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Epibiotic community of the horseshoe crab *Tachypleus gigas*

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Abstract Horseshoe crabs act as moving substrata for simple to complex communities of small marine organisms. Amplexed adult pairs migrate for breeding once every 2 weeks from deep waters towards nearshore waters during highest high tide. Female horseshoe crabs bury themselves to the level of the lateral eyes to deposit eggs while the male crabs fertilize them. Subsequently eggs are buried by the female. *Tachypleus gigas* (Müller) is the most abundant horseshoe crab species above available along the Orissa coast (India). Adults reach terminal anecdysis once sexually mature and live with their carapace for 4 to 9 years. In spite of this, epibiosis is limited. In the current investigation, differences in the epibiotic community (diatoms and macro-epibionts) present on horseshoe crabs, according to gender, were evaluated, and the macro-epibiont population from different regions of the carapace was mapped. In general, female horseshoe crabs harbored fewer epibionts than the males. Among the diatoms, *Navicula* spp., *Nitzschia* spp. and *Skeletonema* sp. were dominant in both sexes. However, the abundance and diversity of diatoms was greater on the carapaces of male crabs. Among the macro-epibionts, the acorn barnacle (*Balanus amphitrite* Darwin) and encrusting bryozoan (*Membranipora* sp.) were the most dominant forms. Barnacles and bryozoans were greater in abundance in the "rough" zone (cardiopthalmic region and anterior region of the opisthosoma). Mapping of the macro-epibionts from different regions of the carapace revealed differential distribution in males and females. Such differentiated distribution of the macro-epibionts can be related to factors such as changing habitat by the horseshoe crabs during breeding, mechanical abrasion and surface availability during mating and nesting periods, requirements of epizootic larvae and surface properties of the carapace (wettability and roughness). In the case of females, mechanical abrasion and surface availability played an important role in the epibiotic community structure and distribution patterns. The surface wettability measurements indicated male carapace to be slightly more hydrophobic than the female carapace. Scanning electron microscopy revealed that the male carapace was comparatively rough compared to the smooth carapace of females. A comparison of surface properties of the carapace indicated that the male carapace is more conducive for epibiosis.

Introduction

In densely populated marine environments, space is often a limiting resource in epibenthic communities (Jackson and Buss 1975). When free space is limiting, living substrata may become important for epibiosis. In the marine environment, a variety of benthic fauna and flora form hard substrates in soft-bottom sediments. Epibiosis of such substrata (i.e. non-symbiotic, facultative association between epibionts and basibionts) becomes a highly valuable phenomenon for the survival of sedentary organisms (Wahl 1989). Some organisms appear to tolerate a considerable load of epibionts (Rützler 1970; Davis and Wright 1989). Epibionts on the hosts have been indicated to have a protective value for the host via camouflage (Ingle 1983; Rasmussen 1973). Only certain biological surfaces resist colonization to variable degrees for more or less extended periods (Fletcher and Marshall 1982). Though there is considerable literature on the mechanisms by which invertebrates deter or shed fouling organisms, there is very little data on natural levels of fouling in invertebrate communities (Davis and White 1994).

Horseshoe crabs act as moving substrata for simple to complex communities of small marine organisms.

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Horseshoe crabs carry a variety of epibionts on their external surface, including green algae, diatoms, coelenterates, flatworms, mussels, oysters, annelids, barnacles, tunicates, bryozoans, isopods, amphipods, gastropods, pelecypods and polychaetes (Humm and Wharton 1942; Roonwal 1944; Rao and Rao 1972; Davis and Fried 1977; Mackenzie 1979; Shuster 1982; Jeffries et al. 1989; Saha 1989; Debnath 1992; Key et.al. 1996). However, taking into account that adults reach terminal anecdysis once sexually mature and live with their carapace for 4 to 9 years, the intensity of epibiosis appears negligible. Mikkelsen (1988) also observed that barnacles are usually seen on males, indicating a potential gender difference. In this investigation an attempt has been made to explore the gender and spatial differences in the epibiosis of the horseshoe crab *Tachypleus gigas* and to postulate the possible causative factors.

Horseshoe crabs live in moderately deep waters and migrate to nearshore waters for breeding. When approaching the beach to nest, a female is almost always accompanied by a male clasping her with his modified claws (Cohen and Brockman 1983). In India, the horseshoe crabs *Tachypleus gigas* and *Carcinoscorpius rotundicauda* are found confined to Orissa and West Bengal coasts. Along the Orissa coast they are found near Burhabalanga estuary and Abana. *T. gigas* is the most abundant of the two species (Vijaykumar 1992; Chatterji 1994).

Materials and methods

Living specimens of *Tachypleus gigas* were collected near the Burhabalanga estuary, Orissa coast (Fig. 1) in March and August of 1997, and again in February and August of 1998. The horseshoe crabs used in the study were haphazardly collected, amplexed pairs so that all crabs were sexually mature and in terminal anecdysis. The specimens were collected during receding high tide and were transported to the laboratory in seawater for the evaluation of diatoms and macro-epibionts.

The horseshoe crabs collected were sexed. The length and width of the prosoma were measured and were used as standard morphometric proxies to determine the size of the horseshoe crabs. Since the carapace (dorsal side) was uneven, the total area was measured by marking it into different geometric figures like triangles and trapeziums (a quadrilateral with only one pair of sides parallel) and summing their areas. The dimensions of the geometric figures differed for different crabs depending on the size of the carapace.

Surface pH of live crabs was determined (six times each for male and female carapaces) at the collection site by placing pH indicator paper (Qualigens, pH 1 to 14) on humid carapaces. Surface wettability of 14 air-dried carapaces (seven each for male and female carapaces) was determined by the drop-spread method as described by Gerhart et al. (1992), using HPLC-grade water and methanol. The spread of 25 µl drops was measured using a series of solutions of 100, 80, 60, 40, 30, 20, 10 and 0% methanol and calculating the standardized harmonic mean (SHM) of the diameter (mm) of the drop. The SHM values strongly correlate with the combined polar components of surface wettability (Gerhart et al. 1992). Using polar solutions of water and methanol, the drop-spread method can detect changes in the polar characteristics of different surfaces with a sensitivity that approaches that of measurements obtained with a contact angle goniometer (Gerhart et al. 1992). The polar solutions used in the drop-spread method also allowed non-destructive testing of the surfaces (Gerhart et al. 1992).

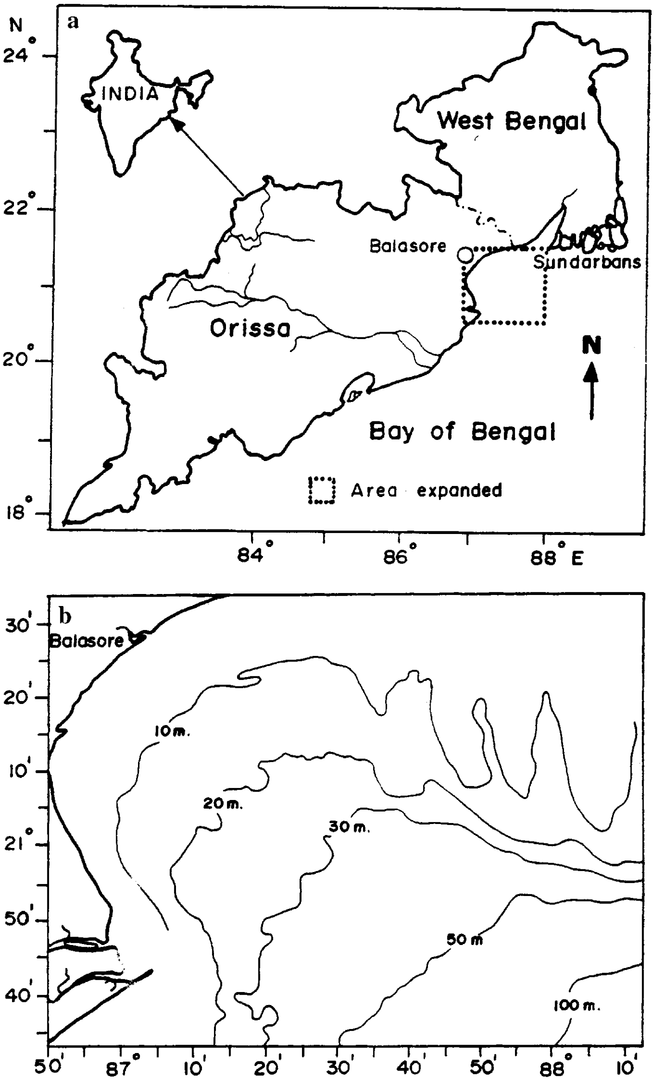

Fig. 1 A Location of the sampling site where the horseshoe crabs *Tachypleus gigas* frequently migrate to the shore for breeding. **B** Area of the sampling locality marked in A

Diatoms

Specimens (two pairs in March 1997, three pairs each in August 1997, February 1998 and August 1998), free of macro-epibionts, were collected for the evaluation of diatom communities. Quantification of diatoms associated with horseshoe crab carapaces was carried out by scraping the entire carapace with a nylon brush into a known volume of filtered seawater. The scraped material was preserved in Lugol's iodine, and its diatom flora was enumerated by the sedimentation technique (Hasle 1978).

The diversity and evenness of the diatom community was evaluated (Shannon–Wiener diversity index, H'). The $\log(x + 1)$-transformed values of diatom abundance were further analyzed using cluster analysis. The dissimilarity levels were measured through squared Euclidean distance and group average method as described by Pielou (1984).

The log-transformed values of abundance of all diatoms (cells dm^{-2}) were subjected to two-way analysis of variance (ANOVA), with unequal but proportional subclass numbers (Sokal and Rohlf

1981), for evaluating the differences with respect to sampling period, sex and the interaction of sampling period and sex (eight subgroups; four samples for each sex).

