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# SIZE AT ONSET OF SEXUAL MATURITY IN *Chasmagnathus granulata* (DECAPODA, BRACHYURA)

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## ABSTRACT

The size at maturity of the estuarine crab *Chasmagnathus granulata* was investigated. The relative growth of morphological characters with reproductive value, in males and females, as well as the gonadal maturity of testes and ovaries at the histological level, were studied. Gonadal maturity, in both sexes, occurs before changes in the relative growth of reproductive characters were noted. The relative growth of the male first pleopod changed upon reaching a carapace width (CW, reference size) of 16.50 mm, while mature testes could be observed at 10.50 mm CW. Spermatozoids were observed in *vasa deferentia* of males as small as 12.08 mm CW. For females, variations in relative abdominal growth occured at 22.70 mm CW, while females as small as 19.00 mm CW had mature ovaries. The size of the smallest ovigerous females collected in the field was close to the size of gonadal maturity, but below the 5 % critical value of normal size distribution of the ovigerous females. The allometric size of maturity was, in turn, above that critical value.

**Keywords**: gonadal histology- relative growth- crabs

The crab *Chasmagnathus granulata* Dana 1851 (Grapsidae) is widely distributed along the Atlantic coast of South America. Dense populations of it can be found in lagoons or salt-marshes, such as Lagoa dos Patos (Brasil), Samborombón Bay (Argentina) and Mar Chiquita (Argentina). The reproductive characteristics of this crab have been recently studied (Stella et al., 1996), as well as ecological aspects of its reproduction (Ruffino et al., 1994). Also, the effects of pollutants on reproductive events of *C. granulata* have been assayed (Rodríguez & Medesani, 1994). The ecological significance of *C. granulata,* as prey to several fish species of commercial value, is also known (Menni, 1983).

Sexual maturity in crabs has been studied by various authors as a function of size (Hartnoll, 1982; Grassé, 1994, for review). In these studies, crabs were found capable of initiating reproductive activity following the so-called puberty molt. Several authors (Hartnoll 1974, 1982; Legrand & Juchault, 1994) associate this molt with a change in the relative growth of certain appendages, or tagmata, of reproductive value (i.e. chelae, abdomen) with respect to a reference body size, such as the length or width of the carapace. Few attempts have been made, however, to associate changes in relative growth with the histological condition of the gonads. The aim of the present study is to determine the onset of sexual maturity from both, the relative growth and the histological points of view.

## METHODS

### Relative growth study

For the relative growth study, 181 males and 375 females with carapace widths (CW) ranging from 8.95 to 29.60 mm and from 9.5 to 30.14 mm, respectively, were randomly chosen from several crab collections made throughout 1994 from Faro San Antonio Beach, at the southern end of Samborombón Bay (36°18'S and 56°48'W). CW was measured as the maximum carapace width, behind the third cephalothoracic spine, by means of a vernier caliper (precision: 0.02 mm). Crabs were fixed in solution of formalin (10 % in water from the collection site). In addition to CW, the following measurements were also obtained: length of first right male pleopod (PL), by means of a micrometric ocular lens (calibrated with 1/100 mm spacing plate); and the maximum female abdominal width (AW, using calipers).

For data analysis, the statistical methods employed by other authors were used (Somerton, 1980; Somerton & MacIntosh, 1983; Minagawa & Higuchi, 1997). After tentative assignment of data to one of two groups representing juvenile and adult crab, an iterative linear regression process was carried out, following log-log transformation of PL or AW plotted against CW. Critical data were re-assigned from one to another group, until the sum of residual squares of the two lines was minimal. The intercept point was considered as the size at which sexual maturity is associated to a change in relative growth. Comparison of data fitting between the two lines with respect to a single line was made by the F-test (Draper & Smith 1966).

### Histological analysis

Crabs were collected from Faro San Antonio beach during the reproductive season (October 1994 and February 1995). CW was taken as the main body size reference, and crabs were selected within a wide range of CW: from 8.76 to 20.72 mm for males (n=23) and 8.54 to 20.64 mm for females (n=16).

