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Annual reproduction and growth of adult crabs *Chasmagnathus granulata* (Crustacea, Brachyura, Grapsidae)

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Abstract: Somatic growth and gonadal maturation of *Chasmagnathus granulata* (Grapsidae, Sesarminae) were studied throughout one year. Spawning and moulting were recorded under controlled laboratory conditions, while gonadosomatic (GI) and hepatosomatic (HI) indexes were estimated from crabs monthly sampled at field (Samborombón Bay, Argentina). Adult females may have at the most four broods during the reproductive season (September to March i.e. Spring-Summer), with one month of incubation period. Brooding and larval eclosion are correlated with the lunar cycle, a fact which favour the larval dispersion to the sea. Females can moult during the reproductive period, with a higher percentage than males. The highest percentage of moulting was observed for both sexes after the reproductive season during April and May (autumn). The seasonal change of the gonadosomatic index indicates that the gonads begin to develop in winter (June to August) after the moulting period.

Résumé : *Reproduction annuelle et croissance de* Chasmagnathus granulata *(Crustacea, Brachyura, Grapsidae).* La croissance somatique et la maturation des gonades de *Chasmagnathus granulata* (Grapsidae, Sesarminae) ont été étudiées pendant un an. La ponte et la mue ont été enregistrées sous des conditions contrôlées de laboratoire, tandis que les indices gonadosomatique (GI) et hépatosomatique (HI) ont été estimés à partir de crabes échantillonnés chaque mois sur le terrain (Baie de Samborombón, Argentina). Les femelles adultes peuvent avoir jusqu'à quatre pontes durant la saison de reproduction (septembre à mars, i.e. printemps-été), avec une période d'incubation d'un mois. La ponte et l'éclosion larvaire sont corrélées au cycle lunaire, ce qui favoriserait la dispersion des larves à la mer. Les femelles peuvent muer pendant la période de reproduction, dans un pourcentage supérieur aux mâles. Le pourcentage de mues le plus élevé a été observé pour les deux sexes après la saison de reproduction en avril et en mai (automne). Le changement saisonnier de l'indice gonadosomatique, indique que la croissance des gonades commence en hiver (juin à août), après la période de mue.

Keywords : reproduction, growth, crabs.

Introduction

The onset of reproduction is a critical event in the life history of animals. It is commonly associated with the "reproductive effort", defined as the proportion of body energy shifted to reproduction. One of the indices for determining the reproductive status of marine invertebrates is based on the observation of gonadal maturation (Giese & Pearse, 1974), both at the macroscopic (colour, weight, shape of gonads) and microscopic levels. Evaluation of the maturity is merely qualitative or may include a quantitative

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analysis. The gonadal index is one of the most common and simple quantitative methods currently used (Haefner & Spaargaren, 1993; Robertson & Kruger, 1994; Arculeo et al., 1995; Chu, 1995; Tsuchida & Watanabe, 1997; Tuck et al., 1997).

The gonadal index (gonadal weight/total body weight) is commonly estimated together with the indexes of other organs that accumulate energy reserves. The main reserve organ in decapod crustacean is the hepatopancreas (see Haefner & Spaargaren, 1993; Chu, 1995; Tuck et al., 1997). Reserves stored in the hepatopancreas are also used for the energy demand of moulting (Passano, 1960), therefore its annual variation in weight is often influenced by both reproduction and growth, two antagonistic processes in brachyuran crabs (Adiyodi, 1988) as well as by starvation, disease and regeneration of autotomized limbs, among other factors.

Chasmagnathus granulata Dana, 1851 (Grapsidae, Sesarminae) is a semi-terrestrial estuarine crab, inhabiting the South American Atlantic coast from Rio de Janeiro (22° S) to San Matías Gulf (41° S) (Boschi et al, 1967). This species forms dense populations in salt-marsh environments, such as Lagoa dos Patos Lagoon, Mar Chiquita Lagoon and Samborombón Bay. Mean seasonal temperature values at Samborombón Bay are: summer, 23-28°C; autumn, 18°C; spring, 17°C and winter, 11-12°C (Rossi, 1982).

During the reproductive period reported for *C. granulata* from Samborombón Bay, i.e. September to March (Rodríguez, 1991), ovigerous females likely migrate to estuarine waters of higher salinity and lower temperature (17°C) suitable for hatching and larval development. After four zoea instars (Boschi et al., 1967), larvae returned to the coast as megalopae, and moult to the first juvenile instar. As the adults, juvenile crabs live in salt-marsh; they reach the adult condition after several moults.

The aim of this work was to characterize the somatic growth and gonadal maturation of adult *C. granulata* throughout one year. Spawning and moulting were recorded under controlled laboratory conditions, while gonadal and hepatosomatic indexes were monthly estimated from field sampling of specimens (Samborombón Bay).

