

**MORPHOMETRIC AND GENETIC VARIABILITY
OF RIVER TERRAPIN, (*Batagur baska*) AND
PAINTED TERRAPIN, (*Batagur borneoensis*)**

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**MORPHOMETRIC AND GENETIC VARIABILITY OF RIVER
TERRAPIN, (*Batagur baska*) AND PAINTED TERRAPIN,
(*Batagur borneoensis*)**

by

NORKARMILA BINTI DULI

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TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	ii
TABLE OF CONTENTS	iv
LIST OF TABLES	viii
LIST OF FIGURES	x
LIST OF PLATES	xi
LIST OF ABBREVIATIONS	xiii
LIST OF APPENDICES	xvi
ABSTRAK	xvii
ABSTRACT	xix
CHAPTER 1 : INTRODUCTION	1
CHAPTER 2 : LITERATURE REVIEW	5
2.1 Species Description	5
2.1.1 Morphology	5
2.1.2 Zoogeography and Habitat	9
2.1.2.1 <i>Batagur baska</i>	11
2.1.2.2 <i>Batagur borneoensis/ Callagur borneoensis</i>	11
2.1.3 Movement	12
2.1.3.1 <i>Batagur baska</i>	12
2.1.3.2 <i>Batagur borneoensis/ Callagur borneoensis</i>	13
2.1.4 Reproduction	13
2.1.4.1 <i>Batagur baska</i>	14
2.1.4.2 <i>Batagur borneoensis/ Callagur borneoensis</i>	15
2.1.5 Studies on Growth and Maturity	18
2.1.5.1 <i>Batagur baska</i>	18
2.1.5.2 <i>Batagur borneoensis/ Callagur borneoensis</i>	18
2.1.6 Dietary Requirement	19
2.1.6.1 <i>Batagur baska</i>	19
2.1.6.2 <i>Batagur borneoensis/ Callagur borneoensis</i>	19

2.1.7	Population Status	20
	2.1.7.1 <i>Batagur baska</i>	20
	2.1.7.2 <i>Batagur borneoensis/ Callagur borneoensis</i>	21
2.1.8	Causes of Decline	22
	2.1.8.1 Human consumption	22
	2.1.8.2 Habitat alteration and destruction	23
	2.1.8.3 Other factors	24
2.1.9	Conservation Effort	24
	2.1.9.1 <i>Batagur baska</i>	25
	2.1.9.2 <i>Batagur borneoensis/ Callagur borneoensis</i>	26
2.1.10	Law Enforcement	27
2.2	Genetic Variation	28
	2.2.1 Conservation Genetics	28
	2.2.2 Molecular Techniques	30
	2.2.3 Genetic Marker: Mitochondrial DNA (MtDNA)	30
	2.2.4 Population and Phylogeographic Analyses of Sequence Data	33
2.3	Morphometric	34
	2.3.1 Statistical Analysis	36
	CHAPTER THREE: MATERIALS AND METHODS	38
3.1	Sampling Site	38
	3.1.1 East Coast of Peninsular Malaysia	42
	3.1.1.1 Kg. Mangkuk & Kuala Baru, Setiu River	42
	3.1.1.2 Lubuk Kawah, Teregganu River	43
	3.1.1.3 Rhu Kudung, Paka River	43
	3.1.1.4 Cherating River	46
	3.1.2 West Coast of Peninsular Malaysia	48
	3.1.2.1 Muda River	48
	3.1.2.2 Bukit Pinang, Kedah River	48
	3.1.2.3 Bota Kanan, Perak River	53
	3.1.2.4 Linggi River	53
3.2	Sampling Method	56
	3.2.1 Morphometric Measurement	56

3.2.2	Blood Sampling Technique	57
3.3	Data Analysis	62
3.4	Molecular Analysis	62
3.4.1	Lysis Buffer Preparation	62
3.4.2	DNA Extraction	64
3.4.3	Amplification of mtDNA gene	65
3.4.3.1	PCR amplification	67
3.4.4	Purification	69
3.4.5	Gel Preparation - Visualisation of DNA Extract and PCR Product	69
3.4.6	Data Analysis	71
	CHAPTER FOUR: RESULT	74
4.1	Morphometric Analysis	74
4.1.1	<i>Batagur baska</i>	74
4.1.1.1	Descriptive Statistic	74
4.1.1.2	Univariate Analysis of Variance (ANOVA)	81
4.1.1.3	Multivariate Analysis	84
4.1.1.3(a)	Principal Component Analysis (PCA)	84
4.1.1.3(b)	Discriminant Function Analysis (DFA)	86
4.1.2	<i>Batagur borneoensis</i> / <i>Callagur borneoensis</i>	91
4.1.2.1	Descriptive Statistic	91
4.1.2.2	Univariate Analysis of Variance (ANOVA)	97
4.1.2.3	Multivariate Analysis	100
4.1.2.3(a)	Principal Component Analysis (PCA)	100
4.1.2.3(b)	Discriminant Function Analysis (DFA)	102
4.2	Molecular Analysis	107
4.2.1	Extraction	107
4.2.2	Polymerase Chain Reaction (PCR)	109
4.2.3	Purification	109
4.2.4	Sequencing Data	109

4.2.5 Population Analysis	112
4.2.5.1 <i>Batagur baska</i>	112
4.2.5.1(a) Maximum Parsimony	112
4.2.5.1(b) Neighbor Joining	116
4.2.5.2 <i>Batagur borneoensis</i> / <i>Callagur borneoensis</i>	123
4.2.5.2(a) Maximum Parsimony	123
4.2.5.2(b) Neighbor Joining	127
4.2.6 Phylogenetic Analysis	133
CHAPTER FIVE: DISCUSSION	136
5.1 Morphometric	136
5.2 Molecular Analysis	139
5.2.1 <i>Batagur baska</i>	139
5.2.2 <i>Batagur borneoensis</i> / <i>Callagur borneoensis</i>	141
5.3 Recommendations for Conservation Program.	145
CHAPTER SIX: CONCLUSION	151
REFERENCES	153
APENDIX A	
APENDIX B	
APENDIX C	
APENDIX D	
LIST OF PUBLICATIONS & SEMINARS	

