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Dynamic metabolic pattern of *Aegla uruguayana* (Schmitt, 1942) (Decapoda: Anomura: Aeglidae): responses to seasonality and ontogeny in a temperate freshwater environment

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ABSTRACT

The trophic activity of freshwater decapod crustaceans varies throughout their ontogenetic process, molt cycle, and daily and annual rhythms. Among freshwater decapod crustaceans, *Aegla uruguayana* (Schmitt, 1942) is an omnivorous, generalist, and opportunistic species. The aim of this study was to analyze the metabolic profile of this species in relation to seasonal and ontogenetic variations. Specimens of different size and sex of *A. uruguayana* were collected in a stream in Entre Ríos province, Argentina during spring, summer, autumn, and winter. Hemolymph samples were collected to determine the levels of glucose, triglycerides, cholesterol, and total proteins. Hepatopancreas and muscle tissue were extracted to quantify glycogen, lipids, and proteins. Maximum concentrations of triglycerides and total proteins in hemolymph were those of spring and summer, respectively, being the size-season interaction significant in both cases. The interaction of the three factors (season, size, and sex) nevertheless showed no significant differences in the set of measurements obtained for glucose and cholesterol. The metabolic parameters analyzed in hemolymph showed no seasonal variations between males and females, except for summer measurements of glucose and cholesterol. The glycogen and lipid measurements in hepatopancreas were significantly affected by the size-season interaction. Instead, proteins showed only seasonal variation. The data obtained for muscle showed that the size-season interaction did not significantly affect the metabolites. The variations observed in the metabolites reflect the use and requirements of nutrients by the species, evidence that *A. uruguayana* is an opportunistic omnivorous species. These variations also reflect the dynamics of the communities and the environments where they live.

Key Words: carbohydrate content, hemolymph, hepatopancreas, lipid content, metabolic energy, muscle, total protein

INTRODUCTION

Feeding and assimilation of nutrients are crucial in heterotrophs such as freshwater decapod crustaceans because of their need to obtain energy to maintain homeostasis and to facilitate the synthesis and degradation of structural macromolecules necessary for maintenance, growth, reproduction, and behavior (Karasov & Martínez Del Río, 2007; Saborowski, 2015). Several studies on trophic ecology have established freshwater decapods as omnivores (Collins & Paggi, 1998; Gutiérrez-Yurrita *et al.*, 1998; Collins, 1999; Williner & Collins, 2002; Albertoni *et al.*, 2003; Collins & Williner, 2003; Bueno & Bond-Buckup, 2004; Santos *et al.*, 2008; Williner, 2010; Williner & Collins, 2013; Williner *et al.*, 2014; Carvalho *et al.*, 2016). It is also known that their consumption varies throughout their ontogenetic process, molt cycle, and daily and annual rhythms (Collins *et al.*, 2007a).

Aegla uruguayana (Schmitt, 1942) is an anomuran crab that inhabits rivers, ponds, shallow lakes, and freshwater streams in Argentina, Brazil, and Uruguay (Bond-Buckup & Buckup, 1994).

Williner (2010) studied the natural diet of this species and indicated that it is similar to that of other freshwater decapods, being an omnivorous, generalist, and opportunistic species. This author also found that juveniles show lower frequency of consumption of plant debris than adults and related these differences to morphological changes of the mouthparts (Williner *et al.*, 2009; Williner, 2010) and with differences in external morphology in the cheliped during ontogeny (M.F. Viozzi, pers. comm.).

Crustaceans, like other animals, use glycogen, proteins, and various types of lipids as the main metabolic fuels (Jimenez & Kinsey, 2015). The hepatopancreas is the principal organ of digestion and storage of lipids and glycogen in crustaceans (Vogt *et al.*, 1985), whereas muscles and gonads seem to be the principal organs for the location of proteins (Claybrook, 1983). In contrast, the hemolymph is involved in the transport of nutrients to tissues. Growth, reproduction, and locomotion involve demands of ATP, leading to changes in the synthesis or mobilization of these energy resources (Harrison, 1990; Chang, 1995; Boyle *et al.*, 2003; Guadagnoli *et al.*, 2005; Sánchez-Paz *et al.*, 2007). Similarly, changes in environmental conditions, including temperature, oxygen availability, and/or salinity, as well as in the accessibility of trophic resources, could alter the metabolic state of the organism (Jimenez & Kinsey, 2015).

The metabolic profile of lipids and carbohydrates in the hepatopancreas and muscle of estuarine and marine brachyuran crabs (e.g. *Neohelice granulata* (Dana, 1851) and *Ocypode quadrata* (Fabricius, 1787)) has acyclic variations to fit the daily and seasonal rhythms (Kucharski & Da Silva, 1991; Vinagre *et al.*, 2007). Studies in members of the family Aeglidae have shown that the storage and degradation of metabolic fuels have seasonal variations, according to the reproductive period, availability of food resources, and seasonality (Oliveira *et al.*, 2003, 2007). Studies on crayfish of the genus *Parastacus* Huxley, 1879 have reached similar conclusions, emphasizing changes during the reproduction period (Silva-Castiglioni *et al.*, 2007) and changes in the availability of trophic resources (Ferreira *et al.*, 2005; Dutra *et al.*, 2008) as main factors influencing the variability of the metabolic profile.

