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# *Tachypleus tridentatus,* Tri-spine Horseshoe Crab

## Errata version

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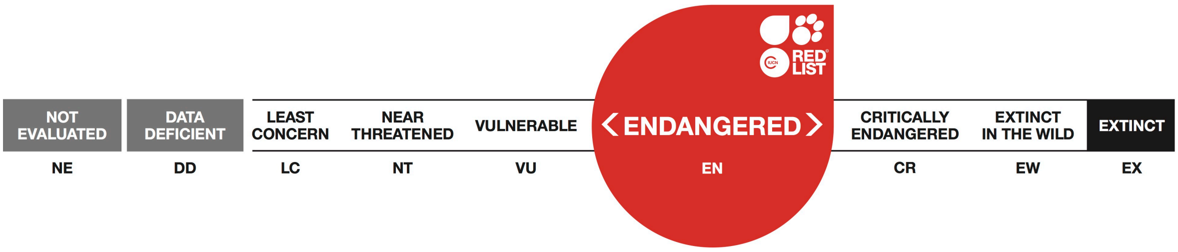


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## Taxonomy

| Kingdom | Phylum | Class | Order | Family |
|---|---|---|---|---|
| Animalia | Arthropoda | Merostomata | Xiphosura | Limulidae |

**Taxon Name:** *Tachypleus tridentatus* (Leach, 1819)

**Synonym(s):**

- *Limulus tridentatus* Leach, 1819

**Common Name(s):**

- English: Tri-spine Horseshoe Crab, Chinese Horseshoe Crab, Horseshoe Crab, Japanese Horseshoe Crab, King Crab

**Taxonomic Notes:**

*Tachypleus tridentatus* and *T. gigas* are sometimes the subject of misidentification. In China, three horseshoe crab nature reserves were established in Guangdong Province in 2004 for the protection of *T. gigas* (Kwan *et al*. 2016), even though *T. gigas* has not been recorded in these areas (Liao and Li 2001, Liao *et al*. 2001a).

In Hong Kong, the occurrence of *T. gigas*, *T. tridentatus* and *C. rotundicauda* was reported (Mikkelsen 1988), but recent population studies did not reveal any presence of *T. gigas* in Hong Kong waters (Li 2008, Shin *et al*. 2009, Morton and Lee 2010) and a recent literature review suggests previous reports of the presence of *T. gigas* in Hong Kong was a case of mistaken identity, based on the wrong labelling of photographs in a popular natural history book at the time (Laurie 2011, unpublished).

On 23 May 2012, five horseshoe crabs were seized at Sam Ratulangi International Airport, Manado, North Sulawesi, Indonesia under PP No. 7/1999 (Indonesia Government Regulation No. 7/1999). Although described as *T. gigas*, photographs indicate the seized animals were *T. tridentatus* (BKIPM 2012).

## Assessment Information

**Red List Category & Criteria:** Endangered A4bcd ver 3.1

**Year Published:** 2019

**Date Assessed:** July 22, 2018

**Justification:**

This assessment integrates information on major threats, habitat and population responses, population genetics, past and predicted future levels of exploitation and management and conservation actions. Population responses, in terms of abundance, geographic range and viability inform risk at the country level, which in turn, informs the assessment of the species' risk of extinction.

**Population Reduction**

*Tachypleus tridentatus* is an inshore, coastal species. Its distribution area is vast and complex, extending in a north to south range, from Japan with a temperate climate in the north, through mainland of the People's Republic of China (hereafter abbreviated to China), Taiwan (province of China, hereafter abbreviated to Taiwan), Hong Kong (a Special Administrative Region of China, hereafter abbreviated to Hong Kong), Viet Nam, Philippines, Brunei Darussalam and East Malaysia to Indonesia, with a tropical climate in the south. There is genetic evidence for subpopulations within its range and it appears to exhibit differing population profiles, with historically dense populations in its northern range (Japan, China, Taiwan, Hong Kong, Vietnam) and low population densities in its southern range (Philippines, Brunei Darussalam, Malaysia, Indonesia).

Its life-history habits with five distinct stages make it vulnerable to a variety of threats at each stage of its development. High tide spawning sites and intertidal juvenile nursery habitat are critical to its survival needs, but the occurrence of such habitats is not universal; they occur intermittently throughout its range based on hydrology, geomorphology, sedimentology and the species' own habitat preferences. Whilst the coastlines where *T. tridentatus* ranges are long, these critical areas of occupancy, comprising the actual used and useable areas utilized as spawning habitat and intertidal juvenile nursery grounds, are relatively small in area. As a consequence, within its range, spawning and intertidal habitats do not equate to all available coastline because only select areas of it constitute suitable habitat. Furthermore, where horseshoe crab species occur sympatrically (such as localities in China and Hong Kong where *T. tridentatus* and *Carcinoscorpius rotundicauda* coexist together) juvenile nursery grounds on intertidal flats are spatially delineated into sandy facies (favoured by juvenile *T. tridentatus*) and muddy facies (favoured by juvenile *C. rotundicauda*), further restricting the extent of useable area available for juvenile *T. tridentatus* to forage.

Where data are available, significant population declines (See Table 1 in the Supplementary Material) and habitat declines (See Table 2 in the Supplementary Material) have been documented in Japan, China, Taiwan, Hong Kong and Viet Nam, with declining population trends being reported by fishermen in the Philippines, Malaysia and Indonesia.

Because of their coastal location, *T. tridentatus* spawning habitat and juvenile nursery grounds are highly susceptible to reclamation, mariculture projects or are suffering from the adverse impacts of coastal infrastructure construction and sea sand extraction. The species has experienced different levels of habitat loss or degradation throughout much of its range, with studies indicating that infrastructure projects have contributed to extirpations, instances of restricted gene flow, low haplotype diversity, and the creation of population bottlenecks indicative of impending extirpation. Whilst many habitats have been lost completely, recruitment has ceased at many previously healthy spawning sites, for reasons that are still not clear. The result is the presence of remnant populations in Japan, China, Taiwan and Hong Kong. In the Philippines, Malaysia and Indonesia population densities are low, common threats occur and population declines have been reported. Population data are not available for Vietnam or Brunei Darussalam. The risk profiles vary between countries.

**Japan**: *T. tridentatus* was once abundant on all of the coasts of the Seto Inland Sea. There is no *T. tridentatus* fishery in Japan. Populations have declined significantly because of habitat loss or degradation. Between 1930 to 1994 over 80% of horseshoe crab habitats disappeared in the Seto Inland Sea and the area of tidal flats in Japan has decreased by over 40% since 1945, whilst between the 1970's to 2000's seagrass beds, some of which support juvenile horseshoe crab populations were reduced by

40% in area. *Tachypleus tridentatus* was assessed to be Critically Endangered in 2006 mainly because of the loss or deterioration of its tidal flat habitats. Reports of extirpations in Japan are not uncommon. *Tachypleus tridentatus* has disappeared from many of its spawning grounds, whilst other sites have recorded significant decreases in visits by spawning pairs. Small, remnant populations remain, some are conservation-reliant for their survival and spawning habitat and juvenile nursery grounds are still threatened with development.

**China**: *T. tridentatus* was once abundant in high densities throughout its range in China. Mass spawning events were a common sight and it was regarded as an important economic species, both to supply the biomedical industry and for consumption. In both cases, all of the animal is used, resulting in 100% mortality. Population declines of ≥90% have been recorded throughout much of its range in China and a number of important fisheries collapsed between the 1970s and 1990s. In parallel to overfishing, land reclamation between the 1950s and the 1990s drastically shrunk intertidal habitats. *Tachypleus tridentatus* was assessed as Endangered in 2004 because populations had seriously declined due to its over-exploitation as a source of raw material for chitin, the utilization of its blood in the medical and biomedical industries and the use of its meat for food. Subsequent to this, between 2008 and 2012 the mangrove area in China decreased by about 66% because of land reclamation and sea sand extraction has caused significant habitat degradation along almost the entire range of *T. tridentatus* on the mainland coast of China. Reports of extirpations are not uncommon. Between 2006 and 2010, no spawning was observed during surveys of 27 nationally recognized spawning sites and a few juveniles were found at only six of the sites, representing an 88% decline in habitat utilization. Dongshan Bay in Fujian went from having abundant adult populations in the early 1980s to no spawning being observed and only juvenile populations being found during surveys between 2006 and 2010. Commercially, limuloid resources are exhausted in China, yet as late as 2011, the Guangxi Government was still promoting the horseshoe crab fishery in Beibu Gulf, demand for *T. tridentatus* as a culinary treasure and to supply the biomedical industry is high and remaining populations are being targeted to meet these demands.

**Taiwan**: *T. tridentatus* was once abundant in high densities throughout its range in Taiwan, but populations have been extirpated or declined significantly because of habitat loss or degradation. On Taiwan Island, 55% of the natural coastline, particularly on the west coast, which was home to the majority of *T. tridentatus* spawning grounds in Taiwan has been lost to reclamation or degraded because of coastal infrastructure construction, to the extent it is extirpated at many localities and is on the verge of extirpation on Taiwan Island. Similar habitat loss and degradation has occurred on Kinmen Island, due to the reclamation, dredging of subtidal areas and sea sand extraction. No adults or mating pairs have been recorded on the intertidal flats of Taiwan Island since the 1960’s, nor more recently from Kinmen. When population surveys commenced in Taiwan in 2003, they were conducted on juvenile populations because of the lack of adults. Where juveniles still exist, populations are declining and most of the remaining sites support non-viable juvenile populations.

**Hong Kong**: *T. tridentatus* was once widespread and abundant in Hong Kong with thriving populations until the 1980s, but by the early 1990s it had disappeared from much of its range. Significant population declines occurred because of overfishing to supply the biomedical industry, combined with significant levels of habitat loss or degradation. Population declines of ≥90% can be inferred in Hong Kong. Spawning was last scientifically observed in 1986 and when population surveys commenced in 2002, they were conducted on juvenile populations because of the lack of adults.

**Viet Nam**: Up to the early 1980s major concentrations of *T. tridentatus* could be found along the coast of Viet Nam, at least as far south as Nha-Trang, but populations have declined significantly through a combination of overfishing to supply the biomedical industry and to meet consumer demand in China, in parallel with significant levels of habitat loss or degradation. *Tachypeus tridentatus* was assessed as Vulnerable in Viet Nam in 2007, based on a population decline of 50%, a fisheries catch decline of 20% and a decline in area of occupancy of 50% between 1990 and 2007. Subsequent to this, Viet Nam lost 40% of its mangroves between 2006 and 2012 and 56% of the remainder are planted mangroves and considered of low ecological value. Seagrass bed coverage in Viet Nam decreased from 40% to 70% between 2009 and 2014 and the coast of Viet Nam's central coastal provinces, where *T. tridentatus* was once most abundant has been transformed since 1999 through a combination of shrimp farming, tourism infrastructure construction and sand mining, which have destroyed thousands of hectares of the ecosystem, so that its ecosystem service functions are now severely diminished. *Tachypleus tridentatus* is a legally exploitable resource in Viet Nam and demand from China to supply the biomedical market and to meet consumer demand remains high.

**Philippines**: Historic information suggests *T. tridentatus* was once widely distributed throughout the Sulu Sea, but it experienced significant declines in numbers in the decades preceding 2000. Spawning sites and nursery grounds are threatened by coastal construction and sand mining and because of their close proximity to human population centres, they suffer from high rates of incompatible habitat use. Surveys in 2001 and 2002 indicated the presence of small juvenile populations at two renowned nursery beaches in Palawan.

**Malaysia**: *T. tridentatus* is only found in Sabah and Sarawak. Where surveys have been conducted in Sabah, they indicate the presence of small, low density populations. Horseshoe crabs, including *T. tridentatus* form an important income component for poor fishermen and are a dietary component of Bajau Laut fishermen. Population declines have been reported by fishermen. The adverse impact of selective fishing practices on adult populations is likely evident in the male-biased Operational Sex Ratios (OSR) observed in Sabah, which is believed to reflect the selective targeting of gravid females by coastal and artisanal fisheries. Because of their close proximity to human population centres, spawning sites and nursery grounds suffer from high rates of incompatible habitat use. No population, habitat or threat data is currently available for Sarawak,

**Brunei Darussalam**: Formal studies have just commenced, so population, habitat and threat data are not available. Because of its small geographic area, it is unlikely significant populations are resident here.

**Indonesia**: Formal studies have just commenced, so population, habitat and threat data are not available. Population declines have been reported by fishermen. Because of their close proximity to human population centres, spawning sites and nursery grounds suffer from high rates of incompatible habitat use. Land reclamation of spawning habitats and juvenile nursery grounds has occurred in Indonesia.

A significant contributor to past population declines and the future near-term threat to *T. tridentatus* is the unmanaged, unsustainable harvest of adults to supply the biomedical industry and the selective targeting of gravid females to meet consumer demands (See Table 3 in the Supplementary Material).

The long-term threat to *T. tridentatus* is the loss or degradation of spawning habitat and juvenile nursery grounds by reclamation, mariculture projects or the adverse impacts of coastal infrastructure construction and sea sand extraction, because these factors are leading to local populations being extirpated and their impacts are not reversible.

The generation length of *T. tridentatus* in its northern range has been estimated to be 20.25 years (giving a 3-generation length of 60.75 years [rounded to 60 years]). There is evidence to suggest different age-to-maturity rates at different latitudes, so that the generation length for southern populations could be considerably shorter.

In terms of future exploitation, the increasing demand driven by the projected growth of the Amebocyte Lysate industry and the categorization of *T. tridentatus* as a culinary treasure in China constitute high risk threats that will have considerable impact on remaining populations, and with the exception of Japan which has no horseshoe crab fishery, it will be aggressively targeted to meet these demands. In its southern range, artisanal fisheries, particularly the selective harvesting of gravid females in small or depleted populations can and in future will have significant negative impact, as evidenced by the male-biased Operational Sex Ratio (OSR) recorded in eastern Malaysia.

Furthermore, once recruitment at a site has stopped, the chances of recolonization and natural population recovery are low. Specific spawning and juvenile nursery habitat preferences, limited dispersal capabilities of larvae and juveniles, limited adult migratory behavior, topographic barriers to migration, genetic isolation and restricted or limited gene flow between geographically close populations and evidence of endemism means natural recruitment to abandoned spawning locations could take generation length time spans for populations to re-establish themselves and at many sites, because of lasting changes to local hydrology and sedimentology, recolonization may not occur because the changes may not be reversible.

Because of the combination of over-harvest and habitat loss, an ongoing population reduction, based on past estimates starting circa 1980 and future projections extending at least for the next two decades, suggest a reduction of ≥50% over a period of about 60 years or 3 generations in a significant portion of the species range.

**Risk Assessment**

*Tachypleus tridentatus* is facing relentless and unremitting anthropogenic threats from multiple stressors on a range of fronts. Major population declines have been reported throughout much of its range, all life-history stages face a combination of threats, including targeting of adults for its high value blood, chitin and eggs, high levels of habitat degradation, loss or abandonment, low resilience to exploitation including long age-to-maturity time, low juvenile recruitment rates, specific spawning and juvenile nursery habitat preferences and largely unregulated, continuing fishing pressure and these threats have not ceased and in some cases they are not reversible.

Up to the early 1980s, when survey data are first available, *T. tridentatus* was abundant with high population densities in much of its northern range, but since then, it has experienced significant population declines throughout much of its range, caused by a combination of unmanaged exploitation and significant levels of habitat loss or degradation It is extirpate at many localities and is still threatened with habitat loss throughout much of its range. The increasing demand for *T. tridentatus*, driven by the projected growth of the Amebocyte Lysate market and to meet consumer demands following its categorization as a culinary treasure in China constitute high risk threats that will result in future exploitation of remaining populations, and with the exception of Japan, it will be aggressively targeted to meet these demands. Its exploitation as an all-parts-use animal means populations have little chance of recovery. *Tachypleus tridentatus* has historically been subject to high levels of exploitation and it is predicted to be the subject of future high levels of exploitation, compounded by significant levels of habitat loss and degradation in the past and likely losses of spawning habitat and juvenile nursery grounds in the future. In summary, these threats have not ceased, in many instances they are not reversible and the future risk and impact of these threats are assessed to be high to extremely high throughout its range. Based on these factors and because of an ongoing population reduction of ≥50% over a period of about 60 years (three generations), *T. tridentatus* is assessed as Endangered.

In addition to the Endangered global assessment, a number of country level assessments have been designated for *T. tridentatus* as follows: Critically Endangered in Japan (2006), Endangered in China (2009) and Vulnerable in Viet Nam (2007).

For further information about this species, see Supplementary Material.

### Previously Published Red List Assessments

1996 – Data Deficient (DD)
http://dx.doi.org/10.2305/IUCN.UK.1996.RLTS.T21309A9267047.en

1994 – Insufficiently Known (K)

1990 – Insufficiently Known (K)

1988 – Insufficiently Known (K)

1986 – Insufficiently Known (K)

1983 – Insufficiently Known (K)

## Geographic Range

### Range Description:

Horseshoe crab fossils are found in deposits ranging from the Palaeozoic to the Cenozoic. The earliest fossil forms date back 475 million years ago (mya) (Van Roy *et al*. 2010) and by 310 mya the unmistakable horseshoe crab body form was clearly established (Shuster 2001, p. 36). Modern horseshoe crabs have existed for more than 200 million years (Botton and Ropes 1987, Shuster 2001, Tanacredi 2001, Anderson and Shuster 2003, Blazejowski 2015).

There are four extant species of horseshoe crab found in two regions of the world. *Limulus polyphemus* (Linnaeus 1758), the American Horseshoe Crab, is found along the Atlantic and Gulf of Mexico coastlines of North America ranging from the Gulf of Maine, USA (42°N) to the Yucatán Peninsula, México (19°N) and west to the northcentral Gulf of Mexico, USA (30°N, 88°W). Three species—*Tachypleus tridentatus* (Leach 1819), *T. gigas* (Müller 1785) and *Carcinoscorpius rotundicauda* (Latreille 1802)—inhabit the coastal waters of Asia (Sekiguchi 1988, p. 1).

The first formal surveys of the range, abundance and populations of *T. tridentatus* were conducted between 1975 to 1981 in its southern range and 1980 to 1982 in its northern range (Sekiguchi 1988, pp. 24–45). *Tachypleus tridentatus* broadly ranges in distribution from the north to the south (Sekiguchi 1988, p. 34), extending from Japan in a southerly direction through China (Zhejiang, Fujian, Guangdong, Hong Kong, Guangxi, Hainan), Taiwan, Vietnam, Philippines (Palawan and Sulu Sea), Malaysia (Sarawak and Sabah), Brunei Darussalam and Indonesia, while *T. gigas* and *C. rotundicauda* range from the east to the west (Sekiguchi 1988, p. 35).

All recent horseshoe crabs, including *T. tridentatus* are limited to relatively narrow areas and their distribution ranges are discontinuous (Sekiguchi 1988, p. 410). During surveys conducted between 1975 and 1982, population densities were highest on the China coast in its northern range, especially in Fujian and on the west coast of Hainan, whilst population densities in its southern range were extremely low (Sekiguchi 1988, pp. 24–25, 34). In the south eastern part of its range *T. tridentatus* overlaps with both *T. gigas* and *C. rotundicauda* (Sekiguchi 1988, p. 28), where the sympatric occurrence of all three species sometimes occurs (Sekiguchi 1988, p. 28, 35; Robert *et al*. 2014; Mashar *et al*. 2017a).
A single female *T. tridentatus* was recorded from Udo Island, Jeju, South Korea in 1979 (Yang and Ko 2015), but because this was not associated with a known spawning or juvenile nursery habitat, it is not considered within the species range occurrence in this assessment.

**Northern extent**: The northern most range of *T. tridentatus* is Kasaoka in Okayama Prefecture on Honshu Island in Japan and the Japanese populations represent the northernmost range for *T. tridentatus*. Spawning habitats are distributed intermittently along the coastal regions of Southern Honshu, Shikoku and Northern Kyushu, with a historic concentration around the Seto Inland Sea (Sekiguchi 1988, pp. 34, 39–45; Nishida *et al*. 2015).

**Southern extent**: Horseshoe crabs, including *T. tridentatus* inhabit the north coast of Java, Indonesia but no horseshoe crabs have been recorded from South Java (Nishida 2012a), the possible reason being the sea south of Java is deep close to shore (Laurie/Meilana 2015, pers. comm.).

**Eastern extent**: Manado in North Sulawesi, Indonesia (Sekiguchi 1988, pp. 32, 36; Yamasaki *et al*. 1988; BKIPM 2012) represents the eastern most occurrence of *T. tridentatus* populations. The Wallace Line, a boundary separating Asian and Australasian ecozones, may represent a barrier to the significant eastward expansion of *T. tridentatus* populations (Laurie/Meilana 2015, pers. comm).

**Western extent**: Sibolga in North Sumatra Province and Padang in West Sumatra Province, Indonesia (Sekiguchi 1988, pp. 30, 36; Yamasaki *et al*. 1988, Fig. V-21, p. 96) represent the western most occurrences of *T. tridentatus* populations.

For a more detailed description of the distribution of *T. tridentatus* within its range states, and the spread of the species through transportation and introductions, see the 'Geographic Range' section in the Supplementary Information.

For further information about this species, see Supplementary Material.

### Country Occurrence:

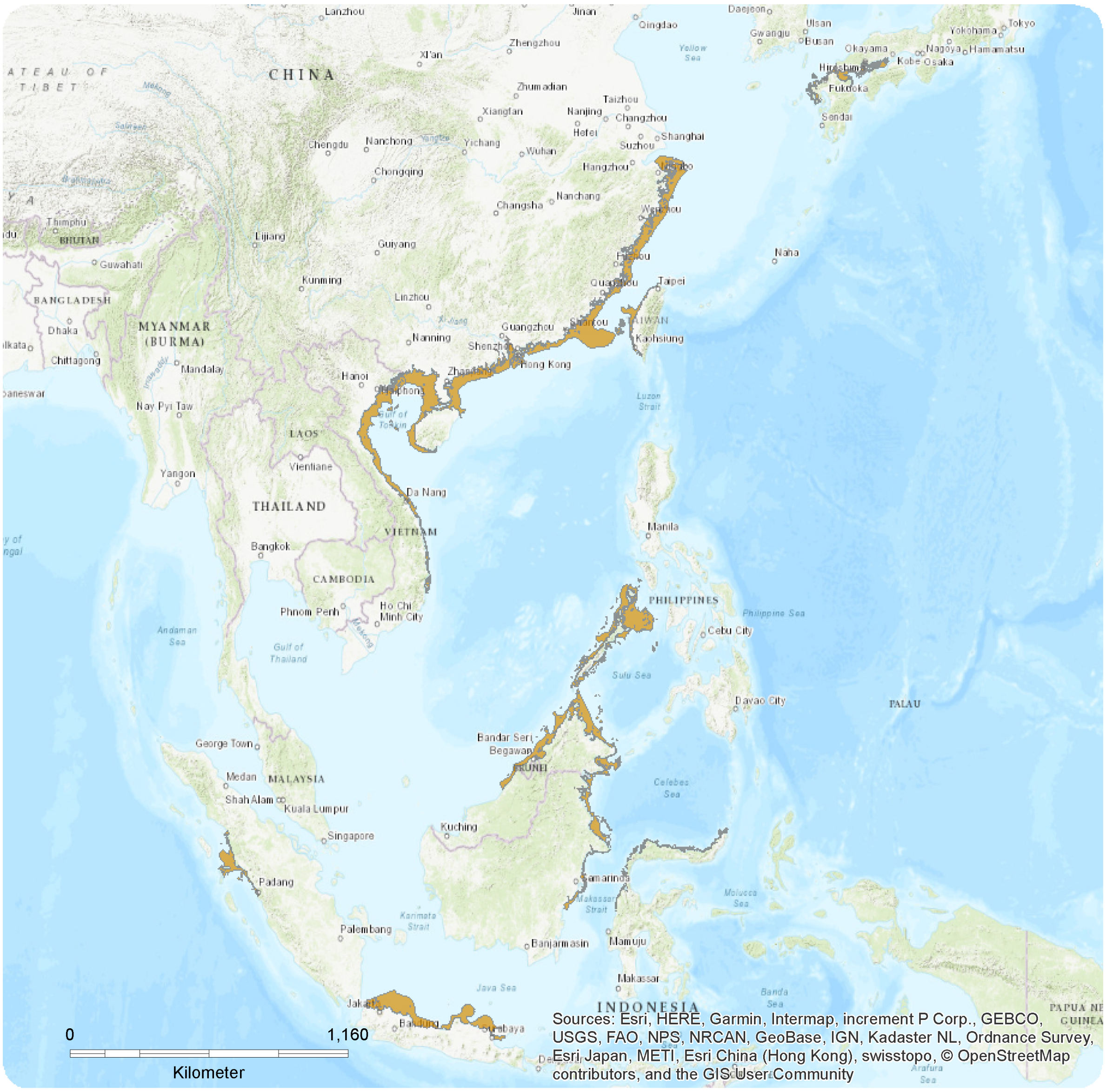
**Native:** Brunei Darussalam; China; Hong Kong; Indonesia; Japan; Malaysia; Philippines; Taiwan, Province of China; Viet Nam

### FAO Marine Fishing Areas:

**Native:** Indian Ocean - eastern, Pacific - western central, Pacific - northwest

# Distribution Map

*Tachypleus tridentatus*

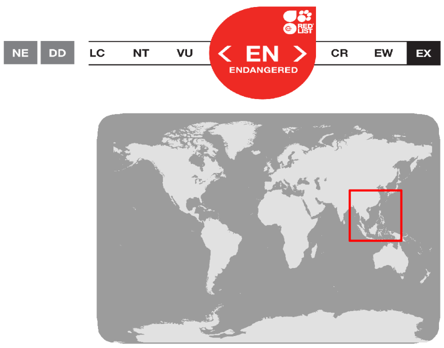



Range

Extant (resident)

Compiled by:

IUCN (International Union for Conservation of Nature)




The boundaries and names shown and the designations used on this map do not imply any official endorsement, acceptance or opinion by IUCN.


http://dx.doi.org/10.2305/IUCN.UK.2019-1.RLTS.T21309A149768986.en

## Population

The population is split into a series of genetically distinct subpopulations across the range of *T. tridentatus* (for a full discussion of genetic diversity and dispersal patterns, see the 'Population' section in the Supplementary Information).

A number of factors may contribute to the presence of subpopulations in *T. tridentatus*. In terms of its ecology, *T. tridentatus* exhibits limited larval dispersal (Botton and Loveland 2003), confined juvenile mobility (Kawahara 1982, Kaiser 2002, Almendral and Schoppe 2005, Kwan *et al*. 2015b), limited adult movement (Wada *et al*. 2016) and spawning beach fidelity (Wada *et al*. 2010, Chan *et al*. 2016, unpublished), which may all be factors contributing to observed patterns of local genetic variation.

Peninsulas

Where noticeable genetic differentiation has been detected, it may be due to geographic separation due to peninsulas including the differences in the eastern and western populations in Japan, separated by the Itoshima Peninsula (Nishida *et al*. 2015) or differences in the Beihai and Zhanjiang populations, separated by the Leizhou Peninsula in southwest China (Weng *et al*. 2013).

Enclosed Bays

Another geographic factor influencing genetic differentiation appears to be the geographic isolation of populations in enclosed bays leading to restricted gene flow. Examples of this type of isolation include the population in Hirao Bay in the eastern Japan group, which exhibit unique genetic features when compared with neighbouring sites (Nishida *et al*. 2015), the limited genetic diversity in the Tiexianwei population in the semi-enclosed Magong Bay in Taiwan (Yang *et al*. 2007), the genetic differentiation seen in the Zhangpu population from the large but semi-enclosed Dangshan Bay in China (Weng *et al*. 2013), and the low haplotype diversity observed in populations on the enclosed north western coast of Lantau Island in Hong Kong (Chan *et al*. 2016, unpublished). In terms of bathymetrics, South-east Asia comprises shallow marginal seas, island arcs and marine basins bounded by deep sea trenches (Hutchinson 1986, 1989) and it is important to note, this underwater 'topography' is equally, if not more important in limiting aquatic dispersal, but in respect of *T. tridentatus* this potential limitation has not yet been tested.

