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การเพาะเลี้ยงตะพาบม่านลาย Chitra Chitra Nutphand, 1986 ซึ่งเป็นตะพาบที่มีขนาดใหญ่ที่สุดในโลกและอยู่ในสถานภาพใกล้สูญพันธุ์อย่างยิ่ง ได้ดำเนินการที่ศูนย์วิจัยและพัฒนาประมงน้ำจืดกาญจนบุรี จังหวัดกาญจนบุรี ผลการศึกษาพบว่าตะพาบม่านลายวางไข่ในหาดทรายเทียมระหว่างเดือนกุมภาพันธ์และเมษายน แม่ตะพาบวางไข่ได้ถึง 4 รังต่อปี มีไข่รังละ 40-88 ฟอง ไข่ (n= 220) มีความกว้าง 31.94±1.57 มม ความยาว 33.16±1.54 มม และมีน้ำหนัก 19.00±1.67 กรัม ใช้เวลาในการฟักไข่เฉลี่ย (n = 256) 59±3 วัน อยู่ในช่วง 55-65 วัน ที่อุณหภูมิอากาศ 24-42 °C และอุณหภูมิทราย 24-39 °C ลูกตะพาบม่านลายมีขนาด (n=297) กระดองหลังกว้าง 38.46±1.52 มม กระดองหลังยาว 42.97±1.59 มม และมีน้ำหนัก 13.10±1.03 กรัม อัตราการฟักอยู่ในช่วง 3-94 เปอร์เซ็นต์ เมื่ออนุบาลลูกตะพาบม่านลายด้วยลูกปลายี่สกเทศ Labeo rohita และลูกปลานิล Oreochromis niloticus เป็นเวลา 14 สัปดาห์พบว่าลูกตะพาบม่านลายมีค่าเฉลี่ยของกระดองหลังกว้าง 86.70±5.17 มม กระดองหลังยาว 91.72±5.75 มม และมีน้ำหนัก 103.97±18.08 กรัม มีอัตรารอด 90.64 เปอร์เซ็นต์

ผลการเปรียบเทียบสัณฐานของกะโหลกศีรษะและกระดองหลังของตะพาบม่านลายไทย Chitra chitra Nutphand, 1986 และตะพาบม่านลายอินเดีย Chitra Indica (Gray, 1831) โดยใช้สัดส่วนของกะโหลกศีรษะ 27 ลักษณะ และสัดส่วนของกระดองหลัง 53 ลักษณะ ชี้ให้เห็นความแตกต่างของตะพาบม่านลายไทยและม่านลายอินเดียและยืนยันว่า C. chitra เป็นชนิดที่แตกต่างจาก C. indica

การศึกษาลักษณะเปลือกไข่ของตะพาบม่านลาย C. chitra จากธรรมชาติ พบว่าผลของ SEM แสดงว่าเปลือกไข่มีสามชั้นคือ ชั้นนอก(calcareous sheet) ชั้นกลาง (crystalline layer) และชั้นใน (fibrous layer) เปลือกไข่ประกอบด้วย ออกซิเจน 52.98±4.81 เปอร์เซ็นต์ คาร์บอน 35.03±9.17 เปอร์เซ็นต์ แมกนีเซียม 5.55±0.34 เปอร์เซ็นต์ แคลเซียม 5.37±7.16 เปอร์เซ็นต์ ซิลิกา 2.87±1.64 เปอร์เซ็นต์ อลูมิเนียม 2.30±1.07 เปอร์เซ็นต์ โพแทสเซียม 0.17±0.1 เปอร์เซ็นต์และโซเดียม 0.74±0.3 เปอร์เซ็นต์ โดยเปลือกไข่เป็น $CaCO_3$ ในรูปของ aragonite

การศึกษาครั้งนี้ยืนยันว่ายังพบตะพาบม่านลาย C. Chitra ในลุ่มน้ำแม่กลองและลุ่มน้ำเจ้าพระยาแต่มีจำนวนน้อยมาก และพบตะพาบม่านลายชนิดอื่นอีกคือตะพาบม่านลายพม่า Chitra burmanica Jaruthanin, 2002 หรือ Chitra vandijki McCord & Pritchard, 2002 ในลุ่มน้ำสาละวิน การศึกษาครั้งนี้ได้รายงานการพบ C. Chitra ในแม่น้ำปิงเป็นครั้งแรก การลด

จำนวนประชากรของตะพาบม่านลาย C. Chitra ในธรรมชาติอย่างรวดเร็ว ทำให้มีความจำเป็นต้องมีการจัดการด้านการอนุรักษ์อย่างรีบด่วน

คำหลัก : ตะพาบม่านลาย, Chitra chitra, การเพาะพันธุ์, ธรรมชาติวิทยา, เปลือกไข่

Project Code : BGJ4380020

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A captive breeding program of the Siamese Narrow-headed Softshell Turtle, Chitra chitra Nutphand, 1986, the world's largest softshell turtle and a critically endangered species, was conducted at Kanchanaburi Inland Fisheries Research and Development Center, Kanchanaburi Province. C. chitra laid eggs from February through April in artificial sandbanks. Each female produced up to 4 clutches/year with 40-88 eggs/clutch. Egg sizes (n=220) were 31.94±1.57 mm in width, 33.16±1.54 mm in length and 19.00±1.67 g in weight. The mean incubation time of C. chitra eggs was 59±3 days (n = 255) with a range of 55-66 days at 24-42 °C air temperature and 24-39 °C sand temperature. Hatchling sizes (n=297) were 38.46±1.52 mm in carapace width, 42.97±1.59 mm in carapace length and 13.10±1.03 g in weight. The hatching success in each clutch varied from 3 to 94 %. The hatchlings were fed with fry fishes of Labeo rohita and Oreochromis niloticus. After 14 weeks, the mean hatchling size was 86.70±5.17 mm in carapace width, 91.72±5.75 mm in carapace length and 103.97±18.08 g in weight. The survival rate of juveniles was 90.64%.

Morphometric comparisons of skulls and carapaces of C. chitra and C. indica, based on 27 skull ratio characters and 53 carapace ratio characters, showed that there were clear osteological differences between Indian and Thai forms. The magnitude of the variation supports the argument that Thai animals warrant specific status.

The eggshell structure of wild C. chitra was studied. The result of SEM showed that the eggshell had three layers; an outer calcareous sheet, a middle crystalline layer and an inner fibrous layer. The eggshells were composed of oxygen (52.98±4.81%), carbon (35.03±9.17%), magnesium (5.55±0.34%), calcium (5.37±7.16%), silica (2.87±1.64%), aluminum (2.30±1.07%), potassium (0.17±0.1%), and sodium (0.74±0.3%). The eggshell was the aragonite form of $CaCO_3$.

This study confirms that C. chitra still exists in the Mae Klong and Chao Phraya river systems but is very rare. Another species, C. burmanica Jaruthanin, 2002 or C. vandijki McCord & Pritchard, 2002, was found in the Salween river system during the survey. A new record of C. chitra in the Mae Ping River was also reported in this study. Due to the rapid decline of the natural population of C. chitra, the conservation management is urgently needed.

Keywords: Siamese Narrow-headed Softshell Turtle, Chitra chitra, Captive Breeding, Natural History, Eggshell

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Chapter 1

Introduction

The Siamese Narrow-headed Softshell turtle, Chitra chitra Nutphand, 1986 is one of the five native softshell turtles in Thailand. Its numbers have decreased rapidly in recent decades. In the year 2000, The International Union for the Conservation of Nature and Natural Resources (IUCN) listed C. chitra as a Critically Endangered Species (CR) (Hilton-Taylor, 2000). The main causes of its endangerment may be mainly related to the construction of the Vajiralongkorn, Srinagarind and Mae Klong Dams that changed the environment of the Kwae Noi, Kwae Yai and Mae Klong Rivers, respectively, and to its exploitation for food consumption and the pet trade. This critically endangered species has been protected under the Wild Animals Reservation and Protection Act B.E. 2535 (WARPA) but it was not protected by the World Conservation Monitoring Centre, 1998 (CITES). To date, knowledge of its biology and ecology is very incomplete. Therefore, the focus of this study is to collect data concerning the natural history of wild C. chitra and to study its biology in captivity. Knowledge gained in this study will provide useful information which can be applied toward the successful captive breeding, hatchlings rearing, reintroduction, reestablishment and long term conservation of this species in Thailand.

Objectives

1. To study the natural history of C. chitra including; skeletal morphology, distribution range, population status, habitat characteristics, breeding season and nesting site characteristics.

2. To study the breeding biology of C. chitra in captivity including; mating and egg-laying activities, clutch size, egg size, incubation time, hatching success and growth and survival rate of hatchlings

Anticipated benefit

The knowledge of the natural history and biology of C. chitra can provide useful information for the conservation and management of this critically endangered species in the future.

Chapter 2

Literature Review

Turtles (Testudines) are reptiles, armored with shell above and below, and capable of withdrawing the head, neck, limbs, and tails either partially or fully within the armor. No other tetrapod has a bony shell that encloses both the pectoral and pelvic girdles. The upper shell, the carapace, is formed from fusion of the eight trunk vertebrae and ribs to an overlying set of dermal bones; the lower shell, the plastron, arises from the fusion of parts of the sternum and pectoral girdle with external dermal bones. The shell is robust in some taxa, such as in tortoises and mud turtles, with only small openings for the head and appendages. In other turtles, such as leatherback sea turtles and softshell turtles, the shell is lightly built and has lost or reduced bony elements. The neck, whether long or short, is extremely flexible and consists of eight cervical vertebrae in all turtles. Extant turtles are divided into two clades based on the movement or retraction pattern of the neck. The Pleurodira or sideneck turtles retract the head and neck by laying it to the side; thus, the sides of the neck and head are exposed in the gap between the carapace and plastron. The Cryptodira or hidden-neck turtles retract the neck posteriorly into a medial slot within the body cavity; the neck forms a vertical S-shape when viewed laterally, and only the tip of the nose is exposed between the shielding forearms. In spite of the different mechanics of neck retraction, the structure of the cervical vertebrae in the two groups is very similar (Zug et al., 2001).

The turtle shell is composed of dermal bony elements that are covered externally by keratinous scutes or, in a few instances, leathery skin (Trionychidae, Dermochelyidae, Carettochelyidae). The scutes do not have the same pattern as the underlying bony elements, and the misalignment of sutures in the bony and keratinous portions of the shell adds strength to the structure (Pough, 2001).

All turtles are oviparous. The number of eggs deposited by females of different species ranges from one to more than a hundred. The number of eggs in a clutch is generally positively associated with female size; small turtles lay one or two eggs and larger turtles lay a dozen or more. Most turtles possess a stereotypic nest-digging behavior. Egg chambers are dug with the hindlimbs, which work alternately to scoop out a flask-shaped chamber as deep as the hindlimbs can reach. Fertilization is internal and, because the shell surrounds the body in both sexes, copulation can be hazardous to the male, who must balance his plastron on top of the female's carapace. Males of many species have a slightly concave plastron to facilitate mating (Zug et al., 2001).

The softshells of the family Trionychidae are a group of turtles that lack epidermal laminae and their bony shell is somewhat reduced. The shell is low, usually nearly circular in outline, and covered with a leathery skin. The neck is long and retractile, the lips are fleshy (not covered with a horny beak as in other turtles), and the snout is drawn into a fleshy proboscis with the nostrils at the tip. The limbs are paddlelike, with three claws on each foot (Goin and Goin, 1962).

Softshell turtles are one of the most ancient of living families, having a fossil record extending at least as far back as the Cretaceous. Even fragmentary remains can usually be assigned to this family since the bones of both carapace and plastron are usually heavily pitted in a characteristic fasion. Early representative include the genus Plastomenus, known from about eleven species from the Cretaceous and Eocene of North America, with a single newly-discovered form (P. mlynarskii) from the Eocene of eastern Kazakhstan (Pritchard, 1979). While Ernst and Barbour (1989) state that softshells' fossil record indicates they were previously more widespread, once occurring in Europe and South America. The oldest known fossil trionychid is Sinaspideretes wimani, which dates possibly from the Late Jurassic.

There are more than 285 turtle species world wide (Zug et al., 2001). Modern Softshell turtles are found in North America, Africa, southern and eastern Asia, and the East Indies to New Guinea (Figure 2.1). There are 23 species world wide, nine species are found in Southeast Asia (Iverson, 1992). Saemathong and Thirakhupt (1994) recorded that there are five native species, Amyda cartilaginea, Dogania subplana, Lissemys scutata, Pelochelys cantorii, Chitra chitra, and one introduced species, Pelodiscus sinensis, in Thailand.

