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Epibiont Infestation on Horseshoe Crab *Tachypleus gigas* (Müller) at Pantai Balok in Peninsular Malaysia

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Abstract
A one year study was conducted at Pantai Balok in the east coast of Peninsular Malaysia to investigate the epibiont infestation on horseshoe crab *Tachypleus gigas*. Number of horseshoe crabs landed was enumerated and each prosoma size (prosoma length and width) was measured. Prosoma width of the horseshoe crabs was used as a morphometric proxy to estimate the approximate age and instar stage of the individual crabs. All horseshoe crabs examined were adult individuals. The carapaces of horseshoe crabs were carefully examined for epibiont infestation. The epibiont was identified to genus level. In this study, acorn barnacle *Balanus*, pedunculate barnacle *Octolasmis*, conical slipper shells *Calyptraea* and flat slipper shells *Crepidula* were found on the carapaces of horseshoe crab *Tachypleus gigas* collected from Pantai Balok.

***Key words*:** Horseshoe crab, *Tachypleus gigas*, epibiont, Peninsular Malaysia

Introduction

Four horseshoe crab species were reported, extant throughout the world, namely *Limulus polyphemus*, *Tachypleus tridentatus*, *T. gigas* and *Carcinoscorpius rotundicauda* (Sekiguchi, 1988). The latter three species are found in the coastal waters of Malaysia (Christianus and Saad, 2009).

At Pantai Balok, the local fishing community harvests the females *T. gigas* to make soup with the prosoma carapace and make salad with the eggs. Some fishermen treat horseshoe crabs as by-catch. The horseshoe crabs are discarded from the fishing nets and tossed onshore. The horseshoe crabs are left onshore and die of desiccation. Therefore, horseshoe crab carcasses could be found along the beach.

Horseshoe crab carapace serves as substrate for the diatoms, coelenterates, flatworms, annelids, bryozoans, isopods, amphipods, gastropods, green algae, mussels, oysters, barnacles, tunicates, pelecypods, and polychaetes (Key *et al*., 1996a). Horseshoe crabs *T. gigas* examined from inshore regions of West Bengal were infested by sea anemones *Metridium*, polychaete *Gattyana*, barnacles *Balanus* and *Chthamalus*, amphipod *Cheiriphotis*, isopod *Cleantis*, bivalves *Ostrea*, brachyuran *Thalamita*, and bryozoans *Membranipora* (Rao and Rao, 1972).

The American horseshoe crab *L. polyphemus* is known as the “walking museum” because it carries many mobile and sessile organisms on its carapace. The organisms are either commensals or pathogenic such as the triclad flatworms *Bdelloura* and fast growing blue mussels

Mytilus edulis (Shuster, 2009). Other Asian horseshoe crab species do not harbor epibionts as many as the American horseshoe crab. Bryozoans, barnacles, tube-building polychaetes and mollusks (mussels, oysters, and slipper limpets) are the most common epibionts reported on *T. gigas*, *C. rotundicauda* and *L. polyphemus* (Botton, 2009).

In most case, the epibionts cause significant damage to the host, as in this case, the horseshoe crabs. For example, *Mytilus* attaching to the walking appendages and book gills of the horseshoe crabs, impeding the movement and breathing system of the host (Botton, 2009). The marine triclad flatworm *B. candida* deposit stalked egg shells into the gill lamellae of *L. polyphemus*. These flatworms were found in the last pair of cleaning legs, book gills, and joint of the walking appendages (Huggins and Waite, 1993). Meanwhile, the growth of barnacles, algae, or bryozoans on the lateral eyes of the horseshoe crabs could impact the vision of the host. However, severe green algal infection could also result the loss of ommatidia, making it difficult for the males to locate female for spawning purpose (Botton, 2009).

This study was conducted to investigate the epibiont species infested on carapace of *T. gigas* examined at Pantai Balok.

Materials and methods

Pantai Balok (3°56.257'N, 103°22.568'E) (Fig. 1), a sandy estuarine beach in Kuantan located in the east coast of Peninsular Malaysia had been chosen as our study site because horseshoe crab *Tachypleus gigas* uses to come ashore to spawn during high tides of full moon and new moon (personal communication).

