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Ontogenetic changes in the digestive system of *Pleoticus muelleri* (Decapoda, Penaeoidea)

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Summary

The present study describes the ontogenetic changes observed in the histology and in total protease, trypsin, and chymotrypsin activities of the digestive system in the shrimp *Pleoticus muelleri* (Decapoda, Penaeoidea) under culture conditions. The stomach development follows the typical pattern described for other decapods. The gland filter develops during larval stages, while the gastric mill takes the adult shape during postlarval metamorphosis and juvenile stages. During early larval stages, the histological structure of the anterior and lateral caeca is similar to that of the adult midgut gland, with R, F and B cells. During late larval stages, the anterior caeca decline and take a structure similar to that of the mesodeum and the lateral caeca expand to form the adult midgut gland by proliferation of tubules in antero-posterior direction and from the cortical region to the medullar region. Total protease activity was higher in postlarvae 45, no significant differences was found in the others larval and postlarval stages. Trypsin activity was lowest in early postlarval stages (PL1 and PL6), coinciding with the metamorphosis; enzyme activity increased in postlarvae 10 followed by a significant decrease in postlarvae 26. Chymotrypsin showed a significantly lower activity in protozoea 3, a peak of activity between postlarvae 1 and 10, and a decrease in the following postlarval stages. The inhibition of trypsin and chymotrypsin activities were confirmed the presence of these serine proteases during developmental stages. The ontogenetic pattern of *P. muelleri* digestive system morphology is similar to those described of others penaeoids. The recorded variation in enzyme activity during developmental stages may be associated with the unique postlarval life history. This research has implications for artificial diet development in crustacean culture and understanding of dietary shifts during larval development.

Key words: Crustacea, ontogeny, digestive system

Introduction

The red shrimp *Pleoticus muelleri* has a large distribution area in southwest Atlantic waters from 20°S, Espíritu Santo, Brazil, to 50°S, Santa Cruz, Argentina; the regions of greater abundance occurs in the temperate waters of Argentine, at temperatures ranging from 6 to 23°C, and salinities between 31.5 and 33.5‰ (Boschi, 1986). This species is considered one of the most important fishing resources from the Argentine Sea because of its value in the national and international

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markets and the excellence of its meat. However, catches are variable with annual and seasonal fluctuations; for this reason its culture is very important, making possible a continuous supply of the product in the market (Díaz, 2001).

In penaeid shrimps, the ontogenetic development involves several states that are evidenced by morphological, anatomical, physiological, and biochemical changes (García Carreño and Hernández Cortés, 1997). *P. muelleri* larval development comprises six naupliar stages, three protozoea stages and two mysis stages (Iorio et al., 1990). Postlarval stage extends up to the moment larva reaches 20 mm total length, between 20 and 100 mm total length are considered juveniles (Boschi, 1989). Under massive culture conditions, at 20°C, the naupliar stage lasts from 2 to 3 days, protozoea stage between 7 and 10 days, and mysis stage between 5 and 8 days, reaching the first postlarvae at 14–21 days (Mallo et al., 1999).

Penaeid larvae are temporary members of the zooplankton community and as such derive their nutrition from within the planktonic ecosystem; they employ either filter or raptorial feeding methods depending upon their trophic feeding level. Since the stage of protozoea use either phytoplankton alone or a combination of phyto- and zooplankton as their major food resource. The benthic habit in *P. muelleri* appears in the postlarvae 10–12, under culture, they begin to feed at the bottom of the tank (Mallo, 2005).

The crustacean digestive system is basically a tubular structure extended along the body and divided into three regions: an anterior region or stomodeum, from ectodermic origin and covered by a chitinized cuticle that comprises the esophagus and the stomach with two chambers (the anterior chamber or cardiac stomach and the posterior one or pyloric stomach); a medial region or mesodeum, from endodermic origin, with caeca and diverticles, and a posterior region or proctodeum, also chitinized and from ectodermic origin. Midgut gland or mid-gut gland opens in the posterior part of the stomach (Díaz et al., 2006). Most of the studies about the morphology and function of crustacean digestive system were based on observations made in adult individuals (see Felgenhauer and Abele, 1985; Factor, 1995). The studies of ontogenetic changes of the digestive system are restricted to a few species, which includes among penaeoids: *Penaeus monodon* (Jones et al., 1987), *Marsupenaeus japonicus* (Nakamura and Tsuru, 1987) and *Litopenaeus setiferus* (Lovett and Felder, 1989, 1990).

In penaeids, proteolytic enzymes synthesized in the midgut gland play a key role in assimilation of food protein. Trypsin and chymotripsin are the most abundant proteolytic enzymes and are responsible for 60% of protein digestion (Muhlia-Almazán et al., 2003). From previous reports, the activity of the digestive enzymes changes according to size and developmental stage (Gonzalez et al., 1994; Lemos et al., 1999), this knowledge is necessary to formulate appropriate feeds to the physiology of each stage of culture. The present study aims to examine the histological changes of the digestive system during the ontogeny and to describe the proteases activity in larval and postlarval stages of the shrimp *P. muelleri* under culture conditions, and they will be compared with the functional morphology of adults shrimps, characterized in previous work (Fernández Gimenez et al., 2001; Díaz et al., 2006).

Material and Methods

Animal supply and maintenance

Specimens of *P. muelleri* were reared from hatchery-raised postlarvae (wild broodstock from Mar del Plata, Argentina) at Nagera Station, Marine Science Department, Mar del Plata National University, Argentina. Nauplii were transferred to 3.5 ton upwelling tanks and maintained at 21°C (±1°C). Larvae were reared on a diet of algae (*Chaetoceros gracilis*, *Tetraselmis chuii*) and *Artemia salina* nauplii. During the larviculture, the animals were fed microencapsulated feeds of different particle size: protozoea 1 (0.30 µm), protozoea 2 and 3 (30–90 µm), mysis and early postlarvae (80–150 µm) (Mallo et al., 1999).

Light microscopy

At each developmental stage (M2, PL 1, 6, 10, 26, 30, 45, and juveniles), 10 specimens were preserved in Davidson fluid (Bell and Lightner, 1988) for 24 h, dehydrated in ethanol, and embedded in butyl-paraffin and paraffin. Serial sections of 5 µm were cut on a rotary microtome and stained with haematoxylin-eosin.

Enzyme analysis

To compare the proteolytic activities during ontogenetic development of the shrimp *P. muelleri*, samples of larvae and postlarvae were collected at the following stages: nauplius (N), protozoea 3 (PZ), mysis 2 (M), postlarvae (PL) 1, 6, 10, 26, 30, 45 and juvenils (J). At stages of N, PZ, M and PL the samples had consisted of 10,000 and 20,000 intact individuals. The sample of juvenile shrimp consisted of 15 midgut gland removed from decapitated animals.

