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DIGESTIVE PHYSIOLOGY OF THREE SPECIES OF DECAPOD CRUSTACEANS OF ARGENTINA

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ABSTRACT This review has the available information about the digestive physiology and morphology of three decapod species from Argentina: *Pleoticus muelleri*, *Artemesia longinaris*, and *Neohelice granulata*. The anatomy of the foregut may be modified in closely related species with similar feeding habits; however, the main features of the decapod crustacean digestive tract are similar to other species. Crustaceans express a set of highly active digestive enzymes that hydrolyze the major food items and include proteinases, lipases, esterases, and glucanases. The close relationship between diet and the range of digestive enzymes produced is well documented; however, digestive responses to specific nutrients differ widely among the species. Variations in digestive enzyme expression during early development reflect changes in digestive capabilities and can be used to identify early developmental feeding transitions. This information provides a deeper insight into nutrition, dietary preferences, and strategies of resource utilization, making possible the development of new aquacultural practices and providing data about the ecological niche that species occupy in aquatic systems. There is no doubt that the occurrence and activity of digestive enzymes are influenced by many internal and external factors, such as diet, molting, and development. The research of three Argentine decapod species is discussed in this review and provides a better understanding of basic digestive physiology. This information important to investigations of nutrition and feeding ecology of crustaceans.

KEY WORDS: Crustacea, diet, digestive enzymes, histology, molting, ontogeny, *Pleoticus*, *Artemesia*, *Neohelice*

INTRODUCTION

In crustaceans, nutrient assimilation depends on their complex digestive system. Numerous studies in morphology, histology, biochemistry, and molecular biology have generated interesting results about the process of digestion and food use. The main functions of the digestive tract of crustaceans are food selection, ingestion, mechanical digestion, enzyme activity, cellular absorption, and storage as well as transfer of excreta (Ceccaldi 1998). The understanding of basic digestive physiology is important for investigations on nutrition and feeding ecology of marine invertebrates. Studies on the kinetic and structural characteristics of invertebrate enzymes and their functions may also help elucidate evolution pathways (Jiwani & Liebman 1994). We review the available information about the digestive physiology and morphology of three decapod species of Argentina: *Pleoticus muelleri* (Bate, 1888); *Artemesia longinaris* Bate, 1888; and *Neohelice granulata* (Dana, 1851).

The endemic shrimp *Pleoticus muelleri* and *Artemesia longinaris* inhabit southwestern Atlantic waters, and they both have an important commercial value and play an essential role in the trophic web of coastal marine waters. There are many studies about these species in relation to fisheries, such as distribution and ecology, physiology of nutrition, molting, and reproduction (Fernández Gimenez 2002). The burrowing semiterrestrial crab *Neohelice granulata* is found in the intertidal zone of estuaries, salt marches, and mangroves. Since 1989, an explosion of publications appeared in journals dealing with its ecology, physiology, toxicology, and behavior; for this reason, this species is considered an emergent animal model for biochemical, physiological, and ecological research (Spivak 2010).

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Several investigations were conducted on Argentine decapod species with regard to digestive physiology and morphology in relationship to diet, molting cycle, and ontogeny (Fernández Gimenez et al. 2001, Fernández Gimenez et al. 2002, Díaz et al. 2008, Fernández Gimenez et al. 2009a, Velurtas et al. 2011, Lancia et al. 2012,), Fernández Gimenez et al. 2009b and this information is presented in the following.

DECAPOD CRUSTACEANS OF ARGENTINA

More than 250 species of decapods are found in the southwestern Atlantic between 25°S and 55°S (Spivak 1997), and their distribution is related to two main biogeographical subregions, according to the different characteristics of the water masses. A warm, subregion from shore to 40–60 m and between 23°S and 44°S latitude, and a cold subregion 150–200 km from the continent in the north at a depth of 80 m or more and extending from 34°S–35°S latitude (Boschi 1979).

The shrimp *Artemesia longinaris* is distributed from Rio de Janeiro, Brazil (21°37′S) to Rawson, Argentina (43°S), whereas *Pleoticus muelleri* has a southern distribution, reaching Santa Cruz Province, Argentina (50°S). These species are distributed mainly from the littoral zone to 30-m isobaths, but occurrences at deeper waters (68 m) have been reported (Boschi 1963). Both species play an important role in the trophic web of coastal marine waters because they are preyed on intensively by several fish species (Dumont & D'Incao 2008). The shrimp *P. muelleri* represents one of the most important fisheries in the Argentine Sea and is, along with *A. longinaris*, an important benthic predator of other crustaceans, molluscs, and polychaetes. Studies on penaeid diet have evidenced that shrimp are omnivorous and carnivorous in coastal habitats and some species exert a direct influence on the abundance of small benthic macrofauna (Roux et al. 2009).

The burrowing crab *Neohelice granulata* (previously known as *Chasmagnathus granulata* and *Chasmagnathus granulatus*) is

found in the intertidal zone of estuaries, salt marshes, and mangroves of the southwestern Atlantic Ocean, from Rio de Janeiro, Brazil, to Patagonia, Argentina (Spivak 2010, Araujo Barutot et al. 2011). Salt marshes are very important environments because they are sites for feeding and reproducing invertebrates and vertebrates. The crab *N. granulata* plays important ecological roles in structuring plant communities, in nutrient recycling, and in decreasing the amount of organic matter exported from salt marshes (Araujo Barutot et al. 2011). It is considered herbivorous and detritivorous because it feeds on halophytic grasses (*Spartina* spp.) in salt marshes, but shifts to sediment rich in organic matter in unvegetated tidal flats (Iribarne et al. 1997). The organic matter found in sediment comes mainly from the degradation of species of the cordgrass *Spartina*, and from some protein-rich items such as small crustaceans, polychaetes, and nematodes (Botto et al. 2006).

DIGESTIVE MORPHOLOGY AND PHYSIOLOGY

The anatomy and cellular composition of the digestive tract of decapod crustaceans is, in many aspects, considerably different from the vertebrate system. These differences include primarily the gastric mill and a sophisticated filter apparatus in the stomach and tubules of midgut gland. Additional differences are the lack of strongly acidic pH and pepsin in the stomach. Consequently, many of the physiological processes are fundamentally different as well, particularly the physical and chemical processing of food, and the synthesis, storage, and mode of action of digestive enzymes (Vogt 1996). Detailed studies on the digestive system of decapod crustaceans underscore the importance of such information for understanding their feeding biology. There is evidence that the anatomy of crustaceans' foregut may be modified in closely related species with similar feeding habits (Icely & Jones 1978). However, the main features of their digestive tract are coincident among species. The gut of decapods consists of the foregut, midgut, and hindgut, and extends the length of the body from the anterior mouth to the posterior anus. Foregut and hindgut are derived ectodermally and are lined with cuticle whereas the midgut, with a nonchitinous glandular epithelium, comes from endoderm (Felgenhauer 1992).

