Emergence Behavior of Juvenile *Tachypleus tridentatus* Under Simulated Tidal Conditions in the Laboratory and at Two Different Sediment Temperatures

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Abstract Previous local studies estimated that the densities of emerged, feeding, juvenile *Tachypleus tridentatus* (prosomal width = 17.1–36.9 mm), obtained from surface counts at Ha Pak Nai, Hong Kong, varied from 4 individuals · 1,000 m^{-2} in summer (July–September) to 0 individuals · 1,000 m^{-2} in winter (December) 2002. To determine if such figures reflected true densities, juveniles were kept in tanks with sediment from the nursery ground at temperatures of between 15–20°C (winter) and 25–30°C (summer) under simulated tidal cycles. After a week's acclimation, their emergences were recorded, as was the depth of sediment to which they burrowed. No individuals emerged under imitated conditions of low tide at winter temperatures whereas 23% emerged at summer ones, indicating that sediment temperatures override circatidal activities when they fall below 20°C. The estimated abundance of juveniles on a nursery beach in the summer of 2002 should therefore be 4.16/0.23 = 18 individuals · 1,000 m^{-2}. During the imitated low tide, nearly all juveniles, which did not emerge at the substratum surface, buried themselves to a depth of <3 cm, irrespective of sediment temperature. Our results also showed that only 5% of the tested juveniles, regardless of temperature, were ever identified above the substratum during high tides. Overall, the present study confirms that field estimations of juvenile *T. tridentatus* abundance should include temporal patterns because emergence varies with temperature and tidal state.

1 Introduction

Horseshoe crabs have been identified as indicator species of healthy and clean intertidal sandy beaches (Morton and Morton, 1983). Three species have been recorded from Hong Kong, that is, *Carcinoscorpius rotundicauda* (Latreille, 1802), *Tachypleus gigas* (Müller, 1785), and *Tachypleus tridentatus* (Leach, 1819) (Hill et al., 1978; Hill and Phillipps, 1981; Morton and Morton, 1983;

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Mikkelsen, 1988). In the wake of habitat destruction and commercial fishing, *T. gigas* is now believed to be locally extinct, whereas the former two species are now similarly at risk from various impacts upon their shrinking populations (Chiu and Morton, 1999a, b, 2003a; Morton and Lee, 2003).

Population studies are indispensable to determining the current plight of horseshoe crab species in Hong Kong. Contrary to anecdotal documentation of adults coming ashore to breed, systematic samplings of juveniles during receding tides at their nursery grounds have been conducted by Chiu and Morton (1999a) and Morton and Lee (2003). These two studies suggested that in Hong Kong, situated in the subtropical transition zone between the temperate Northern Pacific and tropical Indo-Pacific and, therefore, subject to the alternation of hot, humid summers and cold, dry winters, sediment temperature might serve as one of the critical local factors determining the emergence of juveniles from the sediment to feed (Zhou and Morton, 2004). Hence, this might also affect estimates of population numbers.

At Shui Hau, Chiu and Morton (1999a) recorded only six juvenile *T. tridentatus* per sampling trip in winter, as compared with 47 juvenile *T. tridentatus* per sampling trip at the same place in summer. Similarly, in Ha Pak Nai, Morton and Lee (2003) recorded no juvenile *T. tridentatus* at winter sediment temperatures of $<20°C$ as compared with an average summer density of four juvenile *T. tridentatus*$\cdot$1,000 m^{-2}. Although such temporal variations in abundance could be thought of as an indication of the summer spawning season, or of an offshore migration (e.g., Botton et al., 2003), these results also raise questions about whether different temperatures solicit various behaviors. The aims of the present study were thus to investigate and compare the emergence behavior of juvenile *T. tridentatus* at the times of low tides at temperatures of 25–30 and 15–20°C, reflecting summer and winter sediment temperatures in Hong Kong, respectively.

