Materials and Methods

Six Chitra chitra, two females and four males, were maintained in two outdoor enclosures at Kanchanaburi Inland Fisheries Research and Development Center (KIFRDC), Tha Moung District, Kanchanaburi Province, Thailand. The breeding ponds, 400 square-meter (16x25 m), were constructed near the Mae Klong River and were climatically similar to the natural habitat. The water depth varied from 60 to 80 cm, and a 30 m^2 artificial sandbank ascending 80-90 cm above water level was constructed for nesting in each enclosure. Roofs were constructed over the sandbanks for protection from rain-induced mortality of eggs and hatchlings (Kraemer and Bell, 1980). Fences were also constructed around the ponds to protect turtles and their eggs from predators, such as dogs, snakes, and monitor lizards (Figure 7.1). A female and two males were placed in each pond (Table 7.1). Foods provided for C. chitra in the captive ponds were mainly live Nile tilapia Oreochromis niloticus; native fishes, such as Bardodes gonionotus, were available, entering the ponds during water exchange with the Mae Klong River daily Monday – Friday. Air and water temperature in the captive ponds were recorded throughout the year (Figure 7.2).

Pond A

Pond A was completed in early 2000. The first female, 111 kg, was released on 26 February 2000. It was captured from the Kwae Noi River, Kanchanaburi Province, in 1997 and was separated from males for three years. The first male, 44 kg, was captured from the Mae Ping River, Nakhon Sawan Province, in June 1998. It was released in the breeding pond on 31 March 2000. The second male, 60 kg, was captured from the Kwae Noi River in April 1999 and was released into this pond on 2 November 2000.

Pond B

Pond B was completed in November 2001. One female, 71 kg, and a male, 88 kg, were caught from the Mae Klong River system in 1997. The second male, 88 kg, was caught from the agricultural canal (joining the Mae Klong River and the Tha Chin River) in Nakhon Pathom Province on 3 May 1999. All of them were released in the Pond B in November 2001.

Figure 7.1 Chitra chitra rearing Pond A at Kanchanaburi Inland Fisheries Research and Development Center.

Terrestrial Activity and Breeding Behavior

Terrestrial activity and breeding behavior of C. chitra in the breeding ponds were monitored through the year. Terrestrial activity was nocturnal, and its occurrence and frequency data derived from tracks in the sand. Walking tracks indicated terrestrial movement without nesting activity. The track dimensions allowed identification of the individual(s). Nest tracks led to

blank nests or nests with eggs. Mating behavior and copulation duration were also observed and recorded.

Table 7.1 Size, locality, date of capture, and date of release into breeding pond of C. chitra in captivity.

Pond	Sex	Shell length (cm)	Shell width (cm)	Weight (kg)	Locality (river)	Capture time	Released time
A	Female	98.5	81	111	Kwae Noi	1997	26/2/2000
	Male 1	76	66	44	Mae Ping	6/1998	31/3/2000
	Male 2	82	70	60	Kwae Noi	4/1999	2/11/2000
B	Female	84	75.5	61	Kwae Noi	1997	19/11/2001
	Male 1	97	85	88	Kwae Noi	1997	19/11/2001
	Male 2	103	85	88	Mae Klong river system	5/1999	11/2001

Incubation methods

Nests were excavated by hand within 48 hours after nesting. When the first egg was uncovered, the depth to the top of the egg chamber was measured. The depth to the bottom and the width of the chamber were also measured. Eggs of the first and the second clutch of each breeding pond were weighed to the nearest 0.01 g with an electronic balance and linear measurements of width and length measured to the nearest 0.01 mm with digital calipers. The number of eggs and clutches of each female were recorded.

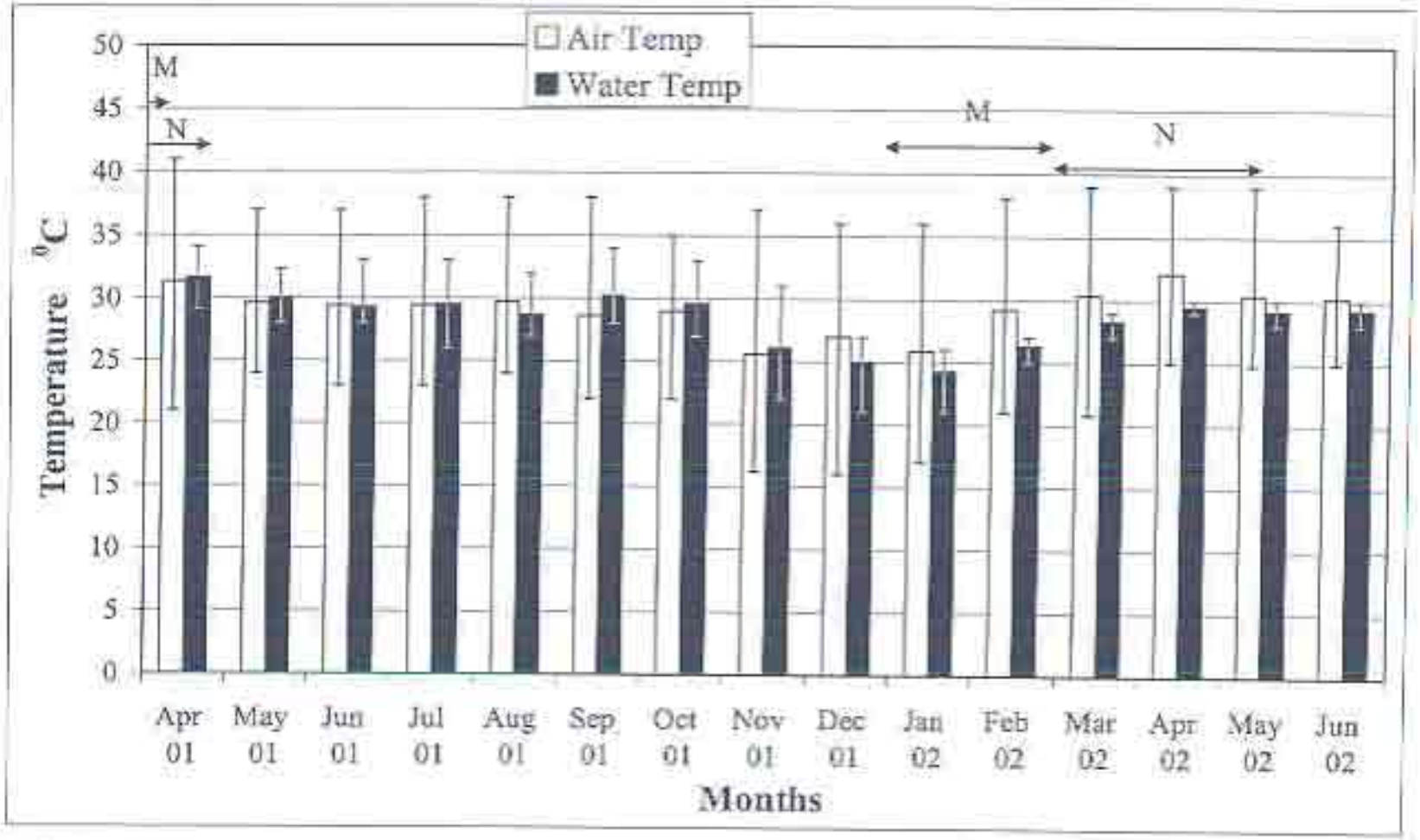

Figure 7.2 Means of water and air temperature in pond A during April 2001 to June 2002. The vertical bars show maximum and minimum of air temperature and water temperature in each month. The arrows show periods of mating (M) and nesting (N).

The eggs were carefully removed and handled to prevent horizontal rotation and reorientation of their vertical axes. Three methods were used for incubation. In the first method, one clutch from each pond was transported to the Department of Biology, Faculty of Science, Chulalongkorn University.

Eggs were put in a plastic container, 40x70x37 cm^3, on 10 cm thick of sand and were covered with 20 cm of additional sand. Moisture in the container was maintained by spraying water on the sand each morning and evening.

In the second method, one clutch from each pond was incubated in circular fiber containers 75 cm in diameter. Eggs were placed on 10 cm of sand and again were covered with 20 cm of additional sand at KIFRDC. The fiber containers were placed in water to protect the eggs from ants and other insects. Water was sprayed on the sand every morning and evening to control the sand moisture content. Water loss was minimized by placing a plastic cover securely over the top of each container.

In the third method, two clutches from each pond were incubated at the nesting area in situ. After 30 days of incubation, the nests were surrounded by mesh to restrain the hatchlings for counting, measuring, and protection from predators.

In each method, the number of hatchlings emerging was recorded and the respective hatching success rates calculated. Incubation times were also recorded in each method. The hatchlings of all treatments were measured (CW and CL) and weighed. Max-Min mercury thermometers were inserted horizontally near the nests to monitor incubation temperature throughout the incubation period. Air temperatures and sand moisture content were also recorded in each method during the studies. Sand moisture content (%) was calculated from [(wet weight – dry weight) / dry weight] x 100.

Growth of hatchlings

Twenty hatchlings, from A1/KIFRDC (Table 7.3), were divided into two groups. They were reared indoors in two glass aquaria, 50x105x40 cm. Sand covered the bottom of the aquaria to a depth of about 4 cm. The hatchlings in each aquarium were provided with 3 cm water. Water was exchanged every Tuesday and Friday. Fry fish of Labeo rohita (Hamilton, 1822), (23.27±1.57 mm length and 0.12±0.05 g weight), Oreochromis niloticus (Linnaeus, 1758), (24.21±1.34 mm length and 0.29± 0.04 g weight), were fed to C. chitra hatchlings in this study. One hundred fish within the first two months and 200 fish in the third month were provided daily in each aquarium. The total number of fish eaten was recorded daily and was divided by hatchlings in order to estimate the average amount consumed per individual. Weight, carapace width and carapace length of hatchlings were measured every two weeks for fourteen weeks.

Results

Reproduction

Timing of reproductive activity

Reproductive behaviors occurred in both ponds over the nine-month period between August and April in both years of the study. Tracks of the females were observed from August through April, with their frequency increasing in November, and from February through April. Nesting tracks and nests were observed from February through April (Figure 7.3).

In the first year of our study, the first copulation observed involved the 44 kg male and female in Pond A on 6 September 2000, at 6.00 A.M. about four months after his release into the pond. Other copulations occurred between 3 December 2000 and 3 April 2001 mainly between 6.15 A.M. and 8.30 A.M. (Table 7.1 and 7.2), with the males being most active between

December and February. At this time, males often chased the females and attempted to copulate.

In the second year, copulation occurred between December and February (Table 7.2). Copulations lasted between 10-15 minutes in both years of study (Table 7.2).

Table 7.2 Date of copulation and mating duration of C. chitra in pond A.

Nesting year	Copulation times	Mating duration
2001	- 6 September 2000; 6.00 am	~10 min
	- 3 December 2000; 8.00 am	~10 min
	- 22 February 2001; 7.30 am	~10 min
	- 2 April 2001; 8.30 am	~10 min
	- 3 April 2001; 6.15 am	~10 min
2002	- 29 December 2001; 2.00 p.m.	~10 min
	- 27 February 2002; 9.40 am	~15 min

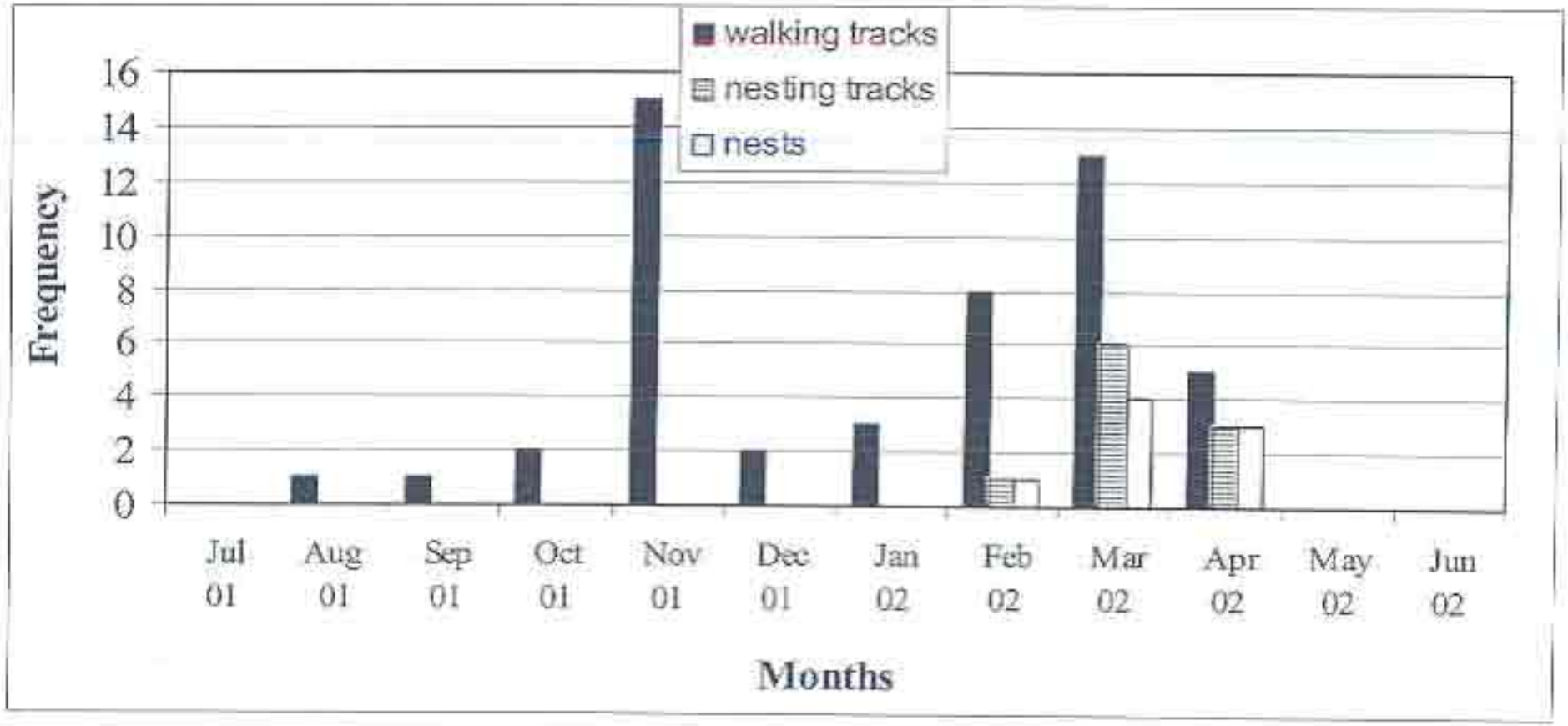

Figure 7.3 Terrestrial activities of C. chitra in pond A; walking tracks, nesting tracks and nests between July 2001 and June 2002.

Nesting

Nesting occurred on the artificial sandbanks. Walking tracks, which may represent exploratory behavior by the females, were found before nesting actually occurred. The first nesting track was found in the morning of 22 February 2001 in Pond A, but the actual nest site was not located. Several additional nest tracks and presumed nests (i.e. large areas of disturbed sand) were found during March to April. On 7 June 2001, after heavy rains and rising water levels within the pond enclosures, Chitra eggs were discovered on the sand surface near the water's edge. Subsequently the sandbank was excavated and four clutches of eggs were removed from flooded nests.

One year later, nesting tracks were found in the morning in both Pond A and Pond B from 27 February to 20 April 2002. Nests were excavated within 48 hours after they were constructed. The nest hole of C. chitra is flask-shaped. The mean of four egg chambers of Pond A was 23.5 cm width, 32.5 cm height from sand surface to top of egg clutch, and 54 cm height from sand surface to nest bottom. The average of four egg chambers of Pond B was 22.8 cm, 32 cm, and 49.3 cm, respectively. The nest dimensions of the smaller female were less than those of the larger female (Table 7.3).

Table 7.3 Hatching success and other parameters for the three incubation methods. Abbreviations: KIFRDC, Kanchanaburi Inland Fisheries Research and Development Center; CU, Chulalongkorn University; ASB, Artificial sandbank;, Size of egg chamber, width (W), height of ground surface to egg top (ET), height of nest bottom to ground surface (NB). Number in parentheses indicates the number of eggs which were found in the original nests.

Pond/Method	Nesting Date	Hatching Date	Egg chamber (W, ET, NB) cm	No. of eggs incubated	Sand Temp. (°C)	Air Temp. (°C)	No. of hatchlings emerged	Incubation time (days)	Hatch rate (%)
A1/KIFRDC	16/3/2002	10-14/5/2002	23, 40, 58	44(45)	24-38	24-42	35	55-59	80
A2/CU	21/3/2002	15-19/5/2002	23, 28, 49	87(88)	26-33.5	25-34	78	55-59	90
A3/ASB	4/4/2002	3/6/2002	22, 30, 54	79(79)	26-35	24-39	74	60	94
A4/ASB	20/4/2002	19-21/6/2002	26, 32, 55	65(68)	26-35	24-39	57	62-64	88
B1/KIFRDC	21/3/2002	18/5/2002	23, 30, 48	40(40)	24-39	24-41	1	58	3
B2/CU	27/2/2002	3/5/2002	25, 35, 50	*22(40)	26-33.5	25-34	1	65	5
B3/ASB	?	16-21/5/2002	22, 30, 50	67(67)	26-35	24-39	44	?	66
B4/ASB	3/4/2002	3-7/6/2002	21, 33, 49	58(58)	26-35	24-39	9	61-65	16

? Actual nesting date not observed.

