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Co-occurrence of juvenile horseshoe crabs *Tachypleus tridentatus* and *Carcinoscorpius rotundicauda* in an estuarine bay, southwestern China

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ABSTRACT: Spawning and nursery habitats are critical in the conservation of horseshoe crabs. We examined the abundance and distribution of juveniles of 2 species of horseshoe crabs, *Tachypleus tridentatus* and *Carcinoscorpius rotundicauda*, in a mangrove-vegetated estuary (Pearl Bay, Guangxi, China). Intertidal sampling and measurement of environmental variables were carried out in October 2014 at 4 sites spanning ~6.3 km from the inner to the outer bay. Both species were found at the 2 innermost mangrove sites but not at the outer 2 sites. Densities were higher than recorded in previous studies, 0.24 ind. m^{-2} for *C. rotundicauda* and 0.05 ind. m^{-2} for *T. tridentatus*. Although only 12 of the latter species were found, they appeared to be more abundant in more seaward, energetic environments in poorly sorted sediments, whereas *C. rotundicauda* occurred in sediments with higher silt-clay and organic carbon content, and aggregated at the mangrove forest edges. These small-scale distribution differences are consonant with the species' life histories, since only *T. tridentatus* migrates to the sea while *C. rotundicaudata* spends its entire life within or near mangrove habitats. International and national conservation strategies for these species should include further efforts to inventory critical habitats, such as Pearl Bay, in southeast Asia.

KEY WORDS: Juvenile horseshoe crab · Distribution · Mangrove · Tidal flat · Estuarine bay · Southern China

INTRODUCTION

Coastal ecosystems provide essential habitats for many commercially and ecologically valuable fishes and macroinvertebrates, including horseshoe crabs. Beaches, tidal flats, creeks and shallow waters are used by them to fulfil nursery, feeding or reproductive functions (Martin 2014, Seitz et al. 2014). *Tachypleus tridentatus* once thrived on the coasts of the South and East China Sea but has severely declined for decades along the coasts of Japan, Taiwan and China (Itow 1993, Botton 2001, Chen et al. 2004, Hu et al. 2009, Nishida & Koike 2009, Shin et al. 2009, Yang et al. 2009, Morton & Lee 2010). *Carcinoscropius rotundicauda* populations in Hong Kong and Singapore are also threatened and declining (Shin et al. 2009, Cartwright-Taylor et al. 2011, Morton & Lee 2010). The declines in *T. tridentatus* are attributed to high demands for *Tachypleus* amoebocyte lysate, loss of coastal habitats and harvesting for human

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consumption. There is a deep concern that these horseshoe crab populations may be decimated if no effective actions are taken to save them, particularly with respect to *T. tridentatus* (IUCN 2012, P. K. S. Shin unpubl. data). Habitat protection has been considered one of the best strategies to conserve horseshoe crabs (IUCN 2012). Therefore, identification and inventory of critical Asian horseshoe crab habitats at both local and regional scales is a vital first step in these species' conservation (IUCN 2012, P. K. S. Shin unpubl. data), despite the fact that low abundance reported for *T. tridentatus* makes ecological sampling difficult.

Horseshoe crabs requires 3 types of coastal zone habitat to complete its life cycle: adult *T. tridentatus* spawn on open, sandy beaches with coarse sands near the high-tide zone, juveniles forage on adjacent mudflats, and sub-adults and adults continue to grow and mature in deeper subtidal areas (Sekiguchi 1988, Loveland 2001, Chiu & Morton 2003, Chen et al. 2004, Almendral & Schoppe 2005, Hsieh & Chen 2009). In contrast, *C. rotundicauda* nests at the high-tide level of mangrove-penetrating tidal creeks just beyond the edges of terrestrial land (Cartwright-Taylor & Hsu 2012). In addition, *C. rotundicauda*, the so-called mangrove horseshoe crab, is known to spend its life within mangrove swamps or sometimes moves to nearby deeper water but does not migrate to the sea (Davidson et al. 2008, Cartwright-Taylor et al. 2012).

With respect to the habitat availability, mangrove-vegetated estuarine bays appear to meet the needs of both *T. tridentatus* and *C. rotundicauda*. For example, in areas adjacent to the southeastern part of the Pearl River Delta in China, both juvenile *T. tridentatus* and *C. rotundicauda* have been recorded to coexist on a few tidal flats along the shallow coasts surrounded by Deep Bay and Lantau Island, Hong Kong (Chiu & Morton 1999, Shin et al. 2009, Morton & Lee 2010). In Morton and Lee's (2010) study, juvenile *T. tridentatus* were found to prefer seagrass beds, but there have been no reports of either species' spatial associations with respect to nearby mangroves. The distribution trend observed for juvenile *T. tridentatus* illustrates that they tend to live in seaward rather than landward zones, as the former supports seagrasses while the latter has mangroves.

