Chapter 10
Studies of Horseshoe Crabs Around Singapore

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Abstract Results from students' research on horseshoe crabs are not often readily available to other workers. A review of these studies has shown that during 6 years since the first published studies, the distribution of horseshoe crabs around Singapore's main island has remained unchanged, the density of mangrove horseshoe crabs, *Carcinoscorpius rotundicauda*, at two sites has remained stable, and periods of high and low reproductive activity during the year have been confirmed. The student studies indicated that juvenile *C. rotundicauda* concentrated at the high tide zones of the mudflats along the Strait of Johor, and smaller juveniles were found on the mud surface and in the small streamlets. Adults were found spread more evenly over the mudflats from the high to low tide zones, on average about 3 m apart, and commonly buried or found in the larger streams among the mangroves. Feeding preference studies suggest mangrove horseshoe crabs are not particularly selective feeders. Sex ratios remained approximately 1:1 all year and at all sites, and mean percentage growth during ecdysis was 34 %, with little difference between the smallest crab of 1.1 cm and the largest of 90.1 cm. A mortality study found death rates of mangrove horseshoe crabs higher at a polluted site (19.8 %) than at a less polluted site (7.5 %). Island wide distribution studies showed that while there is a thriving population of mangrove horseshoe crabs around the north-west, by contrast, *Tachypleus gigas*, the coastal horseshoe crab, is still very rare around Singapore, but an encouraging finding was the first reported sighting of *T. gigas* juveniles at one site. These studies, although small and of short duration, are important for the regular monitoring of the current status of horseshoe crab populations around Singapore and offer a major contribution to the understanding of horseshoe crab ecology in the country.

Keywords Singapore • Mangrove • Coastal • Density • Distribution • Mudflats • *Carcinoscorpius rotundicauda* • *Tachypleus gigas* • Demography • Habitat • Diet preferences • Growth • Conservation

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10.1 Introduction

The Mandai mudflats at Kranji in the north of the main island of Singapore along the Strait of Johor are home to a thriving population of mangrove horseshoe crabs, *Carcinoscorpius rotundicauda*. This area is the largest expanse of mudflats remaining in Singapore and is also an important site for resident shore birds and migratory birds travelling along the East Asian Australasian Flyway.

Fortuitously, the site is accessible, and horseshoe crabs are easy to find. Horseshoe crabs do not run away when approached and they do not sting, bite or pinch, so they make an ideal subject of study for students needing a topic for their degree dissertations. The site offers a valuable educational resource for fieldwork. Most studies are of short duration and are designed so that they can be completed in just a couple of months. So far no student in Singapore has remained involved in horseshoe crab research long enough to continue to a PhD or other studies that would require a longer-term project with more in-depth research, using more sophisticated techniques. In most cases once the study is completed and the student moves on, the dissertation is filed in the library of the university or college without publication in a peer-reviewed journal or other publicly accessible source. Thus, there is a view that there are no or very few data on the biology of horseshoe crabs in Singapore, and knowledge that is based on empirical data rather than anecdotes is fragmentary and not easy to find. In many cases the students have put in much hard work and the studies are thorough and well supervised. Although there is often an element of repetition, these small studies help to confirm earlier findings and provide frequent monitoring of horseshoe crab sites, which can be valuable in determining emerging threats. Hence, if made accessible, the work in these dissertations has potential to make an important contribution to the overall understanding of horseshoe crab ecology in Singapore.

Since the Nature Society of Singapore (NSS) embarked on a research project, born out of a horseshoe crab rescue program, larger studies of longer than a few weeks duration have been undertaken. Unlike students who move on, most of the NSS members have a long-standing involvement in the Society and an abiding interest in nature and wildlife, providing continuity in the NSS work. Once the NSS horseshoe crab program was underway, the NSS soon became able to field a large number of volunteers (students and staff from schools and colleges, NSS members, and the public) to help with field searches and data collection. This effort allowed more ambitious studies to be done and more sophisticated equipment, such as acoustic tracking devices, to be used. Gathering together the data from the short, unpublished works as well as these longer published studies will give a fuller picture of what is known and not known about the status of the two species of horseshoe crabs (*C. rotundicauda* and *T. gigas*) in Singapore. It will then be possible to guide research to fill the gaps in the knowledge of the ecology of these species such that practical conservation measures can be taken to protect them.

All the field studies on the horseshoe crabs in Singapore have used the same methods of search for *C. rotundicauda* and *Tachypleus gigas*, which is occasionally

seen around Singapore. At low tide the site is searched visually for surface crabs on the substrate or in small streams and, for buried crabs, by probing the surface with fingers or small sticks. Individuals of all sizes, from juveniles of approximately 0.5 cm in carapace width to adults of approximately 16 cm and crabs in amplexus can be found at any site visit throughout the year. Juveniles are considered to be those less than 8 cm in carapace width, based on the smallest size of males found in amplexus. There is no mass spawning event on a seasonal basis as with the American species of horseshoe crabs, and spawning individuals are very rarely seen. Sex determination in the adults is always based on the structure of the first pair pedipalps. Sexing of juveniles using genital pore structure is not undertaken in the field because of the difficult conditions under which the work has to be done.

10.2 Distribution

10.2.1 NSS Surveys

In March 2009 the NSS team conducted the first island-wide survey to determine if there were populations elsewhere around Singapore (Cartwright-Taylor et al. 2011). Loss of habitat and pollution are listed as the two main threats to horseshoe crabs in Singapore (Davidson et al. 2008), so periodic surveys of possible remaining habitats are important in monitoring any emerging threats to the remaining populations or, indeed, in identifying if individuals are returning to formerly deserted sites. This first survey was conducted at eight sites around the main island including the Mandai mudflats near Kranji, where horseshoe crabs have been well studied (Fig. 10.1).

