REVIEW ON BIOLOGY, DISTRIBUTION AND CONSERVATION CHALLENGES FOR HORSESHOE CRABS IN INDIA

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Abstract: Although horseshoe crabs have ecological and biomedical value, their restricted distribution in the northeast coast (Odisha, West Bengal) to a limited number of researchers have made them an understudied arthropod in India. With an objective that uses 'desktop review' for horseshoe crab ecology, biology and their life cycle in India, it is learnt that actions to conserve T. gigas and C. rotundicauda are still in implementing stages. In Asia, Tachypleus gigas and Carcinoscorpius rotundicauda are present in sandy and muddy intertidal zones, which are termed nursery and feeding grounds in West Bengal, India. Broad tolerance to temperature (19-41°C) and salinity (2-36‰) variations indicate a nonpaused horseshoe crab presence and reproduction for West Bengal. Horseshoe crabs are not harvested for human consumption in India but, industrial demands for embryonic perivitelline (growth enhancer and stimulus for stem cell differentiation) and novel therapeutic applications (from anti-microbial and anti-carcinogenic proteins) are present in small scales. Although horseshoe crabs are safeguarded by the Wildlife Protection Act, 1972, the definitions are limited to *T. gigas*. Therefore, the output of this study contributes data for IUCN Red List assessments because fisheries records have revealed novel horseshoe crab distribution sites, incidences of by-catch, poor by-catch management and also coastal interventions that risk horseshoe crab nursery grounds in India into a deletion.

Keywords: Arthropod, ecology, fisheries, habitat, industry, strategy.

Introduction

The *Tachypleus* gigas (Müller) Carcinoscorpius rotundicauda (Latreille) are restricted to northeast India (Odisha, West Bengal) where they co-exist in sandy and muddy intertidal areas (Annandale, 1909; Rao & Rao, 1972; Lazarus et al., 1990; Tripathy et al., 2013; Yennawar, 2015; Pati et al., 2015). Yet, intertidal zones should not be assumed as horseshoe crab habitat but rather, an indication of natal-homing into nursery grounds for their routine spawning (Nelson et al., 2015; 2016a; b; Fairuz-Foziet al., 2018; John et al., 2018; Nelson et al., 2019; Zauki et al., 2019a; b). In fact, horseshoe crabs were witnessed to co-exist in mangrove coasts of Sundarban, Hukitola and Dhamra because sand, clay and silt in the ratio of 7:2:1 are preferred by both species for spawning (Chatterji *et al.*, 1992a; Chatterji *et al.*, 1996a). Research developments has updated existing knowledge on spatial-temporal patterns with transitional weather, population size, spawning cycles, habitat mapping and spawning migrations (Annandale, 1909; Seawell, 1912; Rao & Rao, 1972; Debnath *et al.*, 1989; Lazarus *et al.*, 1990; Chatterji *et al.*, 1992b; Chatterji *et al.*, 1996b; Chatterji, 1999; Chatterji.&Shaharom, 2009; Tripathy *et al.*, 2013; Yennawar, 2015; Pati *et al.*, 2015), embryonic development of *T. gigas* and *C. rotundicauda* (Roonwall, 1944; Debnath,

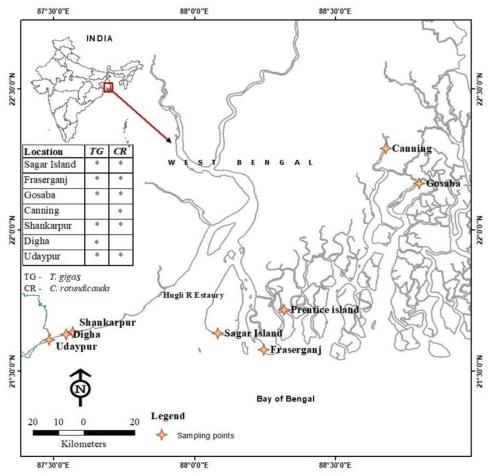


Figure 1: Horseshoe crab spawning and nursery grounds in West Bengal (India). The horseshoe crabs are abbreviated as TG = *Tachypleus gigas* and CR = *Carcinoscorpius rotundicauda*