The coasts of the Beibu Gulf in Guangxi, China, harbor a number of bays which are renowned for their fisheries and shellfisheries, a consequence of the various and numerous habitats they support, including those for horseshoe crabs (Liang 1985, Records of Bays in China 1993, Hu et al. 2009). Liang (1985) reported a nesting beach of *T. tridentatus* on an eastern shore of the Gulf, suggesting that sandy beaches in bays may serve as critical spawning grounds for *T. tridentatus*. Likewise, mangroves in bays are essential for *C. rotundicauda*.

The study area, Pearl Bay, is a mangrove-dominated estuarine ecosystem with patchy seagrass beds and extensive sandy beaches located on the western shore of Beibu Gulf. At low tide, it is common to find adult *C. rotundicauda* in amplexus (male clasping female's opisthosoma) and burying in the mud along the edges of mangrove-penetrating tidal creeks (authors' pers. obs.). However, no *T. tridentatus* are observed, as mangroves are not suitable spawning sites for *T. tridentatus*.

Based on what is known from the aforementioned literature and observations on the habitat requirements for *T. tridentatus* and *C. rotundicauda* throughout their life cycle, we predicted that juvenile *T. tridentatus* would inhabit areas closer to the outer region of the bay and at some distance from the nearest mangroves, whereas juvenile *C. rotundicauda* would tend to use the inner region of the bay and be more closely associated with mangroves. We also predicted that the juveniles of both species would cooccur at some locations within the bay. Pearl Bay appears to be an ideal study site for testing these predictions. The objectives of the present study were to examine the following (1) the spatial distributions of the *T. tridentatus* and *C. rotundicauda* juvenile populations in Pearl Bay, (2) whether the juveniles of the 2 species co-occured on the same tidal flats, and if so, where these sites were located in the Bay, and (3) the habitat characteristics of the nursery grounds for these 2 horseshoe crab species in relation to environmental variables.

MATERIALS AND METHODS

Study area

Pearl Bay is located on the west coast of Beibu Gulf, Guangxi, southwestern China. The bay covers a total area of 94.2 km^2, with the coastline stretching for approximately 46 km. The bay opens into Beibu Gulf at its southern end through a relatively narrow and shallow mouth approximately 3.5 km in width (Fig. 1). Pearl Bay is categorized as a well-mixed bay (Records of Bays in China 1993). The tidal regime of the Bay is characterized as macro-tidal and diurnal, with an average tidal amplitude of 2.24 m and a maximum amplitude of 5.05 m. Tidal currents flow to the northeast, and near high tide, flow velocities reach a

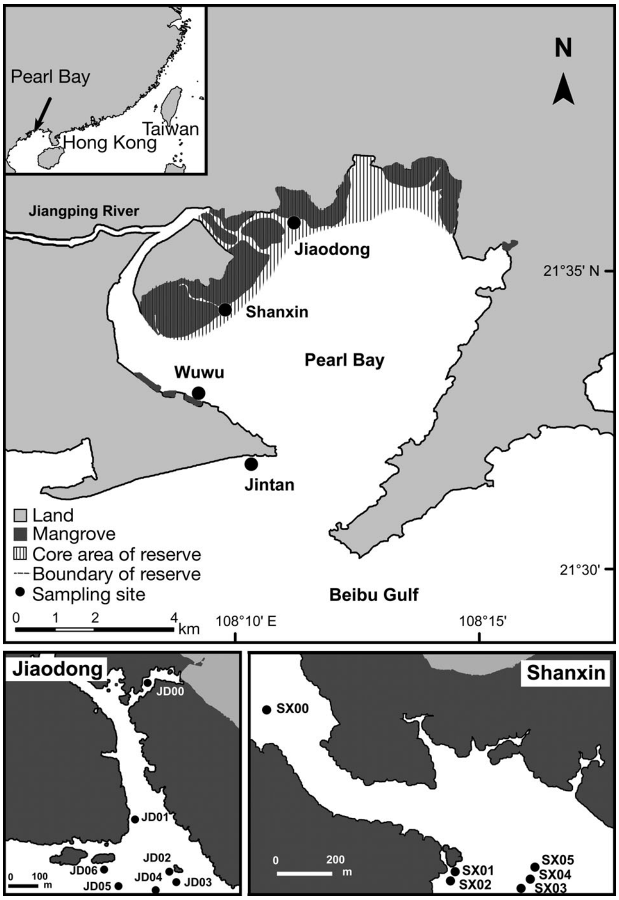

Fig. 1. Study sites at Pearl Bay. Jiaodong and Shanxin are located along the inner bay and have mangrove vegetation, while Wuwu is located in the outer bay and Jintan is located in the coastal zone of the bay. Areas hatched with vertical lines are the planned core areas for mangrove reserves (China Mangrove Conservation Network, www.china-mangrove.org/point/27, accessed on 26 March 2015)