All crabs were cold-anaesthetized and killed at -20 °C during 15 min. Gonads were quickly dissected and fixed in Bouin's solution for 4 h at 20 °C. The organs were then sequentially passed through 90 % ethanol for 20 min, 96 % ethanol for 20 min, 96% ethanol plus buthylic alcohol (1:1 v/v) for 30 min and buthylic alcohol for 30 min, after which gonads were embedded in paraffin and 5-%m sections prepared and stained with haematoxylin-eosin. No less than 10 slides were analyzed for each crab, in order to determine the degree of gonadal maturation.

Criteria used for determining gonadal maturity were: existence of testicular lobulation and presence of spermatids in the lobes, for males, and presence of oocytes undergoing secondary vitellogenesis (Meusy & Charniaux Cotton, 1984), for females. These oocytes could be distinguished because of their eosinophilia, larger size and presence of yolk platelets. These criteria have been used previously by other authors (Johnson, 1980, Krol *et al.*, 1992). The presence of spermatophores or spermatozoids in *vasa deferentia* of males was also employed as additional criterion for maturity.

Field measurements: ovigerous females of *C. granulata* were measured on the middle of the reproductive season (December, 1996) at Faro San Antonio beach, in order to determine their size distribution. A representative area of about 300 $m^2$ was systematically inspected, recording the carapace width of all the ovigerous females (a total of 597 crabs).

## RESULTS

Morphological data obtained is expressed in Figs. 1 and 2, along with the best-fitting lines. For males, the two lines intersected at CW=16.50 mm, whereas for females intersection occurred at CW=22,70 mm. In both cases, the fit of the two-line model was significantly better ($p< 0.001$) than the fit of a single straight line for all data. The equations for each line are shown in the corresponding figures.

Neither testicular lobes nor spermatids can be observed in inmature testes (Fig. 3a, b). Spermatogonia and spermatocytes are the only germinative cells visible. Upon gross morphological inspection, the fresh inmature testes appeared as transparent, thin structures, not clearly differentiated from the anterior *vas deferens.* On the other hand, fresh samples of mature testes were opaque while *vasa deferentia* appeared transparent and folded. The mature testes of *Chasmagnathus granulata* presented large numbers of testicular lobes, most of which contained spermatids (Fig. 3 c, d).

The results of the histological characterization of male crabs analyzed can be seen in Table I. In one case, a minimal CW =10.28 mm showed a mature testis, but another crab of 10.36 mm CW was defined as immature. At size 10.94 mm CW and higher, the testes of all crabs were mature. As a result, histological maturity in male crabs can be associated with a CW ranging of 10 to 10.5 mm. Presence of spermatozoids in *vasa deferentia* could be recognized in males as small as 12.08 mm.

Micrographs of both immature and mature ovaries are shown in Fig. 4. Germinative centers containing numerous oogonia could be observed in the inmature ovary (Fig. 4 a, b). Oocytes undergoing primary vitellogenesis can be seen surrounding oogonia; usually with abundant basophilic and homogenous cytoplasm and a nucleus presenting a central or slightly excentric nucleolus. Numerous round-shaped follicular cells can also observed. These cells appear in transversal sections randomly arranged in the stroma, but not enclosing the oocytes. Fresh preparations of ovaries showed a transparent gonad, with anterior lobes that did not overlap the hepatopancreatic anterior edge. Mature females show the typical features of a brachyuran crab: large vitellogenic oocytes with eosinophilic cytoplasm, containing yolk droplets (Fig. 4 d). A centrifugal gradient from oogonia to primary oocytes and to secondary pink-coloured oocytes, can be observed in ovaries at the start of the maturative phase (Fig. 4 c). Flat, pycnotic follicular cells are present surrounding each