Figure 2.1 Distribution of softshell turtles in the world (after Pough, 2001)

A diagnosis of Genus Chitra was provided by Taylor (1970) who found that the orbits are very close to each other (the distance between them is about half their diameters). The skull is narrow and its length is twice that of its width. The outer part of the nuchal plate overlies the second dorsal rib; 8 neural plates form a continuous series; 8 costal scales are on each side and the last pair medially in contact. Hyoplastron and hypoplastron are distinct from each other. A post orbital arch is about double the diameter of the orbit; the posterior border of pterygoid is free, without an ascending process.

There are 3 species currently recognized, Chitra indica Gray, 1831, Chitra chitra Nutphand, 1986, and Chitra burmanica Jaruthanin, 2002 or Chitra vandijki McCord & Pritchard, 2002, in this genus. The distribution of the Genus Chitra is widespread across southern Asia (occurring in the rivers Indus, Ganga, Godavari, Padma, Mahanadi, and Colercon, as well as in Pakistan, Nepal, India and Bangladesh) and Southeast Asia (Myanmar, Thailand, Malaysia and Indonesia) (Liat and Das, 1999).

Smith (1931) and Taylor (1970) recorded Chitra sp. in Thailand as Chitra indica. In 1986, it was described as a new species, Chitra chitra, by Nutphand. He stated that it was probably the largest softshell turtle in the world and was known only from the Mae Klong river (Ratburi Province), and Khwae Noi and Khwae Yai Rivers (Kanchanaburi Province). Their dimensions could reach or exceed 110 cm in carapace width, 140 cm in carapace length, 150 kg in weight, and females could lay clutches of 60-100 eggs. C. chitra has many dark stripes on a yellow-brown carapace and neck with white plastron (Nutphand, 1990). More recently Chitra populations in Malaysia and western Indonesia have been considered to represent this species also (McCord and Pritchard, 2002).

In Thailand, Chitra chitra occurs only in broad, deep sections of the Mae Klong Basin, from the Vajiralongkorn (formerly called Khao Laem) and Srinagarind Reservoirs to Ratburi Province and the Mae Ping River, both upper and lower Bhumipol Dam (Kitimasak and Thirakhupt, 2002). C. chitra is severely threatened here by hunting, water pollution, reservoir construction and other forms of habitat destruction and alteration (Thirakhupt and van Dijk, 1994).

In 2000, The International Union for the Conservation of Nature and Natural Resources (IUCN) listed C. chitra as one of 27 Critically Endangered

Species (CR) of turtles in the world (Hilton-Taylor, 2000). Its status is classified from the criteria CR A1cd, B1+2c as below;

CR; A taxon is Critically Endangered when it is facing an extremely high risk of extinction in the wild in the immediate future, as defined by any of the following criteria:

A1cd; Population reduction in the form of either of the;

1; An observed, estimated, inferred or suspected reduction of at least 80% over the last 10 years or three generations, whichever is the longer, based on (and specifying) either

c; a decline in area of occupancy, extent of occurrence and/or quality of habitat ; or

d; actual or potential levels of exploitation

Taechachareonsukchera (1991) dissected a dead female C. chitra on 15 March 1990. This female contained 97 white hard-shell eggs and 270 enlarged follicles. Eggs were incubated in a glass-box at 25-35 ^{0}C., and 80-100 % relative humidity. The incubation period was 62 days. The mean of hatchling carapace length was 4.5 cm and 4.0-4.2 cm carapace width. Hatchling groups, ten per group, were raised in 60 cm diameter tanks, with 5-7 cm water depth and without sand at the bottom. Within ten days, many baby softshell turtles were injured by their kin. There were 50 % which survived.

Chapter 3

Morphometric Comparisons of the Skull and Carapace of Chitra chitra Nutphand, 1986 and Chitra indica (Gray, 1831) (Testudines: Trionychidae)

Abstract

Skull and carapace measurements were compared between Chitra indica of the Indian Subcontinent and Chitra chitra of Thailand using discriminant analysis. Based upon 27 skull ratio characters from 6 skulls of C. indica and 9 skulls of C. chitra and 53 carapace ratio characters from 6 carapaces of C. indica and 10 carapaces of C. chitra compared, these analyses indicate that there are clear osteological differences between Indian and Thai forms. The magnitude of the variation displayed by these analyses supports the argument that Thai animals warrant specific status.

Key words: Chitra chitra, Chitra indica, Morphometric Comparison, Discriminant Analysis, Skull, Carapace

Introduction

The Siamese Narrow-headed softshell turtle of the Mae Klong river system of Western Thailand, probably the largest softshell in the world (Pritchard, 2001), was long recognized as only a disjunct population of the Indian subcontinent species, Chitra indica (Nutphand, 1979, Smith, 1931; and Taylor, 1970). More recently, Nutphand (1986) in a magazine article described the population inhabiting Thailand as a new species, Chitra chitra, based solely on its color, stripe pattern and larger adult size – all characters which he believed separated it from C. indica of the Indian subcontinent. Thirakhupt and van Dijk (1994) and van Dijk and Thirakhupt (1995) lent support to this designation by referring to this animal as C. chitra in their accounts of its status and conservation requirements in their reviews of the diversity and conservation of the turtles of western Thailand. C. chitra has since been designated as a Critically Endangered Species (CR) by IUCN (1996), but its specifics status remains controversial. To further clarify the level of differentiation between Indian and Thai Chitra, an array of osteological characteristics of the skull and carapace of both taxa were examined and analysed. The results of these comparisons and their significance are provided below.

Materials and Methods

Specimens of C. chitra and C. indica were examined at BNHM (The Natural History Museum, London), MCZ (Museum of Comparative Zoology, Harvard University), FMNH (The Field Museum, Chicago) and CUB MZ (Chulalongkorn University Bangkok, Museum of Zoology) (table 3.1).

Table 3.1 Specimen types, localities, catalog numbers, museums and collectors of Chitra specimens in this study.

No.	Specimen type	Localities	Catalog Number	Museum	Collectors
1	carapace	Thailand	1974.2451	NHM	M. A. Smith
2	skull, carapace	Thailand	1962.12.16.1	NHM	M. A. Smith
3	skull, carapace	India	86.2.1.1	NHM	-
4	skull	India	-	NHM	Falconer
5	skull, carapace	India	87.3.30.11	NHM	W. Theobald
6	skull, carapace	India	1984.1276	NHM	Rothshild
7	skull, carapace	Thailand	29486	MCZ	M. A. Smith
8	skull	Thailand	29487	MCZ	M. A. Smith
9	carapace	Thailand	29488	MCZ	M. A. Smith
10	skull, carapace	India	-	MCZ	-
11	carapace	India	224234	FMNH	E. O. Moll
12	skull, carapace	India	224228	FMNH	E. O. Moll
13	carapace	Thailand	1994 - 4 -21.1	CUBMZ	Thirakhupt
14	skull, carapace	Thailand	CUBMZ R 2001.10	CUBMZ	Thirakhupt & Kitimasak
15	skull, carapace	Thailand	CUBMZ R 2001.11	CUBMZ	Thirakhupt & Kitimasak

Table 3.1 (continued). Specimen types, localities, catalog numbers, museums and collectors of Chitra specimens in this study.

No.	Specimen type	Localities	Catalog Number	Museum	Collectors
16	skull, carapace	Thailand	CUBMZ R 2001.12	CUBMZ	Thirakhupt & Kitimasak
17	skull, carapace	Thailand	CUBMZ R 2001.13	CUBMZ	Thirakhupt
18	skull	Thailand	CUBMZ R 2001.14	CUBMZ	Thirakhupt & Kitimasak
19	skull, carapace	Thailand	CUBMZ R 2001.15	CUBMZ	Thirakhupt & Kitimasak
20	carapace	Thailand	CUBMZ R 2001.16	CUBMZ	Thirakhupt

Six skulls and 6 carapaces of 7 adult C. indica and 9 skulls and 10 carapaces of 13 adult C. chitra were examined. Twenty-seven characters of each skull (Figure 3.1) and 53 characters of each carapace (Figure 3.2) were measured for this study. These characters and their abbreviations are listed in the appendix.

Skull and carapace variables were divided by SW (Skull Width) and BDL (Bony Disc Length), respectively in order to decrease error due to size variation. Best discriminating variables were selected with a forward stepwise discriminant analysis, using the highest F Value as entrance criteria. The discriminant function was established employing the polled covariance matrix and proportional prior probabilities of membership, since sample sizes were unequal. Statistical analyses were carried out using SPSS program (Ver. 10).