2 Methods

2.1 Experimental Aquaria

Sets of aquaria comprised a small tank ($40 \times 40 \times 40$ cm^3) contained within a bigger one ($55 \times 55 \times 55$ cm^3) (Fig. 1). The bigger tank essentially acted as a seawater storage for the smaller one. The smaller, inner, tank had holes drilled along the upper portion of its walls so as to share its water volume with the bigger, outer, one. The bottom also had a perforated platform to allow aeration of the contained sediment from beneath. A 10-cm layer of sand, excavated from a horseshoe crab nursery ground, was put onto the platform and changed every week. Two horizontal slits, each ~3-mm high and ~30-mm apart, were made on one wall of the inner tank. Each set of tanks also included a closed seawater circulation system with its own reservoir. Water temperatures of some sets of

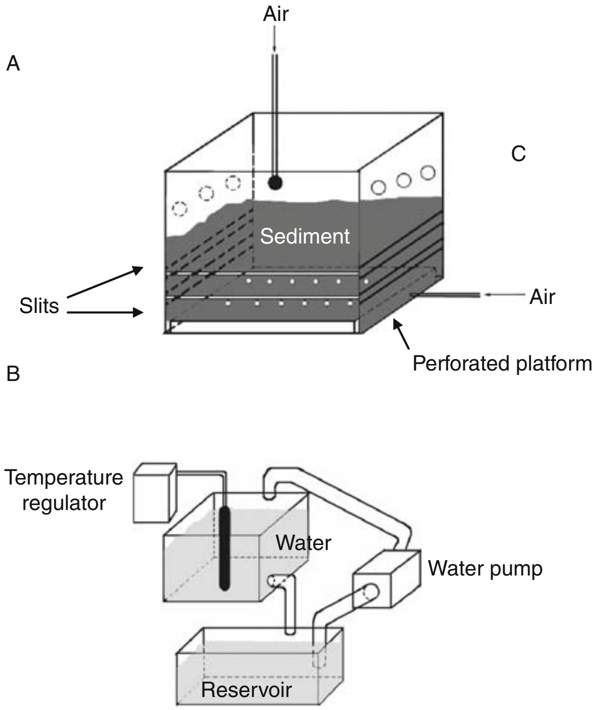

Fig. 1 The experimental setup for the study. (**A**) The inner aquarium; (**B**), the outer bigger one with a reservoir

tanks were maintained at 25–30°C to mimic summer conditions in Hong Kong, while remaining, winter, ones experienced temperatures between 15 and 20°C. The temperatures of the tanks were allowed to stabilize for 24 h before experimentation under the dark:light cycle of 12:12.

2.2 Acclimation Procedures

As it is generally believed that juvenile horseshoe crabs emerge from the sediment primarily to forage, 22 captive individuals used in this study, with prosomal widths ranging from 17.1 to 36.9 mm, were all fed with a regular supply of minced squid and prawn for 2 weeks at 25–30°C. They were then admitted to aquaria at either 15–20 or 25–30°C for a week's starvation, depending on the temperature category they were going to be experimented upon, that is, 15–20°C for the 11 individuals in winter tanks and 25–30°C for another 11 in the summer ones.

2.3 Experimental Procedures

Juveniles were placed in their own smaller, inner, tank with a plastic plate covering both the inner and outer tanks. After living in the experimental tank for 48 h, the emergence (or burrowing) behavior of each juvenile was recorded. Subsequently, water inside each smaller tank was drained off to expose the sediment surface. Further, the water level of the outer tank was lowered to slightly below the sediment surface of the inner one. This allowed the temperature regulator to continue to operate in the residual water of the outer tank, under the cover of the plastic plate over both the inner and outer tanks, so as to maintain the constant temperature of the sediment in the inner one. Ninety minutes later, the location (emerged or buried) of each juvenile was again recorded. If the juveniles were buried, the smaller tank was removed from the outer one and two plastic plates were inserted into the slits on its side to separate the sediment layers, as designed, into ~30-mm thick sections thereby halting the vertical movement of the horseshoe crabs inside the sediment. Each sediment layer was then removed to record how deep the horseshoe crabs had burrowed into it.

At the end of the experiment, the tested juveniles went through the same acclimation procedures again, that is, a week of being regularly fed before another week of starvation at the temperature at which they were experimented. The two groups of animals were then interchanged, that is, those experimented upon in the winter aquaria were put into the summer ones and vice versa. To investigate if the order of temperature treatments affected their burrowing behavior, the chi-square test with Yates' correction was employed.

To study whether or not juveniles would emerge from the sediment during every simulated low tide in their favored conditions, those which did so were, following re-acclimation, put into the same temperature aquaria once again in a repeat of the initial experiment, as described above.