Studies of metabolism during ontogeny in aeglids are practically non-existent and researches on the biochemistry during ontogeny have been undertaken in crustaceans that do not have direct development (Schatzlein & Costlow, 1977; Anger & Harms, 1990; Chu & Ovsianico-Koulikowsky, 1994; Lemos & Phan, 2001). The aim of this study was to analyze the metabolic profile of *A. uruguayana* in three different tissues and its variations due to seasonality and ontogeny. We hypothesized that the metabolic profile varies with seasons and age as in changes in the morphology of mouthparts during ontogeny.

MATERIALS AND METHODS

Sampling

Specimens of different sizes and both sexes of *Aegla uruguayana* were collected in El Espinillo stream (31°47′09.16″S and 60°18′57.46″W), Entre Ríos province, Argentina, during spring (October-November 2013), summer (December 2013 to January-February 2014), autumn (April-May 2014), and winter (July-August 2014). The river is characterized by alternating rocky with sandy stretches along its longitudinal section, and, although it presents floating vegetation, most of the vegetation is allochthonous (riparian). The water flow also varies between rapid and slow, mainly due to rainfall. A multi-parameter sensor (Hanna Instruments, Woonsocket, RI, USA) was used to record water temperature, dissolved oxygen, pH, and dissolved solids (Table 1).

Specimens were collected under rocks with a hand net and transported alive to the laboratory where they were acclimated before being processed during the same day of collection.

Table 1. Seasonal environmental parameters of El Espinillo stream, Entre Ríos province, Argentina (31°47′09.16″S and 60°18′57.46″W) during the sampling period.

Season	Parameters of the water			
	Temperature (°C)	pH	Dissolved oxygen (ppm)	Conductivity (μS cm^{-1})
Autumn	19.3	7.7	8.39	776
Winter	17.4	6.8	7.45	803
Spring	19.8	7.6	10.32	780
Summer	25.2	7.7	8.27	1074

Oxygen and a shelter (rocks or artificial shelters) were provisioned to duplicate natural conditions. 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 a digital caliper (± 0.01 mm) and wet weight with a digital precision balance (± 0.001 g) after removing excess water with absorbent paper. Sex was determined by the presence or absence of modified pleopods (Martin & Abele, 1988). The animals were grouped in four size ranges taking into account the length of the carapace: 5–10 mm, 10–15 mm, 15–20 mm, > 20 mm (Viau *et al.*, 2006; Collins *et al.*, 2008), denominating them as R1, R2, R3, and R4, respectively.

Tissue samples and biochemical analyses

Crabs were anesthetized by hypothermia before removing the tissues. The hemolymph was extracted from the arthrodial membrane in the intersection with the cephalothorax and an appendage with a syringe previously rinsed with an anticoagulant solution of sodium citrate (10%). The hepatopancreas and muscle tissue were removed under a stereomicroscope. Tissues were immediately frozen and subsequently stored at –80 °C until the biochemical determinations were done.

Glycogen, lipid, and protein contents were measured in the hepatopancreas and muscle. Glycogen was estimated using 20 mg of hepatopancreas and 60 mg of muscle tissues treated with 1 ml KOH 30% subsequently with 0.5 ml KOH 60% at 100 °C. After alkaline tissue disruption, glycogen was precipitated by ethanol, and glucose was determined using the anthrone reagent method according to Seifter *et al.* (1950). Lipid content was extracted using chloroform:methanol (2:1) by the method described by Folch *et al.* (1957) and total protein concentration was estimated in tissue homogenates according to Lowry *et al.* (1951), using bovine serum albumin as standard. Specimens of the same size range were pooled when the amounts of tissue per individual crab were not sufficient.

Hemolymph levels of total protein, cholesterol, triglycerides, and glucose were determined with enzymatic colorimetric methods, using the appropriate kits and according to the protocols of the manufacturer (Wiener Laboratorios, Rosario, Argentina). Total plasma protein concentration was measured with a reagent containing the EDTA/Cu complex in an alkaline medium, which reacts with peptide bonds to yield a purple-blue complex (Gasbarro *et al.*, 1972). Plasma levels of total cholesterol and triglycerides were analyzed by using a peroxidase-coupled method (Fossatti & Prencipe, 1982; McGowan *et al.*, 1983). Plasma glucose was determined by a test based on the glucose oxidase method (Trinder, 1969).

Statistical analysis

The statistical tests used were chosen according to the normality and homoscedasticity of the data. The difference between sexes for each size range was tested with the Mann-Whitney U-test. A two-way ANOVA was conducted to examine the effects of size of individuals and season on the values of each metabolite, followed by a

Tukey post-hoc test. When there were differences between sexes, sex was included as an additional factor. Differences were considered significant ($P < 0.05$). All analyses were performed with the PAST software (Hammer *et al.*, 2001).

The values of hemolymph in juveniles were not taken into account due to insufficient sample quantity. The muscle tissue of juveniles was not extracted because of the difficulty of the procedure in small individuals.

RESULTS

Environmental conditions during the study period showed relatively small variations in temperature, pH, dissolved oxygen, and conductivity between seasons. The minimum values of temperature, pH, and dissolved oxygen were recorded during winter (Table 1), when river macrophytes were scarce or practically absent and the vegetation was mainly decaying leaves of riparian vegetation. This situation was reversed during spring and summer, with the presence of macrophytes and an increase in the values of the physico-chemical parameters.

