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ARTICLE

Tissue composition and haemolymphatic metabolites during gonadal development in *Aegla platensis* (Crustacea, Decapoda) maintained in experimental culture

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ABSTRACT: (Tissue composition and haemolymphatic metabolites during gonadal development in *Aegla platensis* (Crustacea, Decapoda) maintained in experimental culture). This study describes the variation of tissue composition and haemolymphatic metabolites in the anomuran crab *A. platensis*, which was maintained for 90, 120, 150, and 180 days under laboratory conditions, and relates the data collected to reproductive aspects of this species. Individuals were collected during the winter from Mineiro Creek, in Rio Grande do Sul, Brazil. Some of the individuals were killed when collected and the remaining animals were maintained in a laboratory for different time periods using a commercial diet. Haemolymph, hepatopancreas and gonads were removed at different times of cultivation to determine their biochemical composition using spectrophotometric methods. Hepatosomatic and gonadosomatic indexes were determined. Statistical analysis revealed significant differences between the sexes in all metabolites except in the proteins levels in the haemolymph. We observed an increase of ovigerous females kept under the same culture conditions between 120 and 150 days and we found an increase in the gonadosomatic index and a decrease in the hepatosomatic index in both sexes for the same times. Results showed that the regular food supply caused an increase in the gonadosomatic index in both sexes during all the time periods when compared to the results of animals from the natural environment. We also observed that the females used part of their hepatopancreatic reserves for vitellogenesis and gametogenesis, but the nutrients obtained from the other tissues and the diet were very important for supporting reproduction. Males used the metabolic reserves for growth, gametogenesis, and reproductive behaviors. This study indicates that reproductive events depend on a regular food supply.
Keyword: Crustacea, Aeglidae, biochemical composition, *Aegla platensis*, diet, reproduction.

RESUMO: (Composição tecidual e metabolitos hemolinfaticos durante o desenvolvimento gonadal de *Aegla platensis* (Crustacea, Decapoda) mantidas em cultura experimental). Este estudo descreve a variação da composição tecidual e metabólitos hemolinfáticos nos caranguejos anomuros *A. platensis* mantidos por 90, 120, 150 e 180 dias no laboratório, e relaciona esses dados com os aspectos reprodutivos. Os animais foram coletados no arroio do Mineiro, Rio Grande do Sul, Brasil durante o inverno. Parte dos animais foi sacrificado em campo, e os restantes animais foram mantidos em laboratório por diferentes períodos com uma dieta comercial. A hemolinfa, o hepatopâncreas e as gônadas foram retiradas em diferentes épocas de cultivo para determinar a sua composição bioquímica por métodos espectrofotométricos. Os índices hepatossomático e gonadossomático foram determinados. A análise estatística revelou diferenças significativas entre os sexos em todos os seus metabolitos, exceto nos níveis de proteínas na hemolinfa. Observamos um aumento de fêmeas ovígeras mantidas sob as condições de cultivo entre 120 e 150 dias e encontramos um aumento do índice gonadossomático e uma diminuição do índice hepatossomático em ambos os sexos no mesmo periodo. Os resultados mostraram que o fornecimento regular de alimentos provocou um aumento no índice gonadossomático em ambos os sexos durante todo o período, quando comparado com os resultados dos animais do ambiente natural. Também foi observado que as fêmeas utilizadas parte das suas reservas do hepatopâncreas para a vitelogênese e a gametogênese, mas os nutrientes obtidos a partir de outros tecidos e da dieta foram muito importantes para apoiar a reprodução. Os machos utilizaram a reserva metabólica para o crescimento, gametogênese e comportamentos reprodutivos. Este estudo indica que os eventos reprodutivos dependem de um fornecimento regular de alimentos.
Palavras-chave: Crustacea, Aeglidae, composição bioquímica, *Aegla platensis*, dieta, reprodução.

INTRODUCTION

Aeglids (with more than 60 species) are anomuran crustaceans that inhabit rivers, streams, creeks and lakes. All the species in this group are endemic to the temperate and subtropical regions of continental South America (Bueno & Shimizer 2008), and live in clean, well-oxygenated fresh water. *Aegla platensis* Schmitt 1942 (Crustacea, Decapoda, Aeglidae) occurs in Southern Brazil and Argentina (Bond-Buckup 1994).

