Spatial and temporal distributions of juvenile horseshoe crabs (Arthropoda: Chelicerata) approaching extirpation along the northwestern shoreline of the New Territories of Hong Kong SAR, China

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Juvenile horseshoe crabs, Tachypleus tridentatus and Carcinoscorpius rotundicauda, with prosomal widths of between 11.5 and 102.5 mm, occur along Hong Kong’s northwestern shoreline abutting Deep Bay where dissolved oxygen (DO) has been identified as the principal factor determining distribution and survival. Unreported upon hitherto, however, Tachypleus tridentatus occurred consistently in association with sea-grass (Halophila beccarii) beds, with DO concentrations of 8–14 mg·L⁻¹, a median grain size of >180 µm, interstitial water contents of <36% (sediment wet weight) and organic contents of <3.2% (sediment dry weight). Most individuals occurred between 60 and 120 m down from the shoreline although there appeared to be a trend of down-shore migration in summer, possibly to alleviate thermal stress. No individuals were found at sediment temperatures ≤20°C, i.e. in sub-tropical Hong Kong’s winter, when they adopt buried repose. Abundances of emerged T. tridentatus, peaking in November, were synchronized with interstitial salinities of 22–26‰ and averaged 1–2 individuals per 100 m². Mean population density of T. tridentatus was 4–9 individuals per 100 m². Only four individuals of C. rotundicauda were found in August and September, suggesting a recent dramatic decline in numbers. The natal crèche environment for juvenile horseshoe crabs in Hong Kong is deteriorating and threatening their survival.

Keywords: Tachypleus tridentatus; Carcinoscorpius rotundicauda; distribution; habitat; sediment physiography; granulometry; beach geomorphology; sea grasses

Introduction

Virtually all research papers on horseshoe crabs agree that there is a general global population decline in all four extant species. For Limulus polyphemus in the USA, this is attributed to shore protection projects and pollution in Chesapeake Bay (Bell and Henderson 1993) and over-harvesting in New Jersey (Botton and Haskin 1984; Shuster 1985; Michels 1996; Swan et al. 1996). Flourishing human activities alongside the breeding grounds on the coast of India (Chatterji 1994) have reduced numbers of Tachypleus gigas, as with Tachypleus tridentatus in Japan (Shinohara 1989; Itow et al. 1991; Kannan et al. 1995; Itow 1998). Habitat degradation, pollution and over-fishing in Taiwan (Yeh 1999) and a similar complex of causes in Hong Kong (Chiu and Morton 1999a, b; Shin et al. 2009) are threatening locally occurring species of horseshoe crabs in Chinese waters.
Three species of horseshoe crabs have been documented to occur in Hong Kong, that is, *Tachypleus tridentatus* (Leach, 1819), *Tachypleus gigas* (Müller, 1785) and *Carcinoscorpius rotundicauda* (Latreille, 1802) (Cheung et al. 1975; Hill and Phillipps 1981; Morton and Morton 1983; Mikkelsen 1988). As a synergistic repercussion of habitat degradation, coastal developments and commercial fishing, *T. gigas* is now believed to be locally extinct (Chiu and Morton 1999a, b, 2003). Populations of the other two species have also diminished locally over recent decades (Cheung et al. 1975; Liang and Zhou 1987; Chiu and Morton 2003). Regular counts of known horseshoe crab populations are therefore needed to estimate their on-going status and the rate of population change over time.

Abundance estimations for the Atlantic horseshoe crab, *Limulus polyphemus* Linnaeus, 1758, in the USA can be traced back to 1870, using inferences made from annual harvest data by the Federal Fisheries Agency (Shuster 1985) and, more recently, from counts of adults coming ashore to breed (Shuster and Botton 1985). The segregation of juveniles from adult horseshoe crabs has, however, long been a hindrance to population estimates (Carmichael et al. 2003), inhibiting comprehensive management plans (Botton et al. 2003; Carmichael et al. 2003). Hence, most studies of horseshoe crab abundances have focused upon adults, with the only exception of a more comprehensive survey, covering adults, juveniles and eggs undertaken upon *L. polyphemus* by Carmichael et al. (2003).

In Asia, however, where it is thought four horseshoe crab species occur (Mikkelsen 1988), it is possible to evaluate the abundance of beach-specific juveniles, because it is estimated that they stay on intertidal mudflats for >10 years and emerge from the substratum at predictable times (Sekiguchi 1988; Lee and Morton 2005). Chiu and Morton (1999a, b) first estimated abundances of horseshoe crab juveniles in Hong Kong in terms of numbers counted per beach visit. A more standardized sampling method is, however, necessary for a more accurate monitoring programme. The focus of this study, therefore, at a location (Deep Bay) where horseshoe crabs have been historically considered most abundant, was to conduct a systematic survey of juvenile population size and to identify spatial-temporal distributions. All individuals were also measured to obtain a size frequency distribution. Concurrently, a study of habitat granulometry was undertaken to identify beaches especially favoured by horseshoe crab juveniles. This study was also initiated in anticipation of the construction of a cross-border bridge between Hong Kong and mainland China and which when completed in 2007 would straddle Deep Bay and, probably, it was believed, destroy some of the horseshoe crab crèches earlier (Morton and Lee 2002) and herein identified and reported upon along this stretch of Hong Kong’s coastline. This study, therefore, provides the first comprehensive analysis of horseshoe crab numbers along this stretch of Deep Bay shoreline prior to a major developmental impact and which can be used in future studies as a baseline to assess not just its but other impacts.

