Horseshoe Crabs: "Living Fossils" Imperiled in the Anthropocene

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Abstract

The four species of horseshoe crabs are true "living fossils" with an evolutionary history that pre-dates the dinosaurs. One species, *Limulus polyphemus*, is found along the Atlantic and Gulf of Mexico coasts of North America. Three other species, the tri-spine horseshoe crab, *Tachypleus tridentatus*; coastal horseshoe crab, *Tachypleus gigas*; and mangrove horseshoe crab, *Carcinoscorpius rotundicauda*, live in shallow-water coastal and estuarine environments in Asia from Japan to India. Horseshoe crabs are valuable for human health because their blood is used to test for the presence of bacterial contamination in vaccines and other biomedical products. Today, all four horseshoe crab species are imperiled by overharvesting, shoreline development, and the loss and degradation of their essential spawning and nursery habitats. Despite these challenges, we remain optimistic that efforts to enhance conservation, including increased fishery management practices, marine protected areas, scientific research, public awareness, and citizen science engagement may together curtail further damage to their populations and enable recovery of these irreplaceable animals.

Introduction

At first glance, the imperative to conserve horseshoe crabs may appear to be paradoxical because this group of animals has lived on Earth for more than 450 million years and survived mass extinction events that long ago claimed their cousins, the trilobites and giant sea scorpions (eurypterids). Horseshoe crabs are not true crabs, but rather chelicerate arthropods, more closely related to spiders and scorpions. Although there are many fossil relatives of today's horseshoe crabs, just four species have survived to the present, bearing notable resemblance to their extinct ancestors (Fig. 1). The American horseshoe crab, *Limulus polyphemus*, inhabits the Atlantic coasts of the United States and portions of the Gulf of Mexico, including the Mexican portion of the Yucatán peninsula. The tri-spine horseshoe crab, *Tachypleus tridentatus*; coastal horseshoe crab, *Tachypleus gigas*; and mangrove horseshoe crab, *Carcinoscorpius rotundicauda* are found in shallow-water marine and estuarine habitats in Asia. Despite their remarkable evolutionary lineage, past survival does not ensure future success in a changing world.

Our current human-dominated era, the Anthropocene, poses a unique set of challenges for horseshoe crabs that stem from overexploitation and habitat loss. Perhaps the best-known factor contributing to commercial exploitation of horseshoe crabs comes from their biomedical importance. Blood cells (amoebocytes) from horseshoe crabs are part of their innate immune system that safeguards them against invading pathogens such as bacteria, and scientists have taken advantage of this property to protect human

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Fig. 1 A specimen of one of the four living species of horseshoe crabs, *Carcinoscorpius rotundicauda* (top) next to a Late Jurassic (ca. 150 million year old) fossil of *Mesolimulus walchi* (bottom), showing a remarkable morphological resemblance. From the collection of Kevin Laurie, photo by Mark Botton.

health by developing a highly sensitive test for bacterial contamination (Gauvry, 2015). This test, manufactured from the horseshoe crab's blue blood, has become the worldwide industry standard to detect gram-negative bacterial endotoxins in pharmaceutical products such as injectable fluids (including vaccines) and implantable medical devices. Endotoxins are part of the bacterial cell wall and induce symptoms of disease such as fevers and sepsis, and can even cause death in patients. The endotoxin test is mass produced using the blood from the American horseshoe crab (*Limulus* amoebocyte lysate, or LAL) and tri-spine horseshoe crab (*Tachypleus* amoebocyte lysate, or TAL). The use of LAL and TAL has improved the lives of countless people worldwide, but as we will discuss below, this use may be unsustainable.

Additional threats to horseshoe crabs come from a combination of sources. Commercial fisheries in North America and Asia harvest horseshoe crabs for bait and human consumption, respectively. These fisheries were largely unregulated until recent times, and in some cases, remain so. The overconsumption of horseshoe crabs as an exotic food, coupled with their over collection for blood and bait, places a pressure on their survival that may eventually eclipse their long-term resilience to extinction. Horseshoe crabs are subtidal through most of the year, but they migrate from deeper waters to intertidal beaches to spawn (Fig. 2). The males jostle for positions around the female and release their sperm as the female deposits her eggs. Females select a spawning site that has clean well-oxygenated sand to provide the best chance for her eggs to survive. The integrity of these shoreline environments is impacted by encroaching human activities worldwide. Consequently, the best spawning habitats for horseshoe crabs are shrinking and degrading throughout their range due to ever-growing coastal human population pressures on the shoreline ecosystem.

"Flagship species" for conservation are typically large, charismatic vertebrates such as pandas, tigers, or polar bears. Horseshoe crabs lack the aesthetic appeal of such captivating megafauna to most of the general public. Yet, the ecological and economic importance of these animals deserves greater attention, and raising awareness of these values can be a way to mobilize efforts to conserve the estuarine and mangrove habitats that support them and the myriad flora and fauna that coexist there (Mattei et al., 2021). Horseshoe crabs are important in the food web of coastal and estuarine ecosystems as both predators and prey. Most notably, *L. polyphemus* eggs provide important food for fishes and migratory shorebirds, including threatened birds such as the Red Knot that depend on horseshoe crabs as part of the food resources needed to double their body weight during the northward migration to the Arctic (Botton, 2009).

