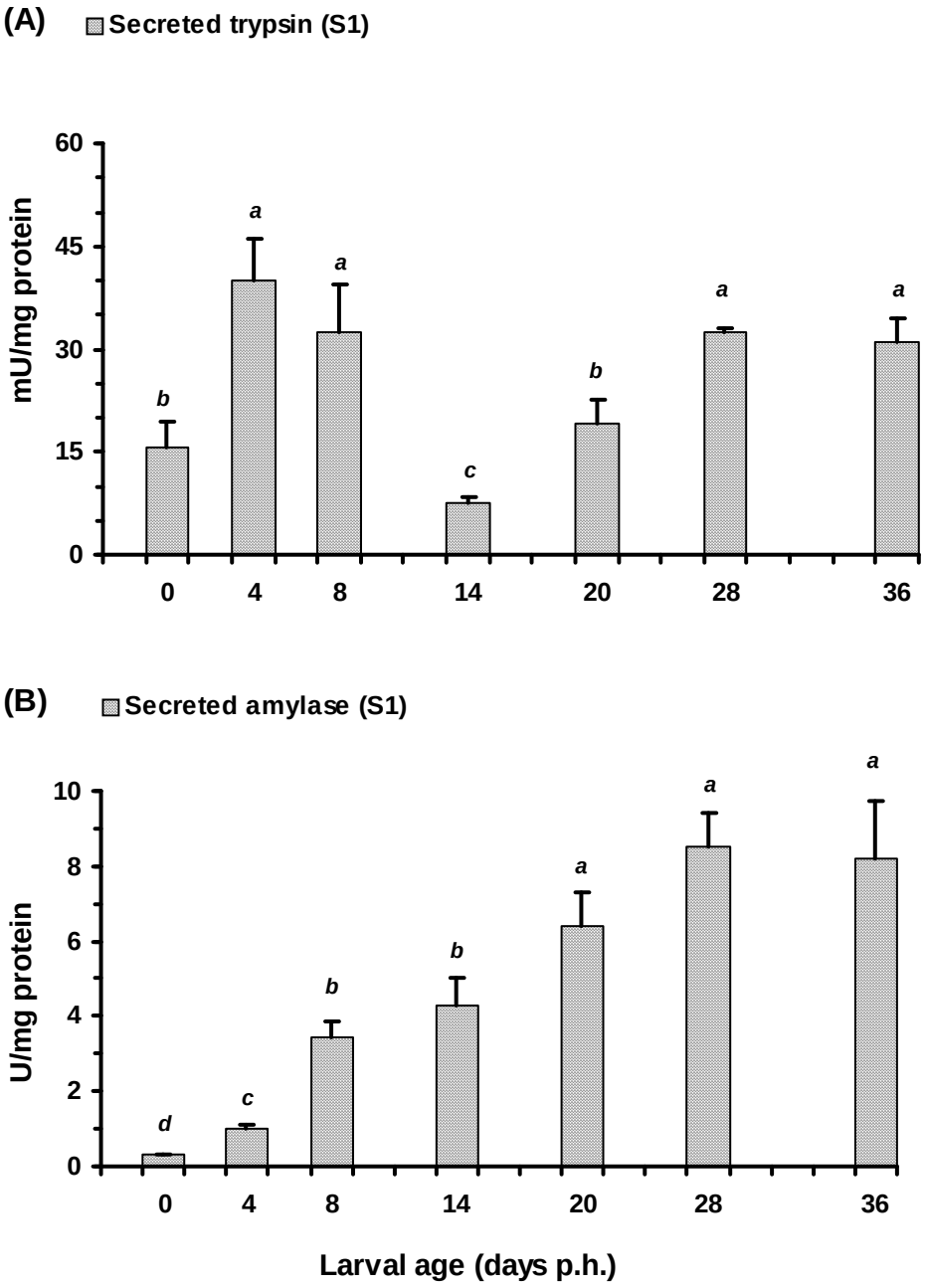

Fig. 4: Specific activities of secreted trypsin (A) and amylase (B) during larval development of *C. labrosus* larvae. Data are given in mean ± S.D (n=3). Different letters indicate significantly different values (α=0.05) according to Tukey's test.

