Received: 7 May 2018 | Revised: 21 August 2018 | Accepted: 15 September 2018
DOI: 10.1111/are.13858

ORIGINAL ARTICLE

WILEY Aquaculture Research

Development of enzymes during ontogeny of two freshwater Decapoda: *Aegla uruguayana* (Aeglidae) and *Macrobrachium borellii* (Palaemonidae)

Gabriela E. Musin[1] | **Andrea Rossi**[1,2] | **Valeria P. Diawol**[1] | **Pablo A. Collins**[1,3] | **Verónica Williner**[1,2]

[1]Instituto Nacional de Limnología (CONICET – Universidad Nacional del Litoral), Santa Fe, Argentina

[2]Facultad de Humanidades y Ciencias, Universidad Nacional del Litoral, Santa Fe, Argentina

[3]Escuela Superior de Sanidad Dr. Ramón Carrillo, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina

Correspondence
Verónica Williner, Instituto Nacional de Limnología (CONICET – Universidad Nacional del Litoral), Santa Fe, Argentina.
Email: vwilliner@inali.unl.edu.ar

Funding information
Proyecto de Investigación Plurianuales - CONICET from Consejo Nacional de Investigaciones Científicas y Técnicas., Grant/Award Number: PIP 790

Abstract

The similarities or differences among species of the same family or among different families suggest a relationship with their feeding habits or a preference for the various types of food. The aim of this study was to identify the presence of digestive enzymes lipases, total protease, amylases and cellulose, and the effects of sex and size in these activities in two decapod freshwater crustaceans. Specimens of different sizes and sexes of *Aegla uruguayana* and *Macrobrachium borellii* were collected in the natural environment. In laboratory, enzymatic preparation consisted in homogenising the digestive gland in a buffer Tris-HCl. The homogenates were centrifuged and the lipid layer was removed. Enzyme activities were determined using standard protocols. There were no statistically significant differences between sexes in any of the species under study. However, there were changes in the activity of amylases during the ontogeny of *A. uruguayana*, indicating that in juveniles the values of said activity are significantly higher than in the rest of the groups. *M. borellii* showed ontogenetic differences in total proteinase activity: organisms of range 2 (intermediate sizes) presented enzymatic activities much smaller than organisms of range 4 (larger sizes). The comparisons of enzymatic activities between both species yielded significant differences, being higher the values in the species *A. uruguayana* with respect to *M. borellii*. The results obtained in this work indicate that although both species are omnivorous and consume similar items, the activity of these digestive enzymes could be an evidence of different strategies of utilisation of the resource.

KEYWORDS
adults, aeglids, digestion, hepatopancreas, juvenile, palemonids

1 | INTRODUCTION

Decapod crustaceans present a wide range of feeding habits, diets and food processing routes although most species combine the predatory type of feed and intake of decomposing material (Saborowski, 2015; Watling, 2013). In the freshwater decapods, the shrimps and crabs are considered as omnivorous predators. In turn, they are also prey and play an important role in trophic webs of freshwater ecosystems (Collins, Williner, & Giri, 2007). Within these, although the anomuran crab *Aegla uruguayana* and the prawn *Macrobrachium borellii* are omnivores; both have preferences for different items. Plant remains and algae are preferred for *A. uruguayana*, while *M. borellii* prefers oligochaetes and dipterous larvae (Collins & Paggi, 1998; Williner, 2010). Both species cohabit in inland waters of the Paraná River system; however each one seems to have a preference for different sectors. Thus, *A. uruguayana* is

 © 2018 John Wiley & Sons Ltd

usually found under rocks or within mollusc shells (Viau, López Greco, Bond-Buckup, & Rodríguez, 2006) while *M. borellii* frequents the littoral zones of abundant vegetation, as evidenced by the components of its diet, belonging to the littoral-benthic community (Collins & Paggi, 1998).

Once the food has been captured and brought to the mouth by the feeding appendages, mainly chelipeds, and mandibles, it is ingested and then digested and assimilated. At this point in the physiological process, digestive enzymes allow animals to convert the complex macromolecules of the diet into forms that can be absorbed and processed into usable forms. However, the digestion of specific nutrients requires particular enzymes (Saborowski, 2015). This specificity in digestive enzymes determines that a species can only digest those food molecules for which it has enzymes capable of hydrolysing specific types of molecular bonds.

In crustaceans, most digestive enzymes belong to the type of hydrolase enzymes (EC3), that are able to cleave various molecular bonds such as ester, glycosidic or peptide bonds, through reactions involving the addition of water. Some of these enzymes, which have been identified in different crustaceans, are trypsin, chymotrypsin, collagenase, astacin, cathepsin, exopeptidases, lipases, chitinases, amylases, cellulase and laminariase (Saborowski, 2015). The composition of enzymes seems to favour the specific feeding behaviour as well as the utilisation of trophic resources (Johnston & Freeman, 2005).

In decapod crustaceans, the profile of digestive enzymes can vary according to the habitats organisms frequent, the trophic habits and/or trophic level that they occupy in the ecosystems where they live (Figueiredo & Anderson, 2009). Moreover, organisms of the same species have the potential to modulate their enzymatic activities according to sex, type and availability of food (Lancia, Fernández Giménez, Bas, & Spivak, 2012), composition of the diet (Lopez-Lopez, Nolasco, Villarreal-Colmenares, & Civera-Cerecedo, 2005; Pavasovic, Anderson, Mather, & Richardson, 2007), stage of moulting (Fernández, Oliva, Carrillo, & Van Wormhoudt, 1997; Fernández Giménez, García-Carreño, Del Toro, & Fenucci, 2001, 2002; Figueiredo & Anderson, 2003; Muhlia-Almazán & García-Carreño, 2002; Perera et al., 2008), ontogenetic development, genetic control and intestinal morphology (Lemos, Hernández-Cortés, Navarrete, Garcia-Carreno, & Phan, 1999; Vega-Villasante et al., 1999). Figueiredo and Anderson (2009) also concluded that enzyme activity seems to adjust to seasonal feeding patterns.

Several comparative studies provided evidence about the composition of digestive enzymes, which varies considerably between species and may probably show specific patterns for certain taxa. More clearly, the separation of specific groups is evident in the expression of proteolytic enzymes (endopeptidases) (Saborowski, 2015). The differences between species of the same family or between different families suggest a relationship with their eating habits or a preference for the different types of food present in the environment (Figueiredo & Anderson, 2009).

Regarding the variations of digestive enzymes during ontogeny, Figueiredo and Anderson (2003) determined ontogenetic changes in proteases and carbohydrases in the freshwater red claw *Cherax quadricarinatus* and found that juveniles have a greater ability to digest proteins than larger individuals, also presenting the first capacity to digest cellulose. In addition, ontogenetic changes were detected for the lobsters *Jasus edwardsii* and *Panulirus argus*, indicating that these changes respond to physiological needs based on particular components of a diet (Johnston, 2003; Perera et al., 2008). In *A. uruguayana*, Williner, Giri, and Collins (2009) found differences in the consumption of plant remains during ontogeny, a phenomenon that is reflected in the use of the mouthparts and in the external morphology in the chelipeds and mandibles (Viozzi, 2016).

