Chapter 4

Results and Discussion

4.1 Sexual dimorphism

In order to minimize size bias, every morphological characters of *Amyda cartilaginea* were transformed to various parameters in relative to carapace length (CL). It was found that the male softshell turtle exhibited significantly higher degree of plastron to cloaca (PC/CL), tailbase to cloaca (TC/CL), cloaca to tail tip (CT/CL), tail length (TL/CL), tail width (TW/CL), and weight (W/CL). The females showed significantly higher degree of bony disc length 1 (BDL1/CL), bony disc length 2 (BDL2/CL), plastron length 2 (PL2/CL), height (H/CL), head length without snout (HL/CL), and head length with snout (HLs/CL). While other parameters were not significantly different between sex. Table 4-1 presents mean and S.E.M. of the mentioned parameters of male and female softshell turtle.

In addition to the above parameters, tail length beyond carapacial margin was also compared between sexes. The length was transformed from subtraction of plastron to rear margin of carapace from addition of plastron to cloaca and cloaca to tail tip {(PC+CT)-PR}. The results were transformed into relative numbers to capapace length and compared between sexes. It was found that the males showed significant higher tail length beyond carapacial margin than females as displayed in table 4-1.

Body outline of softshell turtle was determined from subtraction of carapace length by plastron width (CW-PW) in relative to carapace length. It was found that there was no significant difference between the two sexes as shown in table 4-1.

Cloacal position was determined from division of tailbase to cloaca with tail length (TC/TL). It was found that cloacal position of the males was significantly more posterior than the females as shown in table 4-1.

Table 4-1 Mean and S.E.M. of parameters of morphological characters of *Amyda cartilaginea*.

Parameters	Mean ± S.E.M.	
	Males (n = 80)	Females (n = 54)
Bony Disc Length 1/CL	$0.6548^{a} \pm 0.0035$	$0.6722^{b} \pm 0.0052$
Bony Disc Length 2/CL	$0.5570^{a} \pm 0.0042$	$0.5814^{b} \pm 0.0061$
Carapace Width/CL	$0.7222^{a} \pm 0.0044$	$0.7292^{a} \pm 0.0053$
Plastron Length 1/CL	$0.5155^{a} \pm 0.0029$	$0.5257^{a} \pm 0.0046$
Plastron Length 2/CL	$0.6645^{a} \pm 0.0034$	$0.6771^{b} \pm 0.0050$
Plastron Width/CL	$0.6818^{a} \pm 0.0055$	$0.6895^{a} \pm 0.0052$
Height/CL	$0.2638^{a} \pm 0.0023$	$0.2764^{b} \pm 0.0039$
Plastron to Rear margin of carapace/CL	$0.3526^{a} \pm 0.0021$	$0.3738^{a} \pm 0.0049$
Plastron to Cloaca/CL	$0.3015^{a} \pm 0.0026$	$0.2625^{b} \pm 0.0034$
Tailbase to Cloaca/CL	$0.0690^{a} \pm 0.0022$	$0.0301^{b} \pm 0.0016$
Cloaca to Tail tip/CL	$0.0712^{a} \pm 0.0014$	$0.0623^{b} \pm 0.0022$
Tail Length/CL	$0.1394^{a} \pm 0.0029$	$0.0923^{b} \pm 0.0027$
Tail Width/CL	$0.0963^{a} \pm 0.0017$	$0.0783^{b} \pm 0.0016$
Head Length without snout/CL	$0.2730^{a} \pm 0.0020$	$0.2795^{b} \pm 0.0026$
Head Length with snout/CL	$0.2961^{a} \pm 0.0025$	$0.3088^{b} \pm 0.0027$
Head Width/CL	$0.1748^{a} \pm 0.0022$	$0.1755^{a} \pm 0.0019$
Head Height/CL	$0.1399^{a} \pm 0.0009$	$0.1398^{a} \pm 0.0013$
Weight/CL	$0.3615^{a} \pm 0.0180$	$0.2447^{b} \pm 0.0136$
Tail length beyond Carapace/CL	$-0.010^{a} \pm 0.0037$	$-0.049^{b} \pm 0.0037$
Carapace Width-Plastron Width/CL	$0.0404^{a} \pm 0.0040$	$0.0394^{a} \pm 0.0021$
Tailbase to Cloaca/Tail Length	$0.4901^{a} \pm 0.0091$	$0.3255^{b} \pm 0.0143$

Remark Significant differences ($p<0.05$) of the means between males and females are indicated by differences in superscript letters.

Every morphological characters of each sex were plotted against carapace length (CL). The regression analysis revealed significant linear regression line ($p<0.05$) of both sexes in every pair of morphological characters and CL. Slopes and elevation of regression line in every pairwise were compared between sexes. The results are presented in figure 4-1 to 4-20.

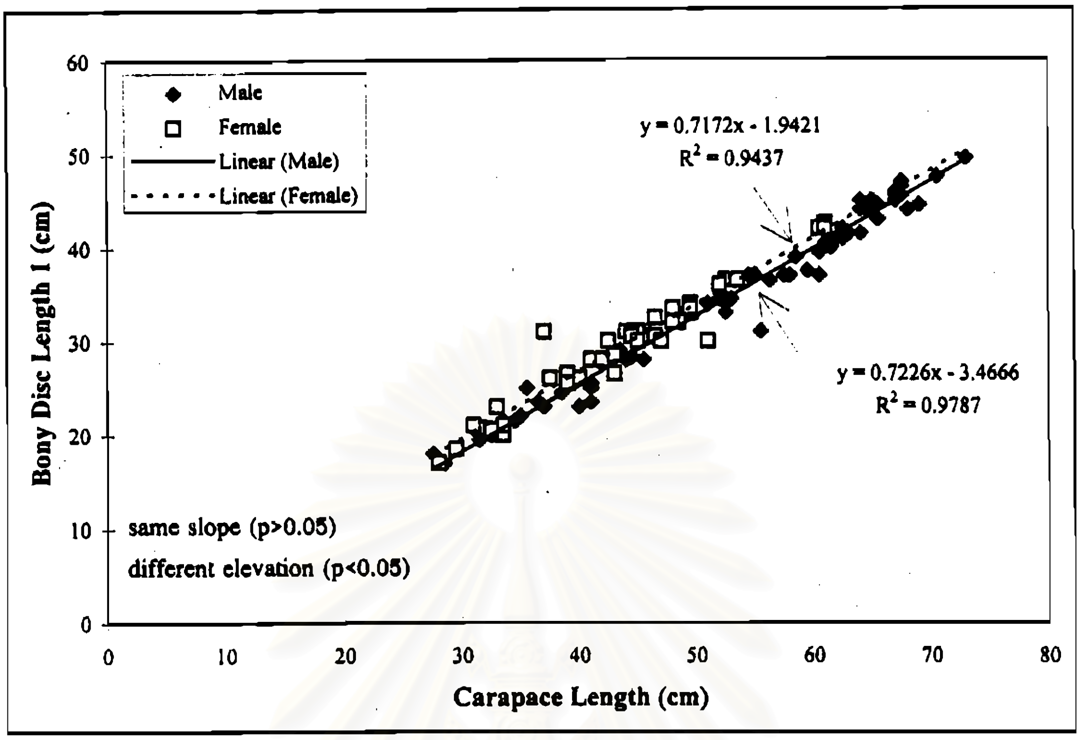

Figure 4-1 Linear regression lines relating bony disc length 1 to carapace length of *Amyda cartilaginea* (p<0.05).

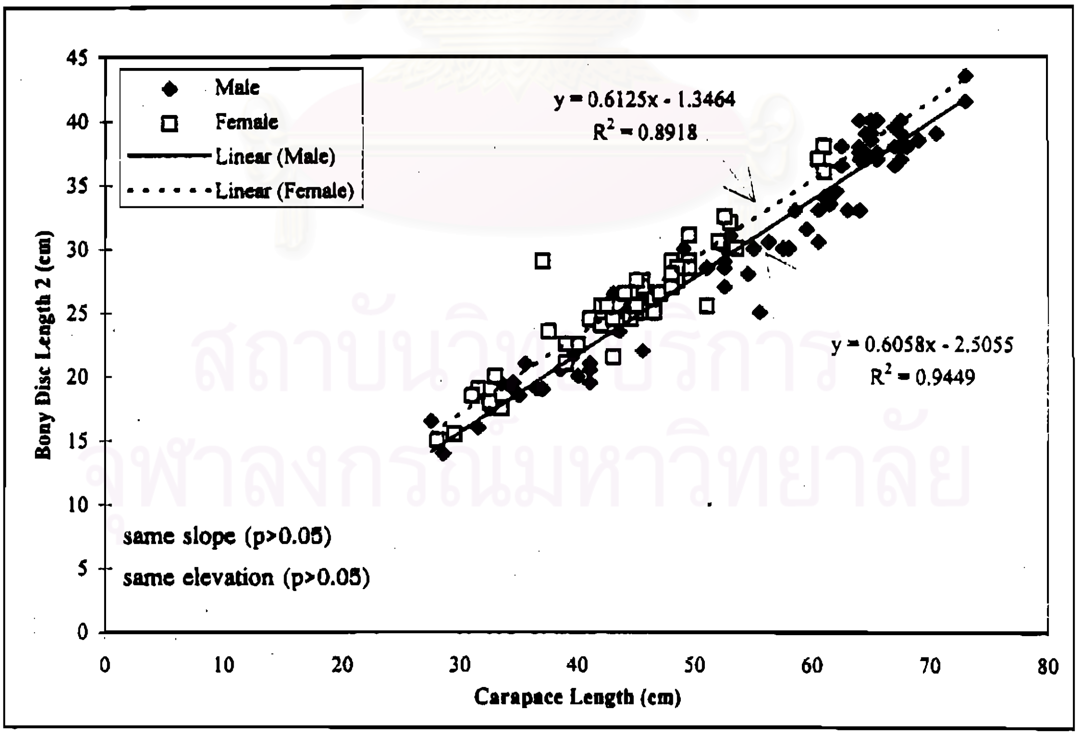

Figure 4-2 Linear regression lines relating bony disc length 2 to carapace length of *Amyda cartilaginea* (p<0.05).

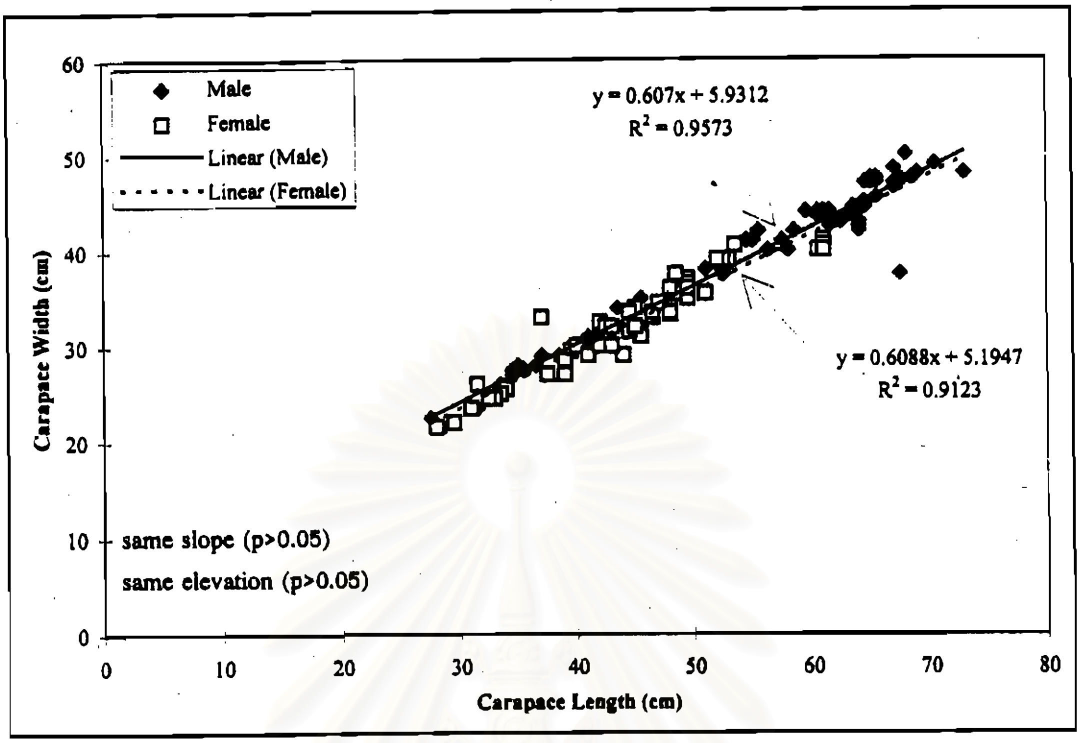

Figure 4-3 Linear regression lines relating carapace width to carapace length of *Amyda cartilaginea* ($p<0.05$).

