

Female reproductive cycle of the Southwestern Atlantic estuarine crab *Chasmagnathus granulatus* (Brachyura: Grapsoidea: Varunidae)*

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SUMMARY: The female reproductive biology of a *Chasmagnathus granulatus* population inhabiting the area near the mouth of Mar Chiquita coastal lagoon, Argentina, was studied. An increase in air temperature during the spring is related to the start of the breeding period, when well defined egg-laying and hatching pulses were observed. Hatching is synchronic during the whole summer but the egg production was not, probably due to the gradual incorporation of young females to the reproductive population. Neither egg-laying nor larval release showed a clear relation to moon phase or tidal cycles, suggesting that reproduction is not rigidly programmed in this unpredictable habitat. Females moult at the beginning of autumn, after releasing the last larvae. However, a new cohort of ovocytes, which was in primary vitellogenesis before moulting, completed the secondary ovogenesis after moulting. Consequently, ovaries remained fully developed throughout the winter.

Key words: *Chasmagnathus granulatus*, breeding cycles, hepatosomatic index, gonadosomatic index, hatching.

RESUMEN: CICLO REPRODUCTIVO DE HEMBRAS DEL CANGREJO ESTUARIAL DEL ATLÁNTICO SUDOCCIDENTAL *CHASMAGNATHUS GRANULATA* (BRACHYURA: GRAPSOIDEA: VARUNIDAE). – Fue estudiada la biología reproductiva de hembras de una población de *Chasmagnathus granulatus* en la boca de la laguna Mar Chiquita, Argentina. El incremento de la temperatura del aire en la primavera está relacionado con el inicio del periodo de cría, momento en el cual se observan pulsos de extrusión de huevos y eclosión de larvas bien definidos. La eclosión de larvas es sincrónica durante todo el verano, pero no así la producción de huevos, probablemente debido a la gradual incorporación de hembras jóvenes a la población reproductiva. Ni la extrusión de huevos ni la eclosión de larvas muestran una clara relación con las fases de la luna o los ciclos de marea, sugiriendo que la reproducción no está sujeta a un programa inflexible en un hábitat impredecible. Las hembras mudan a principios del otoño, después de las últimas eclosiones larvales. Sin embargo, una nueva cohorte de ovocitos, que estaban en vitelogénesis primaria antes de la muda, completan la vitelogénesis secundaria después de mudar. Consecuentemente, los ovarios están completamente desarrollados durante todo el invierno.

Palabras clave: *Chasmagnathus granulatus*, ciclo reproductivo, índice hepatosomático, índice gonadosomático, eclosión.

INTRODUCTION

The existence of a conflict between growth and reproduction is a common premise in studies of reproduction and life history patterns. Although long-lived iteroparous malacostracan females allo-

cate resources “toward reproduction in times of scarcity and toward growth in times of abundance”, scheduling of growth and reproduction is not necessarily determined by resource competition or physiological antagonisms (Nelson, 1991). In fact, crustacean growth is the result of periodic moults of the exoskeleton, and since females of most malacostracan species incubate their eggs externally, they

*Received January 7, 2003. Accepted September 22, 2003.

should not moult during the breeding period simply to avoid the egg loss (Nelson, 1991).

The reproductive activities of a female crab (Crustacea: Brachyura) begin before the maturity moult; the initial growth and development of oocytes is based on autochthonous material (primary vitellogenesis). After this moult, the oocyte growth involves the contribution of allochthonous material (secondary vitellogenesis). It has been shown that reserves stored in the hepatopancreas are mobilised to the ovaries (Pillay and Nair, 1973; Paulus and Laufer, 1987; Kyomo, 1988). Later, oocytes are fecundated and extruded, eggs are attached to the long pleopodal setae and the embryonic development takes place in a sort of incubation chamber. Extrusion and larval release may be synchronic in intertidal crabs, in agreement with circadian, circatidal and circalunar cycles (Saigusa, 1981, 1982, 1992; Forward, 1987; Zimmerman and Felder, 1991; Morgan and Christy, 1994; Saigusa and Kowagoye, 1997), according to species and particular habitat.

Multiple broods within an instar are possible (Cheung, 1969), and the number may vary with season and size (age) (see Nelson, 1991 for a review). Since the development of a new cohort of oocytes may begin before the first clutch is extruded, the ovigerous females may have ovaries in primary or secondary vitellogenesis. When hatching occurs, secondary vitellogenesis of the second cohort is nearly complete, so a new clutch may be soon extruded (Nelson, 1991). Several cycles of ovarian activity could occur during a reproductive season. The existence of these cycles is evidenced by the successive peaks in the proportion of females with mature ovaries, and of ovigerous females, observed during field studies (González-Gurriarán, 1985; Zimmerman and Felder, 1991; Sudha and Anilkumar, 1996; Mantelatto and Fransozo, 1999). Finally, the last cohort of oocytes of the current season arrest their development at the end of primary vitellogenesis and a premoult process begins (Nelson, 1991).

The interval between mating and fecundation is highly variable: mating may be postecdysial, involving soft-shelled females usually protected by the male, or anecdysial, involving hard-shelled females. Within the latter, it may occur anywhere in the long intermoult period or may be restricted to a short period prior to egg extrusion, when the genital pore operculum is decalcified (Fukui, 1993). Females of many Brachyuran species produce multiple egg batches from single matings (Morgan *et al.*, 1983;

González-Gurriarán, 1985; Fukui and Wada, 1986; Elner and Beninger, 1992; Haddon and Wear, 1993, Gao *et al.*, 1994; McDermott, 1998).