maximum of 48 cm s^{-1} at the bottom of the water column and 55 cm s^{-1} at the surface. Ebb currents flow to the southwest, and near low tide, currents increase, reaching a velocity of 71 cm s^{-1} at the bottom of the water column and 82 cm s^{-1} at the surface. The average wave height reaches 0.52 m and the maximum wave height is 4.1 m. The bay is shallow, and its bottom consists of tidal creeks and troughs. The water depth ranges from 5 to 10 m in the trough areas, with the deepest waters measuring 13 m at the mouth. The northwest and west region of the bay is fed by fresh water from the Jiangping River, which runs through dense mangroves at Jiaodong and Shanxin (Fig. 1). Sand and tidal mudflats in the area are large and generally extend 2 to 3 km down shore, thus providing habitats for numerous economically important invertebrates, whereas in the southwest region, tidal sand flats are much narrower and stretch approximately 50 to 150 m down shore.

The mangrove forests in Jiaodong and Shanxin cover approximately 544 ha (Guangxi Mangrove Research Center unpubl. data) and are dominated by *Aegiceras corniculatum* and *Avicennia marina*, with some *Bruguiera gymnorrhiza* and *Kandelia obovata*. Beds of the seagrass *Zostera japonica* are distributed seaward and extend from the edges of the mangrove forests on tidal flats, while *Halophila beccarii* is distributed in small patches on tidal flats, typically along tidal creeks (Qiu et al. 2013, 2014). All mangrove-vegetated areas in Pearl Bay are included in the Beilun Estuary Mangrove Nature Reserve (Ma et al. 2011, China Mangrove Conservation Network, www.china-mangrove.org/point/27). Due to the high energy of the tides, waves, winds and the effects of mangrove energy attenuation, the bay is subject to a highly dynamic erosion and sedimentation regime. Annual water temperatures in Pearl Bay average 23.5°C and range from a minimum of 9.4°C in February to a maximum of 33.6°C in July. During the present study period in October, the water temperatures ranged from 22.7 to 31.6°C (see Table S1 in the Supplement at www.int-res.com/articles/suppl/b024p117_supp.pdf).

Sampling design

To determine the spatial distributions of juvenile *T. tridentatus* and *C. rotundicauda*, 4 sites at Pearl Bay were sampled from October 23 to 25, 2014: 2 inner bay (Jiaodong and Shanxin), 1 outer bay (Wuwu) and 1 coastal location (Jintan). All samples were taken during low tides and a total of 296 m^2 was sampled (Fig. 1, Table S1). Approximately 3 h prior to low tide, juveniles are most active and emerge from the substrata (Chen et al. 2004, Morton & Lee 2010). Emerged juveniles found on the sediment surface and having a size >1 cm in carapace length were sampled. Juveniles of the 2 species were distinguishable from adult forms in that they lacked secondary sexual characters (Yamasaki et al. 1988).

Three to 7 sampling stations were set up for each study site. At each station, juvenile abundance was counted and sizes were measured. Environmental variables in the substrata and the shortest distances between the stations and mangrove edges were also measured. Both plot and transect sampling were used to estimate juvenile densities beyond the initial density levels observed in the field. At each station, 4 plots, each 2 × 2 m, were haphazardly chosen around a location where horseshoe crabs were found. Where densities at each station were too low to be counted using plot sampling, 2 transect lines, each 20 m long and 1 m wide, were used. In addition, 3 transects of the same size were set up parallel to the mangrove forest edge 15 m apart at Stn JD01 of the Jiaodong site with a total sampling area of 60 m^2.

Measurement of body size, estimation of juvenile horseshoe crab density and spatial relation with the mangrove

At all plots (16 m^2 in total) and transects (either 40 or 60 m^2) at each station, juvenile horseshoe crabs were collected, identified to species level, counted to establish abundance, and measured with a digital caliper to determine each crab's maximum prosomal width (Absolute™, Mitutoyo©). The numbers of spines growing along the central ridge on the dorsum of the opisthosomal carapace were used to distinguish between the 2 species. There are 3 spines on *T. tridentatus* whereas *C. rotundicauda* has only 2 spines.

For each species, the spatial relationship between juvenile size and distance to the nearest mangrove was analyzed by plotting a size-frequency distribution of prosomal widths against the distance to the nearest mangrove. When appropriate, the density and the width were expressed as mean ± SE ind. m^{-2} and mean ± SE mm, respectively.

