



A review on fisheries and conservation status of Asian horseshoe crabs

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Received: 12 October 2017 / Revised: 18 August 2018 / Accepted: 24 September 2018 /
Published online: 29 September 2018
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Abstract

Horseshoe crabs are the only extant xiphosurans and are believed to be morphologically unchanged for more than 200 million years. Of the four extant species namely, *Limulus polyphemus*, *Tachypleus tridentatus*, *Tapinauchenius gigas* and *Carcinoscorpius rotundicauda*, the latter three are found in Asian waters. Recent evidences showed that Asian horseshoe crabs are facing serious threats such as degradation of their spawning grounds and habitat, environmental pollution, overexploitation as a culinary delicacy and biomedical bleeding practices. Baseline data on the distribution and existing population of the wild horseshoe crabs remain poorly known in several Asian regions. Several studies have clearly revealed that pressure due to over-fishing of wild stock has increased tremendously in the last decade. Due to an increase in demand for *Tachypleus* Amebocyte Lysate (TAL) analogous to *Limulus* Amebocyte Lysate (LAL) in the United States, there is an urgent need to comprehensively address their fishing and conservation measures in the Asian region. This review addresses the overall studies on three species of Asian horseshoe crabs in relation to their fishing practices, local exploitation of their wild stock either for human consumption (or) by biomedical industries. The authors have structured the discussion on an international scale to address the existing problems in fishing and conservation of horseshoe crabs. Since no specific regulatory force or legislative protection act or a policy to preserve their natural stock are available to this date, this paper strongly recommends representative countries to include horseshoe crabs under their wildlife protection act to avoid further unsustainable exploitation of their wild populations.

Communicated by Angus Jackson.

This article belongs to the Topical Collection: Coastal and marine biodiversity.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10531-018-1633-8>) contains supplementary material, which is available to authorized users.

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Keywords Horseshoe crabs · Habitat degradation · Distribution · Threats · Living fossil

Introduction

Horseshoe crabs are fascinating creatures that have been attracting the attention of conservation scientists, biotechnologists and pharmaceutical industries towards the conservation of their dwindling population size and sustainable utilization of their blood for human welfare. These ancient creatures have changed little over 200 million years and the evolutionary lineage dates back at least ~510 million years (Rota-Stabelli et al. 2013; Souji 2015). Recent fossil evidences revealed that the morphological similarities of extant *Limulus* sp. with *L. darwini* from the upper Jurassic period which is regarded as the oldest known representative of *Limulus* (Kin and Błażejowski 2014). Genetically, horseshoe crabs are the only sexually reproducing (external fertilization) chelicerate arthropods experiencing Whole Genome Duplication (WGD) (Kenny et al. 2016). The WGD could be used for the comparison of genome evolution with other animals, especially vertebrates (Kenny et al. 2016). The four known extant species of horseshoe crabs are *Limulus polyphemus*, *Tachypleus tridentatus*, *Tapinauchenius gigas* and *Carcinoscorpius rotundicauda*. The latter three are found in South East Asian waters from India to Japan including East Indies and the Philippines. The distribution of *L. polyphemus* is restricted to the North American Atlantic coast from Florida to Maine and some along the Gulf States, including Alabama, USA while another genetically distinct population is on the Yucatán Peninsula, Mexico (Sekiguchi and Shuster 2009). Unlike the US, the implementation of horseshoe crab fishery management and action plan for the sustainable utilization of Asian species is challenging due to complexity in environmental priorities designed by host countries besides the transboundary and cross border distribution of the three species. In this context, the Ecological Research & Development Group (ERDG) and IUCN's horseshoe crabs Species Survival Commission (IUCN SSC) are actively involved in conservation of the world's four extant horseshoe crab species. The approval of the SSC committee by IUCN in June 2012 paved a way for greater collaborations among the South East Asian researchers and also with their American counterparts. These collaborations have triggered a tremendous increase in research reports on all four species of horseshoe crabs (Tanacredi et al. 2009; Carmichael et al. 2015). However, the pressure on harvesting wild horseshoe crabs from the Asian region has increased in recent years due to high market demand for *Tachypleus* Amebocyte Lysate (TAL) production by biomedical industries and for human consumption (Manca et al. 2017). In addition, severe alteration of natural nesting habitat by various anthropogenic activities continues unabated (Akbar John et al. 2011a, b). Factors like developments in coastal areas resulted in loss of suitable nesting habitat of horseshoe crabs particularly in South East Asia (Akbar John et al. 2011a, 2012; Nelson et al. 2016a; Lee and Morton 2016). In the Asian countries, there are no specific regulations or management plans for harvesting wild horseshoe crabs for either biomedical or for human consumption. The horseshoe crab was moved from Endangered to Critically Endangered in the Regional Red List of Japan, but despite being special status as a natural monument, no particular protection is provided for this species in Japan.

An assessment of the population size of Asian horseshoe crabs is highly challenging due to the lack of baseline data and the absence of any standard procedure for sampling and assessment techniques. Consequently, the IUCN Red List assessment on three Asian horseshoe crabs listed all three species under Data Deficient. The recent

evidence from IUCN SSC and the research by Asian researchers showed that horseshoe crabs inhabiting Asian waters are facing serious threats such as habitat degradation, shrinking of spawning and nesting grounds and overexploitation of wild population that eventually leads to population decline (Tanacredi et al. 2009; Carmichael et al. 2015). It should be noted that unlike their American counterpart (*Limulus polyphemus*), Asian horseshoe crabs are not used as fertilizer or as bait in eel and conch fishery (Faridah et al. 2015). However, biomedical harvesting of *T. tridentatus* from China causes 100% mortality due to the complete extraction of its blood until death (Gauvry 2015) and the same is true for *T. gigas* from Vietnam transported to China. There is very large demand for horseshoe crabs in China and *T. gigas* exported from Vietnam to China are used first for biomedical purposes, then meat is extracted for food and finally the empty carcasses are sun-dried and sold for chitin (Tom Novitsky pers.comm). Currently, there is not a single specific fishery regulation on export of Asian horseshoe crabs. Other reports have revealed that the Asian crabs were exported to the Atlantic coast for bait fishery (Botton et al. 2015). However, due to a strong fishery enforcement law enacted by the Atlantic State Marine Fishery Commission (ASMFC) and also due to the active role of IUCN SSC efforts in identifying illegal transport of Asian horseshoe crabs to the Atlantic coast, the crabs were not used as bait in USA (Berkson et al. 2009).

In this review, the authors comprehensively address the existing fishery and conservation status of Asian horseshoe crabs in each representative country or region. The distribution map (Fig. 1), wild population size and possible nesting grounds of *T. tridentatus*, *T. gigas* and *C. rotundicauda* in Asian waters are presented (supplementary file 1). Priority was given to address the existing government policies, legislations and Wildlife Protection Acts (WPA) in South East Asian Countries to conserve the three species of Asian horseshoe crabs. The stakes of international consortia on the conservation of horseshoe crabs such as ERDG and IUCN SSC and their future directions in preserving Asian horseshoe crabs are also discussed.

Materials and method

Information retrieval

Data retrieval and meta-analysis were done with the adoption of detailed searches in electronic databases such as SCOPUS, PubMed, ProQuest, ERDG (exclusive website on horseshoe crab) and also in various University Library databases. Following key words in combination were used to screen published resources from different countries: horseshoe crab, Xiphosuran, habitat degradation, population structure, species conservation, *Tachypleus gigas*, *Tachypleus tridentatus* and *Carcinoscorpius rotundicauda*. In addition, all search items were screened together with individual Asian countries where horseshoe crabs are recorded (Malaysia, Indonesia, Thailand, Singapore, Vietnam, Japan, China, Taiwan, Hong Kong, India, Bangladesh, Cambodia and the Philippines). A manual search from the retrieved data was concluded by removing duplicate references and further screened by title and abstracts. No time limits were set during the search and all articles published up until the end of April 2018 were considered.

