NATURE IN SINGAPORE Supplement 1: e2022126

Date of Publication: 18 November 2022 DOI: 10.26107/NIS-2022-0126 © National University of Singapore

# Preliminary efforts to conserve native horseshoe crab species in Singapore

Jasmin Lim\*, Yi Long Ng & Laura-Marie Yap

School of Applied Science, Republic Polytechnic, Singapore, Republic of Singapore; Email: <a href="mailto:jasmin\_lim@rp.edu.sg">jasmin\_lim@rp.edu.sg</a> (\*corresponding author)

**Abstract.** Although the national conservation statuses of the mangrove horseshoe crab (*Carcinoscorpius rotundicauda*) and the coastal horseshoe crab (Tachypleus gigas) are listed as "Vulnerable" and "Endangered" respectively in the Singapore Red Data book, there are currently minimal efforts in place to conserve the natural habitats of these species. Republic Polytechnic (RP) has taken a multi-pronged approach since 2015 in its horseshoe crab conservation strategies: 1) field surveys; 2) captive rearing; 3) education and outreach. Surveys were conducted across seven different sites in Singapore. The proportions of live Tachypleus gigas specimens found were low at Punggol Settlement (13%), Coney Island (28%) and Changi Beach (31%). The percentages of live Carcinoscorpius rotundicauda found at Coney Island and Pasir Ris Park Beach were also low at 8% and 15% respectively. In comparison, there are healthier populations of Carcinoscorpius rotundicauda at Selimang Beach (75%), Mandai mudflats (79%) and Sungei Buloh Wetland Reserve (86%). The data suggests that there is need for active conservation of horseshoe crabs. Ex-situ conservation of Carcinoscorpius rotundicauda was conducted at RP with successful breeding and rearing of Carcinoscorpius rotundicauda larvae from the 1st to the 7th instar stages. The elucidation of optimal conditions for breeding them in captivity can help dwindling populations through release of captive-bred Carcinoscorpius rotundicauda. RP has also raised awareness on the threats to native horseshoe crabs through exhibitions at preschools, secondary schools, institutes of higher learning and public events. With greater awareness of threats such as urbanisation, pollution and entanglement in abandoned nets, the public can better contribute to protecting the horseshoe crab habitats.

Key words. Singapore, distribution, ex-situ conservation, Tachypleus gigas, Carcinoscorpius rotundicauda

**Recommended citation.** Lim J, Ng YL & Yap L (2022) Preliminary efforts to conserve native horseshoe crab species in Singapore. Nature in Singapore, Supplement 1: e2022126. DOI: 10.26107/NIS-2022-0126

## INTRODUCTION

Horseshoe crabs are keystone species that play important ecological roles in their habitats. Their foraging behaviour releases trapped nutrients in their local environments (Jackson et al., 2005). As important predators that forage on benthic organisms, they connect the energy transfer within the coastal food web (Carmichael et al., 2004). Horseshoe crabs feed on a plethora of benthic invertebrates, including crustaceans, bivalves and gastropods and their ecological importance in estuarine and coastal communities has been reviewed by Botton (2009). They are scavengers, preying on worms and other infauna such as the invasive Charru mussels (*Mytella strigata*) (Shuster et al., 2003). Chatterji et al. (1992) had also reported that in addition to bivalves, sand, insects and plant detritus was found in the gut contents of the coastal horseshoe crab (*Tachypleus gigas*) when the number of molluscan species was low in the area. Horseshoe crabs also contribute to the food web as prey. The interrelationship between the horseshoe crab and many species of shorebirds, fish and crustaceans has been well documented, although the literature mainly focuses on the Atlantic horseshoe crab (*Limulus polyphemus*) in Delaware Bay (Warwell, 1897; Perry, 1931; deSylva et al., 1962; Price, 1962; Karpanty et al., 2006; Mizrahi & Peters, 2009). The Asian horseshoe crab eggs are a food source for birds as well, with reports of crows (*Corvus splendens*) predating on the eggs of both the mangrove horseshoe crab (*Carcinoscorpius rotundicauda*) and *Tachypleus gigas* in India (Debnath and Choudhury, 1988; Mishra, 2009). Hence, a decline in horseshoe crab populations may result in negative impacts to the ecosystems they live in.

Wild horseshoe crabs are also of economic importance. They are used commercially in biomedical science, as amoebocyte lysates from their hemolymph are used to test for the presence of bacterial contamination in drugs, blood products and pharmaceutical devices (Levin & Bang, 1968; Rao & Bhagirathi, 1989; Levin et al., 2003). In addition, both *Tachypleus* species are commonly used for chitin production after collection of the *Tachypleus* Amoebocyte Lysate (TAL), thus resulting in mortality of the individuals harvested (Gauvry, 2015). Horseshoe crabs are also used for human consumption in Asia. Their eggs are a delicacy and gravid females are harvested to be cooked (Morton & Blackmore, 2001). They are also used in traditional remedies for treating joint pains and fever (Basudev et al., 2013). When caught as by-catch in Malaysia, they are sold and exported to Thailand as food and decorative ornaments (Christianus & Saad, 2009).

Tachypleus gigas and Carcinoscorpius rotundicauda are distributed in the Indo-Pacific region from the Bay of Bengal to Indonesia. Horseshoe crabs are encountered when they come ashore as a mating pair in amplexus, as can be seen when a male crab attaches itself to the rear end of a female crab using its pedipalps. Solitary males and females can also be seen on beaches. Each female can lay 200–300 eggs and spawning activity is governed by season, sediment and water conditions (Chatterji & Abidi, 1993; Nelson et al., 2019). Information regarding the distribution, population structure, spawning activities and threats to both species have been reported in Singapore, Hong Kong, Peninsular Malaysia and India (Shin et al., 2009; Cartwright-Taylor et al., 2011; Zaleha et al., 2012; Faridah et al., 2015; Nelson et al., 2015).

Research work on *Tachypleus gigas* in Singapore is very limited, and only Cartwright-Taylor et al. (2011) have reported the capture of six live adult *Tachypleus gigas* females at Changi Point. Sightings of *Tachypleus gigas* have been rare and their distribution in Singapore is unknown. Thus, there is a need for more studies on this species (Cartwright-Taylor et al., 2011, Cartwright-Taylor, 2015). Literature on *Carcinoscorpius rotundicauda* in Singapore is also sparse, and has been mainly focused at Mandai mudflats at Kranji, as it is the only known location with a substantial breeding population. Live *Carcinoscorpius rotundicauda* have also been reported at Changi Point, Lower Seletar, Lim Chu Kang, Sarimbun North, Sarimbun South as well as Pandan Reservoir (Cartwright-Taylor et al., 2009; 2011; Cartwright-Taylor & Hsu, 2012; Cartwright-Taylor & Ng, 2012).

