MORPHOMETRIC ANALYSIS AND REPRODUCTIVE BIOLOGY OF THE CRAB *CHARYBDIS AFFINIS* (DECAPODA, BRACHYURA, PORTUNIDAE) FROM THE ZHUJIANG ESTUARY, CHINA

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ABSTRACT

Morphometric characters and organ indices were analyzed in *Charybdis affinis* collected at different seasons from the Zhujiang estuary, China. The crab is heterochelous, with pronounced right handedness in both sexes. There is a marked sexual difference in relative growth of the cheliped. The female abdomen increases in size at puberty, which is also accompanied by a reduced allometric growth rate. Analysis of gonad development gave the estimate of 50% gonadal maturity at 42 and 36 mm carapace width for females and males, respectively. The hepatosomatic index of female crabs is inversely related to the gonadosomatic index indicating the role of the hepatopancreas in storage of nutrients necessary for ovarian development in the reproductive season.

RÉSUMÉ

Les caractères morphologiques et les indices relatifs aux organes ont été analysés chez *Charybdis affinis*, récolté à différentes saisons dans l'estuaire du Zhujiang, Chine. Le crabe présente une hétérochélie, la pince droite étant très développée dans les deux sexes. Il existe une différence sexuelle marquée dans la croissance relative du chélipède. L'abdomen femelle s'accroît en taille à la puberté, ce qui s'accompagne aussi d'un taux de croissance allométrique réduit. L'analyse du développement des gonades a donné une maturité estimée à 50% pour des femelles et mâles, respectivement, ayant une largeur de la carapace de 42 et 36 mm. L'indice hépatosomatique des crabes femelles est inversement proportionnel à l'indice gonadosomatique ce qui indique le rôle de l'hépatopancréas dans le stockage des nutriments nécessaire au développement ovarien lors de la reproduction.

INTRODUCTION

The crab *Charybdis affinis* Dana, 1852 occurs in the Indo-West Pacific from Taiwan to India (Dai & Yang, 1991; Wee & Ng, 1995). A recent survey in the Zhujiang (Pearl River) estuary (22°00′-22°45′N 113°30′-114°00′E) in southern China shows that *C. affinis* is the dominant brachyuran species (Chu et al., 1996). The estuary is one of the largest estuarine systems in the South China

Sea with an area of over 2,000 km^2. This paper reports on the basic aspects of morphometrics and reproductive biology of *C. affinis* in the Zhujiang estuary. There is little information on these biological aspects of any members in the genus *Charybdis*. Sumpton (1990a, b) reported the morphometric growth and reproductive biology of *C. natator* (Herbst, 1794) in Moreton Bay, Australia.

MATERIALS AND METHODS

Crabs were captured from 6 to 10 sites in the Zhujiang estuary by trawling during 10 sampling cruises from March 1993 to July 1996. The crabs were separated from the other catches on board and immediately frozen in dry ice. In the laboratory, *Charybdis affinis* were sorted and sexed based on the narrower abdomen of the males. Measurements of the following body parts were recorded to the nearest 0.1 mm: carapace width (CW, excluding the lateral spines), height (Chh) and length (Chl) of the propodus of both right and left chelipeds, and width of the sixth abdominal segment (AW). The specimen was then blotted dry and its body weight was measured using an analytical balance to the nearest 0.01 g. Individuals missing one of the limbs were not weighed and excluded from analyses using the body weight.

After dissection, the hepatopancreas and gonads were isolated and weighed to the nearest 0.01 g. Hepatosomatic and gonadosomatic indices (HSI and GSI) were calculated by dividing the wet weight of the hepatopancreas or gonad by the total wet weight of the crab, and expressed as percentage values. The stage of ovarian maturation of the females was noted based on the colour of the ovaries. Four stages were recognized: stage I, colourless; stage II, white to ivory; stage III, yellow to yellowish orange; and stage IV, orange. Ovigerous females were designated as such without regard to the maturation stage of their ovaries. In addition there were females with no visibly developed ovaries. Statistical analyses followed Zar (1984).

