CHAPTER IV

RESULTS

Sexual dimorphism

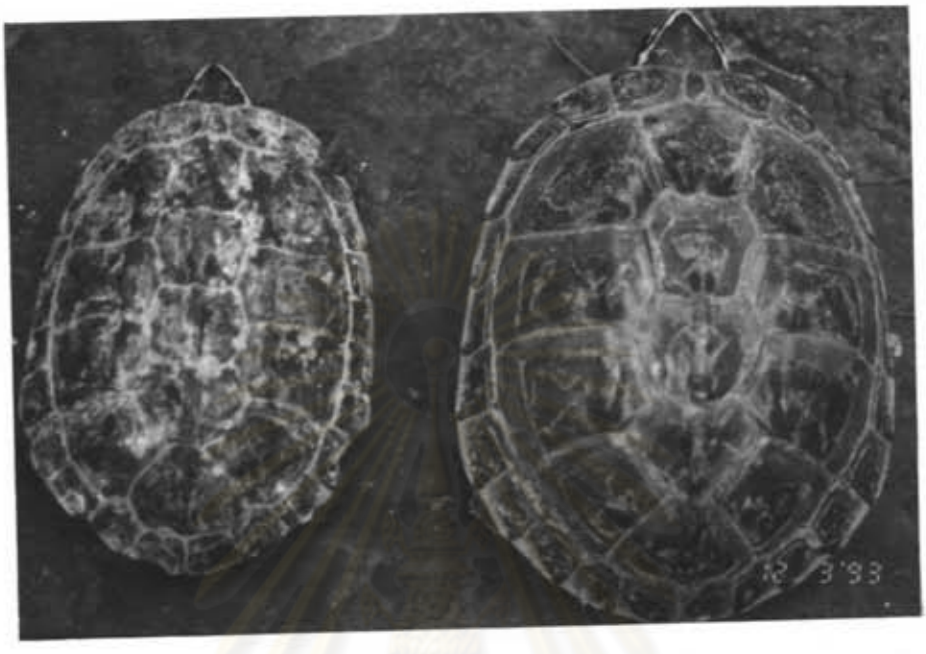
1. Mean shell morphology and tail width

It was found that means of carapace length (CL), carapace width (CW), plastron length (PL), height (Ht), and midline length of: gular (G), humeral (H), pectoral (P), abdominal (Ab), femoral (F), and anal (A) of adult male turtles were significantly less than that of female turtles (t-test $p<.05$). However, the mean tail width of males was significantly greater than that of female (t-test,$p<.05$). Results are demonstrated in table 4-5 and figure 15.

2. Mean ratios of shell morphology and tail width and carapace length

Table 6 shows that mean ratios of CW/CL, PL/CL, Ht/CL, H/CL, P/CL, Ab/CL, and A/CL of male turtles were significantly less than that of female turtles, whereas mean ratio of TW/CL of males was significantly greater than that of females. Mean ratios of G/CL and F/CL between sexes were not significantly different (t-test, $p>.05$).

a



Male Female

b

Figure 15. Aduilt male and adult female of *M. subtrijuga* (a) male has more slender carapace shape than female. (b) male has thicker tail than female.

Table 4. Ranges and means of carapace length,carapace width, plastron length, height, and tail width of adult *M. subtrijuga* found in this study. Differences in superscript letters between males (n=14) and females (n=25) indicate that there is significant difference at $p<.05$.

Morphological characters	Range (mm)	Mean ±SD (mm)
Carapace length		
Male	100.75-133.00	112.20 ± 09.83^{a}
Female	116.50-220.00	155.48 ± 27.91^{b}
Carapace width		
Male	74.00- 99.00	80.04 ± 07.41^{a}
Female	87.00-167.00	118.66 ± 22.29^{b}
Plastron length		
Male	82.00-111.00	90.13 ± 09.34^{a}
Female	102.50-188.00	137.78 ± 24.43^{b}
Height		
Male	41.00- 56.50	44.75 ± 05.17^{a}
Female	47.75- 88.00	67.14 ± 11.62^{b}
Tail width		
Male	15.00- 21.00	19.18 ± 01.80^{a}
Female	11.00- 20.00	15.06 ± 02.43^{b}

Table 5. Ranges and means of gular, humeral, pectoral, abdominal, femoral, and anal scute sizes of adult *M. subtrijuga* found in this study. Differences in superscript letters between males (n=14) and females (n=25) indicate that there is significant difference at $p<.05$.

Midline length of plastron scutes	Range (mm)	Mean ± SD (mm)
Gular		
Male	08.00-13.00	10.20 ± 01.29^{a}
Female	11.00-23.00	15.90 ± 03.13^{b}
Humeral		
Male	10.00-20.00	13.05 ± 03.00^{a}
Female	13.50-28.00	19.03 ± 03.70^{b}
Pectoral		
Male	08.50-15.25	10.84 ± 01.77^{a}
Female	10.50-32.00	18.08 ± 04.34^{b}
Abdominal		
Male	19.50-30.00	23.70 ± 03.14^{a}
Female	24.50-48.50	36.83 ± 07.65^{b}
Femoral		
Male	14.00-19.50	16.09 ± 01.56^{a}
Female	17.00-29.00	22.72 ± 03.23^{b}
Anal		
Male	12.00-17.00	13.71 ± 01.74^{a}
Female	15.75-32.00	21.18 ± 04.37^{b}

Table 6. Mean ratios of various morphological characters to carapace length of *M. subtrijuga*. Differences in superscript letters between males (n=30) and females (n=30) indicate that there is significant difference at $p < .05$.

	Males (n=30) Mean ± SD	Females (n=30) Mean ± SD
CW/CL	0.73 ± 0.03^a	0.77 ± 0.03^b
PL/CL	0.83 ± 0.04^a	0.88 ± 0.03^b
Ht/CL	0.42 ± 0.03^a	0.44 ± 0.03^b
TW/CL	0.19 ± 0.02^a	0.10 ± 0.10^b
G/CL	0.10 ± 1.12^a	0.10 ± 0.01^a
H/CL	0.11 ± 0.02^a	0.12 ± 0.01^b
P/CL	0.11 ± 0.02^a	0.12 ± 0.01^b
Ab/CL	0.21 ± 0.02^a	0.23 ± 0.02^b
F/CL	0.15 ± 0.01^a	0.15 ± 0.01^a
A/CL	0.13 ± 0.01^a	0.14 ± 0.01^b

Abbreviation : CW = Carapace width, CL = Carapace length, PL = Plastron length, Ht = Height, TW = Tail width, G = Gular, H = Humeral, P = Pectoral, Ab = Abdominal, F = Femoral, A = Anal, n = no. of turtles

3. Correlation and regression analysis of shell morphology and tail width

For both sexes, CL was significantly correlated with CW, PL, Ht, H, P, Ab, F, A, and TW (Pearson correlated test, T-test, $p<.05$). Results are shown in table 7. When using CL as indepedent variable and CW, PL, Ht, G, H, P, Ab, F, A, and TW as dependent variables, analysis of variance indicated that regression equations of both males and females were linear ($p<.05$). The 95% confidence interval for slopes of each sex were shown in table 8. The slope of regression equations between sexes were significantly different (ANOVA, $p<.05$), indicating that *M. subtrijuga* is sexually dimorphic (Table 8., Figures 16 a-j).

Table 7. Pearson correlation coefficient (r) between each of the morphological characters and carapace length in different sexes of *M. subtrijuga*. Each correlation coefficient is significant at $p<.05$. n is the number of turtles.

		r	
		Males (n=30)	Females (n=30)
Carapace width	vs Carapace length	0.95	0.99
Plastron length	vs Carapace length	0.93	0.98
Height	vs Carapace length	0.88	0.96
Gular	vs Carapace length	0.59	0.90
Humeral	vs Carapace length	0.71	0.90
Pectoral	vs Carapace length	0.43	0.87
Abdominal	vs Carapace length	0.89	0.94
Femoral	vs Carapace length	0.76	0.92
Anal	vs Carapace length	0.80	0.94
Tail width	vs Carapace length	0.49	0.85

Table 8. Regression equations between each of the morphological characters and carapace length in different sexes of *M. subtrijuga*. Differences in superscript letters between males and females indicate that there is significant difference in slopes of regression equations at $p < .05$. Each regression equation is significant at $p < .05$.