Table 2 shows the results of the two-way ANOVA for the effects of season and specimen size on the values of each metabolite.

Hepatopancreas

The values for glycogen, lipids, and proteins measured in the hepatopancreas were similar between males and females. The concentrations of glycogen were significantly different between sizes and between

Table 2. Results of the two-way ANOVA for the effects of the season and crustacean size on the values of each metabolite measured.

Tissue		Type III sum of squares	d.f.	Mean square	F	Sig
Hepatopancreas *						
Glycogen (84)	Season	1130.619	3	376.873	16.718	**0.000**
	Size	223.064	3	74.355	3.298	**0.026**
	Season*Size	432.110	8	54.014	2.396	**0.024**
Lipids (71)	Season	27444.146	3	9148.049	11.219	**0.000**
	Size	5470.330	3	1823.443	2.236	0.094
	Season*Size	25081.102	8	3135.138	3.845	**0.001**
Proteins (71)	Season	28264.698	3	9421.566	14.779	**0.000**
	Size	2840.224	3	946.741	1.485	0.228
	Season*Size	5797.978	8	724.747	1.137	0.354
Hemolymph **						
Glucose (80)	Season	0.523	3	0.174	80.887	**0.000**
	Size	0.027	2	0.013	6.204	**0.003**
	Sex	0.008	1	0.008	3.520	0.065
	Season*Size	0.091	5	0.018	8.475	**0.000**
	Season*Sex	0.010	3	0.003	1.618	0.194
	Size*Sex	0.001	1	0.001	0.439	0.510
	Season*Size*Sex	0.000	1	0.000	0.196	0.659
Triglycerides (78)	Season	0.859	3	0.286	8.244	**0.000**
	Size	0.004	2	0.002	0.055	0.946
	Season*Size	0.409	5	0.082	2.355	**0.050**
Cholesterol (90)	Season	0.184	3	0.061	10.249	**0.000**
	Size	0.035	2	0.017	2.913	0.061
	Sex	0.019	1	0.019	3.178	0.079
	Season*Size	0.054	5	0.011	1.802	0.123
	Season*Sex	0.006	3	0.002	0.354	0.786
	Size*Sex	0.000	1	0.000	0.021	0.885
	Season*Size*Sex	0.002	1	0.002	0.334	0.565
Total proteins (80)	Season	12010.574	3	4003.525	18.501	**0.000**
	Size	3673.451	2	1836.726	8.488	**0.001**
	Season*Size	2982.512	4	745.628	3.446	**0.013**
*Muscle***						
Glycogen (54)	Season	977.947	3	325.982	19.012	**0.000**
	Size	41.738	1	41.738	2.434	0.126
	Season*Size	24.175	3	8.058	0.470	0.705
Lipids (53)	Season	22.067	3	7.356	6.304	**0.001**
	Size	0.146	1	0.146	0.125	0.725
	Season*Size	5.015	3	1.672	1.433	0.246
Proteins (52)	Season	15804.044	3	5268.015	3.530	**0.022**
	Size	1359.766	1	1359.766	0.911	0.345
	Season*Size	1002.630	3	334.210	0.224	0.879

The size range used was * R1, R2, R3, and R4; ** R2, R3, and R4; ***R3 and R4. The values in parentheses below each metabolite represent the number of specimens examined. d.f.: degrees of freedom. Significant values of the season*size interaction are shown in bold.

seasons (Fig. 1A), indicating statistically significant effects of the size-season interaction ($F = 2.396$; $P = 0.024$; Table 2). The same pattern was observed with lipids ($F = 3.845$; $P = 0.001$; Table 2) (Fig. 1B), but not with proteins. For all sizes, the protein content measured in spring was different among the other seasons, being lower than that obtained in autumn, winter, and summer ($P < 0.05$) (Fig. 1C).

None of the hepatopancreas metabolites showed significant differences between the different size ranges for each season. Instead, the size ranges R3 (15–20 mm) and R4 (> 20 mm) showed variations in glycogen, lipids, and protein measurements throughout the seasons. For R3, glycogen for autumn-summer, winter-spring, and spring-summer ($P < 0.05$); lipids for autumn-summer ($P < 0.05$); proteins for winter-spring and spring-summer ($P < 0.05$). For R4, glycogen for winter-spring ($P < 0.05$); lipids for autumn-summer ($P < 0.05$); proteins for spring-summer ($P < 0.05$) (Fig. 1A, B, C).

Hemolymph

The highest values of glucose were recorded in spring ($P < 0.005$), whereas the highest values of cholesterol were recorded in summer ($P < 0.005$) (Fig. 2A and 2B, respectively).