1991; Chatterji *et al.*, 1992b; Chatterji *et al.*, 1996a; Khan, 2003; Chatterji *et al.*, 2004a), dietary and feeding habits (Debnath *et al.*, 1989; Chatterji *et al.*, 1992c) and exploitation of horseshoe crabs (Debnath & Choudhury, 1988; Pati& Dash, 2016).

After several man-made activities, like the excavation of sand, construction of jetties on seashore areas, the shore geomorphology (<1 m deep) was composed of different proportions of fine sand, and abrupt sediment nomenclature changes were decreasing the spawning emergence of horseshoe crabs in West Bengal over the last two decades (Chatterji *et al.*, 2004b; Chatterji & Shaharom, 2009; Behera *et al.*, 2015; Pati *et al.*, 2017). Although the

circulation of fine sand in Sundarban, Hukitola and Dhamra coasts is under control during the 'no-fishing season' under Indian Fisheries Act 1897 regulations, shore rehabilitation is a present challenge because local communities are regularly using estuaries for capture fisheries and recreational activities (Pati et al., 2017). With horseshoe crabs included in Schedule IV of the Wildlife Protection Act 1972, these arthropods are treated as by-catch in India (Chatterji, 1999; Behera et al., 2015). Therefore, researchers excluded capture fisheries from their attention and focused on horseshoe crab population assessments by comparing their spawning emergence during different seasons (John et al., 2018). A new finding emerged, where T. gigas preferred summer months (March-May, salinity 25-33 ‰) whereas C. *rotundicauda* gravitated towards the monsoon season (June-September, salinity 0-16 ‰) for peak spawning activity (Behera *et al.*, 2015; Techera, 2020; Table 1).

Industrial activities have expanded the conversion of marine resources into products like health tonics and handycrafts, particularly in West Bengal (Northeast India) where traditional practices remain prevalent (Mondayil et al., 2006; Mondal & Bandyopadhyay, 2014; Pati et al., 2020a). For instance, horseshoe crab carapace oil is used as an aphrodisiac (Mishra, 2009a, b), whereas the powdered carapace is effective for lacerations, burn wounds, skin ailments and for food preservation (Kumar et al., 2015; Pati et al., 2018; Ibrahim et al., 2019; Pati et al., 2020b). Meanwhile, the perivitelline fluid that buffers T. gigas embryo from the external environment was fractionated to derive the presence of proteins (hemagglutinin, hemocyanin, 27 kDa lectin and L6 subunit), peptides and buffer salts (Mirshahi et al., 2011). Considering that perivitelline fluid function is similar to amniotic fluid, it can trigger the development of epithelial cells as well as clot blood (c.f. Oppenheim et al., 1974; Weaver et al., 1988). Modern applications include the use of T. gigas embryonic perivitelline fluid to enhance cell growth, ionic regulation (for biological filtering) and treat cardiovascular diseases, while its hemolymph is used as a tumor marker, indicate endotoxin presence and as an antibiotic (Srimal et al., 1985; Ghaskadbi et al., 2008; Mirshahi et al., 2011; Pati et al., 2015; Chinnari et al., 2015; Nelson et al., 2020; Nong et al., 2020).