Materials and method

I. Reproduction and growth under laboratory conditions

1. Collection of material

- a) During the reproductive period (RP) : ovigerous females can be easily seen in the coast during this period. 55 ovigerous females and 25 adult males were collected in October 1995 at Faro San Antonio beach, southern point of the Samborombón Bay (36°8' S, 56°47' W) nearly 200 meters form the sea. The crabs were carried to the laboratory in plastic containers filled with saline water from the collecting site (12 P.S.U.). The carapace width (cw) of the specimens was measured with a vernier caliper at 0.02 mm precision.

In the laboratory, ovigerous females were placed in saline water at 30 P.S.U. (optimal salinity for hatching, according to Rodríguez, 1991) and at a temperature of 21 ± 1°C until larvae hatched (about 20 days). Then, they were transferred to 12 P.S.U. (mean value for the sampling site of such specimens) at the same temperature.

Males were transferred in saline water at 12 P.S.U., and 21 ± 1°C.

Saline waters were prepared, from salts for artificial marine water and de-chlorinated tap water, at appropriate dilutions.

- b) During the non reproductive period (nRP) : non-ovigerous females are found in the coast. Adults males and females, respectively 35 and 75, were collected in March 1996 at Faro San Antonio beach. Animals were carried to the laboratory, and carapace width was measured on each specimen as previously. Males were separated from females and both sexes were acclimatized during two weeks at 12 P.S.U. saline water, 21 ± 1°C and a photoperiod of 12 : 12 (L : D).

2. Experimental conditions

- a) During the reproductive period: five experimental groups were defined, each one comprising eleven post-hatching females and five males. Crabs were randomly assigned to the groups, each crab was identified by a number on the carapace.

Each experimental group was kept in a glass aquarium of 40x30x20 cm, filled with 2 l of 12 P.S.U. water. Artificial burrows (10 to 14 holes of 4.5 cm diameter each) made in a polystyrene foam were put in the half bottom of aquaria, as in previous works (De la Iglesia et al., 1994). Crabs were fed *ad libitum* with rabbit pellet food and cow liver twice a week, and water was renewed 4 to 6 hours after. Photoperiod was kept at 14:10 (light : darkness L:D) and temperature was 21 ± 1°C. The assays lasted 170 days, (October 14 1995, to March 31 1996).

- b) During the non reproductive period : after the acclimatization period, crabs were randomly assigned to 5 experimental groups comprising 15 females and 7 males. They were fed as the preceding group. The assays lasted 180 days (March 25 to September 25, 1996).

3. Observations and checking

Crabs were daily checked and the following variables were recorded: - number of ovigerous females; - duration of the egg incubation period; - proportion of females whose eggs

hatched; - lunar phase during spawning and hatching; - spawning intervals; - number of moults; - size increment at each moult ((final cw -initial cw) / initial cw).
- a) Ovigerous females were immediately isolated in a glass recipient filled with 250 ml of 30 P.S.U. water at temperature 21 ± 1°C. They were not fed during the egg incubation period, and saline water was renewed once a week. After larval hatching, females were returned to their experimental group (12 P.S.U. water and 21 ± 1°C).
- b) When an imminent ecdysis was detected (by colour change of carapace and cuticular apolysis), the crab was isolated in a glass recipient filled with 250 ml of 12 P.S.U. water, to avoid predation by other crabs. After moulting, each crab was maintained during 15 days under the same regime of usual feeding and water replacement, its carapace width was measured and it was finally returned to its assay group.

4. Statistical analysis

Percentages were analysed by means of the Fisher exact test (Sokal & Rohlf, 1979). Size increment was analysed, after angular transformation of data, by two-way ANOVA (sex and period).

II. Gonadosomatic and hepatosomatic indexes

1. Collection of material

Adults of both sexes were collected during 1993-1997, in order to obtain representative samples. A total of 20 to 95 crabs of each sex (cw of females ranging from 22.70 to 34.12 mm and males ranging from 22.15 to 33.68 mm) were sampled every month during each year. Crabs were randomly sampled, but those with any loss of locomotory limbs were discarded, to avoid bias due to limb regeneration (Robertson & Kruger, 1994).

Sampled crabs were treated as mentioned above and transferred to aquaria at 12 P.S.U. and 21 ± 1°C water. Crabs were processed 2 to 3 days after sampling.

2. Processing of material

Each crab was initially weighed at a precision of 0.01 g (total body fresh weight : BFW) and its carapace width was measured with a vernier caliper at a precision of 0.02 mm. Gonads and hepatopancreas were then dissected and the gonad (GFW) and hepatopancreas (HFW) fresh weights were measured at 0.1 mg precision.