Overharvesting, habitat and spawning area loss as well as coastal pollution are the main threats to Asian horseshoe crabs (Chen et al., 2004; Hajeb et al., 2009). Sustained anthropogenic disturbances are affecting the breeding and nursery grounds of horseshoe crabs, so their survival is of concern and there is pressing need for increased conservation efforts (Shin et al., 2009; Nelson et al., 2016; Pati, 2017). The observations of global decline in wild populations and destruction of the natural habitats of this arthropod are also relevant in Singapore, where they have been rapidly displaced due to high demands for land reclamation and development, coupled with other factors such as pollution and entanglement in drift nets (Cartwright-Taylor et al., 2011).

Currently, both species are classified as Data Deficient in the IUCN Red List of Threatened Species (IUCN, 2021). However, according to the Singapore Red Data Book, the national conservation statuses of *Carcinoscorpius rotundicauda* and *Tachypleus gigas* are "Vulnerable" and "Endangered" respectively (Davison et al., 2008). There is also little awareness amongst Singaporeans, especially the younger generations, regarding their existence, behaviour, habitats, the roles they play in the ecosystem, as well as the urgent need for their conservation.

More extensive and collaborative efforts to conserve the native horseshoe crab species with structured measures are crucial. Republic Polytechnic (RP) is an Institute of Higher Learning (IHL) and is one of the five polytechnics in Singapore. Under guidance from their lecturers, students from the Diploma in Environmental Science and Diploma in Marine Science and Aquaculture were trained to conduct field surveys and perform captive breeding as part of the Final Year Projects and internships. Students from interest groups—Conservation Interest Group and Marine Science Interest Group—were also trained to educate the public in outreach events.

This paper documents the multi-pronged approach taken by RP since 2015 in its horseshoe crab conservation strategies:

1) field surveys of horseshoe crabs to better understand their distribution patterns and population sizes; 2) rearing mangrove horseshoe crabs in captivity to understand their life cycle in detail and; 3) through education and outreach, i.e., raising awareness about the existence of these chelicerates on our island and the current threats that they face. It is pertinent that such conservation efforts be sustained over a long term to help protect the native horseshoe crab species in Singapore.

## **MATERIAL & METHODS**

**Study sites for field surveys.** Seven sites were chosen based on literature review (Yap, 2009; Cartwright-Taylor et al., 2011) and collaborative projects with the National Parks Board (NParks). These sites were surveyed to determine the spatial distribution of coastal horseshoe crabs (*Tachypleus gigas*) and mangrove horseshoe crabs (*Carcinoscorpius rotundicauda*) around Singapore.

Table 1. Sites of the island-wide survey of coastal horseshoe crab (*Tachypleus gigas*) and mangrove horseshoe crab (*Carcinoscorpius rotundicauda*) on the northern shores of Singapore's main island and off the shore of Coney Island.

Survey Site	Substrate Type	Sampling Shore Length (m)	Sampling Period
Sungei Buloh Wetland Reserve	Mangrove	1000	November 2017 to November 2018
Punggol Settlement	Sandy beach	800	November 2017 to November 2018
Pasir Ris Park Beach	Sandy beach	3400	November 2017 to November 2018
Changi Beach	Sandy beach	3300	May 2017 to March 2019
Coney Island	Sandy beach with small mangrove area	2000	October 2015 to May 2019
Mandai mudflats at Kranji	Mangrove	100	November 2017 to November 2018
Selimang Beach	Sandy beach	500	April 2019 to December 2019

Visual Search. Prior to each survey, surveyors were briefed to walk abreast of one another in a line, at a distance of 1 m from each other, from one end of the survey area to the other, so as to cover the entire survey area. Data were collected at low tides during the sampling periods stated in Table 1. Each survey site was sampled within one day in order to minimise the chance of re-counting the same individuals. Surveys were mostly conducted at low tides of 0.8 m Chart Datum and below, although there were a few occasions in which the team performed surveys at tide levels of 1.5 m and above to explore the possibility of horseshoe crab sightings in high tides. When a horseshoe crab was sighted, information such as species, status (alive, e.g., those with epibionts; or dead, e.g., not moving, emitting a foul smell or infested with houseflies), sex, weight, carapace length and substrate type were recorded. The team also looked out for other features such as whether the female was gravid and the presence of epibionts on the carapace. Distinguishing morphological characteristics between the two species mentioned in Sekiguchi & Nakamura (1979), such as differences in shape and size of carapace, telson cross section, as well as length of marginal spines, were used to help differentiate between the specimens found in the study. The sex of the adults was determined based on the shape of the first two pairs of claws, which males use to attach to females in amplexus (Fig. 1).

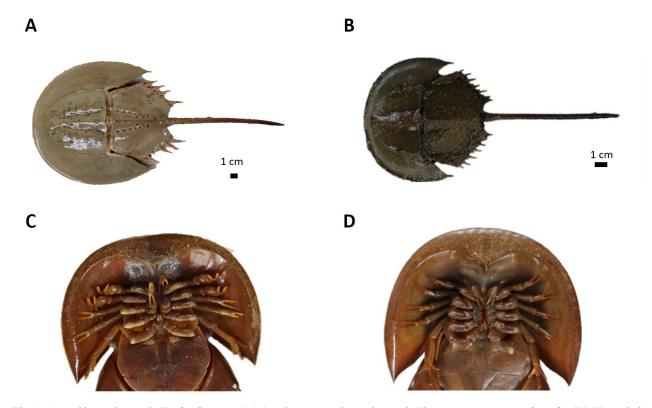


Fig. 1. Coastal horseshoe crab (*Tachypleus gigas*) (A) and mangrove horseshoe crab (*Carcinoscorpius rotundicauda*) (B). Ventral view of male *Carcinoscorpius rotundicauda* (C) and female *Carcinoscorpius rotundicauda* (D). (Photographs by: Linus Mak, Republic Polytechnic).

Rearing Carcinoscorpius rotundicauda in captivity. A horseshoe crab breeding facility was set up in RP's Aquaria, a marine and aquaculture research laboratory, to gain insights into the spawning behaviour, egg and larvae developmental processes and foraging behavior of Carcinoscorpius rotundicauda. The motivation to conserve Carcinoscorpius rotundicauda stemmed from RP's participation in the horseshoe crab research and rescue programme (HSCRR) at the Mandai mudflats at Kranji, which was organised by the Nature Society (Singapore). The plan was to establish a successful protocol to breed Carcinoscorpius rotundicauda in captivity and extend this to Tachypleus gigas later.

