

## Demographics of Horseshoe Crab Populations in Kota Kinabalu, Sabah, Malaysia with Emphasis on *Carcinoscorpius rotundicauda* and Some Aspects of its Mating Behaviour

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### ABSTRACT

Survey results of two populations of *Carcinoscorpius rotundicauda* at a fishing ground and within a protected area were compared so as to establish effects of human activities on the species. *Tachypleus tridentatus* and *Tachypleus gigas* were also found in both sites but in substantially less abundance. The operational sex ratios were male-biased at both sites; 5.50 ( $n = 52$ ) at the fishing ground (Site 1) and 2.58 ( $n = 68$ ) at the protected area (Site 2). Size distributions at both sites were similar of which females were approximately 16% larger than males. A captive experiment was conducted where pair-forming behaviour of *C. rotundicauda* was observed for 30 days. Amplexus were most frequently formed when the sex ratio was balanced, lasting for  $2.44 \pm 2.03$  days, and eight days maximum. Female body size and amplex-forming frequency were positively correlated ( $r = 0.678$ ,  $n = 7$ ,  $p = 0.10$ ), attributable to fitness projected by the phenotype. Solitary males did not exhibit aggression towards paired males, allowing the latter to retain mate exclusivity for considerable periods. Anthropogenic pressures caused an unnatural shift in the population structure of local horseshoe crabs that could lower their reproductive success, making eventual extirpation possible.

*Keywords:* *Carcinoscorpius rotundicauda*, horseshoe crab, mating behaviour, population structure, *Tachypleus gigas*, *Tachypleus tridentatus*

### ARTICLE INFO

#### Article history:

Received: 16 December 2013

Accepted: 6 June 2014

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### INTRODUCTION

All three Asian species of horseshoe crab are present in the waters of Sabah, namely, the tri-spine horseshoe crab, *Tachypleus tridentatus*, the Indian horseshoe crab,

*Tachypleus gigas*, and the mangrove horseshoe crab, *Carcinoscorpius rotundicauda* (Waterman, 1958; Sekiguchi, 1988a). A survey conducted from 2004 through 2006 along the coastal fishing villages in Peninsular Malaysia and Sabah indicated that the population of horseshoe crabs in Malaysia is undergoing a rapid decline (Christianus *et al.*, 2008). Horseshoe crabs exhibit site-specificity especially in areas where eggs and juveniles have developed successfully, making them vulnerable to overexploitation. In addition to being philopatric, the populations of horseshoe crabs have low genetic connectivity and high genotypic variations based on molecular genetics data (Obst *et al.*, 2012; Rozihan *et al.*, 2013) attributed to low migratory ability and hence demographic exchange between them. Unlike two other Asian species, *C. rotundicauda* does not travel out to deeper water after spawning since adults can be found buried 2 - 3 cm deep in the mud, while juveniles remain near the surface (Cartwright-Taylor *et al.*, 2011). Habitat loss is particularly hazardous to the genetically and ecologically constrained *C. rotundicauda* in Kota Kinabalu city where rapid coastal developments and reclamation over the past few decades have greatly decimated its mangrove cover.

All Asian horseshoe crab species share a similar habit of travelling upstream following incoming tides during full moon and new moon to spawn (Sekiguchi *et al.*, 1988; Chatterji *et al.*, 1992). A five-year survey on *C. rotundicauda* in Mandai Mudflats at Kranji in Singapore found that

spawning occurred year-round and the sex ratio was balanced, showing consistently little change (Cartwright-Taylor *et al.*, 2009; Cartwright-Taylor & Hsu, 2012). Male dominant populations are regarded as a rarity in Asian horseshoe crabs, while male-biased populations are a norm in the American horseshoe crab, *Limulus polyphemus* (Sekiguchi *et al.*, 1988; Brockmann & Smith, 2009). A mating pair of horseshoe crab consists of a male mounting the back of a female and clasping the latter at the margin of its opisthosoma, forming an amplexus.