Whole-shrimps at the same development stage were immediately lyophilized and stored at −20°C. Samples were homogenized in chilled distilled water (1:3 w/v) and centrifuged at 10,000 g for 30 min at 4°C to remove

the lipid layer. Total soluble protein was evaluated in the supernatants (Bradford, 1976), with chicken egg albumin as standard (Sigma).

Detailed procedures for enzyme assays are discussed elsewhere (Fernandez Gimenez et al., 2001). Total protease activity was measured using the azocasein in hydrolysis method (Garcıa Carreño, 1992). Trypsin activity was measured with Na-benzoyl-DL-arginine *p*-nitroanilide (BAPNA) (Erlanger et al., 1961). Chymotrypsin activity was evaluated using 0.1 mM Suc–Ala–Ala–Pro–Phe *p*-nitroanilide (SAPNA) (del Mar et al., 1979). The enzyme extracts were incubated with protease inhibitors: TLCK (tosyl-lysine chloromethyl ketone) for trypsin and TPCK (tosyl-phenylalanine chloromethyl ketone) for chymotrypsin. Each assay included blanks and commercial enzymes (1 mg/ml) as internal controls. Total protease, trypsin, and chymotrypsin units of activity were expressed as change in absorbance per min per mg of protein of the enzyme used in the assays.

Statistical analysis

Data are expressed as the mean ± standard deviation. We applied arc sine transformation to percentages. ANOVA and Student's *t*-test were used to find differences among means. In all cases, significance was set as $P < 0.05$ (Sokal and Rohlf, 1979). All assays were replicated three times.

Results

Histology

At mysis 2 stage, the stomodeum is relatively well developed (Fig. 1). The oral region is lined by a single cylindrical epithelium covered by cuticle. The mouth connects with a short esophagus that during the successive stages increments in size. The most evident changes occur in both stomach chambers, separated by an outline of pyloric valve (Fig. 2). The stomach is divided into an anterior (cardiac) and posterior (pyloric) chambers, the latter divided again into a dorsal and ventral subchamber (gland filter). The cardiac stomach does not evidence a developed gastric mill. In the pyloric stomach, the outline of the gland filter can be observed; it consists of two pairs of longitudinal channels that join in a posterior channel (Fig. 3). After the metamorphosis, in the successive postlarval stages, the significant changes comprise an increment in the relative size and chitinization of the gastric mill formed by osicles, and an increase in the complexity of the gland filter with new longitudinal channels formed by setae (Figs. 4 and 5). At the stage of PL45 stomodeum presents the adult shape. Both regions of the stomach are lined with a layer of cuticle, only calcified in the grinding surface (gastric mill). The gland filter became a complex structure of cuticular setae and grooves (Fig. 6).

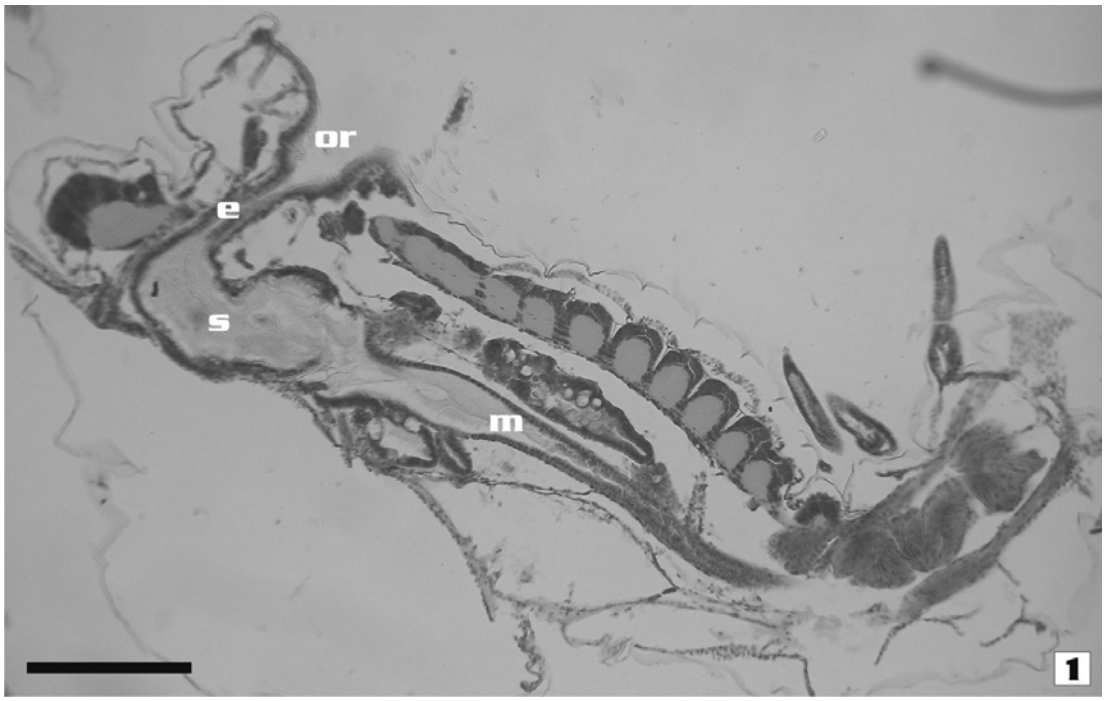

Fig. 1. Longitudinal section of digestive system at mysis 2 stage of *P. muelleri*. e: esophagus, or: oral region, s: stomach, m: mesodeum. Scale bar: 200 µm.