Unfortunately, nursery and feeding grounds in India have been degraded, whereby the poor environmental health impacts the growth and development of larvae (Pati et al., 2021). Therefore, researchers were collecting *T. gigas* with weight (0-130 g), size (0-20 % w/w), density (±3 g cm⁻¹) and haemolymph viability (70-88 %) in negative trends from the same sources (Chatterji *et al.*, 1988; 1992; 1994; Joshi et al., 2002; Vijayakumar et al., 2000; Sahu& Dey, 2013; Pati *et al.*, 2018). With an

objective to compile information on horseshoe crab ecology, biology and life cycle, the findings were used to address conservation challenges for *T. gigas* and *C. rotundicauda* in India. Since *L. polyphemus* and *T. tridentatus* are listed as "Vulnerable" and "Endangered" while both, *T. gigas* and *C. rotundicauda* remain as "Data Deficient", information on novel horseshoe crab distribution adds to existing data for the Red List (IUCN) assessment of these arthropods.

Methodology

Through a 'desktop review', words like horseshoe crabs, research, India, Bengal, northeast, West, conservation, threats, biology, spawning behaviour, Tachypleus gigas and Carcinoscorpius rotundicauda were used (independent or in combination) to search for literature in Google Scholar, Scopus, Web of Science, PubMed and NCBI. The library construction adopts the practice of John et al. (2018) where the inclusion criteria comprise published materials in journals, reports, book chapters, abstracts, symposium transcripts and theses between January 1900 and July 2020, and having touchpoints on horseshoe crabs from India. Yielding more than 100 published materials, an exclusion criterion focusing on biology, distribution and conservation challenges further limited the final library to only 56 information sources, which are available in this review.

Results and Discussions

Horseshoe Crab Ecology

Horseshoe crabs were studied for their distribution, biology, ecology, spawning behaviour through the 56 published materials in this review. In fact, the first publication was released by the National Museum about a strange crab called L. moluccanns (c.a.T. gigas) that occupies a 700 km continental shelf stretch situated between Bhadrak and Kendrapada (Odisha, East India) where depths are 20 fathoms (30-40 m). This arthropod emerges into shallow water in pairs during rising tides and the larger crab carries pale-yellow eggs

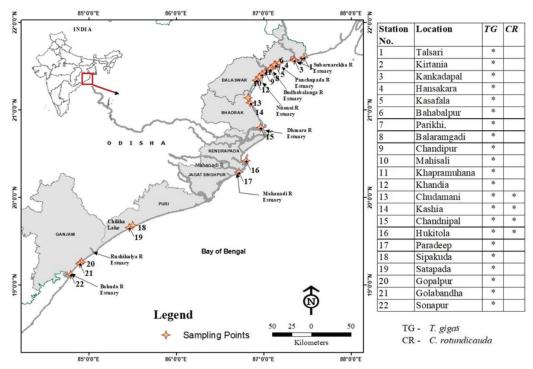


Figure 2: Horseshoe crab spawning and nursery grounds in Odisha (India). The horseshoe crabs are abbreviated as TG = *Tachypleus gigas* and CR = *Carcinoscorpius rotundicauda*

in the ventral section of its head (Annandale, 1909). Comparatively, the C. rotundicauda is described as a benthic crab that lingers in shallow and moderately deep (4-10 m) freshwater habitats some 60 miles upstream the Hoogly River (Seawall, 1912; Figs. 1-2). The taxonomic description of L. moluccanns was revised to T. gigas in 1944 and it was locally called raj kakraor 'king crab' for its larger appearance than decapod crustaceans (Roonwal, 1944). Later research discovered that T. gigas spawning activity in northeast Bengal coincides with one circa tidal lunar clock, the full moon ebb tide (Rao & Rao, 1972; Sekiguchi et al., 1976). Then, hydrographic definitions like wave characteristics, seabed slope, and near-shore gradient were integrated into lunar ebb tide events as a response to enhance understanding on T. gigas spawning and larval recruitment (Chatterji et al., 1996b).