* Eggs were found at 2 weeks old. The infertile eggs were removed.

Table 7.4 Size of eggs and hatchlings of captive C. chitra. Numbers in brackets of no. of eggs and no. of hatchlings indicate the number of eggs and hatchlings which were measured. Numbers in parentheses indicates standard deviation (SD).

Pond	Year	Clutch	No. of egg	Egg width (mm)	Egg length (mm)	Egg Wet mass (g)	No. of hatchling	Hatchling CW (mm)	Hatchling CL (mm)	Hatchling Wet mass (g)
A	2001	A1	74[-]	-	-	-	-	-	-	-
		A2	64[-]	-	-	-	-	-	-	-
		A3	76[76]	33.08(0.43)	33.46(0.62)	20.41(0.77)	-	-	-	-
		A4	1[-]	-	-	-	1[1]	-	-	13.34
	2002	A1	45[44]	32.67(0.44)	33.05(0.51)	19.72(0.70)	35[35]	38.86(1.60)	43.07(1.11)	13.48(0.65)
		A2	88[35]	32.19(0.53)	32.68(0.50)	19.14(0.72)	78[78]	38.22(1.26)	43.98(0.93)	13.65(0.54)
		A3	79[-]	-	-	-	74[74]	39.32(1.27)	43.12(0.89)	13.65(0.49)
		A4	68[-]	-	-	-	57[57]	38.22(1.56)	42.99(1.56)	12.79(0.70)
Mean of pond A				32.76(0.58)[a]	33.18(0.75)	19.93(0.90)[a]		38.65(1.47)[A]	43.36(1.20)[B]	13.43(0.68)[a]
Range				30.10-34.37	30.99-36.67	15.58-21.60		33.32-41.67	39.20-46.66	11.27-14.89
B	2002	B1	40[25]	28.87(1.47)	35.50(2.59)	16.70(0.66)	1[1]	32.02	38.23	10.76
		B2	40[40]	30.68(0.95)	31.63(1.02)	16.85(0.66)	1[1]	35.70	40.48	11.57
		B3	67[-]	-	-	-	44[42]	37.82(0.99)	41.70(1.68)	11.82(0.88)
		B4	58[-]	-	-	-	9[9]	37.72(2.62)	39.33(1.99)	10.57(0.95)
Mean of pond B				29.98(1.46)[b]	33.12(2.60)	16.79(0.78)[b]		37.61(1.73)[A]	41.20(1.96)[B]	11.59(0.99)[b]
Range				26.89-33.40	28.39-40.49	15.31-18.40		32.00-41.17	36.27-45.41	9.09-13.43
Total mean				31.94(1.57)	33.16(1.54)	19.00(1.67)		38.46(1.52)	42.97(1.59)	13.10(1.03)
Total range				26.89-34.37	28.39-40.49	15.31-21.60		32.00-41.67	36.27-46.66	9.09-14.89

Clutch size

Clutch size ranged from 1 to 88 eggs, although it seems likely that the single egg clutch represents aberrant "nesting". The total eggs from the Pond A female were 215 eggs, from clutches of 74, 64, 76, and 1 egg in 2001. In 2002, the female of Pond A laid 280 eggs from clutches of 45, 88, 79, and 68, respectively. Also in 2002, the female of Pond B laid 205 eggs with clutches of 40, 40, 67, and 58 eggs, respectively (Tables 7.3 and 7.4). Both females deposited four clutches each in nesting season of 35 days at intervals of 5-23 days (Table 7.3).

Egg size

Most C. chitra eggs were white and spherically shaped, but a few were more oblong. The smaller female produced more elongate eggs. Eggshells were brittle and easily broken. The mean dimensions of the eggs (n = 220) were 31.94±1.57 mm width (range 26.89-34.37 mm) and 33.16±1.54 mm length (range 29.39-40.49 mm). Mean egg weight was 19.00±1.67 g (range 15.31-21.60 g) (Table 7.4). Comparison of egg size between the two females revealed that egg width and weight were significantly different between the two females although egg length was not (Table 7.4). The larger female laid wider and heavier eggs than the smaller female.

Incubation and Hatching Success

Mean incubation time of C. chitra eggs was 59±3 days (n = 255) with a range of 55-65 days (Table 7.3) at 24-42 °C air temperature and 24-39 °C sand temperatures (Figure 7.4).

Within a clutch, hatching varied from 1-5 days between first and last egg pipping. Only in the A3/ASB clutch that was incubated in the nest area did all hatchlings (n = 74) emerge at the same time (60 days). In the other clutches the eggs hatched over a 3-5 day period. For the 2002 year,

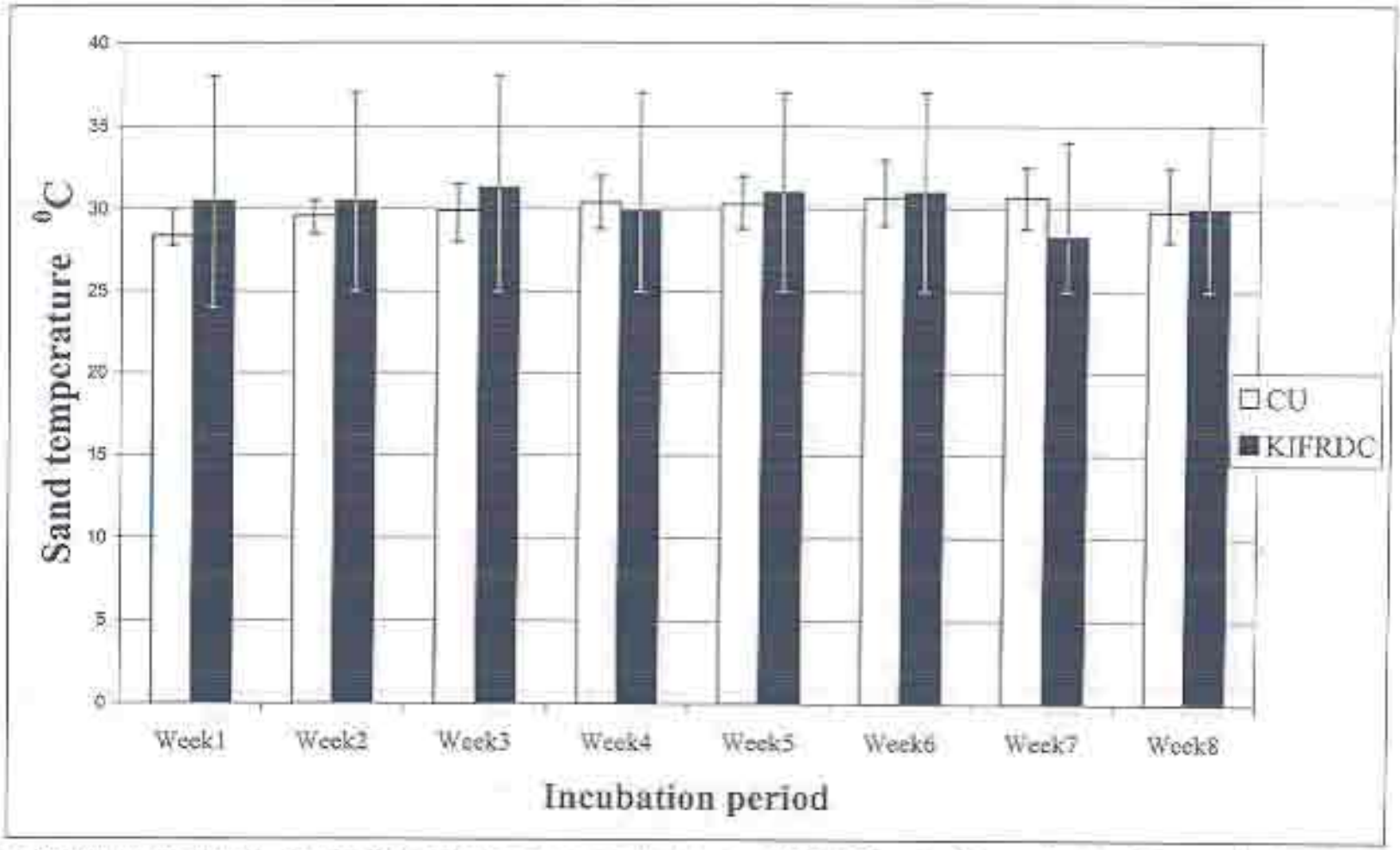

Figure 7.4 Sand temperature during C. chitra's eggs incubation in KIFRDC and CU methods. The vertical solid bars show maximum and minimum of sand temperatures. Sand temperature of ASB was recorded as only max-min throughout the study.

the hatching success rates of the larger female's clutches incubated with the three methods ranged from 80 to 94 % (Table 7.3). Hatching success of the smaller female's clutches was much less, 3 to 66 % (Table 7.3). The hatch rates of the Pond A female were not significantly different among incubation methods. The hatch rate of Pond B was highest in B3/ASB1 clutch (66%) while hatch rates of the other clutches were very low. Air temperature ranged widely in all incubation methods; 24-42, 25-34 and 24-39 °C of KIFRDC, CU and ASB, respectively (Table 7.3). While sand temperatures in the CU method varied less than in the KIFRDC and ASB methods 24-39, 26-33.5 and 26-35 °C of KIFRDC, CU and ASB, respectively (Table 7.3). However, sand temperature in the ASB method was recorded as only max-min throughout the study (Figure 7.4).

Moisture contents of sand in CU, KIFRDC and ASB were 4.64±0.53 (range = 4-5.8), 5.16±0.36 (range = 4.8-6) and 3.78±0.19 (range = 3.6-4.2) %, respectively. The moisture content of ASB is lower than in the CU and KIFRDC methods (Figure 7.5).

Hatchling size

The mean hatchling sizes were 38.46±1.52 mm carapace width (range 32.00-41.67 mm), 42.97±1.59 mm carapace length (range 36.27-46.66 mm) and 13.10±1.03 g weight (range 9.09-14.89 g). Hatchling sizes from Pond A and Pond B were compared by t-test analysis. Hatchlings from Pond A were significantly wider, longer, and heavier than Pond B hatchlings (Table 7.4).

Growth

The mean sizes of 21 day-old hatchlings were 41.35±0.57 cm carapace width, 44.53±0.62 carapace length and 13.99±0.60 g weight. After 14 weeks, mean hatchling size was 86.70±5.17 mm carapace width, 91.72±5.75 mm

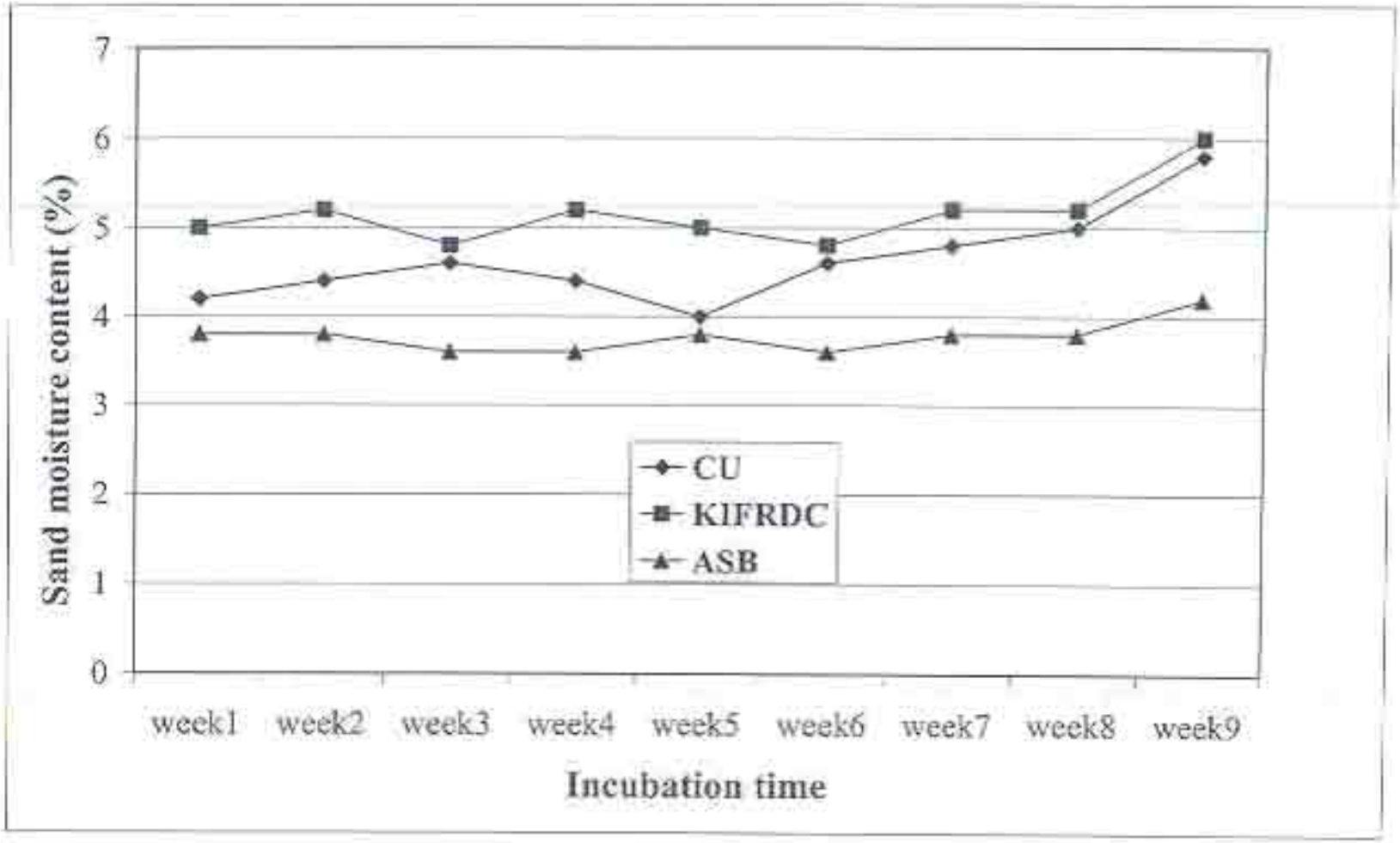

Figure 7.5 Moisture content of sand during incubation in CU, KIFRDC and ASB methods for egg treatments from pond A.

carapace length and 103.97±18.08 g weight, respectively. Moreover, the survival rate of these hatchlings was 90.64% for the test interval.

Figure 7.6 shows the relationships between CW, CL, Wt and age for 19 hatchlings (1 of them died after 2 weeks). It shows that CL and CW are strongly correlated with one another as well as with age, and that weight increase is strongly correlated with age (Figure 7.6). Another significant relationship was established between weight of food consumed per day and hatchling weight over a 14-week observation period (Figure 7.7).

Discussion

Mating and Nesting

The peak mating time of C. chitra observed in captivity, from December to February (Figure 7.2), is similar to the January to February mating season observed in nature (Taechacharernsukchera, 1991). Walking tracks (Figure 7.3) were found from August to April, with two peaks in November and March. The first terrestrial activity occurs before mating time and may be an exploration for suitable nesting sites. The second activity was for nesting, and nesting tracks and nests (Figure 7.3) were found from February to April. Some nests were constructed, but eggs were not deposited. These empty nests presumably result from disturbance of the nesting female and her abandonment of the nesting behavior. Similar occurrences of empty Chitra nests have been observed in nature. Fishermen and local people believe that Chitra constructs false nests to distract the egg predator *Varanus salvator* from locating the true nest. All captive C. chitra nested nocturnally, perhaps to avoid the high daytime temperatures (34-42° C) during the nesting season (Figure 7.2). The captives nested in late winter to late summer (February to May) when warmer temperatures provide appropriate thermal conditions for egg incubation. The captives' nesting season is probably similar to that in

nature. However, we found one clutch of wild C. chitra in Vajiralongkorn Reservoir (formerly Khoa Lam Reservoir) on 14 January 2000.

Knowledge of egg and clutch parameter is relatively well known in sea turtles, but these data are still lacking for many other turtle species (Moll, 1979; Gibbons and Greene, 1990). In this study, two C. chitra produced four clutches each in one breeding season after the larger female produced three or four (depending on whether the one egg clutch is counted) clutches the preceding year. Taechacharernsukchera (1991) dissected a dead female C. chitra that contained 97 hard-shelled oviducal eggs and 270 follicles. The size differences between follicles observed (3-4 groups) suggest that four to five clutches are laid by females each nesting season. The factors influencing the number of clutches per year are unknown. Clutch frequency of C. chitra in this study (based upon two years from Pond A and one year from Pond B) provides the first direct evidence that Chitra can lay multiple clutches (up to four) within a nesting season.

Multiple clutches hatching at intervals would provide some Chitra hatchlings an opportunity to avoid egg and hatchling predators and/or unfavorable weather conditions during portions of the hatching season.