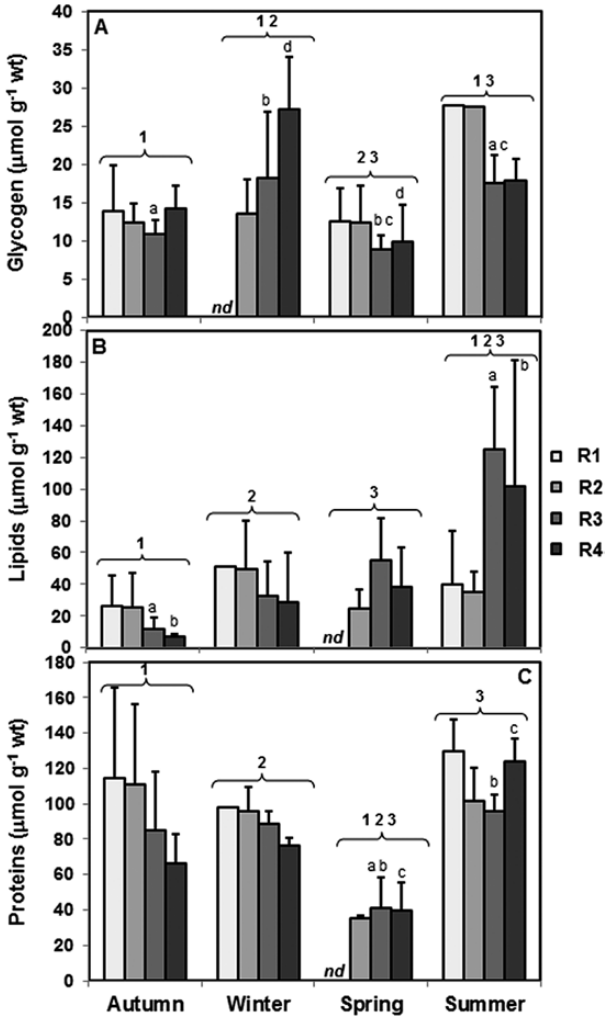

Figure 1. Seasonal concentrations of glycogen (A), lipids (B), and proteins (C) in the hepatopancreas of *Aegla uruguayana*. Individuals were classified in four ranges according to their carapace size: R1 (juveniles, 5–10 mm), R2 (adults, 10–15 mm), and R3 (adults, 15–20 mm), R4 (adults, >20 mm). The same letter represents significant difference between the seasons in a same size range. The same number with the key represent significant difference between seasons assessed by the Tukey post-hoc test; nd, no data.

Maximum concentrations of triglycerides and total proteins were those for spring and summer, respectively (Fig. 2C, D), being the size-season interaction significant in both cases

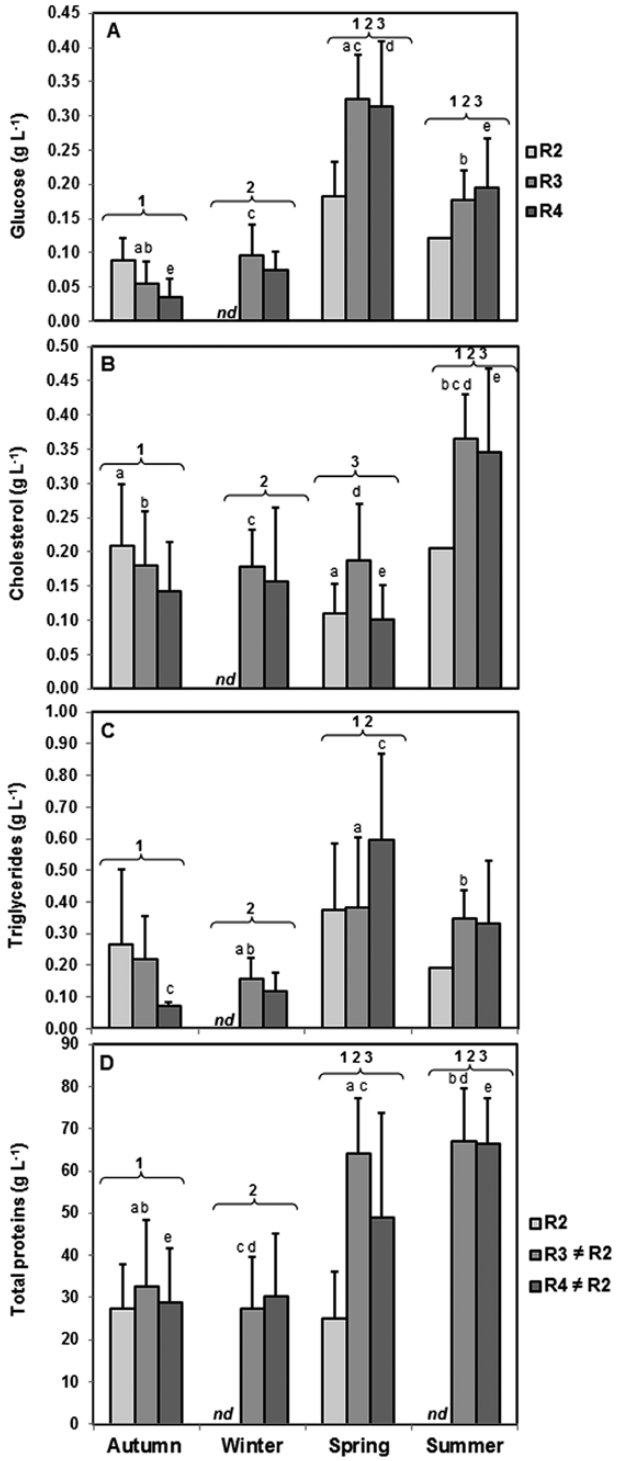

Figure 2. Seasonal concentrations of glucose (A), cholesterol (B), triglycerides (C), and total proteins (D) in the hemolymph of *Aegla uruguayana*. Metabolites were recorded in three size ranges of carapace: R2 (adults, 10–15 mm), R3 (adults, 15–20 mm), and R4 (adults, >20 mm). The same letter represents significant difference between the seasons in a same size range. The same number with the key represent significant difference between seasons thrown by the Tukey post-hoc test. In the legend, the significant differences between sizes (≠) assessed by the Tukey post-hoc test were marked; nd, no data.