Our purpose here is twofold. First, we expand on the reasons why these ancient animals are directly and indirectly threatened by human activities in North America and Asia. Second, we draw attention to the worldwide conservation and restoration actions on their behalf. Although the long-term evolutionary survival of horseshoe crabs is no guarantee of continued success in the Anthropocene, ongoing efforts by many agencies, academic institutions, and conservation organizations give us a basis for optimism.

Distribution, life history and natural mortality

Distribution and life history

The American horseshoe crab (*Limulus polyphemus*), ranges along the east coast of North America, from Maine to the Gulf of Mexico, where its southern limit is Mexico's Yucatán Peninsula. They are most abundant along the middle Atlantic coast of the United States, especially in the Delaware Bay region (Shuster Jr. and Botton, 1985; Botton and Ropes, 1987). The species occurs in bays and estuaries throughout the region, and some individuals may migrate to the inshore continental shelf, where they are primarily encountered at depths less than 30 m (Botton and Ropes, 1987). The tri-spine (*Tachypleus tridentatus*), coastal (*T. gigas*), and

Fig. 2 Typical spawning activity of American horseshoe crabs, *Limulus polyphemus*, in Jamaica Bay, New York, United States. The animal in the center of the photograph has a disc tag, part of a long-term project to study the migratory patterns of the animals. Photo by Mark Botton.

mangrove horseshoe crabs (*Carcinoscorpius rotundicauda*) are distributed in shallow marine and brackish waters throughout southeast Asia, ranging from the Bay of Bengal (India) to southern Japan (John et al., 2018). Horseshoe crabs are "ecological generalists" with a wide tolerance of salinity, temperature and other environmental variables (Botton and Itow, 2009). They consume a diverse diet of clams, marine worms, small crustaceans, and other invertebrates (Botton, 2009; Carmichael and Brush, 2012). Age and growth studies in horseshoe crabs suggest that they require about 9–11 years to attain sexual maturity, and have an estimated life expectancy of at least 14–18 years (Shuster Jr. and Sekiguchi, 2003).

Natural mortality

It is important to understand human impacts to horseshoe crab populations against the reference point of natural mortality. Predation on adult crabs is relatively rare, as their large size and hard exoskeleton offer them a great deal of protection. Consumption of adults has been reported to occur by raccoons and wild hogs on land, and by sea turtles and alligators in the water. American horseshoe crabs that become overturned and stranded on a beach during spawning activity are often predated upon by large sea gulls (Botton, 2009). Predation on horseshoe crab eggs and larvae by birds, crustaceans and shore zone fishes can be intense, and it has been estimated that as few as one out of 100,000 American horseshoe crab eggs survive to the end of their first summer of life (Botton, 2009). *Limulus* eggs provide essential nutrition for migratory shorebirds in the spring, most notably in the Delaware Bay region. By comparison, there is far less information about the causes and extent of natural mortality among Asian horseshoe crabs, a topic that deserves further study. The high natural mortality of juvenile horseshoe crabs combined with the long time to reach sexual maturity and reproduce (approximately 10 years), results in high risk and a low scope for recovery of impacted populations. Regardless of species, the addition of human-induced mortality, the majority of which is focused on adults, makes horseshoe crabs particularly vulnerable to extirpation. The severity of these effects can vary widely depending on mortality pressure and the size of local populations.

Threats to horseshoe crabs

Habitat loss

In the United States and Mexico, spawning habitats are size-limited and threatened (eliminated, degraded or blocked) by anthropogenic shoreline alteration, erosion, development, and sea level rise, with expectation for increased losses in the future (Smith et al., 2016). Some direct mortality has been associated with construction or restoration activities and roadway placement and use, primarily in the mid-Atlantic and Cape Cod, Massachusetts areas of the United States. The trapping of horseshoe crabs in power plant intakes is a concern on the Florida Atlantic coast, United States, and possibly elsewhere where power plants take in water from areas with horseshoe crabs. Tourism, vehicles on beaches, and severe storms (or hurricanes) are concerns throughout the range. Water quality, including various forms of pollution, toxic algal blooms, and changes in salinity dynamics have been recognized as concerns in the United States and Mexico (Smith et al., 2016).

Fig. 3 A typical armored shoreline along the coast of Japan. Such habitats are unsuitable for horseshoe crab spawning. Photo courtesy of Satoquo Seino.