Dense populations of the semi-terrestrial estuarine burrowing crab *Chasmagnathus granulatus* (Brachyura: Grapsoidae: Varunidae) are found from Paranaguá Bay (Brasil) to San Antonio Bay (Argentina) (Spivak, 1997). This species breeds during most of spring, all summer and the beginning of autumn (Ruffino *et al.*, 1994; Spivak *et al.*, 1994, 1996, 2001; López Greco and Rodríguez, 1999). The absence of ovigerous females in Mar Chiquita, an Argentinian coastal lagoon, during winter has been correlated with a decrease in feeding activities caused by low air temperatures (Spivak *et al.*, 1996). In addition, egg laying has been observed in the laboratory at high and constant temperatures ($21 \pm 1^\circ\text{C}$), even during the non-reproductive season (López-Greco and Rodríguez, 1999). On the other hand, *C. granulatus* females mated in the intermoult stage of the moult cycle, multiple ovipositions after one mating were observed in laboratory reared crabs (López-Greco and Rodríguez, 1999), and hatching pulses synchronised with nocturnal ebbs were detected in the Mar Chiquita population (Anger *et al.*, 1994). This synchrony allows Mar Chiquita larvae to be immediately exported to the sea.

In the present study the female reproductive biology of *C. granulatus* in Mar Chiquita was studied. Specifically, we analyzed the effect of air temperature on the starting and duration of the breeding period, the synchrony of extrusion and hatching pulses, the putative relationships of these pulses with tides and lunar phases, and the possible mobilisation of reserves accumulated in the hepatopancreas to the ovary. Finally, we propose the schedule of moulting and reproduction in this habitat.

MATERIALS AND METHODS

Study area

Mar Chiquita coastal lagoon is located between $37^\circ32'$ and $37^\circ45'S$ and $57^\circ19'$ and $57^\circ26'W$, 35 km NE of the city of Mar del Plata, Argentina. It has an area of 46 km² and a maximum depth of ca. 1.2 m (Fasano *et al.*, 1982), and is divided into a wide northern freshwater part and a narrow southern estuarine part. Water temperature and salinity show great seasonal, daily and local variations (Anger *et al.*, 1994, Reta *et al.*, 2001). The mean water tem-

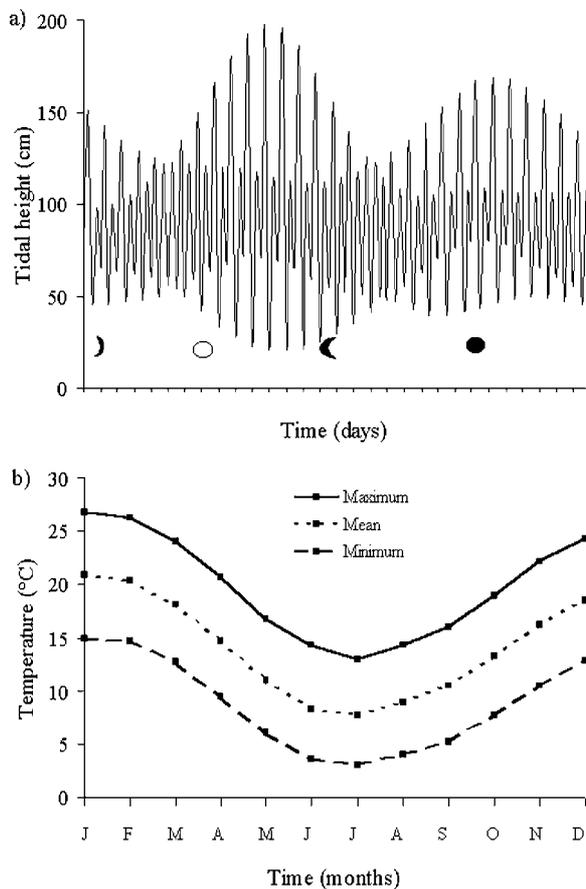


FIG. 1. – *Chasmagnathus granulatus*. a) Tidal regime and lunar phases in Mar del Plata during January, 2001; b) Maximum, mean and minimum monthly air temperatures registered in Mar del Plata between 1981 and 1990.

perature of this shallow water lagoon corresponds closely to air temperature cycles (Reta *et al.*, 2001). Semidiurnal tides (amplitude < 1m) superimposed by irregular, often strong, wind action cause an irregular pattern of influx and outflux of water, which is hampered by a sandbar at the entrance to the lagoon (Anger *et al.*, 1994). Intertidal environments at the lagoon comprise mud flats and large surrounding cordgrass *Spartina densiflora* areas (Olivier *et al.*, 1972). The more abundant crab species of Mar Chiquita lagoon are *Chasmagnathus granulatus*, *Cyrtograpsus angulatus* and *Uca uruguayensis*. *C. granulatus* and *U. uruguayensis* inhabit the upper intertidal; *C. angulatus* inhabits the lower intertidal and subtidal (Spivak *et al.*, 1994; Spivak *et al.*, 1996).

Few data are available on the hydrographic and climatic conditions of Mar Chiquita. The tidal regime of the study area corresponds to a mixed, dominant semi-diurnal type: “large tides” (LT) and “half tides” (HT) alternate with a regular time inter-

val of ca. 7 days. The astronomic tides of January 2001, tabulated for Mar del Plata Harbour by the Servicio de Hidrografía Naval, Argentina, are shown in Figure 1a as an example. Air temperatures of Mar del Plata Airport, 35 km south of Mar Chiquita, were obtained from the Servicio Meteorológico Nacional, Argentina, and the National Climate Data Center, U.S.A. The highest temperatures occurred in January 2000 and February 2001, the lowest in July 2000. During the period 1981-1990, the maximum and minimum mean monthly temperatures were 26.8 and 3.1°C respectively (Fig. 1b). The number of days with temperature $\leq 0^\circ\text{C}$ was 19.2 ± 2.71 per year during the period 1997 to 2002.

