Spatiotemporal Distribution of Asian Horseshoe Crab Eggs Are Highly Intermingled with Anthropogenic Structures in Northern Beibu Gulf, China

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Abstract  Identification, protection and restoration of spawning habitats are vital for protecting the depleted species. Asian horseshoe crabs are ecologically important macroinvertebrates in coastal and estuarine ecosystems. However, their spawning habitat studies were limited to several reports in tropical regions, possibly due to the lack of modified survey methods, particularly in habitats with a lower density of spawning adults, and/or intermingled with anthropogenic structures. In this study, the year-round egg distribution and spawning habitat baselines of Carcinoscorpius rotundicauda and Tachypleus tridentatus were determined in the northern Beibu Gulf, China. Our findings demonstrated that the peak spawning occurred in June–July and ceased in November–January when the average water temperature dropped below 20°C. Egg aggregations were found <10 cm beneath the sediment surface with regular tidal inundation, regardless of seasonal changes, in the vicinity of natural and artificial structures with elevated, mildly sloping substratum within the high tide zones. The nests were characterized by medium-sized sediment grains (0.5–0.9 mm), high temperatures (31–34°C), low water contents (0.8%–0.9%), and total organic carbon contents (0.5%–0.7%), which might maximize the hatching success. The identified nesting beaches were close to nursery habitats for juveniles, and tidal creeks were present as the possible corridor connecting these two important habitats through the dominant mangrove forests. The findings provide valuable insights in the scope of spawning behavior and nest-site selection of Asian horseshoe crabs under a mixture of natural and artificial structures, which could benefit future management efforts for the exploited spawning populations.

Key words  Carcinoscorpius rotundicauda; Tachypleus tridentatus; mangrove; tidal creek; sediment texture; slope; elevated substratum

1 Introduction  Coasts and estuaries are thriving but delicate ecosystems for a myriad assortment of living species. Horseshoe crabs are typical estuarine denizens inhabiting along the west coast of the North Atlantic and Pacific Oceans (Smith et al., 2017; John et al., 2018). Commonly referred to as ‘living fossils’, four extant horseshoe crab species bearing nearly unchanged physical appearance for more than 450 million years (Rudkin and Young, 2009). Horseshoe crabs are ecologically important in the estuarine food web as both predators and prey (Botton, 2009). Recent studies also pointed out their potential role as an indicator species in reflecting the general health of ecosystems (Kwan et al., 2018, 2021). However, the long-term survival of horseshoe crabs does not ensure their future persistence in a changing world. A combination of threats derived from coastal development and human-induced disturbance have severely affected the remaining horseshoe crab populations (Wang et al., 2020). Most notably, commercial exploitation of horseshoe crabs for Tachypleus or Limulus amoebocyte lysate production (Gauvry, 2015; Yan et al., 2018) and human consumption (Fu et al., 2019) pose considerable pressures on their survival. The Atlantic horseshoe crab Limulus polyphemus and tri-spine horseshoe crab Tachypleus tridentatus have been listed as ‘Vulnerable’ (Smith et al., 2017) and ‘Endangered’ (Laurie et al., 2019), respectively, based on the recent extensive reviews of the population status and trends across the ranges. The other two Asian species, the coastal horseshoe crab T. gigas and the mangrove horseshoe crab Carcinoscorpius rotundicauda remain ‘Data Deficient’, but their status are under reassessment due to the
increasing reports depicting their declining trends and the imminent threats to their populations and habitats.

Identification, protection and restoration of critical habitats are the first step for reversing the declining trend of exploited marine organisms (Collins et al., 2000). Horseshoe crabs utilize a unique combination of habitats during their entire life cycle: 1) nearshore areas as foraging ground for adults, 2) intertidal flats as nurseries for juveniles, and 3) sandy beaches between the low-tide terrace and the high-tide water line for spawning and egg incubation (Smith et al., 2017). The life history, ecology and habitat of *L. polyphemus* have been studied extensively, but the collection of habitat baselines for these three Asian species were disproportional (Wang et al., 2020). Some Asian countries and territories, including Mainland China (Xie et al., 2017), Hong Kong region (Kwan et al., 2016), Taiwan region (Hsieh and Chen, 2015), Singapore (Cartwright-Taylor, 2015) and Indonesia (Meliana et al., 2021), had gathered relatively complete population information from the intertidal nursery habitats. However, the data of spawning ecology and behavior, which can provide particular baseline information on egg deposition and nest parameters, were largely restricted to several studies in tropical countries such as Malaysia (Fairuz-Fozi et al., 2018; Mohamad et al., 2019; Zauki et al., 2019) and Singapore (Cartwright-Taylor, 2015).