($F = 2.355$; $P = 0.050$ for triglycerides; $F = 3.446$; $P = 0.013$ for proteins; Table 3). The minimum values of proteins were observed in individuals having carapace lengths of 10 to 15 mm (R2) ($P < 0.005$).

Significant differences were found between glucose ($P < 0.05$) and total protein ($P < 0.05$) (Fig. 2A, D) and size. The size ranges R2 (10–15 mm), R3 (15–20 mm), and R4 (> 20 mm) showed variations in the measurements of glucose, cholesterol, triglycerides, and total proteins throughout seasons. For R2, cholesterol for autumn-spring. For R3, glucose for autumn-spring, autumn-summer, and winter-spring ($P < 0.05$); cholesterol for autumn-summer, winter-summer, spring-summer; triglycerides for winter-spring and winter-summer ($P < 0.05$); total protein for autumn-spring, autumn-summer, winter-spring and winter-summer ($P < 0.05$). For R4, glucose for autumn-spring; cholesterol for spring-summer, and autumn-summer ($P < 0.05$); triglycerides for autumn-spring ($P < 0.05$); total proteins for autumn-summer ($P < 0.05$) (Fig. 2A, C, D).

Glucose and cholesterol concentrations showed statistically significant differences between males and females only in summer for the R3 size range ($P = 0.0286$) (Table 3). The interaction of the three factors (season-size-sex) showed no significant differences in the set of measurements obtained for glucose and cholesterol. The size-season interaction was nevertheless significant in the glucose measurements ($F = 8.475$; $P < 0.005$; Table 3).

Muscle

The values of muscle protein remained constant throughout the seasons, with the average maximum in summer (Fig. 3C). Data obtained for muscle showed that the size-season interaction did not significantly affect the concentration of macromolecules (Table 2). No significant differences were recorded between the different size groups in each season. Size ranges R3 and R4 nevertheless showed significant differences in glycogen measurements throughout the seasons: R3 for autumn-spring, winter-spring, spring-summer ($P < 0.05$); R4 for autumn-spring and spring-summer ($P < 0.05$) (Fig. 3A). The maximum values of glycogen and lipid were recorded in spring ($P < 0.005$).

Figure 4 summarizes the metabolic pathway of biomolecules among the tissues according to seasons and size.

DISCUSSION

The metabolic profile of *A. uruguayana* varied according to size and seasonality. These variations were nevertheless not always caused by the simultaneous interaction of these two factors. Glycogen and lipid measurements in the hepatopancreas were influenced by the simultaneous interaction of size and season, as in the case of glucose, triglycerides, and total protein in hemolymph. In contrast, muscle measurements were not influenced by size and season. We found that in all tissues the metabolites always varied as a function of the season of the year. This was not the case when only size was taken into account.

These findings were affected by several factors. One such factor is the particular function of the analyzed organs play in the organism. The hepatopancreas is the main organ for the storage of macromolecules in the following order: lipids, proteins, and glycogen. Maximum levels of glycogen were recorded during winter and summer. This suggests the existence of a possible storage in this tissue during these seasons. This reserve seems to be mobilized during autumn and spring. This matches with the increased glucose concentration observed in the hemolymph during spring. Also related to the increase in glucose is the availability of food with high carbohydrate content at this time of the year (Sabattini & Lallana, 2007). Plant debris and algae (mainly diatoms) are the main items in the diet of *A. uruguayana* (Williner, 2010). Resources such as leaf litter and unicellular, filamentous and colonial algae occur throughout the year in temperate streams (Benfield, 1997). After this organic input, leaves begin to be degraded by fungi and other microorganisms. In tropical rivers, such as Brazilian streams inhabited by *Aegla longirostri* (Bond-Buckup & Buckup, 1994), the

Table 3. Seasonal concentrations of glucose, cholesterol, triglycerides, and total proteins in the hemolymph. Results represent the mean±standard deviation of the mean.