Macro-epibionts

In the evaluation of macro-epibionts, 6 pairs of crabs were used in March 1997, 15 pairs in August 1997, 15 pairs in February 1998 and 10 pairs in August 1998. The macro-epibiont populations from the dorsal sides of the carapaces (prosoma and opisthosoma) were enumerated. Solitary forms like barnacles (*Balanus amphitrite*, Darwin), false oysters (*Anomia* sp.) and sea anemones were counted and represented in terms of individual counts per square decimeters. Area covered by barnacles and bryozoans was determined in terms of percentage coverage. Dimensions of the barnacles, i.e. basal rostro-carinal and latero-lateral diameters in millimeters, were measured by using a Vernier caliper to calculate the basal area. Basal diameter (mm) of barnacles was considered as a measure of size to determine the growth of barnacles. The individual basal area was used in evaluation of total area covered by barnacles. Size-frequency distribution was evaluated by grouping barnacles in 2-mm intervals (individuals <2 mm were considered spats).

Mapping of the macro-epibiont distribution on carapaces was carried out by marking the carapaces into different zones based on the zones exposed during the nesting period in the nearshore waters (Fig. 2). The prosoma was categorized into three regions: (1) the cardiopthalmic ridge (Prs_1), (2) the anterior prosoma up to the level of lateral eyes (Prs_2) and (3) the two flanks of the prosoma (Prs_3). The opisthosoma was categorized into two regions: (1) the anterior (uncovered region; Opt_1) and (2) the posterior (covered region; Opt_2).

The arcsine transformed values of the total macro-epibiont, barnacle and bryozoan percentage coverages were separately subjected to two-way ANOVA with replication (Sokal and Rohlf 1981). This was done in order to understand the differences associated with different regions of the carapace (prosoma and opisthosoma) versus those associated with gender. These were analyzed further using cluster analysis, to better understand the dissimilarity pattern. The dissimilarity level was measured through squared Euclidean distance and the group average method as described by Pielou (1984).

Scanning electron microscopy (SEM)

The different regions of male and female horseshoe crab carapaces (dorsal side) were examined by SEM to evaluate the surface characteristics. Replicate samples from different regions (Prs_1, Prs_2, Prs_3, Opt_1 and Opt_2) of the carapace were mounted on stubs, gold-coated and examined at 15 kV with a JEOL JSM-5800 LV scanning electron microscope. The observations were repeated with three male and female specimens.

Results

As with all the horseshoe crabs, females were larger than males (Fig. 3). The surface area of the carapace of female horseshoe crabs was 55 to 60% more than that of males. The surface area of the prosoma was approximately 75% greater than the opisthosoma in both sexes. The surface area of the female's prosoma and opisthosoma was 55 to 65% greater than that of the male's.

Surface properties of the carapace

The whole body is covered by tough chitinous exoskeleton, which is sage green in color. The males were lighter

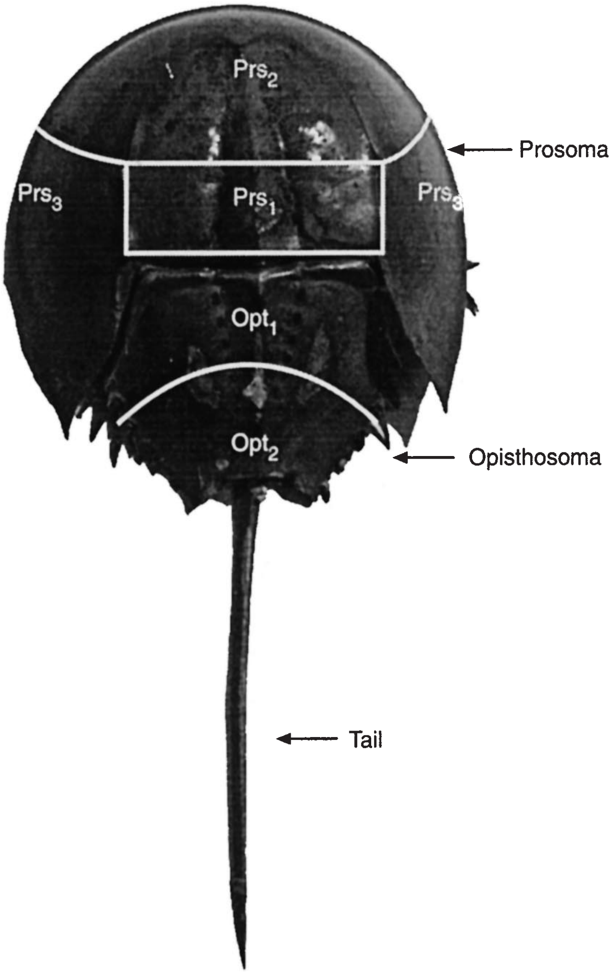

Fig. 2 *Tachypleus gigas*. Carapace demarked for epibiosis evaluation [*Pr* prosoma: Prs_1 cardiopthalmic region, Prs_2 anterior prosoma up to the level of lateral eyes, Prs_3 flanks of the prosoma; *Opt* opisthosoma: Opt_1 anterior opisthosoma (uncovered region), Opt_2 posterior opisthosoma (covered region)

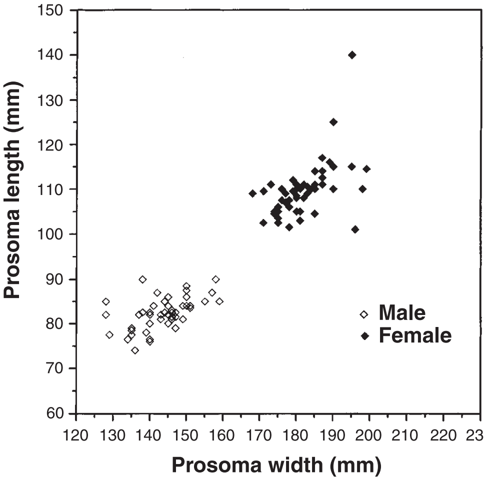

Fig. 3 *Tachypleus gigas*. Prosoma size of male and female horseshoe crabs used in the study

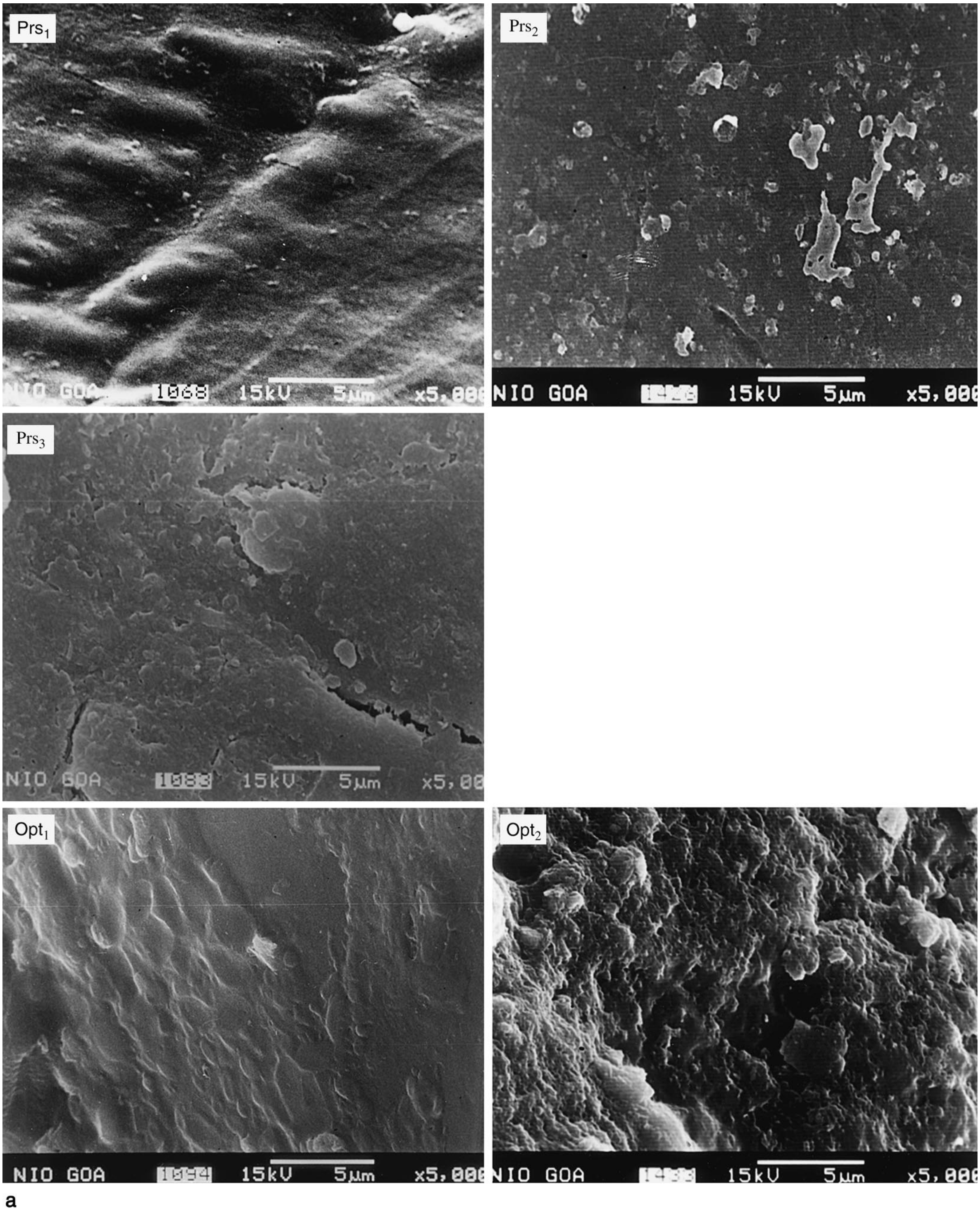

Fig. 4 *Tachypleus gigas*. Scanning electron micrographs of horseshoe crab carapaces. **a** Male carapace: surface texture is rough in Prs$_1$, Opt$_1$ and Opt$_2$ regions. **b** Female carapace: surface texture is comparatively smooth. Star-shaped openings were seen in Prs$_1$ and Prs$_2$ regions. Location of Prs$_1$, Prs$_2$, Prs$_3$, Opt$_1$ and Opt$_2$ on the carapace is as in Fig. 2

in color than the females. The results obtained for surface wettability indicated the male carapace to be slightly more hydrophobic (SHM = 14.5 ± 2.34) than the female carapace (SHM = 18.7 ± 1.8). The surface pH did not vary much between male and female carapaces and ranged from 8 to 8.5.

