

International Workshop on the Science and Conservation of Asian Horseshoe Crabs

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Abstracts of Poster Presentations

Theme 1: Conservation of Asian Horseshoe Crabs: Challenges and Opportunities

(P1) Three Decades of Horseshoe Crab Rearing: A Review of Conditions for Captive Growth and Survival

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Threats to wild populations and growing interest in use of horseshoe crabs for research, education and biomedical applications, has prompted demand for improved techniques to rear and maintain crabs in captivity. To identify and define a consistent set of conditions for the best growth and survival of horseshoe crabs in culture, we surveyed available literature to compile and analyze the variety of available techniques. We divided our analyses into three age classes; embryo, juvenile, adult. We included extant species (*L. polyphemus*, *C. rotundicauda*, *T. gigas*, *T. tridentatus*) and considered time in culture, water temperature and salinity, enclosure size, frequency and timing of water changes, diet, and substrate type. We discovered relatively few published studies that clearly related husbandry conditions to growth and survival, and comparison among studies was complicated by inconsistent data collection and reporting techniques. Most studies reported data for younger age classes and more for *L. polyphemus* than Asian species. Growth showed expected differences between Asian and American species, and was relatively consistent despite variation in temperature or salinity. Survival decreased as individuals reached the third or fourth instar, and by instar two, Asian crabs showed consistently lower survival than *L. polyphemus*. Cultured crabs subsisted on foods that were not typical to known natural diets, but artemia and mixed invertebrates yielded best growth and survival. These data emphasize a need for consistent controlled study of factors that affect horseshoe crab growth and survival in culture, particularly, substrate type, diet composition, maintenance schedules, and possible interactions among environmental attributes.

(P2) Heavy Metal Pollution in Estuaries : Part 1. Effects on *Limulus polyphemus* Embryo Survival, Developmental Rate, and Oxidative Stress Response

C.J. Esposito, M. Malin, M.L. Botton, and M.G. HAMILTON (Fordham University, New York, NY, USA)

Horseshoe crabs are regarded as living fossils, surviving for over 400 million years with relatively constant morphology. During its early developmental stages, horseshoe crabs are subjected to many environmental stressors, including heavy metals pollutants such as Cu and Cd. The literature suggests that these metals generate reactive oxygen species causing lipid peroxidation and protein carbonylation. In this study, we exposed stage 20 embryos of the American horseshoe crab, *Limulus polyphemus*, to concentrations of Cu (as CuSO₄) and Cd (as CdCl₂) from 0.01 to 100 mg/L at four different time intervals ranging from 4 h to 24 h. Levels of oxidative damage to proteins and lipids were measured using assay kits (Cayman Chemical). Increasing duration of metal exposure led to a general delay in developmental rate, but increased mortality was only noted at the highest concentration of Cu, 100 mg/L. We found that embryos exposed to both metals showed oxidative stress damage, as evidenced by a general increase in protein carbonylation and lipid peroxidation relative to unstressed controls. Since the superoxide dismutase enzymes (SODs) are one mechanism by which organisms can survive oxidative stress caused by metal pollution, we also assayed for SODs in these extracts. Those results are presented in the companion abstract (Malin et al.).

(P3) Hemocyanin in the Developing Embryos of *Limulus polyphemus*

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Horseshoe crabs use hemocyanin as their respiratory protein. We tested for the presence of hemocyanin in early *Limulus polyphemus* developmental stages including oocytes, early post-fertilization, limb bud, stage 20, and trilobites. Our experimental plan was to extract proteins from these stages of development, then, to use the indirect immunological method, with an anti-*Limulus* hemocyanin antibody, to detect and quantitate hemocyanin on Western blots. One gram of each stage (ca.300 embryos) was homogenized in T-PER (a non-ionic detergent) to break open the cells and extract the soluble proteins. For the Western blots, the proteins were resolved on SDS-PAGE gels. The primary antibodies used were rabbit anti-*Limulus* hemocyanin and, as a loading control, mouse anti- β -actin. The secondary antibodies were anti-rabbit IgG linked to a dye absorbing at 680 nm and anti-mouse IgG linked to a dye absorbing at 800 nm for scanning in LICOR's Odyssey infrared scanner. Densitometry was done with the UNSCAN program (Silk Scientific). We detected hemocyanin at all stages but the relative amounts differed when normalized to actin with the largest amount in the trilobites, when yolk and other proteins have been depleted. As expected, the subunits of hemocyanin were labeled at about 80 kDa. An interesting finding was that actin occurred in different size classes. In oocytes, early post-fertilization and limb bud samples, the band had a molecular weight ca. 60 kDa, while in Stage 20 embryos it was 48 kDa. Thus, actin may undergo modification during development.