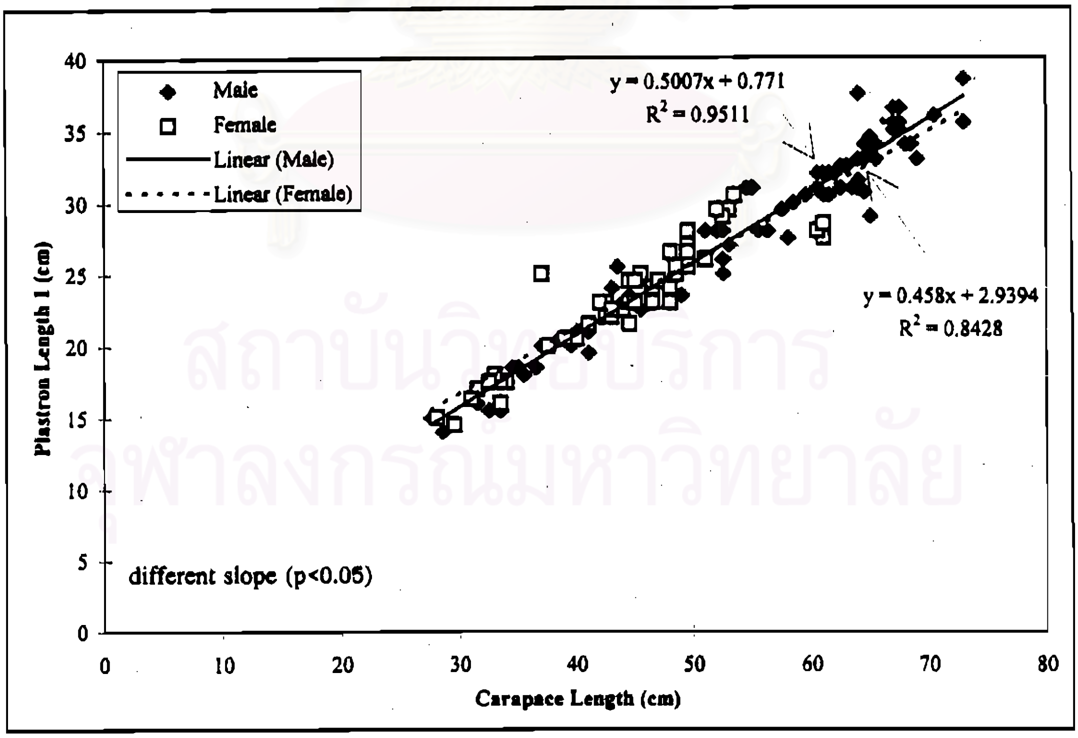

Figure 4-4 Linear regression lines relating plastron length 1 to carapace length of *Amyda cartilaginea* ($p<0.05$).

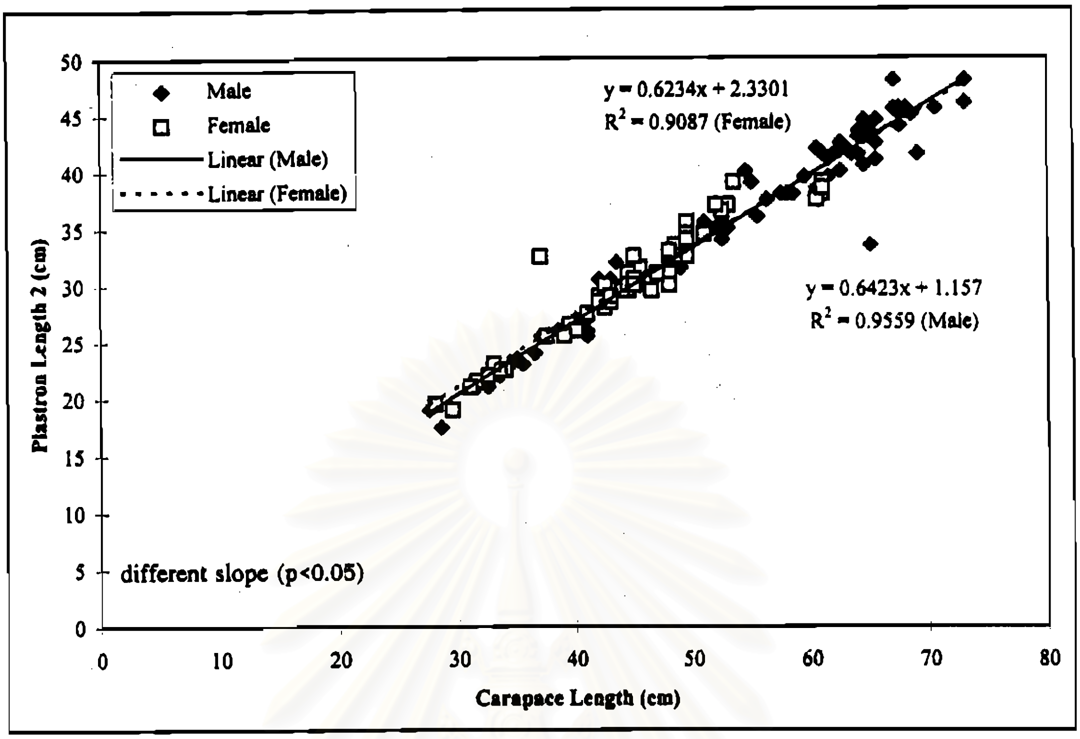

Figure 4-5 Linear regression lines relating plastron length 2 to carapace length of *Amyda cartilaginea* ($p<0.05$).

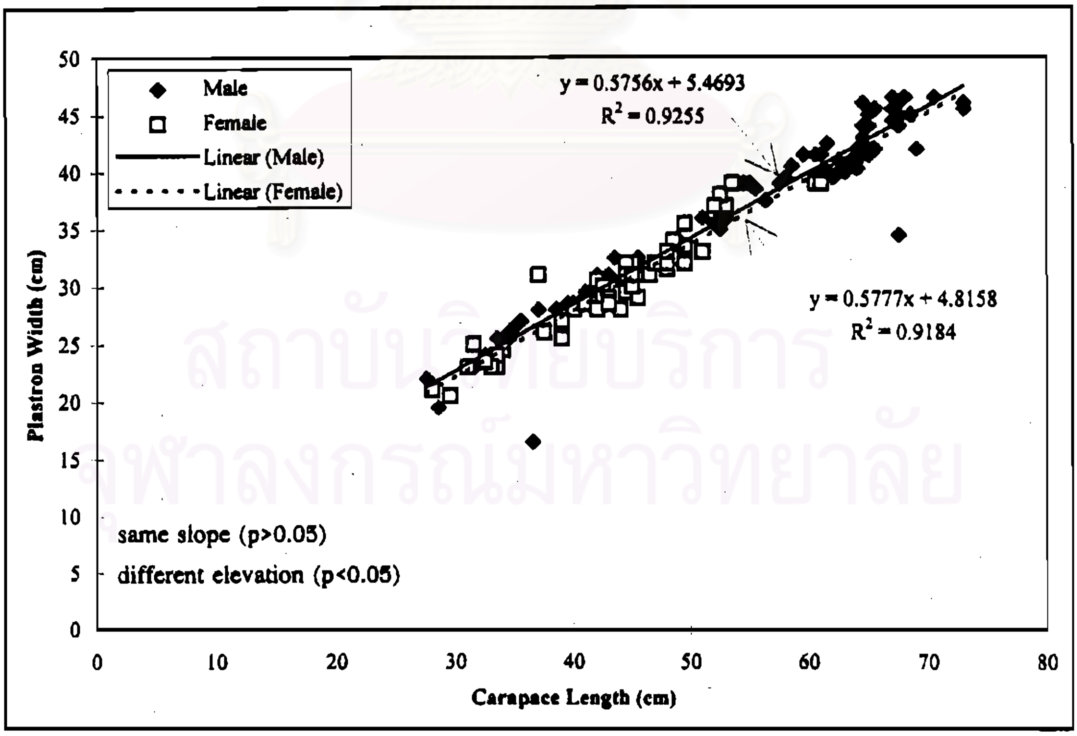

Figure 4-6 Linear regression lines relating plastron width to carapace length of *Amyda cartilaginea* ($p<0.05$).

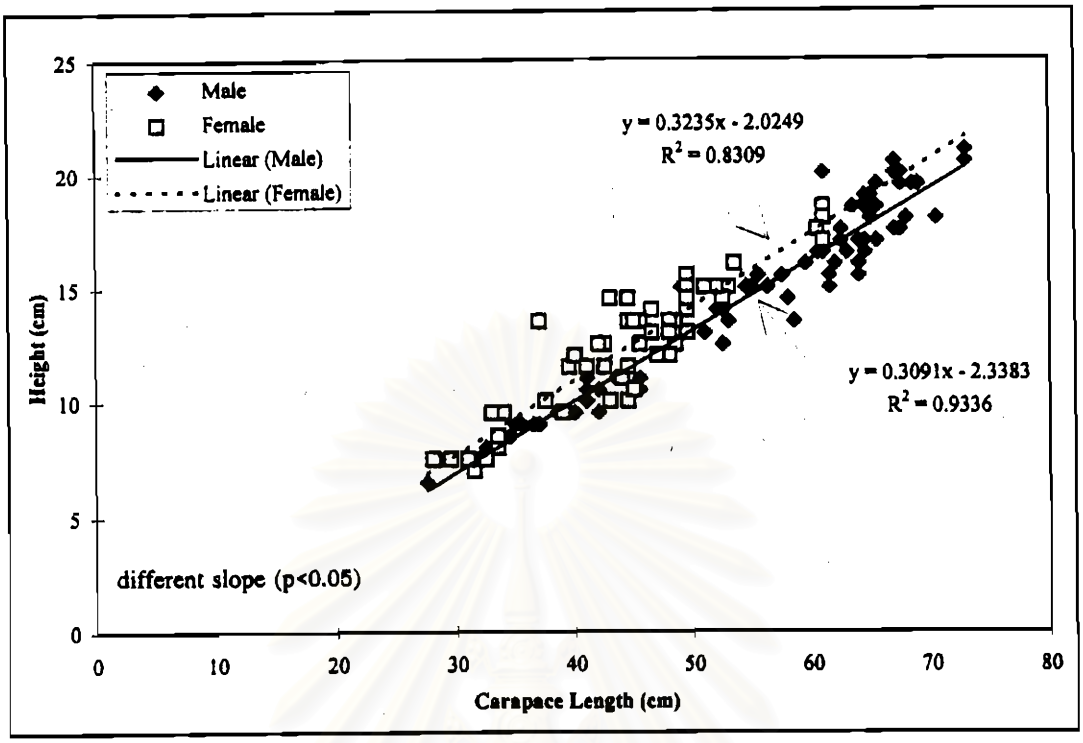

Figure 4–7 Linear regression lines relating height to carapace length of *Amyda cartilaginea* ($p<0.05$).

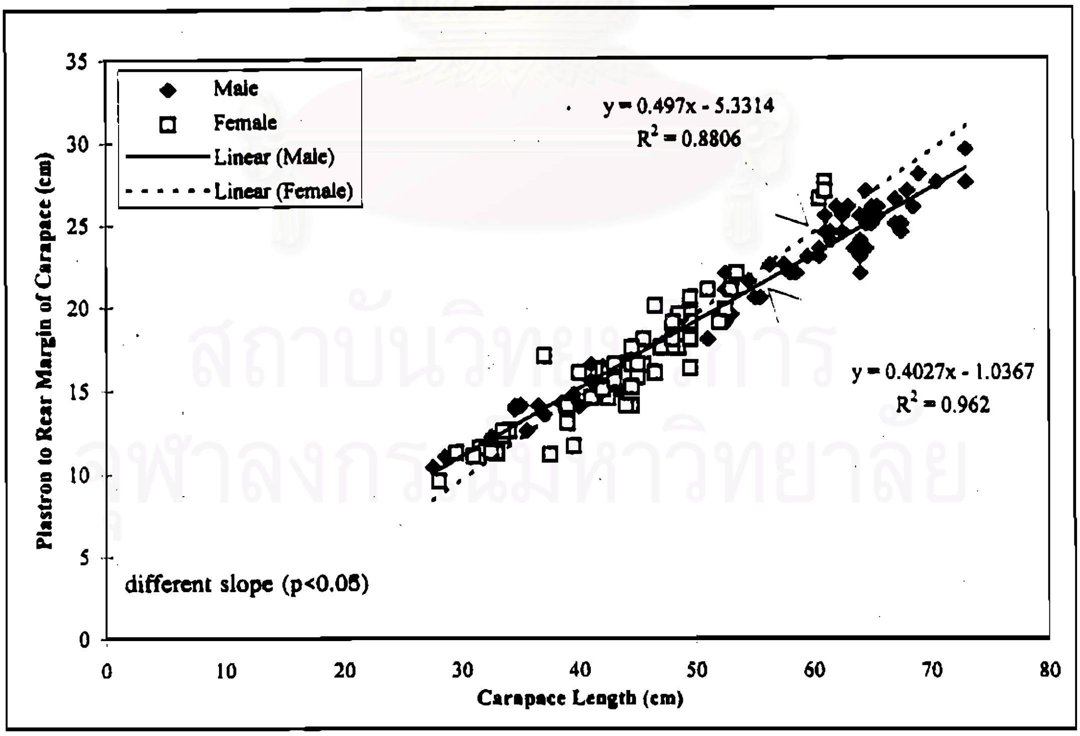

Figure 4–8 Linear regression lines relating plastron to rear margin of carapace to carapace length of *Amyda cartilaginea* ($p<0.05$).

