

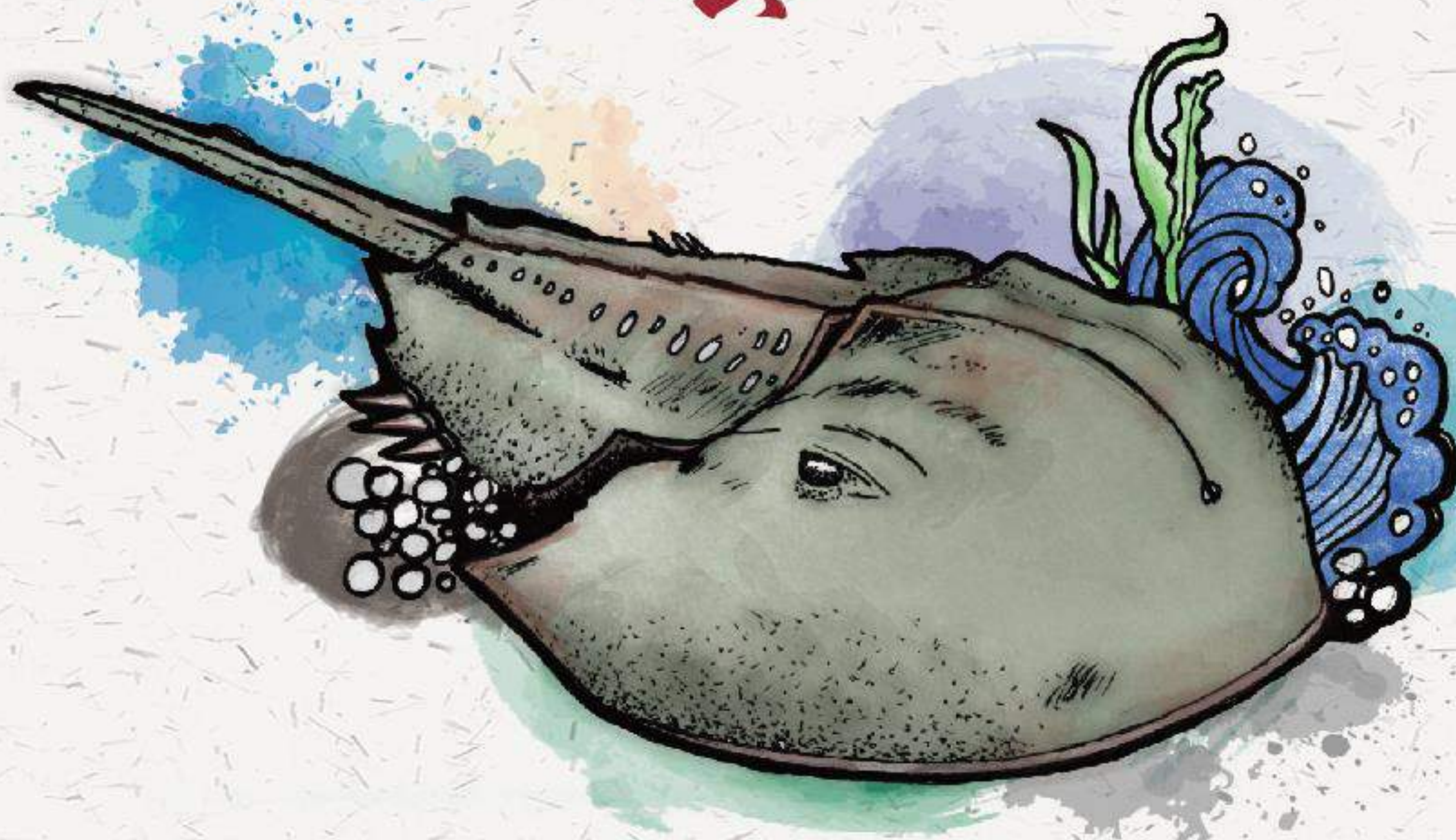
第四届国际鲎科学与保护研讨会

The 4th International Workshop on the Science
and Conservation of Horseshoe Crabs

科学 / 保护 / 公众教育

Integrating Science, Conservation & Education

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摘要手册 | ABSTRACT BOOK

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PLENARY TALKS

特邀报告

特邀报告 | PLENARY TALK



Paul K.S. SHIN | 单锦城

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(retired)

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(退休)

Hope for Horseshoe Crab Conservation in Asia-Pacific

Abstract. Horseshoe crabs in Asia-Pacific are under various threats, including loss of spawning/nursery habitats due to coastal development and overharvest for food and biomedical use, which cause their decline in populations across their distribution range. While horseshoe crabs are on the “Red Data” list and/or under protection regulations in some countries, they may still face eventual extirpation in some areas if current situation continues. Across Asia-Pacific, conservation efforts vary and much can be implemented to raise public awareness on the importance of horseshoe crabs in evolutionary history and ecosystem health, apart from ongoing scientific studies. This presentation addresses the conservation status of Asian horseshoe crabs and reports on the work of the IUCN Red List Assessment of *Tachypleus tridentatus* through the coordination of the Horseshoe Crab Specialist Group. Suggestions to initiating international collaborations towards a common goal to protect Asian horseshoe crabs are presented.

Keywords. Asian horseshoe crabs, conservation status, Red List Assessment, *Tachypleus tridentatus*, international collaborations

特邀报告 | PLENARY TALK

亚太区鲨物种保护的希望

单锦城 香港城市大学化学系 (退休)

摘要： 在亚太地区鲨种群正遭受繁育地丧失的威胁，导致其在分布范围急速地下降。海岸带的发展和以食物及生物医药用途为目的的过度捕捞是导致鲨繁育地丧失的主因。虽然鲨在部分国家被列入红色名录或受当地法律的保护，但在部分地区若情况无法获得改善，鲨种群仍面临灭绝。除了持续开展的科研活动，亚太地区针对鲨所实施的保护措施是多样的，推广鲨演化史和其在生态维护的重要性在提升公众意识上也有很大的可探索空间。本次报告着重于亚太区鲨种群的保护现状以及在鲨专家组协调下中国鲨在世界自然保护联盟红色名录评估的进展。同时建议启动国际合作以为亚太区鲨种群的保护共同努力。

关键词： 亚洲区鲨种群、保护状况、红色名录评估、中国鲨、国际合作

特邀报告 | PLENARY TALK



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Jennifer MATTEI

Department of Biology,
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Keywords. Long Island Sound, *Limulus polyphemus*, citizen science, horseshoe crabs, large data set analysis using R, mapping movement patterns, conservation, education

The Power of Citizen Science: Twenty Years of Horseshoe Crab Community Research Merging Conservation with Education

Abstract. In 1997, very little was known about the horseshoe crab population (*Limulus polyphemus*) in Long Island Sound (LIS) an urban estuary in northeastern USA. Over 23 million people live within an 80 km radius of LIS and the 965km coastline of this large estuary is shared by the states of New York and Connecticut. The most logical way to study the population dynamics of the horseshoe crab in LIS was to involve trained volunteers to tag and record observations of spawning adults on local beaches throughout LIS, and so Project Limulus was established. Project Limulus educates volunteers of all ages to gather data on horseshoe crab population dynamics. Public and school based educational programs recruit new volunteers each year, promote conservation of horseshoe crabs and help make the connection to the importance of LIS ecosystem and general human health. Citizen Science, the active public involvement in scientific research, originated in China and can be traced back several millennia where residents tracked crop pest outbreaks. Today, citizen scientists help generate very large data sets which are not possible to obtain by other means on thousands of projects worldwide. Hundreds of citizen scientists, through Project Limulus, tagged and reported re-sightings of horseshoe crabs on both sides of LIS since 1998. Coding language R was used to map the movements of ~14,000 horseshoe crabs. LIS was divided into five regions where population genetic data was collected and an interactive application was made to allow researchers/managers understand the need for a comprehensive management plan for the species. We have found that the use of a flagship species, even one that is not a charismatic megafauna, has lasting conservation value for participants. Participation in Project Limulus has resulted in local conservation efforts of estuaries and active restoration of coastal habitats 18 around LIS.

特邀报告 | PLENARY TALK

公民科学的力量： 回顾保育与教育相结合的二十年社区鲎研究

Jennifer MATTEI

Department of Biology, Sacred Heart University

摘要：在1997年，人们对美国东北部河口的长岛海峡（LIS）的美洲鲎种群知之甚少。长岛海峡的965公里海岸线由纽约州和康涅狄格州共有，海峡半径80公里内有超过2300万人居住。研究LIS鲎种群动态变化最合理的方式是让受过培训的志愿者参与对当地海滩上岸产卵的成年鲎进行标记和记录，Limulus项目便是因此而设立。Limulus项目旨在培训所有年龄段的志愿者以便收集鲎种群动态的数据。基于公众和学校的教育项目每年招募新的志愿者，促进鲎的保护，并将LIS生态系统的重要性和人类的健康紧密联系在一起。公民科学是公众积极参与科学研究的重要形式。它起源于中国，可追溯到几千年前，古人以此追踪农作物虫害的爆发。如今，全世界数千个项目借助公民科学家的力量获得了其他方式无法企及的大数据。自1998年以来，数百名公民科学家通过Limulus项目标记并报告了LIS两岸重复出现的鲎。R语言被用于绘制大约14000只鲎的动态。LIS被分成五个区域，收集种群遗传数据并进行交互式应用，使研究者或管理者了解制定一个针对该物种的综合管理计划的必要性。在这个过程中，我们发现旗舰物种的应用对于参与者来说具有持续的保护价值，哪怕这个物种并非有魅力的大型动物。Limulus项目的公众参与，也促进了河口地区的在地保护，并带动了长岛海峡一带滨海生境的有效恢复。

关键词：长岛海峡；美洲鲎；公民科学；鲎；使用R的大数据分析；动态模式绘制；保护；教育

特邀报告 | PLENARY TALK



Jayant K. MISHRA

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Studies and Marine
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Horseshoe Crabs in the face of Climate Change; Future Challenges and Conservation Strategies for their sustainable growth and existence

Abstract. Intertidal habitats are very complex, which is considered as the most dynamic living shore line endowed with rich biodiversity. It envisages variety of habitat types including sandy beaches, mud flats, marsh lands, mangroves, grass lands, creeks etc.. These heterogenic coastal habitats not only support vivid biodiversity but also provide innumerable ecosystem based services. Though these habitats are independent, but they are interdependent and function in synchrony with the environmental processes and ecological conditions. These coastal habitat specificity are well documented in case of four extant species of horseshoe crabs *Tachypleus gigas*, *Carcinoscorpius rotundicauda*, *Tachypleus tridentatus*, and *Limulus Polyphemus*, which are considered as endangered species in the world. These severe threat. Each of these species are adapted to specific environmental conditions for their egg laying by making nests in their preferred location in the intertidal zone. The breeding process found to be very unique with the selection of a specific nesting breeding zone with the involvement of specialized sensory mechanism. However, structural characteristics of breeding beach sediments play pivotal role in providing optimal incubating conditions for the development of eggs till they attend trilobite stage.

But in recent years, the impact of global climate change and its inflicting long term irreversible damage on different habitat types in the coastal environment has become major concern. The major threat being the ecosystem and bring changes in coastal geomorphology. As a result the loss of breeding habitats due to the compounding effect of disturbed global climatic conditions and human interference is increasing day by day. It is thus paramount to protect the habitat structure by adopting proper habitat management strategy and protect this highly fragile environment in terms of their utilization and stratification pattern to withstand any impact in the face of the global climate change, which is undeniably imminent due to frequent natural calamities and increased unregulated anthropogenic interference. The probable strategies towards the conservation of breeding beaches of horseshoe crabs are discussed.

Keywords. Climate change, coastal geomorphology, habitat stratification, horseshoe crab nesting ground

特邀报告 | PLENARY TALK

气候变化下鲎可持续增长和生存的挑战与相应保护策略

Jayant K. MISHRA

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摘要：潮间带生境非常复杂，因其丰富的生物多样性被认为是最具活力的海岸生态系统。它涵盖了多种生境类型，包括沙滩、泥滩、盐沼地、红树林、草地、潮沟等。这些高异质性的沿海生境不仅支撑了此处生机勃勃的生物多样性，而且提供着无法估量的生态系统服务价值。虽然这些生境相互独立，但它们也是相互依存、并随着环境过程和生态条件同步发挥功能的。这些沿海栖息地的特殊性也因四种现存的鲎——巨鲎、圆尾鲎、中国鲎和中国鲎——得以详细记录。这四种鲎现今都被认为是濒危物种，都面临着严重威胁。

但是近年来，全球气候变化的影响及其对沿海环境中不同生境类型造成的长期不可逆转的损害已成为人们关注的主要问题。这些威胁可能会影响沿海生态系统，甚至带来沿海地貌的改变。在全球气候变化和人类干扰的综合影响下，鲎繁殖地的丧失日益加剧。由于频繁的自然灾害和越来越多的不受管控的人为干扰带来的全球气候变化迫在眉睫，通过采取适当的生境管理策略来保护生境结构、并从利用和分层模式的角度来保护极度脆弱的环境，以抵御全球气候变化的影响，显得至关重要。本文讨论了鲎繁殖地可行的保护策略。

关键词：气候变化, 海岸地理形态, 生境破碎化, 鲎, 繁殖地

特邀报告 | PLENARY TALK



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Keywords. China, *Tachypleus tridentatus*, *Tachypleus* amebocyte lysate, laboratory culture, conservation biology, tachyplestin, food poisoning due to horseshoe crab consumption

The Research of Science and Conservation of Horseshoe Crabs in China

Abstract.

Research on *Tachypleus tridentatus* amebocyte and the production of *Tachypleus* amebocyte lysate (TAL) started in the 1980s by Zhenmin Xia, Youling Ding and Hongwei Wu, after the transfer of LAL manufacture techniques into China in 1975. Guangyao Liang from the Guangxi Marine Institute conducted captivity breeding of *T. tridentatus* in 1982, their artificial culture in 1984 and population investigation within Beibu Gulf in 1985, and first discovered *Carcinoscopius rotundicauda* in China in 1986. He is considered as the first scientist in China who clarified the distribution pattern of *T. tridentatus* and *C. rotundicauda* in China, and conducted systematic research on the population and artificial rearing of *T. tridentatus*. Prof. Shuigen Hong from the Xiamen University utilized electric stimulation techniques to enhance egg release from *T. tridentatus* in 1985, and researched on the oogenesis process in 1986-1988, hemocyanin in 1997, antibacterial activity of tachyplestin in 1998, and formation of amebocytes in 2000. Prof. S.G. Hong has summarized his research findings and published a book entitled "Biology of Horseshoe Crabs, *Tachypleus tridentatus*" in 2013. He is therefore recognized as the pioneer who conducted systematic research on breeding, physiology and tachyplestin in China. His work has been continued by Prof. Yongyan Liao from the Beibu Gulf University. He worked on artificial fertilization and captivity breeding of *T. tridentatus* in 1997, and researched on tachyplestin in 1998 with Prof. S.G. Hong. Prof. Liao recorded *T. tridentatus* during the winter in 2000, and in the same year he addressed the food poisoning cases due to horseshoe crab consumption. In 2000, he noted the threatened status of *T. tridentatus*, clarified the distribution of horseshoe crab species in the southern waters, and proposed conservation actions. In 2002, he summarized that there are only *T. tridentatus* and *C. rotundicauda* in Chinese waters to overcome the widespread misconceptions that other horseshoe crabs such as yellow-skin horseshoe crab, blue-skin horseshoe crab, ghost crab and black-skin horseshoe crab are other horseshoe crab species present in China. In 2006, he also clarified the species and distribution of horseshoe crabs in Asian waters. Prof. Liao is acknowledged as the first in China working on the conservation biology of *T. tridentatus*, and investigated food poisoning involving horseshoe crabs. Apart from that, Deyao Wang (1983) and Xinyi Cai (1984) from the Xiamen University, P. Liang (1981) and Y.Q. Wu (1993) from the Fujian Medical College implemented cellular research on hemolymph, growth and intestine of *T. tridentatus*; Zhaohong Weng from the Jimei University enhanced the understanding of embryo and cellular development, and ecological perspective of *T. tridentatus*. Since 2010, a research team led by Qiongzhen Li from the Guangxi Academy of Fishery Sciences has been working on the conservation biology and ecology of *T. tridentatus*.

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中国的鲨科学与保护研究

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摘要: 祖述宪等人, 1975年将美洲鲨试剂生产方法引入中国, 上世纪80年代开始, 夏振民、丁友玲、吴洪伟等进行中国鲨血细胞研究, 并开始利用中国鲨生产鲨试剂。广西海洋研究所的梁广耀, 1982年进行中国鲨人工育苗, 1984年进行中国鲨人工养殖, 1985年进行北部湾鲨资源调查, 1986年在中国发现圆尾鲨, 弄清中国有中国鲨和圆尾鲨分布, 是中国系统进行资源调查、养殖和人工育苗的第一人。厦门大学学生的洪水根1985年进行电刺激中国鲨催产, 1986年阐明中国鲨卵膜发生, 1987年阐明中国鲨卵黄的发生, 1988年阐明中国鲨卵子的发生, 1995年阐明中国鲨精子的发生, 1997年进行血蓝蛋白研究, 1998年, 研究鲨素抗菌活性, 2000年, 研究中国鲨血细胞发生, 2013年, 出版《中国鲨生物学研究》。是中国系统进行中国鲨生殖生理和鲨素研究的第一人。北部湾大学廖永岩1997年进行中国鲨人工授精育苗, 1998年将鲨素引入中国, 与洪水根一道, 启动了中国的鲨素研究。2000年在冬季发现中国鲨, 2000年开始系统研究中国的食鲨中毒, 阐明了发生于中国的食鲨中毒问题, 2000年, 发现中国鲨资源濒危, 2001年澄清中国南方海域的鲨的种类及分布, 提出保护策略, 2002年鉴定清楚中国海域的黄皮鲨、蓝皮鲨、鬼鲨、黑皮鲨, 证明中国只有中国鲨和圆尾鲨分布, 2006年澄清亚洲海域的鲨种类及分布。廖永岩是创建中国的鲨保护生物学, 并系统进行中国海域的食鲨中毒研究的第一人。此外, 厦门大学汪德耀(1983)和蔡心一(1984)、福建医学院的梁平(1981)和吴翊钦(1993)对中国鲨的血液和幼体发育、消化道进行了细胞学研究; 集美大学的翁朝红对中国鲨的胚胎发育、细胞发育、生物多样性等生物生态学进行了研究。2010年开始, 广西水产科学研究院的李琼珍团队, 对中国鲨的保护生物学及生物生态学, 进行了研究。

关键词: 中国、中国鲨、鲨试剂、人工育苗、保护生物学、鲨素、食鲨中毒



ORAL PRESENTATIONS

口头报告



THEME 1
HORSESHOE CRAB POPULATION
ECOLOGY AND EVOLUTION

蟹种群生态学和演化

GLOBAL DISTRIBUTIONS OF HORSESHOE CRABS AND THEIR BREEDING AREAS

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Abstract. There is increasing evidence of shrinking horseshoe crab distributions and declining populations globally. However, older literature is still being cited when referring to the global distribution of horseshoe crabs. This poses a problem for conservation, since several studies have shown that previously well-established distributions and breeding areas are now devoid of horseshoe crabs. Thus, in order to conserve suitable habitats for the horseshoe crabs, it is pivotal to identify and map areas that are currently being populated by these species.

In this study, we mapped the global distribution of horseshoe crabs and their breeding areas based on data from the literature, fieldwork, our scientific network, and the online Global Biodiversity Information Facility (GBIF) database. Occurrence records obtained prior to 1995 were excluded from the maps due to changes in horseshoe crab population sizes, their distributions and habitats over the last decades. These changes are for a large part anthropogenic due to the economic growth in Asia with increased human population growth, industrialization and urban development. An overview with updated information on the locations and states of breeding areas (e.g. declining, stable) also shows that many of these are shrinking and disappearing due to anthropogenic activities. Thus, conservation efforts should be directed to those breeding areas that still hold a viable population of horseshoe crabs and the IUCN status of the three Asian horseshoe crab species should change from Data Deficient to Vulnerable.