The foregut comprises the mouth, esophagus, and stomach, and is lined with a simple cylindrical epithelium overlain by cuticle. The esophagus is a muscular tube that drives the food into the cardiac stomach, which is a simple sac with or without calcified structures in the interior, depending on the species. The pyloric stomach has the double structure of the filter, ensuring that only the smallest particles pass into the midgut gland. The outer filter setae press the finest particles toward the inner filter and also eliminate the big particles toward the midgut (Díaz et al. 2006).

In the midgut, the phases of the digestive cycle take place, including digestion of products coming from the cardiac stomach, absorption and processing of digestive products, and removal of residual waste in the form of feces (Icely & Nott 1992).The midgut gland is a large, bilobed organ composed of many blindly ending tubules responsible for food absorption, transport, secretion of digestive enzymes, and storage of lipids, glycogen, and a number of minerals (Gibson & Barker 1979). It is also involved in other processes, such as maintenance of the salt and ion balance, and vitellogenesis, and it also plays an immunological role in the removal of foreign bodies from the blood system, excretion of waste metabolites, and detoxification of metals from foreign organic substances (Cuartas & Petriella 2002). The tubules are made up of 4 basic cell types: E, F, B, and R cells. The E cells, also named embryonic cells, are the small ones found at the blind ends of the tubules and presumably give rise to the other 3 cell types of the gland. They are characterized by a large nucleus with a prominent nucleolus. The F cells, which are called fibrillar cells, have a basally located nucleus and an extensively developed rough endoplasmic reticulum, giving them a fibrillar appearance and a prominent brush border. A wide variety of functions has been attributed to this cell type, such as protein synthesis and storage of minerals. B cells, also named blister cells, are large with a single enormous vesicle surrounded by dense cytoplasm filled with rough endoplasmic reticulum. These cells are the primary producers of digestive enzymes in the midgut gland. The R cells are the most numerous cell type and have a prominent brush border, a centrally located nucleus, and large numbers of storage vesicles in their cytoplasm; these cells function in food absorption and they sequester mineral deposits such as calcium (Felgenhauer 1992).

The midgut gland of *Artemesia longinaris* and *Pleoticus muelleri* has been described by Petriella and Fonalleras (1998) and Cuartas et al. (2002), respectively. Both of these glands are composed of numerous blinded tubules that communicate with the midgut. These tubules are lined with a simple columnar epithelium composed of E, F, B, and R cells, as in other decapods (Fig. 1).

Cuartas and Petriella (2002) described the structure of the midgut gland of *Neohelice granulata*. This gland occupies the full dorsal hemocoel, lateral to the digestive tract and heart, and partially covering the branchial chambers. The cytoarchitecture is similar to those observed in other crustaceans. However, Cuartas and Petriella (2002) indicate that these crab species have a frayed-looking midgut gland because of the delicacy of

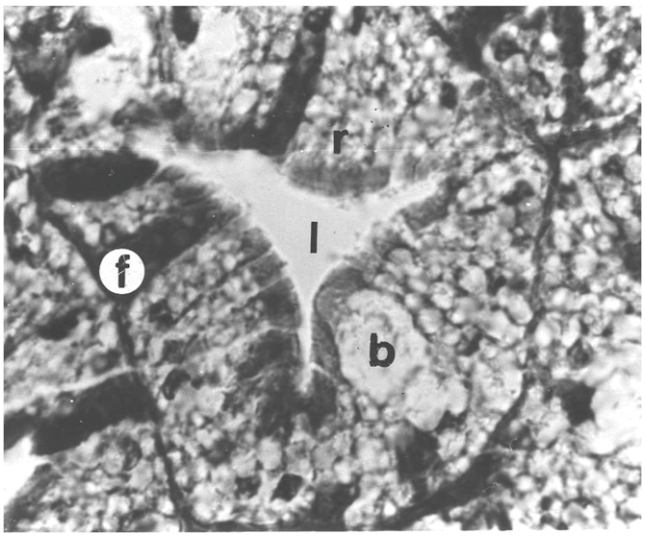

Figure 1. Midgut gland of wild *Pleoticus muelleri*. Transverse section through a tubule showing the types of epithelial cells. Note the narrow lumen and the scarce intertubular space. b, B cell; F, F cell l, lumen; R, R cell. Magnification ×40.

the connective tissue surrounding the tubules and the lack of a limiting external wall of that same tissue.

Crustaceans express a set of highly active digestive enzymes that hydrolyzes major food items. These enzymes include various proteinases, lipases, esterases, and glucanases. In particular, the proteinases appear in many species with high activity. Proteolytic activity, in turn, is often provided by enzymes that belong to the class of serine proteinase, such as trypsinlike and chymotrypsinlike enzymes. The decapods investigated so far for trypsin mostly show high activities of this enzyme (Saborowski et al. 2012). Trypsin is a ubiquitous enzyme in the crustacean digestive system; several trypsins have been isolated and characterized in a number of decapods. Chymotrypsin seems to be less ubiquitous and second in importance, when considering the amount of activity (García Carreño et al. 1994).

It was traditionally thought that crustaceans were largely carnivorous; however, the presence of carbohydrate-degrading enzymes supports the idea that they are able to use plants as major dietary sources. Polysaccharides such as cellulose, laminarin, and starch might be potential alternative food sources (Figuereido & Anderson 2009).

During the past decade, several papers about the digestive physiology of Argentine decapod species have been published. Fernández Gimenez et al. (2001, 2002) evaluated the proteinase activity of *Pleoticus muelleri* and *Artemesia longinaris* in relation to molting. Díaz et al. (2008) evaluated proteinases during ontogenic development of *P. muelleri*, and several studies have researched the effect of food on enzyme activity, such as carbohydrases and proteinases, and apparent *in vitro* digestibility in *P. muelleri*, *A. longinaris*, and *Neohelice granulata* (Fernández Gimenez et al. 2009a, Fernández Gimenez et al. 2009b, Velurtas et al. 2011, Lancia et al. 2012). The results and conclusions of these studies are presented next.