RESULTS

A total of 861 individuals of *Charybdis affinis* were sampled over the four-year period. Table I shows that there are no significant differences in the body size and hepatosomatic index between male and female crabs sampled. Table II shows that carapace length, body weight, and the two organ indices are significantly different in female crabs at different stages of maturation ($P < 0.05$, 1-factor ANOVA). There is a general trend of increase in size from females with no visibly developed ovaries to females with stage III and IV ovaries. This is

TABLE I

Carapace width (CW), body weight (BW), hepatosomatic index (HSI) and gonadosomatic index (GSI) of males and females of *Charybdis affinis* Dana. Values are expressed as mean ± SD with sample size in parentheses; asterisk indicates significant differences between males and females ($P < 0.05$, t-test)

	Male	Female
CW (mm)	41.1 ± 6.6 (379)	40.6 ± 7.4 (482)
BW (g)	16.1 ± 7.9 (216)	15.6 ± 8.8 (244)
HSI (%)	5.1 ± 2.6 (194)	5.0 ± 2.7 (209)
GSI (%)	0.7 ± 0.5* (136)	4.8 ± 3.0 (134)

TABLE II

Carapace width (CW), body weight (BW), hepatosomatic index (HSI), and gonadosomatic index (GSI) of female *Charybdis affinis* Dana at different stages of ovarian maturation. Values are expressed as mean ± SD, sample size in parentheses; different superscripts indicate significant difference between the various stages of sexual maturation ($P < 0.05$, 1-factor ANOVA followed by Student-Newman-Keuls test)

		Stages of ovarian maturation				
	No gonad	I	II	III	IV	Ovigerous
CW (mm)	35.7 ± 6.0^{a} (210)	40.8 ± 6.2^{b} (25)	42.2 ± 5.8^{b} (19)	46.3 ± 4.8^{c} (27)	46.9 ± 5.2^{c} (116)	41.6 ± 5.8^{b} (84)
BW (g)	8.6 ± 4.7^{a} (89)	15.7 ± 9.8^{b} (16)	15.6 ± 6.1^{b} (16)	20.8 ± 5.4^{bc} (10)	21.8 ± 7.3^{c} (68)	19.0 ± 8.8^{bc} (45)
HSI (%)	6.9 ± 2.8^{a} (64)	5.9 ± 2.3^{a} (16)	5.7 ± 2.2^{a} (15)	4.6 ± 2.1^{ab} (9)	4.0 ± 2.2^{b} (67)	3.1 ± 1.5^{b} (39)
GSI (%)	±	1.3 ± 0.7^{a} (7)	2.0 ± 1.2^{a} (13)	3.1 ± 1.3^{a} (9)	5.3 ± 2.5^{b} (65)	5.9 ± 3.3^{b} (40)

accompanied with the increase in GSI reaching a maximum in stage IV and ovigerous females. In contrast, these crabs had lower HSI values than crabs with no or early developing (stages I and II) ovaries. It was noted that 90% of ovigerous females ($N = 40$) had stage III or IV ovaries. When the morphometric characters and organ indices were analysed separately for male crabs with or without testes (table III), it was found that sexually mature males were larger in size than immature males ($P < 0.05$, t-test). Yet there is no significant difference between the HSI between the two groups of males.

It was observed that the right cheliped was generally larger than the left (table IV). More than 70% of the crabs were right-handed. The heterochely was more pronounced with respect to the height than the length of the cheliped in both sexes ($P < 0.05$, t-test). Moreover, the differentials are more prominent in females than in males ($P < 0.01$, t-test). The ratios of the length of both chelipeds to carapace width were analyzed and compared among males and fe-

TABLE III

Carapace width (CW), body weight (BW), hepatosomatic index (HSI), and gonadosomatic index (GSI) of male *Charybdis affinis* Dana with or without testes. Values are expressed as mean ± SD, sample size in parentheses; asterisks indicate significant differences between the crab with and without testes ($P < 0.05$, t-test)

