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Gonadosomatic and Hepatosomatic indexes of the freshwater shrimp *Macrobrachium olfersii* (Decapoda, Palaemonidae) from São Sebastião Island, Southeastern Brazil

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Abstract. *Macrobrachium olfersii* is widely known in the western Atlantic coastal region. However, no studies were found relating the variation of energy reserves of the hepatopancreas during the gonads' development and molt. The aim of this study was to determine the participation of the hepatopancreas in the availability of reserves to the gonadal development and to the molting process. The specimens [104 males (33.12%) and 210 females (66.88%)] were obtained in three samples (November/2007, April and October/2008) at the São Sebastião Island. The correlation between the Gonadosomatic Index (GI) and the Hepatosomatic Index (HI) was significant in both males and females - but only in females in the final stage of ovarian development. Among molt stages there was significant variation of GI but not at HI for females. Opposite result was observed for males, with significant variation of HI and no significant of GI among the molt stages. These evidences are indicative on the involvement of the hepatopancreas in the ovarian development and during the molt cycle.

Key words: gonad, growth, hepatopancreas, molt, reproduction

Resumo. Índices Gonadossomático e Hepatossomático do camarão de água doce *Macrobrachium olfersii* (Decapoda, Palaemonidae) da Ilha de São Sebastião, Sudeste do Brasil. *Macrobrachium olfersii* é amplamente conhecido na região Atlântica costeira ocidental. No entanto, não foram encontrados estudos relacionados à variação das reservas energéticas do hepatopâncreas durante o desenvolvimento das gônadas e estágios de muda. O objetivo deste estudo foi determinar a participação do hepatopâncreas na disponibilidade de reservas para o desenvolvimento gonadal e no processo de muda. Os espécimes [104 machos (33.12%) e 210 fêmeas (66.88%)] foram obtidos em três coletas (novembro/2007, abril e outubro/2008) na Ilha de São Sebastião. A correlação entre o Índice Gonadossomático (IG) e Índice Hepatossomático (IH) foi significativa para machos e fêmeas, mas apenas para fêmeas em fase final de desenvolvimento ovariano. Entre os estágios de muda nas fêmeas, houve variação significativa do IG e não significativa do IH. Nos machos foi observado um resultado oposto, com variação significativa do IH e não significativa do IG entre os estágios de muda. Estas evidências constituem indicativos sobre o envolvimento do hepatopâncreas no desenvolvimento dos ovários e durante o ciclo de muda.

Palavras chaves: gônada, crescimento, hepatopâncreas, muda, reprodução

Introduction

The freshwater shrimp *Macrobrachium olfersii* (Wiegmann 1836) is an abundant species, with a wide geographical distribution from the Atlantic coast of the United States to southern Brazil (Melo 2003). It occurs in the coastal basins and in the lower courses of major rivers, frequently inhabiting regions associated with clean freshwater and requires marine water to complete its life cycle (Melo 2003). As a consequence of its wide distribution, scientific knowledge about *M. olfersii* has been accumulated in recent years and has included studies on physiology (Souza & Moreira 1987, Augusto *et al*. 2007, Mendonça *et al*. 2007, Ribeiro & McNamara 2009), embryonic and larval development (Dugger & Dobkin 1975, Müller *et al.*

2003, Simões-Costa *et al.* 2005), population (Müller & Prazeres 1992, Anger & Moreira 1998), reproductive aspects (Barros 1995, Mossolin & Bueno 2002, Nazari *et al.* 2003, Martins *et al.* 2006), systematic (Pileggi 2009, Mossolin *et al.* 2010) and molecular researches (Rosa *et al.* 2008, Pileggi & Mantelatto 2010, Rossi & Mantelatto, submitted). Despite this favorable scenario about the knowledge of this species, there is a lack of reproductive information addressing the important relationship between the hepatopancreas and the gonadal development.