Collection and analysis

Crabs were collected weekly on a muddy beach located in the southern part of the lagoon (site #3, see Spivak *et al.*, 1994), from November 7, 2000 to March 14, 2002. All crabs present on the surface or in burrows were collected by hand along several 10 m transects approximately perpendicular to the water edge. The number of transects varied according to crab density. Males were discarded, and females (N from 93 to 114) were transported to the laboratory and sorted into ovigerous and non-ovigerous. The percentage of ovigerous to total females (POF) was calculated. A few eggs were removed from each ovigerous female, examined under microscope and classified into one of the 9 embryonic developmental stages (ES₁ to ES₉) proposed by Bas and Spivak (2000). The frequency of ovigerous females carrying embryos in each stage (f_n) was registered and a mean embryonic stage was calculated as follows:

$$\text{MES} = (\sum \text{ES}_n \cdot f_n) * 100 / N_o$$

where n ranges from 1 to 9 and N_o is the number of ovigerous females.

Non-ovigerous females were reared for 7 days in a 60 x 40 x 20 cm aquarium filled with 23 PSU water and examined daily in order to detect extrusion. The water was at ambient temperature and was aerated continuously. The females were not fed during this period. The percentage of females that spawned during a week in comparison with non-ovigerous females (PE) was calculated.

Females with developed embryos (ES₇ to ES₉) were placed individually in 1-litre flasks and examined daily in order to detect the release of larvae.

The percentage of females with these embryos in comparison with all ovigerous females (PH) was calculated.

Morphologically mature females ($n = 50$), characterised by a wide pleon that covers the base of limb coxae, were collected monthly from October 1999 to April 2001, except in July and October 2001. They were transported to the laboratory, frozen to death at -10°C and dissected.

Their ovaries were examined and classified into one of the following 5 stages:

Stage 1: filiform and translucent; pale grey.

Stage 2: both ovarian filaments tubular and narrow ; orange, red or brown.

Stage 3: both ovarian filaments tubular, wider but without differences between the anterior and the posterior part ; sometimes granular; brown to violet.

Stage 4: the anterior part expanded, though not covering either the cardiac stomach or the hepatopancreas; the posterior part with both filaments already visible; always granular; orange to dark violet.

Stage 5: all the organ expanded, covering the cardiac stomach and the hepatopancreas; always granular; bordeaux to dark violet.

The percentage of females with ovaries in each stage was calculated.

Ovaries and hepatopancreas of these 50 morphologically mature females were carefully dissected, dried for 48 h at 80°C , and weighed individually to 0.0001 g; the exoskeleton and remaining organs were also dried for 48 h at 80°C and weighed individually to 0.01 g. Eggs of ovigerous females were previously removed. The gonadosomatic and hepatosomatic index (GSI and HSI) were calculated as the ratio between the dry weight of the organ and the dry weight of the whole crab including the organ, and expressed as a percentage. The mean GSI and HSI were calculated for each sample.

Statistical methods

The null hypotheses of no difference in monthly temperatures between years were compared by Student test. The null hypotheses of no difference in time to larval hatching among females with embryos in stage 7 to 9, and in PH and PE between tidal and lunar phases were analysed by ANOVA, after checking for normality and homocedasticity. A spectral analysis was made for rhythmicity of egg extrusion and larval release, using the Kolmogorov-Smirnov (K-S) d -test for significance. The relationships

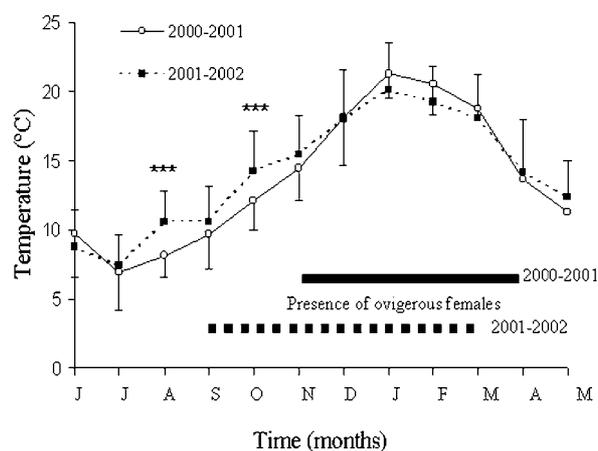


FIG. 2. – *Chasmagnathus granulatus*. Mean air temperatures and reproductive season duration in two successive years (2000-2001 and 2001-2002). *** significant differences between temperatures (t-test, $p < 0.05$). Solid and dotted horizontal bars corresponds to the presence of ovigerous females during 2000-2001 and 2001-2002 respectively.

between the mean GSI and HSI, and between both indexes and the mean air temperature of the current month, were analysed by means of a correlation test. When the statistical power of a performed test was below the desired power of 0.8, negative results should be interpreted cautiously (Norman and Streiner, 1996).

RESULTS

The annual pattern of mean air temperatures presented a similar pattern during the two reproductive seasons (Fig. 2). However, August and October were significantly warmer in 2001 than in 2000; the mean value observed in August 2001 ($\sim 10^{\circ}\text{C}$) was reached only in October 2000 ($\sim 11^{\circ}\text{C}$) (Fig 2). The beginning of a reproductive season coincided with a rise in air temperature (Fig. 2). The minimum air temperature registered during each reproductive season was $> 8^{\circ}\text{C}$ (April 2001) and $> 6^{\circ}\text{C}$ (September 2001) (data not shown).