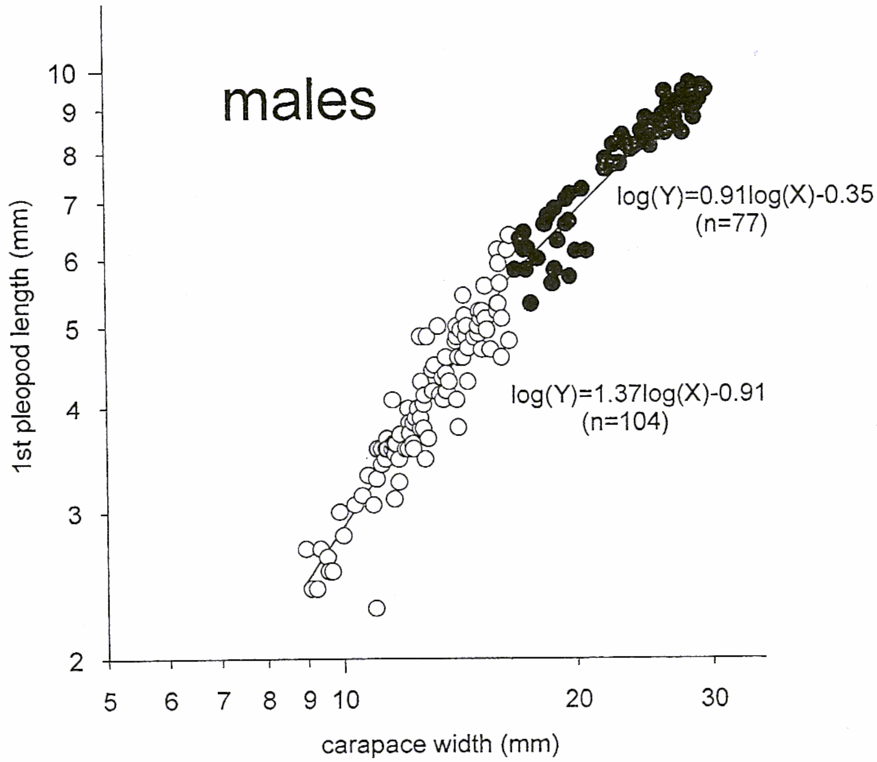


Figure 1: Relative growth of the first pleopod of male *C. granulata*

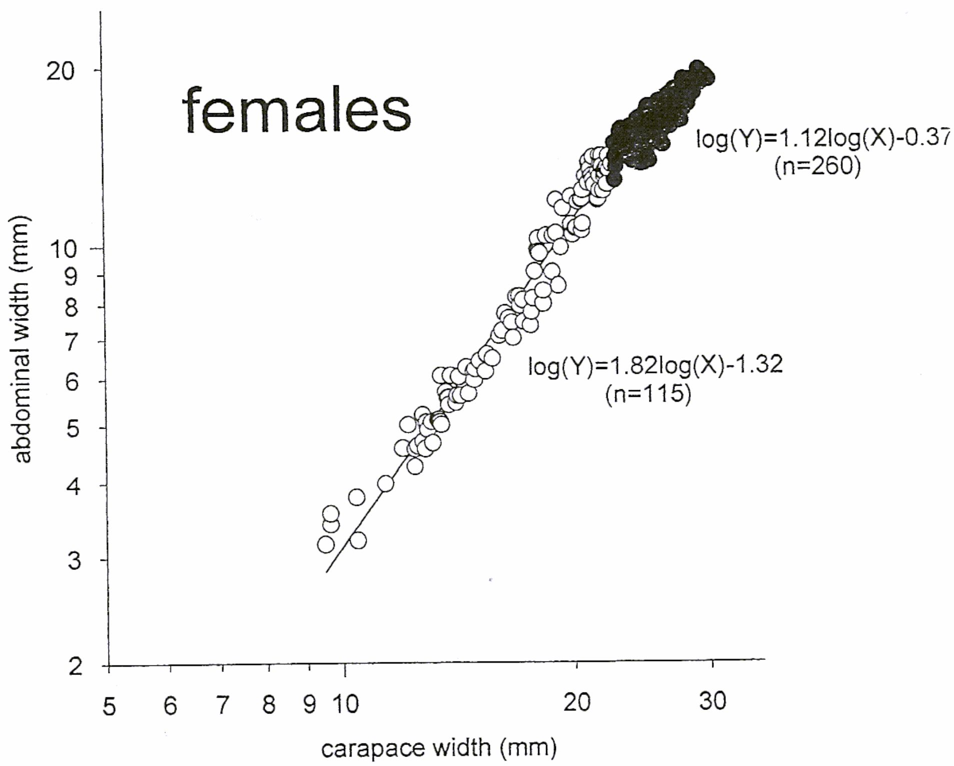


Figure 2: Relative growth of the abdomen of female *C. granulata*

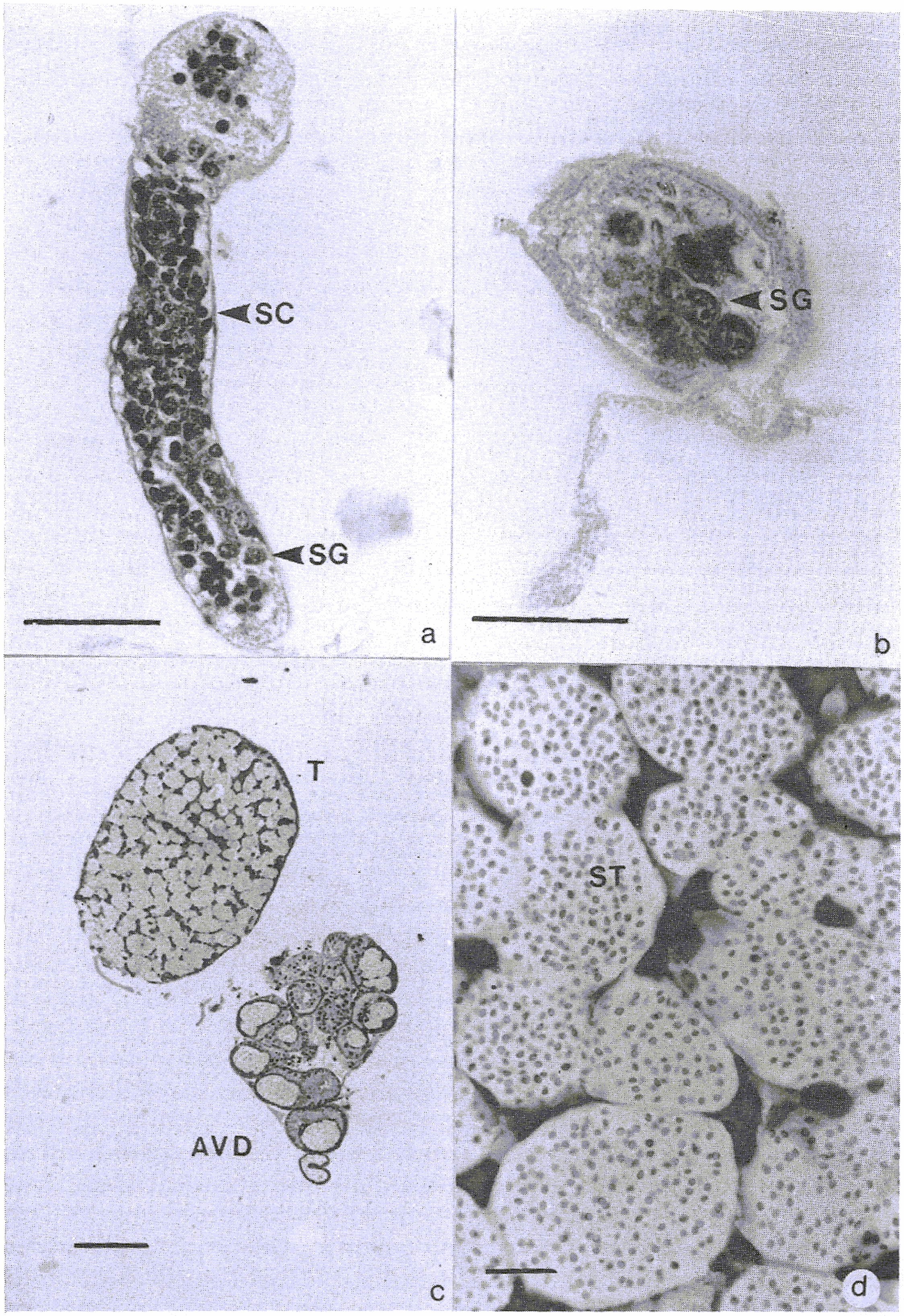


Figure 3: Histological sections of *C. granulata* testes. **Fig. 3a**: longitudinal section of immature testis. Scale: 10 m. **Fig. 3b**: transversal section of immature testis. Scale: 5 m. **Figs. 3 c, d**: transversal sections of mature testis. Scale: 50 and 5 m respectively. SG: spermatogonia, SC: spermatocyte, ST: spermatids, AVD: anterior *vas deferens*, T: testis.

ripening oocyte. The color of fresh mature ovaries ranged from orange to dark brown in the fully mature stage, and these were surrounded by a thin capsule.