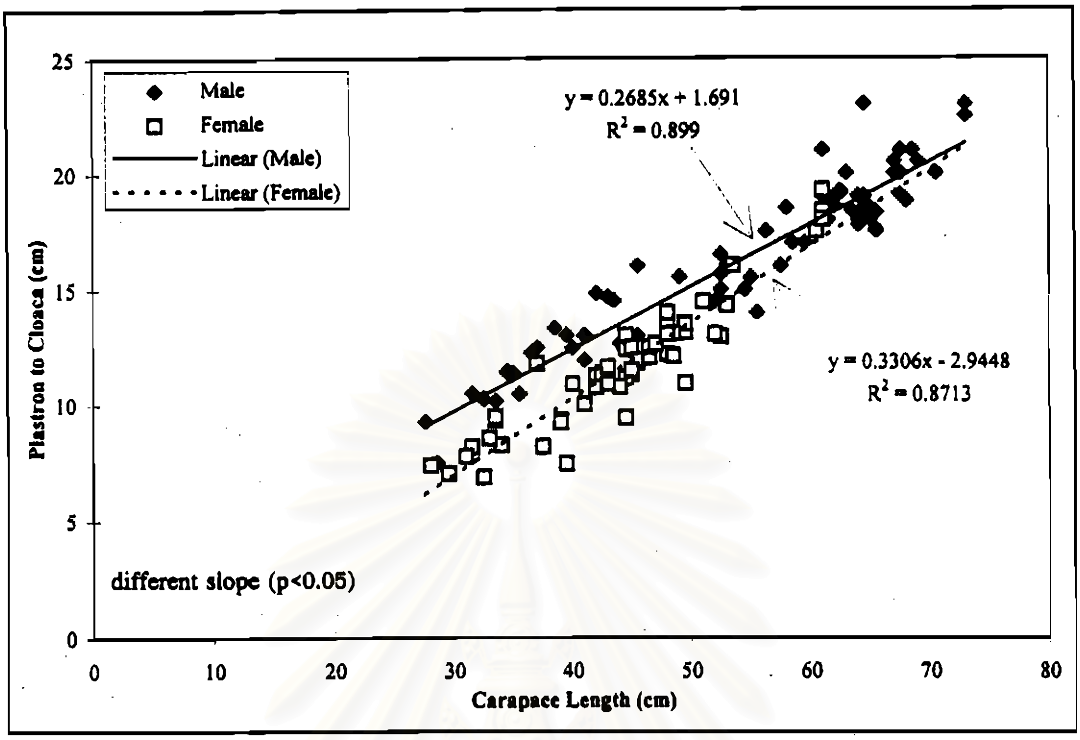

Figure 4–9 Linear regression lines relating plastron to cloaca to carapace length of *Amyda cartilaginea* ($p<0.05$).

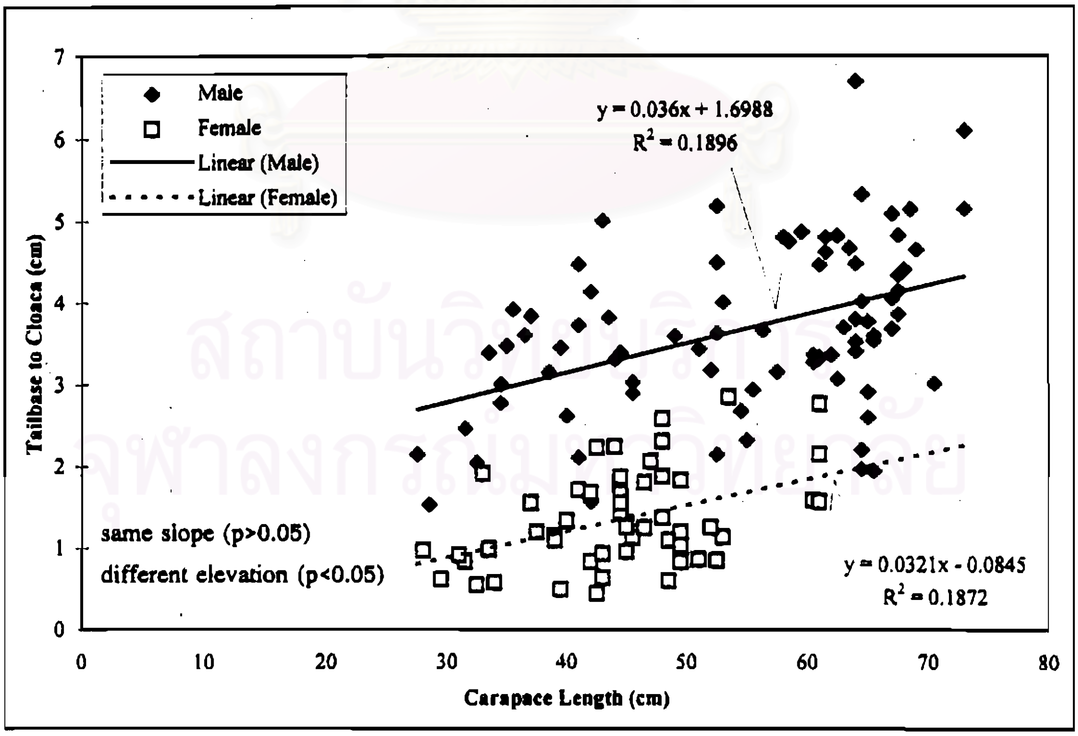

Figure 4–10 Linear regression lines relating tailbase to cloaca to carapace length of *Amyda cartilaginea* ($p<0.05$).

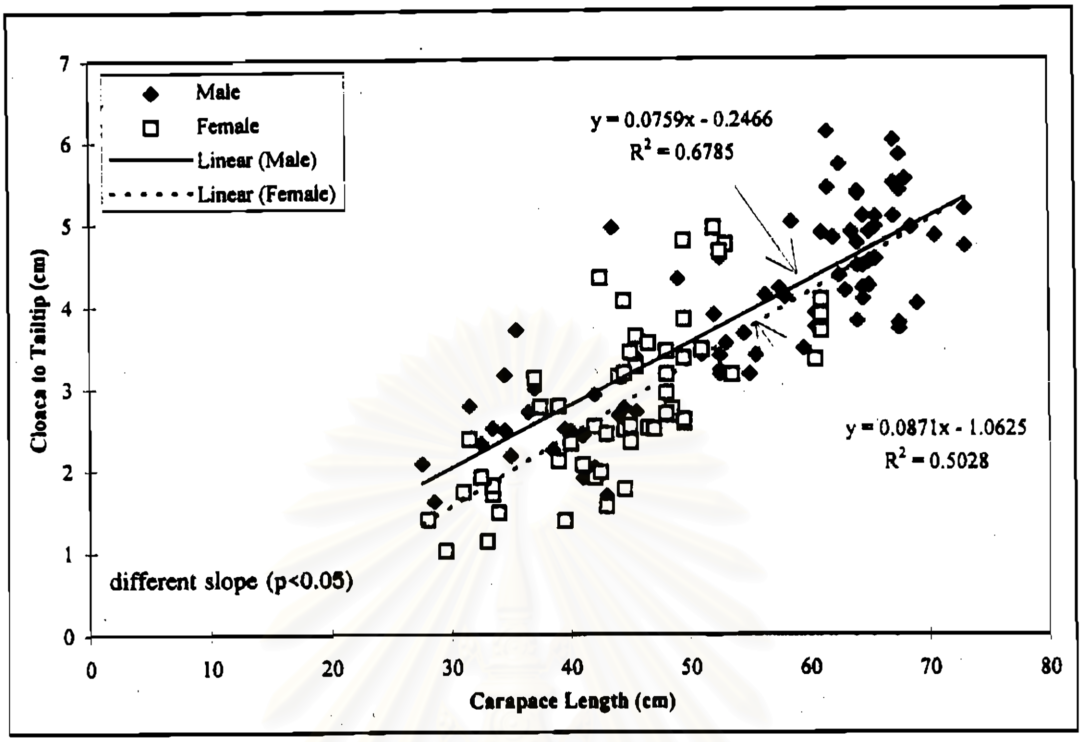

Figure 4–11 Linear regression lines relating cloaca to tail tip to carapace length of *Amyda cartilaginea* ($p<0.05$).

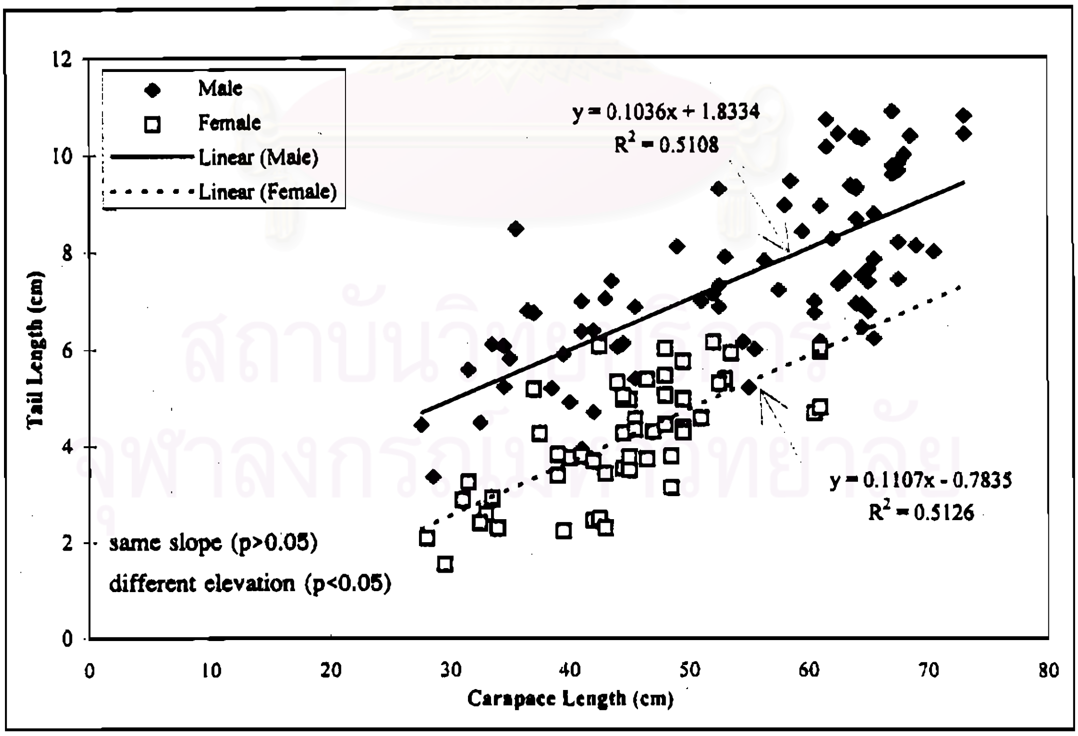

Figure 4–12 Linear regression lines relating tail length to carapace length of *Amyda cartilaginea* ($p<0.05$).

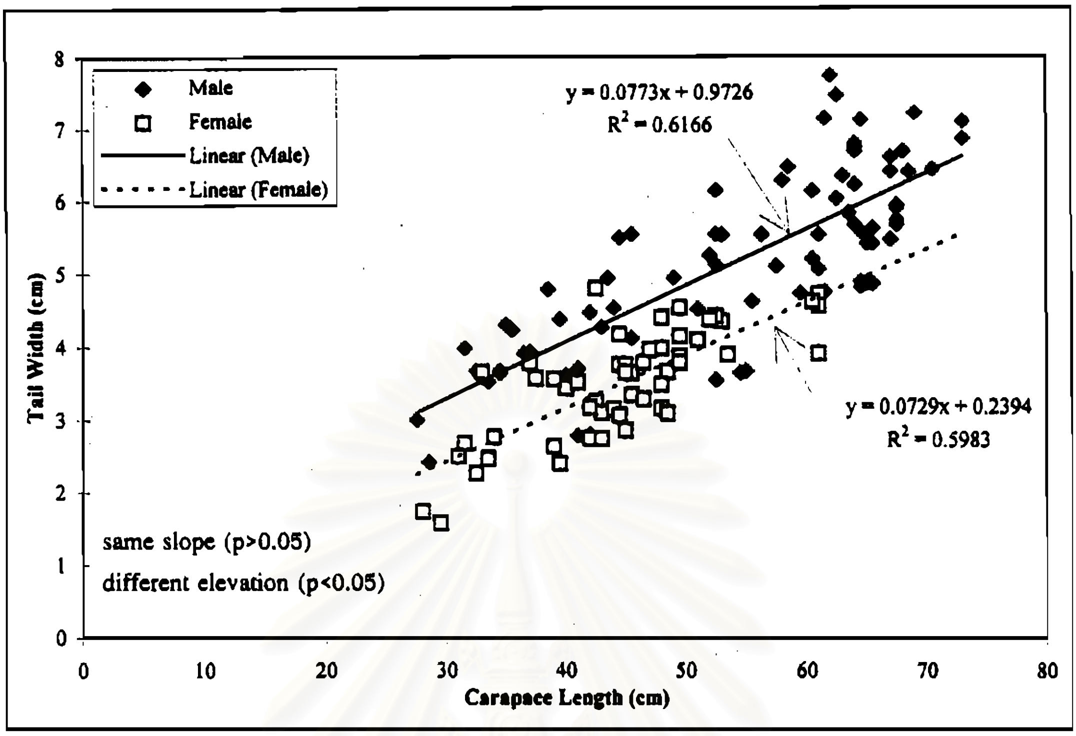

Figure 4–13 Linear regression lines relating tail width to carapace length of *Amyda cartilaginea* ($p<0.05$).

