

**CITY UNIVERSITY OF HONG KONG**  
香港城市大學

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

Submitted to  
Department of Biology and Chemistry  
生物及化學系  
in Partial Fulfillment of the Requirements  
for the Degree of Master of Philosophy  
哲學碩士學位

by

**LI Hiu Yan**  
李曉恩

September 2008  
二零零八年九月

Abstract of thesis entitled

## **The Conservation of Horseshoe Crabs in Hong Kong**

香港馬蹄蟹的保育研究

Submitted by

**LI Hiu Yan**

for the Degree of Master of Philosophy at the City University of Hong Kong

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.

## Table of Contents

<b>Declaration</b>	i
<b>Abstract</b>	ii
<b>Significance of the Study</b>	vii
<b>Thesis Acceptance</b>	ix
<b>Acknowledgements</b>	x
<b>Table of Contents</b>	xii
<b>List of Tables</b>	xvii
<b>List of Figures</b>	xxii
<b>Chapter 1 General Introduction</b>	1
1.1 History of Horseshoe Crabs	1
1.2 Biology of Horseshoe Crabs	1
1.3 Population Study on Horseshoe Crabs	4
1.4 Importance of Horseshoe Crabs	6
1.5 Decline in Horseshoe Crab Populations	9
1.6 Risks Facing by Horseshoe Crabs	10
1.7 Conservation of Horseshoe Crabs	11
1.8 Artificial Breeding of Horseshoe Crabs	12
1.9 Genetic Study of Horseshoe Crabs	13
1.10 Objectives	15
1.11 Organization of the Thesis	16
<b>Chapter 2 Survey of Horseshoe Crabs in Hong Kong</b>	17
2.1 Introduction	17
2.2 General Distribution and Abundance of Juvenile Horseshoe Crabs on Soft Shores in Hong Kong	18
2.2.1 Materials and Methods	18
2.2.1.1 <i>Site Characteristics</i>	18
2.2.1.2 <i>Sample Collection</i>	23
2.2.1.3 <i>Statistical Analysis</i>	26
2.2.2 Results	26
2.2.2.1 <i>Distribution and Abundance of Horseshoe Crabs</i>	26
2.2.2.2 <i>Environmental Parameters of the Study Sites</i>	41

2.2.2.3	<i>Size of the Horseshoe Crabs on the Shores</i>	50
2.3	Temporal Variations in the Population Density of Horseshoe Crabs at Four Nursery Grounds	57
2.3.1	Materials and Methods	57
2.3.1.1	<i>Site Characteristics</i>	57
2.3.1.2	<i>Sample Collection</i>	57
2.3.1.2	<i>Statistical Analysis</i>	58
2.3.2	Results	59
2.3.2.1	<i>Temporal Variations in the Population Density of Horseshoe Crabs</i>	59
2.3.2.2	<i>Environmental Parameters of the Shores</i>	68
2.3.2.3	<i>Size of the Horseshoe Crabs on the Shores</i>	78
2.4	Discussion	102
2.4.1	Updated Distribution of Juvenile Horseshoe Crabs in Hong Kong	102
2.4.1.1	<i>T. tridentatus</i>	104
2.4.1.2	<i>C. rotundicauda</i>	106
2.4.2	Local Distribution Patterns of the Two Horseshoe Crab Species	107
2.4.3	Status of Horseshoe Crabs in Hong Kong	110
2.4.3.1	<i>T. tridentatus</i>	110
2.4.3.2	<i>C. rotundicauda</i>	111
2.4.4	Temporal Variations in the Distribution of Horseshoe Crabs	112
2.4.5	Size Distributions of Horseshoe Crabs	113
<b>Chapter 3</b>	<b>To Differentiate <i>Tachypleus tridentatus</i> from <i>Carcinoscorpius rotundicauda</i> using Morphological and Genetic Studies and to Study Genetic Relationships among Horseshoe Crabs from Various Nursery Grounds in Hong Kong</b>	<b>116</b>
3.1	Introduction	116
3.2	Morphological Study of the Two Horseshoe Crab Species	119
3.2.1	Materials and Methods	119
3.2.1.1	<i>Measurement of Various Body Parts</i>	119
3.2.1.2	<i>Statistical Analysis</i>	119

