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The Conservation of Horseshoe Crabs in Hong Kong
香港馬蹄蟹的保育研究

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Horseshoe crabs in Hong Kong are facing a rapid population decline and local extinction risk. This study focused on some aspects of the conservation of horseshoe crabs in Hong Kong, including 1) most up-to-date information of the present status and distribution of horseshoe crabs; 2) morphological measurements and genetic analysis of two horseshoe crab species *Tachypleus tridentatus* and *Carcinoscorpius rotundicauda*, and geographical variations in population genetics of *T. tridentatus*; 3) the degree of human exploitation of horseshoe crabs; and 4) trials on artificial insemination and breeding of horseshoe crabs and investigations on the optimal environmental conditions for enhancing the survival and hatching success of horseshoe crab eggs.

Juvenile horseshoe crabs were sampled on the soft shores in Hong Kong using a quantitative random quadrat sampling along shore transects at 17 survey sites in Hong Kong. A total of 15 juvenile *Tachypleus tridentatus* were found at Tsim Bei Tsui, Pak Nai, two locations at Ha Pak Nai in northwestern New Territories, and San Tau and Yi O on Lantau Island. In comparison with a similar territory-wide study in 2002 by Morton and Lee (2003), a significant decline in the mean population density was

recorded, with the decrease of 96, 90 and 80% at Pak Nai and two locations in Ha Pak Nai, respectively. No *Carcinoscorpius rotundicauda* was found in the transect study at the 17 sites. Additional walk-through observations on the shores revealed the presence of *T. tridentatus* at other sites including Tsim Bei Tsui in the northwestern New Territories, and Shui Hau Wan, Tai Ho Wan, Sham Wat, Yi O and Tung Chung on Lantau Island. The walk-through study also revealed the presence of *C. rotundicauda* at Tsim Bei Tsui, Sheung Pak Nai, Pak Nai, two locations at Ha Pak Nai in northwestern New Territories, Luk Keng and Lai Chi Wo in the northeastern New Territories, and Tai Ho Wan, Yi O and Tung Chung on Lantau Island.

Further intensive distribution surveys were conducted at four key horseshoe crab nursery grounds, namely Pak Nai, Ha Pak Nai, Shui Hau Wan and San Tau, at monthly intervals from March to August 2005, using both the transect study and walk-through observations. All the sites supported *T. tridentatus* populations with a maximum abundance of 85 individuals at Shui Hau Wan in April. Few (1-2 individuals) *C. rotundicauda* were found at Pak Nai and Ha Pak Nai, while a higher abundance (total 64 individuals) was recorded at San Tau on Lantau Island in the 6-month survey. However, its overall population was much lower as compared with *T. tridentatus*. Among the sites, San Tau and Shui Hau Wan were the key nursery grounds for *C. rotundicauda* and *T. tridentatus*, respectively. The active period for horseshoe crabs lasted from May to July with more individuals being found as compared with the inactive period in March, April and August. A wide range of age groups of horseshoe crabs were recorded, with a maximum prosomal width ranging from 10.1 to 96.1 mm and 10.9 to 43.3 mm for *T. tridentatus* and *C. rotundicauda*, respectively. A significant spatial variation in the abundance of *T. tridentatus* on the shore was obtained with larger individuals on the lower shores. As individuals of *T. tridentatus* <12.0 mm (maximum prosomal width) were recorded in the present survey, these sites were certainly important nursery grounds for juveniles and newly hatched individuals.

Both morphological measurements and genetic analysis were used for the differentiation of the two commonly occurring horseshoe crab species in Hong Kong. For morphological measurements, *T. tridentatus* and *C. rotundicauda* were significantly different in various ratios of body parts. Only 5 out of the 9 parameters were proven important in differentiating the two species; they are the ratios of prosomal length to maximum prosomal width, maximum prosomal width to distance between two compound eyes, carapace length to telson length, first opisthosomal spine length to maximum prosomal width, and sixth opisthosomal spine length to maximum prosomal width. For phylogenetic comparisons, the inter-specific variations of both 18S and 28S rDNA sequence were very small, and was <1% and 1.57%, respectively. Thus, these two rDNA regions may not be powerful enough for the differentiation of *T. tridentatus* and *C. rotundicauda*. The 18S and 28S rDNA of “abnormal” juvenile *T. tridentatus* individuals with only 1 immovable spine on the dorsal surface of the opisthosoma above the insertion of a post-anal spine, and “normal” individuals with 3 immovable spines were also sequenced. Both juvenile groups were genetically closer to *C. rotundicauda* but morphologically closer to adult *T. tridentatus*. There was no significant spatial variation in the genetic pattern of juvenile *T. tridentatus* at five nursery grounds, Pak Nai, Ha Pak Nai, San Tau, Shui Hau Wan and Yi O, based on 18-28S intergenic spacer sequence (ITS) rDNA, suggesting that these horseshoe crab populations may come from a common population.