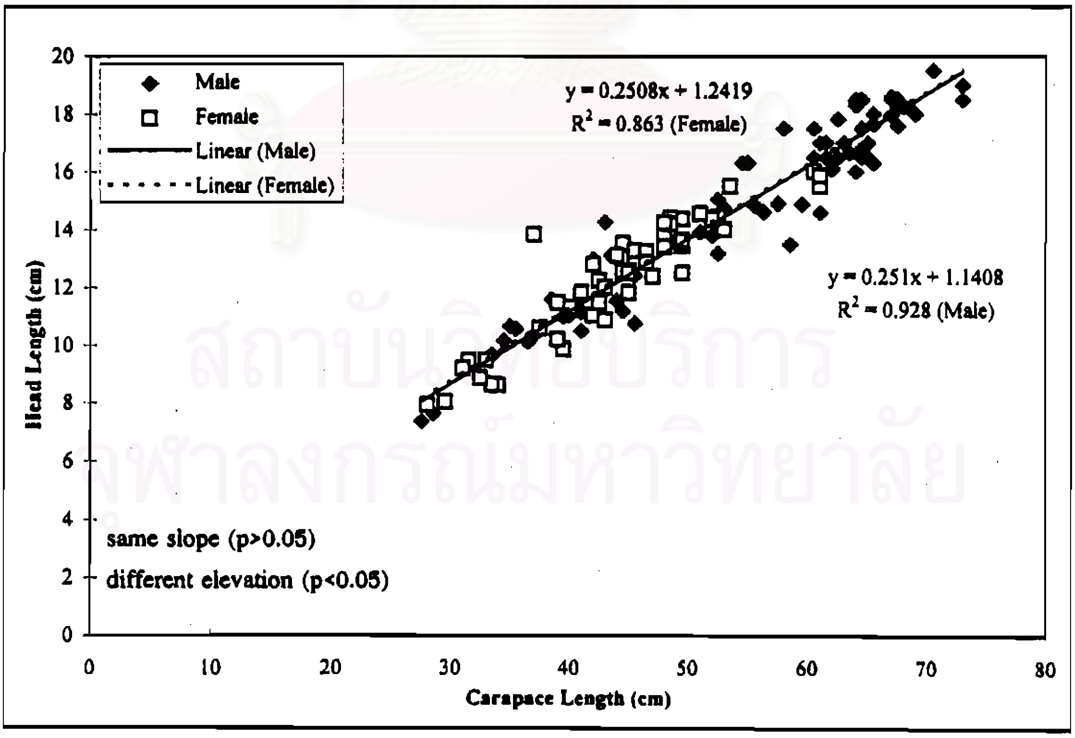

Figure 4–14 Linear regression lines relating head length without snout to carapace length of *Amyda cartilaginea* ($p<0.05$).

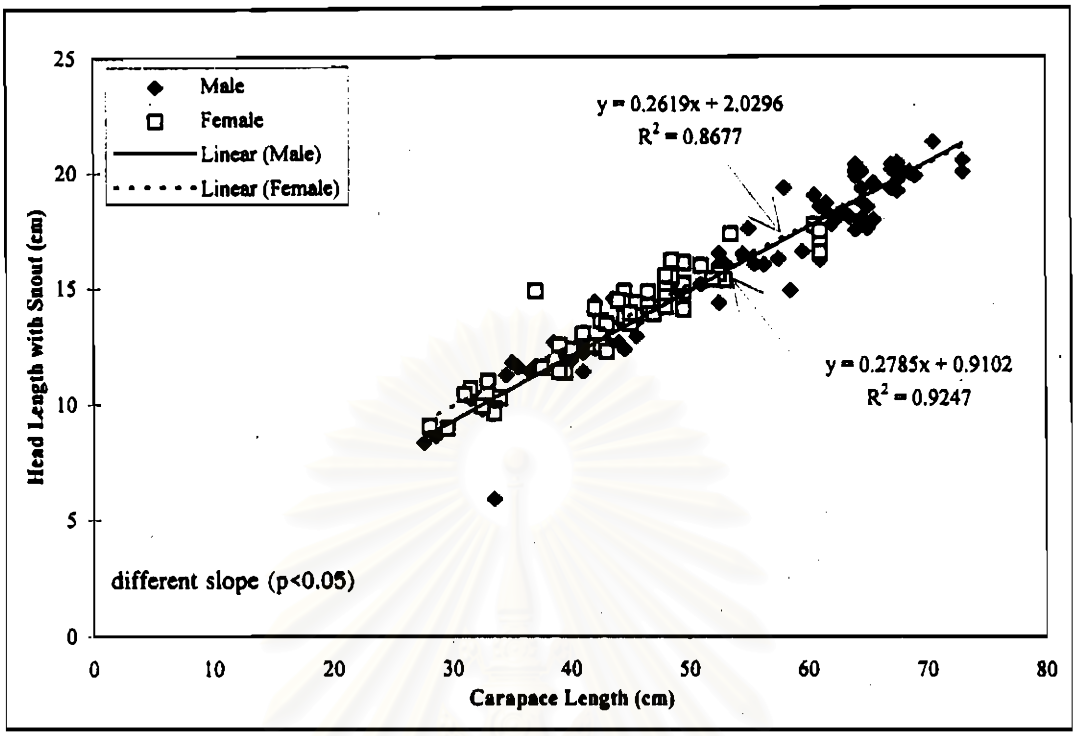

Figure 4–15 Linear regression lines relating head length with snout to carapace length of *Amyda cartilaginea* ($p<0.05$).

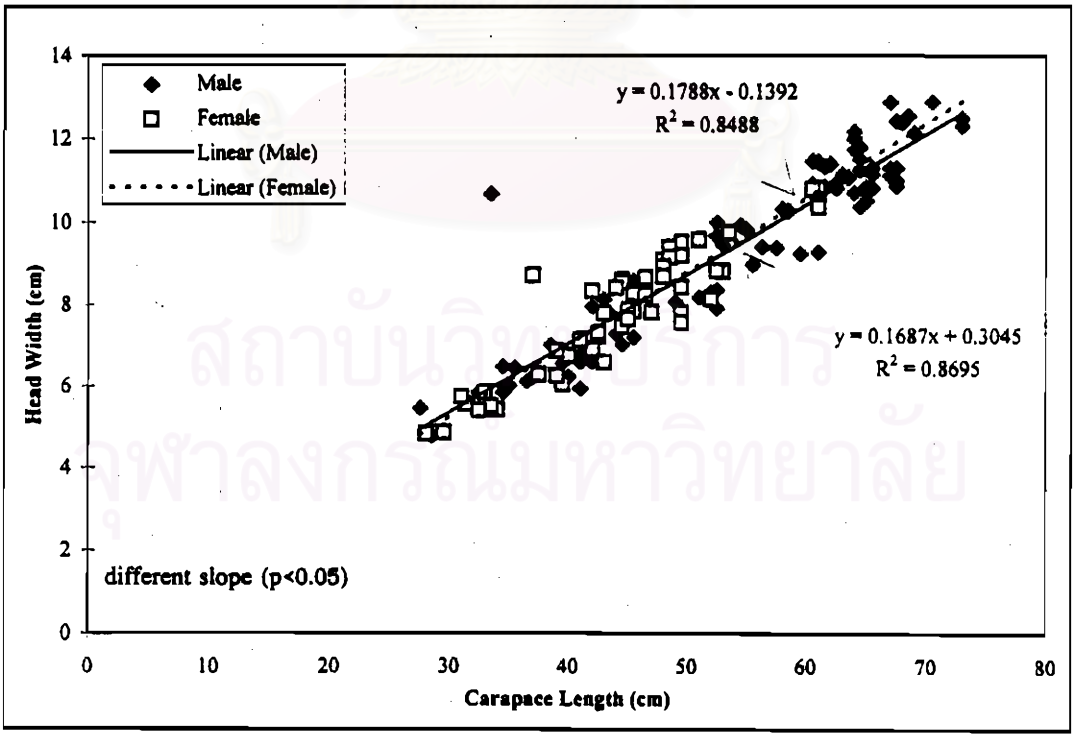

Figure 4–16 Linear regression lines relating head width to carapace length of *Amyda cartilaginea* ($p<0.05$).

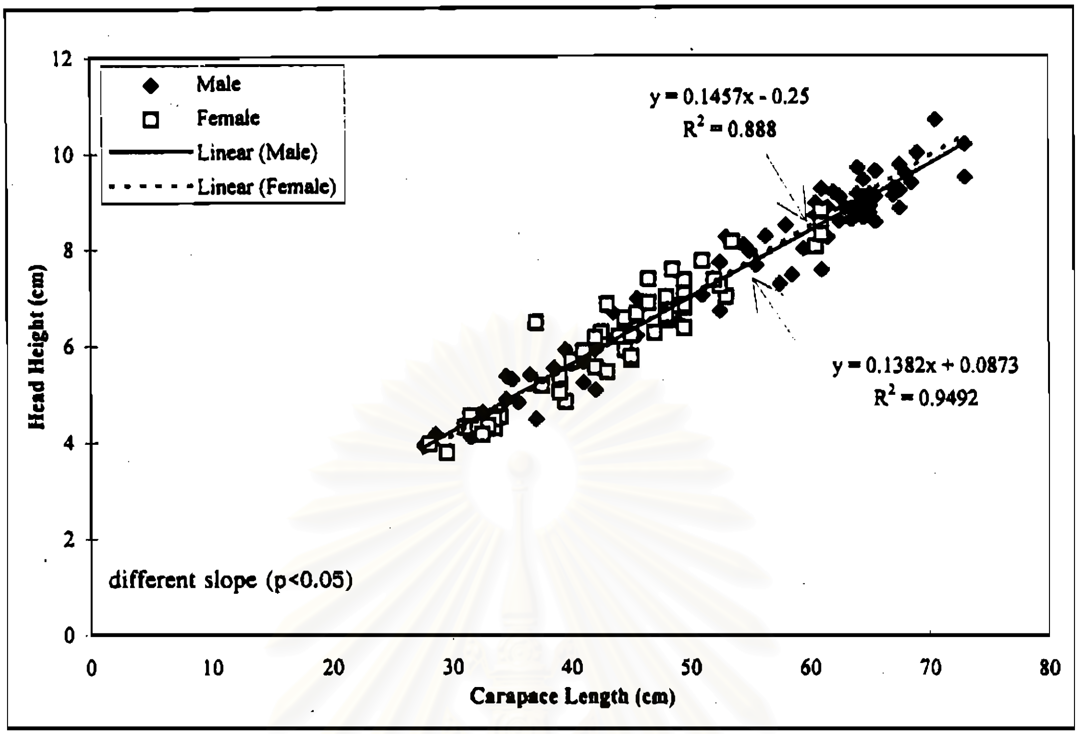

Figure 4-17 Linear regression lines relating head height to carapace length of *Amyda cartilaginea* ($p<0.05$).

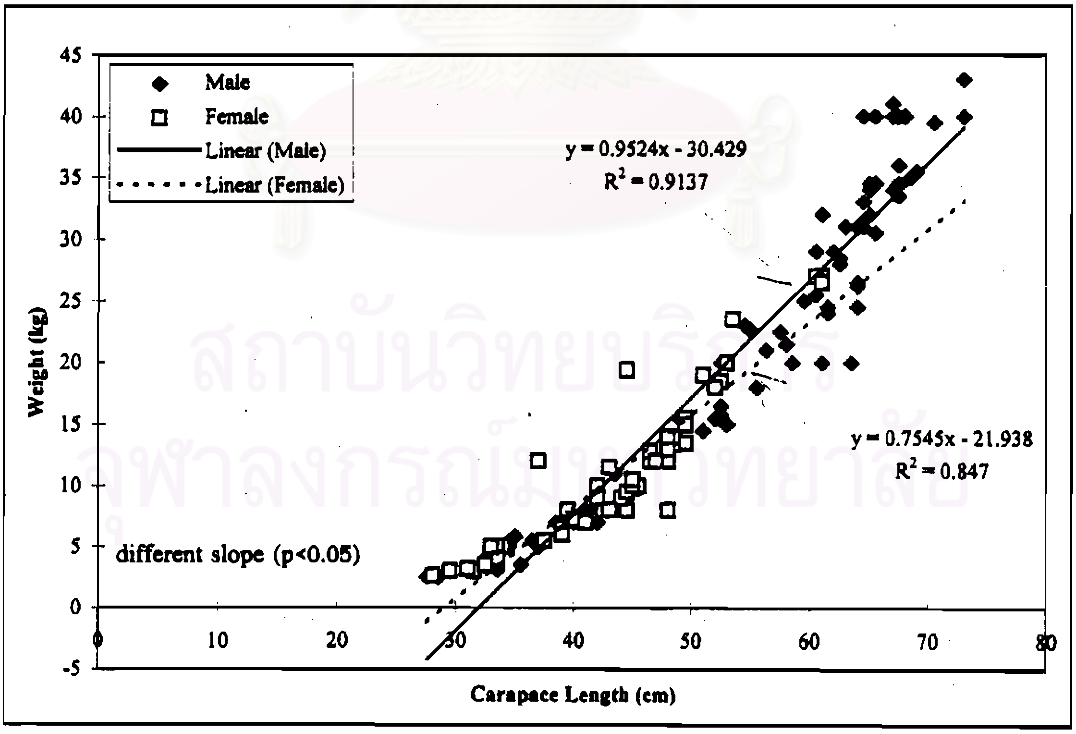

Figure 4-18 Linear regression lines relating weight to carapace length of *Amyda cartilaginea* ($p<0.05$).