3 Results

At simulated low tides, individuals 3, 8, 16, 18, and 20 (Table 1) emerged in the summer aquaria (representing 23% of the tested juveniles), whereas not one did in the winter ones. There was no statistically significant association between their emergence with the type of temperature tanks they first experienced ($\chi^2 = 0.020$; $P > 0.05$). All juveniles which buried themselves in the substratum in both summer and winter aquaria never did so below a depth of 30 mm, except for individual 5, which buried to a depth of between 30 and 60 mm in the latter one.

Under conditions of simulated high tides, only one of the 22 tested individuals was identified above the substrata of the aquaria under both summer and winter conditions ($\chi^2 = 0.095$; $P > 0.05$). Trails made by the juveniles under high-tide conditions, including the emerged ones, were identified more often in summer aquaria (6) than in winter ones (2), irrespective of the type of temperature tanks they first experienced ($\chi^2 = 0.069$; $P > 0.05$). This suggests a relatively higher level of activity in the summer aquaria.

Table 1 *Tachypleus tridentatus*. The emergence of tested juveniles from the substratum in the various experimental setups

Type of tank first experimented		Winter tank: Emergence at high tide	Winter tank: Emergence at low tide (depth to which it buried)	Summer tank: Emergence at high tide	Summer tank: Emergence at low tide (depth to which it buried)
Individuals first experimented upon in winter tank	1	No, but there was a trail	No (0–3 cm)	No	No (0–3 cm)
	2	No	No (0–3 cm)	No	No (0–3 cm)
	3	No	No (0–3 cm)	Yes	Yes
	4	No	No (0–3 cm)	No	No (0–3 cm)
	5	No	No (3–6 cm)	No, but there was a trail	No (0–3 cm)
	6	No	No (0–3 cm)	No	No (0–3 cm)
	7	No	No (0–3 cm)	No	No (0–3 cm)
	8	No	No (0–3 cm)	No, but there was a trail	Yes
	9	No	No (0–3 cm)	No	No (0–3 cm)
	10	No	No (0–3 cm)	No, but there was a trail	No (0–3 cm)
	11	Yes	No (0–3 cm)	No	No (0–3 cm)
Individuals first experimented upon in summer tank	12	No	No (0–3 cm)	No	No (0–3 cm)
	13	No	No (0–3 cm)	No	No (0–3 cm)
	14	No	No (0–3 cm)	No	No (0–3 cm)
	15	No	No (0–3 cm)	No	No (0–3 cm)
	16	No	No (0–3 cm)	No, but there was a trail	Yes
	17	No	No (0–3 cm)	No	No (0–3 cm)
	18	No	No (0–3 cm)	No, but there was a trail	Yes
	19	No	No (0–3 cm)	No	No (0–3 cm)
	20	No	No (0–3 cm)	No	Yes
	21	No	No (0–3 cm)	No	No (0–3 cm)
	22	No	No (0–3 cm)	No	No (0–3 cm)
Total number of emerged juveniles:		1	0	1	5

The five juveniles that emerged from the substratum after drainage in the summer aquaria (individuals 3, 8, 16, 18, and 20 in Table 1) were, following re-acclimation, put back separately into the same condition to repeat the experiment. None of these individuals, however, re-emerged either prior to or after drainage.

4 Discussion

Both Chiu and Morton (1999a; 2004) and Morton and Lee (2003) conducted samplings of juvenile horseshoe crabs at their nursery grounds in Hong Kong at approximately 3 h prior to the predicted time of spring low tides; juveniles are reported to be most active (Rudloe, 1979; Kawahara, 1982). The nursery grounds are so flat that, by this time, about half of the intertidal mud was already exposed. Within the exposed area, a majority of emerged, feeding, *T. tridentatus* were identified at 60 m down from the shoreline (Morton and Lee, 2003). The low estimated abundance of juvenile *T. tridentatus* in summer and virtual absence of emerged juveniles in winter in previous Hong Kong studies lead to a question regarding their behavior in response to sediment temperature.