SEM

Electron micrographs of the carapace of male and female horseshoe crabs are shown in Fig. 4a and b,

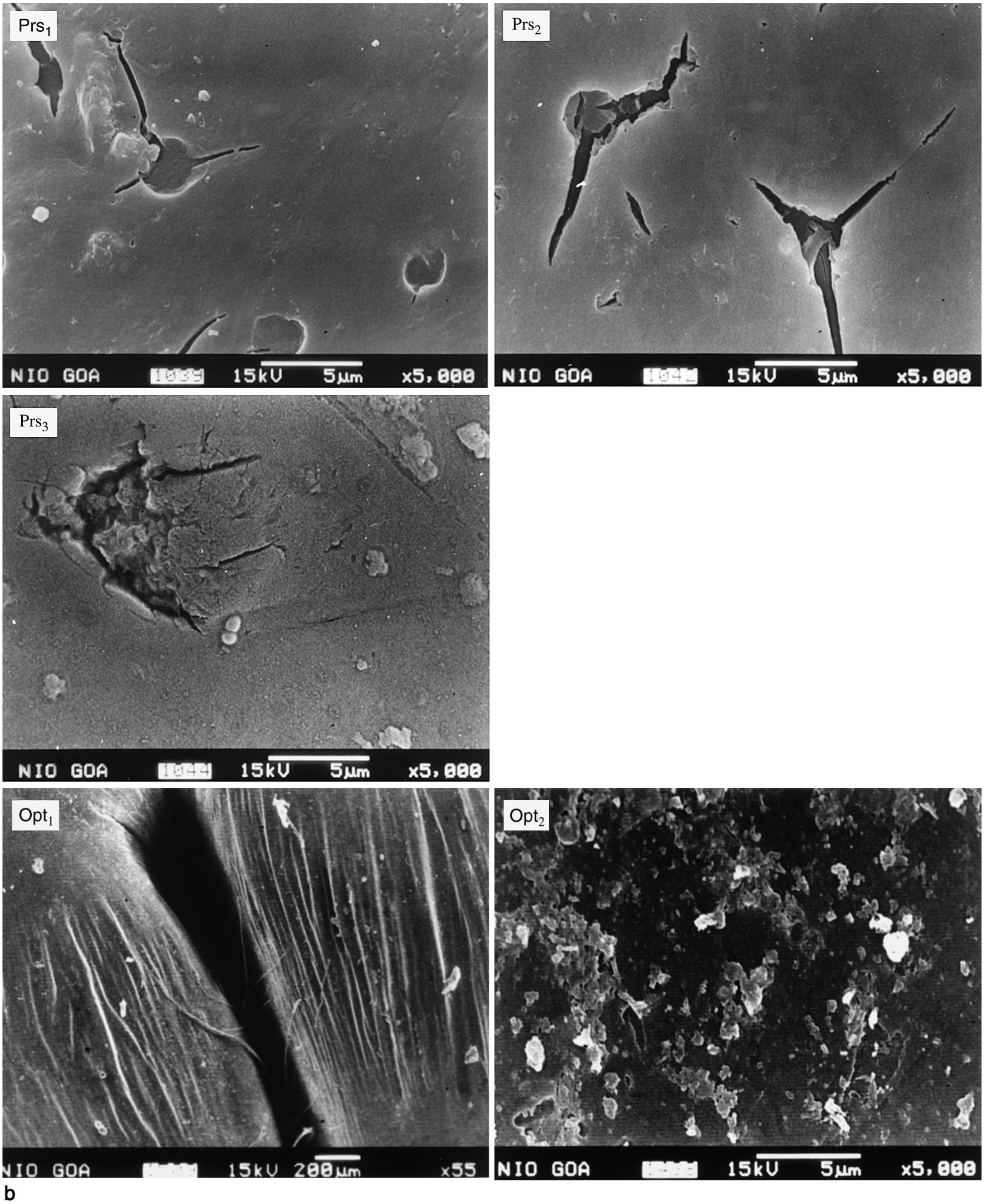

Fig. 4 (Continued)

respectively. SEM revealed that the male carapace was comparatively rough as compared to the smooth carapace of females. Star-shaped openings were observed on the female carapace in the cardiopthalmic region and posterior part of the rim surrounding the cardiopthalmic (posterior prosoma) region (Fig. 4b, Prs$_1$ and Prs$_2$). Such openings were not seen on any of the male carapaces.

Diatoms

Diatoms recorded from male and female horseshoe crabs belonged to 20 (11 pennales and 9 centrales) and 17 (10 pennales and 7 centrales) genera, respectively. The diatom abundance (cells dm^{-2}), generic diversity (H') and evenness values ($H'/H_{\max}$) were lower for females than for males (Fig. 5a, b).

Pennate diatoms dominated the epibiotic community (Fig. 5c). Their dominance in the epibiotic community

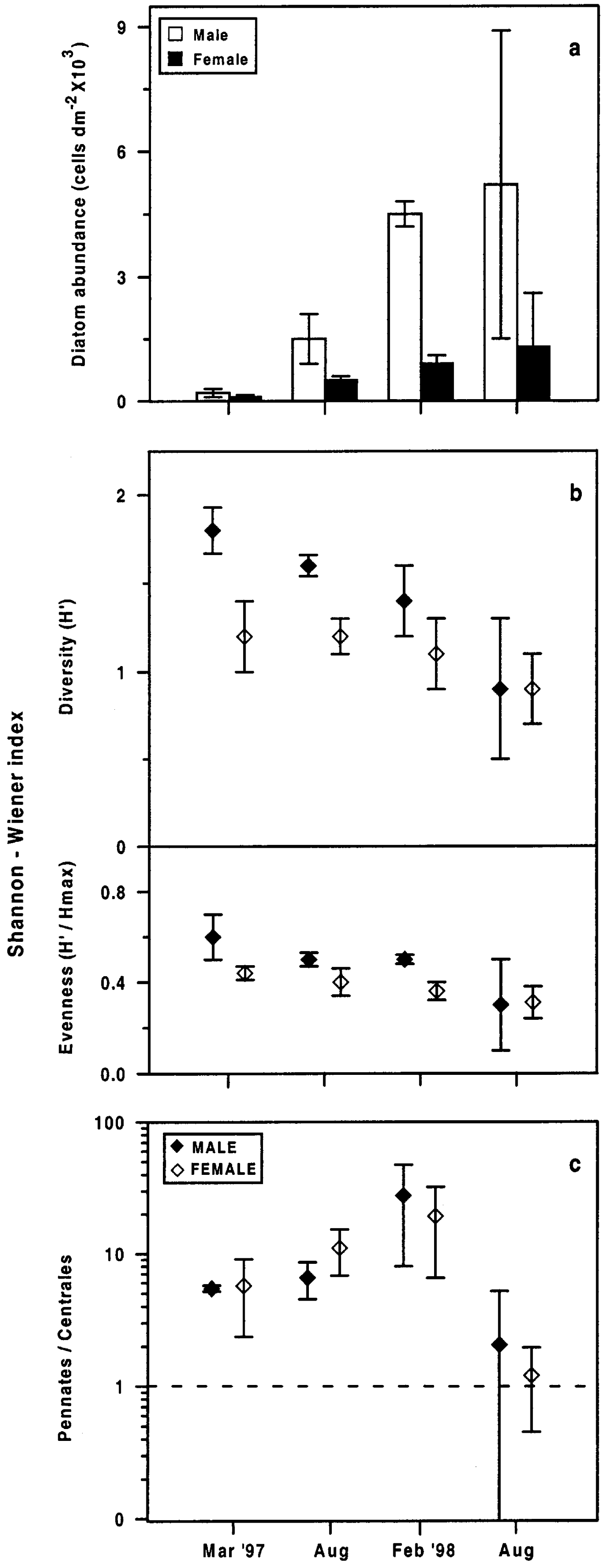

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Fig. 5 *Tachypleus gigas*. A comparison of **a** diatom cell abundance (cells dm^{-2} × 10^3), **b** diatom diversity (H') and evenness (H'/H_{max}) and **c** ratio of pennates/centrales in male and female horseshoe crabs during different occasions. Error bars indicate standard deviation

ranged from 31 to 90% in males and 52 to 93% in females. Among the pennates *Navicula* spp. and *Nitzschia* spp. and among the centrales *Skeletonema* sp. were dominant. Two-way ANOVA of diatom abundance (cells dm^{-2}) revealed that there is a significant variation between the sexes and the sampling period. Interaction of sampling period and sexes revealed insignificant variations, indicating influence of sampling period to be equal in both the sexes (Table 1).

Generic clustering of the diatom population revealed that *Navicula* spp., *Nitzschia* spp. and *Skeletonema* sp. were the most dissimilar forms encountered in both males and females (Fig. 6a, b). However, in the case of males these three forms merge with the rest of the community at a greater dissimilarity level (63.5), whereas in the case of females the merger was complete at lower dissimilarity level (34).