Table I. Gonadal condition (GC) of males and females of *C. granulata* according to histological analysis. IT: immature testes, MT: mature testes, IO: immature ovaries, MO: mature ovaries. The minimal size for the presence of spermatozoids in *vasa deferentia* (SVD) is also indicated.

| MALES | | FEMALES | |
|---|---|---|---|
| CW (mm) | GC | CW (mm) | GC |
| 9.26 | IT | 8.54 | IO |
| 9.44 | IT | 14.62 | IO |
| 10.28 | MT | 15.18 | IO |
| 10.36 | IT | 16.48 | IO |
| 10.60 | MT | 16.64 | IO |
| 10.94 | MT | 17.24 | IO |
| 11.56 | MT | 17.76 | IO |
| 12.08 | MT-SVD | 17.84 | IO |
| 12.14 | MT | 18.00 | IO |
| 12.32 | MT | 18.12 | IO |
| 12.76 | MT | 18.82 | IO |
| 12.88 | MT | 19.04 | MO |
| 15.54 | MT | 19.32 | MO |
| 16.04 | MT | 19.94 | MO |
| 16.08 | MT | 20.56 | MO |
| 16.42 | MT | 20.64 | MO |
| 17.54 | MT | | |
| 18.70 | MT | | |
| 18.82 | MT | | |
| 19.68 | MT | | |
| 20.72 | MT | | |

The minimal CW value associated with female histological maturity was within the range 18.5 - 19 mm (Table I). The size distribution of ovigerous females, determined from the field measurements (Fig. 5) shows that the minimal CW measured was 18.74 mm, while the mode corresponded to the range 26-27 mm CW. The mean one standard deviation ranged from 23.03 to 28.32. The 5 % of size normal distribution of the ovigerous females corresponded to crabs smaller than 21 mm (Fig. 5). Therefore, the histological size of maturity was below that critical value, while the allometric one was above that value.