At the extreme end of the range, the presence of the population at Budai, Taiwan with a single haplotype and no genetic diversity is indicative of a population on the verge of extinction (Yang *et al*. 2009a).

The limited dispersal potential and the subdivision of at least some *T. tridentatus* populations to local embayments parallels the documented population structure in the American Horseshoe Crab (*Limulus polyphemus*), especially in the northern and southern portions of its geographic range (Smith *et al*. 2016).

**Population Trends**

Data availability determined whether quantitative analysis was valid for trend estimation. In the absence of a quantitative analysis, descriptive summaries of observations were included to infer qualitative trends in relative abundance and distribution (Smith *et al*. 2016). Population trends are here assessed comparing previous records, which usually involve mortalities together with recent survey data.

Japan

There is a consensus among Japanese scientists that *T. tridentatus* populations have decreased precipitously throughout Japan since the 1950s and populations within the Seto Inland Sea, where they were once common are almost extinct (Botton 2001, p. 44; Nagasaki Prefecture 2001; Iwaoka and Okayama 2009, p. 581). The word 'decimation' has been used to describe these declines (Botton 2001, p. 48). In particular, *T. tridentatus* was once abundant on all of the coasts of the Seto Inland Sea, but coastal change due to human activities has resulted in habitat loss, leading to reduced ranges and reduced populations (Sekiguchi 1988, p. 39).

During the reclamation of Tomioka Bay in 1958, the remains of approximately 10,000 adult *T. tridentatus* were found stranded (Nishii 1973, Seino *et al*. 2003, p. 8) whilst when Kasaoka Bay was reclaimed commencing in 1969, approximately 100,000 adult *T. tridentatus* and millions of juvenile horseshoe crabs were believed to have perished (Seino *et al*. 2003, p. 8).

In the 1970s, it is estimated that the spawning *T. tridentatus* population at Tatara Beach (a protected horseshoe crab breeding area in Imari Bay on northern Kyushu) may have been 500 pairs (Sekiguchi 1988, p. 44). Surveys conducted at Tatara Beach during the 1979 and 1980 peak spawning season recorded a daily peak count of 23 pairs on one day at diurnal high tide, with normally fewer than 20 pairs spawning at any one time, with the largest number of spawning pairs being recorded during a season being less than 30 pairs. A maximum number of 48 spawning pairs were recorded on 22 July 1982 during a night high tide (Sekiguchi 1988, p. 63-64), whilst a similar survey at the same location in 1994 recorded a total of 29 mated pairs, with a daily peak count of five pairs on two consecutive days (Botton *et al*. 1996).

Sone Tidal Flat is 517 hectares (5.17 km²) when exposed and hosts the largest juvenile *T. tridentatus* intertidal nursery ground in Japan. Annual surveys from 1995 to 2013 recorded 120 pairs in 1995 to a maximum of 1581 pairs in 2005, with fluctuating counts, ranging from 513 pairs in 2006; 265 pairs in 2007; 738 pairs in 2012; and 1,079 pairs in 2013 (Hayashi 2015). In 2016, the adult *T. tridentatus* population was estimated to be around 2,400 individuals at the Sone-Higata tidal flat according to the Fukuoka branch of the Horseshoe Crab Preservation Society of Japan (Nihon Kabutogani o Mamoru Kai) (Takahashi 2016).

In Japan, cooperation has been sought from fishermen to assist in horseshoe crab research by reporting or contributing their catches. Between 2002 and 2005, fishermen at three locations of the Kujukushima Islands in Nagasaki Prefecture reported their catches year-round. During this period, 81 adults were caught, with most being collected between May and October, which covered the pre-spawning, peak-spawning and post-spawning periods (Iwaoka and Okayama 2009, pp. 577–578. Table 1, p. 578).

Between 1999 and 2016, annual *T. tridentatus* surveys including records of offshore capture by fishermen and intertidal surveys at known spawning sites were conducted in Etajima and Takehara cities in Hiroshima Prefecture. At Takehara City, during intertidal surveys, a total of seven adults were recorded, including one amplexed spawning pair in 2015, two amplexed spawning pairs and a single adult female in 2016. The maximum number of juveniles observed at one time during these surveys was 46 individuals in April 2016. At Etajima City, 37 adults were recorded in the study period, with a maximum count of 15 individuals in 2008 whilst 40 juveniles were recorded, with a maximum count of 16 individuals in 2008 and spawning was observed once in 2000 (Ohtsuka *et al*. 2017, Laurie/Ohtsuka 2018, pers. comm, Laurie/Nishihara 2018, pers. comm.). Both populations are nearly extinct

(Laurie/Ohtsuka 2018, pers. comm).

In parallel, a continuous monitoring survey of known *T. tridentatus* spawning sites conducted on the Tsuyazaki coast in Fukuoka Prefecture reported a decrease in spawning pairs from 139 pairs recorded in 2005, to 70 pairs in 2006, 45 pairs in 2007 and 40 pairs in 2008 (Wada *et al*. 2010), a 70% reduction in three years. The decreases are believed to be due to habitat degradation caused by coastal development in the area.

China

*Nationally*: In the 1970s *T. tridentatus* was once widely distributed and abundant along the southeast coast of China, below the Yangtze River with high population densities (Sekiguchi 1988, Hong 2011). It could be found 'throughout the seas' and was regarded as an important economic species, but subsequently it suffered significant population declines (Liao and Ye 2000; China Species Red List 2009a,b; Li and Hu 2011; Hong 2011, p. 153; Weng *et al*. 2012b).

*Fujian Province*: Xiamen was a major locality for *T. tridentatus*, where they could be seen all along the beaches at high tide in the spawning season, from May to September and in the 1970s, it was always easy to find them in the coastal waters around Xiamen (Hong 2011, p. 153). They were easy to catch, from the high tide line to water 5 m deep, where they could be found by following their bubbles. Artisanal fishermen could easily catch 50 to 60 pairs at a time, amounting to overall productivity of 5,000 pairs or more a year (Hong 2011, p. 160). However, interviews with fishermen, field surveys and other research undertaken between 2002 and 2004 revealed that *T. tridentatus* populations had decreased sharply after the 1970s and had become scarce (Hong 2011, pp. 159-161).

Pingtan in Fujian was once reputed to have the largest population of *T. tridentatus* on the coast of China, but the population has since experienced dramatic declines (Huang 2011). Huang *et al*. (2002) also reported that in the 1970s, the productivity of the horseshoe crab fishery in Pingtan was reduced by approximately 80 to 90% compared to the 1950s and by the 1990s there were not enough individuals to support the local fishery. Based on official records kept by the Pingtan Ocean and Aquatic Bureau, the estimated productivity of *T. tridentatus* in the Pingtan area was 15,000 pairs in 1984; 9,500 pairs in 1995; 3,700 pairs in 1998; and 1,000 pairs in 2002 (Huang *et al*. 2002), representing a reported fisheries capture decline of 93% between 1984 to 2002. This is on top of the 80 to 90% declines experienced between the 1950's and 1990's, representing a shifting baseline in estimates.

Another site in Fujian where *T. tridentatus* abounded up to the early 1980s was the seas around Dongshan Island (Mikkelsen 1988 p. 70), but surveys between 2006 and 2010 observed no spawning and only recorded the presence of juvenile populations (Weng *et al*. 2012b).

Field surveys and interviews with local fishermen in previously healthy horseshoe crab habitats in 2005 and 2006 in Fujian by Weng *et al*. (2007) revealed *T. tridentatus* was extirpated at Qianqi in Fuding County and Shandu Island in Ningde City, whilst spawning horseshoe crabs were no longer seen on beaches at Huangcuo in Xiamen City, Meizhou Island in Putian City or on Pingtan Island. In July 2015, surveys of the benthic macrofauna in the sandy intertidal zone of Shanqi and Tannan Bay in the Chinese Horseshoe Crab Reserve in Pingtan Island, Fujian failed to find any evidence for the presence of adult or juvenile *T. tridentatus* (Li *et al*. 2017).

*Guangdong Province*: In the 1980s, it was estimated up to 1,500,000 *T. tridentatus* pairs were distributed in Guangdong waters, particularly around the Leizhou Peninsula (Hong 2011, p. 208) but by 1990, this was reduced to between 600,000 to 700,000 pairs (Hong 2011, p. 154). Since then, *T. tridentatus* are difficult to find around the Pearl River delta (Hong 2011, p. 153) and one of the justifications for establishing a conservation zone in the Shantou Tidal Zone Wetland included the identification of *T. tridentatus* as a 'rare species' (UNEP 2005).

*Guangxi Zhuang Autonomous Region*: In Guangxi, prior to the 1980s, mass spawning of *T. tridentatus* was frequently encountered on the shores of Beibu Gulf (Weng *et al*. 2012b) and according to fishermen, before the 1990s, *T. tridentatus* were common in Guangxi, being widely distributed and abundant and seen everywhere, but after the 1990s they were rare to see (Hong 2011, p. 202).

Oceanic research reports estimated populations in excess of 600,000 to 700,000 *T. tridentatus* pairs in Beibu Gulf before the 1990s (Hong 2011, p. 154), but by the late 1990s these had dropped to 300,000 pairs or less (Liao and Li 2001; Hong 2011, p. 202). Historically, up to 1,000,000 pairs of adult *T. tridentatus* may have been exploited in Beibu Gulf to satisfy the demand for consumption and biomedical applications (Liao and Ye 2000, Li and Hu 2011) and according to Professor Guangyao Liang of the Guangxi Oceanic Research Institute, the output of *T. tridentatus* in Guangxi dropped by 90% between 1990 and 2000 largely because of human consumption (People's Daily 2000).

Because adult *T. tridentatus* are so rarely encountered, surveys are now conducted on juveniles in Guangxi. In 2009, a survey of three intertidal mudflats in Guangxi (Hu *et al*. 2011) recorded 1,742 juveniles at Xi Bei Ling, with a distribution of 1.79 individuals/100 $m^2$, 2,753 juveniles at Jin Hai Wai, with a distribution of 3.19 individuals/100 $m^2$ and 441 juveniles at Xi Cheng, with a distribution of 0.88 individuals/100 $m^2$.

By 2014, it was estimated populations of *T. tridentatus* in Beibu Gulf had decreased by over 90% and about 10% of the remaining adults were being harvested annually. The local biomedical industry estimated that over 80% of the harvested *T. tridentatus* were being consumed as food and 20% were being bled to produce Tachypleus Amebocyte Lysate (TAL) (Fauna and Flora International 2014). Such a decline was supported by responses to a survey in September 2015 of 407 respondents from 29 communities along Beibu Gulf (Liao *et al*. 2017). Elder respondents recalled commonly seeing *T. tridentatus* spawning on the shores when they were younger, but almost all respondents had noted an overall reduction in *T. tridentatus* populations, with the average daily harvest of adult *T. tridentatus* declining sharply from an approximate range of around 50 to 1,000 individuals in the 1990s to 0 to 30 individuals by 2015. Nearly all respondents opined there had been an overall reduction in the abundance of *T. tridentatus* in the previous five years.

*Hainan Province*: Up to the early 1980s, *T. tridentatus* could be found in major concentrations on the west coast of Hainan (Mikkelsen 1988. p. 71; Sekiguchi 1988. p. 34) and before the 1990s, it was estimated up to 300,000 pairs of *T. tridentatus* were distributed in the seas to the north of Hainan (Hong 2011, p. 154).

From 2006 to 2010, interviews with fishermen and surveys were conducted at 27 nationally recognized *T. tridentatus* spawning sites on the coasts of Zhejiang, Fujian, Guangdong, Guangxi and Hainan (Weng *et al*. 2012b). Fishermen reported populations had decreased greatly in most of the coasts surveyed, no spawning was observed at the 27 sites, a few juveniles were found at only 6 of the 27 sites and no 'new recruit' Instar I to Instar III juveniles were found at Longkou in Dongshan Bay in Fujian. Such data indicate that *T. tridentatus* had almost disappeared from much of its range in China, with the exception of Dongshan Bay in Fujian, where over 1,000 juveniles were found and Beibu Bay in Guangxi. By 2004, it was nationally recognized that *T. tridentatus* populations had seriously declined due to over-exploitation (China Species Red List 2009b), such that it was considered to be on the brink of extinction in Chinese waters (Xie and Weng 2011) and in need of urgent protection (Weng *et al*. 2012b).

Taiwan

*Tachypleus tridentatus* was once widespread and abundant along the west coast of Taiwan Island, in the Penghu Islands and Kinmen with thriving populations, as attested to by evidence from historical records, literature, colloquial language, daily usage and place names (Chen *et al*. 2004, Chen and Chen 2011), but populations in Taiwan are declining (Hsieh and Chen 2015). No individual adults or mating adult pairs have been recorded from the intertidal flats of the Taiwan Island coast since the 1960s, nor more recently from Kinmen Island (Hsieh and Chen 2015). However, over the past decade, each year there have been occasional reports of adult horseshoe crabs being landed at fishing wharfs in Taiwan (maximum four or five individuals ever documented from each report), suggesting the adult population in the waters surrounding Taiwan is limited.

Because adult *T. tridentatus* are so uncommon, surveys have been conducted on juveniles in Taiwan. In 2003, surveys over several weeks on the Penghu Islands found only 20 juveniles (Hsieh and Chen 2015) and on Taiwan Island a small population of juveniles was documented at the Haomeiliao Nature Reserve on the Longgong River Estuary in Budai, Chiayi County between 2004 and 2005. However, none have been found there since 2007 (Yang *et al*. 2009b), whilst one juvenile was reported from Xiangshan intertidal flat at Hsinchu City in Taiwan Island in 2013 (Hsieh and Chen 2015).

Further reported by Hsieh and Chen (2015), a study of juvenile densities at Beishan, Nanshan, Hsiashu on Kinmen between 2003 and 2009 showed rapid and significant declines in the juvenile populations at Nanshan and Hsiashu. Juvenile abundance at Nanshan diminished from 0.18 individuals/m$^2$ in 2003 to 0.01 individuals/m$^2$ in 2009, whilst at Hsiashu, juvenile abundance declined from 1.02 individuals/m$^2$ on average in 2003 to 0.18 individuals/m$^2$ on average in 2009. In contrast, the juvenile population at Beishan changed little and fluctuated between 0.37 individuals/m$^2$ in 2003 and 0.03 individuals/m$^2$ in 2007. Beishan and Nanshan are located within the Kinmen Kuningtou Northwest Intertidal Terrain Horseshoe Crab Conservation Area (Hsieh and Chen 2009) and when data from Beishan and Nanshan are combined, the juvenile density decreased from 0.30 individuals/m$^2$ in 2003 to a range from 0.02 to 0.17 individuals/m$^2$ from 2004 to 2009. In 2006, the Kinmen Fisheries Research Institute purchased 57 adults from fishermen and 335 individuals in 2007, but no adults were offered by fishermen in 2008, whilst the Penghu Fisheries Research Institute purchased 209 adults from fishermen in 2008. A survey for adult *T. tridentatus* using drift gillnets over three nights around Penghu Islands in November 2008 only caught two sub-adult males, whilst a separate survey conducted for one day by Kinmen Fisheries Research Institute using bottom trawl nets in 2008 harvested 12 adults (Hsieh and Chen 2015).

*Tachypleus tridentatus* is now extirpated at many locations along the west coast of Taiwan Island and between 1998 and 2012 surveys found only scattered juvenile populations left (Yang *et al*. 2007, Chen 2011, Chang 2012). It is found at two locations on the Penghu Islands, with viable juvenile populations restricted to a number of sites on Kinmen Island, but these populations are declining, even in the

Kinmen Kuningtou Northwest Intertidal Terrain Horseshoe Crab Conservation Area, a designated reserve (Hsieh and Chen 2015).

Hong Kong

*Tachypleus tridentatus* was once widespread and abundant throughout Hong Kong waters with thriving populations, as attested to by evidence from anecdotal accounts and place names (Shin *et al*. 2014). Major concentrations of *T. tridentatus* could be found in Hong Kong up to the early 1980s (Mikkelsen 1988, p. 71), but populations are declining (Huang *et al*. 1998, Chiu and Morton 1999, Chiu 2003, Shin *et al*. 2014). Fishermen and older residents recalled *T. tridentatus* being abundant in Hong Kong. In 1962 a retired police officer remembered seeing 'hundreds' of pairs of *T. tridentatus* on a beach at Lung Kwu Sheung Tan and from the 1960s to 1970s, fishermen in Ha Pak Nai would collect up to 200 *T. tridentatus* as bycatch in their fishing nets in a single night. There were so many on the mudflats on some days, people had to be careful where they walked, whilst in 1979 another police officer recalled seeing 'thousands' of *T. tridentatus* covering the beaches on Chep Lap Kok, spread out in long chains as they were mating (Laurie/Various pers. comm).

The historic widespread occurrence of horseshoe crabs in Hong Kong may be indicated indirectly from place names (Shin *et al*. 2014) and a study of historical distributions of horseshoe crabs through interviews with local fishermen and elderly villagers revealed mating pairs of *T. tridentatus* were often seen in great numbers on many beaches in Hong Kong during the summer months until the 1980s and adults were evenly distributed throughout the waters of Hong Kong (Chiu and Morton 1999). However, by the early 1990s mating pairs were no longer seen on beaches where they had previously occurred and according to local fishermen in 1998 (Huang *et al*. 1998), the Pak Nai mudflat used to attract large numbers of spawning *T. tridentatus* in summer, but few in the last decade.

*Tachypleus tridentatus* has since disappeared from much of its range, whilst reclamation has claimed many historic spawning and juvenile nursery grounds and only lesser numbers of individuals can be found in Deep Bay as compared with anecdotal records (Chiu and Morton 1999, Chiu 2003). According to monthly reports related to a marine ecological survey by the Provisional Airport Authority in 1995 (Huang 1997), no horseshoe crabs were collected from the Sha Chau area, adjacent to Hong Kong's new airport at Chek Lap Kok, although the area was once said to be a thriving habitat for *T. tridentatus*.

Because adult horseshoe crab densities are very low in the seas around Hong Kong (Li 2008), the visual spawning survey of mating pairs on the shores or sea-bottom trawling of adults used for estimating populations of American Horseshoe Crabs (Ehlinger *et al*. 2003, Hata and Berkson 2003) are not practical. The last time a mating pair of *T. tridentatus* was scientifically observed in Hong Kong was at Lung Kwu Sheung Tan in 1986 (Huang *et al*. 1998, Chiu and Morton 1999). Surveys at wholesale fish markets, seafood restaurants, fish sellers and with local fishermen indicated 332 adult horseshoe crabs, mainly *T. tridentatus* were caught in Hong Kong waters from 2004 to 2005 (Shin *et al*. 2009), whilst in a series of studies to investigate the effectiveness of a trawling ban in Hong Kong on demersal fish and crustacean communities, only 13 sub-adults of *T. tridentatus* were recovered from systematic trawl surveys conducted at 12 sampling sites in three different areas of Hong Kong waters between 2012 and 2015 (Laurie/Tao 2018, pers. comm). Where fishermen at Ha Pak Nai caught up to 200 *T. tridentatus* in a single night, they now catch two to three pairs annually (Laurie/Various pers. comm). As adult *T. tridentatus* are so rare in Hong Kong, population estimates and trends are by necessity based on juvenile surveys (Kwan *et al*. 2016).

The first set of quantitative data on juvenile *T. tridentatus* in Hong Kong was reported from a study along the northwestern coastline of the New Territories in 2002 (Morton and Lee 2003), taken as the baseline, followed by surveys in 2004 and 2005 (Shin *et al*. 2009), 2007 (Morton and Lee 2010) and 2012 to 2014 (Kwan *et al*. 2016). In 2002, surveys at four shores in Deep Bay recorded displayed mean juvenile densities from 0.10-1.97 individuals/100 m² (Morton and Lee 2010); in 2004, the same localities displayed mean juvenile densities from 0.08-0.23 individuals/100 m² (Shin *et al*. 2009); in 2007 from 0.00-0.86 individuals/100 m² (Morton and Lee 2010); in 2012 from 0.00-1.17 individuals/100 m² (Kwan *et al*. 2016) and in 2014 from 0.1-1.97 individuals/100 m² (Kwan *et al*. 2016). Surveys at other localities found similar low mean juvenile densities and from surveys conducted between 2012 and 2014, no 'new recruit' Instar I to Instar III juveniles were found throughout Hong Kong (Kwan *et al*. 2016).

The presence of small and discrete *T. tridentatus* juvenile populations on only a few shores in Hong Kong, coupled with relatively few new recruits (Kwan *et al*. 2016), slow growth rate and length of time of between 13 to 14 years to reach maturity (Hu *et al*. 2015), suggest these populations are fragile and vulnerable to local extirpation (Kwan *et al*. 2016). In 2016, the population of juvenile *T. tridentatus* was estimated to be between 2,100 to 4,300 individuals on all horseshoe crab juvenile nursery grounds in Hong Kong (Kwan *et al*. 2016), about 60% of which reside on the intertidal mudflats at Pak Nai and Ha Pak Nai (Shin *et al*. 2014).

Viet Nam

Up to the early 1980s major concentrations of *T. tridentatus* could be found along the coast of Viet Nam, at least as far south as Nha-Trang (Mikkelsen 1988), but populations are decreasing. Before 1990 *T. tridentatus* was commonly found on sand banks in tidal areas and coastal estuaries along the coast of Viet Nam, most abundantly in the Central Coastal Province, but between 1990 and 2007 it is estimated the number of individuals declined by 50%, the area of occupancy in Viet Nam declined by 50% and harvest yield declined by 20% (Nguyen 2007). Since then, habitat loss has continued unabated (Vietnam NBSAP 2015) and populations have been targeted to supply the TAL industry and consumer markets in China (Laurie/Novitsky pers. comm. 2014, Laurie/Do pers. comm. 2014). *Tachypleus tridentatus*, which was once historically abundant in the Gulf of Tonkin, is now considered endangered because of over-exploitation (Liu 2013).

Philippines

Historic information suggests *T. tridentatus* was once widely distributed throughout the Sulu Sea, including on both the west and east shores of Palawan, Danjugan Island on the east coast of Negros Occidental, Tangalan on the northwest coast of Panay, Tigbauan on the south coast of Panay, Cagayancillo in the centre of the Sulu Sea and Turtle Island and Tawitawi in the south of the Sulu Sea (Schoppe 2002). However, *T. tridentatus* experienced significant declines in population numbers throughout its range in the decades preceding 2000 (Schoppe 2002). *Tachypleus tridentatus* was also recorded from Zambales on the east coast of Luzon (Sekiguchi 1988 p 35), but surveys in 2011 failed to find any evidence of its presence there (Nishida 2012b).

In Palawan, surveys conducted between 1999 and 2001 indicated *T. tridentatus* was present in coastal municipalities extending the length of Palawan including Quezon, Aborlan and El Nido on the west coast and Brookes Point, Espanola, Narra, Puerto Princesa City, Roxas, Dumaran, Araceli, Taytay and the islands of Agutaya, Magsaysay and Cagayancillo (Schoppe 2002). Juvenile nursery beaches have only been studied at San Pedro, Puerto Princesa (Kaiser 2002) and Aventura Beach, Puerto Princesa on the east coast of Palawan (Almendral and Schoppe 2005). It has also been recorded from Busuanga Island in the northern end of the Palawan Island Chain (WWF 2010; Bautista *et al*. 2016, p. 84).

There is currently no information available on *T. tridentatus* population trends in the Philippines, although the juvenile populations that have been studied at nursery beaches are small. In an eight-month survey of San Pedro nursery beach between May and December 2001, 374 juveniles were recorded (Dorkas 2002), whilst in a five-month study from June to October 2002 at Aventura Beach, 125 juveniles were recorded (Almendral and Schoppe 2005).

Malaysia

Most horseshoe crab research in Malaysia has focused on Peninsula Malaysia on the two horseshoe crab species that occur there, *T. gigas* and *Carcinoscorpius rotundicauda* (John *et al*. 2012, Mohamad *et al*. 2015). *Tachypleus tridentatus* population studies have only recently commenced in Sabah (Robert *et al*. 2014, Manca *et al*. 2017, Mohamad *et al*. 2017) and are yet to be systematically undertaken in Sarawak (Laurie/John 2017, pers. comm). As such, the status of *T. tridentatus* in East Malaysia is still unknown, but anecdotal reports (Manca *et al*. 2017) and interviews with locals (Laurie/Robert 2014, pers. comm.) suggest the populations are decreasing.

Over the course of an adult survey conducted over four days during daytime of the highest spring tide between April and October 2014, 195 adult individuals as amplexed pairs (98 males; 97 females) were visually located along an 8,000 m stretch of a spawning beach  at Tanjung Limau, Sabah. In the same period, in waters 1 to 3 km off the coast of Inderasabah, Sabah, because no spawning beach could be located, 113 non-spawning adult individuals (80 males; 33 females) were caught using gill nets deployed at up to 10 m depth (Mohamad *et al*. 2016). In another 5-month study of *T. tridentatus* in Tawau conducted from October 2014 to September 2015 using gill nets deployed in waters up to 5 m deep approximately 1 to 3 km from the shore,  271 adults, representing 267 individuals (188 males; 88 females) with four recaptures were recovered (Manca *et al*. 2017). From the above two studies, male-biased Operational Sex Ratios (OSR) were recorded, with 2.42:1 at Inderasabah, Sabah (Mohamad *et al*. 2016) and 2.08:1 at Tawau, Sabah (Manca *et al*. 2017).

Brunei Darussalam

Horseshoe crab studies commenced in 2017 in Brunei Darussalam, so apart from confirming occurrence, there is currently no information available on *T. tridentatus* populations (Laurie/Marshall 2018, pers. comm.), although because of its small geographic size, it is unlikely significant populations are resident here.

Indonesia

According to the Biodiversity Action Plan for Indonesia in 1993, all three species of Asian horseshoe crab, *T. tridentatus*, *T. gigas* and *Carcinoscorpius rotundicauda* were considered either Rare (R), Vulnerable (V) or Endangered (E), but were listed as insufficiently known (K) because of lack of information (Indonesia NBSAP 1993, p. 86).

Basic research on horseshoe crabs in Indonesia was conducted from 1990 to 2000, then stopped (Mashar *et al*. 2017b), when the focus was on *T. gigas* and *C. rotundicauda* (Meilana *et al*. 2015). Recent research has focused on establishing baseline distribution data (Nishida 2012, Mashar *et al*. 2017a), including identifying population and stock status, locations of spawning grounds, nursery grounds, adult habitat range and rates of exploitation for all three horseshoe crab species, including *T. tridentatus* (Mashar *et al*. 2017b). In this respect, *T. tridentatus* has until recently been understudied in Indonesia and is therefore still listed as insufficiently known (K), but interviews with fishermen on the north coast of Java revealed horseshoe crab catches are declining (Meilana and Fang 2017).

For further information about this species, see Supplementary Material.

**Current Population Trend:** Decreasing

## Habitat and Ecology **(see Appendix for additional information)**

All four extant species of horseshoe crab have different habitat requirements at different stages of their life cycle and their habitat requirements and life-history characteristics appear to be similar (Shuster 1982). Each of the species has specific and specialized habitat requirements related to spawning, juvenile development and adulthood and the close proximity of different habitats to meet the differing demands of their various stages of development is critical to their survival needs (Shuster and Sekiguchi 2009).

In *T. tridentatus*, beaches are needed for laying eggs along the high tide mark. There must be intertidal mudflats in close proximity, preferably with seagrass beds, so juveniles can develop and mature. It is also essential to have shallow water offshore in which large juveniles and sub-adults can feed and deeper water offshore for adults to forage after the spawning season is over (Itow 1993, Seino *et al*. 2003, Wada *et al*. 2016).