In a behavioral study of *T. tridentatus* in Hong Kong, Chiu and Morton (2004) questioned whether the seasonal abundance pattern of emerged juveniles at their nursery grounds is a result of differential metabolic activities at various temperatures or a seasonal migration into subtidal areas. The virtual disappearance of *T. tridentatus* juveniles from other Asian nursery beaches in winter has been suggested to result from a declining aerobic metabolism under the influence of low temperatures, leading to a period of dormancy (Kawahara, 1982; Chiu and Morton, 1999a, 2004), as has also been asserted by Rudloe (1981) for juveniles of the American horseshoe crab, *Limulus polyphemus*. It is also known that juveniles can endure periods of starvation for as long as 1 year (Sekiguchi, 1988, Weng and Hong, 2001). Juvenile *L. polyphemus* have also been hypothesized to migrate to subtidal areas during the winter in Delaware Bay (Botton et al., 2003). The present study confirms that in Hong Kong *T. tridentatus* juveniles become inactive and remain buried in the substratum of their nursery ground under, simulated, winter temperatures. This is because more trails or emergence should have been observed if juveniles had retreated to deeper waters. The possible difference between the suggested behaviors of *L. polyphemus* and those of *T. tridentatus* in this study might be attributable to the much colder temperatures in Delaware Bay than in Hong Kong in winter.

Activities of *L. polyphemus* trilobite larvae have been shown to have an endogenous circatidal rhythm (Rudloe, 1981; Ehlinger and Tankersley, 2006). In such experiments, however, individuals were held concurrently under both winter and summer temperature conditions, with all other conditions comparable to each other, and still different behaviors were recorded. Our results thus demonstrate that sediment temperature might override such a rhythm when it falls below 20°C.

Even at summer temperatures, however, only 23% of juveniles of *T. tridentatus* became active and emerged from the substratum under simulated low tides. Similarly, of these, none re-emerged from the substratum at the next low tide. This is also known from our fieldwork in 2002. That is, juveniles do not emerge from the substratum on every receding tide, suggesting that such a behavior occurs only at the population level. A similar behavior is seen in adults, which do not come ashore to spawn on consecutive high tides (Sekiguchi, 1988). Such a lack of behavioral pattern might be key to the continued survival of horseshoe crabs over geological time. If all adults and juveniles emerged from the water or sediments, respectively, at a predictable time, the chances of mortality from predation, for example, would be higher. Extrapolating the 23% rate of juvenile *T. tridentatus* emergence from this study, the average density of juvenile *T. tridentatus* at Ha Pak Nai during the summer of 2002 by Morton and Lee (2003) is readjusted from 4.2 to 18 individuals · 1,000 m^{-2}.

Juvenile *T. tridentatus* burrowed to a depth of ~3 mm, irrespective of sediment temperature. This might be due to the possible occurrence of anoxic sediments at deeper levels, although the depth of the redox layer was not determined. They might also need to maintain a constant flow of seawater over their book gills that could be difficult to achieve if they have buried too deep into the substratum. Possibly, also, the juveniles might burrow for protection from predation. Identification of natural predators might provide more insights into such juvenile behavior. The actual reason behind this behavior requires further investigation.

Because of the high sediment load in the water column of the horseshoe crab nursery grounds at Ha Pak Nai (Kot and Hu, 1996; Chiu and Morton, 2003b), attempts to sample here during high-tide periods have never been successful. This leads to questions of whether individuals also emerge from the substratum when submerged. In this study, under simulated high-tide conditions at winter temperatures, however, most individuals not only buried themselves immediately but remained so; only two of the 22 experimental animals were being either seen on the surface of the substratum or had a trail identified. Conversely, 6 of 22 individuals (27%) were either recorded on the surface of the substratum or left a trail at simulated high tides at summer temperatures. Of the six occasions when juveniles were either emerged or produced trails before aquarium drainage (simulated high tide), two re-buried themselves until the end of the experiment. Such a disappearance at trail ends has also been sporadically encountered in the field (Morton and Lee, 2003; Chiu and Morton, 2004). It is unknown if the trails were made soon after the juveniles were put onto the substratum at the beginning of the experiment. Nevertheless, the number of trails seen before drainage approximated the number of emergences identified after drainage at the end of the experiment in summer aquaria. This suggests that juveniles could also occur above the substratum when they are covered by water at the time of high tide. Such an observation contrasts with the generalization that juveniles are active only during low-tide periods (Kawahara, 1982; Sekiguchi et al., 1988; Chatterji, 1994). Overall, the present study identifies the need to take the temperature-linked emergence behavior of juvenile horseshoe crabs into account when estimating their abundances at nursery beaches via surface counts.