		Autumn	Winter	Spring	Summer
Males					
Glucose (g L^{-1})	R2:	0.10 ± 0.03	nd	0.16 ± 0.03	nd
	R3:	0.06 ± 0.04	0.09 ± 0.05	0.28 ± 0.05	0.14 ± 0.03*
	R4:	0.04 ± 0.03	0.07 ± 0.03	0.31 ± 0.09	0.20 ± 0.07
Cholesterol (g L^{-1})	R2:	0.17 ± 0.01	nd	0.09 ± 0.03	nd
	R3:	0.17 ± 0.09	0.17 ± 0.06	0.16 ± 0.08	0.32 ± 0.03*
	R4:	0.14 ± 0.07	0.16 ± 0.11	0.10 ± 0.05	0.35 ± 0.12
Triglycerides (g L^{-1})	R2:	0.13 ± 0.07	nd	0.22 ± 0.10	nd
	R3:	0.16 ± 0.12	0.15 ± 0.06	0.38 ± 0.21	0.31 ± 0.10
	R4:	0.07 + 0.01	0.12 + 0.06	0.60 + 0.27	0.33 + 0.20
Total proteins (g L^{-1})	R2:	28.91 ± 0.99	nd	19.62 ± 12.15	nd
	R3:	35.52 ± 20.87	27.22 ± 12.29	70.25 ± 16.65	61.87 ± 7.38
	R4:	28.71 ± 12.80	30.21 ± 15.00	48.97 ± 24.74	66.55 ± 10.63
Females					
Glucose (g L^{-1})	R2:	0.09 ± 0.04	nd	0.20 ± 0.06	0.12
	R3:	0.05 ± 0.03	0.12	0.35 ± 0.06	0.21 ± 0.02*
Cholesterol (g L^{-1})	R2:	0.22 ± 0.10	nd	0.12 ± 0.05	0.21
	R3:	0.18 ± 0.08	0.22	0.21 ± 0.09	0.41 ± 0.05*
Triglycerides (g L^{-1})	R2:	0.31 ± 0.26	nd	0.47 ± 0.21	0.19
	R3:	0.24 ± 0.14	0.22	0.39 ± 0.27	0.38 ± 0.07
Total proteins (g L^{-1})	R2:	27.04 ± 11.88	nd	29.21 ± 9.67	nd
	R3:	30.49 ± 12.04	nd	59.20 ± 8.53	73.65 ± 16.46

nd: no data; * represents significant differences between sexes

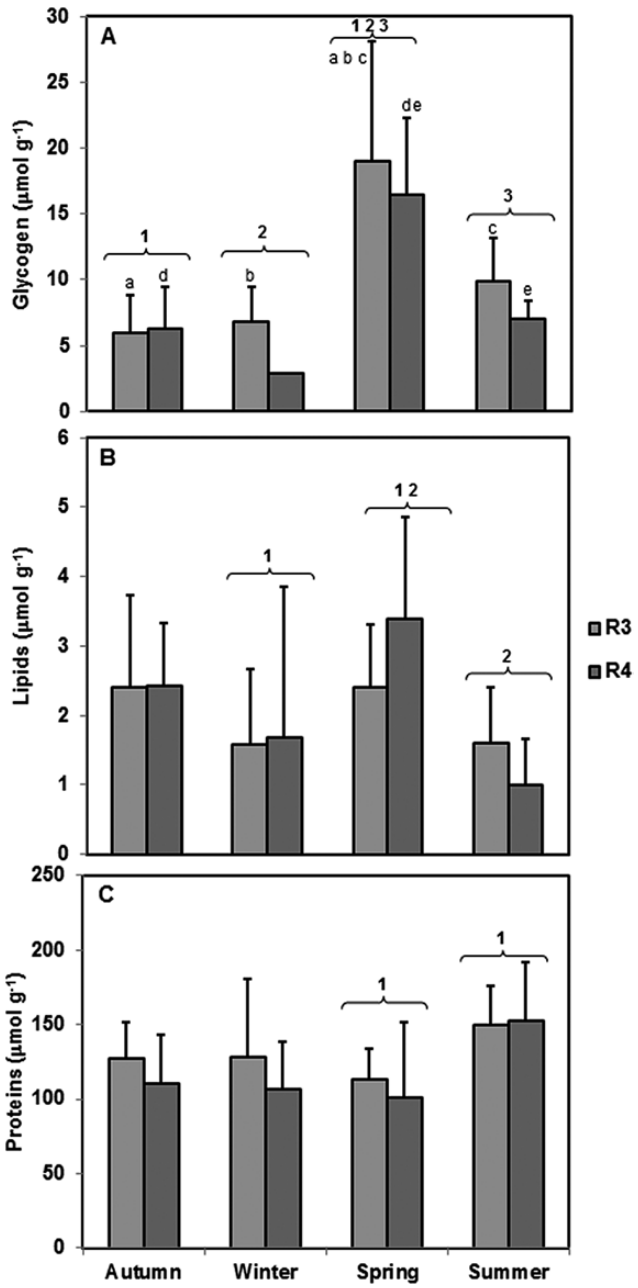

Figure 3. Seasonal concentrations of glycogen (A), lipids (B), and proteins (C) in muscle of *Aegla uruguayana*. The metabolites were recorded in two size ranges of carapace: R3 (adults, 15–20 mm) and R4 (adults, > 20 mm). The same letter represents significant difference between the seasons in a same size range. The same number with the key represent significant difference between seasons assessed by the Tukey post-hoc test.

largest organic allochthonous contribution is recorded in winter (Cogo & Santos, 2013). As in *A. longirostri*, *A. uruguayana* uses mostly vegetable detritus colonized by Ascomycetes fungi and therefore accelerates the breakdown and decomposition of leaf litter (Williner, 2010; Cogo & Santos, 2013).