Ovigerous females were present from November 2000 to April 2001 during the first reproductive season. Several peaks of the percentage of ovigerous females (POF) were observed (Fig. 3a). Three of these peaks include $> 70\%$ of the collected females: 80% in November, 73% in December, and 83% in March. From December to February, POF was between 36 and 70%. The POF reached the lowest value after the first peak (8% in November), and decreased continuously after the last peak, reaching

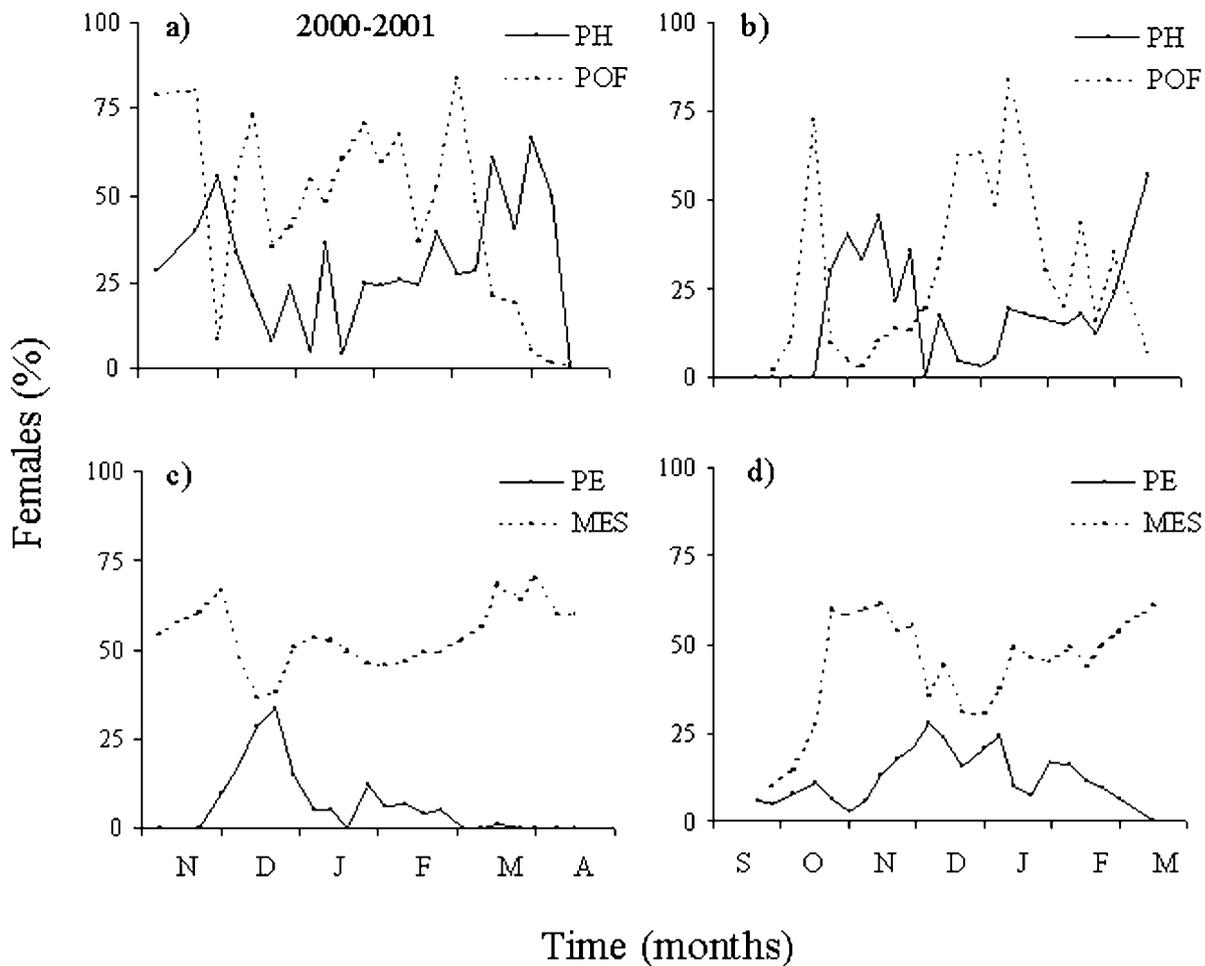


FIG. 3. – *Chasmagnathus granulatus*. Percentage of ovigerous females (POF), mean embryonic stage (MES), percentage of egg-laying occurrence (PE), and percentage of hatching occurrence (PH). a) and c) reproductive season 2000-2001, b) and d) reproductive season 2001-2002.

10% in April. The proportion of ovigerous females that liberated larvae in the laboratory during the week that followed their collection (PH) ranged between 3.8 and 66.7%; the highest values were observed at the beginning and the end of the reproductive season and coincided with minimum POF (Fig. 3a).

The mean embryonic stage (MES) increased during November 2000 to a maximum (66%), and decreased to a minimum (36%) in December. The MES ranged from 45 to 53% during January and February 2001, and increased during March 2001, reaching 70% at the end of this month (Fig. 3c). The proportion of non-ovigerous females that laid eggs during the week following their collection (PE) varied from 0 to 33%. The values of PE increased during the end of spring, reached its maximum in December (33.3%) and decreased in the following 2 weeks. From January to February, PE varied between 3.9 and 12% (Fig. 3c).

During the second reproductive season ovigerous females were present from September 2001 to March 2002. Several peaks of POF were observed, but in a lower proportion than in 2000-2001: 72% in October, 62% in December, 83% in January, 43% in February and 35% in February. POF reached its lowest value after the first peak (November 2%). PH varied from 0 to 57%; the highest values coincided with minimum POF (Fig. 3b).