**Materials and methods**

**Study site**

Situated in a transition region between the temperate Northern Pacific and tropical Indo-Pacific (22°N 114°E), Hong Kong experiences a sub-tropical climate with four distinct seasons. Because of the southeast monsoon, Hong Kong’s summers (June–September) are hot (average air temperature = 27.5°C), with frequent and heavy rain
and the sporadic tropical typhoon (an average of 300–400 mm; about 80% of the annual rainfall occurs between May and September). In contrast, winters (January–February), under the influence of the northeast monsoon from continental China, are cold (15.3°C) and dry (20–40 mm). Sea temperatures generally follow those of the air and range between 16 and 29°C (Morton and Morton 1983).

The study site reported upon herein was the southeastern coastline of Deep Bay, which is located in the northwestern quadrant of the New Territories of Hong Kong (Figure 1A). Deep Bay separates Hong Kong from mainland China to the northwest and, despite its name, is shallow with an average depth of 2.9 m and is nowhere deeper than 6 m (Young and Melville 1993). As a consequence, a huge area of mudflat is exposed as the tide ebbs. The bay is adjacent to and forms part of the Pearl River Estuary with Macao on the opposite bank some 100 km to the west. With a freshwater influx estimated at 308 billion m³·yr⁻¹ and a sediment load of 3.4 × 10⁷ tonnes·yr⁻¹ (Morton and Wu 1975; Kot and Hui 1996), the Pearl is the largest river in southern China and has a dramatic impact upon the hydrography of Hong Kong (Morton and Morton 1983). Streams, of various scales, discharging along the coastline, fuel an additional freshwater influx into Deep Bay. The tides here are mainly mixed semi-diurnal, with a maximum range of ∼2.8 m. (Morton and Morton 1983; Kot and Hui 1996).

The southeastern coastline of Deep Bay (Figure 1B) is mostly vegetated by grasses and mangroves, succeeded seaward by a continuous sheltered sandy beach (∼18 m wide) and, finally, an extensive mudflat of 1.8 km². Due to low beach inclinations, a perpendicular length of up to 400 m of such sediments is exposed at the times of low spring tides. The mudflat supports a few dwarf mangroves and is covered patchily by the sea-grass Halophila beccarii (Ascherson, 1871) and scattered oyster beds (Crassostrea hongkongensis [Lam and Morton, 2003] and Saccostrea cucullata [Born, 1778]) (Lam and Morton 2003). At low spring tides, trails of Cerithidea djadjariensis (K. Martin, 1899), fiddler crabs (Jones and Morton 1994) and, occasionally, horseshoe crabs characterize the mudflat.

Juvenile horseshoe crabs, Tachypleus tridentatus and Carcinoscorpius rotundicauda, have been recorded from along the coastline of Deep Bay at Long Chuk Hang and Ha Pak Nai (Chiu and Morton 1999a, b, 2003; Lee and Morton 2005; Shin et al. 2009) (Figure 1B). The meiofauna here eaten by juvenile horseshoe crabs overwhelmingly comprises nematodes, followed by insect larvae and copepods (Zhou and Morton 2003).

Adjacent to beaches are rural villages, chicken and pig farms and, increasingly, light industries. Improper disposal of domestic and agricultural wastes, sluggish current speeds (0.55–1.10 m·sec⁻¹), ineffective tidal exchanges and a low turnover rate (15 days) in Deep Bay have led to a locally unacceptable water quality (Wong 1975; Morton and Wu 1975; Leung et al. 1975; Wong et al. 1980; Young and Melville 1993; Kot and Hui 1996; Environmental Protection Department 2001a, b; Chiu and Morton 2003). A seasonal pattern with relatively anoxic conditions occurring in summer arising from livestock farm discharges and unsewered village toilets aggravates this problem (Morton and Wu 1975; Environmental Protection Department 2001a, b). The water quality of the rivers here has improved slightly in the last three decades but, still, total inorganic nitrogen concentrations, ranging from 0.23 to 4.04 mg·L⁻¹, have exceeded official objectives for six consecutive years (Environmental Protection Department 2001a, b).
Figure 1. (A) A map of Hong Kong showing the location of the study site in Deep Bay in the northwestern quadrant of the New Territories of Hong Kong. Also shown is the location of the cross-border bridge connecting Hong Kong at Pak Nai with mainland China at Shekou. (B) A more detailed map of the northwestern quadrant of Hong Kong showing the location of the eight sampling stations (A-H) in Deep Bay. Black areas denote mangrove stands, white ones oyster beds. Dotted lines represent streams in the study area.
Over the last few decades, the coastline has also succumbed to the pressure of reclamation for landfill at Nim Wan and a recently completed cross-border bridge between Pak Nai and Shekou connects Hong Kong with mainland China (Figure 1A). A recent visit (2007) to the sampling sites revealed that Station B of this study no longer exists due to coastal development.