Western Sites Further along the coast to the west of Kranji and past the Sungei Buloh Wetlands Reserve (SBWR), a known habitat of horseshoe crabs, is Lim Chu

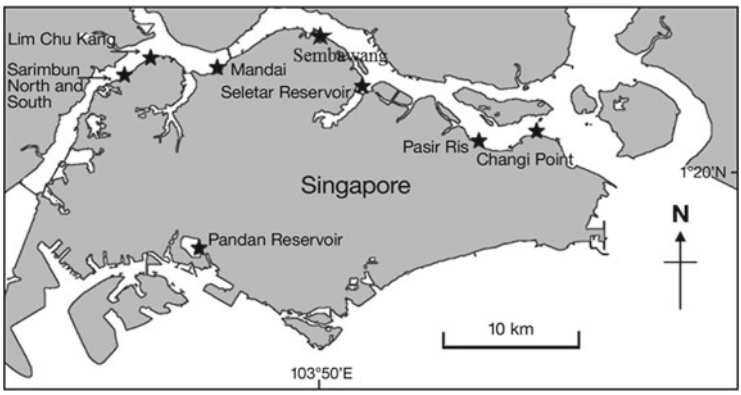

Fig. 10.1 Map of Singapore showing sites where horseshoe crab surveys have been conducted

Kang, a muddy shore with mangrove fringes, where a population of *Carcinoscorpius rotundicauda* was found during the survey. Although this area was known to support a population of *C. rotundicauda*, very little work has been done on the horseshoe crabs at this site. Occasionally *Tachypleus gigas* was also found in the vicinity, and the NSS survey yielded one dead *T. gigas* at this site suggesting some cohabitation with *C. rotundicauda*. The local fishermen also say they occasionally find *T. gigas*. More work at this site would be useful, but the mud here can be very deep, there is a great deal of debris, and moving around the site is not easy.

Further to the west, Sarimbun, a muddy mangrove shore, and the Pandan Reservoir canal to the south were also included in the first survey. *C. rotundicauda* was found at both sites, but neither site is well studied. The mud at the site at Pandan Reservoir, like Lim Chu Kang, is very deep making work here difficult. This site on the south coast is far removed from other known horseshoe crab habitats, all of which are contiguous along the north-west coast, so genetic studies on the Pandan population would be of interest to determine how isolated it has become.

Eastern Sites To the east along the Strait of Johor, no horseshoe crabs were observed at Pasir Ris, a sandy beach with some mud, two live *C. rotundicauda* were found at Lower Seletar, also a mix of sand and mud, and one *C. rotundicauda* was seen at Changi Beach, a true sandy beach more suited to *T. gigas*. The survey also yielded six live *T. gigas* at Changi Beach, all trapped in nets. Clearly there are some *T. gigas* around the shore but where they spawn or forage is unclear. According to elderly fishermen and most Singaporeans in their 50s and 60s, *T. gigas* frequently was seen at all three sites some years ago. However, with coastal development and large areas of land reclaimed from the sea around the south and east of the island, most of the sandy habitats have been destroyed or are now man-made and too steep for horseshoe crabs. Hence, sightings of *T. gigas* are now rare. There is a clear and urgent need for more studies on this species, particularly on spawning sites and timing. Tracking studies, as done on *C. rotundicauda* (Cartwright-Taylor et al. 2012), would help determine how far this species travels and which sites it prefers. Similarly, studies on *T. gigas* molts as a proxy for the live individuals would provide information on population dynamics, molting and growth rates as has been done on *L. polyphemus* in USA (Carmichael et al. 2011; Estes et al. 2015).

10.2.2 Republic Polytechnic Surveys

Many of these same sites around the north of Singapore were again surveyed for horseshoe crabs by Republic Polytechnic students during March and April (Lee 2012) and between September and November of 2012 (Lim 2013). All sites were along the Strait of Johor, with the exception of the Changi site to the east. Sarimbun was replaced with Sembawang because a crocodile had been sighted at Sarimbun and, for safety, access to the shore was denied. Sembawang site is a sandy beach with a substrate suitable for *T. gigas* where a canal opens to the Strait. The other side of the mouth of the canal is an area of inaccessible mangroves that may be suitable for *C. rotundicauda*.

C. rotundicauda was the only species found at Mandai and Lim Chu Kang, while nothing was seen at Changi beach or at Sembawang. However, during the two April surveys, ten and six live juvenile *T. gigas* were found at Sungei Tampines near Pasir Ris. This site is a mix of sand and mud, and although it is also at the mouth of a canal, the location of the search transects was along the Strait. Live *T. gigas* are rarely found and a cluster of juveniles, in particular, may indicate a return of this species to this site, a possibility that needs to be followed up.

10.2.3 National University of Singapore (NUS) Survey

More recently an NUS student (Leng 2013) studied the mangrove horseshoe crab population in the small tidal streams among the mangroves of Sungei Mandai Kechil. This is one of the largest remaining expanses of mangrove forest left in Singapore and is contiguous with and to the east of the Mandai mudflats, yet this area has not been included in previous horseshoe crab surveys or studies largely because of the difficulty of access. The area extends from the outflow of the canal, Sungei Mandai, to the causeway across the Strait to Johor and from the shore inland to the old Malaysia railway line. It is an area that has been untouched by development because the Malaysia railway line running parallel to the shore has protected the mangroves to its seaward side from easy access. However the closure and removal of this line means this area is now more accessible. Although this is good for study of the area, it is of grave concern for the future of the mangroves, as the site is now available for development. The area is not protected by law, and efforts by the NSS in 2009 to include this area in a protected wetland were unsuccessful. The area contains a rich biodiversity of flora and fauna, including locally endangered and rare mangrove species, and the mangroves form an important habitat for mangrove horseshoe crabs and a nursery area for many marine species.