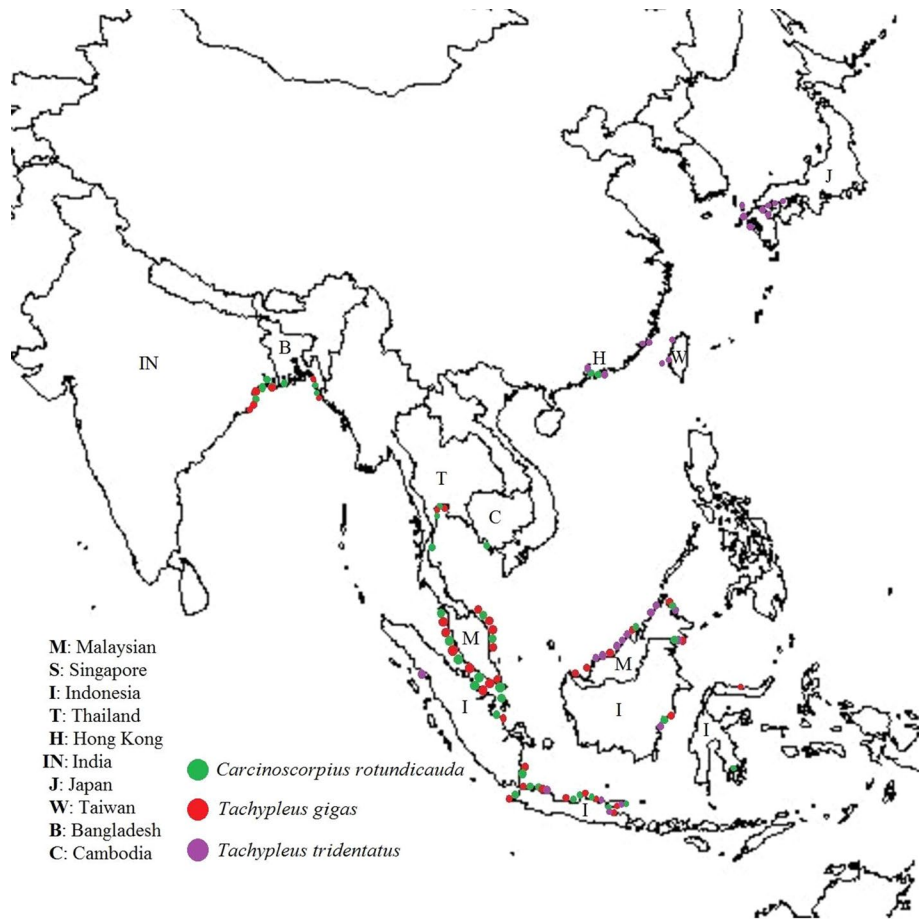


Fig. 1 Distribution and geographic extent map of Asian horseshoe crabs (*Tachypleus tridentatus*, *T. gigas* and *Carcinoscorpius rotundicauda*)

Inclusion and exclusion criteria

Full text articles were considered for further review. In some cases, data collected from university library databases consisting of extended abstract [in case of conference papers], or executive summary [in case of thesis and dissertations] were also considered based on the subject interest. Inclusion criteria includes, (i) approved sampling design, (ii) abundance of horseshoe crab in sampling site, (iii) habitat condition, (iv) outcome interest on species conservation, (v) studies involving genetic population subdivision and (vi) exposure to natural and anthropogenic stress. Exclusion criteria include (i) studies without comprehensive detail on sample collection method and (ii) studies exploring pharmaceutical potential of ameobocytes. When multiple publications from same sampling site were available, the most recent report was given priority (Fig. 2).

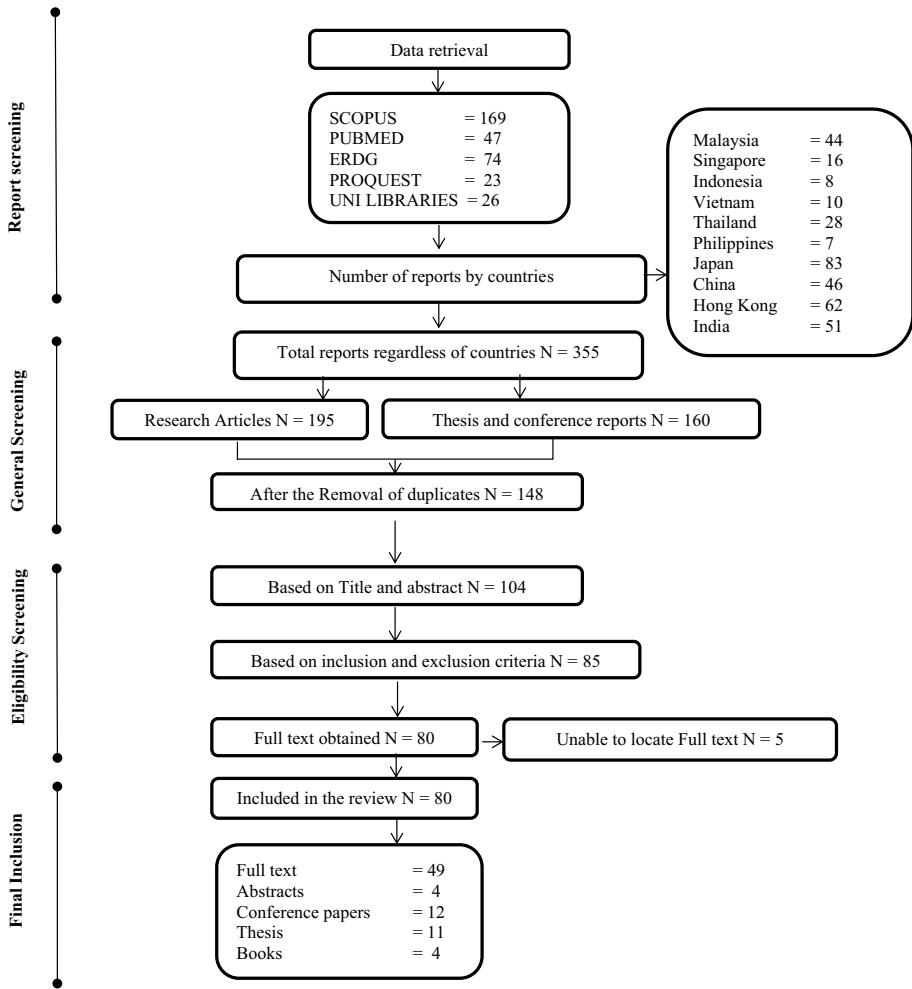


Fig. 2 The flow diagram illustrates article screening and selection criteria for the published/unpublished articles to be included in current review and meta-analysis

Malaysia

Malaysian coastal water harbors three species of horseshoe crabs namely, *Carcinoscorpius rotundicauda*, *Tachypleus gigas*, and *T. tridentatus*. The distribution of *T. tridentatus* is restricted to East Malaysia including Sabah and Sarawak waters. Nesting of these species during peak lunar cycles is well documented in the Malay Peninsula and East Malaysia (Zaleha et al. 2012; Akbar John et al. 2013; Robert et al. 2014; Nelson et al. 2015; Jawahir et al. 2017). Molecular studies indicate that the restricted distribution of *T. tridentatus* is due to continental drift that separated the Malay Peninsula from East Malaysia during the late Triassic period over 200 million years ago (Rozihan and Ismail 2012; Rozihan et al. 2013). Research in Malaysia has addressed the population dynamics and genetic subdivisions of these three species in the Peninsula (Rozihan et al. 2013; Liew et al. 2015) and East Malaysian coasts (Robert et al. 2014). Detailed research on their

biomedical potentials, pharmaceutical significance and impact on the wild population size was addressed by Akbar John et al. (2011a, b). However, studies on their wild population density, abundance and migratory ability between geographically nearest populations are limited (Chatterji et al. 2008; Adibah et al. 2015). Only a few studies have reported the total number of *T. gigas* on the eastern Malay Peninsula, in Cherating, Balok and Setu mangrove swamps (Faridah et al. 2015) and in East Malaysia (Robert et al. 2014). A recent report on the population size of *T. tridentatus* in East Malaysia (Tawau, southeast region of Sabah) based on a tagging study revealed an adult population size of $n = 1095$ (Manca et al. 2017).

