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Seasonal variations in the biochemical composition of the crayfish *Parastacus defossus* (Crustacea, Decapoda) in its natural environment

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Abstract

The crayfish *Parastacus defossus* occurs in Uruguay and the state of Rio Grande do Sul in Brazil. It lives in swamps and lakes, where it digs sloped subterranean tunnels that are used as burrows. Because there is little information about the biology, physiology and ecology of this species, the aim of this study was to identify the seasonal variations of its carbohydrate and lipid metabolism. Crayfish were collected monthly (from November 2002 to February 2004) in the Lami region, Porto Alegre municipality (30°11′41″S — 51°06′00″W). Haemolymph samples, used for determination of glucose, total proteins, triglycerides, total cholesterol and total lipids, were collected in the field using potassium oxalate as an anti-clotting agent. The animals and haemolymph samples were immediately frozen in the field. In the laboratory, the hepatopancreas, gills and abdominal muscles were removed for determination of glycogen, triglycerides, total cholesterol and total lipids. The findings suggest that in *P. defossus*, lipids are an important reserve of energy used during reproduction in both males and females; whereas glycogen may be used during periods of intense activity or environmental stress.

Keywords: Parastacidae; Crayfish; *Parastacus defossus*; Biochemical composition; Carbohydrate metabolism; Proteins; Lipid metabolism; Seasonality

1. Introduction

Crustaceans are exposed to a large number of environmental variables, which follow annual and daily cycles according to the geographical region, and which affect their behavior, feeding and metabolism. Study of the intermediate metabolism in crustaceans has revealed the existence of wide inter- and intra-specific variability, which makes it difficult to determine a standard metabolic profile (Oliveira et al., 2003). This variability can occur because of multiple factors, such as habitat, stage in the molt cycle, sexual maturity (especially in females), feeding state, available food, and seasonality (Schirf et al., 1987; Kucharski and Da Silva, 1991b).

Glucose is the principal monosaccharide present in the haemolymph of crustaceans, and it serves seven main purposes: synthesis of mucopolysaccharides, synthesis of chitin, synthesis of ribose and nicotinamide adenine dinucleotide phosphate reduced (NADPH), formation of pyruvate, synthesis of glycogen and an energy source (Chang and O'Connor, 1983; Herreid and Full, 1988). Stable glucose haemolymph levels are essential for the regular functioning of the nervous, muscle and reproductive systems. Glucose can be accumulated in the form of glycogen in the hepatopancreas and in other tissues, such as the muscles and the gills (Chang and O'Connor, 1983; Loret et al., 1989; Loret and Devos, 1992; Vinagre and Da Silva, 1992; Schmidt and Santos, 1993; Oliveira and Da Silva, 1997; Vinagre and Da Silva, 2002; Oliveira et al., 2003; Marqueze et al., submitted for publication).

The storage-mobilization cycle of glycogen, and the haemolymph glucose reserves vary widely, and depend, with other factors, on the molt stage, season, diet, nutritional state,

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circadian cycle, salinity and dissolved oxygen (Chang and O'Connor, 1983; Herreid and Full, 1988; Loret et al., 1989; Vinagre and Da Silva, 1992; Kucharski and Da Silva, 1991a,b; Morris and Airriess, 1998; Oliveira et al., 2004). In *Aegla ligulata*, males have higher contents of glucose in the haemolymph than females during summer; but in spring, both sexes show hyperglycemia compared with other seasons (Oliveira et al., 2003). In males of *Ocypode quadrata*, the highest glucose levels in haemolymph occur during fall (Vinagre et al., 2007).

In the absence of adipose tissue in crustaceans, the hepatopancreas seems to be the main site of lipid storage (O'Connor and Gilbert, 1968; Chang and O'Connor, 1983; Herreid and Full, 1988; Kucharski and Da Silva, 1991a; Muriana et al., 1993; Garcia et al., 2002), although lipids can also be accumulated in muscle tissue and in the female gonad (Komatsu and Ando, 1992). In the estuarine crab *Chasmagnathus granulatus* (Dana, 1851), for example, Kucharski and Da Silva (1991b) found that total lipids represent more than 20% of the weight of the hepatopancreas.

Several studies have demonstrated that during periods of high energy demand, such as molting and gametogenesis, there is a pronounced degradation of lipids, especially those stored in the hepatopancreas, as observed by Kucharski and Da Silva (1991a) in *C. granulatus*. Rosa and Nunes (2003b) observed a significant increase in the levels of total lipids and cholesterol in gonadal tissue of *Aristeus antennatus*, *Parapenaeus longirostris* and *Nephrops norvegicus* from the Portugal coast. This increase may be related to the stage of ovary maturation.

The muscle is apparently the main protein-storage location in crustaceans. In decapods, the free amino acids in the tissues reach levels tenfold higher than those observed in vertebrates. Several studies suggest that these amino acids participate in osmoregulation and in the control of cell volume (Gilles, 1982; Chang and O'Connor, 1983; Schein et al., 2004). Other studies have demonstrated variation in protein content during ovarian development in crustaceans. These variations may result from increased biosynthesis of several proteins, including enzymes, hormones and lipoproteins involved in gonadal maturation (Yehezkel et al., 2000; Rosa and Nunes, 2003a,b).