The degree of human exploitation of horseshoe crabs in Hong Kong was estimated through interviewing 34 seafood restaurants, 150 fish sellers and fish handlers. A total of 1,023 horseshoe crabs were caught in 2004-05, with 72% from mainland Chinese waters. Of these, 33% were released back to the sea after being caught and only 690 individuals were retained on board. The majority (62%) of them were sold and used for the Chinese traditional set-free rituals, while the remaining were kept and sold in fish stalls and seafood restaurants. An average sale of 17 horseshoe crabs per month was estimated throughout the 13-month study, with 45% of them being obtained from Hong Kong waters. The sale of horseshoe crabs was high by comparing with other popular commercial marine species, in view of the low population density, long maturity period and low breeding rate of horseshoe crabs. Hence, human exploitation is possibly one of the major impacts affecting the long-term survival of this animal in Hong Kong.

In view of the low natural breeding and hatching success, and low juvenile survival, artificial insemination and breeding may be reasonable options to enhance the horseshoe population in the natural environment. Trials of artificial breeding were conducted in the summer of 2004. Several thousand trilobite larvae hatched with some of them further molted into juveniles of second to fifth instars. Mortality rate was high after hatching and varied over time. After almost two years of laboratory rearing, only some 20 individuals of the first batch of trilobites survived to become juveniles. To elucidate the optimum conditions for the survival and hatching of eggs, a two factorial experiment was conducted with combinations of four salinities (15‰, 20‰, 25‰ and 30‰) and three temperatures (20°C, 25°C and 32°C) being studied. Horseshoe crab eggs survived at a wide range of temperatures (20–32°C) and salinities (20–30‰). However, no hatching was observed at 15‰. Although the survivorship at low temperatures and low salinities were relatively high, the developmental and hatching rates were reduced. The highest hatching rate was obtained at 32°C and 30‰ which should be recommended for future artificial breeding practices. Trials on non-invasive artificial breeding methods using electrical stimulation for the collection of unfertilized eggs and sperms were also performed. Several successful trials were undertaken, however, the number of eggs obtained was small. Further studies are required to ascertain the applicability and repeatability of this method.

The present study provides comprehensive baseline information on the horseshoe crabs in Hong Kong that may form a basis for the implementation of conservation measures in the future.

Significance of the Study

Horseshoe crabs have been facing global decline in the past decades. Species specific conservation and management strategy of horseshoe crab are urgently required. The present study provides comprehensive baseline information on the horseshoe crabs in Hong Kong, including:

1) Status evaluation of horseshoe crabs in Hong Kong

The distributions and population densities of the juveniles of the two horseshoe crab species at various nursery grounds in Hong Kong was updated by both extensive and intensive 6-month population studies. The distribution of adult horseshoe crabs was also investigated by interviewing the local seafood markets and fishermen. These distribution studies provided scientific evidence on the sharp decline in local horseshoe crab populations from 2002 to 2005.

2) Threat determination of horseshoe crabs in Hong Kong

Human exploitation, including harvest and sale of horseshoe crabs (purpose for set-free rituals, display and sale for dishes) in Hong Kong was evaluated in the present study. The market survey showed a potential risk of human exploitation on the local horseshoe crab populations.

3) Population enhancement of horseshoe crabs in Hong Kong

Various artificial breeding and rearing practices for *T. tridentatus* have been explored in the present study. It provided important basic knowledge for further artificial breeding and restocking of horseshoe crabs in Hong Kong.

4) Taxonomic and population genetic study of horseshoe crabs in Hong Kong

Differentiating the juvenile forms of the two commonly occurred horseshoe crab species, *T. tridentatus* and *C. rotundicauda* in Hong Kong, using both morphological and genetic approaches, and population genetic of *T. tridentatus* in various nursery grounds have been performed in this study. It provided basic knowledge of the speciation and spatial genetic variation of juvenile horseshoe crabs in local nursery grounds.

Based on these baseline data on local horseshoe crabs, species specific management tools can be designed and undertaken to minimize the threats, conserve the species, and foster sustainability or recovery of it by a dynamic and responsive process. Hence, this study provided vital information for future conservation and management planning of horseshoe crabs in Hong Kong.

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