Clutch size of C. chitra ranged from 40 to 88 eggs in this study, whereas Taechacharernsukchera (1991) found 97 eggs in a wild clutch, Nutphand (1990) stated that C. chitra normally laid 60 to 110 eggs per clutch. The highest number of eggs per clutch in C. chitra was recorded as 117 eggs (Nutphand, 1986). These data support the view that C. chitra should be classified as possessing reproductive Pattern I as defined by Moll, 1979. This pattern is characterized by the production of large clutches; multiple clutches produced during a well-defined nesting season; communal nesting in a well-defined ancestral nesting area; and careful construction of covered nests.

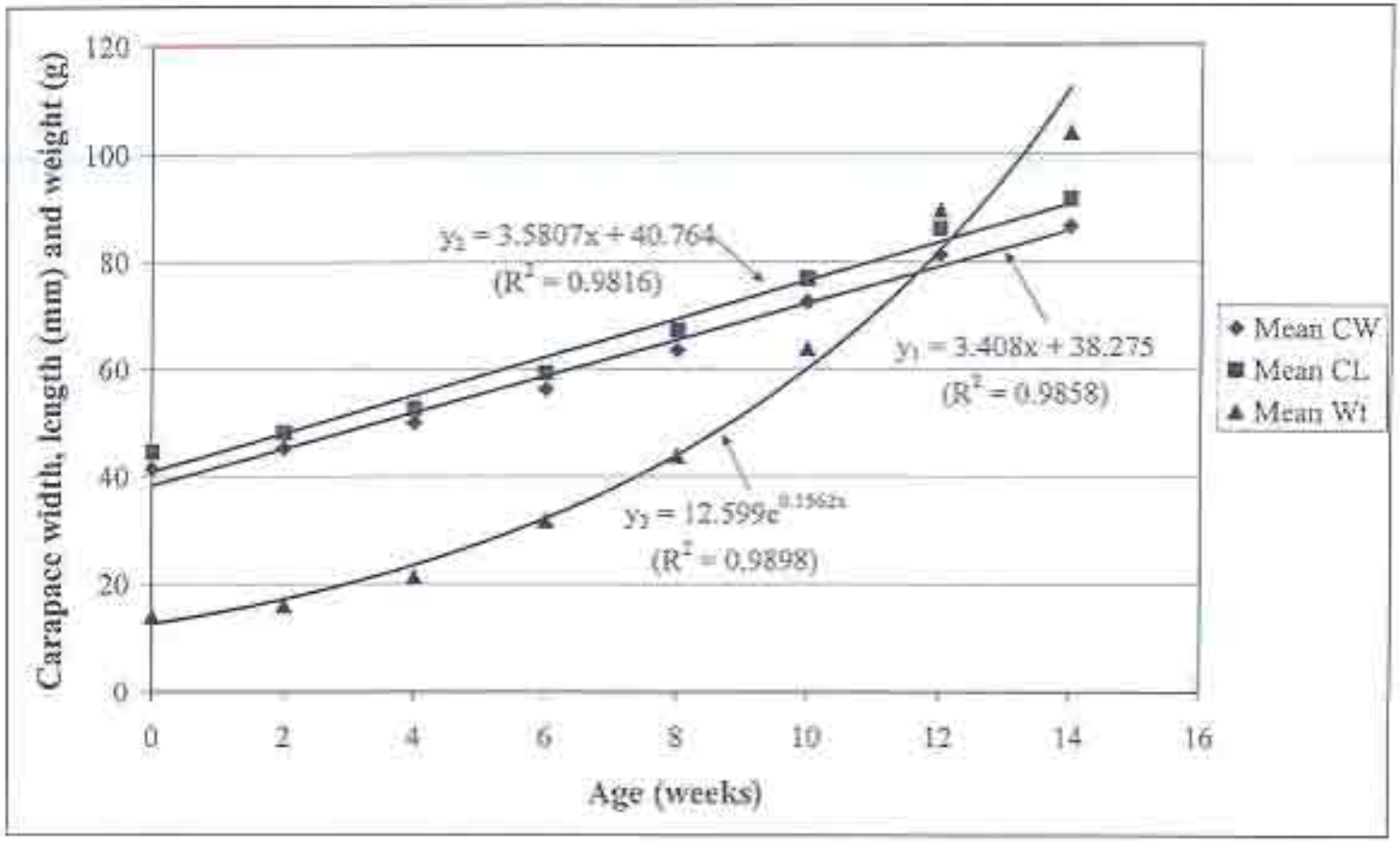

Figure 7.6 Carapace width, carapace length and weight of C. chitra hatchlings for 14 weeks.

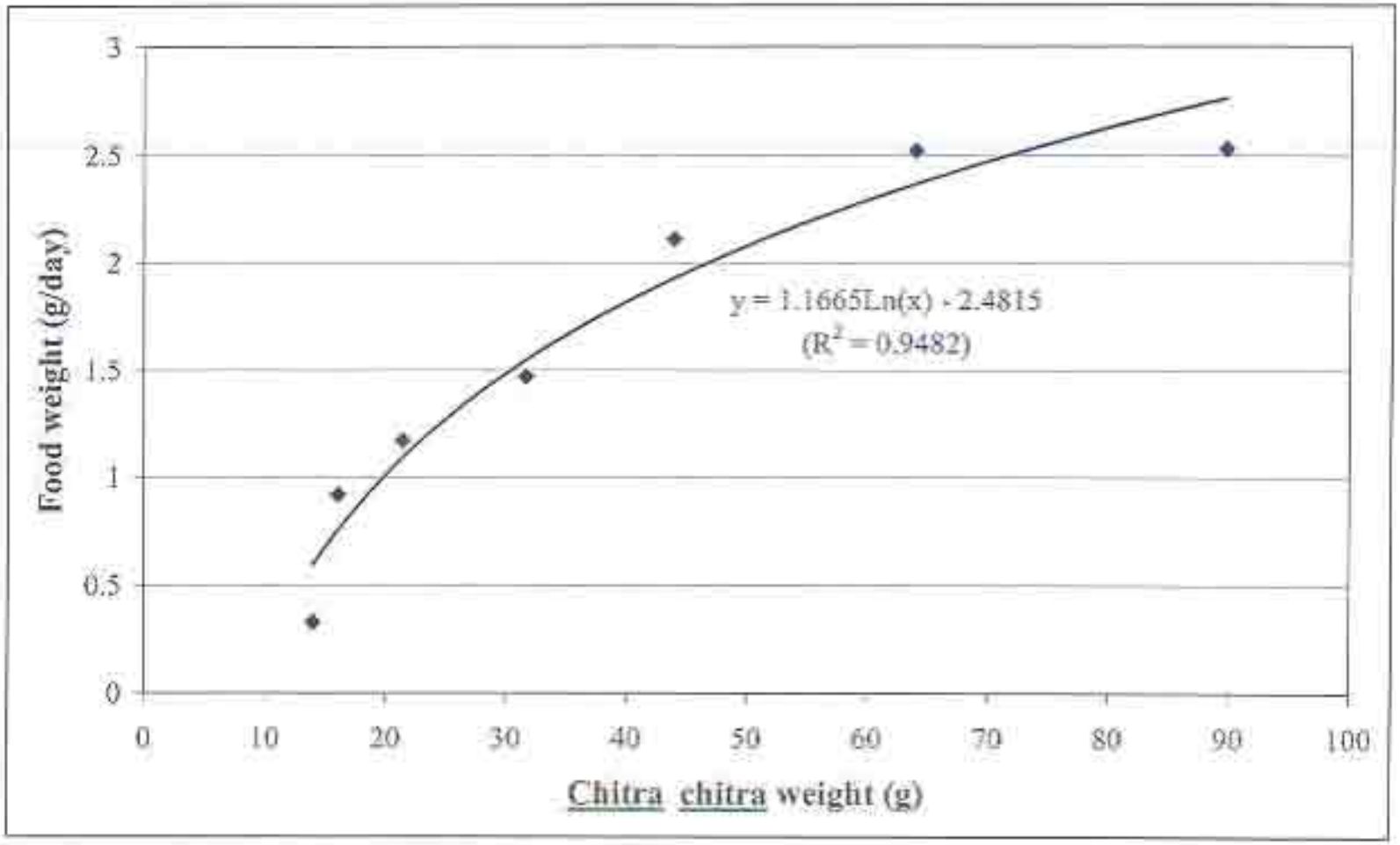

Figure 7.7 Relationship between weight (g) of C. chitra hatchling and food weight (g) per day.

Sea turtles and many other larger riverine turtles also display Pattern I reproductive strategies.

The limited data available suggest that larger Chitra lay larger egg clutches than smaller individuals. This trend has been observed in other turtle species (Moll, 1979; Gibbons, 1982) although environmental influences may also influence clutch sizes to some extent.

Egg size and shape

Chitra chitra laid spherical eggs with white, brittle eggshells morphologically similar to those produced by other softshell turtles (see previous section on egg morphology).

Egg size of C. chitra is small compared to maternal body size. The large C. chitra female laid larger eggs than the smaller female, a trend similar to that seen in sea turtles and other freshwater turtle species (van Buskirk and Crowder, 1994; Miller, 1997; Kuchling, 1999). The smaller female produced more elongated eggs. Perhaps this might reflect the physical constraints by the diameter of the oviducts or a pelvic girdle constraint (Congdon and Gibbons, 1987) of newly matured females.

Incubation time

The incubation times of turtle eggs depend mainly on temperature and humidity. Previous studies showed that incubation times of Testudo hermanni boettgeri eggs incubated at high constant temperatures are shorter than those incubated at low constant temperature (Eendebak, 1995). At the same incubation temperatures, Chrysemys picta eggs in wetter substrates required more time to hatch than those from drier substrates (Packard et al., 1991). Temperatures and soil moisture levels in incubation methods employed in this study were not controlled. The eggs were incubated in fluctuating

temperatures (24-39 ° C sand temperature), and relatively moist substrates so that the effects of these and perhaps other environmental influences on incubation time cannot be determined from this study. However, the moisture content in the last week of each method is higher due to water infused from hatching eggs (Figure 7.5).

Hatching Success

The successful hatching of turtle eggs depends upon temperature, humidity, and air circulation. The hatch rate of C. chitra eggs in this study in Pond A did not differ among the three methods used (Table 7.3). The hatch rates were all high, > 80%, therefore, we conclude that conditions in all incubating methods were appropriate for incubation of C. chitra eggs. Although the hatching success of eggs from Pond B was lower than that from Pond A (3-66 vs. 80-94%), both sets of eggs were incubated under similar conditions. Perhaps the smaller (71 kg) female was ovipositing for the first time (anecdotal evidence suggests that C. chitra females mature at 60 kg or more) and she was physiologically deficient for effective reproductive success.

Hatchling size

Hatchling sizes of C. chitra are very small and similar to sizes recorded for *C. indica* hatchlings (43 and 38 mm CL and CW and 10 g Wt and 39 and 33 mm of CL and CW and 7 g Wt) (Sachsse, 1971). Larger C. chitra eggs produced larger hatchlings (Table 7.4), a trait also known in sea turtles and other freshwater turtle species (van Buskirk and Crowder, 1994; Kuchling, 1999).

Growth

Growth of C. chitra including (CW, CL and Wt) is very rapid during the first 14 weeks. The result was compared with growth of C. indica hatchlings that were studied by Sachsse (1971). The initial sizes of C. indica were 43

and 38 mm of CL and CW and 10 g Wt and 39 and 33 mm of CL and CW and 7 g Wt. After 2 months, their measurements were 44 and 38 mm of CL and CW and 11 g Wt and 40 and 33 mm of CL and CW and 10 g Wt, respectively. In this study the growth rate of C. chitra was much higher in the first 2-3 months than observed in C. indica. Hatchlings from captive breeding have a better chance than animals from the wild. However, growth of C. chitra is probably not maintained at this rate. Two hatchlings of wild caught C. chitra were maintained at Kanchanaburi Inland Fisheries Research and Development Center for nine years. Their sizes are 59 and 63 cm CW, 68 and 71 cm CL and 29 and 36 kg Wt, respectively (Kitimasak, unpublished data) on August 2002.

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Chapter 8

Conclusions and Recommendations

Osteological comparisons between C. chitra and C. indica indicated that skull ratio characters and carapace ratio characters were different among Thai and Indian species. The magnitude of the variation displayed by these analyses supports the argument that Thai animals warrant specific status.

C. chitra is presently found in the Mae Klong and Mae Ping river systems, at Bhumiphol, Srinagarind and Vajiralongkorn Reservoirs and in the river below the dams. In this study, the new distribution range of C. chitra has been recorded to cover the area of Mae Ping river system of Northern Thailand. However, the natural population of C. chitra in both river systems is depleted and their status is certainly very rare. Moreover, live specimens of the other congener, C. burmanica Jaruthanin, 2002 or C. vandijki McCord & Pritchard, 2002, were also found in this study from the Salween river system.

The captive-breeding program of C. chitra conducted in this study has been proven to be quite successful. Mating has been observed in the experimental ponds. Females laid eggs on the artificial sandbank during February to April with up to 4 clutches/year. Clutch size varies from 40-88 eggs/clutch. The mean incubation time of eggs is 59±3 days. The hatching success in each clutch varies from 3 to 94 %. 297 hatchlings were obtained from two females in 2001. The 14 weeks-old hatchlings were 91.72±5.75 mm in carapace length and 103.97±18.08 g in weight with 90.64% of survival rate.

The data on the structure and composition of eggshells from a wild caught female will be useful for comparison with eggshells produced by captive females. These comparisons may help to determine whether the nutritional requirements of the captives are being met.

C. chitra is considered a top carnivore and a keystone species in its habitat. It should receive the highest priority for conservation because at present it is under the threat of extinction in the wild. Its population status in Malaysia and Indonesia is also very rare or unknown. The management programs, including the legal protection of its habitat, the maintenance of its viable population, the development of the dam water-releasing system to protect nesting sites from flooding during breeding season, breeding in captivity, rehabilitation and reintroduction as well as public awareness, education and cooperation are urgently needed. These programs, along with the intensive study of its population ecology and genetics, should be established immediately and conducted as vigorously as possible.

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Captive Breeding of the Giant Narrow-headed Softshell Turtle Chitra chitra Nutphand, 1986 -6th Graduate Congress, December 1st , 2001, National University of Singapore, Singapore.

Eggs and Hatchlings of the Siamese Narrow-Headed Softshell Turtle Chitra chitra Nutphand, 1986- 7th Biological Science Graduate Congress, December 9-11, 2002, Chulalongkorn University, Thailand.

APPENDICES

APPENDIX A

a.

b.

Figure 1 Male (a, LCL= 85 cm) and female (b, LCL= 107 cm) of C. chitra in Pond A at Kanchanaburi Inland Fisheries Research and Development Center, Kanchanaburi Province.

Figure 2 Pond B (16x25 m) of Chitra chitra at Kanchanaburi Inland Fisheries Research and Development Center, Kanchanaburi Province

Figure 3 Mating activities of C. chitra in Pond A at Kanchanaburi Inland Fisheries Research and Development Center, Kanchanaburi Province (Photographed on 27 February 2002 at 9.40 a.m.)

Figure 4 C. chitra eggs in a nest at Kanchanaburi Inland Fisheries Research and Development Center, Kanchanaburi Province

a.

b.

Figure 5 C. chitra hatchlings emerging from its egg (a) and from its nest (b).

Figure 6 One-day old C. chitra hatchling, LCL= 38.23 mm.

APPENDIX B

RESEARCH ARTICLE

ScienceAsia 29 (2003): 1-5

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

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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±0.02 mm and 0.13±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=53.0±4.8%, carbon=35.0±9.2%, magnesium=5.55±0.34%, calcium=5.4±7.2%, silica=2.9±1.6%, aluminum=2.3±1.1%, potassium=0.17±0.1%, and sodium=0.74±0.3%. The eggshell was the aragonite form of $CaCO_3$.

KEYWORDS: *Chitra chitra*, softshell turtle, eggshell structure, eggshell composition, SEM

Introduction

The Siamese narrow-headed softshell turtle *Chitra chitra* Nutphand, 1986 is one of the largest and the least known turtles in the world. Since its recent recognition as a distinct species[1], its population size has almost certainly continued to decline across its limited range in Thailand and elsewhere in Southeast Asia[2-4] due to over fishing, pet trade, population 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 by the authors is designed to rectify this deficiency. As part of our 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 our analysis are presented here, and compared with the few other structural analyses of turtle eggshells which have been published.[5-12]

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 JSM-6400 scanning electron microscope, operated at 15 kV. 3) **Energy dispersive x-ray analysis (EDX)**; the JSM-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 ±0.02 mm and 0.12±0.02 mm, respectively (Table 1). The thicknesses of the outer and inner layers of the eggshells were significantly different ($p < 0.05$).

