

Population and spatial ecology of horseshoe crabs (Limulidae) in a nursery ground at Pulau Bedukang, Brunei Bay, Borneo

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Abstract. Rozaini AF, Laurie KH, Marshall DJ. 2023. Population and spatial ecology of horseshoe crabs (Limulidae) in a nursery ground at Pulau Bedukang, Brunei Bay, Borneo. *Biodiversitas* 24: 4485-4492. Suitable nursery habitat to support populations of juvenile horseshoe crabs is becoming threatened globally. There are currently no formal studies on juvenile populations of the horseshoe crabs (Limulidae) for Brunei Darussalam (Brunei, Borneo). We investigated body size structure, population cohort and ecological distribution of juvenile horseshoe crabs associated with a mudflat on Pulau Bedukang, in Brunei Bay. Seventy juveniles were retrieved during four sampling days covering two seasons and four study sites. *Carcinoscorpius rotundicauda* and *Tachypleus gigas* were common, whereas only two individuals of *Tachypleus tridentatus* were found. Carapace width (size) was similar in the common species and there was no difference in juvenile size between the seasons and study sites. Body weight to carapace width relationships showed that the species grew at similar rates, although *C. rotundicauda* developed faster, producing 14 compared to the eight pre-migration mudflat instars produced by *T. gigas*. Species densities were similar, and there was no spatial separation of species or juvenile size across the mudflat or in relation to the mangrove forest edge. Nearest-neighbor (NN) analysis showed that individual crabs were generally dispersed. Their mudflat cohabitation suggests physiological and behavioral plesiomorphies for common optimal developmental salinity and temperature conditions. This study provides baseline information for comparison with other regional studies and for local conservation planning.

Keywords: Asian horseshoe crabs, ecological distribution, nursery habitat, sympatric occurrence

INTRODUCTION

Coastal development is causing habitat loss, which impacts the nursery grounds of juvenile horseshoe crabs and threatens their local distributions. The Asian horseshoe crabs *Carcinoscorpius rotundicauda* (Pocock 1902), *Tachypleus gigas* (O. F. Müller 1785) and *T. tridentatus* (Leach 1819) are listed in several countries as being locally vulnerable. *Carcinoscorpius rotundicauda* is Vulnerable in Bangladesh (IUCN Bangladesh 2015) and in Singapore (Davison et al. 2008). *Tachypleus gigas* is Endangered in Singapore (Davison et al. 2008) and shows reduced egg counts and spawning activity in Malaysia (Nelson et al. 2015). Similarly, the juvenile nursery grounds of *T. tridentatus* are in decline. In Japan, *T. tridentatus* is listed as Critically Endangered due to the loss or deterioration of its tidal-flat habitat (Japan NBSAP 2014, p.15). Coastline development and environmental degradation there are causing historically large populations of *T. tridentatus* to become locally extinct (Iwaoka and Okayama 2009). *Tachypleus tridentatus* is listed as Vulnerable in Vietnam, with occupancy declining by 50% between 1990 and 2007 (Laurie et al. 2019). Globally, *T. tridentatus* is listed as Endangered in the IUCN Red List of Threatened Species, whilst *C. rotundicauda* and *T. gigas* are currently listed as Data Deficient (Laurie et al. 2019).

In most regions, only two of the three Asian horseshoe crab species usually co-exist, though sympatric occurrence of all three species is seen in the south and eastern regions

of South East Asia (Sekiguchi 1988), including at Sabah (Robert et al. 2014), East Kalimantan (Sekiguchi et al. 1981; Meilana et al. 2021) and East Java (Sekiguchi et al. 1981; Mashar et al. 2017). All three species are reported to occur in Brunei, but no formal study has been conducted on their local distributions and abundances (Laurie et al. 2019; Wang et al. 2019). The IUCN Red List assessment for *T. tridentatus* noted that information on horseshoe crab populations and nursery grounds in Brunei remains limited (Laurie et al. 2019). Locally, *T. gigas* is known from Kuala Belait, Anduki and Panaga beach, whereas adults and beached mating pairs of horseshoe crab have been reported from Danai and Muara. The wall of a local fishermen's house at Kampong Ayer, a water village in Bandar, is adorned with the carapaces of all three Asian horseshoe crab species. Multiple visits over the past two decades to Pulau Bedukang, an island in the inner Brunei Bay, have confirmed that this is a horseshoe crab nursery ground (Marshall et al. 2016).