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(P4) Dietary Protein and Energy Requirements of *Tachypleus tridentatus* and *Carcinoscorpius rotundicauda*

M.H. HU, Y.J. Wang, S.G. Cheung and P.K.S. Shin (City University of Hong Kong, Hong Kong, China)

A feeding trial including 9 formulated feeds with 3 digestible protein (DP) levels (36%, 40% and 44%) and 3 digestible energy (DE) levels (14, 16 and 18 MJ kg⁻¹) was conducted to investigate the optimum protein and energy requirements of two Asian horseshoe crabs, *Tachypleus tridentatus* and *Carcinoscorpius rotundicauda*. One group of juveniles was fed with frozen brine shrimp (*Artemia salina*) to serve as a control. For each species, juveniles were fed once daily (17:00 h) *ad libitum* for 84 days. At the end of the experiment, survival rate of *T. tridentatus* ranged from 67% to 100%, while that of *C. rotundicauda* ranged from 44% to 100%. Final body weight (FBW), thermal-unit growth coefficient (TGC), feed efficiency ratio (FER), nitrogen retention efficiency (NRE) and energy retention efficiency (ERE) of *T. tridentatus* fed with 40% DP and 14 MJ kg⁻¹ DE were significantly higher than that of *T. tridentatus* fed with the control diet. For *C. rotundicauda*, significantly higher FER, NRE and ERE were found in juveniles fed with 40% DP and 14 MJ kg⁻¹ DE than the control diet. The results of this study indicated that the formulated feed containing 40% DP with 14 MJ kg⁻¹ DE would be optimal for good growth and effective feed utilization of the two juvenile horseshoe crab species. The present findings also revealed that the optimum dietary DP/DE ratio for the two species was 28.16 g protein MJ⁻¹.

(P5) Artificial Rearing of Juvenile *Tachypleus tridentatus*

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Horseshoe crabs were once flourished at the west coast of Taiwan but now can only be found on Kinmen Island. In order to regain its wild population, this research conducted various rearing experiments on its juveniles. All juveniles were reared in 0.3 tons of sea water table ($92 \times 64 \times 33 \text{ cm}^3$) with 30-35‰ salinity and 28-30°C temperature. Different levels of seawater dissolved oxygen (controlled by degrees of aeration) were given to 4 groups of fertilized eggs. Approximately 67% of the egg with strong aeration and only 12.5% eggs with no aeration hatched within the first 45 days after fertilization. Different feeding ration with artemia (10 individuals/ml) was given to three trilobite groups: feed 5 days/week, feed 3 days/week and no feeding. Approximately 64% of the trilobites successfully molted into the second instar stage from all three groups, suggesting that dissolved organic matter presumably present in sea water do not enhance the growth of the trilobites. Different feeding rations with artemia (10 individuals/ml) including feed 3 times/day, 2 times/day and 1 time/day was assigned to the second instars. The group that received most food grows fastest: molted to the third instar about 10 days earlier than the group that received next abundant food and 20 days earlier than the group that received the least food. According to these rearing techniques, we were be able to raised horseshoe crabs from fertilized eggs (July 2008) to the 11th instar (carapace width 9.9cm, Feb. 2011) within 32 months.

(P6) Heavy Metal Pollution in Estuaries: Part 2. Oxidative Stress Response: Effects on Superoxide Dismutase levels in *Limulus polyphemus* Embryos