Leng (2013) indicated that there is a healthy population of mangrove horseshoe crabs of all sizes in the tidal streams including adults in amplexus. This finding suggests that horseshoe crabs were breeding in the area, and the tidal streams are an important habitat for all stages of the life cycle.

10.2.4 National Parks Department Survey

The third island-wide survey was conducted by the National Parks Department of Singapore between May and December 2013 using belt transects and visual searches at 18 sites around the main island (Cheo and Lee 2014). *C. rotundicauda* was found at only 6 of the 18 sites where the species has been observed in previous surveys, all along the Strait of Johor on the north coast. *T. gigas* was observed only at one site, Changi Creek Mangrove, close to Changi Beach where *T. gigas* has been seen in previous surveys. With the possible exception of the canal at the Pandan reservoir in

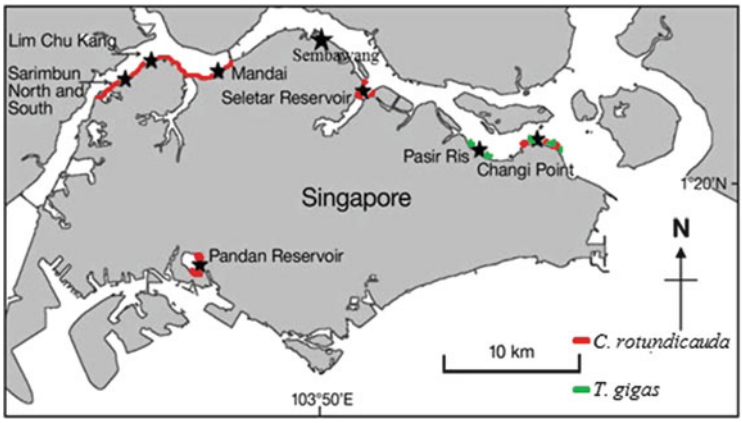

Fig. 10.2 Map of Singapore showing sites where horseshoe crabs have been observed during island-wide surveys

the south west, it seems clear that horseshoe crabs are no longer breeding and can no longer be found around the south coast of the main island, except perhaps for occasional sightings of single individuals washed up on the shore or trapped in nets. Figure 10.2 shows the distribution of horseshoe crabs from all surveys in Singapore.

10.2.5 Summary

C. rotundicauda From the surveys over time described above, it is clear that the situation for the mangrove horseshoe crab has not deteriorated over the years. There are still a few sites where *C. rotundicauda* is thriving, mainly concentrated around the north west of Singapore island. A contiguous population of mangrove horseshoe crabs exists from the causeway to the west past the Mandai mudflats, the SBWR, Lim Chu Kang, Sarimbun and down the west coast, probably along the untouched military reserve site as far as the second link bridge at Tuas across to Malaysia. In addition, a discontinuous population of this species exists along the east of the Strait of Johor. It is not known how much integration there is among all these populations or with sites across the Strait along the Malaysia coast. Genetic comparisons between individuals from all these sites and with crabs from sites across the Strait would be interesting to determine the genetic diversity, if any. Fragmentation of the habitat of the horseshoe crabs means that it is ever more important to protect and preserve the whole area of the north and west where there is a healthy breeding and probably contiguous population.

T. gigas By contrast, *T. gigas*, has been a cause for concern around Singapore for many years, and the situation has not improved. Individual sightings do occur and two adults in amplexus were caught by fishermen during tracking studies of mangrove horseshoe crabs in the Strait of Johor (Cartwright-Taylor et al. 2011) but were

not included in the report, which concentrated on *C. rotundicauda*. A tag was attached to the male of this pair and was tracked for 6 days as he travelled a few miles along the Strait to the west. It is not clear if such individuals represent a small breeding population around Singapore or if they wandered across from Malaysia or elsewhere. Encouragement can be drawn from the fact that juvenile *T. gigas* have been seen on two occasions where they were not seen for many previous years. There also is an intermittent presence of *T. gigas* along the Strait particularly towards the extreme east. This species has been rather neglected as it has been so difficult to find, so work concentrating on *T. gigas* is urgently needed to determine why this species is so uncommon when it seems to co-habitat with *C. rotundicauda* in the Strait. Future work should also consider repopulation of suitable sites with individuals from Malaysia before they also disappear.

10.2.6 Density of Horseshoe Crabs

Only two sites in Singapore have populations of *C. rotundicauda* sizeable enough for density studies, Lim Chu Kang and Mandai mudflats at Kranji. Several studies have been conducted at Mandai since 2007, three by students and two by NSS volunteers, using transects of different sizes and shapes. All studies included juveniles and adults, both solitary and in amplexus, of all sizes as these form the normal population on the mudflats. All studies used collections from measured transects on the mudflats, but the size and shape of the transects differed among studies. Some studies used round quadrats but these present more practical difficulties on the mud surface than the more conventional rectangular or square shapes. These sampling differences could account for the lower density findings in some studies. All studies used visual searches for crabs on the surface of the mudflat and probing with fingers or small sticks for buried crabs. Rakes cannot be used on this surface.