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 (Fig. 1). The outer surface was composed of a nearly continuous calcareous sheet that covered the whole egg (Fig. 1a). It was a very thin layer that could not be

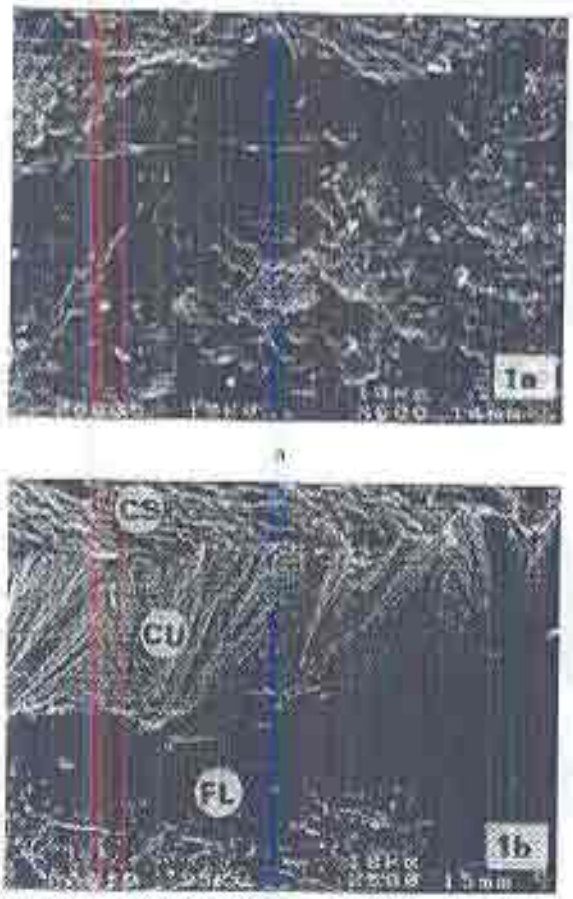

Fig 1. SEM showing the calcareous sheet (CS) covering the outer layer of eggshell, middle layer crystalline unit (CU), and an inner fibrous layer (FL).
a = top view, b = side view. Bar = 10 μm

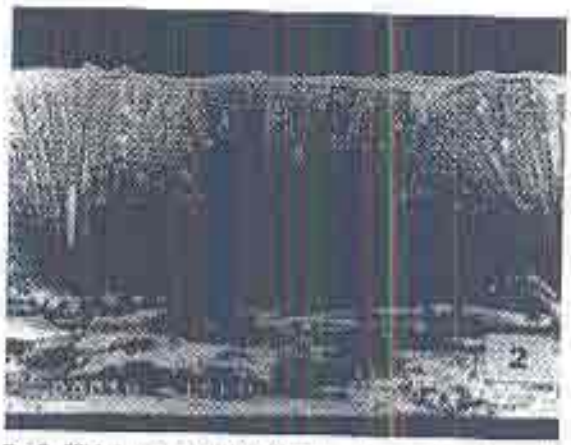

Fig 2. SEM showing the radially fractured appearance of crystalline units of the middle layer. Bar = 10 μm

Fig 3. SEM showing the inner layer of the eggshell.
a = side view (Bar = 10 μm), b = top view (Bar = 10 μm)

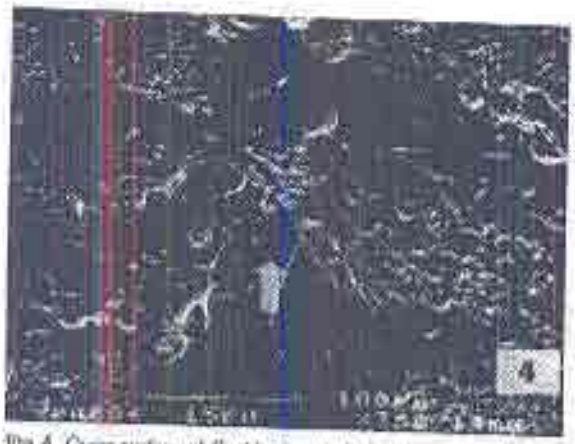

Fig 4. Outer surface of *C. chitra* eggshell showing a pore (arrow) at the intersection of crystalline units. (Bar = 100 µm)

observed under a stereo microscope. The middle layer was composed of fan-shaped crystalline units packed together throughout the layer (Fig 1b; 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 (Fig 1b; 2, 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 (Fig 4).

Energy Disperative 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 1).

X-ray Diffraction Analysis

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

Table 1. Outer layer thickness, inner layer thickness (mm), Energy dispersive x-ray analysis (EDX) (%) and X-ray Diffraction of wild *Chitra chitra* eggs.

Analysis Method	n	Mean ± SD
Outer layer thickness (mm)	6	0.14±0.02
Inner layer thickness (mm)	6	0.12±0.02
Energy dispersive x-ray analysis (EDX) (%)	9	O = 52.96±4.81 C = 35.03±2.17 Mg = 5.56±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

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*[10], spiny softshell turtles *Apalone spinifera*[8], olive ridley turtles *Lepidochelys olivacea*[11], Euphrates softshells *Rafetus euphraticus*[5], European pond turtles *Emys orbicularis*[5], and radiated tortoises *Geochelone radiata*.[5]

Similar to the finding of our study of *C. chitra*, three layers were recorded in snapping turtles *Chelydra serpentina*.[9] 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*[9] and *Trionyx spiniferus*.[8]

Young (1950)[5] 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.[5]

Solomon and Baird (1976)[8] analyzed the elements in eggshells of *Chelonia mydas* and found that the shell was 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.[8,12]

The authors are conducting a captive breeding program for *C. chitra* at Kanchanaburi Inland Fisheries 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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Captive Breeding of the Siamese Narrow-headed Softshell Turtle *Chitra chitra* Nutphand, 1986 (Testudines: Trionychidae)

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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 Development Center (KIFDC), 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 = 235) 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: *Labeo rohita*, *Oreochromis niloticus*, 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). 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

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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 indequate 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.

Materials and Methods

Six *Chitra chitra*, two females and four males, were maintained in two outdoor enclosures at Kanchanaburi Inland Fisheries Development Center (KIFDC), Tha Muang District, Kanchanaburi Province, Thailand. The breeding ponds, 400 square-meter (16×25 m), were constructed near the Mae Klong River and were climatically similar to the natural habitat. The water depth varied from 60 to 80 cm, and a 30 m^3 artificial sandbank ascending 80-90 cm above water level was constructed for nesting in each enclosure. Roofs were constructed over the sandbanks for protection from rain-induced mortality of eggs and hatchlings (Kraemer and Bell, 1980). Fences were also constructed around the ponds to protect turtles and their eggs from predators, such as dogs, snakes, and monitor lizards (Figure 1). A female and two males were placed in each pond (Table 1). Foods provided for *C. chitra* in the captive ponds were mainly live *Nile tilapia, Oreochromis niloticus*; native fishes, such as *Bardodes gonionotus*, were available, entering

Table 1 Size, locality, date of capture, and date of release into breeding pond of *C. chitra* in captivity

Pond	Sex	Shell length (cm)	Shell width (cm)	Weight (kg)	Locality (river)	Capture time	Released time
A	Female	98.5	81	111	Kwae Noi	1997	28/9/2000
	Male 1	78	66	44	Mae Ping	6/1998	31/3/2000
	Male 2	82	70	60	Kwae Noi	1/1999	2/11/2000
B	Female	84	75.5	61	Kwae Noi	1997	19/11/2001
	Male 1	97	85	88	Kwae Noi	1997	19/11/2001
	Male 2	103	85	85	Mae Klong river system	6/1999	11/2001

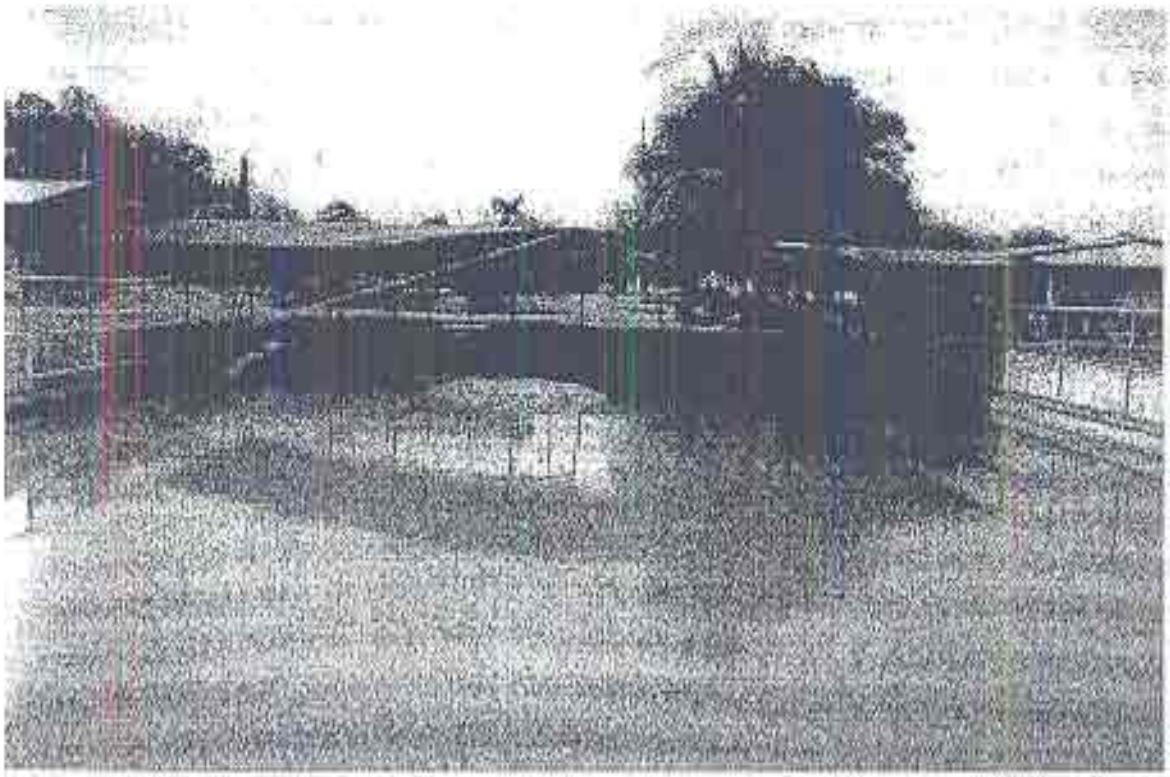

Figure 1 *Chitra chitra* rearing Pond A at Kanchanaburi Inland Fisheries Development Center.

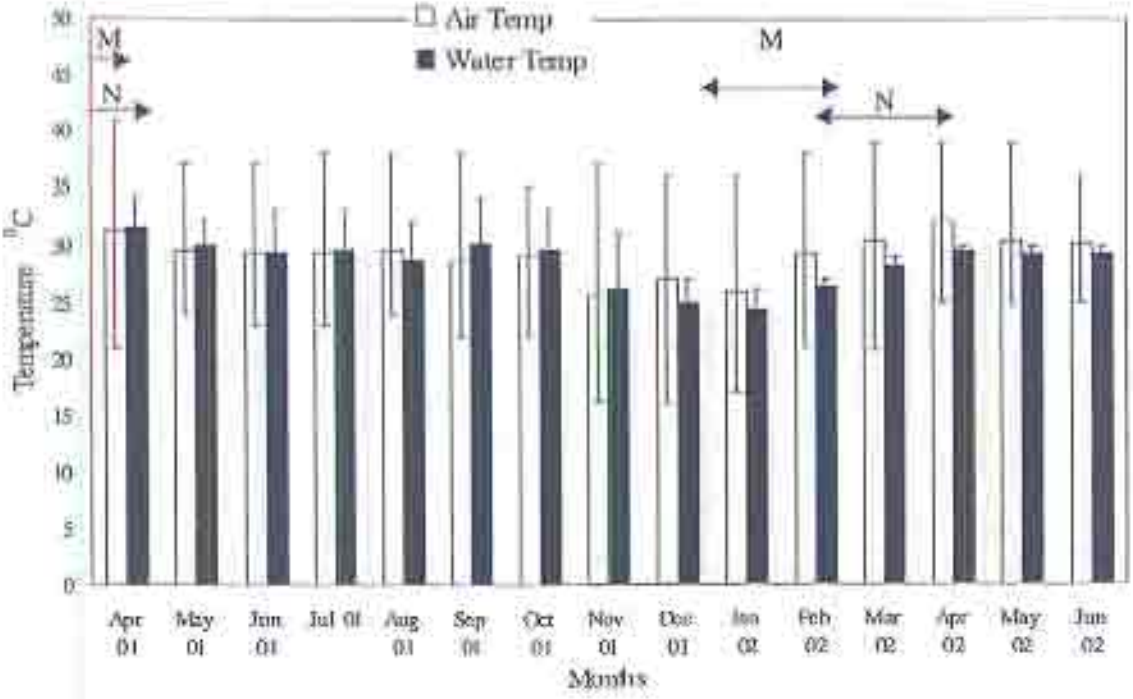

Figure 2 Means of water and air temperature in pond A during April 2001 to June 2002. The vertical bars show maximum and minimum of air temperature and water temperature in each month. The arrows show periods of mating (M) and nesting (N).

the ponds during water exchange with the Mae Klong River daily Monday – Friday. Air and water temperature in the captive ponds were recorded throughout the year (Figure 2).

Pond A

Pond A was completed in early 2000. The first female, 111 kg, was released on February 26, 2000. It was captured from the Kwae Noi River, Kanchanaburi Province, in 1997 and was separated from males for three years. The first male, 44 kg, was captured from the Mae Ping River, Nakhon Sawan Province, in June 1998. It was released in the breeding pond on March 31, 2000. The second male, 60 kg, was captured from the Kwae Noi River in April 1999 and was released into this pond on November 2, 2000.

Pond B

Pond B was completed in November 2001. One female, 61 kg, and a male, 88 kg, were caught from the Mae Klong River system in 1997. The second male, 88 kg, was caught from the agricultural canal (joining the Mae Klong River and the Tha Chean River) in Nakhon Pathom Province on May 3, 1999. All of them were released in the Pond B in November 2001.

Terrestrial Activity and Breeding Behavior

Terrestrial activity and breeding behavior of *C. chitra* in the breeding ponds were monitored through the year. Terrestrial activity was nocturnal, and its occurrence and frequency data derived from tracks in the sand. Walking tracks indicated terrestrial movement without nesting activity. The track dimensions allowed identification of the individual(s). Nest tracks led to blank nests or nests with eggs. Mating behavior and copulation duration were also observed and recorded.

Incubation methods

Nests were excavated by hand within 48 hours after nesting. When the first egg was uncovered, the depth to the top of the egg chamber was measured. The depth to the bottom and the width of the chamber were also measured. Eggs of the first and the second clutch of each breeding pond were weighed to the nearest 0.01 g with an electronic balance and linear measurements of width and length measured to the nearest 0.01 mm with digital calipers. The number of eggs and clutches of each female were recorded.

The eggs were carefully removed and handled to prevent horizontal rotation and reorientation of their vertical axes. Three methods were used for incubation. In the first method, one clutch from each pond was transported to the Department of Biology, Faculty of Science, Chulalongkorn University. Eggs were put in a plastic container, 40×70×37 cm on 10 cm thick of sand and were covered with 20 cm of additional sand. Moisture in the container was maintained by spraying water on the sand each morning and evening.

In the second method, one clutch from each pond was incubated in circular fiber containers 75 cm in diameter. Eggs were placed on 10 cm of sand and again were covered with 20 cm of additional sand at KUFDC. The fiber containers were placed in water to protect the eggs from ants and other insects. Water was sprayed on the sand every morning and evening to control the sand moisture content. Water loss was minimized by placing a plastic cover securely over the top of each container.

In the third method, two clutches from each pond were incubated at the nesting area in situ.

After 30 days of incubation, the nests were surrounded by mesh to restrain the hatchlings for counting, measuring, and protection from predators.

In each method, the number of hatchlings emerging was recorded and the respective hatching success rates calculated. Incubation times were also recorded in each method. The hatchlings of all treatments were measured (CW and CL) and weighed. Max-Min mercury thermometers were inserted horizontally near the nests to monitor incubation temperature throughout the incubation period. Air temperatures and sand moisture content were also recorded in each method during the studies. Sand moisture content (%) was calculated from [(wet weight-dry weight) / dry weight] × 100.

Growth of hatchlings

Twenty hatchlings, from A1/ KIFDC (Table 3), were divided into two groups. They were reared indoors in two glass aquaria, 50×105×40 cm. Sand covered the bottom of the aquaria to a depth of about 4 cm. The hatchlings in each aquarium were provided with 3 cm water. Water was exchanged every Tuesday and Friday. Fry fish of *Labeo rohita* (Hamilton, 1822), (23.27±1.57 mm length and 0.12±0.05 g weight), *Oreochromis niloticus* (Linnaeus, 1758), (24.21±1.34 mm length and 0.29±0.04 g weight), were fed to *C. chitra* hatchlings in this study. One hundred fish within the first two months and 200 fish in the third month were provided daily in each aquarium. The total number of fish eaten was recorded daily and was divided by hatchlings in order to estimate the average amount consumed per individual. Weight, carapace width and carapace length of hatchlings were measured every two weeks for fourteen weeks.

Results

Reproduction

Timing of reproductive activity

Reproductive behaviors occurred in both ponds over the nine-month period between August and April in both years of the study. Tracks of the females were observed from August through April, with their frequency increasing in November, and from February through April. Nesting tracks and nests were observed from February through April (Figure 3).

In the first year of our study, the first copulation observed involved the 44 kg male and female in Pond A on September 8, 2000, at 8.00 A.M. about four months after his release into the pond. Other copulations occurred between December 3, 2000 and April 3, 2001 mainly between 8.15 A.M. and 8.30 A.M. (Table 1 and 2), with the males being most active between December and February. At this time, males often chased the females and attempted to copulate.