A better interpretation of the mechanisms of digestion can be useful in the understanding of the nutritional requirements and formulation of diet for species that are the destiny of maintenance in aquaculture. The potentiality and feasibility for management in culture systems of the species under study were evaluated and confirmed in *M. borellii* in floating cages installed in natural conditions (Collins, 1999). At present, extensive cultivation of *A. uruguayana* is maintained, and is used by a private aquarist enterprise (J. Milillo, personal communication). Thus, it is essential to know the enzymatic composition of the organisms in order to favour the specific feeding behaviour and indicate the use of trophic resources, as shown in brachyuran crabs (Johnston & Freeman, 2005). These authors found that the stomach contents of six different species of crabs varied among themselves and that the digestive enzymes present were consistent with the food items that were being ingested. In this way, digestive enzymes are a useful complementary tool that allows determining which components of the diet are metabolised more effectively by these organisms (Hu, Hagen, Jeng, & Saborowski, 2012).

Although the decapods of the Río de la Plata system are not traditional organisms for human consumption, *A. uruguayana* is marketed as an ornamental animal (Panné Huidobro, 2010), also having —together with *M. borellii*—great potential to be used as live feed in aquaculture, production of crustacean meal, extraction of astaxanthin for the nutrition of farmed fish, as well as extraction of chitosan for various purposes. *Macrobrachium borellii*, on the other hand, has a high nutritional quality (P. A. Collins, *unpublished data*) due to the contribution of proteins (approximately 75% of wet weight), with an insignificant level of lipids (Boschi, 1981). Therefore, a better understanding of the mechanisms of digestion can be useful for the knowledge of the nutritional requirements of both *A. uruguayana* and *M. borellii* and whether different formulations of diets are required for juveniles and adults of both species. In addition, the relationship between different enzymes (e.g., amylase/protease) is a tool of interest as it provides information on the digestive capacities of the specimen, as well as of physiological changes during ontogeny (Ribeiro & Jones, 2000).

The aim of this work was to identify the presence of digestive enzymes lipases, total protease, amylases and cellulose, and evaluate the effects of sex and, size in these activities in two decapod freshwater crustaceans, from the natural environment: crab *A. uruguayana* and prawn *M. borellii*.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Specimens of different sizes and both sexes of *A. uruguayana* and *M. borellii* were collected in "Espinillo" stream (31°47′09.16″ S and 60°18′57.46″ W), Entre Ríos (Argentina). To ensure sample representativeness, these were taken in the four seasons during 1 year. This inland water is characterised by alternating rocky sandy stretches along its longitudinal section, although it presents floating and riparian vegetation. The water flow also varies between rapid and slow, mainly due to rainfall.

The specimens were collected under rocks and among the vegetation with a hand net and transported alive by INALI track in plastic aquaria with aeration to the laboratory where they were processed. Carapace length (from the tip of rostrum to the beginning of the first pair of pleopods) and wet weight of each animal were measured. Carapace length was measured with an electronic digital caliper (Schwyz, Swiss, ±0.01 mm) and wet weight with a digital precision balance (±0.001 g) after removing excess water with absorbent paper. Sex was determined in *A. uruguayana* according to Martin and Abele (1988) and in *M. borellii* according to Pinheiro and Hebling (1998). For both species, the animals were grouped in four size ranges according to the length of the carapace: 5–10, 10–15, 15–20, >20 mm (Collins & Paggi, 1998; Collins, Giri, & Williner, 2008; Viau et al., 2006), here denominated as R1, R2, R3 and R4 respectively.

2.2 | Extraction of digestive gland and enzymatic preparation

Animals were anaesthetised by hypothermia before removing the hepatopancreatic tissue. After these were removed under a stereomicroscope, they were immediately frozen and stored at −80°C. Enzymatic preparation consisted in homogenising the digestive gland in a buffer Tris-HCl (50 mmol L^{-1}, pH 7.5), in a 1:4 ratio (1 g of digestive gland and four volumes of Tris-HCl). The homogenates were centrifuged at 10,000 *g* (4°C) for 30 min. Lipid layer was removed and the supernatant (enzyme extract) was collected and stored at −80°C.

2.3 | Digestive enzymes analysis

2.3.1 | Lipases activity determination

Extract was determined according to Versaw, Cuppett, Winters, and Williams (1989). The reaction mixture consisted of 100 μl of sodium taurocholate (100 mmol L^{-1}), 920 μl Tris-HCl (50 mmol L^{-1}, pH 8), 10 μl of the enzymatic extract and 10 μl β-naphthyl caprylate as substrate (100 mmol L^{-1} dimethyl sulfoxide [DMSO]). The mixture was incubated at 25°C for 30 min. Then, 10 μl Fast Blue BB (100 mmol L^{-1} in DMSO) was added and the mixture was incubated at the same temperature for 5 min. The reaction was stopped with 100 μl trichloroacetic acid (TCA) (0.73 N), clarified with 1.350 ml ethyl acetate: ethanol (1:1 V/V). The absorbance was recorded spectrophotometrically in Metrolab 330 (made in Argentina) at 540 nm.

2.3.2 | Total protease activity determination

It was determined according to García-Carreño (1992), using azocasein as substrate. The reaction mixture consisted of 20 μl of enzyme extract, 230 μl of Tris-HCl (50 mM, pH 7.2) and 500 μl of azocasein (0.5% in Tris-HCl). The mixture was incubated at room temperature for 30 min. The reaction was stopped with 500 μl of 20% TCA and clarified by centrifugation (20,000 *g*, 4°C for 30 min). The absorbance was recorded spectrophotometrically in Metrolab 330 (made in Argentina) at 440 nm.

2.3.3 | Amylases activity determination

It was determined according to Vega-Villasante et al. (1999), with modifications. The reaction mixture consisted of 500 μl Tris-HCl (50 mmol L^{-1}, pH 7.5), 5 μl enzymatic extract and 500 μl of a starch solution (1% in Tris-Cl, 50 mmol L^{-1}, pH 7.5). It was incubated at environment temperature for 10 min. Immediately after incubation, 200 μl of sodium carbonate (Na_2CO_3) (2 N) and 1.5 ml of dinitrosalicylic acid reagent (DNS) were added to the reaction mixture and boiled at bain-marie for 15 min. The volume was adjusted to 10 ml with distilled water and the coloured solution was recorded spectrophotometrically in Metrolab 330 (made in Argentina) at 540 nm.

2.3.4 | Cellulase activity determination

This enzymatic activity was evaluated by determining the production of reducing sugars from the hydrolysis of microcrystalline cellulose, according to Linton and Greenaway (2004). For this, 50 μl of enzyme extract was mixed with 100 μl of a 2% cellulose solution in a 0.1 M acetate buffer solution, pH 5.5. It was incubated with shaking (100 *g*) at 35°C for 60 min. The reaction was stopped by adding 25 μl of TCA 0.3 M. The excess acid was neutralised with 5 μl of 2.5 M K_2CO_3 and the proteins were precipitated by centrifugation at 10,000 *g* for 10 min. In addition, a blank was prepared for each sample by placing 100 μl of the acetate buffer solution and 50 μl of enzyme extract. Subsequently, 100 μl of supernatant was taken, to which 500 μl of distilled water and 500 μl of DNS were added. This mixture was incubated for 15 min in a water bath at 100°C. The final volume was adjusted to 5 ml with distilled water and the absorbance was recorded spectrophotometrically in Metrolab 330 (made in Argentina) at 540 nm.