Horseshoe crab spawning and juvenile intertidal habitats are subject to spatial and temporal variations because of the complex interrelationship between hydrology, geomorphology, light, temperature, nutrients and other environmental factors, as well as chemical variations, caused by factors such as variable river discharge, tributaries with different input composition, precipitation and influx of materials at the sediment-water interface. At a given time, environmental conditions and hydrographic features together may influence certain biological processes including distribution, abundance, physiology and reproduction, as well as the phytoplankton which supports the benthic macro- and meio-fauna at the base of the food chain (Rathod 1992). Such factors guide site selection and site avoidance for all life-style stages.

Populations of *T. tridentatus* in the northern part of its range are found in coastal muddy bottom regions, whilst the southern populations live along sandy coasts and often in coral reefs, indicating adaption to different living environments through its range (Sekiguchi 1988, pp. 32, 53, 414).

For more details on spawning, larval development, growth, mortality, and biology and ecology, see the 'Habitat, Biology & Ecology' section in the Supplementary Information.

**Generation Length**

Generation length in *T. tridentatus* has been estimated based on the following:

Although there is variation in generation length among horseshoe crab species, all are relatively slow maturing, exhibiting particularly slow growth rates, and age-to-maturity for *T. tridentatus* is the longest of the four extant species.

Studies of *Limulus polyphemus* indicated it takes 9-10 years to reach maturity (Shuster and Sekiguchi 2003); the longest live retrieval in an adult tagging study in a published report was 10 years (Swan 2005), whilst in the United States Fish and Wildlife Services adult Horseshoe Crab Tagging Program 13 crabs were retrieved alive 15-17 years after they were tagged (Laurie/Newhard 2018, pers. comm.), suggesting at least similar times can be used for age-to-maturity and age-at-maturity estimates.

In *T. tridentatus* there is evidence to suggest different age-to-maturity rates at different latitudes, but in Japan and China, where data are available, estimates for age-to-maturity are between 13 to 14 years, the female taking longer than the male to reach maturity (Sekiguchi 1988, p. 194; Hu *et al*. 2015).

Based on the retrieval times recorded in tagging studies for *L. polyphemus*, this assessment adopts a conservative approach and uses similar times for age-to-maturity and age-at-maturity, giving a generation length of 20.25 years (maturity plus one half reproductive longevity) for *T. tridentatus*, resulting in a 3-generation length of 60.75 years (rounded to 60 years).

For further information about this species, see Supplementary Material.

**Systems:** Terrestrial, Marine

# Use and Trade

**Biomedical and Pharmaceutical Industries**

***Tachypleus Amebocyte Lysate (TAL)***

Horseshoe crabs are harvested by the biomedical industry for the manufacture of Amebocyte Lysate, which is used to test for gram-negative bacterial contamination in injectable drugs and implantable medical devices. The LAL test was commercialized in the 1970s and is currently the global standard for screening medical equipment for bacterial contamination (Levin *et al*. 2003). The studies of the blood-coagulating proteins of horseshoe crabs and their reaction with bacterial endotoxins aroused the interest of Japanese medical and pharmaceutical researchers and in October 1976 a symposium was held at Kasaoka, Japan entitled 'Contribution of the Horseshoe Crabs to Medical Science', followed two years later by a similar symposium held in the United States (Cohen 1979).

The global Amebocyte Lysate market is largely supplied from two sources, Limulus Amebocyte Lysate (LAL), derived from *L. polyphemus*, and Tachypleus Amebocyte Lysate (TAL), derived from *T. tridentatus*. Whilst blood from *L. polyphemus* for LAL production in the USA is obtained by collecting adult crabs and extracting a portion of their blood, then releasing them (Smith *et al*. 2016), the practice in Asia is to drain all of the animals blood resulting in its death (Hong 2011, p. 154; Laurie/Novitsky 2014, pers. comm).

There are two TAL producers in Japan: Seikagaku Corporation, which is the parent company of Associates of Cape Cod, USA and Wako Pure Chemical Industries Limited, which is the parent company of Wako Chemical, USA (Laurie/Novitsky pers. comm. 2014). Seikaguku Corporation commercialized a TAL reagent in 1978, but did not pursue a US licence until 1995 (Easter 2003, pp. 129-130). In China, there are eight manufacturers of TAL reagent (Cai *et al*. 2017). Four of them are Zhejiang Pukang Biotechnology in Zhejiang, Xiamen Bioendo Technology (Xiamen Chinese Horseshoe Crab Reagent Manufactory), Fuzhou Xinbei Biochemical (Fuzhou Xinbei Biological Industrial) in Fujian and Zhanjiang Bokang Marine Biological (Zhanjiang A&C Biological), a subsidiary of Charles River Laboratories of the USA in Guangdong.

The over-exploitation and unrestricted capture of *T. tridentatus* to supply blood to the lysate and biomedical industry is recognized as being a major contributor to significant population declines in China (China Species Red List 2009b, Gauvry 2015, Mizumura *et al*. 2017). When *T. tridentatus* is used in the TAL industry, all of the animal's parts are used, because there is demand for its blood, meat and the chitin in its shell, making *T. tridentatus* an 'all parts use animal' (Hong 2011, p. 154; Laurie/Novitsky 2014, pers. comm).

In terms of recent annual usage, in the 2010's, in Fujian, China a facility on Dongbi Island, Fuqing Bay, Fuzhou held and bled about 40,000 *T. tridentatus* each year which were sourced from Guangxi, Guandong, Hainan (Laurie/Novitsky 2014, pers. comm.) and Viet Nam (Laurie/Novitsky 2014, pers. comm.; Laurie/Do 2014, pers. comm.), where they were collected and penned in Beihei. Then when there was enough stock, they were transported to Dongbi by truck. In Guangxi, a lysate factory near Beihei bleeds about 60,000 crabs each year, whereas a facility in Dongxing, Fangchenggang supplies 20,000 to 30,000 pairs to Zhanjiang A&C Biological Ltd, 8,000 pairs to Xinbei and because of their higher price, 20,000 to 30,000 pairs are supplied directly to restaurants. In Hainan, several thousand pairs of crabs are supplied from a holding facility in Danzhou to Zhanjiang Bokang Marine Biological Co. Ltd. in Zhanjiang. All crabs held at these facilities are bled completely, then what remains are processed for sale as food and supply of carapaces for chitin (Laurie/Novitsky 2014, pers. comm). According to the National Institute for Food and Drug Control in Beijing, limuloid resources are exhausted in China (Pei *et al*. 2014).

In Hong Kong, between 1980 and 2001, a Japanese pharmaceutical company set up a clandestine, but legal horseshoe crab bleeding facility, to extract TAL from local populations of *T. tridentatus*. This facility was set up in a shark fin warehouse and horseshoe crabs were delivered on a daily basis from the local trawling fleet. The crabs were bled dry, then the carcasses were returned to the fishermen for disposal. In parallel with these activities, adult populations of *T. tridentatus* in Hong Kong collapsed, with conservative scientific estimates putting population declines at around 90% (Shin *et al*. 2014). At this time, the Hong Kong trawling fleet comprised approximately 1,200 trawlers, of which about 650 large trawlers mainly operated in Chinese waters, while the remaining 550 small-to-medium size trawlers operated partly or wholly in Hong Kong waters (LegCo 2010). It is therefore likely many of the horseshoe crabs captured to supply this bleeding operation were caught in Chinese waters, contributing to population collapses there.

In the Klong Yai district of Trat Province in Eastern Thailand a horseshoe crab processing factory operates, which in addition to other functions appears to be a *T. tridentatus* bleeding facility. The horseshoe crabs are sourced from Vietnam and once processed, products are sent abroad (Human Trafficking Organization 2007).

New research into TAL alternatives is being fueled by the realization the current industry is being supported through the unsustainable requirement for raw materials (Mizumura et al. 2017) Although a recombinant Factor C (rFC) based endotoxin test is now available, the industry has been slow to adopt it as an alternative to LAL or TAL (Li *et al*. 2015).

***Chitin***

Chitin and its derivative chitosan are of considerable interest to the biomedical, food, biotechnology and pharmaceutical fields because of their beneficial properties including biocompatibility, safety, non-toxicity, non-allergenicity and biodegradability, as well as antifungal, antibacterial, antitumor, immune-adjuvant, anti-thrombogenic, anti-cholesteremic and bio-adhesivity capabilities (Younes and Rinaudo 2015). Applications include use as cationic agents for polluted wastewater treatment, agricultural materials, food and feed additives, hypo-cholesterolemic agents, biomedical and pharmaceutical materials, wound-healing materials, blood anticoagulant, anti-thrombogenic and hemostatic materials, cosmetic ingredients, textile, paper, film and sponge sheet materials, chromatographic and immobilizing media, and analytical reagents (Hirano 1996). Horseshoe crab chitin has thus received much attention in particular because of its particular non-toxicity and biodegradability (Balasubramanian and Khan 2007).

**Consumption and Use**

In Japan, *T. tridentatus* is neither used as food nor bait (Botton 2001, p. 44) and historically, there was no target fishery for horseshoe crabs. However, those caught as bycatch were dried and used as compost (Tsuchiya 2009, pp. 560-561).
In the coastal provinces of China, *T. tridentatus* was historically a popular dish, being regarded as good and rare, and treated as a delicacy. Consumption was limited to the coastal provinces where it was found, due to the lack of transportation networks along the coast, so it was not traditionally consumed elsewhere. The opening up of coastal areas with the construction of roads has made it easier to transport *T. tridentatus* to new markets, so that considerable quantities are now transported between coastal provinces to meet demand. Furthermore, as it becomes rarer, it is being accorded the status of a culinary 'treasure', making it more expensive, and the rarer it becomes, the more sought after it will become, incentivising fishermen to target it as catch (Hong 2011, p. 154).

In Fujian and Guangxi, whilst some *T. tridentatus* are exploited for medicinal uses, the majority are harvested as food, where *T. tridentatus* is considered as a delicacy and highly nutritious food. In Guangxi, horseshoe crabs are sold in seafood restaurants, and some cook books and magazines teach people how to cook them and advertise the high nutritional value and good taste of their meat, particularly in soup prepared from them (Li *et al*. 2011). Surveys of markets and restaurants along the coastal provinces of China in 2006 to 2007 showed that despite being protected at the provincial level, *T. tridentatus* was widely available and to meet consumer demand in Zhejiang and Fujian, where horseshoe crabs were scarce, the crabs were smuggled across provinces from Guangdong and Guangxi (Weng *et al*. 2012).

In Taiwan, historically horseshoe crabs were consumed on Taiwan Island and Kinmen but as so few adults are now caught, the unpredictable catch can no longer meet the demand of restaurants (Hsieh and Chen 2015).

In Hong Kong, surveys of fish markets between 1995 and 1998 revealed adult *T. tridentatus* were sourced from local waters, the South China Sea and Indonesia (Chiu and Morton 1999). A further comprehensive market survey was conducted from 2004 to 2005 at wholesale fish markets, seafood restaurants, fish sellers and with local fishermen for information on the source, sale and utilization of adult horseshoe crabs. Of the 1,023 adult horseshoe crabs recorded, mainly comprising *T. tridentatus*, 691 individuals (68%) were caught in Chinese waters and 332 individuals (32%) were caught in Hong Kong, by shrimp trawlers and occasionally by netting and cage fishing methods. In terms of seasonal variations, more individuals were caught from September to December 2004 than in the remaining months in 2005. About one-third of the horseshoe crabs collected from trawl nets were released after capture, whilst the remaining two-thirds, about 690 individuals, were kept and sold to wholesale fish markets or seafood restaurants in Hong Kong. Of these, 425 (about 62%) were sold for use in Chinese traditional 'set-free' rituals, whilst 265 were retained by fish stalls and seafood restaurants, either for use as photographic props as a way to attract customers and for photograph taking by tourists, or they were served as food. 'Set-free' rituals are practices by Chinese Buddhist followers who believe if they release live animals back to the wild, they will have relinquished their sins of killing animals in their daily life (Shin *et al*. 2009).

There is currently no target fishery of horseshoe crabs in Hong Kong, although adults are sometimes caught entangled in fishing nets which are erected across the coastline of some shores, including across river mouths at high tides for harvesting fish, crabs or shrimp (Shin *et al*. 2014).

In Viet Nam, horseshoe crabs are not protected, but are recognized as a legally exploitable resource (Nguyen 2007), where they are collected for local consumption and for supplying demand in China, as well as for international trade (Laurie/Do 2014, pers. comm).

**Targeting of gravid females**

In artisanal fisheries, gravid females are targeted for their eggs in the Philippines (Schoppe 2002), Indonesia (Meilana *et al*. 2015) and Sabah in Malaysia, where female-biased harvesting to fulfil high demands from locals and consumers in neighbouring countries may contribute to male-biased OSR (Christianus and Saad 2007, Manca *et al*. 2015, Laurie/Mohamad 2017, pers. comm).

**Talisman and other uses**

In addition to being a source of food, *T. tridentatus* is used for decoration, for sale to tourists or for other uses such as talisman, spiritual or medicinal properties and dead horseshoe crabs are frequently found decorating local homes in the Philippines (Schoppe 2001), including as wall decorations to protect children from vampires (Kaiser 2002, p. 113). In certain areas of Sabah, Malaysia, locals hang *T. tridentatus* in their homes for protection from bad spirits (Manca *et al*. 2017), and when a new plantation is opened a horseshoe crab, which represents fertility, may be sacrificed to ensure a good harvest (Laurie/Mohamad 2017, pers. comm). *Tachypleus tridentatus* is also sold for use in traditional healing remedies in Indonesia (Meilana *et al*. 2015).

In Philippines, adult *T. tridentatus* are sold in the Puerto Princesa city market both as live animals and as decorative items (Schoppe 2001). There is also an international trade in dried *T. tridentatus* carapaces from Palawan, which are sold on eBay and other online trading platforms via seashell and marine curio suppliers, although the extent of this trade has not been quantified (Laurie 2009-2014, pers. obs).

**Illegal Trade**

Sabah, Malaysia has strict regulations relating to the collection, study and export of biodiversity effectively meaning no one can access or export biodiversity without licences issued by the Sabah Biodiversity Council (SaBC 2017). However, commencing in 2017, Thai tourists visiting Inderesabah started buying gravid female T. tridentatus, extracting their eggs, then taking them back to Thailand in almost certain violation of these regulations (Laurie/Mohamad 2018, pers. comm).
In Indonesia, horseshoe crabs were protected in Indonesia by Law No. 5 of 1990 of the Republic of Indonesia on the Conservation of Living Resources and their Ecosystems, under which it is illegal to take, keep, transport or trade in a protected species, including exporting or transporting internally within Indonesia (Indonesia Law No. 5/1990). This law only applied to *T. gigas*, but because of look-alike and interpretation issues, it was applied to all three horseshoe crab species in Indonesia (Laurie/Meilana 2015, pers. comm., Laurie/Wardiatno 2017a, pers. comm.). Despite this, and despite the best efforts of Indonesia law enforcement agencies, there is a substantial illegal trade in horseshoe crabs, which are usually smuggled via ports on the northeast coast of Sumatra to Malaysia, for onward transmission to Thailand. Whilst the principal focus of this trade is gravid *T. gigas* for consumption in Thailand, seizures of *T. tridentatus* are also made (Laurie/Meilana 2015, pers. comm.; Laurie/John 2017, pers. comm). Traders are also visiting remote fishing villages in Indonesia to recruit fishermen to capture horseshoe crabs for the international trade (Laurie/Meilana 2015, pers. comm). As an example, on 23 May 2012, five *T. tridentatus* were seized at Sam Ratulangi International Airport, Manado, North Sulawesi under PP No. 7/1999 (Indonesia Government Regulation No. 7/1999) and although described as T, gigas, photographs indicated the seized animals were *T. tridentatus* (BKIPM 2012).

In June 2018, Indonesia’s Ministry of Environment and Forestry upgraded PP No. 7/1999 (Indonesia Government Regulation No. 7/1999) and issued new regulations which made all three horseshoe crab species, including *T. tridentatus* protected species in Indonesia (Indonesia Government Regulation 20/2018).

## Threats **(see Appendix for additional information)**

The following sub-sections summarize the country level assessment and list the potential threats of *T. tridentatus* throughout its range. As concluded by Hu *et al*. (2015) from a study of three juvenile nursery grounds in Beibu Gulf, China, once a local horseshoe crab population is extirpated, even under ideal conditions, it would take a long period of time for them to re-establish themselves.

**Assessments of Country Threats** Country level assessment on *T. tridentatus* has been conducted only in Japan, China and Vietnam thus far and a synopsis is given below.

In Japan, *T. tridentatus* was assessed to be Critically Endangered (CR + EN) in the Red List and the Red Data Book of Japan in 2006 (Ministry of the Environment Japan 2006), mainly because of the loss or deterioration of its tidal flat habitats (Japan NBSAP 2014, p. 15) and marine and coastal ecosystem habitat and biodiversity loss in Japan has not been contained or halted. It is a continuing trend (Japan NBSAP 2014, p. 22).

In China, *T. tridentatus* was assessed in 2004 (year of assessment) to be Endangered (EN A2ac) in the China Species Red List because populations had seriously declined due to its over-exploitation as a source of raw material for chitin, the utilization of its blood in the medical and biomedical industries and the use of its meat for food. Annual yields in the 1970’s amounted to 200,000 pairs but by 2004, it had become difficult to obtain (China Species Red List 2009a, b).

In Viet Nam, *T. tridentatus* was assessed to be Vulnerable (VU A1c, Ba, b, c) in the Viet Nam Red Data Book in 2007 because it was estimated its population declined by 50% and its area of occupancy declined by 50% between 1990 and 2007 (Nguyen 2007).

**Biomedical Harvest**

Horseshoe crabs are harvested by the biomedical industry for the manufacture of Amebocyte Lysate and because of its size, *T. tridentatus* is the primary source of Amebocyte Lysate in Asia, where the practice is to drain all of the animal's blood resulting in its death (Hong 2011, p. 154; Laurie/Novitsky 2014, pers. comm).

According to market analysts, demand for Amebocyte Lysate is high and globally, the Amebocyte Lysate market maintained an average annual growth rate of 7.25% from US$231 million in 2013 to US$285 million in 2016. It is predicted the market will further expand, so that by 2021, the Amebocyte Lysate market size will reach US$386 million, a 35% increase in market size in five years (BisReport Information Consulting 2017).

Having largely depleted Chinese waters of commercially viable catches, the TAL industry is targeting Viet Nam as a supply source (Laurie/Novitsky 2014, pers. comm., Laurie/Do 2014, pers. comm.) and is also looking farther afield for new sources of supply, such as Indonesia (Laurie/John 2017, pers. comm). Scientists in Malaysia are also exploring the opportunities of capitalizing on horseshoe crab stocks, including *T. tridentatus* in Sabah to produce TAL and other pharmaceutical products (Noraznawati 2015).

Horseshoe crab carapaces are also in demand in China and overseas as a source of chitin, which is satisfied as a by-product of the TAL industry (China Species Red List 2009a, b, Hong 2011, p. 154) as well as collection by children and adults to supply this demand (Hong 2011, p. 154). This is one of the factors resulting in horseshoe crabs being an 'all parts use animal' (Hong 2011, p. 154, Laurie/Novitsky 2014, pers. comm.). The demand for carapaces for chitin is considered to represent a significant threat to *T. tridentatus* in China (China Species Red List 2009b).

The absence any sort of harvest regulations is considered to be a significant cause of the decline in *T. tridentatus* population numbers in Asia (Gauvry 2015).

**Consumption**

The demand for *T. tridentatus* in China for consumption is high (Li *et al*. 2011; Hong 2011, p. 154; Weng *et al*. 2012; Laurie/Do 2014, pers. comm.) and moderate in Taiwan (Hsieh and Chen 2015), Hong Kong (Chiu and Morton 1999, Shin *et al*. 2009, 2014) and Viet Nam (Laurie/Do 2014, pers. comm).

Gravid females are the target of capture throughout its range by artisanal fisheries as a food source in the Philippines (Schoppe 2002), Indonesia (Meilana *et al*. 2015) and Sabah, Malaysia (Wood and Habibah 2014; Manca *et al*. 2015; Laurie/Mohamad 2017, pers. comm.), where female-biased harvesting may be contributing to male-biased OSR (Christianus and Saad 2007; Manca *et al*. 2015;

Laurie/Mohamad 2017, pers. comm).

**Trawling**

Two complementary advances in the late 20th century had considerable detrimental impact on *T. tridentatus* populations in China. Before the 1980s, fishing boats in China were largely reliant on man power, which limited the methods, capability and efficiency of fishing fleets. In the 1980's, old fleets were gradually replaced with mechanized boats, accompanied by the introduction of trawls which could fish in water as deep as 20 m, opening up the seabed and therefore adult horseshoe crab habitat for exploitation (Hong 2011, p. 155). Capture on a commercial scale is still being promoted. According to the Department of Agriculture in Guangxi in 2011, there was an abundance of resources in the shallow seas around Guangxi, including dozens of thousands of tons of horseshoe crabs, with an annual output of 200,000 pairs (Department of Agriculture, Guangxi 2011).

Of all of the fishing methods, bottom trawling poses the highest risk to adult *T. tridentatus* populations because of its indiscriminate nature and the fact that *T. tridentatus* is an economically viable bycatch.

**Artisanal Fisheries**

In Palawan, Philippines artisanal fishing activities including push netting, gill netting and the construction of closed lines forming fish corrals in subtidal areas facing spawning beaches pose major threats to *T. tridentatus* populations (Schoppe 2002). Increasing fishing activities around Puerto Princesa pose a serious threat to remaining populations (Almendral and Schoppe 2005).

In Malaysia, horseshoe crabs, including *T. tridentatus*, are collected for consumption, both locally and for export, forming an important income component for 'poor' fishermen in some areas (Mohamad et al. 2015) and commencing in 2017, instances of Thai tourists visiting Inderesabah in Sabah to buy gravid female *T. tridentatus*, extract their eggs, then take them back to Thailand have been reported (Laurie/Mohamad 2018, pers. comm).

The Bajau Laut, nomadic sea-farers along the south east coast of Sabah, consume horseshoe crabs as a component of their diets (Wood and Habibah 2014). They are specialist marine hunter-gatherers who make a living from freediving (Cullen *et al*. 2007) and represent one of the most widely dispersed Indigenous groups in Southeast Asia. They comprise a total population of approximately 1.1 million, with around 200,000 living in the islands of eastern Indonesia, 347,000 in Sabah, Malaysia and 564,000 in the Philippines (Stacey et al. 2018). Whilst such artisanal fisheries are low technology in nature, the impact of targeting gravid females can be high and have considerable negative impact, particularly when small or already depleted populations are involved. Their fishing activities may help explain the male biased OSR of 2.42:1 observed at Inderasabah (Mohamad *et al*. 2016) and OSR of 2.08:1 observed at Tawau (Manca *et al*. 2017).

**Bycatch**

Throughout Asia, horseshoe crabs are often considered as a nuisance by local fishermen and disposed of, because of their nuisance value in entangling nets, including in Japan (Iwaoka and Okayama 2009, p. 574; Nishida *et al*. 2015), China (Hong 2011, p. 155), Hong Kong (Laurie pers. obs.) and Malaysia, where they are killed as bycatch, or get trapped and die in abandoned ghost nets (Mohamad *et al*. 2015).

**Smuggling**

*Tachypleus tridentatus* eggs are being targeted for smuggling in Sabah, Malaysia (Laurie/Mohamad 2018, pers. comm.) and in Indonesia, where even though they are protected, there is a substantial illegal trade in horseshoe crabs, which are usually smuggled via ports on the northeast coast of Sumatra to Malaysia, for onward transmission to Thailand (Laurie/Meilana 2015, pers. comm.; Laurie/John 2017, pers. comm.). Whilst the principal focus of this trade is gravid *T. gigas* for consumption in Thailand, seizures of *T. tridentatus* are also made (BKIPM 2012).

**Habitat Loss through Reclamation**

Reclamation and related activities pose a considerable threat to *T. tridentatus* populations, through destruction or degradation of all lifestyle stage habitats.

In Japan, following World War II, extensive land reclamation was carried out as a national policy to increase agricultural output (Seino *et al*. 2003, p. 6). Tidal flats, which often exist in deeply indented bays tended to be developed. Hence, the area of tidal flats nationally was reduced by more than 40% between 1945 and 1995 due to landfilling and draining for land reclamation (Japan NBSAP 2014, p. 15) and following large scale reclamations, many small scale land reclamations were also carried out (Seino *et al*. 2003, p. 6). These activities were more prevalent in some areas than others. For example, over 80% of horseshoe crab habitats disappeared in the Seto Inland Sea from 1930 to 1994 (Shuster and Sekiguchi 2009), due to reclamation or revetment projects, which greatly diminished or totally destroyed spawning beaches there (Itow 1993, Tsuchiya 2009).

The effects of such land reclamations had a devastating effect on horseshoe crab populations. During the reclamation of the 106 ha Tomioka Bay in 1958, the remains of approximately 10,000 horseshoe crabs were found stranded (Nishii 1973; Seino *et al*. 2003, p. 8). When 1,800 ha of land were reclaimed in Kasaoka Bay, commencing in 1968, approximately 100,000 adult horseshoe crabs were estimated to be affected, and whilst efforts were made to transport the stranded adults to the sea, it was assessed that millions of juvenile horseshoe crabs perished (Seino et al. 2003, p. 8). In terms of long-term impact, a comparison of horseshoe crab spawning grounds on the Tenjin and Azuma coasts of Kasaoka showed a decrease from a maximum of around 400 breeding sites in the 1970s to no breeding sites after 1981 (Seino *et al*. 2003, pp. 5−6, Fig. 3).

In parallel, it is estimated seagrass beds in Japan were reduced by 40% in area from the 1970s to 2000s, but in addition to loss of habitat, rising sea temperature has been suggested as one of the causes for this decrease (Japan NBSAP 2014, p. 15). Threats to *T. tridentatus* habitat in Japan have not ceased. In 2017, the local government at Sone Tidal flat, home to possibly the largest remaining horseshoe crab population in Japan was considering proposals to reclaim the intertidal flat to build a wind turbine farm

(Laurie/Iwasaki 2017, pers. comm).

China recognizes that land reclamation from wetlands undertaken from the 1950s to the 1990s has drastically shrunk wetland habitat and the area of land reclamation from tidal flats is continuing (China NBSAP 5 2014). From the same report, the area of land reclamation from the seas from 2008 to 2012 reached 650.6 km² and as a result of land reclamation from tidal flats, mangrove area in China decreased by about two-thirds (66%), causing direct damage to habitats and reproduction sites for some important protected species. Before the 1990s, many coastal areas in China were remote and isolated because they lacked paved roads, but with economic development, new road networks were put in place to open up the coastal areas, which in turn allowed fishermen to transport their catches, including *T. tridentatus* to new markets (Hong 2011, p. 155). Despite being illegal, there is now considerable inter-province trade in which *T. tridentatus* is being internally smuggled from Guangdong and Guangxi to meet consumer demand in Zhejiang and Fujian (Weng *et al*. 2012). In the past, *T. tridentatus* spawning beaches were also protected in many areas in Fujian because they were located in restricted military zones, but a relaxation of these restrictions has led to the opening up of these coastal areas to development (Hong 2011, p. 160).