	Testes absent	Testes present
CW (mm)	35.2 ± 4.5 (109)	43.5 ± 5.7* (270)
BW (g)	8.8 ± 3.8 (44)	17.9 ± 7.6* (172)
HSI (%)	5.9 ± 3.6 (32)	5.0 ± 2.3 (162)
GSI (%)	±	0.7 ± 0.5 (136)

TABLE IV

Ratio of measurements of right to left cheliped (mean ± SD) and percentage of right handedness based on length and height in *Charybdis affinis* Dana. No significant difference between males and females ($P > 0.05$, t-test) observed

	Length ratio	%	Height ratio	%	N
Males	1.04 ± 0.06	76.0	1.10 ± 0.13	76.5	217
Females	1.06 ± 0.11	73.6	1.15 ± 0.22	74.4	246

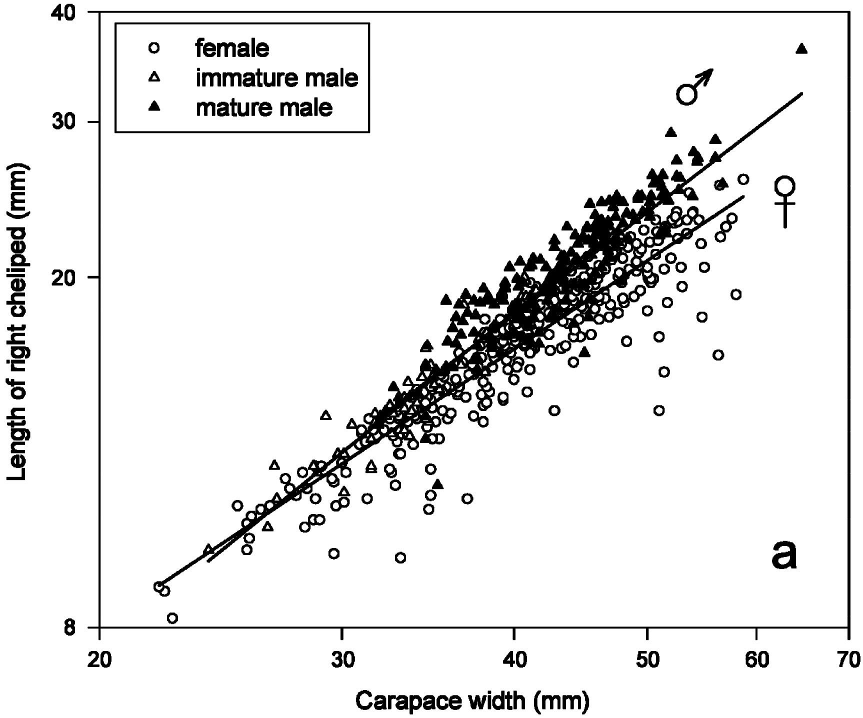
TABLE V

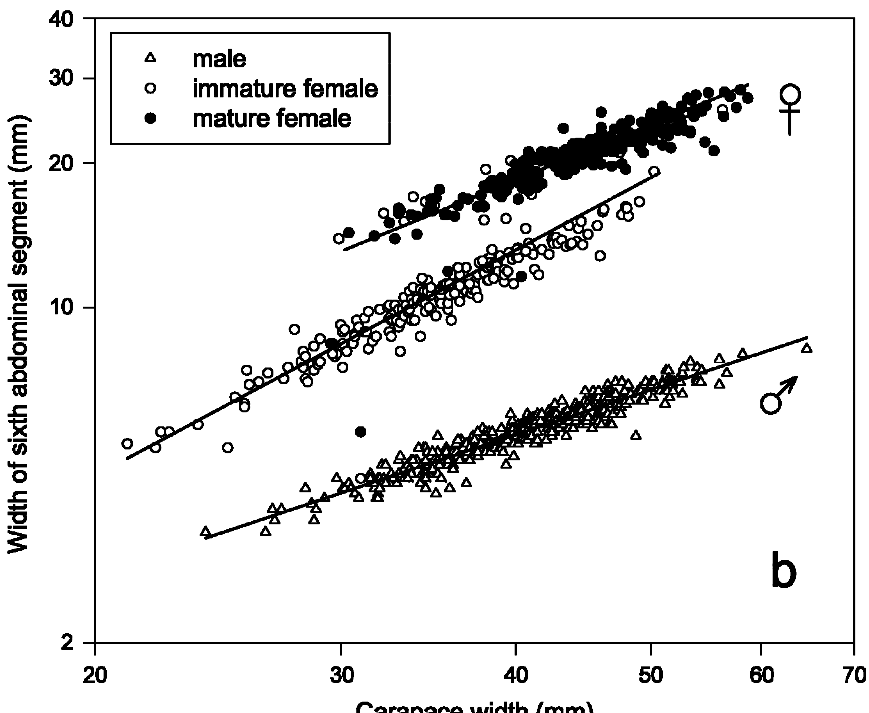
The ratios of length of left or right cheliped (LChl, RChl) to carapace width (CW) and width of the sixth abdominal segment (AW) to CW in male and female *Charybdis affinis* Dana at different stages of sexual maturation. Values are expressed as mean ± SD, sample size in parentheses; different superscripts indicate significant difference between the various stages of sexual maturation in males and females in the same column ($P < 0.05$, 1-factor ANOVA followed by Student-Newman-Keuls test)

		$\frac{\text{LChl}}{\text{CW}}$	$\frac{\text{RChl}}{\text{CW}}$	$\frac{\text{AW}}{\text{CW}}$
Male	Testes absent	0.418 ± 0.028^{a} (59)	0.438 ± 0.029^{a} (65)	0.137 ± 0.006^{a} (109)
	Testes present	0.453 ± 0.036^{b} (208)	0.469 ± 0.034^{b} (204)	0.136 ± 0.006^{a} (268)
	No gonad	0.389 ± 0.023^{c} (121)	0.410 ± 0.029^{c} (126)	0.318 ± 0.064^{b} (210)