The population structure of juvenile horseshoe crabs should reflect the number of individuals that grow to maturity. Thus, the persistence of a dense juvenile population in a nursery ground indicates local sustainability over time. Morphometric and gravimetric data are useful for determining population/cohort structure and species-level attributes, allowing the understanding of population sustainability (Mohamad et al. 2016). Horseshoe crab size and weight can vary under different environmental conditions (habitat physiochemistry) and food availability.

The distribution and dispersion of individuals within an area are informative of the availability and use of habitat by juveniles. Information on the population and spatial ecology of juvenile horseshoe crabs at Pulau Bedukang is considered important for planning and conservation and facilitates comparison with horseshoe crab demographics from neighboring regions (Indonesia, Malaysia, and Singapore). With the aim of providing baseline data for the juvenile horseshoe crabs at the Pulau Bedukang, the objectives of the present study were to determine (1) species composition, (2) individual size structure and population cohorts, (3) size-weight relationships and (4) the distributions, densities and dispersion of the species. This represents the first formal record of the fundamental ecology of these crabs in Brunei.

MATERIALS AND METHODS

Study site and habitat

Pulau Bedukang (PB; 4.9784° N 115.0622° E) is located in the inner Brunei Bay (Figure 1) of the Brunei Estuarine System (BES) and is sheltered from the wave

action of the South China Sea. The island is approximately 0.4 km² and is fringed with dense mangroves. The mangroves are surrounded by an extensive intertidal mudflat, which experiences a semi-diurnal tide ranging between 0 and 2.5 m Chart Datum (CD) (Figure 1, Marshall et al. 2016). The mudflat supports prominent communities of benthic organisms, including juvenile horseshoe crabs, edible mangrove crabs, penaeid shrimps, gastropods, bivalve mollusks, anemones, corals (Marshall et al. 2016). The BES experiences a tropical climate with minor temperature variation between summer (Dec-Jan) and winter (Jun-July). There are two monsoon periods and two slightly wetter and two slightly drier periods each year, though monthly and annual rainfall are highly variable and influenced by El Niño-Southern Oscillation cycles. Shaded air temperature varies between 25 and 35°C and seawater temperature varies annually between 27 and 30°C. The mudflat sediment constitutes proportions of sand, silt and clay of 90.5%, 2.8%, 6.7%, respectively, and organic matter as a volume of around 2.5% (Hossain et al. 2014). The salinity and pH of the tidal water have been well documented and vary between 19.9-31.2 ‰ and 7.7-8.3 units, respectively (Marshall et al. 2008; Proum et al. 2018).