Hepatopancreas has an important role in the metabolism of the crustaceans, related to digestion and absorption, synthesis and secretion of digestive enzymes and carbohydrate metabolism (McLaughlin 1983). Moreover, it is the largest center of organic and inorganic reserves of decapods (Adiyodi 1969) and is also the largest organ of absorption and storage of lipids derived from the food, presenting an important role in their metabolism (Garcia *et al.* 2002). Hepatosomatic index (HI) and Gonadosomatic Index (GI) are common tools used as quantitative methods to verify the gonadal development (López-Greco & Rodríguez 1999, Sokolowicz *et al*. 2006) and represent the percentage of these organs to the total weight of the animal. Some authors observed the mobilization of the reserves in the hepatopancreas during the gonadal development through variations of GI and HI, or even by variations in the quantities of components of these organs (Kyomo 1988, Haefner & Spaargaren 1993, López-Greco & Rodríguez 1999, Yamaguchi 2001, Beatty *et al.* 2005, Sokolowicz *et al.* 2006). Other authors, however, found no significant mobilization among the hepatopancreas' reserves and the gonads, with no clear mechanism of transference (Pillay & Nair 1973, Omori *et al.* 1997, Rosa & Nunes 2003, Castiglioni *et al.* 2006). In addition, several studies have shown that the growth (molting process) and the development of decapod crustaceans are related to the storage of energy reserves in the hepatopancreas, which are partially transferred during the molt cycle (Passano 1960, Adiyodi 1969, Kyomo 1988, Yamaguchi 2001, Marcolin *et al.* 2008).

Considering the lack of information on the relationship between energy reserves of the hepatopancreas and the developmental processes, the objective of this study was to evaluate the relevance of the hepatopancreas in the gonadal development and in the molt, using a population of *Macrobrachium olfersii* as a model of study. These aspects constitute a good source of characters that can help the understanding of some reproductive problems present in many groups of decapods, including the palaemonid members.

Material and Methods

Specimens of *Macrobrachium olfersii* were obtained from three different surveys carried out at the rio Cachoeira da Toca (23° 49' 11,1"S; 045° 21' 40,0"W), on the São Sebastião Island, São Paulo State, Brazil. Sieves (mesh of 2mm) passing with fast and upward movement in submerse marginal vegetation and removing some pebbles in the area of great current (Mossolin & Bueno 2002) were used to capture the animals.

Once collected, the animals were kept alive and transported to the laboratory facilities of the Center of Marine Biology, University of São Paulo (CEBIMar/USP), where the ovarian coloration and molt stages were immediately checked under stereomicroscopy and external observation. Subsequently, they were frozen and transported to the Laboratory of Bioecology and Systematic Crustaceans (LBSC), where weighing and analysis were performed.

Ovary development was classified in three stages, according to its color and location (Mossolin & Bueno 2002): Stage I (initial), colorless or yellowish ovary, extending from the dorsal posterior margin to a maximum edge of the hepatopancreas; Stage II (intermediate), pale green ovary, extending from the posterior dorsal margin and slightly surpassing the hepatopancreas; Stage III (final), olive green or dark green ovary, extending from the middle of the first abdominal segment to the penultimate dorsal tooth of the rostrum.

Molt stages were also recorded in some individuals, based on morphological observations of the internal and the external bristles of the uropods, and on the rigidity of the cuticle. In accordance with these observations, three main stages were established (modified from Pinheiro & Hebling 1998 and Hayd *et al.* 2008): Stage I (post-molt) – the cuticle is not fully formed; the lumen of the bristle is with granular protoplasm and there is a thin and soft exoskeleton. Stage II (intermolt) - the cuticle and the inner cone of bristles are fully formed, the hard exoskeleton and the granular protoplasm lumen do not appear distinctly, Stage III (pre-molt): a strip between the cuticle and epidermis appears from the retraction in the process of apolise and the exoskeleton is not so hard.

Males and females had the carapace length (CL) and total length (TL) measured with a digital caliper (0.01 mm) and weighed on an electronic scale (precision 0.001 g) without the chelipeds, due to the presence of heterochely in the species (see

Mossolin & Bueno 2003). Subsequently, individuals were dissected and both the gonad and the hepatopancreas were removed and immediately weighed. The Gonadosomatic Index (GI) and Hepatosomatic Index (HI) were calculated using the weights of each organ (WG and WH, respectively), divided by the weight without chelipeds of the animal (WWC), then multiplied by 100, following the method described in Grant & Tyler (1983) and later all data were analyzed using Zar (1996) as a reference for the tests. These were carried out using Sigma Stat® - Windows Version 2.03 and adopted for all the *p*-value with significance level of 5%. All data were tested to the normality, performing the Kolmogorov-Smirnov test followed by the Pearson or Spearman correlation when data were parametric or non-parametric, respectively. Moreover, when multiple groups were compared, the test of variance analysis (ANOVA) with Tukey's test was subsequently performed for parametric data, and the Kruskal-Wallis test was used for the non-parametric.