Specific enzyme activities were expressed as the number of enzyme units per milligram of protein (U/mg protein). A unit of enzyme activity was defined as the amount of enzyme required to increment per minute 0.01 units of optical density at the wavelength corresponding to the evaluated enzyme (López-López et al., 2005).

2.3.5 | Total proteins

Protein determination of enzyme extracts from the digestive gland was performed using the method of Bradford (1976), using bovine serum albumin as standard.

2.4 | Statistical analysis

Values of enzymatic activity are expressed as mean ± *SD*. The effect of the sex-size interaction on the measurements of enzymatic activities for each species was examined using a two-way analysis of variance (ANOVA), followed by Tukey's multiple comparisons posttest. When the interaction of sex-size factors was not significant, the influence of the factors was assessed separately by one-way ANOVA analysis.

To analyse the relationship between enzymatic activities and length of the cephalothorax, correlation analysis and subsequent regression were performed using the method of minimum squares.

Furthermore, for each range of the size of each species, the amylase/protease ratio was calculated in order to determine the digestive capacity of organisms.

Finally, to evaluate the enzymatic activities of the two species, Kruskal–Wallis and Dunn post-test were analysed.

Differences were considered statistically significant at $p < 0.05$. All the statistical analyses were carried out using R software (R Development Core Team, 2008).

3 | RESULTS

Enzyme activities of lipases, total proteinase and amylases were detected in the two species of crustaceans. The values of these activities were higher in *A. uruguayana* than in *M. borellii* ($\chi^2 = 4.046$; $p = 0.044$ for lipases; $\chi^2 = 28.548$; $p = 0.000$ for total protease; $\chi^2 = 32.757$; $p = 0.000$ for amylase) (Figure 1 a–c). For both species, lipases activity showed the greatest value: 255.65 (±109.69) U/mg protein for *A. uruguayana* and 179.53 (±51.04) U/mg protein for *M. borellii*, followed by amylase (19.23 ± 15.93 U/mg protein) and proteinase (6.76 ± 3.51 U/mg protein) in the case of the anomuran crab, and by proteinase (1.49 ± 0.68 U/mg protein) and amylase (0.18 ± 0.16 U/mg protein) in that of prawn. Despite having made the pertinent adjustments of the technique determinations made in the hepatopancreas of *A. uruguayana*, cellulase enzymatic activity was not detected. In *M. borellii*, the determinations corresponding to this enzyme were not made.

There were no statistically significant differences between sexes in any of the species under study.

3.1 | Lipases activity

In both *A. uruguayana* and *M. borellii*, there was no significant interaction between sex and size factors in the activity of this enzyme, as well as there was no effect of the factors separately in this activity ($p > 0.05$). No significant differences in enzymatic activity were recorded between the different size ranges for any of the two species ($p > 0.05$) (Figures 2 and 3). In *A. uruguayana*, a minimum value of 113.53 U/mg protein in R2 (10–15 mm) and a maximum of 462.84 U/mg protein in R4 (>20 mm) were recorded. Meanwhile, in *M. borellii*, minimum value was 93.31 U/mg protein and maximum value was 320.85 U/mg protein, both registered for R2 (10–15 mm).

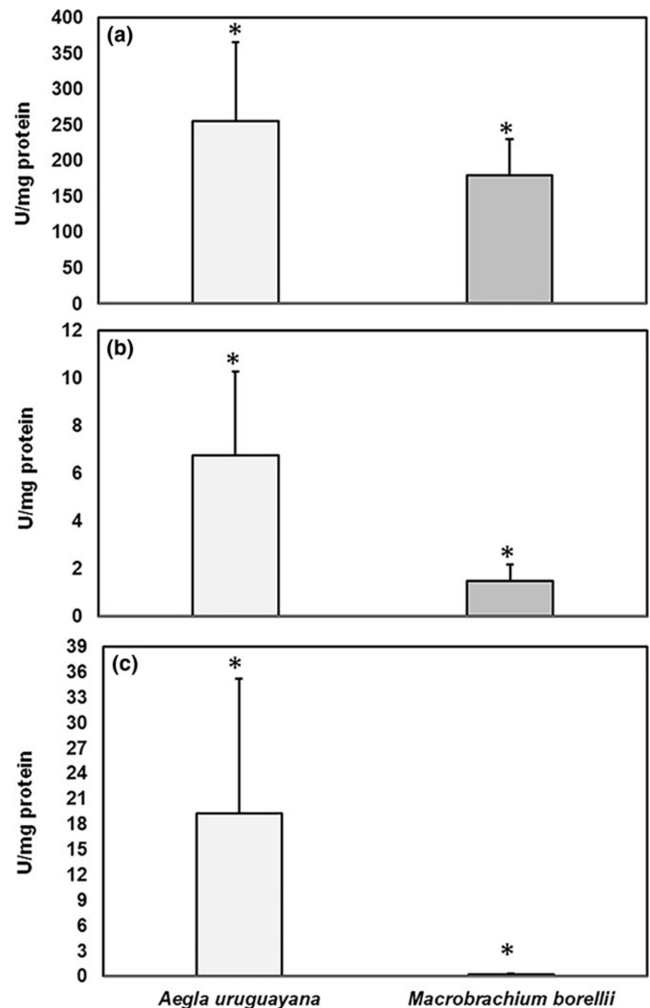

FIGURE 1 Enzymatic activities of lipases (a) total proteinase (b) and amylases (c) in the species *Aegla uruguayana* (from lipases $n = 30$, total proteinase $n = 27$, amylases $n = 33$) and *Macrobrachium borellii* (from lipases $n = 23$, total proteinase $n = 19$, amylases $n = 21$). The asterisk on the bar indicates significant differences. Bars represent mean + *SE*

3.2 | Total protease activity

The interaction of sex and size factors was not significant in this enzymatic activity, nor was the effect of each factor separately for *A. uruguayana* ($p > 0.05$). No significant differences of this enzyme were recorded between the different size ranges in both species ($p > 0.05$) (Figures 2 and 3). The minimum mean value registered in this species was 4.94 (±3.15) U/mg protein. In the case of *M. borellii*, only the effect of size was significant ($F = 4.314$; $p = 0.032$),