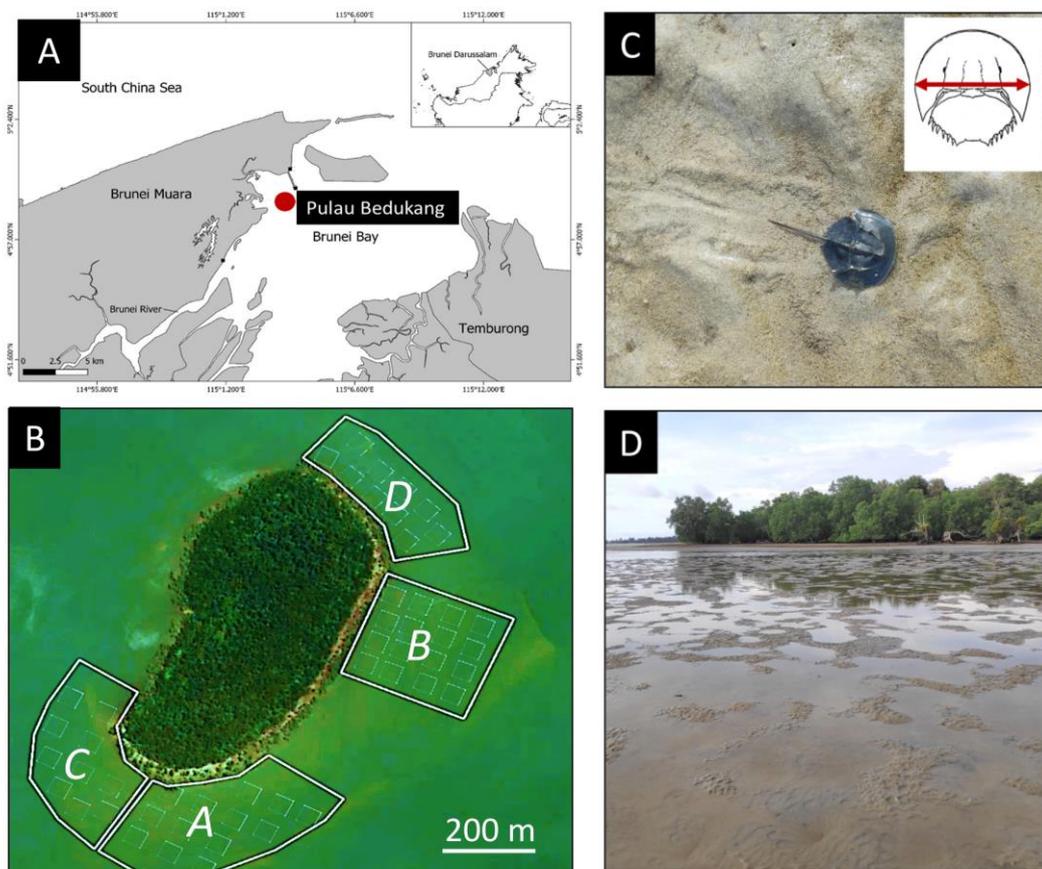


Figure 1. A. Location of the Pulau Bedukang, Brunei Bay (red dot). B. Satellite image of the island (Google Earth Pro, 6 Dec 2021) showing the sites A, B, C and D, with areas of 119,000 m², 100,000 m², 90,000 m² and 75,000 m², respectively; quadrats are shown in the sites. C. A horseshoe crab juvenile moving over the muddy substratum; inset shows how carapace width was measured. D. Mudflat at P. Bedukang during low tide showing uneven surface and tidal pools in the foreground and the mangrove forest edge in the background

Procedures

Field sampling (objectives 1, 2 and 3)

Four study sites were selected based on access to different parts of the mudflat. Using Google Earth Pro software, the area of each site was calculated for A, B, C and D as 119,000 m², 100,000 m², 90,000 m² and 90,000 m², respectively (Figure 1). Sampling was conducted on four occasions, once in each area on 21 Dec 2021, 6 Jan 2022, and 13 and 14 Jun 2022. During each sampling period, the mudflat was searched by the same researcher at low tide (0.1–0.4 m chart datum) for approximately three hours. Juvenile horseshoe crabs within the study area were retrieved, identified to species, photographed and weighed. They were then assigned a reference number, and a GPS reading was taken. Species identification followed Meilana et al. (2021); *C. rotundicauda* was identified by the absence of spines at the dorso-medial posterior of the opisthosoma and a round tail in cross-section, *T. gigas* by a single spine near the base of the triangular tail, and *T. tridentatus* by three opisthosomal spines and a triangular tail.

The juveniles were laid on a piece of a whiteboard to contrast their dark carapace and photographed using a Nikon COOLPIX P520. Each photograph was accompanied by a plastic ruler and a label including specimen number and date. Prior to photography, the juveniles were sprayed with seawater to facilitate their relaxation and to remove excess sediment on the carapace. Following this, the specimens were dried with air blow and a soft cloth, then both dorsal and ventral sides of the body were photographed. A portable electronic balance was used to weigh them to the nearest 0.1 g. Landmark coordinates were attained using a smartphone (Xiaomi Pocophone F1) with a built-in GPS (accurate to one meter). The handling process for each juvenile took around three to five minutes to complete, after which the juveniles were released back into their habitat.

Morphometric and cohort determination (objective 2)

Individual size structure and population cohorts were determined from the photographs of crabs. Morphometric aspects were estimated using CellSens Standard v.1.6. and focussed on carapace width (see Figure 1). This was compared between the species and across seasons and sites. Carapace width was also related to crab body weight for each species. Instar stages were determined by comparison of carapace widths for other studies (Table 1; Sekiguchi et al. 1988; Zadeh et al. 2009; Hu et al. 2015; Kwan et al. 2016).

Density, distributions and dispersion (objective 3)

Distribution refers to the position of individuals on the mudflat and relative to the mangrove edge, density to the number of individuals per area, and dispersion to the degree of clustering of the individuals. The coordinates assigned to each individual were transcribed into Google Earth Pro and QGIS Software and were mapped. From the map, the density of juveniles in each study area was determined. The population density was obtained using quadrats (50 x 50 m²). A total of 49 quadrats were distributed among the study sites (Site A, 13; Site B, 16; Site C, 10; and Site D, 10) using Google Earth Pro software without considering the distances and spacing between each

quadrat (Figure 1). We predicted that the mudflat niche might differ between the species and in relation to distance from the edge of the mangrove forest and that smaller individuals might occur closer to the forest edge. Generally, finer sediments occur closer to the mangrove forest (less current movement), but habitats near the forest edge are higher on the shore and thus are exposed for slightly longer in the air during low tides. For each individual, we measured the distance to the mangrove forest edge.

