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LETTER TO THE EDITOR

Conservation of Asian horseshoe crabs on spotlight

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Unlike the American horseshoe crab (*Limulus polyphemus*), whose fishery and harvest are well monitored and managed by the Atlantic States Marine Fisheries Commission, the conservation of Asian species, i.e., the tri-spine horseshoe crab (*Tachypleus tridentatus*), the coastal horseshoe crab (*T. gigas*) and the mangrove horseshoe crab (*Carcinoscorpius rotundicauda*), is a more formidable challenge, due to the intricacies of regional laws and inconsistent enforcement (John et al. 2018). In the near future, four scenarios will intensify the negative pressure on the wild populations of Asian horseshoe crabs: (1) unsustainable harvest pressure on *T. tridentatus* and its cross-border trade from Vietnam to the mainland China for biomedical bleeding practice and human consumption, (2) legal or illegal export of *T. gigas* and *C. rotundicauda* between ASEAN countries for consumption as a local delicacy, (3) spawning and nesting habitat degradation due to coastal reclamation, industrialization and climate change, and (4) at the genomic level, ritual release and confiscated illegally exported *Tachypleus* spp. released into non-native habitat or far from the home range of respective species might trigger 'genetic bottle-neck' and the 'founder effect' (Herborg et al. 2007; Yang et al. 2009).

Destruction of horseshoe crab spawning grounds has led to the extinction of adult *T. tridentatus* in Kinmen Island, Taiwan. Similarly, with $\geq$ 90% population decline of juvenile *T. tridentatus* in Hong Kong will likely end up in its local extirpation. Gravid female-biased harvesting of *T. gigas* from Indonesia and Malaysia exported to Thailand for local delicacy has steeply increased in the last decade, resulting in imbalanced sex ratio in the wild (Mat Zauki et al. 2019a, b). Owing to continued population decline, *T. tridentatus* biomedical bleeding harvest for *Tachypleus* amebocyte lysate (TAL) production in mainland China has dropped from 600,000 pairs during the 1990s to 100,000 pairs currently (Gauvry 2015). *T. tridentatus* has recently been listed as 'Endangered' on the IUCN

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Red List based on its overexploitation, declining population size, and loss of critical habitat.

Though TAL supports only 20% of the world's bacterial endotoxin test (BET) market (Zion Market Research 2019), the harvesting numbers of *T. tridentatus* should not be considered insignificant. The recent decision by the US Pharmacopeia declining to place synthetic recombinant factor C (rFC) as an equivalent to LAL/TAL will mean, at least in the short term, greater harvest pressure on both *Limulus* and *T. tridentatus* to meet BET market demand (USP 2020). Even prior to the COVID-19 pandemic, the growing healthcare market was projected to double the demand for LAL/TAL by 2024 (EPC 2020). Considering the fact that any medical treatment protocol requires endotoxin validation test that might accelerate the harvest pressure on horseshoe crabs for their biomedical bleeding practice. Urgent inter-governmental collaboration is thus essential to resolve the issue of horseshoe crab smuggling and cross-border legal export for biomedical bleeding and human consumption.

We propose three possible measures to protect dwindling horseshoe crab populations in Asia. (1) BET users can control the supply chain by adopting the animal-free rFC testing method in countries where this use has been approved (EPC 2020) as well as the turbidimetric and chromogenic LAL/TAL testing methods which require smaller volumes of raw product instead of using TAL based gel-clot method to reduce the bleeding pressure on *T. tridentatus*, (2) as part of corporate environmental responsibility, BET users are encouraged to contribute additional expenses and labor to cross-validate the process of rFC test method against LAL so as to utilize rFC instead of direct animal-based LAL/TAL, and (3) inter-governmental coordinated conservation efforts through IUCN SSC (Species Survival Commission) on horseshoe crabs are needed to protect horseshoe crab spawning grounds and control human consumption.

Horseshoe crabs are legally protected country-wide in Bangladesh, Indonesia, Singapore, Vietnam, India and in specific provinces or regions in mainland China and Japan. However, the rigor of law enforcement is questioned (John et al. 2018). There are only fragmentary data on horseshoe crab abundance and population trends from many areas in Asia, which makes it difficult to develop effective conservation or restoration strategies (John et al. 2018). We do know, however, that critical spawning habitats (sandy beaches for *Tachypleus* spp. and mangroves for *C. rotundicauda*) are imperiled by coastal development and sea level rise. Given the ongoing threats caused by overexploitation and habitat destruction, we simply do not have the luxury of time in waiting to take precautionary measures to protect Asian horseshoe crabs. Immediate research should focus on wild stock assessment and the development of standardized survey protocols to implement harvest regulations. Field observations in Asia have clearly demonstrated that the data from legal/illegal horseshoe crab trade coupled with local fishery by-catch data of horseshoe crabs from the wild could offer added value in determining population baseline data. Due to the fragmented population size and high exploitation rate, closed system depletion and change in ratio models may be employed for wild stock assessment (Smith and Addison 2003).

To speed up the population baseline collection in developing countries, horseshoe crab researchers can collaborate with established, local conservation organizations, so that horseshoe crab surveys can be added on to their existing monitoring programmes with minimal extra cost. Market-oriented approaches have been successful in fishery management (Jacquet et al. 2010). However, such approaches may not be applicable in the conservation of horseshoe crabs, as in some countries horseshoe crabs are seen as a nuisance that damages fishing nets and impedes the fishing operation. It would be better to work

with local fishing communities so as to help them in alleviating such a by-catch problem and at the same time to compile the by-catch data of horseshoe crabs for population assessment use.

Public perspective change through citizen science programs and awareness campaigns especially among school and university students have shown positive impact on horseshoe crab conservation efforts (Yang et al. 2019; Mat Zauki et al. 2019a). Through these activities, the general public should be educated that for ritual release activities local species are preferred. For the confiscated illegally exported horseshoe crabs, they can also be returned to their native breeding place through the collaboration between law enforcement and/or conservation agencies. The IUCN SSC Horseshoe Crab Specialist Group is currently revising the Red List status of *T. gigas* and *C. rotundicauda* (currently "Data Deficient") which would also help in convincing the law makers to impose strict measures on cross-border trade and habitat protection. To this end, effort should be made to control international trade of horseshoe crabs through CITES.

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