In China, spawning activities of Asian horseshoe crabs have not been documented since 1984 (Cai et al., 1984), possibly due to the lack of modified spawning and egg survey methods, particularly in areas with a lower density of spawning adults as well as complicated natural and anthropogenic structures along the shoreline. The nesting pattern of horseshoe crabs that lay clusters of eggs at depths of 10–20 cm beneath the sediment layer also imposes significant challenges for the spawning baseline collection (Botton et al., 2021).

While the spawning behavior of horseshoe crabs shares many common features, their nesting preference can be site-specific. For the well-studied Atlantic species, spawning beaches in Delaware and New Jersey, USA are typically coarse-grained, while Florida beaches comprised typically fine-grained and poorly drained substratum (Penn and Brockmann, 1994; Smith et al., 2017). For the Asian species, *T. tridentatus* and *T. gigas*, similar to *L. polyphemus*, seemed to prefer sandy areas between upper- and mid-intertidal zones with fine and medium-sized sediment grains (Nelson et al., 2015; Mohamad et al., 2019). Itaya et al. (2019) also observed high spawning activities of *T. tridentatus* at the moderate slopes near the high tide line. *Carcinoscorpius rotundicauda* was frequently cited as an exception since their spawning adults are widely observed in the muddy mangrove areas even during low tides (Cartwright-Taylor, 2015; Fairuz-Fozi et al., 2018; Liao et al., 2019a). This, therefore, poses an intriguing question on the possibly different spawning behavior and nesting preference of *C. rotundicauda*.

The northern Beibu Gulf is a semi-closed gulf along the coast of southern China and northern Vietnam. The major estuarine ecosystem is fringed by extensive intertidal beaches (713 km²), and include large areas of pristine mangrove forests (74 km²) and seagrass beds (0.8 km²) (Fan et al., 2016). On the other hand, the coastline configuration has been largely altered by the construction of aquaculture ponds (344 km²), artificial salt pans (23 km²), ports (18 km²) and seawalls (654 km) (Li et al., 2017). The gulf is frequently claimed to accommodate high densities of *C. rotundicauda* and *T. tridentatus* populations in Asia-Pacific regions (Brockmann and Smith, 2009; Sekiguchi and Shuster, 2009; Fu et al., 2019; Liao et al., 2019a). The intertidal habitats for horseshoe crab egg deposition can be influenced by the man-made shoreline features (Botton et al., 1988; Jackson et al., 2015; Nelson et al., 2015). In this study, we examined 1) year-round variation of nest and egg densities, 2) temporal changes in nest distributions, and 3) collected environmental physiochemical parameters of Asian horseshoe crab spawning habitats along the shoreline intermingled with anthropogenic structures. The findings can provide valuable baseline data to develop scientifically important conservation measures by accounting for critical habitat distribution and peak spawning season of Asian horseshoe crabs in the region.

2 Methods

2.1 Egg Surveys

Preliminary investigation of Asian horseshoe crab spawning habitats was conducted at 18 potential beaches along the northern Beibu Gulf shoreline (Fig. 1) during June–August 2019 at day-light low tides around the time of the new and full moons. The sites were chosen based on the identified nursery habitat distribution (Xie et al., 2020) and fishing community reports (Liao et al., 2019a). Given that complex natural (e.g., open sandy-mudflats, mangrove forests, tidal creeks and intertidal sand bars) and artificial structures (e.g., seawalls, aquaculture ponds and other coastal physical infrastructure) were present along the shoreline, a modified egg survey method was required. Asian horseshoe crab numbers are much low than those in the USA, and egg densities might be too low to be detected using traditional random cores (Botton et al., 2021). Nest marking method by tracking spawning adults as described in Mohamad et al. (2019) has been tried. However, none was observed possibly due to the relatively smaller adult *T. tridentatus* population in the gulf. It is also a challenge to closely follow the spawning trail of *C. rotundicauda* during rising tides when the estuarine water became turbid.