For each crab, the gonadosomatic index (GI) was calculated as GI= (GFW/BFW)x100, and the hepatosomatic index (HI) as HI=(HFW/BFW)x100. Monthly mean values of both indexes were then calculated.

Gonads and hepatopancreas were finally dried at 70 °C until constant weight, to determine their respective dry weights (GDW) and (HDW). Water percentage in gonad and hepatopancreas respectively was estimated as GWP=((GFW-GDW)/GFW)x100, and HWP=((HFW-HDW)/HFW)x100.

The following colorimetric scale was established for the female gonads, according to their degree of maturity: T, transparent = immature gonad; TO, transparent-orange; O, orange; OB, orange-brown and B, brown = fully mature gonad. The relative percentage of these stages was calculated for each month and the correlation between the colorimetric scale, the indexes GI and HI and the percentages of gonadal and hepatopancreatic water were analysed.

3. Statistical analysis

Indexes and percentages were analysed by one-way ANOVA after angular transformation of data. Percentages corresponding to each month, but obtained from different years, were first compared. If no significant differences were detected, such percentages were pooled in order to increase statistical significance when comparing among months. Tuckey test was employed for multiple mean comparisons (Sokal & Rohlf, 1979).

Results

I. Reproduction and growth under laboratory conditions

The carapace width (cw) of females collected during the reproductive period ranged from 24.06 to 28.34 mm, that of males from 27.40 to 31.18 mm. Cw of females collected during the non reproductive period ranged from 23.70 to 28.70 mm that of males from 26.86 to 30.40 mm.

Results concerning the occurrence of moulting and spawning events are shown in Table 1, for both the reproductive (RP) and non-reproductive periods (nRP). The results of the main statistical comparisons made are referred below.

1. Spawning

- a) During the reproductive period (September-March), a total of 19 females (34.6 %) spawned under laboratory conditions ; it was in fact the second spawning, since all females were collected as ovigerous at the beginning of that period. Percentages of females with a third and fourth spawning were 10.9 % (n=6) and 1.8 % (n=1), respectively. Mean duration of spawning intervals was 59.2 ± 6.7 days (n=6). Volume of the spawned egg mass was decreasing throughout the successive spawning (qualitative observations). During the assay, larval hatching was successful in 69.2 % of ovigerous females, and the mean egg incubation duration was 28.9 ± 3.4 days (n=26).
- b) During the non-reproductive period (March-September) 9 females spawned (12 %) under laboratory conditions. This percentage is significantly ($p<0.05$) lower than that

observed during the reproductive period. These females mainly spawned from June to September. Larval hatching occurred in five ovigerous females (55.6 %) and the mean incubation period was 22.8 ± 1.6 days. The other four females lost their eggs.

2. Moulting of females

- a) During the reproductive period 18 females (32.7 %) had at least one moulting. The moulting appeared at 56th day of the observation period i. e. about two months after the hatching of their first spawning, and ended by March. No significant differences ($p>0.05$) were detected in the percentage of females that only moulted, with respect to that of females that only spawned. The percentage of females that moulted and spawned was significantly ($p<0.05$) lower than the preceding percentages (table 1).
- b) During the non reproductive period, a significantly higher percentage of moulting females (77.3 %, n=58) was registered (Table 1). Females moulted from April to September, mainly in autumn; the highest percentages corresponded to April (55,2 %, n=32) and May (34,5 %, n=20). The percentage of moulting females was significantly ($p<0.5$) higher than that of spawning females. No significant differences ($p>0.05$) was found between the percentage of females that only spawned and the percentage of females that spawned and moulted.

Table 1. Under laboratory conditions, percentages of adults of *C. granulata* having spawned and/or moulted, during the reproductive period (RP) and non-reproductive period (nRP). Percentages of crabs which have neither moulted nor spawned are also indicated. Number of crabs is specified between brackets.

Tableau 1. Dans les conditions de laboratoire, pourcentages d'adultes de *C. granulata* ayant pondu et/ou mué pendant la période de reproduction (RP) et hors période de reproduction (nRP). Les pourcentages de crabes qui n'ont ni mué ni pondu sont aussi indiqués. Le nombre de cas est indiqué entre parenthèses.

	Females				Males	
period	only spawning	only moulting	spawning and moulting	no spawning no moulting	moulting	no moulting
RP	27.3 (n=15)	25.4 (n=14)	7.3 (n=4)	40.00 (n=22)	12.00 (n=3)	88.00 (n=22)
nRP	5.3 (n=4)	70.6 (n=53)	6.7 (n=5)	17.3 (n=13)	31.4 (n=11)	68.6 (n=24)

3. Moulting of males

12 % (n=3) of males moulted by the end of the reproductive period (March); no significant differences ($p>0.05$) was found with the percentage of females that moulted during the same period.