Measurement of environmental variables

A total of 12 environmental variables were measured (Table S1 in the Supplement). Collections and analyses of sediment variables followed the procedures described in Hsu et al. (2009). Sediments were collected using an acrylic tube with 2.6 cm diameter. During transport to the laboratory, all sediment samples were kept cool at ~4°C, while those for chlorophyll *a* (chl *a*) content measurement were also kept in the dark. The top 3 cm of the sediment were sampled and analyzed for granulometry, pH, salinity, total organic carbon (TOC) and total nitrogen (TN) contents, and chl *a* samples were taken from the top 0.5 cm. In the laboratory, samples for measurement of TOC, TN and chl *a* content were kept at −80°C until cryo-dried. Granulometry (including grain size, silt and clay content and degree of sorting) was determined following a protocol developed by Hsieh & Chang (1991). Sediment pH values were measured using a glass electrode pH meter in a 1:2 ratio of sediment to deionized water by weight. Sediment salinity was determined using a refractometer on the strained interstitial water after the sediment was centrifuged. Sediment TOC and TN contents were analyzed using an element analyzer (Perkin-Elmer 2400 Series II CHNS/O analyzer) after sediments were treated by cryo-drying, passed through a 0.5 mm sieve to remove large animal pieces and plant debris, acidified with 1 N HCl to remove inorganic carbons, and ground to a fine powder. Chl *a* was extracted with 90% acetone and analyzed using a fluorescence spectrophotometer (Eclipse GMJL006, Varian) and concentrations were expressed as µg cm^{-2}. In addition, the shortest distances between the stations and the mangrove edges (DisM) were determined using Google Earth based on the geographic coordinates recorded for each station during field sampling.

Statistical analysis

Spearman correlations were used to analyze the relationship between juvenile densities of *T. tridentatus* and *C. rotundicauda* and DisM. The correlation statistics were based on the data from 7 stations at the 2 inner sites, Jiaodong (JD01, JD05-06) and Shanxin (SX01-04), and performed using SAS 9.1 software (SAS Institute 2003). Multivariate ordination techniques were used to examine additional relationships between the juvenile densities for each species and other environmental variables. Two biotic variables, measured as the densities of the 2 species, and 12 environmental variables collected from all 4 sites were included in the analysis. The juvenile densities and environmental variables were log transformed before analysis. A prior de-trended correspondence analysis (DCA) of horseshoe crab density was conducted separately to assess the gradient length of the first DCA axis. The length of the gradient was 1.414 (in SD units). Based on this information, a linear model, also known as a redundancy analysis (RDA), was chosen for our data set (ter Braak & Šmilauer 2012). We utilized the automatic forward-selection mode in our analysis and only included environmental variables that explained a significant proportion of the remaining variation (based on a Monte Carlo test with 999 permutations at $p \leq 0.1$). An ordination analysis was performed using CANOCO for Windows v.5.0 (ter Braak & Šmilauer 2012).

RESULTS

Spatial distribution of juvenile horseshoe crabs

A total of 65 juvenile horseshoe crabs were recorded throughout this study, consisting of 12 *Tachypleus tridentatus* and 53 *Carcinoscorpius rotundicauda*. Spatial distribution patterns were revealed across 3 scales: large, intermediate and micro. At the largest spatial scale, which includes all 4 sampling sites, juvenile *T. tridentatus* and *C. rotundicauda* co-occurred at the 2 inner sites (Jiaodong and Shanxin) within the bay, while none were found at the outer site (Wuwu) or the site beyond the bay (Jintan) (Table 1, Fig. S1 in the Supplement at www.int-res.com/articles/suppl/b024p117_supp.pdf). At the 2 inner sites, *C. rotundicauda* exhibited higher densities than *T. tridentatus* (0.24 ± 0.05 vs. 0.05 ± 0.03 ind. m^{-2}). In addition, *T. tridentatus* was found in lower densities at Jiaodong than at Shanxin (0.02 ± 0.02 vs. 0.11 ± 0.07 ind. m^{-2}), while *C. rotundicauda* had similar densities at both inner sites (0.25 ± 0.02 ind. m^{-2} at Jiaodong and 0.22 ± 0.04 ind. m^{-2} at Shanxin, Fig. S1).

At the intermediate (among stations between sites) and micro spatial scales (among stations within a site), the densities of *T. tridentatus* and *C. rotundicauda* revealed different trends in the species' relative proximities to mangroves and tidal flats. Densities juvenile *T. tridentatus* increased from the locations close to the mangrove edges to the locations on the open, non-vegetated tidal flats, but those of *C. rotundicauda* decreased along the same spatial gradient (Table 1; from JD01 through JD06 to JD05 in Fig. S2 in the Supplement and from SX01 through SX02, SX04 to SX03 in Fig. S3 in the Supplement). For *C. rotundicauda*, the same distribution trend along the mangrove–tidal flat gradient was found at an even smaller scale. Among transects at Stn JD01, the spatial range of *C. rotundicauda* stretched across 3 parallel transects, each 15 m apart. Across these transects, the densities of *C. rotundicauda* decreased sharply from the mangrove edge to the tidal flat (0.65, 0.15 and 0 ind. m^{-2}, respectively, Table 1; Fig. S4 in the Supplement).

Tachypleus tridentatus densities were significantly and positively correlated with distance from the nearest mangroves, while the correlation of *C. rotundicauda* densities was significantly negative (Spearman r = 0.83, p = 0.02 vs. r = –0.86, p = 0.01, n = 7, Fig. 2).