LIST OF TABLES

Table		Page
2.1	Thirty four rivers in Peninsular Malaysia surveyed by Moll (1990) for the occurrence of <i>Batagur baska</i> and <i>Batagur borneoensis</i> .	10
3.1	Summary of sampling sites with total number of sample.	41
3.2	Lysis Buffer preparation	63
3.3	Primer sequences amplifying a partial region of the mtDNA genes, (Cytochrome-b and D-loop)	66
3.4	Concentration and volume of PCR chemicals used	68
3.5	PCR programme used for both sets of primer	68
3.6	Composition of Agarose gel	68
4.1	Number of samples used for morphometric analysis	75
4.2	Descriptive analysis of five populations of <i>B. baska</i> in Peninsular Malaysia (cm)	76
4.3	ANOVA results of 10 characters measured for <i>B. baska</i>	82
4.4	Character loading on the rotated actor matrix of the 1st and 2nd component in <i>B. baska</i>	85
4.5	Extraction of discriminant function based on DFA in <i>B. baska</i>	87
4.6	Standardized canonical discriminant function coefficients in <i>B. baska</i>	87
4.7	Predicted classification generated by DFA in <i>B. baska</i>	90
4.8	Pairwise group comparison based on Mahalanobis method in <i>B. baska</i>	90
4.9	Descriptive analysis of four populations of <i>Batagur borneoensis</i> in Peninsular Malaysia (cm)	92
4.10	ANOVA results of 10 characters measured for <i>B. borneoensis</i> .	98
4.11	Character loadings on the rotated factor matrix of the 1st and 2nd component in <i>B. borneoensis</i>	101

4.12	Extraction of discriminant function based on DFA in <i>B. borneoensis</i>	103
4.13	Standardized canonical discriminant function coefficients in <i>B. borneoensis</i>	103
4.14	Predicted classification generated by DFA in <i>B. borneoensis</i>	106
4.15	Pairwise group comparison based on Mahalanobis method in <i>B. borneoensis</i>	106
4.16	Number of samples applied in the molecular analysis	108
4.17	The range and mean genetic distance within and among populations of <i>B. baska</i> generated by MP analysis	113
4.18	The range and mean genetic distance within and among populations of <i>B. baska</i> generated by NJ analysis	117
4.19	Nucleotide and gene diversity, polymorphic sites and number of haplotypes of the D-loop gene segment of <i>B. baska</i> among all populations	120
4.20	Polymorphic sites observed for mtDNA D-loop haplotype of <i>B. baska</i> .	121
4.21	Distribution of number of individuals for each <i>B. baska</i> haplotype	122
4.22	The range and mean genetic distance within and among populations of <i>B. borneoensis</i> generated by MP analysis	124
4.23	The range and mean genetic distance within and among populations of <i>B. borneoensis</i> generated by NJ analysis	128
4.24	Nucleotide and gene diversity, polymorphic sites and number of haplotypes of the D-loop gene segment of <i>B. borneoensis</i> among all populations.	131
4.25	Polymorphic sites observed for mtDNA D-loop haplotype of <i>B. borneoensis</i>	131
4.26	Distribution of number of individuals for each <i>B. borneoensis</i> haplotype	132

LIST OF FIGURES

Figure		Page
3.1	Project study sites along several rivers in Peninsular Malaysia.	40
3.2	Measuring method for turtle shell and tail	61
3.3	(a) DNA extraction method (b) Purification method	70
4.1	Population means for morphometric variables of <i>Batagur baska</i>	78
4.2	Summary results of SNK tests on five populations of <i>B. baska</i>	83
4.3	Scatterplot of individual distribution generated based on PCA in <i>B. baska</i>	85
4.4	Scatterplot of individual distribution generated based on DFA in <i>B. baska</i>	88
4.5	Population means for morphometric variables of <i>B. borneoensis</i>	94
4.6	Summary result of SNK test on four populations of <i>B. borneoensis</i>	99
4.7	Scatterplot of individuals' distribution generated based on PCA in <i>B. borneoensis</i>	101
4.8	Scatterplot of individuals' distribution generated based on DFA in <i>B. borneoensis</i>	104
4.9	Phylogenetic tree generated by MP analysis of <i>B. baska</i>	115
4.10	Phylogenetic tree generate by NJ analysis of <i>B. baska</i>	118
4.11	Phylogenetic tree generated by MP analysis of <i>B. borneoensis</i>	126
4.12	Phylogenetic tree generated by NJ analysis of <i>B. borneoensis</i>	129
4.13	Phylogenetic tree generated from combined <i>B. baska</i> and <i>B. borneoensis</i> by NJ analysis	134
4.14	Phylogenetic tree generated from combined <i>B. baska</i> and <i>B. borneoensis</i> by MP analysis	135

LIST OF PLATES

Plate	Page
2.1 (a) Male of <i>B. borneoensis</i> (b) Male of <i>B. baska</i>	8
2.2 (a) Female of <i>B. borneoensis</i> (b) Female of <i>B. baska</i>	8
2.3 <i>B. baska</i> male showing breeding colouration	17
2.4 <i>B. borneoensis</i> male showing breeding colouration	17
3.1 Lubuk Kawah sand island	44
3.2 <i>B. baska</i> track at Lubuk Kawah	44
3.3 Rhu Kudung nesting site at Paka. In red circle is the TNB Power Station	45
3.4 <i>B. borneoensis</i> track at Rhu Kudung beach shown by the arrow.	45
3.5 Cherating River mouth	47
3.6 Construction of a new dam at Muda River just few km ahead of the Jajar Dam	50
3.7 Jajar Dam across the Muda River at Kuala Muda site, which block the turtles' movement to the upper stream to nest	50
3.8 A net set across the former nesting beach during nesting season to catch nesting females.	51
3.9 A view of B. Pinang river bank at the hatchery which shows overgrown bank vegetation, unsuitable for nesting activity.	51
3.10 <i>B. baska</i> and <i>B. borneoensis</i> kept in the same pond at the Bukit Pinang Hatchery Center, Kedah.	52
3.11 Bota Kanan nesting beach (Pantai Jabatan): the beach was cleared up from overgrown grass before the nesting season	55
3.12 One of the turtles' nesting site in Pengkalan Balak area, Melaka	55
3.13 Sampling and measurement method: (a-g) measurement, (h) tagging, (i) release of sample back to its natural habitat	58

3.14	Sampling method- Blood sampling technique: (a & b) blood drawn from the tail, (c) blood drawn from the forelimb elbow, (d) blood preserved in the microcentrifuge tube filled with 1000µl of lysis buffer	60
4.1	DNA extraction of <i>B. baska</i>	108
4.2	PCR product of <i>B. baska</i> partial D-loop gene	110
4.3	Purified product of <i>B. baska</i> partial D-loop gene	110
4.4	Forward sequence of individual (SP1) (<i>B. baska</i> , Bota Kanan population)	111