A water recirculating system was used for the culturing of fertilised eggs, subsequent juveniles as well as adult horseshoe crabs (Fig. 2). The recirculating system consisted of a protein skimmer, ceramic rings, ultraviolet (UV) light and filter cotton. Salinity of the water was maintained at 28 to 30 parts per thousand (ppt) in accordance with the salinity of the brackish water collected from the site. The sand and mud substrate were also collected from the site to be placed at the bottom of the tank for the horseshoe crabs to burrow in. The water was changed regularly, and the filtration pump washed weekly to prevent a build-up of ammonia. A blended mixture of prawns, clams, oysters and mussels was fed to the adults, sub-adults and juveniles once in two days. The 1<sup>st</sup> to 4<sup>th</sup> instar stages were fed rotifers, brine shrimp and mashed blood worms, while 5<sup>th</sup> to 8<sup>th</sup> instar stages were fed with bloodworms, shrimps and clams. The adult horseshoe crabs, eggs and larva were reared in the outdoor section of the Aquaria, hence the ambient temperature ranged from 28–35 °C with natural lighting.

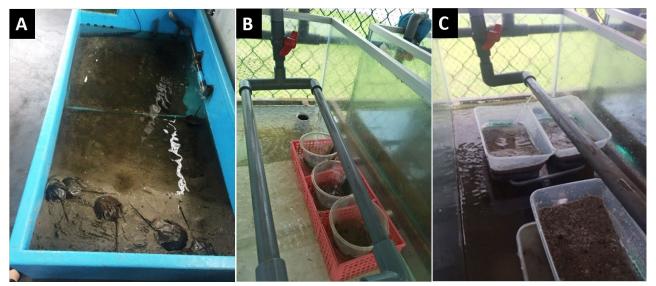


Fig. 2. The main tank for rearing the adult horseshoe crabs (A), the subsidiary tank used for rearing the eggs and 1<sup>st</sup> to 4<sup>th</sup> instar stages with its own sprinkler and heating system (B) and separate tanks for rearing 5<sup>th</sup> to 7<sup>th</sup> instar stages (C). (Photographs by: Laura Yap).

Outreach to raise awareness on the existence and threats faced by native horseshoe crabs. Outreach events were conducted to raise awareness, particularly in the younger generation (pre-schoolers, primary, secondary and polytechnic students), about the importance of conserving the natural habitats of horseshoe crabs in Singapore. Campaign collaterals that were used comprised of educational posters, brochures, posters, videos, stickers, games and craft activities. All collaterals were uniquely designed by final-year project students and interns.

## **RESULTS & DISCUSSION**

**Field surveys.** Survey results showed that there was little overlap in distribution between both species. Live horseshoe crabs of both species were only found on Coney Island (Fig. 3A). It is important to note that coastal horseshoe crabs (*Tachypleus gigas*) were found on Coney Island, with four females and six males found alive, with some individuals in amplexus during the full moon. Two breeding *Tachypleus gigas* pairs were sighted in November 2015 which led to a hypothesis that Coney Island might be a breeding site for the coastal species. Interestingly, an adult female mangrove horseshoe crab (*Carcinoscorpius rotundicauda*) and an adult male *Tachypleus gigas* were found in amplexus in October 2018. Despite no reported sightings of *Carcinoscorpius rotundicauda* previously at Coney Island (Cheo Pei Rong, pers. comm.), there is a small, inaccessible mangrove which could be a possible habitat with suitable muddy substrate for the species. This could account for the presence of the female *Carcinoscorpius rotundicauda*. There have been no previous reports of live *Carcinoscorpius rotundicauda* in Coney Island because the species does not move out to sea with the receding tide, and thus the probability of sighting live specimens is low.

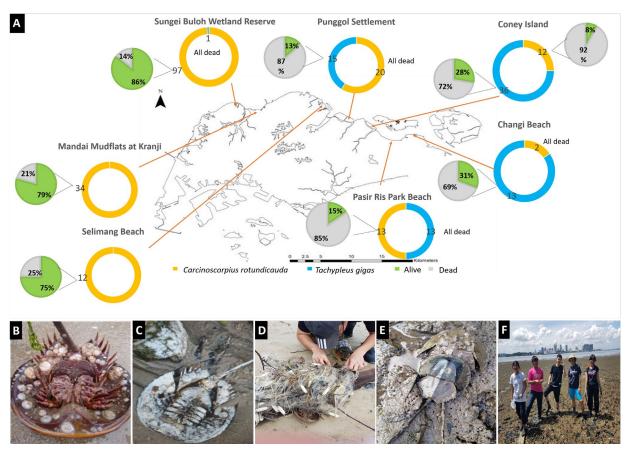


Fig. 3. Field surveys. A, Pie chart showing distribution of coastal horseshoe crabs (*Tachypleus gigas*) and mangrove horseshoe crabs (*Carcinoscorpius rotundicauda*) around Singapore; B–C, *Tachypleus gigas* in Coney Island found covered in epibionts (B) and a white chemical compound (C); D, Horseshoe crabs found entangled in a drift net at Selimang Beach; E, *Carcinoscorpius rotundicauda* in amplexus; F, Students and staff at a field survey in Mandai mudflats at Kranji. (Photographs by: Laura Yap).

There is a worrying suggestion that there may be a possible decline in the *Tachypleus gigas* population at Coney Island. Eight out of the 10 live *Tachypleus gigas* were observed in 2015, whilst the remaining two were seen in 2018, out of a total of 30 surveys made to the island. In addition, the majority of *Tachypleus gigas* were found dead (72%). Some of the *Tachypleus gigas* were found heavily infested with epibionts (Fig. 3B). Common epibionts of horseshoe crabs include bryozoans, barnacles, tube-building polychaetes and molluscs (mussels, oysters, and slipper limpets). Depending on the type of epibiont and where they are attached on the body of the horseshoe crab, epibiont infestation can cause harm to the organism, including locomotory impediment, as well as vision and respiratory impairment (Botton, 2009). Reduced mobility of the host can eventually lead to mortality (Key et al., 1996). Back in December 2015, the team also found a number of *Tachypleus gigas* covered in a white substance, which could possibly be an unknown chemical compound (Fig. 3C). No further tests/analysis were conducted on the white substance. An increase in anthropogenic activities on Coney Island after it was opened to the public in October 2015 might also have impacted the *Tachypleus gigas* numbers present.

Live *Tachypleus gigas* were also observed at Changi Beach and Punggol Settlement. Pasir Ris Park Beach, which is between both locations, had only dead *Tachypleus gigas* recorded. Both Changi Beach and Punggol Settlement also had high numbers of *Tachypleus gigas* carcasses found, at 64% and 87% respectively. The results for *Carcinoscorpius rotundicauda* were also dismal, with Punggol Settlement and Changi Beach having no live specimens, and only two out of the 13 mangrove horseshoe crabs found at Pasir Ris Park Beach were alive.

Although Fig. 3A suggests a healthy population of *Carcinoscorpius rotundicauda* at Selimang Beach, the mangrove horseshoe crabs in Selimang Beach were actually found caught in abandoned fishing nets (Fig. 3D). Hence, the data regarding the population density and health of the mangrove horseshoe crabs there needs further investigation and confirmation.