Voucher specimens were deposited in the Crustacean Collection of the Biology Department (CCDB) of the Faculty of Philosophy, Sciences, and Letters of Ribeirão Preto (FFCLRP), University of São Paulo, Brazil (Access Numbers: 2201 and 2447).

Results

In total, 314 individuals were collected, of which 104 were males (33.12%) and 210 females (66.88%), with the sex ratio of 2.02 females per male. The studied population showed a unimodal pattern with a normal distribution of size classes. The size in males ranged between 4.31 and 25.51 mm of CL (10.37 ± 3.96) and 19.27 to 75.37 mm of TL (37.28 ± 11.62). In non-ovigerous females (N = 103) the CL ranged from 5.68 to 17.04 mm (11.68 ± 2.69) and TL 23.55 to 57.81 mm (41.99 ± 7.80). The CL of ovigerous females (N = 107) ranged from 7.52 to 17.66 mm (12.36 ± 2.20) and TL 28.98 to 58.90 mm (43.78 ± 6.24) (Fig. 1). Due to this similarity between non-ovigerous and ovigerous females, both categories were presented together in the other analyses.

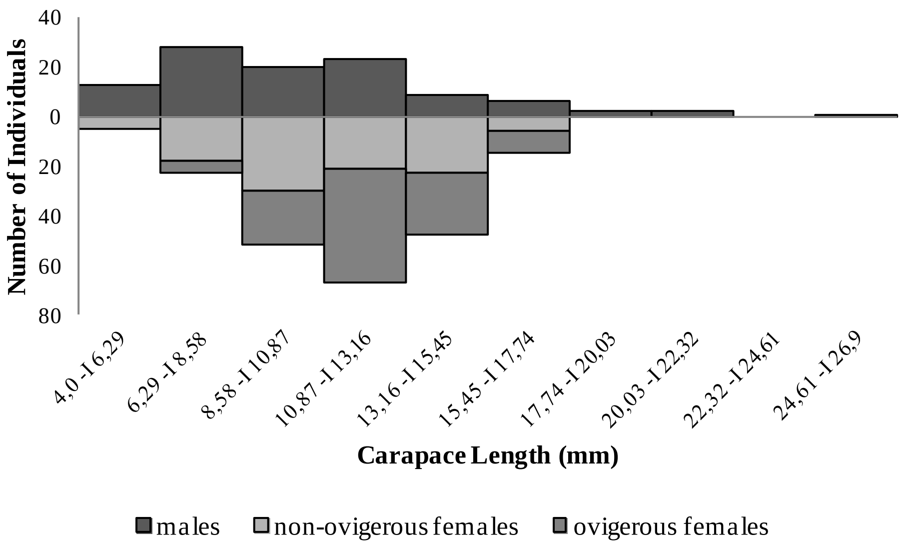

Figure 1. *Macrobrachium olfersii*. Distribution of frequency classes in class sizes of individuals collected in November 2007 and in April and October 2008, on the São Sebastião Island, Ilhabela (SP).

Male. GI / HI

The variation of the GI (mean ± sd = 1.0516 ± 0.8820; minimum = 0.2433; maximum = 2.7370) and HI (mean ± sd = 5.1546 ± 1.9589; minimum =1.4396; maximum = 9.9234) in males, from 0.24 to 2.73, and 1.44 to 9.92, respectively, is presented in the Figure 2. A significant inverse association (p<0.05) between the two variables was observed, with the highest GI values tending to lower levels of HI.

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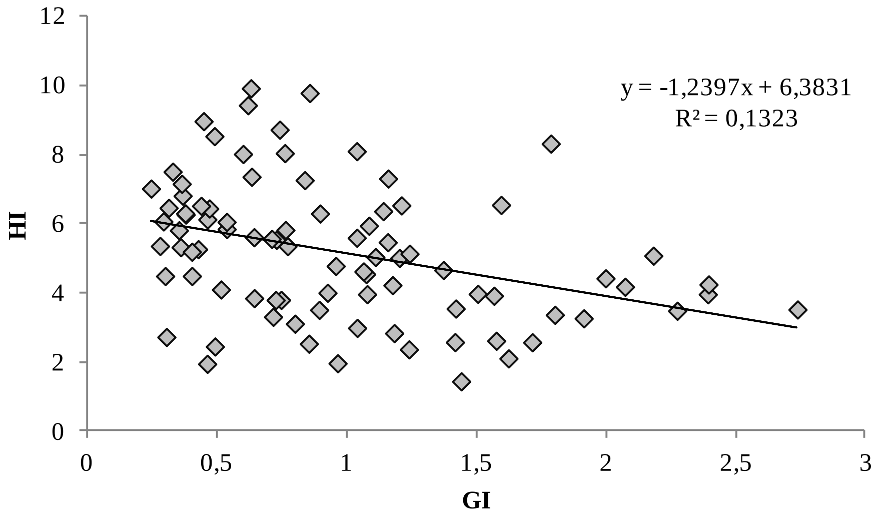

Figure 2. *Macrobrachium olfersii*. Regression analysis between Gonadosomatic Index (GI) and Hepatosomatic Index (HI) of males collected in November 2007, April and October 2008, on the São Sebastião Island, Ilhabela (SP).