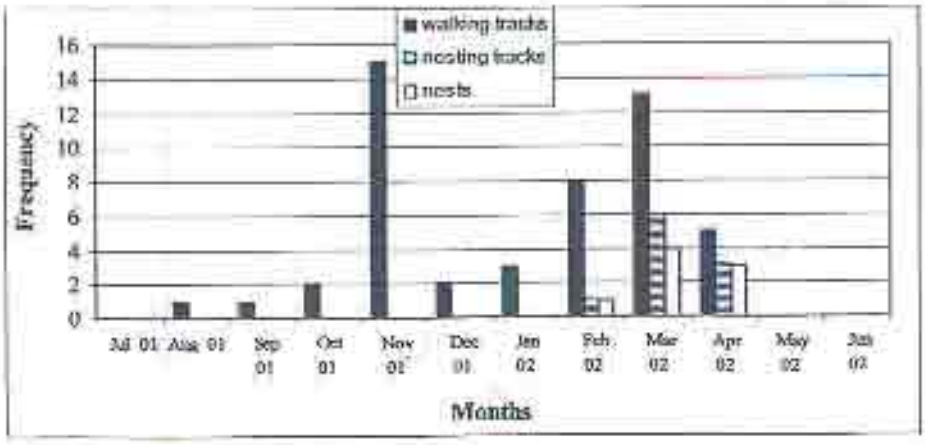

Figure 3 Terrestrial activities of *C. chitra* in pond A; walking tracks, nesting tracks and nests between July 2001 and June 2002.

Table 2 Date of copulation and mating duration of *C. chitra* in pond A.

Nesting year	Copulation times	Mating duration
2001	- 8 September 2000; 6.00 a.m.	~10 min
	- 3 December 2000; 6.00 a.m.	~10 min
	- 22 February 2001; 7.30 a.m.	~10 min
	- 2 April 2001; 8.30 a.m.	~10 min
	- 3 April 2001; 6.15 a.m.	~10 min
2002	- 29 December 2001; 2.00 p.m.	~10 min
	- 27 February 2002; 9.40 a.m.	~15 min

In the second year, copulation occurred between December and February (Table 2). Copulations lasted between 10-15 minutes in both years of study (Table 2).

Nesting

Nesting occurred on the artificial sandbanks. Walking tracks, which may represent exploratory behavior by the females, were found before nesting actually occurred. The first nesting track was found in the morning of February 22, 2001 in Pond A, but the actual nest site was not located. Several additional nest tracks and presumed nests (*i.e.* large areas of disturbed sand) were found during March to April. On June 7, 2001, after heavy rains and rising water levels within the pond enclosures, *Chitra* eggs were discovered on the sand surface near the water's edge. Subsequently the sandbank was excavated and four clutches of eggs were removed from flooded nests.

One year later, nesting tracks were found in the morning in both Pond A and Pond B from February 27 to April 20, 2002. Nests were excavated within 48 hours after they were constructed. The nest hole of *C. chitra* is flask-shaped. The mean of four egg chambers of Pond A was 23.5 cm width, 32.5 cm height from sand surface to top of egg clutch, and 54 cm height from sand surface to nest bottom. The average of four egg chambers of Pond B was 22.8 cm, 32 cm, and 49.3 cm, respectively. The nest dimensions of the smaller female were less than those of the larger female (Table 3).

Clutch size

Clutch size ranged from 1 to 88 eggs, although it seems likely that the single egg clutch represents aberrant "nesting". The total eggs from the Pond A female were 215 eggs, from clutches of 74, 64, 76, and 1 egg in 2001. In 2002, the female of Pond A laid 280 eggs from clutches of 45, 88, 79, and 68, respectively. Also in 2002, the female of Pond B laid 205 eggs with clutches of 40, 40, 67, and 58 eggs, respectively (Tables 3 and 4). Both females deposited four clutches each in nesting season of 35 days at intervals of 8-23 days (Table 3).

Egg size

Most *C. chitra* eggs were white and spherically shaped, but a few were more oblong. The smaller female produced more elongate eggs. Eggshells were brittle and easily broken. The mean dimensions of the eggs (n=220) were 31.94 ± 1.57 mm width (range 26.89-34.37 mm) and 33.16 ± 1.54 mm length (range 29.39-40.49 mm). Mean egg weight was 19.00±1.67 g (range 15.31-21.80 g) (Table 4). Comparison of egg size between the two females revealed that egg width and weight were significantly different between the two females although egg length was not (Table 4). The larger female laid wider and heavier eggs than the smaller female.

Table 3 Hatching success and other parameters for the three incubation methods. Abbreviations: KIFDC, Kanchanaburi Inland Fisheries Development Center; CU, Chulalongkorn University; ASB, Artificial sandbank;, Size of egg chamber, width (W), height of ground surface to egg top (ET), height of nest bottom to ground surface (NB). Number in parentheses indicates the number of eggs which were found in the original nests

Pond/Method	Nesting Date	Hatching Date	Egg chamber (W, ET, NB) cm	No. of eggs incubated	Sand Temp. (°C)	Air Temp. (°C)	No. of hatchlings emerged	Incubation time (days)	Hatch rate (%)
A1/KIFDC	16/3/2002	10-14/5/2002	28, 40, 38	44(43)	24-28	24-42	35	55-59	80
A2/CU	21/3/2002	15-19/5/2002	50, 29, 49	57(58)	28-33.5	25-34	73	53-60	80
A3/ASB	4/4/2002	4/6/2002	22, 22, 64	79(79)	25-33	24-35	74	60	94
A4/ASB	20/4/2002	14-21/6/2002	28, 32, 35	58(58)	25-35	24-35	57	62-64	98
B1/KIFDC	21/3/2002	16/5/2002	22, 30, 43	40(43)	24-29	24-41	3	55	8
B2/CU	27/2/2002	3/5/2002	26, 35, 50	*25(40)	28-33.5	22-34	1	66	5
B3/ASB	?	15-21/5/2002	22, 30, 30	67(67)	25-33	24-38	44	?	66
B4/ASB	9/4/2002	5-7/6/2002	21, 33, 44	58(58)	28-34	24-39	8	61-65	16

? Actual nesting date not observed.

* Eggs were found at 2 weeks old. The infertile eggs were removed.

Table 4 Size of eggs and hatchlings of captive *C. chitra*. Numbers in brackets of no. of eggs and no. of hatchlings indicate the number of eggs and hatchlings which were measured. Numbers in parentheses indicates standard deviation (SD)

Pond	Year	Clutch	No. of egg	Egg width (mm)	Egg length (mm)	Egg Wet mass (g)	No. of hatchling	Hatchling CW (mm)	Hatchling CL (mm)	Hatchling Wet mass (g)
A	2001	A1	74[-]	-	-	-	-	-	-	-
		A2	64[-]	-	-	-	-	-	-	-
		A3	78[74]	33.08(0.43)	33.46(0.85)	20.41(0.77)	-	-	-	-
		A4	1[-]	-	-	-	1[1]	-	-	13.34
	2002	A1	45[44]	32.97(0.44)	33.06(1.01)	19.70(0.70)	35[35]	38.88(1.60)	42.07(1.11)	13.45(0.55)
		A2	58[34]	32.19(0.33)	38.64(2.00)	18.14(0.75)	78[78]	38.22(1.28)	42.88(0.93)	13.68(0.64)
		A3	79[-]	-	-	-	74[74]	39.32(1.27)	43.12(0.89)	13.05(0.49)
		A4	88[-]	-	-	-	67[67]	38.22(1.16)	42.92(1.55)	12.79(0.70)
			Mean of pond A	32.56(0.34)^a	33.16(0.78)	19.92(0.90)^a		38.60(1.47)^a	43.06(1.20)^a	13.43(0.68)^a
			Range	30.10-34.37	33.99-38.47	18.55-21.80		35.35-41.67	39.25-46.88	11.27-14.59
B	2002	B1	40[38]	28.57(1.47)	34.50(2.35)	15.75(0.85)	1[1]	32.01	38.22	10.75
		B2	40[40]	30.88(0.31)	31.62(1.09)	14.65(0.56)	1[1]	30.30	40.45	11.17
		B3	87[-]	-	-	-	44[42]	37.23(0.99)	47.70(1.08)	11.82(0.88)
		B4	65[-]	-	-	-	7[6]	37.72(2.23)	39.38(1.99)	10.57(2.35)
			Mean of pond B	29.48(1.46)^b	33.02(2.80)	15.79(0.75)^b		37.51(3.73)^b	41.20(1.90)^b	11.09(0.89)^b
			Range	25.89-33.40	25.38-40.46	14.31-18.43		33.00-41.17	36.27-46.41	9.29-13.43
			Total mean	31.34(1.37)	33.16(1.84)	18.00(1.67)		38.46(1.82)	42.97(1.59)	13.20(1.03)
			Total range	25.89-34.37	29.38-45.48	13.31-21.80		32.00-41.67	34.27-45.68	9.09-14.88

Incubation and Hatching Success

Mean incubation time of *C. chitra* eggs was 59±3 days (n =255) with a range of 55-65 days (Table 3) at 24-42°C air temperature and 24-39°C sand temperatures (Figure 4).

Within a clutch, hatching varied from 1-5 days between first and last egg piping. Only in the A3/ASB clutch that was incubated in the nest area did all hatchlings (n = 74) emerge at the same time (60 days). In the other clutches the eggs hatched over a 3-5 day period. For the year 2002, the hatching success rates of the larger female's clutches incubated with the three methods ranged from 80 to 94 % (Table 3). Hatching success of the smaller female's clutches was much less, 3 to 66 % (Table 3). The hatch rates of the Pond A female were not significantly different among incubation methods. The hatch rate of Pond B was highest in B3/ASB1 clutch (66%) while hatch rates of the other clutches were very low. Air temperature ranged widely in all incubation methods; 24-42, 26-34 and 24-39°C of KIFDC, CU and ASB, respectively (Table 3). While sand temperatures in the CU method varied less than in the KIFDC and ASB methods 24-39, 26-33.5 and 26-35°C of KIFDC, CU and ASB, respectively (Table 3). However, sand temperature in the ASB method was recorded as only max-min throughout the study (Figure 4).

Moisture contents of sand in CU, KIFDC and ASB were 4.64±0.53 (range =4-5.8), 5.16± 0.36 (range = 4.8-6) and 3.78±0.19 (range = 3.6-4.2) %, respectively. The moisture content of ASB is lower than in the CU and KIFDC methods (Figure 5).

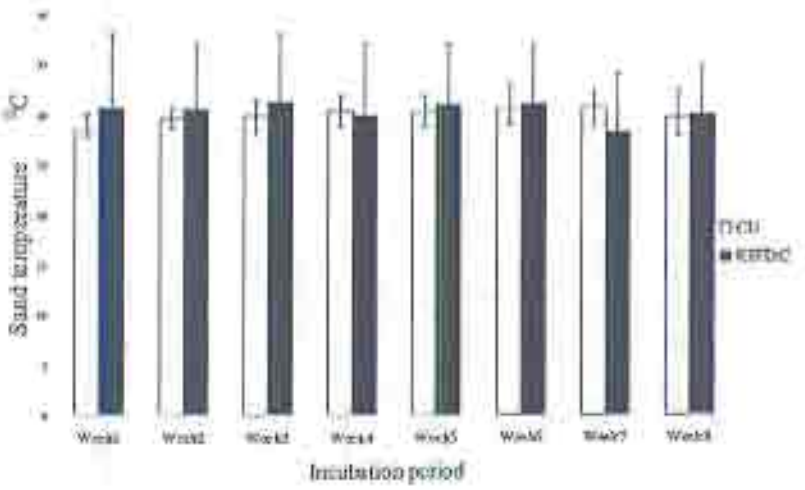

Figure 4 Sand temperature during *C. Chitra*'s eggs incubation in KIFDC and CU methods. The vertical solid bars show maximum and minimum of sand temperatures. Sand temperature of ASB was recorded as only max-min throughout the study.

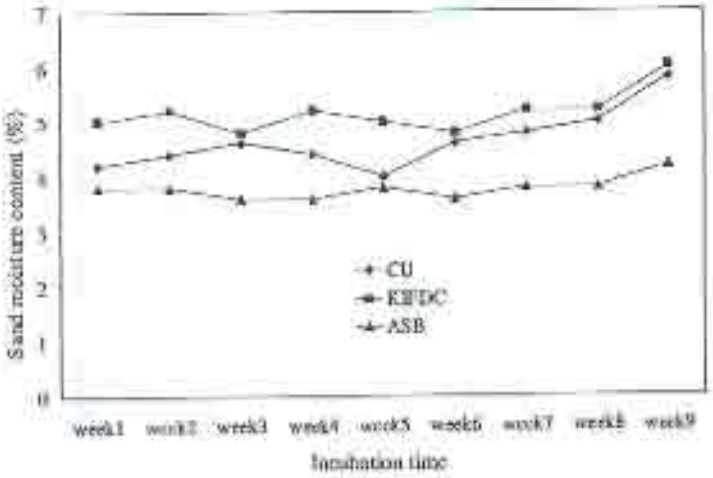

Figure 5 Moisture content of sand during incubation in CU, KIFDC and ASB methods for egg treatments from pond A.

Hatchling size

The mean hatchling sizes were 38.46±1.52 mm carapace width (range 32.00-41.67 mm), 42.97±1.59 mm carapace length (range 38.27-46.66 mm) and 13.10±1.03 g weight (range 9.00-14.89 g). Hatchling sizes from Pond A and Pond B were compared by t-test analysis. Hatchlings from Pond A were significantly wider, longer, and heavier than Pond B hatchlings (Table 4).

Growth

The mean sizes of 21 day-old hatchlings were 41.35±0.57 cm carapace width, 44.58±0.62 carapace length and 13.99±0.60 g weight. 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. Moreover, the survival rate of these hatchlings was 90.64% for the test interval.

Figure 6 shows the relationships between CW, CL, Wt and age for 19 hatchlings (1 of them died after 2 weeks). It shows that CL and CW are strongly correlated with one another as well as with age, and that weight increase is strongly correlated with age (Figure 6). Another significant relationship was established between weight of food consumed per day and hatchling weight over a 14-week observation period (Figure 7).

Discussion

Mating and Nesting

The peak mating time of *C. chitra* observed in captivity, from December to February (Figure 2), is similar to the January to February mating season observed in nature (Tacchacharernsukchera, 1991). Walking tracks (Figure 3) were found from August to April, with two peaks, are in November and March. The first terrestrial activity occurs before mating time and may be an exploration for suitable nesting sites. The second activity was for nesting, and nesting tracks and nests (Figure 3) were found from February to April. Some nests were constructed, but eggs were not deposited. These empty nests presumably result from disturbance of the nesting female and her abandonment of the nesting behavior. Similar occurrences of empty *Chitra* nests have been observed in nature. Fishermen and local people believe that *Chitra* constructs false nests to distract the egg predator *Varanus salvator* from locating the true nest. All

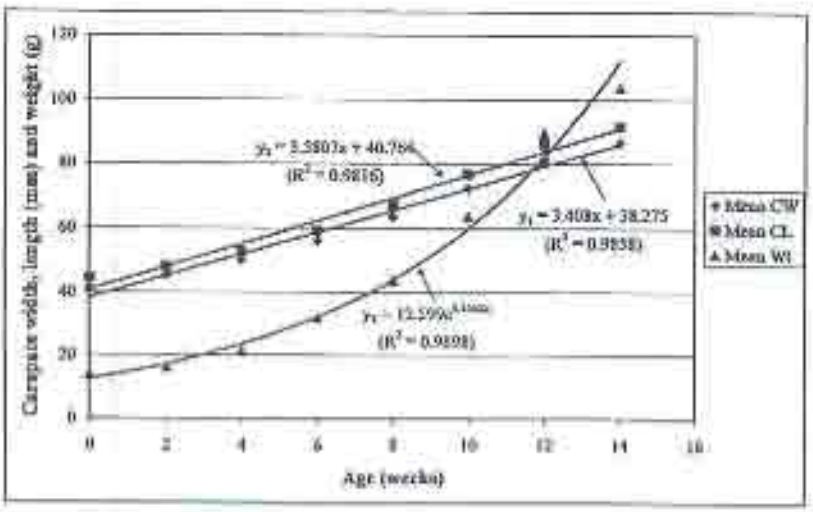

Figure 6 Carapace width, carapace length and weight of *C. chitra* hatchlings for 14 weeks.