The MES was high from October to November 2001 (~60%), decreased to a minimum (30%) in December, and increased to 49% in January 2002. It remained at similar values during January and February and increased again, reaching 61% in March. Several peaks of PE were observed, coinciding with the lowest MES (Fig. 3d).

Larvae hatched successfully from all females with developed embryos (stages 7, 8 and 9) in 41 of the 45 observed samples. The time elapsed from collection to hatching differed significantly among

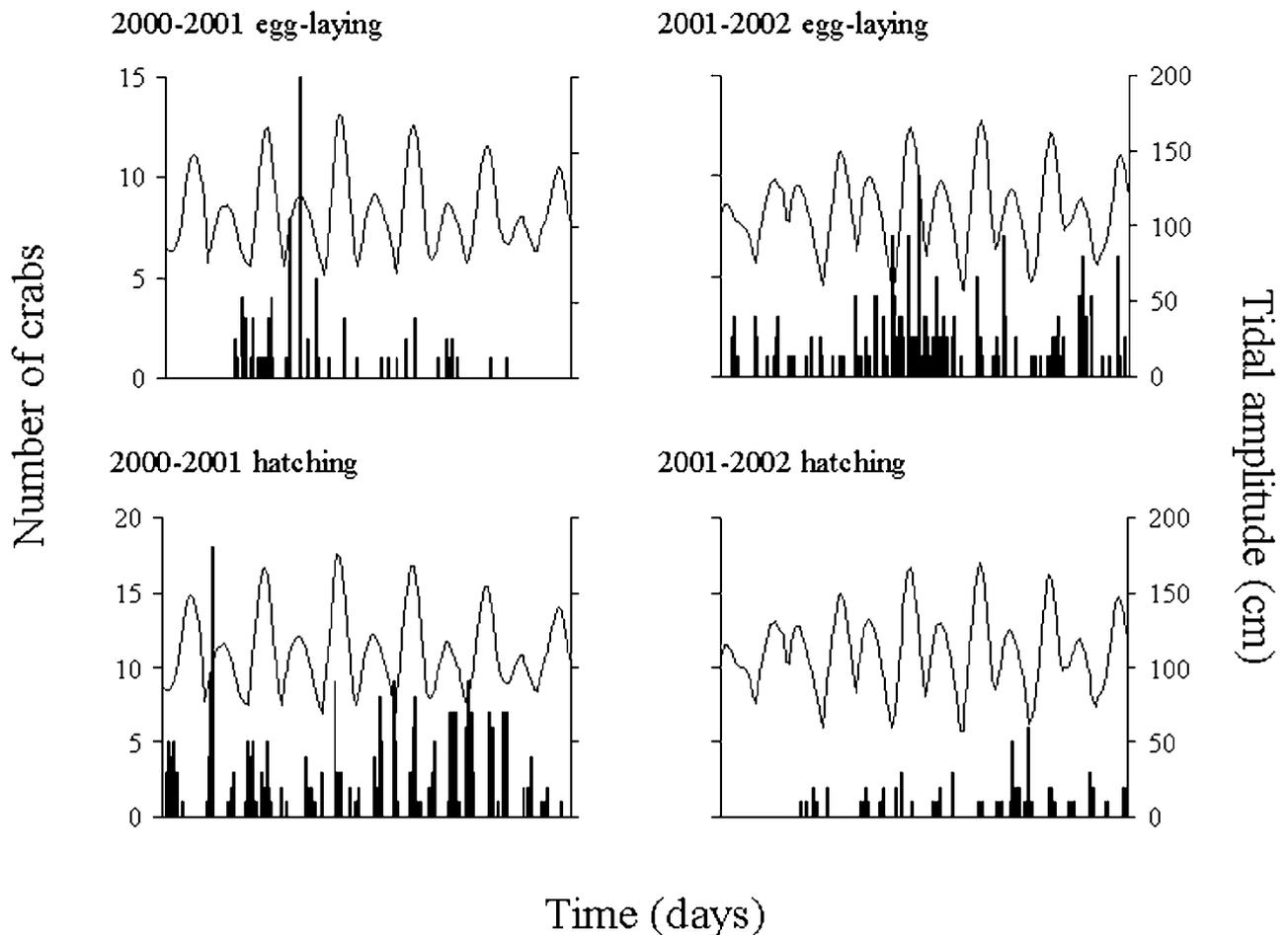


FIG. 4. – *Chasmagnathus granulatus*. Tidal amplitude and the number of females that laid eggs and that released larvae in the laboratory each day during both reproductive seasons.

embryonic stages: 3.6 ± 1.22 d (ES_7), 2.9 ± 1.29 d (ES_8) and 1.2 ± 0.81 d (ES_9) (ANOVA, $p < 0.0001$).

The differences in hatching and egg laying proportions (PH and PE) observed between 4 tidal conditions (spring LT, spring HT, neap LT, neap HT) and between 4 lunar phases were non-significant (ANOVA: PH-lunar phases, $P = 0.1550$, PE-lunar phases, $P = 0.6771$; PH-tides, $P = 0.6537$, PE-tides, $P = 0.4213$). However, the power of the performed tests was < 0.8 .

Larval release showed significant rhythmicity in the 2000-2001 (Fisher Kappa: 5.825; Barlett K-S d : 0.2686) and 2001-2002 (Fisher Kappa: 12.10; Barlett K-S d : 0.3308) reproductive seasons (Fig 4). The periodogram peaks corresponded to periods of 8, 15, and 27 days. Egg extrusion showed significant rhythmicity in the 2000-2001 (Fisher Kappa: 9.807; Barlett K-S d : 0.1595) but not in the 2001-2002 reproductive seasons (Fig. 4). The periodogram peaks of the 2000-2001 season corresponded to periods of 4, 10, and 20 days.