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
bp	Basepair
CI	Consistency Index
CITES	Convention on International Trade in Endangered Species
CR	Critically endangered
Dept.	Department
DFA	Discriminant Function Analysis
DF1	Discriminant function one
DF2	Discriminant function two
DNA	Deoxyribonucleic acid
dNTP	Dinucleotide triposphate
DoFM	Department of Fisheries Malaysia.
DWNP	Department of Wildlife and National Parks.
E	Endangered
EDTA	Ethylenediamine tetra-acetic acid
ESU	Evolutionary Significant Group
EtBr	Ethidium Bromide
H	Height
HI	Homoplasy Index
ICZN	International Code of Zoological Nomenclature
IUCN	International Union for The Conservation of Nature
LB	Length of Bridge
mM	Millimolar
MtDNA	Mitochondrial DNA
MU	Management Unit

NaOH	Sodium hydroxide
NaCl	Sodium chloride
nDNA	Nuclear DNA
PCA	Principal Component Analysis
PCR	Polymerase chain reaction
PC1	Principal component one
PC2	Principal component two
Pers. Communication	Personal communication
PHL	Plastral Hind Lobe
POST	Post-cloacal Tail Length
PTL	Pre-anal Tail Length
RFLP	Restriction fragment length polymorphism
RI	Retention Index
rpm	Revolutions per minute
SCL	Straight Carapace Length
SCW	Straight Carapace Width
SDS	Sodium dodecyl sulfate
SNK	Student Newman-Keuls
SPL	Straight Plastron Length
STD	Sex-Temperature Dependant
TBE	Tris borate EDTA
TE	Tris EDTA
TTL	Total Tail Length
TUMEC	Turtle and Marine Ecosystem Center
Unpub. Data	Unpublished data
Ver.	Version

W	Weight
μg	Microgram
μl	Microlitre
μM	Micromolar

LIST OF APPENDICES

- Appendix A Morphometric Data of *Batagur baska*
- Appendix B Morphometric Data of *Batagur borneoensis*
- Appendix C Sequence Alignment of *Batagur baska*
- Appendix D Sequence Alignment of *Batagur borneoensis*
- Appendix E Interpopulation Genetic Distance of *Batagur baska*
Generated by Neighbor Joining Analysis
- Appendix F Interpopulation Genetic Distance of *Batagur*
borneoensis Generated by Neighbor Joining Analysis

**KEVARIABELAN GENETIK DAN MORFOMETRIK TUNTUNG SUNGAI,
(*Batagur baska*) DAN TUNTUNG LAUT, (*Batagur borneoensis*)**

ABSTRAK

Maklumat tentang struktur genetik populasi adalah penting untuk pemuliharaan spesies-spesies terancam. Ini merupakan penyelidikan pertama ke atas genetik populasi tuntung air tawar di Malaysia. Penyampelan dilakukan di Sungai Terengganu, Sungai Setiu, Sungai Paka, Sungai Cherating, (Pantai Timur Semenanjung), Sungai Kedah, Sungai Muda, Sungai Perak, Sungai Linggi (Pantai Barat Semenanjung), dan empat pusat penetasan dan pemuliharaan tuntung di Linggi, Bota Kanan, Bukit Pinang, dan Kuala Berang. Lapan puluh dua sampel betina *B. baska* liar dan tiga puluh tujuh betina *B. borneoensis* liar telah disampel semasa musim bertelur 2003 dan 2004. Empat puluh dua sampel betina *B. baska* dan sembilan *B. borneoensis* dalam kurungan di pusat-pusat pemuliharaan tersebut telah disampel. Kesemua sampel telah diukur untuk analisis morfometrik, dan darah diambil untuk analisis genetik. Sepuluh parameter morfometrik yang diukur dianalisa menggunakan Analisis Varians (ANOVA) Satu Hala, Analisis Prinsipal Komponen (PCA), dan Analisis Diskriminasi (DFA) menggunakan perisian SPSS ver.11.5. Kedua-dua spesies menunjukkan corak pemisahan antara populasi Pantai Barat dan Pantai Timur Semenanjung, di mana tuntung dari kawasan pantai timur secara puratanya lebih besar daripada tuntung di pantai barat. Variasi saiz, (yang mana selalunya di pengaruhi oleh faktor persekitaran) memainkan peranan penting dalam pembezaan populasi berdasarkan sumbangan yang tinggi dalam PC1. Proses penjujukan DNA telah berjaya ke atas 59 ekor *B. baska* dan 35 ekor *B. borneoensis*, untuk analisis genetik daripada jumlah keseluruhan. Analisis filogeni dalam perisian

PAUP berdasarkan kaedah NJ dan MP menunjukkan penstrukturan geografi di mana Pantai Timur dan Pantai Barat yang dipisahkan oleh Banjaran Titiwangsa, diasingkan dengan nyata bagi *B. baska*. Oleh itu setiap kawasan hendaklah diuruskan sebagai unit pengurusan (MU) yang berasingan. Terdapat tanda-tanda percampuran komposisi genetik bagi *B. borneoensis* antara populasi pantai timur dan pantai barat yang perlu dikaji dengan lebih teliti. Hanya sebanyak enam haplotip didapati dalam semua populasi *B. baska* dan empat haplotip dalam populasi *B. borneoensis* berdasarkan analisis dalam perisian Arlequin ver.2.0. Variasi genetik adalah amat rendah atau hampir tidak wujud antara tuntung-tuntung dalam populasi dan antara populasi-populasi dalam kawasan sempadan yang sama. Terdapat sedikit perselisihan yang boleh dilihat tetapi ini dipercayai disebabkan oleh aktiviti translokasi. Kajian yang lebih terperinci ke atas DNA mitokondria dan DNA nukleus spesies ini dicadangkan untuk merancang pengurusan pemuliharaan yang efektif.