	I	0.392 ± 0.028^{c} (20)	0.411 ± 0.032^{c} (18)	0.344 ± 0.062^{c} (25)
Female	II	0.398 ± 0.022^{c} (17)	0.420 ± 0.025^{ac} (18)	0.428 ± 0.077^{d} (19)
	III	0.406 ± 0.016^{ac} (16)	0.419 ± 0.030^{ac} (18)	0.470 ± 0.045^{e} (27)
	IV	0.393 ± 0.026^{c} (88)	0.419 ± 0.030^{c} (83)	0.470 ± 0.026^{e} (116)
	Ovigerous	0.396 ± 0.028^{c} (58)	0.411 ± 0.041^{c} (59)	0.456 ± 0.044^{e} (83)

males at different maturation stages (table V). The ratios are significantly higher in males with testes than those without testes, or females ($P < 0.05$, 1-factor ANOVA followed by Student-Newman-Keuls test). Moreover, the ratios are not significantly different among females at different maturation stages. The results indicate that mature males have larger chelipeds than females or immature males. Relative growth of the cheliped was studied by log-log plots of the length of the right cheliped to carapace width (fig. 1a). An analysis of the slope of the regression lines, which represents the allometric growth constant (Hartnoll, 1982), for females (1.04 ± 0.02), immature (1.27 ± 0.05) and mature males (1.20 ± 0.04) shows that they are all significantly different from one ($P < 0.01$, t-test), indicating positive allometry of the cheliped. The degree of allometric growth is more evident in males than in females, as allometric growth constants for males are significantly greater than that for females ($P < 0.01$). The constants for immature and mature males are not significantly different, indicating their chelipeds have the same allometric growth rate.