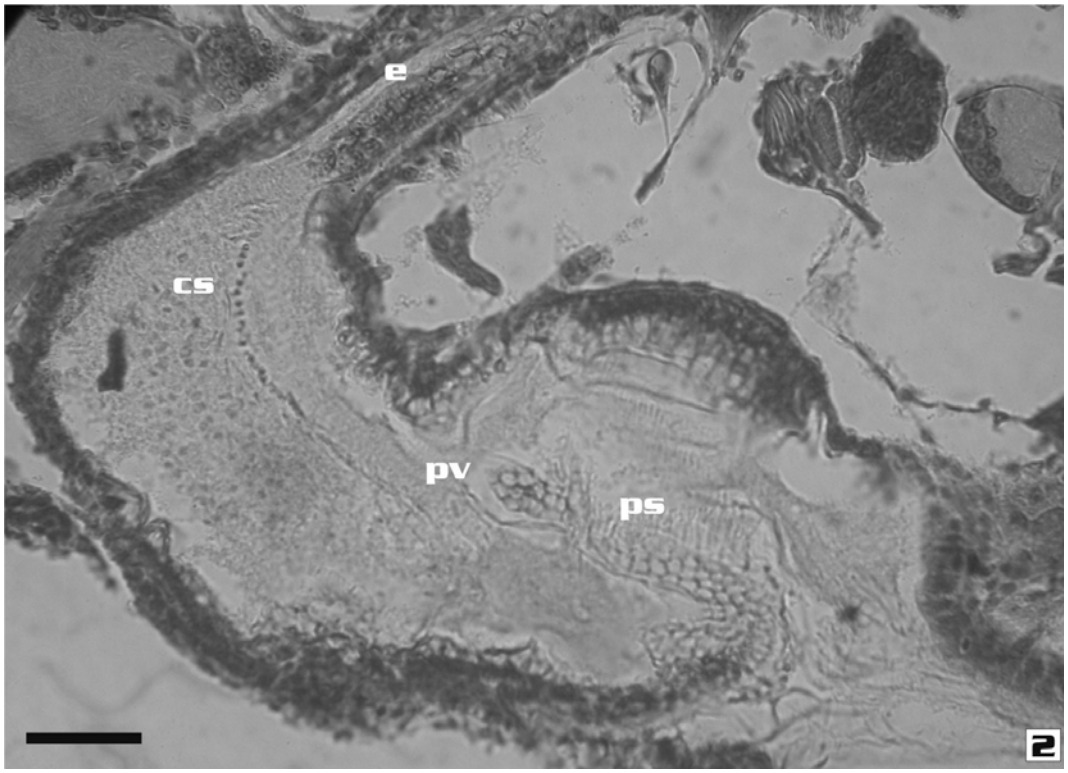

Fig. 2. Stomach chambers with an outline of pyloric valve. e: esophagus, cs: cardiac stomach, ps: pyloric stomach, pv: pyloric valve. Scale bar: 50 µm.

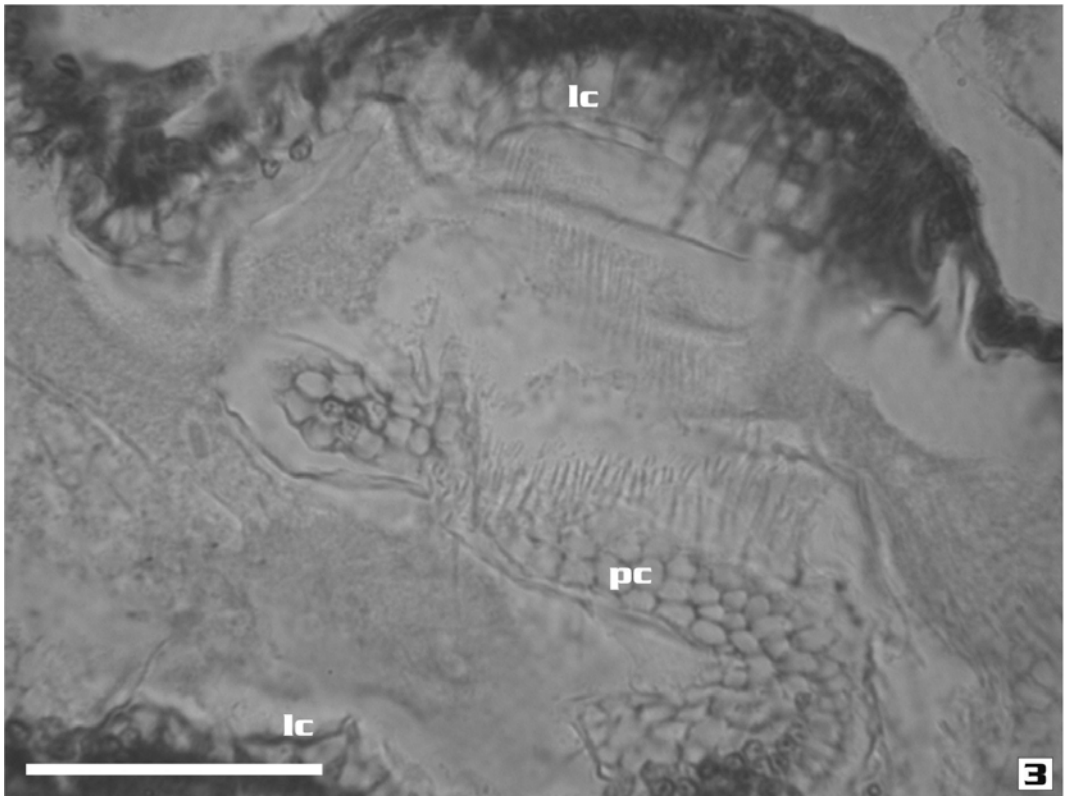

Fig. 3. Pyloric stomach of mysis 2 stage of *P. muelleri* showing the outline of the gland filter. lc: lateral channel, pc: posterior channel. Scale bar: 50 µm.

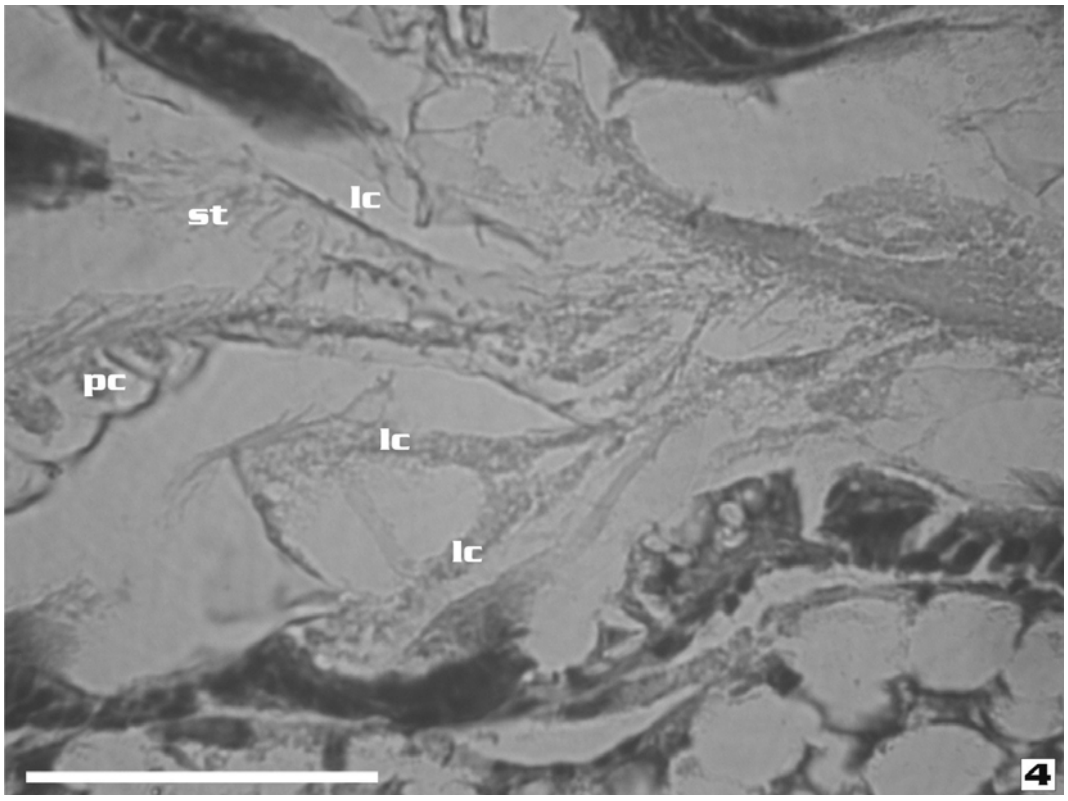

Fig. 4. Pyloric stomach of postlarvae 1 of *P. muelleri* showing the gland filter. lc: lateral channel, pc: posterior channel, st: setae. Scale bar: 50 µm.