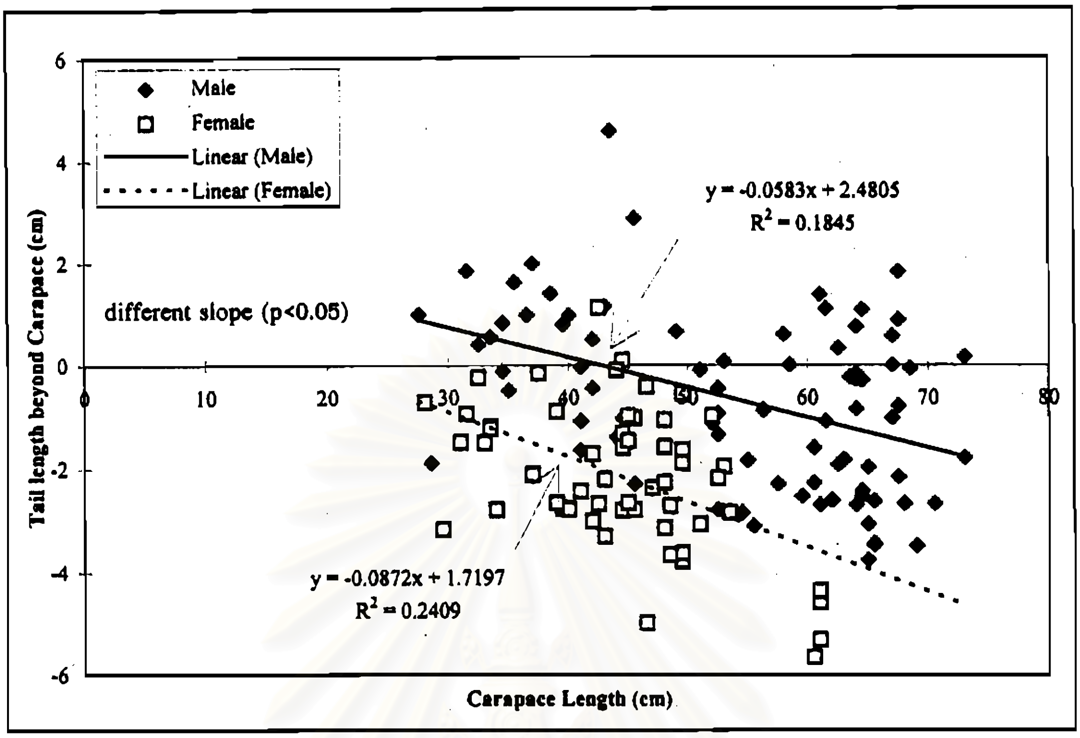

Figure 4–19 Linear regression lines relating tail length beyond carapace to carapace length of *Amyda cartilaginea* ($p<0.05$).

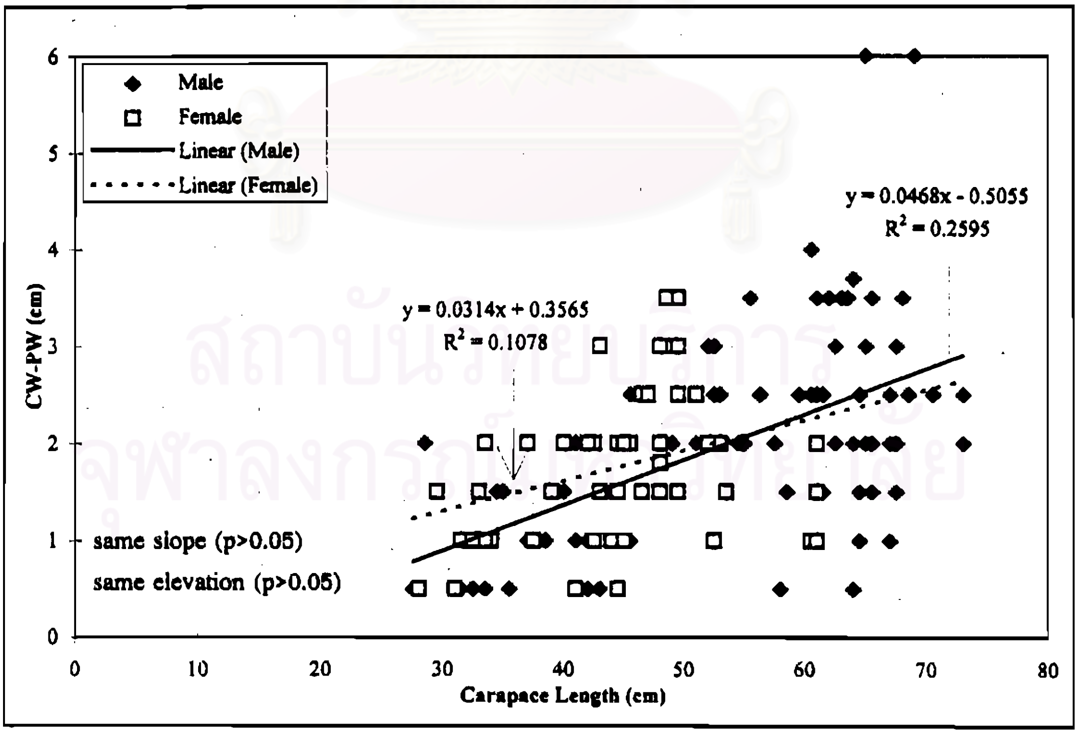

Figure 4–20 Linear regression lines relating CW-PW to carapace length of *Amyda cartilaginea* ($p<0.05$).

To investigate position of cloacal opening, tailbase to cloaca (TC) were also plotted against Tail length (TL). The regression analysis revealed a significant linear regression line ($p<0.05$) of this relationship. Comparison of slope and elevation of the regression lines indicated that the slopes were significantly different between sexes as shown in figure 4-21.

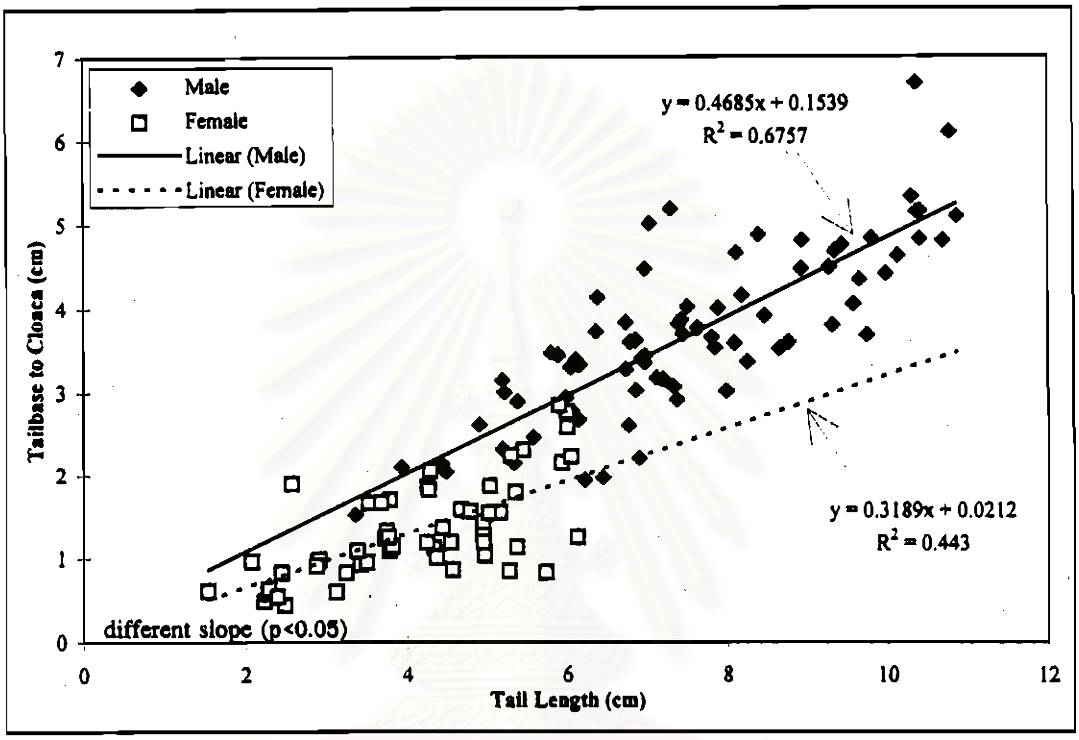

Figure 4-21 Linear regression lines relating tailbase to cloaca to tail length of *Amyda cartilaginea* ($p<0.05$).

Results from regression analysis and comparison of slope and elevation are summarized in table 4-2.

Table 4-2 Regression analysis of *Amyda cartilaginea* morphological characters and comparison of slope and elevation between sexes.

Sex	Linear regression equations	p	Significant difference in	
			Slope	Elevation
Male Female	BDL1 = 0.7226 CL - 3.4666 BDL1 = 0.7172 CL - 1.9421	0.000 0.000	× ($p>0.05$)	✓ ($p<0.05$)
Male Female	BDL2 = 0.6058 CL - 2.5055 BDL2 = 0.6125 CL - 1.3464	0.000 0.000	× ($p>0.05$)	× ($p>0.05$)
Male Female	CW = 0.607 CL + 5.9312 CW = 0.6088 CL + 5.1947	0.000 0.000	× ($p>0.05$)	× ($p>0.05$)
Male Female	PL1 = 0.5007 CL + 0.771 PL1 = 0.458 CL + 2.9394	0.000 0.000	✓ ($p<0.05$)	-

Table 4–2 (cont.) Regression analysis of *Amyda cartilaginea* morphological characters and comparison of slope and elevation between sexes.

Sex	Linear regression equations	p	Significant difference in	
			Slope	Elevation
Male Female	$PL2 = 0.6423\ CL + 1.157$ $PL2 = 0.6234\ CL + 2.3301$	0.000 0.000	✓ ($p<0.05$)	-
Male Female	$PW = 0.5756\ CL + 5.4693$ $PW = 0.5777\ CL + 4.8158$	0.000 0.000	✗ ($p>0.05$)	✓ ($p<0.05$)
Male Female	$H = 0.3091\ CL - 2.3383$ $H = 0.3235\ CL - 2.0249$	0.000 0.000	✓ ($p<0.05$)	-
Male Female	$PR = 0.4027\ CL - 1.0367$ $PR = 0.497\ CL - 5.3314$	0.000 0.000	✓ ($p<0.05$)	-
Male Female	$PC = 0.2685\ CL + 1.691$ $PC = 0.3306\ CL - 2.9448$	0.000 0.000	✓ ($p<0.05$)	-
Male Female	$TC = 0.036\ CL + 1.6988$ $TC = 0.0321\ CL - 0.0845$	0.000 0.001	✗ ($p>0.05$)	✓ ($p<0.05$)
Male Female	$CT = 0.0759\ CL - 0.2466$ $CT = 0.0871\ CL - 1.0625$	0.000 0.000	✓ ($p<0.05$)	-
Male Female	$TL = 0.1036\ CL + 1.8334$ $TL = 0.1107\ CL - 0.7835$	0.000 0.000	✗ ($p>0.05$)	✓ ($p<0.05$)
Male Female	$TW = 0.0773\ CL + 0.9726$ $TW = 0.0729\ CL + 0.2394$	0.000 0.000	✗ ($p>0.05$)	✓ ($p<0.05$)
Male Female	$HL = 0.251\ CL + 1.1408$ $HL = 0.2508\ CL + 1.2419$	0.000 0.000	✗ ($p>0.05$)	✓ ($p<0.05$)
Male Female	$HLs = 0.2785\ CL + 0.9102$ $HLs = 0.2619\ CL + 2.0296$	0.000 0.000	✓ ($p<0.05$)	-
Male Female	$HW = 0.1687\ CL + 0.3045$ $HW = 0.1788\ CL - 0.1392$	0.000 0.000	✓ ($p<0.05$)	-
Male Female	$HH = 0.1382\ CL + 0.0873$ $HH = 0.1457\ CL - 0.25$	0.000 0.000	✓ ($p<0.05$)	-
Male Female	$W = 0.9524\ CL - 30.429$ $W = 0.7545\ CL - 21.938$	0.000 0.000	✓ ($p<0.05$)	-
Male Female	$TBC = -0.0583\ CL + 2.4805$ $TBC = -0.0872\ CL + 1.7197$	0.000 0.001	✓ ($p<0.05$)	-
Male Female	$(CW-PW) = 0.0468CL-0.5055$ $(CW-PW) = 0.0314CL+0.3565$	0.024 0.015	✗ ($p>0.05$)	✗ ($p>0.05$)
Male Female	$TC = 0.4685\ TL + 0.1539$ $TC = 0.3189\ TL + 0.0212$	0.000 0.000	✓ ($p<0.05$)	-

Sexual dimorphism of *Amyda cartilaginea* was determined from both mean comparison of morphological characters and regression analysis of morphological characters relationships. Combination of the two analysis revealed significant difference between sexes in mean, or slope and elevation of regression line, for almost every morphological characters except carapace width (CW) and its transformed parameter (CW-PW). Thus it can be concluded that *Amyda cartilaginea* is sexually dimorphic.

The sexually dimorphic traits of softshell turtle could be divided into 3 groups according to degree of difference between sexes including male larger traits, female larger traits, and indistinctly different traits.