The ratio of width of the sixth abdominal segment to carapace width in male and female crabs at different maturation stages were also analysed (table V). It is apparent that female crabs had a broader abdomen than male crabs ($P < 0.05$, 1-factor ANOVA followed by Student-Newman-Keuls test). Whilst there is no difference in this ratio between male crabs with or without testes, the ratio increases from crabs with no discernable ovaries to those with stage III ovaries, beyond which the ratio remains unchanged. The data indicate an increase in abdominal growth relative to carapace width as the crabs become mature and the puberty moult occurs in concordant with the ovarian development from stage II to III. Thus females with stage III and IV ovaries, regardless of whether they are ovigerous, were considered mature in subsequent analysis. When the width of the sixth abdominal segment is plotted against carapace width in the log scale (fig. 1b), the allometric constant for males (0.97 ± 0.02) is not significantly different from one ($P > 0.05$, t-test), indicating isometric growth of the male abdomen relative to the carapace. On the other hand, in females, the allometric growth constants for immature (1.56 ± 0.06) or mature females (1.19 ± 0.04) are significantly greater than one ($P < 0.001$), indicating positive allometry of the female abdomen. The discontinuity of the two regression lines represents the occurrence of a puberty moult over the size of range of 30 to 50 mm CW, with an increase of relative abdominal width of about 40%. The two allometric growth constants for pre-and post-puberty females are also significantly different ($P < 0.05$, t-test) from each other, indicating a decrease in allometric growth rate of the abdomen in relation to the carapace after the puberty moult.


female
immature male
mature male
♂
♀
a
Length of right cheliped (mm)
Carapace width (mm)
40
30
20
8
20
30
40
50
60
70


male
immature female
mature female
♀
♂
b
Width of sixth abdominal segment (mm)
Carapace width (mm)
40
30
20
10
2
20
30
40
50
60
70

Based on the degree of gonad development (development of testes in male and stage III ovaries in female), the median size of sexual maturity could be determined. The percentage of males with developed testes increased with size (fig. 2a). When the data were fitted to a logistic curve, the median size of maturity estimated is 36.0 mm CW. Carapace width of the smallest male with testes is 31.8 mm. In females, the percentage with stage III or IV ovaries increased with body size (fig. 2b). The median size of maturity is estimated to be 42.1 mm. The smallest female with stage III ovaries has a CW of 32.5 mm.

The percentage of males with testes was the highest in January, with almost all males carrying testes, and decreased toward October (fig. 3). The percentage of ovigerous females peaked in March/April, when more than 50% of all females caught were ovigerous. Gonadosomatic indices of mature males and females varied seasonally (fig. 4). The GSI values of both male and female peaked in March/April and were at the lowest in October. The HSI of females was the lowest in March/April and was the highest in October. The correlation coefficient, r, between GSI and HSI is significant in females (-0.48, $P < 0.001$), but not in males (0.14).

DISCUSSION

Charybdis affinis is heterochelous, with right handedness in both sexes. This dominance of right handedness has been reported in *C. natator* (cf. Sumpton, 1990a) and other crabs such as *Carcinus maenas* (Linnaeus, 1758) (cf. Abby-Kalio & Warner, 1989), *Potamon potamios* (Olivier, 1804) (cf. Gherardi & Micheli, 1989), *Geryon trispinosus* (Herbst, 1803) (cf. Attrill et al., 1991), and *Liocarcinus holsatus* (Fabricius, 1798) (cf. Lee & Seed, 1992). Yet other heterochelous crabs have no preference in handedness, being equally likely to have the right or left cheliped enlarged (Hartnoll, 1982; review). It has been suggested that right handedness is related to the predatory behaviour for handling asymmetric prey (Abby-Kalio & Warner, 1989). In *Charybdis affinis*, males have bigger chelipeds than females. Such a sexual difference in cheliped size is common in crabs, although the degree of sexual dimorphism varies with

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Fig. 1. a, Relationship between length of right cheliped, RChl, and carapace width (CW) in *Charybdis affinis* Dana, 1852 females ($\text{RChl} = 0.90\text{AW}^{1.04}$, $N = 322$), immature males ($\text{RChl} = 0.17\text{AW}^{1.27}$, $N = 65$) and mature males ($\text{RChl} = 0.22\text{AW}^{1.20}$, $N = 204$); b, relationship between width of the sixth abdominal segment, AW, and carapace width for males ($\text{AW} = 0.15\text{AW}^{0.97}$, $N = 377$), immature females ($\text{AW} = 0.04\text{AW}^{1.56}$, $N = 235$), and mature females ($\text{AW} = 0.23\text{CW}^{1.19}$, $N = 245$).