*Limulus polyphemus* is the most intensely-studied marine invertebrate in the world and its mating ecology has been well documented relative to the Asian species. According to Botton *et al.* (1996), although the Asian horseshoe crabs and *L. polyphemus* have over 100 million years of isolation in their lineages, their mating system are fundamentally the same. Studies showed that the species' behaviour in selecting mates is governed by a number of sensory cues which include visual, chemical and tactile cues (Hassler & Brockmann, 2001; Barlow, 2009; Saunders *et al.*, 2010). Size was suspected to play a role in mating selection as males tended to form amplexus with larger females (Suggs *et al.*, 2002). The same could be said of the Asian species since all the four extant species of horseshoe crabs share a lot of common features in terms of their biology and life history.

In Sabah, there have been very few studies done to determine the population status of horseshoe crabs. Demographic

data on vulnerable species are vital in understanding the mechanisms that are driving their decline, especially factors that perturb the balance between fecundity and longevity (see Owens & Bennett, 2004). Thus, this study aimed to compare the ecological status of *C. rotundicauda* populations at a fishing ground and a protected area in the vicinity of Kota Kinabalu to demonstrate the effects of anthropogenic pressure on a population and to provide information on some aspects of its mating behaviour. A brief discussion on *T. tridentatus* and *T. gigas* in Sabah is also included here so that the results of this study can be used as baseline data to be adopted in future appraisals of horseshoe crabs at other localities.

## MATERIALS AND METHODS

### *Study Sites*

Surveys were conducted at two sites, Menggatal River (Site 1) (6° 3' 52.9"E, 116° 7' 21.1"N) and Kota Kinabalu Wetland Centre (Site 2) (5° 59' 6.07"N, 116° 5' 14.48"E) (Fig.1). Site 1 is a river that is approximately 60 m wide and lined with narrow (5 to 10 m) strips of mangroves that, at most parts, have been cleared by fishermen living along the banks. Fishing activities are intense in the area for subsistence and commercial harvest of a myriad of species including bivalves and gastropods that form the major dietary components of horseshoe crabs (see Akbar John *et al.*, 2012). Site 2 is a 2.4 km<sup>2</sup> patch of mangrove which was once part of an extensive mangrove forest lining the coastline of Kota Kinabalu. Site 2

was accorded Bird Sanctuary status in 1996 and declared a State Cultural Heritage Site in 1998. A small stream that is approximately 10 m in width connects the mangrove patch to Likas Bay, where horseshoe crabs have been frequently sighted. Samples collected were brought to Borneo Marine Research Institute (BMRI) of Universiti Malaysia Sabah, Kota Kinabalu, for further analyses and captive experiment.

### *Sampling Technique*

Periodicity of breeding activities of *C. rotundicauda* at both sites coincided with lunar phases, as reported by Sekiguchi (1988b). In order to ensure sampling efficiency, horseshoe crabs were intercepted when they were swimming upstream into the mangrove to spawn, usually in the evening following the rising tide. Sampling was conducted twice per month, i.e. during spring tides of full moon and new moon for two consecutive days in the span of four months (a total of 16 nights) from October 2008 through January 2009 at Site 1 and from January through April 2013 at Site 2.

Horseshoe crabs at Site 1 were caught using three-layer trammel nets that had been deployed along the banks of the river with the help of a local fisherman. The nets were left overnight and checked for horseshoe crabs in the morning. Meanwhile, samples from Site 2 were collected through direct capture because accumulation of debris in the nets deployed made them difficult to be hauled. The conditions in which the individuals from Site 2 were found (solitary or amplexed) were also recorded to

provide supplementary data for the mating behaviour study. Amplexus in Site 1 were often dissociated upon entanglement in the net, and thus, it could not be ascertained whether the individuals caught were solitary or amplexed. Captures were brought to BMRI for species identification, sexing, and to be measured.