The mean density for all studies combined was 0.46 per m^2 with a minimum and maximum of 0.16 per m^2 and 0.90 per m^2, respectively. The consistency of findings among studies and across months suggests there was no apparent seasonal pattern to the densities (Fig. 10.3). It is encouraging to note that the findings of the studies in 2012 closely match those of the earlier in studies in 2007–2009 (Cartwright-Taylor et al. 2011), suggesting that density has changed very little in the intervening years. The site is an important habitat for small juveniles as well as mature and mating adults. Any urbanization of this coastline, therefore, is expected to have a detrimental impact on this thriving population.

At Lim Chu Kang, further along the coast from Mandai, densities of 0.21 and 0.15 per m^2 were recorded by Lim (2013) at two site visits in March 2009. At the same site in Sept and Oct densities of 0.09 and 0 were recorded by Lee (2012). These are only slightly different from densities of 0.012 and 0.05 per m^2 recorded in two transects at the same site by NSS volunteers in March 2009 (unpublished data). However, in all these studies only two small transects were used each time at this site.

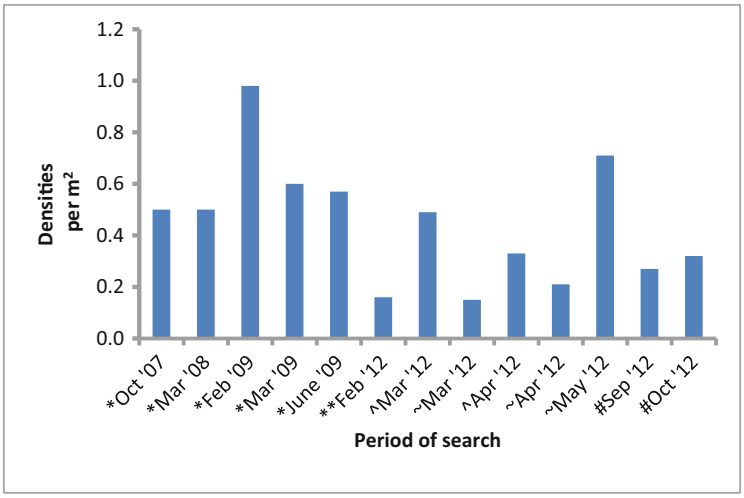

Fig. 10.3 Densities of *Carcinoscorpius rotundicauda* at Mandai mudflat (Compiled from *Cartwright-Taylor et al. 2011; ^Lee 2012; #Lim 2013; **NSS unpublished data; ~Saad 2013)

One recent study (Leng 2013) examined the density of *C. rotundicauda* in two main tidal streams in the mangroves at Sungei Mandai Kechil, further east of the mudflats at Kranji near the causeway to Johor where several streams branch throughout the mangroves. This area has a very different habitat from the open mudflat and comprises stands of mature mangroves cut by tidal streams draining into the Strait. From ten sampling dates between December 2012 and February 2013 at different moon phases, Leng calculated mean densities of adults and juveniles together of 0.037 per m^2 in one stream and 0.068 per m^2 in the other. The range for Stream 1 was 0.011–0.056 per m^2 and for Stream 2 slightly higher at 0.033–0.099 per m^2. It is interesting to note that these densities are an order of magnitude lower than those at the open Mandai mudflats about 200 m away (compare to Fig. 10.3). Indeed the mangrove forest of Sungei Mandai Kechil adjoins the open mudflats so there is unbroken habitat for the horseshoe crabs from the open mudflat site to deep into the mangroves until the causeway. It is not clear why the density should be so much higher just a short distance along the shore, but studies on the salinity of the tidal streams and other environmental parameters may provide an answer. Nevertheless, even these densities are higher than most population studies conducted elsewhere for Asian Pacific horseshoe crabs (Hu et al. 2009; Morton and Lee 2011).

Leng's findings on density showed a trend towards a link with moon phase such that both adult and juvenile densities tended to increase during new and full moons and decrease during half moon phases in both the streams studied. This trend was not confirmed by statistical analysis, possibly because the sample numbers were small, and therefore, require further confirmation. Moon phase and tidal changes are so inextricably linked, however, that it would be difficult to determine which of these may be the driver for any density change. This work certainly is worth repeating on a larger and longer scale and at more sites. These fluctuations in density

suggest that there may be movement to and from the tidal streams, possibly to the nearby mudflats, triggered by moon phase, tidal height or changes in salinity in the streams. This study appears to be the first in Singapore to correlate population density with moon phases, a topic that warrants further work. More work is needed on the horseshoe crabs in the mangrove streams and the mudflats as a single entity to determine the link between the two habitats.

Another student study examined, among other things, the spatial and temporal distribution of *C. rotundicauda* on the Mandai mudflats (Ong 2012a). While adults were evenly distributed on the mudflats from the shore to the low water zone, the juveniles were concentrated 1–23 m from the high tide line. Since the eggs are laid at the high tide level it is to be expected that the juveniles would also be found in this region on the mudflats. The exposed mudflat from the high to low spring tide line covers a distance of about 250 m with a maximum tide height of about 2.5 m, a wide expanse where the horseshoe crabs can be found. Thus 23 m from shore is a very small part of the total area exposed at low tide. However, this distribution means that any coastal development that includes the high tide area of the shoreline will have a profound effect on the population because there will be no place for the eggs and young juveniles.

The students also found a trend toward decreasing numbers of both adults and juveniles as the distance from shore increased, with the highest population density between 10 and 50 m from shore. In addition, they observed that from March to December, excluding June to August, the mean distance between a total of 437 *C. rotundicauda* individuals at Mandai was 313 cm (SD 401.78), demonstrating solitary rather than social behavior. This observation supports the view of Shuster et al. (2003) that it is in the nature of horseshoe crabs to avoid one another. Clustering of mangrove horseshoe crabs is not seen on the open mudflats (personal observation).