According to López-Greco and Rodríguez (1999), the beginning of reproduction is a critical event in the life history of animals, and is related to reproductive effort, defined as the proportion of body energy transferred to reproduction. Analysis of biochemical composition and its seasonal variations is important for reproductive biology, because it is fundamental to understand how different organs can act to store and transfer organic reserves to support gonadal maturation, reproductive period and the maintenance of the animal (Pillay and Nair, 1973; Rosa and Nunes, 2003a). Studies of the reproductive biology of members of the genus *Parastacus* are few. In Brazil, studies have been carried out with *Parastacus defossus* (Almeida and Buckup, 1999), *Parastacus brasiliensis* (Fontoura and Buckup, 1989b; Almeida and Buckup, 1997, 2000) and *Parastacus varicosus* (Castiglioni et al., 2007).

The family Parastacidae is represented in South America by the genera *Parastacus*, *Samastacus* and *Virilastacus*. Only members of *Parastacus* occur in Brazil, preferentially in marshy lentic environments on the plains, and in small, slowly flowing streams (Buckup and Rossi, 1980; Fries, 1980; Fontoura and Buckup, 1989a). Most of the species, including *P. defossus*, construct underground habitations in the form of simple or branched tunnels that reach groundwater level and have one or more openings on the surface. The animals are nocturnal, when they leave their burrows to hunt for food in or near the water (Buckup, 1999). Noro (2007), studying the gastric contents of *P. defossus*, found that, like other parastacids, this species is an opportunistic omnivore with a diet based on plants. The reproduction of this species begins in winter and peaks in spring (Noro, 2007).

In comparison with the extensive literature about biochemistry and reproductive aspects of marine and estuarine crustaceans, fewer studies have considered freshwater crustaceans. The objective of the present study was to evaluate, in the natural environment, the effect of seasonal variations on the biochemical composition of the freshwater crayfish *P. defossus*. The purpose was to obtain basic physiological data to support adequate conservation of these populations.

2. Materials and methods

The animals were cared for in accordance with Brazilian laws, and were used with the permission of the Ethics Committee of the Pontifícia Universidade Católica do Rio Grande do Sul (License 0002/03).

2.1. Sampling

Crayfish (*P. defossus* (Crustacea, Decapoda, Parastacidae) were collected monthly (November 2002 to February 2004) in the Lami region, Porto Alegre municipality (30°11′41″S — 51°06′00″W). Twenty adults of *P. defossus* in stage C or D of the intermolt cycle (Drach and Tchernigovtzeff, 1967) were collected in each season from the Guaíba estuary.

Haemolymph samples were collected (0.8 mL) in the field, by puncturing the membrane at the base of the chelipeds and pereiopods with a syringe containing 10% potassium oxalate as an anti-clotting agent. The samples and the animals were transported to the laboratory (Laboratório de Carcinologia of the Universidade Federal do Rio Grande do Sul) in insulated containers with ice (4 °C). In the laboratory, the crayfish were separated by sex, weighed on an electronic balance (0.001 g), and numbered. The animals were then frozen for later extraction of the main metabolite storage tissues (hepatopancreas, gills and abdominal muscle) and gonadal analysis to confirm their sex.

2.2. Biochemical assays

2.2.1. Haemolymph measurements

The metabolic parameters of the haemolymph sample of each animal were determined in triplicate using spectrophotometric methods.

Glucose levels were measured by the glucose-oxidase method, using a Bioclin Kit (glucose GOD-CLIN). Results are expressed in mmol/L.

Total lipids were measured by the sulfophosphovanillin method (Frings and Dunn, 1970), with the results expressed in mg/L.

Triglycerides were measured by the reactions of lipase, glycerokinase, 1-P-glycerol oxidase and peroxidase enzymes (Biodiagnostic Kit/GPO Trinder). Results are expressed as mg/L of animal.

The levels of total cholesterol were measured by the reactions of cholesterol esterase, cholesterol oxidase and peroxidase enzymes (Labtest Kit/Liquiform) and are expressed in mg/L.

Proteins were measured following the method described by Lowry et al. (1951), using bovine albumin (Sigma Chemical Co./St. Louis, MO, USA) as the reference substance. Results are expressed in mg/mL.

2.2.2. Tissue measurements

The metabolic parameters of the tissue sample from each animal were determined in triplicate using spectrophometric methods.

Glycogen was extracted from tissues following the method described by Van Handel (1965). Glycogen levels in the animals were determined as glucose equivalent, after acidic hydrolysis (HCl) and neutralization (Na_2CO_3), following the method of Geary et al. (1981). Glucose was quantified using a Biodiagnostic kit (glucose-oxidase method). Results are presented as mg/g of tissue.

Free glucose was determined according to Carr and Neff (1984). The tissue samples were weighed and homogenized with Ultra-Turrax in a 100 mM sodium citrate solution, boiled for 10 min, agitated, cooled to ambient temperature, and stored at −20 °C for at most one week. The samples were mixed in a 2:1 (v/v) chloroform–methanol solution in the ratio of 2:1 (w/v) and centrifuged for 10 min at 800 *g* to separate the lipid fraction. Free glucose concentration was determined by the glucose-oxidase colorimetric method (Labtest Kit) in the intermediate fraction obtained after centrifugation. The results are expressed as mg/g of tissue.