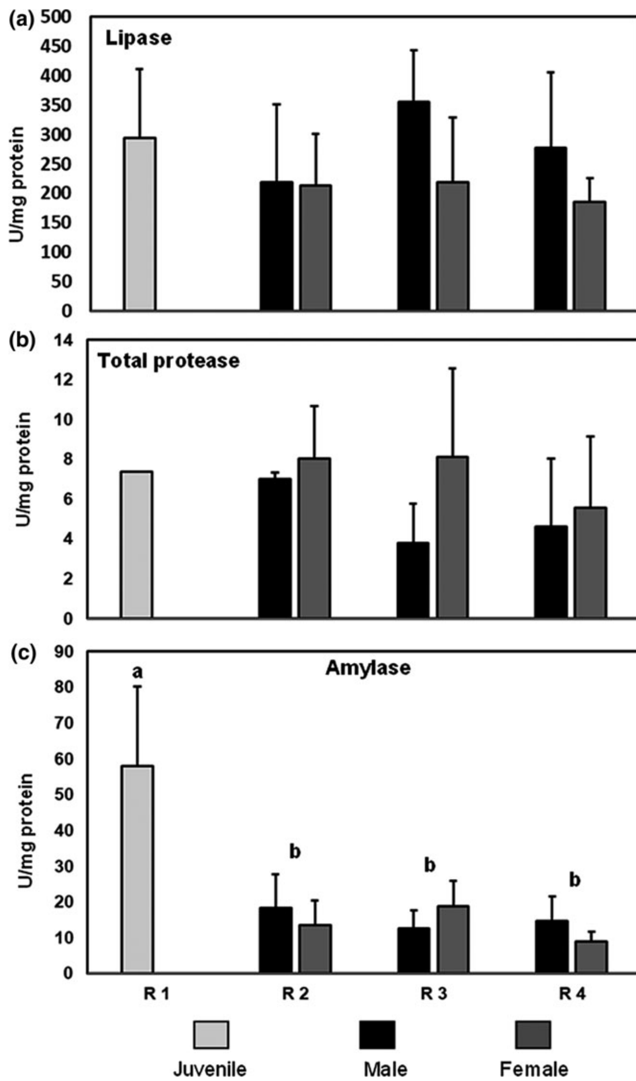

FIGURE 2 Enzymatic activities in the species *Aegla uruguayana* of lipases (a) R1 (LC 5–10 mm) juvenile n = 2, R2 (LC 10–15 mm) male n = 3, female n = 5, R3 (LC 15–20 mm) male n = 5, female n = 6, R4 (LC > 20 mm) male n = 5, female n = 2 male, total proteinase (b) R1 (LC 5–10 mm) juvenile n = 1, R2 (LC 10–15 mm) male n = 3, female n = 5, R3 (LC 15–20 mm) male n = 5, female n = 6, R4 (LC > 20 mm) male n = 4, female n = 2 male, and amylases (c) R1 (LC 5–10 mm) juvenile n = 3, R2 (LC 10–15 mm) male n = 4, female n = 5, R3 (LC 15–20 mm) male n = 5, female n = 6, R4 (LC > 20 mm) male n = 5, female n = 2 male. Different letters in each bar indicate significant differences ($p < 0.05$). Bars represent mean + *SE*

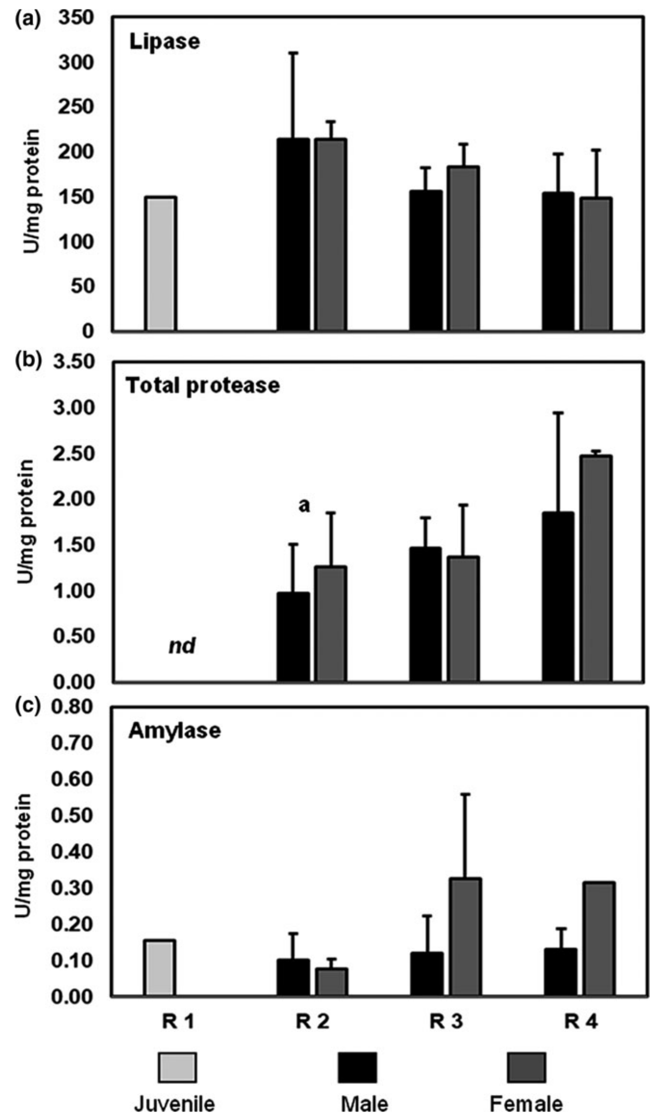

FIGURE 3 Enzymatic activities in the species *Macrobrachium borellii* of lipases (a) R1 (LC 5–10 mm) juvenile n = 1, R2 (LC 10–15 mm) male n = 4, female n = 3, R3 (LC 15–20 mm) male n = 4, female n = 5, R4 (LC >20 mm) male n = 3, female n = 2 male, total proteinase (b) R2 (LC 10–15 mm) male n = 4, female n = 2, R3 (LC 15–20 mm) male n = 4, female n = 4, R4 (LC > 20 mm) male n = 3, female n = 2 male, and amylases (c) R1 (LC 5–10 mm) juvenile n = 1, R2 (LC 10–15 mm) male n = 3, female n = 2, R3 (LC 15–20 mm) male n = 4, female n = 5, R4 (LC > 20 mm) male n = 3, female n = 2 male. Different letters in each bar indicate significant differences ($p < 0.05$). nd: no data. Bars represent mean + *SE*

indicating that the organisms of R2 (10–15 mm) expressed enzymatic activities much lower (1.07 ± 0.51 U/mg protein) than R4 (>20 mm) (2.10 ± 0.84 U/mg protein), those of the R3 (15–20 mm) are located in an intermediate position (1.42 ± 0.43 U/mg protein) (Figure 3b).

3.3 | Amylase activity

In *A. uruguayana*, the factors sex and size failed to explain significantly together with the activity of this enzyme, as well as the factors separately. The amylase enzymatic activity of the juveniles was significantly different from the rest of the groups (F = 19.807; $p < 0.001$) (Figure 2c) (57.93 ± 18.14 U/mg protein). In *M. borellii*, the highest values of enzymatic activity were recorded in R3 (15–20 mm) (0.23 ± 0.21 U/mg protein), but there were no significant differences between any of the ranges (Figure 3C). Together, both factors—sex and size—did not have significant interaction in the activity of this enzyme, nor did the factors separately ($p > 0.05$).