During the non reproductive period, males moulted only during April (36.4 %, n=4) and May (63.6 %, n=7). Percentage of moulting males during the nRP was significantly ($p<0.05$) lower than that of moulting females (Table 1).

4. Size increment at moult

This is shown in Table 2, for both sexes. No significant difference ($p>0.05$) was detected neither between periods for each sex, nor between sexes for each period (RP or nRP).

Table 2. Mean percentage of size increment at moult (carapace width), for both sexes. RP: reproductive period ; nRP: non-reproductive period. Number of crabs is specified between brackets.

Tableau 2. Pourcentage moyen d´accroissement de taille de la carapace à la mue pour les deux sexes. RP : période de reproduction ; nRP : hors période de reproduction. Le nombre de crabes est indiqué entre parenthèses.

period	males	females
RP	3.89 ± 0.94 (n=3)	2.71 ± 0.35 (n=18)
nRP	2.68 ± 0.13 (n=11)	3.09 ± 0.18 (n=53)

II. Synchronization of reproductive events with the lunar cycle

During the reproductive period, spawning occurred more frequently during full moon, while hatching was more frequent during the first quarter moon (Table 3). The lowest percentage of both spawning and hatching was recorded for

Table 3. Percentage of spawning females and percentage of females with successful hatching, for each phase of the lunar cycle under laboratory conditions during the year. RP: reproductive period; nRP: non-reproductive period. Number of crabs is indicated between brackets. FQ and LQ: first and last quarter respectively.

Tableau 3. Pourcentage de femelles ovigères et pourcentage d'éclosion, pour chaque phase du cycle lunaire, dans les conditions de laboratoire et pendant une année. RP : période de reproduction ; nRP : hors période de reproduction. Le nombre de crabes est indiqué entre parenthèses. FQ et LQ : premier et dernier quartier respectivement.

Period	Event	New moon	FQ moon	Full moon	LQ moon
RP	spawning	23.1 (n=6)	26.9 (n=7)	34.6 (n=9)	15.4 (n=4)
	hatching	27.8 (n=5)	44.4 (n=8)	27.8 (n=5)	0 (n=0)
nRP	spawning	22.2 (n=2)	55.6 (n=5)	22.2 (n=2)	0 (n=0)
	hatching	60.0 (n=3)	20.0 (n=1)	20.0 (n=1)	0 (n=0)

the last quarter moon. Hatching was rarely observed during the day hours.

During the non reproductive period, a small number of females spawned and a phase shift was observed: spawning had a maximum in the first quarter moon, while hatching peaked during new moon.

III. Gonadosomatic and hepatosomatic indexes (GI and HI)

Figures 1 to 4 show the annual variation of gonadosomatic and hepatosomatic indexes, for males and females. Data from different years were pooled, because no significant differences ($p>0.05$) were detected between years. The highest value of GI was observed during September-October, while a second peak of lower amplitude was seen in January. Monthly variation of HI was lower than that of GI. In males, monthly variations of both GI and HI were significantly lower ($p<0.05$) than those of females.

IV. Ovarian maturity stages

The percentage of each ovarian maturity stage throughout the year is shown in Figure 5. Fully mature ovaries were observed each month in a variable number of specimens. A minimum monthly percentage of 26-29% for mature ovaries were observed in May and June (nRP); these specimens would be ready to spawn, nevertheless no ovigerous females were found in the field during the non reproductive period.

Table 4 shows ovarian stages together with gonadosomatic and hepatosomatic indexes (GI and HI), as well as with the percentage of gonadal and hepatopancreatic water (GWP and HWP). Correlation coefficients (r^2) for GI vs GWP and for HI vs HWP were 0.96 and 0.60 respectively ($p<0.05$). The maximum GI value for the fully mature ovaries corresponded to the lowest percentage of gonadal water, demonstrating that a high GI is not associated with a high water content. On the other hand, the highest GI was associated with the lowest HI value.

Table 4. Mean values (± standard error) of gonadosomatic (GI) and hepatosomatic index (HI), and percentages of gonadal and hepatopancreatic water (GWP and HWP respectively) corresponding to each ovarian maturity stage (observations made during five years). T, transparent = immature; TO, transparent-orange; O, orange; OB, orange-brown and B, brown = fully mature; n= numbers of sampled crabs.