EFFECT OF DIET ON DIGESTIVE PHYSIOLOGY

Although most studies on the feeding habits of decapods are based on the observation of stomach contents, they do not provide any information on dietary preferences with regard to the suitability of the diet for maintaining the animal. Digestive enzyme studies, however, may be a complementary tool, useful for determining which dietary components are metabolized most effectively (Johnston & Freeman 2005). The close relationship between diet and the range of digestive enzymes produced is well documented in crustaceans; however, digestive responses to specific nutrients appear to vary widely among species (Muhlia Almazán et al. 2003, Johnston & Freeman 2005, Figuereido & Anderson 2009,).

DIGESTIVE ENZYMES AND DIETARY PROTEIN SOURCES

In aquaculture, the knowledge of digestive physiology is important to formulate artificial diets with optimal dietary ratios to maximize crustacean production and growth. Protein is the major and the most expensive ingredient in shrimp feeds, and also is growth limiting. The high amount of protein required in shrimp feeds increases the cost and makes the feed a major expense in shrimp production. Therefore, it is very important to identify low-cost, protein-rich ingredients to reduce the production costs of shrimp. Variations in protein requirements in marine shrimp are attributed mainly to different sources of proteins used in the formulations. In recent years, an interest in identifying and using alternative protein sources for food in aquatic species has increased. Alternative protein ingredients were used to supplement a formulated feed containing fishmeal as the main protein source (Fernández Gimenez et al. 2009a).

Fishmeal is the preferred protein source because it is an excellent source of essential nutrients; however, its limited availability and high demand make it an expensive ingredient. Therefore, the search for alternative low-cost protein sources continues. For example, meat and bone meal, soybean meal, and squid meal may be used as protein sources for growing Argentine shrimp species (Fernández Gimenez et al. 2009b).

Fishmeal has traditionally been used as an ingredient in commercial shrimp feeds as the major source of protein (Tacon & Akiyama 1997). However, fishmeal is more expensive than nontraditional feed ingredients such as meat and bone meal. Shrimp feed cost generally accounts for a large proportion of total farm production costs. To sustain the aquatic food industry, a great part of nutritional research has focused on the search for alternative proteins. Therefore, research is needed to identify and use less expensive and more sustainable ingredients, such as meat and bone meal within shrimp feeds, while maintaining nutritional quality equal to or better than those based mainly on fishmeal (Forster et al. 2003).

Meat and bone meal obtained as by-products of terrestrial animals is a good source of indispensable amino acids, with a content of 45%–65% crude protein. The quality of meat meal as a protein supplement depends on the production process as well as the raw material. Tan et al. (2005) showed that up to 60% of fishmeal protein can be replaced by meat and bone meal with no adverse effect on growth, survival, and body composition of *Litopenaeus vannamei* (Boone, 1931). Similarly, Cruz Suarez et al. (2007) determined that poultry by-product mealpet food grade can adequately replace up to 80% of fishmeal in commercial diets for white shrimp.

Díaz and Fenucci (2002) demonstrated that *Pleoticus muelleri* fed diets containing ≤25% meat and bone meal as replacement for fishmeal had a higher growth rate. Methionine is the first limiting amino acid in meat and bone meal; high levels of this ingredient in experimental diets for shrimp may result in low dietary methionine. Thus, the deficiency of this amino acid may be the main factor that reduces the growth of shrimp. Feed experiments suggest that meat and bone meal could be used as a suitable replacement for fishmeal in a formulated diet for penaeid species.

Soybean meal is the most important plant protein source currently used to supplement feed for cultured shrimp, but it cannot be used as the sole source of protein because it lacks certain essential amino acids and contains antinutritional factors such as lectins and proteinase inhibitors (Cordova Murueta and García Carreño, 2002). Lemos et al. (2004) found in *Farfantepenaeus paulensis* that soybean meal exhibited reduced digestibility and a high degree of protease inhibition; the occurrence of a trypsin inhibitor can be attributed to insufficient heat during processing. Medina Marti et al. (2005) proposed that soybean meal can replace up to 44% of the fishmeal in diets for *Artemesia longinaris* with no adverse effect on growth or survival.

Díaz and Fenucci (2002) maintained that squid protein can be used as a protein source in feed with favorable responses in

penaeids, and, considering that the squid *Illex argentinus* is an abundant resource in the southwest Atlantic ocean, it has great potential as well protein source. In addition, Argentina is one of the main producers of meat and bone meal and soybean meal on a commercial scale. The use of these protein sources as part of shrimp diets has been shown to be of economic importance in areas where they can be obtained at low cost.

Fernández Gimenez et al. (2009b) evaluated the effect of different protein sources on the digestive proteinase activity of *Artemesia longinaris*. Three isoproteic feeds were compared: a base diet containing 48% fishmeal and 17% soybean meal (diet 1), a base diet containing meat and bone meal as partial replacement of the fishmeal (diet 2), and a base diet containing additional soybean meal and squid protein concentrate as partial replacement of the fishmeal (diet 3). Shrimp fed diets 1 and 3 exhibited similar patterns of low enzymatic activity. Diet 2 induced the highest proteinase, trypsin, and chymotrypsin activity (Table 1) that shrimp can compensate its digestive activity when the diet is protein deficient, as well, digestive activity can increase in Crustacea given low nutritional quality food (Le Vay et al. 2001). Proteolytic enzymatic activity in the midgut gland of *A. longinaris* can be influenced by dietary protein quality, and the inclusion of fishmeal in commercial feeds for shrimp can be minimized without affecting production.

A formulated feed for crustaceans should be well balanced and should contain all dietary essential nutrients but still may not effect adequate growth because its nutrients may not be readily available. Determination of digestibility can be used to select ingredients that optimize the nutritional value and reduce the costs of formulated feeds. The digestibility of feed can be affected by the relative ratio of nutrients as well as the presence of inhibitory components in the ingredients.

Fernández Gimenez et al. (2009a) determined the protein apparent digestibility in the shrimp *Artemesia longinaris* using feeds with 0.25% chromic oxide, and animal (fishmeal, meat and bone meal and squid protein concentrate) and vegetable (soybean meal) ingredients. In addition, the rate of protein hydrolysis has been measured *in vitro* (Lan & Pan 1993) using midgut gland enzyme extract from shrimp fed the respective feeds and was compared with those found with enzyme extract of wild shrimp. Results showed no significant differences in *in vitro* protein digestibility among the experimental feeds; however, apparent digestibility coefficients of protein varied, revealing significant differences among treatments. Fishmeal feed presented the highest digestibility (92%), intermediate digestibility (84%) was found for meat and bone meal feed, and the lowest digestible feed (63%) was that containing soybean meal and squid protein concentrate (Table 1). Because the ingredients tested were obtained from the feed manufacturers, the poor digestibility observed for soybean meal in *A. longinaris* would be a reason to increase the quality control of the feed products before it is used in shrimp feed.