Lipids were extracted from tissue homogenized with an Omni Mixer Homogenizer in a 2:1 (v/v) chloroform–methanol solution, according to Folch et al. (1957). Total lipids in this homogenate were determined by the sulfophosphovanillin method (Frings and Dunn, 1970). Triglycerides were measured by the reactions of lipase, glycerokinase, 1-P-glycerol oxidase and peroxidase enzymes (Biodiagnostic Kit/GPO Trinder). The levels of total cholesterol were measured by the reactions of cholesterol esterase, cholesterol oxidase and peroxidase enzymes (Labtest Kit/Liquiform). Results are expressed as mg/g of tissue.

2.3. Statistical analysis

All the metabolic parameters were homogeneous (Levene test), and were normally distributed (Kolmogorov–Smirnov test). For statistical analysis of the seasonal variations, a one-way ANOVA test was used, followed by a Bonferroni test. For comparison between curves obtained during the year for males and females, a two-way ANOVA was applied. The significance level adopted was 5%. All tests were done with the program Statistical Package for the Social Sciences (SPSS- 11.5) for Windows.

3. Results

The glucose levels in haemolymph are shown in Table 1. During summer, males and females showed the highest values ($p<0.05$) of haemolymphatic glucose, whereas in autumn the values were lowest. There was no significant difference in glucose levels between sexes ($p\geq0.05$).

In both males and females, there were no significant seasonal variations in the levels of total proteins ($p\geq0.05$) in the haemolymph. No significant difference between sexes in total protein content was noted ($p\geq0.05$) (Table 1).

The concentrations of total lipids in the haemolymph (Table 1) in males and females reached minimum values in winter ($p<0.05$), and rose in the following seasons. There was no significant difference in total lipids content between the sexes ($p\geq0.05$). The concentrations of total cholesterol in the haemolymph (Table 1) in males and females reached minimum values in summer, and gradually rose in the following seasons, with a peak in spring. There was no significant difference in total cholesterol content between sexes ($p\geq0.05$). The concentrations of triglycerides in the haemolymph (Table 1) in males and females reached minimum values in summer, and gradually rose in the following seasons, with a peak in spring. There was no significant difference between the sexes in triglyceride content ($p\geq0.05$).

During autumn, hepatopancreatic glycogen (Fig. 1A) and muscle glycogen (Fig. 1C) decreased significantly ($p<0.05$) in

Table 1
Seasonal concentration of glucose (mmol/L), total proteins (mg/mL), total lipids (mg/L), total cholesterol (mg/L) and triglycerides (mg/L) in the haemolymph of *Parastacus defossus* collected in the natural environment

	Spring	Summer	Autumn	Winter
Males				
Glucose (mmol/L)	0.44 ± 0.07^{a}	0.97 ± 0.20^{abc}	0.38 ± 0.06^{b}	0.43 ± 0.03^{c}
Total protein (mg/mL)	8.51 ± 0.52	8.09 ± 0.07	8.08 ± 0.51	8.96 ± 0.57
Total lipids (mg/L)	295.9 ± 21.3	318.9 ± 39.4^{a}	259.1 ± 39.1	163.7 ± 46.9^{a}
Total cholesterol (mg/L)	51.2 ± 3.7^{abc}	22.9 ± 1.4^{a}	23.3 ± 1.7^{b}	40.2 ± 2.9^{c}
Triglycerides (mg/L)	41.5 ± 2.8^{abc}	10.4 ± 0.9^{ad}	22.6 ± 3.9^{be}	21.0 ± 2.4^{cde}
Females				
Glucose (mmol/L)	0.44 ± 0.07^{a}	1.46 ± 0.30^{abc}	0.36 ± 0.02b	0.44 ± 0.03^{c}
Total protein (mg/mL)	8.86 ± 0.28	8.69 ± 1.21	7.84 ± 0.55	8.44 ± 0.66
Total lipids (mg/L)	260.8 ± 22.5^{ad}	371.3 ± 30.3^{bd}	287.2 ± 43.5^{e}	89.5 ± 13.1^{abc}
Total cholesterol (mg/L)	50.5 ± 3.8^{abc}	14.6 ± 1.4^{ad}	22.6 ± 2.4^{bd}	40.0 ± 4.1^{c}
Triglycerides (mg/L)	38.9 ± 2.5^{bd}	14.8 ± 1.1^{cd}	24.6 ± 1.9^{ab}	21.4 ± 2.2^{ac}

All results represent the mean±standard error of the mean. The same letter represents a significant difference between the seasons.