In Taiwan, which comprises Taiwan Island, the Penghu Islands, Kinmen and the Matsu Islands, two-thirds of Taiwan Island is hilly or mountainous, bisecting the island along a centre line into east and west corridors. The east of the Taiwan Island is scarcely populated, whilst 95% of the population has settled along the west coast which comprises flat, plain terrain (Chang 2008), which was home to almost all *T. tridentatus* spawning grounds in Taiwan (Hsieh and Chen 2015). On Taiwan Island, habitat destruction resulting from landfill projects and the construction of breakwaters and fishing ports has led to the loss of 55% of the natural coastline, particularly of intertidal areas on the west coast (CPAMI 2007), so almost all *T. tridentatus* spawning grounds have been degraded or lost (Hsieh and Chen 2015). On Kinmen, horseshoe crabs were historically afforded some protection because they were located in restricted military zones (Hsieh and Chen 2009). However, as restrictions were relaxed, the major threat to *T. tridentatus* on Kinmen was habitat loss due to the construction of a commercial port on spawning and nursery grounds in 1997 through reclamation of intertidal flats and dredging of subtidal areas, destroying the ecological integrity of the whole bay (Hsieh and Chen 2015).

In Hong Kong, early maps showed uninterrupted coastlines, natural streams, pristine beaches and extensive and undisturbed mudflats, and the historic widespread occurrence of horseshoe crabs may be indicated indirectly from the names of some locations such as Hau Dei (Ground for Horseshoe Crabs) and Hau Hok Wan (Horseshoe Crab Carapace Bay), but Hau Dei was reclaimed in the 1960's and is now part of a market town (Shin *et al*. 2014). No juvenile or adult horseshoe crabs have been observed at Hau Hok Wan in recent years (Kwan *et al*. 2016). Major reclamation projects since the 1970's resulted in entire suites of *T. tridentatus* habitats disappearing (Shin et al. 2009), including the habitat at Nim Wan, where juvenile *T. tridentatus* were previously found, but which was developed as a landfill site in 1993.

Horseshoe crabs in Indonesia face a series of common threats, including land reclamation of spawning habitats and nursery grounds, houses built along spawning beaches, mangrove loss and degradation, and a high utilization rate of spawning areas and nursery grounds (Meilana *et al*. 2015), whilst in Palawan, Philippines all sandy shore lines in the vicinity of Puerto Princesa City have been subject to coastal construction (Schoppe 2002).

**Impingement of Coastal Infrastructure**

The slow growth of the juveniles and their long life span on the shores make them highly susceptible to direct loss of habitat through construction of coastal defences, such as seawalls and reclamation works and disturbances caused by urban developments in the vicinity of these spawning/nursery shores (Shin *et al*. 2014). Modifications of the hydrography of nearby waterways from coastal developments can also adversely affect the return of adult horseshoe crabs to the shores for spawning, thus resulting in less recruitment and decrease in juvenile populations (Sekiguchi and Shuster 2009).

In Japan, artificial coastlines rapidly increased in the 1960s and 1970s. The length of coastlines, where banks, revetments and other protection structures were developed, reached about 10,000 km, accounting for about 30% of the total length of the country's coastline. Meanwhile, the length of natural coastlines which do not have artificial structures decreased to about 50% of the total length of coastlines by 1998 (Japan NBSAP 2014, p. 15). As reported in Itow (1993), around the Seto Inland Sea the construction of landfills and revetments has caused habitat deterioration through the loss of silt and mud from some tidal flats, turning them into pebble beaches, making it impossible for juvenile horseshoe crabs to survive in these areas.

In Taiwan, horseshoe crabs are also facing habitat deterioration due to construction of dikes close to their spawning grounds (Hsieh and Chen 2015). For example, the extirpation of *T. tridentatus* at Budai, one of the last spawning habitats and nursery grounds on the west coast of Taiwan Island was believed to have been caused by the blocking of the Longgong Estuary by an expansion of Budai Harbour in 2004 (Yang et al. 2009), construction of sea walls adjacent to the Haomeiliao Nature Reserve and excessive opening of fish ponds (BirdLife International 2001). Such alterations in land use contributed to changing the local hydrology so that the sandy substrate that comprised the juvenile nursery ground was replaced by the deposition of a muddy substrate instead (Laurie/Yang 2017, pers. comm).

Even small-scale infrastructure projects can have significant impacts, such as the construction of coastal footpaths and piers at Nim Shue Wan and Lai Chi Wo in Hong Kong (Laurie 2014, 2016 pers. obs). Such footpaths and piers cover high tide spawning locations or bisect intertidal flats and can cause profound effects to the local hydrology and sedimentology, resulting in the extirpation of once thriving juvenile populations.

**Coastal Modification and Mariculture**

In Viet Nam, marine and coastal waters support the livelihoods of approximately 20 million people and demand for fisheries-related products has placed increasing pressure on already over-exploited natural stocks. This has resulted in further aquaculture expansion which has caused a continuous decline in the quality of intertidal areas, mangroves and sea grass ecosystems and points towards future "coastal desertification" (Viet Nam NBSAP 2015). Mangrove degradation has been significant, leading to areas of natural mangrove almost completely disappearing through destruction or conversion to aquaculture to support shrimp farming, whilst mangrove afforestation has increased on tidal flats, to support shellfish aquaculture, with vast areas of intertidal estuaries dwindling in size due to the construction of clam ponds. In 1943, Vietnam had more than 408,500 ha of mangroves, by 1990, the area was about 255,000 ha, declining to 209,741 ha in 2006, 140,000 ha in 2010, so that by the end of 2012 only 131,520 ha of mangrove forests remained. Of the remainder, 56% are considered as "planted mangroves" and therefore of low ecological value (Vietnam NBSAP 2015). In parallel, sea-grass bed coverage across Vietnam has decreased from between 40 to 70% according to area, with a suite of three nationally recognized sites losing over 50% of their coverage between 2009 and 2014, some of which was due to water degradation as a result of mariculture development. The coastal sandy ecosystem, a typical ecosystem of Vietnam's central coastal provinces, has also been remarkably changed, so that its ecosystem service functions, including preventing sand movement, protecting against coastal erosion and maintaining fresh water quality, are now severely diminished. From Vietnam NBSAP (2015), older statistics recorded the area of the coastal sandy ecosystem between Ha Tinh and Ninh Thuan as 85,100 ha, but since 1999 activities such as shrimp farming, sea sand mining and tourism infrastructure construction have destroyed thousands of hectares of the coastal sandy ecosystem in the central provinces.

In certain areas of its range, intertidal mudflats have been converted to oyster cultivation. In Taiwan on Kinmen Island (Chen 2009) and Hong Kong (Morton and Lee 2010), a traditional method of oyster farming involves bottom planting using rock plates, concrete stakes or large stones deployed in rows as protruding arrays on intertidal flats, which have created obstacles for adult *T. tridentatus* when they move toward high tide zones for spawning (Chen 2009). Furthermore, studies in Hong Kong have demonstrated where oyster cultivation occurs, juvenile *T. tridentatus* avoid the areas with cultivated oyster beds, including the significant areas of debris which have been created through the harvesting of oysters (Morton and Lee 2010, Kwan *et al*. 2017).

**Sand and Gravel Extraction**

The use of sea sand and gravel to support reclamation is a common practice in Asia. In the Seto Inland Sea, Japan the disappearance of sandbanks caused by sea gravel extraction may have led to a decrease in the populations of cornerstone species in the food chain (Japan NBSAP 2014, p. 15), and sea sand mining for supplying material for concrete is considered to be one of the factors contributing to the destruction of adult horseshoe crabs habitats there (Nishida *et al*. 2015) and of adult horseshoe crab habitat in Kasaoka in Okayama Prefecture (Seino 2011).

Sand or gravel extraction can not only have a biological impact, leading to a change in benthic communities, it can also change a stable area of seabed into an area of erosion and if undertaken where currents are not strong, this can lead to the preferential erosion and deposition of fine sediments from surrounding areas through a winnowing effect, to replace the sometimes coarser sediment that has been extracted (Desprez 2000). This is what appears to have happened in China, where sea sand extraction has been identified as a significant cause of the degradation of *T. tridentatus* spawning beaches along China's tropical and sub-tropical southeast coast between its border with Vietnam and latitude 28°N, corresponding with the border of Fujian and Zhejiang provinces (UNDP 2000) which encompasses most of *T. tridentatus* range along the coast of China. It has also been identified as a primary cause of degradation in the Dongshan-Nan'ao migratory species corridor between Fujian and Guangdong (Ferguson and Wang 2009), it is implicated in the degradation of *T. tridentatus* habitats at Budai, Taiwan (BirdLife International 2001), in the degradation of adult, sub-adult and juvenile *T. tridentatus* habitats at Kinmen Island, Taiwan (Laurie/Yang 2017, pers. comm.) and of *T. tridentatus* spawning beaches and intertidal juvenile nursery grounds at Puerto Princesa in Palawan, Philippines (Schoppe 2002).

### Habitat Disturbance

Throughout Asia, many *T. tridentatus* spawning and nursery grounds are either situated next to population centres or are easily accessible and popular locations for a variety of activities, including in China (Weng *et al*. 2012), Hong Kong (Shin *et al*. 2014, Kwan *et al*. 2016), Philippines (Schoppe 2002), Malaysia (Robert *et al*. 2014, Mohamad *et al*. 2015) and Indonesia (Meilana *et al*. 2015). From a home range study in analysis of area utilization, juvenile *T. tridentatus* were noted to exhibit limited residential and nomadic movement patterns, which might be associated with the distribution of food patches on the mudflat (Kwan *et al*. 2015b). These results indicate the restricted movements of juveniles and suggest their potential for high susceptibility to localized habitat disturbance.

In Hong Kong, as well as oyster farming and traditional fishing with erected nets across the shoreline, unregulated and uncontrolled recreational activities including clam digging, shellfish collection and sunset watching have caused considerable damage to nursery grounds in intertidal areas, including *H. beccarii* seagrass beds where juvenile horseshoe crabs forage (HKBSAP Report 2014, Kwan *et al*. 2016). Similar threats have been reported in Palawan, Philippines (Schoppe 2002), Sabah, Malaysia (Robert *et al*. 2014, Mohamad *et al*. 2015) and Indonesia (Meilana *et* al. 2015), whilst intense fishing activities in the Menggatal River, Malaysia for subsistence and commercial harvest of a myriad of species including bivalves and gastropods (Robert et al. 2014) may lead to a substantial decrease in these major dietary components of horseshoe crabs.

### Water Quality and Pollution Events

In Japan, several studies have suggested that the susceptibility of eggs and embryos to pollutants is a contributing factor in the decline or extinction of local *T. tridentatus* populations (Botton 2001, p. 46). This is especially so along the coasts of the heavily industrialized Seto Inland Sea, where up to 42% of embryos from some locations were found to be malformed. Pollution in Japan appears to be having a greater impact on *T. tridentatus* than on *L. polyphemus* in the United States. The reasons for this are not clear, but *T. tridentatus* could be more sensitive to pollutants than *L. polyphemus*, or it could be the types and levels of contaminants in Japan are especially harmful (Botton and Itow 2009). The long incubation period of *T. tridentatus* of about 43 days from insemination to hatching (Sekiguchi 1988, p. 148) and the choice of spawning location along the high tide line (Sekiguchi 1988, p. 54) may lead to such high malformation incidences.

Apart from having a direct impact on eggs, pollution may also cause abnormalities during the process of egg formation in the mothers' body, after polluting materials enter the mothers' system from ingesting polluted silt or feeding on polluted prey, transferring environmental contaminants to the eggs before they are laid (Botton and Itow 2009). Another associated issue may be a decrease in prey animals due to the effects of pollution, so it is possible that healthy egg formation may be inhibited by lack of sufficient nourishment in the environment. Itow (1993) suggested the major source of pollution responsible for causing these abnormalities is believed to be from landfills, although the run-off of agricultural chemicals may also have had an impact.

Adverse impacts of poor water quality and changes in local hydrology on horseshoe crab habitats are also apparent. In 1928, Oe-Hama Beach, Kasaoka, Japan was designated as a 'Horseshoe Crab Spawning Ground National Monument', but despite this protection, the area was drained during the 'Kasaoka Bay Land Reclamation Project', a large-scale land reclamation which commenced in 1969 (Sekiguchi 1988, p. 40; Tsuchiya 2009, p. 559). After this, a sandy area along the Konoshima Channel was designated a protected breeding place for *T. tridentatus*, but in parallel the channel became the only passage for wastewater from Kasaoka City, resulting in rapid pollution and a corresponding decrease in horseshoe crab populations (Sekiguchi 1988, p. 41; Botton 2001, p. 45).

Also, before land reclamation, seawater exchange occurred in Kasaoka Bay due to tidal currents passing through the opening of the bay and the Konoshima Channel. However, this seawater exchange was cut off by land reclamation, resulting in the deterioration of water quality and Kasaoka Bay became uninhabitable for many marine organisms. The large-scale alteration of the bay shape has modified local hydrology and exerted a distinct effect on the wave field and tidal currents in the area, leading to changes in overall habitats of horseshoe crabs. As a result, sand was transported and deposited along the shores of Kasaoka Bay by littoral drift, causing the formation of some new spawning sites and the loss of others, especially where beaches became unsuitable because of a lack of wave action to stir and sort the surface sediments. The subsequent accumulation of fine materials remaining near the sand surface thus prevented sufficient exchange of groundwater around the eggs (Seino *et al*. 2003, p.12).

Another consequence of the narrowing of the Konoshima Channel was that the wake from passing ships was not only affecting the distribution of sand along the shorelines but at certain times also swamping the spawning locations along the high tide line (Seino *et al*. 2003, p. 6). The size and speed of ocean going vessels have increased in recent years, resulting in abnormally large waves repeatedly surging onto beaches, which has changed their nature, making them unsuitable for spawning. If ships are passing during a spawning event, the waves generated may also interfere with the egg laying act itself (Itow 1993).

In China, environmental pollution caused by anthropogenic activities (China NBSAP 5 2014) has been cited as one of the causes of *T. tridentatus* population declines (Xie and Weng 2011) and in Vietnam, pollution-related horseshoe crab die-offs have been observed (Laurie/Do 2014, pers. comm.). In Hong Kong, illegal pollution discharges into *T. tridentatus* spawning streams have been observed to be timed to coincide with mainly night time maximum high tides (Laurie 2009, 2011, 2013, pers. obs.), which may not only affect deposited eggs, but also coincides with the peak time when planktonic larvae choose to move from the spawning location to the intertidal area (Sakemi 1997; Botton 2001, p. 47).

**Invasive Species**

In recent years, *T. tridentatus* nursery grounds on Kinmen, Taiwan have been lost to the invasive smooth cordgrass *Spartina alterniflora* (Hsieh and Chen 2015) and the *H. beccari* seagrass beds and tidal flats at Ha Pak Nai/Pak Nai in Hong Kong are being encroached upon by the common cordgrass *Spartina anglica* (HKBSAP Report 2014).

**Climate Change**

Horseshoe crabs have proven resilience. They have survived five mass extinction events, numerous minor extinction events (Tanacredi 2001 p. 2, Rudkin and Young 2009 p. 41) and lived in geological periods which have experienced significant climate extremes (Zachos *et al* 2001, Moritz and Agudo 2013). However, *T. tridentatus* is now facing the threats of climate change with severely depleted populations throughout much of its range, which pose two obvious threats to its populations: loss of spawning habitat due to rising sea levels and rising temperatures (Arkema *et al*. 2013).

*Rising sea levels*
Sea-level rise will increase the risk of flooding of spawning habitats and, in turn, the rate at which these habitats disappear, including increasing the likelihood that spawning habit becomes compressed between the rising sea and existing housing and other infrastructure (Loveland and Botton 2015). This is a recognized risk in Asia, because many *T. tridentatus* spawning sites and nursery beaches are shared with resident human populations (Weng *et al*. 2012b, Kwan et al. 2016, Schoppe 2002). At the extreme, in Japan, spawning habitats such as Tatara Beach, a 16 m by 28 m (448 $m^2$) patch located where a breakwater meets a sea-wall (Sekiguchi 1988, Fig. IV-10, p. 62) already requires periodic replenishment to maintain the sand (Botton 2001, p. 45) and like many spawning habitats in Japan is now conservation-reliant, depending on human support for its continued functioning.

*Rising temperatures*
Many aspects of *T. tridendatus* biology and ecology are temperature influenced. Spawning is seasonal and temperature triggered (Nishii 1975), so increasing water temperatures, particularly in its sub-tropical range, could extend the spawning period.

Eggs and trilobite larvae are the two lifestyle stages most at risk from elevated temperatures and whilst the Earth's average annual temperature has increased at a steady pace in recent decades, global warming has increased the severity of the hottest month and day of the year (Diffenbaugh *et al*. 2017), with an alarming jump in the severity of the hottest days of the year during the same period. This results in short-term, extreme-heat events whose lethal effects are being caused by exposure to high ambient environmental temperatures (Papalexiou *et al*. 2018), and it is these events, more than gradual temperature rises, which are most likely to impact eggs and trilobite larvae.

Eggs are deposited along the high tide line, where the covering sand is periodically exposed to sub-aerial temperatures. Optimal water temperatures for incubating eggs range from 22– 31°C in Japan (Sekiguchi *et al*. 1988 p. 67) to 28-31.8°C in Taiwan (Chen *et al*. 2004). Hence, if eggs are subject to temperatures outside of these ranges, the risk of non-incubation or mortality will increase.

Trilobite larvae initially develop in their nest area, before moving to the inner shores of their natal beaches to develop (Kawahara 1982, Yeh 1999, Hu *et al*. 2011b). Both of these areas are periodically subjected to tide induced sub-aerial exposure, sometimes for extended periods of time, increasing the risk that these larvae will be subject to elevated levels of thermal stress. This may result in higher mortality as temperatures suddenly rise.

Juvenile foraging activity varies with latitude and climate, the emergence of juveniles and ecdysis being temperature related (Lee and Morton 2005, Morton and Lee 2010), so that rising temperatures could result in extended foraging seasons, resulting in juveniles taking a shorter time to reach sexual maturity, because they can moult more frequently at higher sediment or water temperatures (Lee and Morton 2005).

Adult horseshoe crabs have wide ranges of thermal tolerance (Mayer 1914), so the impact of rising sea temperatures on *T. tridentatus* is unlikely to pose a direct major threat to their survival and their exposure to elevated sub-aerial temperatures is likely to be limited, due to their spawning in the intertidal zone (Mohamad *et al*. 2016), or their spawning during nocturnal flood tides (Sekiguchi 1988, p. 58). However, indirect threats could result.

It is assessed the 490 dead adult *T. tridentatus* that were found on the shores of the Kitakyushu/Sonehigata tidal flat in Japan between January and August 2016 (Takahashi 2016) could be victims of rising sea temperatures. According to the Fukuoka Fisheries and Marine Technology Research Center's Buzen Sea Laboratory, the temperature of the Buzen Sea off of the tidal flats from May through August 2016 was between 0.9-1.6° C higher than in a typical year (Takahashi 2016). It is believed the horseshoe crabs had succumbed to predation by the Naru Eagle Ray, *Aetobatus narutobiei* (Kabutogani Jimankan Museum 2016), whose range could have been extended due to warming seas.

Apart from threatening *T. tridentatus*, rising temperatures also pose a threat to its supporting food chain, including seagrass beds as well as the intertidal meiofauna which comprises its trilobite larvae and juvenile prey. In Japan, some of the 40% reduction in area of seagrass beds from the 1970s to 2000s is believed to have been caused by rising sea temperature (Japan NBSAP 2014, p. 15).

*Country impacts*
Viet Nam recognizes it is likely to be one of the countries most impacted by climate change. Under current climate change scenarios, Viet Nm is predicted to house fragmented ecosystems that will undergo a high rate of loss of biological diversity resources compounding its vulnerability to climate change (Vietnam NBSAP 2015). It is because climate change combined with the degradation and depletion of forests of key watersheds can change the use of water resources, which may lead to more frequent floods, flash floods and landslides and cause heavy damage to the environment, all of which can adversely impact on coastal ecosystems. In addition, Vietnam's main delta areas are likely to be severely impacted through sea level rise, leading to the loss of significant areas of coastal habitat (Vietnam NBSAP 2015).

*Land subsidence*
An issue linked to rising sea levels, which leads to flooding but not factored in to many assessments, is land subsidence. This is being recognized an emerging issue in Asia. On Taiwan Island, serious submergence of the ground layer of the southwest coastline and rising sea levels due to global weather changes have contributed to an increase in coastal infrastructure, inundation of sea water and shoreline erosion (CPAMI 2010). A total of 1,150 km² of the coastal region, representing 3.2% of the total land area, has been prone to seawater flooding due to land subsidence (Chang 2008), which has undoubtedly contributed to *T. tridentatus* habitat loss. This threat to *T. tridentatus* habitats exists elsewhere, but has yet to be quantified.

For further information about this species, see Supplementary Material.

## Conservation Actions (see Appendix for additional information)

### Direct Species Protection

*Tachypleus tridentatus* is designated “Grade II Protected Animal of China” in the “List of State Key Protected Wildlife” and “Key Protected Aquatic Wildlife” in Zhejiang, Fujian, Guangdong and Guangxi (Huang *et al*. 2002). However, there is no effective management to protect the species due to a lack of scientific knowledge on its population status (Xie and Weng 2011). Despite the fact *T. tridentatus* is listed as a Key Protected Aquatic Wildlife in Guangxi, in a survey in September 2015 of coastal communities along Beibu Gulf in Guangxi, interviews revealed most people in the region, regardless of their age, consumed horseshoe crabs (Liao *et al*. 2017).

In Indonesia, horseshoe crabs were protected in Indonesia by Law No. 5 of 1990 of the Republic of Indonesia on the Conservation of Living Resources and their Ecosystems, under which it is illegal to take, keep, transport or trade in a protected species (Article 21.1. a), including exporting or transporting internally within Indonesia (Article 21.1. b) (Indonesia Law No. 5/1990), supplemented by Government Regulation No 7/1999 on Preserving Flora and Fauna Species (Indonesia Government Regulation No. 7/1999). Horseshoe crabs were also protected under Forestry Ministerial Decree No. 12/Kpts-II/1987 (Meilana and Fang 2017), which prohibits their exploitation or trade. Law No. 5 of 1990 Appendix II only identifies *T. gigas* as protected aquatic biota, although it appears to be applied on a look-alike basis to *T. tridentatus* using the Indonesian generic term for horseshoe crab ‘Ketam Tapak Kuda’ (Laurie/Wardianto 2017a, pers. comm.). In June 2018, Indonesia’s Ministry of Environment and Forestry upgraded Government Regulation No. 7/1999 and issued new regulations to protect all three horseshoe crab species, including *T. tridentatus* in Indonesia (Indonesia Government Regulation 20/2018).

### Indirect Species Protection

Malaysia is keen to prevent bio-piracy, where biological resources are accessed and extracted without permission and developed for commercialization, so that bio-prospecting initiatives must be carried out with Prior Informed Consent (Malaysia NBSAP 5 2014). In particular, both Sarawak and Sabah have very strict regulations relating to the collection, study and export of biodiversity and each state has established a Biodiversity Council to oversee such approvals. In Sarawak no person shall collect or export biological resources for research and development or for propagation to support such research and development without permits issued by the Sarawak Biodiversity Council (SBA 2016), whilst in Sabah, no researcher or institution can access or export biodiversity without licences issued by the Sabah Biodiversity Council (SaBC 2017). In both states, these regulations are strictly enforced and apply to horseshoe crabs, and because of this, *T. tridentatus* is not seriously exploited for foreign export (Laurie/John 2017, pers. comm).

Similarly, in Brunei Darussalam, a permit from the Fisheries Department is required for the export of all water organisms, including horseshoe crabs (BDNSW. 2014).

### Habitat Protection

In Japan in 1928, Oe-Hama Beach, Kasaoka was designated as a 'Horseshoe Crab Spawning Ground National Monument', but the inadequacies of this designation was demonstrated when this area was drained during the Kasaoka Bay Land Reclamation Project, a large scale land reclamation which commenced in 1969 (Sekiguchi 1988, p. 40, Tsuchiya 2009, p. 559). Areas of spawning site at Toyo region, Ehime Prefecture and Imari City are also protected as a natural monument by each local government, respectively (Nishida *et al*. 2015).

In China, there are two broad categories of Marine Protected Areas in China, No-take Marine Nature Reserves (No-take MNRs) and Multiple-use Marine Special Protected Areas (MSPA), but as a result of great user pressure and lack of enforcement capacity, the zoning schemes are often poorly recognized and implemented, so that even fully protected MNRs are implemented as multiple-use areas, so that certain levels of fishing and industrial activities are usually tolerated within them (Qiu *et al*. 2009). In Fujian, a 700 km² Ocean Special Protection Area, the Xiamen National Nature Reserve for Rare Marine Species, was designated in 2000 to protect a variety of coastal biodiversity including horseshoe crabs, with the objectives of preventing, minimizing and controlling negative human influences on horseshoe crab resources (Qiu *et al*. 2009; Hong 2011, p. 196). However, in the early 2000's, an extensive sandy-mud beach planned for a local horseshoe crab reserve in Pingtan, Fujian was taken over by a businessman for a clam hatchery. Although this business failed, the reserve is gone, demonstrating the often difficult conflict that arises between conservation and economic development in China (Huang 2011), whilst in Guangdong, seven city, county or province level horseshoe crab nature reserves have been established since 2001 (Huang *et al*. 2002).

In Taiwan, *T. tridentatus* is protected on Matsu Island, part of an offshore archipelago in the northern end of the Taiwan Strait (Hsieh and Chen 2015) and the Kinmen Kuningtou Northwest Intertidal Terrain Horseshoe Crab Conservation Area was designated as a no-take zone in 1999 under the Fisheries Law of Taiwan (Taiwan Fisheries Agency 2016). However, there are no penalties for violations within the designated area for activities such as habitat destruction or pollution (Chen *et al*. 2004).

In Hong Kong, there are no designated areas for *T. tridentatus* conservation, but three spawning and nursery grounds, Tsim Bei Tsui, Pak Nai and San Tau and Lai Chi Wo, are listed as Sites of Special Scientific Interest (SSSIs) to protect other species, in a land-use planning system in which new development is not permitted in most circumstances because of their ecological significance (Lai 1998, Chiu and Morton 2003).

In Malaysia, Kota Kinabalu Wetland in Sabah was accorded Bird Sanctuary status in 1996 and declared a State Cultural Heritage Site in 1998 (Robert et al. 2014), whilst in Indonesia, there is no conservation area dedicated to the protection of horseshoe crabs (Meilana and Fang 2017).

**Temporal Conservation Measures**

Apart from special measures, China has also tried to introduce temporal measures to protect *T. tridentatus*. In 2000, the Pingtan government in Fujian issued orders to protect horseshoe crabs, including restricting any unit from catching, selling or dealing in horseshoe crabs and horseshoe crab related products, and anyone was restricted from catching horseshoe crabs between 1 May and 31 August (Hong 2011, p. 197). In 2000, fishermen in Lianzhou Bay, Beihai, Guangxi were asked to remove their nets and fish cages, which were blocking the passage of spawning horseshoe crabs, to open up passageways between the shore and the sea, to allow horseshoe crabs to reach their spawning grounds, then return to the sea (People's Daily 2000).

**Indirect Habitat Protection from Trawling**

In Hong Kong, adult *T. tridentatus* were vulnerable to bottom trawling, but since this was banned in December 2012, this threat to adult horseshoe crabs no longer exists, which could result in a stabilization of adult populations (Shin *et al*. 2014). Inshore bottom trawling is also banned in Indonesia (CITES 2000) and in Taiwan, bottom trawling was once one of the most important coastal fisheries, but annual production started to decline due to overfishing and because of bycatch problems in the 1980s, inshore bottom trawling was banned from within three nautical miles of the shoreline in 1989 (CITES 2000, Shao *et al*. 2012).

**Habitat-Based Conservation Actions**

The Japanese Society for the Preservation of the Horseshoe crab (Kabutogani wo Mamoru kai) was established at 1978 for conservation of *T. tridentatus*, and has branch offices at some habitats/prefectures. Some local non-governmental organizations (NGOs) and groups, including the above society, are conducting conservation activities such as field surveys, beach clean-ups, social education and other activities. Release programs/events of juveniles and/or sub-adults which are reared from eggs are carried out at some habitats for recovery of population by local government/NGO/group, although there is an ongoing discussion about its effectiveness.