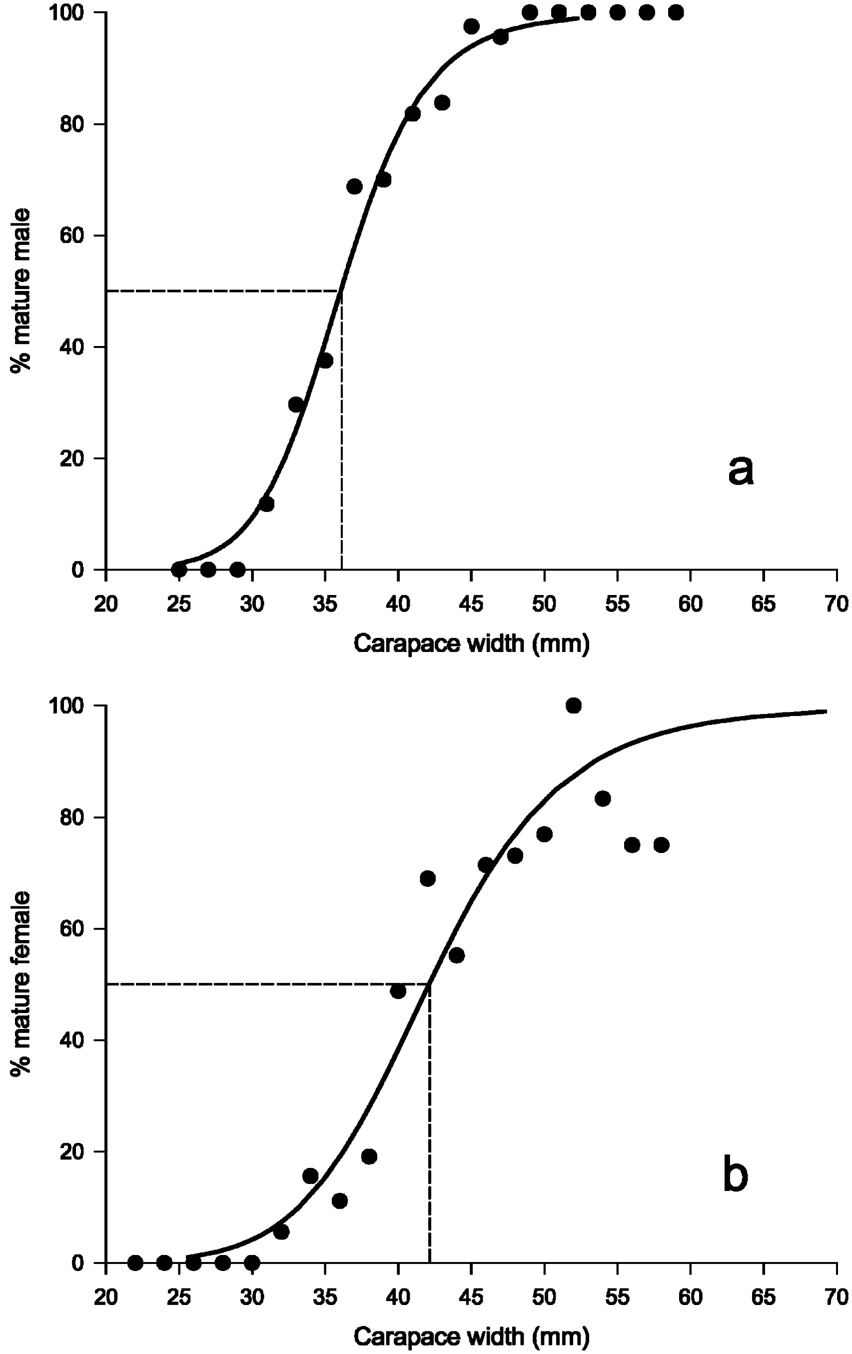

Fig. 2. *Charybdis affinis* Dana, 1852. a, the percentage of males with developed testes; b, the percentage of females with stage III and IV ovaries in classes of 2-mm carapace length range. Each size class consists of 1-45 individuals. The solid lines represent the logistic curves fitted to the data. The broken lines indicate the estimates of 50% gonad maturity.