10.3 Demography and Population Structure

All demography studies have been done on *C. rotundicauda* because *T. gigas* is too rare now for such studies. An NSS study conducted during 1 year starting in 2007 (Cartwright-Taylor et al. 2009) with annual follow up for a further 3 years (Cartwright-Taylor and Hsu 2012) demonstrated a pattern of monthly changes in the population structure of *C. rotundicauda* at the Mandai mudflats.

The proportion of small juveniles (1–3 cm prosomal width) was highest in November 2007 and January 2008, 25 % and 30 % respectively. In June and July only 8 % and 4 % respectively were in these same size classes. By June and July recruitment to the larger size classes (10–12 cm) was approximately 35 %. In captivity it takes at least 369 days for hatching and growth to 1.7 cm prosomal width (Zadeh et al. 2009). At Mandai, juveniles less than 2 cm were not found in June, suggesting that there may be a rest period of low or no breeding activity from May to July each year resulting in none of the smallest sizes in June and July of the fol-

lowing year. The follow-up work (Cartwright-Taylor and Hsu 2012) indicated that this was not just a 1 year phenomenon but that it occurred year-after-year.

These findings are supported by more recent student studies (Ong 2012a; Saad 2013) on this population of *C. rotundicauda* at the same site, during the same time period (Figs. 10.4 and 10.5). Both studies also reported increasing proportions of adults (>7.9 cm carapace width) in the population coupled with decreasing proportions of juveniles (<8 cm carapace width) during visits from March to May 2012. Saad (2013) recorded carapace widths in only three size classes.

Site visits were repeated between September and December 2012 (Ong 2012b) during which time adult horseshoe crabs continued to dominate the population. A total of 507 horseshoe crabs were collected during these 4 months, of which 89.2 % were adult (45 % males, 44.2 % females) and only 10.8 % were juveniles, compared with 51.3 % adults (21.4 % males, 28.9 % females) and 49.7 % juveniles from a total of 532 horseshoe crabs collected between March and May 2012 (Ong 2012a).

One student study (Leng 2013) focused on the population structure of *C. rotundicauda* in the small tidal streams deep among the mangroves at Sungei Mandai Kechil, 200 m along the shore to the east of the Mandai mudflats at Kranji, and a very different habitat from the open mudflats. There, the population structure of horseshoe crabs in two streams in Sungei Kechil from December 2012 to February 2013 (Leng 2013) showed differences in spite of the proximity of the streams to one another; 30 % in one stream were juveniles (<8 cm carapace width) compared with 69 % in the other stream. However, the numbers collected from the first stream were substantially smaller than the second stream, and this may have affected the proportions to some extent. Nevertheless these figures support those of Cartwright-Taylor and Hsu (2012) where higher proportions of juveniles occurred from December to March. These streams, therefore, may be important nursery areas for this population, and coastal urbanization or other destruction of this habitat could be detrimental to the population of *C. rotundicauda* on the nearby mudflats.

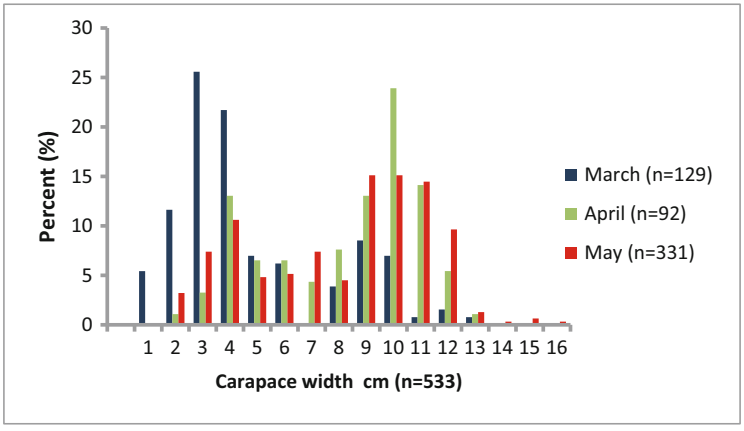

Fig. 10.4 Frequency of size classes (prosomal width) of *Carcinoscorpius rotundicauda* collected from March to May 2012 at Mandai mudflats (From raw data in Ong 2012a)

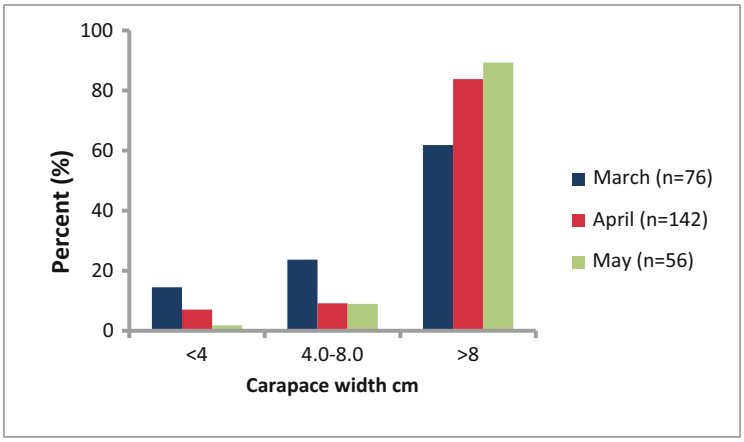

Fig. 10.5 Frequency of size classes (carapace width) of *Carcinoscorpius rotundicauda* collected from March to May 2012 at Mandai mudflats (From raw data in Saad 2013)