Tableau 4. Valeurs moyennes (± erreur standard) des indices gonadosomatique, (GI) et hépatosomatique (HI) et pourcentages d'eau dans les gonades et dans l'hépatopancréas (GWP et HWP respectivement), correspondant à chaque état de maturité de l'ovaire (observations faites sur cinq années). T, transparent = immature ; TO, transparent orange ; O, orange ; OB, orange-marron et B, marron = pleine maturité ; n = nombre de crabes échantillonnés.

Maturity stage	n	GI	GWP	HI	HWP
T	69	0.243 ±0.013	79.46 ±1.03	4.684 ±0.150	74.12 ±0.65
TO	39	0.286 ±0.019	79.88 ±1.47	4.871 ±0.157	74.00 ±0.97
O	144	0.523 ±0.018	78.99 ±0.60	4.511 ±0.103	75.78 ±0.51
OB	48	0.953 ±0.081	71.87 ±1.12	5.400 ±0.198	70.23 ±0.97
B	432	3.245 ±0.103	60.51 ±0.36	4.667 ±0.060	71.60 ±0.32

Discussion

I. Reproduction

According to our results, three to four spawnings per female may occur during the reproductive season, the spawning interval being of about two months. These values are similar to those of other grapsid crabs, such as *Sesarma cinereum* Rathbun, *Sesarma reticulatum* Say and *Sesarma pictum* de Haan (Pillay & Ono, 1978; Seiple, 1979). Nevertheless, our results were obtained under laboratory conditions and although those conditions represented the mean values of temperature, photoperiod and salinity of the natural environment, they did not reflect the natural oscillations of such variables (Rossi, 1982). The crabs *Gaetice depressus* de Haan and *Eriocheir sinensis* Milne Edwards (Sesarminae) had in the laboratory a number of ovipositions higher than in the field conditions (Fukui, 1990; Junyao, personal communication).

The reproductive effort of *Chasmagnathus granulata* females seems to decrease during successive spawnings. The same pattern was reported for *Ranina ranina* Linnaeus (Kennely & Watkins, 1994) and *Sesarma reticulatum* (Zimmerman & Felder, 1991). The egg incubation time for *C. granulata* ranged from 3 to 4 weeks, as in the crabs *Pachygrapsus crassipes* Randall (Hiatt, 1948), *Sesarma pictum* (Pillay & Ono, 1978), *S. cinereum* (Seiple, 1979), *Hemigrapsus sanguineus* de Haan (Mc Dermott, 1997) and *Plagusia dentipes* de Haan (Tsuchida & Watanabe, 1997).

Some spawnings were detected in the laboratory during the non-reproductive period (April to September). This is probably an effect of laboratory conditions because ovigerous females were never observed in the field during this period. In the field, *C. granulata* females did not show a period of ovarian quiescence during autumn and winter (see Figure 5) and the triggering of ovarian development leading to oviposition could be due to laboratory conditions. The constancy of temperature maintained all the year round in the laboratory could be the main factor involved in these spawnings (see for comparison, the variations of mean seasonal values at Samborombón Bay, indicated in Introduction).

II. Moulting

Both males and females mainly moulted at the beginning of the non-reproductive period (April and May). These results

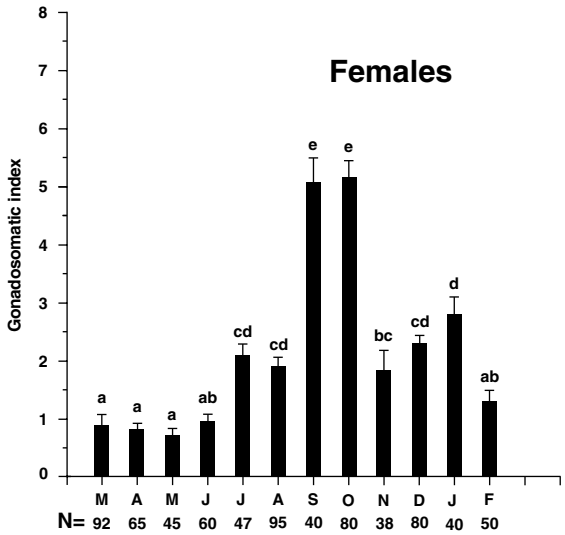


Figure 1. Gonadosomatic index of *C. granulata* adult females, for each month (from March to February). Same single letters indicate no significant differences (p>0.05); N= number of sampled crabs.

Figure 1. Indice gonadosomatiques des femelles adultes de *C. granulata* pour chaque mois (de mars à février). La présence de lettres identiques signifie qu'il n'y a pas de différences significatives (p>0.05) ; N = nombre de crabes échantillonnés.