The natural diet of *A. platensis* consists principally of larvae of insects and aquatic macrophytes, and the consumption of these organisms varies according to

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when they are available. This species was classified as an omnivorous, generalist, and opportunist by Bueno & Bond-Buckup (2004); the population density was 8.7 to 15.3 individuals per m^2 in Mineiro Creek. The reproductive cycle of *A. platensis* is unusual compared to most decapods because it reproduces throughout the year (Bueno *et al.* 2000). Although males and females with immature gonads can be found during every month of the year, the gonadal indexes are highest during autumn (Sokolowicz *et al.* 2006), which indicates that the majority of the individuals in a population exhibit gonadal preparation for the winter, when ovigerous females are more abundant (Bueno & Bond-Buckup 2000).

According to López-Greco & Rodríguez (1999), the beginning of reproduction is a critical event in the life history of animals, and is related to reproductive effort, which is defined as the proportion of body energy transferred to reproduction. Among the many parameters that are fundamental to understanding reproductive biology, is the analysis of the biochemical variations of intermediate metabolism, in as much as different organs can act to store and transfer organic reserves to support gonadal maturation during the reproductive period, complementing the food intake of the animal (Pillay & Nair 1973, Rosa & Nunes 2003a, Oliveira *et al.* 2007).

Oliveira *et al.* (2007) studied the circadian and seasonal variations in the biochemical composition of these aeglids in the environment, and observed that the storage and breakdown of energy substrates are seasonal. The authors reported that these aeglids experience an increase in energy demand, possibly for the production of gametes during summer, egg laying and incubation during autumn and winter, and parental care during spring and summer.

According to Hasek & Felder (2006), typically the organs with the highest lipid content are the hepatopancreas and the ovary. In females, the content of total lipids in the ovary is influenced by the stage of ovarian development. During gonadal maturation and vitellogenesis, lipids are deposited in the ovaries (Morris 1973, Gehring 1974, Mourente *et al.* 1994, Lubzens *et al.* 1995, Spaziani & Hinsch 1997). It appears that these ovarian lipids may be derived from the diet in some decapods; alternatively, lipids may be accumulated in other tissues, principally the hepatopancreas, and later transported to the ovaries during gonadal maturation (Spaargaren & Haefer 1994). While the hepatopancreas is the universal organic reserve organ in crustaceans, not all decapods shuttle measurable lipid reserves from it to the ovaries (Heath & Barnes 1970, Pillay & Nair 1973, Castille & Lawrence 1989).

Hernandez-Vergara *et al.* (2003) evaluated the effect of different concentrations of lipids in artificial diets offered to the crayfish *Cherax quadricarinatus* von Martens 1868 and concluded that males invest their lipid reserves in growth, whereas females, with a higher hepatosomatic index, invest in gonadal development and vitellogenesis. Rosa & Nunes (2003b) and Oliveira *et al.* (2007) demonstrated in decapods that triglycerides and other forms of lipids are allocated to the synthesis of sex hormones and to vitellogenesis.

Rapid environmental degradation and changes of the natural habitats of the aeglids, including *A. platensis*, as a result of agricultural and industrial development have caused these species to be included in the "vulnerable" conservation category (Amaral *et al.* 2008). Studies about nutrition and its relationship to reproductive patterns are very important for population management and conservation of these species.

The present study had the objective of evaluating the effect of diet on the biochemical composition of the freshwater anomuran crab *A. platensis*, which was maintained for different time periods under laboratory conditions, and to correlate the data collected with the gonadal development (hepatosomatic and gonadosomatic indexes) of this species.

MATERIALS AND METHODS

The animals were collected and cared for in accordance with Brazilian laws and with the permission of the Ethics Committee of the Pontifícia Universidade Católica do Rio Grande do Sul (License 0003/03).

Aegla platensis (85 individuals) were trapped during the winter (July, 2004) in Mineiro Creek, located in the municipality of Taquara (29°30'0.2''S, 50°46'50''W), Rio Grande do Sul, Brazil. Ten of the individuals were killed when collected for use as a control group. Thirty adult males and 35 adult females, in stage C or D of the intermolt cycle (Drach & Tchernigontzeff 1967), were retained for use in the experiments. The anomurans were transported in containers with cold water (10°C) to a laboratory at the Universidade Federal do Rio Grande do Sul, where they were placed in aerated aquariums (270 L) for 24 hours without food.