Table 1. Juvenile densities of *Tachypleus tridentatus* and *Carcinoscorpius rodunticauda* at different spatial scales in Pearl Bay, China. Sampling took place at low tides in October 2014. Additional stations were also monitored, but only those stations that served for density comparisons at intermediate and micro spatial scales are given in this table. See Table S1 in the Supplement at www.int-res.com/articles/suppl/b024p117_supp.pdf for detailed sampling information. DisM: distance to the nearest mangrove

Spatial scale	Site	Stn	DisM (m)	Density (ind. m^{-2}) *T. tridentatus*	Density (ind. m^{-2}) *C. rotundicauda*
Largest (from inner bay to coastal zone)	Jiaodong			0.02 ± 0.02	0.25 ± 0.02
	Shanxin			0.11 ± 0.07	0.22 ± 0.04
	Wuwu			0	0
	Jintan			0	0
Intermediate (among stations between sites)	Jiaodong	JD01	30.2	0	0.40
		JD06	46.1	0.05	0.25
		JD05	187.8	0.10	0.23
	Shanxin	SX01	1.6	0	0.31
		SX02	22.8	0.06	0.25
		SX04	275.3	0.19	0.13
		SX03	233.0	0.38	0.13
Micro (among stations within a site)	Jiaodong (JD01)	T1	15.0	0	0.65
		T2	30.0	0	0.15
		T3	45.0	0	0

Relationship between juvenile body size and proximity to the nearest mangrove

The juveniles of the 2 horseshoe crab species measured between 15 and 65 mm in prosomal width (Table S2 in the Supplement at www.int-res.com/articles/suppl/b024p117_supp.pdf). The majority of juvenile *T. tridentatus* had a prosomal width between 25 and 45 mm, whereas most of the juvenile *C. rotundicauda* had a prosomal width between 30 and 40 mm (Table S2). The prosomal width of *T. tridentatus* averaged 36.2 ± 11.4 mm at Jiaodong and 35.9 ± 2.7 mm at Shanxin, while that of *C. rotundicauda* averaged 31.7 ± 2.1 mm at Jiaodong and 34.7 ± 1.5 mm at Shanxin, showing that the juvenile sizes of both species were similar, with *T. tridentatus* being slightly larger.

The size distribution profiles, plotted against the distance to the nearest mangrove edge, varied between species. The smaller juveniles of *C. rotundicauda* tended to aggregate in closer proximity to the mangrove forest edges than did the larger individuals (Fig. 3). Small individuals of 15 to 45 mm

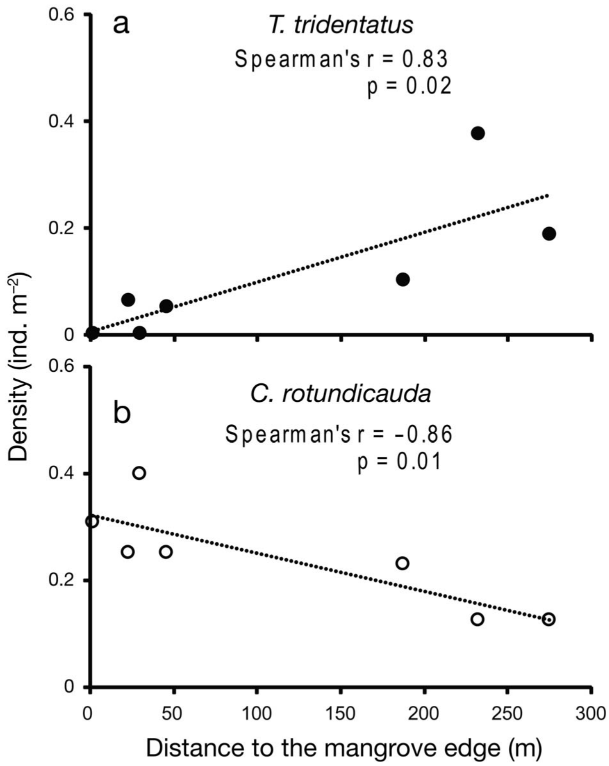

Fig. 2. Spearman correlation analyses showing the relationships of density and the distance to the nearest mangrove in juvenile (a) *Tachypleus tridentatus* and (b) *Carcinoscorpius rotundicauda*. Data points were from Stns JD01, JD05-06, and SX01-04. Dashed lines show correlation trends