Specimens were placed in culture tanks with aerated seawater that was partially exchanged once every day. In captivity, they were fed chopped fish and squid, as

recommended by Lee and Morton (2005). The samples were then identified and sexed using key morphological characteristics as described by Yamasaki *et al.* (1988). The operational sex ratios (OSRs) defined as the ratio of adult individuals (Emlen & Oring, 1977) was computed as the number of males per female. Size was determined by measuring the maximum prosomal carapace width to the nearest 0.02 mm using a Vernier calliper. To prevent duplications in the results, the captures were released at

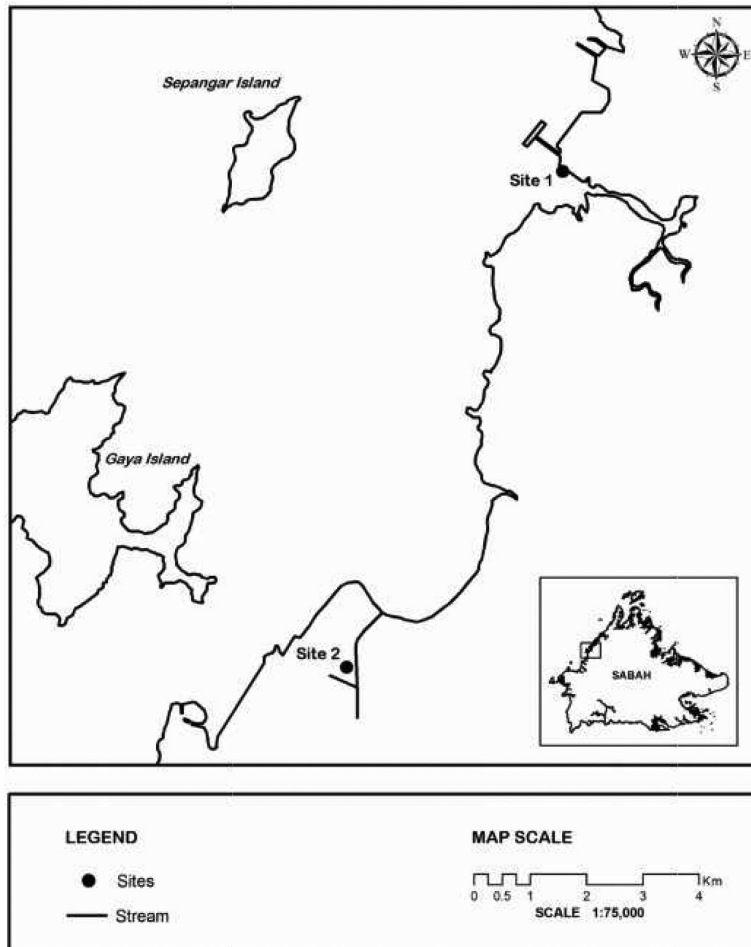


Fig.1: Map showing the sampling sites for horseshoe crabs in Kota Kinabalu

the original sites where they were collected only after sampling ceased.

#### *Experiment of Mating Behaviour*

A captive experiment to investigate the mating behaviour of *C. rotundicauda* was carried out using randomly selected individuals from Site 2. They were marked using oil paint with serial numbers and their sizes were measured and recorded, as shown in Table 1. Three circular HDPE tanks of 2m diameter, filled with 0.5 m deep seawater, were used (Tanks A, B, and C). Four males were put in each tank before four females

were placed in Tank A, two in Tank B, and one in Tank C to give a male:female ratio of 1:1, 2:1 and 4:1, respectively. The experiment commenced when the females were added into the tanks. Observation was made for the first two hours to determine the duration for the formation of the first amplexus and subsequent observations for pair formations were made once every day at 1100 hours for 30 consecutive days.

## RESULTS AND DISCUSSION

Adult *C. rotundicauda*, *T. gigas*, and *T. tridentatus* were found in Site 1 during

TABLE 1

Serial number, size (in mm) and maturity conditions of the male and female individuals used in the captive experiment on mating behaviour