The hepatopancreas, gills, and muscles store glycogen in both males and females (Jimenez & Kinsey, 2015). Such a wide distribution of glucose among tissues allow a rapid respond to environmental stressors (Hochachka *et al.*, 1970). Oliveira *et al.* (2007) observed seasonal fluctuations in glycogen in females but not in males, with maximum values in autumn. In contrast, these authors observed seasonal fluctuations in males but not in females of *A. ligulata* (Bond-Buckup & Buckup, 1994), with maximum values in winter. These data were related to the relatively low locomotory activity of these crabs during winter, when they decrease their metabolism and increase the level of glycogen in the hepatopancreas. The absence of differences between the sexes coincides with the findings for other species of *Aegla* (Oliveira *et al.*, 2003). Metabolic patterns in male and female decapods can be explained by their different reproductive strategies (Hartnoll, 2006). For species with different metabolic pattern between sexes, Hartnoll (2006) explained that in order to ensure paternity, males guard females normally without feeding. Females in these species also have a period without feeding due to the molting process, when their appendices are soft. This occurs in decapods where ecdysis is synchronized with mating. Our results do not fit this model because the females are at an intermolt stage (hard reproduction strategy) during copulation as in other freshwater anomurans (Almerão *et al.*, 2010).

Decapod crustaceans mobilize carbohydrates such as glycogen to use as precursors or metabolic intermediates in the production of energy and synthesis of non-essential amino acids. Carbohydrates also play roles in oogenesis, embryogenesis, vitellogenesis, and production of nucleic acids and ovarian pigments (Harrison, 1990). According to previous records in temperate climates, *A. uruguayana* reproduces throughout the year (Viau *et al.*, 2006). As in *A. platensis* (Bueno & Bond-Buckup, 2000; Sokolowicz *et al.*, 2006), reproduction is maximized at particular times of the year. In the case of *A. uruguayana*, this would happen during autumn, a season in which the gonado-somatic index is the highest (V.P. Diawol, pers. obs.). It is likely that the low glycogen values recorded in autumn was due to the use of this metabolite in gonadal maturation, whereas the minimum values in spring could be due to the use of this reserve in parental care.

The energetic cost of asynchronous reproduction and parental care should also be considered. López Greco *et al.*, (2004) argued that this reproductive aspect is advantageous to optimize the survival in changing freshwater environments, in which food availability and nursery sites can fluctuate in small periods of time (Neiff, 1999; Amoros & Bornette, 2002). *Aegla uruguayana* carried its eggs until they hatch as juveniles. The greatest number of young recruits is observed in the springs (V.P. Diawol, pers. obs.). The decrease in glycogen in spring could therefore represent the energetic cost of parental care. Incubation and parental care often involve the loss of feeding opportunities (Hartnoll, 2006). Carbohydrates also play a fundamental role in the production of glucosamine, the precursor in the synthesis of chitin, the main constituent of the exoskeleton (Meenakshi & Scheer, 1961). Carbohydrates could be used in different amounts by individuals of different size according to their molting frequency (Diawol *et al.*, 2015).

Lipids and proteins are the main metabolic reserves mobilized by decapods during reproduction. Several authors have argued that the decrease in total lipids in the hepatopancreas is attributed to mobilization to the gonads, which is recorded at the same time as an increase in ovarian lipids during gonadal maturation (Pillay & Nair, 1973; Castille & Lawrence, 1989; Millamena & Pascual, 1990; Fatima *et al.*, 2013). Lipids and carbohydrates in the ovaries are the fuel source for oogenesis and vitellogenesis, as they are accumulated and used in the development of oocytes (Harrison, 1990). The minimum values of lipids recorded in the hepatopancreas of *A. uruguayana* during autumn seem to coincide with its use in the gonads, mainly in the large-size individuals, whereas the maximum values recorded in summer could represent the storage of reserves by this organ for use in the next reproductive event.

Proteins involved in gonadal maturation and reproduction are used in the synthesis of peptide hormones, enzymes, and vitelline proteins. These macromolecules also provide nitrogen for the genetic material and coenzymes that can be metabolized as energy substrate (Harrison, 1990; Yehezkel *et al.*, 2000). The dietary protein requirements are much higher for juveniles, which require greater and faster tissue synthesis (Harrison, 1990). While