Macro-epibionts

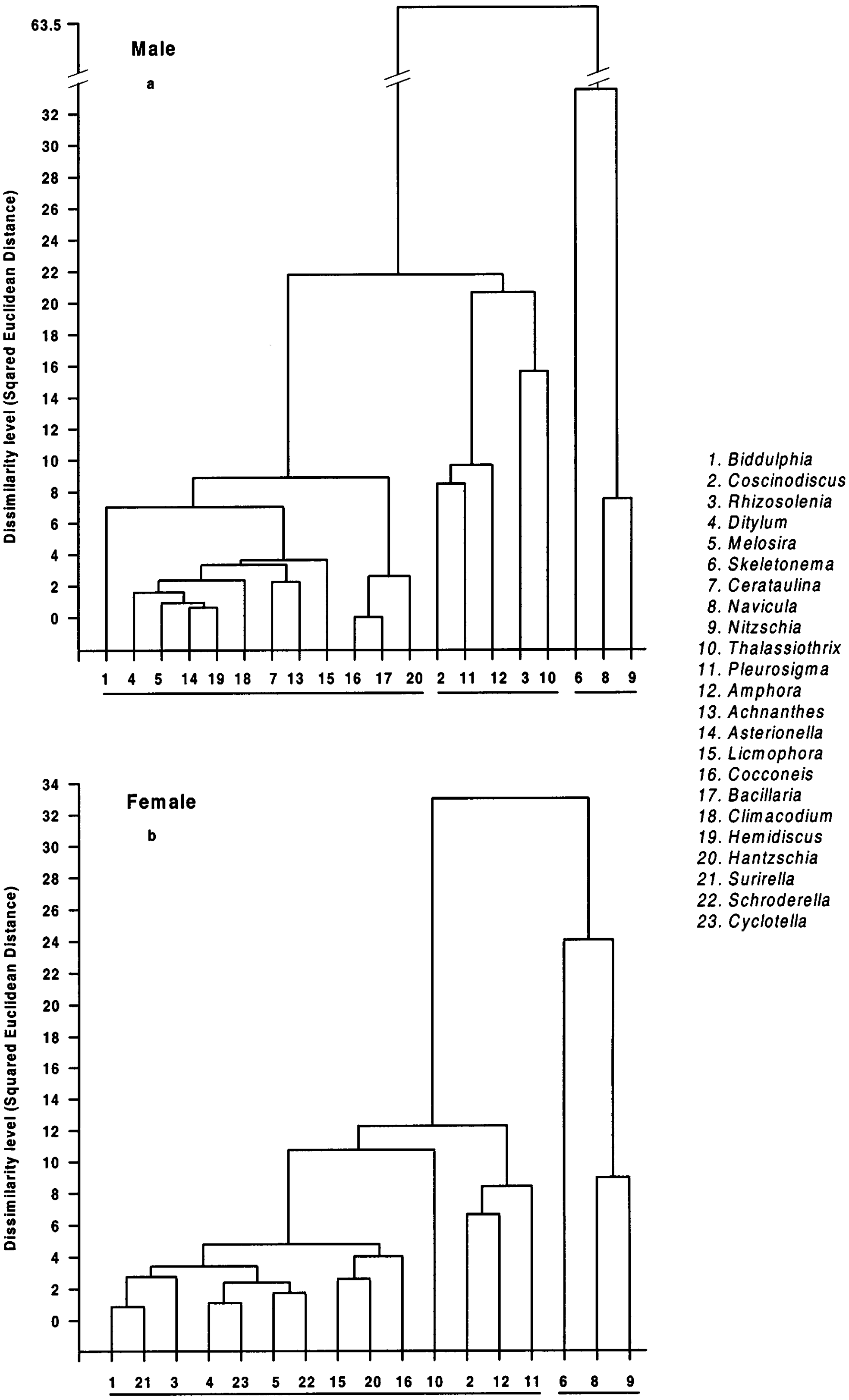
Macro-epibionts consisted of coelenterate *Metridium* sp. (sea anemone), the bryozoan *Membranipora* sp., the barnacle *Balanus amphitrite* (Darwin) and the bivalves *Anomia* sp. (false oyster) and *Crassostrea* sp. Taxon frequency and diversity of macro-epibionts were determined on the carapaces (dorsal side) of live specimens during the periods of sampling. Acorn barnacles (*B. amphitrite*) and encrusting bryozoans (*Membranipora* sp.) were the most abundant forms encountered in terms of coverage (Fig. 7).

Coverage of macro-epibionts was greater on male carapaces than on female carapaces (Fig. 8a). The macro-epibiont coverage was greater on the opisthosoma than prosoma (Fig. 8b, c). The telson of the horseshoe crab was free of macro-epibionts. The total area covered by macro-epibionts (which includes all forms recorded), when subjected to two-way ANOVA, revealed that there is a significant variation between the genders and between the prosoma and opisthosoma of

Table 1 *Tachypleus gigas*. Results of two-way ANOVA with uneven sample sizes comparing diatom density (cells dm^{-2}) between sexes (male and female) over period of sampling

	df	SS	MS	F_s	*p*
Subgroups (8: 4 samples for each sex)	7	5.9	0.8	6.5	≤0.005
Sampling period (4)	3	4.1	1.4	10.4	≤0.001
Sexes	1	1.6	1.6	12.4	≤0.005
Sampling period × Sexes	3	0.2	0.1	0.6	ns
Error	14	1.8	0.1		
Total	21	7.7			

Fig. 6 *Tachypleus gigas*. Generic cluster dendograms of the epibiotic diatom community from **a** male and **b** female horseshoe crabs



the carapace (Table 2). Cluster analysis revealed the total macro-epibiont coverage to be different between the sexes. The least dissimilarity was seen in the case of female prosoma and opisthosoma (Fig. 9a).

Barnacle abundance (ind dm^{-2}) and coverage was also greater on the male carapaces than on the female carapaces (Fig. 7). Barnacle coverage was less on the prosoma than on the opisthosoma in both the sexes

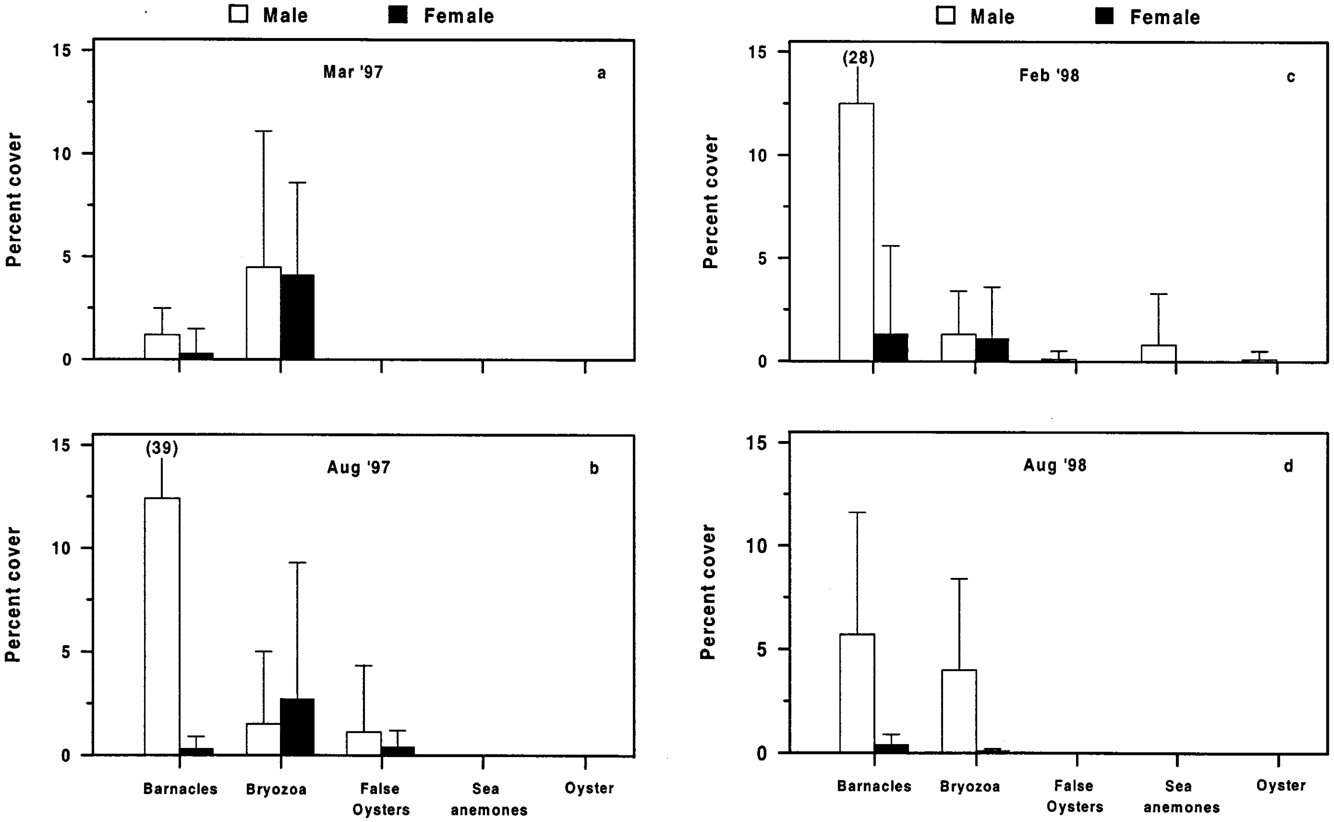


Fig. 7 *Tachypleus gigas*. Comparison of coverage of macro-epibionts on male and female horseshoe crab carapaces during different sampling periods (**a** to **d**). Error bars indicate standard deviation

(Fig. 10). ANOVA revealed a significant difference in barnacle coverage between the sexes and between the prosoma and opisthosoma of the carapace in both the sexes (Table 3). Cluster analysis of barnacle coverage showed a pattern similar to that of total macro-epibiont coverage, whereby the sexes separated out as different clusters. Male opisthosoma was the most dissimilar form, followed by male prosoma, and the least dissimilar were observed in the cases of female opisthosoma and prosoma (Fig. 9b).

Mapping studies revealed that the barnacle distribution (both adults and spat) on male and female carapaces was not uniform. In the case of females, no barnacles were found on the rim surrounding the cardiopthalmic region (i.e. the anterior portion of the prosoma which is subjected to mechanical abrasions) and in the posterior area of the opisthosoma (i.e. the area covered by males) (Table 4). The abundance of barnacles was highest in the rough zone (Prs_1 and Opt_1) of both female and male carapaces.

Barnacle abundance was less on female carapace than on male carapace during all the sampling periods. Fewer barnacles in the 6 to 8 mm size range (reproductive size of *Balanus amphitrite*) were seen on females than on males (Fig. 11). The size-frequency distribution of barnacles on female and male carapaces reveals that the recruitment of larvae to the carapace is high, but few recruits survive to maturity (as indicated by size) (Fig. 11).