Tank (OSR)	Horseshoe crab no.	Prosomal width (mm)	Maturity condition
Tank A (1:1)	CRM1	137.00	Mature
	CRF1	151.38	Ovigerous
	CRM2	123.62	Mature
	CRF2	152.00	Ovigerous
	CRM3	138.00	Mature
	CRF3	162.26	Ovigerous
	CRM4	128.40	Mature
	CRF4	159.10	Ovigerous
Tank B (2:1)	CRM1	133.34	Mature
	CRF1	136.70	Ovigerous
	CRM2	124.82	Mature
	CRF2	159.30	Ovigerous
	CRM3	124.34	Mature
	CRM4	135.40	Mature
Tank C (4:1)	CRM1	126.28	Mature
	CRF1	150.26	Ovigerous
	CRM2	138.52	Mature
	CRM3	127.50	Mature
	CRM4	126.88	Mature

surveys in October 2008 through January 2009. However, only adult *C. rotundicauda* and juvenile *T. tridentatus* were found during the January through April 2013 surveys in Site 2. Nonetheless, this finding confirmed the previous report by Sekiguchi (1988a) on the overlapping habitat of all three species in Sabah. A female *T. tridentatus* from Site 1 was 421.0 mm, larger than the 393.0 mm reported by Yamasaki (1988) who noted that *T. tridentatus* from Kota Kinabalu were the largest compared to its conspecifics in Southeast Asia. Of the 61 individuals from Site 1, 53 were *C. rotundicauda* (52 adults and a small juvenile). The OSR of *C. rotundicauda* in Site 1 was male-biased, with only eight (15%) adult females and 44 (85%) adult males to give an OSR of 5.50. The size of the males was averaged at 130.0  $\pm$  5.3 mm ( $\pm$  *S.D.*; *n* = 44), ranging from 120.4 mm through 145.3 mm, while the females were averaged at 150.8  $\pm$  7.7 mm ( $\pm$  *S.D.*; *n* = 8) and ranged from 139.6 mm through 160.9 mm (Figures 2 and 3). This indicates that the females are 16% larger than the average males.

Slightly more *C. rotundicauda* were caught in Site 2. The captures comprised of 68 adult *C. rotundicauda* and four juvenile *T. tridentatus*. There were 49 (72%) males and 19 (28%) females, giving a less-skewed OSR of 2.58. The average female size ( $\pm$  *S.D.*) was 148.7  $\pm$  10.7 mm, which is approximately 16% larger than the average male ( $\pm$  *S.D.*; 128.2  $\pm$  5.9 mm) (see Fig.2). The largest male was 143.9 mm, while the smallest was 115.4 mm, and the sizes of the females ranged between 129.0 mm through

171.2 mm (the largest *C. rotundicauda* in this study) (Fig.3). Adults from Site 2 had a wider size range but they were on average smaller than the adults in Site 1 (Fig.2); this is attributable to inter-population variations. Eighteen amplexed pairs were collected at Site 1. The mean ( $\pm$  *S.D.*) size of the females in amplexus was 149.4  $\pm$  10.6 mm (*n* = 18). The mean size ( $\pm$  *S.D.*) of the amplexed males was 129.3  $\pm$  6.8 mm (*n* = 18), which was slightly larger than the size of solitary males ( $\pm$  *S.D.*; 127.6  $\pm$  5.4 mm, *n* = 31). Only one pair was size-matched as the amplexed females were always bigger than their mate, with the mean percentage difference ( $\pm$  *S.D.*) of 15.8  $\pm$  10.5% (*n* = 18) ranging from as little as 3% to 37%.

In the captive experiment, the first amplexus was formed 55 minutes after the experiment had commenced in Tank B. The amplexus were more frequently formed in Tank A, observed in 18 days out of 30. The longest lasted for eight days between CRF3 and CRM4 in Tank A. They were a recurrent pair, forming amplexus on three separate occasions for a total 11 days. The biggest female in Tank A (CRF3) formed amplexus with the highest frequency and it was the only female which was courted by every male. The biggest female in Tank B (CRF2) was the only one paired, while the other female was ignored. Some individuals did not form amplexus throughout the experiment. The average number of days ( $\pm$  *S.D.*) for every pairing episode was 2.44  $\pm$  2.03 days.