Sex	Regression equation	p	95% Confidence interval for slope
Male	CW = 0.61 CL + 12.00[a]	0.0000	0.53-0.69
Female	CW = 0.74 CL + 03.41[b]	0.0000	0.70-0.76
Male	PL = 0.69 CL + 14.15[a]	0.0000	0.58-0.79
Female	PL = 0.91 CL - 03.85[b]	0.0000	0.83-0.98
Male	Ht = 0.30 CL + 11.47[a]	0.0000	0.24-0.37
Female	Ht = 0.39 CL + 06.75[a]	0.0000	0.34-0.43
Male	TW = 0.07 CL + 11.98[a]	0.0064	0.02-0.11
Female	TW = 0.07 CL + 03.54[a]	0.0000	0.06-0.09
Male	G = 0.05 CL + 05.14[a]	0.0008	0.03-0.07
Female	G = 0.09 CL + 01.31[b]	0.0000	0.07-0.11
Male	H = 0.15 CL - 03.52[a]	0.0001	0.09-0.21
Female	H = 0.11 CL + 01.50[a]	0.0000	0.09-0.13
Male	P = 0.06 CL + 04.58[a]	0.0166	0.01-0.11
Female	P = 0.13 CL - 01.52[b]	0.0000	0.10-0.15
Male	Ab = 0.25 CL - 04.33[a]	0.0000	0.20-0.30
Female	Ab = 0.26 CL - 04.17[a]	0.0000	0.23-0.29
Male	F = 0.10 CL + 04.65[a]	0.0000	0.07-0.14
Female	F = 0.12 CL + 04.17[a]	0.0000	0.10-0.14
Male	A = 0.11 CL + 01.56[a]	0.0000	0.08-0.14
Female	A = 0.14 CL - 00.04[a]	0.0000	0.12-0.16

Abbreviation : CL = Carapace length
CW = Carapace width
PL = Plastron length
Ht = Height
G = Gular
H = Humeral
P = Pectoral
Ab = Abdominal
F = Femeral
A = Anal

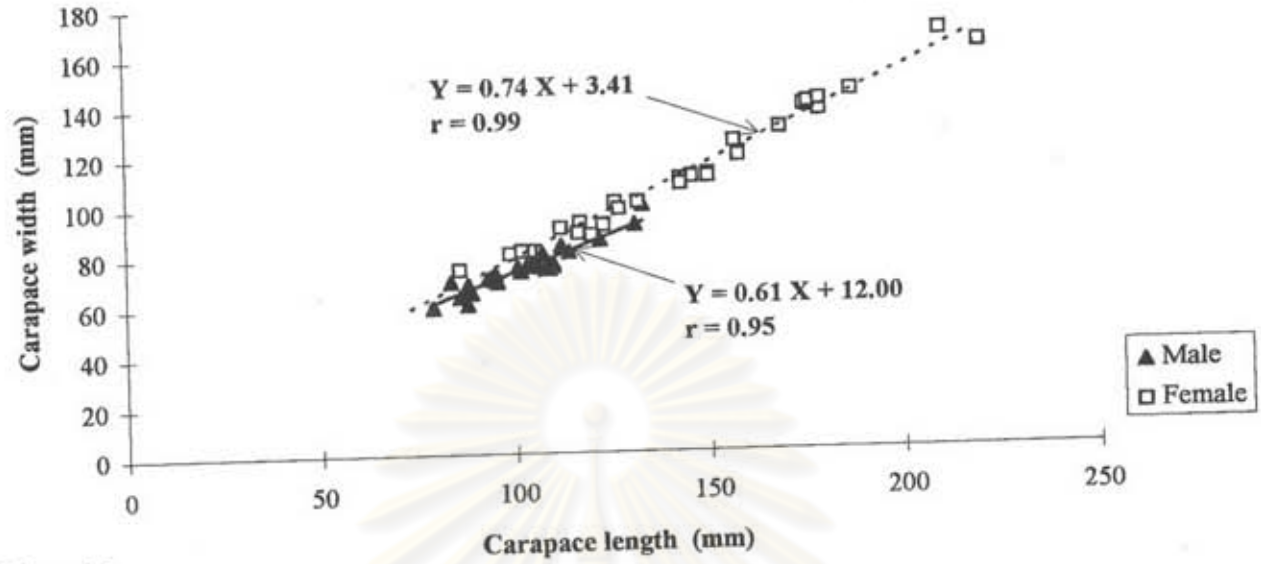


Figure 16a.

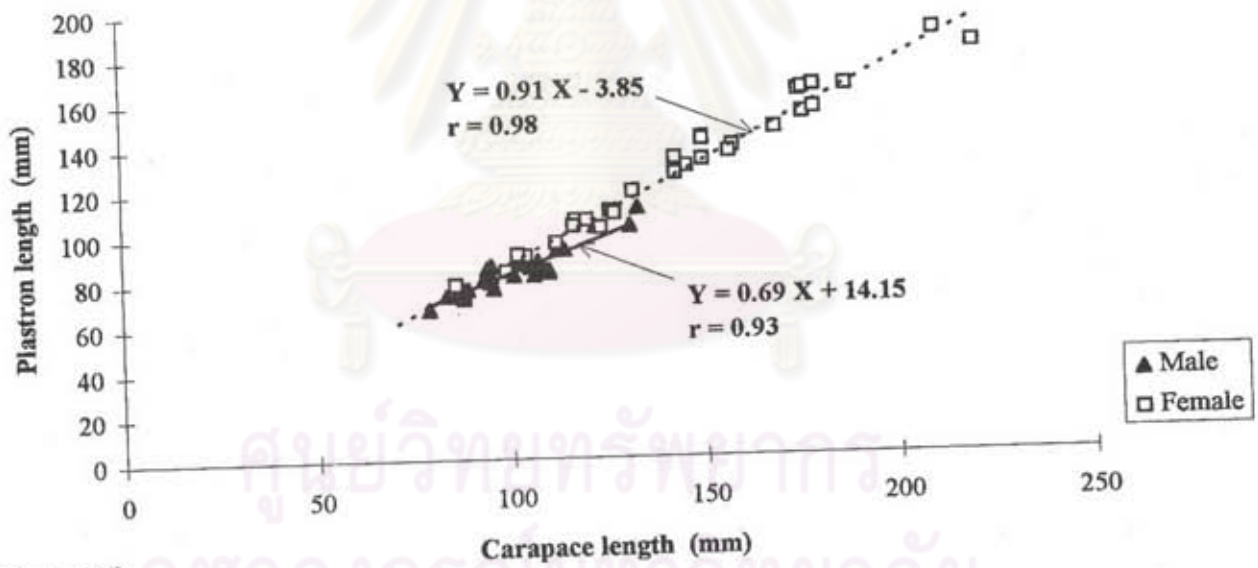


Figure 16b.

Figure 16. Linear regression equations of the relationship between (a) carapace width and carapace length (b) plastron length and carapace length in both sexes of *M. subtrijuga*

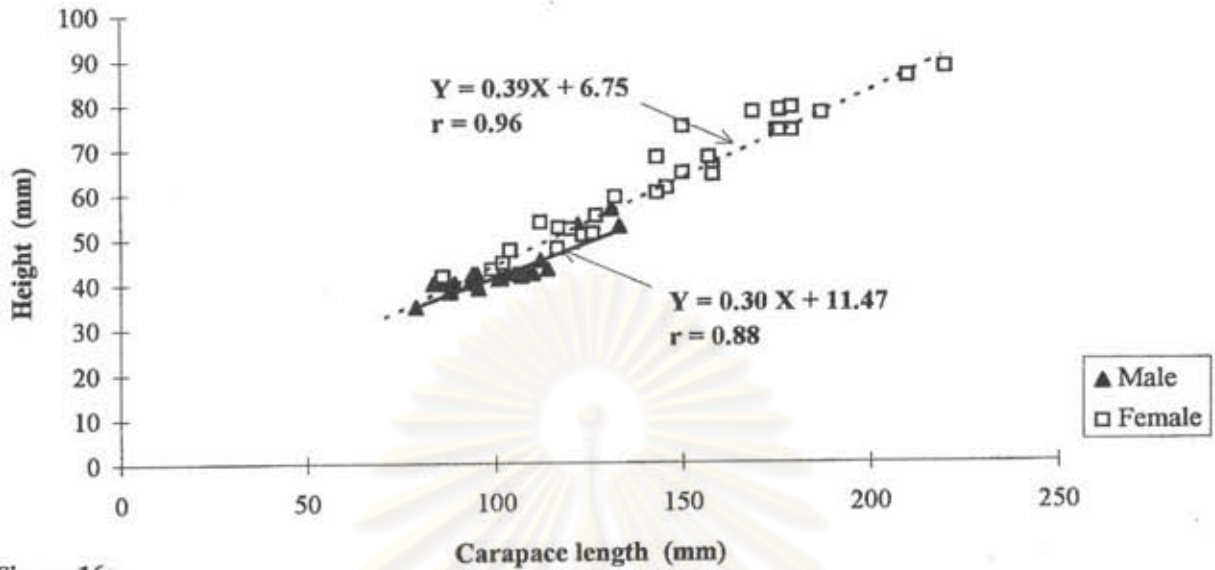


Figure 16c.