The location of Pantai Balok was recorded using Garmin eTrex Vista. Pantai Balok in east coast of Peninsular Malaysia is experiencing north-east monsoon during November to February (Chua, 1984). This study site also experiences mix semi-diurnal tides (two unequal high and two unequal low tides per day). According to the Department of Survey and Mapping Malaysia (JUPEM, 2010), the tidal amplitude recorded was approximately 2.28 m during spring tide and reaching 3.0 m at maximum during full and new moon phases.

Figure 1. Location of Pantai Balok at east coast of Peninsular Malaysia

Days with high tide were selected by referring to tide table of Peninsular Malaysia for sampling activities. Sampling was conducted during both day and night. However, searches during night are quite challenging and time-consuming due to lack of lighting.

This study was conducted for one year, between May, 2009 and April, 2010.

Water parameters measured *in situ* were temperature, salinity, dissolved oxygen content and pH. Equipment used was a handheld multiparameter instrument YSI 556 MPS and parameters were measured at 0.1 m depth.

Visual search technique for horseshoe crab was based on its sighting along the beach at high tide line. Searches were made for approximately 1.5 hrs prior to each high tide of new and full moons and covering an area of 294.02 m. The horseshoe crabs could be seen partly buried in the sand (particularly the *Prosoma carapace* of the female) or rapid swimming searching for the right spot to nest. The horseshoe crabs were handpicked out of the sand and handled with care. Number of horseshoe crab was enumerated. Gender of the horseshoe crab was determined by checking the shape of the first pedipalps.

The prosoma sizes (length and width) of each horseshoe crab were measured using measurement tape and were employed as a morphometric proxy to estimate the approximate age and instar stage of horseshoe crab based on Sekiguchi (1988). Prosoma length was measured at the length of the median line of prosomatic carapace, to the nearest mm. Prosoma width was measured at the maximum width of prosomatic carapace, to the nearest mm (Yamasaki *et al.*, 1988).

The horseshoe crabs were carefully examined for epibiont infesting on the carapaces. Number of fouled horseshoe crabs was counted. All epibionts on each horseshoe crab were then removed using scalpel and preserved in 10% formalin and brought back to the laboratory for further taxonomic identification. Tagging of horseshoe crab was not conducted in this study due to budget constraint. Upon completion of sampling and data collection, the horseshoe crabs were released back into the water.

Results and discussion

Table 1 shows the mean and range of water quality parameters measured *in situ* between May, 2009 and April, 2010 to further describe the study site as a spawning site for horseshoe crab.

Table 1. Water parameters of Pantai Balok.

Parameters	Mean	Range
Temperature (°C)	30.22	28.41-32.73
Salinity (ppt)	30.60	27.70-31.93
DO (mg L^{-1})	6.45	5.13-7.86
pH	7.22	6.73-7.65

Table 2. Summary on instar stage estimation of *Tachypleus gigas* examined at Pantai Balok (based on Sekiguchi, 1988).

Prosoma width range (mm)	Instar stage	Number of crabs	
		Male	Female
154-194	14	55 (30)	1 (1)
194-244	15	19 (9)	42 (10)
> 244	16	11 (0)	33 (2)

Number of fouled crabs (bracketed values)

A total of 161 horseshoe crabs (86 males and 75 females) were collected and examined in this study. Based on Sekiguchi (1988), the horseshoe crabs examined at Pantai Balok were at instar stages 14 and 15, with prosoma width ranging from 154-194 mm and 194-244 mm, respectively. However, crabs with prosoma width more than 244 mm were categorized into instar stage 16 (Tab. 2).

Male horseshoe crabs at instar stage 14 were more prone to epibiont infestation (Tab. 2). This was mainly due to the mating and nesting behavior of female crabs (Cohen and Brockmann, 1983) and females molt more frequent than males. Male *T. gigas* reached maturity on the ninth year and female on the tenth year after 12 and 13 molts, respectively (Sekiguchi, 1988). All of the fouled horseshoe crabs in this study were adults in terminal anecdysis because juvenile horseshoe crabs molt frequently and would not be able to provide suitable substrate for the epibionts (Key *et al*., 1996a).