In captivity, the females in amplexus were always bigger than the males attached

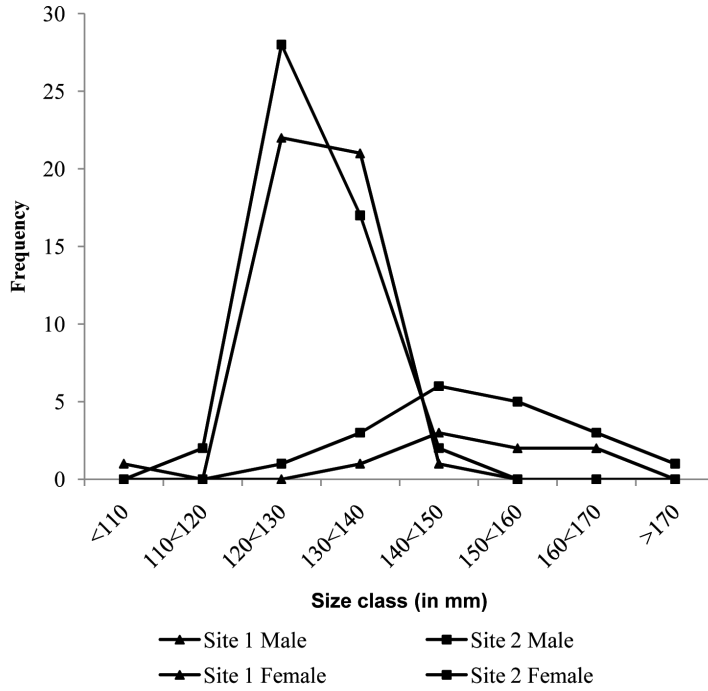


Fig.2: Frequency of size class intervals of the male and female *Carcinoscorpius rotundicauda* from Site 1 and Site 2

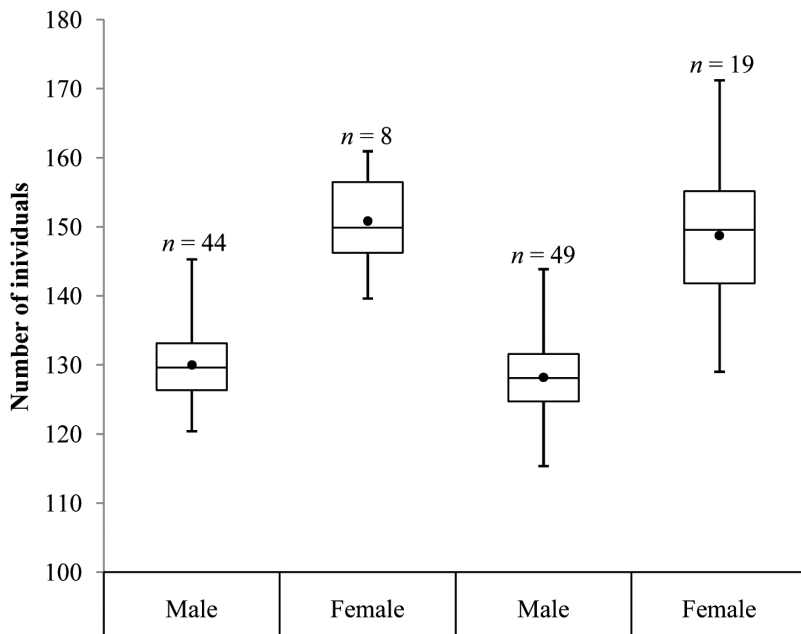


Fig.3: Size distribution of male and female individuals caught from Site 1 and Site 2

to them, reflecting the conditions in the wild. Correlation analyses with levels of significance of 0.10 were employed to illustrate the relationship between size of individuals and their propensity to form amplexus. Although the correlation was weak, the negative coefficient ( $r$ ) value ( $r = -0.184$ ;  $p = 0.10$ ,  $d.f = 10$ ) suggested that smaller males formed amplexus more frequently as compared to larger ones (Fig.4).

On the contrary, the frequency of a female to form amplexus was positively correlated with size ( $r = 0.678$ ;  $n = 0.10$ ,  $d.f = 5$ ), and this indicated the preference towards large-sized females in the mating system (Fig.5). The largest female in Tank

A was the only one which had formed a tandem when an additional male grasped on a paired male, which was never observed in the wild, supporting our theory that mating is size-assortative.