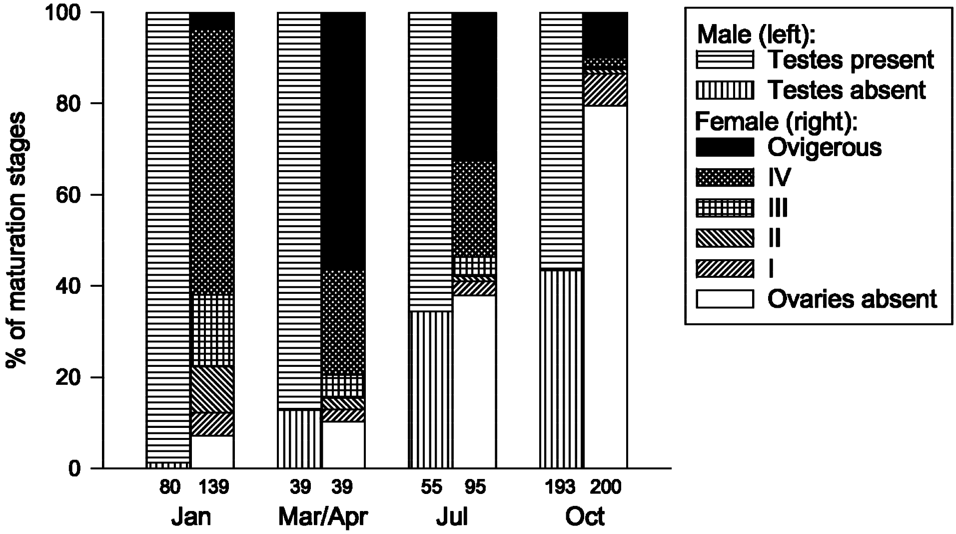

Fig. 3. Seasonal variation in the percentage of *Charybdis affinis* Dana, 1852 males and females at different sexual maturation stages. Sample size is given below the bars.

species (Lee, 1995). This character may give a selective advantage in competition for mate, food or space (Hartnoll, 1982; Shine, 1989; reviews). While the growth of the female cheliped of *C. affinis* is close to isometric throughout, that of the male cheliped is distinctly allometric, but no change in the allometric growth rate can be discriminated. It has been documented in crabs that a pubertal moult is often associated with an increase in cheliped size and allometric growth rate (Hartnoll, 1974, 1982; reviews). In *C. affinis*, due to the wide scattering of the data (fig. 1a), a slight increase in cheliped size and a minor discontinuity in relative growth during the puberty moult cannot be excluded. Yet variations from the general pattern in relative growth of the male cheliped have been reported in other crabs (e.g., Haley, 1973; Fielding & Haley, 1976; Du Preez & McLachlan, 1984; Attrill et al., 1991; see also Hartnoll, 1978 for a review).

Besides the cheliped, the most distinct sexual dimorphic character of crabs is the broad abdomen of the female which serves as the brood chamber (Hartnoll, 1982). In contrast to the isometric growth of the male abdomen, the growth of female abdomen in *C. affinis* is allometric. Moreover, there is a marked increase in the relative abdomen width at puberty, followed by a decrease in allometric growth rate in post-puberty crabs. This is similar to the general pattern of female abdominal growth of most crabs (Hartnoll, 1974). However, in *C. natator*, while there appears to be an increase in the abdominal width relative to carapace length