The Mandai mudflats at Kranji are not open to the public, and are under the jurisdiction of the Police Coast Guard. Hence, it is reassuring to know that the inaccessibility of Mandai mudflats at Kranji to the general public and the protected status of Sungei Buloh Wetland Reserve have provided a somewhat safe sanctuary for the horseshoe crabs to breed, resulting in relatively healthy populations of *Carcinoscorpius rotundicauda* at both sites. Amplexus pairs were also found at both locations (Fig. 3E), which could indicate that breeding activities are helping to maintain the population numbers.

Anecdotal discussions with locals from Pulau Ubin as well as fish farms along the Johor Straits revealed that the locals encounter *Tachypleus gigas* individuals as by-catch (which are usually released). However, it is an infrequent occurrence. NParks officers working in the Conservation division at Pulau Ubin have also reported sightings of both mangrove and coastal horseshoe crabs. Taken as a whole, while it is interesting and encouraging to be able to find *Tachypleus gigas* at multiple locations in Singapore, the low proportion of live horseshoe crab sightings highlights the importance of conserving the native horseshoe crabs.

The local habitats of native horseshoe crabs are clearly under threat. Between the 1920s and 1990s, the majority of Singapore's mangrove forests and sand/mudflats have disappeared (Lai et al., 2015). It has been shown that urbanisation alters shorelines by reducing the area of sandy beaches, resulting in habitat degradation. Spawning behaviours, nursery and foraging habitats will subsequently be impacted (Hopkinson & Vallino, 1995; Nelson et al., 2015; Nelson et al., 2019). Urban pollutants such as heavy metals, oils and organic compounds can also impair water quality resulting in slowed development and deformities in juvenile horseshoe crabs (Botton & Itow, 2009; Hajeb et al., 2009). There needs to be further investigation into how anthropogenic activities are affecting the horseshoe crabs in Singapore. The survey locations in this paper were noted to be exposed to intensive anthropogenic activities. The recreational beaches face issues like human disturbance and trampling, which has been shown to impact biodiversity and distribution of organisms (Huang et al., 2006). In addition, the beaches, mangroves and mudflats in Singapore face exposure to the effects of oil spills that occur along the Johor Straits (Koh, 2017; Tan et al., 2018). Such oil spill episodes are detrimental to horseshoe crabs, as they are benthic and burrow in sediments where dense crude oil precipitates and lingers. Exposure to oil directly affects their development and their diet is also based on bivalves, which are known to accumulate pollutants (Strobel & Brenowitz, 1981; Gold-Bouchot et al., 2007; Botton & Itow, 2009). Zaldívar-Rae et al. (2009) has attributed the decline of horseshoe crab populations in Mexico to oil exposure as well. Owing to Singapore's geographic location, marine trash is often washed ashore during the Southwest Monsoon season which occurs from June to September. This exposes horseshoe crab habitats to plastic pollution, which can lead to smothering, ingestion of plastic or entanglement, with potentially fatal consequences (Tan, 2020; Wong, 2020). Hence, regular monitoring of the horseshoe crab populations through field surveys (Fig. 3F) is necessary, so that there can be a better understanding of horseshoe crab ecology and how anthropogenic threats might be endangering them.

**Ex-situ conservation of horseshoe crabs at Aquaria, Republic Polytechnic.** Successful breeding and rearing of *Carcinoscorpius rotundicauda* larvae from the 1<sup>st</sup> to the 7<sup>th</sup> instar stages allowed observation of their growth over time. The opisthostoma becomes more defined and the telson (tail) becomes longer with each instar. There is an increase in body length from 1<sup>st</sup> instar (3.96 mm) to the 7<sup>th</sup> instar (64 mm) stages. The data of the larvae reared from the egg stage was consolidated with data from the wild-caught juveniles that were also reared in Aquaria. The overall developmental graph showed a large increase in body length (measured from tip of telson to tip of carapace) between the 6<sup>th</sup> and 7<sup>th</sup> instar stages (Fig. 4A). Greater increases in body weight were also observed from 6<sup>th</sup> instar stage onwards (Fig. 4B).

The success in rearing *Carcinoscorpius rotundicauda* larvae for up to 18 months in captivity up to the 7<sup>th</sup> instar stage is important as threats to wild horseshoe crab populations means that there is a growing need for improved protocols to rear and maintain horseshoe crabs in captivity. By adjusting system parameters such as water flow, salinity, dissolved oxygen concentrations, temperature of the water and diet of the juveniles and adult horseshoe crabs, they can be successfully reared and bred under controlled conditions and be used for propagation and restoration activities (Carmichael & Brush, 2012). Chen et. al (2010) reported that enhanced growth of *Tachypleus tridentatus* can potentially be attributed to high seawater temperature, sufficient living space and constant water flow. A study conducted by Lee & Morton (2005) indicated that Hong Kong horseshoe crabs take a shorter time to reach sexual maturity, as compared with conspecifics in Japan, because they can moult more frequently at higher sediment/water temperatures (~28 °C) if food is freely available.

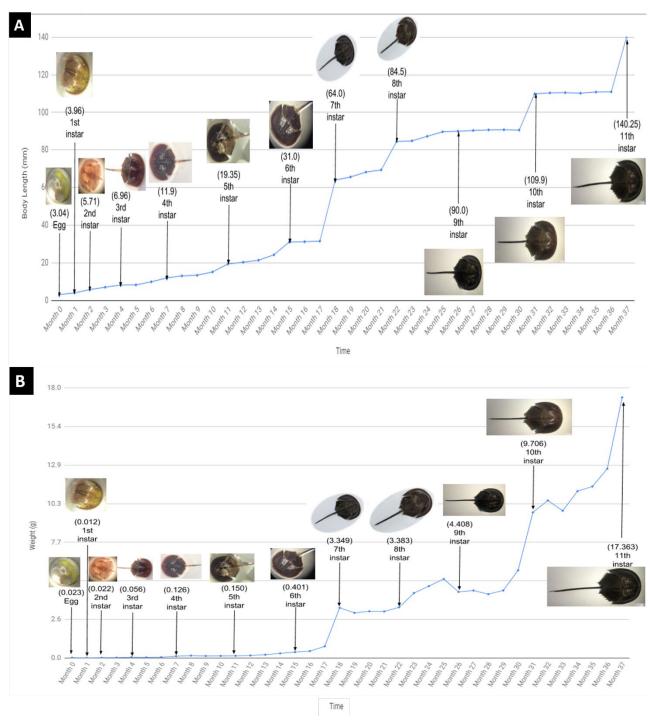


Fig. 4. Growth of *Carcinoscorpius rotundicauda* larvae, showing the developmental graphs in terms of body length (A) and weight (B) from the egg to 11<sup>th</sup> instar stage.