According to Cartwright-Taylor and Hsu (2012), breeding of *C. rotundicauda* in Singapore was year-round with no apparent seasonal pattern. However, the breeding period of *T. tridentatus* and *T. gigas* in Malaysia is thought to be seasonal (personal communication, Christianus, 2013), with marked increase in spawning activities towards the end of the year starting from September in Sabah (Muhammad Ali S. H.; personal observation). The presence of juvenile *T. tridentatus* at Site 2 from January

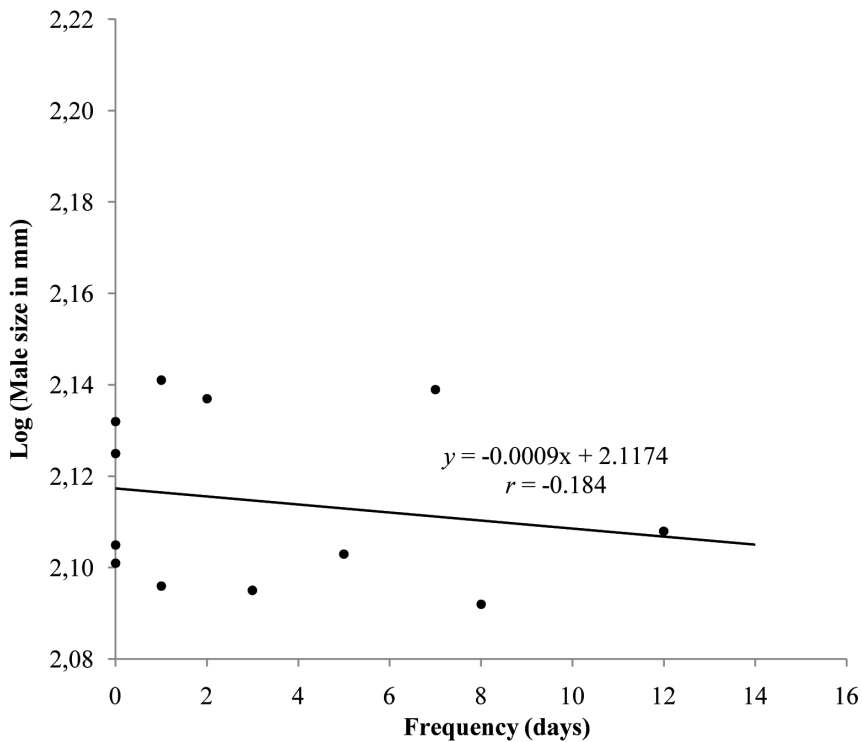


Fig.4: Correlation analysis of male body size and the frequency of forming amplexus



through April may be the product of recent spawning activities at the latter half of the previous year that resulted in recruitment of new individuals in the following months.

The OSRs of *C. rotundicauda* at both Sites 1 and 2 were male-biased but it was even more extreme at the former. Skews as high as 16 males per female (see Mattei *et al.*, 2010) in *L. polyphemus* were caused by selective harvest of larger and ovigerous females for the biomedical and bait industry (Carmichael *et al.*, 2003). Unlike *L. polyphemus*, large-scale commercial harvest of horseshoe crabs in Sabah is hitherto non-existent. However, during a survey carried out late November 2012 or before the survey at Site 2 was commenced,

female tachypleans were seen being sold at a local market in Kota Kinabalu, purportedly caught opportunistically. In Southeast Asia, horseshoe crab eggs are highly sought-after despite documented cases of lethal poisoning after consuming toxic *C. rotundicauda* (Ngy *et al.*, 2007; Kanchanapongkul, 2008). The fishing communities in Kota Kinabalu are aware of this, and this explains why *C. rotundicauda* are not sold to the public. Nonetheless, they claimed to know the organs that need to be removed when preparing a dish of *C. rotundicauda* admitting that the females have been captured occasionally for their own consumption. Perhaps, the extremely low number of females in Site 1 was due to