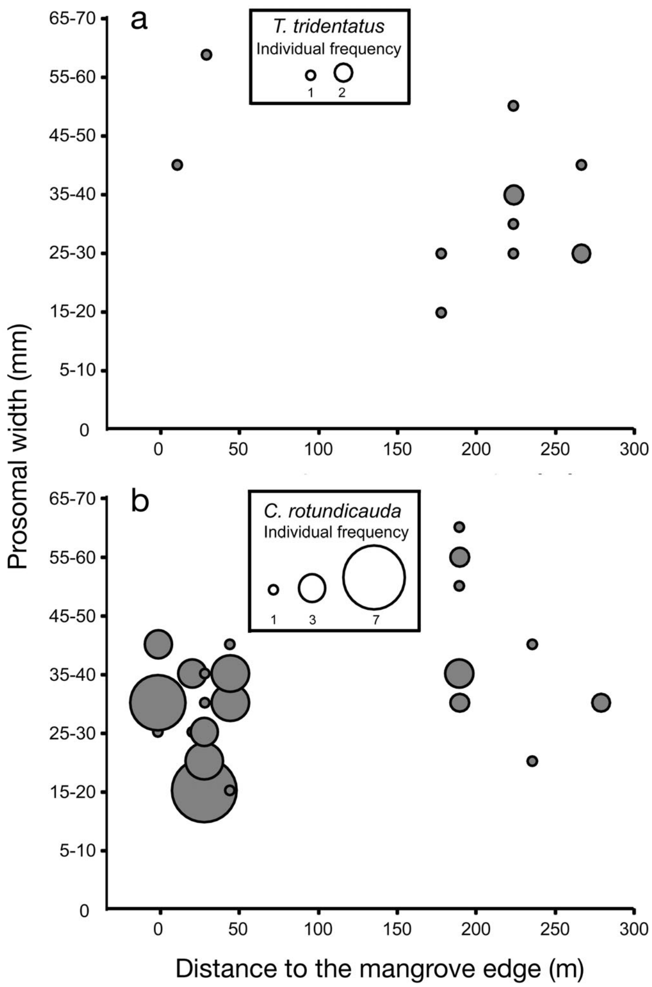

Fig. 3. Spatial relationships between different body sizes (prosomal width) and the distance to the nearest mangrove for juvenile (a) *Tachypleus tridentatus* and (b) *Carcinoscorpius rotundicauda* using the data combined from the Jiaodong and Shanxin sites

prosomal width — 75% of the crabs observed — were distributed within ~50 m of a mangrove edge, while large individuals of 30 to 65 mm — the remaining 25% of the crabs — were distributed 188 to 275 m away from the mangrove edge (Fig. 3, Table S2). In contrast, no trend was evident for *T. tridentatus* size classes (Fig. 3).

Relationships of juvenile densities with environmental variables

The redundancy analysis with automatic forward selection showed that the juvenile densities of *T. tridentatus* and *C. rotundicauda* were significantly correlated with the studied environmental variables, including TOC content, silt-clay content, degree of sorting, chl *a* concentration and DisM (all p values ≤ 0.1, Monte Carlo permutation with 999 permutations, Table 2). These 5 environmental variables explained 68% of the total variation in juvenile densities of both species (F = 5.9, p = 0.002, Monte Carlo permutation test with 999 permutations, Table 3).

Table 2. Redundancy analysis results showing the environment variables that significantly (Monte Carlo permutation tests, p ≤ 0.1) explained changes in the density of juvenile horseshoe crabs *Tachypleus tridentatus* and *Carcinoscorpius rotundicauda* in Pearl Bay. Environmental variables that best explain these density changes are ranked first, while the remaining variables are ranked on the basis of the additional fit from the model selection. TOC: total organic carbon content; DisM: distance to the nearest mangrove

Selected variables	F	p	Variance explained	Correlation Axis 1	Correlation Axis 2
TOC	10.3	0.002	0.37	0.72	−0.02
Silt−clay content	4.3	0.01	0.13	0.72	−0.23
Sorting coefficient	2.4	0.09	0.07	0.46	−0.37
Chl *a*	2.8	0.06	0.06	−0.24	−0.11
DisM	2.2	0.1	0.06	−0.35	−0.01

Table 3. Summary of the statistics from the redundancy analysis of the relationships between environmental variables and the juvenile densities of the horseshoe crabs *Tachypleus tridentatus* and *Carcinoscorpius rotundicauda* in Pearl Bay

	Ordination axis 1	2	3	4	Total variance 1.00	Full model F	p
Eigenvalue	0.52	0.16	0.20	0.12		5.9	0.002
Species–environment correlation	0.85	0.75	0	0			
Cumulative% variance explained							
by species data	52.1	68.1	87.6	100.0			
by species–environment correlation	76.4	100.0	0	0			
Sum of all canonical eigenvalues					0.68		