Horseshoe crabs are considered as fishery commodity in Malaysia and there are no specific regulations implemented to preserve natural stocks. Almost all the studies on their wild population have concluded that nesting grounds are shrinking due to various anthropogenic activities (Akbar John et al. 2013). This has eventually led to a decrease in number of adult crabs reaching the nesting grounds during peak mating seasons (Nelson et al. 2016b). On the other hand, sex specific catch (especially of gravid females) has increased tremendously in the Peninsula. These have been exported for human consumption since 2012, due to the increasing choice of horseshoe crab as a delicacy. Adult and gravid female crabs were handpicked or caught using gill nets deployed parallel to the nesting grounds (Nelson et al. 2016a). More than 50 fishermen are actively involving in fishing *T. gigas* on the Peninsula especially on the east (Pahang and Johor States) and west (Melacca Strait) coasts. The crabs are then exported from Tanjung Sedili (Johor) Hub point to Thailand for human consumption as it creates lucrative business profit for the fishermen. The crabs are procured at a cost of 1.1USD/crab and are then sold at 5.6USD/crab on export. Recent reports have shown that *T. gigas* collected from the west coast of Peninsula (Malacca) are being transported to Japan via Thailand for biomedical bleeding practices (Christianus and Saad 2009).

Currently, there is no strict enforcement either by Department of Fisheries (DoF), Malaysia, the commerce ministry or by other stakeholders in the regulation and prevention of export of horseshoe crabs to other countries (Kamaruzzaman et al. 2012). Despite a number of reports showing that habitat degradation and shrinking of nesting grounds, overexploitation of horseshoe crabs for local consumption and export purposes continues unabated. No specific licenses for harvesting horseshoe crabs are available from the coastal region due to the fact that they are gazetted as a fishery commodity in Malaysia. A strong wildlife protection act and its enforcement is in place in Borneo (Sabah and Sarawak), so *T. tridentatus* is not exploited for foreign export. Any revision of the Wildlife Conservation Act in Malaysia should consider including all three species of xiphosurans (*Tachypleus gigas*, *T. tridentatus* and *Carcinoscorpius rotundicauda*) under the protected insect category to abate illegal export of these species, as they be important raw materials for future Malaysian pharmaceuticals and to maintain healthy ecological conditions in Malaysian coastal waters.

Singapore

Field explorations to assess horseshoe crab populations

Singapore first documented its horseshoe crab research in 1929 (Smedley 1929). The horseshoe crab specimens obtained between 1988 and 1994 from Kallang Basin and Mandai

mangroves are archived by Raffles Museum of Biodiversity Research (Fiona 2004). The National University of Singapore mapped mangrove topography at Kranji and Mandai to determine distribution patterns and sub-habitat characterization (Fiona 2004). The map was used to identify adult *C. rotundicauda* distribution areas favored by juvenile horseshoe crabs and the extent of their distribution. This mapping helped many researchers to establish and identify the presence of horseshoe crabs in areas throughout Singapore. However, recent reports have led to proper identification of areas where horseshoe crabs are commonly sighted. Species-wise, *C. rotundicauda* emerges to spawn at Changi Beach, Pasir Ris, Seletar and the mangrove areas Kranji estuary, Mandai, Lim Chu Kang, Serimbun and Pandan (Cartwright-Taylor et al. 2011). *T. gigas* seeks wave protected areas for their routine spawning such as Changi Beach, Chek Jawa, East Coast Park and the islets Sekudu, Semakau, Ubin and St. John (Tuan. 2011). Of the various forms of field assessments, the Nature Society of Singapore decided to record horseshoe crab spawning emergence using four longitudinal belt transect (5×50 m). In the first assessment, a total of 500 *C. rotundicauda* gathered in the Mandai mangrove area. Interestingly, the results of October 2007 assessment appeared similar with the following assessment in March 2008, which also recorded 500 crabs (Cartwright-Taylor et al. 2011). The longest field assessment spanned 15 months, (March 2007 and July 2008) at Kranji estuary and counted 1298 mature *C. rotundicauda* (Cartwright-Taylor et al. 2009). This survey highlighted that Kranji estuary is an active nursery ground for this species in Singapore.

However, when the same survey was repeated by the Nature Society of Singapore in March 2009, only 15 horseshoe crabs were gathered from Mandai mangrove area (Cartwright-Taylor et al. 2009). Due to poor yield of spawning adult *C. rotundicauda*, an island-wise survey was organized covering Sarimbun, Lim Chu Kang, Mandai, Seletar, Sembawang, Pasir Ris and Changi Beach. A total of 532 horseshoe crabs were retrieved from all sites. Lim Chu Kang and Changi beach were the only areas that showed coarse grain sand and this allowed *T. gigas* and *C. rotundicauda* to co-exist (Cartwright-Taylor. 2015). Follow-up surveys between October 2008 and October 2011 were done by the Nature Society of Singapore to fully understand the biology of horseshoe crabs. Throughout the field visits at Mandai mangrove area, a total of 3016 juveniles and 3556 adults (1888 male and 1688 female) *C. rotundicauda* were recorded (Cartwright-Taylor et al. 2012). Large inter-annual variability in numbers of adult horseshoe crabs was apparent, with numbers decreasing severely in 2009 and gradually recovering in 2010 and 2011 (Cartwright-Taylor and Ng 2012).

Alternative approach and recent explorations of horseshoe crab assessments

Acoustic telemetry was used to track the movements of adult *C. rotundicauda* at Mandai mangrove area between December 2010 and June 2011. During this 7-month period of observations, horseshoe crabs were discovered to linger at the site of release, and these arthropods did not display homing instincts (Cartwright-Taylor and Ng 2012). Apart from population assessments, initiatives to identify the intertidal distribution of horseshoe crabs were carried out by Republic Polytechnic, Singapore (Lee 2012; Lim 2013). *C. rotundicauda* exhibit spatial and temporal distributions, where the adults usually occur from shore to low-tide zone while the juveniles are usually found on the upper shores, 1–23 m from the high-tide line (intertidal distance is > 250 m at low tide) (Ong 2012). Estimates of horseshoe crab populations prompted research about rates of growth and ecdysis juveniles in the laboratory. This led to an observation that *C. rotundicauda* exhibit incremental growth

of 35.4% annually (Cartwright-Taylor 2015). A survey at small tidal streams of Mandai River led to the discovery of many juvenile (density: 0.068–0.99 per m²) *C. rotundicauda*, which suggests that the river streams are nursery grounds (Leng 2013). During the months of March–May and September–December, more adult *C. rotundicauda* (51.3–89.2%) were found on the shores compared to the juveniles (10.8–49.7%) which may indicate annual spawning seasons (Lee 2012; Ong 2012; Lim 2013; National Parks 2015). To establish whether populations of horseshoe crab (*C. rotundicauda* and *T. gigas*) were dwindling (after a large-scale survey in 2012 which retrieved only 532 crabs), National Parks carried out an island-wide survey between May and December 2013 (Cartwright-Taylor 2015). The presence of *C. rotundicauda* was much less than in previous years and were recorded in six out of the 18 sites surveyed. *Tachypleus gigas* was also discovered but only in Changi Beach. The absence of horseshoe crabs at Pandan mangrove means that they are now restricted to the northern sector of Singapore’s main island (Cheo and Lee 2014). The last large-scale horseshoe crab survey was conducted in 2015 by Nature Society Singapore (Vanitha 2015). Only one *T. gigas* juvenile was discovered along with some moults/dried carapace at Pasir Ris and Changi Beach. The abundance of *C. rotundicauda* was also small, with only 45 adults (18 female and 27 male) and 21 juveniles were discovered at Changi Beach, Kranji River mouth, Pandan and Seletar (Vanitha 2015).