Experimental procedure

After this 24-hour period, the animals were maintained in the aquariums with a mean temperature of 16.51±0.55 ºC and a photoperiod of 12:12 hours of light/dark. The individuals were fed *ad libitum* with the diet (pellet ration) in late afternoon when most of them were active (the remaining ration was removed the next morning) for periods of 90, 120, 150, or 180 days. The diet consisted of the following: proteins (31.33%), lipids (6.73%), carbohydrates (45.76%), water (8.16%), ash (8.02%), and calcium (1.03%). During these time periods, we observed no mortality in the anomurans; therefore, we worked with 15 animals (7–8 males and 8–9 females) in each period of culture.

After the 90-day period, samples of haemolymph were collected with a syringe containing 10% potassium oxalate (as an anti-clotting substance), which were frozen for later determination of glucose, total proteins, total lipids, and triglycerides. The animals were cryoanaesthetised and weighed, and the different tissues (hepatopancreas and gonads) were weighed and dissected on an electronic balance (± 0.001). The mean carapace length of females

was 7.05 ± 0.16 cm, and the mean carapace length of the males was 9.30 ± 0.37 cm. The mean weight of females was 0.15 ± 0.03 g, and of the mean weight of the males was 0.31 ± 0.11 g. Tissues were stored and frozen at -80°C until they were used to determine the biochemical parameters. This procedure was repeated after 120, 150, and 180 days.

Hepatosomatic and Gonadosomatic Index

We calculated the index according to Grant & Tyler (1983) and Vazzoler (1996): GI= GW/AW X 100 (GW = gonad weight, and AW = animal weight); multiplied by 100 to obtain the percentage, and HI= HW/AW X 100 (HW = weight of the hepatopancreas).

Haemolymph Measurements

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

Glucose levels were measured by the glucose-oxidase method, using a Labtest Kit (glucose PAP Liquiform reference 84). The results are expressed in mmol/L.

Total lipids were measured by the sulfophosphovanillin method (Meyer & Walter 1980), with the results expressed in mg/dl.

Triglycerides were measured by the reactions of lipase, glycerokinase, 1-P-glycerol oxidase, and peroxidase enzymes (Biodiagnostic Kit / Triglycerides Liquiform reference 87). The results are expressed as mg/dl of animals.

Proteins were measured following the method described by Lowry *et al.* (1951), using bovine albumin as the reference substance. The results are expressed in mg/dl.

Tissue Measurements

The metabolic parameters of the hepatopancreas and gonad samples from each animal were determined in triplicate, using spectrophometric methods.

a. Glycogen was extracted from the tissues following the method described by Van Handel (1965). Glycogen levels in the animals were determined as glucose equivalent, after acidic hydrolysis (HCl) and neutralisation (Na_2CO_3), following the method of Geary *et al.* (1981). Glucose was quantified using a Labtest Kit (glucose PAP Liquiform reference 84 – glucose oxidase method). The results are presented as mmol/g of animal.

b. Lipids were extracted from tissue homogenised with an Omni Mixer Homogeniser 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 (Meyer & Walter 1980). This method consists of oxidising cellular lipids to small fragments after chemical digestion with hot concentrated sulfuric acid. After the addition of a solution of vanillin and phosphoric acid, a red complex is formed, which is measured with a spectrophometer (530 nm).

c. Triglycerides were extracted with the same method used for total lipids, and were measured by the reactions of the enzymes lipase, glycerokinase, 1-P-glycerol oxidase, and peroxidase (Labtest Kit / Triglycerides Liquiform reference 87). The results are expressed as mg/g of animal.