The ovary of all ovigerous females studied during both reproductive seasons were in stage 1 to 3 (Fig. 5a). Stage 1 (post-extrusion) predominated in most samples and the ovaries of all ovigerous females were at stage 1 at the beginning of the second reproductive season (September 2001). Ovarian stages 4 and 5 were observed only in non-ovigerous females. Most females had ovaries in stage 3 at the beginning of the non-reproductive period (April, 2000), but during the rest of this period (May to August) ovaries were mostly in stage 5 (Fig. 5b).

The GSI ranged between 0.2 and 1 during most the reproductive season, except in September 2000 (Fig. 6). It increased at the beginning of the non-reproductive season (April) and reached the highest values during winter (ca. 3). The hepatosomatic index was always higher than the gonadosomatic index but showed a similar temporal pattern, with an increase during the non-reproductive season (Fig. 6). In fact, a significantly linear relationship between mean HSI and GSI was detected (analysis

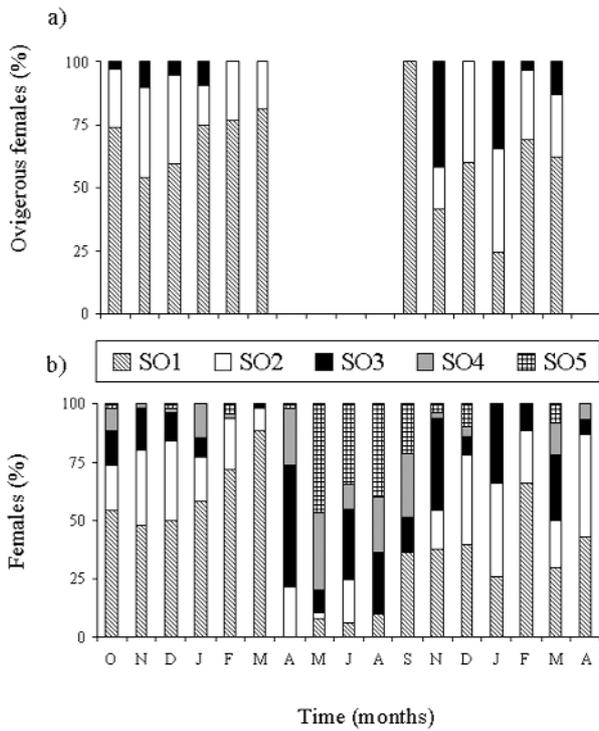


FIG. 5. – *Chasmagnathus granulatus*. Stages of ovarian development (SO1-SO5) of ovigerous (a) and total (b) females from October 1999 to April 2001.

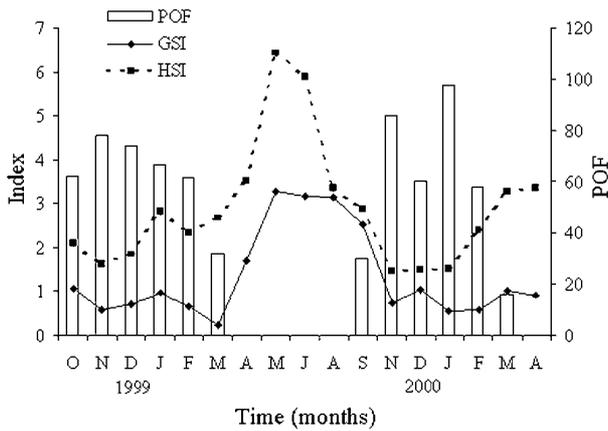


FIG. 6. – *Chasmagnathus granulatus*. Monthly variation in the mean hepatosomatic index (HSI) and gonadosomatic index (GSI). The percentage of ovigerous females (POF) indicates the reproductive seasons 1999-2000 and 2000-2001.

of variance of regression, $P = 0.0005$; Fig. 7a). However, the HSI began to increase earlier than the GSI at the end of both reproductive seasons (February vs March 2000 and January vs February 2001 respectively). On the other hand, the differences between GSI and HSI were high during most of the first reproductive season, diminished from May to December 2000, and increased again by the end of the second reproductive season (Fig. 6). A significantly linear relationship was detected between

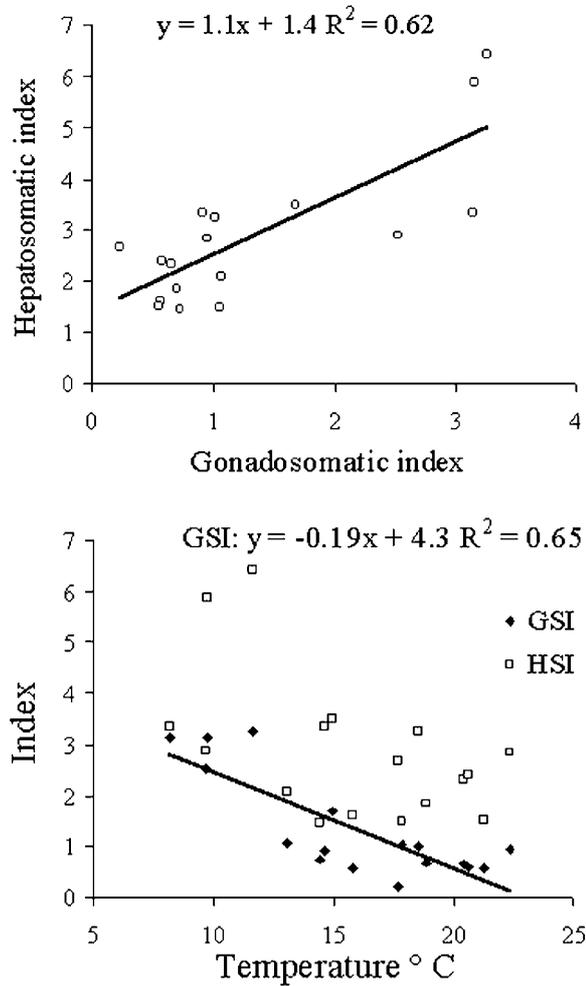


FIG. 7. – *Chasmagnathus granulatus*. Relationships of mean hepatosomatic index (HSI) and gonadosomatic index (GSI) with the monthly mean air temperature. Data from the reproductive season 1999-2000.