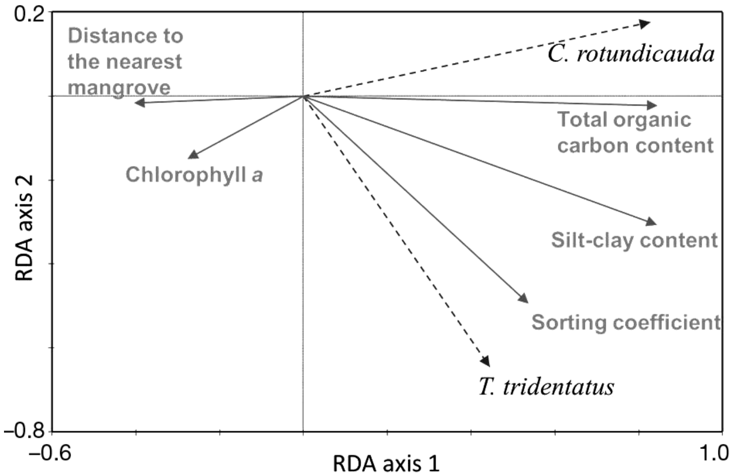

Fig. 4. Redundancy analysis (RDA) showing the ordination diagram of juvenile densities for *Tachypleus tridentatus* and *Carcinoscorpius rotundicauda* (dashed arrows) with environmental variables (solid arrows)

The redundancy analysis results showed that the relationship between juvenile density and each of the 5 environmental variables varied with species. The density of juvenile *T. tridentatus* increased with decreasing degree of sediment sorting, whereas juvenile *C. rotundicauda* densities exhibited greater positive relationships to TOC content and silt-clay content (Fig. 4). Although DisM and chl *a* concentrations in the sampled sediments explained the juvenile density changes to a lesser extent (variances explained), the densities of both species were negatively correlated with these 2 factors. Moreover, *C. rotundicauda* remained closer to the mangroves than *T. tridentatus* (ordinations on RDA axis 2, Fig. 4). The average DisM for *C. rotundicauda* was 113.8 m, while that for *T. tridentatus* was 153 m (calculated from Table 1 data).

DISCUSSION

In the present study, only 12 *Tachypleus tridentatus* juveniles were collected, thus the findings need to be interpreted with caution. However, this low number does highlight that this species is becoming rare and deserves urgent protection and conservation.

The juvenile densities of *T. tridentatus* and *C. rotundicauda* at Pearl Bay are among the highest in comparison to those at other sites (Table 4), revealing that Pearl Bay in southwestern China is a critical habitat for the 2 horseshoe crab species. In particular, the densities of *T. tridentatus* were 25- to 37-fold higher (Table 4). In Pearl Bay, the spatial distribution of juveniles varies according to species, body size, proximity to the mouth of the bay, proximity to mangrove vegetation, and microhabitats. Our findings may facilitate the habitat conservation of the 2 horseshoe crab species and other co-existing commercial species. In particular, such coastal habitats have been considered endangered due to anthropogenic impacts (Martin 2014).

Table 4. Comparisons of juvenile densities of *Tachypleus tridentatus* and *Carcinoscorpius rotundicauda* among different areas. –: no record

Area	*T. tridentatus* (ind. m^{-2}) (range)	*C. rotundicauda* (ind. m^{-2})	Reference
Puerto Bay, Philippines	0.0075–0.015	–	Almendral & Schoppe (2005)
Silver Bay, East Beibu Gulf, Guangxi, China	0.0009–0.024	–	Hu et al. (2009)
Deep Bay and Lantau Island, Hong Kong	0.0008–0.003	Almost zero	Shin et al. (2009)
Deep Bay, Hong Kong	0.011–0.02	Almost zero	Morton & Lee (2010)
Pearl Bay, West Beibu Gulf, Guangxi, China	0.02–0.11	0.22–0.25	This study

The co-occurrence of juvenile *T. tridentatus* and *C. rotundicauda* at the Jiaodong and Shanxin sites in the inner zones of Pearl Bay is consistent with our prediction and can be explained by the specific habitat requirements necessitated by the unique life history of each species. In general, the life histories of *T. tridentatus* and *C. rotundicauda* have been well documented (Sekiguchi 1988, Chen et al. 2004, Cartwright-Taylor et al. 2009, 2012, Cartwright-Taylor & Hsu 2012). These records reveal that the 2 species differ in their demands for spawning and maturation sites, but utilize the same kind of mud and/or mud-sand flats as juvenile nursery grounds.

Pearl Bay is a predominantly mangrove-vegetated estuarine bay. Its inner sections support dense mangroves, which spread along the estuary of Jiangping River. Its outer reaches connect to the sea (Beibu Gulf) and are characterized by open, sandy shores (Records of Bays in China 1993). With this bio- and geomorphological setting, the Bay has the potential to serve as an important migratory path for departing *T. tridentatus* subadults seeking to reach maturation sites in deeper channels (~20 to 30 m depth, Liang 1985, Hsieh & Chen 2015) along the coasts of the Bay and for returning adults who need to access nesting sites on the bay's sandy beaches. The bay also provides suitable habitat in the form of mangrove swamps to allow *C. rotundicauda* to complete its life cycle.