Keywords. Anthropogenic disturbance, conservation, global distribution, habitat loss, occurrence data, Xiphosura

MAPPING OF HORSESHOE CRAB HABITATS IN INDIA

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Abstract. A study was conducted from September 2013 to April 2014 with the objectives of identifying areas of distribution of horseshoe crab population along the east coast of India and mapping their habitats as Ecologically Sensitive Areas (ESAs). The areas of horseshoe crab habitats falling within the coastal regulation zone along the entire Indian Coast including islands were explored, evaluating to prepare the GIS-based maps with boundaries for those habitats along Indian coast. Based on secondary and ground truth information, GIS-maps with boundaries for all horseshoe crab habitats within the coastal regulation zone along the Indian coast including islands were prepared, and the details of habitat attributes and ecological status of each of the “*highly sensitive coastal areas*” was described. Quantitative data generated in the study supplemented with observation based comments on the conservation value and strength of the criteria adopted for identification of horseshoe crab habitats as coastal ESAs. Currently, the distribution of horseshoe crabs is restricted in habitats to small stretches of the east coast of India. Estuarine river mouths with vast mangroves and mudflats of West Bengal, Odisha and Andhra Pradesh along the east coast were identified as breeding sites of the horseshoe crabs. A small portion of the breeding habitats of horseshoe crabs is protected as Gahirmatha Marine Wildlife Sanctuary but the larger portion remains unprotected and is under tremendous pressure from fisheries and other coastal developmental projects. Study results suggest that some of the remaining parts of the potential breeding habitats of horseshoe crabs are to be brought under the ambit of a Protected Area and declared as a Conservation Reserve/Community Reserve that can be better managed in collaboration with local fisherman communities.

Keywords. *Carcinoscorpius rotundicauda*, conservation, habitat mapping, *Tachypleus gigas*, India

Population estimation of horseshoe crab *Tachypleus tridentatus* (Leach, 1819) in Balikpapan waters, East Kalimantan, Indonesia

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Abstract. The tri-spine horseshoe crab (*Tachypleus tridentatus*) is one of marine benthic animals inhabiting East Balikpapan coastal waters. Information on horseshoe crab population in the location is still limited and IUCN broadly classifies the horseshoe crab in deficient data category. This study was aimed at analyzing population of *T. tridentatus* in coastal waters of East Balikpapan. The crabs were collected by local gear of fisherman (dogol – a trawl like fishing-gear and gill net) in different moon phases and different distance from the coast line between January to March 2018. Primary morphological characters in the form of triangular shape of telson and abundant tiny spines on ophisthosoma, genetically ascertained that the collected crab is *T. tridentatus*. Most of these horseshoe crabs found at the study site were adult; with the width of prosoma ranging from 22.5 to 30.5 cm for males and 28.5 to 37.5 cm for females. The highest number of crabs was found at the location of 1 mile from Teritip coastline and 2 miles from Manggar coastline. Variation occurred in number of catch regarding to moon phase, and the highest one was in the third quarter phase. The margin of the population size of *T. tridentatus* in East Balikpapan waters at a 95% confidence interval is between 18 to 2 866 individuals.

Keywords. East Balikpapan, horseshoe crabs, population, *Tachypleus tridentatus*

POPULATION DYNAMICS OF *TACHYPLEUS TRIDENTATUS* IN EYU ISLET INTERTIDAL ZONE, XIAMEN, CHINA

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Abstract. Individual number, cephalothorax length and width, opisthosoma length and telson length of *Tachypleus tridentatus* were measured monthly in the field in Xiamen Eyu Islet intertidal zone from June 2018 to January 2019. The results showed that the individual number of *T. tridentatus* increased from June to September 2018, and decreased from September 2018 to December 2018. No individual was found in December 2018 and January 2019. The mean values of cephalothorax length and width, opisthosoma length and telson length of *T. tridentatus* in June were 26.33 mm, 43.93 mm, 20.76 mm and 42.88 mm respectively, which were all the smallest among six months (from June to November). The mean values of cephalothorax length and width, opisthosoma length and telson length of *T. tridentatus* in July were 37.62 mm, 60.42 mm, 26.28 mm and 71.19 mm respectively, which were all the largest among six months. One-Way ANOVA showed that the cephalothorax length and width, opisthosoma length and telson length of *T. tridentatus* were significantly different between July and June, between July and August, and between July and October (July to June: $F = 6.509$, $P < 0.001$; July to August: $F = 5.280$, $P < 0.05$; July to October: $F = 4.891$, $P < 0.05$). The mean values of cephalothorax length and width, opisthosoma length and telson length of *T. tridentatus* from August to November were 31.91 - 33.92 mm, 48.91 - 52.60 mm, 22.16 - 25.47 mm and 52.52 - 59.10 mm respectively. One-way ANOVA showed that the cephalothorax length and width, opisthosoma length and telson length of *T. tridentatus* were not significantly different among months from August to November. From the above results, we conjecture that summer is a period of rapid growth of horseshoe crabs in Xiamen. Autumn is a period of slow growth. Winter is the hiding period.

Keywords. Eyu Islet, intertidal zone, population dynamics, *Tachypleus tridentatus*

ACOUSTIC TELEMETRY REVEALS CONTRASTING HABITAT CONNECTIVITY ACROSS SEVERAL LIFE HISTORY STAGES OF AMERICAN HORSESHOE CRABS IN COASTAL NEW YORK, USA

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Abstract. Previous studies have examined the seasonal movement patterns of adult American horseshoe crab (*Limulus polyphemus*) populations with sophisticated tracking technologies throughout the US East Coast. None, however, have compared preferred habitat use and quantified habitat connectivity of American horseshoe crabs spanning multiple life history stages within a migratory population. Here, we used passive acoustic telemetry to examine adult (n=70) and juvenile (n=25) horseshoe crab seasonal habitat connectivity and spatial distributions within an *estuarine embayment* (Moriches Bay, NY) to the Atlantic Ocean over a two-year period. Network analysis revealed that both male and female horseshoe crabs exhibited complex, but similar habitat connectivity patterns within each season, as adults expressed limited habitat use in the fall and extensive habitat connectivity in the spring during the spawning season. In contrast, juvenile horseshoe crabs (instars 13+) expressed restricted habitat use and limited exchange between habitats within the same period. Permutation tests applied to network analysis metrics (i.e. degree centrality) demonstrated that habitat connectivity was significantly different between adult and juvenile horseshoe crabs ($p < 0.01$). Juvenile and adult habitat transition probabilities were also calculated across multiple seasons to determine preferred habitat use across temporal scales. Results highlight the seasonal complexity of horseshoe crab spatial distributions and provide novel insight into juvenile habitat preferences.

Keywords. Acoustic telemetry, American horseshoe crabs, habitat connectivity, juveniles, *Limulus polyphemus*, movement patterns, New York

EVALUATING TRENDS IN FLORIDA HORSESHOE CRAB ABUNDANCE USING STATE-SPACE MODELING

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Abstract. Horseshoe crab conservation and management efforts are often hampered by a lack of data on abundance, population trends and habitat preferences. In Florida, American horseshoe crabs (*Limulus polyphemus*) are found in every coastal county, but their population trends and habitat requirements are largely unknown. To begin to fill these gaps, we compiled data from Florida Fish and Wildlife Conservation Commission Fisheries Independent Monitoring (FIM) trawl and seine surveys. The FIM surveys are valuable in that the data were collected from eight sites in the same way every month over 11-20 years (sites vary). The survey data also contain a number of important site variables such as temperature, salinity, and bottom substrate. However, the captures per unit effort are very low for horseshoe crabs, i.e. the time-series from the surveys are highly zero-inflated. Using a multi-year, state-space modeling approach, we evaluate trends in detection probability and abundance. Factors that might affect detection and abundance spatially and over time (e.g. seasonality, water temperature) are included in the models to determine their importance. Our analyses reveal differences in population trends on the West (Gulf of Mexico) and East coasts (Atlantic). Populations appear to be stable or declining on the Gulf and increasing on the Atlantic. State-space modeling also provides insight into the variables influencing horseshoe crab presence and detectability at different sites. For example, we found that warmer temperatures have a positive effect on detection probability in enclosed bays and higher dissolved oxygen improves detection in bays along the Gulf coast. By developing spatially explicit models at annual time steps and combining this modeling with empirical Bayes methods, we highlight spatial variability and temporal changes in abundance, increasing our understanding of local population ecology dynamics. This is a particularly useful approach for evaluating trends in horseshoe crab abundance when data are sparse.

Keywords. Abundance trends, detection, effect of environmental variables, Florida, horseshoe crabs, *Limulus polyphemus*, state-space model.

THE IUCN GREEN LIST ASSESSMENT FOR *LIMULUS POLYPHEMUS*

- INVITED TALK -

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Abstract. The IUCN has developed a Green List Assessment (GLA) to recognize and incentivize conservation achievements that help to conserve and recover species on the Red List, particularly those species designated in threatened categories. After updating the Red List category for *Limulus polyphemus* as Vulnerable, we formed a team to complete the GLA for *Limulus* as a tool to help identify, prioritize, and communicate conservation and information needs throughout the species' range. The *Limulus* GLA team comprises both geographic and topical representation and is an extension of the team that conducted the *Limulus* Red List Assessment. The GLA is conducted by engaging in an informed and structured discussion on past, current, and future conservation efforts and population status based on expert judgment and the existing literature. As an assessment tool, IUCN developed a Google Sheet template to facilitate working on the GLA from an online platform. Our team conducted the assessment remotely over a series of working conference calls and webinars. During the process, the team had in depth discussions on historical range and status, delineation of spatial units for analysis, definition of ecological function, species response to future scenarios with and without conservation, and long-term recovery potential. The GLA complements existing management systems, such as the Atlantic States Marine Fisheries Commission and states agencies. The GLA process helps provide conservation guidance and identify knowledge gaps in areas where management systems are absent or where horseshoe crabs are not receiving high management priority, e.g., in the Gulf of Mexico. In this presentation, we explain the GLA process and illustrate how it was applied to *Limulus*.

Keywords. Conservation assessment and planning, IUCN Red List, IUCN Green List, *Limulus polyphemus*

OCCURRENCE AND MORPHOMETRIC ANALYSIS OF *CARCINOSCORPIUS ROTUNDICAUDA* ALONG BHADRAK COAST OF ODISHA, INDIA

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Abstract. Horseshoe crabs (HSCs) or the King Crabs have been living on earth for more than 445 million years ago. Out of four species distributed globally, *Tachypleus gigas* (Muller, 1785) and *Carcinoscorpius rotundicauda* (Latreille, 1802) are found along the north east coast of Bay of Bengal, India. The study was carried out with an aim to explore the occurrence, distribution and morphometric characteristics of the *C. rotundicauda* along the Bhadrak coast of Odisha, by using standard procedure. The study was conducted in four different places located at the Bhadrak Coast of Odisha, India. These four places were Chandanipal creek (20.7879°N, 86.9595°E), Dosinga creek (20.8139°N, 86.9636°E), Uda bali or Kanika island (20.8038°N, 87.0177°E) and Chudamani ghat (21.1379°N, 86.8074°E). During the study period a total of 117 individuals of *C. rotundicauda* were collected from the four different sites. The morphometric studies of the total individuals belonging to four different sites were carried out taking into consideration of four parameters, that is, total length (TL) of the species, total length of telson (TEL), carapace width (CW), opisthosomal width (OW) and their mean values and standard deviation were calculated from which it is evident that morphometry is an important tool in determining the age and sex of an individual. During sampling, some of the caught horseshoe crabs were physically deformed and damaged such as deformed telson, damaged carapace and some of them were infested with barnacles. Many different sizes (ranges from 18.5cm- 5.9cm of total length) of juvenile form of *C. rotundicauda* were found in Udabali Island. However, fishing activities and other anthropogenic interference such as uses of motor boats and trawlers are the important factors for dwindling the population of horseshoe crabs. As the reported species has ecological, commercial and medicinal importance, necessary steps are warranted for the conservation of the reported species.

Keywords. Bhadrak, conservation, horseshoe crab, morphometric analysis

IDENTIFICATION AND CHARACTERIZATION OF ESSENTIAL NURSERY HABITAT FOR ASIAN HORSESHOE CRABS IN NORTHERN BEIBU GULF

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Abstract. Identification, protection, and enhancement of essential habitats are priority issues for management and restoration of exploited species. We examined evidences for the structuring of juvenile horseshoe crab assemblages along the northern Beibu Gulf shoreline, and the roles of abiotic and spatial factors in determining the assemblage distribution and density. Based on the results of 0.59 km² investigation area on intertidal region, *Tachypleus tridentatus* utilized the shoreline of Jinhaiwan as their core nursery habitats, where a high density of juvenile population (5.27-5.63 individuals/100m²) at mostly 4-7 instars was found. Juvenile *Carcinoscorpius rotundicauda* population, on the other hand, concentrated along coast of the Pearl Gulf, with an exceptional high density of 3.93-5.15 individuals per 100 m² of investigation area. Most juveniles (> 45%) found in the Pearl Gulf are at 6-10 instars. Linear-based generalized linear model demonstrated that area of seagrass, mainly *Halophila beccarii* could explain the juvenile density gradient for both species, in addition to grain size for *T. tridentatus* and chlorophyll a for *C. rotundicauda*. Mangrove area with mostly *Avicennia marina* was shown to be positively correlated with the mean ($R^2 = 0.688$) and standard deviation ($R^2 = 0.586$) of juvenile density of *C. rotundicauda*, despite the facts that a significantly higher juvenile density for both species was found along the outer fridge of mangrove forest and near the mouth of river outflows. These results provide new insights into the nursery habitat requirements of Asian horseshoe crabs in the southern region and practical information for prioritization and conservation of the areas and resource that have great potential for human disturbance.

Keywords. *Carcinoscorpius rotundicauda*, estuary, mangrove, *Tachypleus tridentatus*, seagrass

THE CONSERVATION STATUS OF HORSESHOE CRABS IN VIETNAM

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Abstract. Three species of horseshoe crabs have been recorded in Vietnam, the mangrove horseshoe crab, *Carcinoscorpius rotundicauda*, the coastal horseshoe crab, *Tachypleus gigas*, and the tri-spine horseshoe crab, *T. tridentatus*. In the past few decades, habitat destruction, degradation and pollution of the environment and overexploitation have placed the horseshoe crabs in Vietnam in threatened status. However, the data about the distribution and conservation status of horseshoe crabs in Vietnam are very rare. There are only two studies of morphology, biology and toxicology of horseshoe crabs in Khanh Hoa province, central Vietnam. *T. tridentatus* was assessed as Vulnerable in Vietnam Red Data Book (2007). For a more accurate assessment of the conservation status of the horseshoe crabs in Vietnam, an extensive study of distribution and population dynamics is required. Conservation activities for the horseshoe crabs in Vietnam are really urgent. It requires active participation from the highest management agencies such as the Ministry of Natural Resources and Environment, as well as local governments, coastal communities and scientists. In particular, linking activities of MPAs and wetland areas with horseshoe crab conservation and raising public awareness are the main priorities.

Keywords. *Carcinoscorpius rotundicauda*, conservation, horseshoe crabs, *Tachypleus gigas*, *Tachypleus tridentatus*, Vietnam

LANDING POPULATION AND TOXICITY STUDY OF HORSESHOE CRABS IN PASIR PUTIH VILLAGE, SARAWAK, BORNEO

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Abstract. Horseshoe crabs are usually caught by fishing net with other important fishes and occasionally eaten by local people in Sarawak. Horseshoe crabs are known to possess tetrodotoxin (TTX) which can cause food poisoning and cause adverse effect to human health. In the current study, a total of 297 horseshoe crabs from two species namely *Tachypleus gigas* and *Carcinoscorpius rotundicauda* were collected from intertidal area of Pasir Putih Village, Kuching on March 2014 until December 2016. The specimens were collected manually by hand during the low tide period on new moon day. For landing population analysis, the horseshoe crabs within modified line transect were collected and recorded. The highest density of *T. gigas* and *C. rotundicauda* were recorded in March 2014 (166 inds/100 m²) and December 2016 (48 inds/100 m²), respectively. For toxicity analysis, the tetrodotoxin (TTX) concentration within soft tissues and eggs of horseshoe crabs was analyzed using Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS). Overall, the extracted toxin for both species of horseshoe crabs was shown non-toxic with result less than 10 MU/g as calculated based on TTX standard curve, but certain precaution was still needed since high TTX concentration was detected in certain month, August 2016. Among the tissues, soft tissues were found to be highest in TTX concentration in male (n=5) *T. gigas* (44.27 MU/g) and one individual of female (69.10 MU/g). Therefore, we concluded that both sexes of *T. gigas* might possess TTX in their tissues (eggs and soft tissues) but only with weak concentration which was not sufficient enough to cause human intoxication. The data could be useful for relevant authorities for conservation and protection of horseshoe crab's habitat and also as a guideline for local authorities to ensure public safety in the consumption of horseshoe crabs.

Keywords. *Carcinoscorpius rotundicauda*, landing population, tetrodotoxin, Liquid Chromatography-Mass Spectrometry/Mass Spectrometry, *Tachypleus gigas*

POPULATION GENETIC STUDY ON *CARCINOSCORPIUS ROTUNDICAUDA*

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Abstract. The three extant Asian horseshoe crabs *Carcinoscorpius rotundicauda*, *Tachypleus gigas* and *T. tridentatus* are all categorized as “Data Deficient” according to the International Union for Conservation of Nature (IUCN). This complicates conservation efforts of these species by obscuring the possibility for appropriate management strategies. One of the species, *C. rotundicauda*, inhabits estuaries and mangroves with muddy brackish water and is rarely found in the open seawater. Thus, we hypothesize that this species has limited migration and genetic flow between populations. A previous study showed regional intraspecific differentiation and local adaptations of *C. rotundicauda* across the Thai-Malay Peninsula based on morphometrics. Other studies based on a limited number of molecular markers confirmed this, thus suggesting that the peninsula serves as a biological barrier for dispersal-limited species. In this study, we elucidated the population genetic structure of *C. rotundicauda* by analysing *mitochondrial* COI sequences in combination with microsatellite DNA markers obtained from haemolymph samples collected during fieldwork in 2006-2008 from nine localities in Thailand, Malaysia and Vietnam, representing both sides of the Thai-Malay Peninsula. Our study confirmed low levels of connectivity across the Thai-Malay Peninsula, which implies that conservation efforts should consider and preserve the genetic diversity by protecting breeding areas on both sides of the peninsula.

Keywords. *Carcinoscorpius rotundicauda*, COI, data deficient, genetic diversity, microsatellite markers, population genetics, Thai-Malay Peninsula

HOW CLOSE GENETICALLY THE MANGROVE HORSESHOE CRAB POPULATIONS ON EAST, WEST AND SOUTHERN COASTS OF PENINSULAR MALAYSIA?