Fernández Gimenez (unpubl. data) evaluated the relationship between protein source (fishmeal and meat and bone meal), size (mean weight, 3 g and 8 g), apparent digestibility, and proteinase activity of Argentine red shrimp *Pleoticus muelleri* using similar feeds as those used by Fernández Gimenez et al. (2009b). Formulated feed with fishmeal as the main protein source was significantly more digestible than the feed with meat and bone meal instead of fishmeal, and the highest value obtained was shrimp of 3 g mean weight. Individuals fed diets with fishmeal exhibited lower specific proteinase activity than shrimp fed meat and bone meal, in both sizes (Table 1). Proteinase activity in all samples was inhibited by specific serine proteinase inhibitors, without significant differences. Protein and activity bands were compared by SDS-PAGE, and common patterns were observed: 5 active bands distributed from 17.46–21.9 kDa were detected; 3 trypsins (17.4 and 19.1 kDa), and 1 chymotrypsin (21.9 kDa). According to these results, shrimp weighing more than 8 g could be fed diets containing

TABLE 1.

Enzyme activity and digestibility coefficient in midgut gland of *Artemesia longinaris* and *Pleoticus muelleri* fed different protein sources.

Artemesia longinaris				
	Specific enzymatic activity* (abs/min/mg)			
	Proteinase	Trypsin	Chymotrypsin	Apparent digestibility† (%)
Diet 1	0.3 ± 0.06^{a}	2.1 ± 0.22^{b}	1.3 ± 0.15^{a}	92.2 ± 0.44^{a}
Diet 2	0.4 ± 0.04^{b}	2.6 ± 0.73^{b}	2.9 ± 0.18^{b}	83.8 ± 3.48^{b}
Diet 3	0.3 ± 0.04^{a}	1.9 ± 0.27^{b}	1.6 ± 0.43^{a}	63.1 ± 3.57^{c}
Pleoticus muelleri				
	Shrimp mean weight (g)	Proteinase activity‡ (abs/min/mg)		Apparent digestibility‡ (%)
Diet 1	3	2.5 ± 0.37^{a}		92.6 ± 0.27^{a}
	8	1.2 ± 0.16^{a}		69.9 ± 3.61^{a}
Diet 2	3	3.6 ± 1.12^{b}		47.8 ± 3.78^{b}
	8	3.6 ± 0.54^{b}		47.5 ± 1.13^{b}

* Fernández Gimenez et al. (2009a).
† Fernández Gimenez et al. (2009b).
‡ Fernández Gimenez (unpubl. data).
Values are means of 3 replicate analyses ± SD. Different superscripts in the same column indicate statistical difference ($P \leq 0.05$). Protein sources in diets are measured as grams per 100 g diet. Diet 1: fishmeal, 48; meat and bone meal, 0; soybean meal, 17; and squid protein, 0. Diet 2: fishmeal, 27; meat and bone meal, 23; soybean meal, 17; and squid protein, 0. Diet 3: Fishmeal, 27; meat and bone meal, 0; soybean meal, 23; and squid protein, 10.

meat and bone meal, thereby reducing feed costs in the later stages of intensive culture.

EFFECT OF CARBOHYDRATES ON HEMOLYMPH METABOLIC VARIABLES AND DIGESTIVE ENZYME ACTIVITY

Carbohydrates are not essential for crustaceans; however, they are incorporated in aquaculture feeds for their binding properties during feed manufacturing and to reduce costs. Proteins are the highest reserve substrate in shrimp and are converted to carbohydrates following the gluconeogenic pathway (Campbell 1991).

Polysaccharides such as starch currently represent the most common source of feed for crustaceans. The rate of nutrient absorption depends on the rate at which nutrients come into contact with the absorptive epithelium. Dietary fibers such as cellulose are associated with a delay in stomach emptying and contribute to the efficient use of dietary protein (Gomez Díaz & Nakagawa 1990).

Several metabolic variables of hemolymph, such as, proteins, glucose, and cholesterol, have been proposed to monitor the effect of environmental conditions on wild and cultured shrimp. The type of food is a dominant factor affecting shrimp hemolymph metabolites (Pascual et al. 2003). Baseline levels of hemolymph metabolites were obtained in *Litopenaeus vannamei* by Rosas et al. (2002) to be used as reference parameters. Glucose is the major component of circulating carbohydrates in Crustacea, but its concentration varies markedly among species. Increases in hemolymph glucose levels are also associated with the physiological response of stress in shrimp, but these levels can only be interpreted properly if the nutritional state of the shrimp is controlled carefully. Furthermore, glucose in hemolymph is a indicator of carbohydrate metabolism and the level of this nutrient in the diet.

Velurtas et al. (2011) investigated the dietary nutritional requirements of 2 Argentine penaeid species on the basis of their hemolymph and midgut gland metabolic contents, and conducted an apparent digestibility analysis to determine which of the dietary components were assimilated better. They compared the effect of different cellulose:starch ratios (30:0, 20:10, 10:20, 0:30) on *Artemesia longinaris* and *Pleoticus muelleri* and found no significant differences in plasma metabolites levels in *P. muelleri*. In *A. longinaris*, however, a significant increase was observed in glucose, total protein, and cholesterol in correlation with increased dietary starch (Table 2), although the bibliography suggests that *A. longinaris* and *P. muelleri* present a similar nourishing regime (Boschi 1989, Gavio & Boschi 2004). Velurtas et al. (2011) suggest a more herbivorous behavior for *A. longinaris* and more omnivorous habits for *P. muelleri*. They compared the effect of different starch:cellulose ratios at the levels of plasma metabolites (glucose, total protein, cholesterol), enzymatic activities, and apparent digestibility in two species of penaeids. No significant increase was observed in metabolites in correlation with increased dietary starch. However, the ratio of amylase activity to protease activity declined in *A. longinaris* when the percentage of dietary starch increased; the ratio for *P. muelleri* increased with higher starch concentrations. The apparent digestibility coefficient increased from 51.5% to 83.7% (*A. longinaris*) and from 7.6% to 71.9% (*P. muelleri*) as the dietary starch levels increased (Fig. 2). This result is in accordance with previous results obtained by Fenucci et al. (2009), who proposed that, in cultured conditions, the 2 species of penaeid shrimp have a 92% apparent digestibility of proteins with diets having fishmeal as the main ingredient; when this ingredient is replaced by soybean meal, the digestibility is 83% for *A. longinaris* and 47.7% for *P. muelleri*.