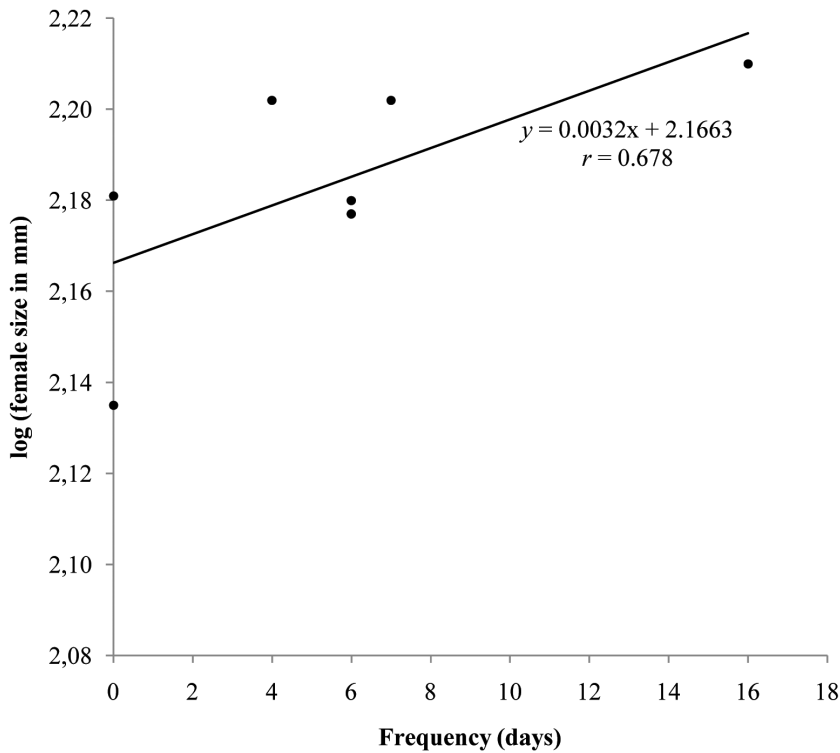


Fig.5: Correlation analysis of female body size and the frequency of forming amplexus

the selective removal of females trapped in fishing nets set up around Site 1.

Even though the adults in Site 2 were not subjected to the same anthropogenic influences as that of Site 1, there was still a slight imbalance in OSR that might be caused by sex-specific spatio-temporal segregation as previously reported in *L. polyphemus*. During spawning periods, unpaired females remained offshore while amplexed ones would travel to spawning grounds above the high tide mark accompanied by solitary males which clustered around spawning pairs (Brockmann, 1990). Of the 19 adult females caught in Site 2, only one was solitary, while others were in amplexus that lent credence to the theory that solitary females did not travel upstream. Female *L. polyphemus* would leave to deeper water after egg-laying, while solitary males would frequent the spawning beaches (Cohen & Brockmann, 1983; Brockmann, 1990; Smith *et al.*, 2013), indicating that over a spawning cycle, the number of males at a spawning beach would be consistent while the number of females would decline.

Despite the differences in the average sizes of individuals at both sites attributable to population variations, the proportion of sexual size dimorphism was similar at both sites. The females were 16% larger than the males, whereas ranges previously reported by Yamasaki *et al.* (1988) and Srijaya *et al.* (2010) were from 4% through 15%. Coincidentally, the mean size of the amplexed females was also 16% larger than the amplexed males. Adult males in all

species of horseshoe crabs are smaller than females and tend to be within a narrower range of sizes. Male horseshoe crabs reach terminal moulting upon attaining sexual maturity where their pedipalps (in all extant horseshoe crabs) and the first ambulating legs (in Asian horseshoe crabs) are modified to form claspers. The reproductive benefit of having smaller body size is that males have better mobility for mate searching ergo increasing their chances of finding a mate.