Imari Bay on northern Kyushu was considered by Sekiguchi (1988) to have the largest population of *T. tridentatus* in Japan, but the abundance of horseshoe crabs is limited by the scarcity of suitable spawning habitat. One of the few breeding areas is Tatara Beach, which is a mere 16 m by 28 m patch located where a breakwater meets a seawall (Sekiguchi 1988, Fig. IV-10, p. 62). This site requires periodic replenishment to maintain the sand (Botton 2001, p. 45), meaning that the horseshoe crab populations are conservation-reliant (dependent on human support for survival) at this location. Similar habitat-based conservation measures have been taken to combat the effects caused by the wake of passing ships on horseshoe crab spawning grounds along the Konoshima Channel, Kasaoka City, where a series of groins were built and beach nourishment programs were initiated to minimize the impact of land alteration in the area (Seino *et al*. 2003, p. 6, Table 2).

**Restocking**

In the past, thousands of Instars I and II have been released into the wild on Kinmen, Taiwan and in Xiamen, Quanzhou and Guangdong, China, but the efficacy of such release programs has not been evaluated and juvenile survival rates after release are not known (Hsieh and Chen 2015). Between 2005 and 2009, more than 40,000 juvenile *T. tridentatus* were released in the vicinity of Xiamen as part of a biological resources and rare species protection and recovery program (Oceanweek.Org 2009) and in 2010 the Guangdong Provincial Government jointly held a mass release at Yangjiang City of juvenile marine animals of economic, rare and endangered species, including juvenile horseshoe crabs (Farmers Daily 2010). Although scientifically supervised juvenile release programs have also been undertaken in Taiwan (Hsieh and Chen 2015) and Hong Kong (Shin *et al*. 2011), their efficacy is still being assessed.

**Law Enforcement**

Horseshoe crabs, including *T. tridentatus*, are protected in China and Indonesia. Despite the significant size of both countries, periodic seizures involving *T. tridentatus* are reported, in both China (Weng *et al*. 2013, SCMP 2017) and Indonesia (BKIPM 2012), although the effectiveness of enforcement effort has not been evaluated.

**Harvest Management**

The absence of harvest regulations is considered the main cause of the decline in *T. tridentatus* population numbers throughout much of its central range (Gauvry 2015), specifically in China (China Species Red List 2009b).

For further information about this species, see Supplementary Material.

# Credits

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## Disclaimer

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## External Resources

For Supplementary Material, and for Images and External Links to Additional Information, please see the Red List website.

# Appendix

## Habitats

(http://www.iucnredlist.org/technical-documents/classification-schemes)

| Habitat | Season | Suitability | Major Importance? |
|---|---|---|---|
| 9. Marine Neritic -> 9.1. Marine Neritic - Pelagic | Non-breeding season | Suitable | Yes |
| 9. Marine Neritic -> 9.4. Marine Neritic - Subtidal Sandy | Non-breeding season | Suitable | Yes |
| 9. Marine Neritic -> 9.5. Marine Neritic - Subtidal Sandy-Mud | Non-breeding season | Suitable | Yes |
| 9. Marine Neritic -> 9.10. Marine Neritic - Estuaries | Non-breeding season | Suitable | Yes |
| 12. Marine Intertidal -> 12.2. Marine Intertidal - Sandy Shoreline and/or Beaches, Sand Bars, Spits, Etc | Breeding season | Suitable | Yes |
| 12. Marine Intertidal -> 12.3. Marine Intertidal - Shingle and/or Pebble Shoreline and/or Beaches | Breeding season | Suitable | Yes |
| 12. Marine Intertidal -> 12.4. Marine Intertidal - Mud Flats and Salt Flats | Non-breeding season | Suitable | Yes |

## Threats

(http://www.iucnredlist.org/technical-documents/classification-schemes)

| Threat | Timing | Scope | Severity | Impact Score |
|---|---|---|---|---|
| 11. Climate change & severe weather -> 11.1. Habitat shifting & alteration | Ongoing | Whole (>90%) | Unknown | Unknown |
| | Stresses: | 1. Ecosystem stresses -> 1.1. Ecosystem conversion<br>1. Ecosystem stresses -> 1.2. Ecosystem degradation<br>1. Ecosystem stresses -> 1.3. Indirect ecosystem effects | | |
| 5. Biological resource use -> 5.4. Fishing & harvesting aquatic resources -> 5.4.1. Intentional use: (subsistence/small scale) [harvest] | Ongoing | Minority (50%) | Negligible declines | Low impact: 4 |
| | Stresses: | 2. Species Stresses -> 2.1. Species mortality | | |
| 5. Biological resource use -> 5.4. Fishing & harvesting aquatic resources -> 5.4.2. Intentional use: (large scale) [harvest] | Ongoing | Majority (50-90%) | Rapid declines | Medium impact: 7 |
| | Stresses: | 2. Species Stresses -> 2.1. Species mortality | | |
| 7. Natural system modifications -> 7.3. Other ecosystem modifications | Ongoing | Minority (50%) | Rapid declines | Medium impact: 6 |
| | Stresses: | 1. Ecosystem stresses -> 1.2. Ecosystem degradation<br>1. Ecosystem stresses -> 1.3. Indirect ecosystem effects | | |

# Conservation Actions in Place

(http://www.iucnredlist.org/technical-documents/classification-schemes)

| Conservation Actions in Place |
| --- |
| In-Place Research, Monitoring and Planning |
| Action Recovery plan: No |
| Systematic monitoring scheme: No |
| In-Place Land/Water Protection and Management |
| Conservation sites identified: Unknown |
| Occur in at least one PA: Yes |
| In-Place Species Management |
| Harvest management plan: No |
| Successfully reintroduced or introduced beningly: Yes |
| In-Place Education |
| Subject to recent education and awareness programmes: Yes |
| Subject to any international management/trade controls: No |

# Conservation Actions Needed

(http://www.iucnredlist.org/technical-documents/classification-schemes)

| Conservation Actions Needed |
| --- |
| 1. Land/water protection -> 1.2. Resource & habitat protection |
| 2. Land/water management -> 2.3. Habitat & natural process restoration |
| 3. Species management -> 3.1. Species management -> 3.1.1. Harvest management |
| 3. Species management -> 3.1. Species management -> 3.1.2. Trade management |
| 4. Education & awareness -> 4.3. Awareness & communications |

# Research Needed

(http://www.iucnredlist.org/technical-documents/classification-schemes)

| Research Needed |
| --- |
| 2. Conservation Planning -> 2.1. Species Action/Recovery Plan |
| 2. Conservation Planning -> 2.3. Harvest & Trade Management Plan |
| 3. Monitoring -> 3.1. Population trends |
| 3. Monitoring -> 3.2. Harvest level trends |

| Research Needed |
| --- |
| 3. Monitoring -> 3.3. Trade trends |
| 3. Monitoring -> 3.4. Habitat trends |

# Additional Data Fields

| Distribution |
| --- |
| Lower depth limit (m): 0 |
| Upper depth limit (m): 40 |
| **Habitats and Ecology** |
| Continuing decline in area, extent and/or quality of habitat: Yes |
| Generation Length (years): 20.25 |
| Movement patterns: Full Migrant |

# Errata

**Errata reason:** This errata version of the 2018 assessment was created to add the distribution map, correct the Assessor name "Shin, N." to "Nishida, S.", and to make the following corrections to the Supplementary Information document: the common name of the species changed from "Japanese Horseshoe Crab" to "Tri-spine Horseshoe Crab"; the affiliation of Billy Kwan Kit Yue changed to "Ocean College of Beibu Gulf University, Beibu Gulf University, Guangxi Zhuang Autonomous Region, China"; and the text "In this report Mainland of the People’s Republic of China (is hereafter abbreviated to China), Taiwan (province of China, is hereafter abbreviated to Taiwan), Hong Kong (a Special Administrative Region of China, is hereafter abbreviated to Hong Kong)" added to the Geographic Range.

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# Tri-spine Horseshoe Crab (*Tachypleus tridentatus*)

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# Geographic Range

In this report, Mainland of the People's Republic of China is hereafter abbreviated to China; Taiwan (province of China) is hereafter abbreviated to Taiwan; and Hong Kong (a Special Administrative Region of China) is hereafter abbreviated to Hong Kong.

## Occurrence within range states

**Japan**: In Japan, the range of *T. tridentatus* is limited to the coasts of the Seto Inland Sea and northern Kyushu (Sekiguchi 1988, p. 39–45). Recognized spawning habitats are found at Kasaoka on Honshu Island in Okayama Prefecture; Hiroshima Bay and Takehara in Hiroshima Prefecture; Chidori Beach, Yamaguchi Bay and Hirao Bay in Yamaguchi Prefecture; in Toyo region on Shikoku Island in Ehime Prefecture; Morie Bay on Kyushu Island in Oita Prefecture; Kafuri Bay, Hakata Bay, Tsuyazaki tidal flat, Sone tidal flat, Kanda and Nakatsu in Fukuoka Prefecture; Imari Bay in Saga Prefecture and Omura-Bay, Kujukushima-Sasebo and Iki Island in Nagasaki Prefecture (Nishida *et al*. 2015).

**China**: *Tachypleus tridentatus* occurs in the coastal waters of China, from its northern most range limit in the Zhoushan Archipelago (Wang 1984) on the south side of the Yangtze River (Chang Jiang) in Zhejiang Province, to all coastal areas to the south, including Fujian Province, Guangdong Province, Guangxi Zhuang Autonomous Region and Hainan Province (Zhejiang, Fujian, Guangdong, Guangxi and Hainan) (Hong 2011, Weng *et al*. 2012b),

**Hong Kong**: The Hong Kong Special Administrative Region (HKSAR) is situated in the centre of Guangdong Province. *Tachypleus tridentatus* occurs sympatrically with *Carcinoscorpius rotundicauda* at seven shores in Hong Kong, namely Tsim Bei Tsui and Ha Pak Nai/Pak Nai and at Tung Chung, San Tau, Sham Wat, Yi O (Shin *et al*. 2009, Kwan *et al*. 2016) and Shui Hau (Kwan *et al*. 2016).

**Taiwan**: *Tachypleus tridentatus* is found on the west coast of Taiwan Island, in the Penghu Islands Archipelago and Kinmen Island. Recognized habitats include: Xiangshan and Budai on Taiwan Island, Dongwei and Tiexianwei on the Penghu Islands and Xiyuan, Beishan, Nanshan, Wujiang Estuary, Hsiashu, Butou and Shanglin in Kinmen and the Matsu Islands (Hsieh and Chen 2015).

**Vietnam**: *Tachypleus tridentatus* occurs throughout the coastal waters of Vietnam, but is more abundant in the Central Coastal Provinces (Nguyen 2007), including Nha Trang (Waterman 1953, 1958, cited in Sekiguchi 1988, pp. 34, 38; Mikkelsen 1988).

**Philippines**: Apart from Palawan (Sekiguchi 1988, p. 34), there are limited data available on the distribution of *T. tridentatus* in the Philippines, with suggestions that it might occur sporadically in the Sulu Sea. In Palawan, surveys conducted between 1999 and 2001 indicated *T. tridentatus* was present in coastal municipalities extending the length of Palawan including Quezon, Aborlan and El Nido on the west coast and Brookes Point, Espanola, Narra, Puerto Princesa City, Roxas, Dumaran, Araceli, Taytay and the islands of Agutaya, Magsaysay and Cagayancillo (Schoppe 2002), as well as Busuanga Island in the northern end of the Palawan Island Chain (WWF 2010, Bautista et al. 2016, p. 84).

**Malaysia**: All three Asian horseshoe crab species occur in Malaysia, but the distribution of *T. tridentatus* is limited to Sarawak and Sabah in East Malaysia (Laurie/John 2017, pers. comm., Laurie/Mohamad 2017, pers. comm). In Sarawak it has been recorded from the Lutong River, Miri (Nyanti et al. 2012) and Kuala Nyalau Beach (Laurie/John 2017, pers. comm.) and in Sabah from Papar (Chatterji and Pati 2014), Tanjung Limau, Jambongan Island (Manca *et al*. 2015, Sofa *et al*. 2015, Mohamad *et al*. 2016), Kota Belud (Sekiguchi 1988, p. 53, Ow 2007), Kota Kinabalu (Sekiguchi 1988, p. 28, Yamasaki *et al*. 1988, p. 94 and Fig. V-21, p. 96; Robert *et al*. 2014), Mengattal River (Ng 2009, Robert *et al*. 2014), Sandakan (Sekiguchi 1988, pp. 28-29), Tun Sakaran Marine Park/Semporna Islands Park (Marine Conservation Society 2006) and Inderasabah (Mohamad *et al*. 2016).

**Brunei Darussalam**: In Brunei a live female was reported from Panaga Beach in 2006 (McIlroy and Dols 2009). The wall of a fisherman's hut in Kampong Ayer Fishing Village has a display of locally caught horseshoe crabs including nine male and five female T. tridentatus (Laurie/Smith 2017, pers. comm.), of which three female carapaces show distinct opisthosomal scars, suggesting they were caught during the spawning season. A mating pair of *T. tridentatus* were captured at Muara Beach in 2017 (Laurie/Marshal 2018, pers. comm).

**Indonesia**: *Tachypleus tridentatus* has been recorded from Sibolga in North Sumatra Province and Padang in West Sumatra Province (Sekiguchi 1988, pp. 30, 36; Yamasaki *et al*. 1988, p. 94 and Fig. V-21, p. 96); Manado in North Sulawesi (Sekiguchi 1988, pp. 32, 36; Yamasaki *et al*. 1988, Fig. V-21, p. 96; BKIPM 2012); Tarakan (Sekiguchi 1988, p. 30; Yamasaki *et al*. 1988, Fig. V-21, p. 96), Nenang, Babulu Laut and Telake (Nishida 2012a), Mangar, Muala Pantuan (Sekiguchi 1988, p. 31) and Balikpapan (Sekiguchi 1988, p. 31, Laurie/Wardiatno 2017b, pers. comm.) in East Kalimantan; Banten (Meilana 2015), Indramayu (Mashar *et al*. 2017a) and Subang (Meilana 2015, Mashar *et al*. 2017a) in West Java; Semarang in Central Java (Meilana 2015); and Gresik (Meilana 2015), Pansuruan (Sekiguchi 1988, p. 31), Tuban and Surabaya (Mashar *et al*. 2017a) in East Java.

## Transport and Introduction

Commercial trade, accidental release from stocking pens and intentional release of *T. tridentatus* can cause artificial movements of individuals and mix up of genetic structure with local horseshoe crab populations, as shown in the following cases.

In Japan, a non-native population comprising 62 individuals (13 adults/49 juveniles) was captured in 12 years commencing 2001 from Ise-Mikawa Bay, Chubu Region, Central Japan which is out of the range of their natural habitat. Genetic analysis suggested these individuals originated in China and interviews with locals revealed that in 1996 approximately 1,000 horseshoe crabs were imported to the area from Pintan in Fujian Province, China and about 200 had escaped from their pens as a result of broken nets (Nishida *et al*. 2015).

In China, artificial movement of individuals due to commercial trade may be a factor contributing to the poor structure (nuclear genetic homogeneity) of *T. tridentatus* at microsatellite markers and confused population structure pattern at mtDNA control region sequences. Because *T. tridentatus* is consumed in China and with the sharp decline in *T. tridentatus* populations along the China coast, *T. tridentatus* was extensively transported from the south coast to the north, mainly from Beibu Bay. Because this activity is illegal, large numbers of *T. tridentatus* were confiscated and released where they were intercepted, mixing them with local populations or even resulting in them replacing depleted local populations. This phenomenon is common along the China coast, but due to the lack of available data, it is difficult to estimate the exact contribution these activities have had on the genetic structure of *T. tridentatus* (Weng *et al*. 2013).

In Hong Kong, a market survey conducted in 2004 to 2005 indicated of the 1,023 adult horseshoe crabs, mainly *T. tridentatus*, caught, 691 individuals (68%) were from Chinese waters. About one-third of these adult crabs were released after capture, whilst the remaining two-thirds, some 690 individuals, were kept and sold to wholesale fish markets or seafood restaurants. Of these, 425 (about 62%) were used in ‘set-free’ rituals, in which Chinese Buddhists believe if they release live animals back to the wild, they will have relinquished of their sins of killing animals in their daily life (Shin et al. 2009). Such a practice results in the transport and release of animals many miles from their original points of capture.

# Population

## Population genetics

Genetic diversity is recognized to be a basic precondition for populations to react to environmental changes and keep their reproductive fitness, which will ensure their long term survival (Frankham *et al*. 2002, Allendorf and Luikart 2007).

Based on their morphology, horseshoe crabs are considered to be well-known living fossils with a reputation for extreme conservatism in morphotypic evolution, being regarded as the ‘archetype of bradytely’, a ‘classic example of arrested evolution’ (Fisher 1984). Owing to their morphological similarity to mid-Mesozoic taxa, they are considered to be evolutionarily static, being referred to as phylogenetic relics (Selander *et al*. 1970). However, close inspection has revealed the presence of considerable variability and geographic differentiation in morphology (Shuster 1979, Riska 1981) and at the molecular level, horseshoe crabs appear to have intraspecific genetic variation and patterns of population differentiation in allozymes, mitochondrial DNA and microsatellites (Selander *et al*. 1970, Saunders *et al*. 1986, King *et al*. 2005).

Furthermore, sequencing of the genomes of *T. tridentatus*, *Carcinoscorpius rotundicauda* and *Limulus polyphemus* reveals multiple copies of different sets of investigated genes in all three species, indicating the occurrence of Whole Genome Duplication, which elsewhere is recognized as a major evolutionary force in speciation and diversification of morphological structures, but its occurrence in horseshoe crabs suggests that genomic diversity is not always reflected phenotypically (Kenny *et al*. 2016).

## Divergence time estimates

Phylogenetic and sequence analyses of the Hox and other homeobox genes of *T. tridentatus*, *C. rotundicauda* and *L. polyphemus*, which encode crucial transcription factors, strongly suggest that Whole Genome Duplication happened before the last common ancestor of these species, in or before the early Cretaceous Period, about 135 mya, when a divergence of Limulus from the Asian species occurred. Following on from this, *Tachypleus* and *Carcinoscorpius* diverged around 45 mya in the mid-Eocene Period and *T. tridentatus* and *T. gigas* diverged around 25 mya in the Late Oligocene (Kenny *et al*. 2016).

Similar results have been produced based on the dating of Asian horseshoe crabs and *L. polyphemus* using fossil and biogeographic records of common ancestors and genetic variation of the COI (cytochrome c oxidase subunit I) sequence (Yang 2014), which suggests that there was a deep divergence around 24.7 and 33.6 mya in the early to late Oligocene Period between *C. rotundicauda* and the two *Tachypleus* species and between *T. gigas* and *T. tridentatus* around 20.3 and 27.6 mya in the late Oligocene/early Miocene Periods and the phylogenetic trees constructed by each marker all exhibited that only *T. tridentatus* had shallow genealogy relative to *T. gigas*.

Furthermore, Obst *et al*. (2012) applied a new set of molecular genetic data in combination with a wide geographic sampling of the intra-specific diversity to re-investigate the evolutionary history among the four extant horseshoe crabs. Their results revealed strong support for a monophyletic genus *Tachypleus* and a diversification of the three Asian species during the Paleogene Period, 66 to 23 mya, with speciation events well separated in time by several million years.

## Point of origin

Haplotype phylogeny constructed by a neighbour-joining algorithm, the network derived from the minimum spanning network and dating by fossil records demonstrated a significantly deep divergence between the populations in the Indian and Pacific Ocean, in the late Oligocene to early Miocene, circa 21-27 mya (Yang 2004). Tree topology also suggested the three Asian species originated in central South-east Asia from a marine stem group that inhabited shallow coastal waters among the Andaman Sea, Viet Nam, and Borneo (Obst *et al*. 2012), with a possible origin in the Indian Ocean (Yang 2014).

## Dispersal patterns

Assuming a southern origin in the shallow coastal waters near the Andaman Sea, Viet Nam, and Borneo, *T. tridentatus* appears to exhibit a pattern of dendritic dispersal, similar to patterns observed in *L. polyphemus*. In this model, the populations at the ends of its range are more differentiated from nearby populations than are populations in the middle of the range (Smith *et al*. 2016).

## Genetic diversity

Genetic diversity found in *T. tridentatus* is similar to that of *L. polyphemus*, which is characterized by six genetically defined subpopulations (Smith *et al*. 2016). Thirty-six *T. tridentatus* haplotypes have been reported and archived with GenBank; nine in the AT series (AT01 to AT09) from Japan (Nishida and Koike 2009, 2010), 27 in the HC series (H1 to H27) from China (Weng *et al*. 2013) and 10 in the H series (H01 to H10) from the Taiwan Strait (Yang *et al*. 2007).

<u>Japan</u>

In Japan, two genetically distinct western and eastern groups have been identified, with three haplotypes, AT02, AT03 and AT07 being shared between Japan and the China/Taiwan populations (Nishida *et al*. 2015).

Nine *T. tridentatus* haplotypes (AT01 to AT09) have been identified in Japanese waters (Nishida and Koike 2010). An analysis was undertaken of over 600 individuals from ten localities: Kujukushima-Sasebo Bay (N=127) in Nagasaki Prefecture; Imari Bay (N=107) in Saga Prefecture; Kafuri Bay (N=51), Hakata Bay (N=138), Tsuyazaki tidal flat (N=19), and Sone tidal flat (N=51) in Fukuoka Prefecture; Wama tidal flat (N=7) and Morie Bay (N=34) in Oita Prefecture; and Yamaguchi Bay (N=22) and Hirao Bay (N=61) in Yamaguchi Prefecture (Nishida *et al*. 2015). Based on a haplotype network constructed using the median joining method (Bandelt *et al*. 1999), all haplotypes were closely related and connected by a single substitution, and two genetically distinct western and eastern groups were clearly defined, their geographic boundary being the Itoshima Peninsula (Nishida *et al*. 2015).

The degree of genetic differentiation was further measured using fixation indices FST and ΦST (Allendorf and Luikart 2007, Frankham *et al*. 2010), then microsatellite markers were applied to re-evaluate the genetic structure of the Japanese populations and to detect genetic differences in the eastern genetic group, which were almost completely monomorphic according to mitochondrial DNA (mtDNA). More than 250 individuals from the same ten localities were analysed (Nishida and Koike 2009, 2010). Results of mtDNA analysis revealed that AT01 was the dominant haplotype, observed from individuals in all regions with high frequency. AT02, AT03 and AT07 were shared between Japan and China/Taiwan populations and the remaining six haplotypes, including AT01, were endemic in Japan, showing Japanese populations are genetically independent from those in China and Taiwan, even though they are genetically close. AT02 and AT03 were posited to be intermediate between haplotypes from China/Taiwan and Japan and AT01 was posited to have arisen from AT02, indicating that these two haplotypes were the ancestral haplotypes for the Japanese populations. AT02 and AT03 were only detected from individuals in the west of the area, which defined the western group and comprised Kujukushima-Sasebo Bay and Imari Bay. AT08 and AT09 were only found in the population in Imari Bay. AT06 was only found from the population in Kafuri Bay with relatively high frequency, initially suggesting that it belonged in the western group and four individuals with the AT04 haplotype were from either Kafuri Bay or Morie Bay, which are geographically separate. The eastern group, comprising Hakata Bay, Tsuyazaki tidal flat, Sone tidal flat, Wama Bay, Morie Bay, Yamaguchi Bay and Hirao Bay was almost monomorphic for AT01. AT05 and AT07 were detected only in the Sone and Tsuyazaki tidal flat (Nishida *et al*. 2015).

High FST and ΦST values (population differentiation) were detected between the western and eastern groups and were statistically significant (Nishida *et al*. 2015), supporting that these two genetic groups are distinct. The Kafuri Bay population, located at the border region between the western and eastern groups, was significantly different from its neighbouring sites of Imari Bay and Hakata Bay and only the population from Kafuri Bay had AT06, suggesting this is an independent population. There were no significant differences among sites in the eastern group, because this group was almost monomorphic for AT01.

Microsatellite analysis failed to detect genetic differences between the Kafuri and Hakata Bay populations and contrary to the mtDNA analysis, it appeared that individuals from Kafuri Bay belonged to the eastern group. This conflict in results may be explained by incomplete sorting of microsatellite alleles or male mediated gene flow (Nishida *et al*. 2015). It therefore appears that the Kafuri Bay population on the border region has elements of both the western and eastern groups. Microsatellite analysis also strongly suggested limited gene flow between populations in Hirao Bay and those from neighbouring sites.

From Nishida *et al*. (2015), genetic diversities (h: haplotype diversity/heterozygosity) of local populations in Japan are also relatively low, 0 (some of eastern populations) to 0.51 (Kujukushima-Sasebo Bay) from mtDNA AT-rich region data, and 0.41(Wama Bay) to 0.57 (Kujukushima-Sasebo Bay) from microsatellite data.

The above results suggest that the Japanese population of *T. tridentatus* formed recently, with a relatively low dispersal rate, leading to the formation of genetically distinct populations (Nishida and Koike 2009).

<u>China</u>

In China, twenty-seven haplotypes (H1 to H27) have been defined in Chinese waters, but genetic variation was found to be moderate at nine of these locations along the

China coast (Weng *et al*. 2013) and genetic diversity was mainly caused by individual differences within local populations (Xu *et al*. 2011).

A study of five geographic populations covering the full distribution range of *T. tridentatus* along the China coast, from Ninghai in Zhejiang (in the northeast); Meizhou and Zhangpu in Fujian; Beihai in Guangxi and Danzhou in Hainan (in the southwest) was undertaken on 28 individuals to examine variations in mtDNA COI sequences and elucidate population genetic diversity and historical demography (Weng *et al*. 2012a). Sequence variation was relatively low with a total of seven transitions observed. In all localities, haplotype H3 was the dominant type observed among eight haplotypes (H1 to H8) and was at the centre of radiation in a Median-Joining network. The prolonged star-like network suggests a signature of population expansions. The study uncovered a low level of diversity/weak genetic structure of *T. tridentatus* in total, but significant genetic divergence between the populations at Ninghai and Danzhou. Both mismatch distribution analysis and Fu's Fs neutrality test provided consistent inference of historic population expansion.

The genetic homogeneity of *T. tridentatus* along the China coast could result from artificial transport, because *T. tridentatus* is frequently smuggled from Beibu Bay in Guangxi to other places in China, but if intercepted, are released where they are intercepted by local law enforcement agencies or animal protectors. These behaviours may cause genetic exchange and eliminate genetic variations among these localities (Weng *et al*. 2012a).

In a study of *T. tridentatus* from nine locations covering its distribution range along the China coast (Ninghai and Wenzhou in Zhejiang; Lianjiang, Pingtan, Meizhou Island and Zhangpu in Fujian; Zhanjiang in Guangdong; Beihai in Guangxi and Danzhou in Hainan), H1 was the dominant haplotype in 49 individuals (43%), and present in all locations (Weng *et al*. 2013). Microsatellite loci revealed weak population structure, with no genetic differentiation among the different locations, indicating nuclear genetic diversity was moderate. No evolutionarily significant unit was found, but mtDNA markers revealed distinct subpopulations, denoting significant genetic variance among locations. Haplotype network pattern also indicates *T. tridentatus* in China underwent historic population expansion and recent historic population recession, whilst mismatch distribution analysis reveals evidence of historic demographic expansion. In terms of population variance, individuals from Ninghai were significantly different from those in Zhangpu and Zhanjiang, and Beihai and Danzhou around Beibu Bay, suggesting that this genetic differentiation is due to geographical distance. However, the Zhangpu population was significantly different from Meizhou, although they are only about 200 km apart. This difference may be a result of genetic isolation as the Zhangpu population was sampled from Dongshan Bay, a semi-enclosed bay 500 km in diameter. Likewise, the Leizhou Peninsula might separate the Zhanjiang population from that in Beihai for genetic exchange.