Outreach activities to raise awareness on the existence and threats faced by native horseshoe crabs. There were numerous activities conducted to raise awareness of native horseshoe crabs to the general public (Table 2). At the event booths, educational materials such as posters and horseshoe crab moults and carcasses were displayed so that there could be an interactive experience when visiting the booth, with RP student volunteers explaining the differences between *Tachypleus gigas* and *Carcinoscorpius rotundicauda* anatomy and habitats to visitors (Fig. 5). Posters and newspaper articles detailing the threats faced by horseshoe crabs, such as entanglement in drift nets, plastic pollution and the presence of the invasive mussel species *Mytella strigata* in their habitats were also highlighted to the public. Games such as horseshoe crab word search and tangrams were used to engage both the young and old. Collaterals such as horseshoe crab bookmarks, horseshoe crab badges and horseshoe crab fact sheets were made available so that the public could bring back mementos from these outreach events. More targeted conservation awareness efforts were conducted via educational talks arranged at preschools, secondary schools and polytechnics.

Table 2. Description of the outreach activities on native horseshoe crabs, conducted by Republic Polytechnic (RP) staff and students from 2016–2021.

Event number	Year Description of Event		Estimated number of people who visited the booth/event	
1	2016	up to raise awareness on horseshoe crab threats. Visitors participated in horseshoe		
2	2017	crab origami whilst learning more about Singapore's native horseshoe crabs. West Grove Primary School. An exhibition booth was set up that allowed the primary school students to view horseshoe crab eggs. RP students shared about horseshoe crab conservation efforts that were conducted by the school.	100	
3	2017	Festival of Biodiversity at Nex Mall. An exhibition booth was set up to share on RP's horseshoe crab conservation efforts.	150	
4	2018	RP Open House. An exhibition booth was set up to share with the public about horseshoe crab conservation efforts by RP and threats faced by native horseshoe crabs. The visitors were mostly secondary school students, so the outreach was conducted through the use of posters.	200	
5	2018	"The Fun Odyssea", International Year of the Reef 2018 Community Launch. An exhibition booth was set up to share with the public about horseshoe crab habitats. The visitors were mostly young families, so fun activities such as jigsaw puzzles and bookmarks were used to engage the young children who visited the booth.	100	
6	2018	Talk at Kranji Secondary School. A short presentation about conservation efforts of native horseshoe crabs was delivered to secondary students, with moult specimens used to help highlight their characteristics.	70	
7	2018	Asia Dive Expo. An exhibition booth was set up to raise awareness about horseshoe crab conservation. Collaterals such as crossword puzzles, posters and bookmarks were used to generate interest amongst the visitors and educate them about the characteristics and threats of horseshoe crabs.	300	
8	2018	Family Aqua Carnival at Water Venture (Sembawang). An exhibition booth was set up to educate the public of horseshoe crab conservation and project efforts.	250	
9	2018	S.E.A. Aquarium Ocean Fest event. An exhibition booth was set up within the aquarium to educate the visitors on the consequences of plastic pollution on horseshoe crab habitats and RP's efforts on conservation.	400	
10	2018	Festival of Biodiversity at Tampines Mall. An exhibition booth was set up to share on RP's horseshoe crab conservation efforts.	170	
11	2018	Lecture at RP. A short presentation about wildlife ecology & conservation of native horseshoe crabs was given to RP students.	65	
12	2019	RP Open House. An exhibition booth showcased RP's horseshoe crab project to the public, explaining about how monitoring of our local populations of horseshoe crabs was important in understanding the impact of urbanisation and human activities on them.	200	
13	2019	Kranji Secondary School. A short presentation was given to secondary school students to educate them on native horseshoe crabs, including threats to their habitats and what we can do to help save them.	50	
14	2019	Asia Dive Expo event. An exhibition booth was set up to raise awareness about horseshoe crab conservation. Collaterals such as posters, word search activity, horseshoe crab shaped badges and horseshoe crab carcasses were used to generate interest amongst the visitors and educate them about the characteristics and threats of horseshoe crabs.	200	
15	2019	Science carnival event at RP. Beneficiaries from Woodlands Social Centre, Pacific Activity Centre and Anglican Senior Centre visited the carnival booth where they played games to learn about horseshoe crab threats and conservation.	70	
16	2019	Lecture at RP. A short presentation about wildlife ecology & conservation of native horseshoe crabs was given to RP students.	40	
17	2020	RP Open House. Horseshoe crab posters were presented to the public and videos about their threats were screened.	200	
18	2021	Festival of Biodiversity at Singapore Botanic Gardens. An exhibition booth was set up to raise awareness on the threats faced by native horseshoe crabs, such as the plastic pollution of their habitats and entanglement in fishing nets. The physical copy of 'Bini the Horseshoe Crab' storybook was also displayed at the event and the visitors could scan a QR code to gain access to the digital version of the book.	100	
19	2021	Reading of 'Bini the Horseshoe Crab' storybook at Forest Discovery Centre @ OCBC Arboretum. The authors of the book (RP students) did a book reading to young families.	20	

Lim et al.: Conservation of native horseshoe crab species

Event number	Year	Description of Event	Estimated number of people who visited the booth/event
20	2021	Virtual Storytelling session @ Evangel Kindergarten. RP students did a book reading to the preschool children and educated them about threats to native horseshoe crabs.	20
21	2021	Central Library at National Library Board (Bugis). A static display booth was set up to raise awareness on the threats faced by native horseshoe crabs, such as the plastic pollution of their habitats and entanglement in fishing nets. The physical copy of 'Bini the Horseshoe Crab' storybook was also displayed at the event and the visitors could scan a QR code to gain access to the digital version of the book.	NA
		Total estimated number	2855

Two in-house videos which were produced by Republic Polytechnic students were also showcased at the outreach events (e.g., <a href="https://youtu.be/QCINIhrlmh8">https://youtu.be/QCINIhrlmh8</a>).

In partnership with NParks, a horseshoe crab story book (both physical and online versions) was written and illustrated by RP students (Adi et al., 2020; National Parks Board, 2021). This helps to have a greater reach to the public and it is useful in the Covid-pandemic situation when physical outreach opportunities are limited.

Social media platforms such as Instagram were also utilised to promote conservation of horseshoe crab by creating a dedicated Instagram page (<a href="https://instagram.com/nrf\_msrdprp\_hsc">https://instagram.com/nrf\_msrdprp\_hsc</a>).

Many pre-schoolers, primary and secondary school children were unaware of Singapore having native horseshoe crabs prior to visiting the booth or attending the lectures. Hence, the outreach events helped them to realise that interesting fauna which are being threatened by plastic pollution and improper disposal of fishing nets can be found on our "little red dot". As for the adults, a number of them had shared with RP students that while they were growing up, encounters with horseshoe crabs along beaches were not uncommon, but they had personally noted their decline in numbers over the years, and that they rarely sighted them now. Many of the participants and visitors at these events expressed willingness to contribute to the conservation of native horseshoe crabs, but were unaware of how they could play a part. The outreach programmes therefore introduced them to events such as the Horseshoe Crab Research and Rescue Programme organised by the Nature Society (Singapore) and the International Coastal Cleanup, Singapore by the National University of Singapore so that they could help conserve the horseshoe crabs. Parents of young children were also very excited to download a copy and read the horseshoe crab storybook to them.