**MORPHOMETRIC AND GENETIC VARIABILITY OF RIVER TERRAPIN,
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ABSTRACT

Information on the population genetic structure is pivotal to the conservation of endangered species. This is the first investigation on the population genetics of the Malaysian freshwater terrapins, *Batagur baska* and *Batagur borneoensis*. Sampling was done in the Terengganu, Setiu, Paka, and Cherating Rivers (East coast), and Kedah, Muda, Perak, and Linggi Rivers (West coast), and four hatchery and conservation centres in Linggi, Bota Kanan, Bukit Pinang, and Kuala Berang. Eighty two wild females of *B. baska*, and thirty seven wild females of *B. borneoensis* were sampled during the nesting season of 2003 and 2004. Forty two captive females of *B. baska* and nine captive females of *B. borneoensis* were sampled in four hatchery and conservation centers. All samples were measured for morphometric analysis and blood was drawn for molecular analysis. The ten morphometric parameters measured were analysed based on analysis of variance (Oneway ANOVA), Principal Component Analysis (PCA), and Discriminant Function Analysis (DFA) using SPSS ver. 11.5 software. Both species showed a trend of separation between the east and the west coast populations, where the east coast turtles were on the average larger than the west coast turtles. Size variation (which is largely influenced by environmental factors) plays a major role in the population differentiation based on their high loadings on PC1. Fifty nine samples were successfully sequence for *B. baska* and 35 for *B. borneoensis* for molecular analysis. Phylogenetic analysis conducted on the PAUP software based on NJ and MP methods showed geographic structuring where the east and the west coast populations which is divided by the Banjaran Titiwangsa,

were clearly differentiated in *B. baska*. Thus each region should be managed as separate management unit (MU). There were some signs of admixture of genetic composition in *B. borneoensis* between east and west coast populations which need further investigation. Six haplotypes were observed in the total *B. baska* populations, and four haplotypes in *B. borneoensis* populations based on Arlequin ver.2.0 software. Genetic variability was very low or even non existent within populations and between populations within regions. Some discrepancies were observed but this was believed to be attributable to translocation events. More detailed study of the mtDNA and nDNA of these species is suggested in planning effective conservation management.

CHAPTER 1

INTRODUCTION

Turtles and tortoise have existed since the Triassic Era, nearly 300 million years ago and long before most of the popularly recognized dinosaurs. Their survival to the present is a story of successful adaption and resiliency. Although they have overcome many catastrophes, they are now facing the most serious threat to their existence due to human greed. Thus, their continued survival is dependent on our concern and assistance.

The chelonia (freshwater and marine turtle, and tortoise), comprise 14 families and 295 species (www.chelonia.org) and of this, approximately 265 species are freshwater turtles and tortoise. Southeast Asia is known to hold the richest diversity of freshwater turtles and tortoises in the world encompassing over 25% of the total number. The two species of freshwater turtles or terrapins studied in this project, *Batagur baska* and *Batagur borneoensis* are two large river turtles that inhabit the Malaysian estuarine. The former is locally known as ‘tuntong sungai’ and the latter as ‘tuntong laut’, based on their nesting preferences. *Batagur baska* is also known as ‘The Royal River Terrapin’ because its eggs were regarded as being a luxurious and special dish to the royal families of the Malay Peninsular especially in Perak. Both species are probably the most heavily exploited terrapins in Malaysia, and appear to show serious decline in numbers.

When this study was started, the turtles were classified into different genera; ‘Tuntong Sungai’ as *Batagur baska* and ‘Tuntong Laut’ as *Callagur borneoensis*. However in 2007, the taxonomy of these turtles were revised; *Callagur* is now included in the genus *Batagur* (Praschag *et. al.* 2007), and therefore renamed as

Batagur borneoensis. Therefore in this thesis, *Callagur borneoensis* will be replaced by the newly recognized generic placement, *Batagur borneoensis* referring to the same species, Tuntung Laut, or painted terrapin.

These large estuarine turtles always occur in sympatry in areas of tidal influence. However, *B. baska* which may attain a bigger body size (SPL=60cm) is found only in large rivers while the smaller *B. borneoensis* (SPL=50cm) is found in both medium and large rivers. *Batagur baska* has a more widespread distribution within Southeast Asia. It occurs from West Bengal, India, Myanmar, Thailand, Malaysia, Sumatra, Cochin China and South Vietnam (Moll, 1978). *Batagur borneoensis* occurs naturally in Borneo, Sumatra, Peninsular Malaysia, and Southern Thailand (Moll, 1985). The distributions and population status of both species in Malaysia are not completely known and no in depth study of any aspects has ever been conducted but, perhaps the most comprehensive study was done by Siow and Moll (1982).

In Peninsular Malaysia, the Perak River has been reported to hold the largest population of *B. baska* where it was documented that 5000 to 7000 eggs were collected each night prior to World War II (Moll, 1980). More recent data recorded by the Hatchery and Conservation Center of Bota Kanan, Perak showed that the number is seriously declining over the years, with only 35 wild clutches (856 eggs) laid at DWNP licensing beaches such as Pantai Jabatan in Bota Kanan in the 2002/2003 season (Unpub. Data; Perak Dept.of Wildlife and National Parks). In 1985, *Batagur borneoensis* was reported to be highly abundant in five rivers in Peninsular Malaysia with the Setiu River supporting the largest population (Sharma, 1997), but today all populations are declining precipitously. Although hatcheries have been set-up for *B. baska* in the states of Perak, Kedah, and Terengganu, and for

B. borneoensis in Terengganu and Melaka, only populations from selected rivers and nesting sites are being managed. Turtle eggs in other rivers are almost fully harvested by unlicensed collectors. Adults are also traded for food. In addition numerous other factors have also set back conservation efforts such as loss and destruction of habitat and nesting beaches due to human activity, and imperfect regulatory laws and lack of implementation. With population sizes rapidly decreasing and habitat destruction uncontrollable, immediate management steps should be implemented before any of the declining wild populations reaches extirpation in Malaysia. In such management actions, genetic information is now recognised as a crucial factor in all biological management programmes (Bowen & Karl, 2007; O'Brien, 1994).

This study was aimed at investigating genetic and morphological variation among nesting female population using mitochondrial DNA (mtDNA) sequencing and morphometric techniques respectively. MtDNA markers are selected due to its rapid rate of evolution, suitable for a study of populations. Morphometric variation data has been documented in a few chelonian studies (Claude *et. al*, 2003) and has been frequently used to delineate stocks of several other organisms such as fish (Neat *et. al*, 2003; Walker, 1997). Females ultimately govern the reproductive output of a population, so knowledge of female dispersal and stock structure would be extremely important in defining management priorities. Furthermore, sampling males is highly problematical; costly, time consuming and at times may be dangerous in fast flowing rivers. It is well accepted that there may be both environmental and genetic components to this variation that may reflect environmental induction of the morphological differences, or rapid genetic change in the morphological characters due to selection. Nevertheless, the morphometric assessment here seeks to examine

the impact, if any, of river basins or other potential geographic barriers that could be influencing the historical connectivity of these, now fragmented, populations.