During the preliminary surveys, a team of four people searched for the eggs according to spawning imprints, i.e., shallow depressions left on sediment surface by the females as they deposit their eggs (see John et al., 2012), within mid- to upper-intertidal areas (<1 km from seawalls or roadsides) during low tides. All likely depressions were attempted using a handheld shovel to an approximate depth of 20 cm from the sediment surface, which can assure that most *C. rotundicauda* and *T. tridentatus* egg clusters can be detected (Fairuz-Fozi et al., 2018; Mohamad et al., 2019). All eggs found within the sediment under the same spawn-
ing imprint were considered as a ‘nest’. The sampling of Asian horseshoe crab eggs was carried out across a variety of natural habitat types and man-made structures. *C. rotundicauda* eggs were distinguished from *T. tridentatus* by their discernably smaller size (diameter 1.9–2.2 mm versus 3.0–3.3 mm for *T. tridentatus*) with greenish-yellow color. Sampling was not conducted during night-time due to safety reasons.

Six shores, including Shanxin (SX), Jiaodong (JD), Yuzhouping North (YZPN), Yuzhouping South (YZPS), Zhongsandun (ZSD) and Shajiao (SJ), were identified as the active spawning habitats for Asian horseshoe crabs (Fig.1). The results of adopted survey method are highly dependent on the people who searched for the nests. Therefore, the same team of four people conducted the monthly egg samplings, except during the outbreaks of COVID-19 pandemic in Mainland of China (February–April 2020), at the designated survey areas with standardized survey efforts (i.e., person-hour per km² survey area; Fig.1, Table 1). Only one nest of *C. rotundicauda* and one nest of *T. tridentatus* were found at XGS respectively, therefore XGS was not included in the monthly egg surveys. All identified nests were marked using a colored stick, and all measurements were conducted after the end of the allowed survey time. The GPS locations, nest depth, species, total counts of eggs and their developmental stages (undeveloped eggs, embryos at stage 20–21, or hatched trilobite larvae) for each nest were recorded. Only eggs without apparent signs of decay, including those embryos and larvae remained within the beach sediments, were included in the counts. The

![Fig.1 Maps showing the locations of the northern Beibu Gulf, China, and the 18 potential spawning sites of Asian horseshoe crabs. Monthly egg density surveys were conducted at the six study shores (yellow triangles) within the designated survey areas (white contours), except during the outbreaks of COVID-19 pandemic in Mainland China (February–April 2020). The high-density egg areas are indicated in orange rectangles. The scale bar on the map is in 200 m. Satellite images were acquired from National Platform for Common Geospatial Information Services (Beijing, China). The site abbreviation is mentioned in Table 1.](image)

![Table 1 Asian horseshoe crab egg survey site coordinates, dates and efforts at six study sites in the northern Beibu Gulf, China](image)

| Site        | Abbreviation | Coordinates         | Survey date                | Total survey area (km²) | Survey time (h) |
|-------------|--------------|---------------------|----------------------------|-------------------------|-----------------|
| Shanxin     | SX           | 21°34’N, 108°10’E  | 2019 Sep 14, Oct 10, Nov 7, Dec 23, Jan 13, May 23, Jun 3, Jul 2, Aug 1, 2020 Jan 14, May 24, Jun 4, Jul 3, Aug 2 | 0.08 | 2.8 |
| Jiaodong    | JD           | 21°36’N, 108°11’E  | 2019 Sep 15, Oct 8, Nov 8, Dec 24, Jan 14, May 24, Jun 4, Jul 3, Aug 2 | 0.10 | 3.5 |
| Yuzhouping North | YZPN     | 21°38’N, 108°22’E  | 2019 Sep 17, Oct 12, Nov 10, Dec 26, Jan 16, May 26, Jun 6, Jul 5, Aug 4 | 0.06 | 2.1 |
| Yuzhouping South | YZPS    | 21°38’N, 108°22’E  | 2019 Sep 16, Oct 11, Nov 9, Dec 23, Jan 15, May 25, Jun 5, Jul 4, Aug 3 | 0.10 | 3.5 |
| Shajiao     | SJ           | 21°37’N, 108°51’E  | 2019 Sep 28, Oct 26, Nov 24, Dec 13, Jan 10, May 8, Jun 19, Jul 19, Aug 17 | 0.02 | 0.7 |
| Zhongsandun | ZSD          | 21°38’N, 108°51’E  | 2019 Sep 28, Oct 26, Nov 25, Dec 14, Jan 11, May 9, Jun 20, Jul 20, Aug 18 | 0.03 | 1.0 |

Note: The survey time was assigned according to the corresponding total survey area at each site with the consistent number of people in survey team.
eggs/larvae were returned back to the original pit and covered by the sediment once the measurements were completed. Similar approach of egg samplings and measurements were conducted in another study of T. tridentatus at Tsuyazaki Cove, Japan (Itya et al., under review), and found that the survival and hatching rates of ‘disturbed’ eggs (i.e., eggs which were repeatedly sampled, measured and returned back to the original pit) were similar to those ‘control’ eggs in the same region. Since the nest/egg density data derived from the present survey were largely based on the team of people involved in the egg searching, the information was only used to demonstrate the spatiotemporal patterns, but not for comparing the ‘absolute’ values among studies.