To ascertain whether individuals were more clustered in suitable niche areas (non-random resource distribution) or were more dispersed (to reduce resource competition), we undertook a nearest-neighbour (NN) analysis. Dispersion patterns of juveniles were determined to assess clustering and whether it varied seasonally (Dec-Jan and Jun; Waugh 2000). Averages of the distance between an individual and its nearest neighbor (NN) were used to derive the NN index for species and seasons. This index is calculated from the observed mean distance between each individual and its nearest neighbor (Do), divided by the expected mean distance for the individual in a random pattern (De) (Waugh 2000). An NN index value of < 1 refers to clustering, whereas an NN value of > 1 refers to relative dispersion. These values were compared using a two-tailed hypothetical test at a 5 % significance level (Waugh 2000).

Otherwise, statistical analyses involved One-way or Factorial ANOVAs in cases where normality and variance homogeneity (Cochran C test) were satisfied, and Kruskal-Wallis tests in cases where these criteria were not satisfied. All statistical analyses were conducted and all figures were prepared using Statistica (v12, Statsoft, New York, USA).

RESULTS AND DISCUSSION

Species, individual sizes, and population cohorts

Seventy (70) juvenile horseshoe crabs representing three species were retrieved from the four sampling sites. *C. rotundicauda* (n = 40) and *T. gigas* (n = 28) were common, whereas *T. tridentatus* was rare (n = 2) and was therefore excluded from further study. The carapace widths of the juveniles ranged between 18.7 and 69 mm, and were normally distributed (normality test, $\chi^2 = 7.6$; p = 0.17). Carapace widths were similar for *C. rotundicauda* (mean = 34.6 mm; range = 18.6–69.1 mm) and *T. gigas* (mean = 40.1 mm; range = 20.9–66.4 mm; ANOVA, $F_{(1,61)} = 3.18$, p = 0.08). Factorial analyses revealed no significant differences in carapace width between the species, seasons, and sites (Table 2). Notably, however, the largest individuals of both species in the Jan-Dec cohort were missing from the Jun cohort (Figure 2).

The species showed similar relationships for carapace width against body weight, which were typically exponential (*C. rotundicauda*: weight = 0.238*exp (0.071*carapace width); *T. gigas*: weight = 0.151*exp (0.081*carapace width) (Figure 3A). A striking difference between the species was found regarding instar gains relative to carapace width. For a 50 mm increase in carapace width (~20–70 mm), *C. rotundicauda* gained eight instars (6–14), whereas *T. gigas* gained only four instars (Figure 3B).

Table 1. Instar stage and mean carapace width (\pm SE, standard error) for juvenile cohorts defined for the present study and previous studies of Sekiguchi et al. (1988), Zadeh et al. (2009), Hu et al. (2015) and Kwan et al. (2016).

Instar stage	Mean prosomal width (mm)									
	<i>T. gigas</i>			<i>C. rotundicauda</i>						
	Sekiguchi (1988)	Zadeh et al. (2009)	This study	Sekiguchi (1988)	Zadeh et al. (2009)	Hu et al. (2015)	Kwan et al. (2016)		This study	
			Pulau Bedukang			Xi Chang	Luk Keng	Pak Nai	Sha Tau Kok	Pulau Bedukang
1	8.33	6.98 \pm 0.26		5.02	3.67 \pm 0.13					
2	12.1	10.87 \pm 0.58		7.57	5.00 \pm 0.23					
3	14.8	15.39 \pm 0.6		10.2	6.98 \pm 0.34					
4	19.2	20.93 \pm 0.77		12.4	9.42 \pm 0.80					
5		28.04 \pm 1.04	27.18 \pm 2.53		12.59 \pm 0.84	15.5 \pm 0.9		14.5 \pm 1.7		
6		36.85 \pm 1.51	35.84 \pm 2.57		17.08 \pm 1.42	20.8 \pm 0.9	21.7 \pm 0.8			20.91 \pm 1.6
7			49.15 \pm 2.3		23.39 \pm 2.51	27.2 \pm 1.5	31.9 \pm 1.6	30.0 \pm 1.9	28.5 \pm 1.3	26.16 \pm 0.66
8			60.44 \pm 4.33			30.9 \pm 0.6				30.44 \pm 0.45
9						35.9 \pm 0.9				35.69 \pm 1.6
10						40.9 \pm 0.8	43.3 \pm 1.2	44.8 \pm 4.7	40.8 \pm 1.4	42.93 \pm 1.32
11						44.5 \pm 1.2	48.3 \pm 1.1			
12						50.5 \pm 0.8			53.6 \pm 0.6	48.74 \pm 0.46
13						53.7 \pm 0.6			61.5 \pm 2.5	
14						61.8 \pm 2.5			73.0 \pm 1.2	63.41 \pm 3.91
15						75.3 \pm 3.2				