Encrusting bryozoans were the other dominant organisms contributing to total macro-epibiont coverage (Fig. 7). The bryozoan coverage was greater in the case of males than females (Fig. 7). Encrusting bryozoan coverage was found to be less on the prosoma than on the opisthosoma in both sexes (Fig. 12). Coverage of encrusting bryozoans did not vary significantly between the sexes, but was significantly different between the parts of the carapace (Table 5). Cluster analysis for bryozoan coverage revealed less dissimilarity in comparison to total macro-epibiont coverage and barnacle coverage. The least dissimilarity was found among regions rather than sexes (Fig. 9c).

Discussion

Horseshoe crabs are slow-growing animals having a life span of 15 to 18 years. Their development to sexual maturity requires 9 to 10 years for males and 10 to 11 years for females (Mikkelsen 1988). After attaining sexual maturity crabs are in terminal anecdysis; the carapace covering the body is not shed. In spite of this, the carapace of the horseshoe crab is comparatively free of epibionts. In an environment where substratum availability is sparse in soft-bottom sediments, these hard-shelled organisms may serve as suitable substrata for epibionts.

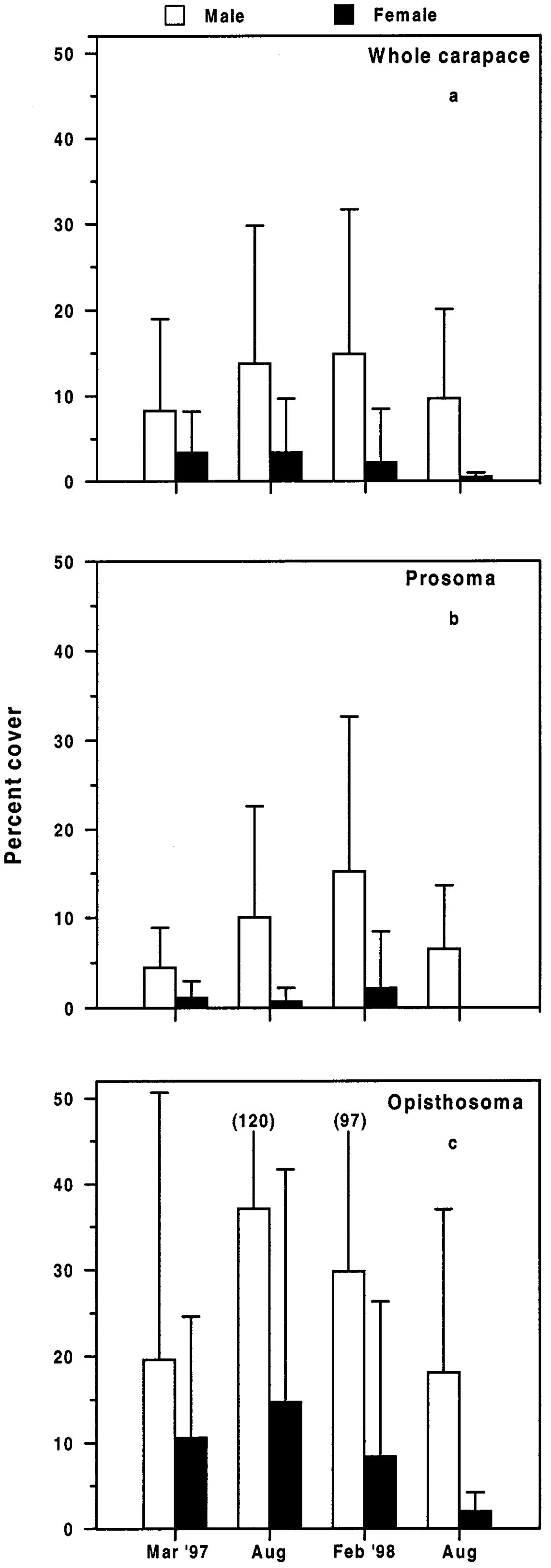


Fig. 8 *Tachypleus gigas*. Comparison of total macro-epibiont coverage between male and female horseshoe crabs on **a** the whole carapace, **b** the prosoma and **c** the opisthosoma during different sampling periods. Error bars indicate standard deviation

Table 2 *Tachypleus gigas*. Results of two-way ANOVA comparing macro-epibiont coverage between sexes (male and female) and between different parts (prosoma and opisthosoma) of carapace (*Prs* prosoma; *Opt* opisthosoma)

	df	SS	MS	F_s	*p*
Parts (Prs & Opt)	1	189.9	189.9	14.6	≤0.001
Sexes	1	3 555.4	3 555.4	27.4	≤0.001
Parts × Sexes	1	712.4	712.4	5.5	≤0.050
Error	180	23 394.4	129.9		
Total	183	29 561.0			

In this investigation we explored the differences in the epibiotic community of *Tachypleus gigas*, according to gender. The dominant diatom forms were *Navicula* spp., *Nitzschia* spp. and *Skeletonema* sp. in both the sexes (Fig. 6a, b). However, there were significant differences between the sexes in diatom abundance and diversity. The abundance, diversity and evenness values of diatoms were lower for females than for males (Fig. 5a, b).

Among the macro-epibionts, barnacles (*Balanus amphitrite*) and bryozoans (*Membranipora* sp.) were dominant on the carapaces of both the sexes (Fig. 7). Both of these organisms are important constituents of the macrofouling community in Indian waters (Karande 1965; Anil 1986). There were significant differences in coverage of macro-epibionts between the male and female carapaces. Total macro-epibiont coverage and coverage of barnacles and bryozoans were also lower on the carapace of female crabs.

The size-frequency distribution of barnacles indicated that settlement does take place in high numbers, but few barnacles remain attached until reproductive age. This conclusion is drawn from the observation made earlier that *Balanus amphitrite* attains sexual maturity at a size (basal diameter) of 7.3 mm. *B. amphitrite* reaches this stage in about 20 to 22 d under normal submerged conditions (Iwaki and Hattori 1987; authors' personal observations). The arrow marks in Fig. 11 indicate the abundance of barnacles that are in this size group in the epibiont community of the horseshoe crab. Here again, the larger barnacles were greater in abundance on the male carapace. Mapping of the macro-epibiont population belonging to this group also indicated considerable differences with different regions of the carapace (Fig. 2; Table 4). For both barnacles and bryozoans, the macro-epibiont coverage on the prosoma is less than on the opisthosoma. Differences in the structure of the epibiont community between sexes or among regions of the carapace may be related to: (1) changing habitat, (2) mechanical abrasion and surface availability, (3) requirements of epizootic larvae and/or (4) surface properties of the carapace.

Changing habitat

Tachypleus gigas is a marine species occurring on sandy beaches and muddy bottoms from the tide-line to depths

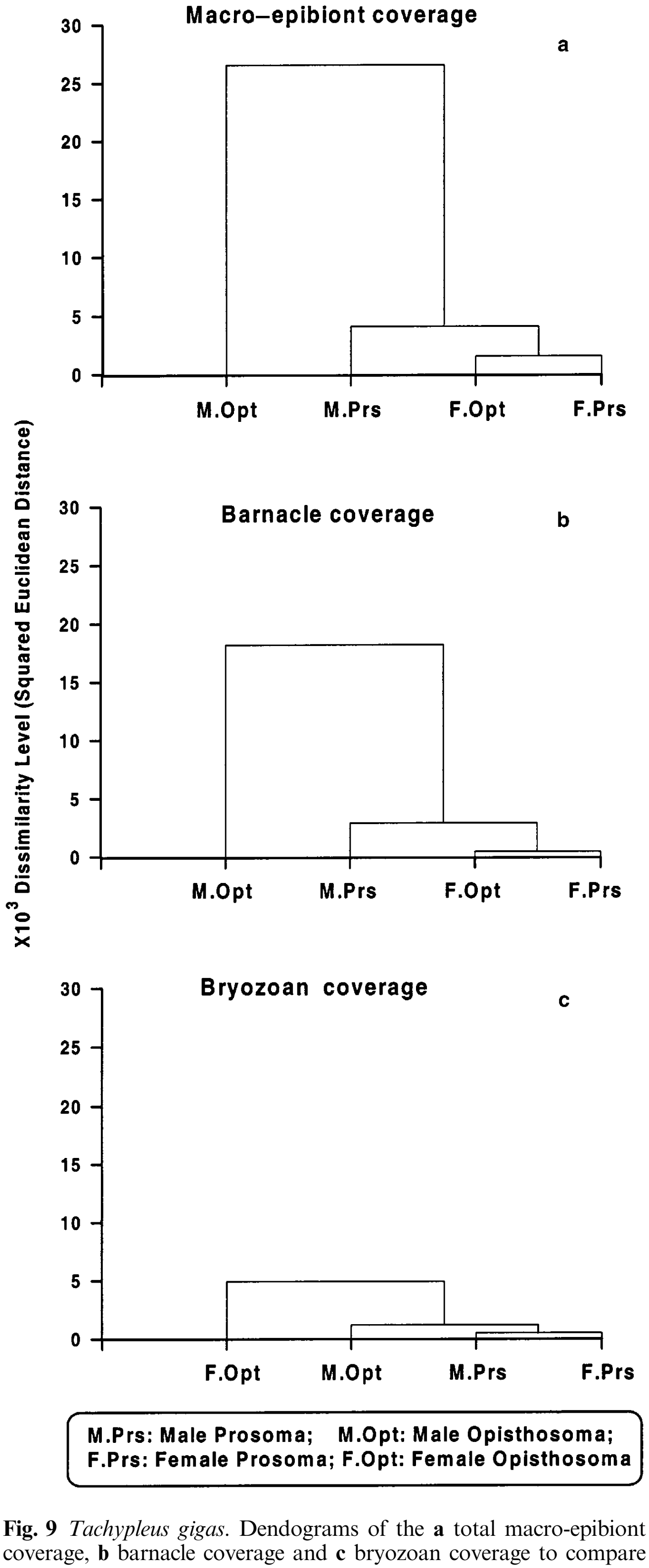


Fig. 9 *Tachypleus gigas*. Dendograms of the **a** total macro-epibiont coverage, **b** barnacle coverage and **c** bryozoan coverage to compare gender differences and that between the prosoma and opisthosoma