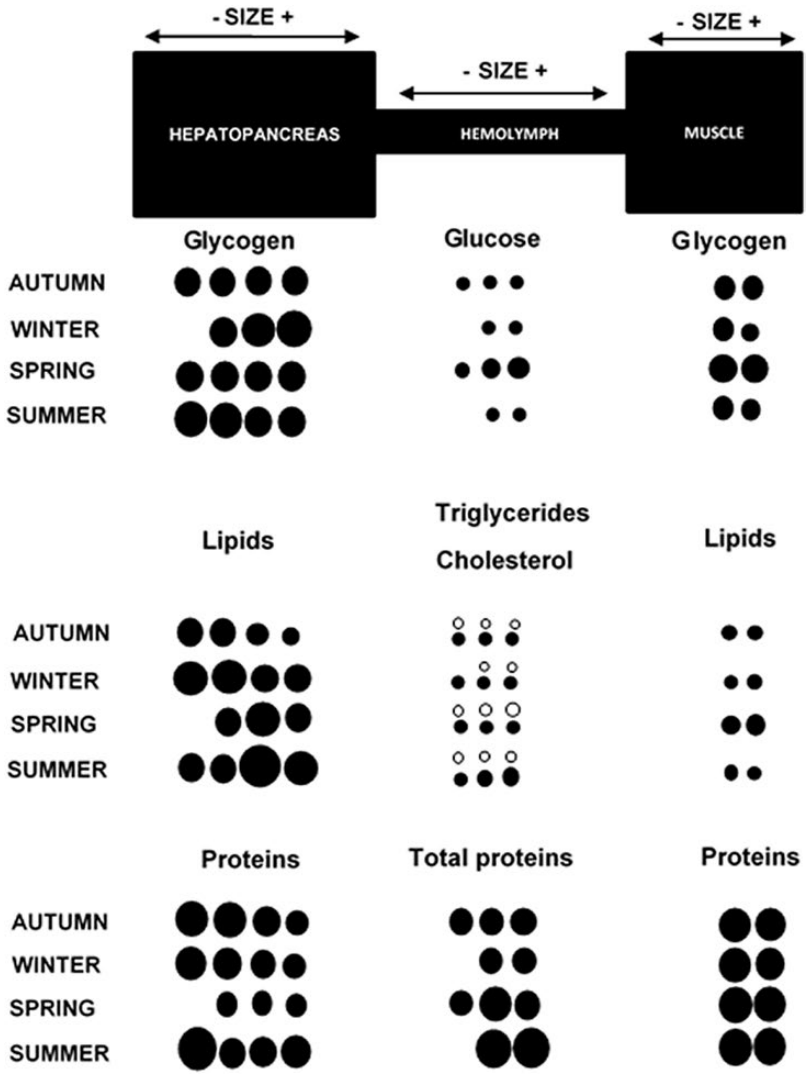

Figure 4. Seasonal movement concentrations of glycogen/glucose, lipids/triglycerides /cholesterol, and proteins among different tissues and in range sizes of *Aegla uruguayana*. Each circle represents the concentration of a metabolite in a particular size range, where size is proportional to the quantity measured. In each tissue, size varies from juveniles to adults (> 20 mm) from left to right.

the protein values recorded for the hepatopancreas of *A. uruguayana* tend to remain stable throughout the year, the decrease during spring is marked in all size ranges.

The glucose recorded in the hemolymph is the result of direct release by the stomach as a product of digestion, and from the glycogenic pathway (Chang & O'Connor, 1983). The maximum glucose values recorded in the hemolymph during spring and its seasonal variation are related to the mobilization of glycogen from the hepatopancreas and muscle, in which maximum values are recorded in spring. The higher energy requirements during parental care, locomotion, and reproductive behavior were observed at this time of the year. These results differ from those obtained for other crustaceans in subtropical Brazil, in which maximum glucose values were recorded during winter and summer (Vinagre *et al.*, 2007; Oliveira *et al.*, 2007; Dutra *et al.*, 2008; Silva-Castiglioni *et al.*, 2007). These differences could be related to food availability or species-specific differences.

The maximum cholesterol values recorded in summer and the corresponding triglycerides values in spring would explain the mobilization of these biomolecules from the hepatopancreas, the site of lipid storage. This metabolite is used during gonadal maturation prior to the reproductive event. Cholesterol is a structural component of cell membranes and acts as a precursor of hormones involved in the reproduction and molting process of crustaceans (Teshima & Kanazawa, 1971). The constant low values during autumn, winter, and spring, and the maximum values in summer would be explained by their use in the gonads, coinciding with reproductive events. The marked decrease in triglycerides during winter and the subsequent increase during spring are associated with reproduction, in which triglyceride reserves are essential in spermatogenesis and vitellogenesis (Harrison, 1990). Such is the case in the freshwater crayfish *Parastacus brasiliensis* (Von Martens, 1869) (Dutra *et al.*, 2008).

The high concentrations of total proteins in the hemolymph during spring and summer coincided with the decreased protein content in the hepatopancreas during spring, and a greater flow of this metabolite in the organ, which peaks again during summer. It also indicates the mobilization of these macromolecules to muscle tissue, one of the sites where proteins are stored. The increase in proteins in the hemolymph during the warmer months could be related to the abundance of food resources with high protein content at this time of the year (Popchenko, 1971). *Aegla uruguayana* ingests insect larvae, oligochaetes, copepods, and ostracods, which provide high amounts of proteins (Williner, 2010). Environments with high temperatures and low rainfall with a subsequent decrease in the stream level provide the highest diversity of invertebrates (Juárez, 2011).

Glycogen levels in muscle showed the greatest variation, whereas levels of lipids and proteins were more stable. The seasonal variation of glycogen coincides with the fluctuation of trophic resources rich in carbohydrates and the use of energy in relation to parental care and reproductive behavior, being higher during spring. Proteins were present in greater amounts in muscle compared to the hepatopancreas, remaining stable during the different seasons. The molting cycle and somatic growth require proteins, increasing the size of muscles (Kuballa *et al.*, 2011; Jimenez & Kinsey, 2015).

A possible resource pathway is hepatopancreas > hemolymph > muscle (see Fig. 4). This pathway is relatively constant in comparison to the reproductive pathway (e.g. hepatopancreas > hemolymph > gonads), which is more seasonal in temperate environments (Pillay & Nair, 1973). Our observations of metabolite variations reflect the use and requirements of nutrients by the species, indicating the dynamics of the communities and their environments.

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