3.2.2	Results	123
3.2.2.1	<i>Qualitative Comparisons</i>	123
3.2.2.2	<i>Quantitative Comparisons</i>	125
3.3	Genetic Differentiation of Horseshoe Crab Species	133
3.3.1	Materials and Methods	133
3.2.1.1	<i>Sample Collection</i>	133
3.2.1.2	<i>DNA Extraction, PCR Amplification and Sequencing</i>	135
3.2.1.3	<i>Statistical Analysis</i>	135
3.3.2	Results	136
3.2.2.1	<i>18S rDNA Gene Comparison</i>	136
3.2.2.2	<i>28S rDNA Gene Comparison</i>	141
3.4	Genetic Relationships among Horseshoe Crabs from Various Nursery Grounds in Hong Kong	146
3.4.1	Materials and Methods	146
3.4.1.1	<i>Sample Collection</i>	146
3.2.1.2	<i>DNA Extraction, PCR Amplification and Sequencing</i>	148
3.2.1.3	<i>Statistical Analysis</i>	148
3.4.2	Results	148
3.5	Discussion	153
3.5.1	Morphological and Genetic Differentiation in Horseshoe Crabs	153
3.5.2	Genetic Relationships among Horseshoe Crabs from Various Nursery Grounds	155
<b>Chapter 4 Assessment of Human Exploitation of Horseshoe Crabs in Hong Kong</b>		159
4.1	Introduction	159
4.2	Materials and Methods	159
4.2.1	Site Characteristics	159
4.2.2	Data Collection	159
4.3	Results	159
4.3.1	Catch of Horseshoe Crabs	161
4.3.2	Sale of Horseshoe Crabs	166
4.3.2.1	<i>Set-free Rituals</i>	166

4.3.2.2	<i>Display of Horseshoe Crabs</i>	168
4.3.2.3	<i>Serving Horseshoe Crabs for Dishes</i>	170
4.3	Discussion	173
4.4.1	Population of Horseshoe Crabs in Open Waters	173
4.4.2	Human Exploitation of Horseshoe Crabs	175
4.4.3	Potential Risks to Horseshoe Crabs	179
<b>Chapter 5 Trials on Artificial Breeding of Horseshoe Crabs</b>		181
5.1	Introduction	181
5.2	Artificial Breeding of Horseshoe Crabs	182
5.2.1	Materials and Methods	182
5.2.2	Results	185
5.3	Effects of Temperature and Salinity on Egg Development	190
5.3.1	Materials and Methods	190
5.3.1.1	<i>Fertilization of Eggs</i>	190
5.3.1.2	<i>Incubation under Different Combinations of Temperature and Salinity</i>	190
5.3.1.3	<i>Statistical Analysis</i>	190
5.3.2	Results	192
5.3.2.1	<i>Survival Rate of Horseshoe Crab Eggs</i>	192
5.3.2.2	<i>Hatching Rate of Horseshoe Crab Eggs</i>	197
5.4	Preliminary Study on Alternative Artificial Breeding Method	199
5.4.1	Materials and Methods	199
5.4.1.1	<i>Check for the Maturity of Horseshoe Crab Eggs</i>	199
5.4.1.2	<i>Electrical Stimulation</i>	199
5.4.2	Results	201
5.5	Discussion	203
5.5.1	Trials on Artificial Insemination	203
5.5.2	Incubation of Eggs in the Laboratory	205
5.5.3	Survival of Trilobites and Juveniles	206
<b>Chapter 6 General Discussion</b>		208
6.1	Recommendations for Further Study	210

6.1.1	Walk-through Method and Mark-recapture Method for Distribution Study	210
6.1.2	Study on Adult Horseshoe Crabs	212
6.1.3	In-depth Studies on Phylogenetic and Geographical Genetic Variations in Juvenile Horseshoe Crabs	213
6.1.4	Risk Assessment of Horseshoe Crabs	215
6.1.5	More Studies on <i>Carcinoscorpius rotundicauda</i>	216
6.1.6	Non-destructive Artificial Breeding Technique	217
6.1.7	Further Study on the Requirements for Juvenile Rearing	218
6.2	Proposed Conservation Measures	219
6.2.1	Re-introduction Programme	220
6.2.2	Ban on Fishing and Possession of Horseshoe Crabs in Hong Kong	221
6.2.3	Designation of Protected Areas	222
6.2.4	Regular Monitoring Study on Horseshoe Crabs Distribution	223
6.2.5	Public Education	223
<b>Chapter 7 Conclusion</b>		<b>225</b>
<b>Chapter 8 References</b>		<b>227</b>
<b>Appendix 1 Questionnaire for Assessing Human Exploitation of Horseshoe Crabs in Hong Kong</b>		<b>250</b>