Habitat loss is also a dire threat to Asian horseshoe crabs. In Japan, concrete shoreline protection structures along the nesting grounds (Fig. 3) imperil populations of *T. tridentatus* (tri-spine horseshoe crab). In Taiwan Province of China (hereafter abbreviated as Taiwan), armored shoreline construction, pollution and invasive cordgrass negatively impact horseshoe crab populations, and the adult horseshoe crabs are locally extirpated (Hsieh and Chen, 2015). Shoreline hardening, the practice of constructing bulkheads, breakwaters, and similar shore-protection structures, is also found in many other coastal areas of Asia, including the Hong Kong Special Administrative Region of China (hereafter abbreviated as Hong Kong) and Mainland of the People's Republic of China (hereafter abbreviated as China). Aquaculture development (e.g., shrimp and finfish pond culture), shoreline erosion, uncontrolled tourism, and other human activities have degraded the spawning habitat of the coastal horseshoe crab, *T. gigas* (Wang et al., 2020). The mangrove horseshoe crab (*C. rotundicauda*) uses the muddy creek banks of mangrove forests as a spawning ground and is acutely vulnerable to the ongoing loss of mangroves in Southeast Asia.

Fisheries

The American horseshoe crab is harvested throughout its range for various purposes, except in the northern Gulf of Maine and northern Gulf of Mexico. In the Delaware Bay area, horseshoe crabs were annually killed by the millions during the 1800s and early 1900s to produce livestock feed and fertilizer (Shuster Jr. and Botton, 1985). In addition, due to concern that horseshoe crabs consume commercially valuable shellfish such as mussels and clams, in some parts of the range, a bounty was placed on them to control their populations. At the present time in the United States, about 1 million animals per year are used as bait in traps for eels and whelks, and in Mexico, it is estimated that tens of thousands are used as bait in the octopus fishery each year. In the United States, the biomedical uses of horseshoe crabs (discussed above) also contribute to mortality, but at a lower level than the bait fishery. Collection of blood from American horseshoe crabs for biomedical purposes is not typically lethal, and federal regulations mandate that the bled crabs be released into the natural environment within 72 h of collection. Studies have shown that mortality associated with biomedical bleeding is about 15%. Hence, out of some 500,000 crabs bled annually, roughly 75,000 die (Smith et al., 2020). In comparison, harvest for bait results in 100% mortality of the 1 million animals harvested. Small numbers of adult horseshoe crabs are collected for research purposes, and about 20,000 crabs (mainly juveniles) are removed annually for the marine aquarium market. In contrast to Asia, human consumption of horseshoe crabs in North America is not popular. In the United States, there is concern that harvest for bait and biomedical purposes will expand to outlying areas of the range where harvest currently does not occur. The quantity of "bycatch" associated with trawls and dredges used in various fisheries remains poorly defined in the United States, but it is recognized by the principal regulatory authority, the Atlantic States Marine Fisheries Commission (ASMFC) as a potentially major threat that requires study.

Populations of Asian horseshoe crabs face serious threats from overfishing, but the causes and the intensity of threats vary by species and nation. Tri-spine horseshoe crabs (*T. tridentatus*) are primarily harvested for biomedical bleeding for TAL production and as an exotic dish in China. In contrast to the United States, horseshoe crabs bled in China are subsequently sold to secondary markets for local consumption and the preparation of chitin, which leads to 100% mortality (Gauvry, 2015). Other than local harvest, *T. tridentatus* is imported (legally and illegally) from neighboring countries such as Vietnam and Cambodia, broadly impacting horseshoe crab populations in the region. Although TAL production is estimated to support 20% of the bacterial endotoxin testing market, the harvest pressure on the wild horseshoe crab population is significant (John et al., 2021). This species

Fig. 4 Horseshoe crabs entangled in discarded fishing nets in Singapore (left) and India (right). Photos courtesy of Nature Society (Singapore) and Association of Biodiversity Conservation (India).

is also commonly used in release rituals in China and Hong Kong, in which adult horseshoe crabs are collected and released away from their native habitat during the ritual procedure. This practice may cause genetic drift in local populations and result in the loss of genetic variation at locations where horseshoe crabs are introduced (John et al., 2021).

There are fewer reports of harvest impacts on the coastal horseshoe crab (*T. gigas*) and mangrove horseshoe crab (*C. rotundicauda*). The coastal horseshoe crab, which occurs in the southern parts of Southeast Asia, is primarily fished for human consumption, with major demand from Thailand. More than 95% of the horseshoe crabs harvested in Malaysia are exported to Thailand freshly frozen via road transport from a single hub, with estimated monthly trade of at least 9000 female crabs. Some live crabs may also be exported from Indonesia to Thailand via Malaysia, with suspected further export for biomedical bleeding practices. Trading or commercial fishing of the mangrove horseshoe crabs (*C. rotundicauda*) is almost unheard of, possibly owing to its smaller size and the risk of poisoning from tetrodotoxin accumulated in its tissues, the same deadly toxin that is sometimes found in the Japanese puffer fish (fugu). Overall, evidence of overfishing and illegal trading, particularly for *T. gigas*, comes mainly from local news, with little conclusive data.

