Aspects of Ecology and Biology of Two Sympatric Horseshoe Crab Species
(Tachypleus tridentatus and Carcinoscorpius rotundicauda)
同地共棲兩種鱟的生態和生物學研究

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Horseshoe crabs are chelicerates, distant relatives of spiders. They are often referred to as living fossils, as they have changed little morphologically in the last 445 million years. There are four extant species of horseshoe crabs. The species *Limulus polyphemus* (Linnaeus, 1758) occurs only along the eastern coast of the USA. The other three species, *Tachypleus tridentatus* (Leach, 1819), *Tachypleus gigas* (Müller, 1785) and *Carcinoscorpius rotundicauda* (Latreille, 1802) live along the coast of the Indo-West Pacific. In Asian waters, habitat degradation especially the loss of spawning and nursery grounds, marine pollution and human exploitation have resulted in a decline in horseshoe crab populations.

The present research investigated two co-existing horseshoe crab species, *T. tridentatus* and *C. rotundicauda*, with a focus on some aspects of their ecology and biology. These included 1) relationship of environmental variability and their spatial distribution on three important nursery beaches on Beibu Gulf, southern China; 2) their distribution,
abundance and population structure at these nursery beaches; 3) diet and trophic relationships of the two species within the food web of the beach ecosystems; 4) effects of starvation on body weight and blood indicators; 5) effects of starvation on the energy budget; 6) nutritional requirements and 7) development of an optimal artificial feed formulation. The overall study objectives were to decipher the ecological and biological differences of the two sympatric horseshoe crab species and to develop laboratory culture practices that would enhance future conservation of these animals and restore their declining populations.

As compared with the adults, juvenile horseshoe crabs are relatively immobile and restricted to a smaller geographic area, making them easier to count. Juvenile abundance data are useful in predicting their population structure and growth development in the field. A comprehensive field study was conducted from May to June 2009, to better understand abundance, population structure, growth development and the relationship of environmental variables and spatial distribution of juvenile horseshoe crabs. This study restricted its survey area to 1.6 m to 0.6 m above chart datum of three nursery beaches, Xi Bei Ling (XBL), Jin Hai Wan (JHW) and Xi Chang (XC), along Beibu Gulf, southern China.

Hydrography and sediment characteristics of each transect at the three nursery beaches were measured. Results showed that four environmental variables were found to be positively correlated with the abundance of juvenile horseshoe crabs in the following order: sediment TOC > sediment median diameter > DO of interstitial waters > tidal height of the transect. The remaining two variables, temperature and salinity, were found to be negatively correlated with the abundance of juvenile horseshoe crabs.
Only *T. tridentatus* was found at XBL, whereas both *T. tridentatus* and *C. rotundicauda* co-existed on JHW and XC beaches. The species-specific growth and mortality of each species were estimated based on the size-frequency data of the juveniles. *T. tridentatus* at 7th instar stage was in the majority at XBL, while *T. tridentatus* at 8th instar stage was dominant at both JHW and XC. However, *C. rotundicauda* at 7th instar stage was only abundant at XC. The relationship between pre-moult and post-moult sizes of juvenile horseshoe crabs was generally fitted with the Hiatt linear model. The model data showed that the increments of juvenile *T. tridentatus* were significantly larger than juvenile *C. rotundicauda*, indicating species-specific growth characteristics. Results from the present study added information to the current database on the growth allometry of the two sympatric horseshoe crab species recorded in Asian waters. From the field data, cumulative mortality of *T. tridentatus* from 9th to 11th instars at JHW (71.0%) was lower than that at XBL (83.8%) and XC (84.8%). For *C. rotundicauda*, cumulative mortality from 10th to 11th instars was 47.7% at XC. Estimated species-specific mortality rate at a given instar stage ranged from 23.0% to 64.9% for *T. tridentatus* and 13.0% to 59.5% for *C. rotundicauda*. The present findings suggested that natural mortality for both juvenile horseshoe species was comparable to data reported in literature.

In order to evaluate the food sources and trophic relationship between these co-occurring *T. tridentatus* and *C. rotundicauda* populations, carbon and nitrogen stable isotopic signatures (δ13C and δ15N) were analyzed on the two species and their potential prey specimens. The statistical differences in the isotopic signatures, among different size groups per horseshoe crab species, revealed location-specific differences. Stable isotope signatures revealed the food sources of the two species, including bivalves,
crustaceans, gastropods and polychaetes. The present results indicated that juvenile horseshoe crabs are generalist feeders. The two sympatric species exhibited trophic segregation at JHW, but they showed an overlap in trophic niche at XC. This indicated that the two species at XC were overlapped not only in their spatial but also in their trophic niches.