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Among the many environmental stressors that horseshoe crabs are subjected to during their early development is exposure to heavy metal pollutants such as Cu and Cd. These metals differ in their biological roles. Cu is essential to many enzymes and hemocyanin, the respiratory protein. Cd, however, is truly “toxic” with no role in metabolism. To cope with oxidative stress, all organisms use the superoxide dismutases (SODs) that convert the superoxide radical (produced in mitochondria) to O₂ and H₂O₂. In this study, stage 20 embryos of the American horseshoe crab, *Limulus polyphemus*, were exposed to concentrations of Cu (as CuSO₄) and Cd (as CdCl₂) from 0.01 to 100 mg/L at four different time intervals ranging from 4 h to 24 h. The proteins were extracted by bead-beating in the non-ionic buffer T-PER (Pierce Chemical Co.). As reported in the accompanying Abstract (Esposito et al.), levels of oxidative damage to proteins and lipids were measured using assay kits (Cayman Chemical). The activity of SOD was assayed using a colorimetric kit (Dojindo Molecular Technologies), and the amounts of SOD by immunodetection on Western blots. In general, the levels of SOD were higher in metal treatments compared to controls, although there was no clear correlation between SOD levels and the concentration of Cu or Cd. Overall, increases in the levels of SOD appears to be one mechanism by which developing *L. polyphemus* embryos can survive oxidative stress caused by metal pollution.

(P7) The Effect of Culture Method on the Growth and Molting Frequency of *Tachypelus gigas* Reared from 6 to 11 Month-old

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Information on the growth of *Tachypelus gigas* is not well established as compared to its temperate counterpart *Limulus polyphemus*. Lack of documented study on *T. gigas* has further encouraged the research the growth and molting frequency under different culture method. This report compares the size and weight increments, and molting frequency of *T. gigas* larvae cultured using convention (80-90% water change/day) and non-conventional (recirculating water system) methods. Size increment was measured based on prosomal width and weight increments of the larvae. Molting frequency was determined for the larvae from 6 to 11-month old. The larvae culture using these two methods molted 3 times during the culture period. Result of T-test showed that there were no significant differences ($P > 0.05$) in the molting frequency of the larvae between the two methods. The final prosomal width achieved for *T. gigas* larvae cultured using conventional method was 23.50 mm which is slightly smaller as compared to 27.99 mm using non-conventional method (recirculating system). Similarly the final weight of the 11-month-old larvae cultured using conventional method was 0.61 g which is comparatively lower than those culture under recirculating system, 0.92 g. However, T-test showed non-significant difference ($P < 0.05$) for increments on prosomal width and weight of *T. gigas* larvae for both culture methods. Water parameters (pH, salinity, temperature, dissolved oxygen and ammonia) for both systems were monitored and it was found that the conventional and non-conventional methods of culture do not differ in salinity, temperature and pH except for dissolved oxygen (DO) and ammonia. Recirculating water system produces higher DO due to the continues flow of water in the system, while lower ammonia was basically due to the biofilter installed in the system. Therefore, it can be concluded that there is no difference in the prosomal width and weight increment, and molting frequency of *T. gigas* larvae culture in conventional and non-conventional method. This study showed that *T. gigas* larvae can be cultured under recirculating water system without negative effects on its growth and molting frequency.

(P8) Development of Optimal Artificial Feed for Laboratory Culture of Juvenile Mangrove Horseshoe Crab *Carcinoscorpius rotundicauda*

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A 84-day laboratory feeding trial was conducted to assess the suitability of a combination of rendered protein ingredients, including poultry by-product meal (PBM), meat and bone meal (MBM) and blood meal (BM), as fishmeal (FM) substitutes in the formulation of artificial diets for the culture of juvenile mangrove horseshoe crab *Carcinoscorpius rotundicauda* in the laboratory. Nine isonitrogenous (digestible protein 40%) and isoenergetic (18 MJ kg⁻¹) diets were formulated to feed juvenile *C. rotundicauda*. Diet 1 (control) was a high quality juvenile horseshoe crab diet based on a previous study, containing 28% FM as the sole animal protein source. In the other eight diets (Diet 2 – 9), 25–100% of FM was replaced by a blend of PBM, MBM and BM with different proportions of PBM and MBM (2:1 or 1:1). The final body weight (FBW), thermal-unit growth coefficient (TGC), feed efficiency ratio (FER), nitrogen retention efficiency (NRE) and energy retention efficiency (ERE) of juveniles fed with the feed in which 100% of FM protein was replaced by the blend of PBM, MBM and BM was significantly lower than other feed groups. The highest FBW and TGC were recorded in juveniles fed with the feed which contained the combination of 12% PBM, 6% MBM and 4.5% BM. This combination was equivalent to an optimal replacement of 75% FM protein in the culture of juvenile *C. rotundicauda*.