2.2 Habitat Physicochemical Parameter Measurements

Measurements of nest temperature, elevation, inclination and oxidation-reduction potential (ORP) were conducted in situ during low tides. Nest temperature and ORP were measured with a portable ORP meter (Harveson 100P, Suzhou, China), whereas inclination data were collected using a digital inclinometer (Syntek, Suzhou, China). Elevation on the nest surface was determined by a satellite-based real-time kinematic positioning system (Hi-Target, V90, Guangzhou, China).

Sediment samples for determination of grain size, water content, total organic carbon (TOC) and chlorophyll a (Chl a) were collected in the vicinity of the high-density nest areas at the depth of egg cluster using a 7 cm-diameter sediment core. Sediment samples were stored on ice and under dark conditions before they were taken to the laboratory or nearby accommodation within an hour. The grain size was determined according to the Blott and Pye (2001) protocol; TOC was analyzed following wet dichromate oxidation method; the water content was calculated from the difference of weight before and after the samples were dried at 60°C for 72 h until achieving a constant weight; and the Chl a measured using Wisconsin State Laboratory of Hygiene’s ESS Method 150.1 with modification. All technical details were described in Xie et al. (2020).

To collect more complete environmental baselines regarding spawning habitats, surface water physicochemical parameters were also determined at a minimum of three areas per study site, where higher densities of Asian horseshoe crab nests were recorded. The water samples were collected during rising tides when the seawater level reached at approximately 40 cm, and a high number of mating pairs was observed within the investigation areas. Water temperature, salinity, dissolved oxygen (DO), pH, water turbidity and ORP were measured in situ, whereas suspended particulate matter (SPM), total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) were quantified in the laboratory. Water temperature and DO were measured by a portable DO meter (YSI ProODO, OH, USA), salinity was determined by an optical refractometer (ATAGO, WA, USA), pH was measured using a handheld pH meter (Thermo Scientific, Orion Star A211, MA, USA), and turbidity was determined with the portable turbidimeter (HACH, 2100Q, CO, USA). SPM concentration was determined by calculating the weight difference between the particulate matter retained on the dried and pre-filtered blank filters, as described by Dan et al. (2019). TDP was quantified using an auto-analyzer after the decomposition of samples in an autoclave at 120°C for 90 min with boric acid-persulfate oxidation solution (Lin et al., 2012). TDP was determined by digesting the samples with acidified 50 g L⁻¹ K₂S₂O₈ solution (pH = 1) in an autoclave sterilizer at 120°C for 90 min (Cai and Guo, 2009). The environmental baseline data for T. tridentatus at SJ and ZSD were not collected due to the very limited number of nests.

2.3 Data Analysis

All data were tested for normality and homogeneity of variance using Shapiro-Wilk test and Levene’s test, respectively. While abundance data of eggs per nest did not meet the normality requirements after all attempts for arithmetic transformations, the differences among study sites and sampling months were analyzed using non-parametric Scheirer-Ray-Hare extension of Kruskal-Wallis test. The above calculations were performed in SPSS Statistics (v 26, IBM, NY, USA), and Scheirer-Ray-Hare extension of the Kruskal-Wallis test was conducted following the modified SPSS protocol described in Shen et al. (2013).

3 Results

3.1 Temporal Variations of Nest and Egg Densities

Only four T. tridentatus nests were found at SJ and one at ZSD during September – November. Monthly changes in densities of C. rotundicauda nests and eggs were evident (Fig.2). Nest and egg densities at SX and YZPS peaked in June, whereas those at JD, YZPN and SJ peaked in July (Figs.2A – B). Both the nest and egg densities declined gradually after July and entirely ceased in November or December (Figs.2A – B). Spawning of C. rotundicauda seemed to begin in the coming year in May or earlier in April. The egg and nest densities at ZSD were generally low throughout the year with a peak in August.