DIGESTIVE ENZYME ACTIVITY IN RELATION TO SEX, HABITAT, AND NATURAL FOOD

The feeding pattern of crabs in their natural environment includes herbivorous, carnivorous, scavengers, deposit feeders, and sometimes filter-feeding species, although most of them have the ability to deal with a variety of diets and could be considered opportunistic omnivores (Warner 1977). Different food types (e.g., whole animals with different skeletal parts, animal carrion, macroalgae, siliceous microalgae, vascular plants, etc.) require different digestive processing strategies, such as different mechanical processing, enzymes, time of digestion, and so forth, and an understanding of the digestive process constitutes a mechanistic bridge between the physiological

Table 2.

Metabolic variables in haemolymph and midgut gland of *Artemesia longinaris* and *Pleoticus muelleri* fed different cellulose/starch ratio in diet.

	Artemesia longinaris				*Pleoticus muelleri*			
	30/0	**20/10**	**10/20**	**0/30**	**30/0**	**20/10**	**10/20**	**0/30**
Cellulose/starch	**Haemolymph**				**Haemolymph**			
Glucose	0.7 ± 0.05^{c}	0.8 ± 0.07^{b}	0.9 ± 0.03^{a}	1.2 ± 0.13^{a}	0.7 ± 0.02^{a}	0.7 ± 0.01^{a}	0.7 ± 0.03^{a}	0.9 ± 0.04^{a}
Protein	2.2 ± 0.01^{b}	2.1 ± 0.18^{b}	3.1 ± 0.02^{a}	3.4 ± 0.28^{a}	2.2 ± 0.21^{a}	2.6 ± 0.14^{a}	2.5 ± 0.40^{a}	2.9 ± 0.39^{a}
Cholesterol	0.2 ± 0.08^{b}	0.2 ± 0.01^{b}	0.3 ± 0.23^{a}	0.4 ± 0.26^{a}	0.3 ± 0.06^{a}	0.3 ± 0.02^{a}	0.3 ± 0.01^{a}	0.3 ± 0.05^{a}
	Midgut gland				**Midgut gland**			
Glucose	0.3 ± 0.04^{c}	0.5 ± 0.01^{b}	0.4 ± 0.01^{c}	0.4 ± 0.01^{b}	0.5 ± 0.11^{a}	0.5 ± 0.05^{a}	0.7 ± 0.06^{a}	1.0 ± 0.01^{b}
Protein	1.2 ± 0.32^{a}	1.4 ± 0.05^{a}	1.4 ± 0.11^{a}	2.3 ± 0.67^{a}	1.2 ± 0.26^{b}	2.0 ± 0.25^{a}	1.5 ± 0.17^{b}	1.1 ± 0.19^{b}
Cholesterol	2.9 ± 0.24^{b}	2.4 ± 2.65^{b}	6.2 ± 0.03^{b}	19.4 ± 2.6^{a}	0.02 ± 0.0^{b}	0.02 ± 0.0^{b}	1.4 ± 1.89^{b}	0.01 ± 0.0^{b}

Glucose, protein and cholesterol values are in mg/ml. Different letters in the same row for each species indicates statistical differences ($P \leq 0.05$).

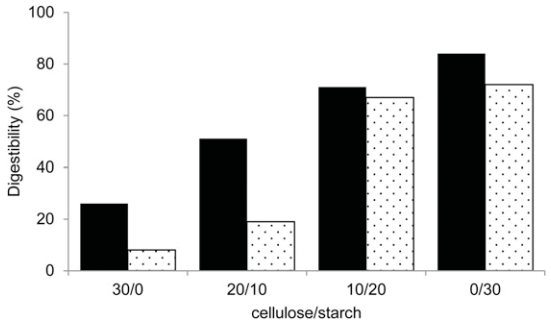

Figure 2. Apparent protein digestibility at different levels of dietary cellulose/starch in *Artemesia longinaris* ( ) and *Pleoticus muelleri* ( ).

processes that occur in the digestive tract, feeding, and nutritional ecology (Karasov & Martínez del Rio 2007).

By understanding the digestion and assimilation of specific dietary components, we can identify the type of prey that the animals prefer, and those they are best equipped to digest. For example, carnivorous species exhibit a wide range and high activity of proteolytic enzymes to process their high-protein diet, whereas herbivores and omnivores ingest large amounts of carbohydrates (Johnston & Freeman 2005).

In this regard, Lancia et al. (2012) evaluated the digestive physiology in relation to sex and microhabitat of the crab *Neohelice granulata*. The aims of their study were 2-fold: (1) to detect the differences in digestive enzymes related to sex and (2) discern the microhabitat of crabs. To achieve the first goal, the specific activity of proteolytic, cellulolytic, and amylolytic enzymes was compared between males and females collected in winter, when males are in a resting, postreproductive period, whereas females, which do not feed during the summer ovigerous period, are developing ovaries and preparing for the next reproductive season (Ituarte et al. 2006, Luppi et al. 2012). To accomplish the second goal, the specific activity of the same enzymes was measured in crabs collected in the saltmarsh and the mudflat, which were fasted during 5 days and then fed *Spartina densiflora* and sediment from the natural habitat, respectively, to be confident of the quality of the food ingested.

The crab *Neohelice granulata* is considered herbivorous and detritivorous because it feeds on halophytic grasses (*Spartina* spp.) in salt marshes, but shifts to sediment rich in organic matter in unvegetated tidal flats (Iribarne et al. 1997). The organic matter found in sediment comes mainly from the degradation of species of the cordgrass *Spartina* and from some protein-rich items such as small crustaceans, polychaetes, and nematodes (Botto et al. 2006). In the intertidal area, *N. granulata* is exposed to a great number of physical changes, such as temperature, humidity, salinity, and dissolved oxygen in water, and biological changes such as food items available, all of which can produce changes affecting feeding and metabolism (Oliveira et al. 2004). In addition, crabs feed on sediment when tides recede, but they feed on plants during flooding tides (Bas, pers. obs.). As a consequence, they face different sets of conditions when feeding at each habitat.