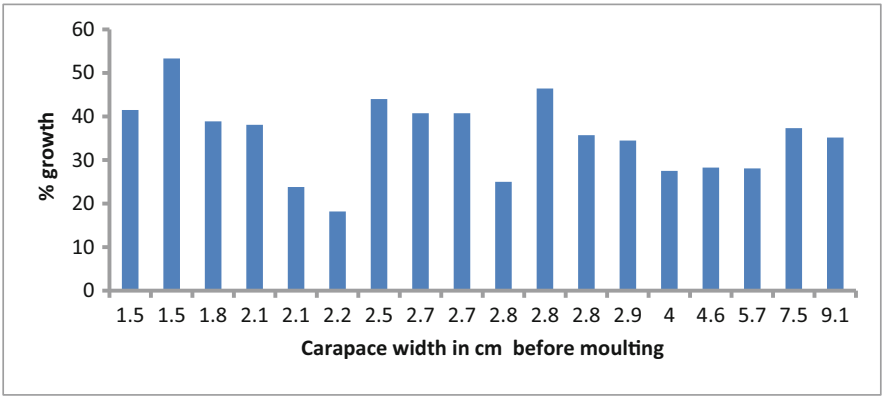

Fig. 10.6 Growth rates during ecdysis of 18 individual *Carcinscorpius rotundicauda* from Mandai mudflats

10.3.1 Growth

From 2007 to 2012 a total of 18 individual *C. rotundicauda* from the wild population at Mandai mudflats were collected while in the process of ecdysis. The prosomal width was measured before and after complete ecdysis, and the percentage growth recorded. Pre-molting widths varied from 1.5 to 9.1 cm, and mean incremental growth was 35.4 % (SD 8.8) (Fig. 10.6). Percentage growth in the largest crab was not very different from that in the smallest. Further studies on molting and growth in the wild population would be interesting to compare with such studies on captive individuals.

Table 10.1 Sex ratios of *Carcinscorpius rotundicauda* at Mandai mudflats from student studies

	Mean (range)	
	March–May	Sept–Dec
Lim (2013)		0.74 (0.60–0.88)
Lee (2012)	0.89 (0.86–0.92)	
Saad (2013)	1.05 (0.4–1.7)	
Ong (2012a, b)	0.74[a]	1.02[a]
Goh (2010)		0.82[b]

[a]Four months combined
[b]Single site visit Sept 2009

10.3.2 Sex Ratios

Sex ratios of *C. rotundicauda* at the Mandai mudflats from all the student studies in 2012 (Table 10.1) support published figures (mean 1.28; SD 0.78) from the same site during a 4-year period prior to 2011 (Cartwright-Taylor and Hsu 2012). The ratios altered little from 1 throughout the year. All studies confirmed that mating activity was not strongly seasonal, as occurs in temperate climates. In addition pairs in amplexus were seen year-round in varying numbers and with no discernible monthly pattern.

10.4 Habitat and Foraging

10.4.1 Habitat Preferences

Two studies have been conducted on sub-habitat preferences and size class among the mangroves that are flooded at high tide. Between 5th February and 3rd March 2004 one study was conducted along the Kranji Nature Trail (KNT) (Hong 2004) a few miles west of the Mandai mudflats. The second study (Leng 2013) was conducted from December 2012 to February 2013 at Sungei Mandai Kechil, 200 m to the east of the Mandai mudflats. Both sites comprise a range of sub-habitats deep within the mangroves, and the studies included streams over mud where water is fast flowing, streamlets over mud that drain into the streams and where water velocity is slower and the bed muddier, small pools, mudflats, and, in the eastern site, sea grass, *Halophila beccarii*, over mud, and sand, a grittier substrate than mud. Mud and sand were differentiated by the texture of sediment. In both studies, adults and juveniles of different size classes showed different preferences for these microhabitats.

Juveniles (<8.6 cm) comprised 70 % of 162 individuals found at KNT (Hong 2004). Later studies on *C. rotundicauda* at Mandai have used 8 cm as the threshold for adults based on sizes of pairs in amplexus, and indeed this may be too high because rarely smaller males in amplexus have been found during recent visits to

Mandai (7.5–7.9 cm; unpublished data). The high percentage of juveniles is not surprising as we now know from the studies on population structure over the years, that February and March are months when the proportion of juveniles to adults is high (as previously described).

In both studies, adults (above 8.0 cm) and the larger juveniles (up to 7.0 cm) were more commonly found in larger streams and mud, while smaller juveniles (down to 1.0 cm) were more numerous in small pools, shallow streamlets, and on the mud surface. Hong (2004) observed no juvenile <6.0 cm carapace width was buried in the mud, but juveniles larger than this size were found buried and were thus absent from streams. She postulated that the small juveniles were not strong enough to bury themselves in mud.

At the Sungei Mandai Kechil site, deep in the mangroves, among juveniles, size was significantly different among habitats (Kruskal Wallis $p = 2.33 \times 10^{-7}$), indicating that juveniles of different sizes preferred different habitats (Leng 2013). Smaller juveniles (10–42.6 mm) tended to be in sea grass habitats, sand (10–40.4 mm) or in small streams (10–60.3 mm), while larger juveniles (12.6–76.7 mm) were found in faster flowing streams. Leng (2013) postulated that this preference was due to the higher oxygen content in streamlets and sea grass that juveniles prefer. Morton and Lee (2011) found higher numbers of *T. tridentatus* juveniles in substrates with high oxygen concentration (8–14 mg/L). Water velocity in streams, however, may wash away the small juveniles resulting in a higher proportion of larger juveniles in streams.