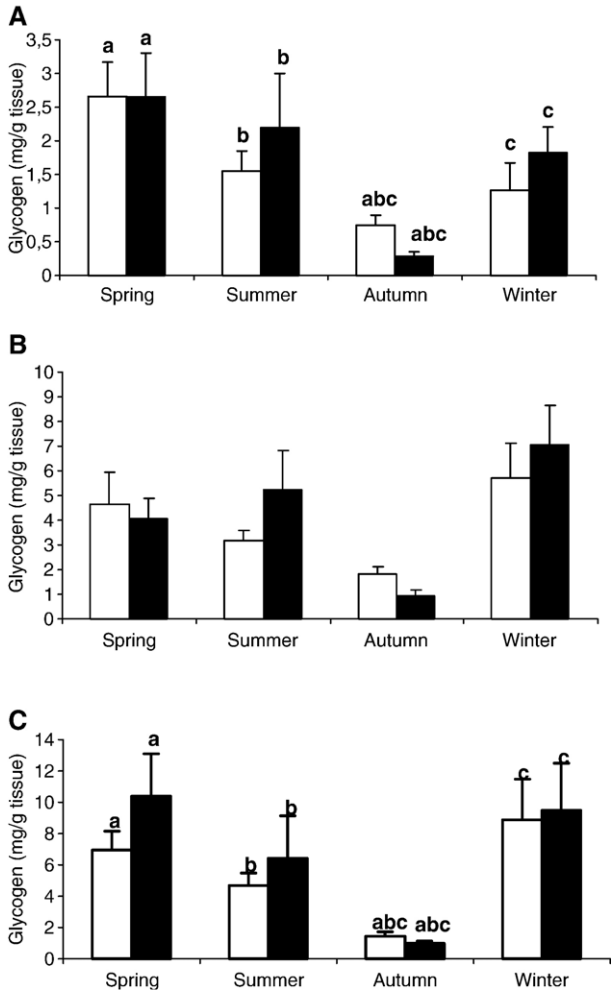

Fig. 1. Seasonal concentration of glycogen in the hepatopancreas (A), gills (B) and muscle (C) of *Parastacus defossus* collected in the natural environment. Males: white bar; Females: black bar. Columns represent the mean, and bars the standard error of the mean. Results are expressed in mg/g. The same letter represents a significant difference between the seasons. * Significant difference between sexes.

both sexes, with no differences between males and females ($p \geq 0.05$). No variation in the gill glycogen concentration (Fig. 1B) between seasons, or between males and females was found; a tendency to decrease during fall was not significant ($p \geq 0.05$).

The levels of free glucose in the hepatopancreas, gills and abdominal muscle are shown in Fig. 2. The content of free glucose in the hepatopancreas (Fig. 2A) of males and females showed no significant seasonal variations ($p \geq 0.05$). There was no significant difference in free glucose content between sexes ($p \geq 0.05$). The free glucose levels in gills (Fig. 2B) of males showed no seasonal variations ($p \geq 0.05$), but in females reached minimum values in summer ($p < 0.05$) and peaked in spring ($p < 0.05$). There was no significant difference between free glucose content of both sexes ($p \geq 0.05$). The levels of free glucose in abdominal muscle tissue (Fig. 2C) of males did not show any seasonal variation; in females, a significant ($p < 0.05$) increase in this parameter occurred in spring, and was four-fold lower in summer. There was no significant difference in free glucose content between sexes ($p \geq 0.05$).

The concentrations of total lipids in the hepatopancreas of males (Fig. 3A) showed the highest values in autumn and lowest in summer; in females, the content of total lipids was highest in spring, with a decrease of approximately 50% in summer. There was no significant difference in total lipids content between sexes ($p > 0.05$). In males, the content of total lipids in the gills (Fig. 3B) reached its highest values in autumn and lowest values in spring ($p < 0.05$); females showed no seasonal variation ($p \geq 0.05$). There was a significant difference in total lipids content in gills between sexes: males showed higher levels than females during the year. In muscle tissue of both sexes (Fig. 3C), the levels of total lipids showed no seasonal difference ($p \geq 0.05$). There was a significant difference during the year in the total lipids content between sexes: males showed higher levels in summer and autumn in relation to females.