Male softshell turtles exhibited higher degree of plastron to cloaca (PC), tailbase to cloaca (TC), cloaca to tail tip (CT), tail length (TL), tail width (TW), tail length beyond carapace (TBC), cloaca position and weight (W). Except the weight, all parameters are related to tail size and position of cloacal opening which mainly involves in reproductive performance of softshell turtle. This is consistent with previous reports in many species of turtle including softshell turtle (Graham, 1979; Wirot Nutaphand, 1979; Gibbon and Lovich, 1990; van Dijk, 1992; Meylan, Moll and van Dijk, 1995). The longer, thicker tail and relative posterior position of cloacal opening might be benefitial to the males in order to extend the penis from cloacal opening to inseminate the females efficiently. Furthermore the longer and thicker tail could be useful to locate the female cloaca and assist in balancing during copulation.

Although the males showed higher degree of tail length beyond carapacial margin (TBC), plots of TBC against carapace length (CL) revealed that the tail length beyond carapacial margin is less obvious in the large male softshell turtle. Therefore the previous sexing technique indicating male from extension of tail beyond carapace (Anon Sirisuriyakamolchai, 1993; Sujin Nukwan, Panu Tavarutmaneegul and Anusin Inkuan, 1995) could lead to missidentify in large specimen. Hence other sexually dimorphic characters should be considered in addition to this criteria.

According to plots of weight (W) against carapace length (CL), the large softshell turtle seemed to exhibit much higher weight than linear proportion. Another regression models were tested and found that power and exponential regression lines also showed significant relationships ($p<0.05$). This might indicated the plentiful of food in this turtle pond. Therefore the higher weight of males could be related to higher number of large males instead of the sexual difference.

Female softshell turtles showed higher degree of bony disc length 1 (BDL1), bony disc length 2 (BDL2), plastron length 2 (PL2), height (H), head length without snout (HL), and head length with snout (HLs). These parameters are less conspicuously related to reproductive behavior like the males. Larger in bony disc length, plastron length and height, which are related to larger female size, might be accounted for benefits in increasing female fecundity such as capable of producing more eggs (Berry and Shine, 1980).

The larger in female head length which is related to head size of softshell turtles might be related to difference in microhabitat utilization in order to decrease intersexual competition for resource. The finding offer support for the competition avoidance hypothesis (Darwin, 1889; Slatkins, 1984; Shine, 1989, 1990).

The indistinctly different traits included plastron length 1 (PL1), plastron width (PW), plastron to rear margin of carapace (PR), head width (HW), and head height (HH). Regression analysis of these parameters suggested a sexual dimorphism of softshell turtles but the patterns of difference remained invivid.

Plastron length, plastron width and plastron to rear margin of carapace which are account for size of softshell turtles might be related either to female fecundity or male combat. Thus the study for sexual size dimorphism of softshell turtles should be investigated in order to assimilate the dimorphism.

Plots of head width and head height against carapace length revealed the significant difference in slope of the regression lines between sexes. This restated the difference in head size which might be related to difference in microhabitat usage to decrease intersexual competition for resource, and offer support for the competition avoidance hypothesis (Darwin, 1889; Slatkins, 1984; Shine, 1989, 1990).

4.2 Annual reproductive cycle

4.2.1 Male reproductive cycle

Mean steroids levels in each sample of male softshell turtles are shown in table 4-3 and figure 4-22.

Table 4–3 Mean steroids levels of male *Amyda cartilaginea* in each sample.

Sample	n	Mean ± S.E.M.		
		Testosterone (ng/ml)	**Estradiol (pg/ml)**	**Progesterone (ng/ml)**
1/2 October 1996	3	22.4842^{de} ± 2.3495	40.7296^{cd} ± 16.3395	0.3942^{c} ± 0.0906
2/2 Octobet 1996	-	-	-	-
1/2 November 1996	4	25.0340^{e} ± 2.9028	53.1612^{d} ± 3.6206	0.4888^{c} ± 0.1376
2/2 November 1996	6	16.9881^{bcde} ± 4.3699	32.2805^{bc} ± 3.1655	0.3499^{abc} ± 0.0939
1/2 December 1996	7	11.5782^{abc} ± 4.5941	36.1937^{cd} ± 9.5368	0.3263^{abc} ± 0.1122
2/2 December 1996	5	20.9805^{cde} ± 3.5287	40.5056^{cd} ± 6.6185	0.3446^{abc} ± 0.0480
January 1997	5	9.2848^{ab} ± 3.2832	38.9026^{cd} ± 13.0646	0.2286^{ab} ± 0.0732
1/2 February 1997	7	14.0072^{abcd} ± 3.0406	24.8409^{abc} ± 4.6282	0.2506^{ab} ± 0.0275
2/2 February 1997	7	9.5862^{ab} ± 3.4424	19.6181^{abc} ± 5.4018	0.1788^{ab} ± 0.0665
March 1997	6	7.6590^{ab} ± 2.0728	10.7294^{a} ± 2.4199	0.1771^{ab} ± 0.0269
April 1997	5	8.2008^{ab} ± 3.2975	24.8698^{abc} ± 5.7844	0.1671^{a} ± 0.0342
May 1997	5	9.7507^{ab} ± 3.0756	17.1941^{abc} ± 5.9587	0.1561^{a} ± 0.0199
June 1997	8	5.2029^{a} ± 1.1999	11.7681^{ab} ± 2.2272	0.1400^{a} ± 0.0323
July 1997	4	9.7254^{ab} ± 3.0688	14.5271^{ab} ± 2.2683	0.1829^{ab} ± 0.0619
August 1997	7	3.7492^{a} ± 0.9758	11.1681^{ab} ± 1.2685	0.1478^{a} ± 0.0240
September 1997	1	7.0349	16.3404	0.0818

Remark * Significant differences ($p<0.05$) among each sample are indicated by differences in superscript letter.

** There was no male in the second sample (2/2 October 1996) and only one male in the 16th sample (September 1997).

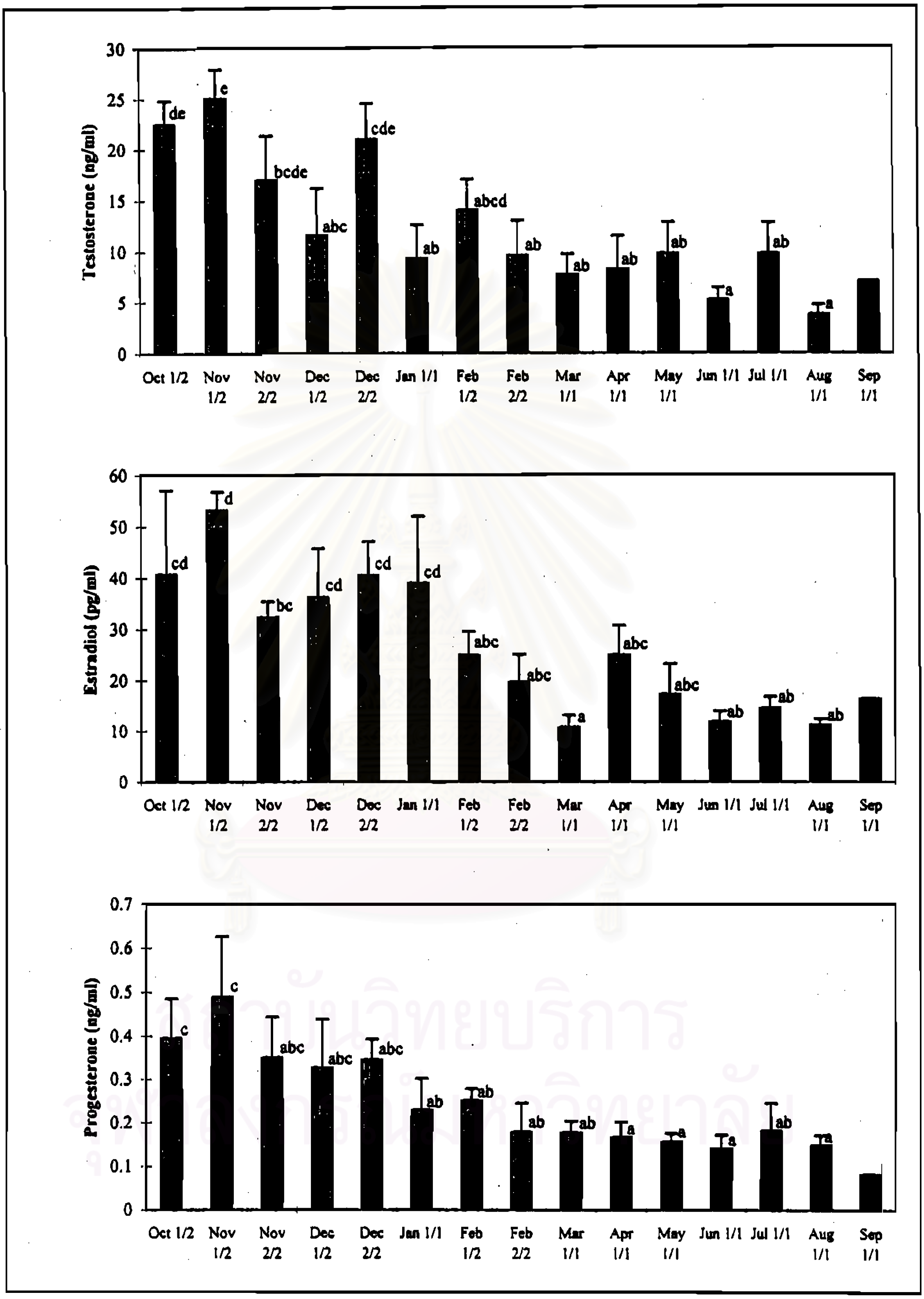

Figure 4–22 Plasma steroids profile of male *Amyda cartilaginea* in each sample. Error bar indicate standard error of the mean (S.E.M.). Difference in the above letter indicate significant difference (p<0.05) among samples.

According to hormonal profiles of male softshell turtles, significant difference ($p<0.05$) in means of the three plasma steroids were evident among samples suggesting temporal fluctuations of fertility year-round.

To monitor monthly changes of steroids levels, data of the two samples of October, November, December and February which were sampled twice in each month were combined and calculated for the new mean and S.E.M.. The monthly hormonal profiles of male softshell turtle are shown in table 4-4 and figure 4-23.

Table 4–4 Mean steroids levels of male *Amyda cartilaginea* in each month.

Month	**n**	**Mean ± S.E.M.**		
		Testosterone (ng/ml)	**Estradiol (pg/ml)**	**Progesterone (ng/ml)**
October 1996	3	22.4842^{d} ± 2.3495	40.7296^{b} ± 16.3395	0.3942^{b} ± 0.0906
November 1996	10	20.2065^{cd} ± 3.0357	40.6328^{b} ± 4.0884	0.4055^{b} ± 0.0773
December 1996	12	15.4958^{bcd} ± 3.2489	37.9903^{b} ± 5.9989	0.3346^{ab} ± 0.0621
January 1997	5	9.2848^{ab} ± 3.2832	38.9026^{b} ± 13.0646	0.2286^{ab} ± 0.0732
February 1997	14	11.6628^{abc} ± 2.2900	22.2295^{ab} ± 3.4931	0.2147^{ab} ± 0.0360
March 1997	6	7.6590^{ab} ± 2.0728	10.7294^{a} ± 2.4199	0.1771^{a} ± 0.0269
April 1997	5	8.2008^{ab} ± 3.2975	24.8698^{ab} ± 5.7844	0.1671^{a} ± 0.0342
May 1997	5	9.7507^{ab} ± 3.0756	17.1941^{ab} ± 5.9587	0.1561^{a} ± 0.0199
June 1997	8	5.2029^{ab} ± 1.1999	11.7681^{a} ± 2.2272	0.1400^{a} ± 0.0323
July 1997	4	9.7254^{ab} ± 3.0688	14.5271^{a} ± 2.2683	0.1829^{a} ± 0.0619
August 1997	7	3.7492^{a} ± 0.9758	11.1681^{a} ± 1.2685	0.1478^{a} ± 0.0240
September 1997	1	7.0349	16.3404	0.0818

Remark * Significant differences ($p<0.05$) among each month are indicated by differences in superscript letter.

** There was only one male in September 1997.

Nesting season of *A. cartilaginea* was reported to coincide with the dry and hot seasons in Thailand, from February to July with a peak in April (Wirot Nutaphand, 1979; Meylan, Moll and van Dijk, 1995). The nesting of softshell turtle population at Prayurawongsawas temple was found during March and May (Wichase Khonsue, 1993; Wachira Kitimasak, 1996). This nesting period was overlayed to the monthly steroids profile as shown in figure 4-23.