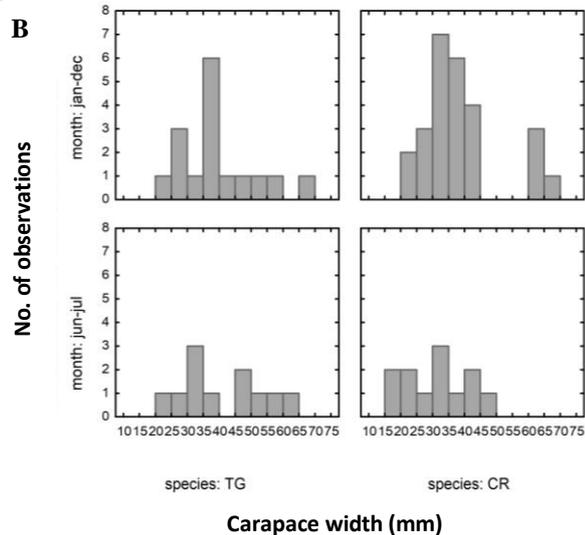
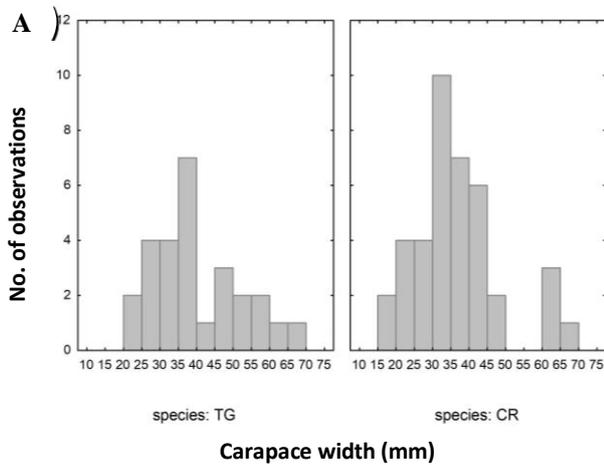


Figure 2. A. Carapace width frequency distributions for *Tachypleus gigas* (TG) and *Carcinoscorpium rotundicauda* (CR). B. Carapace width distributions for the species separated for each season. Carapace widths were not different across species, sites, and seasons (see Table 1)

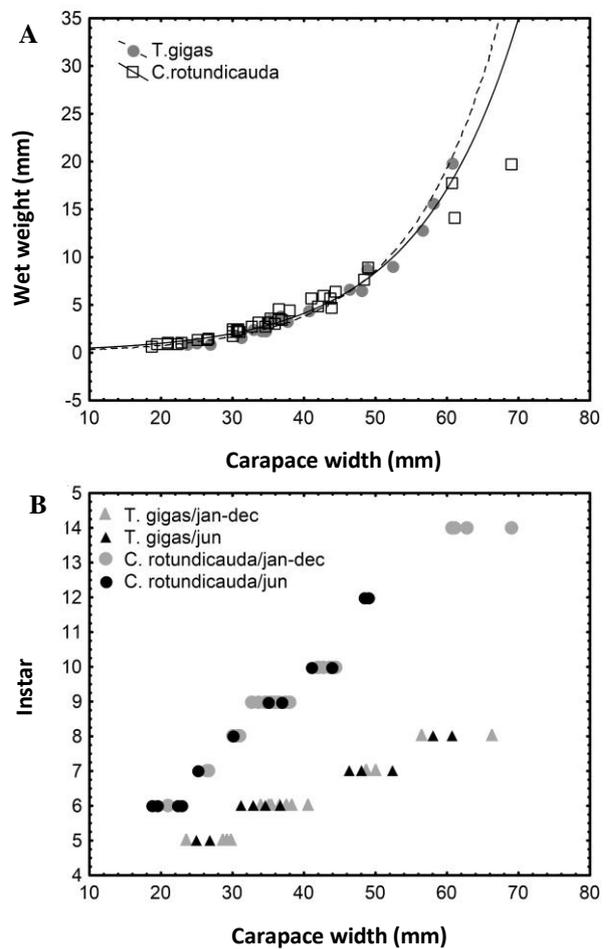


Figure 3. A. Relationship between wet weight and carapace width for *Carcinoscorpium rotundicauda* (weight = $0.2376 \cdot \exp(0.0713 \cdot x)$, squares and solid line) and *Tachypleus gigas* (weight = $0.1508 \cdot \exp(0.0808 \cdot x)$, circles and dashed line). B. Relationship between instar stage and carapace width of *C. rotundicauda* and *T. gigas*. Symbols indicating the species and time of sampling are given in the key