Most significantly, the bay, with its large intertidal sand and mud flats, provides nourishment for the juveniles of the 2 horseshoe crab species. Analysis of sediment characteristics reveal that the inner bay experiences deposition while the outer and coastal areas are subject to erosion. In addition, prior measurements revealed that during flood tides, the prevalent flow direction was to the northeast with a high velocity, while at ebb tides, the flow direction shifted to the southwest and had an even higher velocity (Records of Bays in China 1993). Given such a flow regime, we reason that the first instars of *T. tridentatus* hatch from their nests when high tides wash the sandy beaches and are very likely to be conveyed by the northeastern currents toward the inner Bay to settle in areas subject to deposition. Those areas also happen to be ideal habitat for mangroves and the mangrove horseshoe crab *C. rotundicauda*.

The distinct spatial associations between juvenile size and the DisM for each species can be attributed to the differences in spawning sites and the separation between adult spawning and juvenile nursery sites. *C. rotundicauda* nests on tidal creeks within mangroves; therefore, the hatchlings are able to easily transfer to the adjacent mudflats within the mangroves (Cartwright-Taylor & Hsu 2012). Consequently, smaller juveniles appear to aggregate in a close proximity to mangroves. In contrast, the spawning sites of *T. tridentatus* are not located in the mangroves but on sandy beaches as seen at Beihai coast, Guangxi (Liang 1985), Hakata Bay, Japan (Sekiguchi 1988) and Kinmen, Taiwan (Chen et al. 2004). Sand bars are well developed in the outer regions of the bay, which include the study sites Wuwu and Jintan (Records of Bays in China 1993). In addition, some outer edges of the sand flats at the Jiaodong and Shanxin sites are slightly more elevated than the adjacent tidal flats (authors' personal observation). These sandy beaches very likely serve as nesting sites for *T. tridentatus* and are separated from the mangroves; consequently, the localities that juvenile *T. tridentatus* inhabit do not necessarily occur in close proximity to the mangroves.

Our data reveal that juvenile *T. tridentatus* can explore more habitat types than juvenile *C. rotundicauda*. Juvenile *T. tridentatus* are distributed on intertidal fine sand flats in mangrove- and seagrass-vegetated estuarine bays or coasts (such as Pearl Bay of this study and Silver Bay coast studied by Hu et al. 2009), differing from those also found in open, non-mangrove vegetated headland bays (such as bays studied by Chen et al. 2004, Hsieh & Chen 2009 and Weng et al. 2012). In contrast, *C. rotundicauda* has been recorded persistently within or near mangrove habitats (this study, Cartwright-Taylor & Hsu 2012, Cartwright-Taylor et al. 2012), reflecting its habitat specificity.

The juveniles of both *T. tridentatus* and *C. rotundicauda* exhibit a preference for localities with poorly sorted sediments that have high organic carbon content. These physico-chemical properties of sediments suggest that various forms of food are available there. Organic matter buried in sediments is found to benefit many detritivores and through conversion and transfer contributes to the sustenance of many consumers at higher trophic levels within mangrove ecosystems (Hsieh et al. 2002). With respect to food sources for juvenile *T. tridentatus* and *C. rotundicauda*, the data from $\delta^{13}C$ signatures showed that seagrass-derived organic matter, rather than that of mangroves, was assimilated by the juveniles of both *T. tridentatus* and *C. rotundicauda* in Pearl Bay (authors' unpubl. data). A mix of polychaetes, bivalves and gastropods that are supported by seagrass biomass comprised the diet of juvenile *T. tridentatus* studied in Hong Kong (Kwan et al. 2015). Benthic microalgae were also found to contribute to the diet of *C. rotundicauda* studied in Malaysia (Newell et al.

1995). Benthic microalgae primarily grow on open sandy tidal flats and are rarely found on mud flats or under the canopy of mangroves (Wainright et al. 2000, Hsieh et al. 2002). Given these food sources and their locations, the critical nursery grounds of *T. tridentatus* and *C. rotundicauda* should consist of a variety of habitats, including open sandy and muddy tidal flats, seagrass beds and mangroves.

We recommend the following strategic guidelines for horseshoe crab conservation. (1) Identify critical habitats for horseshoe crabs. Nesting beaches and migratory routes for *T. tridentatus* and nesting tidal creeks for *C. rotundicauda* are key habitats. It is essential to inventory and identify these habitats on the southern coast of China. (2) Increase legal protection of critical habitats. In bays, harvest activities that adversely affect habitats include rearing free-range ducks in mangroves, bottom trawling and collecting clams with rakes. These activities should be monitored and managed. Alternatively, the conservation of critical habitats can be incorporated into current laws mandating mangrove protection. Recently, the IUCN Species Survival Commission for horseshoe crabs has launched protection programs to put these species on the IUCN Red List. (3) Raise public awareness of horseshoe crab conservation efforts. Education programs could include (i) advising local fishermen to cease with bottom trawling and to avoid deploying fishing nets and cages in mangrove protected areas, (ii) advising the public not to consume ducks reared in mangroves, (iii) empowering local non-government organizations to monitor the stressors of horseshoe crab habitats and populations, and (iv) making the public aware of the use *Tachypleus* amoebocyte lysate for medicinal puposes.

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