As in North America, Asian horseshoe crabs are unintentionally killed or damaged as bycatch. In parts of Asia, accidental entanglement of horseshoe crabs in gill nets is common (Fig. 4), and can lead to considerable mortality. Other uses of horseshoe crabs in Asia include the use of the exoskeleton in traditional medicine in India, Bangladesh and China, as agriculture compost and fertilizer in Japan, and to ward off evil in a few rural villages in Malaysia and Brunei (John et al., 2018). Even though these other uses may occur at a very small scale, the cumulative effect of multiple threats leads to the decline of Asian horseshoe crab populations.

We have summarized the major threats to the four species of horseshoe crabs in each country, where information is available, in Table 1.

Historical abundance and shifting baselines

Shifting baselines pose a major challenge to horseshoe crab conservation and management. The "shifting baseline syndrome" in conservation biology means that our perception of a baseline or pre-impacted condition is biased by the lack of reliable long-term data on the abundance and distribution of a species. Even in the United States, where mid-Atlantic horseshoe crab populations have been studied intensively for four decades, we have limited data attesting to the much larger populations in the 1800s (Shuster Jr. and Botton, 1985). The lack of adequate baseline data on the Asian horseshoe crab populations is even more evident. In-depth ecological studies are generally lacking on Asian horseshoe crabs, particularly for Malaysia, Indonesia, Philippines, Vietnam, Thailand, Myanmar, Cambodia, India and Bangladesh, where data on population density and abundance are sporadic and limited (John et al., 2021). Empirical data from field surveys, however, demonstrate that all three Asian species were once abundant but eventually disappeared in many locations due to the various natural and anthropogenic influences described above (Wang et al., 2020). Though overall decline in juvenile and adult populations have been observed in all habitat types across the globe, some recovery has been documented among populations in the United States (Smith et al., 2016).

Conservation actions

The IUCN horseshoe crab specialist group

The International Union for the Conservation of Nature (IUCN) is one of the world's largest global professional environmental organizations. The IUCN Species Survival Commission oversees the incorporation of data on species' status, population trends and

Table 1 Country-specific status, threats and protection actions for horseshoe crab species.

Country	*Available species*	*Species Protection status (if any)*	*Major threats in order of intensity*	*Regional conservation agencies and groups*	*Ongoing conservation actions*
United States	*L. polyphemus*	Regional fishery management plan, Atlantic States Marine Fisheries Commission	Loss of spawning habitat, bait fishery, LAL production, bycatch	Atlantic States Marine Fisheries Commission Ecological Research and Development Group Project *Limulus* Center for Environmental Research and Coastal Oceans Monitoring, Molly College	Carl N. Shuster horseshoe crab reserve Public awareness programs Citizen science tagging and monitoring programs Captive rearing programs Basic and applied research
Mexico	*L. polyphemus*	Protected by Federal law ("in danger of extinction")	Poaching, loss of spawning habitat, shoreline development	Federal Secretariat for the Environment and Natural Resources and National Commission for Natural Protected Areas State Secretariats for the Environment and Sustainable Development (States of Campeche, Yucatán and Quintana Roo) Project Mex Anáhuac Mayab, Center for Research and Advanced Studies of the National Polytechnic Institute (Mérida Unit) South Border College (El Colegio de la Frontera Sur), (Chetumal Unit) Mexico Institute of Technology (Tizimin and Chetumal Units)	Implementation of sea and coastal ecosystem conservation Public awareness programs Tagging and monitoring Basic and applied research
China	*T. tridentatus*, *C. rotundicauda*	Regional protection (e.g., in Guangdong and Fujian Province) Both species now listed as National Grade 2 Protected Wildlife (2021)	TAL production, loss or fragmentation of spawning and nursery habitat, local delicacy, ritual release	Fauna and Flora International Guangxi Beihai National Wetland Park Guangxi Institute of Oceanography Guangxi Hepu Dugong National Nature Reserve Guangxi Mangrove Research Center Quanzhou Wetland Society	Implementation of protected areas Public awareness programs Captive rearing programs
Hong Kong, (Special Administrative Region of China)	*T. tridentatus*, *C. rotundicauda*	Partial protection through establishing Sites of Special Scientific Interest Trawling ban in Hong Kong waters since 31 December 2012	Urbanization and land reclamation projects, pollution	City University of Hong Kong Ocean Park Conservation Foundation Hong Kong World Wide Fund for Nature Hong Kong	Proposed marine protected areas Public awareness programs Captive rearing programs Basic and applied research
Taiwan (Province of China)	*T. tridentatus*	Not protected; adult population is locally extirpated	Armored shoreline construction, invasive plant (*Spartina alterniflora*), pollution	Kinmen Horseshoe Crab Conservation Society Chiayi Ecological Environment Conservation Association	Establishment of Guningtou Horseshoe Crab Conservation Area on Kinmen Island Public education programs Captive rearing programs
Japan	*T. tridentatus*	Regional protection e.g., in Kasaoka City (Okayama Prefecture), Imari City (Saga Prefecture) and Saijo City (Ehime Prefecture)	Shoreline development and loss of spawning and nursery grounds	Kujukushima Aquarium *Nihon kabutogani wo mamoru kai* (Japanese Society for the Preservation of Horseshoe Crabs) Horseshoe Crab Museum, Kasaoka City Yamaguchi horseshoe crab research meeting	Horseshoe crabs are considered "natural monument" in Japan Public awareness programs Field monitoring by Japanese Society for the Preservation of Horseshoe Crabs Captive rearing programs