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Abstract. The mangrove horseshoe crab, *Carcinoscorpius rotundicauda* has a restricted distribution throughout Asia. In order to identify its distinct populations within Peninsular Malaysia, the present study observed both morphometric data and genetic diversity (through *Cytochrome Oxidase 1* - CO1 sequencing) from Perak (west), Pahang (east) and Johor (south) states. At first, gill nets were used to catch the crabs every month during full and new moon periods (Jan 2016 - Jan 2017), and obtained the adult crab measurements. Second, blood (0.5 ml) sample from the crabs and freshly deposited eggs (6-8 nos.) from the nesting sites were analysed for DNA sequencing. Due to no observed nesting activity at Perak, only blood samples were considered. The prosomal width of *C. rotundicauda* varied considerably among Johor (10.8-12.9 cm), Pahang (12.40-14.2 cm) and Perak (10.6-12.25 cm) populations. The gene isolations were analysed against to the NCBI GenBank® and found similar to the reports available from India, Thailand and Vietnam. From the phylogenetic tree of resemblance (produced with MEGA v.6 software), it was possible to distinguish two major clades separating West and East Malay Peninsula. While West Malay Peninsula with Perak (Straits of Malacca) sample has a lineage of Odisha (Bay of Bengal) and Phuket (Andaman Sea), the East Malay Peninsula with Pahang (South China Sea) sample has a lineage of Bac Lieu (South China Sea) and Bang Pu (Gulf of Thailand). The southernmost Johor (Straits of Johor) sample was found to be a sub-clade of East Malay Peninsula (with 78% support) and suggests a historical connectivity with the South China Sea, but separated from the direct influence. Overall, *C. rotundicauda* populations on the west and the east coasts of Peninsular Malaysia are distinctly separate for which local topography on the south (as land barrier) along with sea surface currents could be responsible.

Keywords. *Carcinoscorpius rotundicauda*, CO1 sequencing, genetic diversity, living fossil, Malaysia, morphometric data

POPULATION GENOMICS OF TWO HORSESHOE CRAB SPECIES IN SINGAPORE

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Abstract. There are two species of horseshoe crabs, *Tachypleus gigas* and *Carcinoscorpius rotundicauda*, sharing the habitats along the coast of Singapore. Recent coastal modification, pollution and invasion of exotic species have greatly threatened the survival of these horseshoe crabs. We sampled 121 individuals of the two horseshoe crab species, 38 *T. gigas* and 83 *C. rotundicauda*, around Singapore and used double digest RAD sequencing to collect their genomic information. We harvested ~30,000 genetic markers throughout the horseshoe crab genome to examine the relationship among sampled individuals. With the genomic data, we aim to check the population structure which provides us with critical information such as genetic diversity, population sub-structuring, inbreeding and genetic connectivity among habitats around Singapore. These results will help us identify important - population clusters and corridors to connectivity for maintaining a healthy status of regional populations in Singapore.

Keywords. *Carcinoscorpius rotundicauda*, conservation genomics, ddRADseq, horseshoe crabs, *Tachypleus gigas*

CONSERVATION GENETIC ASSESSMENT FOR HORSESHOE CRABS AROUND EAST ASIA

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Abstract. Three of four species horseshoe crabs distributed in Asia were under long-term stress due to coastal development, marine pollution and overharvest. The conservation assessments for their population and habitat are evaluated by IUCN SSC Horseshoe Crab Specialist Group. Otherwise, loss of genetic variation within a species may affect its ability to adapt to changing environmental conditions. However, there is a current lack of explicit considerations of genetic condition in conservation assessments. In this study, range-wide population demography, habitat and genetic condition of horseshoe crabs in Asia were compared. Generally, the populations with related higher genetic diversity were found in south east Asia compared to northwestern Pacific area. The full loss of genetic diversity was found in some populations in Taiwan and Japan due to population crash. Temporal haplotypes shift and loss of genetic connectivity in *T. tridentatus* at Kinmen were also found due to construction of port through a 16-years genetic data tracked from 2002 to 2017. Marine protected area network can also be designed based on genetic connectivity along China coast. Long-term and regional scale population monitoring are not easily applied on each location, but conservation genetic assessment may make a contribution on a broader view of population status.

Keywords. Conservation genetic assessment, genetic connectivity, horseshoe crabs, loss of genetic diversity

GENETIC AND PHENOTYPIC VARIATION IN THE AMERICAN HORSESHOE CRAB (*LIMULUS POLYPHEMUS*): A CASE STUDY FROM NORTHEAST FLORIDA

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Abstract. American horseshoe crabs, *Limulus polyphemus*, show both genetic and phenotypic variation across their broad range. Analysis of microsatellite (nuclear DNA) variation shows genetic differentiation into discrete populations with a deep genetic discontinuity along the southeast coast of the US. Here the species is separated into two distinct clades: “Florida East” and “Southeast.” The “Florida East” horseshoe crabs are among the smallest (mean prosoma widths: female 18.7 cm, male 14.9 cm) on the Atlantic coast. They appear to be mostly confined to the Indian River Lagoon system of central Florida, a shallow, microtidal estuary with highly variable environmental (e.g. temperature, salinity, dissolved oxygen) conditions. “Southeast” horseshoe crabs occur in coastal north Florida, Georgia and South Carolina where tides are exceptionally high (2.5 m) and environmental conditions relatively stable. Individuals in this population are large (female 30 cm, male 23 cm) and some have a unique, “extra” pair of spines on the anterior margin of the opisthosoma. When first observed in 1815, Thomas Say proposed that these horseshoe crabs should be considered a separate species, *Limulus australis*. Our fine-scale analysis of horseshoe crabs from “Southeast” and “Florida East” sites reveals a region of overlap and admixture in northeast Florida with random mating and interbreeding between two populations of one species, *Limulus polyphemus*. Large size and “extra” spines are associated with individuals that have a higher proportion of alleles from the “Southeast” population. The frequency of large size, “extra” spines and “Southeast” population alleles gradually declines from southern Georgia to central Florida. Given that there is some overlap and interbreeding, the maintenance of these two genetically and phenotypically distinct populations indicates strong adaptation to local environmental conditions. Genetic structuring and phenotypic differences between horseshoe crab populations also suggests that management efforts should be targeted within regions at a local scale.

Keywords. Florida, genetic differences, habitats, *Limulus polyphemus*, population overlap, size, spine polymorphism



THEME 2
ADVANCES IN HORSESHOE CRAB BIOLOGY

蟹生物学进展

INFECTIOUS DISEASES *IN TACHYPLEUS GIGAS* DURING CAPTIVE BREEDING AND REARING

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Abstract. Captive breeding and rearing of horseshoe crab is a promising step towards its conservation and sea-ranching. However, infectious and non-infectious diseases are the main challenges in determining the health condition of horseshoe crabs under captivity. Both developing eggs as adult horseshoe crabs are susceptible to various pathogens such as protozoa, bacteria, fungi, algae and other micro and meiobenthos. We address a number of pathogens that caused mortality in captive adult and juvenile horseshoe crabs. Samples were taken from gill fluids, gill tissue, and eggs of captive *T. gigas*. The microbes were cultured in various media (Tryptic Soy agar, Muller-Hinton agar and Nutrient agar) at different culture conditions (25 °C for 4 days or 37 °C for 24 hrs) with and without salt (1% NaCl) to facilitate the growth and viability of wide range of microbes. Genomic DNA extraction, PCR amplification and DNA sequencing were carried out to target molecular identification of the pathogens using partial sequencing of 16S rRNA gene for bacteria and ITS gene for fungi.

Aeromonas caviae strains were isolated from the gill fluid of an infected animal that died within a day of exposure. *Lysinibacillus fusiformis* strains were isolated from gill tissues of animals kept under prolonged captivity (>6 months). *Aspergillus aculeatus* strains were screened from eggs reared in captivity where development was retarded or ceased post infection. The diverse, frequency and varying targets of these microbial infections requires more studies on the culture condition, feeding and other parameters used for maintaining horseshoe crabs in laboratory settings.

Keywords. Captive breeding, captive rearing, conservation, infections, *Tachypleus gigas*

QUANTIFYING THE FEED UTILISATION ON ALTERNATIVE FEEDS BY ADULT HORSESHOE CRABS *CARCINOSCORPIUS ROTUNDICAUDA* IN CAPTIVITY

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Abstract. From the increasing interest in horseshoe crabs rearing for research purposes triggered a new trend of studies in developing alternative feed for horseshoe crabs in captivity. Adult *C. rotundicauda* was used since it is easily available from local fishermen. A feeding study was undertaken for 5-weeks, and separated into three parts: 1. The development of soft-dry, semi-moist pellets for horseshoe crabs; 2. The acceptance level of horseshoe crabs towards food; 3. The horseshoe crabs' growth performance and feeding utilisation. Triplicate groups of *C. rotundicauda* were fed with 3 treatments (2 alternatives and 1 natural feed). The alternative feeds (pellets) were composed fishmeal and cockle at a ratio of 1:1 (namely A) and conjugating a local cuisine fish cake or known locally as Keropok (B) as another alternative food treatment. Control treatment on the other hand was raw cockles (C). *C. rotundicauda* preferred A the most (56.21 %). However, there were no differences in body weight (BW) changes within alternative food-fed *C. rotundicauda* (treatment A and B). Also, feeding rate was lower (the highest was 1.88 % BW day⁻¹ in A-fed horseshoe crabs) than previously cited 3% BW day⁻¹ in many literatures. The feeding conversion ratio (FCR) in A- and B-fed *C. rotundicauda* (0.064-0.071) were different from C-fed *C. rotundicauda* (0.024; $p=0.001$). Besides, specific growth rate (SGR, % week⁻¹) also showed the same pattern of results as FCR. On the other hand, all treatments showed no differences in absorption efficiency (>95%; $p>0.05$). These results showed that *C. rotundicauda* displayed clear preference towards alternative food apart from exhibited better growth performance and feeding utilisation than the natural food.

Keywords. Alternative food, *Carcinoscorpius rotundicauda*, feeding utilisation, food preference, growth performance, horseshoe crabs

FEEDING ECOLOGY AND FOOD PREFERENCES OF *TACHYPLEUS GIGAS* FROM MALAYSIAN WATERS

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Abstract. Horseshoe crabs are opportunistic foragers that can take advantage of a wide range of locally available prey. However, in the wild habitat, they prefer to feed on preferential food over other available feeds. Gut content analysis of *Tachypleus gigas* collected from east coast of Peninsular Malaysia showed monthly and seasonal variations in the food preferences of *T. gigas*. They prefer lesser number of polychaetes than bivalves during non-monsoon while it was the reverse during monsoon. Gut content analysis revealed that mollusks were highly preferred by *T. gigas*. It was also found that horseshoe crabs feed intensely during their peak mating season)May-August (in Pahang coast. Male prefers to feed less compared to the female crabs. In this study we also addressed electivity index (EI) and Gastro Somatic Index (GSI) of the crabs collected during 12 months of the sampling period .

Keywords. Captive culture, electivity index, gastro somatic index, horseshoe crabs, *Tachypleus gigas*

CHEMORECEPTION IN THE AMERICAN HORSESHOE CRAB, *LIMULUS POLYPHEMUS*: IS THERE A “NOSE”?

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Abstract. The use of multiple sensory cues is crucial for prey detection, predator avoidance and mate location. Among arthropods, pancrustaceans and crustaceans including insects are well studied. They typically have well-developed vision, mechano- (touch and vibration sensing), and chemoreception. Chemoreception is normally mediated by numerous setae all over the body but olfaction is placed in special setae on the antennae. Interestingly, chelicerates, including horseshoe crabs lack antennae. Although researchers have found some evidence for structures being involved in chemoreception, the knowledge of the olfactory system in horseshoe crabs is limited. Previous studies have indicated that chemoreception in the horseshoe crab *L. polyphemus* is associated to different structures including the gnathobase, the chela, the flabellum and the gills. Here we present both neuroanatomical and physiological data from the chelae, pedipalps and walking legs. Electrophysiological studies were carried out on appendages of juvenile *L. polyphemus* using juice from mussels as a food stimulus. The results indicate the highest sensitivity in the pedipalps, but the dose dependent responses still require rather high concentrations. The nerves were examined in the same appendages of two adult *L. polyphemus* (female and male) using light- and transmission electron microscopy. The results revealed several hundred axons of varying size in each nerve. The size of some of the axons indicates the presence of both mechano-, and chemoreceptor cells. Further, there was a difference in the number of axons according to appendages with the pedipalps having the most and the chela the least. In total our results point to the pedipalps being the most sensitive chemosensory limb of the horseshoe crab.

Keywords. Chemoreception, neuroanatomical, olfaction, physiology, the nose of *Limulus polyphemus*

BEHAVIORAL RESPONSE OF HORSESHOE CRAB TO GREEN LED LIGHTS

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Abstract. Horseshoe crabs are one of the protected animals that are found as bycatch in fishing activities in Indonesia and has a high sensitivity to light. One effort to approach the bycatch reduction is to understand the behavior of horseshoe crabs. In the trilobite larva phase, horseshoe crab shows a positive response to bright light. However, in the adult phase, it does not prefer light. Green LEDs are one of the smart fishing gears, but the response of adult horseshoe crabs to green LED lights is unknown. This study aimed to observe the behavioral response of *Tachypleus gigas* against green LED lights. The method used in this study was a laboratory experiment with 22 *T. gigas* individuals, each with a total length ± 34 cm and under similar conditions: having hard carapace, no shell damage, no molting, and not in the spawning phase. The experiment was carried out in 86 repetitions in artificial ponds and observed under infrared camera. The data were collected by the number of responses, time, and patterns. The results showed that 77% horseshoe crabs gave a positive response to light and 23% gave a positive response to dark areas. Horseshoe crab pattern to light response was divided into Straight to Light (41), Side to light (9), Light to dark (13) and to dark areas without light Straight to dark (12), Side to dark (5) and dark to light (2). The fastest direct straight response was that under green LED lights horseshoe crabs moved quickly and responded immediately to light and remained under the green light. Comparison of the response time between Straight to Light and Straight to Dark was 18.66 sec/m and 26.92 sec/m respectively. This study showed that adult horseshoe crabs have positive phototaxis and are attracted to green LED lights by moving in a faster time with direct response patterns.

Keywords. Green LED, horseshoe crab, response, positive phototaxis

SEMILUNAR PERIODIC ALTERNATION OF ACTIVITY RHYTHMS IN THE TRI-SPINE HORSESHOE CRAB *TACHYPLEUS TRIDENTATUS*

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Abstract. Activity rhythms of the tri-spine horseshoe crab *Tachypleus tridentatus* was examined to understand the mechanisms of biological clocks and the ecological functions in its environment. An acceleration data-logger attached on each animal recorded whether the animal was active or inactive at 1-min interval up to consecutive 45 days. The locomotive activity of 19 animals exposed to light/dark and tidal cycles were monitored in unrestrained conditions. Activity rhythms (either 24-h or 12.4-h cycles) was determined in each trial. For 9 of 19 animals, activity rhythms with both 12.4-h and 24-h cycles were appeared in an experimental trial. The periodic alternation of activity rhythms was investigated with generalized linear mixed models. The fittest model suggested that activity rhythms of *T. tridentatus* periodically alternated from 24-h and 12.4-h cycles and vice versa in 14.8-day periods (semilunar cycles). The animals switched its activity from 24-h to 12.4-h cycles during spring tides and return to 24-h cycles toward neap tides. In this presentation, the mechanisms of biological clocks and its evolutionary functions will be discussed.

Keywords. Biological clock, bio-logging, chronotype, circadian rhythm, circatidal rhythm, Zeitgeber

GENETIC VARIATION IN COI GENE OF INDIAN HORSESHOE CRABS (*TACHYPLEUS GIGAS*) POPULATION ALONG THE COASTAL AREA (BAY OF BENGAL) OF DISTRICT BALASORE, ODISHA, INDIA

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Abstract. Horseshoe crab (*Tachypleus gigas*) is an archaic group of marine creature which plays an important role in saline ecosystem. Many researchers are emphasizing and enhancing the knowledge about the basic biology, morphology and ecology of horseshoe crab whereas very little information is available about its population genetics. This study was attempted to develop a baseline database about the ecology, phylogeography and genetic variation among the horseshoe crab population from India. We collected 127 samples of horseshoe crab from the coastal area of district Balasore in the Bay of Bengal. The generated Cytochrome C Oxidase Subunit I gene (COI) sequences of *T. gigas* were compared with the seven sequences of *T. gigas* obtained from the GenBank. These seven GenBank sequences were of two populations from South China and Malaysia. A total of 14 unique haplotypes were observed in three populations of *T. gigas*. Pairwise F-statistic distance (F_{ST}) between South China-India was 0.708; Malaysia-India was 0.608; and South China-Malaysia was 0.136. It indicated that South China population was closely related to Malaysian population and Indian population was appeared to be genetically distinct from other two populations. It signifies the ecological value of Indian population. The migrant per generation (Nm) was 0.16, which is an indication of low gene flow among *T. gigas* populations. The haplotype diversity (H_d) and nucleotide diversity (π) were 0.58826 and 0.00393, respectively. We further need to examine more samples from other locations of India to examine the presence of other populations in its range (if any). The findings from this study may have important implications for future strategy and conservation of horseshoe crabs among the certain area of Bay of Bengal coast in India.

Keywords. COI gene, genetic variation, horseshoe crab, phylogeny, *Tachypleus gigas*

MOLECULAR IDENTIFICATION OF HORSESHOE CRABS IN JAVA AND MADURA ISLANDS, INDONESIA

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Abstract. Resource management needs molecular information as a basis to determine which species that require conservation and which species are introduced from other places, and to decide on protection areas. Horseshoe crabs are one of those conservation species that is protected by law in Indonesia, but the research about genetics of this species still limited. The study aimed to validate horseshoe crabs based on 16S rRNA gene from Java and Madura Islands, Indonesia. Specimens were collected from 7 locations in north of Java Island (i.e. Indramayu, Subang, Semarang, Demak, Rembang, Tuban, Lamongan, and Surabaya) and 1 location in Madura Island. Molecular identification was conducted by DNA barcoding method. Amplification was used with specific primer of 16S rRNA designed by Nurlisa Alias Butet. The results of the study showed that the primer successfully identified and confirmed species taxonomy based on molecular characteristics. Thirty-two sequences of partial 16S rRNA gene confirmed as *Carcinoscorpius rotundicauda* (98.44-99.03%), while eleven sequences confirmed as *Tachypleus gigas* (98.92-99.35%). The nucleotide base length of 43 sequences ranged 503 to 522 and had been submitted to GenBank as base data of horseshoe crab nucleotides from Indonesia. There were 10 specific nucleotide bases as markers for *C. rotundicauda* and 5 specific nucleotide bases as markers for *T. gigas* in Indonesia. The findings based on 16S rRNA have important implications for exact taxonomy, stock identification, and geographic variation of horseshoe crabs in Indonesia.

Keywords. 16S rRNA, *Carcinoscorpius rotundicauda*, DNA barcoding, horseshoe crabs, *Tachypleus gigas*

GENOME SEQUENCING AND TRANSCRIPTOME STUDY FOR *TACHYPLEUS TRIDENTATUS*

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Abstract. Chinese horseshoe crabs (*Tachypleus tridentatus*), ancient marine arthropods dating back to the mid-Palaeozoic Era, have provided valuable resources for the detection of bacterial or fungal contamination and the conquest of cancer and HIV virus. However, excessive exploitation for the amoebocyte lysate of *Tachypleus* has dramatically decreased the population of the Chinese horseshoe crabs. Thus, we present sequencing, assembly and annotation of *T. tridentatus*, with the hope of understanding the genomic feature of the living fossil and assisting scientists with the protection of this endangered species. And more, the sequencing of horseshoe crabs genome will help us understand the evolution of arthropods more deeply. The final genome contained a total size of 1.943Gb, covering 90.23% of the estimated genome size. The transcriptome of three larval stages was constructed to investigate the candidate gene involved in the larval development and validate annotation. The completeness of the genome and gene models was estimated by BUSCO, reaching 96.2% and 95.4%, respectively. The synonymous substitution distribution of paralogues revealed that *T. tridentatus* had undergone two rounds of whole-genome duplication. All genomic and transcriptome data have been deposited in public databases, ready to be used by researchers working on horseshoe crabs.