exceeding 37 m (Mikkelsen 1988). Amplexed pairs migrate from deeper regions to nearshore waters, with the male riding on the female crab for spawning during highest high tide. Migration occurs once every 2 weeks during highest high tide, and then crabs move back to their natural habitat with ebbing tide. Breeding of these crabs is year round (Chatterji 1994). Such a habitat change may exert stress on the epibionts. This has been suggested as a problem for epizoans on sea snakes (Key et al. 1995), sea turtles (Caine 1986) and epizoic bryozoans on the horseshoe crab *Carcinoscorpius rotundicauda* (Key et al. 1996). Desiccation and inhospitable depths may cause dislodgment/mortality of epibionts. This investigation revealed the dominance of barnacles and bryozoans in the epibiotic community. *Balanus amphitrite* is a eurytolerant organism, cosmopolitan in distribution, whose range extends to fringes of the marine environment including the supra-littoral zone. *B. amphitrite* has been reported to occur to depths of 40 m (Hutchins 1952). Owing to this broad distribution the physiological stresses caused by changing habitat may be considered negligible. During nesting, crabs spend considerable time on the beach laying eggs. Bryozoans are sessile, colonial animals commonly encountered in subtidal regions (Menon 1972). Menan (1973) also observed that *Membranipora* sp. generally prefer lower levels, i.e. 0.5 to 3 m below tidal levels. This natural distribution range indicates that subaerial exposure can have a negative impact on the epizoic bryozoans. In the case of diatoms such exposure may not markedly affect the community as several diatom genera are known to tolerate desiccation even at higher temperatures, due to the production of exopolysaccharides which function as antidesiccants (Evans 1959; Hostetter and Hoshaw 1970; Davis 1972; Hoagland et al. 1993). On the other hand, migration of the crabs to deeper waters may curtail the proliferation of diatoms due to light limitations.

Mechanical abrasion and surface availability

During mating, the male, which is almost always smaller, grasps the posterior half of the carapace of the female with the modified pincers of the second pair of feet, thus covering about 70 to 80% of the female opisthosoma. Macro-epibionts were not found in the posterior area of the carapace (i.e. the covered region) of females. The availability of undisturbed surface for macro-epibionts is less on female carapace, while the whole of the male carapace is exposed for epibiont colonization. The amplexed pairs remain in such a position for a considerable time, which further reveals that mating activities have the potential to prevent further epibiosis in the protected region of females. In the case of *Limulus polyphemus*, another species of horseshoe crab seen along the Atlantic and Gulf coasts of North America, amplexed pairs never separate, even after spawning (Barnes 1980). This phenomenon of prolonged amplexus, in which the male remains joined to the female during non-reproductive periods, has been explained as a mechanism to ensure access to the female by the male at the time of spawning (Rudloe 1980).

Fig. 10 *Tachypleus gigas*. Comparison of *Balanus amphitrite* **A**, **B** % cover and **C**, **D** abundance (ind dm^{-2}, of different size groups) between male and female horseshoe crabs on **A**, **C** prosoma and **B**, **D** opisthosoma during different sampling periods. Error bars indicate standard deviation

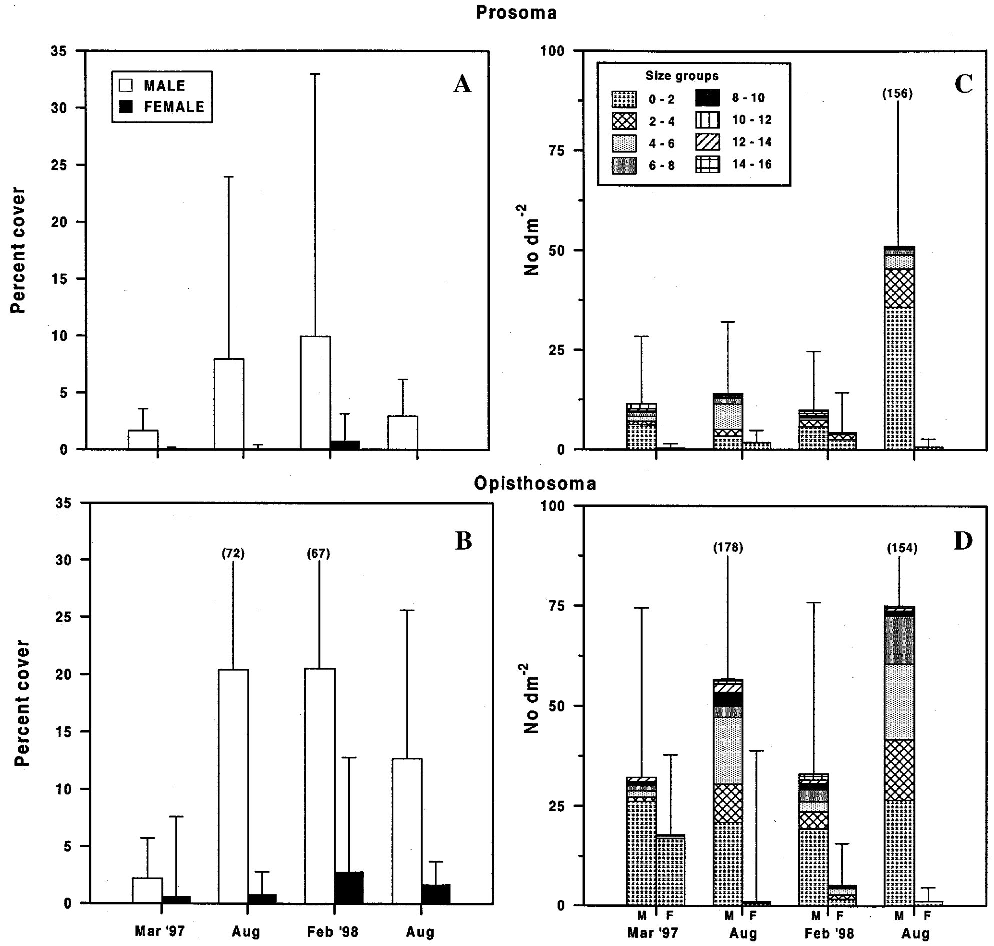


Table 3 *Tachypleus gigas*. Results of two-way ANOVA comparing barnacle (*Balanus amphitrite*) coverage between sexes (male and female) and between different parts (prosoma and opisthosoma) of carapace (*Prs* prosoma; *Opt* opisthosoma)

	df	SS	MS	F_s	*p*
Parts (Prs & Opt)	1	772.7	772.7	8.3	≤0.005
Sexes	1	2 477.5	2 477.5	26.5	≤0.001
Parts × Sexes	1	525.1	525.1	5.6	≤0.025
Error	180	16 806.9	93.37		
Total	183	20 582.2			

In the case of turtles, mating activities probably do not markedly affect carapace communities. Both the sexes have similar distributions of epibionts (Caine 1986). Turtles amplex only during breeding season and separate after mating so that both the sexes are exposed wholly for epibiosis.

During nesting, female horseshoe crabs generally bury themselves to the level of the lateral eyes. Females remain in this position, with occasional digging movements, for some time until egg laying and external fertilization occurs (Cohen and Brockman 1983). Once the fertilization is over, the female buries the eggs and begins to excavate the next nest. The

Table 4 *Tachypleus gigas*. Intensity of macro-epibiont distribution on marked areas of carapace. Observations from 46 carapaces for each sex during different sampling periods, March 1997 to August 1998. Numbers relate to the number of individuals in the case of barnacles and percentage occurrence in the case of bryozoans [*M* male; *F* female; *Prs₁* cardiopthalmic region; *Prs₂* anterior prosoma up to the level of lateral eyes; *Prs₃* flanks of the prosoma; *Opt₁* anterior opisthosoma (uncovered region); *Opt₂* posterior opisthosoma (covered region)]

Macro-epibionts	Prs$_1$		Prs$_2$		Prs$_3$		Opt$_1$		Opt$_2$	
	M	F	M	F	M	F	M	F	M	F
Barnacle spat	12 ± 58	2 ± 58	1 ± 5	0.3 ± 2	0.2 ± 1	0.2 ± 1	7 ± 16	4 ± 8	2 ± 8	1 ± 7
Adult	6 ± 11	1 ± 2	4 ± 8	0.1 ± 1	1 ± 3	0.1 ± 2	7 ± 15	2 ± 7	5 ± 12	0.2 ± 13
Bryozoa (%)	15	2	15	2	24	14	33	30	28	16