A decline in adult *T. gigas* retrieval over a series of population assessment studies

motivated the inclusion of horseshoe crabs (specifically *T. gigas*) into schedule IV of the Wildlife Protection Act 1972 (Chatterji, 1999; Behera et al., 2015). Separately, laboratory experiments on adult T. gigas revealed that low salinity (2-16‰) maintained their body weights but, reduced their haemocyte:haemolymph composition (viability) by 8% (male) and 10% (female) (Chatterji et al., 1992b). Also, an exsitu assay involving T. gigas trilobite larvae showed that 10% and 40% salinity increased the rate of embryogenesis by 7 days if compared to ambient (20-33‰) gradients (Chatterji et al., 2004a). Together, these experiments related ecology conditions with the quality of crabs, particularly during the sourcing of horseshoe crabs for haemolymph and chitosan extraction (Pati et al., 2018). In addition, research collaboration between India and Malaysia produced novel understanding for relationships between riverine mangrove vegetation, bedload and fluvial sediment transport, the relationships between sediment compaction and nest depth,

Species	Area	Salinity (‰)	pН	Nest (cm)						Larva	
				Width	Depth						
T. gigas	Sandy	11-36	7.29- 8.35	12-30	10-30	60- 720	3.7	Trilobites swimming to the sea with the low tide			
C. rotundicauda	Mangrove & muddy	2-33	6.90- 7.55	-	3-7	80- 200	2.3	Juveniles found in the mangroves mud flat			

Table 1: The spawning ground conditions in Odisha (Saha, 2010)

the importance of osmotic and temperature shocks and also methods to identify *C. Rotundicauda* nests (Nelson *et al.*, 2015; Pati *et al.*, 2017; Fairuz-Fozi *et al.*, 2018; Nelson *et al.*, 2019; 2020).

Horseshoe Crab Biology

The anatomy of horseshoe crabs was first described using L. polyphemus (Heinbecker, 1932; Shuster Jr., 1948; Dumont et al., 1965). Later revisions used T. gigas for mid-gut gland labels and also classification of its J-shaped digestive tract into fore-gut (mouth, oesophagus and proventriculus), mid-gut (comprising stomach and intestine with hepatic (yellow) connective tissue extending to the opisthosoma, whereas the diverticulum (branching hepatic ducts) almost occupies the entire prosoma) and hind-gut (rectum and anus) (Debnath et al., 1989). Separately, T. gigas is recognized by its deep green shell with depressed lateral spines, six opisthosoma spines (equally long = male; 3 long and 3 short = female), movable spur on the 4th segment of the sixth prosomatic appendage and a triangular-shaped telson, whereas the C. rotundicauda has a deep brown-green shell, six prosomal spines of equal length for both genders, spurs are absent on the 4th segment of the sixth prosomatic appendage, its genital operculum extends distally to the tip of gill lamellae and has a smooth-rounded telson (Debnath, 1991).

With the discovery of histamine as a neurotransmitter in L. polyphemus (Battelle et al., 1991), the T. gigas brain (collar around the oesophagus) was examined and classified into anterior (suprapharyngeal ganglion) and (suprapharyngeal tritocerebrum posterior ganglion) nerve cords where olfactory nerves connect to lateral eyes, and cheliceral nerves communication to its appendage (Debnath, 1991). Neurological and anatomical descriptions of T. gigas were later used to completely describe the body plan of L. polyphemus in Walls et al. (2002). The T. gigas feeds on decayed organic matter, detritus, scaphopods, gammarid amphipods, foraminifera, vascular plant, molluscs, polychaetes and crustaceans, all of which relate to its benthic lifestyle (Debnath et al., 1989; Table 2). The presence of acid protease (gizzard>oesophagus), alkali protease (intestine>gizzard), esterase and cellulose (oesophagus), invertase and amylase (digestive gland) suggests that T. gigas is a generalist that can digest plant and animal materials (Debnath et al., 1989; Chatterji et al., 1992c). This arthropod does not have fixed diets and it would feed more during the winter months (December-January) (Nair et al., 1990).