National Parks of Singapore agreed that shoreline developments (reclamation or improvement schemes) made the beaches of Singapore less favorable for *T. gigas* spawning (National Parks 2015). Due to an extensive decline in the abundances of *T. gigas* during field surveys up to 2008, it was stated as ‘Vulnerable’ in Singapore Red Data Book (Davison et al. 2008). However, between May and December 2013, the National Parks of Singapore decided to assess the abundance of horseshoe crabs that linger or spawn on the islands. It involved field surveys assisted by National Biodiversity Centre where belt-transect (250 m²) and visual assessment methods were employed. It was carried out at 18 intertidal sites on the coastlines of Singapore. Major highlights of that survey included the presence of discarded nets that had trapped many spawning adult horseshoe crab encrusted with fouling organisms (MyGreenSpace 2014). Due to the near absence of adult *T. gigas* during field visits, the National Parks of Singapore decided to reinstate horseshoe crabs to the Singapore Red Data book; *C. rotundicauda* is listed as ‘Vulnerable’ whereas *T. gigas* are listed as ‘Endangered’ (National Parks 2015).

Indonesia

The coastal waters of Indonesia from the west to central regions especially Sumatra, Java, Kalimantan and Sulawesi have three species of Asian horseshoe crabs, namely *Carcinoscorpius rotundicauda*, *Tachypleus gigas*, and *T. tridentatus* (Rubiyanto 2012; Meilana et al. 2016; Mashar et al. 2017). Horseshoe crabs were reported in Maluku waters along the eastern Indonesian coast in 1898 (Dolejš and Vaňousová 2015). Since then, no scientific reports have addressed the occurrence of Asian horseshoe crabs in eastern Indonesia.

Horseshoe crabs in Indonesia are considered as by-catch and are not an important fishery. However, many coastal communities consume meat and eggs of horseshoe crabs, making them a tradable commodity at local level. The crabs are valued at approximately US\$ 0.4–0.8/crab. This leads to overexploitation of the horseshoe crabs, especially females, leading to reduction in the wild population. Therefore, conservation efforts are necessary in order to sustain and improve wild horseshoe crab populations.

The Decree of Indonesian Ministry of Forestry No. 12/KPTS.II/1987 is to protect *C. rotundicauda* and *T. tridentatus*, and PP (Government Regulations) No. 7/1999 is to protect *T. gigas*. However, these regulations provide protection only from potential extinction and are not enforced enough to prevent overexploitation (Safrida 2010). The Indonesian Ministry of Marine Affairs and Fisheries in 2013 issued a list of endangered marine biota of Indonesia and a priority is set to protect *T. tridentatus* and *C. rotundicauda*. Recent incidents of the illegal transport of horseshoe crabs collected from Indonesian waters for an export to Thailand via Malaysia have attracted the attention of policy makers (<https://www.youtube.com/watch?v=Z9-XWxKAq0A>). However, there is no regulation to enforce an effective sustainable management and utilization of horseshoe crabs in inland waters.

Implementation and enforcement of regulations to prevent overexploitation of horseshoe crabs might decrease the number of collectors and exporters of horseshoe crabs. The demand for female horseshoe crabs for their eggs for human consumption is high in coastal communities (Safrida 2010). Besides the exploitation for human consumption, the horseshoe crab populations in Indonesian waters are also under pressure from development activities in coastal areas, which can cause direct destruction of habitat.

Although the geographic distribution of horseshoe crabs in Indonesian waters is extensive, basic studies on them, such as population assessment and reproductive cycles are still scant. Scientific studies on horseshoe crab populations in Indonesia have only been done in Kuala Tungkal, Tanjung Jabung Barat, Jambi (Rubiyanto 2012) and in Bintan Bay (Anggraini et al. 2017). There is no information about the population of horseshoe crabs in other coastal areas of Indonesia.

Information about the reproductive aspects of Indonesian horseshoe crabs, such as nesting ground, spawning ground, and spawning season, is either lacking or very limited. Basic morphometric and DNA studies on the distribution and identification of horseshoe crabs in Indonesia have been done recently (Meilana 2015; Meilana et al. 2016; Mashar et al. 2017). There was significant variation in morphological parameters in samples collected from different sampling stations and low genetic variation among samples. Recommendations are proposed to the Indonesian government by the Indonesian Institute of Science (LIPI) in the year 2017 to make a strong policy for the sustainable fishery management and conservation of horseshoe crabs. Recently, the Ministry of Environment and Forestry of Indonesia has classified all three species of horseshoe crabs under protected animals (MEFI 2018a, b).

Thailand

Two species of Asian horseshoe crabs are found along the coasts of Thailand, namely *T. gigas* and *C. rotundicauda*. They are locally referred as ‘*mangda*’ and ‘*hera*’ respectively. Horseshoe crabs were caught from Ang-Sila, Bang Pakong, Chon Buri, Ko Chang, Mahachai, Phetchaburi, Samut Prakan and Surat Thani (Kungsuwan et al. 1987). Since Thailand has a long coastline, there are plentiful places for horseshoe crabs to feed and come to spawn of which many are yet to be investigated. Unlike in other parts of Asia (i.e. Malaysia, Indonesia and China), horseshoe crabs in Thailand are caught only for local consumption, and they are easily available at local fish markets (Banner and Stephens 1966). Horseshoe crabs were of interest in Thailand during 1925 after several people died from consuming horseshoe crab. The root cause was the poor

understanding by fishermen about horseshoe crab biology compounded by misidentification of *C. rotundicauda* as *T. gigas*, leading to poisoning (Kanchanapongkul and Krit-tayapooisipot 1995).

Researchers at the University of Hawaii used bioassays to quantify toxicity of the gut and hepatopancreas of *C. rotundicaudata* (Banner and Stephens 1966; Fusetani et al. 1983). The poison was identified as tetrodotoxin (TTX) (Kungsuwan et al. 1987) and the probable causative agent was *Vibrio* spp. (Kungsuwan et al. 1988). Poisoning by TTX occurred only between the months of December and March annually and only some *C. rotundicauda* contain this nerve inhibiting poison (Kanchanapongkul 2008). There were about 6 reports of poisoning between 1994 and 2006 with 457 people hospitalized and about 8 deaths from TTX poisoning (Joob and Wiwanitkit 2015). The reason for the presence of the poison only during the winter monsoon months from December to March (Northeastern winds) remains unclear. The spawning activity, total population size, total number of nesting grounds and the rate of consumption of *C. rotundicauda* and *T. gigas* by the humans are a few issues that urgently need to be addressed in Thailand urgently. Such information may serve as evidence to determine whether or not to include horseshoe crabs under Thailand Fisheries or Wildlife Act governance.