Horseshoe crabs are omnivorous, preferring diets containing marine phytoplankton, microalgae and benthic invertebrates. Under natural conditions, horseshoe crabs have to tolerate and overcome the shortage or total absence of food during for short and long periods in the wild due to environmental perturbations, such as marine pollution and seasonal changes in the structure of benthic communities. The scarcity or even total absence of food during shorter or longer periods may affect the physiology of the animals in the marine ecosystem. In order to understand their feeding strategies and energy utilization, the effect of prolonged starvation on blood indicators and energy budgets of adult _T. tridentatus_ and _C. rotundicauda_ were studied under two food treatments (fed and starved) for seven weeks.

During the experimental period, no significant differences of body weight were detected between the starved and fed groups in the two species. Plasma alkaline phosphatase increased slightly and then decreased. Plasma calcium remained fairly constant during the experimental period. However, there was a continuous decrease in plasma glucose in the starved treatment. Additionally, cholesterol, lipase and triglycerides in the plasma increased significantly later in the study, while plasma total protein remained fairly constant during the experiment. The results suggested that, for adult horseshoe crabs, starvation might stimulate a “hunger response”, which in turn might lead to some
internal nutrients consumption. By consuming internal nutrients, adult horseshoe crabs can withstand prolonged starvation.

In terms of energy budget, significant interactive effects among species, feeding regimes (fed or starved) and time course on respiration rate (RR), ammonia excretion rate (ER), oxygen: nitrogen (O:N) ratio and scope for growth (SfG), were evident during the seven–week experiment. No significant effects of species and time course, or their interaction on absorption efficiency, were observed in the fed treatments. For each species, RR and ER of the starved treatment significantly decreased, while their O:N ratio significantly increased, as compared to the fed treatment, which remained relatively stable during the experiment. In the starved treatment of each species, a rapid reduction in SfG was apparent at the start of the experiment; thereafter, SfG remained stable over the study period. SfG of fed *T. tridentatus* was significantly lower than that of fed *C. rotundicauda* throughout the experiment, while no significant difference between species was found in starved treatments from Week 1 to Week 7. *C. rotundicauda* showed a greater decrease in SfG under starvation than *T. tridentatus*, suggesting that they may have a more competitive life history strategy for adjusting to different nutritional conditions.

The above research led to further studies on nutritional requirements, particularly for the rearing of juvenile horseshoe crabs by artificial feed, in the laboratory, with an aim to restock their declined populations in the field. In order to assess dietary protein and energy requirements of the two horseshoe crab species, a feeding trial including nine formulated feeds with three digestible protein (DP) levels (36%, 40% and 44%) and three digestible energy (DE) levels (14, 16 and 18 MJ kg\(^{-1}\)) was conducted. The control
group fed on frozen brine shrimp (*Artemia salina*). For each species, juvenile horseshoe crabs were randomly distributed into 30 tanks and fed once daily (17:00 h) to satiation level for 84 days. At the end of the experiment, the survival rate of *T. tridentatus* ranged from 67% to 100%, while that of *C. rotundicauda* ranged from 44% to 100%. For the two species, the growth and feed utilization of juveniles strongly correlated with the dietary DP and DE. The results of this study indicated that the formulated feed containing 40% DP with 14 MJ kg$^{-1}$ DE was the best, in terms of growth and feed utilization for the two juvenile horseshoe crab species. 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. However, for *C. rotundicauda*, only 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 present findings revealed that the optimum dietary DP/DE ratio for the two species was 28.16 g protein MJ$^{-1}$.

A further 84-day experiment progressed 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 substitutes for fishmeal (FM) in experimental diets for the two species. A suitable substitution for fishmeal would help improve the cost-effectiveness of the culturing of juvenile horseshoe crabs. The optimal replacement levels of FM by the combination of PBM, MBM and BM were shown, by the third-order polynomial regression analysis, to be 50% and 75% in protein for *T. tridentatus* and *C. rotundicauda*, respectively. A nutritionally balanced, practical artificial feed for each juvenile horseshoe crab species was formulated successfully.
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