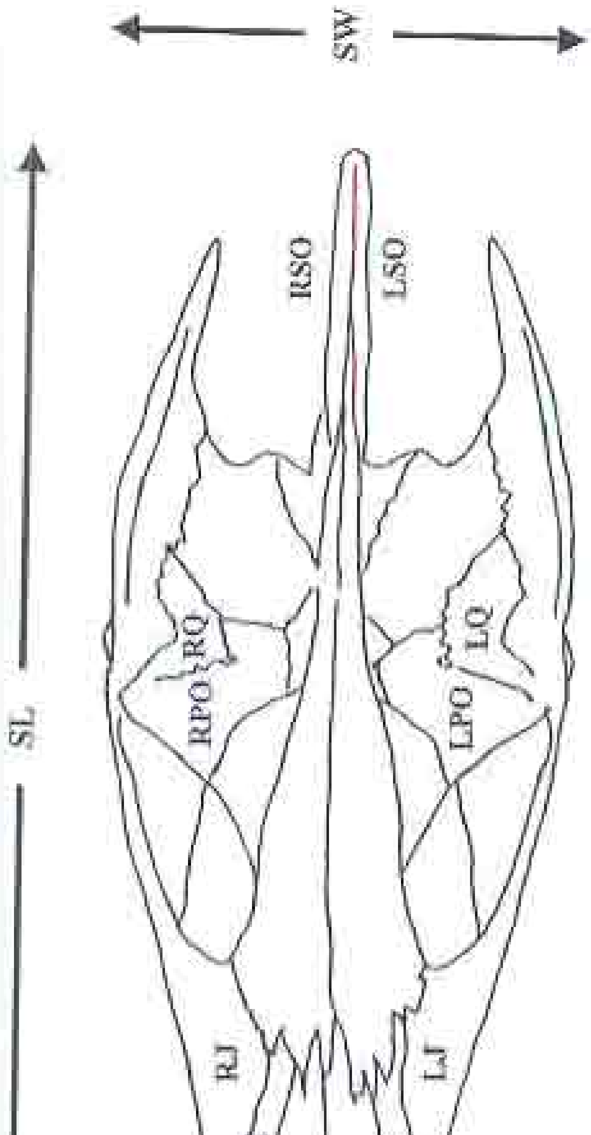

Figure 3.1 Skull variables of C. chitra and C. indica

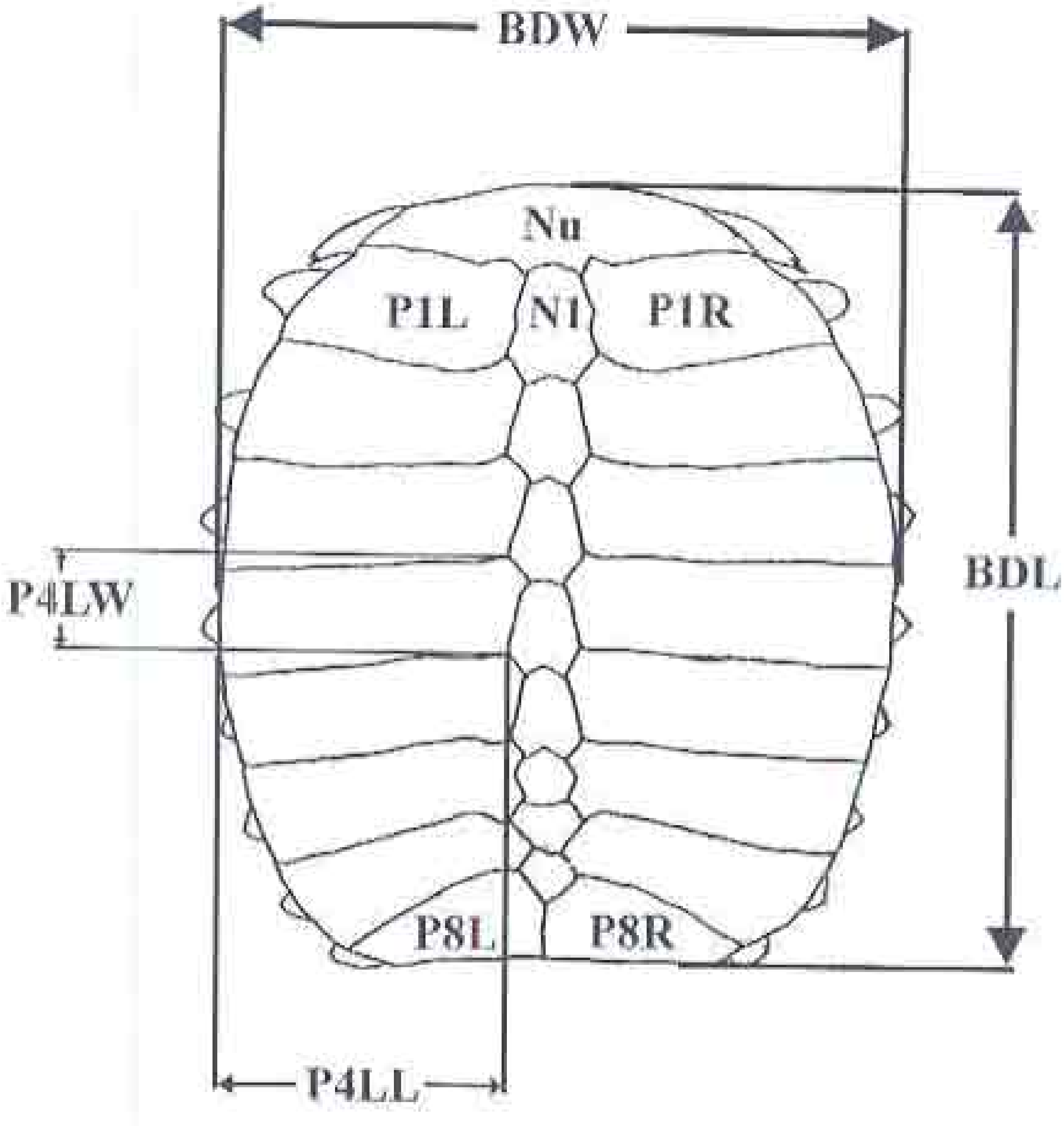

Figure 3.2 Carapace variables of C. chitra and C. indica

Results

Skull Analysis

Seven from 26 skull ratio characters were chosen in this study. In the stepwise discriminant procedure the ratio of RPFL/SW, RPFW/SW, LPOL/SW, NW/SW, SH/SW, RPOW/SW and LPFW/SW were the best discriminating variables, achieving 100% original grouped cases correctly classified (Table 3.2).

Table 3.2 Statistics of the skull variables selected by forward stepwise discriminant analysis for species discrimination between C. chitra and C. indica. The variables were listed in order of entrance in the model.

Variable	step	Wilks' Lambda	Exact F	P
RPFL/SW	1	0.570	7.531	0.021
RPFW/SW	2	0.278	11.706	0.003
LPOL/SW	3	0.109	21.763	<0.001
NW/SW	4	0.059	28.151	<0.001
SH/SW	5	0.029	39.865	<0.001
RPOW/SW	6	0.012	69.687	<0.001
LPFW/SW	7	0.005	107.880	<0.001

The discriminant scores of skulls vary from +10 to +12.5 in C. chitra and -13.5 to -16 in C. indica. There was a non-overlapping distribution of discriminant scores of skulls between C. chitra and C. indica (Figure 3.3). As a result, the discriminant scores of skulls in Figure 3.3 have distinguished C. chitra from C. indica effectively.

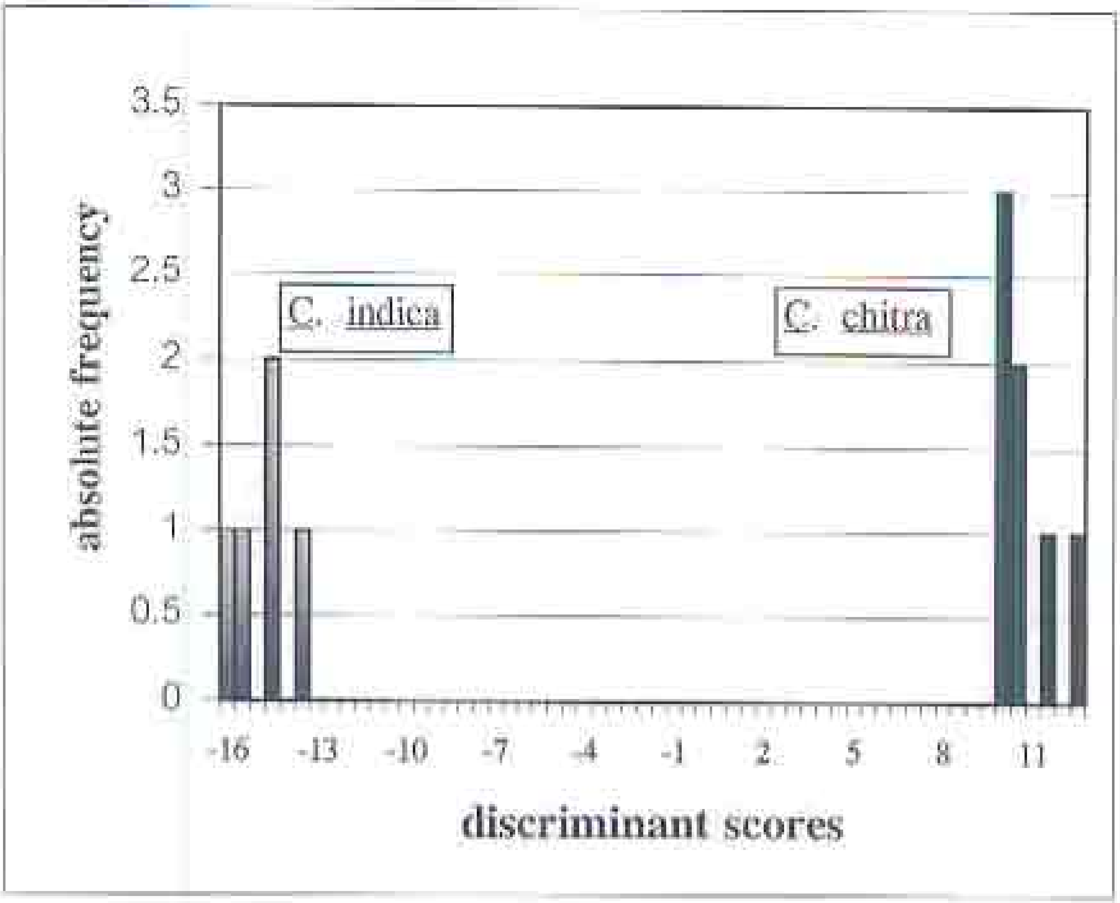

Figure 3.3 Discriminant scores of skulls vary from +10 to +12.5 in C. chitra and -13.5 to -16 in C. indica.

Therefore only those variables were employed for further function:

D (skull) = 211.481(SH/SW) – 519.047(NW/SW) – 673.041(RPFL/SW) + 639.521(RPFW/SW) +138.364(LPFW/SW) + 271.269(LPOL/SW) – 100.089(RPOW/SW) + 38.768

Where D $\geq$ -2.12 = C. chitra and D < -2.12 = C. indica

Carapace Analysis

The ratios of P3RW/BDL, P3LW/ BDL, N3W/ BDL and P7LW/ BDL were chosen as the best stepwise discriminant variables with 100% original grouped cases correctly classified (Table 3.3).

Table 3.3 Statistics of the carapace variables selected by forward stepwise discriminant analysis for species discrimination between C. chitra and C. indica. The variables were listed in order of entrance in the model.

Variable	step	Wilks' Lambda	Exact F	P
P3RW/CL	1	0.216	43.486	<0.000
P3LW/CL	2	0.108	45.278	<0.001
N3W/CL	3	0.057	54.868	<0.001
P7LW/CL	4	0.027	80.123	<0.001

The discriminant function for carapace variables was then created as the following:

D (carapace) = 160.663(N3W/ BDL) + 111.874(P3LW/ BDL) – 209.154(P3RW/ BDL) – 63.314(P7LW/ BDL) + 5.053

Where D $\geq$ -0.797 = C. chitra and D < -0.797 = C. indica

The discriminant scores of carapaces in Figure 3.4 have distinguished C. chitra from C. indica effectively.

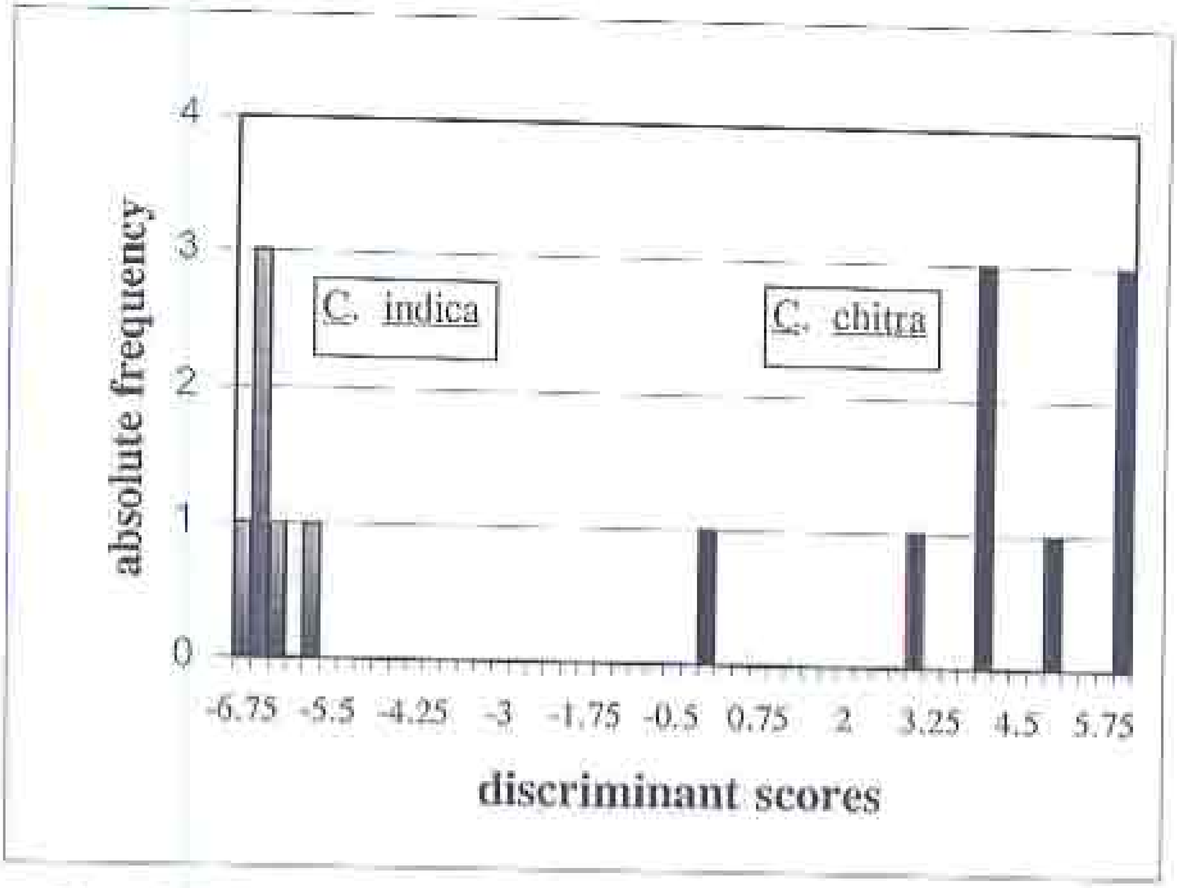

Figure 3.4 Discriminant scores of the carapaces vary from 0 to +6 in C. chitra and -5.5 to -6.75 in C. indica.

Discussion

There were 7 skull ratio characters and 4 carapace ratio characters were chosen as significant difference between C. chitra and C. indica. Discriminant scores of skulls and carapaces between C. chitra and C. indica also show non-overlapping distribution (Figure 3.3 and 3.4). Discriminant analyses of the skull and carapace characters selected provide strong evidence for the separation of C. chitra from C. indica and could be used to support the designation of the former as a valid species. Moreover, the recent study of phylogenetic analysis from the mitrochondial ND4 gene revealed deeply divergence between C. chitra and C. indica (Engstrom et al., 2002).

Furthermore, the discriminant functions of the skull and the carapace could be very useful for the confirmation of identity of unidentified specimens.

Acknowledgments I would like to thank Dr. Brian Greene for reviewing the data presented here. I also thank Dr. Colin McCarthy (The Natural History Museum, London), Dr. James Hanken and Jose' Rosado (Museum of Comparative Zoology, Harvard University), Dr. Harold K. Voris and Dr. Robert F. Inger (The Field Museum, Chicago) for allowing me to examine Chitra collections. Research was supported by The Thailand Research Fund (TRF) for The Royal Golden Jubilee Ph.D. Program (RGJ).