Relative growth relationships between length-weight and length-length were used to indicate *T. gigas* health (or wellbeing) and to identify mature size-classes in their native habitat (Chatterji *et al.*, 1988; 1992; 1994). Yet, merging horseshoe crab samples of different size

Bivalves	Gastropod	Annelids	Arthropods	Plant	Miscellaneous	Teleosts
Anadra sp.	Cerithedia sp.	Gattyana sp.	Mysid shrimps	Casuarina needles	Ammonia beccarii	Goboid fish
Dosinia sp.	Littorina sp.	Phyllodoce sp.	Gammarid amphipods	Plant fruits	Asteroroatalia dentate	
Placenta sp.	Assimenea sp.	Nereis sp.	Copepodas (<i>Microstella</i> sp.)	Algal bodies	A. multispinosa	
Macoma sp.	Nerita sp.	Perinereis Sp.	Decapods (<i>Crangon</i> sp.)		A. trispinosa	
Solen sp.	Cassibula sp.	Glyceria sp.	Cirripeds		Bolivina sp.	
Neosolen sp.	Cymia sp.	Sabellaria sp.	Chrionomus sp.		Elphidium sp.	
Teredo sp.		Polydora sp.	Atylotus agrestis		Ouinquloculina sp.	

Table 2: Food sources discovered from the *Tachypleus gigas* gut (Debnath, 1989)

classes (male and female) and maturity (juvenile, sub-adult and adult) was a common practice and therefore, it implicated the accuracy results for weight ($\pm 24\%$) and size ($\pm 21\%$) analysis (Sahu & Dev, 2013). The male L. polyphemus is smaller than the female because it lacks the 17th moult stage (Levin, 2003). Therefore, male horseshoe crabs become sexually mature earlier than the female crabs, although they weigh much less than their counterparts (Chatterji et al., 1988; Graham et al., 2009). With weight influenced by eggs (for gravid female crabs) and feeding, morphometry studies indicated that prosoma of T. gigas develops symmetrically (p = 0.87-0.95) with its total length (Vijayakumar et al., 2000). Altogether, the T. gigas relative growth assessments were later revised by sub-clustering samples into different gender, size class and habitat groups (Vijayakumar et al., 2000; Khan, 2003; Sahu & Dey, 2013; Chatterji & Pati, 2014; Panda & Naik, 2017). Novelty by studies were recognized and adapted with T. tridentatus, L. polyphemus and T. gigas morphology assessments in Asia (Chiu & Morton, 2003; Srijaya et al., 2010; Smith &Brockmann, 2014; Kumar et al., 2015; Mohamad et al., 2016; Jawahir et al., 2017; Razak &Kassim, 2018; Putri *et al.*, 2019; Syuhaida *et al.*, 2019).

Life Cycle

Researchers learnt that horseshoe crab size varies by gender, but confusion arises when same size and different gender crabs from both species were presented together. It was resolved when researchers compared the hook-like structure of 2nd and 3rd appendages in which, only male crabs possess a degenerate propodiate while the dactylopodiate appears similar for all appendages (Rao & Rao, 1972). Species-wise, male T. gigas possesses an underdeveloped propodiate and its 2nd and 3rd opisthosoma marginal spines are slightly longer than other spines, whereas the C. rotundicauda has a well-developed propodiate and its opisthosoma marginal spines are arranged in reducing order for their length (Debnath, 1991). The marked morphological differences between male and female could also indicate their maturity, where matured crabs appear in dark tans, mating scars and broken spines are indications of sexually active females, while male crabs have bulged lateral prosoma flesh (Debnath & Choudhury, 1988). Though widely accepted that horseshoe crabs will become encrusted by fouling communities if the crab remains dormant for long periods (John et al., 2018), it was previously assumed that epifauna (fouling

Species Name	Class/Order	T. gigas	C. rotundicauda
Metridium schillerianum	Anthozoa	+	+
Gattyana deludens	Polychaeta	+	
Balanus darwini	Hexanauplia	+	+
Chthamalus stellatus	Hexanauplia		+
Cheiriphotis megacheles	Amphipoda	+	
Cleantis natalensis	Isopoda	+	
Ostrea sp.	Bivalvia		+