Mainland China

Horseshoe crab population distribution in China

Tachypleus tridentatus and *C. rotundicauda* were discovered in Fujian (Fuqing, Pingtan, Quanzhou Bay, Xiamen and Kinmen [*T. tridentatus* conservation area]), Hainan (Sanshan Bay), Zhejiang (Zhoushan), Guangdong (Zhanjiang, Pearl Bay, Guangzhou and Beibu Gulf) and Guangxi (Beihai) (Weng and Hong 2001; Liao and Liu 2006; Yang et al. 2007). Phylogeographic observations via expressed sequence tags, microsatellite loci, Cytochrome C Oxidase (CO1) and mitochondrial sequences in GenBank revealed subdivided horseshoe crab populations every 60 km, extending up to 1000 km (Xia 2000; Yang et al. 2007; Li et al. 2009; Yang et al. 2009; Xu et al. 2011; Weng et al. 2012, 2013). With this, horseshoe crab populations in China are assumed to have different adaptation thresholds depending on their environment of origin. For instance, the discovery of juvenile *T. tridentatus* in eastern Beibu Gulf sandy shores and *C. rotundicauda* in muddy mangrove bays gives an impression that different species have specific site preferences (Liang 1985a). However, *T. tridentatus* juveniles were also discovered at *C. rotundicauda* nursery in Pearl Bay, Guangxi (Chen et al. 2015). Hence, interspecies co-existence is environment and food-resource driven which may provide an explanation for horseshoe crab spawning migrations (Hsieh et al. 2002; Kwan et al. 2015a). Since different species of horseshoe crabs can co-exist, their behavior might be ecologically specific. This led studies on horseshoe crab life strategy and distribution inquiries through its morphology (Huang 1994; Liao et al. 2002), juvenile incubation in different substrates, temperature and pH of water (Gao et al. 2003; Hu et al. 2013) as well as feeding regimes (Gao et al. 2003; Zhou and Morton 2004). Sadly, mass culture of horseshoe crabs in the laboratory remains impossible because of difficulties in provision of food (Hu et al. 2013) affect haemolymph quality (Kwan et al. 2014). This obstacle is responsible for historical unregulated harvest of wild horseshoe crab stocks throughout China.

Threats to Chinese horseshoe crabs

Historical records revealed about industrial harvest of horseshoe crabs for aphrodisiacs and to manufacture decorative crafts since the early 1950s. It led to declines in horseshoe crab population of 80–90% in the 1970s (Liang 1985a). After a decade, poor environmental conditions prompted investigations about survival of horseshoe crabs in barren and polluted environments. Horseshoe crabs continued to thrive in some polluted areas, suggesting a degree of ‘hardiness’ and ability to adapt to local conditions (Wu 1982). The ability of horseshoe crab blood to neutralize some microbe toxins (Liang 1985b, 1987; Huang et al. 1995) and the successful isolation of *Tachypleus* Amoebocyte Lysate (TAL) greatly increased demands for horseshoe crab supply (Li et al. 2015). Continued environment degradation and overfishing of horseshoe crabs are responsible for mass reduction of wild stocks in Beibu Gulf (Liao and Li 2001) and in Beihai, South China (Hu et al. 2009).

Other impacts can cause stress and health problems for horseshoe crabs. For instance, juvenile *T. tridentatus* from Xi Bei Ling and Xi Chang (beaches being infringed by humans) and Jin Hai Wan (pristine mangrove reserve) at Beibu Gulf, South China had reduced growth rates compared to *C. rotundicauda* juveniles from the same areas (Hu et al. 2015). In another case, phenotypic diversity of *T. tridentatus* was low at disturbed areas despite the adult populations appearing healthy (Weng et al. 2012). Moreover, deformed juvenile horseshoe crabs with reduced haemolymph quantities were produced after cadmium and tributyltin assays. One possibility is that ecological burdens are making juvenile *T. tridentatus* sacrifice growth in order to tolerate environmental conditions (Kwan et al. 2015b). Yellow connective tissues and nutrient reserves are auto-consumed by juvenile horseshoe crabs in the absence of food which allows this arthropod to forgo feeding and reduce its metabolism (Weng and Hong 2003; Wei et al. 2007; Hu et al. 2010, 2011).

Apart from homeostasis regulations, horseshoe crabs can endure broad ranges of salinities and temperatures because 63% of their carapace mass is fortified with amino acids (aspartic acid glutamine, isoleucine, leucine and glycine) (Wei et al. 2007). This allows them to move between sea and riverine conditions, sometimes across distant geography. However, the release of horseshoe crabs into different geographical regions could upset their livelihood. For instances, religious practices in Southern China encouraged locals to release animals into the wild during festive occasions. However, regardless of species (*T. tridentatus* and *C. rotundicauda*) or origin, these arthropods are pooled together before their mass release (Ng 2016). In addition, as an effort to curb illegal harvest, 276 *T. tridentatus* were rescued in Beihai, Guangxi region and then released back into the wild (Zhang 2017). In each case, the release of horseshoe crabs into non-native localities cause spawning migration, complete migration or loss of the population.

Government effort to manage horseshoe crabs

The drastic decrease of horseshoe crab populations at Pingtan urged Fujian provincial authority to rear horseshoe crabs and restore beach conditions between 1978 and 1980. However, this initiative did not last long because the public failed to cooperate and knowledge of horseshoe crab biology was insufficient for artificial rearing (Gu 1980). Fortunately, insufficient supply of wild horseshoe crabs forced many companies to shift their business direction elsewhere between 1970s and 1980s and this allowed wild stocks to recover reaching an astonishing 15,000 amplexus in 1984 (Huang et al. 2002). Greater

availability of wild horseshoe crabs in the 1990s reverted businesses to its harvest. Wild stocks of Pingtan dwindled from 9500 amplexus in 1995–1000 amplexus in 2002, creating an urgency for the Fujian government to reattempt restocking efforts. However, lack of support from the public continued to foil all efforts (Huang et al. 2002). Apart from Pingtan, recovery of horseshoe crab populations was also carried out at Xiamen between 2000 and 2017. Similar to Pingtan, all recovery efforts failed because of poor public support (Weng et al. 2008).

Public awareness was increased through conferences and awareness programs run by government agencies, but it did not stop the public from overfishing *T. tridentatus* and utilizing coastal areas (Chiu and Morton 2003). Since the public would not alter their behaviour at Pingtan (Fujian) and Beihai (Guangxi), the local government resorting to restore degraded horseshoe crab habitats (Huang et al. 2003; Hu et al. 2009; Li and Hu 2011). Overfishing or illegal harvesting of horseshoe crabs remained difficult to curb and horseshoe crabs were almost extinct throughout China between 2011 and 2016 (Li and Hu 2011; Weng et al. 2012; Liao et al. 2017). For instance, 50–1000 adult *T. tridentatus* were recorded from 45 sites at Beibu Gulf (Guangxi Zhang) between 1991 and 1999 but recent surveys between 2011 and 2016 only recorded 0–30 adult (Liao et al. 2017).

Four decades ago, horseshoe crabs thrived throughout China but, the absence of governance and legislations allowed overharvesting and horseshoe crab populations declined by 90% by 2004 (WWF 2007). In Hong Kong, this situation led to the public voting that horseshoe crabs were in the top-10 animals that should be protected (Ng 2016). Recently, Chinese horseshoe crabs were declared as Grade II protected species in the List of State Key Protected Wildlife as well as Key Protected Aquatic Wildlife at provincial-level, especially in Zhejiang, Fujian, Guangdong and Guangxi Zhuang (Yang et al. 2009). With the introduction of legislative governance, public harvest of horseshoe crabs is prohibited at seven horseshoe crab areas in Fujian and Guangxi Zhang (GEPA 2018).