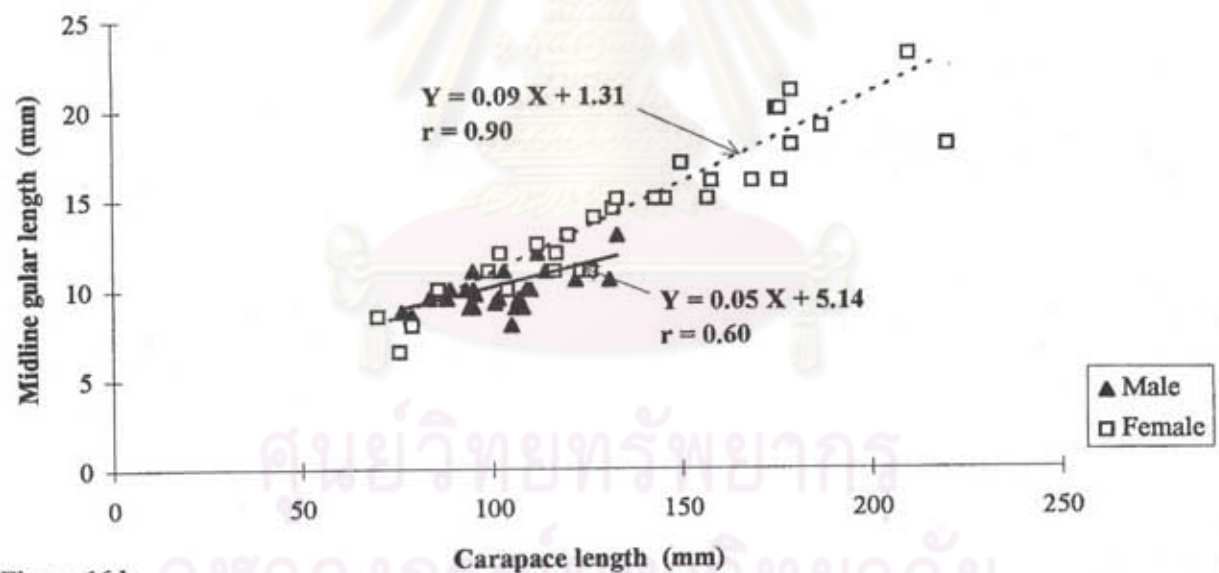


Figure 16d.

Figure 16. Linear regression equations of the relationship (c) height and carapace length (d) midline gular length and carapace length in both sexes of *M. subtrijuga*

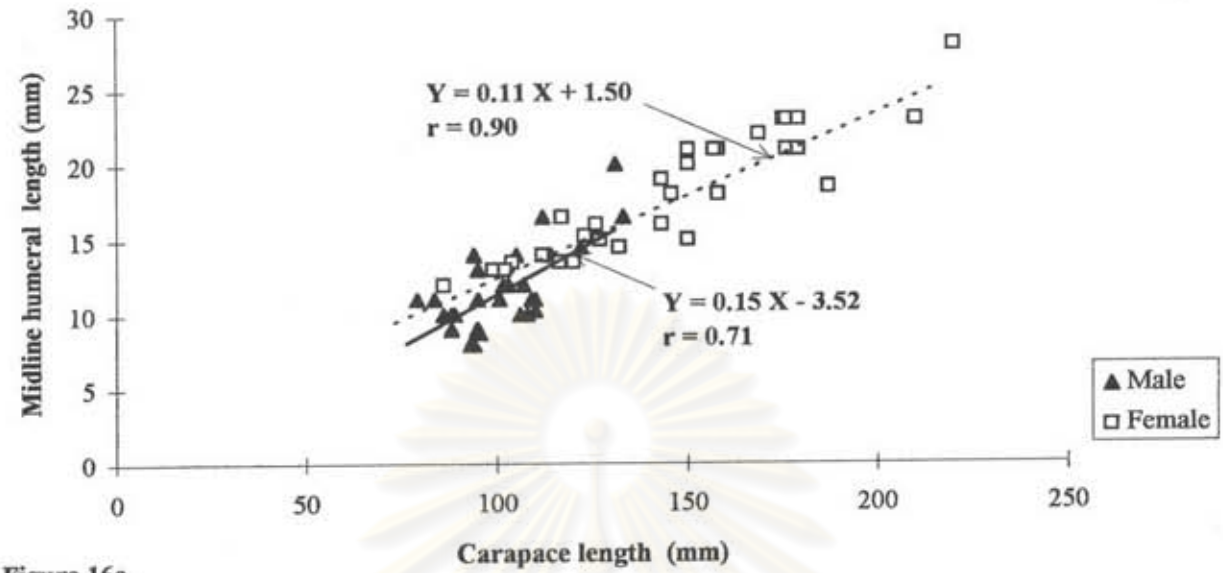


Figure 16e.

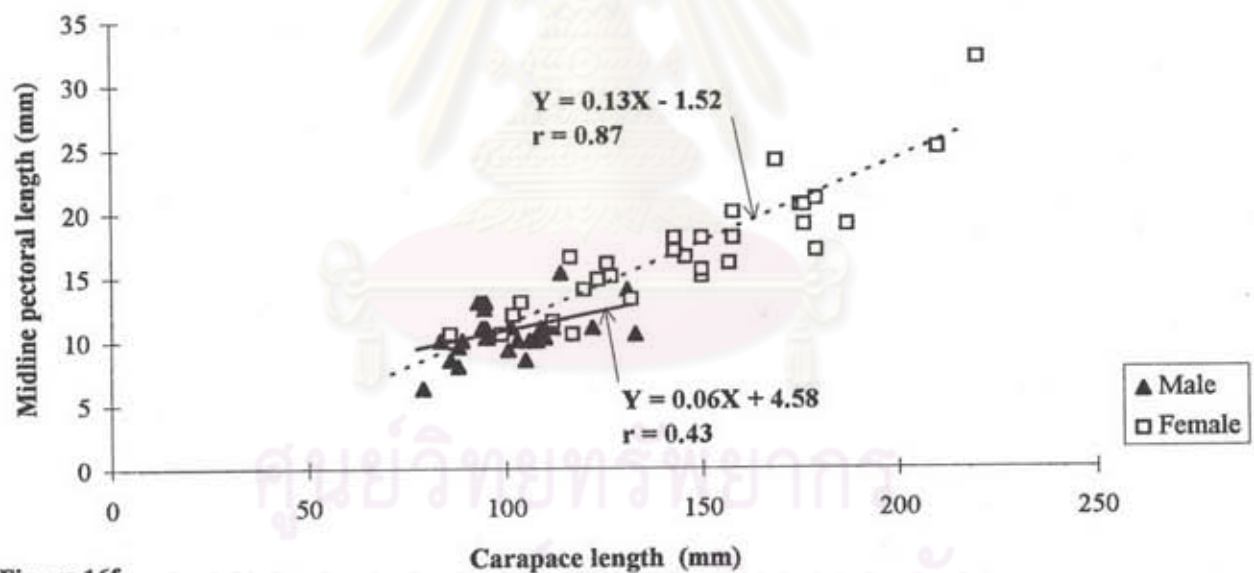


Figure 16f.

Figure 16. Linear regression equations of the relationship (e) midline humeral length and carapace length (f) midline pectoral length and carapace length in both sexes of *M. subtrijuga*

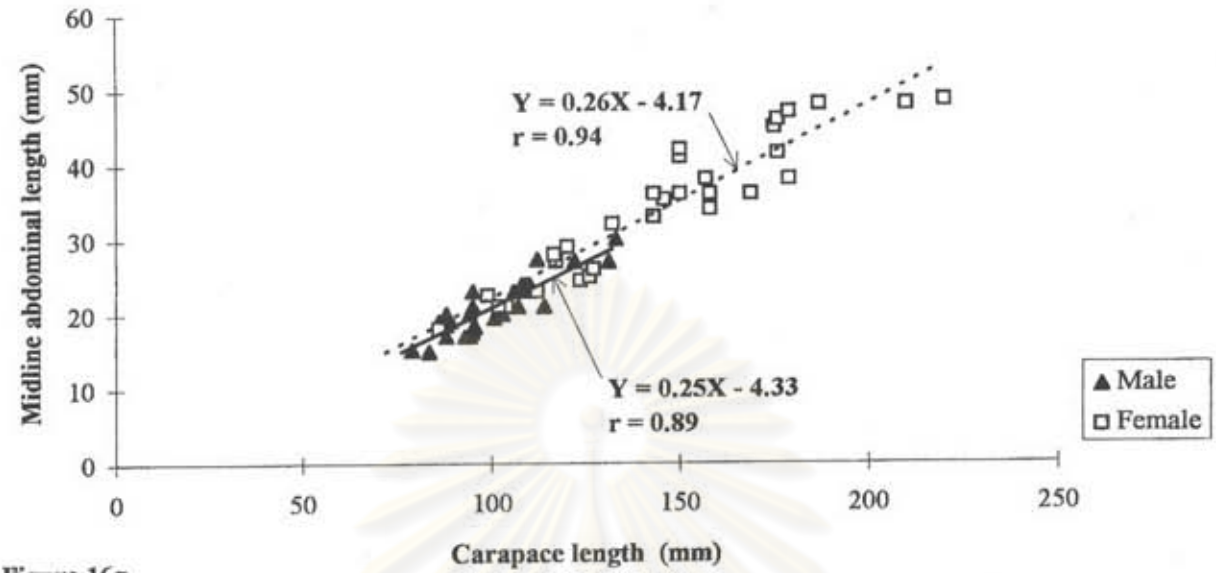


Figure 16g.