Conclusion. Over the past eight years, RP has made efforts to conserve native horseshoe crab species in Singapore through surveys to monitor their populations, breeding and rearing *Carcinoscorpius rotundicauda* in captivity and building local awareness about the existence and threats that they face via outreach programmes to the public. Although Singapore's Wildlife Act states that wildlife, including horseshoe crabs, must not be killed, trapped, taken away or kept unless there is approval from NParks to do so, there continue to be instances of members of the public threatening their populations or harvesting them from the wild (Zheng, 2021). More needs to be done to ramp up current conservation efforts such as continued population monitoring, increased number of rescue programmes, beach clean-ups and research in captive breeding. In particular, the captive breeding programme could be extended to *Tachypleus gigas* as well. It would also be useful to ascertain if any of the sites at which *Tachypleus gigas* was sighted are breeding sites for the species. It is only through having consolidated and assiduous efforts on the ground that these native species can continue to be part of our local natural heritage.

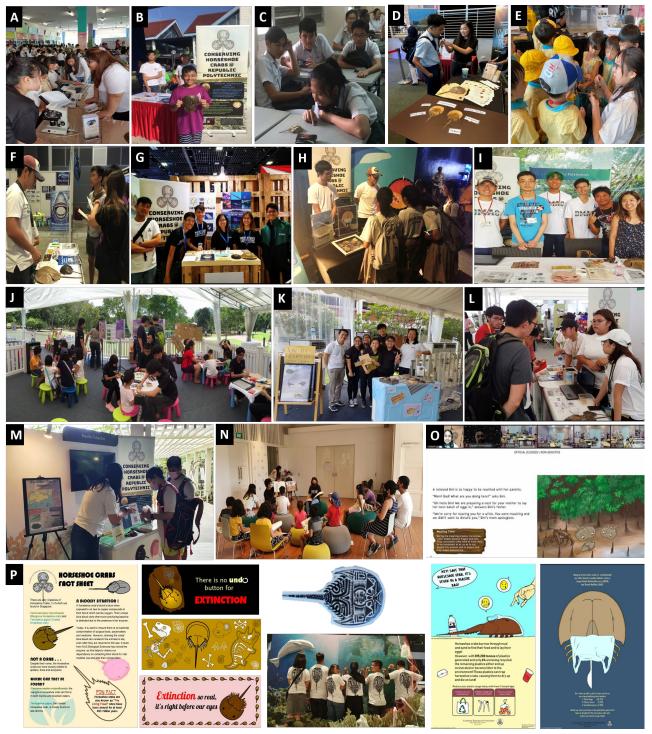


Fig. 5. Outreach activities on native horseshoe crabs, conducted by Republic Polytechnic (RP) staff and students. A, West Grove Primary School (2017), B, Family Aqua Carnival at Water Venture, Sembawang (2018); C, Kranji Secondary School (2018); D- E, Republic Polytechnic Open House (2018, 2019); F-G, Asia Dive Expo (2018, 2019); H, South East Asia Aquarium, Resorts World Sentosa (2018); I, International Year of the Reef (2018); J-M, Festival of Biodiversity (2016, 2017, 2018 and 2021); N, Reading of 'Bini the Horseshoe Crab' storybook at Forest Discovery Centre @ OCBC Arboretum (2021); O, Virtual Storytelling session @ Evangel Kindergarten (2021); P, outreach collateral used in the various events. (Photographs by: Laura Yap & Jasmin Lim).

### **ACKNOWLEDGEMENTS**

The authors would like to thank NParks for giving us permission to conduct surveys at the various sites as well as providing the booth space at various events such as Asia Dive Expo and Festival of Biodiversity. We are also grateful to the Marine Science Research and Development Programme-19 (MSRDP-19) and Wildlife Reserves Singapore Conservation Fund (WRSCF) grant for funding this work. The authors are also grateful for the assistance rendered by all past final-year project students—especially Rina Lee, Yuan Jiao, Celestyn Chee, Javier Loo and Vivien Ng for the video creation—and student interns.