Theme 2: Population Status, Assessment Methods, and Management Approaches

(P9) Conservation Plans for Horseshoe Crabs in Long Island Sound Combining Both Population Genetic and Mark/Recapture Data

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Horseshoe crabs (*Limulus polyphemus*) have global economic importance with local ecological value and are directly tied to improved human health. Three no-harvest zones on spawning beaches in Connecticut were established to conserve the population. This study was developed to determine if this plan was appropriate to conserve the genetic diversity of the population of horseshoe crabs residing in Long Island Sound (LIS). Data from a long-term tagging and monitoring study of horseshoe crabs and a DNA-based genetic survey of the population were collected and analyzed. To date, ~53,000 crabs have been tagged over a ten year period through Project *Limulus*, a research and science literacy program, with an annual average recapture rate of 13%. The tag data revealed that most horseshoe crabs stay within a 16 kilometer radius from where they were originally tagged. Ten to 20% of the recaptured crabs move greater distances including across but rarely leaving LIS. A total of 187 horseshoe crabs, collected from 5 distinct sites, spanning the geographic extent of LIS, were genotyped for 11 microsatellite DNA regions. The genetic data indicates that the LIS horseshoe crab population is in good genetic health, does not exhibit isolated subpopulations, and is genetically homogenous with no signs of inbreeding. The genetic data confirmed that the crabs move around the Sound but generally stay within its boundaries. Therefore, the locations of the established no-harvest zones conserve genetic diversity. However, to continue to harvest horseshoe crabs sustainably, a tri-state management strategy is needed to successfully increase the population.

(P10) Are the Mangrove Horseshoe Crabs in the Strait of Johor Singapore Permanent Residents? Tracking Studies

L. CARTWRIGHT-TAYLOR and H.H. Ng (Nature Society (Singapore), Singapore)

We have been studying a population of *C. rotundicauda* along the Strait of Johor in Singapore. Although adults and juveniles can be found at the site year round data were lacking on their ranging behaviour and whether they migrate to deeper waters or to the open sea. Such information is needed to develop conservation strategies for this population.

We used acoustic telemetry to track the movements of adult *C. rotundicauda* in the Strait. Six acoustic receivers were deployed along the Strait of Johor approximately 1 km apart suspended from the platforms of the floating fish farms in the area. Twelve adults (six males, six females) were tagged. Four were released at one site of capture, four at a second site of capture about 6 km west of the first site, and four were captured at the first site, but released at the second site to determine if they displayed any homing instincts by returning to their site of capture.

Data over four months of tracking suggest that the crabs do not travel far from the release site and do not display homing instincts. This would allow crabs to be taken from one site to restock another suitable site. There is no seasonal imperative for the crabs to migrate away from the site, so these are probably Singapore permanent residents. As this is probably the last resident breeding population of *C. rotundicauda* around the main island of Singapore we recommend that the government takes some measures to protect it.

(P11) Decline of juvenile horseshoe crab (*Tachypleus tridentatus*) populations at the intertidal zones of Kinmen Island

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The juvenile horseshoe crabs slowly disappeared from the intertidal zone in the Taiwan main island and now can only be found on Kinmen Island. A total of 800 ha of coastal zone were designated as “The Horseshoe Crab Protected Area” in Kinmen in December 1999. To monitor the long-term changes of the juvenile population in the Protected Area, two transect lines were set up at each tidal flat of the Beishan, Nanshan and Hsashu. Crab densities were recorded monthly from 2003 to 2009. Most of the juvenile crabs appeared during summer (May - August) therefore the data of these four months were used as yearly representatives. The results showed that juvenile populations decreased remarkably during the study period. At Beishan station, annual peak density was 0.57 ind./m² in 2003 but dropped to 0.21 ind./m² in 2009. The same trend was found at Nanshan (peak: 0.31 in 2003, 0.03 in 2009) and at Hsashu (peak: 1.90 in 2003, 0.38 in 2009). A simple regression analysis was conducted to estimate the density (D) of juvenile horseshoe crab over the year (Y) for each area – Beishan: $D = 0.295 - (0.00671 \times Y)$, Nanshan: $D = 0.130 - (0.0215 \times Y)$, and Hsashu: $D = 1.305 - (0.139 \times Y)$. The result showed that juvenile horseshoe crabs densities decreased significantly at Nanshan and Hsashu, but not at Beishan. The survey indicated that more efforts on conservation and management were needed to restore the declining horseshoe crab population on Kinmen Island.