Average egg counts (± standard deviation) of each C. rotundicauda nest varied monthly between 14 and 30 ± 2 (Fig.2C). However, the egg yields per nest differed neither across sampling month nor among study sites (Month: $H = 3.735$, $p = 0.710$; Site: $H = 1.079$, $p = 0.960$; Month × Site: $H = 6.524$, $p = 1.000$). Egg counts were considerably higher in each T. tridentatus nest (195 – 573) compared to those in C. rotundicauda (13 – 90). The percentage of undeveloped eggs, after excluding those embryos and trilobite larvae, was the lowest in October – November (Fig.2D). C. rotundicauda eggs were deposited at a depth of 2.4 cm (range: 1.4 – 3.7 cm), which was apparently shallower than those of T. tridentatus (6.9 – 7.5 cm).

3.2 Spatial Distribution of Nests

Both C. rotundicauda and T. tridentatus nests were observed to be located primarily within the higher intertidal zones limited by the presence of seawalls and/or coastal shrimp ponds (Fig.3). Most nests were found in unshelter-
Fig. 2 Monthly variations of (A) nest density, (B) egg density, (C) the mean number ± standard error of eggs per nest, and (D) the percentage of undeveloped eggs (excluding those embryos and trilobite larvae) of *C. rotundicauda* at the six study sites. The samplings for Asian horseshoe crab (A) nests and (B) eggs were conducted at the designated survey areas with standardized survey efforts, and their densities were expressed in the amount of sampling work performed by the average person in one hour (person-hour). The data in (C) were analyzed by Scheirer-Ray-Hare extension of Kruskal-Wallis test, but no significant difference among site/month groups was detected. The site abbreviation is mentioned in Table 1.

Fig. 3 The monthly nest distribution of *C. rotundicauda* (red) and *T. tridentatus* (blue) at the six study sites along the northern Beibu Gulf shoreline (within the orange rectangles in Fig. 1). The satellite maps are obtained from the National Platform for Common Geospatial Information Services (https://www.tianditu.gov.cn/). Different sampling months are indicated by the gradients of red/blue. The temporal variation of nest locations was indiscernible. The site abbreviation is mentioned in Table 1.

ed areas between mangrove patches with tidal creeks nearby. At SX, spawning also occurred along the artificial tidal channels of aquaculture ponds, given that the water control gate was remained open. Temporal changes in egg distributions were indiscernible (Fig. 3). Potential nesting points on open sandy mudflats in mid-intertidal zones out-
side the mangrove fringes were also searched during the preliminary surveys, however, no nest was detected.

In terms of beach topography, nests were mostly located at elevated substratum beside mangrove tidal creeks, at the bases of mangrove *Excoecaria agallocha* surface roots or elevated coarse-grained substrate with cobbles, as well as on the sandy slope or narrow band in high tide areas (Fig. 3 and Figs. 4A–F). Nests can also be found not far away from man-made structures, including power transmission facilities, mangrove boardwalks, abandoned construction wastes, cemented walkways, water control gates and man-made tidal channels of aquaculture ponds (Fig. 3 and Figs. 4G–I).

### 3.3 Nest Parameters

The sediment physiochemical conditions of *C. rotundicauda* nests were similar among study sites (Table 2). At low tides, the average nest temperature reached 31–34°C, and the water content was 0.8%–0.9%. The nest sediment was mainly comprised medium sized grains (0.5–0.9 mm) with low TOC (0.5%–0.7%), Chl a (1–3 μg g⁻¹) and ORP (225–252 mV). Spawning occurred at substratum with mild and elevated slope (inclination 5°–14°, elevation −18 m to −20 m; Table 2).

The spawning habitats are regularly inundated by estuarine waters with the relatively stable temperature of 31–33°C, salinity 11–21, DO 5–6 mg L⁻¹, pH 7.3–7.8 and ORP 217–236 mV (Table 3). The water turbidity (36–187 NTU) as well as levels of SPM (77–151 mg L⁻¹), TDN (61–84 μmol L⁻¹) and TDP (2–66 μmol L⁻¹) of the spawning grounds were greatly fluctuated during rising tides (Table 3).