**Sampling strategies**

Eight stations, >500 m apart, were designated along the southeastern (Hong Kong) coastline of the Deep Bay study area (Stations A to H in Figure 1B). At each station, six horizontal transects were laid at 30, 60, 90, 120, 150 and 180 m down-shore from the line of terrestrial vegetation (“the shoreline”), as illustrated in Figure 2. Each transect paralleled the shoreline and was 100 m long and 2 m wide. Such a sequence of long, thin, transects was chosen because they crossed many microhabitats, including streams, sea-grass beds and farmed oyster patches, to reduce variation in a clumped distribution pattern (Krebs 1989). This was of particular concern because microhabitat preferences vary between horseshoe crab species (Mikkelsen 1988). Regular environmental samplings and horseshoe crab counts were carried out fortnightly, during daylight when the tide receded to <-0.7 m Chart Datum (C.D.) from May to December 2002 and ~3 hours prior to the predicted time of low tide when horseshoe crab juveniles are reportedly most active (Rudloe 1979a; Kawahara 1982). The beaches along this stretch of the Hong Kong coastline are so flat that, by this time, over half (>200 m) of the intertidal mudflat was exposed.

**General sediment physiography of the study area**

Sediment temperature, dissolved oxygen concentration and salinity of the interstitial waters were measured at 0, 50 and 100 m along each transect. Approximately 500 g sediment samples were obtained from the 30, 90 and 150 m transects in August/September 2002, again at 0, 50 and 100 m along each transect. Half of each sample was washed through a graded series of sieves (2000, 1000, 500, 250, 125 and 63 µm mesh size) to obtain a picture of grain size distribution. About 6 g of the sediment from each sample was dried at 80°C for 24 hours to obtain estimations of interstitial water content, before undergoing ignition at 550°C for 6 hours to derive sediment organic contents (Buchanan 1984; Penn and Brockmann 1994).

**Distribution of horseshoe crab juveniles**

All emerged horseshoe crab juveniles within the sampling area were identified to species, tallied, maximum prosomal widths measured and each individual tagged by cementing a plastic label (10 mm × 5 mm) onto the dorsal surface of the left opisthosomatic carapace with “Superglue ©”. It has been demonstrated in the laboratory that the tag would remain on such individuals for >7 months and induced neither noticeable growth impediments nor mortality (Lee and Morton 2005) although, clearly, it would be lost at ecdysis. In addition, sediment temperature, salinity and dissolved oxygen concentration in the vicinity of every juvenile were recorded. The shortest distance between each individual and an abutting sea-grass bed and farmed oysters was, finally, also evaluated.
Figure 2. A pictorial illustration of the sampling strategy adopted for the survey of juvenile horseshoe crabs at the eight stations. Sampling areas are indicated by grey rectangles.
Statistical analyses
Spatial differences in sediment temperature, salinity and dissolved oxygen concentration of interstitial waters as well as median sediment size were examined between the eight sampling stations using a one-way analysis of variance (ANOVA) with log-transformed data to avoid deviation from a normal distribution. Non-parametric Spearman correlation analyses were employed to investigate any gradients in salinity and dissolved oxygen concentrations from the shore seaward for each sampling occasion. A two-way, nested design, ANOVA was selected to test for inter-station differences and between shore levels in terms of sediment organic content. Data were arcsine-transformed, for the matrix was not normally distributed.

Weighted mean distances from the shoreline defined the distance from where the core population of horseshoe crabs was distributed using the following formula:

\[ \text{Weighted Mean distance down-shore from the shoreline (m)} = \frac{\sum n_i d_i}{\sum n_i} \]

where:
- \( n_i \) = the number of individuals per 100 m\(^2\)
- \( d_i \) = distance down-shore from the shoreline (m).

Before the analyses, Shapiro Wilk’s and Bartlett’s tests were employed to examine whether the abundances and prosomal widths of horseshoe crab juveniles were normally distributed and with equal variances, respectively. If either of these was disputed, data were log (x+1)-transformed before undergoing a two-way nested ANOVA to examine any differences between stations and shore levels. Similar analyses were undertaken to identify variations in the distribution of horseshoe crab juveniles between sampling stations using one-way ANOVA. All statistical tests were conducted using the SAS software for Windows Release 8.02 from the SAS Institute Inc. A significance level of \( P = 0.05 \) was adopted.

The population size of horseshoe crab juveniles was also estimated using the Jolly-Seber method and was based on the percentages of marked individuals recaptured over time (Hedrick 1984; Krebs 1989; Fowler et al. 1998).

Results
Spatial distribution of sediment physiography
Across the eight sampling locations, inter-station differences in sediment temperature were inconspicuous. Parameters demonstrating discernible inter-station differences included salinity and dissolved oxygen concentrations of interstitial waters, median grain sizes, and water and organic contents of substratum sediments (Table 1). Overall, the eight stations fell into three groups:

(1) Northern stations (Stations A–D). The substrata of this group of stations were characterized by significantly poorest aerated interstitial waters (4.4 ± 3.7 mg·L\(^{-1}\)) \((MS = 31.36; df = 7; F = 105.82; P = 0.0001)\), highest proportions (59.4 ± 7.9%) of silt (sediments with grain size <63 \(\mu\)m) resulting
Table 1. Environmental parameters (means ± standard deviations) of the substrata at the eight stations along the northwestern coast of the New Territories, Hong Kong, between May and December 2002.