mean GSI and air temperature (analysis of variance of regression, $P < 0.0001$); these variables were negatively correlated (Fig. 7b). Although mean HSI diminished with air temperature, a significant relationship was not found ($P = 0.0374$); however, the power of the performed test was < 0.8 .

DISCUSSION

The life of mature female crabs has two outstanding, and externally evident, events: egg laying and larval, or juvenile, release. Both extrusion and hatching are generally programmed “so that brood release occurs at a time most appropriate for the growth of the young” (Adiyodi, 1988). Intertidal and land crabs inhabiting temperate regions are generally seasonal breeders and avoid breeding during the winter months (Sastry, 1983; Adiyodi, 1988: 140).

Furthermore, the duration of the breeding season is negatively correlated with latitude in several grapsoid and ocypodid crabs (Emmerson, 1994; McDermott, 1998; Leme, 2002). *Chasmagnathus granulatus* breeds during most of spring and summer in Mar Chiquita and, apparently, ovigerous females appear one month after the mean air temperature rises above 10°C. Cold “limits the active season, and hence time available for feeding, and therefore, the energy available for growth and reproduction” of land crabs (Wolcott, 1988: 60); the same argument was applied to *C. granulatus* (Spivak *et al.*, 1996), although the annual variations in feeding activity have not yet been quantified. However, photoperiod is another factor that may control the beginning of female breeding seasons, acting alone (Zimmermann and Felder, 1991) or associated with temperature (Knudsen, 1964; Pillay and Ono, 1979; Flores and Negreiros Fransozo, 1998). Only careful experimental evidence could help to separate the effects of photoperiod and temperature on the breeding season in *C. granulatus*.

Female *Chasmagnathus granulatus* reached sexual adulthood in less than one year after their recruitment in the Mar Chiquita lagoon (Luppi *et al.*, 2002). The presence of small ovigerous females was evident at the beginning of summer. They produced more than one clutch during summer and moulted at the beginning of autumn. A higher moult frequency of *C. granulatus* was observed during early autumn in Mar Chiquita (Luppi and Bas, unpublished) and in Samborombón Bay (López-Greco and Rodríguez, 1999). Autumn was an active but unproductive period. The mean air temperature was still above 10°C, and crabs fed and accumulated reserves. During the winter they remained inactive, inside their burrows, and apparently did not feed. At the beginning of spring, they became active and, after a few weeks, these larger females were synchronously ovigerous. During their second breeding season, they could also have produced more than one clutch and moult in autumn. The life span of these females was estimated in less than 3 years (Kittlein *et al.*, 2000). Similar life-phases were described by Kyomo (1986, 1988) in *Sesarma intermedia* in Japan.

Ovocyte development involves the contribution of autochthonous and allochthonous material (Adiyodi, 1988; Quackenbush and Keeley, 1988; Nelson, 1991). The mobilisation of reserves stored in the body fat or midgut gland (= hepatopancreas, *sensu lato*) to the ovary during vitellogenesis was

demonstrated in Malacostraca (Nelson, 1991), including non-brachyuran (Quackenbush, 1989; Harrison, 1990; Haefner and Spaargaren, 1993; Spaargaren and Haefner, 1994) and brachyuran decapods (Pillay and Nair, 1973; Paulus and Laufer, 1987; Kyomo, 1988). The energy partitioning of female crabs means that: i) lipids are stored in the hepatopancreas of crabs (Adiyodi, 1969); ii) there is a compromise between reproduction and growth (Sastry, 1983; González-Gurriarán, 1985; Abelló, 1989; Nelson, 1991; Mantelatto and Fransozo, 1999; López-Greco and Rodríguez, 1999); and iii) nutrients stored in the hepatopancreas can be used for moulting (Zimmerman and Felder, 1991; Koga, 1995) or transferred to the ovaries (Pillay and Nair, 1973; Paulus and Laufer, 1987; Kyomo, 1988). The annual changes in hepatopancreas and ovary of *Chasmagnathus granulatus* females can be explained under these assumptions. During the summer, part of the energy obtained from food must be used to prepare females for the autumn moult, while the ovaries produce several clutches, until reserves are exhausted by the end of March. A transient nutrient accumulation in the hepatopancreas may occur in January, and these reserves could be used during the premoult processes. From February to April, the hepatopancreas accumulated reserves that could be later transferred to the ovaries; the ovaries were thus ready for the first oviposition that took place in September. Interestingly, the eggs of the first annual clutch of *C. granulatus* were larger than those of subsequent clutches (Bas, 2001). Since crabs fed during spring and summer, at least part of the nutrients obtained during this period could be used for successive cycles of ovarian development. The growth of *C. granulatus* ovaries during the non-reproductive season was also observed in northern populations (Ruffino *et al.*, 1994; López-Greco and Rodríguez, 1999).