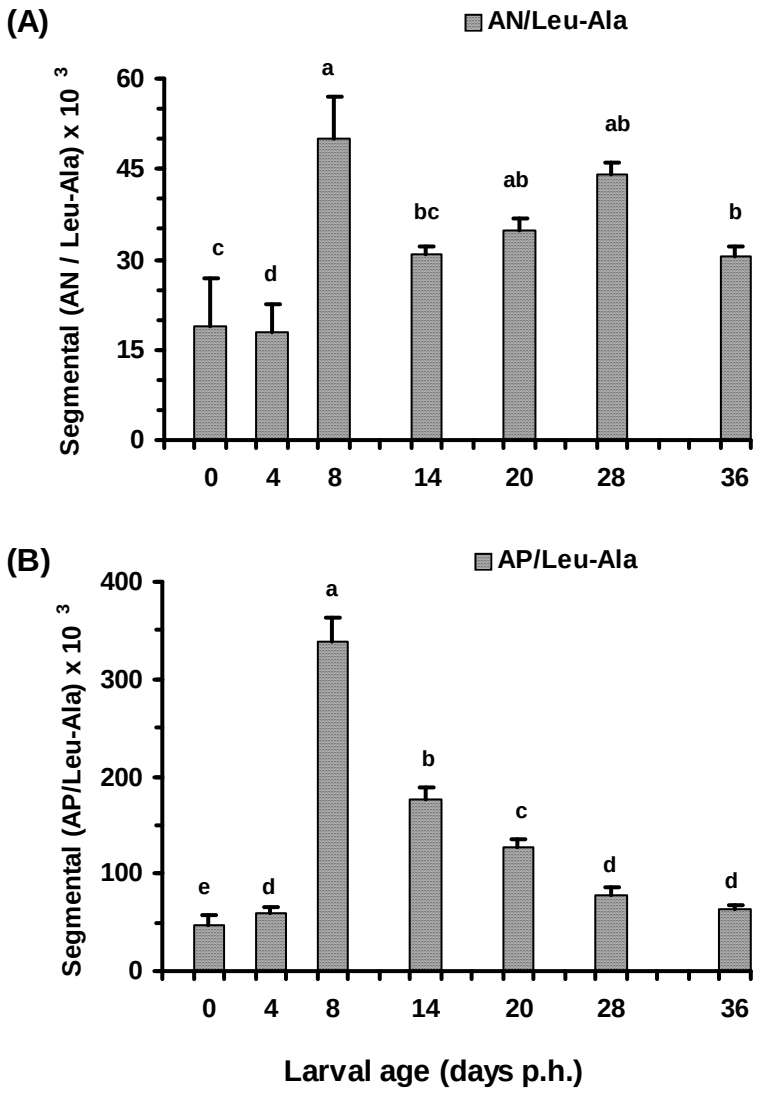

Fig. 5: Ratios of intestinal enzymes, AN/Leu-ala (A) and AP/Leu-ala (B) during larval development of *C. labrosus*. Data are given in mean ± S.D (n=3). Different letters indicate significantly different values (α=0.05) according to Tukey's test.

Tables

	Cytosolic enzyme	Brush border enzymes	
Age (day p.h.)	Leu-ala peptidase (U/mg protein)	Aminopeptidase N (mU/mg protein)	Alkaline phosphatase (mU/mg protein)
0	55,56 ±5,65 (d)	10,30 ±3,55 (d)	26,11 ±3,74 (d)
4	42,30 ±1,70 (e)	7,53 ±1,68 (d)	25,18 ±1,90 (d)
8	33,07 ±2,89 (f)	16,50 ±1,62 (c)	112,69 ±17,55 (b)
14	80,51 ±3,97 (b)	24,77 ±1,69 (b)	140,88 ±5,28 (a)
20	83,28 ±2,03 (b)	28,87 ±1,10 (ab)	106,25 ±5,22 (b)
28	65,16 ±1,71 (c)	28,70 ±1,26 (ab)	51,24 ±6,33 (c)
36	103,56 ±1,47 (a)	31,63 ±2,25 (a)	66,88 ±3,55 (c)

Table 1: Specific activities of intestinal enzymes assayed on the larvae homogenates of *C. labrosus*. Data are given in mean ± S.D (n=3). Different letters indicate significantly different values (α=0.05) according to Tukey's test.