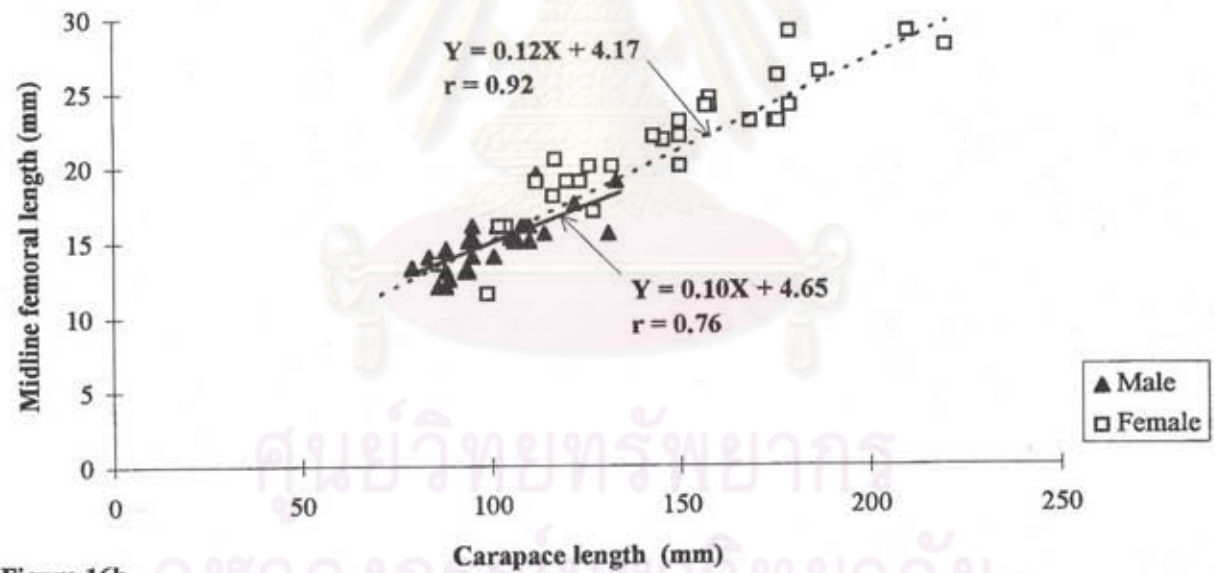


Figure 16h.

Figure 16. Linear regression equations of the relationship (g) midline abdominal length and carapace length (f) midline femoral length and carapace length in both sexes of *M. subtrijuga*

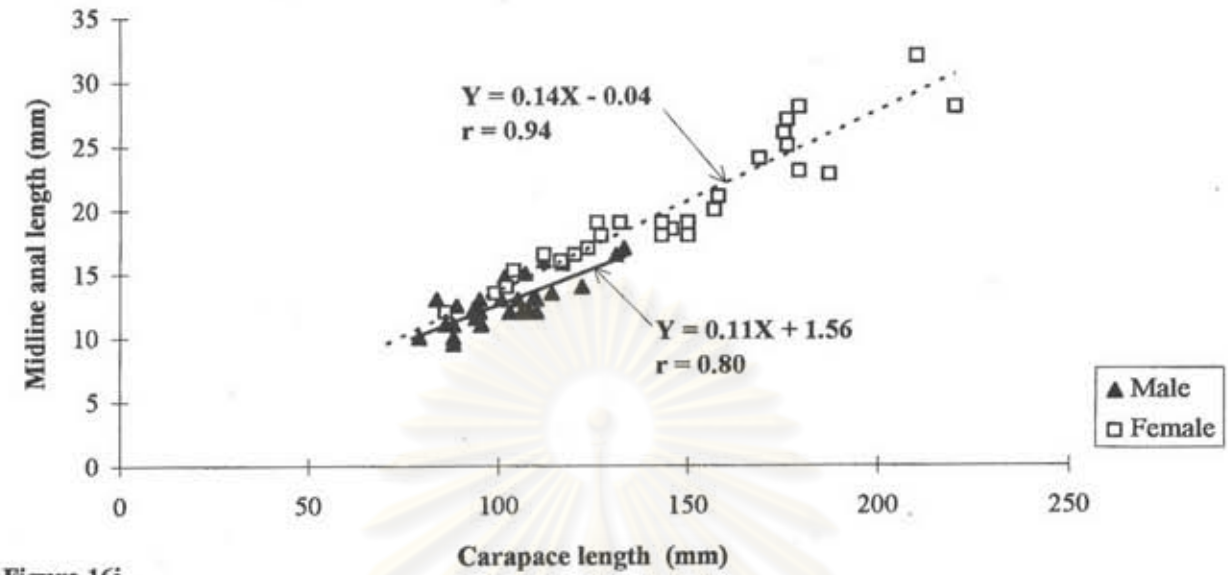


Figure 16i.

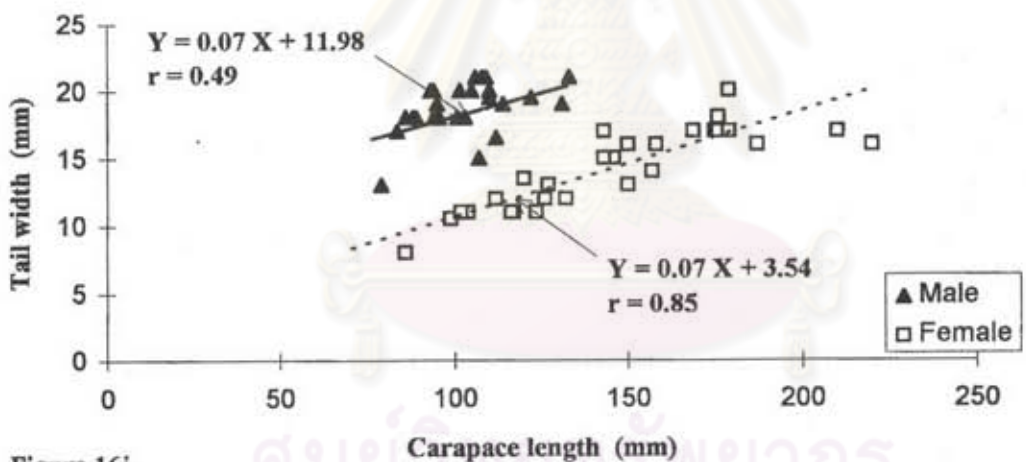


Figure 16j.

Figure 16. Linear regression equations of the relationship (i) midline anal length and carapace length (j) tail width and carapace length in both sexes of *M. subtrijuga*

Growth rate

Figure 17 and table 9 shows that the mean plastron lengths and the estimated mean carapace lengths of newborns of both sexes were not significantly different. After the hatchling year, the mean plastron length and the estimated mean carapace length were significantly greater for females than for males (t-test, $p<.05$). This indicates that the growth rate of females is higher than that of males.

When using plastron midline length as the independent variable and carapace length as the dependent variable, analysis of variance indicated that regression equations of male and female were linear ($p<.05$) in figure 18. The 95% confident interval for slope of each sex is shown in table 10. However, the slopes of regression were not significantly different between males and females (t-test, $p>.05$,Table 10).

Table 9. Mean plastron midline length and estimated mean carapace length of *M. subtrijuga* from hatchling year to 5 years. Differences in superscript letters between males and females indicate that there is significant difference at $p<.05$. n is the number of turtles.