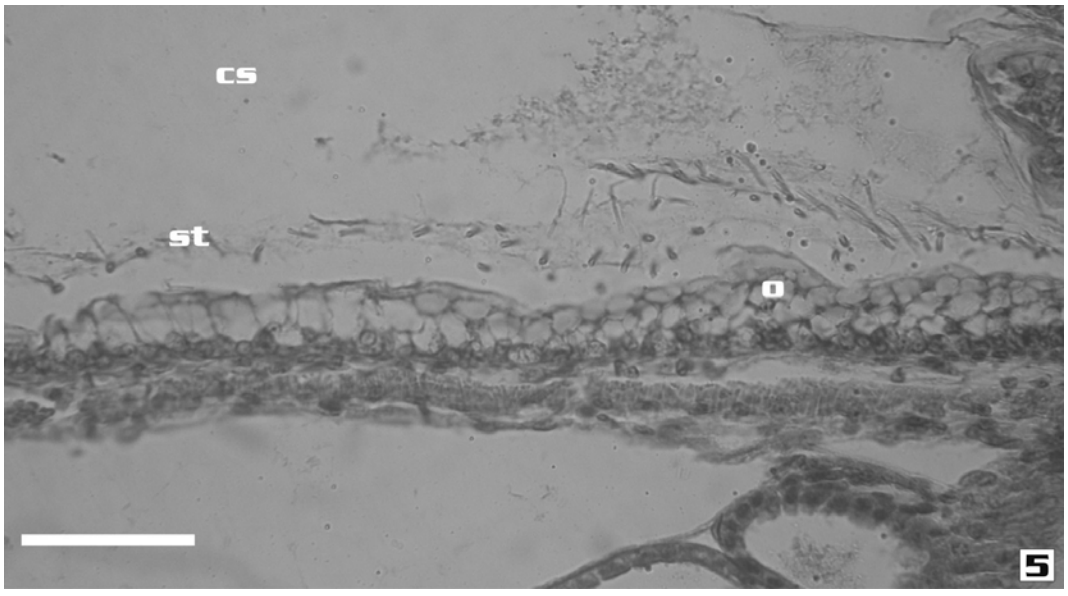

Fig. 5. Detail of epithelium lining the cardiac stomach of postlarvae 1 of *P. muelleri*. cs: cardiac stomach, o: osicles, st: setae. Scale bar: 50 µm.

The adult mesodeum consists of a midgut trunk and two diverticles, the anterior and the posterior. In the larval stages, at the junction of the stomodeum with mesodeum, two pairs of caeca are observed, an anterior pair formed by a simple tubule, and a lateral pair with three lobes (Figs. 7 and 8). Each tubule is lined by a simple epithelium with different cellular types: E (embryonic), F (fibrillar), R (resorptive), and B

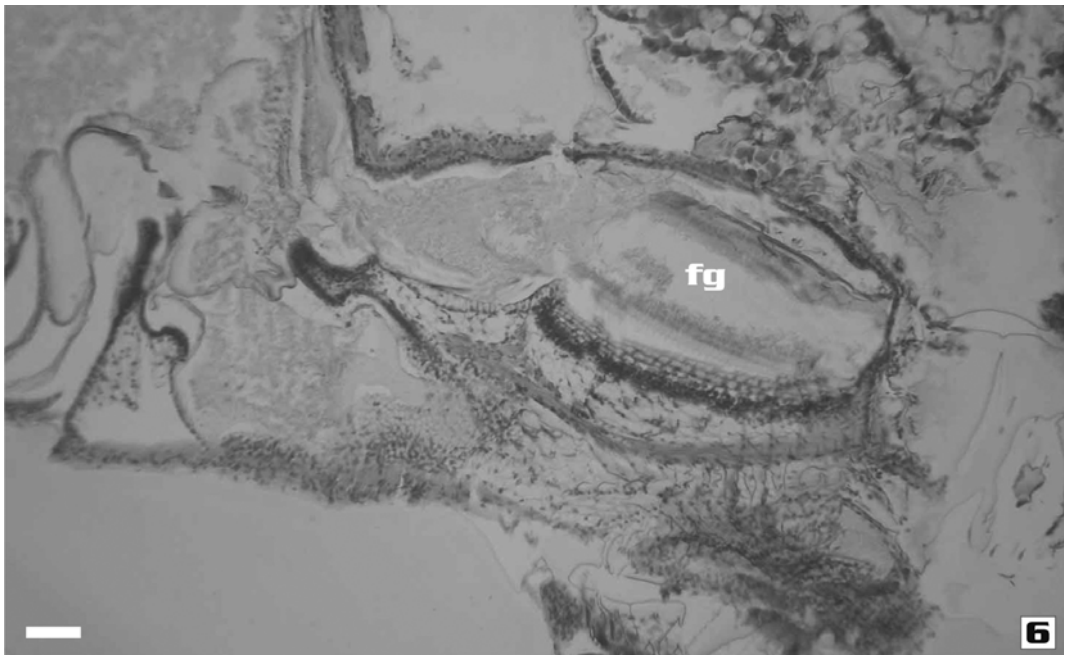

Fig. 6. Pyloric stomach of postlarvae 45 of *P. muelleri*. fg: gland filter. Scale bar: 50 µm.