Lancia et al. (2012) analyzed the specific cellulolytic, amylolytic, and proteolytic enzyme activities in the midgut gland of males and females from each microhabitat fed in the laboratory with *Spartina densiflora* leaves and sediment, respectively, to detect sex-, food-, and microhabitat-related differences. The presence of β-1,4-glucosidase, endo-β-1,4-glucanase, α-amylase, trypsin, and chymotrypsin were confirmed. Specific cellulolytic activity was higher in crabs fed leaves than in those fed sediment or in starved controls, and variable differences between sexes were observed (Fig. 3). Specific amylase activity of crabs fed leaves was the lowest recorded. Total proteinase activity of starved crabs was higher than fed specimens, but the level of activity and the difference between fed groups depended on the site of origin and gender. Activity of starved animals from the saltmarsh was higher than starved animals from the mudflat, and a gender difference existed between specimens from the mudflat. Total proteinase activity of males and females fed sediment was similar to the activity of females fed leaves, and was equally low compared with the activity of males fed leaves. Gender differences were found between crabs fed leaves from the saltmarsh (Fig. 4). The feeding condition was the factor that most influenced the values of proteolytic activity. Trypsin- and chymotrypsin-specific activities were higher in saltmarsh crabs fed leaves than in mudflat crabs fed sediment. Different mechanisms of enzyme regulation to explain the observed differences among groups were suggested. In addition, differences between sexes suggest different metabolic needs related to gonad maturation. It is concluded that *N. granulata* have the ability to adapt digestive enzyme production to support their physiological and metabolic needs based on different food sources.

ONTOGENETIC CHANGES IN DIGESTIVE PHYSIOLOGY AND MORPHOLOGY

Functional larval morphology during ontogenetic development appears to be similar for most decapod larvae. Most larvae are dependent on enzymatic breakdown of ingested food, development of secretor tissue of midgut gland dictates the type of prey that can be consumed (Jones et al. 1997).

Variations in digestive enzyme expression in early development may reflect changes in digestive capabilities and this information can be used to identify early developmental feeding transitions. In addition, these data may also provide insight into nutrition, dietary preferences, and strategies of resource utilization that could lead to improved management strategies for

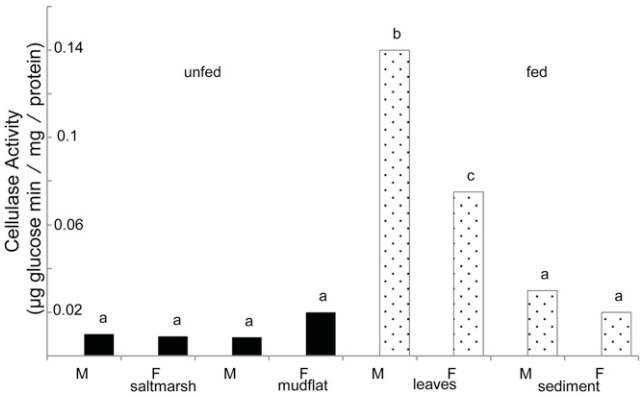

Figure 3. Cellulose activity in the midgut gland of *Neohelice granulata*. Shown are the control (no feed) males (M) and females (F) from the saltmarsh and the mudflat, and males and females from the saltmarsh and the mudflat fed leaves and sediment, respectively. Activity is expressed as micrograms per minute per milligram protein of reducing sugars released. Means of 3 replicate analysis ± SD. Significant differences are indicated by different letters.

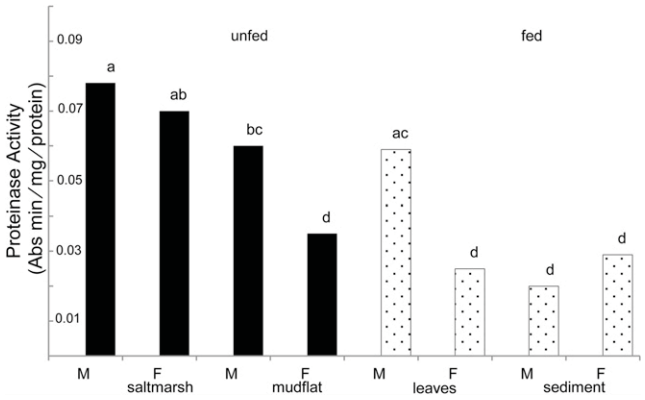

Figure 4. Proteinase activity in the midgut gland of *Neohelice granulata*. Shown are the control (no feed) males (M) and females (F) from the saltmarsh and the mudflat; and males and females from the saltmarsh and the mudflat fed leaves and sediment, respectively. Activity is expressed as the change in absorbance per minute per milligram protein of reducing sugars released. Means of 3 replicate analysis ± SD. Significant differences are indicated by different letters.

aquaculture practices and ecology. This information may also aid in defining the niche that species occupy in aquatic systems (Hammer et al. 2000). Besiot and Capuzzo (1990), Díaz et al. (2008), Fang and Lee (1992), Lemos et al. (1999), Lovett and Felder (1990), and Ribeiro and Jones (2000) studied the development of digestive function by early life history stages of different species of crustaceans.

Díaz et al. (2008) described the ontogenetic changes observed in the histology and in proteinase, trypsin, and chymotrypsin activities of the digestive system of *Pleoticus muelleri*. Stomach development follows the typical pattern described for other decapods; the gland filter develops during larval stages, whereas the gastric mill takes adult shape during postlarval metamorphosis and juvenile stages. During the larval stages, at the junction of the stomodeum with mesodeum, two pairs of caeca are present—an anterior pair formed by a simple tubule and a lateral pair with three lobes (Fig. 5). Each tubule is lined by a simple epithelium with different cellular types—E, F, R, and B cells—typical of the adult midgut gland. During late larval stages, the anterior caeca declines and acquires a structure similar to that of the mesodeum, the lateral caeca decline and take a similar structure to that of the mesodeum and expand to form the adult midgut gland from the proliferation of tubules in the anteroposterior direction and from the cortical region to the medullary region (Fig. 6).

Proteinase activity was higher in postlarvae 45 and no significant differences was found in the others larval and postlarval stages. Trypsin activity was the lowest in early postlarval stages (Postlarvae 1 and Postlarvae 6), coinciding with 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 (Table 3).