Age	Plastron midline length (mm) Mean ± SD		Estimated carapace length (mm) Mean ± SD	
	Males	Females	Males	Females
Hatchling	29.01 ± 1.24[a] (n=20)	28.21 ± 1.74[a] (n=20)	36.99 ± 1.51[a] (n=20)	36.33 ± 1.98[a] (n=20)
1 year	42.70 ± 3.81[a] (n=20)	51.47 ± 7.02[b] (n=20)	53.13 ± 4.45[a] (n=20)	62.84 ± 7.99[b] (n=20)
2 years	53.98 ± 4.39[a] (n=20)	65.63 ± 9.76[b] (n=20)	66.21 ± 5.08[a] (n=20)	78.98 ± 11.13[b] (n=20)
3 years	64.23 ± 5.69[a] (n=19)	80.51 ± 12.90[b] (n=18)	78.14 ± 6.60[a] (n=19)	95.94 ± 14.71[b] (n=18)
4 years	73.21 ± 6.37[a] (n=15)	94.18 ± 13.86[b] (n=16)	88.93 ± 8.80[a] (n=15)	111.53 ± 15.80[b] (n=16)
5 years	86.34 ± 7.37[a] (n=5)	110.80 ± 13.76[b] (n=11)	103.79 ± 8.55[a] (n=5)	127.75 ± 15.53[b] (n=11)

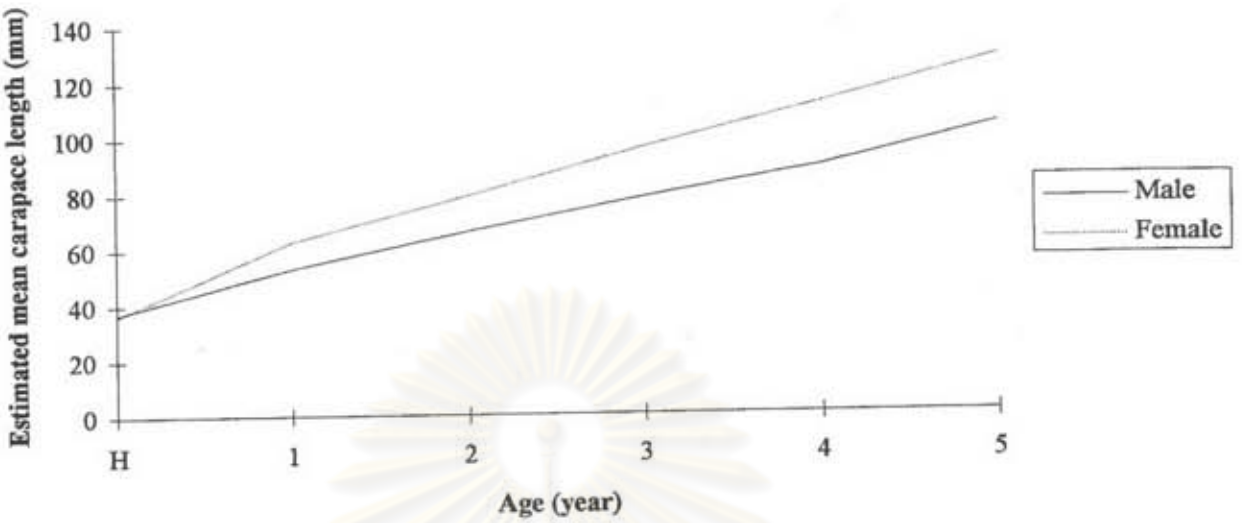


Figure 17. Graph showing the comparisons of growth rates from hatchling year to 5 years old between sexes of *M. subtrijuga*. H means hatchling year.

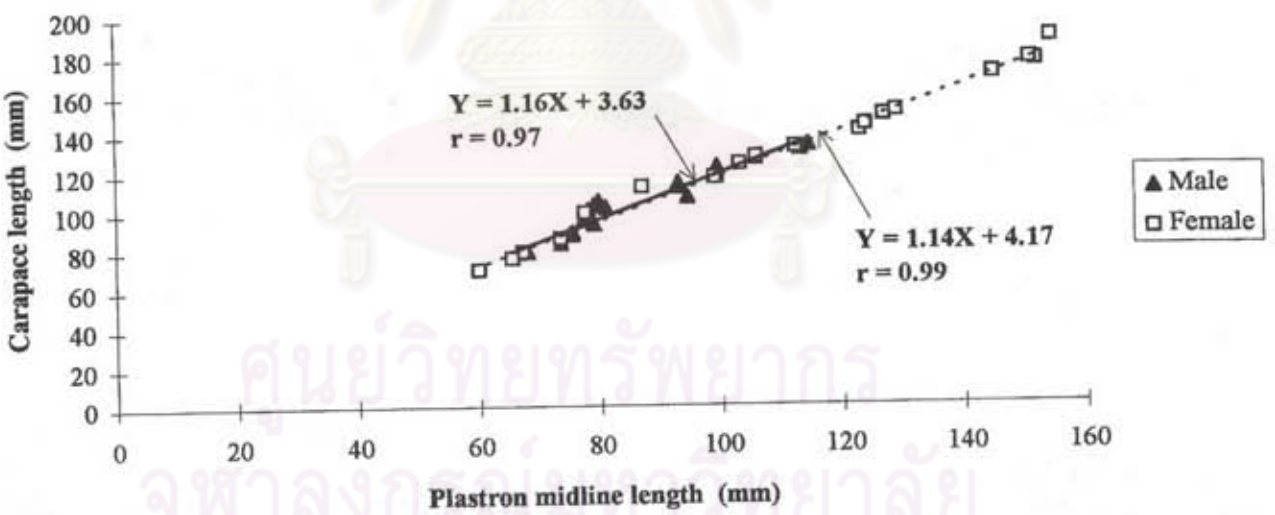


Figure 18. Linear regression equations of the relationship between carapace length and plastron midline length in both sexes of *M. subtrijuga*.

Table 10. Regression equations between plastron midline length and estimated carapace length in different sexes of *M. subtrijuga*. Same superscript letters between males and females indicate that there is not significant difference at $p>.05$. Each regression equation is significant at $p<.05$.

Sex	Regression equation	p	95% Confidence interval for slope
Male	$CL = 1.16\ PML + 3.63^{a}$	0.0000	1.01-1.33
Female	$CL = 1.14\ PML + 4.17^{a}$	0.0000	1.08-1.20

Abbreviation : CL = Carapace length
PML = Plastron midline length

Diet

Examination by dissection of stomach contents from 5 males and 5 females of *M. subtrijuga* showed that the species consumes mainly freshwater molluscs.

Two species of gastropods were identified to species level : *Filopaludina sumatrensis*, which is in the Family Viviparidae, and *Brotia costula*, which belongs to the Family Thiaridae. Both these families are included in the Order Mesogastropoda (Figure 19-22).

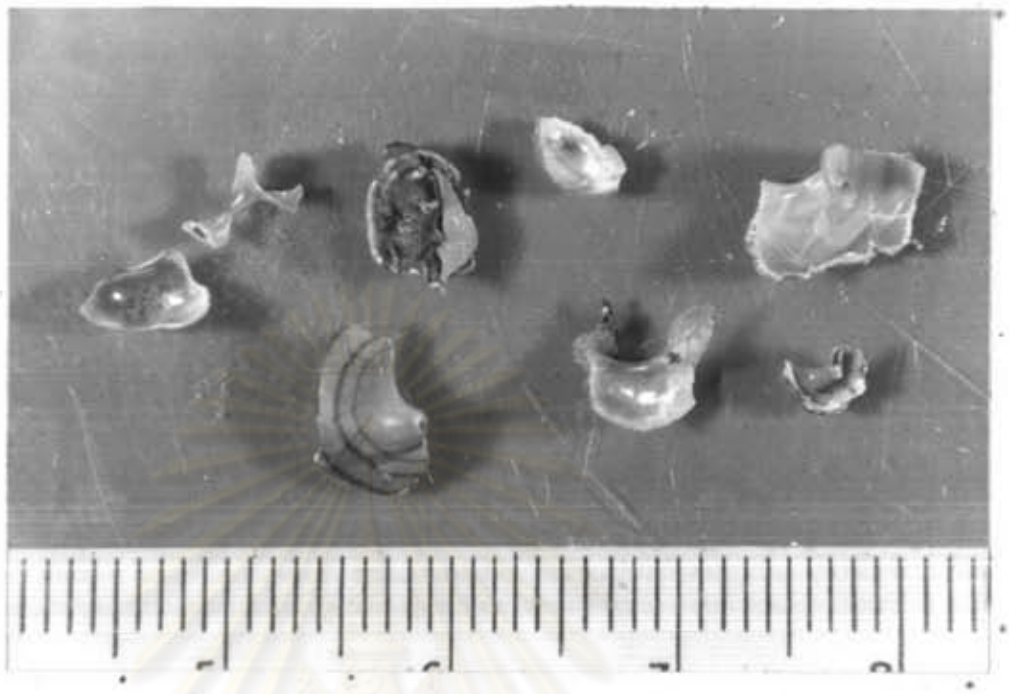


Figure 19 : Shell Fragments of *Filopaludina sumatrensis* found in the gut of *M. subtrijuga*