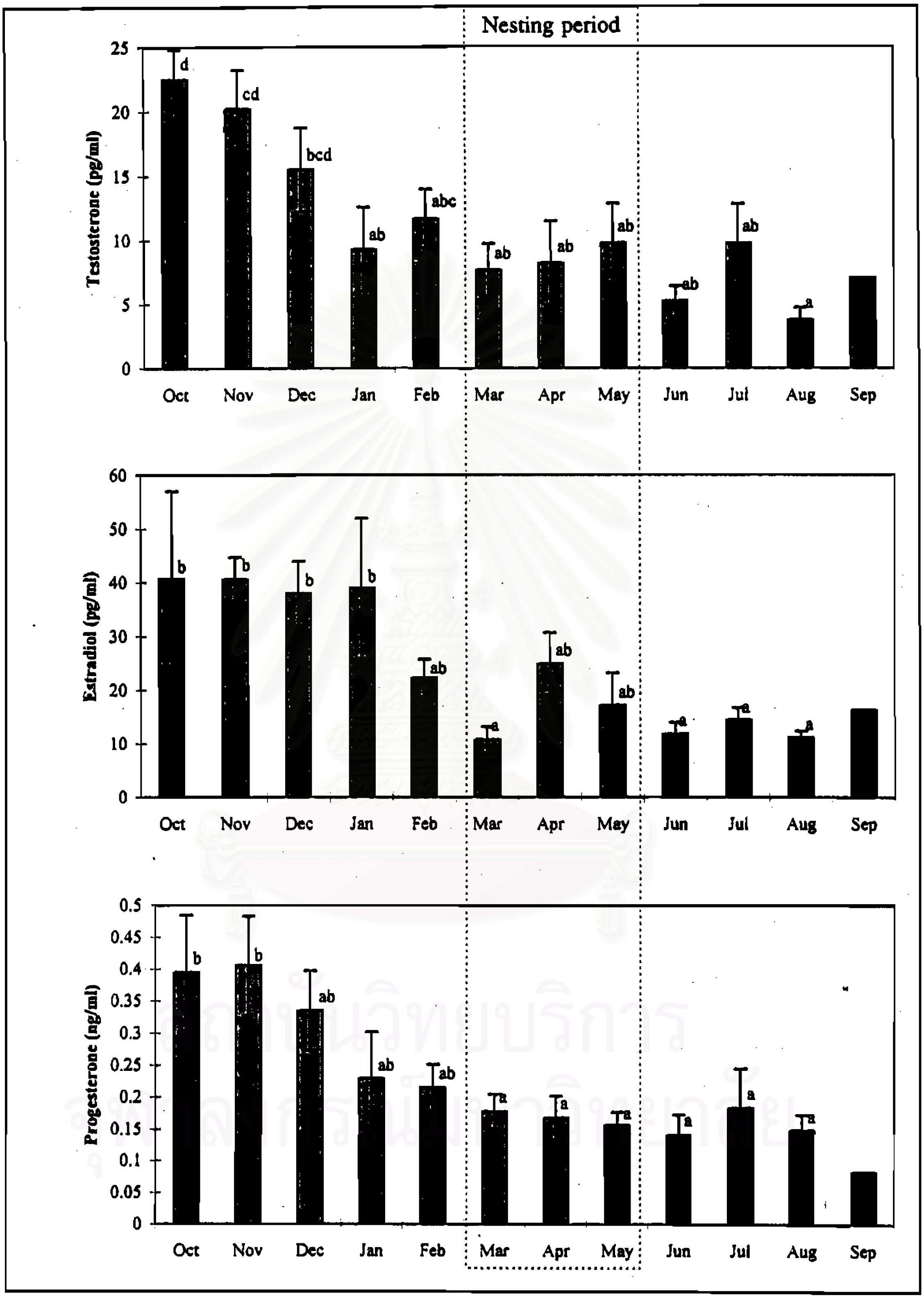

Figure 4-23 Monthly plasma steroids profile of male *Amyda cartilaginea*. Error bar indicate standard error of the mean (S.E.M.). Difference in the above letter indicate significant difference (p<0.05) among months.

Monthly steroids profile revealed that levels of plasma testosterone were high in October to December then gradually decreased to medium levels through February, and declined to almost constant low levels through the year. According to Licht (1982) and Loft (1987), testosterone was reported to play important roles in spermatogenesis and mating behavior in turtles. These high levels might be accounted for spermatogenesis while the medium levels could be related with mating behavior of *Amyda cartilaginea*.

Unlike other freshwater turtles living in temperate zones, *Amyda cartilaginea* exhibited high and medium levels of testosterone during the prenesting period. This is similar to the prenuptial peak of testosterone found in several species of sea turtles (Licht et al., 1979; Licht, Rainey and Cliffton, 1980; Licht, Wood and Wood, 1985; Wibbels, 1990; Rostal et al., 1998).

Although primary male sex steroid is testosterone, estradiol and progesterone also showed the detectable levels year-round. Levels of plasma estradiol were high in October through January then decreased to medium levels during February and May, and declined to relative constant low levels through the year. While levels of plasma progesterone were high in October and November then gradually decreased to medium levels in December to February and declined to nearly constant low levels through the year. Moreover, the result revealed that levels of plasma testosterone, estradiol and progesterone displayed significant correlation ($p<0.05$) among each others as shown in table 4-5.

According to Staub and De Beer (1997), the heterologous sex steroids (androgens in females and estrogens in males) play the roles in synthesis of, or aromatization to other steroid molecules. Estrogens in males are also found to be important in regulating seasonal cycles of testosterone, regulating spermatogenesis, and influencing behavior. In *Amyda cartilaginea*, levels of estradiol as well as its correlation with testosterone also offer support to the above statement. Hence estradiol in the males, though were much lower than in females, provided evidence for functions of heterologous sex steroids in reptile and should be further studied in more details.

Levels of progesterone, existed in less than 4 percent of testosterone, might be resulting from release of intermediate into the blood during the $\Delta 4$ pathway of testicular steroidogenesis (Johnson and Everitt, 1988).

Correlation of various climatic factors of Bangkok Metropolis area and levels of plasma sex steroids were analyzed and shown in table 4-5.

Table 4-5 Pearson's correlation coefficients relating climatic data of Bangkok Metropolis area and plasma sex steroid levels of male *Amyda cartilaginea*. Shaded cells indicate significant correlation ($p<0.05$).

	Max. temp.	Mean temp.	Min. temp.	Max. RH	Mean RH	Min. RH	Daily sunshine	Daily rainfall	T	E_2	P
Max. temp.	1.000										
Mean temp.	0.827	1.000									
Min. temp.	0.559	0.892	1.000								
Max. RH	-0.063	0.344	0.452	1.000							
Mean RH	-0.111	0.218	0.479	0.605	1.000						
Min. RH	0.033	0.311	0.501	0.380	0.749	1.000					
Daily sunshine	0.450	0.119	-0.176	-0.013	-0.408	-0.639	1.000				
Daily rainfall	-0.074	0.098	0.288	0.574	0.837	0.628	-0.367	1.000			
Testosterone	-0.552	-0.448	-0.372	-0.070	0.131	-0.113	-0.278	0.061	1.000		
Estradiol	-0.708	-0.732	-0.725	-0.127	-0.122	-0.297	-0.075	-0.062	0.820	1.000	
Progesterone	-0.621	-0.554	-0.514	-0.205	-0.059	-0.160	-0.310	-0.168	0.936	0.855	1.000

Estradiol profile showed significant correlation with minimum, mean and maximum temperature of Bangkok area ($p<0.05$), while plasma progesterone showed significant correlation with maximum temperature of Bangkok ($p<0.05$). The results are consistent with Whittier and Crews (1987) that photoperiod and temperature both serve as proximate cues for gonadal maturation.

Due to data availability, sunshine duration was used instead of photoperiod and showed no significant correlation with steroids level. The above result as well as the insignificant correlation of testosterone and climatic factors might suggest priority of endogenous over exogenous factors in controlling the reproductive cycle of the softshell turtle. This might be related to different control patterns of tropical organisms which are exposed to relative constant environment year-round.

4.2.2 Female reproductive cycle

Mean steroids levels in each sample of female softshell turtles are shown in table 4-6 and figure 4-24.

Table 4–6 Mean steroids levels of female *Amyda cartilaginea* in each sample.

Sample	**n**	**Mean ± S.E.M.**		
		Testosterone (ng/ml)	**Estradiol (pg/ml)**	**Progesterone (ng/ml)**
1/2 October 1996	5	0.2828^{a} ± 0.2373	207.957^{a} ± 56.107	2.6649^{e} ± 0.3622
2/2 Octobet 1996	5	0.1070^{a} ± 0.1153	127.090^{a} ± 7.0697	1.7587^{abcde} ± 0.6057
1/2 November 1996	2	0.0652^{a} ± 0.0269	215.513^{a} ± 67.468	2.5648^{de} ± 0.8863
2/2 November 1996	3	0.0661^{a} ± 0.0117	101.397^{a} ± 41.557	2.1737^{bcde} ± 0.5822
1/2 December 1996	4	0.0743^{a} ± 0.0100	240.930^{a} ± 70.341	2.4463^{cde} ± 0.4046
2/2 December 1996	4	0.0822^{a} ± 0.0200	187.088^{a} ± 56.075	2.2121^{bcde} ± 0.3060
January 1997	4	0.1250^{a} ± 0.0131	135.010^{a} ± 58.024	1.3112^{abc} ± 0.1506
1/2 February 1997	3	0.1551^{a} ± 0.0441	164.968^{a} ± 48.392	1.2569^{ab} ± 0.1038
2/2 February 1997	2	0.1699^{a} ± 0.0525	239.547^{a} ± 25.033	1.7154^{abcde} ± 0.3474
March 1997	3	0.2047^{a} ± 0.0733	112.685^{a} ± 16.218	1.1295^{ab} ± 0.0618
April 1997	4	0.0486^{a} ± 0.0170	90.075^{a} ± 32.762	1.4631^{abcd} ± 0.2639
May 1997	3	0.0411^{a} ± 0.0038	79.671^{a} ± 22.424	1.4834^{abcd} ± 0.3885
June 1997	2	0.1551^{a} ± 0.0420	70.817^{a} ± 34.298	0.9582^{a} ± 0.1431
July 1997	-	-	-	-
August 1997	3	0.1234^{a} ± 0.0578	77.587^{a} ± 17.346	0.8164^{a} ± 0.0999
September 1997	7	0.0646^{a} ± 0.0160	136.264^{a} ± 22.267	1.0686^{ab} ± 0.2139

<u>Remark</u> * Significant differences ($p<0.05$) among each sample are indicated by differences in superscript letter.
** There was no female in the 14th sample (July 1997).

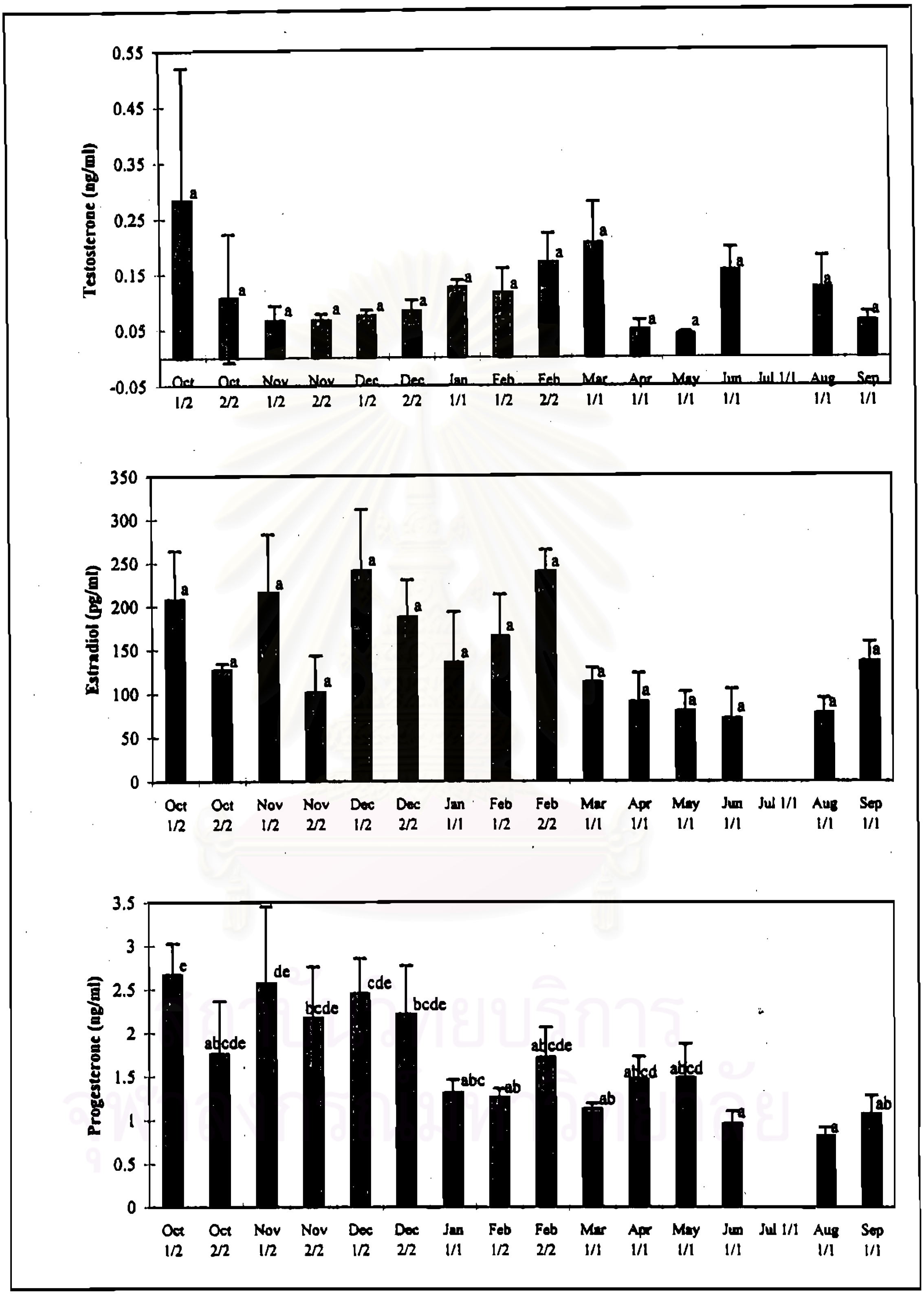

Figure 4-24 Plasma steroids profile of female *Amyda cartilaginea* in each sample. Error bar indicate standard error of the mean (S.E.M.). Difference in the above letter indicate significant difference ($p<0.05$) among samples.