Malaysia	*T. gigas*, *T. tridentatus*, *C. rotundicauda*	Protected only in East Malaysia (Sabah and Sarawak)	Legal cross-border export, shoreline development, local delicacy	International Islamic University Malaysia, horseshoe crab research unit Universiti Malaysia Terengganu, horseshoe crab community outreach program Horseshoe Crab Aquaculture Center, Johor	Public awareness through citizen science approach Captive rearing programs Population baseline data collection
Indonesia	*T. gigas*, *T. tridentatus*, *C. rotundicauda*	Protected species under Indonesian Ministry of Environment and Forestry No. 12/KPTS.II/1987	Loss of spawning and nursery habitat, illegal cross-border export	Institut Pertanian Bogor	Implementation of existing law enforcement Public awareness programs Preparation of baseline data
Singapore	*T. gigas*, *C. rotundicauda*	*T. gigas* (Endangered), *C. rotundicauda* (Vulnerable) under Singapore Red Data Book	Shoreline development, pollution	Nature Society (Singapore)	Protected areas Public awareness programs Monitoring programs by Nature Society (Singapore)
Thailand	*T. gigas*, *C. rotundicauda*	Not Protected	Local delicacy, pollution and shoreline development	Unknown	None
Myanmar	*T. gigas*, *C. rotundicauda*	Not Protected	Local delicacy by tourists especially at Tanintharyi Region	Unknown	None
Vietnam	*T. tridentatus*, *C. rotundicauda*	Listed under Vietnam Red Book "Vulnerable"	Legal export to China for TAL production, land reclamation for shrimp, crab and clam aquaculture, local delicacy	Unknown	None
Cambodia	*T. tridentatus*, *C. rotundicauda*	Not Protected	Export for TAL production, local delicacy	Unknown	None
Philippines	*T. tridentatus*, *C. rotundicauda*	Not Protected	Shoreline development	Unknown	None
India	*T. gigas*, *C. rotundicauda*	Listed under Schedule IV Protected animal, Indian Government	Loss of spawning and nursery habitat, bycatch, traditional medicine	Association for Biodiversity Conservation (ABC) Malkolak Knowledge Center, Hyderabad Sophitorium Group of Institutions in Bhubaneswar, Odisha	Public awareness programs Population baseline data collection Captive rearing programs
Bangladesh	*T. gigas*, *C. rotundicauda*	*C. rotundicauda* is listed as Vulnerable, *T. gigas*—No report available	Tourism activities at Cox's Bazar, bycatch	Unknown	None
Brunei	*T. tridentatus;* occurrence of *T. gigas* and *C. rotundicauda* is uncertain	Not protected	Tourism and bivalve culture activities at Water village (Kampong Ayer)	Unknown	None

Threats are presented in order from highest to lowest concern, based on IUCN Red List Assessments for *Limulus polyphemus* (American horseshoe crab) and *Tachypleus tridentatus* (tri-spine horseshoe crab), and published reviews on *Tachypleus gigas* (coastal horseshoe crab) and *Carcinoscorpius rotundicauda* (mangrove horseshoe crab) (John et al., 2018), with additional information from papers presented at the 3rd and 4th International Workshops on the Science and Conservation of Horseshoe Crabs in 2015 and 2019.

Table 2 Distribution, current IUCN conservation status, and critical habitats for the four species of horseshoe crabs.

Scientific name	*Common name*	*IUCN Red List Status*	*Geographic distribution*	*Spawning habitat*	*Nursery habitat*
Carcinoscorpius rotundicauda	Mangrove horseshoe crab	Data deficient	Hong Kong to India	Mangrove creeks	Tidal flats
Limulus polyphemus	American horseshoe crab, Atlantic horseshoe crab	Vulnerable	United States, Mexico	Estuarine sandy beaches	Tidal flats and shallow subtidal
Tachypleus gigas	Coastal horseshoe crab	Data deficient	Indonesia, Malaysia, Singapore, Thailand, Myanmar, Bangladesh, India	Estuarine sandy beaches	Tidal flats
Tachypleus tridentatus	Tri-spine horseshoe crab	Endangered	Japan, Hong Kong, Taiwan, China, Vietnam, Philippines, Malaysia, Brunei, Indonesia	Estuarine sandy beaches	Tidal flats