Our results agree with the proposed schedule of moulting and reproduction in crabs, based on data from *Neopanope sayi* (Swartz, 1978) summarised by Nelson (1991: 84, Figure 4g), except at the end of the breeding season. Nelson's (1991) figure showed that the last cohort of ovocytes arrested their development during primary vitellogenesis before moulting, and was reinitiated after moulting. In fact, most *Chasmagnathus granulatus* females had their ovaries in stage 1 during March (when adults usually moult, Bas and Luppi, unpublished). However, the presence of developing ovaries during April and May suggests that secondary ovogenesis may begin

soon after moulting and is completed in autumn. The ovaries remain fully developed during the whole winter.

Different patterns of the relationship between the ovary and the hepatopancreas were observed in other intertidal, estuarine, and burrow-building grapsoids. The minimum HSI values of *Sesarma intermedia* females were observed in winter, the maximum in summer and autumn; HSI increase preceded GSI increase. Females of this crab have accumulated reserves in the hepatopancreas by the end of the reproductive season and use these reserves for ovarian development in the following year (Kyomo, 1988). In *Helice tridens* and *H. japonica*, the maximum HSI was observed in summer, together with the minimum GSI; the maximum GSI values were observed in winter and spring (Omori *et al.*, 1997).

The number of broods produced by a crab in a single reproductive season could be as high as 13, as Cheung (1969) observed in *Menippe mercenaria*. A single *Chasmagnathus granulatus* female could hatch 6 times in a season in Mar Chiquita lagoon. The mean period between hatches was 20.8 days; the duration of the embryonic development estimated in the laboratory was 21 days *in vitro* (Bas and Spivak, 2000) and ca. 15 days *in vivo* (Bas and Luppi, unpublished). Our data agree with reports on the incubation period duration (20 to 30 days at a temperature of ca. 20°C) and number of broods per year (2-6) in many grapsoid crabs (Kyomo, 1986; Fukui, 1988; Zimmermann and Felder, 1991; Omori *et al.*, 1997; McDermott, 1998). However, all these figures differed sharply from those reported for another *Chasmagnathus granulatus* population: females collected in Samborombón Bay and reared in the laboratory at 21°C produced 4 hatches separated by 60 days (López-Greco and Rodríguez, 1999). The observed differences between *C. granulatus* populations are probably a consequence of temperature and laboratory rearing. The time between hatching and a new extrusion was 7 days in *Sesarma intermedia* (Kyomo, 1986); no such information is available for *C. granulatus* in Mar Chiquita.

Two pulses of egg laying were observed at the beginning of the breeding season in Mar Chiquita, probably as a consequence of a synchronic ovocyte development during winter. On the other hand, egg production was not synchronic during the rest of the summer, probably due to the gradual incorporation of smaller females, which were extruding their first egg clutch, to the reproductive population. The fecundity and reproductive behaviour of young

ovigerous females have been studied in other decapod species (e.g. Somerton and Meyers, 1983; Paul and Paul, 1996; Mantelatto and García, 1999). The increase in the rate of embryonic development of crabs observed at higher temperatures (González-Gurriarán, 1985; Omori *et al.*, 1997) and the temperature differences between *Chasmagnathus granulatus* microhabitats in Mar Chiquita (Spivak *et al.*, 1994) may also explain the asynchrony of embryonic development during summer.

Well-defined hatching pulses of *Chasmagnathus granulatus* were observed during both breeding seasons. Such synchrony may be expected at the beginning of the season, as a consequence of extrusion pulses. However, hatching continues to be synchronic during the whole summer, suggesting that females or embryos could partially control the incubation duration, as was demonstrated in other decapod species (Sastry, 1983; De Vries and Forward, 1991). The timing of larval release with particular environmental conditions is usually "related to survival of the adult female and her larvae" (Forward, 1987). An export strategy for larvae of *C. granulatus* in Mar Chiquita has been postulated on the basis of the higher frequency of zoea I in the lagoon plankton during nocturnal ebbs (Anger *et al.*, 1994). This strategy would make it possible to avoid the low salinity of the lagoon and the effect of visual oriented predators. In fact, *C. granulatus* zoeae I could not survive for a long time at reduced salinities (Anger and Ismael, 1997). López Greco and Rodríguez (1999) detected a lunar hatching rhythm in Samborombón females kept under constant laboratory conditions.

Reproductive lunar or tidal rhythms were observed in several intertidal or terrestrial grapsoids (Forward, 1987; Zimmermann and Felder, 1991; Quinn *et al.*, 1997; Saigusa and Kawagoye, 1997; Omori *et al.*, 1997; Flores *et al.*, 2002). Neither the percentage of females of *C. granulatus* in Mar Chiquita that spawned during a week in comparison with non-ovigerous females, nor the percentage of females with advanced embryos in comparison with all ovigerous females, coincided with a particular lunar phase or an astronomically predicted tidal condition. Although a circatidal larval release rhythm was detected using spectral analysis, this pattern is not as clear as that observed in several intertidal marine species (e.g. Gove and Paula, 2000, Figs. 4 and 8). The geomorphology and climatology of Mar Chiquita made this habitat unpredictable (Anger *et al.*, 1994), so it is reasonable to expect that repro-

ductive events of *C. granulatus* are not rigidly programmed. Thus, most larvae would hatch under favourable environmental conditions.

ACKNOWLEDGEMENTS

This paper was funded by the Universidad de Mar del Plata (UNMdP, grant 15/E082) and the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, grant PIP 838/98) to EDS. This paper is based on work done by RBI in partial fulfilment of the "Licenciada en Biología" degree at the UNMdP. We are grateful to two anonymous reviewers for helpful comments and advice.

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Scient. ed.: C. Zeng