3.4 | Amylase/protease ratio

In anomuran crabs, the amylases/proteinase ratio was higher in juveniles (7.83), while in the rest of the ranges it remained in the order of 2.06 and 5.17. In *M. borellii*, there were no variations of this proportion between the different size ranges. The range of proportions was 0.07–0.37 (Table 1).

With respect to correlation analysis of enzymatic activity versus size, only two cases were significant. In *A. uruguayana*, it indicated a negative association between size and amylases activity ($\rho = -0.3503893$; $p = 0.05831$). On the other hand, in *M. borellii*, the relationship between carapace length and total protease activity was significant, showing a positive association between both (cor = 0.4688549, $p = 0.04287$). As can be seen, in spite of the significant association between both variables, they were not very high.

4 | DISCUSSION

During the ontogeny of *A. uruguayana* it could be changed in the enzymatic activity of amylase, being greater in smaller organisms, but not in lipase or total protease activities. In contrast, for *M. borellii*, only the total protease enzyme activity showed a change among the different sizes, with the greatest activity in organisms of sizes >20 mm. The enzymatic activity was similar in males and females of both species and in the different ranges of sizes.

Digestive lipases catalyse the hydrolysis of triglycerides producing the release of fatty acids and glycerol (Saborowski, 2015). The greater activity of this enzyme in comparison with the rest of the enzymes detected coincides with the highest proportion of triglycerides registered in the hepatopancreas of *A. uruguayana* in relation to other metabolites (Musin, Rossi, Diawol, Collins, & Williner, 2017). The activity of lipases registered in the two species studied would account for the activity of the digestive lipase, which is found in the hepatopancreas, as well as the intracellular lipase (lysosomal). The former is responsible for hydrolysing the lipids of the diet, while the latter is responsible for the hydrolysis of the reserve triacylglycerols (Rivera-Pérez & García-Carreño, 2011). Both enzymes prove to be constantly active in all sizes of organisms, acting on triacylglycerols from the diet and also in those that allow it to obtain energy to be used or transferred as reserve material. Notwithstanding, in juveniles of *Macrobrachium tenellum*, lower values of lipases were registered (below 50 U/mg protein) (De los Santos-Romero, García-Guerrero, Vega-Villasante, Cortés-Jacinto, & Nolasco-Soria, 2017), but in another experimental study this activity reaches an average of 150 U/mg protein (De los Santos Romero, García-Guerrero, Vega Villasante, & Nolasco Soria, 2017). In *Litopenaeus vannamei* digestive lipases are affected by fasting and increase their activity after 24 hr of treatment, which suggests that lipids are used as energy reserves during fasting (Rivera-Pérez, Navarrete del Toro, & García-Carreño, 2010).

TABLE 1 Amylase/protease ratios for the species *Aegla uruguayana* and *Macrobrachium borellii*

	A. uruguayana	*M. borellii*
R 1 (Juveniles)		
	7.83 (±3.00)	*nd protease*
R 2 (10–15 mm)		
Males	2.52 (±1.67)	0.14 (±0.12)
Females	2.06 (±1.32)	0.07 (±0.06)
R 3 (15–20 mm)		
Males	2.60 (±2.15)	0.10 (±0.03)
Females	4.06 (±2.41)	0.37 (±0.25)
R 4 (>20 mm)		
Males	5.17 (±2.78)	0.09 (±0.08)
Females	2.21 (±1.92)	0.13 (nSD)

Note. *nd protease*: without total protease activity data; (nSD): without standard deviation.

The proteinases are responsible for hydrolysing the polypeptide bonds to generate smaller molecules. According to the results obtained in this work, an increase in proteinase activity was detected in the shrimp as the size increased. The analysis of stomach contents for this species concluded that although it is an omnivorous organism, there is an important carnivorous component in the diet of *M. borellii*. These components are of larvae of diptera and oligochaetes as predominant components with high protein values, although during winter—the season of low temperatures and waters as well as the absence of macrophytes—there is a greater preference for zooplankton organisms (Collins & Paggi, 1998). Nevertheless, in this same study, it was also found that there were no ontogenetic changes in the diet, while Carvalho and Collins (2011) determined that both juveniles and adults have the same capacity for predation on these preys of greater preference and different bioforms. However, it must be considered that not all proteinase activity can be attributed to trophic aspects but may be related to other functions, such as anti-inflammatory purposes, blood coagulation, immunisation, embryonic development, among others (Hernández-Cortés, Rivera-Pérez, García-Carreño & Martínez-Alarcón, 2017). Therefore, the increase in proteinase activity as the size increases may not be due to a preference for protein items in larger sizes, but may account for the mobilisation of reserves to be used in the reproductive period (Antunes et al., 2010; Rosa & Nunes, 2003).

As mentioned above, *A. uruguayana* has a preference—within its omnivorous diet—for plant remains and algae (Williner, 2010), which could justify the great amylase activity recorded in second place, after lipase activity. Amylase is a carbohydrase that hydrolyses the α-D (1,4) glycosidic linkages in polysaccharides such as starch, amylopectin, and glycogen, releasing oligomers and glucose monomers. However, taking into account pattern analysis of the mandible in organisms of different sizes, it was shown juveniles consume plant remains at a lower frequency than adults (Williner et al., 2009). Therefore, it is assumed that in small size organisms there would not be a considerable amount of substrate available in the digestive tract to justify the amounts of the enzyme found in this work. Previous studies indicate that secretion of large quantities of an enzyme against a small component in the diet allows maximising the use of

this resource (Harris, Samain, Moal, Martin-Jezequel, & Poulet, 1986; Lovett & Felder, 1990). According to the mentioned authors, the biochemical components of a food item are best extracted with higher enzyme levels when these decrease in the ingested food. It follows that the enzymatic activity is not only related to the available foods, but also to the ability to satisfy the various nutritional requirements of the organism, being related to the digestion and assimilation of the food more than with the intake of them. Therefore, taking into account the latter and the dietary analysis carried out by Williner (2010), the stomach contents observed in *A. uruguayana* might not be an accurate representation of what this species really assimilate, but a measure of the environmental offer and the selectivity. According to spatial distribution studies, juveniles of *A. uruguayana* usually use more areas of the streams where the size of the sediment particles is smaller and where they can be buried and sheltered, while adults prefer sectors of the stream where there are more deposits of plant remains from the allochthonous vegetation (V. P. Diawol, personal communication). This distribution coincides with that provided by Cogo and Santos (2013), who demonstrated in southern Brazil streams that decapod crustaceans such as *A. longirostri* are of great importance as vegetable material shredders, mainly allochthonous, accelerating the decomposition rate of the leaf. In previous studies, it has been reported that amylase activity increased until the decapods grew, and that the reason for the difference in amylase activity may be caused by different food sources (Chen, Chen, & Tan, 2018; Hammer, Bishop, & Watts, 2000).

The decrease in the proportion of amylases/proteinase as size increases in *A. uruguayana* would show a great ability to digest carbohydrates in the smaller sizes, which could come from plant items in the diet. On the other hand, there would be an opposite trend in the larger sizes. *Macrobrachium borellii* showed no pronounced trend in this relation.