(P12) Genetic Variability of Horseshoe Crab, *Tachypleus gigas* (Müller) in Peninsular Malaysia

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In Malaysia, horseshoe crabs, *Tachypleus gigas* (Müller) have been investigated for their abundance and morphology but very little information on their population size and phylogeny. This study was conducted to investigate the genetic variability and structure of two Malaysian *T. gigas* populations: Cherating, Pahang and Tanjung Dawai, Kedah. Random amplified polymorphic DNA (RAPD) and 18S rDNA gene sequence analysis were utilized to determine polymorphisms in these populations. Information obtained from RAPD and 18S rRNA gene sequences were then used to assess the genetic variation of wild *T. gigas* by constructing a phylogenetic tree analyses and their DNA divergence. The percentage of polymorphic loci for the Cherating and the Tanjung Dawai populations were 83.33% and 44.44% respectively. Based on the UPGMA dendrogram, the two population samples were segregated into two distinct clusters for both groups. From 18S rRNA gene sequence analysis, the net nucleotide divergence among populations was -0.0073 indicating a low polymorphism among the populations studied. Total number of mutations in Tanjung Dawai was higher than Cherating samples, which are 73 and 59 respectively where as shared mutations across the populations were 8, and reveal the evolutionary in the genome of Malaysian *T. gigas*. The tree topology of both populations inferred using NJ method by comparing 1791 bp of partial 18S rRNA sequence revealed that *T. gigas* haplotypes were clustered into seven clades, suggesting that they are genetically diverse among population's derived from a common ancestor.

(P13) Horseshoe Crab *Tachypleus gigas* (Müller) Found at Spawning Site at Pantai Balok in Peninsular Malaysia

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In Malaysia, the population monitoring data for horseshoe crabs in its natural habitats are scarce, therefore the population status is poorly understood. The public community is unaware of the ecological importance of this living fossil. Horseshoe crabs are harvested for local dishes or exported to Thailand, as there is high market demand there. The size frequency distribution, age estimation, sex ratio, and epibiont infestation of *Tachypleus gigas* were investigated in this study. All horseshoe crabs examined at Pantai Balok were adults at age 10-11, 11-12 and > 12 years. Juvenile or young adult was not found. Only mature crabs migrate to the nesting beach for breeding purpose which occurred during new and full moons. There was higher proportion of males than the females because females were harvested as food for the eels and local community. Number of horseshoe crabs sighted was lower during the Northeast monsoon season (November to March) due to the occurrence of heavy precipitation and flooding. The null hypothesis on sex ratio was equivalent to 1 per month of the year cannot be rejected, except in July, September, December 2009, and January to April 2010 where the sex ratios were more than 1. Males were found to be more prone to epibiont infestation. Approximately 78 % of the males sampled were fouled. Four epibiont species found on the carapaces of *T. gigas* are the acorn barnacle *Balanus*, pedunculate barnacle *Octolasmis*, conical and flat slipper snails *Calyptrea* and *Crepidula*.

(P14) Genetic Diversity of Horseshoe Crab (*Tachypleus tridentatus*) distributed along Southeastern Coast of China Mainland Assessed by Microsatellite Markers

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(CA)_n-microsatellite enriched genome library of *Tachypleus tridentatus* was conducted by using FIASCO. Nine polymorphic microsatellite markers were selected and their polymorphic characteristics were analyzed in 37 individuals. The number of alleles was 5-14, the average was 8.1, and observed heterozygosity was 0.1892~0.7838. Seven loci significantly deviated from HWE ($P < 0.05$). Linkage disequilibrium (LD) among these nine loci did not exist ($P > 0.01$). Then these nine polymorphic microsatellite markers were used for genetic diversity analysis of nine populations of *T. tridentatus* from Chinese coast. AMOVA analysis showed that genetic variation existed mostly within the population. Information from fixation index (F_{st}) showed that there was no significant differentiation among these nine populations and gene flow was large among them; thus the nine populations were belonged to the same mating groups. Mantel analysis showed that the genetic distance was not correlated to geographic distance. The weak genetic structure of horseshoe crabs from Chinese coast may be due to frequently artificially transportation for commercial purposes.