habitat availability into the Red List of Threatened Species. The publication of The American Horseshoe Crab (Shuster Jr. et al., 2003) was a milestone in stimulating global conversations about the ecological importance of horseshoe crabs and their potential overexploitation. Growing concerns about horseshoe crab conservation worldwide stimulated the formation of the IUCN Horseshoe Crab Specialist Group (SSG) in 2012 (Botton et al., 2015). The Horseshoe Crab SSG coordinates numerous activities in support of that goal, through sponsorships of International and regional conferences on horseshoe crab biology and conservation, publication, education and public outreach. Four highly productive International conferences have been held, in New York (2007), Hong Kong (2011), Sasebo, Japan (2015), and Qinzhou/Beihai, China (2019), supplemented by numerous regional meetings in North America and Asia.

The SSG is an advocate for science-based conservation of horseshoe crabs and their habitat, and it has spearheaded the updating of the Red List status for the four horseshoe crab species (Table 2). The Red List status of the American horseshoe crab has recently been elevated from "Least Concern" to "Vulnerable," based on an extensive review of the population status and trends throughout the species' range (https://www.iucnredlist.org/species/11987/80159830). A similar review of *T. tridentatus* led to its elevation to "Endangered" status in 2019 from its previous Data Deficient category (https://www.iucnredlist.org/species/21309/149768986). *Tachypleus gigas* and *C. rotundicauda* remain Data Deficient, but the status of both is being brought up-to-date in light of recent publications describing the threats to their populations and habitats (John et al., 2018). Updating these listings may provide some leverage to motivate authorities to initiate and support increasing conservation efforts throughout the range. The SSG is also pursuing the possibility of designating horseshoe crabs as the first "World Heritage Species" under the UNESCO World Heritage Program.

Habitat protection

The largest area of protected horseshoe crab habitat in the United States is the Carl N. Shuster, Jr. Horseshoe Crab Reserve on the inshore mid-Atlantic coast, named in honor of one of the pioneers in horseshoe crab biology. Fishing and possession of horseshoe crabs are prohibited in the reserve. Other areas of spawning habitat along the United States mid-Atlantic coast are protected by limited public access from land, but boat access is not restricted. American horseshoe crabs benefit from overall protection of shoreline areas. For example, most of the shoreline of Jamaica Bay, New York is part of Gateway National Recreation Area where the collection of horseshoe crabs is illegal. Sections of the Delaware Bay, New Jersey beach have been incorporated into the Cape May National Wildlife Refuge or have been acquired by The Nature Conservancy as protected lands. In Mexico, all of the most important coastal lagoons in the Yucatán peninsula, which include critical spawning and nursery habitat for horseshoe crabs and are key reservoirs of genetic diversity, are within Federal or State protected areas. Even in areas where management plans do not explicitly focus on the species, they afford protection to the ecosystems that harbor horseshoe crab critical habitats, such as intertidal beaches, sand bar islands, mangrove forests and aquatic plant communities.

Many marine protected areas (MPAs) have been proposed to preserve dwindling horseshoe crab populations in China, Hong Kong, Taiwan, Japan, Singapore and Indonesia (Hsieh and Chen, 2015; Kwan et al., 2016) (Table 1). At present, there are seven horseshoe crab prefecture (city-based) MPAs in Guangdong and Fujian Province, China and one on Kinmen Island, Taiwan. In Singapore, the Mandai mangrove and mudflat and Sungai Buloh wetland reserve, are conserved as Nature Parks. Habitat protections such as these may be particularly useful in countries where horseshoe crabs are not legally protected.

Fisheries management and monitoring

In the United States, the Atlantic States Marine Fisheries Commission (ASMFC) and the Gulf States Marine Fisheries Commission (GSMFC) oversee management of horseshoe crab harvest along the Atlantic and Gulf of Mexico coasts, respectively. ASMFC sets statewide quotas on bait harvest, and some individual states set additional harvest quotas, daily catch limits, and seasonal or area

closures. GSMFC does not have regulations in place for horseshoe crabs. Some efforts have been made to reduce mortality at power plants in the United States under the federal Clean Water Act. In Mexico, horseshoe crabs are protected by federal law (regarded as "in danger of extinction"), which forbids harvesting. However, enforcement of regulations remains a concern throughout the range in the United States and Mexico, and illegal fishing for horseshoe crabs is a known threat in both countries (Botton et al., 2015).

Some areas in North America remain data poor (or datasets are outdated), particularly at the northern and western edges of the range. On some parts of the USA Atlantic coast there are long-term spawning surveys and tagging and resighting programs, but there is little or no ongoing monitoring in areas without historical harvest activities. In Mexico, horseshoe crabs are included in inventories of federal protected areas, but there are no species-specific monitoring or management programs. A lack of public awareness and education about horseshoe crabs is a hurdle to conservation, particularly among outlying habitats on the Gulf of Mexico coasts.