Bovine albumin and glycogen (from rabbit liver) were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA) and sulfuric acid, sodium carbonate, potassium hydroxide, sodium hydroxide, ethanol, chloroform, methanol, chloridric acid, vanillin, and phosphoric acid

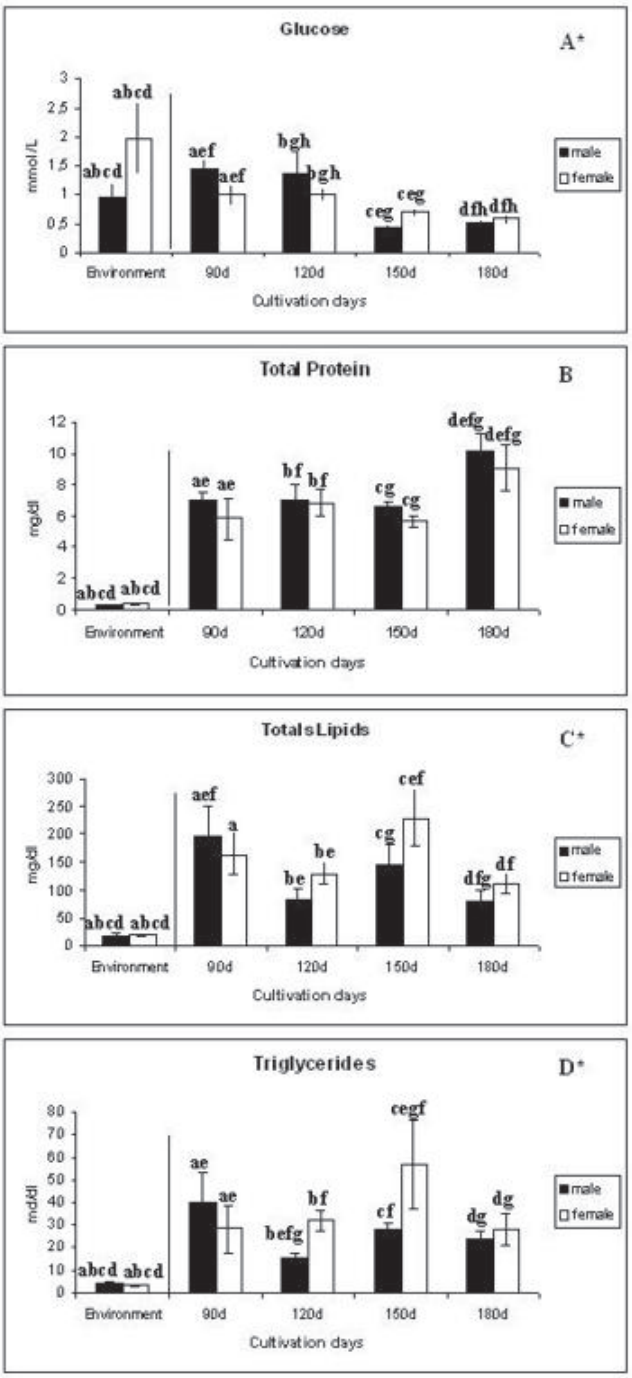

Figure 1. Concentrations of glucose (A), total proteins (B), total lipids (C), and triglycerides (D) in the haemolymph of males and females of *Aegla platensis* on different days of the experiment. The columns show the mean; vertical bars show the standard error of the mean. Same letters indicate significant differences ($p<0.05$). * indicates significant difference between sexes.

were purchased from Merck & Co (Merck & Co., Inc., Whitehouse Station, USA).

Statistical Analyses

All the results were homogeneous (Levene test), and were normally distributed (Kolmogorov-Smirnov test). Statistical analysis between days of experimental culture was carried out by means of a one-way ANOVA test, followed by a Bonferroni test. For comparison between sexes, a two-way ANOVA was used. The significance level adopted was 5%; all the tests were done with the program *Statistical Package for the Social Sciences* (SPSS- 11.5) for Windows.

RESULTS

In males, the haemolymph glucose levels were higher at 90 and 120 days, than in the animals in the natural environment and after 150 and 180 days of culture. In females, the haemolymph glucose levels were higher in the animals in the natural environment, decreased by 50% during 90 days of culture, and had the lowest levels after 180 days of culture (Fig. 1A). When comparing the sexes, we observed a significant difference during period of cultivation ($p<0.05$).