It was found that significant difference ($p<0.05$) in mean of steroids among samples was evident only for progesterone, while testosterone and estradiol showed insignificant difference between samples. According to the sampling, there were only 7 out of 16 samples with more than 3 females while there were 13 samples with more than 3 males. Hence the low number of females as well as high individual variation could be accounted for these insignificant differences.

To monitor monthly changes of steroids levels, data of the two samples of October, November, December and February which were sampled twice in each month were combined and calculated for the new mean and S.E.M.. The monthly hormonal profiles of female softshell turtle were shown in table 4-7 and figure 4-25.

Table 4-7 Mean steroids levels of female *Amyda cartilaginea* in each month.

Month	n	Mean ± S.E.M.		
		Testosterone (ng/ml)	**Estradiol (pg/ml)**	**Progesterone (ng/ml)**
Octobet 1996	10	$0.1949^{a} \pm 0.1171$	$167.5233^{a} \pm 36.9667$	$2.1615^{bc} \pm 0.2256$
November 1996	5	$0.0658^{a} \pm 0.0106$	$147.0435^{a} \pm 41.8886$	$2.3301^{c} \pm 0.4352$
December 1996	8	$0.0782^{a} \pm 0.0104$	$214.0090^{a} \pm 42.8674$	$2.3459^{c} \pm 0.3060$
January 1997	4	$0.1250^{a} \pm 0.0131$	$135.010^{a} \pm 58.024$	$1.3112^{ab} \pm 0.1506$
February 1997	5	$0.1370^{a} \pm 0.0322$	$194.7999^{a} \pm 33.1499$	$1.4403^{abc} \pm 0.1671$
March 1997	3	$0.2047^{a} \pm 0.0733$	$112.685^{a} \pm 16.218$	$1.1295^{a} \pm 0.0618$
April 1997	4	$0.0486^{a} \pm 0.0170$	$90.075^{a} \pm 32.762$	$1.4631^{abc} \pm 0.2639$
May 1997	3	$0.0411^{a} \pm 0.0038$	$79.671^{a} \pm 22.424$	$1.4834^{abc} \pm 0.3885$
June 1997	2	$0.1551^{a} \pm 0.0420$	$70.817^{a} \pm 34.298$	$0.9582^{a} \pm 0.1431$
July 1997	-	-	-	-
August 1997	3	$0.1234^{a} \pm 0.0578$	$77.587^{a} \pm 17.346$	$0.8164^{a} \pm 0.0999$
September 1997	7	$0.0646^{a} \pm 0.0160$	$136.264^{a} \pm 22.267$	$1.0686^{a} \pm 0.2139$

Remark * Significant differences ($p<0.05$) among each month are indicated by differences in superscript letter.
** There was no female in July 1997.

The nesting period of softshell turtle population at Prayurawongsawas temple was overlayed to the monthly steroids profile as shown in figure 4-25.

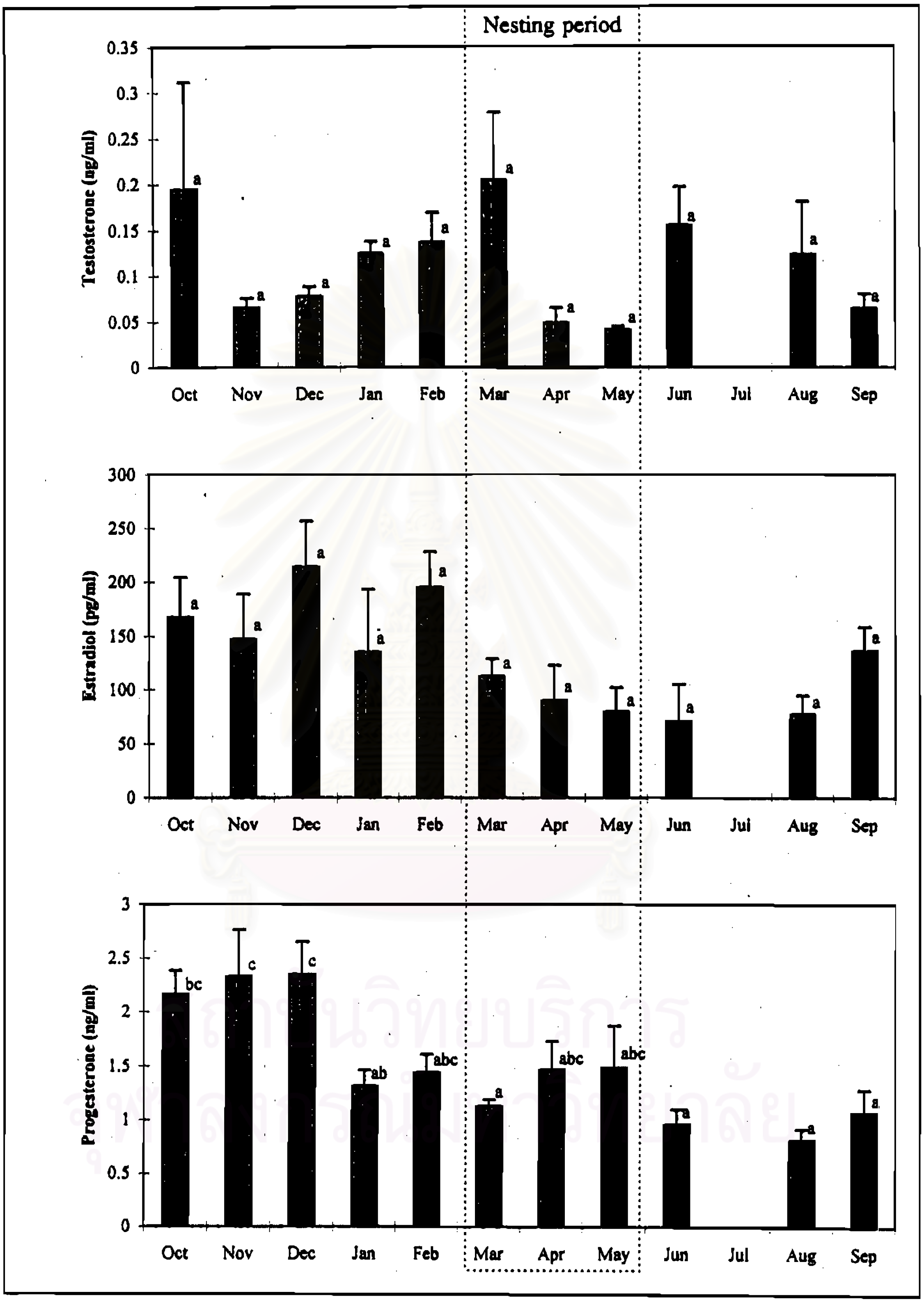

Figure 4–25 Monthly plasma steroids profile of female *Amyda cartilaginea*. Error bar indicate standard error of the mean (S.E.M.). Difference in the above letter indicate significant difference ($p<0.05$) among months.

Only progesterone profile was found to exhibit significant difference among months. Insignificant differences of testosterone and estradiol profiles are likely to be resulting from the low number of females and the high individual variation of *Amyda cartilaginea*. Therefore, reproductive cycle of the softshell turtle was considered from trends of testosterone and estradiol fluctuations instead.

Estradiol levels showed gradual rise from September through March with relative peak in December and February. While plasma progesterone levels were high during October through December then decreased to medium levels through May and declined to relative low levels through the year. It was also found that levels of the two primary female sex steroids showed significant correlation ($p<0.05$) with each other as presented in table 4-8. According to the previous studies in turtles, estradiol was reported to play important roles in vitellogenesis while progesterone play important roles in oocyte maturation and ovulatory process (Licht, 1982; Chieffi and Pierantoni, 1987; Ho, 1987; Nagahama, 1987; Xavier, 1987). The high levels of estradiol during December and February might be related to the prenesting vitellogenesis. While high levels of progesterone during October and December and medium levels through May might indicated period of oocyte maturation and ovulation of the softshell turtles.

In contrast to freshwater turtles living in temperate regions, *Amyda cartilaginea* displayed high levels of these two steroids in accordance with the nesting season of this population which was reported to happen during March and May (Wichase Khonsue, 1993; Wachira Kitimasak, 1996). This agrees with the prenuptial rise of estradiol and progesterone found in several species of sea turtles by Licht et al. (1979), Licht, Rainey and Cliffton (1980), Wibbels (1990), Rostal et al. (1997) and Rostal et al. (1998).

Levels of testosterone were not significantly different among months. Although the testosterone levels in the females are much lower than in the males, the detectable levels year-round provide evidence for functions of heterologous sex steroids as reviewed by Staub and De Beer (1997) that androgens play a significant role in normal female development. Furthermore, role of testosterone on female reproductive cycle was also suggested by Licht (1982) and Chieffi and Pierantoni (1987). This suggested that the functions of heterologous sex steroids in reptiles should be further studied in more details.

Correlation of climatic factors of Bangkok Matropolis area and levels of plasma sex steroids were analyzed and displayed in table 4-8.

Table 4-8 Pearson's correlation coefficients relating climatic data of Bangkok Metropolis area and plasma sex steroid levels of female *Amyda cartilaginea*. Shaded cells indicate significant correlation ($p<0.05$).

	Max. temp.	**Mean temp.**	**Min. temp.**	**Max. RH**	**Mean RH**	**Min. RH**	**Daily sunshine**	**Daily rainfall**	**T**	**E_2**	**P**
Max. temp.	1.000										
Mean temp.	0.827	1.000									
Min. temp.	0.559	0.892	1.000								
Max. RH	-0.063	0.344	0.452	1.000							
Mean RH	-0.111	0.218	0.479	0.605	1.000						
Min. RH	0.033	0.311	0.501	0.380	0.749	1.000					
Daily sunshine	0.450	0.119	-0.176	-0.013	-0.408	-0.639	1.000				
Daily rainfall	-0.074	0.098	0.288	0.574	0.837	0.628	-0.367	1.000			
Testosterone	-0.074	0.011	-0.038	0.112	0.111	-0.079	-0.003	-0.198	1.000		
Estradiol	-0.660	-0.775	-0.657	-0.357	-0.150	-0.488	-0.165	-0.053	0.080	1.000	
Progesterone	-0.471	-0.517	-0.508	-0.376	-0.115	-0.213	-0.351	-0.100	-0.185	0.676	1.000

Only estradiol profile showed significant correlation with minimum, mean and maximum temperature of Bangkok area ($p<0.05$). The finding that temperature serve as succeeding cues for gonadal maturation and reproductive cycle is consistent with the previous review by Whittier and Crews (1987).

An insignificant correlation between sunshine duration and steroids levels as well as the insignificant correlations of testosterone and progesterone, and climatic factors might suggest priority of endogenous over exogenous mechanisms in controlling the reproductive cycle of the softshell turtle. This might be related to different control patterns of tropical organisms which are exposed to relative constant environment through the year.

Changes in fertility year-round in terms of plasma sex steroids profiles of the males and the females indicate a seasonal reproductive cycle of softshell turtle. Unlike freshwater living in the temperate environment, the results suggest that *Amyda cartilaginea* might exhibit a prenuptial reproductive cycle resulted in rising of plasma sex steroids prior to mating season and stable low levels in other period of the year as found in sea turtles of Family Cheloniidae and Family Dermochelyidae living in the tropical environments (Licht, Wood and Wood, 1985; Rostal et al., 1996; Rostal et al., 1998). Furthermore, the data provides evidence in agreement with Licht (1982) that an associated reproductive pattern also appears in turtles as well as a dissociated reproductive pattern which is mainly found in freshwater turtles living in the temperate zones.

According to the present study, the high levels of plasma sex steroids, indicating high gonadal activity, were found during winter while mating and nesting periods were evident in summer. This provided an appropriate environment for the softshell turtle offspring that after a period for incubation, the hatchling would born during rainy season which is the most fertile period of the tropical region. This offer support to Whittier and Crews (1987) that timing of reproduction in a population is determined by 1) when the most offspring survive and 2) when parents are capable of energetically supporting the production of viable young at the least cost to themselves.

In order to certify the findings, annual reproductive cycle of softshell turtles should be conducted by other means of reproductive indicators such as changes in level of vitellogenin, plasma gonadotropins, and gonadal development including spermatogenesis and follicular development.

The high individual variation in plasma sex steroids levels which was evident in the softshell turtle could be beneficial for an artificial selection of breeder males and females which is important for economic animal development programs. Moreover, the finding that endogenous factors might dominate over exogenous factors in controlling the reproductive cycle suggest a possibility to enhance fertility of the softshell turtle by mean of hormonal stimulation. This could be usefully applied to both economic animal development programs as well as endangered turtles recovery programs.