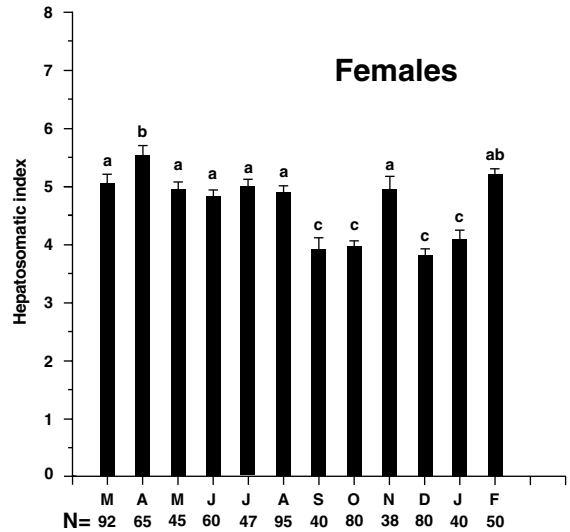


Figure 2. Hepatosomatic index of *C. granulata* adult females, for each month (from March to February). Same single letters indicate no significant differences (p>0.05); N= number of sampled crabs.

Figure 2. Indice hépatosomatique des femelles adultes de *C. granulata,* pour chaque mois (de mars à février). La présence de lettres identiques signifie qu'il n'y a pas de différences significatives (p>0.05) ; N = nombre de crabes échantillonnés.

corroborate previous observations made during several years, both in the laboratory and the field. On the other hand, a moulting synchronization between sexes is evident. This kind of synchronization was also reported for *S. pictum,* but at the end of the reproductive period, and for *Hemigrapsus penicillatus* de Haan, at the beginning and the end of that period (Pillay & Ono, 1978). For *C. granulata,* the occurrence of moulting out of the reproductive period could minimize the intra- and inter-sex cannibalism and could be related to further mating as well. In this respect, *C. granulata* has a hard-female mating pattern (López, 1997), which requires that both sexes are in inter-moult. In *Cyclograpsus punctatus* Milne Edwards, another crab with hard-female mating pattern, the same synchronization as in *Chasmagnathus granulata* was observed (Broekhuysen, 1941).

Moulting was also observed during the reproductive period, but in a lesser extent than in the non-reproductive period. All females were ovigerous at the beginning of the reproductive period. Under the control of both endocrine and environmental factors, some females had several spawnings during the reproductive period, while others, during the same period had a moult after a spawning. A relationship of these observations with the possible existence of a terminal moult in some specimens cannot be excluded. Nevertheless, since *C. granulata* acquired the sexual maturity at a small size (López et al, 1997) and that a very wide size range was reported for ovigerous females (Stella et al., 1996), the possibility that *C. granulata* has a terminal moult, as other brachyuran species (Hartnoll, 1983), seems improbable. On the other hand, the possibility that adult females of *C. granulata* have resting periods in their reproductive effort, as suggested for *Callinectes sapidus* Rathbun (Havens & McConaugha, 1990), cannot be discarded.

Males began to moult at the end of the reproductive period (end of March) and continued moulting until the end of May. In this sense, males showed an annual pattern of moulting different from females, who began to moult in the middle of the reproductive season (December). This suggest that energy investment of males is shifted to insure gamete production during the entire reproductive period. Male crabs of *Hemigrapsus penicillatus,* moult simultaneously with females at the beginning and the end of the reproductive period, but their moult percentage is lower than that of females (Pillay & Ono, 1978; Fukui, 1990; Fukui, 1993).

According to the rule reported for crustaceans (Hiatt, 1948), the size increment after puberty was lower in both males and females than in juvenile crabs (López, 1997). On the other hand, the absence of difference in the size

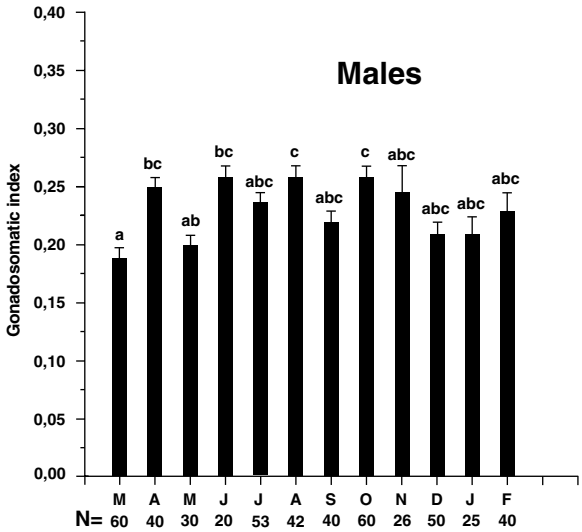
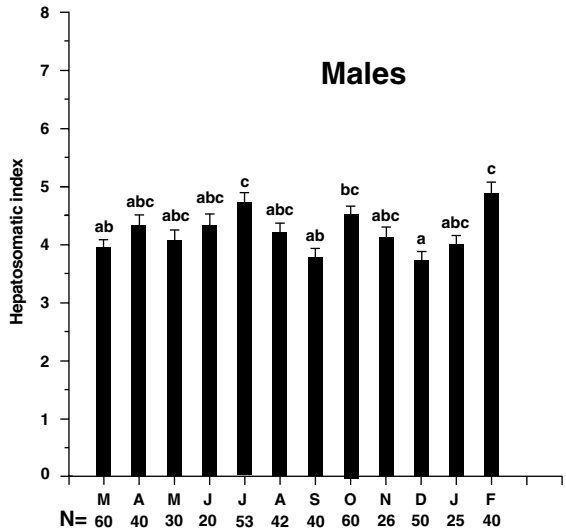