## List of Tables

Table 2.1	Information on the 17 sampling sites in New Territories, and on Lantau Island and Lamma Island in Hong Kong.	21
Table 2.2	Total number and density of <i>T. tridentatus</i> and various environmental parameters of the 17 sampling sites in New Territories, and on Lantau Island and Lamma Island in Hong Kong in the summer distribution study.	28
Table 2.3	Total number and density of <i>T. tridentatus</i> and various environmental parameters of the 17 sampling sites in New Territories, and on Lantau Island and Lamma Island in Hong Kong in the winter distribution study.	30
Table 2.4	Total number of <i>T. tridentatus</i> at various tidal levels in New Territories, and on Lantau Island and Lamma Island in Hong Kong.	32
Table 2.5	Results of the Kruskal-Wallis test for the differences in the population density of juvenile <i>T. tridentatus</i> at the 17 sites in the summer and winter.	36
Table 2.6	Abundance and population density (individual hour <sup>-1</sup> person <sup>-1</sup> ) of juvenile <i>T. tridentatus</i> obtained by the walk-through survey at the 17 study sites in the summer and winter.	38
Table 2.7	Abundance and population density (individual hour <sup>-1</sup> person <sup>-1</sup> ) of juvenile <i>C. rotundicauda</i> at the 17 study sites in the summer and winter.	39
Table 2.8	Results of the 3-way ANOVA test for the differences in temperature at the 17 sites in the summer and winter (tidal level × site × season).	43

Table 2.9	Results of the 3-way ANOVA test for the differences in salinity at the 17 sites in the summer and winter (tidal level $\times$ site $\times$ season).	46
Table 2.10	Results of the 3-way ANOVA test for differences in the DO level at the 17 sites in the summer and winter (tidal level $\times$ site $\times$ season).	49
Table 2.11	3-way ANOVA (tidal level, site and season) results on the prosomal width of juvenile <i>T. tridentatus</i> recorded by the walk-through survey.	52
Table 2.12	3-way ANOVA (tidal level, site and season) results on the prosomal width of juvenile <i>C. rotundicauda</i> recorded by the walk-through survey.	55
Table 2.13	Results of the Kruskal-Wallis test for differences in the population density of <i>T. tridentatus</i> at the 4 sites from March to August 2005.	63
Table 2.14	Abundance (total number of individuals) and population density (individual hour <sup>-1</sup> person <sup>-1</sup> ) of juvenile <i>T. tridentatus</i> and <i>C. rotundicauda</i> recorded by the walk-through survey at the four sites from March to August 2005.	65
Table 2.15	Results of the 3-way ANOVA test for differences in temperature at the four sites from March to August 2005 (tidal level $\times$ site $\times$ time).	70
Table 2.16	Results of the 3-way ANOVA test for differences in salinity at the four sites from March to August 2005 (tidal level $\times$ site $\times$ time).	74

Table 2.17	Results of the 3-way ANOVA test for differences in DO level at the four sites from March to August 2005 (tidal level $\times$ site $\times$ time).	77
Table 2.18	Prosomal width (PW) of <i>T. tridentatus</i> found at the 4 sites.	79
Table 2.19	Results of the Kruskal-Wallis test for the differences in prosomal width of <i>T. tridentatus</i> at the four sites from March to August 2005.	84
Table 2.20	Average prosomal width ( $\pm$ SD) of individual cohorts of <i>T. tridentatus</i> at the four sites as identified by FiSAT II.	91
Table 2.21	Measurements of prosomal width (PW) of <i>C. rotundicauda</i> found at the four sites.	94
Table 2.22	Results of the Kruskal-Wallis test for the differences in prosomal width of <i>C. rotundicauda</i> at the four sites from March to August 2005.	98
Table 2.23	Records of juvenile horseshoe crabs obtained from previous surveys by Chiu and Morton (1999a), Morton and Lee (2003) and the AFCD monitoring survey (AFCD field data).	103
Table 3.1	The means ( $\pm$ SD) and ranges of various morphological ratios in juvenile <i>T. tridentatus</i> and <i>C. rotundicauda</i> .	127
Table 3.2	Results of the test of equality of discriminant analysis for differences in various morphological ratios between <i>T. tridentatus</i> and <i>C. rotundicauda</i> .	128
Table 3.3	Comparisons of the morphology between <i>T. tridentatus</i> and <i>C. rotundicauda</i> .	129