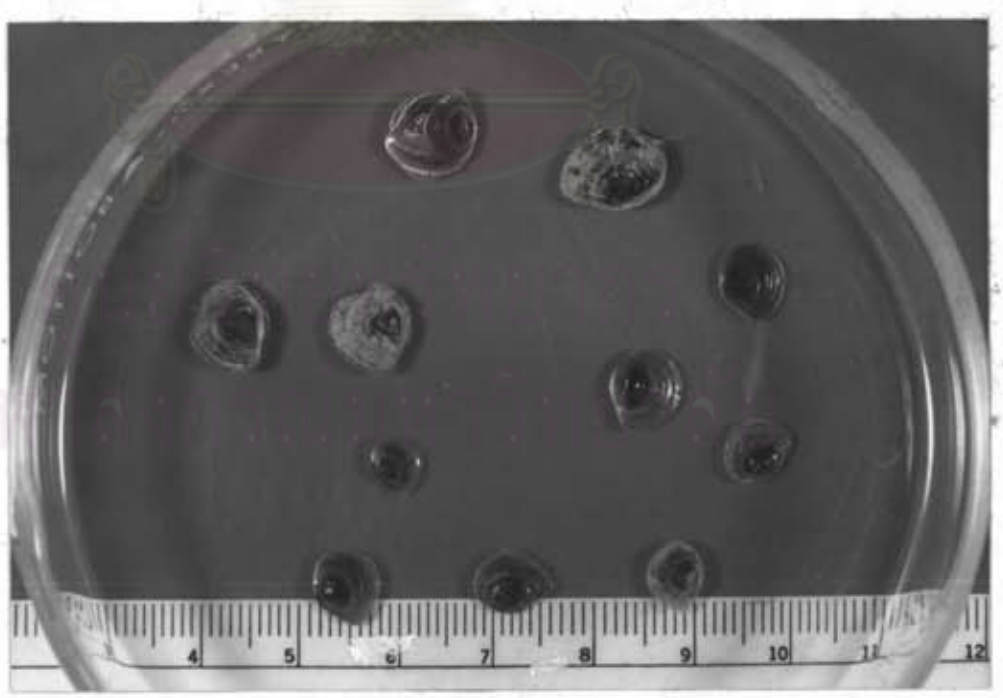


Figure 20 : Operculums of *Filopaludina sumatrensis* found in the gut of *M. subtrijuga*

Figure 21 : Shells of *Filopaludina sumatrensis*

Figure 22 : Shells of *Brotia costula*

Reproductive biology

1. Egg sizes and clutch sizes

Clutch sizes at Tharang district, Phetchaburi Province varied from 3 to 6 eggs per clutch ($\bar{x} = 3.86 \pm 1.08$, $N = 22$). Egg sizes from each clutch are shown in table 11 and figure 23). There was significant difference in mean egg length between clutch size 3 and 4, 3 and 6, 4 and 6, and 5 and 6; whereas only clutch size 4 and 6 were significantly different in mean egg weight (ANOVA, P<.05, table 11). However, both mean egg length and egg weight were slightly positively correlated with clutch size but not significant (Pearson correlation test= 0.26, 0.27 (t-test, p>.05)).

Table 11. Ranges and means of eggs length and egg weight for different clutch sizes. *N* is number of clutches and n is total number of eggs. Differences in superscript letters between clutch sizes indicate that there is significant difference at p<.05.

Clutch size	*N*	n	Egg length (mm)		Egg weight (g)	
			Range	Mean ± SD	Range	Mean ± SD
3	11	32*	36-45	40.36±2.77^{b}	6.3-15.4	10.76±2.57^{ab}
4	6	24	32.5-44	37.91±2.68^{a}	7.8-14.5	9.58±7.14^{a}
5	2	10	38-41.25	39.43±1.01^{ab}	9.7-11.8	10.90±0.65^{ab}
6	3	18	39.25-45	42.47±1.70^{c}	9.6-14.6	12.34±1.78^{b}

Note : * One egg was broken and not measured.

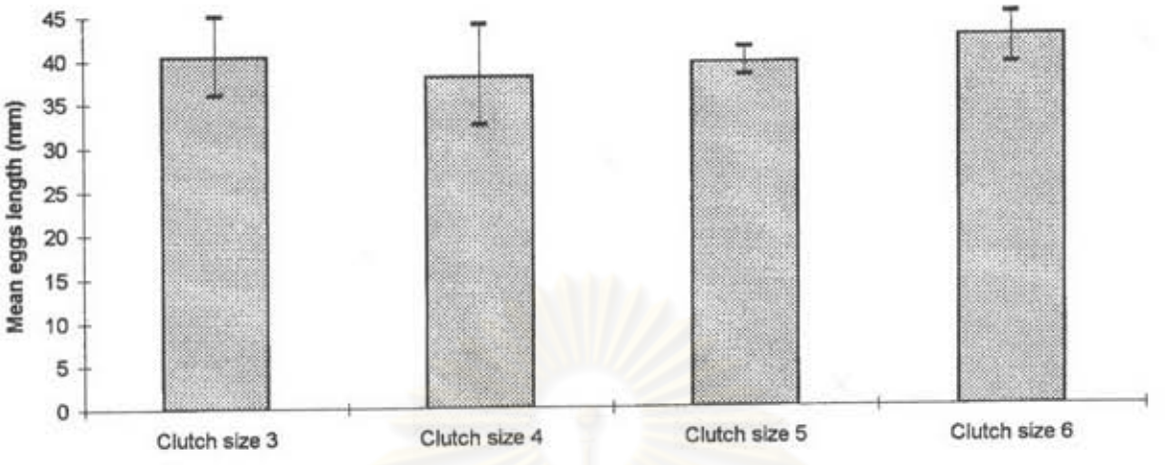


Figure 23a.

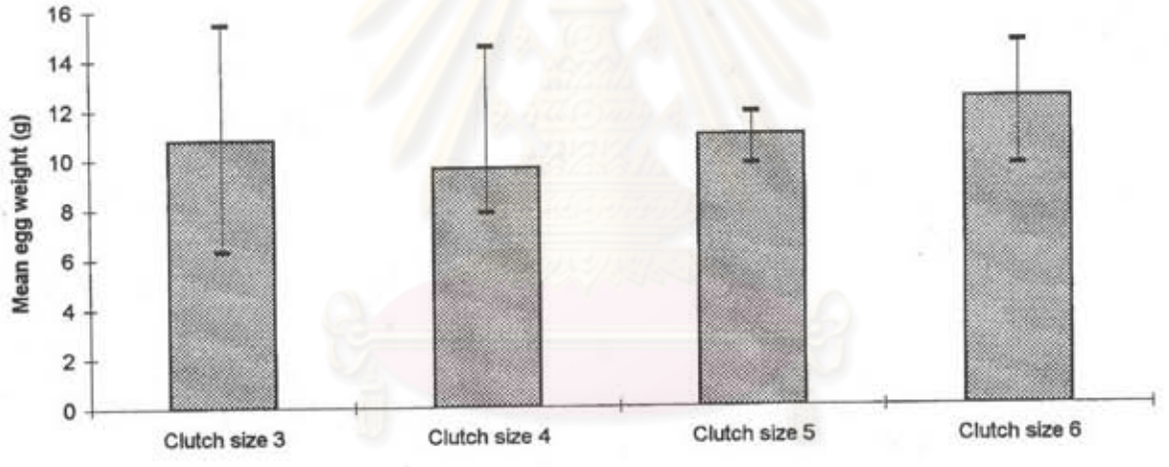


Figure 23b.

Figure 23. Bar diagram showing the relationship between (a) clutch size and mean egg length in (mm) (b) clutch size and mean egg weight in (g) of *M. subtrijuga*.

2. Incubation periods and hatching success

Incubation periods of eggs incubated under 26-32°c from each clutch are shown in table 12-15. It is found that one egg from clutch size 4 had the longest period of 292 days, whereas one egg from clutch size 6 has the shortest period of 97 days. However, means of all incubation periods of every clutch size were not significantly different (ANOVA, $p<.05$). Result are presented in table 16 and figure 24 a.

Percentage of success in each clutch are shown in table 12-15. It was shown that mean percentage of success from clutch size 4 was 70.83% whereas mean percentage of success from clutch size 6 was 38.90%. However, mean percentages of success among all clutch sizes were not significantly different (ANOVA, $p>.05$)(Table 16 and figure 24 b).

Table 12. Means and ranges of incubation periods and hatching success for eggs from clutches containing 3 eggs.

Clutch size	Clutch number	Incubation periods (days) egg (n=20)			Mean ± SD	Hatching success (%)
		1	2	3		
3	1	131	140	172	147.67 ± 21.55	100.00
	2	200	-	-	-	33.33
	3	225	-	-	-	33.33
	4	121	129	221	157.00 ± 55.57	100.00
	5	108	115	178	133.67 ± 38.55	100.00
	6	181	-	-	-	33.33
	7	123	134	185	147.33 ± 33.08	100.00
	8	146	-	-	-	33.33
	9	136	-	-	-	33.33
	10	195	222	222	213.00 ± 15.59	100.00

Table 13. Means and ranges of incubation periods and hatching success for eggs from clucthes containing 4 eggs.

Clutch size	Clutch number	Incubation periods (days) egg (n=17)				Mean ± SD	Hatching success (%)
		1	2	3	4		
4	1	292	-	-	-	-	25.00
	2	131	131	137	162	140.25 ± 14.77	100.00
	3	121	122	137	182	140.50 ± 28.62	100.00
	4	131	223	229	-	194.33 ± 54.93	75.00
	5	193	158	-	-	175.50 ± 24.75	50.00
	6	102	119	171	-	130.67 ± 35.95	75.00

Table 14. Means and ranges of incubation periods and hatching success for eggs from clutches containing 5 eggs.