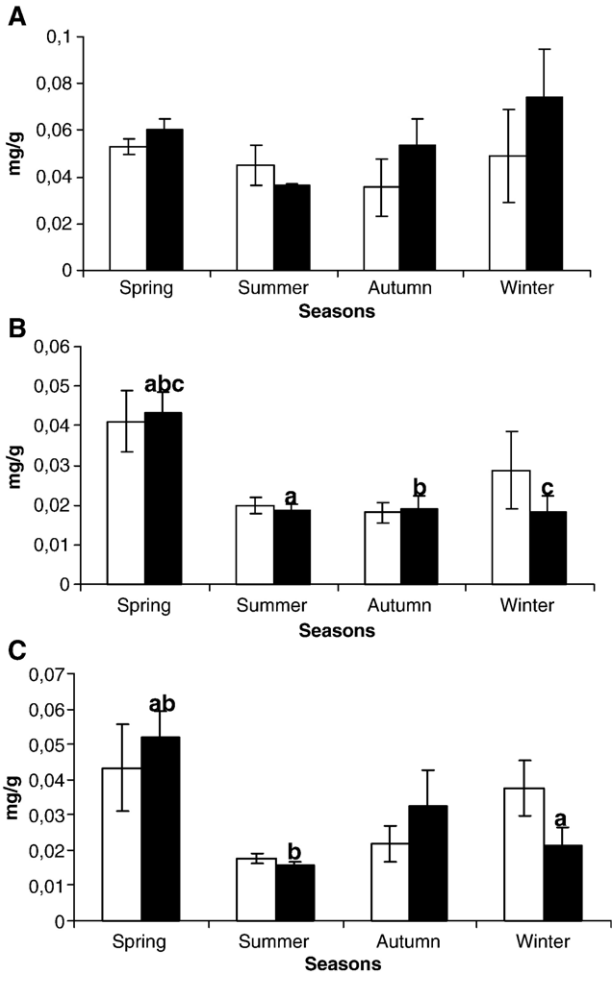

Fig. 2. Seasonal concentration of free glucose in the hepatopancreas (A), gills (B) and muscle (C) of *Parastacus defossus* collected in the natural environment. Males: white bars; Females: black bars. Columns represent the mean, and bars the standard error of the mean. Results are expressed in mg/g. The same letter represents a significant difference between the seasons.* Significant difference between sexes.

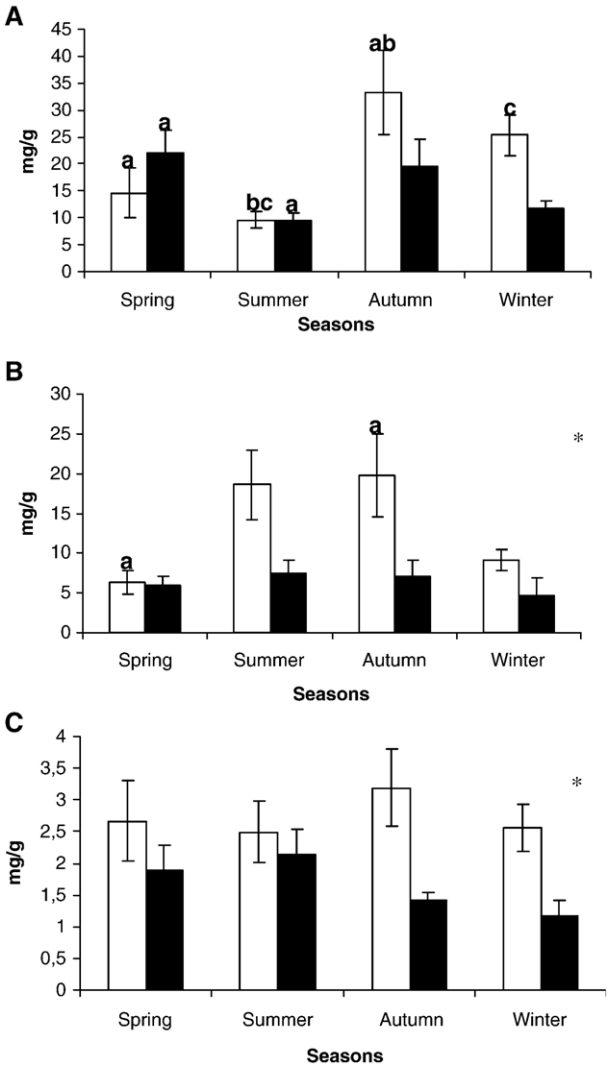

Fig. 3. Seasonal concentration of total lipids in the hepatopancreas (A), gills (B) and muscle (C) of *Parastacus defossus* collected in the natural environment. Males: white bars; Females: black bars. Columns represent the mean, and bars the standard error of the mean. Results are expressed in mmol/g. The same letter represents a significant difference between the seasons. * Significant difference between sexes.

The levels of triglycerides in the hepatopancreas, gills and abdominal muscle are shown in Fig. 4. Triglyceride levels in the hepatopancreas (Fig. 4A) of males showed no significant seasonal variations; females reached minimum values in summer, and peaked in autumn ($p<0.05$). There was no significant difference in triglyceride content between sexes ($p\geq 0.05$). The triglyceride levels in gills (Fig. 4B) and abdominal muscle (Fig. 4C) of both sexes showed no seasonal variations ($p\geq 0.05$). There was no significant difference in triglyceride content between sexes in both tissues ($p\geq 0.05$).

The concentrations of total cholesterol in the hepatopancreas (Fig. 5A) in males and females showed the highest values in autumn and the lowest in summer ($p<0.05$). There was a significant difference between total cholesterol content between sexes. In males, the content of total cholesterol in the gills (Fig. 5B) showed highest values in spring and winter, whereas in females the highest values were in autumn and the lowest values in summer ($p<0.05$). There was no significant difference in total lipids content between sexes. In males, the content of total cholesterol in muscle tissue (Fig. 5C) was lowest in summer. In females, muscle total cholesterol was highest in spring ($p<0.05$). There were no significant differences ($p\geq 0.05$) in total lipids content between sexes.