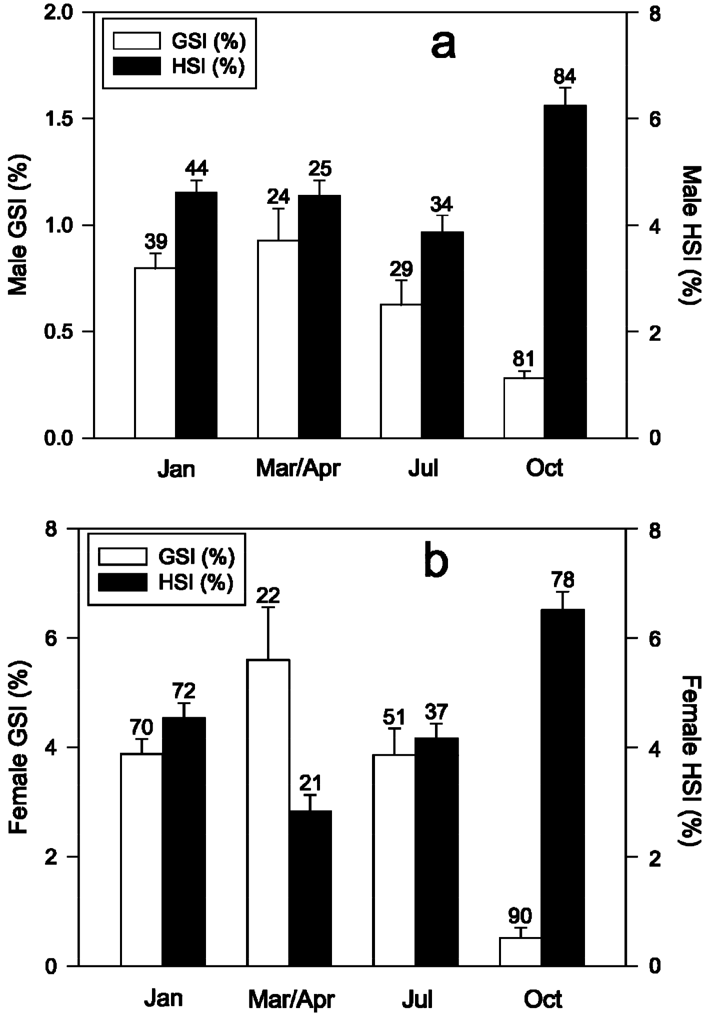

Fig. 4. Seasonal variation in gonadosomatic (open bars) and hepatosomatic (closed bars) indices of: a, males, and, b, females of *Charybdis affinis* Dana, 1852. Sample size is given above the bars.

at the pubertal moult, there is no increase in the relative abdominal width after the moult (Sumpton, 1990a). In *C. affinis*, the pubertal moult occurs over a wide range of carapace width, indicating that the carapace width is not an accurate indicator of sexual maturity. This is also true for the same analyses in *C. natator* (cf. Sumpton, 1990a). It was shown in *C. affinis* that the changes in relative growth of the abdomen sexual characters coincide with distinct phase of gonad development, based on which the median size of maturity was estimated (see Results).

As samplings were conducted seasonally, it is difficult to define precisely the spawning season of *C. affinis*. Both the GSI values and the percentage of ovigerous females peak in March/April. In October, both the GSI values and percentage of males with testes are at the lowest. Our data thus suggest that *C. affinis* possibly has one peak of spawning activity in spring which continues to early summer. The occurrence of developing and mature ovaries in most ovigerous females suggests sequential broodings occur. Sumpton (1990b) also provided evidence that sequential brooding occurs in *C. natator*.

The hepatosomatic index of *C. affinis* females at early stages of sexual maturation is higher than in those with developed and mature ovaries. The index is at its lowest in ovigerous females. Similar changes of HSI with the female reproductive cycle have been documented in other crabs (e.g., Kyomo, 1988). It was also found in *C. affinis* that the HSI peaks in non-reproductive season, as reported in some other crabs (e.g., Shih, 1993). The results are in line with data from biochemical studies that energy reserves in the hepatopancreas are mobilized to the ovary for oocyte maturation in crustaceans (e.g., Mourente & Rodróguez, 1991; Spaargaren & Haefner, 1994). In contrast, the HSI of *C. affinis* males is unaffected by the development of testes, presumably related to the lower energy requirement for testes development (Kyomo, 1988).

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