Hong Kong

Tachypleus tridentatus and *C. rotundicauda* are found in coastal waters of Hong Kong. Although they appeared in the literature as early as the late 1970s, the first detailed and extensive study of their distribution in Hong Kong was conducted in 1995 (Chiu and Morton 1999, 2003). Since 2002, surveys of juvenile populations have been conducted on a number of mudflats and beaches once every 2–3 years. In the most recent surveys in 2012 and 2014, juvenile populations were small and discrete, suggesting that their status in Hong Kong is fragile and vulnerable to local extirpation. In fact, horseshoe crabs were commonly found throughout Hong Kong waters in the past, according to scattered historic information in articles as well as interviews with fishermen and rural villagers (Chiu and Morton 1999). The current population of juvenile *T. tridentatus* and *C. rotundicauda* in Hong Kong is estimated at 2100–4300 and 2400–3000 individuals, respectively with half of *C. rotundicauda* distributed at Luk Keng in the northeast of Hong Kong and half of *T. tridentatus* at Ha Pak Nai in the west (Kwan et al. 2016).

The juveniles of the two species of horseshoe crabs occur on discrete beaches in small numbers. This indicates the limited number of nursery beaches as well as small adult populations. The distribution and abundance of adult horseshoe crabs in Hong Kong waters, however, is virtually unknown. They may be caught in fishnets on breeding shores by accident but no information is available on where in sea they live. Owing to the small number

of individuals, the adult population cannot be estimated using the capture-recapture method for mating pairs going to the shore during the breeding season. This becomes an obstacle in understanding the reproductive potential and long term sustainability of the species.

In the past 30 years, Hong Kong has experienced rapid industrialization, technological innovation and economic growth and was thus named as one of the four Asian dragons. The economic success, however, deterioration of the environment through habitat destruction and degradation, pollution and human exploitation is the most common way to create land for development, particularly on soft shores which are the natural habitat of juvenile horseshoe crabs. It is estimated that over 35% of the developed land area in Hong Kong has been reclaimed from the sea (<http://www.oldhkphoto.com/coast/>). A typical example is Tolo Harbour which is a semi-estuarine embayment located in the northeastern quadrant of Hong Kong. It is a harbour with a “bottle-neck” topography, resulting in very poor flushing. With a rapid increase in catchment population since the early 1970s, the harbour was seriously polluted with sewage and farming wastes, causing anoxia in the bottom sediments. The new town development along the coast of the harbour also destroyed the breeding beaches of horseshoe crabs. As a result, while they once flourished in the harbour in the 1980’s (Mikkelsen 1988), horseshoe crabs have now disappeared (Chiu and Morton 2003).

Hong Kong is located in the east of the Pearl River Delta (PRD), which is one of the most densely urbanized regions in the world and is an economic hub of China with a population of 120 million people. It has been identified as possibly the biggest pollution hot spot in East Asia, with major impacts spilling over into the South China Sea (The World Bank 2016). Western Hong Kong, where the largest population of juvenile *T. tridentatus* is located (Kwan et al. 2016), is directly influenced by the Pearl River. Additional impacts come from large scale ongoing construction projects in this area. These include the 50 km long Hong Kong—Zhuhai—Macao Bridge (a mega-size sea crossing linking Hong Kong, Zhuhai City of Guangdong Province and Macao) and the third runway of Hong Kong International Airport. Dredging and reclamation may cause the resuspension of bottom sediments and release pollutants to the water column, resulting in an increase in turbidity and a deterioration of water quality (Morton et al. 1996). The construction work may change the water flow hence alter the shore profile which is an important factor determining the spawning activity and the growth of the juvenile horseshoe crabs (Botton et al. 1988).

Compared to habitat destruction and pollution, the impact of human exploitation on horseshoe crabs in Hong Kong is relatively small as they have a low market value. A market survey between September 2004 and September 2005 showed that 30% of the horseshoe crabs caught in the net of fishing boats were released immediately back to the sea (Li 2008). Those which were sold in the market (a total of 690 individuals) served three major purposes, namely set-free ritual, display and food. The set-free ritual is a Buddhist practice where release of seafood back to the sea, is believed to create good karma by saving the lives from ending up on people’s dinner plates. This activity was responsible for 62% of the sale of horseshoe crabs in the market. Horseshoe crabs are not commonly consumed by people in Hong Kong with only three restaurants serving dishes with horseshoe crabs. A small number of horseshoe crabs were kept in a tank at seafood restaurants for display and as a tourist attraction.

Although horseshoe crabs as a taxon are not protected by law in Hong Kong, partial protection is offered by the establishment of Sites of Special Scientific Interest (SSSI) for protecting other species. For example, San Tau on Lantau Island and Pak Nai in the north-western New Territories, where juvenile horseshoe crabs are found, were designated as SSSI for the protection of seagrass. The permanent trawl ban in Hong Kong waters was introduced by the Government in December 2012 to conserve the fisheries resources. As

an indirect effect, adult horseshoe crabs living in the sea bottom become free from the risk of being harvested. Encouraging news comes from a more positive attitude of the Government recently towards the conservation of horseshoe crabs. Tung Chung Bay near the airport on Lantau Island is one of the most important breeding and nursery grounds of *T. tridentatus*. Reclamation in the bay was proposed by the Government for expansion of the new town. The plan to reclaim 14 hectares to the west of the town centre, inhabited by juvenile horseshoe crabs, has been abandoned after environmental groups voiced fears that it would affect the ecology of Tung Chung Bay (South China Morning Post 2014). An even more encouraging piece of news is on the recently released Hong Kong Biodiversity Strategy and Action Plan (BSAP) 2016–2021 (Hong Kong SAR Government 2016) where the Government has identified horseshoe crabs as one of the five taxon groups with the species action plan to be prepared. Additional studies will also be conducted to collect information that is essential for the implementation of the plan.

India

Annandale (1909) described the habits, habitats and breeding ecology of Indian king crabs (*Tachypleus gigas*) and this was perhaps the first scientific documentation of horseshoe crabs in India. Roonwal (1944) reported on breeding biology, colour, shape, size, weight and perivitelline fluid (PVF) of the eggs. Significant work on distribution pattern and taxonomy has been done (Debnath and Choudhury 1989; Vijayakumar et al. 2000; Chatterji 2005). Specific studies like population estimation by capture-recapture methods (Debnath and Choudhury 1988), diagnostic study on morphology and sexual dimorphism of *T. gigas* and *C. rotundicauda* (Debnath 1991), feeding and food selection patterns (Debnath and Choudhury 1989; Chatterji and Mishra 1992), fecundity, seasonal variation in the volume of the haemolymph (Chatterji et al. 1992a, b) and body weight, spawning and migration (Sahu and Dey 2013) have also been documented. In 2014, the Zoological Survey of India (ZSI 2014) under the Man and Biosphere Programme (MBP) conducted studies on the horseshoe crab breeding biology and other associated study along the east coast of India where *T. gigas* and *C. rotundicauda* have been reported along the Bay of Bengal in the selected coastal stretches between Ganga to Godavari river mouth. Based on 300 carcasses examined by Basudev et al. (2013) along the upper east coast of India, *T. gigas* was the most abundant species recorded, while *C. rotundicauda* occurred at selected coastal beaches of Sundarban and Digha in West Bengal and Balaramgadi, Hukitola and the area of Odisha.

The degradation of breeding ground by human activities such as fishing and shifting of beach sands for construction purposes causing damage to nesting sites has been reported by Pati et al. (2017). Detritus-rich muddy shores along with mangrove vegetation are being lost for construction of fishing jetties and other coastal development, which gradually degraded the horseshoe crab breeding habitats (Basudev et al. 2013). They also pointed out that several anthropogenic activities like sand mining, near-shore mechanized fishing; illegal prawn farming, destruction of mangrove and destruction of coastal vegetation are major threats to Indian horseshoe crabs.