Design Type(s)	transcription profiling by high throughput sequencing design • sequence assembly objective
Measurement Type(s)	whole genome sequencing • transcription profiling assay
Technology Type(s)	DNA sequencing • RNA sequencing
Factor Type(s)	developmental stage
Sample Characteristic(s)	<i>Tachypleus tridentatus</i> • skeletal muscle tissue • ocean biome • larva

Keywords. Annotation, evolution, horseshoe crabs, protection, sequencing

SCREENING OF ANTIMICROBIAL ACTIVITY OF INDIAN HORSESHOE CRAB HAEMOLYMPH ON FOODBORNE PATHOGEN

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Abstract. Foodborne diseases are an extensive and a rising communal health problem. In order to explore the process and means of regulating the spread of clinical pathogens, we focus on screening and recognizing marine bioactive elements, especially those having antimicrobial activity against foodborne pathogens. Our study investigates the antimicrobial activity of cell free haemolymph of Indian horseshoe crab against the isolated foodborne pathogenic bacteria. Isolation of food pathogenic bacterial strain was carried out by spread plate method. Prominently visible colonies were taken for molecular identification through DNA isolation and phylogenetic analysis which revealed the species as *Micrococcus cseolyticus* having genbank accession number KR821154. Horseshoe crabs collected from Chandipur coast, Odisha, India was identified through DNA isolation and partial sequencing (~650bp) of mitochondrial cytochrome c oxidase subunit I gene (COI) using “Universal” DNA primers for DNA barcoding. Phylogenetic and BLAST analyses exposed that the collected species was *Tachypleus gigas* having genbank accession number KU880543. The hemolymph of horseshoe crab offers a large catalogue of evolutionarily conserved proteins which are immensely responsive to pathogens. In this experiment, the horseshoe crab haemolymph was collected, extracted and screened for antimicrobial activities against isolated bacteria. This investigation was carried out using haemolymph supplemented agar plate of concentration ranging 50 to 500 µl. Representative strain was diluted 10⁻¹ to 10⁻¹⁰ times in double distilled water and 0.1 ml from these diluted sample was utilized for spreading onto the plates and incubated at 30°C for 48 hours. Throughout the incubation the plates were examined and it showed a declination of bacterial growth in the occurrence of particular concentrations of haemolymph. An integrated management with the incorporation of societal involvement, education and better awareness is essential in the conservation of horseshoe crabs.

Keywords. Antimicrobial activity, foodborne Pathogen, *Micrococcus cseolyticus*, Molecular Characterization, *Tachypleus gigas*

NOVEL ANTI-BACTERIAL COMPOUND FROM SHELLS OF FERTILISED EGGS OF INDIAN HORSESHOE CRAB *TACHYPLEUS GIGAS* (MÜLLER)

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Abstract. Identification of new anti-microbial compounds has become one of the frontier areas in biomedical research due to an alarming rise in the occurrence of antibiotic-resistant bacterial strains. *Tachypleus gigas* (Müller) one of the major species of Indian horseshoe crab migrates regularly towards sandy beaches along the north-east coast of Odisha. They lay their gametes in nests which are built on sandy beaches. Surprisingly, developing embryos in nests do not show any infection during their incubation period of 40-45 days although breeding beaches are found to be heavily infested with pathogens. Thus an attempt was made to identify, isolate and characterize the active anti-bacterial component present in the outer membrane of the fertilised eggs of *T. gigas*. Different solvent extracts of egg membrane were prepared in ethanol, methanol, hexane, chloroform and PBS. All these extracts were subjected to activity-based biological screening for the presence of anti-bacterial properties using nine strains of human pathogenic bacteria. Extract prepared in ethanol showed potentially a high and broad-spectrum activity against all these bacterial strains. The final purified and most active fraction was obtained following a standard activity-based fractionation scheme and all these fractions were screened independently for the presence of anti-bacterial properties. Fraction (Et-2.1.a) isolated from the crude ethanolic extract showed a maximum activity where 120% zone of inhibition was recorded as compared to known antibiotic. Anti-bacterial compound present in the outer egg membrane of the fertilised eggs of the horseshoe crab will be a potential product for chemical synthesis that will be useful commercially for biomedical applications, especially for treating diseases caused by multi drug resistant and extreme drug resistant bacterial strains.

Keywords. Antibiotic, activity-based fractionation, egg shells, extract, Horseshoe Crab, *Tachypleus gigas*

EFFECTS OF DIETARY COPPER SUPPLEMENTATION ON NON-SPECIFIC IMMUNE ENZYME ACTIVITIES, BLOOD-CHEMISTRY AND HEMOCYANIN CONCENTRATION OF CHINESE HORSESHOE CRAB (*TACHYPLEUS TRIDENTATUS*)

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Abstract. Copper is considered to be an essential trace element for horseshoe crabs that is vital to their health. In this study, the effects of different levels dietary copper (0, 50, 100 mg kg⁻¹ amino acid chelated copper) on non-specific immune enzyme activities, blood-chemistry and hemocyanin concentration of adult male Chinese horseshoe crab (*Tachypleus tridentatus*) were evaluated through four weeks experiment. Nine parameters: Cu-Zn *superoxide dismutase*, malondialdehyde, *glutathione peroxidase*, total antioxidant capacity, lysozyme, acid phosphatase, alkaline phosphatase, triglyceride, cholesterol and hemocyanin concentration, were detected. The present findings revealed healthy status of the adults fed with diets containing 50 mg kg⁻¹ amino acid chelated copper was better, and hemocyanin concentration of this group was significantly higher, than other groups at the end of the experiment. The optimum amino acid chelated copper supplementation for adult horseshoe crab was around 50 mg kg⁻¹.

Keywords. Amino acid chelated copper, blood-chemistry, Chinese horseshoe crab, hemocyanin, non-specific immunity enzyme

POSTEMBRYONIC DEVELOPMENT AND POPULATION SIZE OF *TACHYPLEUS TRIDENTATUS* IN A NURSERY HABITAT ON PALAWAN AND FIRST DATA ABOUT THE BREEDING SEASON IN THE PHILIPPINES

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Abstract. Despite the iconic nature of horseshoe crabs, very little is known about the development of the group in the tropics. To understand the population demographic of *Tachypleus tridentatus* populations and judge the status thereof in the Philippines, we conducted a mark-recapture experiment in a nursery habitat on Palawan Island twice within 16 years. The results obtained provide the first consecutive and near comprehensive dataset on the stepwise growth of the species in the Philippines. The size of the juvenile population in 2001 was compared with the population size assessed in 2017 and differences were highlighted. Moreover, between 2015 and 2016, adult horseshoe crabs were caught over a 12-month period to assess the morphometric parameters and explore the breeding period of *T. tridentatus* in the study area. The data support the assumption that moulting continues year-round in the tropics and also indicate that the average age of mature male and female *T. tridentatus* in the Philippines ranges from three to four years. Our data align within results from Japan and suggest that 14 postembryonic stages characterise the development of natural populations of the species. Though more data are needed, the results provide a sound baseline for future horseshoe crab studies in the Philippines.

Keywords. Juvenile population dynamics, ontogeny, Philippines, Tri-spine Horseshoe Crab

CHANGES OF INTESTINAL MICROBIOME DURING THE DEVELOPMENT OF CHINESE HORSESHOE CRAB (*TACHYPLEUS TRIDENTATUS*) LARVAE

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Abstract. Increasing scientific evidence points to an important role of intestinal microbiota composition and function in many physiologic and immunologic processes. Changes of the microbiota composition has been considered to link to many conditions, such as diseases, developmental stages, diets. In this study, the differences in the intestinal microbiome of Chinese horseshoe crab (*Tachypleus tridentatus*) larvae of different developmental stages (1st instar stage; 2nd instar stage with or without feeding brine shrimp) were compared. The results indicated horseshoe crab larvae of different stages contained specific microbiota, with significantly different composition and abundance of the dominant microbiota. Even at the same stage (2nd instar stage), diet feeding influenced intestinal microbiota profiles and marked changes were found. Recent studies have found new mechanisms by which components of food impact the development of intestinal system, thus offering new ideas to develop food-based intestinal modulators in the future.

Keywords. Chinese horseshoe crab, Brine Shrimp, Intestinal microbiome, 1st instar stage, 2nd instar stage



THEME 3
HORSESHOE CRAB IN BIOTECHNOLOGY

蟹生物技术

BIOTECHNOLOGICAL SIGNIFICANCE AND BIO-PROSPECTING OF HORSESHOE CRABS

- INVITED TALK -

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Abstract. Horseshoe crabs are unique type of arthropods having a lot of biomedical, biotechnological and evolutionary significance. There are four extant species of horseshoe crabs found globally namely: *Limulus polyphemous*, *Tachypleus tridentatus*, *Tachypleus gigas*, and *Carcinoscorpius rotundicauda*. The circulatory fluid of the horseshoe crab is known as haemolymph. The haemolymph and amoebocytes contain some large granules which have very important factors like Factor B, C, D and G, Proclotting enzyme, coagulogen, Anti- LPS factor, Big Defensins, Tachylectin 1-5, Cystatin, Alpha-2 macroglobulin, LICI-123 as well as some small granules those have Tachypleusins, Big Defensins, Tachycitins and Tachystatins etc. The *Amoebocytes Lysate* of horseshoe crab has been used extensively for detection of minute quantity of bacterial endotoxin. The perivitelline fluid of the horseshoe crab eggs also contains some important primitive type of proteins which plays an important role during embryogenesis by promoting cell proliferation. Lectin is the constituent of the perivitelline fluid that helps in the proliferation of chick embryonic heart cells, in wound healing activity and differentiation of human bone marrow stem cell into cardiomyocyte. The role of such molecules for growth stimulating effect can generate millions of rupees and enrich the global economy in future. The antimicrobial peptides (AMPs) i.e. the tachypleusin I and II obtained from haemocytes of horseshoe crabs are currently upcoming new bio-active compounds known for its unique method of killing cancer cells. Chitin is a substance found in the shells or exoskeleton of dead horseshoe crab carapace. It is nontoxic and biodegradable and can be processed to produce another substance called chitosan which has enormous commercial value.

Keywords. Antimicrobial peptides, biotechnological, bio-prospecting, chitosan, endotoxin, haemolymph, horseshoe crabs

CLINICAL USE OF TAL FROM CHINA HORSESHOE CRABS IN EARLY DIAGNOSIS OF IFD AND THEIR CONSERVATION

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Abstract. Invasive fungal disease (IFD) is a life-threatening disease that affects millions of people worldwide. We have used *Tachypleus amebocyte lysate* (TAL) of China horseshoe crabs (*Tachypleus tridentatus*) from Guangxi Province, China for investigating the clinical significance in the early diagnosis for IFD. TAL extracted from China horseshoe crabs contains G factor, a protease that can enzymatically digest (1,3)- β -D-glucan (BDG) in fungal cell walls. BDG is known as a biomarker for fungal infection. IFD could be caused by several fungal pathogens including invasive aspergillosis (IA), invasive candidiasis (IC), Pneumocystis pneumonia (PCP), etc. Conventional diagnosis has limited capacity to early detect most causes of IFD. However, using BDG as a pan-fungal screening test has demonstrated significant clinical value in the early detection of IFD in variety of clinical settings. Recent ROC analyses showed that Dynamiker Fungus (1,3)- β -D-glucan (D-BDG) has achieved promising performance. That has showed over 80% sensitivity and specificity, respectively for the early diagnosis of IA, IC and PCP cross several high-risk patient cohorts of 425 serum samples. Also, it provides comparative results with the Associate for Cape Cod Fungitell assay. In conclusion, the D-BDG provides a useful tool to aid the early diagnosis of IFD. In addition to continuing the clinical use, we need to protect the China horseshoe crabs from their declining. We have the following solutions: 1. have established a series of farming pools for artificial breeding horseshoe crabs in Beihai and Qinzhou cities, Guangxi Province; 2. have set up the regulations and policies for culturing and releasing horseshoe crabs back to the ocean; 3. have been strengthening education on horseshoe crab conservation awareness; 4. have been promoting harmonious development of conservation and social economy.

Keywords. Chinese horseshoe crab, invasive fungal disease, *Tachypleus amebocyte lysate*

THE LONG-TERM EFFECT OF BLEEDING FOR LIMULUS AMEBOCYTE LYSATE ON ANNUAL SURVIVAL: AN ANALYSIS OF TAGGING DATA

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Abstract. Between 2013-2017, over 525,000 horseshoe crabs, on average, have been captured and brought to biomedical facilities for bleeding to produce Limulus ameocyte lysate (LAL). Based on a meta-analysis of 47 experimental studies, Atlantic States Marine Fisheries Commission estimates short-term bleeding-induced mortality to be 15% (4% to 30%) and, furthermore, reports that LAL activities including handling and bleeding effects resulted in mortality of over 67,000 annually in recent years. This is not a high level of mortality coastwide relative to other sources of mortality, such as the bait harvest, or relative to the coastwide abundance of horseshoe crabs. However, the long-term effect on annual survival is not included and has not been studied. To address this knowledge gap, we used capture-recapture models fit to data from the U.S. Fish and Wildlife horseshoe crab tagging database to estimate the differences in recapture rates and survival rates of bled and unbled horseshoe crabs that were tagged in the same years and geographic area. The underlying hypothesis was that bled horseshoe crabs would have a lower annual survival compared to unbled crabs, all else equal. Tagging occurs after nearshore capture by trawl. Crabs are then released within 48 hours onto spawning beaches near capture location. We fit Cormack-Jolly-Seber (CJS) capture-recapture models for the subset of data tagged and released in the coastal region of Delaware, Maryland, and Virginia from 1999 to 2017. There were 87,436 tagged animals with known sex and bleeding status: 8,449 unbled females, 20,435 bled females, 14,998 unbled males, and 33,554 bled males. Models, which were fit using RMark package in R, included covariates for sex, bleeding status, and time for apparent survival and capture probability. The best fitting models included effects on survival due to bleeding and sex. Survival also varied with time; however, year-specific survival was not estimable for many of the years. Thus, we binned years into periods defined by 2, 3, or 4 consecutive years and estimated average survival over the multiple year periods. The model with 3-year periods fit best. Contrary to the underlying hypothesis, the estimated apparent survival was higher in most time intervals for crabs that had been bled, particularly for females.

On average, females had a lower survival rate than males, but the difference was higher for unbled crabs (70% for females and 73% for males) than for bled crabs (75% for females and 76% for males). Preliminary analysis presents some evidence for a short-term reduction in survival due to bleeding based on first year returns. In contrast, annual survival considering multiple years does not indicate a reduction in survival due to bleeding. The observed pattern of higher survival for bled crabs could be due to confounding factors related to local harvest pressure on unbled crabs tagged on coastal beaches or due to the culling of biomedical catches for selection of high-condition individuals. Biomedical culling could result in biomedically tagged individuals representing a healthier subset of the overall population and thus having higher survival, all else equal. These are preliminary analyses, and we recommend continued evaluation of available tagging data.

Keywords. Horseshoe crab tagging, LAL, *Limulus* ameocyte lysate, mortality, survival rate

LAL/TAL AND ALTERNATIVE ENDOTOXIN TESTS: THEIR CHARACTERISTICS AND IMPACT ON THE HORSESHOE CRAB POPULATIONS IN THE USA AND ASIA

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Abstract. The global demand for endotoxin testing is projected to double over the next seven years. However, in light of the serious decline of the Asian horseshoe crab species, and regulated harvesting restrictions on the American horseshoe crab, it is unlikely that LAL and TAL alone can meet the markets demand. Viable alternative animal-free endotoxin tests are available, such as recombinant Factor C (rFC) assays. However, misinformation on their efficacy and use has kept them on the fringe of the endotoxin test market and it is only recently that pharmaceutical industry and regulatory authorities have started to implement rFC. Although the pharmaceutical and medical device industries reliance on the horseshoe crab for endotoxin testing does not pose the greatest threat to the species survival, they are the only user groups with the power to transcend geographic boundaries, government malaise, linguistics, social and cultural indifference. For these industries have the ability through supply chain management, to augment their endotoxin test requirements with alternatives, and in doing so, put an end to the use of TAL, and reduce the harvesting pressure on LAL production. In this paper we will discuss the status and threats posed to the world's four extant horseshoe crab species, what conservation measures are in place and being considered. Furthermore we will review the state of the global endotoxin test market, the characteristics and misconceptions of the various endotoxin tests and the role the pharmaceutical and medical device industries can play in the survival of the world's four horseshoe crab species.

Keywords. Alternative methods, best practice, conservation, endotoxin testing, ethical supply chain, horseshoe crab, LAL, pharmacopoeia, recombinant Factor C, TAL



SECONDARY SCHOOL SESSION

中学主题报告

THE FEASIBLE METHODOLOGY OF STIMULATING THE SPAWNING AND INCUBATING THE EGGS UNDER SECONDARY SCHOOL ENVIRONMENT

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Abstract. The horseshoe crabs (*Tachypleus tridentatus*) conservation program has been launched in Sai Kung Sung Tsun Catholic School since 2016. Some previous studies showed that mating of horseshoe crabs only took place in laboratory tanks with sand collected from the site where the pair of horseshoe crabs were caught, together with the specific tidal pattern. In addition, some previous studies showed the fertilized eggs should be incubated under higher temperatures for better development. However, after consulting some fishermen in Hong Kong, our students learnt that the adult horseshoe crabs appeared in the estuarine beaches for mating after three consecutive days of heavy rain. The first hypothesis is ‘the diluted seawater due to heavy rainfall can stimulate the spawning of horseshoe crabs’. The second hypothesis is ‘horseshoe crabs can develop well under optimum temperature’. The third hypothesis is ‘horseshoe crabs can develop well in optimum density’. In 2016 summer, the adult horseshoe crabs were successfully stimulated to spawn under simulated seawater conditions in estuarine beaches after heavy rain. The adult horseshoe crabs were reared in seawater with a salinity of 30 ppt which is the normal condition for the seawater (ranging from 30 to 33 ppt in Hong Kong) in the beginning. After one month, the salinity was reduced to 18 ppt that stimulated the spawning in July 2016. The steps were repeated and spawning took place in August 2017, September 2018 and October 2018. The eggs were collected and cultivated in aerated sieves under different temperatures (22°C, 26°C, 30°C and 34°C). The result showed that the embryos developed slowly in 22 °C and showed “premature hatching” in 34°C. Besides, by adjusting the aeration bubbling system, the death of eggs due to infection can be reduced significantly. The tank showed no egg infection even in an overcrowded condition (density of 20 eggs/cm³). Further investigation is required to find out the effect of bubbling action on the hatching rate of horseshoe crabs.

Keywords. Embryonic development, horseshoe crabs, incubating, premature hatching, salinity, spawning, *Tachypleus tridentatus*

THE RELATIONSHIP BETWEEN LIVING DENSITY AND MORTALITY OF JUVENILE HORSESHOE CRABS IN CAPTIVITY

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Abstract. The horseshoe crabs (*Tachypleus tridentatus*) conservation program has been launched in Sai Kung Sung Tsun Catholic School (Secondary Section) since 2016. After consecutive successful trials, more than 20,000 juvenile horseshoe crabs developed from the 'spawning-hatching through-train processes' were reared in the school laboratory in October 2018. There was an immediate need to find an optimum density for the rearing the juvenile horseshoe crabs in order to solve the problem of overcrowding. In the investigation, three immersed sieves each of 5cm², 10cm² and 15m² in size, were placed just below the water surface of a seawater tank. A sand layer of 2cm thick was placed in each sieve. In each sieve 20 third-instar horseshoe crabs were cultivated for three months. The whole investigation was repeated three times. The results showed that the mortality rate decreased as the density of juvenile horseshoe crabs decreased. The mortality rate in the most-crowded 5cm² sieve was the highest (40%) while the mortality rate dropped to 0% in the least-crowded 15cm² sieve. Besides, molting was recorded only in the 15cm² sieve. Meanwhile, owing to over-crowding and the limited thickness of sand layer, not all the juvenile horseshoe crabs in the 5cm² and 10cm² sieves were able to burrow into the sand which was their instinctive behavior.

Further investigation is required to ascertain the effect of total surface area covered by juvenile horseshoe crabs and the thickness of sand layer on the mortality rate of the juvenile horseshoe crabs. In addition, investigation to find out the effect of thickness of sand layer on the molting of juvenile horseshoe crabs is also required.