<table>
<thead>
<tr>
<th>Station</th>
<th>Sediment</th>
<th>Interstitial water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temperature (°C)</td>
<td>Median grain size (µm)</td>
</tr>
<tr>
<td>A</td>
<td>29 ± 2</td>
<td>&lt; 63 ± N.A.</td>
</tr>
<tr>
<td>B</td>
<td>27 ± 7</td>
<td>138 ± 138</td>
</tr>
<tr>
<td>C</td>
<td>26 ± 6</td>
<td>63 ± 1</td>
</tr>
<tr>
<td>D</td>
<td>29 ± 3</td>
<td>94 ± 39</td>
</tr>
<tr>
<td>E</td>
<td>27 ± 6</td>
<td>238 ± 165</td>
</tr>
<tr>
<td>F</td>
<td>28 ± 6</td>
<td>341 ± 204</td>
</tr>
<tr>
<td>G</td>
<td>29 ± 4</td>
<td>105 ± 43</td>
</tr>
<tr>
<td>H</td>
<td>26 ± 7</td>
<td>240 ± 140</td>
</tr>
</tbody>
</table>
in finest median grain sizes of 90 ± 76 µm for 24 of 36 samples, as well as
greatest water (50.4 ± 10.2%) and organic contents (5.4 ± 1.5%). Within this
group, Stations A and B were notably less saline (16%) as compared with the
remaining six stations (18–21%) (MS = 1.48; df = 7; F = 4.89; P = 0.0001).

(2) The majority of the southern stations (Stations E, F and H). Sediments in
this cluster of stations were significantly better aerated, with dissolved oxygen
concentrations consistently >4.5 mg·L\(^{-1}\) and an average of 10.3 ± 2.8 mg·L\(^{-1}\);
largest median grain sizes (273 ± 172 µm, except for one sample from the
lower shore at Station H with a median grain size <63 µm) (MS = 1.41; df = 3; F = 5.94; P = 0.0028); lowest percentages of silt (21.78 ± 7.9%); and
minimum water (28.8 ± 9.4%) and organic contents (2.5 ± 1.2%).

(3) Station G was the only exception among the southern stations that had
sediment characteristics which fell between the above two groups (dissolved
oxygen concentration=8.3 ± 3.0 mg·L\(^{-1}\); median grain size=105 ± 43 µm
(with two samples from the lower shore with values of <63 µm); proportion
of silt=28.8 ± 14.6%; water content=37.2 ± 7.2%; organic content=3.3 ± 0.7%).

Possibly due to the low inclination of the intertidal flat, the physical parameters
recorded were not correlated with tidal gradients for any of the eight sampling stations
(sediment temperature (P > 0.05), median grain size (P > 0.05), water (MS = 99.57; 
df = 16; F = 1.25; P = 0.2697) and organic contents (MS = 2.94; df = 16; F = 1.25; 
P = 0.2697).

Spatial distribution of horseshoe crabs

Of a total of 169 horseshoe crab individuals recorded throughout this study, 165 were
identified as *Tachypleus tridentatus*, occurring exclusively at Stations E, F and H.
Although the averaged occurrence at Station H was lower (1.14 \(T. tridentatus\)·100m\(^{-2}\))
than at Stations E and F (1.97 and 1.55·100m\(^{-2}\), respectively) (Figure 3), this was not
statistically significant (MS = 0.96; df = 2; F = 1.83; p = 0.1707).

*Tachypleus tridentatus*

A significant pattern was identified with regard to the intertidal distribution of
*Tachypleus tridentatus* for Station E (MS = 3.79; df = 5; F = 6.80; p = 0.0001) only,
the species being relatively more abundant (>3-100 m\(^{-2}\)) at 60, 90 and 120 m down-
shore than at 30, 150 and 180 m (<0.71·100 m\(^{-2}\)) (Figure 3). The distributions of
*T. tridentatus* at the three stations (E, F and H) were approximately similar with peak
occurrences at between 60 and 90 m down-shore (MS = 1074.67; d.f. =2; F = 2.15; 
p = 0.1335).

Sampled *Tachypleus tridentatus* ranged in prosomal width from 11.5 to 102.5 mm,
with the majority generally ranging from 12 to 40 mm (Figure 4). Prosomal width did
not correlate with sampling time and individuals with values <20 mm were recorded
sporadically at all three stations from July to November, except September 2002.
Conversely, those individuals with a prosomal width >80 mm were recorded occa-
sionally at the three stations from July to October 2002. Individuals at Station F
were significantly larger than those at the other two stations (MS = 1.15; df = 2;
Figure 3. The spatial distribution in terms of mean abundance of juvenile *Tachypleus tridentatus* at Stations E, F and H with mean abundances ± standard deviations at the three stations also shown.

\[
F = 4.42; \quad P = 0.0135.
\]

Values did not change significantly with distance down from the shoreline at all three sampling stations (MS = 0.17; df = 13; F = 0.58; P = 0.8674).

The peak occurrences of individuals with prosomal widths of <40 mm was greatest 60 m down-shore at all three stations (Figure 5). Individuals with prosomal widths of between 41 and 70 mm occurred in greatest numbers at 90 m down-shore at Stations E and F and at 60 m down-shore at Station H. Individuals with prosomal widths of >70 mm occurred in greatest numbers at between 60 and 90 m down-shore at Station E and at between 30 and 90 m down-shore at Station F but were rarely encountered at Station H (Figure 5).