In a separate study, the AFLP (amplified fragment length polymorphism) technique was used to analyse the genetic diversity of three populations of 92 adult specimens of *T. tridentatus* collected from the south-eastern coast of China: Pingtan in Fujian (N=55); Hong Kong (N=17) and Beihai in Guangxi (N=20) (Xu *et al*. 2011). All three populations had a high degree of genetic similarity and may belong to a large group and the genetic diversity that was detected was mainly caused by individual differences within each population. Each of the 92 individuals displayed a unique AFLP band pattern. Dendrograms constructed on genetic diversity indices and genetic distance showed that the rational division of these three geographic populations of *T. tridentatus* was not significant, in which the genetic distance was not proportional to the geographic distance and the high level of genetic diversity obtained from the AFLP analysis may be due to the effective population size of the species in Chinese waters.

The genetic structure of three populations of *T. tridentatus* from the Taiwan Strait was investigated using mitochondrial (mt) AT(Adenine and Thymine)-rich region DNA sequences by examining 23 individuals from Kinmen, 12 from Tiexianwei and 12 from Dongwei near Magong Island in the Penghu Archipelago (Yang *et al*. 2007). DNA sequence analysis of 369 base pairs (bp) of the mt AT-rich region revealed 10 haplotypes among the 47 individuals and pairwise F-statistics (FST) revealed significantly high gene flow between Kinmen and Dongwei, but marked population subdivision and restricted gene flow between Kinmen and Tiexianwei. Mismatch distribution analysis indicated that the relatively low haplotype and nucleotide diversity observed in the Tiexianwei population can be attributed to a recent bottleneck, probably due to the isolation of Tiexianwei in semi-closed Magong Bay, which prevents gene flow from neighbouring populations.

In a further study of the genetic variation of mt AT-rich region of 114 *T. tridentatus* individuals from eight populations in the northern South China Sea and East China Sea (Zhoushan in Zhejiang, Yangjing in Guangdong, Beihai in Guangxi and in Taiwan, Kinmen Island, Penghu Island, Tiexiawei and Dongwei in the Penghu Archipeligo, Budai and the northern coast of Taiwan Island), pairwise tests of genetic differentiation (FST) indicated that two populations from Budai and Tiexianwei were significantly different from the others (Yang *et al*. 2009a). The genetic connectivity of the other six populations showed a pattern consistent with an isolation-by-distance model of gene flow, in which mismatch distribution analyses indicated that three populations (Beihai, Yangjing and Zhoushan) had a pattern consistent with range expansion and three populations (northern Taiwan Island, Kinmen and Dongwei) appeared to be in equilibrium. Individuals from Budai and Tiexianwei, which are located in semi-enclosed embayments, had patterns of nucleotide substitution consistent with recent population bottlenecks. Populations along the China coast appear to have undergone recent range expansion and could have been affected by glacial sea-level fluctuations along the northern South and East China Seaboard, whilst populations estimated to have undergone a bottleneck may have experienced reduced gene flow due to geographic barriers that contributed to inbreeding depression based on evidence of lower genetic diversity.

Surveys conducted between 2003 and 2007 also indicated the existence of one small *T. tridentatus* population in the Haomeiliao Nature Reserve of the Longgong River, which represents the only *T. tridentatus* nursery ground on the west coast of Taiwan (Yang *et al*. 2009b). However, the Longgong River estuary was blocked by expanding construction of Budai Harbour in 2004. Genetic data revealed by the mt AT-rich region (Yang *et al*. 2007) and COI (Folmer *et al*. 1994) indicated that both nucleotide and haplotype diversities of the Budai population in 2007 were 0 (Yang *et al*. 2009b).

This unexpected genetic homogenization is worse than for horseshoe crab populations that are isolated within natural embayments, resulting in decreased genetic diversity due to restricted gene flow and the bottleneck effect (Pierce *et al*. 2000, Yang *et al*. 2007) and was further supported when the population at Budai was compared to the density of *T. tridentatus* juveniles in Hsiashu, Kinmen in 2005 and 2006 which averaged 0.169 individuals/m² based on annual transect surveys (Hsieh and Chen 2009). However, the density at Budai was too low to be detected, even based on monthly transect surveys throughout 2007 (Yang *et al*. 2009b). This loss of genetic variability and the disruption of current flow by harbour construction and other developmental projects near the

Haomeiliao Nature Reserve indicated this remnant population is not sustainable and may be about to become extirpated (Yang *et al*. 2009b).

<u>Hong Kong</u>

In Hong Kong, the genetic diversity of *T. tridentatus* was investigated by sequencing a mitochondrial gene region from 33 samples collected from six locations (Chan *et al*. 2016, unpublished). Eight haplotypes were identified and the relationship among haplotypes was inferred using Network Analysis. Despite the small distances involved, the results showed the presence of three differentiated populations (Deep Bay, Tung Chung and Shui Hau). The results indicate *T. tridentatus* had a high level of endemism, raising the possibility that *T. tridentatus* exhibits beach fidelity. Given the short geographic distances involved, these genetic patterns suggest that *T. tridentatus* has limited dispersal ability and the low haplotype diversity observed around Tung Chung implies the presence of a population bottleneck, which may eventually result in local extinction.

<u>Philippines</u>

At Palawan Island, Philippines, 24 individuals of *T. tridentatus* were analysed for estimating genetic diversity, using mtDNA AT-rich region. Three unique haplotypes were detected, with one haplotype being dominant (22 individuals), leading to significantly low genetic diversity, h = 0.16 (Nishida *et al*. unpublished data, Laurie/Nishida 2018, pers. comm).

## Conservation implications

Because of national boundaries, it is not possible to mandate region wide conservation management units, but the genetic data support the necessity for range states to take into account genetic subdivisions when drafting conservation management plans.

In Japan, two genetically distinct western and eastern groups were detected and six tentative local management units were defined (Nishida *et al*. 2015).

In China, taken alone, the microsatellite survey data does not distinguish separated evolutionary significant units and mandate the species along the China coast be treated as a single management unit. However, mt DNA surveys indicated significant difference among these locations, so that four management units were implied, including representative locations from Ninghai in Zhejiang, Meizhou Island and Zhangpu in Fujian, Beihai in Guangxi and Danzhou in Hainan, which are on opposite shores of Beibu Bay (Weng *et al*. 2013).

In Hong Kong, an investigation of *T. tridentatus* genetic diversity from six locations, tentatively identifying three local management units (Chan *et al*. 2016, unpublished).

Furthermore, in countries where genetic studies have not been undertaken, but obvious geographic sub-divisions are observed, similar principles should apply.

# Habitat, Biology & Ecology

## Spawning

*Tachypleus tridentatus* deposit their egg clusters along the high tide line of protected sandy beaches, where the spawning environment acts like a humidor (Sekiguchi 1988, pp. 66-67). The eggs enjoy a narrow, controlled temperature range. They are occasionally submerged for relatively short periods of time at high tide, when they are washed with oxygen rich water rising through the sand interstices that hold tiny air bubbles during low tide, and which ensures an exchange of the interstitial water around the eggs. For the rest of the time, the eggs are exposed to the air.

In Japan, a study of 12 spawning sites on the Tsuyazaki Coast, Fukuoka, Japan from 2004 to 2008 (Wada *et al*. 2010) indicated that almost 70% of reproductive females laid eggs at just four sites, in which the silt-clay content of the sand fraction of sediment samples ranged from 0% to 6.1%, the median diameter of sediments ranged from -2.32 to 2.32 phi (ϕ) (medium to very coarse sand) and size-sorting of sediments ranged from 0.37 to 1.98 ϕ (moderately to highly heterogeneous). There was a significant positive relationship between the median diameter of sediments and the number of reproductive visiting pairs at each spawning site.

In Taiwan, potential spawning grounds for *T. tridentatus* have been experimentally confirmed. In Kinmen, they are located at the high tide zone and comprise coarse sand sediment (1.5 mm diameter grain size) with 15% saturated water content in the top 10 to 45 cm of substrate at low tide, 2.4 to 5.0 m tidal amplitude and 0.09 to 0.12 beach slope (Chen *et al*. 2004, Hsieh and Chen 2009).

In Hong Kong, *T. tridentatus* usually nests on the high tide mark of protected sandy beaches and in areas where suitable beaches are not available, or have been diminished, *T. tridentatus* may spawn in the lower reaches of tidal streams. Where the two species occur sympatrically, *C. rotundicauda* does not spawn on sandy beaches, but lays its eggs in muddy substrate in streams and rivers associated with mangrove stands (Shin *et al*. 2014).

In terms of spawning site selection, *T. tridentatus* is capable of locating small patches of suitable habitat amongst larger areas of armoured shoreline, such as the spawning habitat at Tatara Beach in Japan, which is a 16 m by 28 m (448 m² in area) patch located where a breakwater meets a seawall (Sekiguchi 1988, Fig. IV-10, p. 62, Botton 2001, p. 45), to spawning along the pristine five metre wide intertidal zone of an 8,000 m length of beach (40,000 m² in area) at Tanjung Limau in Sabah, Malaysia (Sofa *et al*. 2015).

## Larval development

In all horseshoe crab species, eggs take up to 43-45 days to develop after spawning (Sekiguchi 1988 pp. 148, 163) and a number of phases of development take place inside the egg prior to hatching, including four embryonic molts (Sekiguchi 1988, pp. 145–163, p. 195). After hatching, the trilobite larvae initially develop in the spawning area, then when developed, they planktonically move to a nursery area in the intertidal zone (Sekiguchi 1988, p. 67; Tsuchiya 2009, p. 563). Larval dispersal, based on studies with the American Horseshoe Crab, appears to be quite limited with most planktonic larvae being captured within a few metres of the shoreline (Botton and Loveland 2003).

## Juvenile habitat

Location

*Tachypleus tridentatus* nursery grounds occur on intertidal flats (Sekiguchi 1988 p. 51; Chiu and Morton 1999; Hsieh and Chen 2009; Shin *et al*. 2014), where they are subject to a host of environmental variables, each of potential ecological importance, either alone, or in combination with others (Vestbo *et al*. 2018).

In Hong Kong, juvenile *T. tridentatus* are currently only found at seven shores on the west side of Hong Kong, all of which experience lower salinity in the summer owing to increased freshwater discharge from the Pearl River, a large river system during the summer rains in southern China (Shin *et al*. 2009). All such shores are in sheltered bays largely protected from strong wave action (Kwan *et al*. 2016), all nursery flats are fed by freshwater streams (Chiu and Morton 2004), but not all intertidal flats support juvenile horseshoe crab populations. Where juveniles occur in Hong Kong, whilst all *T. tridentatus* nursery beaches are found in the vicinity of mangrove stands, not all intertidal flats associated with mangrove stands support *T. tridentatus* nursery beaches; some are only inhabited by *C. rotundicauda* (Laurie/Various 2009–2018, pers. obs). Likewise, in southern China, *T. tridentatus* and *C. rotundicauda* are often found sharing the same intertidal flats in the vicinity of mangroves (Chen *et al*. 2015, Xie *et al*. 2017).

Area

In Japan, Sone Tidal Flat is 517 hectares (5.17 km²) when exposed and hosts the largest juvenile *T. tridentatus* intertidal nursery ground in the country (Hayashi 2015). In Hong Kong, juvenile *T. tridentatus* intertidal nursery grounds are delineated in extent by the low-tide mark and range in size from 21,000 m² at Sham Wat; 35,904 m² at San Tau; 39,284 m² at Yi O; 78,676 m² at Shui Hau; 93,100 m² at Pak Nai (Long Chuk Hang); to 114,000 m² at Ha Pak Nai (Li 2008). Studies show that where the two species occur sympatrically, these are spatially delineated into sandy facies, favoured by *T. tridentatus* and muddy facies, favoured by *C. rotundicauda* (Zhou and Morton 2004, Hu *et al*. 2011b, Kwan *et al*. 2015a, Chen *et al*. 2015), further restricting the extent of useable area available for juvenile *T. tridentatus* to forage.

In Puerto Princessa, Palawan, Philippines, the juvenile foraging ground exposed at low tide at the Bernardo Marcelo intertidal flat including seagrass beds is 130 m wide by 600 m long, covering an estimated 78,000 m² in area (Kaiser 2002).

Sediment

Sediment size spectrum governs a range of physico-chemical parameters (Kwan et al. 2016), including drainage, organic content, interstitial oxygen content, microbial activity, warmth (Shuster 1985, Penn and Brockmann 1994) and interstitial water salinity (Morton and Lee 2010). These in turn determine whether juveniles can burrow easily during high tide periods, possibly to avoid predators, and keep themselves cool and moist in summer (Sekiguchi 1988) and warm in winter.

In China, in a study of three nursery beaches in Beibu Gulf (Hu *et al*. 2011b), results showed juvenile *T. tridentatus* preferred living on sandy mudflats, while *C. rotundicauda* preferred muddy shores. Grain size and dissolved oxygen were negatively correlated with the densities of *T. tridentatus*, whereas salinity was positively correlated with their distribution. For *C. rotundicauda*, temperature and salinity were positively correlated with their distribution, whilst grain size, total organic content and tidal height were negatively correlated.

In Taiwan, a study of *T. tridentatus* nursery grounds at Kinmen (Hsieh and Chen 2009) indicated juveniles preferred sediments comprising fine sand with a grain size of 0.14-0.27 mm in diameter, silt/clay content ranging from 13.7-36.2%, water content from 16.9-23.2% when tides receded, total organic carbon content from 0.23-0.41%, total organic nitrogen content from 0.04-0.07%, total chlorophyll a content from 2.3-2.8 μg/cm² and poorly sorted substrates with sorting coefficients from 1.87-2.76 ϕ. Furthermore, juvenile density increased based on the amount of chlorophyll a content in the sediment, which reflected the amount of microalgae available as a food source for higher trophic levels, including polychaetes, which were an important food source for the juveniles, suggesting they prefer nursery grounds containing abundant prey and its supporting food web.

In Hong Kong, large juvenile populations of *T. tridentatus* were located on sandy flat or sandy-mudflat (median particle size: 0.10-0.92 mm) with consistently lower water salinity (12-14 ‰) and higher dissolved oxygen (6.8-7.7 mg/l), while higher densities of juvenile *C. rotundicauda* were found on mudflats which generally had low dissolved oxygen level (3.8-5.5 mg/l), small particle diameter (0.08-0.14 mm) and high water content (32.1-51.1%) (Kwan *et al*. 2016). This is consistent with findings from Pearl Bay, Guangxi where higher abundances of juvenile *C. rotundicauda* were generally observed at high tide mark near mangroves, approximately 1.6 m above Chart Datum, whilst juvenile *T. tridentatus* populations were evenly distributed in the area in between these tidal heights (Chen *et al*. 2015). Such different microhabitat preferences can thus create segregation and avoid competition when similar juvenile species are found occurring sympatrically at the same location (Arakaki and Tokeshi 2012).

Drainage (water content)

Sediment water content seems to restrict the occurrence of juvenile *T. tridentatus*. In terms of sediment water content, values of 11.6-66.3% at *T. tridentatus* intertidal nursery mudflats in Hong Kong (Morton and Lee 2010) far exceeded counterparts identified for Taiwan, where water content for similar mudflats ranged from 9.8-11.4% (Yeh 1999).

Total organic content

Similarly, in terms of organic content, values of 0.7-7.6% at *T. tridentatus* intertidal nursery mudflats in Hong Kong (Morton and Lee 2010) far exceeded counterparts identified for Taiwan, where organic content for similar mudflats ranged from 0.1-0.4% (Yeh 1999).

Dissolved oxygen content

A critical factor in determining the distribution of juvenile *T. tridentatus* appears to be dissolved oxygen (DO) levels. Sediment with low DO directly affects habitat quality by increasing microbial activity (Wong *et al*. 1980) and hence exacerbating the degree of organic pollution (Morton and Lee 2010). Although juvenile horseshoe crabs appear able to tolerate heavy organic pollution loads, they cannot endure low DO levels, as evidenced by their greater occurrence in areas with interstitial oxygen levels of between 8 and 14 mg/l, linked to the proximity of relatively cleaner streams (Morton and Lee

2010). Furthermore, from Morton and Lee's study along the northwestern shoreline of the New Territories of Hong Kong, 80.9% of *T. tridentatus* juveniles were recorded from sediment with well-aerated interstitial waters and relatively better aeration might explain not just the occurrence of *Halophila beccarii* seagrass beds in such areas, but also account for the close interactive relationship identified between these and juvenile *T. tridentatus*, in addition to the habitat structural complexity contributed to by their presence.

Interstitial water salinity

A majority of *T. tridentatus* juveniles were recorded at salinities of 22-26‰, suggesting they have a high salinity tolerance. This compares with the optimal salinity of 8-35‰ recorded for *L. polyphemus* eggs (Jegla and Costlow 1982, Shuster 1985), 5-32‰ for *L. polyphemus* juveniles (Shuster 1982) and 6-31‰ for *L. polyphemus* adults (Robertson 1970), as well as 6-31‰ for adult *T. gigas* (Chatterji 1994).

Temperature (thermal tolerance)

Horseshoe crabs are known to tolerate a wide thermal range (Shuster 1982, Jegla and Costlow 1982, Chatterji 1994), which in *L. polyphemus* changes with latitude (Mayer 1914). Temperature is important to horseshoe crabs because it affects them in terms of blood pH (Howell *et al*. 1973), hatching time, juvenile intermolt duration, carapace length (Yeh 1999), juvenile foraging seasons and foraging time (Morton and Lee 2010), as well as nutrition and reproduction (Shuster 1979).

In Hong Kong, a laboratory study on emerging behaviour of juvenile *T. tridentatus* (Lee and Morton 2009) showed that no juvenile emerged from the sediment under simulated tidal cycles when the water temperature was below 20°C. In contrast, 23% of the juveniles emerged during simulated low tides at 25-30°C, whilst 5% of the juveniles emerged during simulated high tides at 25-30°C.

## Biology and Ecology

Biological studies indicate all four horseshoe crabs species generally follow the same life history protocol (Sekiguchi 1988). Information on *T. tridentatus* is summarized in the following sub-sections.

### Reproduction

*Spawning*

Spawning in *T. tridentatus* varies with latitude. In Japan the spawning season is from the end of June to August (Sekiguchi 1988, pp. 54 and 63); in east China from June to October, and earlier in the Beibu Gulf (Weng *et al*. 2012); in Hong Kong from April to September (Shin *et al*. 2014); in Vietnam from March to September (Nguyen 2007); in Malaysia from March to September in Sarawak (Jawahir *et al*. 2017) and from April to October in Sabah (Mohamad *et al*. 2016).

In Japan, spawning is associated with diurnal and nocturnal high tides (Sekiguchi 1988, p. 58), with peaks being associated with spring tides (Sekiguchi 1988, p. 63, Fig. IV-12, p. 64). Spawning also appears to show lunar periodicity (Sekiguchi 1988, p. 63, 65, Figs. IV-12 and IV-13, p. 64) and is characterized by numerous pairs of *T. tridentatus* approaching spawning beaches at the same time (Sekiguchi 1988, pp. 57-66), where the desirable temperature for adult T. tridentatus to approach the shore to spawn is approximately 18°C (Nishii 1975). Another factor affecting breeding activity appears to be rainfall which affects salinity levels. When the salinity at a breeding beach is exceptionally low, the number of spawning pairs approaching the spawning beach is also low, reductions in salinity being affected by the amount of rainfall received (Sekiguchi 1988, p. 65). In Malaysia, Tan *et al*. (2012) reported the spawning season for all horseshoe crab species is governed by the northeast monsoon, which occurs from November to February annually, when fewer horseshoe crabs are sighted because of strong waves, heavy precipitation and turbulent river flows.

*Mating*

Females arrive at the spawning beach with a male attached to her back (Sekiguchi 1988, p. 54). The males attach to the females by grasping the lateral margin of the female's opisthoma with the claspers at the distal ends of the second and third prosomatic appendages. The female and male swim while coupling and approach the spawning beach on the rising tide (Sekiguchi 1988, p. 61).

Evidence for an OSR of 1:1 is strong, as the following observations show. In Japan, spawning is characterized by pairs of *T. tridentatus* approaching spawning beaches at the same time (Sekiguchi 1988, pp. 57-66; Botton *et al*. 1996; Wada *et al*. 2010), in China, fisheries catch records are counted in pairs (Liao and Li 2001; Huang *et al*. 2002; China Species Red List 2009b; Hong 2011, pp. 154, 160, 202, 208), inshore fishermen in Hong Kong catch adult *T. tridentatus* as mating pairs (Laurie/Various pers. comm.) and in Sabah, Malaysia only amplexed *T. tridentatus* pairs were observed during a survey in 2014, with no tandem or unpaired females, or satellite males, indicating monogamous behaviour in the spawning population (Mohamad *et al*. 2016), suggesting satellite behaviour is almost non-existent in *T. tridentatus* (Brockman and Smith 2009, p. 211).

*Egg deposition*

Horseshoe crabs are the only extant marine arthropod with external fertilization of their eggs (Brusca and Brusca 2003). In Japan, the female climbs to the high tide line where she excavates a cavity in the sand and deposits some of her eggs, after which the male releases sperms to fertilize them. The pair then advances 15 to 20 cm to repeat the procedure. When the female digs out the second nest, the sand that she excavates covers the eggs in the first nest. They repeat this procedure several times, then retreat from the beach on the ebbing tide (Sekiguchi 1988, p. 54).

*Tachypleus tridentatus* fecundity in Japan ranges from 13,000 eggs (Tsuchiya 2009, p. 563) to about 20,000 eggs (Sekiguchi 1988, p. 66) and field observations suggested that the number of eggs in a cluster can vary from between 100 to 200 (Nishii 1975) to 500 (Tsuchiya 2009, p. 563) to between 561 to 888 and eggs can be laid in as many as ten clusters at a single spawning event (Sekiguchi 1988, p. 61), the eggs being deposited at a depth of between 10 and 20 cm, indicating not all eggs are deposited during a single spawning event (Sekiguchi 1988, p. 66).

In Sabah, Malaysia spawning amplexed *T. tridentatus* pairs at Tanjung Limau were located in the tidal zone five metres from the high tide line on an 8,000 m length of beach by searching for the formation of spawning foam created by streams of bubbles that are released from the sediments as the female horseshoe crabs excavate their nests (Mohamad *et al*. 2016). In a parallel study at the same location, 24 nests created by six pairs of *T. tridentatus* were all excavated close to shore, within the intertidal zone, between 1.73 and 4.79 m from the high tide mark. Single females made from between two to seven nests during a single spawning event, which lasted from ten to 56 minutes, depositing between 283 and 2,136 eggs per cluster (Sofa *et al*. 2015). In China, fishermen also use streams of bubbles to locate spawning pairs near the shore line (Weng *et al*. 2012).

## **Growth**

*Egg development*

In all horseshoe crab species, eggs take up to 45 days to develop after spawning (Sekiguchi, 1988). In laboratory conditions, the time from insemination to hatching is about 14 days for *L. polyphemus*, 34 days for *C. rotundicauda*, 37 days for *T. gigas* and 43 days for *T. tridentatus* (Sekiguchi 1988, p. 148). A number of phases of development take place inside the egg prior to hatching, including four embryonic molts (Sekiguchi 1988, pp. 145-163 and 195).

Optimal water temperatures for incubating eggs range from 22-31°C in Japan (Sekiguchi *et al*. 1988) to 28-31.8°C in Taiwan (Chen *et al*. 2004).

*Larval to planktonic phase*

After the final embryonic moult, the embryos hatch out as swimming Instar I, called trilobite larvae (Sekiguchi1988, p. 163). Hatched Instar I have only two pairs of branchial appendages (Yeh 1999) and after hatching, the trilobite larvae initially develop in the spawning area, or close to it. In Japan, the hatched larvae spend winter in the nest and emerge the following spring, when they planktonically move to a nursery area in the intertidal zone (Sekiguchi 1988, p. 67; Tsuchiya 2009, p. 563). Surveys of the dispersal patterns of trilobite larvae in *L. polyphemus* suggest their capability for long-range dispersal is limited (Botton and Loveland 2003) and similar patterns are seen in *T. tridentatus*. Sekiguchi (1988 p. 67) observed that after hatching, *T. tridentatus* larvae move very little compared with *L. polyphemus*, whereas Sakemi (1997) reported that at a spring tide event in Imari Bay, Japan, most larvae moved planktonically from the hatching during the night at high tide to the intertidal area on the ebbing tide.

Based on laboratory observations of *T. tridentatus* in Taiwan (Hsieh and Chen 2015), the hatching rate of Instar I is as high as 80 to 90%. Instar I does not feed and moults into Instar II approximately three months later. The molting rate of Instar II to Instar III, however, is low, around less than 10%. After Instar III, juveniles possess better foraging and defence abilities and capacity to adapt to environmental changes, thus achieving better survival in natural habitats than Instars I and II, although Instar III still exhibit weak locomotive ability and cannot swim against strong currents. The trilobite larvae may also exhibit phototaxis (Sakemi 1997).

*Juvenile*

During juvenile development *T. tridentatus* undergoes stepwise growth shedding of its exoskeleton, producing between 13 to 16 moults for males and 14 to 17 moults for females as they grow from the larval stage to sexual maturity (Harada 2003, Sekiguchi

1988, pp. 192 and 194). Hence, prosomal width is correlated with either age or developmental stage (Shuster 1954).

Horseshoe crabs typically experience six moults (ecdyses) in the first three years after fertilization (Rudloe 1981, Shuster 1982, Mikkelsen 1988, Sekiguchi 1988 p. 194, Carmichael *et al*. 2003). Thereafter, ecdysis may take place once a year until maturation (Shuster 1954, 1958, 1982; Mikkelsen 1988; Sekiguchi 1988 p. 194; Chatterji 1994). Exuvia always occur on the sediment surface, indicating that individual horseshoe crabs emerge from the substratum to moult (Shuster 1958).

During growth, Instar I pass the winter and moult three times in the second year, twice in the third year and once in the fourth year, reaching the Instar VII stage. After this, they molt once a year, so that males become mature in the 13th year and females in the 14th year (Sekiguchi 1988, p. 194). In China, *T. tridentatus* has a similar growth rate and the length of time to reach maturity is between 13 to 14 years (Hu *et al*. 2015). *Tachypleus tridentatus* therefore grows at a much slower rate than its American counterpart *L. polyphemus* (Sekiguchi 1988, p. 185).

In respect of *T. tridentatus* in Japan, various rates of development at different locations have been documented. Goto and Hattori (1929) described *T. tridentatus* to take 13 moults to reach maturity, whereas Kawahara (1984) reported males to molt 14 times in nine years and females to moult 15 times in 10 years. Based on an allometric growth pattern identified in laboratory culture over a period of nine years, Sekiguchi (1988 pp. 192 and 194) accounted males to moult 15 times in 13 years and females to moult 16 times in 14 years. An earlier study by Asano (1942) also reported *T. tridentatus* to spend approximately 16 years to moult 18 times before reaching maturity. Given the wide range of variables that could affect development, particularly temperature, all such development rates could be correct.

In terms of measuring growth rates, a laboratory study in Hong Kong (Lee and Morton 2005) showed that after every ecdysis juvenile *T. tridentatus* prosomal width increments increased from between 38.9% for a 25-30 mm juvenile to 8.1% for a 75-80 mm juvenile, with an average prosomal width growth increment of 24.2%. Similarly, after every ecdysis, juvenile *T. tridentatus* wet weight gains varied from between 40 and 90.7%, with average percentage weight gains of 71.5%. It is apparent as juveniles get larger, prosomal width increments and wet weight gains decrease with size. Both sets of results also demonstrated that individuals with similar pre-moult sizes show a wide range of growth increments even though they were kept under the same conditions. These gains are similar to data from a field tracking study conducted in Hong Kong between 2010 and 2012, which showed prosomal width growth rate ranged from 0.08 to 0.25 mm per day in 16 juvenile *T. tridentatus* that were released and recovered (Kwan *et al*. 2015b).