Clutch size	Clutch number	Incubation periods (days) egg (n=4)					Mean ± SD	Hatching success (%) Mean
		1	2	3	4	5		
5	1	187	222	223	-	-	210.67 ± 20.50	60
	2	171	-	-	-	-	-	20

Table 15. Means and ranges of incubation periods and hatching success for eggs from clutches containing 6 eggs.

Clutch size	Clutch number	Incubation period (days) egg (n=7)						Mean ± SD	Hatching success (%) Mean
		1	2	3	4	5	6		
6	1	210	-	-	-	-	-	-	16.67
	2	97	144	146	172	204	-	152.60 ± 39.46	83.33
	3	221	-	-	-	-	-	-	16.67

Table 16. Means of incubation period and means of hatching success for different clutch sizes. Same superscript letters between clutch sizes indicate that there is no significant difference at $p > .05$.

Clutch size	Incubation periods (days) Mean ± SD	Hatching success (%) Mean ± SD
3	164.20 ± 40.33^a	66.67 ± 35.14^a
4	161.24 ± 49.64^a	70.83 ± 29.23^a
5	200.75 ± 25.95^a	40.00 ± 28.28^a
6	170.57 ± 44.61^a	38.89 ± 38.49^a

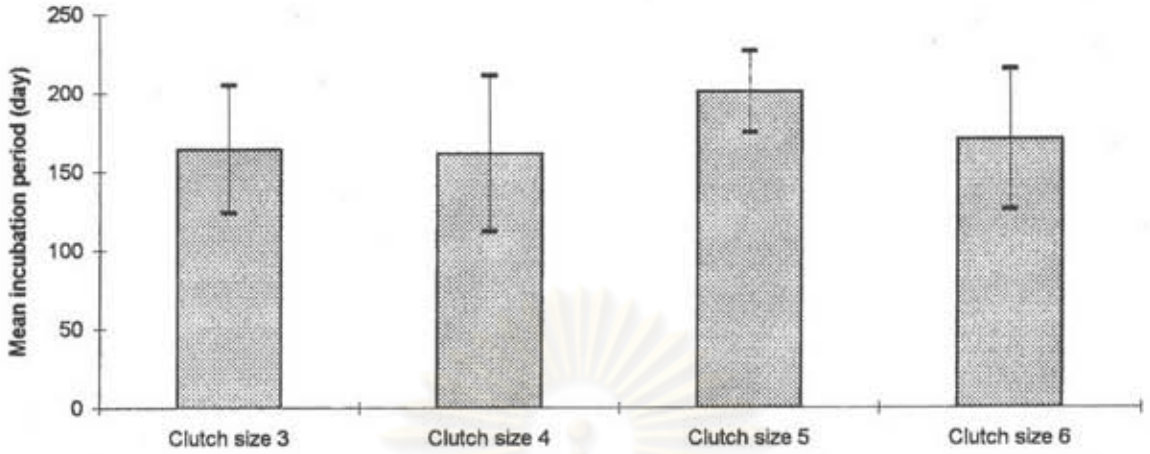


Figure 24a.

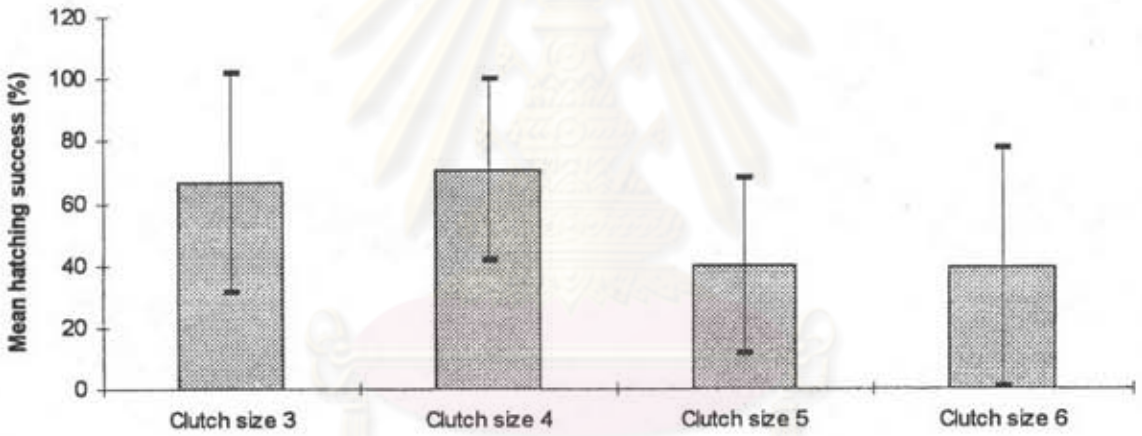


Figure 24b.

Figure 24. Bar diagram showing the relationship between (a) clutch size and mean incubation period (b) clutch size and mean hatching success (%) of *M. subtrijuga*.

Mean of hatched eggs (n = 48) and mean of unhatched eggs (n = 36) were 39.68, and 40.49 mm in length and 10.45 and 11.28 g in weight respectively. However, there were no significant differences between hatched and unhatched egg means (t-test, P>.05, Table 17). In addition, egg weights(n = 48) and weight of hatchlings (n = 48) were 7.3-15.40 g (x = 10.45±2.11) and 4.20-11.12 g ($\bar{x}$ = 8.04±1.61) repectively. The mean weight of hatchlings was positively correlated with egg weight (Pearson correlation test = 0.78 (t-test,p <.05).

Table 17. Means of length and weight of hatched and unhatched eggs. Same superscript letters between hatched eggs and unhatched eggs indicate that there is no significant difference at p>.05.

	Egg Length (mm) Mean ± SD	Egg Weight (g) Mean ± SD
Hatched eggs	39.68 ± 2.86[a]	10.45 ± 2.11[a]
Unhatched eggs	40.49 ± 2.83[a]	11.28 ± 2.34[a]

3. Hatchling survival rate

Survival rates of the 48 hatchlings, when fed with 4 species of natural food, freshwater snail (*Filopaludina sumatrensis* and *Brotia costula*) (Figure 25), small shrimp (*Macrobrachium lanchesteri*), and small fish (*Oreochromis nilotica*) (Figure 26 a-b) for the continuous period of 5 months under laboratory conditions, were 100% for the first 2 months, 96% in the third month, 79% in the fourth month, and 65% in the fifth month (Figure 27).

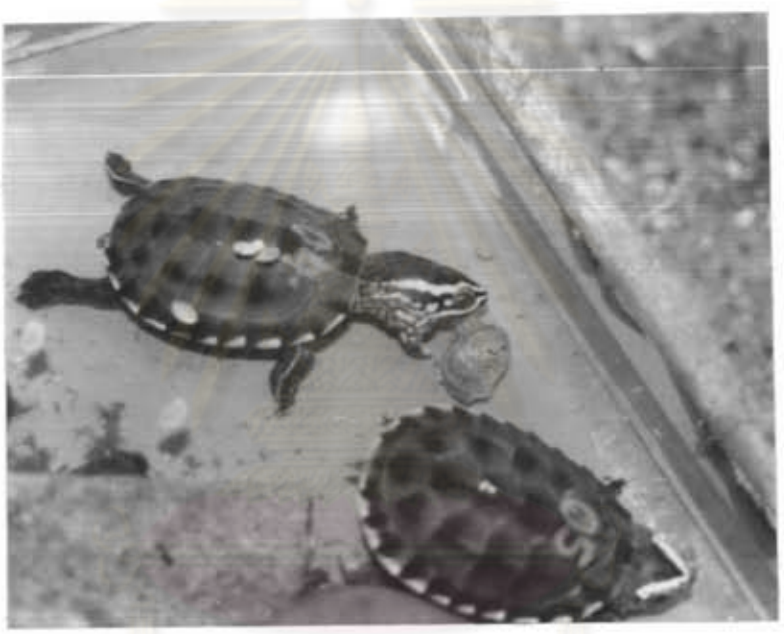


Figure 25. A hatchling was engulfing a freshwater snail, *Filopaludina sumatrensis*.