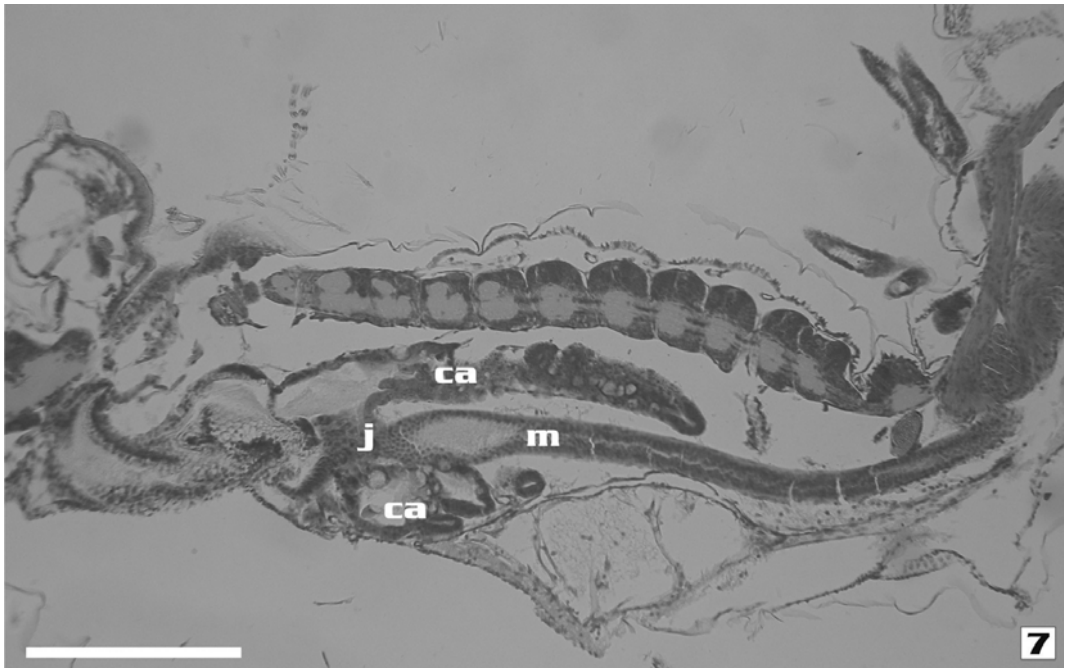

Fig. 7. Longitudinal section of mysis 2 stage of *P. muelleri* showing the caeca (ca) at the junction (j) of the stomodeum with mesodeum (m). Scale bar: 200 µm.

(blisterlike) cells, typical of adult midgut gland (Cuartas et al., 2002). The posterior caecum starts development during late larval stages and reaches the adult structure at the stage of PL45. According to Lovett and Felder (1989), we apply the term diverticulum to the reduced form that occurs after the metamorphosis, and caecum to the form found in larvae.

After postlarval metamorphosis, the lobes of the lateral caeca start to proliferate to form midgut gland tubules. Ramifications of tubules are observed in antero-

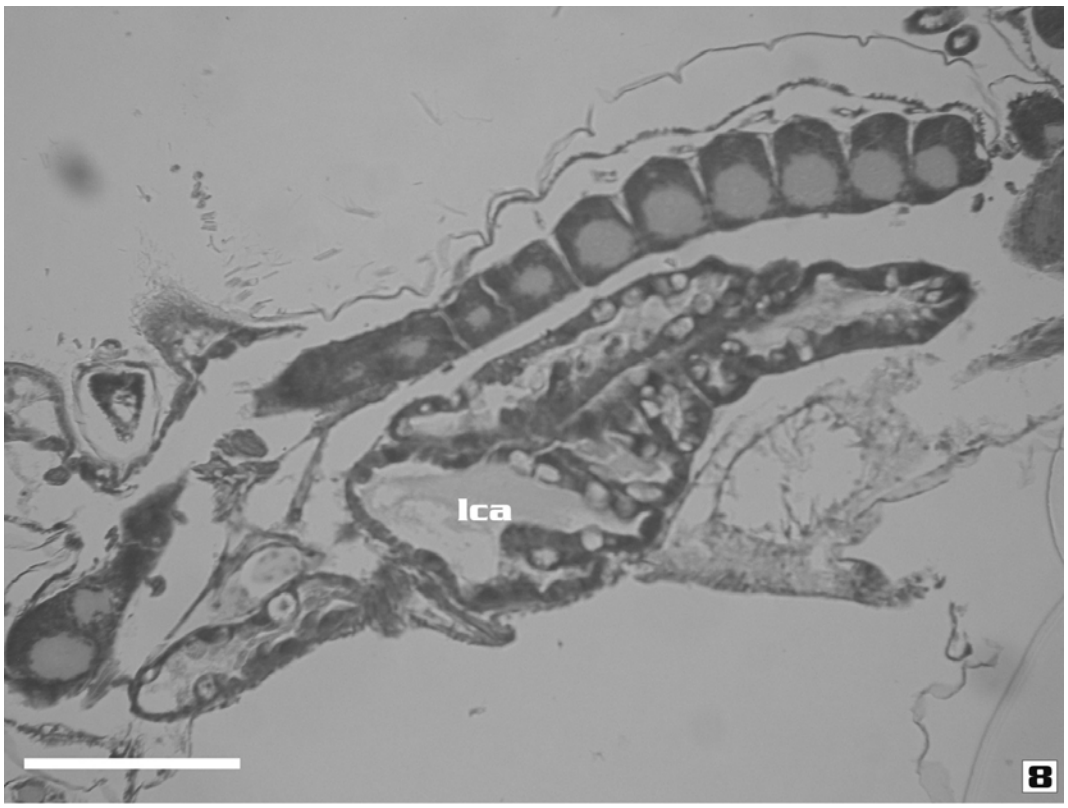

Fig. 8. Longitudinal section of mysis 2 stage of *P. muelleri* showing a detail of the lateral caecum (lca) with three lobes. Scale bar: 200 µm.