*Carcinoscorpius rotundicauda*

Only four *Carcinoscorpius rotundicauda* individuals were recorded from two stations (C and E) over the course of the study period. At Station C, two individuals occurred at 150 m down from the shoreline. The other two were identified closer to the shoreline at 60 m and 90 m at Station E. Prosomal width measurements were obtained for three of these individuals and were 62 mm at Station C and 29.2 mm and 39 mm at Station E.

*Temporal pattern of sediment physiography*

The study period extended over three seasons:

1. Spring (April–May 2002) was characterized by relatively lower sediment temperatures (26.0–28.2°C) and relatively stable salinities (14.3–20.9‰).
Figure 4. The size frequency distribution, in terms of prosomal width, of juvenile *Tachyleus tridentatus* individuals obtained from Stations E, F and H, with means and standard deviations.
Figure 5. The spatial distribution of juvenile *Tachypleus tridentatus* individuals of different prosomal width ranges at Stations E, F and H.
(2) Summer (June–September 2002) resulted in the highest sediment temperatures ranging from 27.9 to 33.8°C. Interstitial waters in the (characteristically rainy) summer were also characterized by low, fluctuating, salinities (7.3–20.2‰) with the lowest absolute value of 0.3‰ being recorded at Station E in September, and more variable inter-station dissolved oxygen concentrations (1.3–18.4 mg·L⁻¹).

(3) Autumn (October–December 2002) was characterized by wide-ranging but progressively declining (as winter approached) sediment temperatures (11.5–31.2°C), more saline interstitial waters (18.9–27.2‰) and comparatively stable dissolved oxygen values (8.6–13.7 mg·L⁻¹).

Lee and Morton (2009) have shown that *Tachypleus tridentatus* assumes buried repose in the Hong Kong winter months from December to March and these months were thus not investigated in the present study.

**Temporal distribution of horseshoe crabs**

*Tachypleus tridentatus* individuals were recorded from late April 2002 to the end of November 2002, i.e. from early spring to autumn in subtropical Hong Kong. Qualitative samplings were also made prior to the study period in early April when not one individual was seen. In late April, however, ~12 juveniles were identified at Stations E and F. No individuals were identified from December to March, i.e. Hong Kong’s winter. Peak occurrence overall was from mid-August to the end of November (Figure 6), although an additional peak in abundance was recorded in late May but only at Station H. The four individuals of *Carcinoscorpius rotundicauda* were identified from Station C in September and Station E in August and September.

Temporal changes in the distribution of *Tachypleus tridentatus* were not generally discernible overall (Figure 7). However, individuals appeared to have most noticeably moved down-shore from ~60 m in May to ~90 m in September at Station F. Similarly, most individuals were recorded 135 m down-shore from the shoreline in July and August at Station E. At Station H, most individuals occurred at 90 m and 110 m down-shore in August and September, respectively. There thus seemed to be a general trend for individuals to move down-shore in the hotter summer months in Hong Kong (Figure 7).

**Relationships between horseshoe crab distribution and environmental parameters**

Figure 8A shows that 45.0% of *Tachypleus tridentatus* individuals were recorded when sediment temperatures lay between 28 and 32°C. A bimodal distribution was identified in relation to interstitial water salinity (Figure 8B). Few individuals (9.9%) were recorded from interstitial waters with salinities of between 10 and 20‰, while the majority were recorded at salinities of 22–26‰ and 4–10‰. Finally, 80.9% of individuals were recorded from well-aerated interstitial waters (8–14 mg·L⁻¹) (Figure 8C).

*Tachypleus tridentatus* also appeared to prefer natural sea-grass (*Halophila beccarii*) to cultivated oyster (*Crassostrea hongkongensis*) beds as most individuals, over half (64.2%), were identified within one metre of the former (Figure 9A). Conversely, of all the individuals recorded, <~6% were associated (at a distance of <1 m) with cultured oysters (Figure 9B). Of these, 4% were moving in the thin film of surface water and
Figure 6. Temporal variations in the total abundance of juvenile *Tachypleus tridentatus* individuals at Stations E (♦), F (■) and H (Δ).

Figure 7. Temporal variations in the distribution of juvenile *Tachypleus tridentatus* individuals downshore from the shoreline at Stations E, F and H with means and standard deviations also shown.
Figure 8. Frequency distributions of (A) sediment temperature, (B) salinity and (C) dissolved oxygen concentration of interstitial waters where juvenile *Tachypleus tridentatus* individuals were obtained in the study area.
only 1% of such individuals, with prosomal widths of between 12.5 and 41.0 mm, were not inundated.

**Tagging experiment**

Of the 165 *Tachypleus tridentatus* tagged during the course of the study period, 17 were recaptured (10.3%) between July and November 2002 from Stations E and F (Table 2). All were recaptured only once. It took 10–58 days to recover the tagged individuals exclusively from ± 30 m down-shore. Not one individual was recaptured from Station H. Prosomal widths of recaptured individuals varied between 12 and 91 mm. In terms of body weight, approximately half exhibited weight gains of between 1 and 3 g. Most
experienced weight gains of between 2.6 and 33.3% of their original body weight. A few even reached >100%.