Table 3.4	Significant morphological ratios and their discriminant function coefficients of <i>T. tridentatus</i> and <i>C. rotundicauda</i> , derived from the stepwise multiple discriminant analysis.	131
Table 3.5	Grouping of horseshoe crab specimens used in the study of 18S and 28S rDNA.	134
Table 3.6	Percentage of pair-wise difference in 18S rDNA gene of horseshoe crab individuals and number of transitions/transversions.	138
Table 3.7	Percentage of pair-wise difference in 18 rDNA gene within and among each horseshoe crab species (mean $\pm$ SD).	139
Table 3.8	Percentage of pair-wise difference in 28S rDNA gene of horseshoe crab individuals and number of transitions/transversions.	143
Table 3.9	Percentage of pair-wise difference in 28 rDNA gene within and among each horseshoe crab species (mean $\pm$ SD). Bracketed values indicate the range of the data.	144
Table 3.10	Grouping of horseshoe crab specimens used in the study of 18-28S intergenic spacer sequence (ITS) rDNA.	147
Table 3.11	Percentage of pair-wise difference in the 18-28S ITS rDNA gene of horseshoe crab individuals and number of transitions / transversions.	150
Table 3.12	Percentage of pair-wise difference in the 18-28S ITS rDNA gene for <i>T. tridentatus</i> within each study site and among different study sites (mean $\pm$ SD).	151

Table 4.1	Historical records of adult horseshoe crabs in open waters in Hong Kong with reference to Chiu and Morton (1999a).	174
Table 5.1	Different combinations of temperature and salinity were used in culturing the eggs of <i>T. tridentatus</i> .	191
Table 5.2	Results of the two-way ANOVA test followed by the Tukey multiple comparison test for differences in the survival rate of the horseshoe crab eggs at 3 temperatures and 4 salinities.	193
Table 5.3	Results of the multiple comparisons of the effect of temperature at individual salinities and the effect of salinity at individual temperatures on the survival rate of horseshoe crab eggs.	196
Table 5.4	Number of juvenile horseshoe crabs hatched under different combinations of temperature and salinity for 90 days.	198

## List of Figures

Figure 1.1	Three major body parts of horseshoe crab ( <i>Tachypleus tridentatus</i> ).	3
Figure 2.1	The 17 distribution study sites in New Territories, on Lantau Island and on Lamma Island.	20
Figure 2.2	Sampling design for the distribution survey of juvenile horseshoe crabs in Hong Kong.	25
Figure 2.3	Mean density (+SE) of juvenile <i>T. tridentatus</i> recorded by the random quadrat sampling.	34
Figure 2.4	Mean density (+SE) of juvenile <i>T. tridentatus</i> found at the four tidal levels recorded by the random quadrat sampling.	35
Figure 2.5	Population distribution of the horseshoe crabs at the 17 sites by the walk-through survey.	40
Figure 2.6	Temporal variations in temperature ( $\pm$ SD) at the 17 sites in the summer and winter.	42
Figure 2.7	Temporal variations in salinity of the interstitial waters ( $\pm$ SD) at the 17 sites in the summer and winter.	45
Figure 2.8	Temporal variations in the DO level ( $\pm$ SD) of the interstitial waters at the 17 sites in the summer and winter.	48
Figure 2.9	Mean (+SE) prosomal width of juvenile <i>T. tridentatus</i> recorded by the walk-through survey at the 17 sites in the summer and winter.	51
Figure 2.10	Mean (+SE) prosomal length of juvenile <i>T. tridentatus</i> found at the four tidal levels.	53

- Figure 2.11 Mean (+SE) prosomal width of juvenile *C. rotundicauda* recorded by the walk-through survey at the 17 sites in the summer and winter. 54
- Figure 2.12 Mean (+SE) prosomal length of juvenile *C. rotundicauda* found at the four tidal levels recorded by the walk-through survey. 56
- Figure 2.13 Mean density of juvenile *T. tridentatus* at the four sites recorded by the random sampling method from March to August 2005. 61
- Figure 2.14 Spatial distribution (tidal levels) (+SE) of juvenile *T. tridentatus* at the four sites recorded by the random sampling method from March to August 2005. 62
- Figure 2.15 Spatial distribution (tidal levels) (+SE) of juvenile *T. tridentatus* at the four sites recorded by the walk-through survey from March to August 2005. 66
- Figure 2.16 Spatial distribution (tidal levels) (+SE) of juvenile *C. rotundicauda* at the four sites recorded by the walk-through survey from March to August 2005. 67
- Figure 2.17 Temporal variations in average temperature ( $\pm$  SD) at the four sites from March to August 2005. 69
- Figure 2.18 Temporal variations in the average interstitial water salinity ( $\pm$  SD) at the four sites from March to August 2005. 73
- Figure 2.19 Temporal variations in the average DO level ( $\pm$  SD) of the interstitial water at the four sites from March to August 2005. 76
- Figure 2.20 Spatial variations (among sites and tidal levels) of the prosomal width (+SE) of *T. tridentatus* from March to August 2005. 81