Theme 3: Exploitation: Managing Sources of Mortality

(P15) Characterization of Amoebocyte Lysates from *Carcinoscorpius rotundicauda* and *Tachypleus gigas*.

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Amoebocyte lysates were prepared from two Singaporean species of horseshoe crab, *Carcinoscorpius rotundicauda* and *Tachypleus gigas*. Whilst chloroform extraction adversely affected the potency of the lysate, no adverse effect was observed using BSA as protein stabilizer. The quality of the prepared lysate was enhanced by the addition of calcium, magnesium and sodium ions. The lysates were comparable to commercial lysates of *Limulus polyphemus* (LAL) and *T. tridentatus* (TAL) in terms of the total protein content and sensitivity of endotoxin detection. Biochemical similarities between the two types of lysates revealed that a major protein of 21 kDa was involved in the gelation reaction. This protein, later determined to be coagulogen, constitutes approximately 40% of the total lysate protein. Upon cleavage of coagulogen, a 17 kDa protein found to be coagulin, was monitored against time and varying concentrations of either lysate protein or endotoxin. However, protein concentration did not appear to be an absolute indicator of lysate potency. The presence of the 17 kDa coagulin proved to be a more definitive way to ascertain the integrity of fresh lysate preparations. Taken together, our preparations were proven effective in the gelation, chromogenic and fluorimetric assay for endotoxin.

(P16) Screening for Tachycitin Alike Substance in Blood Cells of *Carcinoscorpius rotundicauda* and *Tachypleus gigas*

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The main protecting mechanism in horseshoe crab against invasion of microorganisms is its innate immune system. This immunity induces via cellular and humoral defense. Production of antibacterial compounds in the granular blood cell of horseshoe crab is known as humoral immunity. Tachycitin is an antibacterial substance with antibacterial activity against both Gram positive and Gram negative bacteria, which has previously isolated from blood cells of *Tachypleus tridentatus*. Incident and expression of tachycitin gene in granular blood cell of *C. rotundicauda* and *T. gigas* was assessed using RT-PCR and antagonistic abilities of their blood against Gram positive and Gram negative bacteria was evaluated. The amplified genes of *C. rotundicauda* and *T. gigas* were compared to the Gene bank, those exhibited 93% and 95% similarities to the tachycitin gene of *T. tridentatus*, respectively. Fresh blood of *T. gigas* was demonstrated antibacterial activity against *Escherichia coli*, *Vibrio parahaemolyticus* and *Bacillus cereus*, but it exhibited partial antibacterial activity against *Sterptococcus aureus* and *Bacillus subtilis*. While fresh blood of *C. rotundicauda* was exhibited antagonistic properties against all mentioned microorganisms but partially inhibited growth of *Sterptococcus aureus*.

Theme 4: Habitat: Status, Assessment Methods, and Management Approaches

(P17) Relationship of Environmental Variability and Spatial Distribution of Two Horseshoe Crab Species along Nursery Beaches of Beibu Gulf Coast, Southern China

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Beibu Gulf in southern China is an important nursery ground for the Asian horseshoe crabs *Tachypleus tridentatus* and *Carcinoscorpius rotundicauda*. In this study, spatial distribution, densities and population structure of these two species along three known nursery beaches of Beibu Gulf were investigated during the summer of 2009. Physio-chemical parameters, including tidal height, temperature, salinity, pH, dissolved oxygen (DO), particle size and total organic content (TOC) in the sediments of each transect were measured. Results showed that juvenile *T. tridentatus* preferred living on sandy beaches, while *C. rotundicauda* on muddy shores. Grain size and DO were negatively correlated with the densities of *T. tridentatus*, whereas salinity was positively correlated with their distribution. For *C. rotundicauda*, temperature and salinity were positively correlated with their distribution; however, grain size, TOC, DO and tidal height showed the negative relationship. For both species, smaller juveniles (mean prosomal width circa 15.5 mm) lived closer to the shore, while larger ones (mean prosomal width circa 71.4-88.8 mm) were found further offshore. Hence, both *T. tridentatus* and *C. rotundicauda* preferred to move further from their natal area to the lower shore area as they grow, because the offshore sediments tended to have higher TOC which may reflect more potential food sources for them.