### 4 Discussion

Asian horseshoe crabs, *C. rotundicauda* and *T. tridentatus* coexist on many intertidal flats in Asia-Pacific regions (Mohamad et al., 2015; Kwan et al., 2016; Xie et al., 2020; Meilana et al., 2021). In this study, *T. tridentatus* eggs were found in the vicinity of those from *C. rotundicauda* at SJ and ZSD (Fig. 3), despite the fact that the number of identified nests from *T. tridentatus* was limited. In addition to the apparent declines of the local *T. tridentatus* population (Liao et al., 2019a; Wang et al., 2020), we also speculate the nesting of *T. tridentatus* occurs on sand bars or elevated sand substratum in the lower intertidal zones (Cai et al., 1984). Extensive stick nets erected along the
coastline may also prevent the larger-bodied *T. tridentatus* adults, compared to other Asian species, from accessing the upper intertidal areas. *Carcinoscorpius rotundicauda* was observed to spawn throughout the year in tropical regions with a peak in the southwest monsoon period that takes place during May–September (Fairuz-Fozi et al., 2018; Mat Zauki et al., 2019). In another report with the contrasting result, May–August was probably the period with least frequent breeding of the species in Singapore (Cartwright-Taylor, 2015). In our study which was conducted in the subtropical region, the spawning of *C. rotundicauda* begins at least before May and peaks in June–July. The nesting activities in most study sites seem to cease in November or not later than December (Fig.2), when the water temperature typically drops below 20°C (Gong et al., 2019). This is consistent with the findings from both field investigations and laboratory experiments showing that Asian horseshoe crabs have restricted tolerance capability to low temperatures (Chiu and Morton, 2004; Liao et al., 2019b). In October and November, the percentage of un-developed eggs was low, and most had been developed into stage-20 embryos or hatched into trilobite larvae (Fig.2D). In the laboratory, *C. rotundicauda* eggs spent approximately 48 days to develop into trilobite larvae (Zadeh et al., 2009). The hatched larvae would remain in the nests for several more weeks before emerging from the sediment and being transported from the breeding grounds (Rudloe, 1979; Botton et al., 2010). In this case, most eggs were deposited probably around July, which was identified as the spawning peak in this region. Additionally, 5–8 trilobite larvae were found in SJ and SX sediments in May, which suggest that the spawning activities may begin in March–April. The number of eggs per nest of *C. rotundicauda* (13–90) and *T. tridentatus* (195–573) in this study was comparable to those noted in Peninsular Malaysia which was 18–107 for *C. rotundicauda* (Fairuz-Fozi et al., 2018; Zauki et al., 2019), and in Japan which was 200–500 for *T. tridentatus* (Sekiguchi and Nakamura, 1979); however, it was considerably lower than that in Borneo, Malaysia, which was 967 for *T. tridentatus* (Mohamad et al.,

Table 2 Nest environmental characteristics during low tides at identified spawning habitats for *C. rotundicauda* along the northern Beibu Gulf coast

| Site | Temperature (°C) | Elevation (m) | Inclination (°) | ORP (mV) | n | Grain size (mm) | Water content (%) | TOC (%) | Chl a (µg g⁻¹) |
|------|-----------------|---------------|----------------|----------|---|----------------|------------------|--------|--------------|
| JD   | 31.17 ± 2.17    | −20.45 ± 0.88 | 5.53 ± 5.98    | 252.44 ± 64.98 | 32 | 0.79 ± 0.40    | 0.84 ± 0.03       | 0.53 ± 0.18 | 2.90 ± 1.76  |
| SX   | 32.51 ± 2.03    | −20.22 ± 0.55 | 5.53 ± 5.98    | 236.05 ± 50.19 | 15 | 0.65 ± 0.21    | 0.84 ± 0.02       | 0.51 ± 0.19 | 1.52 ± 0.97  |
| SJ   | 34.19 ± 3.03    | −18.24 ± 0.53 | 5.53 ± 5.98    | 240.76 ± 30.99 | 8  | 0.92 ± 0.29    | 0.92 ± 0.17       | 0.65 ± 0.21 | 0.71 ± 0.32  |
| ZYPN | 31.20 ± 1.70    | −19.80 ± 0.52 | 5.53 ± 5.98    | 230.15 ± 37.36 | 7  | 0.47 ± 0.08    | 0.83 ± 0.03       | 0.51 ± 0.09 | 1.00 ± 0.79  |
| ZYPS | 31.73 ± 1.79    | −19.89 ± 0.76 | 5.53 ± 5.98    | 224.57 ± 38.29 | 8  | 0.59 ± 0.09    | 0.86 ± 0.02       | 0.46 ± 0.11 | 1.52 ± 1.24  |
| ZSD  | 31.37 ± 2.06    | −18.30 ± 0.77 | 10.0 ± 0.63    | 230.15 ± 37.36 | 3  | 0.45 ± 0.47    | 0.82 ± 0.86       | 0.47 ± 0.59 | 1.21 ± 1.40  |

Notes: ORP, oxidation reduction potential; TOC, total organic carbon content; Chl a, chlorophyll a. The range data are provided in brackets. The data for *T. tridentatus* nests are not shown in Table 1 because of the very limited number of nests was found. The site abbreviation is mentioned in Table 1. Data are expressed as mean ± standard deviation; n = sample size.