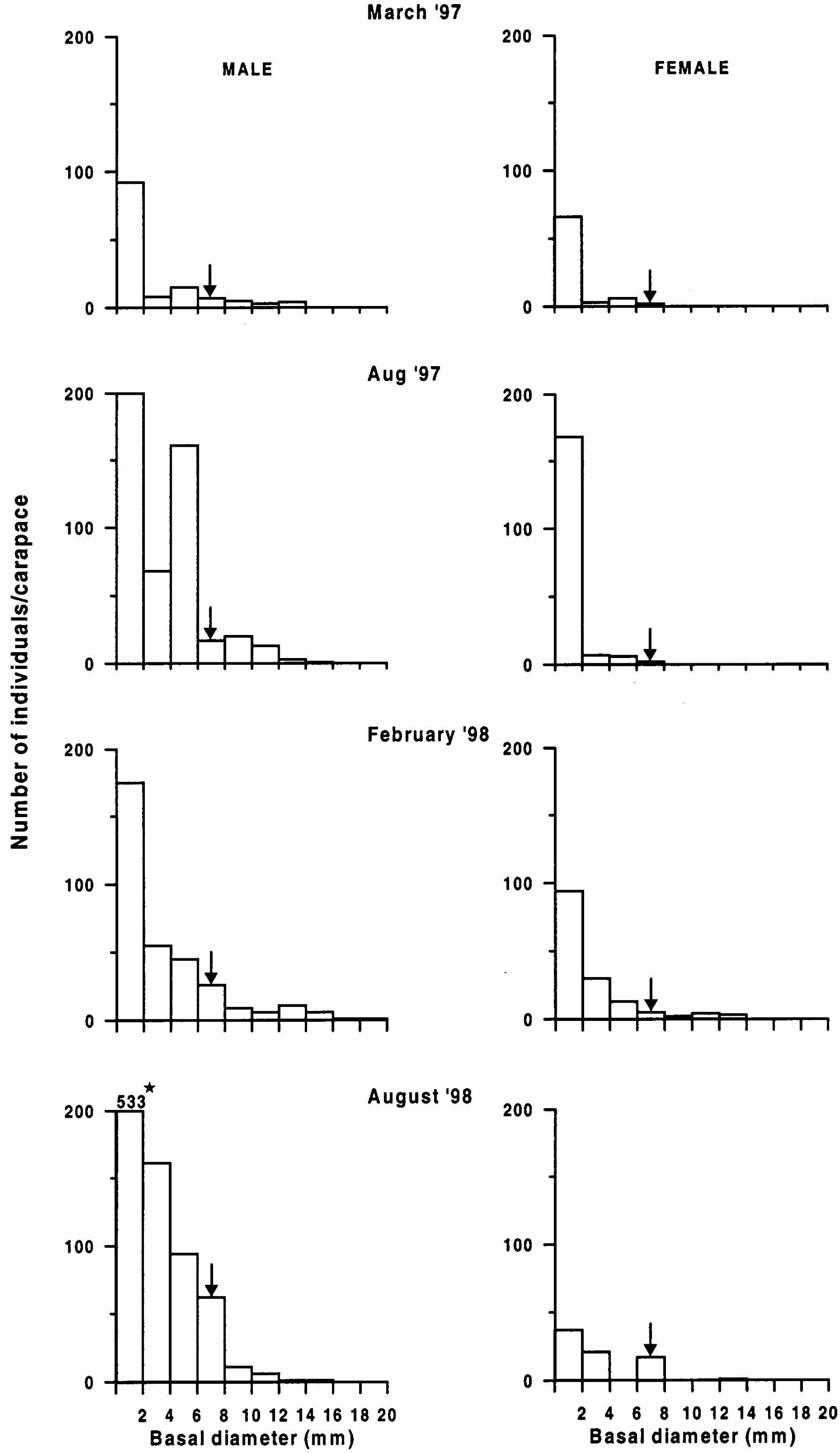


Fig. 11 *Tachypleus gigas*. Size-frequency distribution of *Balanus amphitrite* on male and female horseshoe crabs during different sampling periods (*arrows* sexually mature size class)

friction developed between the sediment and carapace of the female during nesting can dislodge or cause mortality of epibionts on females. Burying behavior of basibionts/hosts adversely affects the settlement and survival of epibionts (Mori and Zunino 1987; Abello et al. 1990; Becker and Wahl 1996). Barnacles were not found on the steep rim surrounding the cardiopthalmic region (Prs_1 and Prs_2) (Fig. 2; Table 4). This portion of the carapace is buried in the sediment during the nesting period.

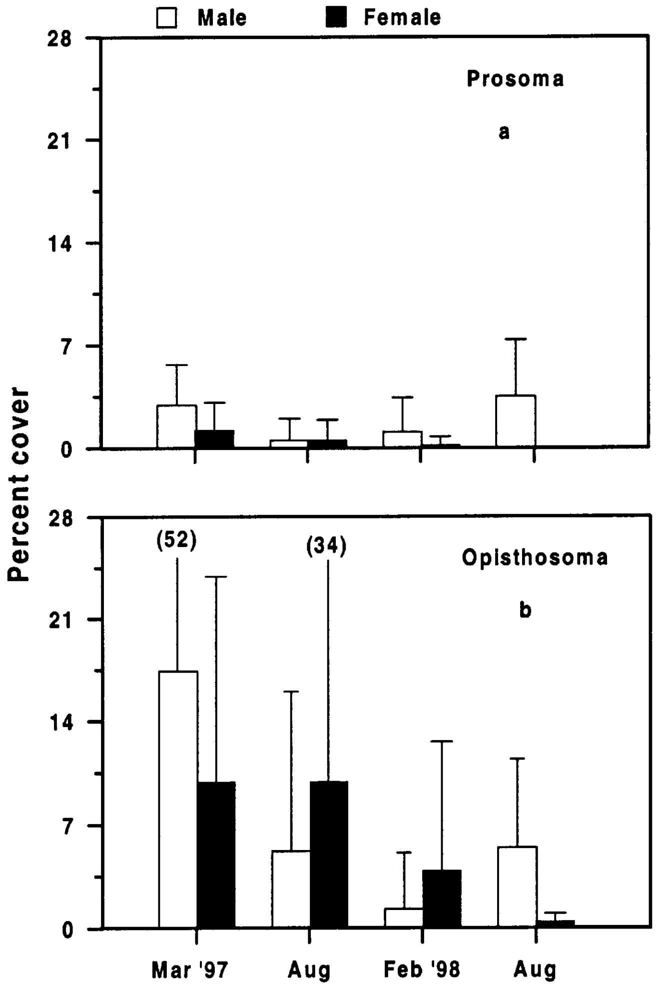


Fig. 12 *Tachypleus gigas*. Comparison of bryozoan coverage (*Membranipora* sp.) between male and female horseshoe crabs on **a** prosoma and **b** opisthosoma during different sampling periods. Error bars indicate standard deviation

Table 5 *Tachypleus gigas*. Results of two-way ANOVA comparing bryozoan (*Membranipora* sp.) coverage between sexes (male and female) and between different parts (prosoma and opisthosoma) of carapace (*Prs* prosoma; *Opt* opisthosoma; *ns* not significant)

	df	SS	MS	F_s	*p*
Parts (Prs & Opt)	1	234.9	234.9	6.6	≤0.01
Sexes	1	0.5	0.5	0.01	ns
Parts × Sexes	1	39.4	39.4	1.10	ns
Error	180	6441.5	35.8		
Total	183	6716.3			

Most shore crabs exhibit grooming activities (cleaning their carapace with the aid of appendages) as a defense mechanism to prevent fouling. Horseshoe crabs do not possess this capability as their appendages do not extend beyond the edge of the carapace.

Requirements of epizootic larvae

Differences in larval requirements for undergoing metamorphosis, for example phototaxis, surface roughness or chemistry, may influence the distribution of epibionts on the carapace. Most of the bryozoan larvae are known to be negatively phototropic at the time of metamorphosis, while most of the barnacle larvae (cyprids) are positively phototropic (Thorson 1964). Such differences in larval behavior have been used to interpret the distribution differences of barnacles and bryozoans on the carapace of portunid crabs, e.g. *Bathynectes piperitus* Manning and Holthuis, 1981 (Gili et al. 1993). Mapping studies in this investigation revealed that most of the barnacles were concentrated around entapophyseal pits on the posterior sloping opisthosoma, and on the opercular pleurite adjacent to the prosomal genal angle, along the prosoma longitudinal furrow and over the ophthalmic ridge, suggesting rugophilic (roughness seeking) and rhaeophilic (turbulence seeking) behavior on the part of the cyprid larva (Fig. 2; Table 4). Bryozoans concentrated closer to movable marginal spines, suggesting rugophilic (groove seeking) behavior. The flanks of the prosoma bore bryozoans, suggesting geophobic (antigravitational) behavior by settling bryozoan larvae (Gore 1995).

Surface properties of the carapace

The properties of the substrata have considerable influence on the metamorphosis of barnacle and bryozoan larvae and are well documented in biofouling studies (Rittschof and Costlow 1989; Maki et al. 1989, 1990, 1994; Anil and Khandeparker 1998). Wettability of a given surface plays an important role in the slime film formation and in attachment of settling larvae (Rittschof and Costlow 1989). The results of the surface wettability measurement indicated the male carapace to be slightly more hydrophobic than the female carapace. The study also revealed that the micro-epibiont population differed on male and female carapaces. Such a difference can also be influenced by the observed variations in the wettability. The electron microscopic evidence revealed that the male carapace is comparatively rougher than the female carapace. Star-shaped openings were also observed in the female carapace in Prs_1 and Prs_2 regions, suggesting the opening of pore glands. Mikkelsen (1988) suggested that horseshoe crabs keep their surfaces clean from ectocommensals and epiphytes by means of a glycoprotein exudate produced by hypodermal glands and secreted through the carapace. Females harbor large numbers of eggs on the ventral surface of the abdominal appendages. The eggs are toxic. The toxicity of the eggs has been related to the production of tetrodotoxin (Ho et al. 1994a, b). Strong alkaloids which are toxic have also been identified in the eggs and tissue of *Tachypleus gigas* and *Carcinoscorpius rotundicauda* (Mikkelsen 1988). Toxic compounds secreted through the carapace by means of pore canals in females may also play an important role in the control of epibiosis.

Summary

A study was carried out to evaluate the pattern of epibiosis in the horseshoe crab *Tachypleus gigas*. The results of this evaluation indicated that female horseshoe crabs harbor fewer epibionts compared to male horseshoe crabs. Pennate diatoms dominated the epibiotic community of micro-organisms. Among the pennates, *Navicula* spp. and *Nitzschia* spp. and, among the centrales, *Skeletonema* sp. were dominant in both the sexes. Among the macro-epibionts, acorn barnacles (*Balanus amphitrite*) and encrusting bryozoans (*Membranipora* sp.) were the dominant forms encountered. The size-frequency of barnacles reveals that settlement takes place in large numbers, but very few settlers grow to reproductive age. Greater numbers of sexually mature barnacles were found on the carapaces of male crabs. Results of the analysis of surface wettability and the scanning electron micrographs revealed that the male carapace was more hydrophobic and rougher than the female carapace. Mapping studies revealed that the distributions of macro-epibionts on male and female horseshoe crabs were not uniform. Such distinct distributions of macro-epibionts between genders and among regions of the carapace may be influenced by ecological and physical characteristics (migratory behavior, mating and nesting behavior, availability of undisturbed substrata during the nesting period, and wettability and roughness of the carapace) of the basibiont as well as the requirements of the settling epizootic larvae. It is also possible that toxic compounds associated with the eggs could be secreted out of the carapace and help in the control of epibiosis.