GIS-based habitats mapping along the Indian Coast

Records of *T. gigas* along the north east coast of Odisha viz. Chandipur, Balaramgadi, Mahisali, Khandia, Khapra, Ekakula, and Bhitarkanika were reported by Roonwal (1944),

Debnath and Choudhury (1989), Lazarus et al. (1990), Chatterji and Abidi (1993), Mishra (2009), Behera et al. (2015) and Pati et al. (2015). *Carcinoscorpius rotundicauda* has been reported from Canning, Digha, Sunderban, Dobanki, Satjelia, Frazerganj, Henery's Island, Pakkiralay and Sudhyanakhali in west Bengal (Rao and Rao 1972; Debnath and Choudhury 1988; Basudev et al. 2013) while in Odisha *C. rotundicauda* was found in Paradeep, Dhamra, Udabali, Balichandrapur, Balramgadi, Ekakula, Talsari, Udaypur, Kashaphala, Chandipur and Hukitola (Basudev et al. 2013).

A total of 69 coastal stretches have been identified as horseshoe crab habitat along the upper east coast of India, of which 28 sites were along the West Bengal coastal stretch, 39 sites along Odisha coast and 2 sites in Andhra Pradesh. In West Bengal, 22 coastal stretches are located inside the Sundarbans Biosphere Reserve and 6 sites are located between Hoogly river mouth to Subarnarekha river mouth. Of these 28 sites, only three co-occurrence of both species; only two of 39 sites are located inside Protected Area i.e. Bhitarkanika and Gahirmatha Sanctuary. Of the 39 sites, occurrence of both species was found at 17 sites and at 9 sites, only *T. gigas* was found. In Andhra Pradesh, only two sites were identified as horseshoe crab habitat, exclusively for *T. gigas*.

Threats to Indian species

Human activities adversely affect the survival of horseshoe crabs by affecting their spawning grounds and their nesting activities, for example, beach development and shoreline modifications prevent them from reaching the nesting sites. Dead specimens found on the shoreline are often due to degradation and destruction of breeding beaches by human activities which pose a serious threat to the survival of horseshoe crabs (Mishra 2009). Very little is known about the predation of Indian horseshoe crabs in their natural habitat except that the predation of *T. gigas* by crows (*Corvus splendens*), domestic pig (*Sus scrofa domestica* or *Susdomesticus*) and dogs has been observed in India (Debnath and Choudhury 1988; Pati and Dash 2016). Natural factors such as these predation, beach stranding and disease cause the death of adult crabs that come to the shore for spawning (Behera et al. 2015). Development of shoreline is another threat that leads to the degradation of spawning habitat of horseshoe crabs (Chatterji et al. 1996a, b). Beach erosion, tsunami and floods also leads to the destruction of nesting grounds (Chatterji and Shaharom 2009).

Japan

Horseshoe crab conservation efforts in Japan (an interview with Mr. Keiji Tsuchiya)

Most literatures on horseshoe crab laboratory culture and assessment of wild population density in Japan is available only in Japanese (Tsuchiya 2009). On 12–13 May 2017, A personal interview was conducted by one of the authors (YI) with Mr. Keiji Tsuchiya who was recognized with a lifetime achiever award for the conservation of horseshoe crab in the 3rd International Workshop on Science and Conservation of Horseshoe crab 2015, in Sasebo Japan. He is also one of the founders of the Japan Society for the Conservation of Horseshoe Crabs. Some of the discussions is translated into English and presented here. For background details of Mr. Tsuchiya's involvement with horseshoe crabs and of Kasakura City, readers might want to refer to Seino et al. (2003).

Some of Mr. Tsuchiya's current concerns, other than ongoing environmental modifications of shorelines by humans, are; (1) using horseshoe crabs as bait for eels and conchs (in the US), (2) consumption of horseshoe crabs as a delicacy in Asia, and (3) the presence of predators such as crows. In order for further protections that might be easier to implement than tackling all these issues, he recommends to plant sea grasses or eelgrasses for horseshoe crabs to spawn and for their juveniles to be protected, and to enforce a regulation to standardize the amount of blood extraction from Asian horseshoe crabs.

Role of the Japan Society on the conservation of the horseshoe crabs

In 1928, horseshoe crabs became a 'natural monument' in Japan. This does not mean that the species are protected everywhere in Japan, but only in designated areas. Kasaoka is one of such areas for this species. Additional areas are Imari (Saga Pref.) and Saijou (Ehime Pref.). In 1978, Mr. Tsuchiya and Dr. Hiroyuki Nishii, founded the Japan Society for the Conservation of Horseshoe Crabs in Kasaoka City, Okayama. Since then, every August, the Society has held a general assembly to report the current status of horseshoe crab populations in Japan. Surveys include counting spawning adults and horseshoe crab juveniles where applicable. Regional branches hold their own meetings as well. Methods to protect horseshoe crabs against crows (*Corvus* spp.) has been one of the important issues, as they are becoming the major predator of horseshoe crabs.

Distribution of *Tachypleus tridentatus* in Japan

Each branch of the Society conducts horseshoe crab population assessment every spawning season, from June to August. The results are presented in "Kabutogani", an annual publication issued from the Society. *Tachypleus tridentatus* is the only species of horseshoe crab in Japan, which is mostly distributed in the Seto Inland Sea to northern Kyushu. There is also a report of the same species in Ise-Mikawa Bay, Aichi Prefecture, outside of the Seto Inland Sea. However, based on a genetic study, this population was found to be an alien, possibly from China (Nishida et al. 2015). This population has appeared each year since the early 2000s, but their ecological impact has not been reported yet.

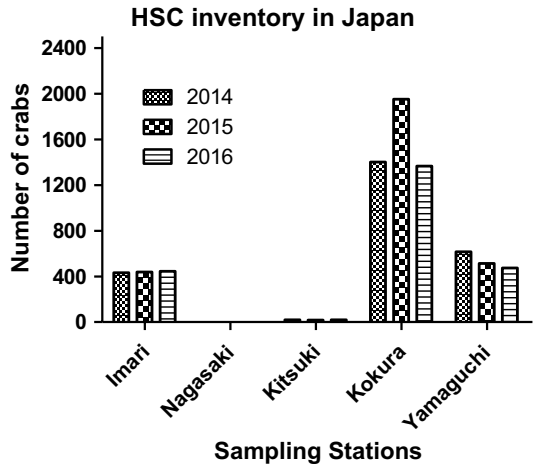
Problems in Kokura, Kita-Kyushu

In the early summer of 2016, Mr. Shungo Takahashi and his colleagues at the Fukuoka Branch of the Society noticed something different about the Sone Tidal Flat where they survey for horseshoe crab population every year. Before the end of summer, they discovered and collected 402 dead horseshoe crabs, excluding badly damaged or aged carcasses that were probably dead before that year. There were slightly more females than males and only 10% were sexually immature individuals.

On average, 50–60 dead horseshoe crabs were found each year, except for 2005 when they found > 300. Kokura, Kita-Kyushu where Sone tidal flat is located is currently the major location for horseshoe crabs coming ashore to spawn in Japan (Fig. 3).

The reason for the mass mortality of horseshoe crabs is still under debate. Along the coast of Japan, degradation in water quality because of industrial pollution is often considered to be one of the major causes of population decline in horseshoe crabs (Botton 2001; Botton and Itow 2009). However, study of water quality in the vicinity suggested that there

Fig. 3 Horseshoe crab sightings in Japan, from 2014 to 2016 for 5 regions, Imari (Saga Pref.), Nagasaki Pref., Kitsuki (Oita Pref.), Kokura (Fukuoka Pref.), and Yamaguchi Pref. In each year, July sees the most population burst, while Kokura is clearly the hub of spawning horseshoe crabs. Based on the data taken from “Kabutogani” Nos. 35–37



were no abnormalities (Takahashi 2017). Because of the unpleasant smell from carcasses in hot summer, and garbage washing in from the ocean and sensationalised reporting by mass media created poor or incorrect awareness of the issue by the public. Local administrations failed to provide enough support to investigate the cause of the mass mortality of this endangered species.