Though horseshoe crabs are protected under law in many parts of Asia, regional law enforcement is highly compromised due to the complex array of stakeholders (John et al., 2018). Inadequate baseline data, particularly for the coastal and mangrove horseshoe crabs, makes it hard to convince regional governments to elevate species protection status. Although horseshoe crabs are not yet protected by law in Hong Kong, the Hong Kong government is preparing a list of threatened species and its two species of horseshoe crabs (*T. tridentatus* and *C. rotundicauda*) are included under the Hong Kong Biodiversity Strategy and Action Plan. In general, population baseline data in Asia are acquired through personal observations (37%), fishermen responses (24%) and systematically executed field surveys (Wang et al., 2020). To facilitate further collecting population data in Asia, several approaches have been proposed, including: (1) enhanced cooperation of researchers with local conservation agencies and NGOs to strengthen monitoring programs, (2) market-oriented approaches in countries where horseshoe crabs are targeted fishery and, (3) documenting and mitigating bycatch issues (John et al., 2021).

In Japan, horseshoe crabs have been recognized as a "natural monument" since 1928. Despite this status, regulations have been implemented to preserve the species in only a few prefectures such as Kasaoka (Okayama Prefecture), Imari (Saga Prefecture) and Saijou (Ehime Prefecture). Since the establishment of the Japan Society for the Preservation of Horseshoe Crabs in 1979, yearly horseshoe crab population surveys have been carried out and data are presented in society general assembly meetings and in annual reports.

Harvest reductions have also been targeted through efforts to reduce demand. Alternative bacterial endotoxin tests have been developed that are based on recombinant factor C (rFC) which mimics the natural coagulation process that occurs when horseshoe crab blood encounters endotoxins. If and when the rFC test is deemed to be an equally valid endotoxin test, its adoption could globally reduce the mortality associated with bleeding natural populations of horseshoe crabs. As of March 2021, the rFC test has been accepted by the European Pharmacopeia, but not as yet by the US Pharmacopeia. LAL producers have developed methodologies that require smaller volumes of horseshoe crab blood for testing purposes. Additionally, the adoption of bait bags by eel and whelk fishermen in the United States has enabled fishermen to bait their traps using fewer horseshoe crabs. This effort reduced horseshoe crab harvest for bait by as much as 50–75% in some cases, during the first few years of implementation (Glenn Gauvry, personal communication).

Habitat restoration and captive rearing

The restoration of sandy beach and mangrove habitats used by horseshoe crabs is costly, and the ecological outcomes are uncertain. Habitat restoration on sandy beaches may involve some combination of the removal of coastal armoring, coupled with the addition of sand to rebuild the beach, a process called beach nourishment. These coastal engineering projects often need to be done on a recurring basis because the sand erodes from the site of deposition, and if sediment grain-size is unsuitable, horseshoe crabs may not make significant use of the nourished beaches (Mattei et al., 2021). "Living shoreline" projects have been implemented to dampen wave energies and reduce erosion, thereby helping to stabilize horseshoe crab spawning beaches (Mattei et al., 2021). Some success has been achieved with small-scale habitat restoration of horseshoe crab habitats in Taiwan (Hsieh and Chen, 2015). Similarly, efforts to restore mangrove areas could be beneficial to mangrove horseshoe crabs, but these well-intentioned projects have often failed because of haphazard replanting of mangroves without proper regard to factors such as elevation, hydrology, and salinity (Mattei et al., 2021). In Mexico, in contrast, mangrove replanting may result in the colonization and disappearance of the few sandy beaches where horseshoe crabs spawn within coastal lagoons. Many factors, from economic and social to ecological, will ultimately determine how successfully habitat restoration efforts can meet horseshoe crab conservation goals.

Threats to wild horseshoe crab populations also have prompted interest in captive rearing for propagation and release, or to support conservation and research programs. Captive rearing has been most popular in Asia as part of restoration and associated public outreach campaigns to raise awareness of declining native horseshoe crab populations (Xu et al., 2021). These efforts have been dovetailed with ongoing research and educational activities in the United States and Asia (Carmichael and Brush, 2012). Since 2009, for example, the Ocean Park Conservation Foundation (OPCF), Hong Kong and City University of Hong Kong have maintained a Juvenile Horseshoe Crab Rearing Program in collaboration with local high schools, so that students can observe and monitor horseshoe crab growth and release them to the wild. The program aims to inspire young generations to engage in conservation actions, and has involved more than 3000 students in conservation education. Similarly, *T. tridentatus* rearing programs in mainland China have resulted in ranching of ~750,000 juvenile and occasionally adult crabs during the last decade, releasing more than 100,000 individuals into the wild. In Japan, juvenile horseshoe crab rearing programs are conducted in limited areas to reintroduce *T. tridentatus* populations, resulting in some population recovery in Kasaoka Bay. In India, *T. gigas* restoration