There was no statistically significant difference in the size of adults among habitats (Kruskal Wallis $p = 7.97 \times 10^{-1}$), indicating that adults were randomly distributed among the different habitats. The sex ratios were similar in both studies, 0.9 in KNT (Hong 2004) and 2.0 and 1.5 in the two streams at Sungei Kechil (Leng 2013), suggesting that males and females had similar habitat preferences. Sand appeared to be the least preferred habitat for adult and juvenile mangrove crabs alike.

The study by Hong (2004) was one of the earliest studies on horseshoe crabs in Singapore. In spite of the generally accepted local view that breeding occurred year round, Hong postulated a seasonality of mating that has now been demonstrated as a period of low breeding activity rather than true seasonal breeding. Her recommendation that year-round monitoring is required to determine such seasonality is ongoing by the NSS. She also noted that the numbers of amplexed pairs, albeit only seven, but with no gravid females, might also imply that January to March is not the mating season. Longer studies now suggest that the time of low breeding activity is probably from May to August. These findings highlight the value of longer and more intensive studies to follow up and corroborate smaller and shorter duration studies.

There also is a need for more studies on the length of time *C. rotundicauda* spends in amplexus and how much of this time is spent nesting and spawning in a region with no seasons. Anecdotal evidence from local people and from pairs caught in the wild but kept in captivity by the Singapore Zoo suggests that *C. rotundicauda* remains in amplexus for months rather than weeks, and much of this time does not seem to involve spawning. This pattern of behavior may be different compared with species in temperate climates, where coupling and spawning are seasonal and hap-

pen in just a few weeks in the spring, and amplexus out of season is uncommon. The assumption that adults in amplexus indicate a breeding season may not be correct if pairs remain together for extended periods. Studies using tracking devices on pairs of crabs in amplexus would be useful to determine how long they stay together.

Observations of Leng (2013) on microhabitat preference support those of Hong (2004), nearly a decade earlier in a different site but with similar microhabitats, making these studies valuable as a baseline for the population of *C. rotundicauda* in this habitat. Within the mangroves the tidal streams are not homogenous, resulting in many different microhabitats. These studies show that adults and juveniles have different preferences for microhabitats. *C. rotundicauda* selects for different environmental parameters at different stages of the life cycle. These tidal streams among the mangroves may be important for spawning and as nursery areas safe from predators such as large crabs, but spawning surveys are needed to determine when and where spawning occurs as it has rarely been seen and few egg nests have been found.

10.4.2 Habitat Degradation and Mortality

The percentage of dead to live *C. rotundicauda* found in defined areas at two different locations of the Mandai mudflat at Kranji have been compared to determine the influence pollution may have on the survivability of horseshoe crabs (Saad 2013). One location was heavily polluted and nearer the shore while the other location, 10 m further down the beach, was not so polluted. In addition to flotsam and jetsam, the mudflats are polluted with oils and road run off from the many small, contaminated rivulets and canals that drain from the town out to the mudflats. Patches of black mud with an unpleasant smell indicating the presence of sulphides can also be seen along the whole of the intertidal zone, particularly near the shoreline.

From seven site visits, the percentage of dead crabs was consistently higher in the polluted location (19.8 %) than in the less polluted location (7.5 %). This difference may be due to greater pollutant loads, which have been recorded along the Strait (Bayen et al. 2005; Cuong et al. 2005) or to entrapment of carcasses by debris at the more littered site. It would be interesting to pursue the mortality study by continuing with chemical or other tests that could suggest possible causes of the higher mortality rate in the high tide zone. Regular monitoring of organic and inorganic pollutants in the horseshoe crabs' habitat is important, particularly if further development of industry along that coast is planned. However, it is a feature of these student projects that, by their nature, there is little or no follow up or subsequent study to build on what was found.

10.4.3 Epibionts

Ong (2012b) compared the number of the common acorn barnacle, *Semibalanus balanoides*, attached to male and female mangrove horseshoe crabs in amplexus to determine if these epibionts would affect amplexus. Of 157 amplexus pairs 56.7 % of the males (89/157) and 32 % (51/157) of the females had barnacles. The higher rate of attachment was attributed to the larger surface area available for epibionts to attach while the pairs are in ecdysis, which may be several weeks. Epibionts do not seem to affect amplexus formation. In addition, the numbers of barnacles were higher on the larger crabs, but they were also more numerous on males than females of the same size. A positive correlation between horseshoe crab size and the size of the largest barnacles of *Ballanus* species had also been observed by Goh (2010) who also observed that females carried larger barnacles (mean 9.3 mm) than males (mean 8.6 mm).

A similar study 25 years ago (Jeffries et al. 1989) of the pedunculate barnacle, (*Octolasmis warwickii*), on both species of horseshoe crab collected from around Singapore, showed three times greater abundance of this species on the 18 female *T. gigas* than on the 38 males, and all were on the ventral prosoma, while none of 19 *C. rotundicauda* carried this barnacle. Twenty five years ago *T. gigas* was still to be found around the Singapore shores, the highest number (43) being at Tuas on the west coast of the main island, an area that has not been included in recent surveys, but should be considered for future work, particularly on *T. gigas*.