Table 3 Surface water physiochemical conditions within the high-density *C. rotundicauda* nest areas during rising tides (water level: about 40 cm) in the northern Beibu Gulf

| Site | Temperature (°C) | Salinity (‰) | DO (µmol L⁻¹) | pH | ORP (mV) | Turbidity (NTU) | TDN (µg L⁻¹) | TDP (µmol L⁻¹) |
|------|-----------------|--------------|---------------|----|----------|----------------|--------------|--------------|
| JD   | 31.24 ± 3.05    | 11.11 ± 5.54 | 5.60 ± 1.75   | 7.35 ± 0.30 | 236.18 ± 73.39 | 71.82 ± 107.13 | 112.35 ± 101.82 | 65.75 ± 156.90 |
| SX   | 31.25 ± 3.56    | 19.26 ± 5.67 | 5.17 ± 1.16   | 7.78 ± 0.32 | 223.10 ± 43.23 | 187.05 ± 62.12 | 103.58 ± 33.96 | 83.72 ± 20.28  |
| SJ   | 33.25 ± 2.22    | 20.90 ± 5.88 | 5.59 ± 1.35   | 7.53 ± 0.46 | 223.10 ± 43.23 | 95.48 ± 68.52  | 151.49 ± 94.10 | 70.63 ± 23.66  |
| ZYPN | 30.50 ± 3.74    | 7.28 ± 6.80  | 6.30 ± 8.40   | 7.19 ± 8.44 | 110 ± 227      | 86.00 ± 32.00  | 51.60 ± 154.00 | 50.97 ± 114.83 |
| ZYPS | 31.42 ± 3.82    | 19.90 ± 5.72 | 4.90 ± 1.04   | 7.74 ± 0.29 | 217.20 ± 41.50 | 35.94 ± 36.64  | 77.35 ± 38.80  | 84.22 ± 43.08  |
| ZSD  | 31.32 ± 4.89    | 19.00 ± 8.24 | 4.65 ± 1.14   | 7.33 ± 0.72 | 233.09 ± 24.96 | 63.17 ± 73.81  | 98.31 ± 82.71  | 63.29 ± 87.1 |

Notes: Data are expressed as mean ± standard deviation; n = sample size. The range data are also provided. The data for *T. tridentatus* nests are not shown in Table 3 because of the very limited number of nests was found. DO, dissolved oxygen; ORP, oxidation reduction potential; SPM, suspended particulate matter; TDN, total dissolved nitrogen; TDP, total dissolved phosphorus. The site abbreviation is mentioned in Table 1.
The nest-site selection by spawning adults can be selective, typically in the upper beaches along the high tide line, to increase developmental success by minimizing exposure of the developing eggs to hypoxia and hydrogen sulfide (Penn and Brockmann, 1994; Vasquez et al., 2015). Despite the high abundance of spawning adults of *C. rotundicauda* was noted in the mangrove areas, their nest selection characteristics were unclear. Fairuz-Fozi et al. (2018) found the egg aggregations on a sand bar with pioneer mangroves *Sonneratia* and *Avicennia* spp. and intertidal areas with domestic discharge points nearby, but not those open flats covered densely with pebbles, shells and pneumatophore roots of mangroves. In the present study, the egg distributions of *C. rotundicauda* showed the following characteristics: 1) in the upper intertidal zones with a tendency of approaching the high tide lines; 2) unsHELtered areas on the edges of mangroves with tidal creeks nearby; 3) at the bases of elevated natural substratum (e.g., sand bars, cobbles and mangrove surface roots) or man-made structures (e.g., construction wastes, power transmission facility and mangrove boardwalks); 4) at the sandy slopes along seawalls or tidal channels of aquaculture ponds (Figs.3 and 4).