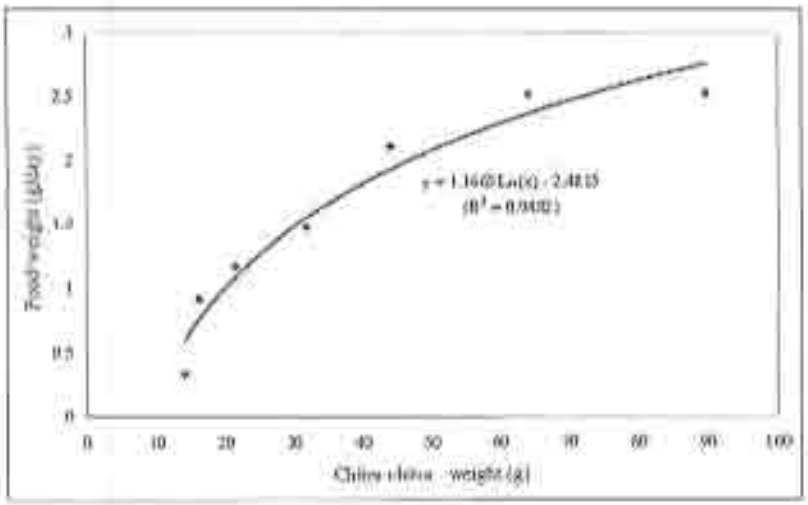

Figure 7 Relationship between weight (g) of *C. chitra* hatchling and food weight (h) per day.

captive *C. chitra* nested nocturnally, perhaps to avoid the high daytime temperatures (34–42°C) during the nesting season (Figure 2). The captives nested in late winter to late summer (February to May) when warmer temperatures provide appropriate thermal conditions for egg incubation. The captives' nesting season is probably similar to that in nature. However, we found on clutch of wild *C. chitra* in Vajiralongkorn reservoir (formerly Khoa Lam reservoir) on January 14, 2000.

Knowledge of egg and clutch parameter is relatively well known in seaturtles, but these data are still lacking for many other turtle species (Moll, 1979; Gibbons and Greene, 1990). In this study, two *C. chitra* produced four clutches each in one breeding season after the larger female produced three or four (depending on whether the one egg clutch is counted) clutches the preceding year. Taechacharernsukchera (1991) dissected a dead female *C. chitra* that contained 97 hard-shelled oviducal eggs and 270 follicles. The size differences between follicles observed (3–4 groups) suggest that four to five clutches are laid by female each nesting season. The factors influencing the number of clutches per year are unknown. Clutch frequency of *C. chitra* in this study (based

upon two years from Pond A and one year from Pond B) provides the first direct evidence that *Chitra* can lay up to four clutches within a nesting season.

Multiple clutches hatching at intervals would provide some *Chitra* hatchlings an opportunity to avoid egg and hatchling predators and/or unfavorable weather conditions during portions of the hatching season.

Clutch size of *C. chitra* ranged from 40 to 88 eggs in this study, whereas Taechacharoensuk-chera (1991) found 97 eggs in a wild clutch, Nutphand (1990) stated that *C. chitra* normally laid 60 to 110 eggs per clutch. The highest number of eggs per clutch in *C. chitra* was recorded as 117 eggs (Nutphand, 1986). These data support the view that *C. chitra* should be classified as possessing reproductive Pattern I as defined by Moll, 1979. This pattern is characterized by the production of large clutches; multiple clutches produced during a well-defined nesting season; communal nesting in well-defined ancestral nesting area; and careful construction of covered nest. Seaturtles and many other larger riverine turtles also display Pattern I reproductive strategies.

The limited data available suggest that larger *Chitra* lay larger egg clutches than smaller individuals. This trend has been observed in other turtle species (Moll, 1979; Gibbons, 1982) although environmental influences may also influence clutch sizes to some extent.

Egg size and shape

Chitra chitra laid spherical eggs with white, brittle eggshells morphologically similar to those produced by other softshell turtles (see previous section on egg morphology).

Egg size of *C. chitra* is small compared to maternal body size. The large *C. chitra* female laid larger eggs than the small female, a trend similar to that seen in seaturtles (van Buskirk and Crowder, 1994; Miller, 1997). The smaller female produced more elongated eggs. Perhaps this might reflect the physical constraints by the diameter of the oviducts or a pelvic girdle constraint (Congdon and Gibbons, 1987) of newly matured females.

Incubation time

The incubation times of turtle eggs depend mainly on temperature and humidity. Previous studies showed that incubation times of *Testudo hermanni boettgeri* eggs incubated at high constant temperatures are shorter than those incubated at low constant temperature (Ecndebak, 1995). At the same incubation temperatures, *Chrysemys picta* eggs in wetter substrates required more time to hatch than those from drier substrates (Packard *et al.*, 1991). Temperatures and soil moisture levels in incubation methods employed in this study were not controlled. The eggs were incubated in fluctuating temperatures (24-30° C sand temperature), and relatively moist substrates so that the effects of these and perhaps other environmental influences on incubation time cannot be determined from this study. However, the moisture content in the last week of each method is higher due to water infused from hatching eggs (Figure 5).

Hatching success

The successful hatching of turtle eggs depends upon temperature, humidity, and air circulation. The hatch rate of *C. chitra* eggs in this study in Pond A did not differ among the three methods used (Table 3). The hatch rates were all high, > 80%, therefore, we conclude that conditions in all incubating methods were appropriate for incubation of *C. chitra* eggs.

Although the hatching success of eggs from Pond B was lower than that from Pond A (3-66 vs. 80-94%), both sets of eggs were incubated under similar conditions. Perhaps the smaller (71 kg) female was ovipositing for the first time (anecdotal evidence suggests that *C. chitra* females mature at 60 kg or more) and she was physiologically deficient for effective reproductive success.

Hatchling size

Hatchling sizes of *C. chitra* are very small and similar to sizes recorded for *C. indica* hatchlings (43 and 38 mm CL and CW and 10 g Wt and 39 and 33 mm of CL and CW and 7 g Wt) (Sachsse, 1971). Larger *C. chitra* eggs produced larger hatchlings (Table 4), a trait also known in seaturtles (van Buskirk and Crowder, 1994).

Growth

Growth of *C. chitra* including (CW, CL and Wt) is very rapid during the first 14 weeks. The result was compared with growth of *C. indica* hatchlings that were studied by Sachsse (1971). The initial sizes of *C. indica* were 43 and 38 mm of CL and CW and 10 g Wt and 39 and 33 mm of CL and CW and 7 g Wt. After 2 months, their measurements were 44 and 38 mm of CL and CW and 11 g Wt and 40 and 33 mm of CL and CW and 10 g Wt, respectively. In this study the growth rate of *C. chitra* was much higher in the first 2-3 months than observed in *C. indica*. Hatchlings from captive breeding have a better chance than animals from the wild. However, growth of *C. chitra* is probably not maintained at this rate. Two hatchlings of wild caught *C. chitra* were maintained at Kanchanaburi Inland Fisheries Development Center for nine years. Their sizes are 59 and 63 cm CW, 68 and 71 cm CL and 29 and 36 kg Wt, respectively (Kitimasak, unpublished data) on August 2002.

Acknowledgements

We would like to thank Mr. Vikrom Kromadit, Mr. Vitoon Kromadit, Mr. Pisit Na Phatthalung (the Director of the Zoological Park Organization), Mr. Somwang Pimombutara (the Director of Nakhon Sawan Inland Fisheries Development Center) for providing the animals in this study. Special thanks to Dr. Jaranthada Karnasuta, Mr. Rungsan Suangchompun (the Director of Kanchanaburi Inland Fisheries Development Center), Mrs. Aurapa Nagachinta and Mr. Chao Damlert for helping and furnishing facilities at KIFDC. This study was supported by the Thailand Research Fund (TRF) for the Royal Golden Jubilee Ph.D. Program (RGJ).

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The Natural History Journal of Chulalongkorn University 2(1):63-65, February 2002

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

WACHIRA KITIMASAK[1,2*] AND KUMTHORN THIRAKHUPT[2]

[1] *Kanchanaburi Inland Fisheries Development Center, Tha Muang, Kanchanaburi 71110, THAILAND*
[2] *Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok 10330, THAILAND*

Chitra chitra Nutphand, 1986 is a 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, river of southern Peninsula, southeastern part, Chao Phraya and Mae-kong river systems (Vidthayanon et al., 1997). *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).

The habitat of *C. chitra* in this record was divided into two areas (Fig. 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 Development Center and was later moved to Kanchanaburi Inland Fisheries 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 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 (Fig. 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.

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 in Chao Phraya river system as well.

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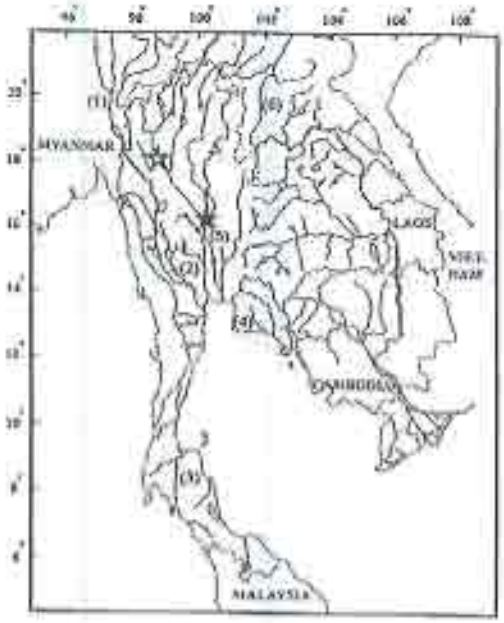

FIGURE 1. Six major river drainages in Thailand; (1) the Salween, (2) Maeklong, (3) river of southern Peninsula, (4) southeastern part, (5) Chao Phraya and (6) Mackong River systems and the new record of *C. chitra* in Mae Ping River, upper Bhumipol Dam (open star) and below Bhumipol Dam (solid star).

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

ACKNOWLEDGMENTS

We are grateful to Mr. Somvung Pimonbutr, Director of Nakhon Sawan Inland Fisheries Development Center, Mr. Jiradet Boonmak and Mr. Chatuporn Tantisunthorn who help us to access *C. chitra* information. We thank Dr. Art-ong Pradatsundarasar, Dr. Wichase Khonsue, Thavit Phupradit, Thongchai Ngamprasertwong and students in Animal Ecology class who joined our field work. This study was supported by The Thailand Research Fund (TRF) for The Royal Golden Jubilee Ph.D. Program (RGJ).

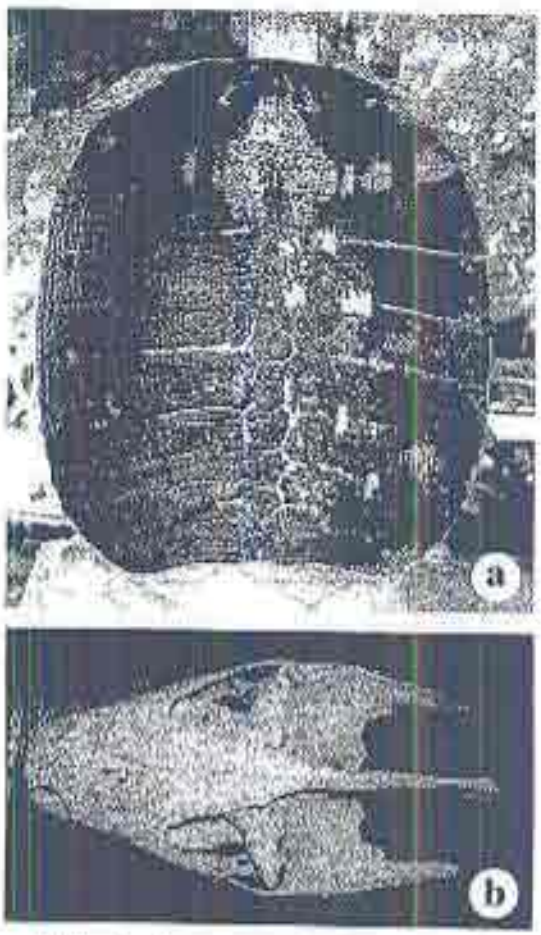

FIGURE 2. Carapace (a) and skull (b) of *C. chitra* in Mae Ping River.

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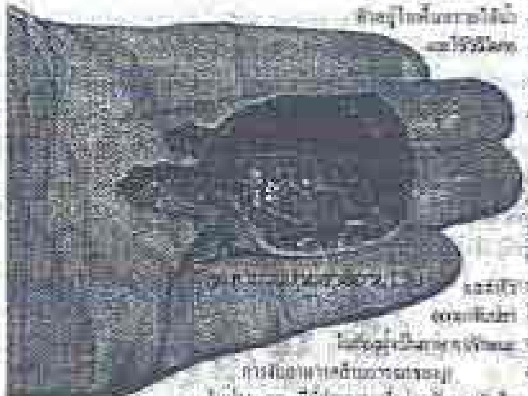
APPENDIX C

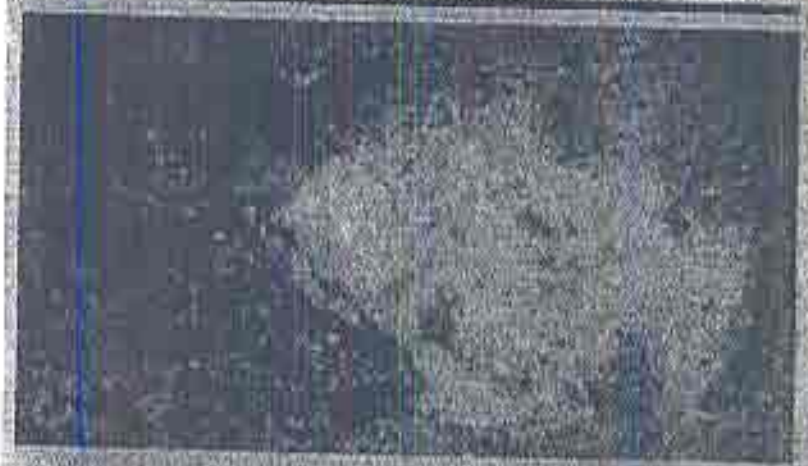
ไทยรัฐ
หนังสือพิมพ์จำหน่ายมากที่สุดของประเทศ
ปีที่ 53 ฉบับที่ 16204 วันจันทร์ที่ 3 มิถุนายน พ.ศ.2545 ราคา 8.00 บาท

"สืบชีวิตตะพาบม่านลาย" ก่อนสูญพันธุ์



[KOMPAS], JUMAT, 31 MEI 2002

KHAZANAH



ASSOCIATED PRESS/DEPARTMENT OF FISHERIES

Pembiakan Penyu — *Penyu berkepala sempit dan bercangkang lunak yang oleh penduduk Thailand disebut chitra-chitra, untuk pertama kalinya berhasil dibiakkan Departemen Perikanan Thailand. Tampak seekor bayi penyu keluar dari telurnya dalam foto yang dikeluarkan tanggal 30 Mei. Penyu yang sudah langka ini dianggap sebagai penyu bercangkang lunak terbesar di dunia. Setelah dewasa, beratnya bisa mencapai 200 kg.*

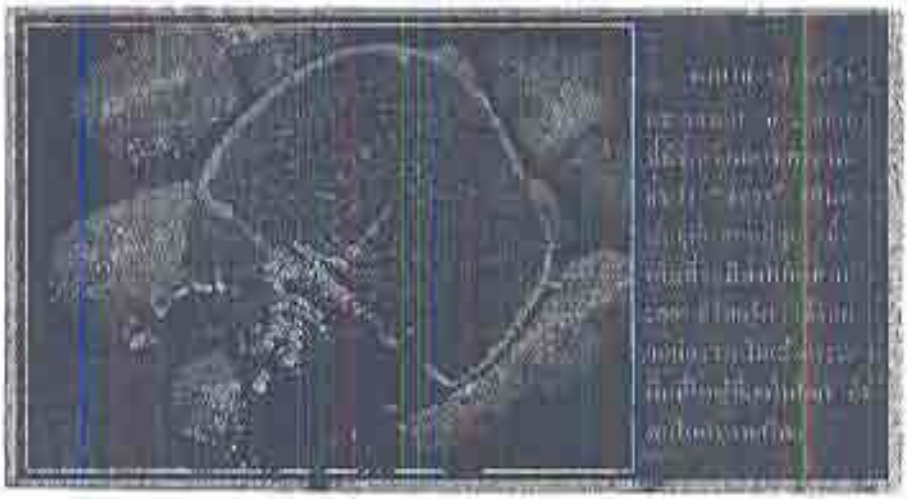
เพื่อชีวิต เจาะข่าวทั่วไทย
คมชัดลึก

เพาะ'ตะพาบม่านลาย'หนึ่งเดียวในโลก

เดลินิวส์
ฉบับที่ 19,224 วันศุกร์ที่ 31 พฤษภาคม พ.ศ. 2545 ราคา 8 บาท DAILY NEWS

ผสมพันธุ์ 'ตะพาบ' น่านสายได้ในบ่อ

เดลินิวส์
ฉบับที่ 19,228 วันพุธที่ 5 มิถุนายน พ.ศ. 2545 ราคา 8 บาท DAILY NEWS



A Fisheries Department official shows a narrow-headed softshell turtle. The success of a captive breeding programme in Kanchanaburi has given rise to hope that the species, threatened with extinction, can be saved. —PATTANAPONG HIRUNARD

Survival hope for rare turtle

Breeding programme heads off extinction

Porpot Changyawa

The success of a captive breeding programme for the very rare narrow-headed softshell turtle could save the species from looming extinction.

Only about 20 of the turtles are known to exist in their natural habit in the Mae Klong and Ping rivers.

After nine years of research a breeding programme run by the Freshwater Fisheries Development Centre in Kanchanaburi is now caring for 100 young turtles and about 100 eggs.

Researcher Wajira Kittimasak said the softshell turtles were bred in an artificial pond and sand dune. It was hoped they would be the foundation of a new breeding stock and save the species from extinction.

"This is only the first step. To be able to reintroduce them in the wild is the ultimate goal," said Kumthorn Tumakupt, a professor at Chulalongkorn University.