Female horseshoe crabs may grow larger than males because of the continuous growth spurred by juvenile hormones (Levin, 2003). Females may moult continuously throughout their lives, enabling them to attain significantly larger sizes than males. In a mating system where females have to carry males, maintenance of a female-biased size dimorphism is necessary due to loading constraints exerted on them (see Otronen, 1997). In captivity, male *C. rotundicauda* in an amplexus may be very persistent, clinging onto the same female usually for more than one day. Extensive copulation time coupled with resistance of aquatic locomotion in the wild would exacerbate loading constraints on female horseshoe crabs. Consequently, they possess larger body sizes to compensate for the higher bioenergetics requirements of mating (Crespi, 1989). In addition, female size was found to be positively correlated with fecundity in horseshoe crabs (Chatterji & Parulekar, 1992; Leschen *et al.*, 2006). The two factors combined were suspected to be key factors why males showed preference for the largest females in captivity, resulting

in size-assortative mating, and in turn, female-biased size dimorphism in the extant horseshoe crabs.

Females exhibited polyandry as they acquiesced to any courting male evident through the lack of resistance to grasping males or attempts to dismount them. Horseshoe crabs have highly-sensitive mechanoreceptors throughout their bodies that enable them to detect important physical cues such as hydraulic pressure and to locate mates (reviewed in Saunders *et al.*, 2010). Females in amplexus grasped by hand during sampling displayed an exaggerated response to this stimulus, ostensibly to forcefully dissociate themselves to ease escape. In captivity, a courting male manoeuvred itself deliberately around a female before grasping the latter's opisthosoma. Grasped females were rarely startled by the males' advances as indicated by their calm demeanour.

Results of the captive experiment showed that smaller males had formed amplexus more frequently than the larger males (Fig.4;  $r = -0.184$ ,  $n = 12$ ,  $P = 0.10$ ), although in the wild amplexed males were of similar size to solitary males, showing size-independent mating. Despite having smaller body size, there was a lack of size constraint for males to form amplexus, as demonstrated by a pair of *C. rotundicauda* from Site 2 that consisted of a female which was 37% larger than the male, twice the average size difference. Younger males that have recently moulted are smaller in size and thus lighter in terms of weight, which is more favourable for the females. Older individuals can be visually discerned

from younger individuals by having larger body sizes and their carapace appears darker due to degrading actions of chitinase bacteria and more heavily encrusted due to attachment of symbiotic epibionts (mainly barnacles and limpets) that contribute to an increase in their weight.

Asian horseshoe crabs have undergone further modifications in their morphology to remain amplexed for longer periods than *L. polyphemus* (Botton *et al.*, 1996). Prolonged precopulatory amplexus ensures that the male retains access to the female when breeding periods commence, and thus, ensuring its reproductive success (Rudloe, 1980). An alternative mating tactic for solitary males, which is common in older *L. polyphemus*, is to engage in sperm competition with amplexed males and amongst themselves without investing in primary access to a female (Brockmann, 1990; Smith *et al.*, 2013). Hordes of solitary males ( $n = 31$ ) were captured swimming along with amplexed pairs in Site 2, and excess males from Site 1 were most probably solitary as well, indicative of an alternative mating tactic in *C. rotundicauda*. Solitary males may still contribute to the genetic diversity of offspring despite being deprived of an exclusive mate in addition to ensuring a higher rate of fertilisation of the eggs.

## CONCLUSION

The two populations studied in this work can be ecologically distinguished from each other mainly from the discrepancies in term of their OSRs. Lack of protection on horseshoe crabs would result in a further

decline of its already low numbers in the vicinity of Kota Kinabalu. Since it takes almost a decade for an individual to attain maturity, loss of adults due to natural and anthropogenic factors may compromise the long-term health of a population and affected populations may require longer time to rebound. Baseline data of horseshoe crabs at other locations in Sabah and studies on their breeding periodicity are therefore necessary to instigate conservation and protection measures of the valuable species.

### ACKNOWLEDGEMENTS

The authors would like to thank Sabah Wetlands Conservation Society (SWCS) for granting the permission to conduct this research in KKWC and for providing logistical support, the staff (especially Ms. Lee Ka Han, Ms. Siti Joanni Matlan and Mr. Abraham) for providing useful data and information in the course of this study, and undergraduate students whom volunteered to assist in the collection of samples.

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