From the tagging experiment, the estimated mean abundances of juvenile Tachypleus tridentatus at Stations E and F were calculated to be 18 and 2 individuals per 1000 m², respectively. No Carcinoscorpius rotundicauda individuals were recaptured.

Discussion

Horseshoe crab habitat preferences

Tachypleus tridentatus

Temperature affects horseshoe crabs in terms of blood pH (Howell et al. 1973), hatching time, juvenile intermolt duration, carapace length (Yeh 1999), nutrition and reproduction (Shuster 1979). The unfavourable time for juvenile horseshoe crabs, as identified in this and an earlier study (Lee and Morton 2009), appears restricted from December to March, i.e. Hong Kong’s winter, when substratum temperatures ranged from 11.5 to 18.0°C, overlapping with the range (4–16°C) which adversely affects the aerobic metabolism of Limulus polyphemus (Mangum and Ricci 1989). Local temperatures, nonetheless, never exceeded the lethal upper (>41°C) and lower (<−1.1°C) limits for adult L. polyphemus (Mayer 1914). For the rest of the study period, the temperature regime (17–38.5°C) encompassed the desirable temperature of ~18°C when adult Tachypleus tridentatus come ashore to spawn (Nishii 1975; Weng and Hong 2001). This agrees with values obtained for T. tridentatus egg viability of between 22 and 31°C (Sekiguchi et al. 1988; Chen et al. 2004) and falls into the optimal thermal range for juvenile L. polyphemus (15–40°C; Shuster 1982) and T. tridentatus (28–31°C; Yeh 1999; Chen et al. 2004). The study area is therefore appropriate for spawning, as well as for egg and larval development and juvenile growth, between late April-May and November in each year. The Deep Bay mudflats are, hence, a suitable natal crèche for T. tridentatus.

Horseshoe crabs are known to tolerate a wide thermal range (Shuster 1982; Jegla and Costlow 1982; Chatterji 1994). The results of the present study confirmed that juvenile individuals of Tachypleus tridentatus are active only when temperatures rise above 20°C, and they remain buried in temporary dormancy at lower temperatures, i.e. in Hong Kong’s winter (Lee and Morton 2009).

Located within the largest local estuary and fed by five large streams, the Deep Bay study area is characterized by a wide salinity range of 4–30‰, with Stations A and B significantly less saline, being furthest from the over-riding influence of the Pearl River. In comparison with the optimal salinity (8–35‰) recorded for Limulus polyphemus eggs (Jegla and Costlow 1982; Shuster 1985), juveniles (5–32‰) (Shuster 1982) and adults (6–31‰) (Robertson 1970), as well as adult Tachypleus gigas (6–31‰) (Chatterji 1994), only Stations A, D, F, G and H had minimum salinity values >5‰; Stations D, G, H >6‰; Station D >8‰. It is, moreover, likely that values would be further subdued during rain at low tide periods. The emergence, therefore, of Tachypleus tridentatus juveniles from the substratum predominantly at salinities of between 22 and 26‰ at Stations E, F and H implies that they have a high salinity tolerance as suggested for Limulus polyphemus by Pearse (1928), Shuster (1957) and Jegla and Costlow (1982) and for T. tridentatus by Chatterji (1994). Individuals appeared
Table 2. Population abundance estimates (individuals per 1000 m$^2$) using the Jolly-Seber method of *Tachypleus tridentatus* juveniles at Stations E and F.

<table>
<thead>
<tr>
<th>Date</th>
<th>Station E</th>
<th></th>
<th></th>
<th>Station F</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Number of tagged individuals</td>
<td>Number of recaptures</td>
<td>Population estimate</td>
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<td>Number of tagged individuals</td>
<td>Number of recapture</td>
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Average
to remain buried during adverse salinity periods but this did not seem to jeopardize survival.

Sediment size spectrum governs drainage, organic content, interstitial oxygen content, microbial activity and warmth (Shuster 1985; Penn and Brockmann 1994) and, in turn, determines whether juveniles can burrow easily during high tide periods (possibly to avoid fish predators), and keep themselves cool and moist in summer (Sekiguchi 1988) and warm in winter. The median sediment size value in the study area (around Station F) in 1999 was 451 $\mu$m (Chiu and Morton 1999a, b), that is, positioned within the range of this study, implying barely discernible annual variations. In selection of nesting grounds, the upper shores of Stations B, E, F, G and H are also suitable for nesting adult Tachypleus gigas (182–203 $\mu$m), as reported by Chatterji (1994), whereas only the mid-shore of Stations E and the upper shores of F and H favoured Tachypleus tridentatus (440–1420 $\mu$m) (Lin 2002). This might help explain the exclusive occurrence of T. tridentatus juveniles at these stations in the current study.

In comparison with an analogous study on Tachypleus tridentatus juveniles in Taiwan (Yeh 1999), sediment size spectra identified for Hong Kong Stations B, E, F, G and H suggest that these sites are appropriate as a nursery crèche for T. tridentatus (110–520 $\mu$m), while the latter four are even more desirable in relation to sediment proportions of $< 63$ $\mu$m (0.53–34.95%).