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Appendix

Characters and their abbreviations

Skull

Skull height, SH; Skull length, SL; Skull width, SW; Right orbit length, ROL; Left orbit length, LOL; Nostril length, NL; Nostril width, NW; Right frontal length, RFL; Left frontal length, LFL; Right frontal width, RFW; Left frontal width, LFW; Right post-frontal length, RPFL; Left post-frontal length, LPFL; Right post-frontal width, RPFW; Left post-frontal width, LPFW; Right jugal length, RJL; Left jugal length, LJL; Right pro-otic length, RPOL; Left pro-otic length, LPOL; Right pro-otic width, RPOW; Left pro-otic width, LPOW; Right supraoccipital length, RSOL; Left supraoccipital length, LSOL; Right quadrate width, RQW; Left quadrate width, LQW; Right quadrate length, RQL; Left quadrate length, LQL.

Carapace

Nuchal width, NuW; Nuchal Length, NuL; Carapace Width, CW; Carapace Midline Length, CML; Carapace Length, CL; Neural1 width, N1W; Neural2 width, N2W; Neural3 width, N3W; Neural4 width, N4W; Neural5 width, N5W; Neural6 width, N6W; Neural7 width, N7W; Neural8 width, N8W; Neural1 length, N1L; Neural2 length, N2L; Neural3 length, N3L; Neural4 length, N4L; Neural5 length, N5L; Neural6 length, N6L; Neural7 length, N7L; Neural8 length, N8L; Pleural1 Right Width, P1RW; Pleural2 Right Width, P2RW;

Pleural3 Right Width, P3RW; Pleural4 Right Width, P4RW; Pleural5 Right Width, P5RW; Pleural6 Right Width, P6RW; Pleural7 Right Width, P7RW; Pleural8 Right Width, P8RW; Pleural1 Left Width, P1LW; Pleural2 Left Width, P2LW; Pleural3 Left Width, P3LW; Pleural4 Left Width, P4LW; Pleural5 Left Width, P5LW; Pleural6 Left Width, P6LW; Pleural7 Left Width, P7LW; Pleural8 Left Width, P8LW; Pleural1 Right Length, P1RL; Pleural2 Right Length, P2RL; Pleural3 Right Length, P3RL; Pleural4 Right Length, P4RL; Pleural5 Right Length, P5RL; Pleural6 Right Length, P6RL; Pleural7 Right Length, P7RL; Pleural8 Right Length, P8RL; Pleural1 Left Length, P1LL; Pleural2 Left Length, P2LL; Pleural3Left Length, P3LL; Pleural4 Left Length, P4LL; Pleural5 Left Length, P5LL; Pleural6 Left Length, P6LL; Pleural7 Left Length, P7LL and Pleural8 Left Length, P8LL.

Chapter 4

Eggshell Structure of the Narrow-headed Softshell Turtle Chitra chitra Nutphand, 1986 (Testudines: Trionychidae)

Abstract

Eggs of Chitra chitra were examined for: 1) eggshell thickness by stereo microscope 2) eggshell structure by scanning electron microscopy (SEM) 3) qualitative and quantitative elements of eggshell structure by energy dispersive x-ray analysis (EDX), and 4) eggshell composition by X-ray diffraction analysis. It was found that the eggshell thickness of the outer layer and inner layer detected by stereo microscope were 0.14 $\pm$ 0.02 mm and 0.13 $\pm$ 0.02 mm, respectively. The result of SEM showed that the eggshell had three layers; an outer calcareous sheet (previously unseen), a middle crystalline layer and an inner fibrous layer. The eggshells were composed of oxygen = 52.96$\pm$4.81%, carbon = 35.03$\pm$9.17%, magnesium = 5.55$\pm$0.34%, calcium = 5.37$\pm$7.16%, silica = 2.87$\pm$1.64%, aluminum = 2.30$\pm$1.07%, potassium = 0.17$\pm$0.1%, and sodium = 0.74$\pm$0.3%. The eggshell was the aragonite form of $CaCO_3$.

Key words: Chitra chitra, Softshell turtle, Eggshell Structure, Eggshell Thickness, Eggshell Composition, SEM

Introduction

The narrow-headed softshell turtle Chitra chitra Nutphand, 1986 is one of the largest, and one of the least known turtles in the world. Since its recent recognition as a distinct species (Nutphand, 1986), its population size has almost certainly continued to decline across its limited range in Thailand and elsewhere in southeast Asia (Thirakhupt and van Dijk, 1994; Engstrom et al, 2002; Kitimasak and Thirakhupt, 2002) due to over fishing, pet trade, and habitat alteration and degradation (mainly due to reservoir construction). Its biology and natural history are very poorly known, and the lack of information seriously limits conservation efforts in its behalf. An ongoing study of this species in the field and in captivity is designed to rectify this deficiency. As part of this investigation of the reproductive biology of this species the structure and composition of the eggshell of eggs from a wild caught female were analyzed. The results of the analysis are presented here, and compared with the few other structural analyses of turtle eggshells which have been published (ie. Young, 1950; Solomon and Baird, 1976; Baird and Solomon, 1979; Packard and Packard, 1979; Packard, 1980; Woodall, 1984; Roberts and Sharp, 1985; Packard and Hirsch, 1986; and Wangkulangkul et al., 2000).

Materials and Methods

A female C. chitra (~80 kg) was caught in Srinagarind Reservoir, Kanchanaburi Province, Thailand in March, 2001. Subsequently she laid 3 eggs in a fiber holding tank. All of these eggs were broken. After that synthetic oxytocin was used to induce oviposition. All of the eggs obtained from oxytocin injection (N=32) were incubated in a styrofoam box. Six undeveloped eggs, two weeks old, were separated for study. Specific eggshell characteristics examined and techniques utilized in the analyses are described below: 1) **Eggshell thickness**; the eggshells were randomly cut into 5 pieces/egg of about 1 x 5 mm. They were dehydrated and stained by 0.5% eosin. Thirty thicknesses (5 locations/egg) were measured under a stereo microscope with an ocular micrometer. The thicknesses of outer and inner layers were compared by t-test analysis using SPSS program (Ver. 10). 2) **Scanning Electron Microscopy (SEM);** the dehydrated eggshells were coated with gold and were examined by a J.S.M.-6400 scanning electron microscope, operated at 15 kV. 3) **Energy dispersive x-ray analysis (EDX);** the J.S.M.-6400 electron microscope was used to examine both qualitative and quantitative elements in the dehydrated eggshells. 4) **X-ray Diffraction analysis;** the eggshells were ground down to powder-sized particles to examine their composition. An X-ray Diffractometer model JDX-8030 was used at 45 kV for this procedure.

Results

Eggshell thickness

Eggshells of C. chitra were examined and were separated into 2 distinct layers through eosin staining. The outer layer was unstained but the inner layer had a pink color from the eosin. It was found that the mean thicknesses of the outer layer and inner layer were 0.14 $\pm$ 0.02 mm and 0.12 $\pm$ 0.02 mm, respectively (Table 4.1). The thicknesses of the outer and inner layers of the eggshells were significantly different ($p < 0.05$).

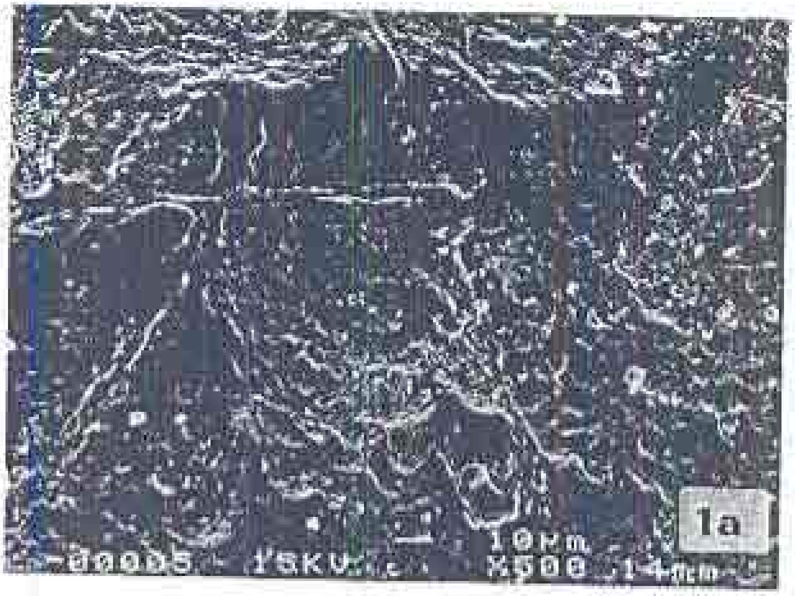
Table 4.1 Outer layer thickness, Inner layer thickness (mm), Energy dispersive x-ray analysis (EDX) (%) and X-ray Diffraction of wild Chitra chitra eggs.

Eggshell Thickness	n	Mean $\pm$ SD
Outer layer thickness (mm)	6	0.14 $\pm$ 0.02
Inner layer thickness (mm)	6	0.12 $\pm$ 0.02
Energy dispersive x-ray analysis (EDX) (%)	9	O = 52.96+4.81 C = 35.03+9.17 Mg = 5.55+0.34 Ca = 5.37+7.16 Si = 2.87+1.64 Al = 2.30+1.07 Na = 0.74+0.3 K = 0.17+0.1
X-ray Diffraction	9	Aragonite

Scanning Electron Microscopy (SEM) Analysis

SEM determined that C. chitra eggshells were divided into three distinct layers – an outer calcareous sheet, a middle crystalline layer, and an inner fibrous layer (Figure 4.1).

The outer surface was composed of a nearly continuous calcareous sheet that covered the whole of the egg (Figure 4.1a). It was a very thin layer that could not be observed under a stereo microscope. The middle layer was composed of fan shaped crystalline units packed together throughout the layer (Figure 4.1b; 4.2). These units displayed the radial fractured symmetry of aragonite projecting from nucleation centers located on the surface of the inner layer. The inner layer of the eggshell was composed of multiple layers of reticular fibers (Figure 4.1b, 4.2, 4.3). Interspersed at intervals along the surface of the calcareous sheet were pore opening which extended downward between the adjacent crystalline units of the middle layer (Figure 4.4).



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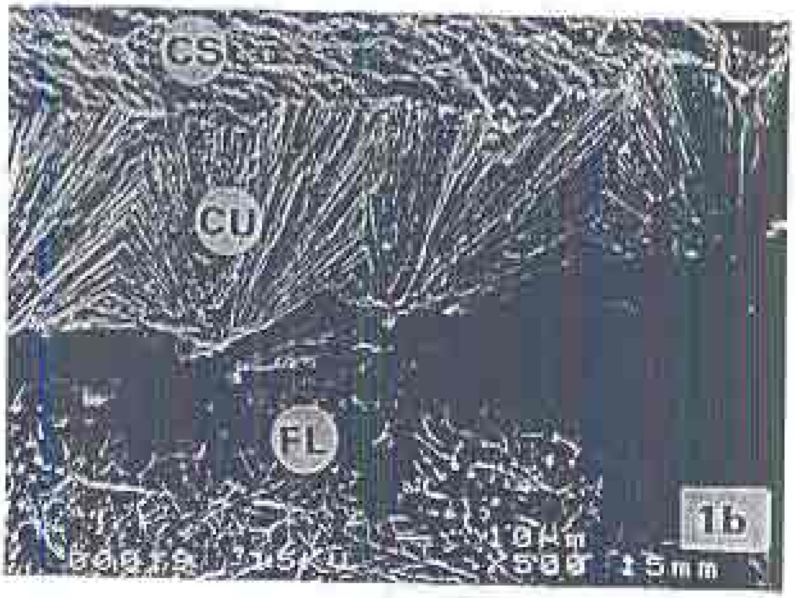


b

Figure 4.1 SEM showing the calcareous sheet (CS) covering the outer layer of eggshell, middle layer crystalline unit (CU) and an inner fibrous layer (FL). a = top view, b = side view, Bar = 10 μm