# LITERATURE CITED

- Adi HZ, Ameryn DMZ & Boo BCK (2020) Adventures of Bini the Horseshoe Crab. National Parks Board, Singapore, 1–44 pp.
- Basudev T, Sivakumar K, Sajan J, Satyarajan B & Choudhury BC (2013) Assessment of the population status and threats to the horseshoe crabs along the northern east coast of India. In: Venkataraman K, Sivaperuman C & Raghunathan C (eds.) Ecology and Conservation of Tropical Marine Faunal Communities. Springer, New York, pp. 137–146.
- Botton ML & Itow T (2009) The effects of water quality on horseshoe crab embryos and larvae. In: Tanacredi JT, Botton ML & Smith DR (eds.) Biology and Conservation of Horseshoe Crabs. Springer, New York, pp. 439–454.
- Botton ML (2009) The ecological importance of horseshoe crabs in estuarine and coastal communities: a review and speculative summary. In: Tanacredi JT, Botton ML & Smith DR (eds.) Biology and Conservation of Horseshoe Crabs. Springer, New York, pp. 45–63.
- Carmichael RH & Brush E (2012) Three decades of horseshoe crab rearing: A review of conditions for captive growth and survival. Reviews in Aquaculture, 4: 32–43.
- Carmichael RH, Rutecki D, Annett B, Gaines E & Valiela I (2004) Position of horseshoe crabs in estuarine food webs: N and C stable isotopic study of foraging ranges and diet composition. Journal of Experimental Marine Biology and Ecology, 299: 231–253.
- Cartwright-Taylor L, Lee J & Chia CH (2009) Population structure and breeding pattern of the mangrove horseshoe crab *Carcinoscorpius rotundicauda* in Singapore. Aquatic Biology, 8: 61–69.
- Cartwright-Taylor L, Yap VB, Hsu CC & Lou S (2011) Distribution and abundance of horseshoe crabs *Tachypleus gigas* and *Carcinoscorpius rotundicauda* around the main island of Singapore. Aquatic Biology, 13: 127–136.
- Cartwright-Taylor L & Ng HH (2012) Tracked mangrove horseshoe crab *Carcinoscorpius rotundicauda* remain resident in a tropical estuary. Aquatic Biology, 17: 235–245.
- Cartwright-Taylor L & Hsu CC (2012) Follow-up study on population structure and breeding pattern of the mangrove horseshoe crab *Carcinoscorpius rotundicauda* in Singapore. Aquatic Biology, 14: 217–222.
- Cartwright-Taylor L (2015) Studies of horseshoe crabs around Singapore. In: Carmichael RH, Botton ML, Shin PKS & Cheung SG (eds.) Changing Global Perspectives on Horseshoe Crab Biology, Conservation and Management. Springer, Cham, pp. 193–211.
- Chatterji A & Abidi SAH (1993) The Indian horseshoe crab—a living fossil. Journal of Indian Ocean Studies, 1: 43–48. Chatterji A, Mishra JK & Parulekar AH (1992) Feeding behaviour and food selection in the horseshoe crab, *Tachypleus gigas* (Müller). Hydrobiologia, 246: 41–48.
- Chen CP, Yeh HY & Lin PF (2004) Conservation of the horseshoe crab at Kinmen, Taiwan: Strategies and practices. Biodiversity Conservation, 13: 1889–1904.
- Chen Y, Lau CW, Cheung SG, Ke CH & Shin PKS (2010) Enhanced growth of juvenile *Tachypleus tridentatus* (Chelicerata: Xiphosura) in the laboratory: a step towards population restocking for conservation of the species. Aquatic Biology, 11: 37–46.
- Christianus A & Saad CR (2009) Traditional uses of horseshoe crabs in Malaysia and Thailand. In: Tanacredi JT, Botton ML & Smith DR (eds.) Biology and Conservation of Horseshoe Crabs. Springer, New York, pp. 616-617.
- Davison GWH, Ng PKL & Ho HC (2008) The Singapore Red Data Book: Threatened Plants and Animals of Singapore. Second Edition. The Nature Society (Singapore), Singapore.
- Debnath R & Choudhury A (1988) Predation of Indian horseshoe crab *Tachypleus gigas* by *Corvus splendens*. Tropical Ecology, 29: 86–89.
- deSylva DP, Kalber FA & Shuster CN (1962) Fishes and ecological conditions in the shore zone of the Delaware River Estuary, with notes on other species collected in deeper water. University of Delaware Marine Laboratories, Information Series Publication, 5: 1–170
- Faridah M, Ismail N, Ahmad BA & Manca A (2015) The population size and movement of coastal horseshoe crab, *Tachypleus gigas* (Muller) on the east coast of Peninsular Malaysia. In: Carmichael RH, Botton ML, Shin PKS & Cheung SG (eds.) Changing Global Perspectives on Horseshoe Crab Biology, Conservation and Management. Springer, Cham, pp. 213–228.
- Gauvry G (2015) Current horseshoe crab harvesting practices cannot support global demand for TAL/LAL: The pharmaceutical and medical device industries' role in the sustainability of horseshoe crabs. In: Carmichael RH, Botton ML, Shin PKS & Cheung SG (eds.) Changing Global Perspectives on Horseshoe Crab Biology, Conservation and Management. Springer, Cham, pp. 475–482.
- Gold-Bouchot G, Zapata-Pérez O, Ceja-Moreno V, Rodriguez-Fuentes G, Sima-Alvarez R, Aguirre-Macedo M, Vidal-Martinez V, Luisa Ros & Nasci C (2007) Biological effects of environmental pollutants in American Oyster, *Crassostrea virginica*: a field study in Laguna de Terminos, Mexico. International Journal of Environment and Health, 1: 171–184.
- Hajeb P, Christianus A, Ismail AZ, Shakiba S & Saad CR (2009) Heavy metal concentration in horseshoe crab (*Carcinoscorpius rotundicauda* and *Tachypleus gigas*) eggs from Malaysian coastline, In: Tanacredi JT, Botton ML & Smith DR (eds.) Biology and Conservation of Horseshoe Crabs. Springer, New York, pp. 455–464.