Keywords. Captivity, density-mortality relationship, juvenile horseshoe crabs, living density, *Tachypleus tridentatus*

SUMMARY AND COUNTERMEASURE OF CAMPUS PROPAGANDA AND EDUCATION ACTIVITIES ON PROTECTION OF HORSESHOE CRABS IN BEIHAI EXPERIMENTAL SCHOOL

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Abstract. The horseshoe crab, which is a living fossil, is older than the dinosaur. However, the number of horseshoe crabs in Guangxi Beibu Gulf has decreased dramatically in just two decades. Based on the above situation, our school has carried out a variety of campus conservation activities to protect horseshoe crabs, mainly including horseshoe crabs' micro-ecological farming experience, protection of community propaganda, cultural transmission, habitat inspection and protection. The results of the campus propaganda and education activities are outstanding, a total of 190 defenders of horseshoe crabs are trained and 22 theme exchange activities of horseshoe crabs are held. "Love and Habitat" technology DV made by our students won the first prize of the National Youth Science and Technology Image Contest and 18 theme paintings won the prize in the ERDG International Youth Competition. And our school was invited to participate in the Children's Calligraphy and Painting Exhibition of the Antalya World Garden in Turkey, leading the culture of horseshoe crabs to the world. Nevertheless, on the basis of summing up the campus propaganda experience of horseshoe crabs, our school has put forward corresponding countermeasures and opinions on possible problems: such as paying more attention to the protection of horseshoe crabs' habitats, raising the protection to the legal level, jointing other government units to protect horseshoe crabs and so on.

Keywords. Conservation, Guangxi, horseshoe crab, school education

The background of the entire page is a soft-focus, misty landscape of rolling mountains. The mountains are rendered in various shades of light green, pale blue, and off-white, creating a sense of depth and atmosphere. The mist is particularly dense in the valleys and between the ridges, giving the scene a serene and ethereal quality. The overall color palette is muted and naturalistic.

THEME 4

EDUCATION AND PUBLIC AWARENESS

公众意识宣教和公众参与

LINKING HORSESHOE CRABS CONSERVATION TO THE SOCIETY - CONSERVATION EDUCATION EFFORT IN HONG KONG

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Abstract. The populations of both species of horseshoe crab *Tachypleus tridentatus* and *Carcinoscorpius rotundicauda* found in Hong Kong are facing intense conservation threats such as loss of spawning ground, habitat degradation and environmental pollution. Direct harvesting of adult horseshoe crabs is in small scale except around religious season for the set-free rituals (mercy release). Statutory measures of protecting the animals and their key habitats are crucial but are often ineffective due to various limitations. In Hong Kong, the coastal/marine nature of horseshoe crabs' habitat has raised the difficulty in protecting the animals via law and enforcement; occurrence of the horseshoe crabs in area with high economical productivity also created enormous challenges to preserve their habitats from urban development; unit manpower and enforcement resources are high due to the wide-range and long lifespan of these species. Therefore the effective conservation of the horseshoe crabs in Hong Kong is hard and demanding from the conservation community.

To achieve the urgent conservation need, Ocean Park Conservation Foundation, Hong Kong (OPCFHK) adopts an approach focusing on conservation education with the intention to reduce the conservation challenges while gaining extra support from the society. We engaged multiple targeted communities in the society via programs such as eco-tours, school based horseshoe crabs rearing program, school and public talks, population surveys, beach cleaning activities and conservationist training program. We incorporated STEAM (Science, Technology, Engineering, Art and Math) elements in the educational program for teenagers to encouraging their participation. Between 2016 and 2019, more than 100,000 students were reached by our effort. This talk will describe how OPCFHK achieves conservation target via conservation education activities and to summarise and evaluate the effectiveness of different activities.

Keywords. *Carcinoscorpius rotundicauda*, conservation education, Hong Kong, *Tachypleus tridentatus*, STEM education

CITIZEN SCIENCE-BASED POPULATION MONITORING PROGRAM HIGHLIGHTS URGENT NEEDS OF COMPREHENSIVE CONSERVATION AND MANAGEMENT FOR HORSESHOE CRABS AND THEIR CRITICAL HABITATS

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Abstract. The Beibu Gulf of Guangxi provides the most important habitats and breeding grounds for two sympatric horseshoe crabs, *Tachypleus tridentatus* and *Carcinoscopius rotundicauda*. However, long-term research on populations and habitats was lacking in this area. Therefore, local NGO BRC has launched a citizen science-based population monitoring program on horseshoe crabs of the Beibu Gulf since 2014, which has engaged citizen scientists to investigate the populations and habitats of horseshoe crabs at seven long-term monitoring sites for five years. This program is aiming to support further studies on horseshoe crabs and establish sound in-situ conservation strategy.

During five years, BRC team has obtained comprehensive data of two horseshoe crabs' juvenile densities, population structures and distributions, as well as habitat condition data of seven selected sites along the Beibu Gulf coast. The results revealed that the densities of juvenile *T. tridentatus* were higher than those of *C. rotundicauda* at six intertidal mudflats, and their densities both fluctuated across years, ranging from 0.08 individuals to 0.89 individuals per 100m². At the mangrove tidal creek site, the population density of *C. rotundicauda* was higher than that of *T. tridentatus*, which showed seasonal dynamic pattern. Meanwhile, seven key habitats of horseshoe crab were facing with different threats, and the main threats were reclamation and development, human interference, pollution and invasive species, which put huge pressures on wild populations of horseshoe crabs.

This five-year monitoring program highlights the urgent needs of comprehensive conservation and management for horseshoe crabs in the Beibu Gulf in Guangxi, and we recommend that relevant government agencies should: (1) pay more attention to horseshoe crab conservation and population management; (2) strengthen the conservation and management on key habitats to reduce major threats; (3) improve enforcement to control the illegal trade of horseshoe crab; and (4) collaborate with research institutes to promote the artificial propagation and releasing, and improve the quality of breeding habitats to restore the wild population. The results will also (5) provide science-base strategies and ideas for further public education, participation, and engagement in actions.

Keywords. Beibu Gulf, *Carcinoscopius rotundicauda*, citizen science, conservation recommendations, horseshoe crabs, population monitoring, *Tachypleus tridentatus*

ARACHNOPHILIA: THE ROLE OF CIVIL SOCIETY IN HORSESHOE CRAB CONSERVATION

Lester J.X TAN, Stephen BENG

Nature Society (Singapore)

Abstract. Nature Society (Singapore) is a dynamic civil society organization, which promotes the protection and preservation of Earth's natural ecosystems. Its Marine Conservation Group supports the NGO's advocacy and outreach functions, and delivers scientific data needed for the conservation and management of our marine and coastal resources. The group's activities are focused on limiting anthropogenic threats through citizen science and ground-up governance. In advocacy and outreach, NSS conducts the Horseshoe Crab Rescue & Research program monthly. Apart from valuable data gathering for *Carcinoscorpius rotundicauda* and *Tachypleus gigas*, the outreach value is tremendous. One of our goals for the program is for the animals to be endeared, and they have been successfully winning the hearts and minds of participants over the years. Students have been inspired to take on more research projects and the horseshoe crab is now an iconic coastal species in Singapore. In citizen science and research, NSS first noticed the invasive Charru Mussel (*Mytella strigata*) on Kranji mudflats in early 2016, alerted authorities and continued to monitor its encroachment and impact on horseshoe crabs over time. This led to an island-wide research project between NSS, NUS and NParks. Collaborative research allows NGOs and academics to react quickly to emerging issues and provide validity to conservation positions. Citizen science connects volunteers to nature, widens research scope through greater participation, and mainstreams science through multiple communication channels.

Keywords. *Carcinoscorpius rotundicauda*, citizen Science, civil Society, horseshoe crabs, invasive alien species, marine debris, *Mytella strigata*, pollution, Singapore, *Tachypleus gigas*

USING COMMUNITY-BASED CONSERVATION PROGRAMS TO PROTECT HORSESHOE CRABS AND THEIR HABITAT: BEST PRACTICES AND APPLICATIONS TO UNDERSERVED REGIONS

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Abstract. Global conservation of horseshoe crab species is linked to preservation of essential habitat and management practices that minimize human impact to horseshoe crab populations. In the U.S., traditional management for the American horseshoe crab (*Limulus polyphemus*) has been directed by government agencies; however, this type of regulatory action is funding-dependent, and habitat protection is limited to state or federal lands. During the late 1990s, the Ecological Research & Development Group (ERDG) introduced two programs, Just flip ‘em![™] and Backyard Stewardship[™], as alternative, cost-effective strategies to reduce horseshoe crab mortality and preserve spawning habitat in the Delaware Bay region of the mid-Atlantic, U.S. These voluntary programs shifted the primary responsibility for natural resource stewardship to the private sector, encouraging members of the public to flip horseshoe crabs stranded upside down on spawning beaches and enabling local residents to define these shared beach habitats as community-based horseshoe crab sanctuaries. Horseshoe crab survey data, collected in the Delaware Bay region during the last two decades, illustrate the effectiveness of these community-based conservation programs to support a viable spawning population on privately owned lands, while also increasing public education and engagement in conservation. In regions where regulatory management is minimal or absent, community-based conservation programs often present the only effective strategies to preserve horseshoe crabs or other vulnerable species and their habitats. Interest and activism generated by community-based conservation measures can, in turn, influence allocation of resources for regulatory action and oversight. By adapting best practices of community-based conservation to specific needs of individual communities and ecologically distinct horseshoe crab populations, these initiatives have the potential to change relationships between communities and their natural resources, enhancing horseshoe crab conservation in the U.S. with transferability to habitats worldwide.

Keywords. Community sanctuary, *Limulus polyphemus*, public engagement, spawning habitat, stewardship

STATUS OF HORSESHOE CRABS RESEARCH IN MYANMAR: PRELIMINARY FIELD SURVEY IN MYANMAR CITIZEN SCIENCE APPROACH

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Abstract. Myanmar as a coastal country of the Bay of Bengal is fully aware of the transboundary effects on the health of the coastal and marine environment. The occurrence of two horseshoe crabs, *Carcinoscorpius roundicauda* and *Tachypleus gigas* in Myeik, Tanintharyi Region was studied based on interview surveys undertaken from January 2019 and onward. The objective of this study was to gather basic data on survey interviews with fishermen to determine the abundance and distribution of *Tachypleus gigas* and *Carcinoscorpius roundicauda* in some coastal areas. During the survey, questionnaire was used to interview 15 fishermen from all sites about sightings and catches of horseshoe crabs from in-shore waters, to provide information on when and where horseshoe crabs may be found. According to interview survey results, the lower number of horseshoe crabs *Carcinoscorpius roundicauda* (23%) in mangrove and higher number of *Tachypleus gigas* (77%) at landing sites of Kyun-su and Ma-san-pa villages were caught in Myeik Archipelago. According to all respondents' statement, fishermen catch horseshoe crabs for earning income, so now the market price is 3-5USD per horseshoe crab. The population of horseshoe crabs is declining (30%) within 3 years (2016-2019) due to daily harvest. Tanintharyi division has 801 islands so ecotourism is more developed than other divisions in Myanmar and especially most visitors treat horseshoe crabs as food, so there is no little knowledge or public awareness on horseshoe crabs in the Myeik environs. The present study was the preliminary interview survey on citizen science of horseshoe crabs. Thus there is a need to use further effective methods for assessing the status of population on horseshoe crabs and their level of threat in other areas of Myanmar.

Keywords. Abundance, landing site, Mangrove horseshoe crab, questionnaire

YOU CAN OBSERVE A LOT BY JUST WATCHING: A TRIBUTE TO CARL SHUSTER'S CONTRIBUTIONS TO HORSESHOE CRAB BIOLOGY, CONSERVATION AND EDUCATION

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Abstract. Dr. Carl N. Shuster Jr. (b. 1919) has been a seminal leader in the horseshoe crab community for seven decades. His M.S. thesis (1948) on the horseshoe crab digestive system, and his Ph.D. thesis (1955) on geographic variation within the American horseshoe crab (*Limulus polyphemus*), laid the groundwork for his long and distinguished career in academia, governmental agencies, and consulting. Carl's many papers and book chapters, including several highly-cited review articles, are required reading for everyone in the field. A remarkably talented artist, Carl's detailed line drawings of horseshoe crab anatomy have been reproduced countless times. Perhaps most significantly, Carl's infectious enthusiasm for horseshoe crabs and his emphasis that one should make careful observations of the crabs in their natural environment have profoundly influenced legions of young scientists. The personal contacts that Carl made in the 1980's with Dr. Koichi Sekiguchi and his colleagues from Japan helped to set the stage for today's International collaborations that, in turn, led to the establishment of the IUCN Horseshoe Crab Specialist Group in 2012. Carl has been a tireless advocate for scientifically based horseshoe crab conservation strategies. He personifies the role of professional scientists in the arena of public education, and the growing appreciation for horseshoe crabs through programs such as Green Eggs and Sand is a tribute to his efforts.

Keywords. Conservation, education, IUCN, *Limulus*, Shuster

CONSERVATION HISTORY OF HORSESHOE CRAB THROUGH SAND ANIMATION AND LACQUER PAINTING

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Abstract. Living in harmony with Nature has been an integral part of Indian culture. This has been abundantly reflected in a variety of traditional practices, religious beliefs, rituals, folklore, arts and crafts, and in the daily lives of the Indian people from time immemorial. Historically, the protection of nature and wildlife was an ardent article of faith, reflected in the daily lives of people. As I belongs to coastal village *Dublagadi-Bahabalpur estuary* and spent my childhood, I used to see thousands of horseshoe crabs coming in pair popularly known as “*Ramlikhan Kankada*”. Ramlikhan, the word is related to Indian myth but at present their population is in danger and after my involvement with Association for Biodiversity Conservation & Research (ABC), we have been trying to initiate socio artistic activities to protect this valuable aquatic creature through Lacquer Art medium. In the course of its conservation programme we had also executed series of laquer painting activities to the school students and young mass along the coastal villages in Odisha on the theme of “Know Me & Save Me” with the aim to sensitize young generation community. I am also doing sand animation on different platform like meeting and workshop to raise public and community awareness.

Keywords. Conservation, lacquer painting, Ramlikhan, Sand animation

MORPHOLOGICAL CHARACTERS FOR HORSESHOE CRAB IDENTIFICATION: ARE THEY PRACTICAL FOR VOLUNTEERS IN MONITORING PROGRAM?

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Abstract. Main morphological characters are one of the ways that can be used in practical species identification. Population size of horseshoe crabs in Indonesia is lacking, and one method to obtain it can be done through voluntary monitoring program. East Balikpapan coastal is one of the locations where *Tachypleus tridentatus* and *T. gigas* are present. These two species for ordinary people are morphologically look the same. This study was to determine the morphometric characters for practical identification of *T. tridentatus* in voluntary monitoring program. In this study two phenotype forms of tiny spines were found in posterior of opisthosoma. 5.29% of the total samples had one tiny spine and 84.71% of the samples had three tiny spines. In most cases, three spines in posterior of opisthosoma have been used in practical identification of *T. tridentatus*. However, analysis of blood samples collected from specimens with one spine and three spine in posterior of opisthosoma using CO1 gene sequence and compared with the database in NCBI showed that the samples are all *T. tridentatus*. This study confirmed that number of tiny spine in posterior of opisthosoma could not be used as morphological characters for *T. tridentatus* identification. Yet, triangular cross section of telson and relatively much small spines on opisthosoma are seems to be good characters for voluntary monitoring program. The later character is useful to distinguish between *T. tridentatus* and *T. gigas* for the monitoring program.

Keywords. Eastern Balikpapan, practical identification, small spines on opisthosoma, *Tachypleus tridentatus*

CONSUME OR BE CONSUMED: COMMUNITY AND THEIR SUSTAINABLE PRACTICES

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Abstract. Before horseshoe crabs became famous in Malaysia from published materials onset year 2006, these arthropods were already on dinner plates of local fishermen and neighbouring communities. Disparity for horseshoe crab consumption was observed between East, West and South Peninsular Malaysia. While folks in Balok, Pahang (East Peninsular Malaysia) focused on *Tachypleus gigas* for its larger size and reduced post-consumption hangovers, folks in Melaka (West Peninsular Malaysia) were consuming both *Carcinoscorpius rotundicauda* and *T. gigas* just like the practice in Pendas, Johor (South Peninsular Malaysia). Owing to different means for meal preparations, traditional practices have allowed fishermen to divide the animal into different categories on basis of ‘intoxication’, chances for being gravid and, morphology difference. Adopting dissimilar approach for community education and awareness, folks in Balok, Pahang cooperated by properly docking their boats and clearing discarded nets at Balok River shore. Folks at Pendas, Johor controlled littering in their village, particularly in the estuary section after made famous by movie production companies. A standout fisher folk known as ‘horseshoe crab father’ taught local folks and their children in Melaka about horseshoe crab biology after combining scientific literature with personal experience. The incorporation of local knowledge with fisheries practices particularly towards horseshoe crab catch are promoting communal-awareness while also actively gathering followers. These followers are then, participating in daily routines that revolve with ecosystem care for the future. With much said and done, the theme ‘consume or be consumed’ reflects on horseshoe crab (*C. rotundicauda* and *T. gigas*) safe-harvest practices, an anticipation opposing their total wipe-out from occurring environments. Safeguarding measures reflect on diligent cooperation by community with researcher heed that are in line with Sustainable Development Goal 12 and 14 of United Nations Development Program, the wakeup call to deter sixth extinction-reality in Malaysia.

Keywords. Citizen science, estuary, fishermen, horseshoe crab, livelihood, resilience

LESSONS FROM THE PUBLIC EDUCATIONS ON HORSESHOE CRAB CONSERVATIONS THROUGH WETLAND SOCIETY PLATFORMS ACROSS TWO SIDES OF THE TAIWAN STRAIT

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Abstract. During recent years, the Chinese native horseshoe crab (*Tachypleus tridentatus*), a coastal living fossil species with excellent medical values, is facing a serious crisis of individual survival, population decline and species endanger, due to destruction of their habitats, indiscriminate killing, environmental pollution, and the demand for a large number of blood stasis preparations. In order to facilitate joint efforts to protect the wetlands across the Taiwan Straits, the Quanzhou Wetland Society signed a memorandum of understanding on development in 2013 with the Taiwan Wetland Society. One of the cooperative goals is to conserve precious and endangered wildlife shared by the two sides of the strait such as Chinese Horseshoe Crab. Since then, we have conducted several rounds of public educations and activities to promote awareness of this horseshoe crab conservations. In this talk, I will share some of lessons we learnt from the public educations on this endangered species through the wetland society platforms across two sides of the strait. I will focus on (1) How to design interesting programs to promote working together to protect horseshoe crabs based on children's cognitive characteristics and hobbies, including horseshoe crab kite drawing DIY, horseshoe crab jigsaw puzzle or paper-cutting game, horseshoe crab clay DIY, small talent speech contests on protecting horseshoe crab environment, marine culture children's cultural performances, horseshoe crab ecology lectures, and horseshoe crab knowledge guessing games, etc.; (2) How to conduct proper activities to promote social responsibility, initiation ability and positivity for horseshoe crab conservation according to adolescent curiosity and knowledge, including participation in the production and distribution of posters and leaflets, popular science lectures, participation in various forms of environmental protection exercises and volunteer services, etc. In addition, the construction of ecological civilization is deeply rooted in China and the awareness of environmental protection among scholars and ordinary citizens in various fields is improved rapidly.