The water and organic contents of the study area seem to restrict the occurrence of juvenile horseshoe crabs. Both values (water content = 11.6–66.3%, organic content = 0.7–7.6%) far exceeded counterparts identified for Taiwan (water content = 9.8–11.4%, organic content = 0.1–0.4%) (Yeh 1999). The reportedly high organic loads at Pak Nai have not been alleviated (Chiu and Morton 1999a) (4.3% in 1997–98, falling within the range obtained at Station F in the current study), implying the absence of pollution improvements over the subsequent and intervening four years. Only the four southern stations, that is, E to H, had reduced values (water content = 11.6–48.5%; organic content = 0.7–5.3%).

The critical factor in determining the distribution of juvenile Tachypleus tridentatus appears to be dissolved oxygen levels, which directly affect habitat quality by increasing microbial activity (Wong et al. 1980) and, hence, exacerbating the degree of organic pollution. Although juvenile horseshoe crabs appear able to tolerate heavy organic pollution loads, they cannot endure low dissolved oxygen levels, as evidenced by their greater occurrence in areas with interstitial oxygen levels of between 8 and 14 mg·L$^{-1}$ (Figure 8C), linked to the proximity of relatively cleaner streams (6.3–7.6 mg·L$^{-1}$; Environmental Protection Department 2001b). The Deep Bay study area has long been rated as polluted with poor aeration (63–84% saturation), high ammonia levels, biological oxygen demand (BOD$_5$) (BOD$_3$) values and heavy metal contamination, including lead, cadmium, nickel and zinc ($> 5$ mg·L$^{-1}$) (Chiu and Morton 1999a, b), probably a consequence of both organic and industrial influxes (Wong et al. 1980). Relatively better aeration might also explain not just the occurrence of sea-grass (Halophila beccarii) beds, but also account for the close interactive relationship identified herein between these and juvenile horseshoe crabs, in addition to the habitat structural complexity added to by the beds. This co-occurrence of sea-grass beds and juvenile horseshoe crabs has not been reported upon hitherto. Conversely, this study shows that juvenile T. tridentatus appear to avoid oyster beds perhaps because they can see and avoid dark objects, as with Limulus polyphemus (Errigo et al. 2001).
Carcinoscorpius rotundicauda

The habitat preference of *Carcinoscorpius rotundicauda* is unresolved by the present study, only four individuals being recorded from habitats ranging from muddy Station C to sandy Station E. *Carcinoscorpius rotundicauda* is apparently not restricted by anoxic substrata (4.2 mg L$^{-1}$) at the former station. Salinity does not seem to be a factor in determining distribution either, as the individuals were recorded from interstitial waters with salinities of between 7.8 and 22.5‰. Notwithstanding, *C. rotundicauda* is known to be tolerant of brackish and freshwaters (Annandale 1909; Størmer 1955; Shuster 1957; Mikkelsen 1988), apparently preferring muddy rivers, swampy estuaries and mangrove stands (Shuster 1985; Sekiguchi 1988; Chatterji 1994; Weng and Hong 2001). This study, thus, implies that parameters other than salinity and substratum granulometry might govern the distribution of *C. rotundicauda* although Stations A, C and D would appear to be locally optimal for *C. rotundicauda*.

**Horseshoe crab size distribution**

Prosomal widths of *Tachypleus tridentatus* obtained in this study (12–102.5 mm, giving ages of between 2 and 10 years old) were much greater than those recorded from Taiwan (8–40 mm, 1–5 years old; Yeh 1999; Lin 2002) and Japan (20–70 mm, 3–8 years old; Kawahara 1982), but are congruent with individuals collected from Shui Hau in Hong Kong (20–142 mm, 3–12 years old; Chiu and Morton 1999a, 2001), confirming that local juveniles spend at least 10 years in their natal crèches. Notwithstanding, latitude and locality (Sekiguchi et al. 1976, 1978, 1988; Yamasaki et al. 1988), together with salinity and other habitat parameters (Shuster 1950; Jegla and Costlow 1982), play a role in development rate and, in turn, the prosomal widths of all four extant horseshoe crab species.

It is generally believed that horseshoe crab eggs, that is, of *Limulus polyphemus*, are laid at the high water line during spring tides. Juveniles inhabit intertidal areas nearby (Shuster 1979; Sekiguchi et al. 1988). Growing juveniles move offshore from their natal crèche areas with increasing age (Shuster 1979). Similarly, it was also reported from Taiwan that *Tachypleus tridentatus* Instars II and III, that is, <2-year-old individuals, tend to occur mostly at <50 m from the highest water mark and Instars III–VII (2–5-year-old individuals) at >150 m (Yeh 1999). Lin (2002) came to a similar conclusion, with individuals with prosomal widths of <5.8 mm recorded mostly from 125 m down-shore from the high water mark. Notwithstanding, both Yeh (1999) and Lin (2002) added that this size-specific distribution pattern was not always absolute and remarked that juveniles might swim and disperse themselves at the times of high tides, resulting in a mixed distribution of assorted-sized juveniles. Such a hypothetical gradation of juvenile size from the coastline to offshore is, actually, overturned by this study. Early juveniles (12–14 mm prosomal width) were also recorded at 120 m down from the shoreline and most individuals of all ages occurred generally at distances of between 60 and 120 m down-shore from the shoreline, although this appeared to change with season.