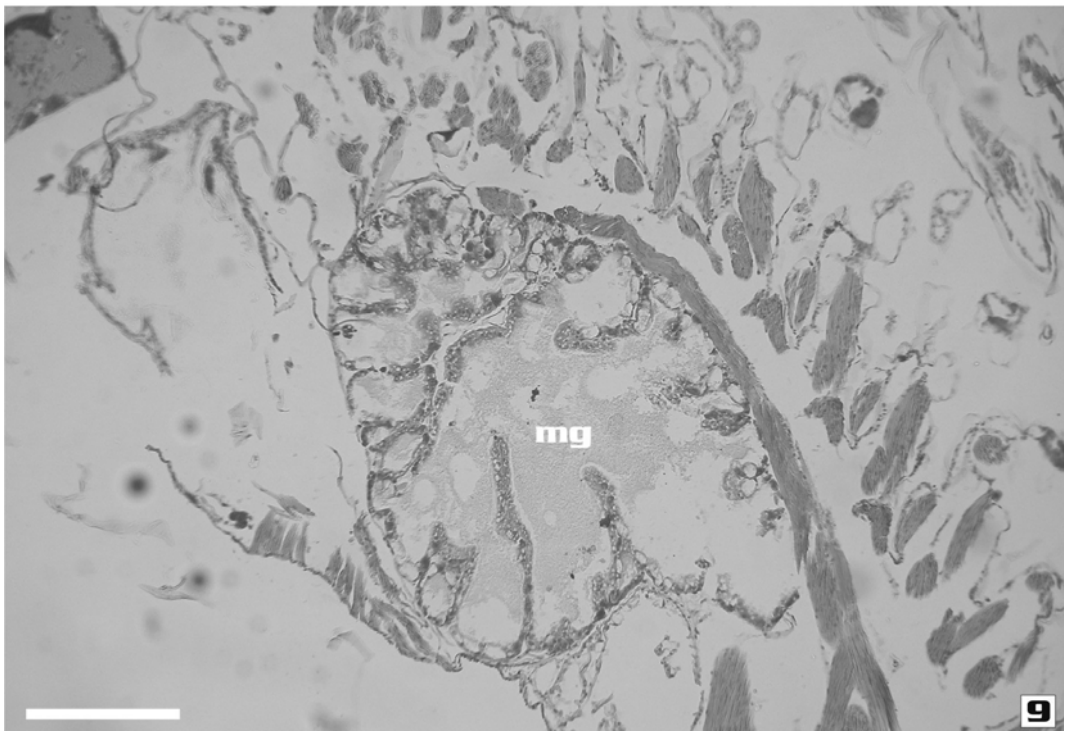

Fig. 9. Longitudinal section of lateral caeca of postlarvae 1 of *P. muelleri*. Photograph shows the ramificacion of tubules in antero-posterior direction and from the cortical to the medullar region of incipient midgut gland (mg). Scale bar: 200 µm.

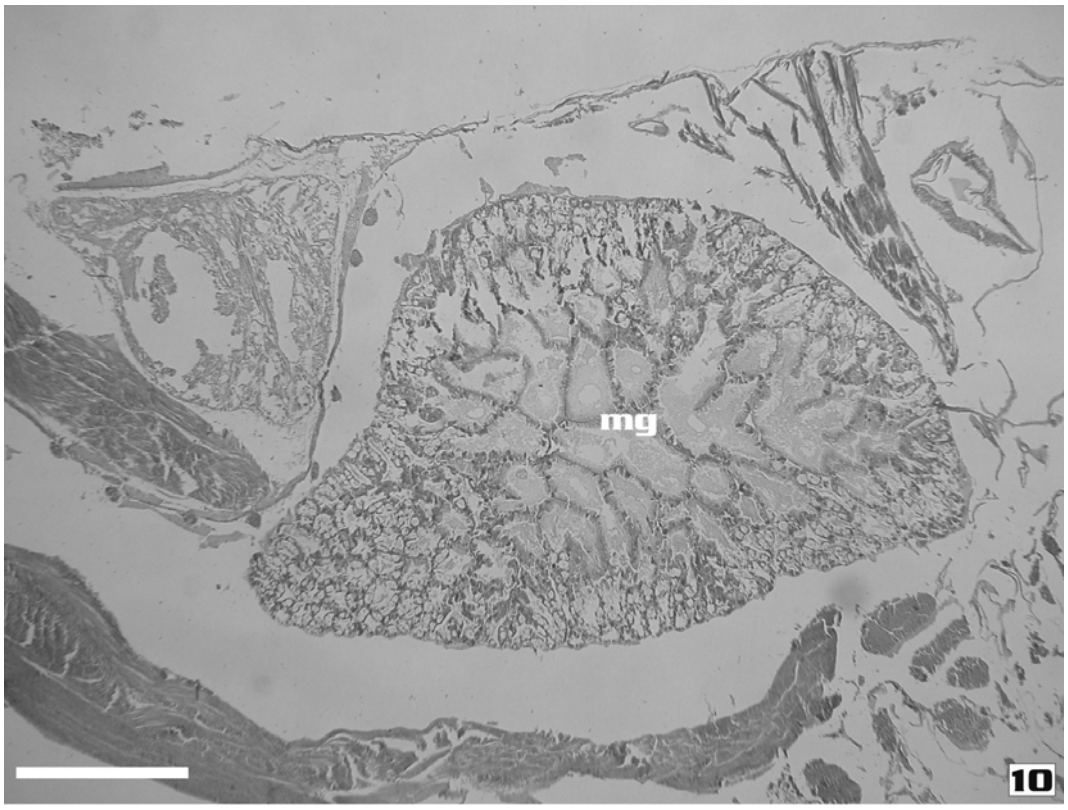

Fig. 10. Longitudinal section of midgut gland (mg) of postlarvae 30 of *P. muelleri*. Scale bar: 200 µm.

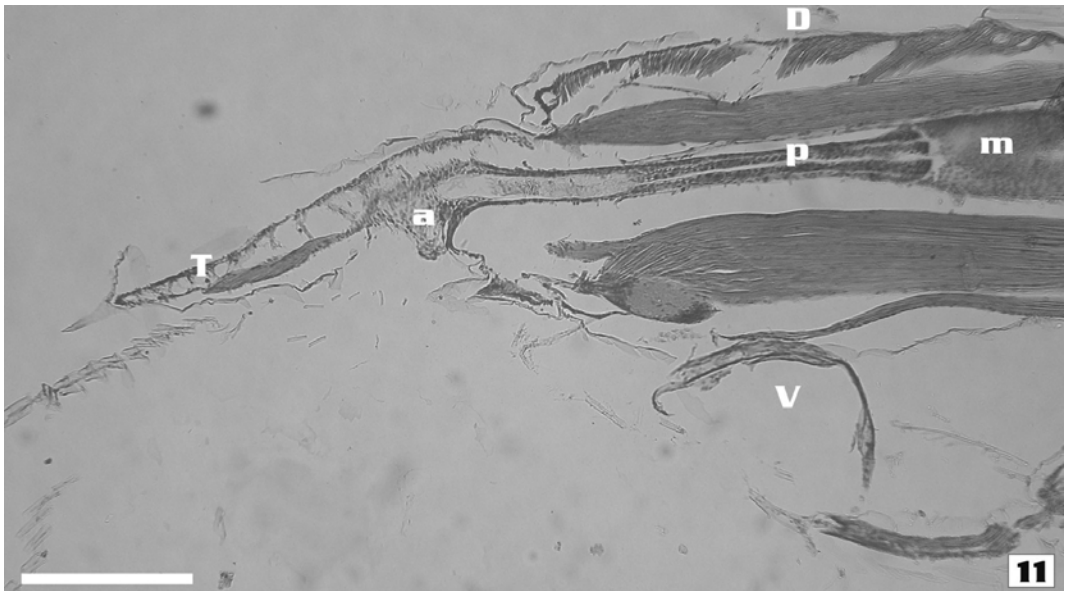

Fig. 11. Longitudinal section of proctodeum of postlarvae 45 of *P. muelleri*. a: anus, D: dorsal, m: mesodeum, p: proctodeum, T: telson, V: ventral. Scale bar: 200 µm.