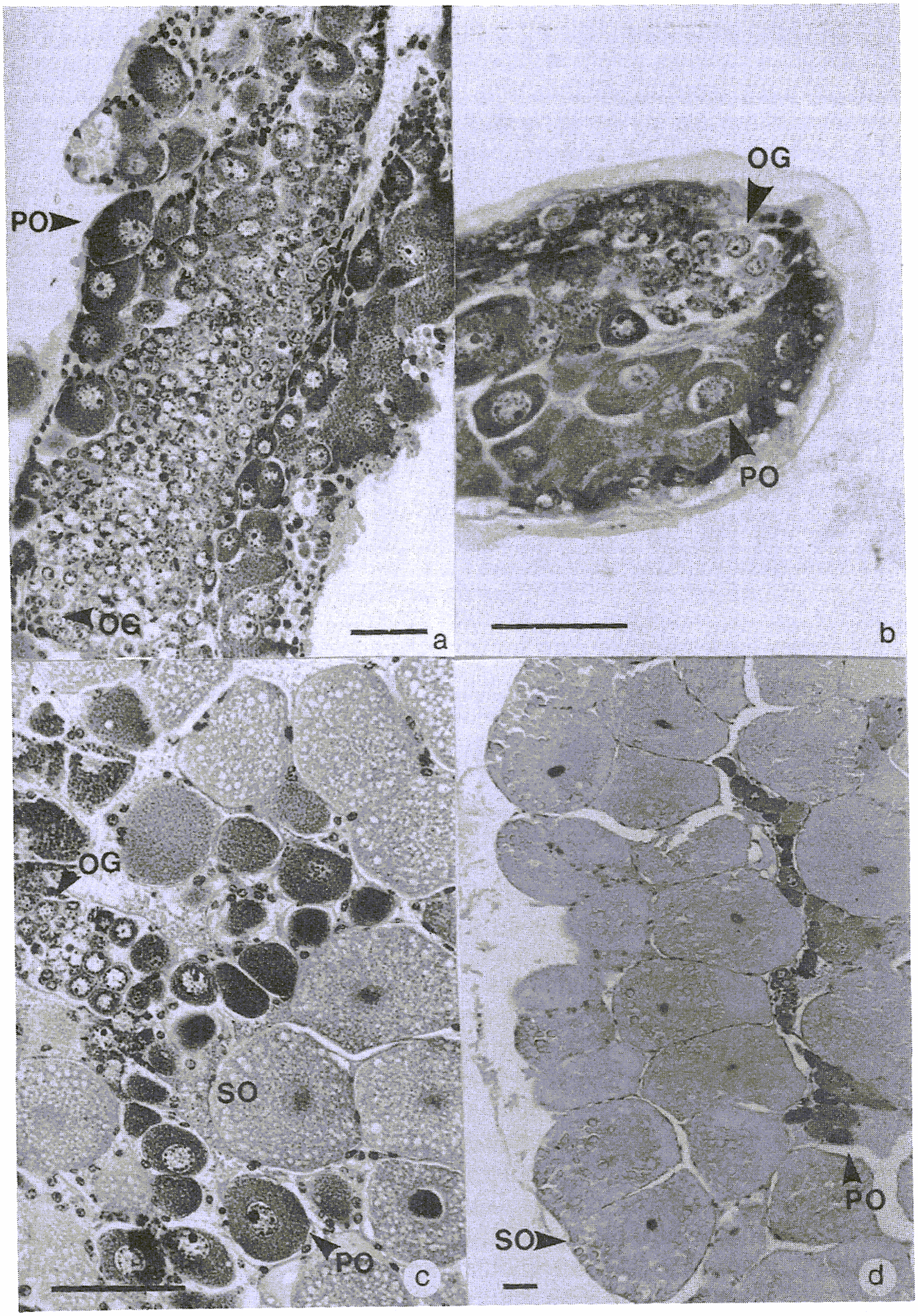


Figure 4: Histological sections of *C. granulata* ovaries. **Fig. 4a**: longitudinal section of immature ovary. **Fig. 4b**: transversal section of immature ovary. **Figs. 4 c, d**: transversal sections of mature ovary. OG: oogonia, PO: primary oocyte, SO: secondary oocyte. Scales: 10 m.

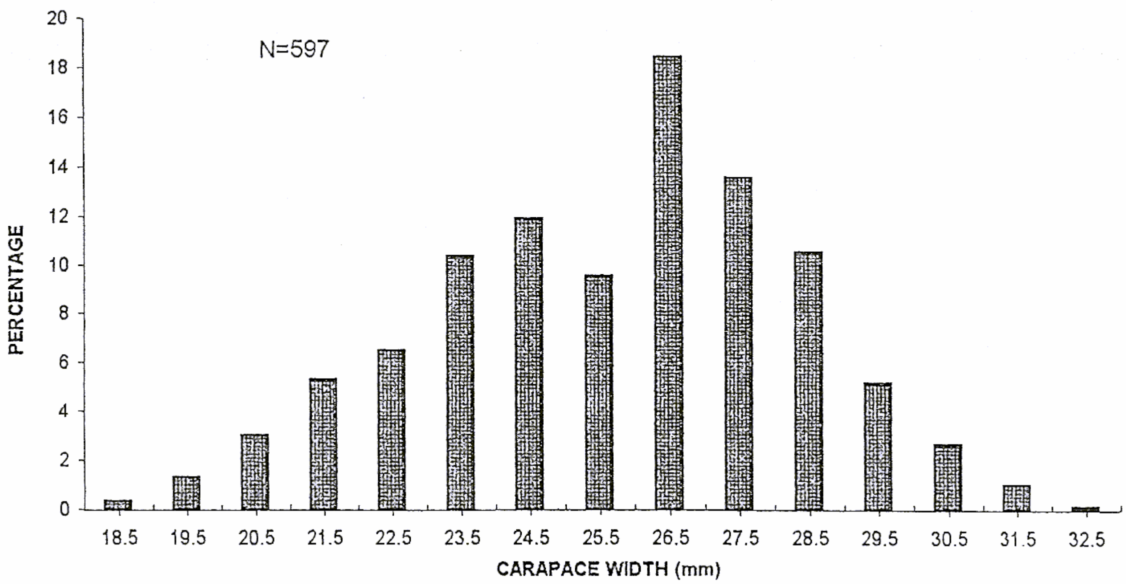


Figure 5: Frequency distribution of size (carapace width) for ovigerous females of *C. granulata*. N= number of measured females (field measurements). White bars= 5% of distribution.