The total protein levels are shown in Fig. 1B. Within the haemolymoph, males and females from the natural environment had the lowest levels of total proteins, which increased after 90 days of culture (approximately 15-fold); the highest levels were observed at 180 days

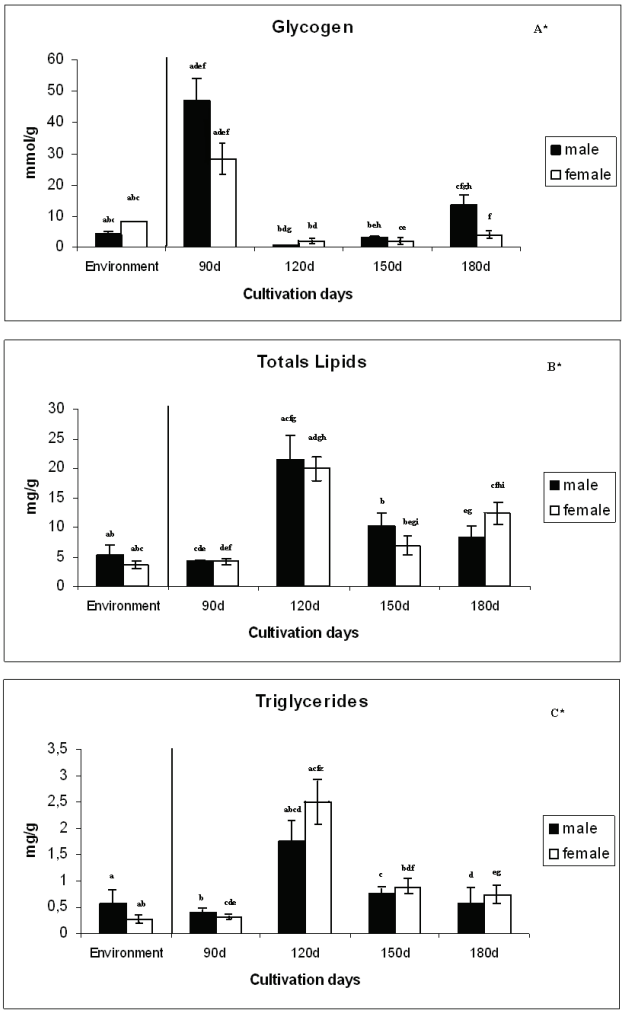

Figure 2. Concentrations of glycogen (A), total lipids (B), and triglycerides (C) in the hepatopancreas of males and females of *Aegla platensis* on different days of the experiment. The columns show the mean; vertical bars show the standard error of the mean. Same letters indicate significant differences ($p<0.05$). * indicates significant difference between sexes.