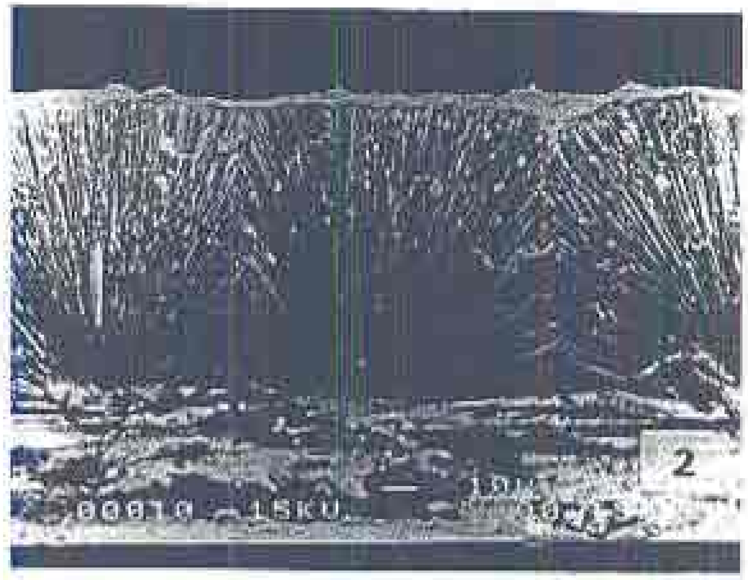
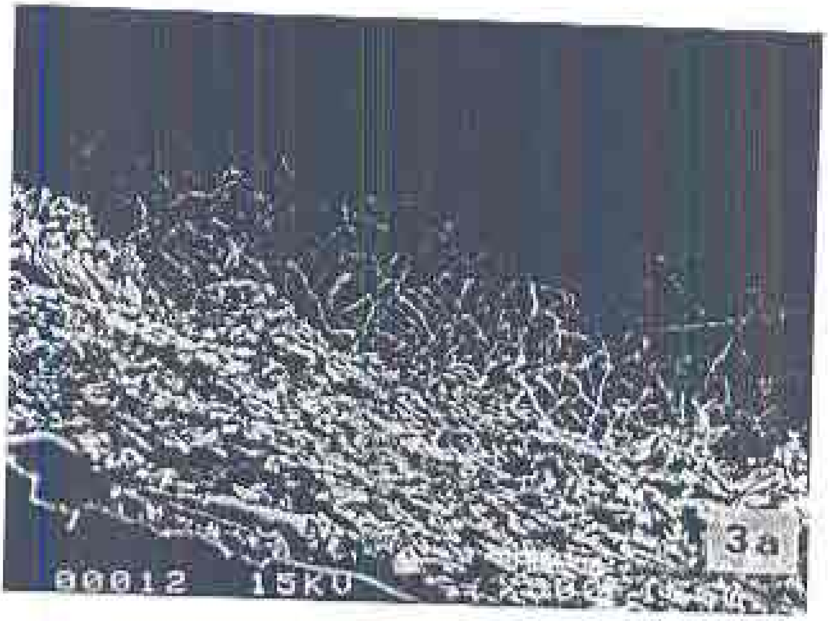


Figure 4.2 SEM showing the radially fractured appearance of crystalline units of the middle layer. Bar = 10 μm



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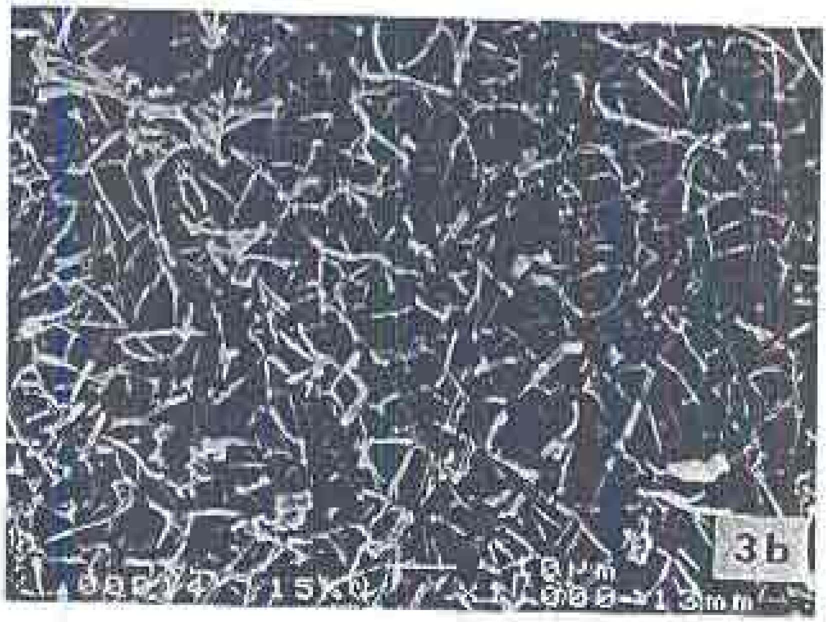

b

Figure 4.3 SEM showing the inner layer of the eggshell.

a = side view (Bar = 10 μm), b = top view (Bar = 10 μm)

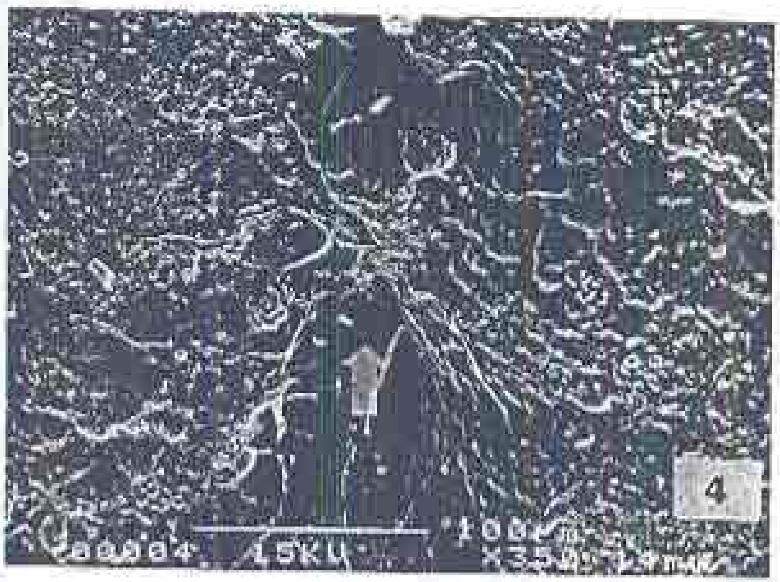

Figure 4.4 Outer surface of C. chitra eggshell showing pore (arrow) at the intersection of crystalline units. (Bar = 100 μm)

Energy Dispersive X-Ray Analysis (EDX)

EDX analysis revealed that C. chitra eggshells were composed of the following elements (ranked in order of relative abundance): oxygen, carbon, magnesium, calcium, silicon, aluminum, sodium and potassium (table 4.1).

X-ray Diffraction Analysis

The powder X-ray photographs indicated that eggshells of C. chitra were composed of the aragonite form of $CaCO_3$.

Discussion

The eggshell of C. chitra displayed only two layers, the middle and inner layers under stereo microscope, but showed 3 layers including an outer calcareous sheet under SEM. Numerous other studies of turtle eggshell structure record the presence of two shell layers. These include broad-shelled river tortoises Chelodina expansa, spiny softshell turtles Apalone spiniferus, olive ridley turtles Lepidochelys olivacea, Euphrates softshells Rafetus euphraticus, European pond turtles Emys orbicularis and radiated tortoises Geochelone radiata (Young, 1950; Packard and Packard, 1979; Woodall, 1984; Wangkulangkul et al., 2000).

Similar to the finding of our study of C. chitra, three layers, were recorded in snapping turtles Chelydra serpentina by Packard (1980). The outer layer of the snapping turtle eggshell was covered by a thick organic calcareous sheet which obscures the tips of the crystalline units of the middle layer. The middle layer was structurally similar to that observed in C. chitra in this study. The outer calcareous sheet may not have been recorded in many turtle eggshells due to the researchers' use of old preserved eggs in their studies. The calcareous sheet may have deteriorated in the preservative over time. The inner layer of C. chitra has multiple layers of fiber similar to those observed in the previous studies of C. serpentina and Trionyx spiniferus (Packard and Packard, 1979; Packard, 1980)

Young (1950) recorded that the eggshell thicknesses of R. euphraticus, Emys orbicularis and G. radiata were 0.23 – 0.29 mm, 0.27 – 0.28 mm and 0.59 – 0.84 mm, respectively. In this study the mean total eggshell thickness of C. chitra was 0.26 mm, falling within the range of eggshell thicknesses of the aquatic turtles Young studied.

Solomon and Baird (1976) analyzed the elements in eggshells of Chelonia mydas. They found the shell composed of ~ 20% Ca, 0.06% Mg, and ~ 1% P. In C. chitra eggshells only ~ 5.37% is composed of Ca but Mg content (5.55%) is substantially higher than that in C. mydas. The reasons for the differences in composition of these species' eggshells are unknown.

The composition of C. chitra eggshells from the wild caught female was $CaCO_3$ in aragonite form. This result was similar to that of other studies of turtle eggshells (Solomon and Baird, 1976; Baird and Solomon, 1979; Packard and Packard; 1979; Packard, 1980; Woodall, 1984; Roberts and Sharp, 1985; and Packard and Hirsch, 1986).

The author is conducting a captive breeding program for C. chitra at Kanchanaburi Inland Fisheries Research and Development Center, Kanchanaburi, Thailand to promote the long-term conservation of this critically endangered species. Knowledge of the structure and elemental composition of the eggshells of C. chitra obtained from wild stock may be useful for comparison with those produced by captive females. The similarity of eggshells of brood stock and wild stock may be a useful indicator to determine nutritional requirements of captives and to increase the likelihood that healthy hatchlings would be produced.

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Chapter 5

New Record of Chitra chitra Nutphand, 1986 in Mae Ping River, Thailand

Abstract

The distribution of the Siamese narrow-headed softshell turtle Chitra chitra Nutphand, 1986 had been known only in Mae Klong river system in Thailand. This study found the distribution range of C. chitra extends to Mae Ping River from the past to present. Life and skeleton (skull and carapace) specimens were found along Mae Ping River both below and upper Bhumipol Dam.

Key words: Chitra chitra, Distribution, locality, Mae Ping River

Introduction

Chitra chitra Nutphand, 1986 is one of five native softshell turtle species in Thailand. It was listed as a Critically Endangered Species by IUCN in 2000. The river systems of Thailand have been classified into six major river drainages; the Salween, Mae Klong, southern Peninsula, southeastern part, Chao Phraya and Mae Kong river systems (Vidthayanon et al., 1997). However, C. chitra has not been previously reported from anywhere other than the Mae Klong river system (Nutphand, 1990; Smith, 1931; Thirakhupt and van Dijk, 1994).

This study shows the discovery of extension range of C. chitra in Thailand's river system.

Materials and Methods

This study was conducted by boat surveys and by interviews with fishermen and local people along the Mae Ping River. The study area began at the mouth of Mae Ping River at Nakhon Sawan Province and extended to Mae Ping National Park, Lamphun Province, a distance of about 100 km. The data including size and locality of the live specimen and osteological specimens (skull and carapace) are shown below.

Results

The habitat of C. chitra in this record was divided into two areas (Figure 5.1). The first is the area below Bhumipol Dam. In this area, one live male C. chitra was caught by the longline hook, usually used for Mystus spp., near the mouth of Mae Ping River at 15°43.020′N 100°08.632′E, Mueang district, Nakhon Sawan Province in June 1998. The hook was attached to its leg. This live specimen was donated to Nakhon Sawan Inland Fisheries Research and Development Center and was later moved to Kanchanaburi Inland Fisheries Research and Development Center, Kanchanaburi Province on April 29, 1999 for the captive breeding program. The shell width, shell length and body weight were 66 cm, 76 cm and 44 kg, respectively.

Other evidence of C. chitra in the Mae Ping River included four carapaces and one skull from the survey in November 2001 and in February 2002. All of these were found in the reservoir of upper Bhumipol Dam at different times. The first carapace with the skull (Figure 5.2) has been kept by a restaurant owner for more than 10 years. Its carapace width, carapace length, skull width and skull length were 61 cm, 66 cm, 13 cm and 27 cm, respectively. The second carapace (CUMZ (R) 2001.11.27, 1) was from a 90 kg C. chitra at Ban Pakveak, Sam Ngao District, Tak Province which was captured by a fisherman in 1987. Its carapace width and carapace length were 64 cm and 71 cm respectively. The third specimen (CUMZ(R) 2001.11.27, 2) was kept by a villager who bought it for food a few years ago. It was a juvenile with a carapace of 21.1 cm wide and 21.7 cm long. The fourth specimen (CUMZ(R) 2002.2.17, 1) was sold to a restaurant owner by a fisherman in 1997 and was reported to have been captured from Mae Toun, Mae Ramat District, Tak Province. It weighed about 11 kg and the carapace width and length were 27.5 cm and 29.1 cm respectively. At present, the last three specimens are deposited at the Chulalongkorn University Museum of Zoology, Bangkok.