The generalization that *Limulus polyphemus* eggs are laid at the highest water line might be true only with an approximate 1 m tidal regime (Rudloe 1979b; Penn and Brockmann 1994). In Delaware Bay where the tidal amplitude is ~2 m, nests occur down from the high tide line to extend over 60% of the exposed intertidal area (Shuster 1982). A wider distribution was recorded for Cape Cod Bay with a tidal amplitude of
∼3 m (Rudloe 1979b; Penn and Brockmann 1994). Hence, the local tidal amplitude of ∼2.8 m in Hong Kong implies scattered nests of *Tachypleus tridentatus* over the wide expanse of the exposed intertidal mudflats of Deep Bay.

**Population estimates and prospects for horseshoe crab conservation**

Capture-and-recapture tagging was employed by Shuster (1950) to estimate the population size of adult *Limulus polyphemus*. In this study, although recapture rates of juvenile *Tachypleus tridentatus* at Station E were comparable to Station F, population estimates using the tagging method (1.8 individuals per 100 m$^2$) approximated those inferred from transect studies at Station E (2 individuals per 100 m$^2$), whereas a large discrepancy was noted at Station F (tagging = 0.2 individuals per 100 m$^2$, transect = 1.6 individuals per 100 m$^2$). Tagging incurs errors because tagged individuals grow, molt and shed their exuviae, which carry the tag, despite frequent samplings, adding to the possibility that such juveniles might move out of the defined sampling area (Rudloe 1981; Chiu and Morton 2004). Nevertheless, both population estimation methods hinge largely on the emergence of juveniles at predicted times, which Lee and Morton (2009) demonstrated varies with temperature. Extrapolating from the calculated emergence rate of 23% in summer, populations of juveniles in the study area might be 2 juveniles per 100 m$^2$ ÷ 23% = 9 juveniles per 100 m$^2$ at Station E, 1.6 juveniles per 100 m$^2$ ÷ 23% = 7 juveniles per 100 m$^2$ at Station F and 1.1 juvenile per 100 m$^2$ ÷ 23% = 5 juveniles per 100 m$^{-2}$ at Station H, on the condition that temperatures remain consistently ≥25°C, although this is not the case. The results of this study might, therefore, not serve as a factual estimate of population size but it can still be used as an abundance indicator for between-year comparative purposes.

Once, but ∼30 years ago, juveniles of *Tachypleus tridentatus*, *Tachypleus gigas* and *Carcinoscorpius rotundicauda* were reported to occur in large numbers in Deep Bay (i.e. the present study area), Tai Po and Tolo Harbour (Figure 1A) (Cheung et al. 1975; Hill and Phillipps 1981; Morton and Morton 1983; Mikkelsen 1988). Today, with urbanization and poor sewage-treatment facilities, juvenile horseshoe crabs have been extirpated from the latter two areas and the population of *T. gigas* has died out locally (Chiu and Morton 1999a), possibly from over-fishing of adults. Just a few years before this study, an average of 32 juveniles per visit was recorded from Pak Nai, i.e. Stations E, F, G and H of this study (Chiu and Morton 1999a, b). In the intervening lapse of three years, the present study demonstrates that horseshoe crab juveniles occur in Deep Bay with an overall maximum density of two *T. tridentatus*-individuals per 100 m$^2$ (tagging experiment) or a site-specific mean density of between four and nine individuals per 100 m$^2$ (transect censuses). After the cross-border bridge over Deep Bay was opened in July 2007, a re-survey of the study area, using the same methodology, in September 2007 yielded only 0.25 and 0.17 *T. tridentatus* individuals per 100 m$^2$ at Stations E and F, respectively, as compared with abundances of 1.33–3.7·100 m$^{-2}$ (average 2.100 m$^{-2}$) in September 2002 (pers. obs.). Furthest from the bridge at Station H, however, such a decline was less apparent; indeed numbers seemed to have increased (2.42 in 2007; 0.33–1.5 in 2002).

No *Carcinoscorpius rotundicauda* individuals were identified in 2007 from any of the three stations (but two *C. rotundicauda* juveniles were identified from the area between Stations G and H). Longer-term studies are hence necessary to confirm the
possible impact(s) of the construction of the bridge on the juvenile populations of both horseshoe crab species and their intertidal crèches. The 2007 results obtained and reported upon here are in broad agreement with those reported upon by Shin et al. (2009) for a 2004 survey of the Deep Bay and other horseshoe crab beaches in Hong Kong. That is, these authors also recorded a decline in the numbers of *T. tridentatus* juveniles in Deep Bay from 0.10, 1.97, 1.55 and 1.14 individuals per m² at Sheung Pak Nai, Pak Nai and two other locations at Pak Nai, respectively, in 2002 (Morton and Lee 2002; this study), to zero, 0.86, 016 and 0.23 individuals per m² at these four locations, again respectively.

The Deep Bay study area in Hong Kong is characterized by both sandy beaches and abutting mudflats that are indispensable for horseshoe crab spawning and natal crèches, respectively. Together with the optimal temperature, salinity and sediment size spectrum, there is no reason why the large expanse of shore, despite the cross-border bridge and other developments, cannot continue as a horseshoe crab nursery ground locally. Once, juvenile horseshoe crabs (of undetermined species) prospered (Mikkelsen 1988; Chiu and Morton 1999a, b, 2003). Today, *Carcinoscorpius rotundicauda* appears close to extirpation locally and the remaining *Tachypleus tridentatus* individuals are not far behind them.

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