- Figure 2.21 Temporal variations of the prosomal width (+SE) of *T. tridentatus* from March to August 2005. 83
- Figure 2.22 Size-frequency distributions of *T. tridentatus* at Site 1 (Pak Nai), Site 2 (Ha Pak Lai), Site 3 (Shui Hau Wan), and Site 4 (San Tau). 87
- Figure 2.23 Spatial variations (both among sites and tidal levels) of the prosomal width (+SE) of *C. rotundicauda* from March to August 2005. 96
- Figure 2.24 Temporal variations of the prosomal width (+SE) of *C. rotundicauda* from March to August 2005. 97
- Figure 2.25 Size-frequency distributions of *C. rotundicauda* at Site 1 (Pak Nai), Site 2 (Ha Pak Lai), and Site 4 (San Tau). 100
- Figure 3.1 Photos of juvenile horseshoe crabs found on the shores. 121
- Figure 3.2 Various body parts of a horseshoe crab were measured to the nearest 0.1mm. 122
- Figure 3.3 The dorsal view of a) *Tachypleus tridentatus*; b) *Carcinoscorpius rotundicauda*. 124
- Figure 3.4 Plot of the stepwise multiple discriminant analysis based on various morphological ratios in *T. tridentatus* (T.t.) and *C. rotundicauda* (C.r.). 132
- Figure 3.5 Neighbor-joining tree for the horseshoe crab 18S rDNA gene sequences. 140
- Figure 3.6 Neighbor-joining tree for the horseshoe crab 28S rDNA gene sequences. 145

Figure 3.7	Neighbor-joining tree for the horseshoe crabs 18-28S ITS rDNA gene sequences.	152
Figure 4.1	Number of <i>Tachypleus tridentatus</i> being caught in Hong Kong and China waters, from September 2004 to September 2005.	163
Figure 4.2	Number of <i>Tachypleus tridentatus</i> being caught in Hong Kong waters, with proportions for sale and set-free, from September 2004 to September 2005.	164
Figure 4.3	Number of <i>Tachypleus tridentatus</i> being caught in China waters, with proportions for sale and set-free, from September 2004 to September 2005.	165
Figure 4.4	The sale of horseshoe crabs for the release and non-release activities from September 2004 to September 2005.	167
Figure 4.5	Number of <i>T. tridentatus</i> displayed in fish stalls and seafood restaurants from September 2004 to September 2005.	169
Figure 4.6	The advertisements of horseshoe crab dishes were shown in a) Cheung Chau seafood restaurant; b) Causeway Bay seafood restaurant.	171
Figure 4.7	Estimated sale of horseshoe crabs for local consumption from September 2004 to September 2005. Individuals being caught from both Hong Kong and China waters are shown.	173
Figure 5.1	The direct extraction of horseshoe crab eggs; a) body view of a female horseshoe crab; b) extraction of eggs from one side of the ovary of a female horseshoe crab.	183
Figure 5.2	Fertilized eggs were incubated in a water table with aeration and temperature control.	184

Figure 5.3	Various developmental stages of horseshoe crab eggs.	188
Figure 5.4	Different instars of the horseshoe crab; a) second instar; b) third instar; c) fourth instar.	189
Figure 5.5	The survival rate ( $\pm$ SE) of the horseshoe crab eggs under different temperatures at the salinity of a) 15‰; b) 20‰; c) 25‰; d) 30‰.	194
Figure 5.6	The survival rate ( $\pm$ SE) of horseshoe crab eggs under different salinities at the temperature of a) 20°C; b) 25°C; c) 32°C.	195
Figure 5.7	Photos showing a) the position of the gonopores on the ventral side of the genital operculum; b) electrical shock applied 1 cm beneath the gonopores of a female <i>T. tridentatus</i> .	200
Figure 5.8	Photos showing a) a wide view; b) a close up of the horseshoe crab eggs released from the gonopores after electrical stimulation.	202