Table 2. Tests for the effect of month and site on the carapace width of the horseshoe crabs. The lower table shows the mean (± 1 SE) carapace width for combinations of species, months and sites. Homogeneity of variance was observed for combinations of all variables (Cochran C, $p > 0.05$). SE, standard error

Parameters	Between months (seasons)			Between sites		
	MS	F	p	MS	F	p
Species	445.5	3.17	0.079	286.9	2.13	0.106
Month (Site)	92.45	0.65	0.419	182.97	1.35	0.248
Species x Month (Site)	282.11	2.013	0.161	192.61	1.42	0.243
Error	140.13			134.79		

Species	Month (site)	Mean	SE	N
TG	Jan/Dec	39.25	2.9	16
TG	Jun	41.13	3.5	11
CR	Jan/Dec	38.12	2.3	26
CR	Jun	31.19	3.4	12
TG	A	40.2	3.5	11
CR	A	43.0	4.3	7
TG	B	37.1	5.1	5
CR	B	36.6	2.7	18
TG	C	48.1	4.7	6
CR	C	34.1	4.1	8
TG	D	32.7	5.1	5
CR	D	29.8	4.7	6

Density, distributions, and dispersion

The Dec-Jan sampling recorded 25 individuals of *C. rotundicauda* and 16 individuals of *T. gigas*, whereas the Jun sampling recorded 15 individuals of *C. rotundicauda* and 12 individuals of *T. gigas*. Density was determined for 32 quadrats of the original 48 that contained at least a single crab. The mean density of *T. gigas* (5.3×10^{-4} individ.2500 m⁻²) did not differ significantly from that of *C. rotundicauda* (7.1×10^{-4} individ.2500 m⁻²; Kruskal-Wallis test, $H(1, n = 44) = 1.3, p > 0.05$; Figure 4). Different sites were used for different sampling sessions to avoid resampling, although in the Dec-Jan survey, a single individual from Site A was recaptured in Site B (based on photographic and morphometric similarity) and was excluded from further analyses.

Contrary to the expectations that the mudflat niche varies relative to distance from the mangrove forest edge and that species are likely spatially separated, we found no difference in the distance of individuals of the species from the mangrove edge (Figure 4; $F_{(1,66)} = 0.14; p = 0.71$). Furthermore, there was no difference in individual size (carapace width, mm) relative to distance (m) from the mangroves for either species (*T. gigas*: $y = 133.9 + 1.4x, R^2 = 0.03; p = 0.37$; *C. rotundicauda*: $y = 132.5 + 1.0x, R^2 = 0.19; p = 0.1$). Regarding dispersion, nearest neighbor (NN) distances for *T. gigas* ranged between 4.1 and 221.9 m, whereas for *C. rotundicauda*, these distances ranged between 2.95 and 78.9 m. Although mean NN values differed greatly between the species and were higher in *T. gigas* (41.3 m) than in *C. rotundicauda* (23.7 m), the data were highly variable and the difference was not significant (Kruskal-Wallis test; $H(1, n = 68) = 1.74, p > 0.1$). For all cases, NN indices were near to or > 1 , suggesting a tendency towards dispersion rather than clustering. Significant NN indices of > 1 (dispersion) were found for

Dec-Jan, but not for Jun when the data of both species were combined, and for each species when seasons were combined (Tables 3, 4 and 5).

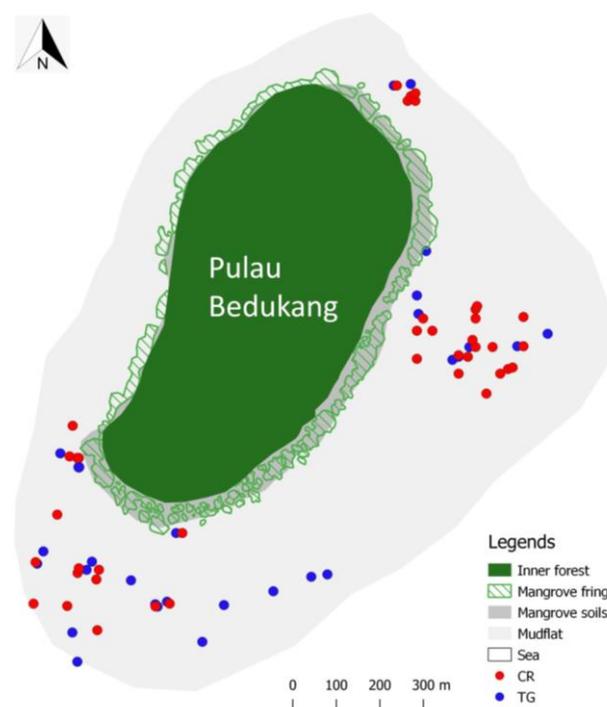


Figure 4. The spatial distribution of individual juveniles of *Carcinoscorpius rotundicauda* (CR; red markers) and *Tachypleus gigas* (TG; blue markers) on the intertidal mudflat at Pulau Bedukang in Dec 2021- Jan 2022 and June 2022