The egg distribution pattern can be explained by the limited availability of suitable substratum for spawning in low-energy, muddy shores with little slope. Organic matter and hydrogen sulfide concentrations are commonly high in these muddy beaches (Zaldiar-Rae et al., 2009; Vasquez et al., 2015). Consequently, Asian horseshoe crabs deposited their eggs at high beach elevations, including natural and artificial structures, where the accumulation of coarse-grained (0.5–0.9 mm) and well drained sediments occurred (Table 2). Those eggs were also observed to experience high nest temperature (31–34°C) and ORP (225–252 mV), but low water content (0.8%–0.9%) and TOC (0.5%–0.7%) during low tides (Table 2). By comparing the present results with previous results from other researchers, relatively finer sediment textures were recorded in spawning habitats of *C. rotundicauda* (0.2–0.4 mm, Zauki et al., 2019) in Malaysia and those of *T. tridentatus* in Japan (0.2–0.3 mm, Itaya et al., 2019). Meanwhile, Mohamad et al. (2019) observed a wider range of grain sizes (0–2 mm) suitable for *T. tridentatus* spawning in the northeastern Borneo. Sediment TOC was consistently low (<3%) in most places where *C. rotundicauda* eggs were found (Fairuz-Fozi et al., 2018; Zauki et al., 2019). The inclination of nests (5°–14°) was also comparable to those recorded in Japan (3°–8°, Itaya et al., 2019). In terms of water physiochemical conditions, it seems that spawning adults can tolerate a broader range of fluctuations in salinity (2–32), DO (5–7 mg L⁻¹) and pH (6–8). The nest depths of *C. rotundicauda* (1–4 cm) and *T. tridentatus* (7–8 cm) were shallower, which can avoid anaerobic conditions of deeper sediments that are known to affect development (Botton et al., 1988; Penn and Brockmann, 1994; Vasquez et al., 2015). The depth data were similar to those reported in previous studies (*C. rotundicauda*: 2–5 cm; Fairuz-Fozi et al., 2018; *T. tridentatus*: 5–22 cm, Botton et al., 1996; Mohamad et al., 2019).

Extensive shore protection structures such as seawalls, bulkheads and revetments that extend below mid-foreshore can reduce, fragment and restrict the availability of suitable spawning habitats for horseshoe crabs (Botton et al., 1988; Jackson et al., 2015). Their interaction with wave and hydrology also alters sediment textures and physiochemical conditions, which reduce the spawning activity of horseshoe crabs (Nelson et al., 2015). In this study, spawning adults and eggs of Asian horseshoe crabs were noted within the artificial tidal channels when the water control gates remained open (Fig.3 SX). We postulate that the presence of seawalls and other coastal anthropogenic structures have obstructed the mating pairs from accessing the high tide line. The amplexed pairs were also aggregated along the seawalls or at the entrance of water control gates, and such behaviors have previously documented by Itaya et al. (2019). On the other hand, the clustering of horseshoe crab spawning near mangrove boardwalks, water control gates and other physical structures may also be attributed to the sheltered areas created by these vertical features extend out onto the intertidal zone. Similar cases were noted in Delaware Bay, USA, where a higher spawning density of *L. polyphemus* occurred within the enclaves between bulkhead segments with low wave energy and decreased current velocities (Jackson et al., 2015). Additionally, the nesting sites were not far away from the identified nursery habitats on sandy mudflats along the outer fringe of mangroves (Xie et al., 2020). Therefore, tidal creeks that present near the identified nests (Fig.3) were probably the corridor connecting nursery and spawning habitats for Asian horseshoe crabs separated by the dominant mangrove forests.

Based on the present results, possible management measures for conservation of Asian horseshoe crab spawning populations include: 1) routinely remove ground cages and erected stick nets in high tide areas, including those along tidal creeks, 2) regulate artisanal bivalve aquaculture and beachcombing activities near mangrove areas, 3) close the upper intertidal areas in June–July during the peak spawning period to minimize the detrimental effects from artisanal fishing with illegal gears, 4) avoid coastal reclamation projects that diminish the beach topography, particularly those elevated, coarse-grained substratum and slopes, and 5) restore the spawning habitats by creating mounds with moderate slope and suitable sediment textures along the shoreline. Future studies will explore the nesting behavior of Asian horseshoe crabs, including the influence of lunar tidal rhythmicity and the daily migration to deeper waters, to further our holistic understanding of their life history and reproduction ecology.

5 Conclusions

Our findings demonstrated apparent temporal variations of nest and egg densities, which peaked in June–July. Aggregations of eggs were found embedded in the shallow surface layer (<10 cm) of coarse-grained sediment in the upper intertidal zones, and in the vicinity of natural and artificial structures with the elevated, mildly sloping sub-
stratum. The nest site selection was to maximize the hatching success by incubating the developing eggs with higher temperature and aerobic conditions. Seawalls and other physical infrastructure seemed to obstruct the access of spawning pairs to the high tide line. The present study provides valuable insights in the scope of spawning and nest site selection of Asian horseshoe crabs under a mixture of natural and artificial structures, which is useful to improve the management efforts of a group of ecologically important and endangered species.

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