In decapods with direct development, like *C. quadricarinatus*, protease enzymes and carbohydrases varied ontogenetically, showing high levels of proteases in smaller sizes, which, as their size increased, decreased their activity and carbohydrases increased, without having this relationship with the diet provided, although it would be related to the greater preference of larger organisms for plant foods (Figueiredo & Anderson, 2003). From this perspective, in crustaceans with larval stages, feeding is not a signal for the production of digestive enzymes, but enzymatic activities detected in the early stages of development would be involved in the hydrolysis of endogenous energy reserves, or else "they would be preparing" for feeding in later stages (Johnston, 2003). On the contrary, in those works where adult specimens were analysed, Johnston and Freeman (2005) concluded that activities of digestive enzymes in each species are consistent with the diets of each of them. The authors say, enzymes favour a specific feeding behaviour and a dietary preference and demonstrate for each species the different strategies of resource use. In the species of the present study, aeglids were characterised by the direct development of the abbreviated type, without free-swimming larval forms, distinguished as juveniles with characteristics of adults (Rodrigues, 1978). The prawn *M. borellii* presents an abbreviated larval development, adapted to freshwater environments, whose first young stage has the general characteristics of adults (Boschi, 1981). Thus, it is possible to affirm that for the adult crustaceans, in which the morphological development has already finished, the enzymes are mainly influenced by the diet, in addition to other physiological factors related to gonadal and reproductive maturation events. However, the circadian cycle, the photoperiod, and the molting cycle also exert effects on the activity of digestive enzymes, as demonstrated by Espinosa-Chaurand, Vega-Villasante, Carrillo-Farnés, and Nolasco-Soria (2017) in juveniles of *M. tenellum*.

On the last enzyme analysed, cellulose, activity has not been recorded in the hepatopancreas of the anomurans. Cellulase activity represents a set of enzymes that act synergistically to degrade cellulose (Xue et al., 1999). Thus, endoglucanases hydrolyse the β-1,4 glycosidic linkages randomly throughout the molecule; the exoglucanases release cellobiose units at the end of the cellulose chain, and the β-glucosidases release glucose from the cellobiose. Regarding the enzymatic activity of the cellulase in anomurans, the fact of not having detected activity of this group of enzymes could be due to several causes. One reason would be that this set of enzymes is not present in the anomura, neither endogenously nor exogenously. However, taking into account that activity of this set of enzymes has been detected and characterised in other decapod crustaceans, such as *C. quadricarinatus* (Xue et al., 1999), it is possible that *A. uruguayana* presents these enzymes but maybe, because of the technique used, particularly the insolubility of the substrate (microcrystalline cellulose), it has not been possible to detect it. In the aforementioned Australian lobster, cellulase activity was evaluated according to different substrates, diets and metabolic states (Figueiredo, Kricker, & Anderson, 2001; Sacristán, Nolasco-Soria, & López Greco, 2014). The hypothesis of the presence of this enzyme in anomurans decapods is based on the high intake that these organisms make of plant matter and could be given as it happens in other decapods (Byrne, Lehnert, Johnson, & Moore, 1999).

5 | CONCLUSIONS

The results obtained in this study on the enzymatic activity in both species in the natural environment are a necessary input to design cultures and artificial diets. In this regard, the absence of large variations in the activity of the enzymes facilitates the design of nutrition requirements and its relation to the administration thereof. However, the difference in activity of the amylase in *A. uruguayana* is a factor that in future studies would be adjusted. According to the behaviour of the protease, in *M. borellii*, the effects of different levels of proteins need to be considered and evaluated. The absence of important variations in the enzymatic activity in organisms of different sex can be a positive aspect to consider for the culture proposal.

ACKNOWLEDGMENTS

This work was supported by grant PIP 790 CONICET from Consejo Nacional de Investigaciones Científicas y Técnicas.

ORCID

Verónica Williner http://orcid.org/0000-0001-7950-6968

REFERENCES

Antunes, G. D. F., Do Amaral, A. P. N., Ribarcki, F. P., Wiilland, E. D. F., Zancan, D. M., & Vinagre, A. S. (2010). Seasonal variations in the biochemical composition and reproductive cycle of the ghost crab *Ocypode quadrata* (Fabricius, 1787) in Southern Brazil. *Journal of Experimental Zoology. Part A, Ecological Genetics and Physiology*, *313*(5), 280–291. https://doi.org/10.1002/jez.593

Boschi, E. E. (1981). *Decapoda Natantia.[Natant Decapod]. Fauna de agua dulce de la República Argentina*. Buenos Aires, Argentina: Fundación para la Educación, la Ciencia y la Cultura.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1–2), 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Byrne, K. A., Lehnert, S. A., Johnson, S. E., & Moore, S. S. (1999). Isolation of a cDNA encoding a putative cellulase in the red claw crayfish *Cherax quadricarinatus*. *Gene*, *239*(2), 317–324. https://doi.org/10.1016/S0378-1119(99)00396-0

Carvalho, D. D., & Collins, P. A. (2011). Ontogenetic predation capacity of *Macrobrachium borellii* (Caridea: Palaemonidae) on prey from littoral-benthic communities. *Nauplius*, *19*(1), 71–77. https://doi.org/10.1590/S0104-64972011000100008

Chen, J., Chen, C., & Tan, O. (2018). Ontogenic changes in the digestive enzyme activities and the effect of different starvation duration on the digestive enzyme activities of larval red swamp crayfish (*Procambarus clarkii*). *Aquaculture Research*, *49*, 676–683. https://doi.org/10.1111/are.13497

Cogo, G. B., & Santos, S. (2013). The role of aeglids in shredding organic matter in neotropical streams. *Journal of Crustacean Biology*, *33*(4), 519–526. https://doi.org/10.1163/1937240x-00002165

Collins, P. A. (1999). Role of natural productivity and artificial feed in the growth of freshwater prawn *Macrobrachium borellii* (Nobili, 1896) cultured in enclosures. *Journal of Aquaculture in the Tropics*, *14*(1), 47–56.

Collins, P. A., Giri, F., & Williner, V. (2008). Sexual maturity and ontogenetic shape variations in the freshwater anomuran crab, *Aegla uruguayana* (Decapoda, Aeglidae). *Invertebrate Reproduction & Development*, *52*(1–2), 113–119. https://doi.org/10.1080/07924259.2008.9652278

Collins, P. A., & Paggi, J. C. (1998). Feeding ecology of *Macrobrachium borelli* (Nobili) (Decapoda: Palaemonidae) in the flooded valley of the River Paraná, Argentina. *Hydrobiologia*, *362*(1), 21–30. https://doi.org/10.1023/a:1003166116086

Collins, P., Williner, V., & Giri, F. (2007). Trophic relationships in crustacean decapods of a river with a floodplain. Chapter 5. In A. M. T. Elewa (Ed.), *Predation in organisms. A distinct phenomenon* (pp. 59–85). Berlin/Heidelberg, Germany: Springer – Verlag.