Table 3. Nearest Neighbor (NN) analyses for the species combined for Dec-Jan and Jun. The NN index revealed that crabs were dispersed in Dec-Jan. * indicates a significant difference

	N	\bar{D}_o	\bar{D}_E	\bar{a} (m ²)	NN index	z	SE	p
Dec-Jan	41	30.6	25.8	109500	1.18	2.26	2.11	p < 0.05*
Jun	27	31.5	28.9	90000	1.09	0.9	2.9	p > 0.1

Table 4. Nearest Neighbor analyses for each species (intraspecific) with seasons combined. The NN index revealed that individuals were dispersed. * indicates a significant difference

	N	\bar{D}_o	\bar{D}_E	\bar{a} (m ²)	NN index	z	SE	p
CR	40	33	25	99750	1.32	3.91	2.06	p < 0.01*
TG	28	54.9	29.84	99750	1.84	8.51	2.95	p < 0.01*

Table 5. Nearest Neighbor analyses for each species (intraspecific) and each season. * indicates a significant difference

	N	\bar{D}_o	\bar{D}_E	\bar{a} (m ²)	NN index	z	SE	p
CR Dec-Jan	25	31.45	33.1	109500	0.95	-0.47	3.46	p > 0.1
Jun	12	35.7	38.7	90000	0.92	-0.58	5.23	p > 0.1
TG Dec-Jan	16	60	41.4	109500	1.45	3.45	5.41	p < 0.01*
Jun	12	48.2	43.3	90000	1.11	0.75	6.53	p > 0.1

Discussion

This paper represents the first formal study of juvenile horseshoe crabs of Brunei and reveals the sympatric cohabitation at Pulau Bedukang of *C. rotundicauda*, *T. gigas*, and *T. tridentatus*. *Carcinoscorpius rotundicauda* and *T. gigas* were common, whereas only two individuals of *T. tridentatus* were found, confirming that all three species breed in the coastal waters of Brunei. Such sympatric occurrence of the three species is only known in the southeastern region of Asia, where their distributions overlap (Sekiguchi 1988). This has previously been reported in Sabah (Robert et al. 2014), East Kalimantan (also on Borneo; Meilana et al. 2021) and eastern Java (Sekiguchi et al. 1981; Mashar et al. 2017). The low population densities of *T. tridentatus* in these regions (Sekiguchi 1988) may be explained by the southeastern geographical limit of this species. This species otherwise occurs at high population densities in the northern part of its distributional range, in the coastal waters of China (Sekiguchi 1988). At Pulau Bedukang, *C. rotundicauda* was more abundant (57.1%; 40/70) than *T. gigas* (40%; 28/70). Local variation in the relative abundances of these species has been observed in other studies (Meilana 2015; Fairuz-Fozi et al. 2018; Zauki et al. 2019), as well as for *C. rotundicauda* and *T. tridentatus* (Kwan et al. 2016), and likely relates to the idiosyncratic nature of habitat distribution.

The size, growth and phenologies of *C. rotundicauda* and *T. gigas* at Pulau Bedukang were remarkably similar. Carapace width (size) was similarly distributed across species, sites and seasons. The body sizes of these species were also similarly exponentially related to wet weight and concordant with this relationship for *T. tridentatus* (described in Lee and Morton 2005). Such similar growth