Figure 3. Gonadosomatic index of *C. granulata* adult males, for each month (from March to February). Same single letters indicate no significant differences (p>0.05); N= number of sampled crabs.

Figure 3. Indice gonadosomatique des mâles adultes de *C. granulata* pour chaque mois (de mars à février). La présence de lettres identiques signifie qu'il n'y a pas de différences significatives (p>0.05); N= nombre de crabes échantillonnés.



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increment between reproductive and non-reproductive periods, for both sexes, as well as between sexes, demonstrates the strong constancy of the size increment after puberty in *C. granulata*.

III. Synchronization with the lunar cycle

Some results obtained on *C. granulata* concerning the relationship between spawning, larval eclosion and lunar cycle were similar to those reported for other grapsid crabs. For instance, *Sesarma cinereum* presented the highest percentage of ovigerous females during full moon, as well as an egg incubation period of 28 days (Seiple, 1979). In the same species, larval hatching was reported to occur at night (De Vries & Forward, 1991). Synchronization of hatching with lunar, tidal and diary cycles was extensively reported for brachyuran larvae (Warner, 1967; Saigusa, 1981; De Vries & Forward, 1991; Zimmerman & Felder, 1991; Saigusa & Kawagoye, 1997). The fact that these spawning and hatching rhythms in *C. granulata* were observed under constant conditions of photoperiod, temperature and water level shows that a significant endogenous component is involved in such rhythms, as suggested for other Sesarminae crabs (Saigusa, 1981; Saigusa & Kawagoye, 1997).

Several intertidal brachyuran crabs present the so called "export type strategy of dispersal and recruitment", so that larval eclosion mainly occur during tidal phases which optimize the larval migration from the coast to the sea (Anger et al., 1994). In this sense, synchronization of hatching with the biggest high-tides (end of first quarter moon and full moon) would favour the larval dispersion to the sea and the further planktonic development (Warner, 1967; Saigusa, 1981; Zimmerman & Felder, 1991; Saigusa & Kawagoye, 1997).

In the case of *C. granulata* the hatching peak occurs near the full moon and at that time, ovigerous females having mature eggs can be easily seen migrating to the sea, hundreds or thousands meter away from waters of high salinities. High salinities are essential for all zoea instars of *C. granulata* (Boschi et al., 1967), therefore a strong larval dispersion from the coast to offshore would be a favourable adaptive strategy.

IV. Gonadosomatic and hepatosomatic indexes

Seasonal gonadosomatic index has been used to investigate the variation of the gonads throughout the year in several species which have a reproductive period ranging from two to three months, such as *Plagusia dentipes* (see Tsuchida & Watanabe, 1997) and *Aristeus antennatus* (see Arculeo et al., 1995). When the reproductive period is more extended, it is expected that the seasonal gonadosomatic index cannot