Males. GI and HI / Molt Stages

There was little variation of the GI means' values among the molt stages I, II and III, which did not differ significantly (*p*>0.05) and corresponded to 1.07 ± 0.60, 1.07 ± 0.68 and 1.60 ± 0.90, respectively in the stages of post-molt (I), intermolt (II) and pre-molt (III). There was a significant variation (*p*<0.05) of the values of HI among each molt stage: 4.90 ± 1.73, 5.66 ± 2.31 and 3.80 ± 1.20, respectively in I, II and III, and they were higher in the intermolt stage.

Females. GI / HI

The correlation between the GI (mean ± sd = 3.3822 ± 3.0295; minimum = 0.3450; maximum = 12.0826) and HI (mean ± sd = 5.2143 ±1.4418; minimum = 2.1550; maximum = 9.3922) during the stage I of ovarian development evidenced that the range of GI values was small, from 0.34 to 1.44. In the development of ovaries in stage II, GI was higher than that in stage I and ranged from 0.55 to 6.37, while in the stage III, the same index's range was from 1.13 to 12.08, the highest one recorded (Fig. 3). The mean values of GI ranged from 0.66 (25% = 0.54 and 75% = 0.82), 1.63 (25% = 1.18 and 75% = 2.66) and 5.04 (25% = 3.47 and 75% = 8.00) in the stages I, II and III, respectively (*p*<0.05).

The variation of HI was quite high in the stage I, ranging from 2.79 to 8.59. In the stage II it remained high, ranging from 2.15 to 9.39; and the amplitude of HI during the Stage III of the ovaries' development was similar to the other stages', ranging from 2.26 to 8.20 (Fig. 3). The mean values of HI was 5.11 ± 123, 5.70 ± 1.76 and 5.06 ± 1.47 in the stages I, II and III, respectively (*p*>0.05). There is no significant direct relationship (*p*>0.05) between GI and HI in the earlier stages of the ovarian development (I and II), while in the final stage (III), there is a significant inverse relationship between these variants (*p*<0.05).

In females with ovaries in stage I of development, the gonad's weight was very low, with minimal interference in the relationship of GI, while in the stage III, the gonads reached large sizes when compared to the gonads in the earlier stages, interfering in the relationship of GI. The Hepatosomatic Index assumes almost constant values over the initial and intermediate stages of the ovarian development, decreasing in the final stage.

Females. GI and HI / Molt Stages

There was significant variation (*p*<0.05) of the GI among the stages of molt, with the mean values ranging from 0.72 (25% = 0.59 and 75% = 0.84), 1.28 (25% = 0.73 and 75% = 3.59) and 5.17 (25% = 1.90 and 75% = 7.71) in stages I, II and III respectively. The values of HI among the three stages of molt did not differ (*p*>0.05) and were 5.62 ± 1.51, 5.95 ± 1.52 and 5.34 ± 1.90, respectively in the molt stages I, II and III, the lowest value appearing in the pre-molt (III) stage.