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References

Botton ML, Loveland RE, Tiwari A (2003) Distribution, abundance, and survivorship of young-of-the-year in a commercially exploited population of horseshoe crabs *Limulus polyphemus*. Mar Ecol Prog Ser 265:175–184

Chatterji A (1994) The horseshoe crab – a living fossil. A Project Swarajya Publication, Orissa, India

Chiu HMC, Morton B (1999a) The biology, distribution and status of horseshoe crabs, *Tachypleus tridentatus* and *Carcinoscorpius rotundicauda* (Arthropoda: Chelicerata) in Hong Kong: recommendations for conservation and management. Final Report to China Light and Power Company Limited. The Swire Institute of Marine Science, The University of Hong Kong, Hong Kong

Chiu HMC, Morton B (1999b) The distribution of horseshoe crabs (*Tachypleus tridentatus* and *Carcinoscorpius rotundicauda*) in Hong Kong. Asian Mar Biol 16: 185–196

Chiu HMC, Morton B (2003a) The status of horseshoe crabs in Hong Kong. In: Morton B (ed) Perspectives on Marine Environmental Change in Hong Kong and Southern China: 1977–2001. Hong Kong University Press, Hong Kong, pp. 741–756

Chiu HMC, Morton B (2003b) The sediment and hydrographic characteristics of three horseshoe crab nursery beaches in Hong Kong. J Ocean Univ Qingdao 2: 35–43

Chiu HMC, Morton B (2004) The behaviour of juvenile horseshoe crabs, *Tachypleus tridentatus* (Xiphosura), on a nursery beach at Shui Hau Wan, Hong Kong. Hydrobiologia 523: 29–35

Ehlinger GS, Tankersley RA (2006) Endogenous rhythms and entrainment cues of larval activity in the horseshoe crabs *Limulus polyphemus*. J Exp Mar Biol Ecol 337: 205–214

Hill DS, Gott B, Morton B, Hodgkiss J (1978) Hong Kong Ecological Habitats, Flora and Fauna. Hong Kong University Press, Hong Kong

Hill DS, Phillipps K (1981) A Colour Guide to Hong Kong Animals – A Naturalist's Book on Local Wildlife. The Hong Kong Government, Hong Kong

Kawahara D (1982) Investigations on ecology of horseshoe crab larvae. Aquabiology 4: 380–382 (In Japanese)

Kot SC, Hu SL (1996) Water flows and sediment transport in Pearl River Estuary and waves in South China Sea near Hong Kong. In: Civil Engineering Department (ed) Coastal Infrastructure Development in Hong Kong Special Administrative Region: A Review. Proceedings of the Symposium on Hydraulics of Hong Kong Waters, Hong Kong 1995. Civil Engineering Department, Hong Kong, pp 13–32

Mikkelsen T (1988) The Secret in the Blue Blood. Science Press, China

Morton B, Lee CN (2003) The biology and ecology of juvenile horseshoe crabs along the northwestern coastline of the New Territories, Hong Kong: prospects and recommendations for conservation. Final Report to China Light and Power Company Limited. The Swire Institute of Marine Science, The University of Hong Kong, Hong Kong

Morton B, Morton J (1983) The Sea Shore Ecology of Hong Kong. Hong Kong University Press, Hong Kong

Rudloe A (1979) Locomotor and light responses of larvae of the horseshoe crab, *Limulus polyphemus* (L.). Biol Bull 157: 494–505

Rudloe A (1981) Aspects of the biology of juvenile horseshoe crabs, *Limulus polyphemus*. Bull Mar Sci 31:125–133

Sekiguchi K (1988) Ecology. In: Sekiguchi K (ed) Biology of Horseshoe Crabs. Science House Co. Ltd., Tokyo, Japan, pp 50–68

Sekiguchi K, Yamamichi Y, Seshimo H, Sugita H (1988) Normal development. In: Sekiguchi K (ed) Biology of Horseshoe Crabs. Science House Co. Ltd., Tokyo, Japan, pp 133–224

Shuster CN (1954) A horseshoe "crab" grows up. Ward's Nat Sci Bull 28:1–6

Weng ZH, Hong SG (2001) The distribution and habit of horseshoe crabs. Chinese J Zool 36(5): 4–8

Zhou H, Morton B (2004) The diets of juvenile horseshoe crabs, *Tachypleus tridentatus* and *Carcinoscorpius rotundicauda* (Xiphosura), from nursery beaches proposed for conservation in Hong Kong. J Nat Hist 38: 1915–1925