The assessment of both genetic and morphological diversity are the main thrust of the study, as these data are highly relevant to a population's health and continued survival. However, molecular genetic studies in conservation need not only be applied to reveal genetic variation, but also for ascertaining pedigrees, reconstructing phylogenies, identifying genetic stocks, estimating migration frequencies, determining trends in population dynamics, and in sex determination (very important for reptiles species in Sex-Temperature Dependent (STD) investigation). Combination of the data is crucial to implementing appropriate conservation programs ensuring the long term survival of both species and providing maximum future options for the recovery of wild population in its natural habitat (Bowen & Karl, 2007; Devon *et. al*, 2006).

Therefore, the main objectives of this study were:

- a) To investigate the genetic variation among and within wild and captive populations of *B. baska* and *B. borneoensis* using mitochondrial DNA (mtDNA) markers.
- b) To investigate the morphometric variation among wild and captive populations of *B. baska* and *B. borneoensis*.

The null hypothesis for this study is that genetic and morphological variations are not correlated to geographical region of east and west of Peninsular Malaysia.

CHAPTER 2

LITERATURE REVIEW

2.1 Species Description

2.1.1 Morphology

The freshwater terrapin, *B. baska* and painted terrapin, *B. borneoensis* are collectively known as tuntong locally; the former as ‘tuntong sungai’ and the latter as ‘tuntong laut’ based on their nesting site preferences. *Batagur baska* is also known as the ‘Royal River Terrapin’, reaching a carapace length that may exceed 60cm (Blanco *et. al*, 1991). *Batagur borneoensis* may attain 50cm in carapace length. The hierarchical taxonomy of these two species can be represented by the following:

Class: Reptilia

Order: Testudines

Family: Geoemydidae

Genus & Species: i) *Batagur baska*

ii) *Batagur borneoensis* (or *Callagur borneoensis*)

Geoemydidae (formerly Bataguridae), is the largest and most diverse family in the order Testudines (turtles) with about 75 species. They usually have webbed toes, and the pelvic girdle articulates with the plastron flexibly. The neck is drawn back vertically. The carapace has 24 marginal scutes. The plastron is composed of 12 scutes and has no mesoplastron. Geoemydids live in the tropics and subtropics of Asia, Europe and North Africa. Most geoemydids are freshwater turtles, but some have adapted to estuarine or terrestrial habitats (Ernst *et. al*, 2000). Although most are herbivorous, there are also some omnivorous and carnivorous species. During the

nesting season, females normally lay several clutches per season. Some species have temperature-dependent sex determination system while others possess distinct sex chromosomes (<http://en.wikipedia.org/wiki/Geoemydidae>). According to Praschag *et. al*, (2007), analysis on the sequence variation of the cytochrome *b* gene provided evidence that the earlier classification of these turtles into different genera of *Batagur* and *Callagur* (and including *Kachuga*) was misleading. The phylogenetic tree assigned these species to one, well-supported monophyletic clade. Therefore, these species were re-classified within a single genus. According to the International Code of Zoological Nomenclature, *Batagur* Gray, 1856, being originally erected at higher rank, takes precedence over the simultaneously published name *Kachuga* Gray, 1856, and the younger name *Callagur* Gray, 1870, resulting in an expanded genus *Batagur*. Thus, *Callagur borneoensis* is now recognized as *Batagur borneoensis* (Praschag *et. al*, 2007).

Batagur baska females have soft parts with varying shades from gray to greenish or bluish-gray dorsally, becoming lighter ventrally. The iris is brown and the mandible is a yellowish gray or brown. The shell varies from brown to olive brown to gray on the upper surface and light yellow on the lower surface. Males have a cream coloured iris and tend to be darker than females in body and shell colouration (Moll, 1980; 1984).

Females of *Batagur borneoensis* are mainly grey-brown but being more yellow brown dorsally. Non-breeding colouration on adult males is described as 'dark phase' where the carapace is brown to grey brown and the head is dark grey to brown with a dull orange to light brown stripe running mid-sagittally from the snout to occiput (Moll, *et. al*, 1981). They also have a distinct seasonal colouration

presumably associated with breeding (refer to section 2.1.4). Plate 2.1 and 2.2 showed the turtle features during non-breeding season.



(a)



(b)

Plate 2.1: (a) Male of *B. borneoensis*
(b) Male of *B. baska*
(Photographed by Norkarmila Duli, on December 2003, Bota Kanan Pond)



(a)



(b)

Plate 2.2: (a) Female of *B. borneoensis*
(b) Female of *B. baska*
(Photographed by Norkarmila Duli, on December 2003, Bukit Pinang Pond)

2.1.2 Zoogeography and Habitat

Batagur baska and *B. borneoensis*, are estuarine dwelling chelonians. In Peninsular Malaysia both species occur in sympatry with the Asian giant softshell turtle, *Pelochelys bibroni* and the Asiatic soft-shell turtle, *Amyda cartilaginae*. However the distribution of *B. baska* is restricted to large rivers whereas *B. borneoensis* is found in large and medium size rivers.

The distributions of both species in Malaysia are not completely known. The distribution map of turtle in Malaysia published by (Hendrickson, 1961 in Moll, 1990) did not clearly distinguish between areas occupied by *B. baska* and *B. borneoensis*. In another study, Siow and Moll (1982) investigated their distribution along rivers from the Thai border to the Pahang River through interviews and river surveys. There is however no accurate data on their present distribution nor population status in Peninsular Malaysia. The only study on the overall distribution in Peninsular Malaysia was conducted by Moll (1990); through museum specimens, visual observation, interviews, and documentation on 34 rivers (Table 2.1). All these rivers were found to be inhabited by *B. borneoensis*, but only 18 were inhabited by *B. baska*. However some of the details are questionable as there was no strong evidence to confirm occurrence.

Table 2.1: Thirty four rivers in Peninsular Malaysia surveyed by Moll (1990) for the occurrence of *B. baska* and *C. borneoensis*. Sources of information range from (i) museum specimen (ii) visual confirmation by investigator (iii) interviews and (iv) scientific literature.