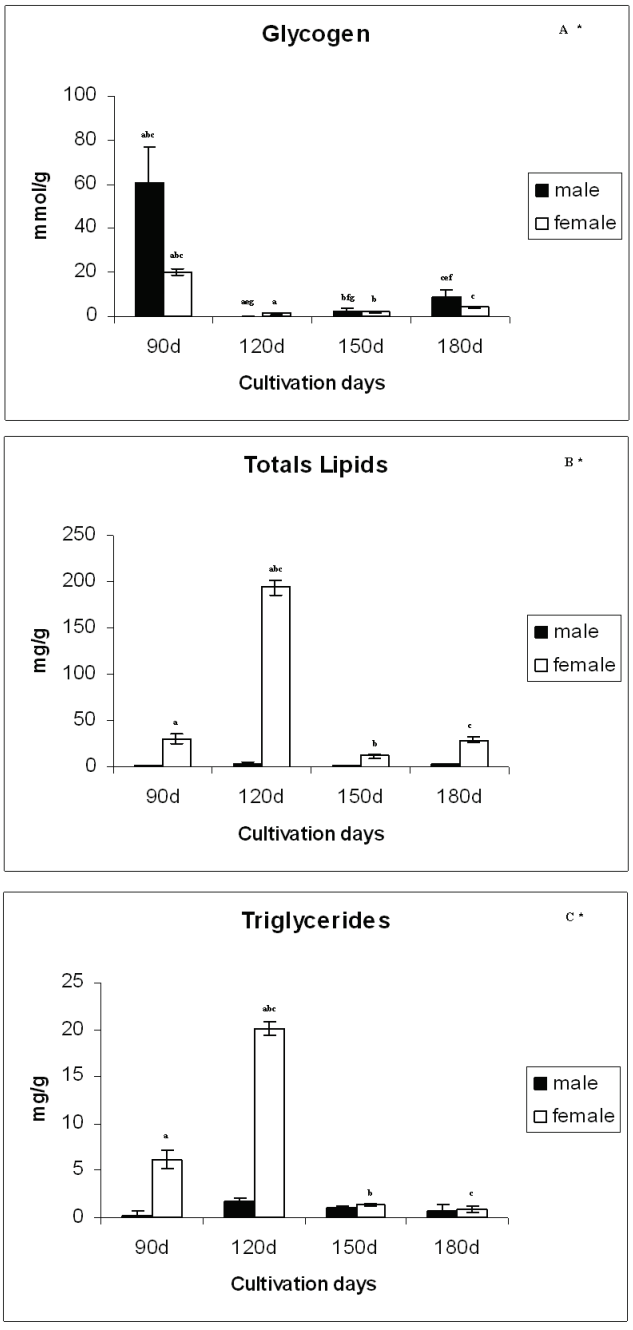

Figure 3. Concentrations of glycogen (A), total lipids (B), and triglycerides (C) in the gonads of males and females of *Aegla platensis* on different days of the experiment. The columns show the mean; vertical bars show the standard error of the mean. Same letters indicate significant differences ($p<0.05$). * indicates significant difference between sexes.

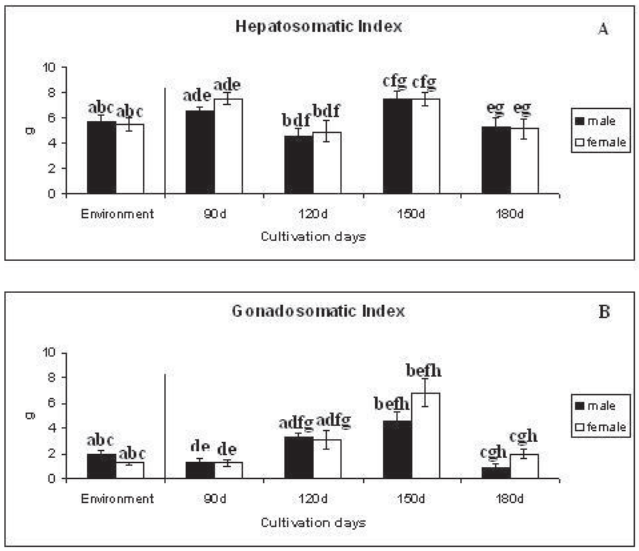

Figure 4. Hepatosomatic index (A) and gonadosomatic index (B) of males and females of *Aegla platensis* on different days of the experiment. The columns show the mean; vertical bars show the standard error of the mean. Same letters indicate significant differences ($p<0.05$). * indicates significant difference between sexes.

of culture. When comparing the sexes, we observed no significant difference ($p>0.05$).

In both sexes, the concentrations of total lipids (Fig. 1C) and triglycerides (Fig. 1D) in the haemolymph showed the same pattern: in individuals from the natural environment, the levels of these metabolites were lower when compared to the animals kept in experimental culture for 90 or more days. Males showed the highest values of total lipids and triglycerides after 90 days of culture, while females showed the highest values after 150 days of culture. When comparing the sexes, we observed a significant difference ($p<0.05$) for both metabolites during cultivation.

The concentrations of glycogen in the hepatopancreas of males were high 90 days after the beginning their diet, decreased (24-fold) after 120 days until they reached lower levels than the animals from the environment, and then rose again (3-fold) after 180 days. In females, glycogen levels decreased after 120 days until they reached lower levels than animals from the natural environment, and then remained low until the end of the experiment (Fig. 2A).

The levels of total lipids in the hepatopancreas of males, after 90 days of culture, were similar to those found for animals in the environment, increased significantly after 120 days (approximately 4-fold) of culture, decreased 50% after 150 days, and then remained constant until the end of the experiment (Fig. 2B). In the hepatopancreas of females, the total lipid levels after 90 days of culture were similar to those found in animals in the environment, increased significantly after 120 days, then returned to similar levels as the control (90 days) after 150 days, and rose again after 180 days (Fig. 2B).

The triglyceride levels in the hepatopancreas of males after 90 days of culture were similar to those found for animals in the environment, around 0.4 mg/g; after 120 days of culture the levels increased 4.5-fold, decreased 50% after 150 days, and then remained constant until the end of experiment. Females showed the same pattern (Fig. 2C). When comparing both sexes, we observed a significant difference ($p<0.05$) in all metabolites.