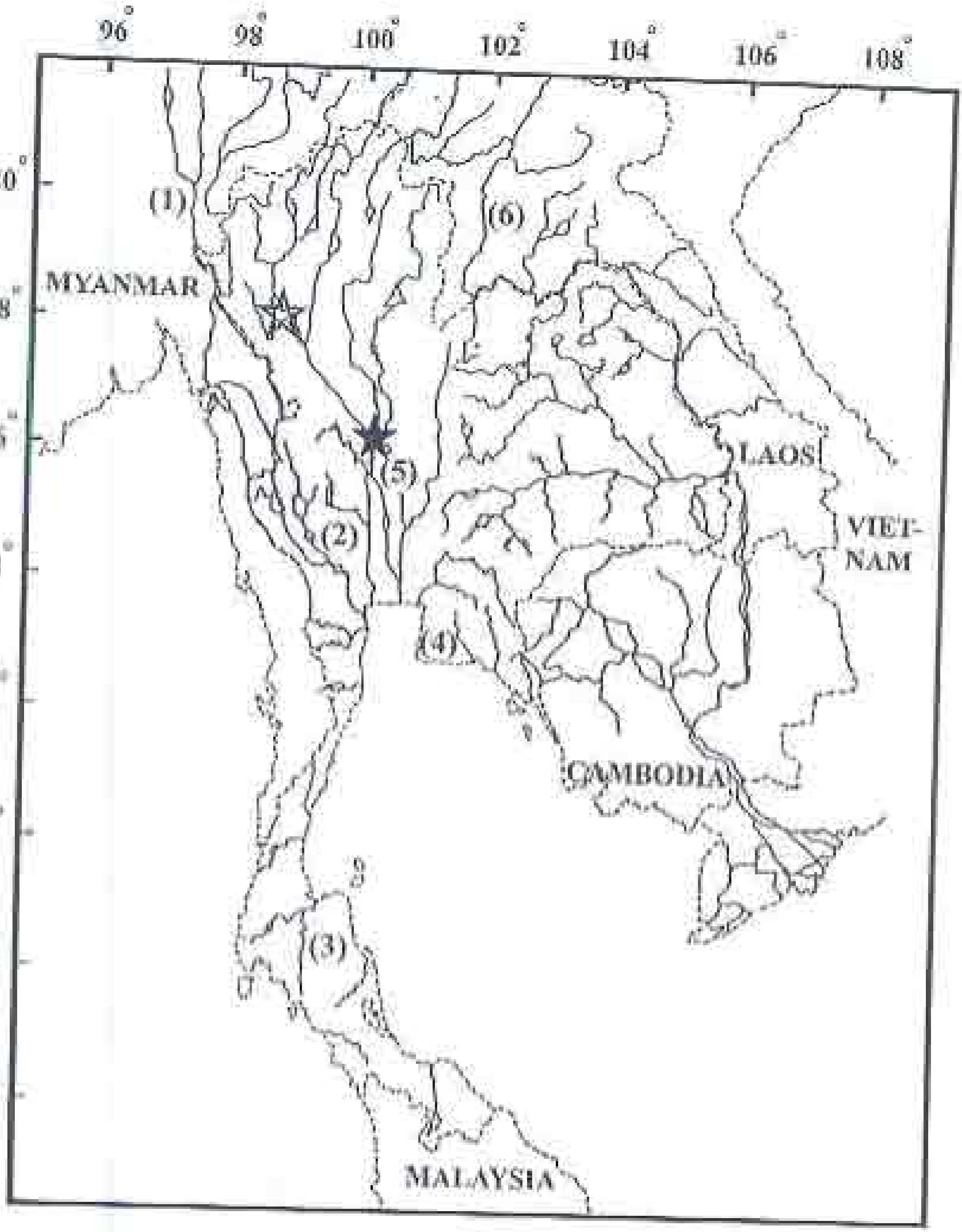
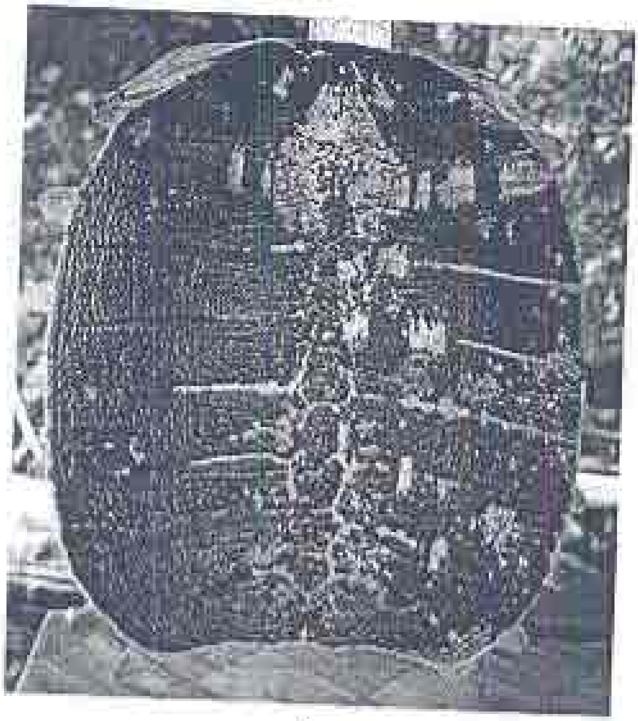


Figure 5.1 Six major river drainages in Thailand; (1) the Salween, (2) Mae Klong, (3) southern Peninsula, (4) southeastern part, (5) Chao Phraya and (6) Mae Kong river systems and the new record of C. chitra in Mae Ping River, upper Bhumipol Dam (open star) and below Bhumipol Dam (solid star).



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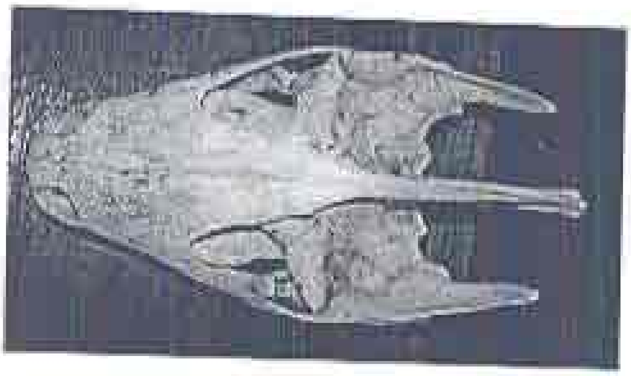

b

Figure 5.2 Carapace (a) and skull (b) of C. chitra in Mae Ping River.

Discussion

Thirakhupt and van Dijk (1994) questioned why C. chitra remains restricted to the Mae Klong, apparently unable to reach the nearby and connected Tha Chin and Chao Phraya Rivers, and this remains a biogeographical mystery. This record may provide the answer that C. chitra did not only inhabit the Mae Klong river system but was once probably more common in the Chao Phraya river system as well. However, the apparent absence of C. chitra in the Tha Chino River is still not understood. Perhaps it was extirpated by human exploitation here before its presence was recognized by zoologists.

This record may be important for the conservation and management of this critically endangered species. An intensive survey on its population status and its breeding sites in the Mae Ping River is in progress. In addition, the study of other aspects of the biology and biogeography of this species is urgently needed.

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Chapter 6

Distribution and Status of the Siamese Narrow-headed Softshell Turtle Chitra chitra Nutphand, 1986 in Thailand

Abstract

The distribution range and status of Chitra chitra Nutphand, 1986 in Thailand were investigated. C. chitra was found in the Mae Klong and Chao Phraya river systems. Another species, C. burmanica Jaruthanin 2002 or C. vandijki, McCord & Pritchard, 2002, was reported to occur in the Salween river system located along the Thailand-Myanmar border. At present, the status of C. chitra is very rare everywhere and the natural population is declining. Conservation and management action in behalf of this species is urgently needed.

Key words: Chitra chitra, Distribution, Status, Thailand

Introduction

The Siamese narrow-headed softshell turtle, Chitra chitra Nutphand, 1986, is probably the largest softshell turtle in the world. Pritchard (2001) estimated the maximum leathery carapace length (LCL) of C. chitra as 122 cm. A female, 152 kg with leathery carapace length of 123 cm, was found in 1967 (Nutphand, 1986). The largest female found in Thailand was recorded as 202 kg in 1986 (The Royal Institute, 1992).

Thirakhupt and van Dijk (1994) reported that C. chitra was an endemic turtle species of Thailand. Information subsequently presented by Engstrom et al. (2002), Engstrom and McCord (2002), and McCord and Pritchard (2002) now indicates that populations representing this species also extend into peninsular Malaysia and Indonesia (to Java) of the six major river drainages recognized in Thailand by Vidthayanon et al. (1997), including the Salween (SW), Mae Klong (Mkl), Chao Phraya (CP), Southern Peninsula (St), Southeastern part (E), and Mae Kong (MK) (Figure 6.1), only the first three are now known to contain Chitra populations. The recently described species called either C. burmanica (Jaruthanin, 2002) or C. vandijki (McCord & Pritchard, 2002) inhabits the Salween river system along the Thailand-Myanmar border. Chitra chitra is known to occur in the Mae Klong river system of western Thailand (Thirakhupt and van Dijk, 1994) and has recently was discovered in the Mae Ping River of the Chao Phraya drainage also (Kitimasak and Thirakhupt, 2002; chapter 5 this volume). Previously Thirakhupt and van Dijk (1994) had questioned why C. chitra was restricted only to the Mae Klong basin although it was connected to the Chao Phraya and Tha Chin rivers. The discovery of C. chitra in the Mae Ping River suggests that its presence in these rivers may have been overlooked by zoologist-perhaps as the result of greatly reduced populations from levels of former abundance due to human exploitation and habitat destruction. Chitra presence in the Tha Chin River has not yet been verified, however, and Chitra's possible former and present distribution elsewhere in Thailand requires further investigation.

From the past to present, C. chitra has been threatened by hunting, water pollution, reservoir creation and other forms of habitat destruction and alteration (Thirakhupt and van Dijk, 1994). These events have negatively affected its natural distribution and population size. In 2000, IUCN listed C. chitra as a critically endangered species, while Thirakhupt and van Dijk (1994) recommended C. chitra as the first priority of turtles requiring conservation action in Thailand. To date, the information on the distribution and status of this species is very incomplete. Greater knowledge of its distribution range and population size as well as its habitat characteristics would be very useful for its conservation and management in the future.

Methodology

This study was conducted by sending 129 questionnaires to the local fisheries offices, Fisheries Department, Agricultural and Cooperative Ministry, throughout Thailand. Intensive investigations were carried out in every province that had positive information of C. chitra. In addition, the locality data of live specimens and preserved specimens from all sources were recorded both in Thailand and abroad. Museum collections containing C. chitra examined in this study are the Natural History Museum, London (BNHM); Museum of Comparative Zoology, Harvard University (MCZ); and Chulalongkorn University Bangkok, Museum of Zoology (CUBMZ).

Results

Eighty-three completed questionnaires (64.34%) were received. There were seventeen (20.5%) with positive information indicating that C. chitra might be, or once was present in the Salween, Mae Klong, Southern Peninsula, Southeastern part and Chao Phraya river systems (Table 6.1). Intensive investigations were conducted in the areas from which positive information was received and the results are described below.

Salween River System

There were no published records of C. chitra from the Salween river system in the past. However, in this study, positive information concerning its presence there was obtained from fisheries officials at Mae Sot District, Tak Province.

The field study was conducted in November 2001. Five specimens of Chitra sp., 4 live specimens (Figure 6.2) and 1 carapace were found. All specimens were C. burmanica Jaruthanin, 2002 or C. vandijki McCord & Pritchard, 2003. They were captured by local people in the river near the Thailand-Myanmar border line but there were no exact data concerning their specific localities.