Seasonal variations in triglyceride ratios ranged from 3.94% to 24.52% of the total lipid content in different tissues of the males, and from 3.92% to 44.71% in females. Females showed a peak in the triglycerides ratio during winter, and a marked decrease in summer, whereas in males this ratio remained constant (Table 2). The proportions of total cholesterol ranged from 1.58% to 27.82% of the total lipid content in males, and from 1.50% to 47.55% in females. Males showed a peak of total cholesterol in abdominal muscle during winter and a marked

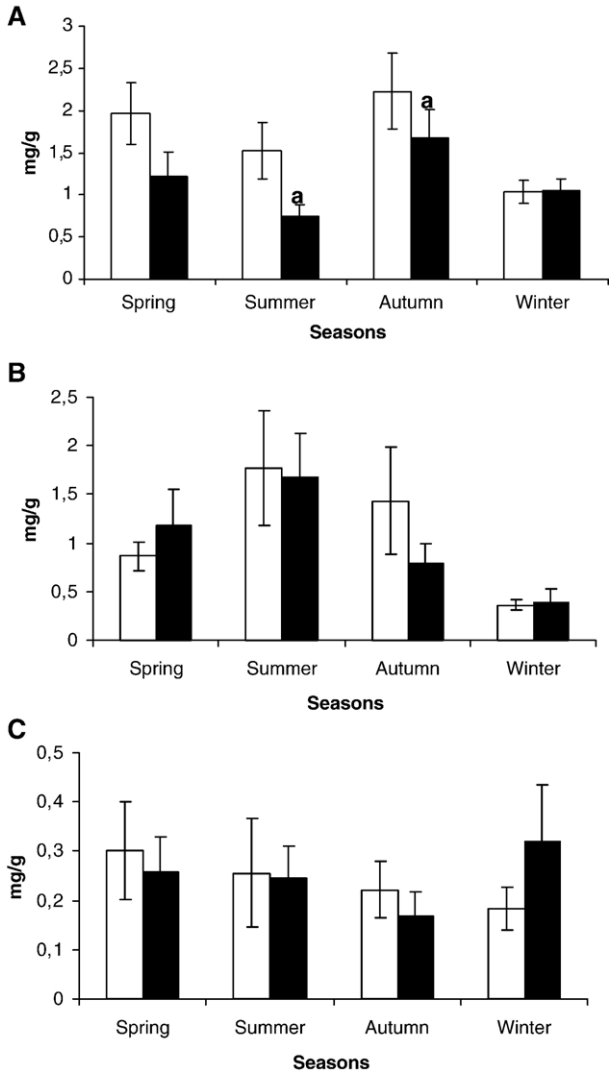

Fig. 4. Seasonal concentration of triglycerides in the hepatopancreas (A), gills (B) and muscle (C) of *Parastacus defossus* collected in the natural environment. Males: white bars; Females: black bars. Columns represent the mean, and bars the standard error of the mean. Results are expressed in mg/g. The same letter represents a significant difference between the seasons. * Significant difference between sexes.

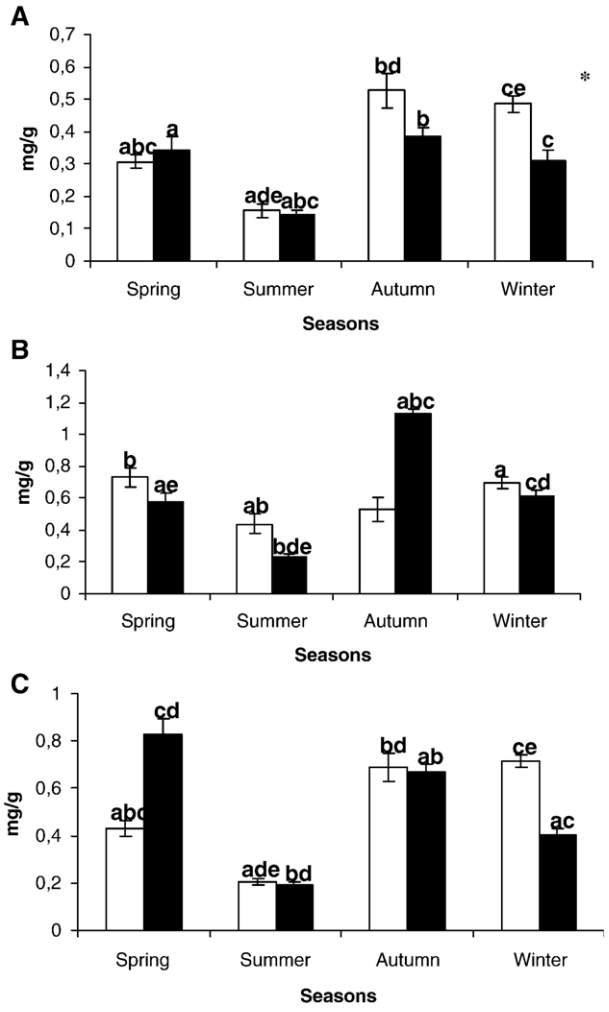

Fig. 5. Seasonal concentration of total cholesterol in the hepatopancreas (A), gills (B) and muscle (C) of *Parastacus defossus* collected in the natural environment. Males: white bars; Females: black bars. Columns represent the mean, and bars the standard error of the mean. Results are expressed in mg/g. The same letter represents a significant difference between the seasons. * Significant difference between sexes.

decrease in summer; whereas females showed a peak in autumn and a marked decrease in summer. Females showed a peak of the total cholesterol proportion during autumn and a marked decrease in summer, whereas in males this proportion was constant (Table 3).