Horseshoe crab specialists from University of Kyushu and Environmental Office in Kita-Kyushu tentatively concluded some possible causes; (1) rapid sea water temperature rise; (2) oxygen depletion in the water column; (3) transmittable diseases; and (4) lack of food sources. However, none of these could cause a mass mortality in such a scale. Some suggest that it was because of the lifespan of horseshoe crabs and was a natural event. Kokura is considered one of the prominent horseshoe crab habitats still existing in the Seto Inland Sea, and in Japan in general. For this reason, one may think that finding many dead horseshoe crabs could be normal because there would be plenty of them in the sea. However, the incident in 2016 may signal something else, but at this point, no one knows for sure what that could mean.

Other Asian countries

Scientific studies on the population dynamics on Asian horseshoe crabs in Bangladesh, the Philippines, Cambodia and Brunei are still insufficient. Studies from Vietnam suggested that at least 50% of the *T. tridentatus* (locally known as “Sam ba gai đuôi”) population has declined from 1990 to 2007 and this trend continuous (Laurie 2017). In the last decade, coastal aquaculture and shoreline developments have destroyed at least 20% of *T. tridentatus* spawning grounds. Horseshoe crabs are recognized as a legally exploited fishery resource in Vietnam and traded cross-border to supply the TAL industry in China (Laurie 2017). *Tachypleus tridentatus*, *C. rotundicauda* were included in the Vietnam Red Data Book of 1992 and 2000. *C. rotundicauda* have been reported for the first time from Tan Hai village and Vung Tau province (Dao et al. 2009) and the distribution of *T. tridentatus* was reported from the Northern Pacific coastal area of Gulf of Tonking (Fischer et al. 1994).

A study on juvenile population density of *T. tridentatus* in Guningtou Horseshoe Crab Conservation Area, Taiwan revealed that the population has declined rapidly from 0.3 individuals/m² in 2003 to between 0.02 and 0.17 individuals/m² in 2004–2009 (Hsieh and Chen 2015). Many strategies have been proposed for the conservation of horseshoe crabs in Kinmen, such as designating them as protected species, habitat protection, population enhancement through sea-ranching techniques and public education (Chen et al. 2004; Hsieh and Chen 2015). Hsieh and Chen (2015) have studied juvenile crab abundance and density at Beishan, Nanshan, and Hsiashu sites during 2003–2009 and concluded that there were decreases in juvenile abundance of 8, 5 and 17%, respectively. Major threats to *T. tridentatus* on Kinmen Island includes habitat degradation due to the construction of a commercial port and invasion by *Spartina alterniflora* (Cordgrass). Adult *T. tridentatus* are considered locally extinct on the main Island of Taiwan although juvenile population is thriving, despite the dwindling population on Kinmen Island.

In Bangladesh, *C. rotundicauda* and *T. gigas* (locally called “deokera”), inhabit the Bay of Bengal. They are usually caught by a dragnet with other commercially important fishes. The eggs of horseshoe crab are occasionally eaten by tribal people, especially at festivals. No food poisoning incident has yet been reported (Tanu and Noguchi 1999). In Bangladesh, *C. rotundicauda* is commonly found along the coast of Cox’s Bazar, St. Martin’s, Sonadia and Moheshkhalī Islands and rivers of the Sundarbans. While *T. gigas* is observed in the intertidal zones of Cox’s Bazar, Teknaf and St. Martin’s Islands; they are absent from the Sundarbans (Chowdhury and Hafizuddin 1980).

In the Philippines, *T. tridentatus* and *C. rotundicauda* have been recorded in Palawan. A survey from northern to central Palawan confirmed the presence of *T. tridentatus* (Schoppe 2002). In Palawan, habitat conversion, destruction, and fishing activities were identified as the major threats to horseshoe crabs (Schoppe 2002). A population survey of *T. tridentatus* at Aventura Beach at Palawan, recorded juvenile density of 1.47 individuals 100 m⁻² (Almendral and Schoppe 2005).

Scientific studies on horseshoe crabs in Brunei remains limited but reports have shown the distribution of *T. tridentatus* at Panaga Beach (McIlroy and Novi 2009). *T. tridentatus* have also been recorded in Kampung Ayer, the water village of Bandar in northwest Borneo. (Personal blog-<http://lifearth.blogspot.in/2017/04/horseshoe-crabs-past-and-present-nw.html>). The mudflat of Brunei bay serves as nursery ground for horseshoe crabs. (Silvestre et al. 1992).

In Cambodia, Ngy et al. (2007) have reported the presence of *C. rotundicauda* along the shore of Sihanouk Ville. Individuals were found to be toxic in the wet and in the dry season.

Conclusion and future direction

Asian horseshoe crabs are facing a serious threats to their wild populations due to unsustainable harvesting for human consumption and for biomedical bleeding practices. Vulnerability to local extinction and the extent of the risk varies from country to country. Additionally, habitat degradation, shoreline development and/or land claim, construction of sea walls across the spawning grounds and loss of coastal mangrove habitat along the nesting grounds can directly affect spawning success for all three Asian horseshoe crab species. Although some Asian countries such as Indonesia, India and Japan have regulations to protect horseshoe crabs, the enforcement and implementation of such regulations are still

questionable. On the other hand, other Asian countries/regions are still considering horseshoe crabs as a fishery commodity and no such strict regulations are in place to ensure sustainable harvest from the wild.

Recently many incidences of horseshoe crab export/smuggling across the borders for biomedical bleeding practice and local delicacy have been reported in Asia. For instance, export of *Tachypleus tridentatus* as bait for the eels and conch fishery in the United States, and smuggling *T. gigas* from Indonesian waters to Thailand via Malaysia, with a final destination in Japan have been documented (Christianus and Saad 2009; Botton et al. 2015; Gauvry 2017). Thus, the conservation of *T. tridentatus*, *T. gigas*, and *C. rotundicauda* must require an international collaboration among the researchers and stakeholders. Since populations trend of Asian horseshoe crabs have declined over the past few decades, an active conservation and protection along with an inter-governmental co-operation at regional as well as international level is essential to protect them from over-exploitation. Declines in populations of horseshoe crabs in Asia might put pressure on *Limulus polyphemus* in the USA for LAL/TAL (Moore 2017). Hence, preservation of their natural habitat by limiting the coastal development, prevention of illegal export and strict regional implementation of legislation is crucial for the protection and sustainable harvesting of this living fossil. Standardization of sampling protocols for either DNA or wild population density assessment is crucial in Asian region due to the fact that variation in sampling techniques can lead to misinterpretation of results. Similarly, regional authorities in Asia can regulate harvesting of horseshoe crabs by implementing catch quotas and issuing permits to authorized fishermen to harvest crabs similar to the one available to protect *L. polyphemus* on the eastern US coastline. Overall, the information presented in this work should be valuable for supporting and/or revising the IUCN Red List assessment for Asian horseshoe crab species.

Acknowledgements Authors extend their thanks to IUCN Horseshoe Crab Species Specialist Group (IUCN SSC) for its inspiration to carry out this extensive review work. Special thanks goes to Ecological Research and Development Group (ERDG), USA for their contribution to conservation of four extant horseshoe crab species.

Author contribution All authors have equal contribution in this manuscript writing. Corresponding author of this manuscript conceived idea and prepared introduction, review flow and drafted final manuscript.

Compliance with ethical standards

Conflict of interest Authors have no conflict of interest.

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