Unfortunately, it was impossible to obtain gonads for the metabolic analyses, because the animals collected for the control group were small and did not contain enough tissue. The concentrations of glycogen (Fig. 3A) in the gonads of males showed high levels after 90 days of the diet, decreased after 120 and 150 days, and increased after 180 days of culture; they remained lower than the levels of the control (90 days). In females, glycogen levels decreased drastically after 90 days of the diet, and remained low until the end of the experiment.

In the gonads of males, the levels of total lipids (Fig. 3B) and triglycerides (Fig. 3C) showed a different pattern of variation ($p<0.05$); these levels were very low (18-fold) in relation to the levels of the females. Females showed an increase after 120 days, and this point differed significantly from the other periods studied. This response was not observed in males. The levels of glycogen, total lipids, and triglycerides showed significant differences between ($p<0.05$) males and females.

In both sexes, the hepatosomatic index (Fig. 4A) decreased after 120 days of experimental culture, and returned to similar levels (compared to individuals from the environment) in 180 days. In addition, in both sexes, the gonadosomatic index (Fig. 4B) showed an increase after 120 and 150 days, and decreased again after 180 days of culture.

DISCUSSION

Significant differences in the levels of total lipids and triglycerides in the gonads and hepatopancreas must be related to the reproductive cycle of these animals. In females, we found the highest levels of these metabolites in gonadal tissue after 120 days, associated with a significant increase in the gonadosomatic index (120 and 150 days), and a decrease in the hepatosomatic index after 120 days of culture. Similar responses of these indexes occurred in males, which was also observed by Sokolowicz *et al.* (2006) for *Aegla platensis* in the natural environment. However, the hepatopancreas reserves were not completely used, because the hepatosomatic index values were always higher than the gonadosomatic index values.

After 150 days of experimental culture, the levels of total lipids and triglycerides decreased in the gonads. These responses may be related to the use of these substrates for the synthesis of vitellogen in the female and its transfer to eggs, as well as an energy investment in reproductive behaviour, as observed by Greco *et al.* (2004) for *Aegla uruguayana* Schmitt 1942. This hypothesis was reinforced by the decrease in glucose levels during the experiment; this metabolite can be used to maintain mi-

nimum values of glycogen in the ovaries. Also, increased glycogen degradation in the hepatopancreas and gonads may make carbon skeletons available for triglyceride synthesis in the gonads of both sexes. A similar response was observed in males, and these results may be related to the decrease in hepatopancreatic glycogen and the increase in gonadal lipids and triglycerides. The subsequent decrease (150 days) in lipid reserves in gonadal tissue reinforced this hypothesis. During this study we observed the highest number of ovigerous females between 120 and 150 days of experiment.

According to Rodríguez-González *et al.* (2006) carbohydrates in the gonads decline during broodstock maturation. Normally, it appears that carbohydrates are an important source of energy during the early stages of gonadal development. The maximum value of carbohydrate indexes corresponded to the primary vitellogenic stage of oocytes. This stage was most frequently observed (44.6%) during the first stages of gonadal development, and progressively decreased as the gonadosomatic index increased. Reduction in the frequency of primary vitellogenic oocytes was accompanied by a decline in the carbohydrate content of the gonad. Low concentrations of carbohydrates have also been observed in eggs and embryos of *Cherax. quadricarinatus* (García-Guerrero *et al.* 2003), suggesting that this component is not used as an important source of energy during embryonic development. Similar observations have been reported for eggs of other decapods (Clarke 1982, Roustaian & Kamarudin 2001).

Rodríguez-González *et al.* (2006) observed changes in the lipid composition of the hepatopancreas and gonad with maturation, and also suggested that this fuel is transferred to the gonad. The lipid contents of maturing oocytes were highly correlated with their developmental stage. Galois (1984) observed in *Penaeus indicus* H. Milne Edwards, 1837 that lipids actively accumulate during the development of the gonad. Several investigators have also reported active mobilisation of lipid reserves from storage tissues (hepatopancreas and adipose tissue) to the gonad for the buildup of gametes in other crustacean species (Galois 1984, Castille & Lawrence 1989, Mourente *et al.* 1994, Rodríguez-González 2001). Mourente *et al.* (1994) reported that lipid accumulation in growing oocytes of *Uca tangeri* Eydoux, 1835 depends mostly on food intake. In *C. quadricarinatus*, this mechanism would also indicate that lipids required for broodstock maturation come from the diet.