posterior direction and from the cortical to the medullar region (Fig. 9). In PL30, the midgut gland presents a structure similar of the adults (Fig. 10). It is a compact bilobed organ that occupies great part of the cephalothorax, it is ventral to the gonad, antero-dorsal to the stomach and covers partially the midgut. The histological study shows that it is a composed tubular gland, surrounded by a continuous layer of laminar connective

Table 1. Protein content and specific activity in *Pleoticus muelleri*

Stage	Protein content (abs_{366} min^{-1} mg^{-1})	Total proteases* (abs_{366} min^{-1} mg^{-1})	Trypsin** (abs_{366} min^{-1} mg^{-1})	Chymotrypsin*** (abs_{366} min^{-1} mg^{-1})
N	4.00[a]	0.01[a]	0.77 ± 0.134[a]	—
PZ3	3.34[a]	0.04[a]	0.94 ± 0.106[a]	0.60 ± 0.007[a]
M2	2.34[b]	0.27[a]	1.35 ± 0.113[a]	1.76 ± 0.714[b]
PL1	2.74[c]	0.26[a]	0.18 ± 0.113[b]	3.91 ± 0.099[c]
PL6	2.80[c]	0.28[a]	0.03 ± 0.007[b]	3.43 ± 0.071[d]
PL10	2.20[b]	0.22[a]	1.48 ± 0.205[a]	3.24 ± 0.049[d]
PL26	6.20[a]	0.07[a]	0.32 ± 0.007[c]	1.64 ± 0.049[b]
PL30	2.80[c]	0.07[a]	0.95 ± 0.170[a]	2.31 ± 0.297[b]
PL45	3.06[c]	0.33[b]	1.04 ± 0.099[a]	2.82 ± 0.099[b]
J	4.60[a]	0.11[a]	0.65 ± 0.163[a]	1.57 ± 0.085[b]
A[#]	7.37	0.63	0.90 ± 0.060	1.20 ± 0.100

Means followed by different superscripts are significantly different (P <0.05). Means of three replicates ± S.D. N: nauplius; PZ: protozoea; M: mysis; PL: postlarvae; J: juvenile; A: adult.
[#]Fernandez Gimenez et al., 2001. Substrates: *azocasein; **BAPNA; ***SAPNA.

tissue that bounds a haemal space. In the medullar region, there are secondary secreting ducts opening into collecting ducts that are connected to the posterior chamber of the stomach by a single primary duct.

The adult proctodeum is relatively short, with serrated folds of different depth and longitudinal spines. It runs from the sixth abdominal somite to the ventral anus (Fig. 11). In early larval and post larval stages, the proctodeum is a simple tube formed by a columnar epithelium overlain by thin chitinous cuticle, which increases its relative length in the successive stages.

Enzyme analysis

P. muelleri presents activity of total proteases in all the studied larval and post larval stages. Total protease activity was higher in postlarvae 45 (0.33 Abs_{366} min^{-1} mg^{-1}), no significant differences were found in the others larval, postlarval stages and juvenile, ranging from 0.01 Abs_{366} $min^{-1}mg^{-1}$ in nauplius stage to 0.28 Abs_{366} $min^{-1}mg^{-1}$ in postlarvae 6 (Table 1).

To identify the different proteolytic enzymes involved in digestive proteolysis, extracts were tested using synthetic substrates. Results are given in Table 1. Using BAPNA as substrate, trypsin-like activity was found in all ontogenetic stages with significant decreases after metamorphosis (PL1-PL6) and PL26. Chymotrypsin was assayed with SAPNA as substrate. The highest chymotrypsin activity was found from PL1 to PL10. Table 2 shows the percentage inhibition by TLCK (BAPNA as substrate) and TPCK (SAPNA as substrate) on enzyme extracts at different ontogenetic stages. Trypsin activity was partially inhibited in nauplius and protozoea 3 (64.2% and 63.4%) and completely inhibited from mysis 2 to PL45 (92.7%–98.2%). However, TPCK had almost no effect on the protease activity (8.2%–39.4%).

Table 2. Effect of inhibitors on trypsin and chymotrypsin activity

Stage	Inhibition (%)	
	TLCK*	TPCK**
N	64.2 ± 4.17[a]	—
PZ3	63.4 ± 3.28[a]	25.6 ± 0.68[a]
M2	95.0 ± 1.13[b]	22.5 ± 6.07[a]
PL1	94.5 ± 0.07[b]	30.4 ± 5.42[a]
PL6	92.7 ± 6.58[b]	30.2 ± 12.66[a]
PL10	93.2 ± 0.71[b]	22.1 ± 14.64[a]
PL26	98.2 ± 0.35[b]	8.2 ± 6.15[a]
PL30	87.9 ± 4.46[b]	11.2 ± 6.51[a]
PL45	93.4 ± 3.08[b]	39.4 ± 10.8[a]
J	78.1 ± 2.82[a]	16.5 ± 4.16[a]
A	86.3 ± 3.73	62.4 ± 4.49

Means followed by different superscripts are significantly different (P <0.05). Means of three replicates ±S.D. N: nauplius; PZ: protozoea; M: mysis; PL: postlarvae; J: juvenile; A: adult. Substrates: *BAPNA; **SAPNA.

Discussion

The inadequate comprehension of feeding biology, dietary requirements and digestive capacity of the different larval stages has been the major impediment to produce crustacean larvae. The observation of the functional morphology of the digestive system during ontogeny can be useful to illustrate dietary changes. Variation in digestive enzyme activity may be related to the characteristic life history during the early onto-

genetic development of penaeids, showing the relative significance of change from a herbivorous to a omnivorous feeding. The morphological aspects of the digestive system can be correlated with the development of oral appendages, changes of habitat and diet, and with the main changes that occur during the metamorphosis (Factor, 1995).

In a previous study about the morphology of digestive system in adult *P. muelleri* (Díaz et al., 2006), was determined that the cardiac chamber is large, with thin and flexible walls, except at the posterior half which is covered by calcified ossicles and chitinous plates, connected by membranous ligaments that permit their movements. The pyloric chamber is much narrower than the cardiac one and is divided in two compartments, a superior compartment that opens into the mesodeum and an inferior with a filter that opens into the midgut gland. The degree of development of the gastric mill is related to the efficiency of the feeding mechanism. The ontogenetic changes of the digestive system are coincident with changes in the external anatomy; decapod larvae employ either filter and raptorial feeding methods depending upon their trophic feeding level; the mouthparts of early and late stage penaeid larvae are capable of selecting particles and exhibit chemosensory discrimination (Jones et al., 1997).