Previous studies in other penaeid species such as *Litopenaeus setiferus, Litopenaeus vannamei, Penaeus monodon*, and *Farfantepenaeus paulensis* demonstrated that digestive enzyme activity is very low in embryos before hatching, then increases during the late zoea and early mysis stages. These changes reflect the

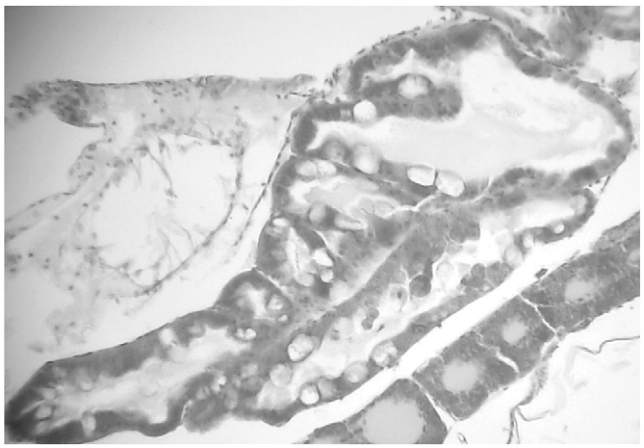

Figure 5. Longitudinal section of mysis 2 stage of *Pleoticus muelleri* showing a detail of the lateral cecum. Magnification ×40.

increased energy turnover associated with intense swimming behavior and food ingestion (Lovett & Felder 1990, Fang & Lee 1992, Lemos et al. 1999, Puello Cruz et al. 2002). During postlarval stages, amylase activity increases in *L. setiferus* and *P. monodon*, whereas protease activity is relatively constant until week 5 of postlarval developmental (Lovett & Felder 1990, Fang & Lee 1992). Changes in enzyme activities among the postmetamorphic stages may be related to changes in body form, habitat, or patterns of energy storage and utilization.

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 ontogenetic development of penaeids, showing the relative significance of change from an herbivorous to an omnivorous feeding pattern. 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 metamorphosis (Díaz et al. 2008). Ontogenetic changes of the digestive system and feeding appendages coincide with behavioral changes occurring during the important transition from a planktonic to benthic life style that take

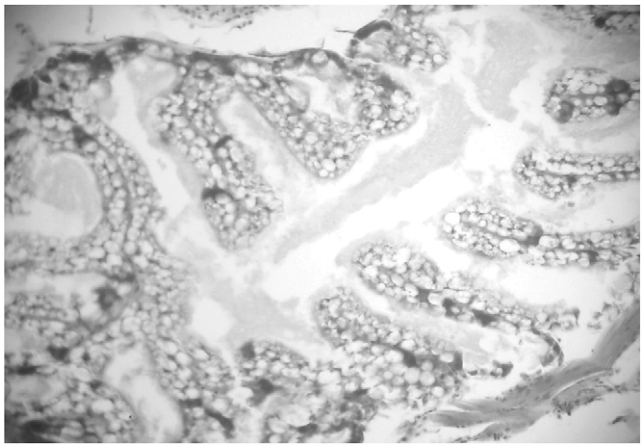

Figure 6. Longitudinal section of the incipient midgut gland of *Pleoticus muelleri* postlarvae. Magnification ×40.

TABLE 3.

Trypsin and chymotrypsin activity in *Pleoticus muelleri* during ontogeny.

Stage	Enzyme activity (abs/min/mg)	
	Trypsin*	Chymotrypsin†
Nauplius	0.77 ± 0.134^{a}	—
Protozoa 3	0.94 ± 0.106^{a}	0.60 ± 0.007^{a}
Mysis 2	1.35 ± 0.113^{a}	1.76 ± 0.714^{b}
Postlarvae 1	0.18 ± 0.113^{b}	3.91 ± 0.099^{e}
Postlarvae 6	0.03 ± 0.007^{b}	3.43 ± 0.071^{d}
Postlarvae 10	1.48 ± 0.205^{a}	3.24 ± 0.049^{d}
Postlarvae 26	0.32 ± 0.007^{c}	1.64 ± 0.049^{b}
Postlarvae 30	0.95 ± 0.170^{a}	2.31 ± 0.097^{c}
Postlarvae 45	1.04 ± 0.099^{a}	2.82 ± 0.099^{c}
Juvenile	0.65 ± 0.163^{a}	1.57 ± 0.085^{b}

Substrates are * BAPNA and † SAPNA. Means in the same column followed by different superscripts are significantly different ($P \leq 0.05$). Means of 3 replicate analysis ± SD.

place after the metamorphosis. In *Pleoticus muelleri* from PL10 to PL12 under culture conditions, they begin to be fed with an artificial diet at the bottom of the tank. Low chymotrypsin activity observed at PL26 may be related to a highly digestible diet (Díaz et al. 2008). However, Lovett and Felder (1989) indicated that during weeks 4 and 5 of postlarval development, important changes occur in digestive system morphology that restrict temporally the communication between the midgut gland and the midgut, affecting gland functioning. This agrees with the important decrease of trypsin and chymotrypsin activities registered by Díaz et al. (2008) in *P. muelleri* at the stage of PL26. From PL30, the values of these enzymes correspond to the rank found in adults and coincide with midgut gland morphogenesis observed in histological studies. The ontogenetic pattern of *P. muelleri* digestive system morphology is similar to that of others penaeids. Some studies showed that the digestive tract morphology depends primarily on 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 a influenced by many external and internal factors. Díaz et al. (2008) demonstrated that the differences in enzyme levels during the ontogeny of *P. muelleri* are related to its unique postlarval life history.

DIGESTIVE ENZYME ACTIVITY IN RELATION TO MOLTING

The body of crustaceans is limited by a rigid exoskeleton that determines a peculiar type of growth. Growth appears as a discontinuous phenomenon that takes place in staggered increments, which outstanding manifestation is the ecdysis (Félix & Petriella 2003). The concept of the molt cycle in Crustacea as a well-defined sequence of stages was first advanced by Drach (1939) on the basis of his classic studies on brachyurans. His subsequent work (Drach 1944) on the macruran *Leander serratus* confirmed there is a certain amount of generality for this concept in decapod crustaceans. The author described the 3 major phases of transformation. One of them is the postmolt, during which individuals become turgid by absorption of water; another is the intermolt, which is a phase of calcification of the integument and tissue growth; and the last stage is the premolt, this is a period when the organisms are preparing for the next molt. Each one of these phases is subdivided into stages of shorter duration based on specific morphological features (Kurup 1964).

The molt cycle is the most critical and challenging phase in crustacean physiology. This metabolic process drives extensive behavioral, integumentary, physiological, and biochemical changes. Besides its role in digestion, the digestive gland participates actively in the molt cycle; it is the major site for storage of glycogen, fats, and calcium during premolt, and thus is a primary factor in the mobilization of these reserves when needed for subsequent molt stages. Knowledge of the digestive capacity of Crustacea through their molt cycle is limited to a few species, such as *Farfantepenaeus notialis* (Fernández et al. 1997), *Pleoticus muelleri* (Fernández Gimenez et al. 2001), *Artemesia longinaris* (Fernández Gimenez et al. 2002), *Litopenaeus vannamei* (Muhlia Almazán & García Carreño 2002), and *Panulirus argos* (Perera et al. 2008), and results are, to some extent, contradictory.