NO.	STATE	RIVER
1	Kedah	^a Kedah River
2		^a Muda River
3	Penang	^a Penang Coast
4	Perak	^a Beruas River
5		^a Perak River
6		^a Bernam River
7	Selangor	^a Selangor River
8		^b Langat River
9	Melaka	^b Linggi River
10		^b Sungei Baru River
11		^b Melaka River
12	Johor	^a Muar River
13		^b Batu Pahat
14		^b Senggarang River
15		^b Sedili Besar
16		^b Endau River
17	Pahang	^b Pontian River
18		^a Rompin River
19		^a Pahang River
20		^a Kuantan River

21	Terengganu	^a Kemaman River
22		^b Kemasik River
23		^b Kerteh River
24		^a Paka River
25		^a Dungun River
26		^b Merchang River
27		^b Marang River
28		*Ibai River
29		^a Terengganu River
30		^b Merang River
31		^a Setiu River
32		^a Besut River
33		Kelantan
34	^a Kelantan River	

^a River inhabited by *B. baska* and *C. borneoensis*

^b River inhabited by *C. borneoensis* only

Modified from Moll, 1990

2.1.2.1 *Batagur baska*

Batagur baska has historically inhabited south-eastern Asia from West Bengal, India, along the coast of Myanmar, Thailand, Malaysia, Sumatra, up to Cochin in China and South Vietnam (Moll, 1976; 1984). It utilises the tidal portion of rivers in all its various stages of growth development. In Malaysia, the only population that has received intensive scientific attention is that inhabiting the Perak River (Moll, 1980). In another survey Moll (1976) found that the Terengganu River also supported *Batagur* populations particularly over a thirty kilometre stretch of the river between Kg. Losong and Kg. Tanggol.

2.1.2.2 *Batagur borneoensis*

The painted terrapin, *B. borneoensis* distribution is restricted to the Sundaland Archipelago Island of Borneo and Sumatera, Peninsular Malaysia, and southernmost Thailand (Moll, 1985). In Peninsular Malaysia, a total of 34 rivers are believed to support this species; 13 rivers were confirmed through Moll's investigation (1990), whereas the presence in the other 21 rivers was based on museum records, interviews, and scientific literature (Table 2.1) (Moll, 1990). The availability of nesting sites is believed to be the limiting factor of the distribution in the west coast, where the short stretches of beaches are generally mangrove lined, flat, muddy, with heavy sea traffic and heavily eroded (Sharma, 1997).

Moll (1985) reported that only five rivers are believed to harbour more than 100 nesting females. The Setiu River in Terengganu is believed to support the largest nesting populations, and Paka River the second largest. Other rivers that possibly support viable populations include Linggi River, Pahang River, Semerak River, and Kemaman River (Sharma, 1995; Sharma, 1997).

2.1.3 Movement

Both species are active during the day, as well as, at night. Their activities appear to correlate with the tides more than any other factors (Moll, 1980). Generally, tuntongs live along those parts of river banks and tributaries that are affected by the tides throughout most of the year. As the tide rises, the tuntongs move up river, frequently into small, but reproductive (plentiful food supply) tributaries. Here they forage until ebb tide when the current carries them down stream. Females, however, will assemble and begin their annual journey upstream (*B. baska*) and downstream (*B. borneoensis*) to nest. Males apparently do not make this trip as none has ever been seen in the nesting areas and many are usually spotted in the river mouth throughout the nesting period (Moll, 1980).

2.1.3.1 *Batagur baska*

Their movement is only limited up and down a particular river. The longest non-migratory movement recorded was 13km by an adult male in Perak River. Juveniles are less vagile than adults. Limited telemetry data indicated juveniles utilise smaller stretches of a river (less than 1km), (Moll, 1980).

Females will move upstream to freshwater areas to nest on the river banks which aptly explains its local name ‘tuntung sungai’ or river terrapin. On the Perak River, the peak nesting area is a 50 to 60 miles journey upstream from their feeding areas, between Lambor and Parit. On the Terengganu River, most nesting takes place on a 24 mile stretch from Pasir Tinggi to Pasir Petaseh. This overlaps in part with their feeding range, so it is difficult to assess the actual extend of the nesting migration. Following nesting, the females return to the river mouth. There is circumstantial evidence indicating that the new hatchlings also move down river to

tidal areas, if not immediately upon emergence from the nest, at least at a very young age (Moll, 1984; 1978; 1976).

2.1.3.2 *Batagur borneoensis*

B. borneoensis, or painted terrapin is locally known as ‘tuntong laut’ because of its characteristic movement down river to the coastal beaches to nest. On the east coast of Peninsular Malaysia, they often migrate to within several kilometres of the river mouths. However, there have been reports by villagers of nesting activity 19km from the estuarine inland in Dungun River (Moll, 1990). On the west coast, females may travel as far as 15 to 20km along the beach (Sharma, 1997). Hatchlings must swim out from the sea, locate and enter freshwater bodies as soon as possible as they can only withstand a limited time of exposure to high salinity (Dunson and Moll, 1980).

2.1.4 Reproduction

Little is known of their reproductive activities especially their courtship behaviour and where it takes place. Blanco *et. al*, (1991) wrote that the silt laden rivers of Southeast Asia obscure movements and do not permit observations of breeding behaviour or social interaction. This is a setback that has to be overcome as behavioural studies may well provide further insight into the intricacies of their breeding biology and offer direction as to how they can best be maintained in captivity. Observations and studies on *B. baska* at the Bronx Zoo recorded many courtship sequences; some components witnessed (for example head swaying and throat pumping) appeared to be novel (Blanco *et. al*, 1991).

The tuntong's reproductive pattern is similar to that of other large aquatic turtles occupying sizable bodies of water, which includes seasonal, communal nesting on ancestral sites, and nocturnal behaviour. Males show a distinct seasonal colouration presumably associated with breeding. Like many other turtle species, it is likely both species possess sex temperature dependent (STD) determination (sex of hatchlings is influenced by the temperature during egg incubation), but no study has been done to confirm this. Limpus (1993) suggested that incubation temperature of turtle eggs at 26-27⁰C would produce all males and at 30-31⁰C only females would be produced.

2.1.4.1 *Batagur baska*

Breeding colouration of male *B. baska* includes; dorsal skin and shell turning jet black while the iris turns an immaculate white (Plate 2.3) (Moll, 1980). Female *B. baska* excavates a body pit and egg cavity during egg-laying, a characteristic of sand-nesting turtles.

According to the Perak Game Department records, (based on observations conducted from 1969 to 1974), nesting may begin in early November and as late as the middle of January after the monsoon season and usually continuing for approximately 3 months. However, on the east coast, nesting begins somewhat later, when the monsoon rainfall is of greater magnitude (from February through the end of March) (Moll, 1976; 1980).