During the ontogeny, in *P. muelleri*, the stomach development follows the typical pattern described for other decapods. Essentially, the gland filter develops during the larval stages while the gastric mill takes the adult form at metamorphosis into the postlarvae and juvenile stages. Mastication of food shifts from the mandibles to the gastric mill at metamorphosis. The adult form is present in PL30; similar results were obtained by Lovett and Felder (1989) in *Litopenaeus setiferus*. In adult individuals, the pyloric filter was shown to act as a sieve that eliminates particles bigger than 1 µm towards the midgut and ensures that only the finest particles pass into the midgut gland (Hopkin and Nott, 1980). However, in the stages from M2 to PL42 of *L. setiferus*, the gland filter does not act as a sieve, but as pump that moves the chime between the cardiac chamber and the mesodeum, emulsifies it with the digestive enzymes and breaks mechanically the particles by turbulence, dispersing lipid droplets (Lovett and Felder, 1990).

In the adult, the endodermic mesodeum consists of a narrow tubule enclosed by circular and longitudinal muscles. The midgut presents two lobular diverticles in dorsal position that open near of the junction with the stomodeum and proctodeum. Presumably, these diverticles increase the absorptive surface and act as a site of cellular proliferation of the midgut epithelium. In *P. muelleri*, cellular division is confined to the distal end of the diverticle where basal cells that function as replacement elements were found (Díaz et al., 2006). The same observation was described in adult *Litopenaeus stylirostris* (Bell and Lightner, 1988).

In *P. muelleri* during early larval stages, anterior and lateral caeca show a histological structure similar of the adult midgut gland, with R, F, and B cells. The presence of these differentiated cells suggests a high functional digestive capacity in early larval stages, though the organogenesis has not been completed yet. Anterior and lateral midgut caeca are believed to be the main enzyme secretion sites in penaeid larvae, reaching the maximum volume at PZ3–M1. A reduction in the size of the midgut caeca begins at the mysis stage and extends through the early postlarval substages (Lovett and Felder, 1989; Icely and Nott, 1992). During late larval stages, the paired anterior caeca decline and take a similar structure to that of the mesodeum, and the lateral caeca origins the midgut gland. The anterior midgut caecum clearly have an important secretory role and represent an adaptation to herbivorous feeding, being absent in carideans and vestigial, without apparent digestive function, in brachyuran and homarid larvae (Le Vay et al., 2001). In coincidence with observations in *L. setiferus* by Lovett and Felder (1989), in *P. muelleri* the lateral caeca consist initially of three lobes each one, and after the metamorphosis they start branching in tubules of small diameter that will originate the adult midgut gland. In *L. setiferus*, the ramification rate of the tubules increases from PL10.

The midgut gland is the main organ of synthesis and secretion of digestive enzymes, absorption of nutrients, storage of lipids, glycogen, distribution of minerals and other organic substances (Icely and Nott, 1992). Van Wormhoudt (1980) affirmed that all the digestive enzymes produced by the midgut gland of adult penaeoids are present in the embryos but at lower levels. In general, in all the studied penaeoid species, including *P. muelleri*, there are low levels of proteolytic activity in nauplius. Lovett and Felder (1989) proposed that the function of digestive enzymes at this stage, in which the animal does not feed, is the mobilization of nutrients from the vitelum. Gonzalez et al. (1994) and Lemos et al. (1999) showed for *Litopenaeus schmitti* and *Farfantepenaeus paulensis*, that protease activity is low in nauplius, reaching the maximum in protozoea and decreasing towards PL5. In several species of penaeids it had been reported peaks in protease activity during PZ3 and M1 and low activity towards the metamorphosis, then the activity increases again from PL35, coinciding with the end of morphogenesis of the digestive system (Lovett and Felder, 1989; Lemos et al., 1999; Gonzalez et al., 1994; Rodríguez et al., 1994). In *P. muelleri*, values of total protease activity in all larval

and post larval stages analyzed were lower than those recorded in adults (0.63 Abs_{366} $min^{-1}mg^{-1}$) (Fernández Gimenez et al., 2001). The percentage of inhibition of trypsin and chymotrypsin activities with specific substrate were confirmed the presence of these serine proteases during developmental stages.

It has been argued that higher trypsin content in species and developmental stages which are dependent on digestion of phytoplankton, declining through the omnivores and with lowest activity in carnivorous feeders (Le Vay et al., 2001). In the present study *P. muelleri* exhibit an increase in trypsin content during the herbivorous protozoeal stages, reaching a peak in mysis stages and declining during omnivorous feeding in the first postlarval stages (PL1–PL6). Similar results were observed in other penaeoid species; in *Farfantepenaeus paulensis* (Lemos et al., 1999) and *Litopenaeus setiferus* (Lovett and Felder, 1990) trypsin activity was low in the first larval stages with an increment in PZ and a decrease towards PL1. In *Fenneropenaeus indicus* (Ribeiro and Jones, 2000), the trypsin activity was low from PL1 to PL14 and increased when post larvae reached 24–31 mm (PL20 to PL22 stages). The "critical period" in early development of postlarvae (PL1–10 days old) has been identified with high mortality in culture (Wickins, 1976; Lovett and Felder, 1990), and coincides with the decrease in size of the anterior caeca and it is characterized by changes in enzyme production levels. Maybe, this is the main reason of the significant decrease of the trypsin activity in *P. muelleri* between the metamorphosis and PL10. In relation with chymotrypsin activity the results found differ from the other penaeoid species studied, showing an increase in this period. Variations in digestive enzyme activity may be related to the characteristic life history during ontogenetic development, which are unique among penaeoids (Boschi, 1969); this species' life cycle occurs completely in the sea without entering coastal estuarine regions.

Ontogenetic changes of the digestive system and feeding appendages coincide with behavioral changes occurring during the important transition from planktonic to benthic life style that take place after the metamorphosis. In *P. muelleri* from PL10–12 under culture conditions, they begin to feed artificial diet at the bottom of the tank. Low chymotrypsin activity observed at PL26 may be related to a highly digestible diet. However, Lovett and Felder (1989) indicated that between the fourth and fifth week of post-larval development important changes occur in the digestive system morphology that restricts temporally the communication between the midgut gland and the midgut, affecting the midgut gland functioning. This agrees with the important decrease of trypsin and chymotrypsin activities registered in *P. muelleri* at the stage of PL26. From PL30, the values of these enzymes correspond to the rank found in the adults and coincide with the midgut gland morphogenesis observed in histological studies.

The ontogenetic pattern of *P. muelleri* digestive system morphology is similar to those described of others penaeoids. Some studies showed that the digestive tract morphology depends primarily on the species phylogeny; however other factors such as dietary preferences can modify its anatomy. There is no doubt that the occurrence and activity of digestive enzymes were influenced by many external and internal factors; in the present study we have demonstrated that the differences in enzyme levels during the ontogeny of *P. muelleri* is related to its unique postlarval life history.

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