Fernández Gimenez et al. (2001, 2002) described the activity and some characteristics of digestive proteinases of *Pleoticus muelleri* and *Artemesia longinaris* during the different stages of the molting cycle. Proteolytic activity of *P. muelleri* was highest between pH 7.5 and 8, and no significant differences were found in total activity when comparing molting stages. Low trypsin and chymotrypsin activities were found during intermolt, and increased during postmolt (Table 4). Three low-molecular weight trypsin forms (17.4 kDa, 19.1 kDa, and 20 kDa) and 1 band of chymotrypsin (21.9 kDa) were found in all molting stages.

Digestive proteinase activity of the Argentine shrimp *Artemesia longinaris* was assayed at different stages of the molting process. Total proteolytic activity in the midgut gland was highest during postmolt. Trypsin and chymotrypsin activities were highest during intermolt (Table 4). Specific inhibitors and zymograms of shrimp midgut gland extracts showed 4 trypsins (14.79 kDa, 15.49 kDa, 16.60 kDa, and 17.38 kDa) and 3 chymotrypsins (21.38 kDa, 22.91 kDa, and 27.54 kDa) (Fernández Gimenez et al. 2002).

Fernández Gimenez et al. (2001, 2002) suggest that proteolytic activity in the midgut gland of Argentine shrimp is influenced by the molting cycle. Types and activity of shrimp digestive enzymes constitute background information to study further on the digestive abilities of these organisms and would lead to an understanding of their nutritional needs and feeding ecology.

In crustaceans, there are alternate episodes of feeding and fasting during development, which occurs through molting and results in growing by sequential steps. This process requires a high amount of energy. Molting involves a series of stages with different feeding behavior. During intermolt, crustaceans feed actively. Before molting, feeding declines until it stops completely during ecdysis, then feeding begins again during postmolt (Sugumar et al. in press). Starvation induction of Crustacea during the intermolt stage has been suggested to be a good model to understand the molecular and enzymatic changes that occur naturally during their growth process, although the effect of hormones must not be forgotten (Sanchez Paz et al. 2006). In this regard, Muhlia Almazán and García Carreño (2002) investigated the effect of starvation as a stimulant of the digestive system on digestive proteinase activities in the white shrimp *Litopenaeus vannamei*. The starved organisms

TABLE 4.

Proteinase, trypsin, and chymotrypsin activity in *Pleoticus muelleri* and *Artemesia longinaris* during the molting cycle.

		Enzyme activity (abs/min/mg protein)		
		Proteinase*	Trypsin†	Chymotrypsin‡
Pleoticus muelleri (Fernández Gimenez et al. 2001)	Postmolt	0.63 ± 0.030^{a}	2.40 ± 0.190^{c}	3.73 ± 0.291^{c}
	Intermolt	0.63 ± 0.025^{a}	0.90 ± 0.061^{a}	1.21 ± 0.101^{a}
	Premolt	0.36 ± 0.020^{a}	1.40 ± 0.070^{b}	2.10 ± 0.072^{b}
Artemesia longinaris (Fernández Gimenez et al. 2002)	Postmolt	1.23 ± 0.027^{b}	3.60 ± 0.171^{b}	4.83 ± 0.251^{b}
	Intermolt	0.82 ± 0.018^{a}	3.91 ± 0.111^{c}	5.40 ± 0.501^{c}
	Premolt	0.91 ± 0.020^{a}	2.80 ± 0.620^{a}	3.90 ± 0.140^{a}

Substrates are * azocasein, † BAPNA, and ‡ SAPNA. Means in the same column and same species followed by different superscripts are significantly different ($P \leq 0.05$). Means of 3 replicate analysis ± SD.

were sampled periodically according to the molting stage and were compared with a continuously fed group; molting stage was included as an independent variable. Most analyzed variables, except for trypsin, were primarily affected by starvation than by molting, indicating that starvation is a stimulant that masks the effect of molting, and shows that food or alimentary stress is more conspicuous than the physiological one. In the starved organisms, trypsin and chymotrypsin activity was similar, suggesting dependence on one another. Changes in proteolytic activity and the number of protein bands elucidated during electrophoresis showed evidence of synthesis regulation in the midgut gland of white shrimp.

The relationship between molt and reproduction in crustaceans has been studied for decades. In some species, mating, spawning, and incubation of eggs are more successful if they are synchronized. The time between mating and spawning varies greatly among the different crustacean species, and this variation is related to the molt stage of the females at the moment of mating, the method used to transfer and store the sperm, and the process of egg hatching. Díaz et al. (2003) investigated the relationship between the molting cycle and gonadal maturation in a *Pleoticus muelleri* population from the coastal waters of Mar del Plata, Argentina. The analysis of molting activity revealed activity patterns that vary conspicuously with both the changes in shrimp reproductive status and the season. Males and females exhibited molt synchronism, and intermolt lengthened during the reproductive season.

Fernández et al. (2007) studied the relation between ovarian maturation and digestive enzyme activity in wild female *Farfantepenaeus notialis* at different stages of the molt cycle. Total proteolytic activity in the digestive gland was high from the latest postmolt to intermolt, and in the stomach the highest value was observed during premolt. For the endopeptidases, the highest value was for trypsin in the stomach during premolt and in the midgut gland during intermolt. The exopeptidases showed peaks in the midgut gland during intermolt. Leucine aminopeptidases showed the lowest proteolytic values, and peaks in stomach were observed during intermolt and later premolt. Several causes could have changed the different activity of digestive enzymes in relation to the molting cycle and ovarian maturation: the absence of feeding during postmolt and late premolt, the coincidence of the beginning of the feeding process with oocyte development, and the influence of stimulating and inhibiting substances derived from eyestalk neurosecretions.

CONCLUSION

There is no doubt that the occurrence and activity of digestive enzymes is influenced by many internal and external factors, such as diet, molting, and development. The research of Argentine decapod species discussed in this review provides a better understanding of basic digestive physiology, and is important for nutrition and feeding ecology investigations.

Interest in the culture of *Pleoticus muelleri* and *Artemesia longinaris* is increasing; therefore, a deeper knowledge of their mechanisms of digestion may be helpful in the formulation of diets. It is also necessary to conduct additional research on *Neohelice granulata* to understand its biochemical and physiological adaptations to different environments.

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