- Hopkinson & Vallino (1995) The relationships among man's activities in watershed and estuaries; A model of runoff effects on patterns of estuarine community metabolism. Estuaries, 18: 598–621.
- Huang D, Todd A, Chou LM, Ang KH, Boon PY, Cheng L & Ling H (2006). Effects of shore height and visitor pressure on the diversity and distribution of four intertidal taxa at Labrador Beach, Singapore. The Raffles Bulletin of Zoology, 54: 477–484.
- IUCN (2021) The IUCN Red List of Threatened Species. Version 2021-3. <a href="https://www.iucnredlist.org/species">https://www.iucnredlist.org/species</a>. Accessed on 29 June 2022.
- Jackson NL, Nordstrom KF & Smith DR (2005) Influence of waves and horseshoe crab spawning on beach morphology and sediment grain-size characteristics on a sandy estuarine beach. Sedimentology, 52: 1097–1108.
- Karpanty SM, Fraser JD, Berkson J, Niles LJ, Dey A & Smith EP (2006) Horseshoe crab eggs determine red knot distribution in Delaware Bay. Journal of Wildlife Management, 70: 1704–1710.
- Key Jr, MM, Jeffries WB, Voris HK & Yang CM (1996) Epizoic bryozoans, horseshoe crabs, and other mobile benthic substrates. Bulletin of Marine Science, 58: 368–384.
- Koh V (2017) Oil spill in Singapore waters after two vessels collide at sea. Today Online, 4 Jan 2017. <a href="https://www.todayonline.com/singapore/spore-registered-container-vessel-involved-collision-johor-coast-oil-spill-spotted-spore">https://www.todayonline.com/singapore/spore-registered-container-vessel-involved-collision-johor-coast-oil-spill-spotted-spore">https://www.todayonline.com/singapore/spore-registered-container-vessel-involved-collision-johor-coast-oil-spill-spotted-spore">https://www.todayonline.com/singapore/spore-registered-container-vessel-involved-collision-johor-coast-oil-spill-spotted-spore">https://www.todayonline.com/singapore/spore-registered-container-vessel-involved-collision-johor-coast-oil-spill-spotted-spore">https://www.todayonline.com/singapore/spore-registered-container-vessel-involved-collision-johor-coast-oil-spill-spotted-spore">https://www.todayonline.com/singapore/spore-registered-container-vessel-involved-collision-johor-coast-oil-spill-spotted-spore">https://www.todayonline.com/singapore/spore-registered-container-vessel-involved-collision-johor-coast-oil-spill-spotted-spore">https://www.todayonline.com/singapore/spore-registered-container-vessel-involved-collision-johor-coast-oil-spill-spotted-spore">https://www.todayonline.com/spill-spotted-spore</a>
- Lai S, Lok LHL, Hilton M, Bouma TJ & Todd P (2015) The effects of urbanisation on coastal habitats and the potential for ecological engineering: A Singapore case study. Ocean & Coastal Management, 103: 78–85.
- Lee CN & Morton B (2005) Experimentally derived estimates of growth by juvenile *Tachypleus tridentatus* and *Carcinoscorpius rotundicauda* (Xiphosura) from nursery beaches in Hong Kong. Journal of Experimental Marine Biology and Ecology, 318: 39–49.
- Levin J & Bang FB (1968) Clottable protein in Limulus; its localization and kinetics of its coagulation by endotoxin. Thrombosis et Diathesis Haemorrhagica, 19: 186–197.
- Levin J, Hochstein HD & Novitsky TJ (2003) Clotting cells and *Limulus* amebocyte lysate: An amazing analytical tool. In: Shuster CN, Barlow RB & Brockmann HJ (eds.) The American Horseshoe Crab. Harvard University Press, Cambridge, pp. 310–340.
- Mishra JK (2009) Horseshoe crabs, their eco-biological status along the Northeast Coast of India and the necessity for ecological conservation. In: Tanacredi JT, Botton ML & Smith DR (eds.) Biology and Conservation of Horseshoe Crabs. Springer, New York, pp. 89–96.
- Mizrahi DS & Peters KA (2009) Relationships between sandpipers and horseshoe crab in Delaware Bay: A synthesis. In: Tanacredi JT, Botton ML & Smith DR (eds.) Biology and Conservation of Horseshoe Crabs. Springer, New York, pp. 65–87.
- Morton B & Blackmore G (2001) South China Sea. Marine Pollution Bulletin, 42: 1236–1263.
- National Parks Board (2021) Adventures of Bini the Horseshoe Crab. Last updated 23 June 2021. <a href="https://www.nparks.gov.sg/learning/teaching-and-learning-resources/recommended-books/adventures-of-bini-the-horseshoe-crab">https://www.nparks.gov.sg/learning/teaching-and-learning-resources/recommended-books/adventures-of-bini-the-horseshoe-crab">https://www.nparks.gov.sg/learning/teaching-and-learning-resources/recommended-books/adventures-of-bini-the-horseshoe-crab</a> (Accessed 4 April 2022).
- Nelson BR, Satyanarayana B, Moh JHZ, Shaharom F, Sukumaran M & Chatterji A (2015) Episodic human activities and seasonal impacts on the *Tachypleus gigas* (Müller, 1785) population at Tanjung Selangor in Peninsular Malaysia. Estuarine, Coastal and Shelf Science, 164: 313–323.
- Nelson BR, Satyanarayana B, Moh, JHZ, Ikhwanuddin M, Chatterji A & Shaharom, F. (2016). The final spawning ground of *Tachypleus gigas* (Müller, 1785) on the east Peninsular Malaysia is at risk: a call for action. Peer J, 4: e2232.
- Nelson BR, Moh JHZ, Zauki NAM, Satyanarayana B & Chowdhury AHJK (2019) Effects of shore sedimentation to *Tachypleus gigas* (Müller, 1785) spawning activity from Malaysian waters. Journal of Sustainability Science and Management, 14: 41–60.
- Pati, S (2017) Cleaning the breeding beaches and awareness event for horseshoe crab conservation along Chandipur coast and nearby estuaries in Odisha. Zoo's Print, 32: 46–48.
- Perry LM (1931) Catfish feeding on the eggs of the horseshoe crab, Limulus polyphemus. Science, 74: 312.
- Price K (1962) Biology of the sand shrimp, *Crangon septemspinosa*, in the shore zone of the Delaware Bay region. Chesapeake Science, 3: 244–255.
- Rao DV & Bhagirathi B (1989) *Carcinoscorpius rotundicauda* amoebocyte lysate for detection of endotoxins-its preparation, stability, sensitivity and comparison with *Limulus* amoebocyte lysate. Indian Journal of Experimental Biology, 27: 26–31.
- Sekiguchi K & Nakamura K (1979) Ecology of the extant horseshoe crabs. In: Cohen E (ed.) Biomedical Applications of the Horseshoe Crab (Limulidae). Alan R. Liss, New York, pp. 37–45.
- Shin PKS, Li HY & Cheung SG (2009) Horseshoe crabs in Hong Kong: current population status and human exploitation. In Tanacredi JT, Botton ML & Smith DR (eds.) Biology and Conservation of Horseshoe Crabs, Springer, New York, pp. 347–360.
- Shuster CN, Barlow RB & Brockmann HJ (2003) The American Horseshoe Crab. Harvard University Press, Cambridge, pp. 133–151.
- Strobel CJ & Brenowitz AH (1981) Effects of Bunker C oil on juvenile horseshoe crabs *Limulus polyphemus*. Estuaries, 4: 157–159.

- Tan A, Lim Y & Chew HM (2018) Oil spill in Johor reaches mainland Singapore shores; AVA issues suspension of sales to 3 fish farms. The Straits Times, 9 Oct 2018. <a href="https://www.straitstimes.com/singapore/environment/oil-spill-in-johor-reaches-mainland-singapores-shores">https://www.straitstimes.com/singapore/environment/oil-spill-in-johor-reaches-mainland-singapores-shores</a> (Accessed 19 March 2022).
- Tan A (2020) East Coast Park beaches lined with piles of trash brought in by Southwest monsoon, Mothership, 30 July 2020. <a href="https://mothership.sg/2020/07/east-coast-park-beach-trash/">https://mothership.sg/2020/07/east-coast-park-beach-trash/</a> (Accessed 19 March 2022).
- Warwell, HC (1897) Eels feeding on the eggs of *Limulus*. The American Naturalist, 31: 347–348.
- Wong Y (2020) Conservationists concerned by PPE showing up at beaches, nature trails. The New Paper, 27 Jul 2020. <a href="https://tnp.straitstimes.com/news/singapore/conservationists-concerned-ppe-showing-beaches-nature-trails">https://tnp.straitstimes.com/news/singapore/conservationists-concerned-ppe-showing-beaches-nature-trails</a> (Accessed 19 March 2022).
- Yap V (2009) Results of the HSC population and distribution survey & quadrat sampling at Mandai. Nature News, May–June 2009: 7.
- Zaldívar-Rae J, Sapién-Silva RE, Rosales-Raya M & Brockmann HJ (2009) American horseshoe crabs, *Limulus polyphemus*, in Mexico: open possibilities. In: Tanacredi JT, Botton ML & Smith DR (eds.) Biology and Conservation of Horseshoe Crabs. Springer, New York, pp. 97–113.
- Zaleha K, John BA, Atika HE, Kamaruzzaman BY & Jalal KCA (2012) Spawning and nesting behaviour of *Tachypleus gigas* along the East Coast of Peninsular Malaysia. International Journal of Biology, 4: 102–111.
- Zheng Z (2021) Group at Sembawang Park seen catching endangered horseshoe crab & other sea creatures. Mothership, 28 June 2021. <a href="https://mothership.sg/2021/06/group-catches-horseshoe-crab-sembawang/">https://mothership.sg/2021/06/group-catches-horseshoe-crab-sembawang/</a> (Accessed 19 March 2022).