(P18) Horseshoe Crab in Fujian: Conflict between Conservation and Economic Development?

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Pingtang, Fujian Province, was once recognized to have the largest population of *Tachypleus tridentatus* along the coast of China. A dramatic decline of the population began at least over half a century ago. Overfishing and beach reclamation are considered the most stressful reasons accounting for such a decline. At the beginning of this century, an extensive sandy-mud beach planned for local horseshoe crab reserve was taken over by a business man for clam hatchery. Although this business did not go far, the reserve gone. It is usually believed that there are conflicts between natural conservation and economic development. It is not always true. There are many successful cases of ecotourism in wildlife conservation. If the beach mentioned above has been reserved for horseshoe crab and the conservation program be incorporated as an element of Pingtang ecotourism industry, it will be expected to be beneficial not only to the ancient marine life, but also the tourism and local economy.

(P19) Diet Analysis for Horseshoe Crab in Japan by $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ Measurements

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Diet analysis using carbon and nitrogen isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) was conducted in different age class and localities. The objectives of this study are 1) to determine the isotopic characteristics of the molted skins, 2) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ changes of this species with growth, 3) trophic position of this species based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, 4) food preference by comparison with location of different environmental conditions. A total of 126 samples consisted of molted skins (exuvia), muscle and whole body of juvenile stage, originally from 7 local populations in Japan: Imari Bay, Kafuri Bay, Hakata Bay, Tsuyazaki tidal flat, and Sone tidal flat, Kitsuki Bay and Yamaguchi Bay. Inference on egg nutrients to embryo and younger stages was examined using artificial hatching and fed individuals. It suggested that animal body was almost composed from their foods after 4th stage. Comparison between muscle and shells using relatively fresh dead animals showed that each individual had higher $\delta^{15}\text{N}$ value in muscle than that of shell with an average difference of +3‰. Differences of $\delta^{13}\text{C}$ values between muscle and shell in each pair were variable, and estimated as average of +1‰ shift. Local variation of the horseshoe crab in ecosystem seems that most of the younger horseshoe crab seems to depend on seagrass ecosystem, and adult horseshoe crab in the Sone tidal lands must depend on seagrass ecosystem.

(P20) Chinese Horseshoe Crab (*Tachypleus tridentatus*) Resources Investigation along Southeastern Coast of China Mainland

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Chinese Horseshoe crab (*Tachypleus tridentatus*) was once widely distributed along the south-east coast of China mainland, which was an important economic species as seafood and its amebocyte lysate was widely used in endotoxin test. Although it has been included as key protected animals in coastal provinces, there is no effective management to protect this species due to a lack of scientific knowledge on its population status. Hence, the outlook for the sustainability of its resource is not optimistic.

In this study, the resource of horseshoe crab (*Tachypleus tridentatus*) was investigated in the Fujian Province by visiting fishermen who practice shallow bottom trawling and by observations on spawning and juvenile growing behavior at intertidal zone. The representative habitats of horseshoe crabs in Zhejiang Province, Guangdong Province, Hainan Province and Guangxi Autonomous Region were also visited and investigated. The results showed that the horseshoe crab resources, which were very abundant along Chinese coast, have rapidly decreased and are at the brink of extinction in Chinese waters. The main reasons for the decline of resources were as follows: overfishing, intertidal habitat destruction, environmental pollution, and so on.

Theme 5: Public Consensus: Building Public Consensus for Conservation Action through Education, Outreach, and Cooperation

(P21) Artificial Breeding of *Tachypleus tridentatus* in a Secondary School Environment

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Recently there has been a sharp decline in horseshoe crabs population in Hong Kong. Studies revealed that horseshoe crabs have been extinct in selected sites. With the support from the City University of Hong Kong, sixty-five two-to-five instars *Tachypleus tridentatus* were bred in our secondary school as a pilot to raise the wild population of horseshoe crabs with the direct participation at school level. A team of fifty secondary school students take up the daily responsibility of feeding, measuring environmental parameters and recording growth. We reviewed the effectiveness of this 15-month breeding and re-introduction exercise. Most horseshoe crabs molted at least once, with maximum of four. Finally 23.1% (15 out of 65, five-to-seven instars) survived and successfully released to a mudflat habitat in Northwestern Hong Kong, a figure comparable to natural breeding. The greatest difficulties of this exercise were: (1) time constraints in monitoring during school hours and (2) inadequate experience in managing horseshoe crabs with healthy situation. We recommended that (1) a shorter duration of breeding exercise and (2) in-depth understanding and practice before breeding would contribute to a greater survival rate.