The citizen in the coastal areas are gradually awakening to the need for protection of this endangered species, so the wetland societies worked together and published a joint appeal of more than 200 scholars and social leaders across the Taiwan Straits for “No sale, No consuming of Horseshoe Crabs” in the Fujian Daily before the QiXi Holiday of 2017. We also promote cooperation on organizing public education activities among related associations and organizations of mainland China, Taiwan, and Hong Kong , such as the Taiwan Wetland Society, the Taiwan Research Institute Biodiversity Research Center, the Taiwan Chiayi County Environmental Protection Bureau, the Taiwan Chiayi County Ecological Conservation Association, the Taiwan Xinyi Elementary School, the Hong Kong Wetland Park, the Hong Kong Ocean Park, and the Hong Kong Chenjie Memorial High School. All of them participated in the Qixi Festival for protecting horseshoe crabs. Finally, we held a workshop and issue a declaration to integrate research, education, enterprise, domestic and foreign NGOs for the conservation of the endangered horseshoe crabs in China, which effectively increased the powerfulness of protection.

THE POWER OF PUBLIC: WHAT WE CAN LEARN FROM THE HORSESHOE CRAB CONSERVATION ACTIONS IN THE BEIBU GULF OF GUANGXI, CHINA

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Abstract. Nature conservation is an important social undertaking, where the public can be its foundation and supporting force. As a local non-governmental organization, BRC proposed this conservation model based on public participation and multi-stakeholder engagement that adapted from a five-year horseshoe crab conservation program in the Beibu Gulf of Guangxi, China. In this model, BRC opens an NGO based platform to engage public to participate, government agencies to lead, research institutes to collaborate, media to deliver voices, enterprises and other parties to act, which has collectively promoting the conservation of horseshoe crabs in the Beibu Gulf.

Since 2014, BRC has obtained population and habitat data of two horseshoe crabs, *Tachypleus tridentatus* and *Carcinoscopius rotundicauda*, by implementing a long-term citizen science-based monitoring program at 7 critical important horseshoe crab habitats along the Beibu Gulf coast of Guangxi, which becomes the first-handed data for us to understand the living status of horseshoe crab in the area. Meanwhile BRC empowered six local voluntary conservation teams across the whole coast, who have become the major civil society crowd powers to influence local conservation of horseshoe crabs and coastal environment. The power of crowds has become the fundamental social force to elevate the horseshoe crab conservation to a higher level and leverage more stakeholders and social resources to join. The “No Horseshoe Crab Consumption Campaign” has also become the first multi-stakeholder engagement conservation action to tackle the over-exploitation issue of Chinese horseshoe crab, which attracts many governmental agencies, media, enterprises and the public to participate and take actions, and lead to over 110 “Horseshoe Crab Friendly” seafood restaurants in Beihai, one of three major coastal cities of Guangxi. This model reveals promising potentials and great power of the public in conservation. We are hoping it can lead us to more effective conservation of horseshoe crabs in larger area and be promoted to serve for the benefits of more living organisms and our blue planet.

Keywords. Beibu Gulf, horseshoe crab, “No Horseshoe Crab Consumption Campaign”, public participation,



THEME 5
HORSESHOE CRAB CONSERVATION
CHALLENGES

蟹栖息地保护面临的挑战

OYSTER RUBBLE REDUCES THE HOME RANGE AND DISTURBS THE FORAGING BEHAVIOUR IN *TACHYPLEUS TRIDENTATUS*

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Abstract. The home range of the juvenile Chinese horseshoe crab *Tachypleus tridentatus* was determined at Ha Pak Nai, where the highest density of this animal was found in Hong Kong. The movement pattern of juvenile *T. tridentatus* was tracked using the coloured plastic tag (CPT) during low tides in the summer of 2018. The survey lasted for 2 months with 130 individuals being tagged. The mean recovery rate of tagged individuals was 44%. The positional fix for each recaptured individual was recorded during each survey and the size of the home range was estimated using the minimum convex polygon (MCP) of the ArcGIS. Ha Pak Nai was divided into regions with high (≥ 1 oyster shell m^{-1}) or low density of oyster rubble (< 1 oyster shell m^{-1}). Eighty-eight percentage of the juvenile horseshoe crabs preferred utilizing the region with less oyster rubble and the home range was also statistically larger (median: 55.75 m^2) as compared with the area with more oyster rubbles (median: 88.9 m^2). The juveniles were also found to prefer foraging during sunny days than drizzling days, and on the sediment covered with a thin layer of water than deeper puddles. Conservation issues related to the findings are discussed.

Keywords. Ha Pak Nai, home range, oyster rubble, *Tachypleus tridentatus*

REINTRODUCTION OF *TACHYPLEUS GIGAS* IN SETIU LAGOON, EAST COAST OF PENINSULAR MALAYSIA

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Abstract. *Tachypleus gigas* population is decreasing in Peninsular Malaysia especially due to huge trade of frozen female adults to Thailand. As part of conservation efforts, Horseshoe Crab Research Group of Universiti Malaysia Terengganu has reintroduced the species into Setiu Lagoon, Terengganu. Community engagement was carried out prior to horseshoe crab release, and ten fishermen were selected to become pioneer “Setiu HSC-Crews”. A total of 662 adult HSCs (307 males, 355 females) were released into the lagoon at various locations between July 2017 and October 2018. Twelve percent of these released crabs were recaptured and more nursery grounds were identified within the Setiu Lagoon area, indicating some positive impacts of this reintroduction effort. Monitoring is still in progress.

Keywords. Conservation, horseshoe crab, *Tachypleus gigas*

MANAGEMENT ON CLAM DIGGING ACTIVITY AND AT HORSESHOE CRAB HOTSPOTS IN HONG KONG

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Abstract. Clam digging is a common recreational coastal activity in Hong Kong. During the summer holiday, hundreds of tourists could be found digging clams on the mudflat. This unregulated recreational activity causes a serious concern towards the environment. Some of the clam digging hotspots in Hong Kong (Pak Nai, Shui Hau and Tung Chung Bay) are critical spawning grounds and nursery sites for the Chinese horseshoe crab *Tachypleus tridentatus* and the mangrove horseshoe crab *Carcinoscorpius rotundicauda*. Due to the habitat overlapping with clam species and horseshoe crab, clam digging activity is one of the considerable threats to horseshoe crab populations in Hong Kong. Visitors may accidentally trample on or disturb juvenile horseshoe crabs during clam digging. Nowadays, horseshoe crab populations are rapidly declining around the world, so protecting these sites are vital to ensure the survival of horseshoe crab population. WWF Hong Kong (WWF-HK) has been calling for 30% of Hong Kong water to be designated marine protected area by 2030. As Shui Hau is one of the seven identified conservation priority sites in Hong Kong, WWF-HK is now implementing a marine conservation project in Shui Hau aiming to demonstrate better sustainable habitat management in terms of striving the balance between the clam digging activity and environmental conservation. We have conducted citizen science ecological surveys for clams and walk through surveys for horseshoe crabs to collect population and distribution information. Based on the results from surveys and literature review, we produced Code of Conduct for clam digging activities and clam gauges to pose minimal impacts to habitats and biodiversity. After that, we also established horseshoe crab mapping and identified ecologically sensitive areas, eventually target to advocate the government to implement conservation measures, which may include set up of no-entry zone, conservation management zoning or seasonal closure during breeding season.

Keywords. Clam digging, habitat overlap, Hong Kong, horseshoe crabs, protected area management, public engagement, Shui Hau, sustainable use of marine resource

THREATS TO THE MARINE BIODIVERSITY IN THE SOUTH CHINA COASTAL WATERS: IMPLICATIONS FOR THE ASIAN HORSESHOE CRAB CONSERVATION

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Abstract. Coastal waters are among the most productive habitats in the widespread ocean, providing mankind with multiple ecosystem services. The coastal habitats, however, also suffer from escalating human stresses which have threatened the marine biodiversity and ecosystems. The lack of an understanding of the threats at mesoscopic scales may be the information gap in biodiversity and ecosystem conservation, and the gap compromises the efficacy prioritization of management efforts in conservation. We examined the threatened species information for those found within 30m water depth in the South China waters based on the IUCN Red List by summarizing their taxon, threatened level, and threats. While Chordata was the primary group accounting for more than 70% of the threatened species identified, most marine invertebrates (36.8%), including Asian horseshoe crabs, were underrepresented or misrepresented to be Data-Deficient. Among the eleven identified categories of threats, biological resource use, pollution, and residential and commercial development were claimed to be the major threats to the marine biodiversity in South China coastal waters, in which these threats should be concurrently imposed on those listed as Data-Deficient species. Based on the findings, we suggest implementing the following conservation management initiatives for maintaining the stocks of Asian horseshoe crabs in South China coastal habitats: 1) Coastal waters and intertidal zones should be regarded as a management unit for the comprehensive conservation planning; 2) Evaluate, mitigate or avoid, and implement long-term monitoring on humans' coastal activities for habitat alterations and biological resource overexploitation to reduce the potential risks imposed on marine biodiversity in the region; 3) Asian horseshoe crabs, in addition to China's national protected Indo-Pacific humpback dolphins, should serve as the surrogates for proactively building a protected area network to cover the essential habitats and ensure the connectivity throughout coastal habitats.

Keywords. Estuary, habitat loss, intertidal zone, overexploitation, surrogate species

KEY ISSUES FOR CHINESE HORSESHOE CRAB, *TACHYPLEUS TRIDENTATUS* CONSERVATION AND MANAGEMENT IN CHINA

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Abstract. The Chinese horseshoe crab, *Tachypleus tridentatus* was historically distributed along the coastline of South China Sea. However, the population has widely been observed to experience apparent decline since the 1980s, particularly in the southeastern part. There are examples of places where *T. tridentatus* were known to abundantly occur in 20 years ago, but is now extirpated. Four key issues, which believed to impede future conservation initiatives for *T. tridentatus* population, have been identified: (1) Poor conservation awareness and enforcement power of the government, and consider horseshoe crab conservation efforts are of low importance; (2) Lack of public awareness, particularly among the coastal and fishing communities on horseshoe crab conservation. Economic benefits derived from fisheries resources seem to outcompete other factors when tackling the environmental issues; (3) Huge research gaps and insufficient baseline data of horseshoe crabs have severely affected the government's decision-making process; (4) Limited and scattered efforts by non-governmental organizations and media to gain public attention on horseshoe crab conservation issues. The joint efforts of various parties, including the government, public engagement, researchers, non-governmental organizations and media influences can enhance the conservation and sustainable use of horseshoe crab resources in China.

Keywords. China, conservation, key issues, management, priorities, *Tachypleus tridentatus*



POSTER PRESENTATIONS

墙报

POPULATION GENETIC STRUCTURE OF JUVENILE CHINESE HORSESHOE CRABS IN HONG KONG

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Abstract. Populations of Chinese horseshoe crabs (*Tachypleus tridentatus*) are declining in Hong Kong primarily due to the destruction of spawning and nursery shores through coastal reclamation and increasing urban encroachment. Understanding their phylogeographic pattern is thus important to protect the remaining sites where juvenile horseshoe crabs are found. The genetic diversity of juvenile *T. tridentatus* was investigated by sequencing a mitochondrial gene region from 33 samples collected from six locations in Hong Kong. Eight haplotypes were identified, and the relationship among haplotypes was inferred using Network Analysis. Results indicated that *T. tridentatus* has a high level of endemism, and the population in Deep Bay is differentiated from that on the northwestern coast of Lantau Island. Horseshoe crabs at Shui Hau on the southern coast of Lantau Island are also geographically isolated. These genetic patterns may indicate the limited dispersal ability of Chinese horseshoe crabs in Hong Kong. A very low haplotype diversity observed on the northwestern coast of Lantau Island implies the problem of a population bottleneck, which may eventually result in local extirpation if an effective conservation plan is not put in place.

Keywords. Conservation management, Juvenile horseshoe crab, Mitochondrial DNA, Population genetic structure, *Tachypleus tridentatus*

POTENTIAL HABITAT REQUIREMENTS OF HORSESHOE CRABS *CARCINOSCORPIUS ROTUNDICAUDA* AND *TACHYPLEUS GIGAS* IN NORTH-EAST COAST, INDIA

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Abstract. In spite of being a marine living resource of immense economic and ecological importance, the population status and habitat requirements of Indian horseshoe crabs are not adequately known in India. The beach-dynamics and extensive mudflat in North-east coast are reportedly sheltered for sympatric occurrences of *Tachypleus gigas* and *Carcinoscorpius rotundicauda* in India. However, they are disjunct in distribution and their populations are confined to isolated habitat of selected rivers mouths and costal mangrove forests. Most of the earlier studies on selective sites in the east coast reported ~4000 individuals/season with densities higher at beaches near river mouths, ranging from 60 to 100 individuals/km/season in Sundarbans, Southern West Bengal, northern and central Odisha coast; and 15–20 individuals/km/season in southern Odisha. The density was as low as 10 individuals/km along the rest of the coasts. Currently, the distribution of horseshoe crabs is restricted to smaller stretches near estuaries, mangrove and mudflats along the east coast are potential breeding ground for horseshoe crabs. Although there is documentation on the population all along the coast, there is no clear demarcation of sites of horseshoe crab habitats with location-specific data on their population. Based on abundance data from past and current surveys along the northeast coast of India, we have categorised and mapped the habitat as low (<50), medium ($\geq 51 < 100$), high ($\geq 101 < 200$) and significant (≥ 201) habitat for *T. gigas* and *C. rotundicauda* in India. A total of 69 coastal stretches have been identified as horseshoe crab habitats along the north coast of India, in which four potential habitats of *C. rotundicauda* and eight for *T. gigas* have been identified as the most significant habitats for distribution and their occurrences. This habitat requirement information can be interpreted by policy makers for formulating a future management and conservation plan for the Indian horseshoe crabs.

Keywords. Bay of Bengal, *Carcinoscorpius rotundicauda*, conservation, habitat mapping, India, *Tachypleus gigas*

PREDICTING THE POTENTIAL DISTRIBUTION OF HORSESHOE CRABS (*TACHYPLEUS GIGAS*) ALONG ODISHA COASTLINE INCORPORATING CLIMATE CHANGE BY USING MAXIMUM ENTROPY MODELING

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Abstract. Delineating a species range is challenging because many factors interact at multiple spatial scales to affect a species distribution. Species distributions models (SDM) can be used to identify factors most associated with a species presence and, therefore, potentially define a range edge. We evaluated the utility of one popular SDM approach, maximum entropy models (e.g., Maxent) for determining the range edge for the horseshoe crab spawning habitat. A total of 61 sighting points was used in the Maxent modelling. The area under curve (AUC) value was higher than 0.987. Using the mapping and analysis software ArcGIS, we constructed and validated SDMs with 19 environmental predictor variables. The potential distribution of horseshoe crabs was predicted by Maxent model for present and the upcoming hypothetical (2028) climatic scenario. The approach used in this study is considered useful in predicting the potential distribution of *T. gigas* species, and can be an effective tool in conservation planning. The results show that there is a significant impact under future bioclimatic scenarios on the potential distribution of *T. gigas* along Odisha coastline in India.

Keywords. Horseshoe crabs, Maxent, SDM, *Tachypleus gigas*

MORPHOLOGICAL ABNORMALITIES IN INDIAN HORSESHOE CRABS, *TACHYPLEUS GIGAS*

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Abstract. Out of the four extant species of horseshoe crabs (HSCs) in the world, *Tachypleus gigas* (Müller, 1785) has the broadest distribution in Thailand, Vietnam, Indonesia, Philippines, Malaysia, Singapore and North-east Coast of India. Abnormalities are the variations from normal structure and function of different organs of organisms. Abnormalities are found in all stages of development, i.e., during embryonic stage, larval stage and adulthood. The embryonic abnormalities include segment-defective embryos, double embryos and embryos with abnormal eyes. The larvae have abnormalities mainly in the *prosoma*, *opisthosoma* and mostly in the telson. Consequently, similar abnormalities are found in the *prosoma*, *opisthosoma* and telson of the adult HSCs. During the survey across the various estuaries and beaches of North-east Coast of India, we found the majority of specimens showed abnormalities in the prosomal, opisthosomal and telson region. The abnormal telson includes bent ends, shortened or damaged telsons with varied diameter. The prosomal abnormalities include deformities and/or injured parts in the frontal ridge of the prosoma and lateral spinal endings. The opisthosomal abnormalities were seen mostly on the lateral spine regions and near the hinge region of the HSCs. Depending on the resemblance of the abnormalities they have been innovatively described as L shaped, V shaped, W shaped, U shaped and C shaped. Gross abnormalities were observed in the opisthosoma and telson regions. Morphometric abnormalities in HSCs are poorly studied. The purpose of the present investigation was to identify valuable discriminating characters of both male and female HSCs. A number of factors including predation, loss of natural habitat and ever increasing anthropological activity are the causes of abnormalities in the Indian Horseshoe crabs. However, the exact causes yet remain wanting. So a detailed study is required whether the abnormalities in the morphology of the HSCs are due to genetic variations or environmental effects, and its importance in evolution.

Keywords. Abnormalities, environmental effects, evolution, genetic variations, *Tachypleus gigas*

DOES INDONESIAN *TACHYPLEUS TRIDENTATUS* IS THE SAME POPULATION STOCK AS OTHER ASIAN *T. TRIDENTATUS* STOCK? : A SIMPLE GENETIC APPROACH

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Abstract. Intensive previous studies on Asian horseshoe crab population stock have increased in East Asia such as Japan, China, and Korea, while in Indonesia such studies are still limited, especially for *T. tridentatus*. Eastern Balikpapan coastal waters as one of the locations known for the existence of *T. tridentatus* do not have further information about the species. This paper aims to investigate the Indonesian and Asian *T. tridentatus* population stock. Four blood samples from Indonesian *T. tridentatus* collected from Balikpapan were analysed molecularly using CO1 gene fragment with PCR technique. This study attempted to compare the result of genetic information of Indonesian *T. tridentatus* with that of Asian *T. tridentatus*. The genetic information of Asian *T. tridentatus* was obtained from NCBI using access code EF460846.1 (Malaysia), FJ860267.1 (China), JQ739210.1 (Korea), U09387.1 (Japan). Results showed that *T. tridentatus* in Balikpapan has a closer relationship with population in Malaysia than that in Korea, China and Japan. Although these five populations as a whole have a close relationship, there is one specific different nucleotide base site (in site 475th). This suggested that populations of *T. tridentatus* in Balikpapan and Malaysia can be distinguished from populations of *T. tridentatus* in East Asia (China, Korea, and Japan). The specific nucleotide site is a substitution mutation. *T. tridentatus* in Balikpapan, however, can also be distinguished from *T. tridentatus* from Malaysia at the 111th and 533th nucleotide base sites. Thus the population stock of *T. tridentatus* in Indonesia and Malaysia are different from *T. tridentatus* in East Asia.

Keywords. East Asia, Eastern Balikpapan, nucleotide base, population stock, *Tachypleus tridentatus*

SPAWNING SITE SELECTION OF THE ENDANGERED HORSESHOE CRAB *TACHYPLEUS TRIDENTATUS* AT TSUYAZAKI COVE IN FUKUOKA, JAPAN

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Abstract. Spawning site selection of *Tachypleus tridentatus* in relation to elevation, gradient and grain size were examined using a topological model combined with field investigation results of spawning frequency and particle size analysis in Tsuyazaki, Fukuoka, Japan. The most abundant spawning activities occurred in the range from 0.61 meters and 5.97 degrees to 0.89 meters and 8.26 degrees, suggesting that *T. tridentatus* might prefer moderate shaped mounds regarding elevation and gradient as their spawning sites. However, some spawning activities were found at a slope with lower elevation, and a lesser gradual area where the original shape of spawning ground was destroyed due to construction works suggesting that the species lay their eggs reluctantly at that site. The medium diameter and size-sorting of sediments ranged from 0.19 to 0.38mm and 0.59 to 1.06 phi respectively. More spawning activities were found at the sites in which the medium diameter and size-sorting of sediments from 0.19 to 0.22 mm and 0.59 to 0.73 phi respectively. Our results indicated that *T. Tridentatus* prefers the areas where the original mound shape was relatively well-preserved offering suitable elevation, gradient and grain size for their spawning. Possible reasons for these results are discussed, and management implications are suggested.