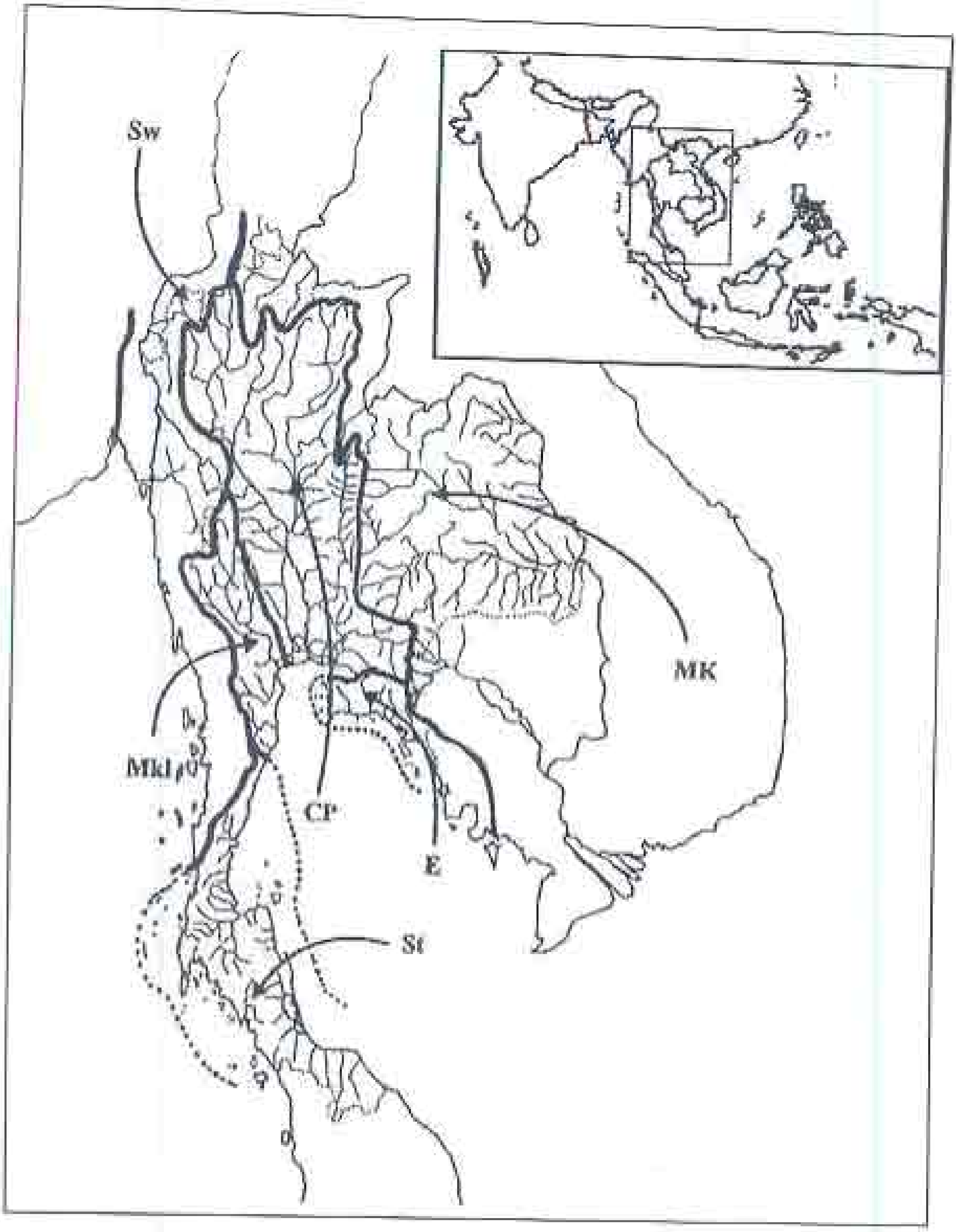

Figure 6.1 The river system of Thailand was classified into six major river drainages; the Salween (SW), Mae Klong (Mkl), Southern Peninsula (St), Southeastern part (E), Chao Phraya (CP) and Mae Kong river systems (MK) (After Vidthayanon, et al., 1997).

Table 6.1 Distribution of Chitra in Thailand

River systems	Sources of Data		
	Literature review	Questionnaire and interview	Field study
Salween	X	√	√
Mae Klong	Kwae Noi, Kawe Yai, Mae Klong, Srinagarind Reservoir	Kwae Noi, Kawe Yai, Mae Klong	Kwae Noi, Kawe Yai, Mae Klong, Srinagarind Reservoir, Vajiralongkorn Reservoir
Southern Peninsula	X	√	X
Southeastern part	√	√	X
Chao Phraya	Chao Phraya	Chao Phraya, Nan	Chao Phraya, Nan, Mae Ping
Mae Kong	X	X	-

√ = positive information

X = negative information

- = not investigated

The external characters of this turtle differed from those of C. chitra and C. indica. The stripe patterns on their carapaces, necks and fore limbs and body color are different from both C. chitra of the Mae Klong river system and C. indica. One of them had a dark-yellow carapace when it was seen the first time (Figure 3), which then changed to light yellow when it was moved to Kanchanaburi Inland Fisheries Research and Development Center (KIFRDC) after a few days (Figure 6.2A). The middle stripes on the necks of all specimens are longer than C. chitra and their stripe patterns on the carapace have more light color than C. chitra. They also have stripes on their forelimbs which are not found in C. chitra. Moreover, they have several unique short stripe patterns along the carapace edge (also see descriptions in McCord and Pritchard, 2002).

Mae Klong River System

The location of the Mae Klong River system is shown in Figure 6.1. The Kwae Yai River and Kwae Noi River join together at Mueang District, Kanchanaburi Province, in the western part of Thailand. The natural habitats of C. chitra in the Mae Klong river system were blocked by several dams. The earlier waterways of the Kwae Noi River and Kwae Yai River were blocked by Vajiralongkorn Dam (formerly called Khao Leam Dam) and Srinagarind Dam, respectively. Moreover, the Kwae Yai River was also blocked by Tha Thung Na Dam below Srinagarind Dam. Furthermore, the Mae Klong River was also blocked by the Mae Klong Dam (formerly called Vajiralongkorn Dam). Field studies were conducted several times in the Mae Klong River and its tributaries, the Kwae Noi and Kwae Yai Rivers during 1998-2001.

Figure 6.2 Stripe pattern of C. burmanica or C. vandijki. (A, B at KIFRDC and C, D at Personal Collection) from Salween river system. The Leathery Carapace Lengths (LCL) of A, B, C and D were 44 cm, 45.5 cm, 36.5 cm, and 28.2 cm, respectively.

Figure 6.3 The color of C. burmanica or C. vandijki was darker before it was moved to KIFRDC. Turtle in this picture is the same turtle in Figure 6.2A.

Kwae Yai River and Srinagarind Reservoir

One female, C. chitra was caught by a fishing hook in the Srinagarind Reservoir, located at 14° 56.360' N 99° 10.764' E, in October 1997 (Figure 6.4). The angler estimated that its weight was 270 kg. It was killed and was sold for food. Follicles, approximately one cm in diameter, were said to be found in the ovaries.

Additional information concerning two other C. chitra, approximately 70 and 150 kg from the Ong Thung area in the Srinagarind Reservoir was obtained from a fisherman. Their sexes were unknown and they are probably living in that area.

Figure 6.4 An adult female of C. chitra caught in Srinagarind Reservoir in October 1997. The site is located at 14^{0} 56.360' N 99^{0} 10.764' E.

In 1998, KIFRDC obtained two C. chitra hatchlings from a fisherman at Srinagarind Reservoir. He reported that 20 hatchlings were found in July 1998 from the small island located at 14^{0} 39.819' N 99^{0} 03.606' E (Figure 6.5). They were observed climbing out from their nest and were moving into the reservoir. He excavated the nest and found that the nest was ~40 cm in depth and ~ 10 m distant from the water's edge. There were approximately 60-70 dead hatchlings in the nest. The eggshells were counted roughly to about 100. It is likely that the female laid its eggs nearer to the water but during the incubation period Srinagarind Dam released water from the reservoir for generating electrical power. Therefore, during the hatching time, the water level had decreased to about 10 m below the initial egg-laying site. There were many hatchlings that could not climb out of the nest but some that did reached the water before the fisherman encountered them.

Figure 6.5 A nest site in Srinagarind Reservoir, located at 14° 39.819' N 99° 03.606' E

In April 2001, one C. chitra female, about 80 kg, was caught by a fishing hook in Srinagarind Reservoir. She laid three eggs in a fiber tank after being moved to Ayuthaya Province. Synthetic oxytocin was injected to induce further oviposition. Later on, 32 eggs were obtained and were incubated in a styrofoam box. However, only one egg hatched after incubation.

There is no record of C. chitra being found below Srinagarind Dam in the Kwae Yai River during the last decade. The area along the river was surveyed and it was found that the habitat was not suitable because there were no sandbanks available for egg laying sites. Besides, the areas along the river bank were mostly settled by humans.

Kwae Noi River and Vajiralongkorn Reservoir (formerly called Khao Laem Reservoir)

There has been no report of C. chitra in Vajiralongkorn Reservoir since the Vajiralongkorn Dam was constructed. However, in January 7, 2000, fishermen found a C. chitra female laying its eggs near the water at midnight on a small island in the Vajiralongkorn Reservoir (located at 14^{0} 59.995' N 98^{0} 33.071' E) (Figure 6.6). It was caught by a spear and was sold to local people. The animal died on the following day, was dissected and about 100 eggs were found in the oviduct. The skull and carapace were donated to CUBMZ (CUBMCZ (R) 2001.14). The nest was excavated and 60 eggs were found in the nest. Two eggs were broken, the rest were incubated under 10 cm of sand in a circular container. Twelve days later, the eggs were investigated and only five fertile eggs were found. However, no egg hatched after being incubated for two months.

Figure 6.6 A nest site in Vajiralongkorn Reservoir, located at 14^{0} 59.995' N 98^{0} 33.071' E

A survey was conducted in the Vajiralongkorn Reservoir in 2000. Two skulls and one carapace of C. chitra were found at a fisherman's house. One skull and one carapace of the same animal were donated to CUBMZ (CUBMCZ (R) 2001.15). The fisherman mentioned that the skull and the carapace were collected more than 10 years ago.

From interviews it was determined that C. chitra was regularly collected along the Kwae Noi River from the past until the present. Surveys were conducted along the Kwae Noi River several times during 1999-2001. It was found that when the water level was low there were many suitable sandbanks for egg laying along the river (Figure 6.7). Local people estimated that more than 100 C. chitra in the Kwae Noi River were caught in the last decade. Most of them were hatchlings and juveniles. However, at present, most sandbanks are flooded when water is released from the dam, thereby rendering them useless as Chitra nesting site.

Figure 6.7 Natural habitat of C. chitra at Kwae Noi River, located at 14° 20.384' N 98° 56.448' E

Mae Klong River and Mae Klong Reservoir (formerly called Vajiralongkorn Reservoir)

Mae Klong (formerly called Vajiralongkorn) Dam was constructed since 1964 and was completed in 1970. The dam is located at about 14 km below the junction of the Kwae Yai and Kwae Noi Rivers. Some local people living along this waterway were interviewed. They provided the information that there were many sandbanks and many C. chitra in this portion of the Mae Klong River before the dam was constructed. After the water level rose and flooded all the sandbanks in the reservoir, C. chitra rapidly disappeared and nobody has caught or seen them in this reservoir for at least the last 10 years.

Below the Mae Klong Dam, C. chitra has been seldom caught and is thought to be continuously decreasing in number. The distribution range of C. chitra in the Mae Klong River extends to Damnoen Saduak Canal, the canal that joins the Mae Klong River to the Tha Chin River. A monk who lived near Damnoen Saduak Canal said that C. chitra was found in the canal when he was young, which was about 30 years ago. In 1999, one adult male was caught in an irrigation canal, located between the Mae Klong Reservoir and Damnorn Sadowg Canal, and was transported to KIFRDC. These results suggest that the range of C. chitra could include at least part of the southern Chao Phraya river system. It is possible that C. chitra can disperse between the Mae Klong river system and Chao Phraya river system through small tributaries that connect the two river systems. However, there is no record that C. chitra's range extends to the brackish water area at the mouth of Mae Klong River.

Southern Peninsula River System

There were three sources of positive information concerning C. chitra from the Southern Peninsula river system; from a questionnaire, a local person in Chumphon Province, and an animal trader in Narathiwat Province. However, field studies and interviews with other local people in the areas did not provide any evidence of C. chitra and there was no record of it presence there in the past.

Southeastern Part River System

One positive questionnaire was received from Sa Kaeo Province. In 1995, van Dijk and Thirakhupt (1995) stated that C. chitra was found in the Bangpakong River at the turn of century. However, the field study in this area did not acquire any positive evidence of Chitra's presence.

Chao Phraya River System

There were 6 positive responses received in questionnaires from Nan, Phichit, Lop Buri, Ang Thong, Phra Nakhon Si Ayutthaya and Sing Buri Provinces, indicating the possibility that fishermen in these provinces had seen C. chitra in the wild.

Field surveys were carried out along the Chao Phraya, Mae Ping and Nan Rivers. It was found that some local people, living along these rivers, have known C. chitra and provided information that the population of C. chitra was very rare 10-20 years ago. A fisherman at Wat Bot District, Phitsanulok Province said that he had caught big softshell turtles in the Nan River just a few years ago. The details of softshells' descriptions mentioned by the fisherman were similar to those of C. chitra, especially the stripe pattern on its neck. The local people called the turtle as "Tao", "Bunlai" or "Kore-Sak-Yan" (tattoo pattern, similar to the shape of a pagoda on its neck).

Discussion

Distribution

It could be concluded that currently the distribution range of Chitra spp. is in three river systems of Thailand, the Salween, Mae Klong and Chao Phraya (Table 6.1). C. chitra was found in the Mae Klong River system (Kwae Noi, Kwae Yai, and Mae Klong Rivers; Srinagarind, Vajiralongkorn and Mae Klong Reservoirs) and Chao Phraya river system (Mae Ping, Nan and Chao Phraya Rivers).

The previous records of the distribution of C. chitra in Thailand were mainly in the Mae Klong river system (van Dijk and Thirakhupt, 1995; Iverson, 1992; Liat and Das, 1999; Nutphand, 1979; Nutphand, 1986; Nutphand, 1990; Smith, 1931; Taechacharernsukchera, 1991; Thirakhupt and van Dijk, 1994; Youngprapakorn, 1993). In addition, all localities of C. chitra in many other collections from Thailand were from the Mae Klong river system (Table 6.2).