(P22) Chinese Horseshoe Crab Conservation, Education and Training Programme in Beihai, Guangxi, China

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Horseshoe crabs have been facing global decline in the past decades. Species specific conservation and management strategies of horseshoe crab are urgently required. Chinese horseshoe crabs are exploited for medicinal uses but a majority of them are harvested as food. Asian residents, especially Fujian and Guangxi of South China, consider horseshoe crabs as a delicacy and highly nutritious food. In Guangxi, horseshoe crabs are sale in local seafood restaurants, some cook books and magazines even teach people how to cook these animals and advertise the high nutritional value and good taste of their meat and the soup prepared from them. Therefore, it is suspected that human exploitation may be one of the major factors contributing to the decline of these animals in Guangxi. Beihai city of Guangxi province which locates in Beibu Gulf is one of ideal habitats of Chinese horseshoe crabs, therefore, it is important to adopt the relevant conservation actions for this animal species. The present study was funded by Ocean Park Conservation Foundation Hong Kong (OPCFHK) during the period from July 2008 to June 2009. Our research team has already produced a series of educational and training materials, such as publications, leaflets and exhibiting panels, which can be used in exhibition and activities related to the conservation of horseshoe crabs

(P23) An Exhibition on Horseshoe Crab Biology, Conservation and Sustainability at Taiwan's National Museum of Marine Biology and Aquarium

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Despite their importance in marine ecosystems, horseshoe crabs are notoriously understudied, particularly in the Indo-Pacific. Taiwan's National Museum of Marine Biology and Aquarium (NMMBA) was interested, then, in promoting the importance and beauty of these creatures to the general public in the form of a novel exhibition at its public facilities in Southern Taiwan. Fortunately, one of the pioneers of horseshoe crab biology and conservation, Dr. Chang-Po Chen, has maintained active in the field despite his retirement last year from Academia Sinica (Taipei, Taiwan) and is currently collaborating with NMMBA towards the creation of such an exhibit. The NMMBA is a multi-faceted institute that is involved in display of Taiwan's richly biodiverse marine life, and, as such, has an exemplary facility for the culturing of fish, marine mammals, and a wide multitude of marine invertebrates. Given the high quality of these facilities and exhibits, NMMBA attracts upwards of 1000,000 tourists each year and sometimes many more. NMMBA also maintains an active research faculty focused on biodiversity and biotechnology of the marine realm, and, through National Dong Hwa University, faculty are able to accept Master's students who wish to conduct their thesis research at a state of the art marine research institute. With these physical and academic resources at its disposal, NMMBA hopes to complete its horseshoe crab exhibit in collaboration with Dr. Chen by the summer of 2011, allowing for the establishment of an effective means to disseminate knowledge of horseshoe crabs to the Taiwanese public.

(P24) Public awareness and local action for conservation of the horseshoe crab, *Tachypleus tridentatus*, in Kinmen, Taiwan

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Since 1996, we have conducted basic researches and public education of horseshoe crabs in Kinmen for more than a decade. Through our efforts and government's recognition, "The Horseshoe Crab Protected Area" enclosed 800 ha of coastal areas at Gugingtou was designated in 1999. In the following year, an annual festival of "releasing juvenile horseshoe crabs to the wild" by local elementary school students was taken place in the protected area. In 2007, one of the natural habitats for these juveniles, Hofung, was decided for harbor construction. Due to self-awareness of the habitat-lose risk causing horseshoe crab extinction, local residents organized a "Wushui River Horseshoe Crab Patrol Team". The team not only monitors juvenile populations but also educates residents, especially students, and improves public awareness such as cooperating with a bakery store to make bread of horseshoe crab shape. All their achievements have been published in a book. In 2010, we invited several specialists and scholars from mainland China to visit the protected area in Kinmen. We discussed the conservation strategies of horseshoe crab and what we can do both in mainland China and Taiwan. We agreed to sign a draft about cross-strait cooperation on protection of horseshoe crabs. We also decided to designate Chinese Valentine's Day as "Cross-strait Horseshoe Crab Protection Day".