De los Santos Romero, R. B., Garcia Guerrero, M., Vega Villasante, F., & Nolasco Soria, H. (2017). Effect of dietary chitin on digestive enzyme activity, growth and survival of *Macrobrachium tenellum* juvenile prawns. *Latin American Journal of Aquatic Research*, *45*(1), 130–138. https://doi.org/10.3856/vol45-issue1-fulltext-12

De los Santos-Romero, R., García-Guerrero, M., Vega-Villasante, F., Cortés-Jacinto, E., & Nolasco-Soria, H. (2017). Effect of photoperiod and temperature on growth and activity of digestive enzymes in juveniles of the long-arm river shrimp *Macrobrachium tenellum* (Smith, 1871) (Caridea: Palaemonidae). *Journal of Crustacean Biology*, *37*(4), 445–452. https://doi.org/10.1093/jcbiol/rux055

Espinosa-Chaurand, D., Vega-Villasante, F., Carrillo-Farnés, O., & Nolasco-Soria, H. (2017). Effect of circadian rhythm, photoperiod, and molt cycle on digestive enzymatic activity of *Macrobrachium tenellum* juveniles. *Aquaculture*, *479*, 225–232. https://doi.org/10.1016/j.aquaculture.2017.05.029

Fernández, I., Oliva, M., Carrillo, O., & Van wormhoudt, A. (1997). Digestive enzymes of Penaeus notialis during reproduction and moulting cycle. *Comparative Biochemistry and Physiology Part A: Physiology*, *118*, 1267–1271. https://doi.org/10.1016/S0300-9629(97)86802-8

Fernández Gimenez, A. V., García-Carreño, F. L., Del Toro, M. N., & Fenucci, J. L. (2001). Digestive proteinases of red shrimp *Pleoticus muelleri* (Decapoda, Penaeoidea): Partial characterization and relationship with molting. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *130*(3), 331–338. https://doi.org/10.1016/S1096-4959(01)00437-7

Fernandez Gimenez, A. V., García-Carreño, F. L., Del Toro, M. N., & Fenucci, J. L. (2002). Digestive proteinases of *Artemesia longinaris* (Decapoda, Penaeidae) and relationship with molting. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *132*(3), 593–598. https://doi.org/10.1016/S1096-4959(02)00080-5

Figueiredo, M., & Anderson, A. J. (2003). Ontogenetic changes in digestive proteases and carbohydrases from the Australian freshwater crayfish, red claw *Cherax quadricarinatus* (Crustacea, Decapoda, Parastacidae). *Aquaculture Research*, *34*(13), 1235–1239. https://doi.org/10.1046/j.1365-2109.2003.00929.x

Figueiredo, M. S. R. B., & Anderson, A. J. (2009). Digestive enzyme spectra in crustacean decapods (Paleomonidae, Portunidae and Penaeidae) feeding in the natural habitat. *Aquaculture Research*, *40*(3), 282–291. https://doi.org/10.1111/j.1365-2109.2008.02087.x

Figueiredo, M., Kricker, J. A., & Anderson, A. J. (2001). Digestive enzyme activities in the alimentary tract of redclaw crayfish, *Cherax quadricarinatus* (Decapoda: Parastacidae). *Journal of Crustacean Biology*, *21*(2), 334–344. https://doi.org/10.1651/0278-0372(2001) 021[0334:DEAITA]2.0.CO;2

García-Carreño, F. L. (1992). The digestive proteases of langostilla (*Pleuroncodes planipes*, Decapoda): Their partial characterization, and the effect of feed on their composition. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, *103*(3), 575–578. https://doi.org/10.1016/0305-0491(92)90373-Y

Hammer, H. S., Bishop, C. D., & Watts, S. A. (2000). Activities of three digestive enzymes during development in the crayfish *Procambarus clarkii* (Decapoda). *Journal of Crustacean Biology*, *20*(4), 614–620. https://doi.org/10.1163/20021975-99990084

Harris, R. P., Samain, J.-F., Moal, J., Martin-Jezequel, V., & Poulet, S. A. (1986). Effects of algal diet on digestive enzyme activity in *Calanus helgolandicus*. *Marine Biology*, *90*(3), 353–361. https://doi.org/10.1007/BF00428559

Hu, M. Y., Hagen, W., Jeng, M. S., & Saborowski, R. (2012). Metabolic energy demand and food utilization of the hydrothermal vent crab *Xenograpsus testudinatus* (Crustacea: Brachyura). *Aquatic Biology*, *15*, 11–25. https://doi.org/10.3354/ab00396

Johnston, D. J. (2003). Ontogenetic changes in digestive enzyme activity of the spiny lobster, *Jasus edwardsii* (Decapoda; Palinuridae). *Marine Biology*, *143*(6), 1071–1082. https://doi.org/10.1007/s00227-003-1154-0

Johnston, D., & Freeman, J. (2005). Dietary preference and digestive enzyme activities as indicators of trophic resource utilization by six species of crab. *The Biological Bulletin*, *208*(1), 36–46. https://doi.org/10.2307/3593099

Lancia, J. P., Fernandez Gimenez, A., Bas, C., & Spivak, E. (2012). Adaptive differences in digestive enzyme activity in the crab *Neohelice granulata* in relation to sex and habitat. *Journal of Crustacean Biology*, *32*(6), 940–948. https://doi.org/10.1163/1937240X-00002090

Lemos, D., Hernández-Cortés, M. P., Navarrete, A., Garcia-Carreno, F. L., & Phan, V. N. (1999). Ontogenetic variation in digestive proteinase activity of larvae and postlarvae of the pink shrimp *Farfantepenaeus paulensis* (Crustacea: Decapoda: Penaeidae). *Marine Biology*, *135*(4), 653–662. https://doi.org/10.1007/s002270050666

Linton, S. M., & Greenaway, P. (2004). Presence and properties of cellulase and hemicellulase enzymes of the gecarcinid land crabs *Gecarcoidea natalis* and *Discoplax hirtipes*. *Journal of Experimental Biology*, *207*(23), 4095–4104. https://doi.org/10.1242/jeb.01252

Lopez-Lopez, S., Nolasco, H., Villarreal-Colmenares, H., & Civera-Cerecedo, R. (2005). Digestive enzyme response to supplemental ingredients in practical diets for juvenile freshwater crayfish *Cherax quadricarinatus*. *Aquaculture Nutrition*, *11*(2), 79–85. https://doi.org/10.1111/j.1365-2095.2004.00305.x

Lovett, D. L., & Felder, D. L. (1990). Ontogenetic change in digestive enzyme activity of larval and postlarval white shrimp *Penaeus setiferus* (Crustacea, Decapoda, Penaeidae). *The Biological Bulletin*, *178*(2), 144–159. https://doi.org/10.2307/1541973

Martin, J. W., & Abele, L. G. (1988). *External morphology of the genus Aegla (Crustacea Anomura, Aeglidae)*. Washington, DC: Smithsonian Institution Press.