Table 3: Epifauna that were fouling the *Tachypleus gigas* carapace in India (Debnath, 1989)

community) encrusting is a parasite infection (Roonwal, 1944; Rao & Rao, 1972). Later, exsitu experiments showed that epifauna-infected crabs were less active and inspection of genital pores after epifauna removal led researchers to conclude that only infertile mature crabs become inhabited by fouling communities (Debnath & Choudhury, 1991; Patil & Anil, 2000; Table 3).

Meanwhile, the absence and presence of nests, closely spaced nests and >1 egg clutch per nest indicated the possibility of multiple spawning incidences per amplexus, fecundity to relative size and also spawning migration (Chatterji & Parulekar, 1992; Chattarji *et al.*, 1992; 1996). On the other hand, carapace-like impressions, nest diameter (14-30 cm), nest depth (2-3 inches) and attaining 20-100 eggs per clutch, were indications that photoperiod, tidal and lunar cycles are influencing the spawning behaviour of *T. gigas* during the summer months (March-May) and *C. Rotundicauda* during the



Figure 3: Liberation of horseshoe crab (Tachypleus gigas) oil by boiling the carapace

monsoon season (June-September) in northeast India (Debnath & Choudhury, 1988; Debnath & Choudhury, 1992; Chatterji *et al.*, 1992a, b; Mishra *et al.*, 1993; Sahu& Dey, 2013; Chatterji *et al.*,1996b; Figs. 1-2). Considering that transitional weather shifts shore geomorphology (0.182-0.203 mm) and sediment temperature (31.8-34.6°C), disparities in spawning yields (eggs and nests) indicate the possibility of spawning migration for *T. gigas* (Chatterji *et al.*, 1996b; Chatterji, 1999), whereby *T. gigas* increases its home range beyond the natal shore boundary (Tripathy *et al.*, 2013).

The eggs of *T. gigas* are green, spherical, protected by a thin elastic euticulablastodermica, encapsulated by a leathery chorion and have nine embryogenesis stages before hatching of the trilobite larvae (Roonwal, 1944). Later, a total of four embryonic moults were discovered for L. polyphemus (Bennett et al., 1972; Bannon & Brown, 1980) and T. tridentatus, including the hatching that commences after 25-32 days of embryogenesis (Yamamichi & Sekiguchi, 1974; Yamamichi et al., 1983). Annotations on T. gigas embryogenesis by Chatterji et al. (1996a) reveals that newly fertilized eggs (size 3.57 mm and weight 24.17 mg; Stage A), rupturing of chorion that enlarges the egg to 3.86 mm (Stage C; Moult 1), separation of leathery chorion after swelling of euticulablastodermica by perivitelline fluid build-up (Stage D; Moult 2), embryonic cleavage for vasculogenesis (Stage E; Moult 3), embryo weighs 138.02 mg and has movable legs (Stage F) and hatching of trilobite larvae that measures 6.99 mm and weighs

156.22 mm (Stage I; Moult 4). This annotation considers all nine development stages described by Roonwall (1944) and adopts the embryonic moults described by Yamamichi *et al.* (1983) to produce complete understanding on *T. gigas* embryonic life stages.