There were two records of C. chitra in the Chao Phraya River system. The first record was from the Chao Phraya River (The Royal Institute, 1992). The latter, a C. chitra female, 202 kg, was caught in a canal, a branch of the Chao Phraya River (CP), at Tambon Soun Yai, Mueang District, Nonthaburi Province on May 19, 1986. It was released at the Phutthamonthon area and was not seen again. Kitimasak and Thirakhupt (2002), also described the extension of the distribution range of C. chitra into the Mae Ping River, tributary of the Chao Phraya River system, providing further evidence of its presence here.

The distribution range of C. chitra above the dams was previously recorded as Srinagarind Reservoir (Thirakhupt and van Dijk, 1994) and

Bhumipol Reservoir (Kitimasak and Thirakhupt, 2002). From this study, the identification of C. chitra in Vajiralongkorn Reservoir provides a new record.

C. chitra is primarily found in large rivers with sandy or muddy bottoms. Its habitat is similar to that of C. indica reported by Das (1991), Das (1995), Ernst and Barbour (1989) and Tikader and Sharma (1985). The distribution range of C. chitra only includes freshwater sections of rivers. There is no record of C. chitra in brackish water in either the Chao Phraya or Mae Klong River estuaries.

C. chitra has traditionally been associated with clean and clear water, as recorded by van Dijk and Thirakhupt (1995). Nevertheless, a few turbid water habitats containing C. chitra in the Mae Klong River were identified in this study, perhaps due to the increasing siltation of the river as a whole, and representing less than optimal habitat conditions for this species.

In this study, a recently described Chitra sp. specimen that has distinguishable characters from C. chitra and C. indica was found from the Salween river system near the Thailand-Myanmar border. Jaruthanin (2002) named it as Chitra burmanica in Fish Zone magazine. He reported that the stripe pattern of C. burmanica differed from C. chitra and C. indica as discussed previously. However, this scientific name, C. burmanica, is rejected by McCord (personal communication) in that the species description of Jaruthanin (2002) is inadequate by ICZN standard and the proposed name should not be accepted. McCord and Pritchard (2002) described this new softshell species as C. vandijki.

Table 6.2 Localities and Catalog Numbers of C. chitra specimens from Thailand.

No.	Localities	Catalog Number	Museums
1	Mae Klong River, Banpong, Ratchaburi Province	1921.4.1.197	BNHM
2	Mae Klong River, Banpong, Ratchaburi Province	1974.2451	BNHM
3	Kanchanaburi Province	1962.12.16.1	BNHM
4	Kanchanaburi Province	29486	MCZ
5	Mae Klong River, Banpong, Ratchaburi Province	29487	MCZ
6	Mae Klong River, Banpong, Ratchaburi Province	29488	MCZ
7	Thailand	1994-4-21.1	CUBMZ(R)
8	Kaew Yai River, Kanchanaburi Province	CUB MZ R 2001.10	CUBMZ(R)
9	Thailand	CUB MZ R 2001.11	CUBMZ(R)
10	Thailand	CUB MZ R 2001.12	CUBMZ(R)
11	Thailand	CUB MZ R 2001.13	CUBMZ(R)
12	Vajiralongkorn Dam, Kanchanaburi Province	CUB MZ R 2001.14	CUBMZ(R)
13	Vajiralongkorn Dam, Kanchanaburi Province	CUB MZ R 2001.15	CUBMZ(R)
14	Thailand	CUB MZ R 2001.16	CUBMZ(R)

This Salween softshell has been supported as a valid species by the study of Engstrom et al. (2002). They studied phylogenetic diversity of Chitra from Bangladesh, Myanmar, Thailand, Malaysia and Indonesia. The results showed that phylogenetic analysis of sequence data from the mitochondrial ND4 gene revealed three deeply divergent patterns within Chitra : C. indica from Bangladesh, C. chitra from Thailand, Malaysia and Indonesia and the third from the Salween River of Myanmar recently described as either C. burmanica or C. vandijki refered to above.

Status

C. chitra was described as a new species in 1986 (Nutphand, 1986). Since 1996, it has been listed as "critically endangered" by IUCN (IUCN, 1996, IUCN, 2000) due to its extremely high risk of extinction.

In Thailand, the main causes of C. chitra's decline are: 1) hunting, 2) dam construction and 3) habitat destruction from sand mining and other human activities.

1. Hunting

C. chitra is protected under WARPA law (Wild Animals Reservation and Protection Act B.E. 2535) in Thailand. However, local people have continuously captured C. chitra for food and for sale in the international or local pet trade. Moreover, most eggs that were found by local people would be collected for sale or for captive hatching, due to the high price of live hatchlings turtles in the pet trade. C. chitra of all sizes have been captured for years, mainly from Mae Klong river system (Table 6.3).

2. Dam Construction

The status of C. chitra in reservoirs and below dams is a serious problem. After the dams were constructed, water flooded all C. chitra's habitats and nest sites. In this study, two nests in Srinagarind Reservoir and Vajiralongkorn Reservoir were investigated. Both nests were laid on the banks of small islands that formerly were hill tops. The banks are composed mainly of gravels and rocks, differ totally from the natural sand bank along the Mae Klong river system (Figures 6.5, 6.6 and 6.7). Moreover, the island bank in Vajiralongkorn Reservoir was covered with shrubbery, which inhibits laying activity. Even though C. chitra could lay eggs along these island banks, the successful hatching rate tended to be very low. The failure of successful incubation of eggs from Vajiralongkorn Reservoir might be because eggs were laid in very humid condition (Kitimasak, 1996). Below the dam, all nest-sites would be flooded due to the fluctuating water levels in the river following the release of water for electrical power and agricultural purposes. It was found that C. chitra naturally laid its eggs on sandbanks at low water levels in the dry season. As a result, all eggs would be flooded embryos drowned before hatching. Therefore, at present, populations of C. chitra cannot successful reproduce either above or below the dams. The C. chitra populations in Thailand continue to decline rapidly as a result.

Changes in river flow patterns and floodplain development may also increase river turbidity and siltation. These changes may negatively affect Chitra by inhibiting its sight-feeding foraging abilities, and by potentially silting over sandy nesting site, thereby rendering them unsuitable for use.

3. Habitat destruction due to sand mining and other human activities

Due to country development, many human activities, such as road and building construction require a large amount of sand. Sand has been removed from both Chao Phraya and Mae Klong riverbanks for decades to serve this purpose. It represents another probable cause of population decline, by eliminating or degrading the quality of sand beach nesting habitats.

Table 6.3 Numbers and localities of C. chitra specimens that were caught from river systems in Thailand. Interview data were obtained from fishermen, local people and traders.

River System	≤1996	1997	1998	1999	2000	2001	2002
Salween	-	-	-	-	-	{20}	{1}
Mae Klong	[8], {20}	[5]	[3], (20)	[2]	[1], (20)	[3], {3}, (22)	-
Southern Peninsula	-	-	-	-	-	-	-
Southeastern Part	-	-	-	-	-	-	-
Chao Phraya	[3], {1}	{1}	{1}	-	-	{1}	-
Mae Kong	-	-	-	-	-	-	-
Total	[11], {21}	[5], {1}	[4], (20)	[2]	[1], (20)	[3],{24}, (22)	{1}

[ ] adult

{ } juvenile

( ) hatchling

Recommendation

The results of this study indicate that C. chitra is now very rare in Thailand. Its populations appear to have declined rapidly in the last two decades and nowadays it is nowhere common. This species is surely going to be extinct in the wild in the near future if it does not receive special conservation action immediately. Field surveys throughout their distribution areas during the study period did not result in the observation of a single live specimen in the wild. Its population is severely affected by hunting and habitat alterations described above in its present status, C. chitra should be considered the first priority of all Thai turtles to receive special concern and action. The long-term conservation of this critically endangered species is to be accomplished by the implementation of the following proposal procedures:

1. Captive Breeding Program

A captive breeding program should be one of the first priorities to be carried out in order to produce eggs, hatchlings, juveniles and breeding adults. Moll and Moll (2000) stated that captive breeding of riverine turtles is expensive, requiring large tracts of land, elaborate enclosures, and a permanent staff. However, captive breeding should be employed after existing habitat has been destroyed or when the population becomes too small and scattered for natural reproduction to be effective. The parental stock of known locality should then be collected and housed in range and their captive-bred offspring are suggested to be released at the same location whenever suitable habitat is judged to remain (and other threats which reduced populations initials are minimized or eliminated).

C. chitra meets most of these requirements and captive breeding is considered to be the best methodology under present circumstances for restoring populations to viable levels.

2. Habitat Protection

The restoration and conservation of suitable habitat is most important for C. chitra, both for maintaining and rebuilding existing populations and for reintroduced populations. Areas where Chitra is protected form hunting, and areas where suitable nesting sites are abundant and protected from flooding, mining, and poaching are vitally important it C. chitra is to be conserved.

Since several rare species may be effectively conserved in protected areas, some existing habitats of C. chitra that are not currently protected should be considered for sanctuary status as well.

3. Translocation and Reintroduction

Translocation refers to the release of an animal outside its original range. This method may have limitations in that C. chitra may have potential negative effects on the new host ecosystem, or may not be able to adapt to the new environment. Reintroduction within the original habitat should be considered as the first choice in the release of captive turtles. In case the existing areas are not suitable due to habitat alteration or other reasons then translocation may be considered. The monitoring of released individuals should be performed regularly after the reintroduction or translocation to the extent possible in order to assess the effects and level of success of the procedure.

4. Public Relations and Education

Public relations and education are vitally important to promote conservation programs for C. chitra. Villagers, fishermen and NGO's should be informed of the requirements and status C. chitra by researchers. Local cooperations and participation in conservation action stimulated by such input is fundamentally important in the conservation of Chitra populations.

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Chapter 7

Captive Breeding of the Siamese Narrow-headed Softshell Turtle Chitra chitra Nutphand, 1986 (Testudines: Trionychidae)

Abstract

A captive breeding program of a critically endangered species, the Siamese narrow-headed softshell turtle Chitra chitra Nutphand, 1986, was conducted in two 400 m^2 ponds at Kanchanaburi Inland Fisheries Research and Development Center (KIFRDC), Kanchanaburi Province, Thailand from 2000 to 2002. Copulations were observed during September to February. Two females laid eggs during February to April in artificial sandbanks. Each captive female produced 3 or 4 clutches/year with 40-88 eggs/clutch. Egg sizes (n=220) were 31.94±1.57 mm in width (range 26.89-34.37 mm), 33.16±1.54 mm in length (range 29.39-40.49 mm) and 19.00±1.67 g in weight (range 15.31-21.60 g). The mean incubation time of C. chitra eggs was 59±3 days (n = 255) with a range of 55-65 days at 24-42 °C air temperature and 24-39 °C sand temperature. Hatchling sizes (n=297) were 38.46±1.52 mm in carapace width (range 32.00-41.67 mm), 42.97±1.59 mm in carapace length (range 36.27-46.66 mm) and 13.10±1.03 g in weight (range 9.09-14.89 g). The hatching success in each clutch varied from 3 to 94 %. The hatchlings were fed with fry fishes of Labeo rohita and Oreochromis niloticus. After 14 weeks, mean hatchling size was 86.70±5.17 mm carapace width, 91.72±5.75 mm carapace length and 103.97±18.08 g weight, respectively. The survival rate of juveniles was 90.64%.

Key words: Chitra chitra, softshell turtle, captive breeding, incubation, growth

Introduction

The Siamese narrow-headed softshell turtle, Chitra chitra Nutphand, 1986, is found in Thailand, Malaysia, and Indonesia (Thirakhupt and van Dijk, 1994; Engstrom et al., 2002; Kitimasak and Thirakhupt, 2002; McCord and Pritchard, 2002). It is probably the largest softshell and freshwater turtle in the world (Pritchard, 2001). All populations of this species are declining, and it is considered very rare in all these countries. IUCN (2000) listed its status as "critically endangered" due to its extremely high risk of extinction in the world. The causes of its decline are due mainly to the reduction of suitable habitat resulting from dam construction and other human-related habitat alterations, and from exploitation for food and the animal trade (Thirakhupt and van Dijk, 1994).

Moll and Moll (2000) stated that captive breeding programs may be an important conservation and management methods which can be used to conserve riverine turtles under certain circumstances – especially when suitable natural habitat remains for the release of hatchlings or head-started juveniles produced by this methodology. Unfortunately the opportunity to apply this approach toward C. chitra's conservation and return toward healthier population sizes in nature has been hampered by our inadequate knowledge concerning its basic biological requirements, and consequently, of the most effective techniques for successful maintenance and breeding of captives. This study was conducted to provide data which will alleviate these deficiencies, and therefore provide a sound protocol for successful captive maintenance and breeding of adult C. chitra, and the maintenance and growth of hatchling C. chitra to a size which is deemed most suitable for release into natural habitats.