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References

Abello P, Villanueva R, Gili JM (1990) Epibiosis in deep sea crab population as indicator of biological and behavioural characteristics of the host. J mar biol Ass UK 70: 687–695

Anil AC (1986) Studies on marine biofouling in the Zuari estuary (Goa), west coast of India. PhD thesis, Karnatak University, Dharwad, India

Anil AC, Khandeparker RDS (1998) Influence of bacterial exopolymers, conspecific adult extract and salinity on the cyprid metamorphosis of *Balanus amphitrite* (Cirripedia: Thoracica). Mar Ecol 19(4): 279–292

Barnes RD (1980) Invertebrate zoology. Saunders College, Philadelphia

Becker K, Wahl M (1996) Behaviour patterns as natural antifouling mechanisms of tropical marine crabs. J exp mar Biol Ecol 145: 49–63

Caine EA (1986) Carapace epibionts of nesting loggerhead sea turtles: Atlantic coast of USA. J exp mar Biol Ecol 95: 15–26

Chatterji A (1994) The horseshoe crab – A living fossil. A Project Swarajya Publication, Cuttack, India

Cohen JA, Brockman HJ (1983) Breeding activity and mate selection in the horseshoe crab, *Limulus polyphemus*. Bull mar Sci 33: 274–281

Davis AR,White GA (1994) Epibiosis in a guild of sessile subtidal invertebrates in south-eastern Australia: a quantitative survey. J exp mar Biol Ecol 177: 1–14

Davis AR, Wright AE (1989) Interspecific differences in fouling of two congeneric ascidians (*Eudistoma olivaceum* and *E. capsulatum*): is surface acidity an effective defense? Mar Biol 102: 491–497

Davis JH (1972) Survival records in the algae and the survival role of certain algal pigments, fats and mucilaginous substances. Biologist 54: 52–93

Davis RE, Fried B (1977) Histological and histochemical observations on *Bdelloura candida* (Turbellaria) maintained in vitro. Trans Am microsc Soc 96: 258–263

Debnath R (1992) Studies on Indian horseshoe crabs (Merostomata: Xiphosura) with special reference to its feeding behaviour. PhD dissertation, Department of Marine Science, University of Calcutta, Calcutta, India

Evans JH (1959) The survival of freshwater algae during dry periods. J Ecol 47: 55–71

Fletcher M, Marshall KC (1982) Are solid surfaces of ecological significance to aquatic bacteria? Adv microb Ecol 6: 199–236

Gerhart DJ, Rittschof D, Hooper IR, Eisenman K, Meyer AE, Baier RE, Young C (1992) Rapid and inexpensive quantification of the combined polar components of surface wettability: application to biofouling. Biofouling 5: 251–259

Gili JM, Abello P, Villanueva R (1993) Epibionts and intermoult duration in the crab *Bathynectes piperitus*. Mar Ecol Prog Ser 98: 107–113

Gore J (1995) Epibiont distributions and experimental flow patterns around horseshoe crabs. In: Grassle JP, Kelsey A, Oates E, Snelgrove PV (eds) Twenty-third benthic ecology meeting. Institute Marine Coastal Sciences, Rutgers the State University, New Brunswick, New Jersey

Hasle GR (1978) Concentrating phytoplankton: the inverted microscope method. In: Sournia A (ed) Phytoplankton manual. Monographs on Oceanographic Methodology 6. UNESCO, Paris, pp 88–96

Ho, -B, Yeo DSA, Ding JL (1994a) Detection and evaluation of tetrodotoxin activity in *Carcinoscorpius rotundicauda* by cell culture technique. In: International Conference Advisory Committee (eds) 3rd International Marine Biotechnology Conference program, abstracts and list of participants. Tromsø University, Tromsø, Norway, p 135

Ho, -B, Yeo DSA, Ng HC, Tok WW, Ding JL (1994b) Marine bacteria from *Carcinoscorpius rotundicauda* produce tetrodotoxin activity. In: International Conference Advisory Committee (eds) 3rd International Marine Biotechnology Conference program, abstracts and list of participants. Tromsø University, Tromsø, Norway, p 135

Hoagland KD, Rosowski JR, Gretz MR, Roemer SC (1993) Diatom extracellular polymeric substances: function, fine structure, chemistry and physiol. J Phycol 29: 537–566

Hostetter HP, Hoshaw RW (1970) Environmental factors affecting resistance to desiccation in the diatom *Stauroneis anceps*. Am J Bot 57: 512–518

Humm HJ, Wharton GW (1942) Ecology of sand beaches at Beaufort, North Carolina. Ecol Monogr 12: 135–190

Hutchins LW (1952) Relations to local environments. In: Marine fouling and its prevention. Woods Hole Oceanographic Institute, Woods Hole, Massachusetts and U.S. Naval Institute Annapolis, Maryland, pp 102–117

Ingle RW (1983) Shallow water crabs. Synop Br Fauna NS 25: 1–206

Iwaki T, Hattori H (1987) First maturity and initial growth of some common species of barnacles in Japan. Bull Fac Fish Mie Univ 14: 11–19

Jackson JBC, Buss LW (1975) Allelopathy and spatial competition among coral reef invertebrates. Proc natn Acad Sci USA 72: 5160–5163

Jeffries WB, Voris HK, Yang CM (1989) Observations on the incidence of the pedunculate barnacle *Octolasmis warwickii* (Gray, 1825) on horseshoe crabs (Xiphosura) in the seas adjacent to Singapore. Raffles Bull Zool 37: 58–62

Karande AA (1965) On cirripede crustaceans (barnacles), an important fouling group in Bombay waters. Proc Symp Crustacea 1965. Ernakulam, Cochin Part IV: 1245–1252 (Mar biol Ass India)

Key MM Jr, Jeffries WB, Voris HK (1995) Epizoic bryozoans, sea snakes and other nektonic substrates. Bull mar Sci 56: 462–474

Key MM Jr, Jeffries WB, Voris HK, Chang M Yang (1996) Epizoic bryozoans, horseshoe crabs and other mobile benthic substrates. Bull mar Sci 58(2): 368–384

Mackenzie CL Jr (1979) Transportation of the oyster drill by horseshoe "crabs". Science 137: 36–37

Maki JS, Rittschof D, Samuelsson MO, Szewzyk U, Yule AB, Kjelleberg S, Costlow JD, Mitchell R (1990) Effect of marine bacteria and their exopolymers on the attachment of barnacle cypris larvae. Bull mar Sci 46: 499–511

Maki JS, Rittschof D, Schmidt AR, Snyder AG, Mitchell R (1989) Factors controlling attachment of bryozoan larvae: a comparison of bacterial films and unfilmed surfaces. Biol Bull mar biol Lab, Woods Hole 177: 295–302

Maki JS, Yule AB, Rittschof D, Mitchell R (1994) The effect of bacterial films on the temporary adhesion and permanent fixation of cypris larvae, *Balanus amphitrite* Darwin. Biofouling 8(2): 121–131

Menon NR (1972) Heat tolerance, growth and regeneration in three North Sea bryozoans exposed to different constant temperatures. Mar Biol 15: 1–11

Menon NR (1973) Vertical and horizontal distribution of fouling bryozoans in Cochin backwaters, southwest coast of India. In: Larwood GP (ed). Living and fossil Bryozoa. Academic Press, London, pp 153–164

Mikkelsen T (1988) The secret in the blue blood. Scientific Press, Beijing, China, pp 1–123

Mori M, Zunino P (1987) Aspects of the biology of *Liocarcinus depurator* (L.) in the Ligurian Sea. Investigación pesq 51 (Suppl 1): 135–145

Pielou EC (1984) The interpretation of ecological data. John Wiley and Sons, New York

Rao KVR, Rao KVS (1972) Studies on Indian king crabs (Arachnid, Xiphosura). Proc Indian natn Sci Acad 38: 206–211

Rasmussen E (1973) Systematics and ecology of the Isefjord marine fauna (Denmark). Ophelia 11: 1–495

Rittschof D, Costlow JD (1989) Bryozoan and barnacle settlement in relation to initial surface wettability: a comparison of laboratory and field studies. In: Ros J (ed) Topics in marine biology Proc 22nd eur mar Biol Symp. Vol. 53 (2–3). Inst-De-Ciencias-Del-Mar, Barcelona, pp 411–416

Roonwal ML (1944) Some observations on the breeding biology and on the swelling, weight, water content and embryonic movements in the developing eggs of the Moluccan king crab, *T. gigas* (Muller) (Arthropoda, Xiphosura). Proc Indian Acad Sci (Sect B) 20: 115–129

Rudloe A (1980) The breeding behavior and patterns of movement of horseshoe crabs, *Limulus polyphemus*, in the vicinity of breeding beaches in Apalachee Bay, Florida. Estuaries 3: 177–183

Rützler K (1970) Spatial competition among Porifera: solution by epizoism. Oecologia 5: 85–95

Saha D (1989) Status survey, breeding biology and some aspects of morphology by SEM study of xiphosurid arthropods in Indian coastal waters. PhD dissertation, Deptartment of Zoology, University of Calcutta, Calcutta, India

Shuster CN Jr (1982) A pictoral view of the natural history and ecology of the horseshoe crab *Limulus polyphemus*, with special references to other Limulidae. In: Bonaventura J, Bonaventura C, Tesh S (eds) Physiology and biology of the horseshoe crabs: studies on normal and environmentally stressed animals. Alan R Liss, New York, pp 1–52

Sokal RR, Rohlf FJ (1981) Biometry, 2nd edn. Freeman W. H & Company, San Francisco

Thorson G (1964) Light as an ecological factor in the dispersal and settlement of larvae of marine bottom invertebrates. Ophelia 1: 167–208

Vijaykumar R (1992) Ecology and biology of the horseshoe crab of the Indian coast. PhD thesis, University of Karnataka, Dharwad, India

Wahl M (1989) Marine epibiosis. 1. Fouling and antifouling: some basic aspects. Mar Ecol Prog Ser 58: 175–189