Table 2
Percentage of triglycerides in relation to the amount of total lipids in males and females of *Parastacus defossus*

	Spring	Summer	Autumn	Winter
Males				
Haemolymph	17.53	7.18	9.01	24.52
Hepatopancreas	13.38	15.84	6.70	4.07
Gills	13.60	9.55	7.26	3.94
Abdominal muscle	11.27	10.28	6.94	7.13
Females				
Haemolymph	19.36	3.92	7.87	44.71
Hepatopancreas	7.57	7.80	6.26	8.85
Gills	19.72	22.17	11.18	8.37
Abdominal muscle	13.65	11.50	11.86	27.23

Results are expressed in %.

Table 3
Percentage of total cholesterol in relation to the amount of total lipids in males and females of *Parastacus defossus*

	Spring	Summer	Autumn	Winter
Males				
Haemolymph	14.03	3.25	8.70	12.85
Hepatopancreas	2.10	1.62	1.58	1.91
Gills	11.50	2.36	2.67	7.60
Abdominal muscle	16.05	10.40	21.53	27.82
Females				
Haemolymph	14.93	3.99	11.32	23.88
Hepatopancreas	1.54	1.50	1.97	2.60
Gills	9.68	3.12	15.90	13.23
Abdominal muscle	44.12	9.08	47.55	34.31

Results are expressed in %.

4. Discussion

The levels of proteins, carbohydrates and lipids are an expression of an animal's adaptive characteristics and its strategies for adaptation. Many biotic (e.g., maturation, reproduction and food availability) and abiotic factors (e.g., photoperiod, temperature, pH and oxygen in water) can strongly affect the biochemistry and physiology of decapod crustaceans (Company and Sardà, 1998; Rosa and Nunes, 2003a,b; Vinagre et al., 2007).

In this study, the glucose contents in the haemolymph of females and males of *P. defossus* ranged from 0.36±0.02 to 1.46±0.30 mmol/L. These levels are similar to those measured in the crab *C. granulatus* maintained in laboratory conditions on a high-carbohydrate diet (Kucharski and Da Silva, 1991a; Oliveira and Da Silva, 1997), and contrast with the results observed in crabs maintained on a protein-rich diet, which showed decreased levels of circulating glucose (Vinagre and Da Silva, 1992). In another freshwater crustacean, *Aegla platensis*, the levels of glucose in haemolymph of the males (from 0.36± 0.03 to 1.15±0.29 mmol/L) and females (from 0.40±0.04 to 1.98±0.63 mmol/L) were similar to the levels observed in this study (Oliveira et al., 2007). Bueno and Bond-Buckup (2004) showed that *A. platensis* is an omnivorous generalist, with aquatic macrophytes the principal items in its natural diet. We can therefore infer that the natural diet of *P. defossus* is high in carbohydrate and low in protein, with plant detritus probably the main item. The same pattern was found by Dutra et al. (submitted for publication) and Castiglioni et al. (2007), studying seasonal variations in the metabolism of *P. brasiliensis* and *P. varicosus*, respectively. Other species of parastacids are often polytrophic omnivorous and opportunist feeders, and plant detritus may be the main item in their natural diet (Goodard, 1998; Hogger, 1988; Hollows et al., 2002).

During the summer, both sexes showed hyperglycemia. During this period of low rainfall, surface water in the study area dried up. This water deficiency must have been a stress factor for this species, explaining the increase of the glucose

levels in the haemolymph. These aspects can be considered in laboratory experiments in the future.

In the present study, the levels of total proteins showed no seasonal variations in both sexes. This pattern contrasts with observations Oliveira et al. (2007) in *A. platensis*, a freshwater anomuran, where haemolymphatic protein levels decreased in both sexes in autumn. This decrease may have been related to vitellogenesis, energy investment in gametogenesis, and the reproductive behavior of males. Castiglioni et al. (2007) observed in *P. varicosus* that protein levels decreased in summer in the haemolymph in both sexes; this may be related to the transfer of these reserves to reproductive behavior in males and development of the gonads in females, which contained high protein levels at this season, reinforcing this hypothesis. Proteins, as well as being structural components of embryonic tissues, can also be used as combustibles in the final stages of development. A similar pattern was observed during the embryonic development of *Cherax quadricarinatus* by García-Guerrero et al. (2003), who reported that proteins are the principal components of the eggs. Several investigators have also suggested that protein levels in haemolymph can be used as a predictive indicator of the spawning capabilities (Gehring, 1974; Chapelle, 1986; Millamena and Pascual, 1990; Mourente and Rodriguez, 1991). In the present study, protein levels were not evaluated in other tissues, so we cannot reject the possibility that a mobilization of the proteins from other tissues, such as hepatopancreas and muscle, may occur during the reproductive period.

In both males and females, glycogen of the hepatopancreas and muscle decreased in autumn, suggesting an increase in the use of this polysaccharide for ATP synthesis in a period subsequent to decrease in environmental oxygen levels (hypoxia) and higher temperatures or during a period of food scarcity. Noro (2007) reported a decrease in levels of dissolved oxygen in water burrows at this season. In the hepatopancreas of *Marsupenaeus japonicus*, phosphoarginine and glycogen levels were far lower than in muscle, and decreased during hypoxia ($1.3 \sim 1.7$ mg O_2/L), suggesting that ATP buffering by phosphoarginine is not effective and glycogen was depleted in the hepatopancreas (Abe et al., 2007).