In China, young juveniles of *T. tridentatus* forage on intertidal mud flats and when their exoskeletons are black local fishermen call them ‘black-skin’ horseshoe crabs. As they moult and grow, they move into shallow subtidal waters and between the 11th and 16th moults, their skins become yellowish, when they are referred to as ‘sallow-skin’ horseshoe crabs (Liao *et al*. 2001b).

Measuring the maximum prosomal width of juvenile *T. tridentatus* on intertidal nursery grounds can give an indication of how long juveniles spend foraging before moving into deeper water to develop into adults. In Hong Kong, the maximum prosomal widths of juvenile *T. tridentatus* recorded on intertidal nursery grounds included 102.5 mm at Pak Nai, indicating an age of about 10 years old (Morton and Lee 2010), 127.20 mm for an exuviae found at Ha Pak Nai (Laurie 2009, pers. obs.) and 142 mm for a juvenile at Shui

Hau, indicating an age of about 12 years old (Chiu and Morton 1999). Such data confirmed that juveniles in Hong Kong spend at least 10 years foraging in their intertidal nursery grounds before migrating into offshore waters (Morton and Lee 2010).

## Factors affecting juvenile growth

*Temperature (Emergence and Activity): Seasons/ Latitudes - Sub-tropical/Tropical*

Juvenile foraging activity varies with latitude and climate and the emergence of juveniles is temperature related. In Japan, most juveniles become inactive around November, only to reappear the following March to April, when they begin foraging again (Sekiguchi 1988, p. 51). During over-wintering, juveniles remain buried in the sandy-mud bottom of the intertidal area (Kawahara 1982).

In China, juveniles in Xiamen, Fujian and Shantou in east Guangdong were observed to be active from May to September; in Shanwei in east Guangdong and Wuchuan in west Guangdong from April to September; in Donghai Island in west Guangdong from March to September and in Qishui and Beihai in Guangxi, all year round, from January to December (Liao *et al* 2001a).

In sub-tropical Hong Kong, field observations of the same intertidal juvenile nursery grounds in summer and winter indicated juvenile *T. tridentatus* do not emerge when sediment temperatures fall below 20°C and in winter, juvenile densities are much reduced in comparison to summer densities for the same area (Chiu and Morton 2004). This emergence temperature of 20°C in Hong Kong has been confirmed in simulated laboratory conditions (Lee and Morton 2009). As a result, juvenile *T. tridentatus* are usually inactive and remain buried in the substratum of their nursery ground during much of the winter months from December to March, when substratum temperatures range from 11.5-18.0°C (Lee and Morton 2009, Morton and Lee 2010). Such burial behaviour may be the result of declining aerobic metabolism in juvenile horseshoe crabs under the influence of low temperatures, leading to a period of dormancy (Kawahara 1982, Chiu and Morton 1999, Chiu and Morton 2004).

In Taiwan, studies at Beishan, Nanshan and Hsiashu showed seasonal changes in juvenile abundance with densities increasing approximately ten fold from May to August, with a peak in June, compared to September to April (Hsieh and Chen 2009), whilst in tropical Palawan in the Philippines there appears to be no seasonality to foraging activities as juveniles can be found foraging year round (Schoppe 2002).

The optimal thermal range for juvenile *T. tridentatus* activity in Taiwan ranged between 28-31°C (Chen *et al*. 2004) and between 28-32°C in Hong Kong (Morton and Lee 2010), compared with the optimal thermal range of juvenile *L. polyphemus* activity of between 15-40°C (Shuster 1982).

Even under laboratory simulated summer temperatures of 25-30°C in Hong Kong (Lee and Morton 2009), only 23% of juvenile *T. tridentatus* became active and emerged from the substratum under simulated low tides and of these, none re-emerged from the substratum at the next low tide, which has also been noted from field observations, suggesting that such behaviour occurs only at the population level, possibly to avoid the emergence of all juveniles at the same predictable time. This might be related to reducing the chances of mortality, such as from predators.  In the same experiments, only 5% of tested juveniles, irrespective of temperature were ever identified above the stratum during simulated high tides, with more activity being recorded at simulated summer temperatures.

Ecdysis in *T. tridentatus* is also affected by temperature, slowing substantially when sediment and water temperatures reach 20°C (Morton and Lee 2003) and in laboratory experiments, 50% of juvenile *T. tridentatus* moulted at approximately 28.8°C, whilst only 10% moulted at approximately 18.8°C, indicating *T. tridentatus* in Hong Kong might take a shorter time to reach sexual maturity, as compared with conspecifics in Japan. Furthermore, since juveniles can moult more frequently at higher sediment or water temperatures, this raises the possibility that ecdysis might persist throughout the year in horseshoe crabs living in the tropics, especially if food is available, suggesting individuals might moult more frequently and take a shorter time to reach sexual maturity, as compared with conspecifics in both subtropical Japan and Hong Kong (Lee and Morton 2005). In *L. polyphemus* temperatures of below 20°C have been found to reduce molting hormone levels to a critical degree and hence suspend the ecdysis process (Jegla 1982).

During simulated low tides, irrespective of sediment temperature, nearly all juveniles which did not emerge at the substratum surface buried themselves to a depth of less than 3 cm, possibly due to the occurrence of anoxic sediments at deeper levels in their natural habitat (Lee and Morton 2009).

*Salinity*

During its life cycle *T. tridentatus* can encounter a wide range of salinity changes and a study of late juvenile *T. tridentatus* ('sallow-skin' horseshoe crabs) indicated they were osmoconformers, being able to tolerate a wide range of salinity changes. This may allow them to adapt gradually from inshore low salinity to offshore high salinity (Liao *et al*. 2012).

*Diet*

There are two different approaches to ascertaining diet in horseshoe crabs: gut content analysis and stable isotope analysis (Botton and Shuster 2003, Botton 2009), by tracking the original site of photosynthetic fixation of carbon atoms that were ultimately assimilated into the animal's tissues (Carmichael *et al*. 2004, Bird *et al*. 2018).

In *L. polyphemus* studies of juveniles indicate their food sources change during ontogeny, from mainly benthic and suspended particulate organic matter for Instar II to Instar III, to crustaceans and polychaetes for Instar V to Instar XI, when they are large enough to feed on small-bodied animals in the sediment (Gaines *et al*. 2002, Carmichael *et al*. 2009) and as their body size increases, they prey on larger animals, increasing their trophic position as they grow (Carmichael *et al*. 2004). Similar patterns are seen in *T. tridentatus*.

In Japan, dual stable isotope analysis (δ13C and δ15N) conducted on 126 samples of juvenile *T. tridentatus* exuvia, muscle and whole body from seven local populations indicated that the isotopic signatures of animal body after Instar IV largely reflected the recent food source intake by the juveniles (Koike 2011). Most of the younger *T. tridentatus* were also found to be dependent on seagrass ecosystems.

In Hong Kong, juvenile *T. tridentatus* have a close interactive relationship with *Halophila beccari* seagrass beds (Fong 1998, Huang et al. 1998, Morton and Lee 2010), where *H. beccari* is recognized as being ecologically significant in supporting a variety of the prey consumed by juvenile horseshoe crabs on intertidal mudflats in summer and winter. A dual stable isotope analysis (δ13C and δ15N) of Instar VI to Instar XI juvenile *T.*

*tridentatus* (Kwan *et al*. 2015a) found they consumed a mixed diet of intertidal invertebrates comprised primarily of polychaetes, crustaceans and bivalves which were seasonally available on the mudflat. The food sources available to juvenile *T. tridentatus* were largely supported by seagrass and its epiphytes, except that some juveniles collected in winter had heavier carbon values, which might represent a mixed food source of seagrass *H. beccari* and common cordgrass *Spartina anglica*.

*Tachypleus tridentatus* and *C. rotundicauda* occur sympatrically at some locations in Hong Kong and dual stable isotope analysis (δ13C and δ15N) of their food preferences indicated that juvenile *T. tridentatus* tend to prey on crustaceans, including amphipods and isopods, gastropods and insect larvae, while juvenile *C. rotundicauda* preferred mainly polychaetes and bivalves. Furthermore, temporal changes in the diet of juvenile *T. tridentatus* were apparent, with 51% seagrass and 31% crustaceans as the main dietary contributors in summer, whilst 35% crustaceans, 23% gastropods and 17% insect larvae as the main contributors in winter (Shin *et al*. 2013, unpublished). Gut content analysis of a range of juveniles of both species at two nursery crèches revealed that both species fed selectively on only a small component of the available benthos (Zhou and Morton 2004). Their diets reflected the locality where they foraged, their diets differed, and in addition to insect larvae, polychaetes and small crabs favoured by juvenile *T. tridentatus*, organic detritus, fragments of *H. beccari* and unidentified eggs were identified. At the localities studied, both species appear to avoid hard-shelled species, including gastropods, chitin-covered nematodes, despite their high abundance in sediment samples, and ostracods. The apparent disparity in results reflected the juveniles are adopting a ‘you are where you ate’ approach to feeding (Bird *et al*. 2018).

**Movement (Behaviour)**

Growing juveniles move offshore from their nursery areas with increasing age (Shuster 1979) and field observations of *L. polyphemus* indicated that juveniles migrate seawards with increasing body size to forage and eventually move to shallow waters where they become adults (Rudloe 1981). This generally holds true for *T. tridentatus*.

In China, a study of three nursery beaches in Beibu Gulf (Hu *et al*. 2011b) showed that smaller juveniles of T. tridentatus and *C. rotundicauda*, with a mean prosomal width of circa 15.5 mm, lived closer to the shore, while larger juveniles, with a mean prosomal width of circa 71.4-88.8 mm, were found further offshore. Such distribution pattern suggested that juveniles of both species move away from their natal area to the lower shore area as they grow, possibly because offshore sediments tend to have higher total organic content, which may reflect the availability of more potential food sources for them. A study in Taiwan also found that *T. tridentatus* Instars II and III, which were less than two years old tended to occur close to shore, mostly less than 50 m from the highest water mark and Instars III to VII, representing individuals two to five years old, were found more than 150 m away (Yeh 1999).

In Japan, the juvenile stage, up to seven cm in prosomal width, is spent in the intertidal area near the hatching beach, and locomotive activity is relatively low, around 50 m per month (Kawahara 1982, Sekiguchi 1988, pp. 51-52). In Hong Kong, a two-month field tracking study of juvenile *T. tridentatus* on an intertidal nursery beach (Kwan *et al*. 2015b) showed that Instars VII to X, with prosomal widths of between 31 and 59 mm, utilize a mean home range of between 269 and 462 m² for foraging, with cumulative recovery rates of tagged juveniles ranging from 70 to 82 %. There was no significant correlation between body size and home range area.

In all horseshoe crab species, juveniles gradually emerge from the sediment when the substratum is exposed during low tides to forage. They forage on the intertidal flat areas covered with a thin layer of surface water (Zhou and Morton 2004, Kwan *et al*. 2015b) or pools of standing water in seagrass beds. The shape of their foraging trails is irregular and distinctive (Rudloe 1981, Chiu and Morton 2004). In Japan, juveniles emerge from the substrate about 1.5 hours after the tide ebbs, forage then bury themselves about three hours after the lowest tide level forms. Fewer individuals forage at night and no individuals were observed foraging in the intertidal area when it was covered in water during high tide (Kawahara 1982; Sekiguchi 1988, p. 51). In the Philippines, juveniles emerge from the substrate about two hours before the lowest tide level and forage for about three to four hours, before burying themselves in the substrate as water levels increase on incoming tides (Schoppe 2002). In studies around Puerto Princesa City, all foraging juveniles were found in the intertidal area below the mean low water line on surfaces which were covered in 2-4 cm of water during the low-tide period (Kaiser 2002) and they preferred foraging in areas with a sandy-muddy substrate (Almendral and Schoppe 2005). In an eight-month study, 88% of juveniles were recaptured in the same area at San Pedro Beach (Kaiser 2002), whilst 40% were recaptured during a five-month study at Aventura Beach (Almendral and Schoppe 2005), suggesting there is a high degree of beach fidelity for juvenile *T. tridentatus*.

**<u>Sub-adult</u>**

As they grow, larger juveniles and sub-adult individuals gradually move to deeper water (Shuster 1982), at depths of 20-40 m, depending on their growth stage (Liao et al. 2001b).

In a study to investigate the effectiveness of a trawling ban in Hong Kong on demersal fish and crustacean communities, systematic trawls surveys were conducted at 12 sampling sites in three different areas of Hong Kong waters between 2012 and 2015. No adult *T. tridentatus* were collected, but sub-adults were recovered at two nearshore, shallow water sampling sites, nine off the West New Territories coast, five on the same day and five off the West Lantau coast, three on the same day. Their close proximity to juvenile nursery crèches suggests these areas are sub-adult foraging grounds (Laurie/Tao 2018, pers. comm).

**<u>Adult</u>**

*Tachypleus tridentatus* is the largest of the four extant species (Sekiguchi 1988, p. 94). Female *T. tridentatus* are considered adult at a prosomal width of circa 27.8 cm; males at a prosomal width of circa 24.4 cm (Sekiguchi 1988, pp. 187 and 191). An adult female with a prosomal width of 42.1 cm has been recorded from Menggatal River, Sabah (Robert *et al*. 2014), a female 85.0 cm in total length was recorded from the same area, whilst males in the area have been recorded with a prosomal width of 31.8 cm, growing up to 68.5 cm in length (Sekiguchi 1988, p. 17). Such data confirmed that *T. tridentatus* from Kota Kinabalu, Sabah, Malaysia are the largest compared to its conspecifics in Southeast Asia (Yamasaki *et al*. 1988).

*Tachypleus tridentatus* is sexually dimorphic, with males being smaller than females (Shuster 1982). In all species of horseshoe crabs, adult males can be distinguished from females because in males the first prosomatic legs form hemichelate claspers, resembling a clenched fist. In *T. tridentatus*, *T. gigas*, and *C. rotundicauda*, the second prosomatic legs are also modified as claspers (Yamasaki *et al*. 1988). In *T. tridentatus* the adult male prosomatic carapace exhibits a pair of wide, but shallow notches on the anterior margin, with long marginal spines in the male, whilst the fourth to sixth marginal spines are degenerated in the female (Sekiguchi 1988, p. 17).

In *T. tridentatus* there is evidence to suggest different age-to-maturity rates at different latitudes, but in Japan and China, where data are available, estimates for age-to-maturity are between 13 to 14 years, the female taking longer than the male to reach maturity (Sekiguchi 1988, p. 194; Hu *et al*. 2015).

Adult individuals winter in deep water, about 20 m or more offshore (Sekiguchi 1988, p. 52). In Japan, they move inshore to shallow waters, 1 to 3 m deep, when the seawater temperature rises to above 18°C around early June, to begin to feed, prior to spawning (Nishii 1975).

## Migration and Dispersal

Capture-recapture studies on adult *L. polyphemus* have demonstrated they can have a restricted movement range close to their original tagging location and tend to repeatedly return to the same beach to spawn in the same or subsequent seasons (Rudloe 1980, James-Pirri *et al*. 2005). Investigations using telemetry technologies have also shown that year-round movements of some adult *L. polyphemus* populations are likely to remain within the same estuary or bay (Watson *et al*. 2009, James-Pirri 2010, Schaller *et al*. 2010).

A 17-year study of 30,432 tagged *L, polyphemus* between 1986 and 2002 in the Middle Atlantic Bight indicated the presence of discrete spawning populations, with released animals returning to their point of release within days during the spawning season. Of the 762 (100%) recoveries of crabs released in a tagging study in Delaware Bay, 75.07% (572 individuals) had travelled 0-20 km, 21% (160 individuals) had travelled 20-50 km, 2.36% had travelled 50-100 km, and 1.57% (12 individuals) had travelled over 100 km (Swan 2005).

Similar patterns of behaviour are evident in *T. tridentatus*. Between 2006 and 2009, two studies which monitored the year round migratory patterns of twenty adult *T. tridentatus* (15 males; five females) in coastal embayments in Tsuyakazi Bay, Japan using acoustic telemetry found 13 out of 20 (60% recapture rate) individuals overwintered within the bay where spawning took place, while three of the females overwintered outside the bay (Wada *et al*. 2016). Movements began in May and lasted until late October, in the post spawning period and there was more activity during periods of higher water temperature. They were also found to be more active at night than during the day, whilst in surveys conducted in Etajima City in Hiroshima Prefecture between 1999 and 2016, 13 out of 29 tagged adults were recaptured in the same area (45% recapture rate), with one individual being tagged in 2007, then recaptured in 2009 and 2014 (Ohtsuka *et al*. 2017, Laurie/Ohtsuka 2018, pers. comm).

An on-going tagging study in Hong Kong suggested adult *T. tridentatus* are generally found on the same or nearby shore within a 5 km radius from their point of release (unpublished data, cited in Kwan *et al*. 2016). In Sabah, Malaysia, a tagging survey to record the movements of *T. tridentatus* in the adjacent bights of Tanjung Limau in Sandakan and Inderasabah in Tawau indicated movements of from 14 km in 26 days, to 24 km in 286 days (Mohamad *et al*. 2017). However, despite the adjacent proximity of Tanjung Limau and Inderasabah, initial results indicated no mixing of populations.

Spawning habit and limited movement and migration patterns of trilobite larvae, juveniles and adults are consistent with the findings of genetic studies, including the formation of genetically distinct subpopulations in Japan (Nishida and Koike 2009), China (Weng *et al*. 2013), Taiwan (Yang *et al*. 2007, 2009a) and Hong Kong (Chan *et al*. 2016, unpublished).

## Mortality

The natural survival rate of juveniles for all species of horseshoe crabs, particularly in the first year of their life is low. On the shores, juvenile horseshoe crabs are vulnerable to predation because of their small body size and relatively softer carapace than the adults. From field data, the mortality rate per instar (moulting) stage for juvenile *T. tridentatus* in Hong Kong may be between 57 to 84% (unpublished data, cited in Shin et al. 2014), whilst in China, a study of three nursery beaches in Beibu Gulf indicated a cumulative mortality rate of circa 50% (Hu *et al*. 2015).

*Natural Predators*

In comparison to *L. polyphemus* (Weber and Carter 2009), *T. tridentatus* appears to lay fewer eggs per cluster (Nishii 1975; Sekiguchi 1988, p. 61; Tsuchiya 2009, p. 563; Sofa *et al*. 2015). This finding, along with the low density of spawning females, may explain the apparent absence of mass natural predator events on its eggs, as has been documented for *L. polyphemus* (e.g. Botton 2009).

In Japan, there appears to be no natural predators of *T. tridentatus* eggs, although eggs laid in ill-drained, muddy sand have been observed to have died and the eggs clusters infected with the nematode *Turbatrix* (Sekiguchi 1988, p. 66).

In Hong Kong, Deep Bay lies at the heart of the East Asian-Australasian Flyway (EAAF), which extends 13,000 km, from the Arctic Circle through Southeast Asia to Australia and New Zealand. The EAAF is a route used by over 50 million migratory water birds and each year these birds fly between their breeding and wintering grounds, stopping off in places such as Deep Bay where they feed and rejuvenate before continuing on their long and arduous journeys. Although Deep Bay and its surrounding provide feeding and roosting habitat for up to 70,000 water birds each winter and another 20-30,000 shorebirds visit the area on passage during their annual migrations in spring and autumn (WWF Hong Kong 2017), none of these migratory events correspond to *T. tridentatus* spawning events in Hong Kong. Thus, unlike Delaware Bay in the USA (Botton 2009), *T. tridentatus* spawning in Hong Kong does not appear to support shorebird migration.

Between January and August 2016, 490 dead adult *T. tridentatus* were found on the shores of the Kitakyushu/Sonehigata tidal flat in Japan (Takahashi 2016). It is believed they had succumbed to predation by the Naru Eagle Ray, *Aetobatus narutobiei*, which may have extended its range due to warming waters (Kabutogani Jimankan Museum 2016).

In Sabah and Sarawak, Malaysia, the Long-tailed Macaque (*Macaca fascicularis*), also known as the crab-eating macaque is a predator of horseshoe crabs (Ang 2016), although it is unlikely to have a significant impact on *T. tridentatus* populations.

*Natural disasters*

Significant earthquakes with epicentres in Sumatra occurred in March and September 2007, and in Padang, Sumatra in September 2009. Prior to these earthquakes, *T. tridentatus* was naturally rare in Padang and after these earthquakes, no specimens of *T. tridentatus* were found during site visits in 2011 and local villagers reported the population size had decreased significantly following the earthquakes (Nishida 2012b, Laurie/Nishida 2018, pers. comm).

**Table 1**. Summary of population data and estimated population declines for *Tachypleus tridentatus* throughout its range based on available literature.

| Nation / region | Time / period | Population data | Source |
|---|---|---|---|
| **Japan** | 1958 | 10,000 adults were stranded during the reclamation of Tomioka Bay. | Nishii 1973, Seino *et al*. 2003 |
| | 1969 | 100,000 adults were stranded during the reclamation of Kasaoka Bay. | Seino *et al*. 2003 |
| | 1970s-1990s | A reduction from 500 to 30 spawning pairs visiting Tatara Beach, N. Kyushu each season. | Sekiguchi 1988, Botton *et al*. 1996 |
| | 1995-2013 | Between 120 and 1581 spawning pairs were recorded visiting Sone Tidal Flat each year. | Hayashi 2015 |
| | 1999-2016 | 37 adults were recorded in surveys at Etajima City, Hiroshima Prefecture. | Ohtsuka *et al*. 2017 |
| | 1999-2016 | Seven adults were recorded in surveys at Takehara City, Hiroshima Prefecture. | Ohtsuka *et al*. 2017 |
| | 2002-2005 | 81 adults were caught by fishermen during surveys in Kujukushima Islands, Nagasaki Prefecture. | Iwaoka and Okayama 2009 |
| | 2006 | *T. tridentatus* assessed as Critically Endangered in the Red Data Book of Japan. | Ministry of Environment Japan 2006 |
| | 2016 | 490 dead adult *T. tridentatus* were found on the Kitakyushu/Sonehigata (Sone) Tidal Flat. It is believed they had succumbed to predation by the Naru Eagle Ray, which may have extended its range due to warming waters. | Takahashi 2016, Kabutogani Jimankan Museum 2016 |
| **China** | 1970s | *T. tridentatus* was once widely distributed and abundant along the southeast coast of China with high population densities. | Sekiguchi 1988, Hong 2011 |
| | 1970s | Pingtan in Fujian was reputed to have had the largest population of *T. tridentatus* on the coast of China. | Huang 2011 |
| | 1970s-1980s | Mass spawning events were seen by villagers in Fujian and Guangxi. | Hong 2011, Weng *et al*. 2012b |
| | 1975-1982 | Surveys throughout Asia found the highest population densities in Fujian and on the west coast of Hainan. | Sekiguchi 1988 |
| | Before 1980s | Up to 1,500,000 spawning pairs were estimated distributed in Guangdong. | Hong 2011 |
| | Early 1980s | *T. tridentatus* abounded in the seas around Dongshan Island in Fujian and major concentrations could be found around Hainan Island. | Mikkelsen 1988 |

| Nation / region | Time / period | Population data | Source |
|---|---|---|---|
| | Before 1990s | In excess of 600–700,000 pairs were estimated in Beibu Gulf. | Hong 2011 |
| | 1990 | Populations in Guangdong province had dropped to 600,000–700,000 pairs. | Hong 2011 |
| | Before 1990s | In excess of 300,000 pairs were estimated in Hainan Island. | Hong 2011 |
| | 2004 | *T. tridentatus* populations had seriously declined due to over-exploitation. It was assessed as Endangered in the China Species Red List. It was now difficult to obtain. | China Species Red List 2009a,b |
| | 2005 | *T. tridentatus* was recognized as a rare species for establishment of a conservation area in Shantou, Guangdong. | UNEP 2005 |
| | 2006-2010 | Surveys at 27 previously renowned spawning sites observed no adult spawning and juvenile populations at only six sites. Surveys are based on juvenile population counts. | Weng *et al*. 2012b |
| | 2006-2010 | No spawning was observed at Dongshan Bay, but 1,000 juveniles were counted. | Weng *et al*. 2012b |
| | 2009 | Surveys in Guangxi are based on juvenile population counts. | Hu *et al*. 2011 |
| | 2014 | *T. tridentatus* populations in Guangxi were estimated to have declined by 90% in recent years. | Fauna and Flora International 2014 |
| | 2015 | No juvenile or adult *T. tridentatus* were found at Shanqi and Tannan Bay in the Chinese Horseshoe Crab Reserve in Pingtan Island, Fujian. | Li *et al*. 2017 |
| **Taiwan** | | *T. tridentatus* was once widespread and abundant on the west coast of Taiwan Island, in the Penghu Islands and Kinmen, with thriving populations. | Chen *et al*. 2004, Chen and Chen 2011 |
| | 1960s | No individual adults or mating adult pairs have been recorded from the intertidal flats of Taiwan Island coast since the 1960s. | Hsieh and Chen 2015 |
| | 2003 | Surveys on the Penghu Islands found only 20 juveniles. | Hsieh and Chen 2015 |
| | 2003-2009 | A study of juvenile densities at Beishan, Nanshan, Hsiashu on Kinmen showed rapid and significant declines in the juvenile populations at Nanshan and Hsiashu. | Hsieh and Chen 2015 |
| | 2004-2005 | A small population of juveniles was documented at the Haomeiliao Nature Reserve in Budai on Taiwan Island. | Yang *et al*. 2009b |

| Nation / region | Time / period | Population data | Source |
|---|---|---|---|
| | 2007 | No juveniles have been found at Budai on Taiwan Island since 2007. | Yang *et al*. 2009b |
| | 2013 | One juvenile was reported from Xiangshan intertidal flat at Hsinchu City on Taiwan Island. Viable juvenile populations are restricted to a number of sites on Kinmen Island. | Hsieh and Chen 2015 |
| **Hong Kong** | | *T. tridentatus* was once widespread and abundant throughout Hong Kong waters with thriving populations. | Shin *et al*. 2014 |
| | 1960s-1970s | Mass spawning events were observed by residents. | Laurie/Various pers. comm. |
| | Up to 1980s | Mating pairs of *T. tridentatus* were often seen in great numbers on many beaches during the summer months and adults were evenly distributed throughout the waters of Hong Kong. | Chiu and Morton 1999 |
| | Early 1980s | Major concentrations of *T. tridentatus* could be found in Hong Kong. | Mikkelsen 1988 |
| | 1986 | The last time spawning was scientifically observed in Hong Kong. | Huang *et al*. 1998, Chiu and Morton 1999 |
| | Early 1990s | By the early 1990's mating pairs were no longer seen on beaches where they had previously occurred. | Huang *et al*. 1998 |
| | 2002 | First formal population surveys were conducted on juveniles due to a lack of adults. | Morton and Lee 2003 |
| | 2004-2007 | Surveys found low mean juvenile population densities. | Shin *et al*. 2009, Morton and Lee 2010 |
| | 2012-2014 | Surveys found small and discrete juvenile populations with low mean densities and no 'new recruit' Instar I to Instar III juveniles were found throughout Hong Kong. | Kwan *et al*. 2016 |
| | 2016 | The population of juvenile *T. tridentatus* in Hong Kong is estimated to be between 2,100 and 4,300 individuals, of which 60% reside on the same coastline at Pak Nai/Ha Pak Nai. | Shin *et al*. 2014, Kwan *et al*. 2016 |
| **Viet Nam** | Before 1990s | *T. tridentatus* was commonly found, being most abundant in the Central Coastal Province. | Nguyen 2007 |
| | Early 1980s | Major concentrations of *T. tridentatus* could be found along the Vietnamese coast, at least as far south as Nha-Trang. | Mikkelsen 1988 |
| | 1990-2007 | Adult populations declined by 50%. | Nguyen 2007 |
| | 2007 | *T. tridentatus* assessed as Vulnerable in the Vietnam Red Data Book, due in part to 50% population declines. | Nguyen 2007 |

| Nation / region | Time / period | Population data | Source |
|---|---|---|---|
| **Southern Range** | 1975-1982 | Surveys throughout Asia found low population densities in *T. tridentatus* southern range. | Sekiguchi 1988 |
| **Philippines** | | Historic information suggested that *T. tridentatus* was once widely distributed throughout the Sulu Sea. | Schoppe 2002 |
| | Before 2000 | *T. tridentatus* experienced significant declines in numbers throughout its range in the decades preceding 2000. | Schoppe 2002 |
| | 2001 | In an eight-month study at San Pedro nursery beach, Palawan, 374 juveniles were recorded. | Dorkas 2002 |
| | 2002 | In a five-month study at Aventura Beach, Palawan, 125 juveniles were recorded. | Almendral and Schoppe 2005 |
| **Malaysia** | | Anecdotal reports and interviews with locals suggest *T. tridentatus* populations are decreasing in Sabah. | Manca *et al*. 2017, Laurie/Robert pers. comm. 2014 |
| | 2014-2017 | *T. tridentatus* population studies commenced at different sites in Sabah. | Robert *et al*. 2014, Manca *et al*. 2017, Mohamad *et al*. 2017 |
| | 2014 | 195 adult individuals as amplexed pairs (97 females; 98 males) were visually located during four days of daytime surveys conducted along a spawning beach at Tanjung Limau, Sabah. | Mohamad *et al*. 2016 |
| | 2014-2015 | 271 adults, representing 267 individuals (188 males and 88 females) were caught using gill nets deployed offshore in a 5-month study in Tawau, Sabah. | Mohamad *et al*. 2016 |
| | 2014-2015 | Male-biased Operational Sex Ratio (OSR) of 2.42:1 was recorded at Inderasabah, Sabah and 2.08:1 at Tawau, Sabah. | Mohamad *et al*. 2016, Manca *et al*. 2017 |
| **Brunei Darussalam** | 2018 | Formal population surveys commenced in 2018. | Laurie/Marshall pers. comm. 2018 |
| **Indonesia** | 1993 | *T. tridentatus* was recognized to be Rare, Vulnerable or Endangered, but listed as insufficiently known because of lack of information. | Indonesia NBSAP 1993 |
| | 2007 & 2009 | Significant earthquakes with epicenters on the west coast of Sumatra were followed by a significant *T. tridentatus* population decline in Padang in the same area. | Nishida 2012b |

| Nation / region | Time / period | Population data | Source |
|---|---|---|---|
| | 2012-2017 | Research has focused on establishing baseline distribution data, including identifying population and stock status, locations of spawning grounds, nursery grounds, adult habitat range and rates of exploitation for all three horseshoe crab species, including *T. tridentatus*. | Nishida 2012; Mashar *et al*. 2017a, Mashar *et al*. 2017b |