programs supported by the Government and Conservation Leadership Program have reared more than 50,000 juvenile crabs, and *T. gigas* have been successfully reared on the Pahang coast of Malaysia since 2018 by adopting rearing technology developed by Associates of Cape Cod Inc., United States under a "Horseshoe Crab Sustainability Project." The Center for Environmental Research and Coastal Oceans Monitoring (CERCOM) on Long Island, NY began horseshoe crab rearing in 2001 as a unit of Dowling College until 2013, when it was transferred to Molloy College (https://www.molloy.edu/academics/undergraduate-programs/biology/cercom). It is the only long-term horseshoe crab breeding laboratory for *L. polyphemus* in the United States, producing more than 10,000 juveniles per year for long-term research on survivability in response to changing environmental parameters, such as ocean acidification. These programs highlight the potential for captive rearing initiatives to significantly enhance conservation education as well as contribute to science and population recovery for horseshoe crabs.

Captive rearing of horseshoe crabs can be logistically challenging and time consuming, and has largely been considered impractical on a commercial scale, in large part due to the time it takes to reach maturity (Shuster Jr. and Sekiguchi, 2003). Data to support captive rearing programs also are limited. Until recently (Xu et al., 2021), much of the information on horseshoe crab laboratory culture practices in Asia for the past four decades was unpublished or available only in the regional language (i.e., Chinese and Japanese), making their knowledge inaccessible in some cases. Despite these challenges, datasets are increasing and there has been an effort in recent years to increase the international exchange of data (Carmichael and Brush, 2012).

Civic engagement and public education

There is a growing network of citizen science initiatives in North America to increase public awareness of the importance of horseshoe crabs. For example, the Ecological Research and Development Group (ERDG) based in Delaware is a leader in horseshoe crab conservation, and coordinates a program in which volunteer beach walkers rescue horseshoe crabs that become stranded during mating activity on the Delaware beaches (https://horseshoecrab.org/conservation/). Another volunteer-based effort called Return the Favor (http://returnthefavornj.org/) leads a similar project in New Jersey. Collectively, these efforts have rescued hundreds of thousands of horseshoe crabs from Delaware Bay that might have otherwise died. Many Atlantic coast states have citizen science programs for counting and tagging horseshoe crabs during the breeding season. Among these, Project *Limulus*, based at Sacred Heart University, has tagged more than 90,000 animals mainly from Long Island Sound (Connecticut and New York), providing valuable data on population trends and migratory patterns. The tags currently used are circular plastic disc tags inserted into a small hole at the rear of the prosoma, the large dome-like anterior portion of the carapace (Fig. 2). Tags are distributed through the U.S. Fish and Wildlife Service, and each is printed with a unique number and information on how to report recaptures. On Long Island, New York, there are volunteer horseshoe crab spawning surveys at numerous beaches, coordinated by researchers at CERCOM and the Cornell Cooperative Extension Service. CERCOM sponsors a "Crab Club" for junior and senior high schools, coupled with a K-12 curriculum designed in collaboration with the ERDG that is distributed to participating teachers.

Public participation and citizen science are gradually changing the perception of horseshoe crabs in Asia and the need for conservation. Community engagement programs throughout Asia, including by OPCF Hong Kong; Guangxi Biodiversity Research and Conservation Association, China; Kinmen Horseshoe Crab Conservation Society, Taiwan; Nature Society (Singapore); and Association for Biodiversity Conservation, India have had a proven impact on species conservation. In the near future, implementation of harvest regulations and licensing, habitat restoration, juvenile captive rearing, and expanding marine protected areas where horseshoe crabs co-exist with other iconic species or habitats could be prioritized to enhance conservation in Asia.

Social media has proven to be valuable in disseminating information among scientists, conservation groups, and the general public. Active Facebook groups, including ERDG (https://www.facebook.com/horseshoecrab.erdg), Project *Limulus* (https://www.facebook.com/groups/109787499063443), CERCOM at Molloy College (https://www.facebook.com/CERCOMMCLI), Horseshoe Crabs Group (https://www.facebook.com/groups/142126905862143), and Asian Horseshoe Crab Group (https://www.facebook.com/groups/406285826215740) are proven platforms to connect different stakeholders to disseminate information at an international level. Ongoing public awareness programs, public participation and species conservation courses embedded in primary and secondary level school curriculum will be essential to support effective conservation strategies globally.

Epilogue

Horseshoe crabs are undoubtedly one of evolution's success stories, but the 450 million year old lineage of these living fossils requires our attention and protection if horseshoe crabs are to survive and flourish in the Anthropocene. Horseshoe crabs have immense ecological and economic importance, and their blood provides the basis of a test for bacterial contamination that is important to protect human health, safeguarding millions of lives each year. They are threatened by overfishing and the loss of essential spawning and juvenile nursery habitats, driven by a burgeoning human population along global coastlines. Despite these challenges, we remain optimistic that efforts to enhance conservation, including increased fishery management practices, marine protected areas, scientific research, public awareness, and citizen science engagement may curtail further damage to their populations and enable recovery on a global scale.

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