## DISCUSSION

Both males and females of *Chasmagnathus granulata* showed two phases of relative growth, as seen for other brachyurans (Somerton, 1980; Hartnoll, 1982). Changes in the length of the first pleopod (gonopod) in male *C. granulata* followed allometric changes similar to those observed in other crab species (Hartnoll, 1974; Davidson & Marsden, 1987), i.e., a decrease in the positive allometric levels observed during the prepuberty or juvenile phase was found after the puberty molt. A change in the relative growth from a positive allometric value (1.37) to a negative allometric one (0.91) could even be observed in *C. granulata*, as reported for several other crab species (Hartnoll, 1974; 1978). Apparently, the gonopods of mature males would not be better adapted for copulation if they continued to increase in size at the same rate as in juveniles. In regard to females, a change from a positive allometric level (1.82) to a lower but nevertheless positive value (1.12) took place after the puberty molt. This type of change seems to be the general rule for female brachyuran crab; since a disproportionate growth of the abdomen through successive adult molts could be disadvantageous for walking (Hartnoll, 1974,1982).

The other aspect analyzed in this study was the minimum size at wich gonadal maturation took place. We found marked differences, in both sexes, between such values and the sizes indicated by changes in relative growth. For males, while testes were clearly mature after 10.5 mm CW, only at 16.5 mm

CW could a change in the relative growth of the first pleopod be detected. The presence of spermatozoids in *vasa deferentia* of males as small as 12.08 mm CW indicates that probably molting once after attaining gonadal maturity, males would be able to reproduce. Nevertheless, such presence is actually necessary but not the only factor needed for succesful mating, as stated for other crabs (Comeau & Conan, 1992; Conan & Comeau, 1986). The change in the relative growth of reproductive, or even meristic characters (like chela size), seems to be another necessary factor to allow mating, as well as competition with other males (Comeau & Conan, 1992, Mac Diarmid, 1989, Moriyasu & Comeau, 1996, Stevens *et al.*, 1993). Therefore, the functional maturity for *C. granulata* males was probably closer to the change in the relative growth of the first pleopod, than to the acquisition of gonadal maturity.

Females showed mature ovaries after 19 mm CW, but changes in allometric growth were not apparent below 22.7 mm CW. Gonadal maturation previous to changes in the allometric growth of meristic characters was also observed in anomurans (Lovrich & Vinuesa, 1993), although opposite results were reported for brachyurans (Vanini & Gherardi, 1988). Our results indicated that such gonadal maturity can occur before changes in the relative growth of characters with a high reproductive value, like abdominal width.

The size at onset of gonadal maturity corresponded to the smallest ovigerous detected, i.e., the size of functional maturity in nature. Nevertheless, not all the females that have reached the gonadal maturity size reproduce in the population. Small adult females probably shift a significant portion of their energy investment for growth, even during the reproductive season. Our results suggest that the reproductive effort begins to be relevant (in terms of percentage of ovigerous females) near the allometrical size of maturity. These results are in accordance with previous ones recently obtained from experimental matings carried out in the laboratory with small females of *C. granulata*. While almost all the females which attained the allometric size of maturity spawned, most of the females having the size of gonadal maturity molted, and only a few of them reached the ovigerous condition (López & Rodríguez, 1996).

Latitudinal differences may strongly influence the size of sexual maturation. *C. granulata* females from Rio Grande (32° 03' S), Brazil, had mature sizes lower than Samborombón females: a CW close to 15 mm was associated to a change in the relative growth of maximal abdominal width, while an ovigerous female as small as 12.2 mm was reported for Rio Grande females (Ruffino *et al.*, 1994). Smaller size at maturity in tropical habitats seems to be a rule for crustaceans (Sastry, 1983). Nevertheles, it should be emphasized that the maximum sizes attained by male and female crabs from Rio Grande were lower than those achieved by crabs from Samborombón (D'Incao *et al.*, 1993). The broad distribution of *C. granulata* would allow the extension of these comparisons to other latitudes, and thus correlate the onset of sexual maturity with levels of environmental factors such as temperature and food.

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