Nesting occurs on the river sandbanks, usually outside of the feeding areas, and tended to be aggregated in particular areas where there is heavy accumulation of washed up sand, such as along sand banks or under bridges. Along the Perak River, nesting occurs from Lambor to Parit some 40 to 60 miles upstream from the mouth

of the river with areas near Bota and Layang-Layang being most heavily utilised. In the Terengganu River, most nesting occurs along a 25-mile stretch between Pasir Tinggi and Pasir Petaseh. Terengganu River is sandier than the Perak River, and extensive sand banks line the lower reaches of the river. With more nesting sites to choose from, *B. baska* nesting tended to be less concentrated than on the Perak River (Moll, 1980; 1984).

The number of clutches lay annually per female is usually two to three per nesting season. Clutches average 26 eggs in nests in the Perak River. Eggs average 6.6 X 4.0 cm, and 64 grams in weight. Incubation time at ambient temperatures of 23⁰C– 33⁰C varies between 66 and 88 days (Moll, 1980).

2.1.4.2 *Batagur borneoensis*

This species does not excavate a body pit, maybe consequent of their small body size. They are more sensitive to disturbances compared to *B. baska* where they would abort nesting even halfway through the process if disturbed and return to the sea at any sign of movement, lights, or presence of any unusual object. Breeding colouration on males is called 'light phase' (Plate 2.4). The carapace turn to greenish white to cream coloured with distinct line of black spots on the vertebral and coastal scutes. The head appears puffy, pure white, with a red bright stripe running mid-sagittally from the snout to the occiput (Sharma, 1997).

B. borneoensis is generally considered a sea beach nester (Moll, 1985) but in some rivers it is also known to nest on estuarine sand and upriver sand banks which are also utilised by *B. baska* (eg: Perak, Muda, Dungun, Terengganu, and Besut Rivers). Sharma (1995) reported it may travel as far as 16km from the Linggi River mouth to nest while those from the Perak River may exceed 18km. Females in Setiu

River are more aggregated on the south of the river mouth, stretching a distance of 3.0km along the beach. On the Paka River, the most productive nesting beach is at Rhu Kudung. The furthest nest recorded was 12.25 km from the river mouth (Sharma, 1997).

The nesting season usually starts later than that of *B. baska*. Nesting season on the east coast normally occurs from June to August, while the west coast season starts from October to January (Moll, 1980) thus hatchlings would emerge during the monsoon season. Peak activity appears to be concentrated during low tide (Moll, 1986). *Batagur borneoensis* lays relatively larger eggs with small clutch sizes (seldom > 15) compared to *B. baska* (Moll, 1980). There is no strong evidence to show whether either or both species lay eggs more than once annually, but a few records do exist (Moll, 1990) that *B. baska* may return after about two weeks (based on a sighting in the Terengganu River) to lay a second clutch.



Plate 2.3: *B. baska* male showing breeding colouration
(Photographed by Norkarmila Duli, on Mei 2004, Bota Kanan Pond)



Plate 2.4: *B. borneoensis* male showing breeding colouration
(Photographed by Norkarmila Duli, on Jun 2004, Bota Kanan Pond)

2.1.5 Studies on Growth and Maturity

Studies on growth rate and maturity have been carried out by several investigators (Wilbur, (1975); Galbraith *et. al*, (1989); Gibbons, (1967); Zuffi *et al* (2006)). There are many kinds of growth measurement techniques that can be employed on turtles, and the application varies with species. Normally, the measurements involve the carapace and plastron using a vernier dial calliper accurate to 0.1mm (Harless & Morlock, 1979). However, growth rings, very useful in many other Emydid growth studies, are shallow and rarely clear in both species. Growth rates of *B. baska* and *B. borneoensis* living under natural conditions have never been adequately studied.

2.1.5.1 *Batagur baska*

Some preliminary but early reports of growth potential are available from various captive *B. baska* groups raised at Batu Gajah since 1968 (Moll, 1976). Size and probably age at sexual maturity varies with sex. Examination of secondary sexual characteristics on 87 Perak males (white eyes, black coloration, elongated tail) indicated that they matured on reaching 40.0 cm CL. Females appeared to mature around 45.0 cm CL.

2.1.5.2 *Batagur borneoensis*

Some limited morphometric data have been recorded for nesting females (Sharma, 1995). *Batagur borneoensis* are generally documented to be smaller than *B. baska* at nesting. Females may attain maximum carapace length of 50cm while males seldom reach 40cm (Sharma, 1995). Males from the Perak River were found to mature at 28 cm SCL on the average (Moll *et. al*, 1980).

2.1.6 Dietary Requirement

Juveniles of both species have been reported to be omnivorous. The young, consume more animal food such as molluscs to gain enough calcium, but appear to be more herbivorous as adults in the wild. Most feeding occur at high tide when vegetation is exposed and fruits from low-hanging limbs dangle in the water (Moll, 1984).

2.1.6.1 *Batagur baska*

The fruit of Berembang (*Sonneratia spp.*), a widely distributed mangrove plant, is its dietary staple which probably supports all its nutritional requirement, but as mentioned earlier, it is omnivorous and eats whatever is available. Laboratory observation on hatchlings obtained from the Kedah Hatchery aged 1-2 years old showed that they grew well on kangkung (*Ipomea asiatica*) and pellet, but did not seem to favour fish (Norkarmila, 2002). This may be because they seldom or had never been fed with fish at the Kedah Hatchery where they were raised. It was observed from that study that hatchlings fed only on vegetable developed soft shell, while individuals fed only on pellets experienced a very low growth rate. Thus a mixture seeking a balanced diet was concluded to be necessary for optimal growth.

2.1.6.2 *Batagur borneoensis*

Dietary requirements for this species are similar to *B. baska*. In the natural environment, it tends to be herbivorous, feeding on riparian plants including stem of grasses, stem of aquatic macrophytes, fruit of *Pandanus spp*, and fruits, flowers, and buds of *Sonneratia spp* (Moll, 1985).

2.1.7 Population Status

The latest update by The IUCN Red Data List label these two species as ‘critically endangered’ (CR) Under the IUCN definition, a species is classified as critically endangered if the taxon is under extreme high risk of extinction in the wild in the immediate future (www.iucnredlist.org). In addition, both are listed in the CITES list.

The most practical population size estimation is based on number of nests and nesting females per season because capture-recapture method is very time consuming, difficult, and might be dangerous in fast flowing rivers. To date no comprehensive study on their population status in Malaysia has been done. Data recorded a few decades ago are presumably no longer relevant.

2.1.7.1 *Batagur baska*

From 1982 until 1996, *B. baska* was labeled as Endangered (E) by the IUCN, but then reclassified as ‘Critically Endangered’ (CR) with the status label CR A1cd (www.iucnredlist.org). It is also listed in the Appendix I of CITES. Appendix I lists species that are the most endangered among CITES listed species and prohibits commercial international trade in specimens of these species.