parameters across the species suggest a phylogenetically constrained attribute. However, this could also be a function of similar foraging opportunities and food quality in their common habitat. Hu et al. (2022) showed that *C. rotundicauda* and *T. tridentatus* foraged on similar food sources when prey was abundant but not when prey was scarce. During food scarcity, *T. tridentatus* primarily fed on bivalves, whereas *C. rotundicauda* was more generalistic, consuming a variety of food types, including particulate organic matter, microalgae, bivalves, polychaetes, crustaceans and gastropods (Hu et al. 2022). One striking difference between our study species refers to the instar stage relative to carapace width. Similar to several other studies that distinguish the instar stage, *C. rotundicauda* showed a higher molting frequency than *T. gigas* (see Table 1). Although the juveniles of both species reached a similar maximum carapace width (~70 mm; Figure 4), the greatest instar of *C. rotundicauda* was 14 compared to 8 for *T. gigas*. This implies faster development by *C. rotundicauda* (Zadeh et al. 2009), but also a difference in the developmental stage that migrates away from the mudflat habitat (see also Sekiguchi 1988; Chiu and Morton 2004; Cartwright-Taylor et al. 2012). Notably, significant growth of both species occurs after migration from the mudflat to achieve between 15 and 17 molts before reaching maturity (Sekiguchi 1988; Hu et al. 2015). Such migration suggests that the mudflat diet does not adequately support the energetics associated with exponential growth during the full course of development. The similarity of maximum juvenile size on the mudflats of the disparate populations and species (reported here and elsewhere) despite differences in habitat conditions (food availability, etc.), implies an evolutionary constraint on the phenology of these horseshoe crab species (Bicknell et al.

2022). Furthermore, habitat separation between juveniles and subadults that have migrated to deeper water in the estuary (*C. rotundicauda*), or to the open sea (*T. tridentatus*), suggests a mechanism for reducing intraspecific competition (Svanbäck and Bolnick 2007).

No clear pattern was found in the distribution of individuals of both species on the mudflat. Although sediment particle size typically becomes finer toward the higher shore, implying different food items relative to shore height, we found no distribution pattern of the horseshoe crabs (abundance variation) in relation to the mangrove edge. There was also no variation in crab body size in relation to distance from the mangrove edge, suggesting that smaller and larger juveniles are similarly distributed on the mudflat. These findings are consistent with another study (Meilana et al. 2021), but contrast with the suggestion that larger individuals often occur lower in the intertidal zone (Chiu and Morton 2004).

Although we show that the species are not spatially separated, we do not know whether they occupy different trophic niches, as the gut analysis was not performed for conservation reasons. Hu et al. (2022) suggest that cohabiting *T. tridentatus* and *C. rotundicauda* selectively feed on different items, which should reduce interspecific competition. Dispersion of individuals assessed using Nearest-Neighbor (NN) analysis for seasons, species, and their combinations revealed little evidence of clustering of individuals in more preferred habitats. The dispersion of individuals could result from the heterogeneity of the mudflat, which comprises scattered shallow pools (~2 m, diameter, and 5 cm depth; Figure 1), or from some mechanism to reduce intra- and interspecific competition. Meilana et al. (2021) found that juveniles of the same species were preferentially distributed in areas with high content of total organic matter, chlorophyll a, and silt.

An important question emerging from this study is why Pulau Bedukang uniquely represents a nursery ground and attracts the spawning and development of the three horseshoe crab species when multiple other islands in Brunei Bay, which to possess similar sediment properties, do not support horseshoe crab populations. Similar observations were made of spawning site selectivity by *C. rotundicauda* in the Indian Sundarbans. In surveys of 40 randomly selected sites during different seasons over a four-year period, *C. rotundicauda* was observed at only 14 sites. The colonization of the Pulau Bedukang mudflat might relate to unique physicochemical conditions for optimal development, including salinity and temperature. Notably, *T. gigas* sub-adults migrate to deeper, more saline waters along the South China Sea coast, whereas *C. rotundicauda* migrates to deeper and less saline waters in the Brunei Bay and estuarine systems. The suggestion that the three species require similar physical environmental conditions for optimal juvenile development implies the possibility of evolutionary-constrained developmental physiology in these ancient marine chelicerates.

In summary, the decline in suitable nursery habitats for Asian horseshoe crabs is reported in many countries, but no formal studies have previously been conducted for Brunei. This study confirms the presence of spawning populations

of all three Asian horseshoe crab species in Brunei, including the IUCN Red-listed species, *T. tridentatus*. This study extends the known range of juvenile cohabitation of the three species. The populations were dominated by *C. rotundicauda*, followed by *T. gigas*, whereas *T. tridentatus* was rare. The species grew at similar rates, although *C. rotundicauda* progressed more quickly through its life stages and matured sooner than *T. gigas*. The juveniles were similarly distributed on the mudflat and their distance from the mangroves was unrelated to body size. Dispersion of the juveniles probably relates to a mechanism to reduce resource competition. The co-existence of juveniles in the same habitat suggests similar optimal environmental conditions for growth and implies evolutionary-constrained physiology. Given the unique importance of the Pulau Bedukang mudflat to these horseshoe crabs, this area should receive priority consideration in future conservation planning and management.

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