Muhlia-Almazán, A., & García-Carreño, F. L. (2002). Influence of molting and starvation on the synthesis of proteolytic enzymes in the midgut gland of the white shrimp *Penaeus vannamei*. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *133*(3), 383–394. https://doi.org/10.1016/S1096-4959(02)00163-X

Musin, G. E., Rossi, A., Diawol, V. P., Collins, P. A., & Williner, V. (2017). Dynamic metabolic pattern of *Aegla uruguayana* (Schmitt, 1942) (Decapoda: Anomura: Aeglidae): Responses to seasonality and ontogeny in a temperate freshwater environment. *Journal of Crustacean Biology*, *37*(4), 436–444. https://doi.org/10.1093/jcbiol/rux048

Panné Huidobro, S. (2010) Ornamental aquatic organisms: Their import and export in 2009 (in Spanish). Informe de la Dirección de Acuicultura Subsecretaría de Pesca y Acuicultura – Ministerio de Agroindustria de la Presidencia de la Nación (p. 14) consulted on October 8, 2018. https://www.agroindustria.gob.ar/sitio/areas/acuicultura/publicaciones/_archivos//000000_Desarrollos%20Acu%C3%ADcolas/100628_Organismos%20Acu%C3%A1ticos%20Ornamentales%20%20(Su%20importaci%C3%B3n).pdf.

Pavasovic, A., Anderson, A. J., Mather, P. B., & Richardson, N. A. (2007). Effect of a variety of animal, plant and single cell-based feed ingredients on diet digestibility and digestive enzyme activity in redclaw crayfish, *Cherax quadricarinatus* (Von Martens 1868). *Aquaculture*, *272*(1–4), 564–572. https://doi.org/10.1016/j.aquaculture.2007.08.027

Perera, E., Moyano, F. J., Díaz, M., Perdomo-Morales, R., Montero-Alejo, V., Rodriguez-Viera, L., … Galich, G. S. (2008). Changes in digestive enzymes through developmental and molt stages in the spiny lobster, *Panulirus argus*. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *151*(3), 250–256. https://doi.org/10.1016/j.cbpb.2008.07.005

Pinheiro, M. A. A., & Hebling, N. J. (1998). Biologia de *Macrobrachium rosenbergii* (De Man, 1879). In W. Cotroni Valente (Ed.), *Carcinicultura de Água Doce. Tecnologia Para Produção de Camarões* (pp. 21–46). Brasília, Brazil: IBAMA.

R Development Core Team. (2008). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org

Ribeiro, F., & Jones, D. A. (2000). Growth and ontogenetic change in activities of digestive enzymes in Fennero *Penaeus indicus* postlarvae. *Aquaculture Nutrition*, *6*(1), 53–64. https://doi.org/10.1046/j.1365-2095.2000.00132.x

Rivera-Pérez, C., & García-Carreño, F. (2011). Effect of fasting on digestive gland lipase transcripts expression in *Penaeus vannamei*. *Marine Genomics*, *4*(4), 273–278. https://doi.org/10.1016/j.margen.2011.07.002

Rivera-Pérez, C., Navarrete del Toro, M., & García-Carreño, F. L., (2010). Digestive lipase activity through development and after fasting and re-feeding in the whiteleg shrimp *Penaeus vannamei*. *Aquaculture*, *300*(1–4), 163–168. https://doi.org/10.1016/j.aquaculture.2009.12.030

Rodrigues, W. (1978). Estudos biológicos em *Aegla perobae* Hebling & Rodrigues, 1977 (Decapoda, Anomura). *Revista Brasileira De Biologia*, *38*(2), 383–390.

Rosa, R., & Nunes, M. L. (2003). Biochemical changes during the reproductive cycle of the deep-sea decapod *Nephrops norvegicus* on the south coast of Portugal. *Marine Biology*, *141*(6), 1001–1009. https://doi.org/10.1007/s00227-002-0911-9

Saborowski, R. (2015). Nutrition and digestion. In E. S. Chang, & M. Thiel (Eds.), *Physiology, the natural history of Crustacea*, Vol. *4* (pp. 285–319). New York, NY: Oxford.

Sacristán, H., Nolasco-Soria, H., & López Greco, L. (2014). Effect of attractant stimuli, starvation period and food availability on digestive enzymes in the redclaw crayfish *Cherax quadricarinatus* (Parastacidae). *Aquatic Biology*, *23*(1), 87–99. https://doi.org/10.3354/ab00611

Vega-Villasante, F., Fernández, I., Preciado, R. M., Oliva, M., Tovar, D., & Nolasco, H. (1999). The activity of digestive enzymes during the molting stages of the arched swimming *Callinectes arcuatus* Ordway, 1863 (Crustacea: Decapoda: Portunidae). *Bulletin of Marine Science*, *65*(1), 1–9.

Versaw, W. K., Cuppett, S. L., Winters, D. D., & Williams, L. E. (1989). An improved colorimetric assay for bacterial lipase in nonfat dry milk. *Journal of Food Science*, *54*(6), 1557–1558. https://doi.org/10.1111/j.1365-2621.1989.tb05159.x

Viau, V. E., López Greco, L. S., Bond-Buckup, G., & Rodríguez, E. M. (2006). Size at the onset of sexual maturity in the anomuran crab, *Aegla uruguayana* (Aeglidae). *Acta Zoologica*, *87*(4), 253–264. https://doi.org/10.1111/j.1463-6395.2006.00239.x

Viozzi, M. F. (2016). Morfología y ontogenia de mandíbulas y quelípedos de *Trichodactylus borellianus* y *Aegla uruguayana* (Crustacea: Decapoda): Un enfoque ecológico y funcional (p. 70). Facultad de Humanidades y Ciencias, UNL: Tesina para obtener el título de Licenciada en Biodiversidad.

Watling, L. (2013). Feeding and digestive system. In: L. Watling, & M. Thiel (Eds.), *The natural history of the Crustacea. Functional morphology and diversity*, (Vol. *1*, pp. 237–260). New York, NY: Oxford University Press.

Williner, V. (2010). Foregut ossicles morphology and feeding of the freshwater anomuran crab *Aegla uruguayana* (Decapoda, Aeglidae). *Acta Zoologica*, *91*(4), 408–415. https://doi.org/10.1111/j.1463-6395.2009.00416.x

Williner, V., Giri, F., & Collins, P. (2009). Ontogeny of the mandible of *Aegla uruguayana* (Decapoda: Anomura) a geometric morphometric approach. *Paleontologia I Evolució*, *3*, 117–118.

Xue, X. M., Anderson, A. J., Richardson, N. A., Anderson, A. J., Xue, G. P., & Mather, P. B. (1999). Characterisation of cellulase activity in the digestive system of the redclaw crayfish (*Cherax quadricarinatus*). *Aquaculture*, *180*(3), 373–386. https://doi.org/10.1016/S0044-8486(99)00213-6

How to cite this article: Musin GE, Rossi A, Diawol VP, Collins PA, Williner V. Development of enzymes during ontogeny of two freshwater Decapoda: *Aegla uruguayana* (Aeglidae) and *Macrobrachium borellii* (Palaemonidae). *Aquac Res*. 2018;00:1–9. https://doi.org/10.1111/are.13858