Horseshoe crabs were generally found burrowing shallowly in the sediment substrates when resting, eating, or spawning (Sekiguchi and Shuster, 2009). The prosoma of the female horseshoe crab was buried at the level of lateral eyes into the sand during nesting (Cohen and Brockmann, 1983), 70 to 80% of the opisthosoma was covered by the male holding onto the female using its first pedipalps. Epibionts were not found in the covered region of the carapace. Therefore in most cases, the male crabs would indirectly expose their carapaces and telson to epibionts (Patil and Anil, 2000).

Even though the bryozoans were not commonly found on mobile substrata where the host randomly exposes its outer surface (Key *et al*., 1996b) but there were two cheilostome bryozoans, *Biflustra savartii* and *Electra angulata* found infesting the carapaces of *T. gigas* living in shallow marine waters of Singapore (Key *et al*., 2000). Acorn barnacle *Balanus amphitrite* and encrusting bryozoan *Membranipora* sp. were infesting the carapaces of *T. gigas* in Burhabalanga estuary in Orissa coast (Patil and Anil, 2000). Other encrusting invertebrates found on the horseshoe crabs carapaces were tube-building polychaetes and mollusks (mussels, oysters, slipper limpets) (Botton, 2009).

In this study, four epibiont species were found infesting on the carapaces of horseshoe crab *T. gigas.* Among the epibionts, barnacles were the most dominant form found on 27.3% of horseshoe crabs whereas 1.8% of the crabs were infested by slipper shells (Tab. 3).

Table 3. Frequency of occurrence of epibiont species on *Tachypleus gigas* at Pantai Balok, May, 2009-April, 2010.

Epibiont species	Frequency of occurrence Both sexes (n=161)	Females (n=75)	Males (n=86)
Barnacles			
Acorn barnacle *(Balanus)*	28 (17.4%)	4 (5.3%)	24(27.9%)
Pedunculate barnacle *(Octolasmis)*	16 (9.9%)	8 (10.7%)	8 (9.3%)
Slipper shells			
Conical slipper shells *(Calyptraea)*	2 (1.2%)	1 (1.3%)	1 (1.2%)
Flat slipper shells *(Crepidula*	1 (0.6%)	0	1 (1.2%)

Figure 2. Conical slipper snails *Calyptraea* and flat slipper snails *Crepidula* on a male horseshoe crab *Tachypleus gigas*. (Scale bar 60 mm)

Conical slipper shells *Calyptraea* sp. and flat slipper shells *Crepidula* sp. were found on the ventral opisthosoma of horseshoe crabs (Fig. 2). Acorn barnacle *Balanus* sp. was found on the dorsal prosoma and opisthosoma whereas pedunculate barnacle *Octolasmis* sp. was found on the ventral prosoma (Fig. 3) and on walking appendages. Epibiont coverage was mainly concentrated on the carapaces.