10.4.4 Diet Preferences

In two small studies to determine food preferences in *C. rotundicauda* collected at Mandai (Ong 2012a, b), an individual horseshoe crab was turned on its back, selected food was put onto the mouth area, and the time taken for the food to be consumed was measured. It is not normal for horseshoe crabs to feed this way so the findings need to be interpreted with caution. Red bristle worms (Polychaeta, species not given) were popular with the adults and juveniles alike, while seaweed (species not given but probably *Halophila beccarii*) and the flesh of blood cockles (*Anadara granosa*) were not consumed at all. Only the adults consumed the flesh of white clams (species not given) but took an average of 40 min to do so, possibly because of the size of the food offered (Ong 2012a).

In a follow up study (Ong 2012b) red bristled worms, blood worms (*Chironomidae tetans*), white clams and mussels (*Perna viridis*) were used. In this study 23 horseshoe crabs were offered each of these items as food, two to three times, and the numbers of crabs feeding on the offering were counted. No preferences were observed among the food sources offered (not significant at the 1 % level), and the

conclusion was that horseshoe crabs were not particularly selective feeders. Generally horseshoe crabs are considered to be omnivorous scavengers feeding on whatever they encounter, such as bivalves, worms, molluscs and even algae (Goh 2001; Ng and Sivasothi 2002).

Ong (2012a, b), however, was offering various food species to the horseshoe crabs in the laboratory to determine preferences. This approach does not identify what horseshoe crabs would actually eat in their normal habitat. By contrast, Akbar et al. (2012) conducted a study in Malaysia on feeding ecology and food preferences by examining gut contents of *C. rotundicauda*. They collected individuals from two known nesting sites on the east coast of Malaysia in Pahang. Pairs of crabs were collected every month for a year shortly after nesting, and in the laboratory gut contents were identified to group level (bivalves, gastropods, crustaceans, polychaetes and other). Macrobenthic samples were also collected to determine the availability of food sources. The feeding choices of the crabs differed according to the monsoon period, with preference changing from polychaetes to bivalves in periods between monsoons and back to polychaetes during monsoon periods. Overall, molluscs, especially gastropods, were preferred over other macrobenthos groups.

It would be useful to conduct similar studies on gut contents of horseshoe crabs from sites in Singapore. Gut contents, however, provide information only on recently consumed food and food that is hard to digest and remains in the gut. A more fruitful method of determining foraging ranges and food sources may be to compare stable isotope ratios in the horseshoe crabs with food sources in different locations as has been done with *Limulus polyphemus* in USA (Carmichael et al. 2004). Not only would such information help with the captive breeding of *C. rotundicauda* and in determining migration patterns, but it would be particularly interesting to determine if the waste food from under the fish farms in the Strait of Johor forms part of the diet of the resident crabs. The whole area along the north west of Singapore, although small, supports a very high density of horseshoe crabs, so it would be useful to know how much the fish farms contribute to this success, if at all.

10.4.5 Dietary Contaminants

The study by Ong (2012b) included one species that was also included in a study on persistent organic pollutants (POPs; Bayen et al. 2005) in marine samples collected nearby along the coast. The green mussel (*Perna viridis*) is a filter feeder at the top end of the trophic scale (3.1) among the species studied. Since horseshoe crabs feed on this species when offered, it can be assumed that horseshoe crabs may be a trophic level above green mussels, and they are likely to have similar concentrations to the higher trophic level species. The highest concentration of POPs was found in predator species in the higher trophic levels and in detritivores (e.g. rodong shell), indicating a biomagnification in the higher trophic levels (Bayer et al. 2005). Since

horseshoe crabs forage on detritus and smaller aquatic species, they may well have POP concentrations similar to those species studied.

10.5 Future Work

Although much research has been published on the biochemistry of the blood of the mangrove horseshoe crab in Singapore, little has been published on the ecology and behavior of this species and even less on the coastal horseshoe crab in Singapore. Hence, any studies, however small, may make a useful contribution to increasing knowledge of these creatures. Although the existing student studies are necessarily small and often of short duration, the findings prove useful and important because they contribute to the continuous monitoring of the horseshoe crab populations around Singapore. This work is particularly important at Kranji where there is a healthy population of mangrove horseshoe crabs. The methodology described in these studies is straightforward, so results through time and among studies can be compared with caveats being considered for experimental differences.

These studies were conducted by students of different institutions, they are not published, and the findings are often not easily available to students or researchers at other institutions, resulting in the risk of duplication. Now that the Nature Society of Singapore has become known for its work on horseshoe crabs through publications, publicity and the NSS journal, some students are sending their dissertations to the NSS horseshoe crab team for comment. This step is very helpful to allow findings to be collated, compared, and used for planning future work, which is required to build on these studies.

The NSS has an outreach program with most of the colleges, so is in a good position to encourage, suggest and guide future work to plug knowledge gaps. With so few habitats in Singapore remaining for either of the horseshoe crabs species, but in particular *T. gigas*, all research findings should be gathered and used to help the efforts of the NSS and other interest groups to preserve what is left of the natural sea shore of Singapore.

The horseshoe crab habitats along the Strait of Johor are important in their own right, but also as support for the protected area of the Sungei Buloh Wetland Reserve (SBWR), the Mandai mudflats, and Sungei Mandai Kechil streams that are vital for the survival of the Reserve. The whole stretch of coastline needs to be considered as an integrated, inter-dependent and inter-connected ecosystem, and there is much ongoing discussion from many interested groups to press for the whole area to be listed as a Ramsar site (The Convention on Wetlands of International Importance). Importantly, the work completed to-date strongly suggests that urbanization or industrial development that destroys any part of this coastline will significantly impair recovery of the remaining horseshoe crab populations and will also risk adversely affecting the SBWR, one of the major wetland systems of the country.

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