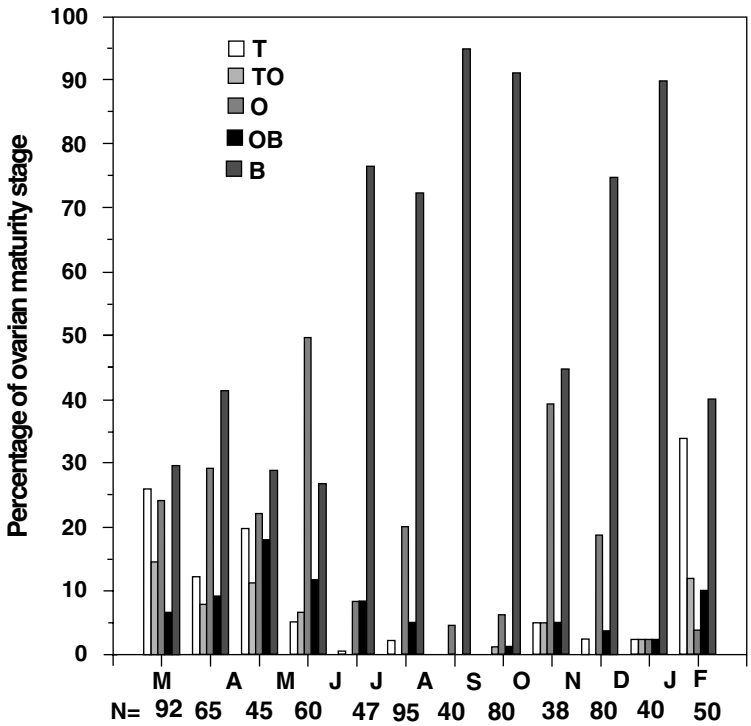

Figure 5. Percentage of the different stages of ovarian maturity for each month. T, transparent = immature; TO, transparent-orange; O, orange; OB, orange-brown and B, brown = fully mature; N= number of sampled crabs.

Figure 5. Pourcentage des différents états de maturité de l'ovaire pour chaque mois. T, transparent = immature ; TO, transparent, orange ; O, orange ; OB, orange-marron et B, marron = pleine maturité ; N = nombre de crabes échantillonnés.

reflect the fluctuations in gonadal maturation during the reproductive season. Nevertheless in *C. granulata,* whose reproductive period comprises six months at least, the monthly gonadosomatic index during that period showed significant fluctuations.

In fact, the energetic investment of females, in terms of gonadal biomass, showed a clear trend to decrease during the reproductive period. The highest values of GI correspond to September and October, and the values decrease later, although a secondary peak can be observed in the middle of the reproductive period (December-January). A similar observation has been reported for the crabs *Hemigrapsus penicillatus, Sesarma pictum* and *Sesarma reticulatum,* whose reproductive annual pattern is similar to that of *C. granulata,* concerning both ovarian maturation and percentage of ovigerous females at field (Pillay & Ono, 1978; Zimmerman & Felder, 1991).

While no difference in the percentage of fully mature ovaries (colorimetric scale) was seen between the beginning and the middle of the reproductive period (about 90 % in both cases) (Figure 5), a significant difference between the primary and the secondary gonado-somatic index peaks of *C. granulata* was detected. This suggests that the mature ovaries of females sampled in the middle of the reproductive period had a lower number of oocytes than the ovaries of females sampled at the beginning of the period, since the egg volume, a species specific feature (Fukui, 1988), is constant. This conclusion is closely in accordance with the decreasing biomass of spawned eggs, observed in ovigerous females, both in the laboratory and at field, as the reproductive period advances (López, 1997).

On the other hand, *C. granulata* females from Samborombón Bay did not show a real state of quiescent ovaries during autumn and winter, since maturing and fully mature ovaries were in fact detected in more than 50% of females during the entire year, including the winter months (Figure 5).

Concerning the hepatosomatic index for females, a significant seasonal variation was observed. The minimum value of HI, corresponding to spring, coincided with the maximum value of GI, while the maximum value of HI, in autumn, corresponded to the lowest GI value. This suggests a mobilization of metabolic reserves from hepatopancreas to ovary for vitellus synthesis. Considering the monthly fluctuations of both HI and GI, the same negative correlation was seen: the minimum values of HI were detected in September, October and December, just when the GI peaked. The HI finally increases by March, at the end of the reproductive period, probably linked to reserve accumulation before moulting, mainly observed during April and May. The influence of tissue hydration on GI or HI values when comparing among seasons or months does not seem to enhance the observed differences, since a negative correlation was found between those indexes and the gonadal or hepatopancreatic water percentage, i.e. the great weight of a mature ovary cannot be attributable to a gain in water content. The body water content, on the other hand, is a very constant parameter in intermoult crabs of this species (Rodríguez & Dezi, 1987).

The gonadosomatic index was one order of magnitude lower in males than in females, stressing the difference between sexes in the energetic investment for reproduction. Besides, both the seasonal and monthly variation of GI was of low magnitude in males, compared to females, probably in relation with the possibility of mating all the year long for the males, except the moulting periods. Interestingly, the maximum values of GI, in winter and spring, were similar in both sexes, confirming that gonadal growth begins in winter, once crabs have moulted in autumn. The annual

pattern of hepatosomatic index for males did not show, as it did for females, significant oscillations related to reproduction or moulting.

Our results suggest a high optimization of the energetic investment between growth and reproduction, especially in females. Gonadal growing of crabs during winter allows a high reproductive output from the beginning of the reproductive period. At the end of the reproductive period the crabs moult, mainly in autumn, before a new investment for the following reproductive period.

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