Keywords. Coastal management, endangered species, habitat restoration, habitat selection

EFFECTS OF MORPHOLOGICAL CHARACTERS ON BODY WEIGHT OF ARTIFICIALLY BRED THE FIRST INSTARS OF HORSESHOE CRABS *TACHYPLEUS TRIDENTATUS*

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Abstract. Artificial release proliferation is not only an important way to recover horseshoe crab, but also protect biodiversity and to promote sustainable development of the fishery. We conducted a preliminary study on artificial breeding technology of horseshoe crabs *Tachypleus tridentatus* in Zhanjiang, Guangdong Province from May to September 2018. During this period, 9 pairs of *T. tridentatus* laid 87000 eggs in total, with a fertilization rate of 56% to 68%. After 45 days, the embryo hatches into the first instars of the juvenile *T. tridentatus*, the hatching rate reaches over 90%, and a total of 48,000 *T. tridentatus* larvae were obtained. In order to study the correlation between phenotypic traits and body weight, five quantitative traits (carapace length X_1 , carapace width X_2 , carapace height X_3 , abdomen length X_4 , and body weight Y) of the first instars of *T. tridentatus* were measured and analyzed from 500 randomly collected individuals by correlation analysis, multiple regression analysis, path analysis and determination coefficient analysis. The results showed that there were significant correlations ($P < 0.01$) among the five measured traits. T Path coefficients of various morphological traits for body mass reached a very significant level ($P < 0.01$), and the largest path coefficient was the width of the head and chest armor, which indicated that the width of the head and chest armor had the greatest direct effect on body mass. The trend of determination coefficient analysis was comparable to that of path analysis. The elevated total determination coefficient ($\Sigma d = 0.863$) between phenotypic features and body weight indicated that the selected traits were practically useful. The multiple regression equation for estimation of body weight was $Y = -0.228 + 0.248X_1 + 0.073X_2 + 0.333X_3 + 0.073X_4$. Conclusion: The carapace width of the first instars of *T. tridentatus* has the greatest influence on body mass. Therefore, the width of cephalothorax should be the primary choice for germ plasm evaluation and seedling screening.

Keywords. Artificial propagation, correlation analysis, morphological trait, path analysis, *Tachypleus tridentatus*

SOCIODEMOGRAPHIC DRIVERS AND PUBLIC PERCEPTIONS OF CONSUMPTION AND CONSERVATION OF ASIAN HORSESHOE CRABS IN NORTHERN BEIBU GULF, CHINA

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Abstract. Overharvesting Asian horseshoe crabs for food is a primary threat to their populations. In the present study, 451 residents were interviewed from the northern Beibu Gulf, the coastal area which is believed to accommodate the highest density of the Chinese horseshoe crab *Tachypleus tridentatus* worldwide, to obtain baseline information on their consumption behaviour and underlying drivers. Based on the survey data, it was estimated that a mean individual consumption rate of ten meals per year. Nearly half of the total respondents (49.7%) claimed that they had previously eaten at least two horseshoe crab meals. Among the six occupation categories (managers, professionals, workers, environmental-related, fishers and farmers, and students), fishers and farmers were identified as the group which had eaten a significantly higher number of horseshoe crab meals, consumed them more frequently, and had a greater intention of eating them than that of students. Participants' age, education level and salary were also the important sociodemographic variables which could relate to their consumption behaviour. More than one-third of respondents decided to consume horseshoe crabs because they perceived them as tasty "seafood" and valuable ingredients of traditional Chinese medicine. Eighty-three percent of participants reported an overall decrease in the number of horseshoe crabs in the region. While the community was supportive towards horseshoe crab conservation, their ecological knowledge of horseshoe crabs was limited. The findings may be useful for providing insights to merge the gap between scientific data and management planning for the horseshoe crab population in the region. Conservation management initiatives, such as the involvement of fishers in monitoring and enforcement, developing a reporting system for landings and bycatch, and implementing long-term conservation education through school curriculum could promote protection and management of the population.

Keywords. Disturbance, estuary, intertidal, invertebrates, overfishing, public perception

THE ACUTE TOXICITY TEST OF POTASSIUM PERMANGANATE ON *TACHYPLEUS TRIDENTATUS*

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Abstract. A total of six potassium permanganate treatments (50, 200, 350, 500, 650, 800mg/L) and a control (no potassium permanganate added) were set up with potassium permanganate and seawater for the acute potassium permanganate toxicity experiment on survival rate and virulence return of *Tachypleus tridentatu*. The mean body weight of *Tachypleus tridentatu* was about 0.025g, and the experiment was for 96h. The results showed that *Tachypleus tridentatu* was significantly different from that in the control group ($P < 0.05$), which was the survival rate of Chinese horseshoe crab seedlings in the test group with potassium permanganate concentration higher than 800mg/L for 48h. At 96h, potassium permanganate mass concentration had a significant effect on the survival rate of Chinese horseshoe crab seedlings ($P < 0.05$), and there was no significant difference among the test groups with potassium permanganate concentration below 200mg/L ($P > 0.05$). At 96h, the LC50 value of potassium permanganate to the Chinese horseshoe crab seedlings was 377.973mg/L, which was the viable concentration of Chinese horseshoe crab seedlings.

Keywords. Potassium permanganate concentration, survival rate, survival time, *Tachypleus tridentatus*

ASIAN HORSESHOE CRAB CONSERVATION: KNOWLEDGE, ATTITUDES AND INTENTIONS OF LOCAL COLLEGE STUDENTS IN BEIBU GULF, CHINA

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Abstract. Community-based programs are an essential strategy in the conservation of Asian horseshoe crabs. Since the participants of conservation awareness programs in Beibu Gulf region are mostly local college students, this study aimed to gain a better understanding of the students' attitudes and behavioral intentions towards horseshoe crab conservation. The results from 391 respondents showed that the students' attitudes towards horseshoe crab conservation were generally neutral to positive (mean score of 3.68 on the 5-point Likert scale). However, they exhibited a poor understanding of basic ecological knowledge regarding horseshoe crabs (4.77 correct responses out of 11). Greater conceptual understanding of horseshoe crabs was positively correlated with scores in students' perceptions and intentions towards horseshoe crab conservation. Students who had previously joined environmental awareness campaigns had significantly greater knowledge of and support for horseshoe crabs and their conservation. Results also indicate that the students' gender and their parents' occupations influence their attitudes and intentions. This study provides a basis for recommendations of how to enhance community participation in future horseshoe crab conservation awareness programs in the region.

Keywords. Asian horseshoe crabs, behavioral intentions, Beibu Gulf, community-based conservation, social perceptions

LOCAL KNOWLEDGE-BASED STUDY ON THE STATUS OF HORSESHOE CRABS ALONG THE INDONESIAN COAST

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Abstract. There is still a dearth knowledge on the population of horseshoe crab and its ecological information in Indonesia. A questionnaire-based survey was conducted with the help of local knowledge to avoid costly sampling and monitoring programs with the aim of collecting potential population status, distribution information and finding associated threats in the 35 identified sites in Indonesia. The results from 237 interviewees showed that horseshoe crabs were found in 51 districts; 98 villages of Java, Sumatra, Kalimantan, and Sulawesi Island. The population of horseshoe crab in these districts is declining (as agreed by 70.5% interviewees) with discarding of by-catch in unsuitable habitat (27.7%) and beach litter (19.8%) topping the list of threats. Further findings also indicate that gillnetting (48.3%) and bottom trawling (42.4%) are main fishing methods. Due to the common practice of consuming adult crabs in these areas (90.0%), mass harvesting of adult horseshoe crabs was observed (29.3%). The study therefore suggests the urgent need of legislation for the protection of these crabs as well as enhancing community education and sensitization.

Keywords. Distribution, horseshoe crabs, Indonesia, local knowledge, threats,

BENTHIC MACROFAUNAL COMMUNITY AT HORSESHOE CRAB HABITAT IN EYU ISLET INTERTIDAL ZONE, XIAMEN, CHINA

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Abstract. *Benthic macrofauna* were quantitatively investigated at 3 sampling stations in Eyu Islet intertidal zone, Xiamen in July 2018 (summer), October 2018 (autumn) and January 2019 (winter). The results showed that 68, 53 and 58 species of *benthic macrofauna* were recorded in summer, autumn and winter respectively. The communities of *benthic macrofauna* in the three seasons were dominated by polychaetes, with 43, 33 and 24 species being recorded in summer, autumn and winter respectively. There was a decreasing trend for the number of polychaete species, and there was an increasing trend for the number of gastropod and bivalve species, and there was a little change for the number of crustacean species. One-way ANOVA showed that the density of *benthic macrofauna* was significantly different among the sampling stations. However, the species number, biomass, abundance index (d), evenness index (J) and diversity index (H') of *benthic macrofauna* were not significantly different among the sampling stations. One-way ANOVA showed that the densities of *Prionospio pacifica*, *Sigambra hanaokai* and *Musculista senhousia* were significantly different among the sampling stations and seasons. In quantitative sampling, no Chinese horseshoe crab, *Tachypleus tridentatus*, was found. However, in qualitative sampling, *Tachypleus tridentatus* was noted at station H. There are high sand content, number of shallow water pool between elevated sediment bumps, high density of bivalves and gastropods at station H, which provides a good habitat and food source for horseshoe crabs. There are stones for culturing oysters at sampling station M, and high clay content at sampling station L. Both such stations are thus unfavorable to the habitat of horseshoe crabs.

Keywords. *Benthic macrofauna*, community, Eyu Islet, habitat of horseshoe crab, intertidal zone

NEMATODE ASSEMBLAGES IN HORSESHOE CRAB HABITAT IN EYU ISLET, XIAMEN, CHINA

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Abstract. In China, horseshoe crab *Tachypleus tridentatus* populations have been declining sharply in recent years. Habitat destruction, environmental pollution and overfishing are the main reasons for the decline of this species, which usually inhabits the upper intertidal zone of pristine sandy beaches. In order to understand the meiofauna assemblages in horseshoe crab habitat, we began to investigate the meiofaunal assemblages in high, middle and low intertidal zones in horseshoe crab habitat of Eyu Islet in Tong'an Bay, Xiamen from October, 2018, and plan to investigate this area for four seasons. In October 2018, meiofaunal density was highest in the mid-intertidal zone, followed by high and low intertidal zones (mean density of 856, 778 and 700 ind. 10 cm⁻², respectively). Average nematode density ranged from 600 to 810 ind. 10 cm⁻². Average copepod density increased from the high intertidal zone to the low intertidal zone (16, 35 and 82 ind. 10 cm⁻² respectively). A total of 47 nematode genera were recorded, but only eight (*Daptonema*, *Dorylaimopsis*, *Metachromadora*, *Metoncholaimus*, *Neochromadora*, *Ptycholaimellus*, *Sphaerolaimus* and *Terschellingia*) occurred in all the samples. *Tripyloides* was only found in the high intertidal, whilst *Bathylaimus*, *Laimella* and *Paramesacanthion* were only found in the mid-intertidal zone and *Quadricoma*, *Vasostoma* and *Syringolaimus* only in the low intertidal zone. *Sabatieria*, a tolerant genus, had low percentage in the study area but was the dominant genus in other intertidal zones in Tong'an Bay, indicating that the study area provides better environmental quality for horseshoe crabs. ANOSIM analysis indicated that nematode assemblages of high, middle and low intertidal zones did not differ significantly from each other. The relationships of nematode assemblages and environmental factors such as temperature, salinity, sediment granulometry, chlorophyll-a content, TOC, PAHs and trace-metals will be discussed.

Keywords. China, environment factors, horseshoe crab, intertidal zone, meiofauna

BENTHIC MACROFAUNAL COMMUNITY AT HORSESHOE CRAB OR SEA TURTLE HABITATS IN THE SOUTHEAST COAST OF CHINA IN SUMMER

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Abstract. Fujian Pingtan Horseshoe Crab Special Reserve (not formally established by government) is located on Pingtan Island, Fujian coast. Gangkou Turtle Nature Reserve is located in Guangdong Huizhou coast. Horseshoe crabs were often found along Guangxi Beihai coast. In this paper, four sampling sections (each section includes three sampling stations, and each station includes five 25 cm * 25 cm * 30 cm quadrats) of sandy intertidal zone in Fujian Pingtan Horseshoe Crab Special Reserve (PA, PB, PC and PD), four sampling sections of sandy intertidal zone in Gangkou Turtle Nature Reserve (DN, DP, DQ and DV), and two sections of sandy intertidal zone along Guangxi Beihai coast (BX and BZ) were investigated for benthic macrofauna in summer 2016 or in summer 2018. The results showed that the mean values of species number, density, biomass, Shannon-Wiener diversity index (H') and richness index (d) of benthic macrofauna on sandy intertidal zone in Gangkou Turtle Nature Reserve (25 species, 90.4 ind./m², 43.71 g/m², 1.592 and 0.918 respectively) were all lower than those in Fujian Pingtan Horseshoe Crab Special Reserve (43 species, 1077.6 ind./m², 47.98 g/m², 1.848 and 1.272 respectively), and in Guangxi Beihai sandy intertidal zone (38 species, 382.9 ind./m², 101.08 g/m², 2.289 and 1.708 respectively) because the mean tidal range in Gangkou Turtle Nature Reserve was lower than that in Fujian Pingtan Horseshoe Crab Special Reserve and in Guangxi Beihai coast. The intertidal width in Gangkou Turtle Nature Reserve was narrower than those in Fujian Pingtan Horseshoe Crab Special Reserve and along Guangxi Beihai coast. The dominant species in Tannan Bay (sampling sections PA and PB) was polychaete *Amaeana trilobata*, in Shanqi Bay (sampling sections PC and PD) was gastropod *Umbonium costatum*, in Gangkou Turtle Nature Reserve were crustacean *Scopimera globose* and bivalve *Donax semigranosus*, in Guangxi Beihai sandy intertidal zone were polychaete *Ceratonereis erythraeensis* and bivalve *Cryptonema producta*. Thus, the dominant species of different sections were different in sandy intertidal zone in the southeast coast of China in summer.

Keywords. *Benthic macrofauna*, horseshoe crab, sandy intertidal zone, sea turtle, southeast coast of China

PUBLIC PARTICIPATION PROTECTION AND PRELIMINARY STUDY ON HABITAT PROTECTION MEASURES OF CHINESE HORSESHOE CRABS IN SHATIAN AREA, BEIBU GULF, CHINA

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Dugong National Nature Reserve, Hepu of the Guangxi Zhuang Autonomous Region

Abstract. Dugong National Nature Reserve, locating at Hepu of Guangxi Zhuang Autonomous Region, is in the Shatian sea area of Beibu Gulf. It is one of the habitats of Chinese horseshoe crabs (*Tachypleus tridentatus*) in China. In the past five years, Dugong National Nature Reserve and Guangxi Biodiversity Research and Conservation Association have conducted long-term surveys of Chinese horseshoe crabs' population resources in Shatian sea area to provide theoretical and biological basis for conserving Chinese horseshoe crabs and their habitats. Having collected data from interviewing fishermen and issuing questionnaires, we compared the encounter rate of Chinese horseshoe crabs five years ago with the rate of today. Basing on the survey results, we found that the population density of Chinese horseshoe crabs in Shatian sea area showed a downward trend. The main reasons for the population decline are: habitat loss and degradation, tidal flat farming, and by-catching and discarding during gill net fishing, etc. We further proposed approaches to address the threats to Chinese horseshoe crabs and their habitats, such as upgrading the Chinese horseshoe crabs' protection class, banning tidal flat farming, strengthening the law enforcement and supervision, and increasing publicity and education. Thus we carried out a lot of publicity activities, such as displaying 8 billboards, 200 posters and 30 banners, and handing out 5000 brochures and 200 buckets for collecting horseshoe crabs at fishing villages. We also implemented an incentive mechanism to encourage fishermen to put the accidentally-caught horseshoe crabs into the collection buckets and return them to the sea. Via calling back, we revealed over 70% utilization of the collection buckets, and a significantly-reduced number of by-caught and discarded Chinese horseshoe crabs.

Keywords. Beibu Gulf, horseshoe crabs, publicity activities

BIVALVE SELECTION AND CONSUMPTION BY HORSESHOE CRAB IN CAPTIVITY

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Abstract. *Tachypleus gigas* and *Carcinoscorpius rotundicauda* are two species of horseshoe crab (HSC) that were once abundant in East Coast of Peninsular Malaysia including Setiu Lagoon, Terengganu. Current decline of HSCs' population leads to reintroduction of HSC in Setiu Lagoon as one of the conservation initiatives. However, this relocation has brought concern especially among the Setiu's local fishermen community that the HSC might affect the abundance of economically important edible bivalves in that area. Feeding trials were performed to determine HSC's selection on four types of bivalve meat from Setiu which are *Scapharca cornea*, *Meretrix meretrix*, *Polymesoda erosa* and *Crassostrea iredalei*. The quantity consumed by HSC on each type was also determined. The HSCs were fed with a mix of four types of bivalves for the food preference experiment whereas single type of bivalves were used for feeding capacity experiment. Both sexes of *T. gigas* shows no preference in bivalves consumed whereas *C. rotundicauda* least preferred *S. cornea* (consumed less than 0.20 individual per day) compared to other bivalves (consumed more than 0.53 individual per day). The highest number of bivalves consumed in five days by a single *T. gigas* was *S. cornea* (21 pieces; 4.84 g) whereas for *C. rotundicauda* was *M. meretrix*, *P. erosa* and *C. iredalei* (8 pieces each; 1.81 g, 6.87 g and 5.81 g respectively). There is also no difference in feeding capacity of each type of bivalves between sexes within the same HSC species. Based on these laboratory findings, we believe that even if reintroducing HSC affects the bivalve abundance in Setiu, the impact would not be as significant as the HSCs only consume very little bivalves. In the wild, they are also free to choose other benthic organisms of less economic importance available in Setiu Lagoon.

Keywords. Captive conditions, *Carcinoscorpius rotundicauda*, feeding consumption, food selection, Southeast Asia, *Tachypleus gigas*

MORPHOMETRIC ALLOMETRY OF HORSESHOE CRAB (*TACHYPLEUS TRIDENTATUS*) IN NORTH AND EAST COAST OF SABAH, MALAYSIA

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Abstract. *Tachypleus tridentatus* can be found in East Malaysian coast including Sabah and Sarawak. Samples were collected from three different locations (Kudat (North coast), Kunak and Semporna (East coast)) by handpicking during full and new moon period and also using gill net from July to August 2018. Morphometric parameters were recorded to determine the allometry relationship within and among the sampling stations. An average prosoma width (PW) of horseshoe crabs for male was 28.91 ± 1.60 cm and female was 27.75 ± 2.68 cm in Kudat respectively. While, at Kunak station, an average PW for male and female crabs were 28.91 ± 1.48 cm, 29.44 ± 5.47 cm respectively. In Semporna, mean PW of male and female crabs were recorded to be 21.73 ± 1.34 cm and 24.42 ± 1.36 cm respectively. No significant difference in the weight of the crabs was observed between Kudat and Kunak station compared to the samples from Semporna ($P > 0.05$). Negative allometry relationship was observed in length-length morphometric parameter at Kudat, Kunak and Semporna, while length-weight relationship had positive allometry value. Significant difference in morphometric parameters was observed between sexes regardless of sampling stations ($P < 0.05$). Results have shown that the population of *T. tridentatus* from Kudat has discrete population while samples from Kunak and Semporna have non-discrete population due to their geolocation. Possible molecular identity together with *morphometric allometry* study could help in developing *T. tridentatus* conservation management measures at East Malaysia.