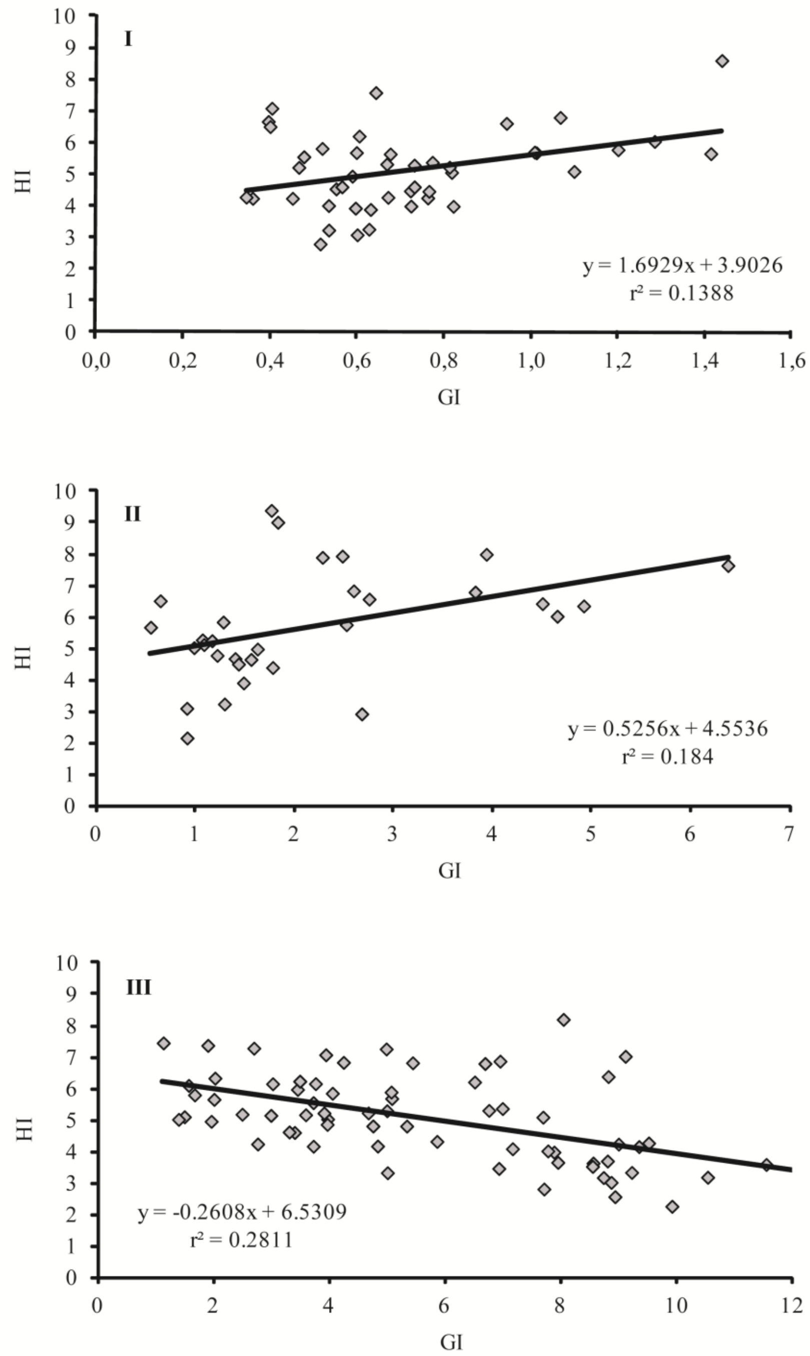

Figure 3. *Macrobrachium olfersii*. Regression analysis between the values of the Gonadosomatic Index (GI) and Hepatosomatic Index (HI) of females with ovaries at stage I (initial), II (intermediate) and III (final) of ovarian development, collected in November 2007, April and October 2008, on the São Sebastião Island, Ilhabela (SP).

Discussion

The present work corroborates the main idea about the intimate relationship between the reproductive and the molting processes of *M. olfersii*. In males, the relationship between the GI and HI reflects the association between the weight of the gonad and the hepatopancreas, it is observed that the lowest values of GI tend to correspond to the highest values of HI, and the reverse can also be observed.

As the major organ of organic and inorganic reserves in crustaceans (Passano 1960), the hepatopancreas is expected to provide reserves for the gonad development and the growth of *M. olfersii.* However, Mossolin & Bueno (2002) observed ovigerous female through a period of two years (June/96 to May/98) and inferred that a close population of *M. olfersii* at the São Sebastião region showed a continuous pattern of reproduction. That result suggested that males of the species showed a continuous production of spermatozoids. That fact was also observed in other decapods, as demonstrated Pillay & Nair (1973), who found spermatophores in the vas deferens throughout the year in the brachyuran *Uca annulipes* (H. Milne Edwards 1837), *Portunus pelagicus* Linnaeus 1766, and shrimp *Metapenaeus affinis* H. Milne Edwards 1837. Omori *et al.* (1997) found that the GI of males of *Helice japonica* Yatsuzuka & Sakai 1980 and *Helice tridens* (De Haan 1835) were relatively constant over the years, and Yamaguchi (2001) studied the seasonal changes in the Hepatosomatic Index of *Uca lactea* (De Haan 1835) and noted that there was a drop in that index due not only to the reproductive period, which might be related to sexually active males throughout the year, but also to the molting process of the animals.