The turtles' natural habitat has spoiled by factory and household pollution, and even though they can survive there they do not reproduce, Mr Wajira said.

Dams were also believed to be a major contributor to the reptile's disappearance.

The dam reservoirs flood the river banks, where the eggs hatch.

Although a protected species, the softshell turtles have been extensively hunted for their elaborately patterned shells — a one-metre long shell could sell for over 100,000 baht.

หนังสือพิมพ์คุณภาพ เพื่อคุณภาพของประเทศ
http://www.matichon.co.th
มติชน
วันศุกร์ที่ 31 พฤษภาคม พุทธศักราช 2545 ปีที่ 25 ฉบับที่ 8848 ราคา 8 บาท

ประมงเจ๋งเพาะ'ตะพาบม่านลาย' ใกล้สูญพันธุ์-ใหญ่สุดถึง200กิโล

นายอำนวยศ์ ประกอบบุญ อธิบดีกรมประมง แถลงเมื่อวันที่ 30 พฤษภาคม ว่ากรมประมงประสบความสำเร็จในการเพาะพันธุ์ตะพาบม่านลายเป็นครั้งแรกในประเทศไทย ซึ่งตะพาบชนิดนี้เป็นสัตว์เลื้อยคลานพื้นเมืองของไทยที่จัดอยู่ในสภาพใกล้สูญพันธุ์อย่างยิ่ง และเป็นตะพาบที่มีขนาดใหญ่และมีน้ำหนักมากที่สุดในโลกและมีความสวยงามมากที่สุดในโลกด้วย ก่อนหน้านี้เคยมีผู้พบน้ำหนักมากกว่า 200 ก.ก. อย่างไรก็ตาม ตะพาบม่านลายขาดใหญ่สุดจะมีกระดองยาว ประมาณ 1 เมตร ราคาจำหน่ายในท้องตลาดกว่า 1 แสนบาท

นายอำนวยศักดิ์กล่าวว่า ความสำเร็จในครั้งนี้เป็นการทำการศึกษาของน.ส.ชิระ กิตติมลักดิ์ นักวิชาการประมง ณ ศูนย์พัฒนาประมงน้ำจืด จังหวัดปทุมธานี ซึ่งน.ส.ชิระได้รับทุนการศึกษาในโครงการปริญญาเอกกาญจนาภิเษก(คปก.) อยู่ที่ภาควิชาชีววิทยา คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย โดยการสนับสนุนของสำนักงานกองทุนสนับสนุนการวิจัย(สกว.) จากการศึกษาทำให้ขณะนี้กรมประมงได้ลูกตะพาบม่านลายที่เกิดจากการผสมพันธุ์ในบ่อเลี้ยงจำนวน 102 ตัว และกำลังขยายจำนวนเพิ่มขึ้นเรื่อยๆ

นายวชิระ กิตติมลักดิ์ นักวิชาการประมง กล่าวว่า ในช่วง 4 ปีที่ผ่านมา ไม่พบตะพาบม่านลายเลย ครั้งนี้ถือเป็นครั้งแรกที่มีการเพาะพันธุ์ได้ โดยตะพาบม่านลายเป็นตะพาบในจำนวน 5 ชนิด ที่พบในประเทศไทยคือตะพาบดาบแดง ตะพาบหิน ตะพาบน้ำ ตะพาบหัวหมู และตะพาบม่านลาย และทั่วโลกมี 25 ชนิด เป็นไปได้ว่าไทยเป็นประเทศเดียวที่มีตะพาบชนิดนี้อยู่ แต่เนื่องจากการเปลี่ยนแปลงสภาพของแม่น้ำทำให้จำนวนลดลง โดยเฉพาะการสร้างเขื่อนเพราะเขื่อนทำให้ระดับน้ำในแม่น้ำเปลี่ยนไป ส่งผลให้ราษฎรรายที่เป็นแหล่งวางไข่ถูกน้ำท่วมในฤดูวางไข่ลดลง จากการที่ตะพาบหายากส่งผลให้ราคาสูงกว่า 1 แสนบาท แม้แต่ลูกตะพาบราคาเกือบ 20,000 บาท ทั้งๆ ที่สัตว์ที่ถูกควบคุมห้ามซื้อขายเด็ดขาด

หนังสือพิมพ์
โลกวันนี้ รายวัน
เปิดโลกกว้างทัศน์ ให้ชีวิตทันโลก

เพาะตะพาบท่านลาย'ตัวละแสนสำเร็จ

เพาะ

วันที่ 5 มิถุนายน 2545

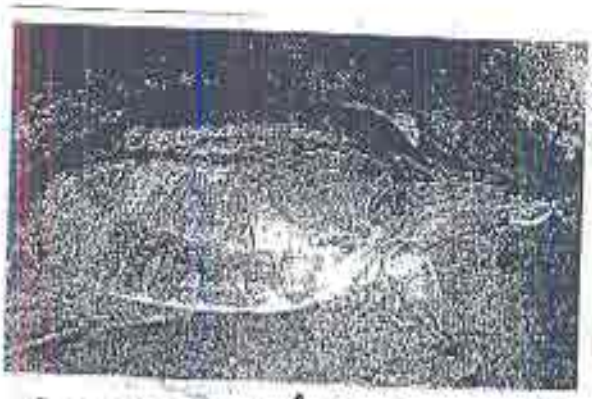
ตะพาบน้ำม่านลายไม่สูญพันธุ์ ประมงผสมพันธุ์ในบ่อสำเร็จ

นายวชิระ กิติมศักดิ์ นักศึกษาโครงการปริญญาเอกกาญจนาภิเษก (คปก.) กรมประมง เผยถึงผลสำเร็จในการผสมพันธุ์และขยายพันธุ์ตะพาบน้ำม่านลายสายพันธุ์แม่น้ำแม่กลองในบ่อเลี้ยงว่า แม่พันธุ์ตะพาบ 2 ตัว ที่ทดลองเลี้ยงไว้ได้วางไข่ทั้งหมด 300 ฟอง และสามารถฟักเป็นลูกตะพาบจำนวน 160 ตัว ซึ่งขณะนี้ลูกตะพาบดังกล่าวมีขนาดความยาวกระดองประมาณ 4-5 ซม. และอยู่ระหว่างการศึกษาสภาพการดำรงชีวิตและการเจริญเติบโตอย่างต่อเนื่อง โดยคาดว่าหากสามารถเลี้ยงลูกตะพาบดังกล่าวจนถึงอายุ 3 เดือน เปอร์เซ็นต์ที่จะเลี้ยงต่อไปจนถึงวัยเจริญพันธุ์ก็จะเพิ่มสูงขึ้นตามไปด้วย

การขยายพันธุ์ตะพาบน้ำในบ่อเลี้ยงครั้งนี้ ถือเป็นงานวิจัยแรกที่ประสบสำเร็จในตะพาบน้ำขนาดใหญ่ กรมประมงและสำนักงานกองทุนสนับสนุนการวิจัย (สกว.) ซึ่งเป็นผู้ให้ทุนสนับสนุนการวิจัยดังกล่าวตั้งแต่ต้น จึงได้ร่วมมือกันสนับสนุนการวิจัยเพิ่มเติม เพื่อทำความเข้าใจสภาพการดำรงชีวิต การเจริญเติบโต และการผสมพันธุ์ของตะพาบน้ำม่านลายอย่างต่อเนื่อง เพื่อให้ความสำเร็จของงานวิจัยครั้งนี้นำไปสู่การพัฒนางานอนุรักษ์ตะพาบน้ำและสัตว์น้ำชนิดอื่น ๆ อย่างยั่งยืนต่อไป

ทั้งนี้ตะพาบน้ำม่านลาย จัดเป็นสัตว์คุ้มครองชนิดหนึ่งที่สามารถพบได้เฉพาะในแม่น้ำแม่กลองและแม่น้ำปิง โดยธรรมชาติจะขุดหลุมวางไข่บริเวณหาดทรายริมแม่น้ำในช่วงหน้าแล้ง และไข่จะฟักเป็นตัวก่อนเข้าหน้าฝน แต่เนื่องจากการใช้ประโยชน์พื้นที่หาดริมน้ำที่เปลี่ยนแปลงไป ประกอบกับการสร้างเขื่อนทำให้แหล่งอาศัย และวางไข่ของตะพาบน้ำม่านลายถูกทำลายไปจำนวนไม่น้อย ปัจจุบันจำนวนตะพาบน้ำชนิดนี้จึงลดลงอย่างต่อเนื่อง สำหรับ IUCN ได้จัดสถานภาพอยู่ในกลุ่มสัตว์ใกล้สูญพันธุ์อย่างยิ่ง

ประจำวันที่ 4 มิถุนายน 2545



รายงาน

'ตะพาบม่านลาย' วิกฤติ ก่อนสิ้นลมหายใจ

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นอกจากสาเหตุข้างต้นแล้ว สาเหตุสำคัญอีกประการหนึ่งที่ทำลายประชากรตะพาบชนิดนี้ในธรรมชาติคือ การสร้างเขื่อน เนื่องจากการมีเขื่อนทำให้ระดับน้ำในแม่น้ำเปลี่ยนไปจากเดิม โดยบริเวณเหนือเขื่อนน้ำจะท่วมหาดทรายที่เป็นแหล่งวางไข่ทั้งหมด ในปัจจุบันตะพาบม่านลายตกตัวละเป็นแสนบาทหรือเกินกว่านั้น หรือแม้แต่ลูกตะพาบที่เพิ่งฟักจากไข่ใหม่ ๆ ก็ขายกันตัวละเกือบสองหมื่นบาท ทั้งที่สัตว์ชนิดนี้ถูกกำหนดเป็นสัตว์คุ้มครองที่ห้ามมีการ

ครั้งแรกของโลก
เพาะตะพาบสายพันธุ์ไทยสำเร็จ

เป็นเรื่องที่น่ายินดีที่นักศึกษาจากกรมประมงสามารถผสมพันธุ์ตะพาบม่านลายสายพันธุ์ลุ่มน้ำแม่กลองในบ่อเลี้ยงได้สำเร็จเป็นครั้งแรกของโลก ถือเป็นจุดเริ่มต้นในการอนุรักษ์ตะพาบพันธุ์ใหญ่ที่สุดในโลกชนิดนี้ไว้ จากพ่อแม่พันธุ์ 6 ตัว ได้ไข่กว่า 300 ฟอง ที่ฟักเป็นลูกตะพาบแล้วกว่า 160 ตัว

"ตะพาบม่านลาย" ถือเป็นตะพาบขนาดใหญ่ที่สุดจากตะพาบทั้งหมด 5 ชนิดที่พบในเมืองไทย (ตะพาบแก้มแดง-ตะพาบหับ-ตะพาบน้ำ-ตะพาบหัวกบ-ตะพาบม่านลาย) และ 24 ชนิดที่พบทั่วโลก โดยเคยมีรายงานว่าบางตัวหนักมากกว่า 200 กิโลกรัม และเชื่อได้ว่าไทยอาจเป็นประเทศเดียวที่มีตะพาบชนิดนี้อยู่ในสภาพธรรมชาติ โดยตะพาบชนิดนี้ปัจจุบันมีรายงานการพบเฉพาะในแม่น้ำแม่กลองและแม่น้ำปิงเท่านั้น มักอาศัยหมกตัวอยู่ใต้พื้นทรายใต้น้ำและใช้วิธียืดคอและหัวออกมาจับปลาในท้องน้ำเป็นอาหาร แต่ในช่วง 20 ปีที่ผ่านมา ที่อยู่อาศัยของมันคือบริเวณหาดทรายริมน้ำของแม่กลอง และแม่น้ำปิง มีการเปลี่ยนแปลงสภาพไปอย่างมากจากกิจกรรมต่าง ๆ ของมนุษย์ ทำให้แหล่งอาศัยและแหล่งวางไข่ถูกทำลายลงไป บางตัวถูกจับเป็นอาหาร อีกทั้งกระดองของตะพาบชนิดนี้มีลวดลายที่สวยงามมาก เหล่านี้ทำให้จำนวนของตะพาบชนิดนี้ได้ลดลงไปจน IUCN จัดสถานภาพอยู่ในสัตว์ใกล้สูญพันธุ์อย่างยิ่ง

ซื้อขายกันอย่างเด็ดขาด จากเหตุผลดังกล่าว การอนุรักษ์ตะพาบม่านลายในธรรมชาติเพียงอย่างเดียวคงไม่สามารถรักษาสัตว์ชนิดนี้ไว้ได้ ด้วยเหตุนี้ คุณวชิระ กิติมาศักดิ์ นักศึกษาโครงการปริญญาเอกกาญจนาภิเษก (คปก.) ซึ่งสนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย (สกว.) จึงได้ทำวิทยานิพนธ์เรื่อง...ประวัติธรรมชาติและชีววิทยาของตะพาบม่านลาย เพื่อศึกษาความเป็นไปได้ของการผสมพันธุ์และขยายพันธุ์ตะพาบชนิดนี้ในบ่อเลี้ยง โดยมี รศ.ดร. กำธร ธีรคุปต์ จากคณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย เป็นอาจารย์ที่ปรึกษาวิทยานิพนธ์ คุณวชิระได้ให้ข้อมูลว่า...ตะพาบพันธุ์นี้จะผสมพันธุ์กันในช่วงเดือนธันวาคม และเริ่มวางไข่ในเดือนกุมภาพันธ์ โดยแม่พันธุ์ตะพาบที่ทดลองเลี้ยงทั้ง 2 ตัววางไข่ตัวละ 4 ครั้ง ได้ไข่ทั้งหมดกว่า 300 ฟอง และฟักเป็นลูกตะพาบระยะเวลาประมาณ 4 เดือน ขณะนี้เหลือแล้วถึง 160 ตัว และจะทำการศึกษาตะพาบรุ่นนี้ไปจนถึงอายุ 3 เดือน ซึ่งคาดว่าหากตะพาบมีอายุขนาดนั้นจะสามารถเลี้ยงต่อไปถึงวัยเจริญพันธุ์ได้ในบ่อเลี้ยงที่ดูแล ซึ่งงานวิจัยชิ้นนี้จะส่งผลในการอนุรักษ์สัตว์ท้องถิ่นของไทยตัวนี้อย่างยั่งยืน และเป็นแนวทางในการอนุรักษ์ตะพาบและสัตว์น้ำชนิดอื่น ๆ ในอนาคต

คนที่เขาเห็นคุณค่าของสัตว์ที่ใกล้สูญพันธุ์ ก็พยายามที่จะอนุรักษ์ไว้ เอ...แล้วคนที่เห็นแต่การ "ฆ่า" เพื่อนำมาบริโภคจะคิดได้ไหมเนี่ย

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ปีที่ ๑๙ ฉบับที่ ๒๒๐
ราคา ๗๐ บาท

โลก ใหญ่

[illegible] : รายงาน / สำนักงานกองทุนสนับสนุนการวิจัย : ถ่ายภาพ

ตะพาบม่านลาย เป็นตะพาบที่มีขนาดใหญ่ที่สุดจากตะพาบ ๙ ชนิดที่พบในเมืองไทย และ ๒๕ ชนิดที่พบทั่วโลก เคยมีรายงานว่าบางตัวมีน้ำหนักมากกว่า ๒๐๐ กิโลกรัม ปัจจุบันมีรายงานว่าพบตะพาบม่านลายเฉพาะที่ประเทศไทยและอินโดนีเซียเท่านั้น แต่มีจำนวนประชากรลดลงอย่างมาก จนกระทั่ง IUCN [illegible] สำหรับประเทศไทย

ตะพาบม่านลายจากบ่อเลี้ยงรุ่นแรกของโลก

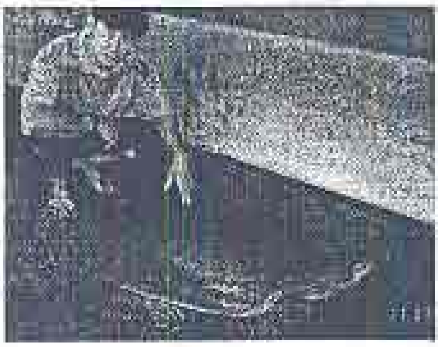
[illegible] สำคัญมาจากการสร้างเขื่อน

กรมประมงประสบความสำเร็จในการเพาะพันธุ์ตะพาบม่านลายเป็นครั้งแรกของโลก [illegible] ชนิดนี้ให้อยู่คู่ธรรมชาติ

วชิระ [illegible] นักวิชาการกรมประมง [illegible] ปริญญาเอกที่จุฬาลงกรณ์มหาวิทยาลัย ในฐานะนักศึกษาโครงการปริญญาเอกกาญจนาภิเษก (คปก.) ซึ่งสนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย (สกว.) และทำวิทยานิพนธ์เรื่อง "ประวัติธรรมชาติและชีววิทยาของตะพาบม่านลาย" กล่าวว่า ปัจจุบันมีรายงานว่าพบตะพาบม่านลายเฉพาะในแม่น้ำแม่กลองและแม่น้ำปิงเท่านั้น และมีประชากรน้อยมาก คาดว่าจำนวนพ่อแม่พันธุ์ที่เหลืออยู่ในธรรมชาติมีไม่เกิน ๒๕ ตัว

"สาเหตุมาจาก [illegible] หรือประชาชนเข้าไปบุกรุกพื้นที่ ทำให้ที่อยู่อาศัยของตะพาบถูกทำลาย [illegible]