In Malaysia, documented evidence on the population sizes was last reported by Moll (1984), and the only fairly complete information is available for only the Perak population. Prior to World War II, between 5000 to 7000 eggs were collected each night from the Perak River for three months, giving a full season production of approximately 450,000 to 630,000 eggs annually. The number of nesting females was calculated to be in the order of 5769 to 8100 (Moll, 1976). The numbers dwindled to an average of 300 nests (presumably n= 100 females) per night; with

approximately 22,500 eggs per season (Momin Khan, 1964). Later, Moll (1987) found only 190 nests on the most popular nesting beaches that had previously been reported. Unfortunately no recent study has been conducted to estimate current population sizes.

There is no complete study on the Terengganu River, thus the population status is not known. However, during the course of this study it was found that the number of nesting females is now higher in the Terengganu River than in the Kedah and Perak Rivers (Unpub. Data; Terengganu Dept. of Wildlife and National Parks). The reason of this current circumstance is not clear and needs to be investigated. Some of the possible explanations include: (1) the destruction of most of the sand beaches along Perak River, (2) released hatchlings have not survived, (3) lack of food due to over exploitation of the river, and (4) Illegal trapping and smuggling of turtles from the Perak to foreign markets as delicacies.

2.1.7.2 *Batagur borneoensis*

From 1982 until 1986, *C. borneoensis* was labelled as 'Vulnerable' (V), but reclassified to 'Endangered' (E) from 1988 to 1994. Then again the status was changed to 'Critically Endangered' (CR) in 1996 with the status labelled CR A1bcd (The IUCN Red Data List of Threatened Species, Webpage). It is also listed in Appendix II of CITES. Appendix II lists species that are not necessarily immediately threatened with extinction but that may become so unless trade is closely controlled.

In 1985, Moll reported that only five rivers in Peninsular Malaysia were believed to support more than 100 nesting females, with Setiu River supporting the largest population, and Paka River coming in second. According to Sharma, (1995

and 1997), other rivers that may also support viable populations include Linggi River, Pahang River, Semerak River, Kerteh River, and Kemaman River.

However, Setiu may no longer have the largest population as in 1993 more than 100 adults were found dead succumbing to chemical fertilizer pollution from a plantation area close by (Pers. Communication, Dionysius Sharma). Total clutch from the Fisheries Department gazetted areas of Setiu in 2003 was only 83; compared to the Paka population of 128 clutches (Unpub. Data; TUMEC). Other populations have also been reported to be declining such as the Chendor and Cherating River in Pahang and Linggi River in Melaka. Linggi population is believed to be almost depleted because large numbers of adults have fallen prey to unscrupulous traders. Within the last few years not more than 10 nests could be found annually (Pers. Communication, villagers).

2.1.8 Causes of Decline

The decline of *B. baska* and *B. borneoensis* in Malaysia is attributable to various forms of natural, accidental and deliberate destruction, overexploitation and alteration of habitat. However the most significant reasons for the decline of *B. baska* throughout its range have been over exploitation of the eggs as a delicacy and alteration of their natural habitats.

2.1.8.2 Human consumption

The turtles are rarely eaten in Malaysia because it is prohibited to Muslims. However, the eggs are highly prized and exploited due to their reputed aphrodisiacal properties. A major decline of *B. baska* population in Malaysia occurred during the Japanese occupation of World War II (Loch, 1950; Momin Khan, 1964) as the eggs

and adults were eaten in large numbers. However, continued exploitation and habitat destruction following the war have prevented recovery of the population to the pre war levels.

Licensing regulation for egg collection in most states are not properly observed nor enforced. Egg poaching is rampant even within licensed areas. In 1993, only an estimated 30%-40% of all *B. borneoensis* eggs laid in Setiu River were sent to the hatchery while the rest presumably went for human consumption (Sharma, 1997). The same problem is believed to be continuing in other hatchery areas.

2.1.8.2 Habitat alteration and destruction

Batagur baska and *Batagur borneoensis* appear to require large deep rivers and large open expanses of sand for nesting. Habitat alteration and destruction adversely affect both kinds of sites. Clearing of forested water sheds, sand and tin mining lead to a greater silt load with associated problems of increased flooding, silt deposition, and reduced productivity. Unseasonal floods are most dangerous to *B. baska* for when they occur during nesting and incubation periods, the entire annual reproductive output could be destroyed. *B. borneoensis* nesting beaches are also heavily disturbed by industrial and tourism development (eg: Chendor, Cherating, Paka, and Linggi Rivers). In addition to the pollution problem, they are extremely sensitive to bright light and movement, and will abort nesting even midway through the process if disturbed (Sharma, 1995). Clearing banks of vegetation eliminates food sources and increases turbidity which in turn reduces growth of aquatic vegetation. Water pollution due to toxic wastes is of course another major threat not only to turtles but affects the ecosystem equilibrium, but remains unquantified despite the potential vector of these contaminants into people via the eggs eaten.

Dams or tidal barrages blocking a major river (such as in the Kedah and Perak Rivers) prevent movement between the turtle feeding and nesting areas (Moll, 1976). They also disrupt the natural displacement of sand for the nesting beaches.

2.1.8.3 Other factors

Terrapins are also directly killed by humans for a variety of accidental and intended reasons. For example, collisions with motorboats occasionally occur and could be fatal. It is common to find terrapins killed in this manner during each nesting season in the Setiu River (Sharma, 1995). They also easily get trapped in the fishing nets or onto hooks and damage fishing equipment and may be harmed by angered fishermen. Increased coastal fishing activity is the most dangerous threat to *B. borneoensis*. Nesting females also face great danger from egg collectors who reportedly catch them (especially *B. baska*) to induce egg laying. If this is unsuccessful they may kill and break open the females to obtain the eggs (Pers. communication, villagers). Killing the effective group of a population will certainly put the population into even greater danger.

2.1.9 Conservation Effort

Efforts to conserve Chelonians in Peninsular Malaysia were initiated in 1961 with the construction of a hatchery by the Department of Fisheries Malaysia (DoFM) to incubate the leatherback, *Dermochelys coriacea* eggs (Siow and Moll, 1982). In the following years, several more hatcheries were set up by DoFM and Department of Wildlife and National Parks (DWNP) for marine turtle and terrapin conservation. Malaysia is the first country to take positive steps towards the conservation of *B. baska* by the setting up of hatcheries and head starting programmes for this species.