Thereby, and considering the absence of seasonal influence on the population, we suggest that the inverse relationship between the weights of the reproductive system and the hepatopancreas of males is not totally related to the reproductive process, but may be related to the molting process, which requires a lot of energy. The analysis of the GI and HI indexes and the molt stages seems to reflect this intimate relationship, since during the different molt stages it was observed that the GI averages ranged less than the HI averages. During the intermolt stage, HI reaches its maximum and, according to Passano (1960), it is when there is a greater accumulation of reserves in the hepatopancreas to be used later in the molting process. At the pre-molt, a decrease of the HI values may indicate the spending of these reserves in the period when the formation of a new exoskeleton and animal growth happens. In that period, Passano (1960) suggested that the decrease of lipids in hepatopancreas might be due to a rapid conversion of fat into sugar to be used in the formation of the chitin. Concerning the stage of post-molt, the values of the index increase, indicating a new accumulation of reserves. The transfer of the hepatopancreas' reserves during the molting process is also suggested by some authors (Passano 1960, Adiyodi 1969, Adiyodi & Adiyodi 1972, Kyomo 1988, Yamaguchi 2001, Marcolin *et al.* 2008).

In the females, the relationship between the GI and HI varied according with the ovaries' development stages. During the earlier stages of ovarian development, there is a direct relationship between the indexes, with the lowest values of GI tending to lower values of HI; and the same was observed in the stage II. Only in the later stage (III), the lowest values of GI tend to correspond to the highest values of HI, and the reverse can be observed.

As pointed, the largest center of organic and inorganic reserves in crustaceans (Passano 1960), the hepatopancreas is expected to mobilize some reserve during the ovarian development, since this is a costly process in females, in which there is a severe biochemical synthesis with mobilization of lipids and proteins for the oocytes' development (Sastry 1983). Furthermore, molt and reproduction are related and require mobilization of organic reserves from storage sites to the epidermis and gonad, a process coordinated by hormones (Sastry 1983).

According to Okumura & Aida (2000), a rapid ovarian development occurs during the vitellogenesis, a process characterized by the appearance of vitellogenin, a protein precursor of the yolk protein, and its accumulation causes rapid increase in the size and diameter of oocytes. Li *et al.* (2006) also observed that the hepatopancreas is the site of extraovarian synthesis of vitellogenin in the crab *Eriocheir sinensis* H. Milne Edwards 1854 and Ding *et al.* (2010) observed that yolk protein is synthesized in both the ovary and the hepatopancreas of the crab *Carcinus maenas* (Linnaeus 1758), but the hepatopancreas appears to be a much more important synthetic site.

Thus, it is believed that the inverse relationship between the GI and HI in the final stage of ovarian development is related to the mobility of the hepatopancreas' reserves to the gonad. This relationship was also observed by Castille & Lawrence (1989) in *Penaeus setiferus* (Linnaeus 1767), by López-Greco & Rodríguez (1999) in the crab *Neohelice granulata* (Dana 1851) and by Sokolowicz *et al.* (2006) in the aeglid *Aegla platensis* Schmitt 1942.

During the analysis of the molt stages and of the indexes GI and HI, it was observed that there was a variation of the GI during the molt stages, when ovarian development happens synchronized with the molt cycle. The same pattern was observed by Okumura & Aida (2000) in congener species, *M. rosenbergii* (de Man 1879). These authors studied the relationship between the level of vitellogenin in the hemolymph and the ovarian development in molt cycles of reproductive and non reproductive individuals and observed high values of GI associated with high levels of vitellogenin, which were higher during the pre-molt stage, the same period observed in *M. olfersii*.

Despite the vitellogenesis and the secretion of a new cuticle during the molting period had generated a competitive resource of the hepatopancreas (Subramoniam 2000), it did not exhibit a clear relation of this organ's mobilization during the molt cycle; only a trend of the highest and the lowest values of HI were related to the period of intermolt (stage II) and pre-molt (stage III), respectively.

The GI and HI show antagonistic behavior in females in the stage III of ovarian development, as observed in relation to the males; however, unlike these, the variations of HI during the process of molt are not significant. These results indicate an investment of the hepatopancreas' energy reserves in the ovarian development by the females, while the males invest those reserves in growth. Thus, our work brings data on the participation of the hepatopancreas in the process of ovarian development and in the process of molt in the studied *M. olfersii* population.

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