[illegible]



[illegible]


วารสารการประมง
THAI FISHERIES GAZETTE
ปีที่ 56 ฉบับที่ 3 พฤษภาคม - มิถุนายน 2546
Volume 56 Number 3 May - June 2003

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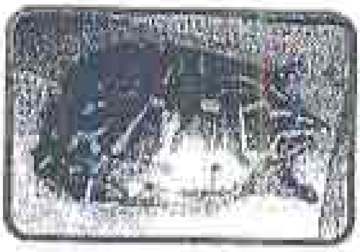
http://www.sc.chula.ac.th/

ปีที่ 25 ฉบับที่ 6 เดือนมิถุนายน 2545

นิสิตปริญญาเอก

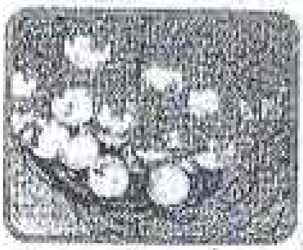
เพาะพันธุ์ตะพาบม่านลายสำเร็จเป็นครั้งแรกของโลก



นายวัชระ กิติมาศักดิ์ นิสิตปริญญาเอก ภาควิชาชีววิทยา คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ซึ่งเป็นนิสิตโครงการปริญญาเอกกาญจนาภิเษก (คปก.) เมื่อปี ๒๕๔๑ ได้ทำการค้นคว้าวิจัย เพาะพันธุ์ตะพาบม่านลายสำเร็จเป็นครั้งแรกของโลก รายละเอียดของการศึกษา คุณวัชระ ได้ให้รายละเอียดว่า ตะพาบม่านลายในธรรมชาติจะขุดหลุมวางไข่บริเวณหาดทรายริมแม่น้ำซึ่งเกิดขึ้นในช่วงหน้าแล้งที่มีน้ำน้อย ไข่จะฟักเป็นตัวก่อนจะถึงหน้าฝน แต่การมีเขื่อนทำให้ระดับน้ำในแม่น้ำเปลี่ยนแปลงไปจากเดิม โดยบริเวณเหนือเขื่อนน้ำจะท่วมหาดทรายซึ่งเป็นแหล่งวางไข่ทั้งหมด ส่วนบริเวณใต้เขื่อนระดับน้ำจะสูงต่ำตามการปล่อยน้ำจากเขื่อน ทำให้หาดทรายที่เป็นแหล่งวางไข่ถูกน้ำท่วมได้ในฤดูวางไข่ ตะพาบจึงไม่สามารถวางไข่ได้ หรือไข่ที่วางไว้แล้วถูกน้ำท่วม เป็นสาเหตุของการลดจำนวนตะพาบม่านลายในธรรมชาติที่สำคัญ ซึ่งความหายากนี้ทำให้ราคาตะพาบเพื่อการค้าสูงมาก ๆ ก็ขายกันตัวละเกือบครึ่งหมื่นบาท ทั้งที่สัตว์ชนิดนี้ถูกกำหนดให้เป็นสัตว์คุ้มครองที่ห้ามมีการซื้อขายกันอย่างเด็ดขาด"

ดังนั้น การอนุรักษ์ตะพาบน้ำในธรรมชาติเพียงอย่างเดียวคงจะไม่สามารถรักษาสิ่งมีชีวิตชนิดนี้ไว้ได้ จึงได้ทำวิทยานิพนธ์เรื่อง "ประวัติธรรมชาติและชีววิทยาของตะพาบม่านลาย" เพื่อศึกษาความเป็นไปได้ของการผสมพันธุ์และขยายพันธุ์ตะพาบชนิดนี้ในบ่อเลี้ยง โดยมี รศ.ดร.กำธร ธีรคุปต์ เป็นอาจารย์ที่ปรึกษาวิทยานิพนธ์

จากข้อมูลที่ได้จากการสำรวจพื้นที่อยู่อาศัยและแหล่งวางไข่ของตะพาบพันธุ์นี้ในธรรมชาติ ผู้วิจัยจึงนำมาออกแบบและดัดแปลงบ่อขนาด ๔๐๐ ตารางเมตร ในศูนย์พัฒนาประมงน้ำจืดกาญจนบุรี ให้ใกล้เคียงกับสภาพธรรมชาติ ก่อนจะปล่อยตะพาบน้ำทั้งตัวผู้และตัวเมียให้อยู่ด้วยกัน และทำการศึกษาพฤติกรรมของมัน เพื่อปรับสภาพแวดล้อมภายในบ่อให้เหมาะกับมันมากที่สุด และจากการศึกษาวิจัยกว่า ๔ ปี สามารถทำให้ตะพาบม่านลายที่เลี้ยงไว้ผสมพันธุ์กันได้เอง และสามารถวางไข่ได้ในบ่อเลี้ยง ทำจนไข่ได้ฟักเป็นตัวออกแล้วมากกว่า ๑๔๐ ตัว ตะพาบพันธุ์นี้จะผสมพันธุ์กันในช่วงเดือนธันวาคม และเริ่มวางไข่ในเดือนกุมภาพันธ์ โดยแม่พันธุ์ตะพาบที่ทดลองเลี้ยงทั้ง ๒ ตัว วางไข่ตัวละ ๔ ครั้ง ได้ไข่รวมทั้งหมดกว่า ๓๐๐ ฟอง และฟักเป็นลูกตะพาบขนาดความยาวกระดองประมาณ ๔ เซนติเมตร ซึ่งเป็นส่วนของงานวิทยานิพนธ์เขาจะทำการศึกษาการอนุบาลลูกตะพาบรุ่นนี้ไปจนถึงอายุ ๓ เดือน ซึ่งก็สามารถเลี้ยงได้จนถึงอายุ ๕ เดือนแล้ว คาดว่าต่อไปลูกตะพาบจะมีอัตราการรอดสูงขึ้น และสามารถเลี้ยงต่อไปได้จนถึงวัยเจริญพันธุ์

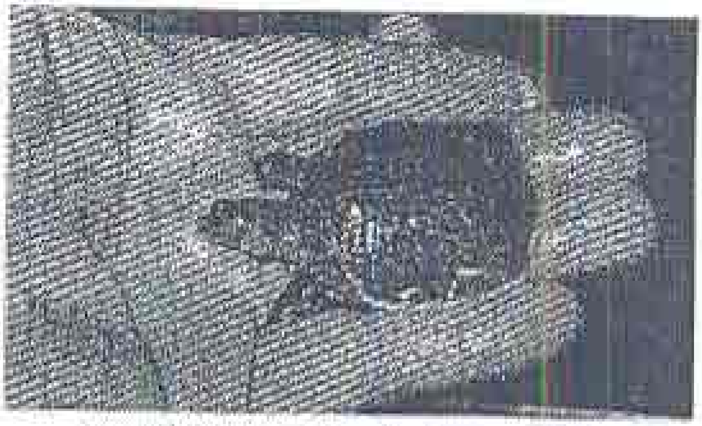


จากการค้นคว้าวิจัยดังกล่าวนี้ทำให้ประสบความสำเร็จในการที่จะอนุรักษ์พันธุ์สัตว์น้ำประเภทตะพาบ และเป็นความสำเร็จครั้งแรกของโลกและของประเทศไทยการเพาะเลี้ยงตะพาบในบ่อเลี้ยง

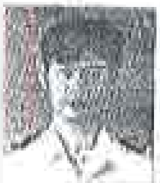
จุฬาสัมพันธ์

ปีที่ ๔๔ ฉบับที่ ๑๐ วันจันทร์ที่ ๗ กรกฎาคม ๒๕๔๔

นิสิตปริญญาเอกจุฬาฯ ขยายพันธุ์ "ตะพาบม่านลาย" เป็นผลสำเร็จครั้งแรก



นิสิตปริญญาเอกสาขาวิชาชีววิทยา คณะวิทยาศาสตร์ จุฬาฯ ประสบความสำเร็จในการเพาะพันธุ์ตะพาบม่านลายได้เป็นครั้งแรกในประเทศไทย [illegible]



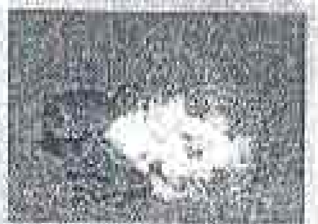
นายวชิระ กิตติมศักดิ์ นิสิตปริญญาเอกคณะวิทยาศาสตร์ และนักวิชาการกรมประมง ผู้ใช้เวลากว่า ๗ ปี ทุ่มเทศึกษาชีวิตของ "ตะพาบม่านลาย" ซึ่งถือเป็นตะพาบขนาดใหญ่ที่สุดจากตะพาบทั้งหมด ๕ ชนิดที่พบในประเทศไทย [illegible] โดยได้ทำวิทยานิพนธ์เรื่อง "ประวัติธรรมชาติและชีววิทยาของตะพาบม่านลาย" ซึ่งได้รับการสนับสนุนจากสำนักงานกองทุนสนับสนุนการวิจัย (สกว.) [illegible]

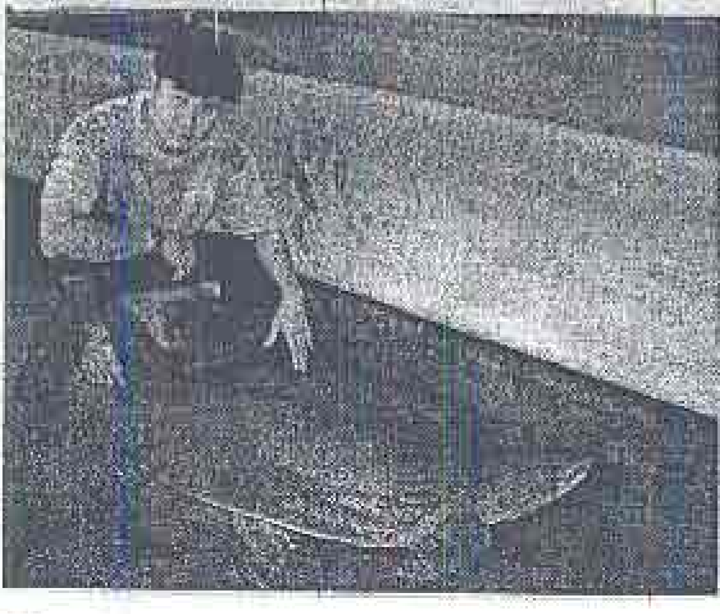
[illegible]

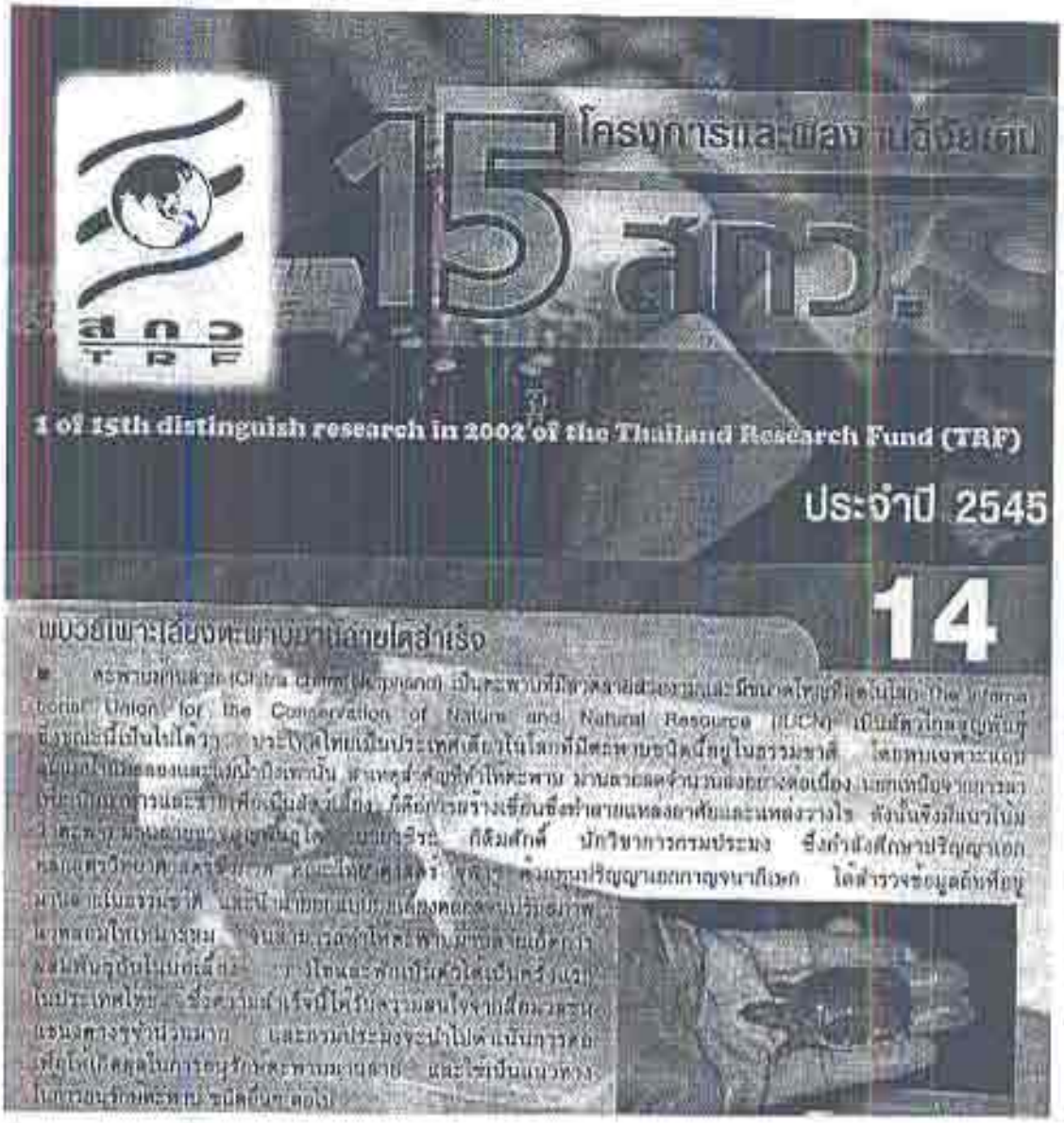
Research Community
ประชาคมวิจัย
จดหมายข่าวราย 2 เดือน สำนักงานกองทุนสนับสนุนการวิจัย (สกว.) ฉบับที่ 46 เดือนพฤศจิกายน 2545

พบวิธีเพาะเลี้ยงตะพาบม่านลายได้สำเร็จ

ตะพาบม่านลาย (*Chitra chitra* Nutphand) เป็นตะพาบที่มีความสวยงามและมีขนาดใหญ่ที่สุดในโลก The International Union for the Conservation of Nature and Natural Resources (IUCN) จัดตะพาบม่านลายเป็นสัตว์ที่อยู่ในสถานภาพใกล้สูญพันธุ์แล้ว ทั้งที่เจอแหล่งที่เป็นไปได้ว่าประเทศไทยเป็นประเทศเดียวในโลกที่มีตะพาบชนิดนี้อยู่ในธรรมชาติ โดยพบเฉพาะบริเวณลุ่มแม่น้ำแม่กลองและแม่น้ำปิงเท่านั้น สถานภาพของตะพาบม่านลายลดจำนวนลงอย่างต่อเนื่อง นอกจากนี้จากการจับเพื่อเป็นอาหารและขายเพื่อเป็นสัตว์เลี้ยง ก็มีการสร้างเขื่อนซึ่งทำลายแหล่งอาศัยและแหล่งวางไข่ และด้วยความหายากทำให้เกิดความต้องการซื้อขาย ดังนั้นจึงมีแนวโน้มว่าตะพาบม่านลายจะสูญพันธุ์ได้ [illegible] นักวิชาการประมง ซึ่งกำลังศึกษาปริญญาเอกในหลักสูตรวิทยาศาสตร์ชีวภาพ คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ด้วยทุนปริญญาเอกกาญจนาภิเษก โดยมี ดร. [illegible] เป็นอาจารย์ที่ปรึกษาวิทยานิพนธ์ และ Professor Dr. Don Moll จาก Southwest Missouri State University, Missouri ประเทศสหรัฐอเมริกา เป็นอาจารย์ที่ปรึกษาร่วม ได้สำรวจข้อมูลถิ่นที่อยู่และการแพร่กระจายของตะพาบม่านลายในธรรมชาติ และนำมาทดลองเพาะเลี้ยง ตลอดจนปรับสภาพแวดล้อมให้เหมาะสม จนสามารถทำให้ตะพาบม่านลายเกิดการผสมพันธุ์ในบ่อเลี้ยง วางไข่ และฟักเป็นตัวได้เป็นครั้งแรกในประเทศไทย ซึ่งความสำเร็จในการเพาะตะพาบม่านลายนี้ได้รับความสนใจจากสื่อมวลชนทางด้านหนังสือพิมพ์ วารสาร วิทยุ และโทรทัศน์ และได้นำไปเผยแพร่ผ่านสื่อดังกล่าวจำนวนมาก และคาดว่าจะนำไปพัฒนาการต่อเพื่อให้เกิดผลในการอนุรักษ์ตะพาบม่านลาย และใช้เป็นแนวทางในการอนุรักษ์ตะพาบชนิดอื่นๆ ต่อไป



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