Conservation Challenges

The dorsal shell (exoskeleton) of horseshoe crabs is armoured by a chitinous carapace, opisthosoma spines and a hard telson while the ventral section has soft tissues. Predators, like shorebirds, teleost, crabs, shrimp, crow (Corvus splendens) and wild pig (Sus scrofa) only consume the crab's soft tissue and would discard the exoskeleton (Debnath& Choudhury, 1988; Pati & Dash, 2016). Tribal (e.g. Nolia, Santhal, Oran and Munda) communities in India that live along estuaries would use T. gigas exoskeleton (carcases) as decorative, produce jewellery, extract medicated oils (rheumatism, muscle aches and arthritis; Fig. 3), develop health tonic broths (aphrodisiac, spondylosis, bronchitis, wrist rheumatism, pneumonia and intestinal colic) or, the exoskeleton is used in rituals (supernatural and superstitious) (Chatterji & Abidi, 1994; Saha, 2010; Tripathy et al., 2013). In 1995-1996, an adult T. gigas is valued at RS. 25 (USD 0.33) but after the nationwide seasonal fishing ban in 1998, enforcement was strengthened on vulnerable species and horseshoe crabs were listed in Schedule IV of the Wildlife (Protection) Act 1972 (Tripathy et al., 2013; Behera et al., 2015; Shinoj & Ramachandran, 2017).

Now, horseshoe crabs are considered as by-catch that damage fishing nets in northeast Odisha and West Bengal. Removal on land or discarding nets containing horseshoe crabs have made these crabs vulnerable to beach stranding from which, dead crabs are gathered by fish farm workers to prepare them as fish meal or compost (Khan, 2003; Tripathy *et al.*, 2013; Fig. 4). Recently, sedimentation from land reclamation, removal of mangrove forests and sand mining are threatening horseshoe crab spawning grounds (Tripathy *et al.*, 2013). Interventions, like rip-rap, Groyne and wave-breakers, were







Figure 4: a. Discarded (by-catch) juvenile horseshoe crab. b. Entanglement in fishing nets resulting in the mortality of horseshoe crab c. Trapped in between stone on constructed jetty constructed near spawning ground

introduced but submerged horseshoe crab spawning grounds, exposed them to high energy currents and therefore, these affected areas are no longer suitable for horseshoe crab spawning (Mishra *et al.*, 2015). In addition, discarded fishing nets on the shore and ghost nets are stranding and suffocating horseshoe crabs (Pati

et al., 2017). Although capture fisheries may not threaten horseshoe crabs in India, human activities in coastal zones are challenging the survival of adults, hatching success of trilobite larvae and also larvae growing into adults which requires 7-11 years of endurance in their natal grounds.

Present Actions in Horseshoe Crab Conservation

Demand for live horseshoe crabs in Maharashtra (West) and Andhra Pradesh (southeast) offers income opportunity (RS. 50 ~ USD 0.67 per crab) to artisanal fishers in northeast India. However, these crabs are retrieved as by-catch and sold to local importers an a very small scale because these middle-men arrive without prior notice (Pati et al., 2020c). Though tribal markets in Odisha have horseshoe crab byproducts for sale, these communities either use carcases or dead crabs which have become stranded. The Wildlife Protection Act 1972 only penalizes possession of more than 3 live crabs (personal consumption). Although tribal communities are exempted from this clause, they only use horseshoe crab carcases for their cultural practices. With fishing season bans (61 days) and fishing gear controls (cast net, bottom trawler net and gill net for shellfish) governed by the Fisheries Act (1897), regulations on net soaking and catch-per-unit gives horseshoe crabs an opportunity to reproduce and sustain their wild populations. Therefore, the only delimiting impact on horseshoe crabs is by-catch. Awareness spreading through questionnaires (2017-2018) have convinced 388 individuals to carefully remove horseshoe crabs from fishing nets and return them to the sea. A series of sea ranching projects in 2017-2019 that involved registered volunteers from the Association for Biodiversity Conservation and Research (ABC) who collected the eggs, incubate them in a closed hatchery setup and released the hatched (95% hatching success) instar 1 larvae during lunar ebb tides have successfully recruited over 7,800 T. gigas larvae into Balaramgadi and Chandipur (Odisha). By far, horseshoe

crab conservation is in the implementing stage because it is challenged by area of coverage and communication. Also, volunteer training is time consuming and thus, the current manpower would require at least another five years to successfully spread and implement sea ranching outreach to the entire northeast coast of India.

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