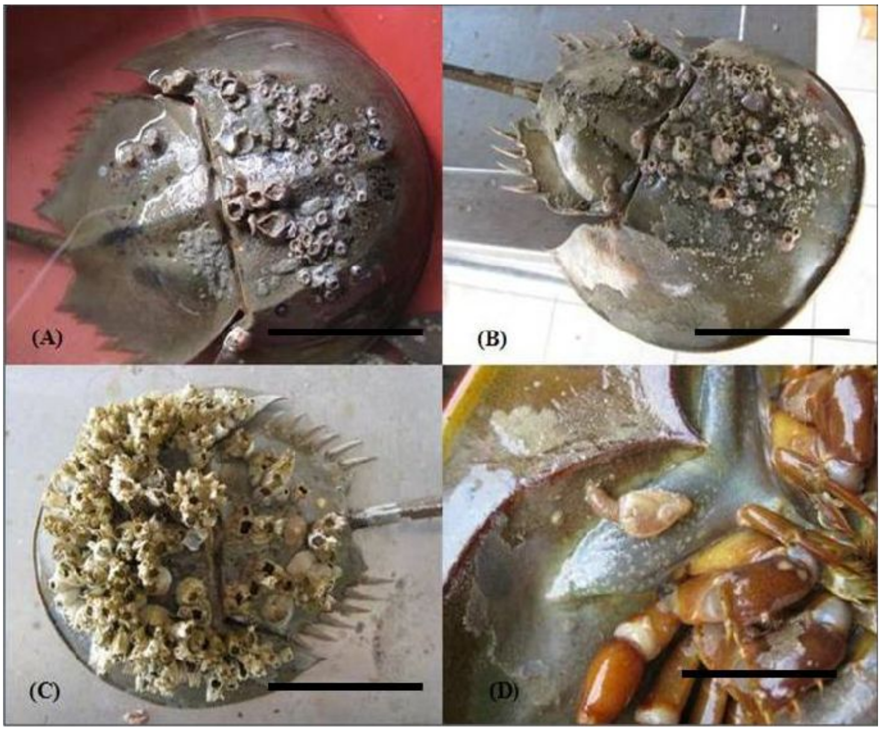

Figure 3. Barnacles infesting carapace of horseshoe crab *Tachypleus gigas*. (A, B) Acorn barnacles *Balanus* living on dorsal carapace, (C) dead acorn barnacles *Balanus* and conical slipper snails *Calyptraea* on dorsal carapace, (D) pedunculate barnacle *Octolasmis* on ventral prosoma. (Scale bar 60 mm)

The telson of the horseshoe crab was free of epibiont.

Acorn barnacle *Balanus* sp. is a coastal and estuarine biofouling organism. It is commonly found on hard surfaces such as rocks, oyster beds and mollusk shells and also on ship hulls, pilings, and seawalls (Cohen, 2005).

Intertidal barnacle species is more tolerant to extreme temperature than non-intertidal species. Ability in temperature tolerance allows the species to live at greater depths (Werner, 1967). *Balanus* is eurythermal (Cohen, 2005). Low temperature restricts reproduction activity (Bishop, 1950). High temperature is a significant factor limiting barnacle zonations on tropical shores. *Balanus* is prone to desiccation and erosion and somewhat mitigated by the growth of shell plates (Foster, 1974). High temperature accelerates water loss in juveniles with larger surface area to volume ratios and poorer integumental permeability which eventually leads to dehydration and death (Foster, 1974).

Balanus is euryhaline and could survive in tropical estuaries where seasonal monsoon leads to salinity declining to as low as 4ppt (Desai *et al*., 2006). Balanomorph barnacle encloses its body surface with fewer tissues directly exposed to the environment to withstand lower salinity waters in contrast to Lepadomorph barnacle exposing its body surface with their long peduncle (Key *et al*., 1996a). Temperature determines the length of brooding season and influences molting rate of epibiont. Observation of gonad development of species thriving in tropical environment is said to be related to chlorophyll a concentration (Desai *et al*., 2006).

Octolasmis hosts are potentially found in shallow littoral or pelagic waters. Octolasmid is distributed widely in tropical and temperate seas (Jeffries *et al*., 1995) and commonly found in shallow water at depths of 10m (Jeffries *et al*., 1989a) and with only a few species are found in depths greater than 1000m (Jeffries and Voris, 1996). Current and the amount of unutilized substrate may be factors attributed to determination of distribution (Jeffries *et al*., 1982).

Pedunculate barnacle *Octolasmis warwickii* was previously reported in horseshoe crab *T. gigas* collected from Singapore. The female crabs carried more individual barnacles than the males (Jeffries *et al*., 1989b). The barnacles were distributed over the ventral prosoma and walking appendages. Very few were found on the opisthosoma and none on the book gills and dorsal prosoma (Jeffries *et al*., 1989b). Most of the species in the genus *Octolasmis* were symbiotic on decapod Crustacea (Voris *et al*., 1994). It was commonly found infesting the gill chambers of mangrove crab *Scylla serrata*. This species was unevenly distributed in the proximal, medial and distal portions of the gills (Jeffries *et al*., 1982). *Octolasmis* also reported distributed on skin of marine snakes (Jeffries and Voris, 1979).

A male horseshoe crab was found entangled in fishing net discarded onshore. The carapace was heavily fouled by dead acorn barnacles and slipper snails (Fig. 3C). Heavy barnacle infestation could reduce mobility of the host and eventually leads to mortality (Key *et al*., 1996a). Desiccation may have caused the mortality of the epibionts when the carapace of the horseshoe crab was exposed out of the water and to high temperature during receding tides. But the horseshoe crab itself was still alive because the book gills were moist.

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