The origin of lipids reaching the ovary is not fully understood. Lipids stored in the hepatopancreas have been shown to be transported to the ovary during vitellogenesis (Teshima *et al.* 1988, Castille & Lawrence 1989, Harrison 1990). However, the amount of lipids accumulated within the ovaries is greater than that stored in the hepatopancreas (Castille & Lawrence 1989). In the present study this behaviour was observed for lipids of the hepatopancreas and gonads in females. Teshima *et al.* (1986a, b) showed that female shrimps double their food consumption, indicating that lipids accumulating in the ovaries must originate from food. It is not known whether these lipids pass *via* the metabolic junction in the hepatopancreas or are taken up directly from the gut.

Almerão *et al*. (2009), studying the reproductive behaviour of *A. platensis,* reported that this behaviour can be divided into three parts: (1) precopulatory phase, (2) copulatory phase, and (3) postcopulatory phase. The first phase is characterised by male agonistic display, male approach, and courtship. Male approach led to display of courtship behaviour (body vibration, thrust, body lifting and abdomen flapping). During the copulatory phase, males and females touched each other with the antennae and males positioned themselves beneath the females. Finally, in the postcopulatory phase, males guard females during the process of egg attachment. All these behaviours require much energy, which may explain the results of the present study for males, principally the initial hyperglycemic levels and their sharp decrease after 150 days, and the complete depletion of glycogen of the hepatopancreas and gonads after 180 days of experimental culture.

In the present study, males and females showed a significant peak in total protein levels of the haemolymph after 150 days of culture, a period that is equivalent to spring in the natural environment. This variation may be related to the reproductive behaviour of males and reproduction in females. However, we cannot reject the hypothesis that females may mobilise proteins in other tissues such as hepatopancreas and muscle, because the protein levels in these tissues were not determined in the present study. Oliveira *et al.* (2007), studying this species in the natural environment, suggested that the increase in total protein concentration of the haemolymph observed during spring and summer may be a result of the decrease of these proteins in the tissues. They also suggested that the decrease in total proteins observed in autumn is probably correlated with the use of this substrate for vitellogen synthesis in the female gonads, and also as an energy investment in gametogenesis and in the reproductive behaviour of males.

In females, the decrease in the hepatosomatic index, total lipids, and triglycerides in the hepatopancreas in both sexes may have determined the increase in the levels of total lipids in the haemolymph after 120 days and the increase in the total lipid levels in the gonads after 120 days of the experiment, as well as the increase in the gonadosomatic index.

Sokolowicz *et al.* (2006) reported that males and females of *A. platensis* showed an inverse relationship of the hepatosomatic and gonadosomatic indexes. Sokolowicz *et al.* (2006) observed that the hepatosomatic index was always higher than the gonadosomatic index, suggesting that these aeglids utilise other energy sources in addition to the hepatopancreas. This hypothesis is supported by observations made by Oliveira *et al.* (2007), while studying these animals in the natural environment, which indicated that energy is derived from other tissues; and

also by the good state of nutrition from an abundance and diversity of food in natural environmental as observed by Bueno & Bond-Buckup (2004). These observations may explain the slight decrease of the hepatosomatic index in females that was observed only after 120 days of culture.

Several reports on changes in biochemistry during reproduction have demonstrated that other tissues and organs besides the hepatopancreas and ovary can accumulate organic reserves, such as haemolymph and muscles (Pillay & Nair 1973, Spaargaren & Hafner 1994, Palacios *et al.* 2000, Cavalli *et al.* 2001, Rosa & Nunes 2002, Castiglioni *et al.*2007).

Our results showed that a regular food supply can be related to an increase in the gonadosomatic index in males and females during the entire period of experimentation, compared with the index of animals taken directly from the natural environment. This pattern can improve the reproduction of *A. platensis* maintained in experimental cultivation. However, the pattern of the seasonality is the same as that observed in the natural environment by Sokolowicz *et al.* (2006). We also observed that the females used part of their hepatopancreatic reserves for vitellogenesis and gametogenesis, but the nutrients obtained from the other tissues and diet were very important to support reproduction. The results for males suggest that metabolic reserves are used for growth, gametogenesis, and reproductive behaviours such as maintaining the young and females. The present study indicates that reproductive events depend on a regular food supply, which is essential for conservation of this anomuran in its natural environment.

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