Keywords. Allometric, Malaysia, morphometric, *Tachypleus tridentatus*

SPAWNING ECOLOGY OF *CARCINOSCORPIUS ROTUNDICAUDA* UNDER REVERSE ANTHROPIC EFFECTS

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Abstract. Investigation at Balok (East coast of Peninsular Malaysia) revealed the co-existence of *Tachypleus gigas* and *Carcinoscorpius rotundicauda* in the vicinity. Unfortunately, the latter species received less attention from inaccessible spawning grounds, reduced commercial value and difficult to acquire. Thus, knowledge on population and ecological statuses of *C. rotundicauda* remains unresolved. Alike the *T. gigas*, spawning sites of *C. rotundicauda* are exposed to anthropic activities like sand mining (2009), wave breaker erection (2011) and fishing jetty (2013). These perturbations transitioned the sediment properties of Balok from medium to fine sand between the years 2009 and 2013. Consequently, spawning *T. gigas* were less attracted to Balok Beach. Surprisingly, *C. rotundicauda* released 5,117 eggs in 91 nests along Balok River (200 to 1000 m from open sea). Breakdown of *C. rotundicauda* spawning yields revealed 2,880 eggs in 56 nests during the Southwest monsoon (SWM), 1,254 eggs in 19 nests during the Northeast monsoon (NEM) and 983 eggs in 16 nests during the Inter-monsoon (IM) seasons. With more eggs discovered in shallow nests, silt and clay (> 20%) and reduced erosion were thought to influence moisture retention in surface shores. On contrary, 50 *C. rotundicauda* in amplexus were attained in SWM, followed by 6 amplexus during NEM and 1 amplexus during IM of year 2016. Positively, Balok Beach was favored by *C. rotundicauda* as a spawning site. The perception of reducing horseshoe crab populations at intervened beaches should be treated differently because ecological balances will counter imbalances.

Keywords. Human intrusion, Limulidae, oviparous spawning, river mouth, transitional seasons

LOCAL ACTIONS FOR CONSERVATION OF THE HORSESHOE CRAB, *TACHYPLEUS TRIDENTATUS*, IN CHIAYI, TAIWAN

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Abstract. The horseshoe crab, *Tachypleus tridentatus*, once thrived but almost extinct for recent decades from the coasts of Taiwan. In 2005, several juvenile horseshoe crabs were found again in the intertidal mud flat in Budai of Chiayi County, southwestern Taiwan. However, no more juveniles were observed since then, pressing local people to call for returns of horseshoe crab populations. Subsequently, local non-government organization, Chiayi Ecological Environment Conservation Association (CEECA) has initiated a series of education activities. In 2006, 'Knowing What Horseshoe Crabs Are' was introduced to elementary school students. In 2007, the horseshoe crab natural habitats were surveyed and data collected were prepared for restocking horseshoe crabs in the future. In 2011, the CEECA rented a discarded salt-pan and gathered volunteers to rehabilitate the salt-pan for horseshoe crabs. In May 2012, a 'Conservation Horseshoe Crab Seminar' was held to stimulate a vision as Budai has been considered a very infertile land. Horseshoe crabs are also nicknamed 'The Couple Fishes' because of the amplexus behavior during reproductive season. Inspired by this legend, the 'Cross-Strait Horseshoe Crab Conservation Day' on the 7th day of the 7th lunar month in the Chinese calendar, which day was also known as the Chinese Valentine's Day, Qixi, was held in Xincen Elementary School, Budai, Chiayi County since 2012. In March 2013, 'Caring Horseshoe Crab Forever' program focused on releasing horseshoe crab juveniles to the coastal water. In 2013 and 2014, conservation teams from Hong Kong and mainland China joined Taiwan's team on Qixi festivals through videoconference. In May 2014, a youtube filmed 'Caring Horseshoe Crab on Qixi' was presented publicly. In 2018, the CEECA further made a project to search for juveniles along the Chiayi coast to assess nature habitats which can serve as juvenile releasing sites and designed an exhibition hall for horseshoe crab conservation.

Keywords. Conservation, horseshoe crab, local non-government organization

TRADITIONAL CULTURE-BASED PUBLIC AWARENESS AND CONSERVATION ACTIONS FOR HORSESHOE CRAB AT KINMEN ISLAND, TAIWAN

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Abstract. During the Marital Law era in 1949-1990s, Kinmen was brought under tight military control and kept people away from the coast beaches filled with mines and enabled the marine animal, horseshoe crabs to thrive. However, most of residents lost their awareness to close to the sea during the long military period- not to mention to care marine life and the environment. During the decade of the 2000s, the construction of the 2.2 million m² port had destroyed fishing grounds and devastated the ecosystem at Kinmen. Meanwhile, the horseshoe crab population has disappeared along with the traditional way of life for the island's fishermen. The homeless horseshoe crab and fisherman were made their first awarded documentary as "The Lost Sea". To seek for conservation support from the locals and public, traditional culture-based public awareness and conservation actions were conducted. Traditional fishery skills were demonstrated by old fisherman with concept of sustainable fishery. DIY course of horseshoe crab model's sticky rice cake and origami enriched participants' knowledge and interests of horseshoe crab. Field tours around intertidal zone were guided for firsthand experience with horseshoe crab juveniles and their habitat. All the efforts contributed to the citizen science and urge government's conservation actions on coast biodiversity and the environment.

Keywords. Citizen science, horseshoe crabs, traditional culture-based public awareness

DESIGNING AN ISLAND PROTECTION PROGRAM FOR HORSESHOE CRABS IN KINMEN

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Abstract. While the horseshoe crab used to be found on the west coast of Taiwan Island, it now can only be seen in Kinmen, which is on the west side of Taiwan Strait and close to Fujian province of China. According to the statistics released in 2013, there are five observation points in Kinmen, including Nanshan, Beishan, Qionglin, Qingyu and Jiangong Islet with the most horseshoe crabs living on it. In line with the "Measures Governing the Establishment of Nature Reserves" amended by the Council of Agriculture of Taiwan in 2015, the planning area is divided into three zones: core zone (established for ecosystem conservation), buffer zone (established for environmental education) and sustainable utilization zone (set up to promote the development of surrounding communities). This paper summarizes some ecological problems of the habitats for horseshoe crabs and then gives several corresponding suggestions to improve the ecological environment. Firstly, since the seagrasses of the existing habitats in intertidal zone for horseshoe crabs are almost disappeared, it must to restore the seagrass bed through seeding method. With the restoration of seagrass beds, water quality will be purified and living condition quality for the horseshoe crabs will be enhanced. Secondly, the mangroves at estuary of the Wu Jiang River are too dense and need to be thinned. It is suggested to replant different species of mangroves according to the depths of water, maintaining the muddy condition in intertidal zone required by the juvenile horseshoe crabs. Thirdly, in order to prevent the overgrowth of *Spartina alterniflora* which will affect the habitat of horseshoe crabs, it is advised to set up an artificial walkway, using the physical way to clear *S. alterniflora*. Lastly, we should establish relationships with Kinmen Fisheries Research Institute, so as to promote the development of industries related to horseshoe crabs, and organize community residents to participate in the training and become environment expositors. Through education, more people will know horseshoe crabs and Kinmen.

Keywords. Horseshoe crabs, Kinmen, marine functional zoning, mangroves, economy, seagrass

HORSESHOE CRAB POPULATION RESPONSE TO NATURAL AND ANTHROPOGENIC PERTURBATIONS THROUGH TIME: HURRICANE, OIL SPILL, AND A DECADE OF CHANGE

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Abstract. Horseshoe crab conservation demands understanding of increasing pressure from interacting natural and anthropogenic forces. The northern Gulf of Mexico (nGOM) coast presents a unique setting to test population level response and resiliency of horseshoe crabs to a range of perturbations. During the past 13 years, the nGOM experienced a major hurricane (Katrina, 2005) and oil spill (Deepwater Horizon, 2010), unusual weather events (2010, 2011, 2012), and altered access to key spawning grounds (2011). To determine how a local horseshoe crab population responded to this suite of natural and anthropogenic changes through time, we compared the number and size of horseshoe crab molts collected from Petit Bois Island, MS during 2008 to 2015. Cohorts of horseshoe crabs, represented by groups of the same size (prosomal width) that were spawned at the same time (estimated back to ~1999) were used to detect effects on individual age classes through time. The number and size classes of horseshoe crabs varied among years, with fewer molts found during 2012 than other years. Spawning periods in 2012 had higher wind and precipitation than other years, suggesting local weather patterns affected occurrence of molts, potentially limiting use of the area by live animals or affecting likelihood of molt detection. Across sampling years, few molts were found in cohorts spawned during 2005 and 2010, regardless of the ability to detect other age classes. This finding suggests animals in these cohorts were underrepresented in the population, potentially due to decreased survival following hurricane or oil spill related disturbance. Although few young of the year (Y0) molts were found in 2011, following a weeks-long freshet and shoreline alteration, molts in this cohort were detected in subsequent years, indicating weather patterns affected detection and not survival. High numbers of Y0 molts were found during 2009 and 2013, and the 2009 cohort provided a bench mark for comparison to the 2010 and 2011 age classes. Overall, these data suggest that major environmental perturbations such as a Category 5 hurricane or oil spill (and associated beach clean-up) have potential to reduce the number of crabs in a given year class. Because earlier and subsequent age classes appeared unaffected, it is likely these events primarily affected eggs and Y0 juveniles. Local weather patterns likely had a greater effect on detection of molts than survival. These data highlight the importance of considering locally relevant factors that may affect detection limitations to use of molt data for demographic analyses and the value of consistent long-term monitoring to understand population level resiliency.

Keywords. Cohort, *Limulus polyphemus*, population dynamics, survival, resiliency, stressor, weather

AIRSOFT SCIENCE: A TEST OF HORSESHOE CRAB MOLT DURABILITY

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Abstract. Molts can provide valuable information on horseshoe crab population demographics and may be particularly useful where live animals are difficult to study. To support this application, information on molt durability of different size classes is needed to help determine if molts on shore are from animals that molted locally or if molts can endure long distance travel from more remote areas. To determine molt durability in or out of the water, we recorded the shape and size of fractures on wet and dry horseshoe crab molts of three age classes (large juveniles: 88 ± 2 , subadults: 147 ± 3 , large subadults/ adults: 233 ± 3 mm prosomal width). We hypothesized that dry molts would break more (have larger fractures or areas of damage) than wet molts and that largest-sized molts would be stronger, having a smaller area of damage than other sized molts.

Juvenile and subadult molts were collected from Petit Bois Island, MS during summer 2011 and adult sized molts came from Delaware Bay during 2003-2005. Molts were soaked for 24-hrs before testing, dried for 24-hrs and retested to simulate wet and dry field conditions. Molt durability was tested by firing a 6mm plastic ball projectile from an AGM M14 socom airsoft rifle M305 (255-265 FPS) from a distance of 10 feet. All molt treatments were damaged by the projectile except wet juveniles, two of which had no damage or only a dent when wet, possibly due to greater pliability compared to the more rigid, harder shells of dry and larger sized molts. Although some dry juvenile molts had high fracture size and maximum affect length, on average these values were similar among all treatments. Based on the point-type of physical stressor in this study, wet juvenile molts may be less likely to suffer fracture, but when they do, the size of damage is comparable to other age classes. Although wave action likely affects molts differently from point-pressure, our study suggests that if a force is strong enough to cause damage, larger juvenile to adult sized molts may be similarly affected.

Keywords. Age class, damage, demographics, *Limulus polyphemus*, prosomal width

TOOLS FOR GETTING PEOPLE TO BE AWARE AND CARE: THE HORSESHOE CRAB TEACHER TOOLBOX REVISITED

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Abstract. Growing interest in horseshoe crab conservation has prompted the need for, and emergence of, many innovative ideas, approaches and materials for cultivating awareness of this ancient animal in various parts of the world. To address this need, at the 2015 International Workshop in Japan, a new on-line *Horseshoe Crab Teacher Toolbox*, designed for promoting global exchange of such resources, was unveiled. In the time since, use and growth of the *Toolbox* has been modest, prompting a need to rekindle interest in all it has to offer. To that end, this poster: highlights how the *Toolbox* works, offers examples of the kinds of materials it contains, and provides instructions for easy upload and download of materials to and from it. The role of the *Toolbox* in enabling educators from parts of the world where horseshoe crab education and public awareness is flourishing to share their tools and successes with people in places where such resources are in great need is emphasized. By making the materials available on-line and in different languages, and allowing for ongoing growth and adaptation through user contributions, the *Toolbox* promises to be a key component in efforts to promote and expand horseshoe crab conservation around the world.

Keywords. Brochures, education, fact sheets, horseshoe crabs, lesson plans, outreach, posters, presentations, public awareness, teaching, videos, website

RIGHTING AND BURROWING BEHAVIOR OF JUVENILE LIMULUS IN DIFFERENT SAND SUBSTRATES

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Abstract. We conducted a laboratory study to understand if burrowing and righting behavior of crabs can explain the distribution of juvenile horseshoe crabs at Plumb Beach (Brooklyn, NY). Righting and burrowing behavior of eighteen juvenile horseshoe crabs (average prosoma width=43.2 mm) was observed in aquaria (36 x 22 cm) with 5 L of artificial sea water and 2 cm of either coarse (> 2 mm) or fine (250 μ m) sand. For the righting experiments, each animal was placed in an aquarium with either no sand, coarse sand, or fine sand. To begin, the juvenile horseshoe crab was placed upside down in the aquarium and timed until it righted. A trial was terminated if the crab failed to right within 5 min. For the burrowing experiments, the crab was placed the crab right side up on either coarse or fine sand, and timed until it burrowed to its lateral eyes. The treatment order in both the righting and burrowing experiments was randomly selected using R and between in Excel and each treatment was run in triplicate. Results indicated 92.6% success rate in burrowing in fine sand compared to 24.1% success rate in coarse sand. The righting success of *Limulus* was the same (33.3%) in both coarse and fine sand, but no crabs were able to right themselves in the absence of sand. Juvenile *Limulus* are vulnerable to predation, and the ability to burrow quickly is advantageous. We have found that most juvenile crabs at Plumb Beach are found on fine sand, which is consistent with the findings of our laboratory burrowing experiments.

Keywords. Behavior, Jamaica Bay, juveniles, *Limulus polyphemus*, sediments

FLORIDA HORSESHOE CRAB WATCH: RESULTS FROM CITIZEN SCIENCE TAGGING AND SPAWNING SURVEYS

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Abstract. American horseshoe crabs (HSC) are found in suitable habitat along the entire 2,170 km coastline of Florida where they are divided into 5-6 genetically distinct populations. These HSC populations face many threats including coastal development, sea level rise and harvesting. The status (e.g. population trends) of Florida HSC populations is completely unknown, hindering effective management. To address this lack of data, university researchers and extension agents partnered with the State Fish and Wildlife Conservation Commission management of HSC to initiate Florida Horseshoe Crab Watch (FHCW). This is a community-based program that relies on citizen scientists to tag and re-sight HSC and to conduct spawning surveys. Coordinators and volunteers at each FHCW site are recruited from local nature centers, universities, and State and federal agencies that have on-going outreach and citizen science programs. A new on-line database was developed for data entry by site coordinators, which self-populates with NOAA data on environmental conditions including water temperature and wind direction. FHCW now samples at 20 sites and covers most HSC populations in Florida. Since 2015 FHCW volunteers have tagged 3,131 HSC and conducted nearly 500 spawning surveys. Our short-term results show that Northeast Florida HSC are divided into two, overlapping populations that differ in body size. With an 8% re-sighting rate, we have found that Florida HSC usually stay within 1 km of the beach on which they were tagged, but a few males travel up to 80 km. We have also found that Gulf coast HSC in north Florida have two breeding seasons: autumn (Sept-Oct) breeding involves fewer pairs, a more male-biased sex ratio and more newly molted individuals than spring (Mar-Apr) spawning. With long-term data, FHCW will be able to estimate abundance, identify population trends and determine the cues that initiate spawning throughout Florida.

Keywords. Breeding season, citizen science, Florida, *Limulus polyphemus*, marine-life harvest, spawning surveys, tagging

INJURIES IN AMERICAN HORSESHOE CRABS (*LIMULUS POLYPHEMUS*) FROM JAMAICA BAY, NEW YORK (USA)

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Abstract. Carapace condition and the frequency of injuries was collected on a sample of 241 live adult horseshoe crabs from Jamaica Bay in Spring 2018. The following data was collected on each individual: size (prosoma width) sex, telson length, eye condition, shell condition, and leg condition. Lateral eye condition was rated on a scale of 0 to 5, ranging from a perfect eye (clear, with all facets intact) to a completely rotted eye, respectively. Lateral eyes that were classified 4 to 5 on this scale, along with missing eyes and eyes covered by fouling organisms were considered injured/abnormal. Carapace condition was scored as 1 (new molt), 2 (intermediate), or 3 (heavily worn and pitted). The mean carapace condition of the adult horseshoe crabs in Jamaica Bay was 2.5. The finding that most of the sample consisted of animals with older, worn-out exoskeletons may indicate low rates of recruitment to the population. Eye and leg injuries occurred more frequently in individuals in poorer carapace condition. Injuries or abnormalities of the lateral eyes (52%), and legs (35%) were the most common anomalies in the Jamaica Bay population. Males were more likely to have damaged pedipalps than females, which we attribute to the use of this appendage as the male “clasper” during amplexus. The frequencies of other kinds of injuries were similar between males and females. A number of unusual injuries and malformations to the prosoma, opisthosoma, and telson were documented, and the probable causes (mechanical injury, disease, or developmental abnormality) are discussed.

Keywords. Abnormalities, carapace, injuries, Jamaica Bay, *Limulus polyphemus*

LARGE SCALE IVF/CULTURE SYSTEM FOR SUPPORTING *LIMULUS POLYPHEMUS* POPULATIONS

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Abstract. Strategies to minimize the impact humans have on horseshoe crab (HSC) populations are a corporate focal point of Associates of Cape Cod, Inc. (ACC). Our business relies upon responsible and creative approaches to sustainable HSC fisheries management. In some geographies where populations have depleted due to unsustainable fishing activities and environmental disturbance, HSC conservation and minimized use may not be sufficient. Of particular concern are problems with the HSC ecosystem that include the removal of available spawning locations due to seafront development; decreased spawning locations translate to continued reductions in HSC populations. Conservation cannot correct this problem and an approach that obviates the need for traditional beach spawning is needed. In addition to developing products that drastically reduce the amount of lysate required to manufacture a robust, reliable Bacterial Endotoxin Test (BET) reagent, ACC has developed large scale capabilities for the in vitro fertilization and culture of *Limulus polyphemus* eggs. This scalable, laboratory based system is capable of producing tens to hundreds of thousands of fully formed, viable juvenile HSCs ready for return to the wild. Application of this technology to *Tachypleus* species may be able to play a role in the timely recovery of this depleted species.

Keywords. Bacterial Endotoxin Test, culture, horseshoe crabs, in vitro fertilization, *Limulus polyphemus*, sustainable, *Tachypleus*

INVESTIGATIONS OF TRANSCRIPT EXPRESSION ON FIRST INSTARS OF THE JUVENILE HORSESHOE CRAB *TACHYPLEUS TRIDENTATUS* REVEAL IMPACTS OF CADMIUM

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Abstract. Cadmium, a high toxic heavy metal, which is a potential threat to juvenile Horseshoe crab *Tachypleus tridentatus*. This experiment was carried out to study the effects of different concentrations of Cd (0, 0.05, 0.5 mg/kg) 15 days on the transcript expression of juvenile *T. tridentatus*. A total of 132,079 transcripts with an average length of 1054.8 bp were identified by RNA-Seq. Most of the transcripts ranged from 0 to 1000 bp. GO annotations found that the transcripts were mainly enriched in cellular process, single-organism process, metabolic process, biological regulation, and regulation biological process. KEGG annotations found that the transcripts were mainly enriched in metabolism, organismal systems, and human diseases, etc. three modules. This study is the first step to understanding the transcriptional regulation of juvenile *T. tridentatus* influenced by cadmium..

Keywords. Cadmium, juvenile *Tachypleus tridentatus*, transcript regulation, annotation



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