**Table 2**. Summary of habitat decline (loss or degradation) data for *Tachypleus tridentatus* throughout its range, from literature review.

| Nation | Period | Habitat data | Source |
|---|---|---|---|
| **Japan** | 1930-1994 | 80% of *T. tridentatus* habitats in Seto Inland Sea were lost to reclamation or from the adverse effects of coastal infrastructure construction. | Shuster and Sekiguchi 2009 |
| | 1945-1995 | 40% of tidal flats nationally were lost to land reclamation. | Japan NBSAP 2014 |
| | 1970s-1981 | Spawning grounds at Tenjin and Azuma in Kasaoka decreased from 400 sites to zero sites. | Seino *et al*. 2003 |
| | 1970s-2000s | 40% of Seagrass beds were lost to reclamation and rising sea temperatures. | Japan NBSAP 2014 |
| | By 1998 | 30% of Japan's coastline was protected by banks, revetments or other structures, so that less than 50% of Japans coastline did not have artificial structures. | Japan NBSAP 2014 |
| | 2005-2008 | Reduction from 139 to 40 spawning pairs visiting the Tsuyazaki Coast, Fukuoka Prefecture each season, suggesting a pattern of site abandonment. | Wada *et al*. 2010 |
| | Before 2011 | Sea sand mining is considered a factor contributing to the destruction of adult horseshoe crabs habitats in Kasaoka in Okayama Prefecture. | Seino 2011 |
| | Before 2015 | Sea sand mining is considered a factor contributing to the destruction of adult horseshoe crabs habitats in the Seto Inland Sea. | Nishida *et al*. 2015 |
| | 2017 | Proposals are made to reclaim Sone Tidal Flat to build wind farms. | Laurie/Iwasaki 2017, pers. comm. |
| **China** | | Many *T. tridentatus* spawning and nursery grounds are situated next to population centers or are easily accessible and popular locations, leading to significant levels of anthropogenic habitat disturbance. | Weng *et al*. 2012 |
| | 1950s-1990s | Reclamation drastically shrunk wetlands, including tidal flats in China. Reclamation of tidal flats is projected to continue. | China NBSAP 5 2014 |

| Nation | Period | Habitat data | Source |
|---|---|---|---|
| | Before 2000 | Sea sand mining is implicated as a significant cause of the degradation of *T. tridentatus* spawning beaches along China’s tropical and sub-tropical southeast coast between its border with Vietnam and the border of Fujian and Zhejiang provinces, which encompasses most of *T. tridentatus* range along the coast of China. | UNDP 2000 |
| | Before 2009 | Sea sand mining is implicated as a primary cause of degradation in the Dongshan-Nan’ao migratory species corridor between Fujian and Guangdong | Ferguson and Wang 2009 |
| | 2006-2010 | Surveys and interviews with fishermen at 27 previously renowned spawning sites observed no adult spawning and juvenile populations at only six sites, an 88% reduction. | Weng *et al*. 2012b |
| | 2008-2012 | Reclamation from tidal flats resulted in 66% decrease of Mangrove area in China. | China NBSAP 5 2014 |
| **Taiwan** | Before 2001 | Sea sand mining is implicated in the degradation of *T. tridentatus* nursery grounds at Budai, Taiwan. | BirdLife International 2001 |
| | Before 2007 | 55% of the natural coastline on Taiwan Island, particularly on the west coast has been lost to reclamation or from the adverse effects of coastal infrastructure construction. | CPAMI 2007 |
| | 1997 | Significant *T. tridentatus* habitat was lost due to the construction of a commercial port on Kinmen Island and dredging of subtidal areas. | Hsieh and Chen 2015 |
| | Before 2017 | Sea sand mining is implicated in the degradation of adult, sub-adult and juvenile *T. tridentatus* habitats at Kinmen Island, Taiwan. | Laurie/Yang 2017, pers. comm. |
| **Hong Kong** | | Many *T. tridentatus* spawning and nursery grounds are situated next to population centers or are easily accessible and popular locations, leading to significant levels of anthropogenic habitat disturbance. | Shin *et al*. 2014, Kwan *et al*. 2016 |
| | 1970s - present | Entire habitat suites were lost to reclamation or the adverse effects of coastal infrastructure construction. | Shin *et al*. 2009 |
| **Viet Nam** | 1990-2007 | According to the Vietnam Red Data Book, *T. tridentatus* area of occupancy (AOO) declined by 50%. | Nguyen 2007 |

| Nation | Period | Habitat data | Source |
|---|---|---|---|
| **Philippines** | | Many *T. tridentatus* spawning and nursery grounds are situated next to population centers or are easily accessible and popular locations, leading to significant levels of anthropogenic habitat disturbance. | Schoppe 2002 |
| | Before 2002 | Sea sand mining is implicated in the degradation of *T. tridentatus* spawning beaches and intertidal juvenile nursery grounds at Puerto Princesa in Palawan. | Schoppe 2002 |
| **Malaysia** | | Many *T. tridentatus* spawning and nursery grounds are situated next to population centers or are easily accessible and popular locations, leading to significant levels of anthropogenic habitat disturbance. | Robert *et al*. 2014, Mohamad *et al*. 2015 |
| **Brunei Darussalam** | | Formal surveys commenced in 2018. No information available. | Laurie/Marshall pers. comm. 2018 |
| **Indonesia** | | Many *T. tridentatus* spawning and nursery grounds are situated next to population centers or are easily accessible and popular locations, leading to significant levels of anthropogenic habitat disturbance. | Meilana *et al*. 2015 |

**Table 3**. Summary of estimated exploitation data for *Tachypleus tridentatus* throughout its range, from literature review.

| Nation / region | Time / period | Population data | Source |
|---|---|---|---|
| | | Demand for Amebocyte Lysate is high and globally, the Amebocyte Lysate market maintained an average annual growth rate of 7.25% from 2013 to 2016. It is predicted the market will further expand. | BisReport Information Consulting 2017 |
| **Japan** | | There is no target fishery in Japan | Botton 2001 |
| | | There are two TAL producers in Japan: Seikagaku Corporation and Wako Pure Chemical Industries Limited. | Laurie/Novitsky pers. comm. 2014 |
| **China** | | *T. tridentatus* could be found throughout the seas and was regarded as an important economic species. | Liao and Ye 2000, Li and Hu 2011, Hong 2011, p. 153, Weng *et al*. 2012b |
| | | The demand for *T. tridentatus* in China for consumption is high. | Li *et al*. 2011, Hong 2011, p. 154, Weng *et al*. 2012, Laurie/Do 2014, pers. comm. |
| | | There are eight manufacturers of TAL reagent in China. | Cai *et al*. 2017 |
| | | In China, all horseshoe crabs are bled completely, then what remains are processed for sale as food and supply of carapaces for chitin. This is one of the factors resulting in horseshoe crabs being an 'all parts use animal'. | Hong 2011, Laurie/Novitsky 2014, pers. comm. |
| | | Horseshoe crab carapaces are in demand in China and overseas as a source of chitin, which is satisfied as a by-product of the TAL industry as well as collection by children and adults. | China Species Red List 2009 |
| | 1950s-1970s | Productivity of the Pingtan fishery, Fujian declined between 80 – 90%. | Huang *et al*. 2002 |
| | 1970s-1980s | Annual yield of *T. tridentatus* in China amounted to up to 200,000 pairs. | China Species Red List 2009b |
| | 1984-2002 | Productivity of the Pingtan fishery, Fujian declined by over 90%. | Huang *et al*. 2002 |
| | 1990s | The *T. tridentatus* fishery in Xiamen, Fujian collapsed. Stocks had to be imported from other provinces and Vietnam to supply demand. | Hong 2011, pp. 159 – 161, Weng *et al*. 2012, Laurie/Novitsky 2014, pers. Comm |
| | 1990s | Up to 1,000,000 pairs were exploited in Beibu Gulf, Guangxi. | Liao and Ye 2000, Li and Hu 2011 |
| | 1990-2000 | *T. tridentatus* output dropped 90%, in Guangxi largely because of human consumption. | People's Daily. 2000 |

| Nation / region | Time / period | Population data | Source |
|---|---|---|---|
| | 2006-2007 | Despite being protected at the provincial level, *T. tridentatus* was widely available to meet consumer demand in Zhejiang and Fujian, the crabs being smuggled from Guangdong and Guangxi. | Weng *et al*. 2012 |
| | 2009 | *T. tridentatus* was assessed as Endangered in the China Species Red List based on its exploitation as a source of food, utilization in the medical and biomedical industries and as raw material for chitin. It was subject to harvest with unlimited catches. | China Species Red List 2009 |
| | 2010s | In Fujian, a facility on Dongbi Island, Fuqing Bay, Fuzhou held and bled about 40,000 *T. tridentatus* each year which were sourced from Guangxi, Guandong, Hainan and Vietnam. | Laurie/Novitsky 2014, pers. comm. |
| | 2010s | In Guangxi, a lysate factory near Beihei bleeds about 60,000 crabs each year. Another facility in Dongxing, Fangchenggang supplies 20,000 to 30,000 pairs to Zhanjiang A&C Biological Ltd, 8,000 pairs to Xinbei and because of their higher price, 20,000 to 30,000 pairs are supplied directly to restaurants. | Laurie/Novitsky. 2014, pers. comm. |
| | 2010s | In Hainan, several thousand pairs of crabs are supplied from a holding facility in Danzhou to Zhanjiang Bokang Marine Biological Co. Ltd. in Zhanjiang. | Laurie/Novitsky. 2014, pers. comm. |
| | 2010s | Having largely depleted Chinese waters of commercially viable catches, the TAL industry is targeting Vietnam as a supply source. | Laurie/Novitsky 2014, pers. comm., Laurie/Do 2014, pers. comm. |
| | 2011 | The Guangxi Government was promoting the horseshoe crab fishery in the Beibu Gulf. | Department of Agriculture of Guangxi Autonomous Region 2011 |
| | 2011-2014 | Food demand for *T. tridentatus* is high. As a culinary treasure in China, as it becomes rarer, it will become more sought after | Li *et al*. 2011, Hong 2011, p. 154, Weng *et al*. 2012, Laurie/Do 2014, pers. comm. |
| | 2014 | It was estimated 10% of the remaining adults in Beibu Gulf were being harvested annually. The local biomedical industry estimated over 80% of harvested *T. tridentatus* were being consumed as food and 20% were being bled to produce TAL. | Fauna and Flora International 2014 |

| Nation / region | Time / period | Population data | Source |
|---|---|---|---|
| | 2014 | According to the National Institute for Food and Drug Control, limuloid resources were exhausted in China. | Pei *et al*. 2014 |
| | 2017 | According to an industry market report, the TAL industry is projected to expand. | BisReport Information Consulting 2017 |
| **Taiwan** | | The demand for *T. tridentatus* in Taiwan for consumption is moderate and as so few adults are caught, the unpredictable catch can no longer meet consumer demand. | Hsieh and Chen 2015 |
| **Hong Kong** | | The demand for *T. tridentatus* in Hong Kong for consumption is moderate. | Chiu and Morton 1999, Shin *et al*. 2009, 2014 |
| | 1980-2001 | A clandestine but legal TAL bleeding facility operated in Hong Kong. | Shin *et al*. 2014 |
| | 1995-1998 | *T. tridentatus* in local markets were sourced from local waters, the South China Sea and Indonesia. | Chiu and Morton 1999 |
| | 2004-2005 | Market surveys revealed 691 (68%) of horseshoe crabs, mainly *T. tridentatus* were caught in Chinese waters and 332 (33%) were caught in Hong Kong waters. Some were released after capture. The remainder were used in set-free rituals, as photographic props or were consumed. | Shin *et al*. 2009 |
| | 2014 | There is no longer a target fishery in Hong Kong, although adults are sometimes caught as bycatch. | Shin *et al*. 2014 |
| **Viet Nam** | | The demand for *T. tridentatus* in Vietnam for consumption is moderate. | Laurie/Do 2014, pers. comm. |
| | | Horseshoe crabs are recognized as a legally exploitable resource in Vietnam. | Nguyen 2007 |
| | 1990-2007 | *T. tridentatus* harvest yield declined by 20%. | Nguyen 2007 |
| | 2007 | In the Klong Yai district of Trat Province in Eastern Thailand a horseshoe crab processing factory operates, which included a *T. tridentatus* bleeding facility. The horseshoe crabs are sourced from Vietnam. | Human Trafficking Organization 2007 |
| | Before 2014 | *T. tridentatus* is being targeted to supply the TAL industry and consumer markets in China. | Laurie/Novitsky pers. comm. 2014, Laurie/Do pers. comm. 2014 |
| **Philippines** | | Gravid females are targeted for their eggs. | Schoppe 2002 |

| Nation / region | Time / period | Population data | Source |
|---|---|---|---|
| | | *T. tridentatus* is used for decoration, for sale to tourists or for other uses such as talisman, spiritual or medicinal properties and dead horseshoe crabs are frequently found decorating local homes. | Schoppe 2001, Kaiser 2002 |
| | 2009-2014 | There is an international trade in dried *T. tridentatus* carapaces from Palawan, which are sold on eBay and other online trading platforms. The extent of this trade has not been quantified. | Laurie 2009–2014, pers. obs. |
| **Malaysia** | | The Bajau Laut consume horseshoe crabs as a minor component of their diets. | Wood and Habibah 2014 |
| | 2014-2015 | Gravid females are targeted for their eggs, where female-biased harvesting may contribute to male-biased Operational Sex Ratio (OSR) of 2.42:1 observed at Inderasabah and of 2.08:1 observed at Tawau in Sabah in 2014 - 2015. | Christianus and Saad 2007, Manca *et al*. 2015, Mohamad *et al*. 2016, Manca *et al*. 2017, Laurie/Mohamad 2017, pers. comm. |
| | | In certain areas of Sabah locals hang *T. tridentatus* in their homes for protection from bad spirits. | Manca *et al*. 2017 |
| | 2017 | Thai tourists visiting Inderesabah have started buying gravid female *T. tridentatus*, extracting their eggs, then taking them back to Thailand. | Laurie/Mohamad 2018, pers. comm. |
| **Brunei Darussalam** | | Dried horseshoe crab carapaces, including *T. tridentatus* are displayed on the walls of fishermen's huts in Kampong Ayer Fishing Village. | Laurie/Smith 2017, pers. comm. |
| **Indonesia** | | Gravid females are targeted for their eggs. | Meilana *et al*. 2015 |
| | | *T. tridentatus* is sold for use in traditional healing remedies in Indonesia. | Meilana *et al*. 2015 |
| | 2016 | Interviews with local fishermen on the north coast of Java revealed horseshoe crab catches are declining. | Meilana and Fang 2017 |
| | 2017 | Having largely depleted Chinese waters of commercially viable catches, the TAL industry is looking farther afield for new sources of supply, including Indonesia. | Laurie/John 2017, pers. comm. |

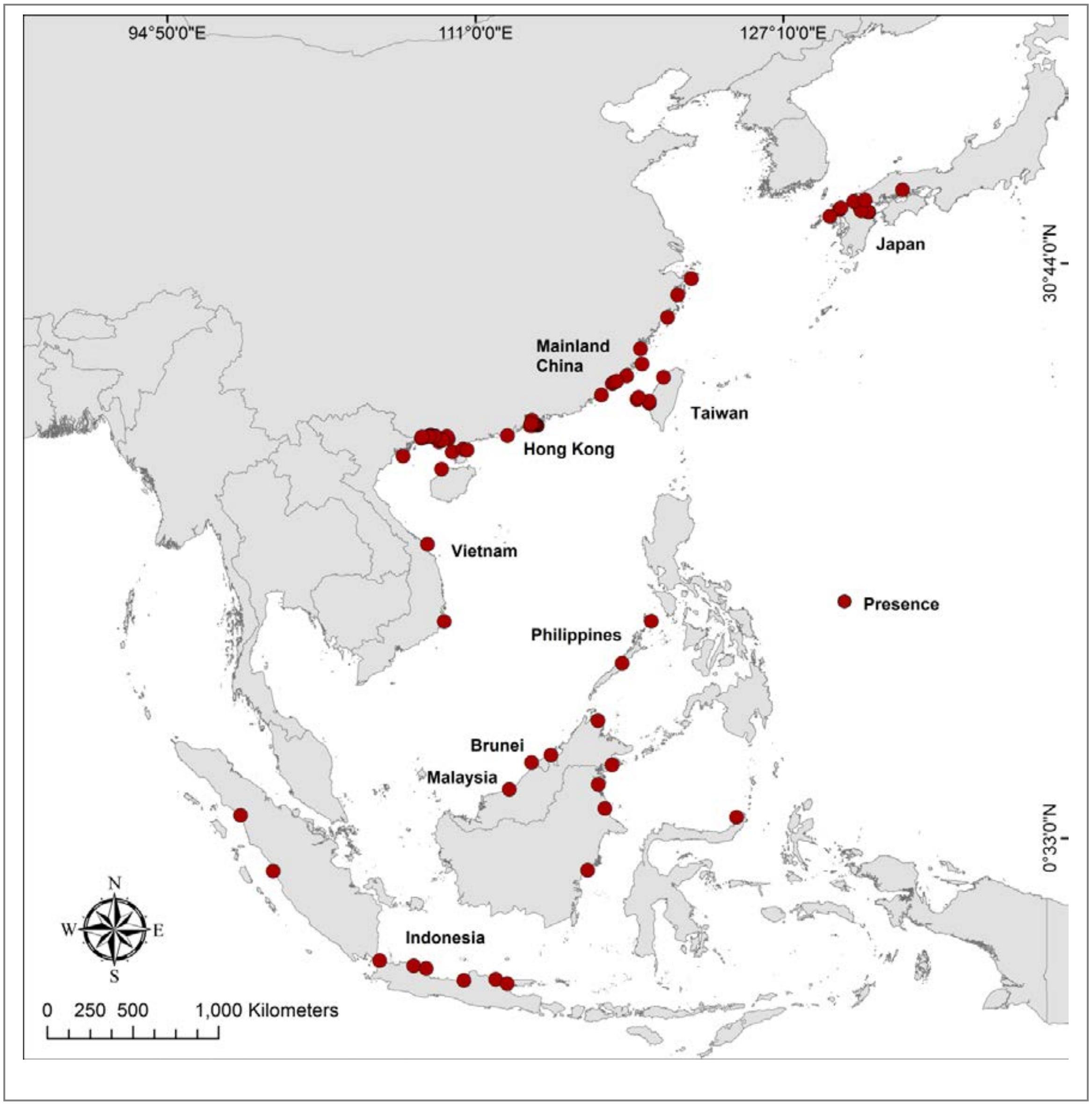


**Figure 1**. Distribution map of *Tachypleus tridentatus*.

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## Map References

The following map references (DMS co-ordinates) to produce distribution maps in GIS format relate to the location of survey sites as recorded in the cited references (in brackets), or are derived from other sources:

### Japan

Kasaoka, Okayama Pref. (34°29'59.99" N 133°29'59.99" E) (Latitude.to). url: http://latitude.to/articles-by-country/jp/japan/152167/kasaoka-okayama.

Hiroshima Bay, Hiroshima Pref. (34°11'60.00" N 132°20'59.99" E) (Latitude.to). url: http://latitude.to/articles-by-country/jp/japan/136243/hiroshima-bay.

Toyo Region, Shikoku Island (nearest large city – Saijo) (33°55'0.01" N 133°10'59.99" E) (Latitude.to). url: http://latitude.to/map/jp/japan/cities/saijo.

Kafuri Bay, Fukuoka (33°32'16.8"N 130°10'01.2"E) (Google Maps). url: https://www.google.com/maps/place/33%C2%B032'16.8%22N+130%C2%B010'01.2%22E/@33.538,130.167,555976m/data=!3m1!1e3!4m5!3m4!1s0x0:0x0!8m2!3d33.538!4d130.167?hl=en.

Imari Bay, Saga Pref. (33°15'60.00" N 129°52'59.99" E) (Latitude.to). url: http://latitude.to/articles-by-country/jp/japan/83137/imari-saga.

Iki Island, Nagasaki Pref. (33°46'59.99" N 129°42'59.99" E) (Latitude.to). url: http://latitude.to/satellite-map/jp/japan/50460/iki-island.

### China

Zhoushan Ahipelago, Zhejiang (northern most limit) (29°40′-30°17′N, 122°30′E) (Wang 1984).

Ninghai (NH), Zhejiang (29°9′29"N, 121°42′54"E) (Weng *et al*. 2013)

Wenzhou (WZ), Zhejiang (27°57′04"N, 121°07′00"E) (Weng *et al*. 2013)

Lianjiang (LJ), Fujian (26°13′56"N, 119°42′19"E) (Weng *et al*. 2013)

Pingtan (PT), Fujian (25°25′33"N, 119°46′07"E) (Weng *et al*. 2013)

Meizhou Island (MZ), Fujian (25°03′40"N, 119°04′39"E) (Weng *et al*. 2013)

Zhangpu (ZP), Fujian (23°48′12"N, 117°33′53"E) (Weng *et al*. 2013)

Zhanjiang (ZJ), Guangdong (20°55′17"N, 110°33′32"E) (Weng *et al*. 2013)

Beihai (BH), Guangxi (21°21′27"N, 109°05′44"E) (Weng *et al*. 2013)

Danzhou (DZ), Hainan (19°51′33"N, 109°07′28"E) (Weng *et al*. 2013)

### Hong Kong SAR

Ha Pak Nai (22° 25' N, 113° 56' E) (Shin *et al*. 2013, Kwan *et al*. 2017)

### Taiwan

Haomeiliao Nature Reserve, Budai, Taiwan Island (23°22'01"N, 120°07'49"E) (Yang *et al*. 2009b)

Hsia Su, Kinmen Island (20°58'58" N, 118°35'49" E) (Yang *et al*. 2007)

Dongwei, Magong Island, Penghu Islands (23°35'00" N, 119°35'40" E) (Yang *et al*. 2007)

Tiexianwei, Magong Island, Penghu Islands (23°32'00" N, 119°35'30" E) (Yang *et al*. 2007)

Matsu Islands (26°09'2.40" N 119°55'22.79" E) (Latitude.to). url: http://latitude.to/articles-by-country/tw/taiwan/9561/matsu-islands.

**Viet Nam**

Nha-Trang (12°14'42.25" N 109°11'39.55" E) (Latitude.to). url: http://latitude.to/map/vn/vietnam/cities/nha-trang.

**Philippines**

Aventura Beach (named after a beach resort), Bancao Bancao, Puerto Princesa City, Palawan at (09°43.804′N, 118°46.370′E) (Almendral and Schoppe 2005)

Busuanga (Dimakya Island) at (12°13.943'N 120°05.304'E) – (WWF 2010).

**Malaysia**

***Sabah***

Menggatal River, Sabah (6°3'52.9" N, 116°7'21.1" E) (Laurie/John 2017, pers. comm.)

Kota Kinabalu, Sabah (5°59'6.07" N, 116°5'14.48" E) (Laurie/John 2017, pers. comm.)

Tanjung Limau, Sandakan (N 06°44.562' E 117°23.528') (Mohamad *et al*. 2016)

Inderasabah, Tawau (N 04°18.043' E 118°14.378') (Mohamad *et al*. 2016)

Papar, Sabah (5°43'48" N, 15°55'48" E) (Laurie/John 2017, pers. comm.)

***Sarawak***

Kuala Nyalau beach, Sarawak (3°38'27.3696" N, 113°22'48.1764" E) (Laurie/John 2017, pers. comm.)

Miri Sandi beach (Lutong), Sarawak (4°23'24.7632" N, 113° 8'35.598" E) (Laurie/John 2017, pers. comm.)

**Brunei Darussalam**

Muara District (4°54'0.00" N 114°54'0.00" E) (Latitude.to). url: http://latitude.to/articles-by-country/bn/brunei/45581/brunei-muara-district.

**Indonesia**

Sibolga, North Sumatra (1°44'24.58" N 98°46'52.21" E) (Latitude.to). url: http://latitude.to/map/id/indonesia/cities/sibolga.

Padang, West Sumatra (0°56'57.26" N 100°21'15.37" E) (Latitude.to). url: http://latitude.to/map/id/indonesia/cities/padang.

Banten, West Java (-6°02'32.82" S 106°09'39.35" E) (Latitude.to). url: http://latitude.to/articles-by-country/id/indonesia/37586/banten-town.

Semarang, Central Java (-6°59'35.52" S 110°25'13.08" E) (Latitude.to). url: http://latitude.to/map/id/indonesia/cities/semarang.

Surabaya, East Java (-7°14'57.01" S 112°45'2.99" E) (Latitude.to). url: http://latitude.to/map/id/indonesia/cities/surabaya.

Manado, Sulawesi (1°28'55.85" N 124°50'56.11" E) (Latitude.to). url: http://latitude.to/map/id/indonesia/cities/manado.

Balikpapan, Kalimantan (-1°16'3.11" S 116°49'43.93" E) (Latitude.to). url: http://latitude.to/map/id/indonesia/cities/balikpapan.

Tarakan, Kalimantan (3°22'0.01" N 117°35'60.00" E) (Latitude.to). url: http://latitude.to/map/id/indonesia/regions/north-kalimantan/kota-tarakan.

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