In different crustaceans, glycogen reserves are used in the molting process, hypoxia and/or anoxia, osmoregulation, growth, different stages of reproduction and during periods of starvation (Chang and O'Connor, 1983; Kucharski and Da Silva, 1991a; Kucharski and Da Silva, 1991b; Oliveira et al., 2001, 2004; Rosa and Nunes, 2003a). Rosa and Nunes (2003a) found in *A. antennatus, P. longirostris* and *N. norvegicus* that glycogen is stored mainly in the hepatopancreas, with the lowest proportion in muscle. Castiglioni et al. (2007), studying *P. varicosus*, observed in males and females a marked decrease of glycogen in muscle tissue during spring and summer, when oxygen levels in the water were lowest. According to Baden et al. (1994), in *N. norvegicus* the depletion of this polysaccharide in muscles during hypoxia and starvation suggests that the muscles can store glycogen, which would then be more easily accessible during this type of stress. These results may agree with the observed peak of glucose in haemolymph observed during summer, in both sexes.

Dehydration is thought to be the single most important factor limiting the success of decapod crustaceans on land (Powers and Bliss, 1983). Wood et al. (1986) reported that the crabs *Cardisoma carnifex* were occasionally seen above ground during the daytime, but usually retreated to burrows containing fresh water during the heat of the day, apparently to avoid dehydration. MacMillen and Greenaway (1978) and Wood et al. (1986) suggested that the depression in metabolic rate observed in the laboratory with *C. camifex* kept in low humidity can be interpreted as a water- and energy-conserving mechanism, allowing the animals to resist prolonged periods of drought in their burrows. In all tissues studied, there was a clear decrease in glycogen during autumn, although in gills this trend was not significant in either sex. We can suggest that *P. defossus* saves this energy resource to be used during dehydration stress in autumn. This suggestion is reinforced by the difficulty of collection in the summer and autumn intense dry period, when these crayfish remain inside their burrows for longer periods of time (Noro, 2007).

Several studies have shown that the hepatopancreas and muscle are the principal sites of lipid storage in crustaceans (O'Connor and Gilbert, 1968; Chang and O'Connor, 1983; Herreid and Full, 1988). According to Jones and Obst (2000), lipids are the main reserves of the hepatopancreas in the Australian crayfish *Cherax destructor*, and this organ can act as a source of nutrient mobilization when food is scarce, and also during gonadal maturation. A similar response pattern was observed in the present study. The reproductive period of *P. defossus* occurs during the winter and spring, which are wet seasons (Noro, 2007). During the winter, the levels of total lipids decrease in haemolymph more intensely in females, suggesting that these reserves are mobilized for vitellogenesis. During the spring, triglycerides and cholesterol levels increase in the haemolymph, as well as free glucose in the muscle. Also, total cholesterol concentration in the haemolymph was depleted in summer, which may be related to synthesis of sex hormones, because both sexes showed similar responses. According to Kanazawa and Teshima (1971), cholesterol is a structural component of the cell membranes and a forerunner of the sex hormones involved in reproductive control in crustaceans.

The proportions of triglycerides in relation to total lipids in the different tissues studied were lower in males (for example, 4.07% in the hepatopancreas of males in winter) than in females, suggesting that triglycerides are not the main type of lipid reserve in males. However, in females, triglycerides can reach 44% of the total lipids. The same pattern was found for the proportion of total cholesterol in relation to total lipids. In the present study, the variations in triglyceride proportions suggest that triglycerides are the important lipid reserves for females, but not for males; these proportions varied among the different tissues studied and the season. The total cholesterol predominated in abdominal muscle in males and females, independently of season, suggesting that this tissue is the main site of cholesterol reserves. Oliveira et al. (2007) showed that the triglycerides content ranged from 6.36 to 26.54% of the total lipid content in *Aegla platensis*. In *Daphnia laevis* and *Moina micrura*, two freshwater crustaceans, Macedo and Pinto-Corilho (2001) observed that most of

the lipid reserves in both cladocerans consisted of triglycerides, corresponding to 49.8% to 68.4% of total lipids.

In summer, the levels of total cholesterol are reduced in the haemolymph, hepatopancreas, muscle and gills in both sexes. Triglycerides are also reduced in the haemolymph of both sexes and in the hepatopancreas of females, indicating that these lipids are used as an energy resource during the dry summer, when the animals reduce their activity and food is less available. The hyperglycemia in this season also indicates a stressful period. In autumn, glucose values in the haemolymph are reduced, but glycogen reserves in muscle and hepatopancreas are also reduced, while the values of lipids are normal or higher than in the other seasons (Table 1, Figs. 3, 4 and 5). These data suggest that carbohydrates are the main source of energy during autumn, and may also indicate that in this period the animals are intensely active. They are probably involved in courtship, since the maturation of the gonads is increasing (Noro, 2007). In the winter, total cholesterol levels were reduced in relation to autumn in all of the tissues studied, including the haemolymph. This may be related to the reproductive peak, since the young are usually more common in the spring. In conclusion, these findings suggest that in *P. defossus*, lipids seem to be an important reserve of energy used during reproduction, both in males and females, while glycogen may be used during periods of intense activity or fasting.

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