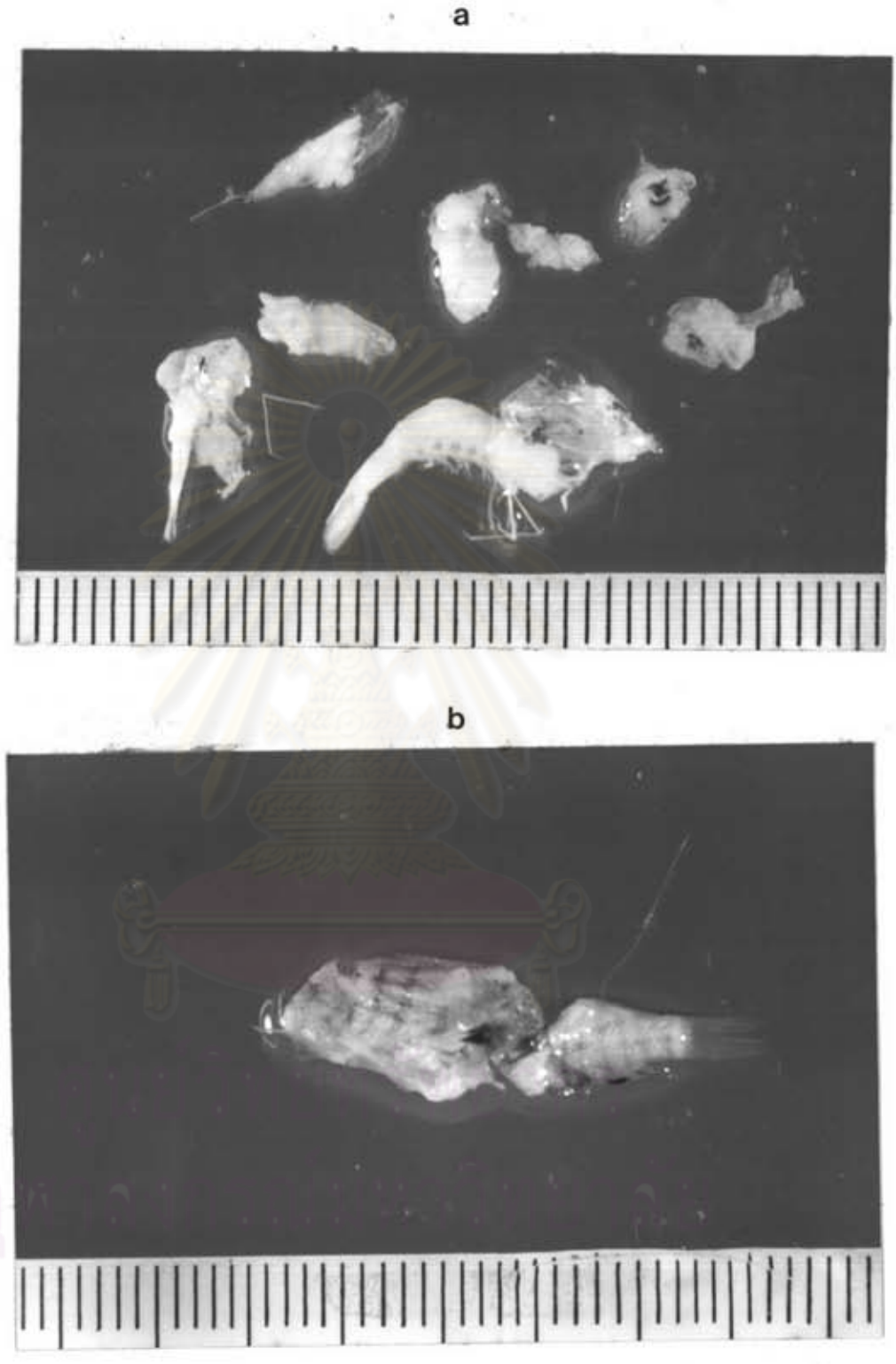


Figure 26 : Fragments of food left in the turtorium : (a) *Macrobrachium lanchesteri* (b) *Oreochromis nilotica*

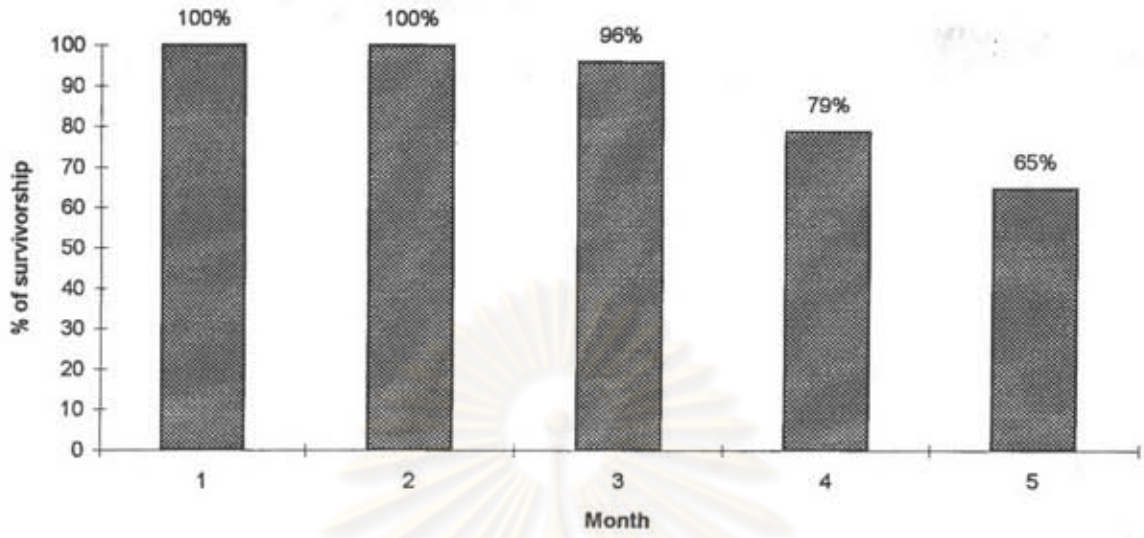


Figure 27. Bar diagram showing the relationship between survivorship (%) and age of hatchling of *M. subtrijuga*.

Parasites

Malayemys subtrijuga had both ectoparasites and endoparasites. The ectoparasites found on the scutes of both carapace and plastron, and on the skin of the neck, body, and four legs were leeches (Figure 28-29). They are in Order Acanthobdellida (Karstner, 1967), Class Hirudinea, Phylum Annelida (Pakpimol Mahannop, pers.comn, 1994). The endoparasites found in the guts (stomach, small and large intestines) were nematodes and flukes. Two species of nematodes were found, both belonging to the Subclass Phasmidia (Secernentea). One is in Family Oxyuridae, Superfamily Oxyuroidea, Suborder Oxyurida, and Order Ascaridida (Figure 30-31). The other is in the Family Rhabditidea, Superfamily Rhabditoidea, Suborder Rhabditata, and Order Rhabditida (Figure 32-33). One species of fluke was also found and classified in the Suborder Prosostomata, Order Digenea, and Class Trematoda, Phylum Platyhelminthes (Figure 34).

Figure 28 : Leech collection found on the scutes of carapace and plastron and on the skin of the body, neck, and legs of *M. subtrijuga*

Figure 29 : Different sizes of the leeches under stereomicroscope (10X)

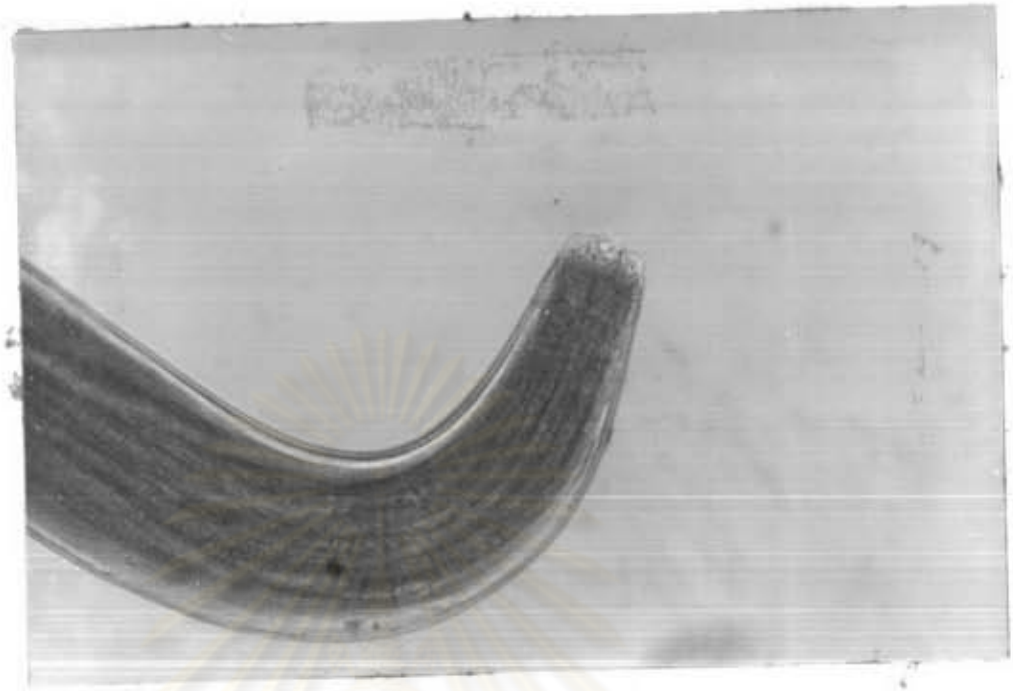


Figure 30 : A nematode in the Family Oxyuridae found in the stomachs, small and large intestines of *M. subtrijuga*, showing the anterior end (40X)

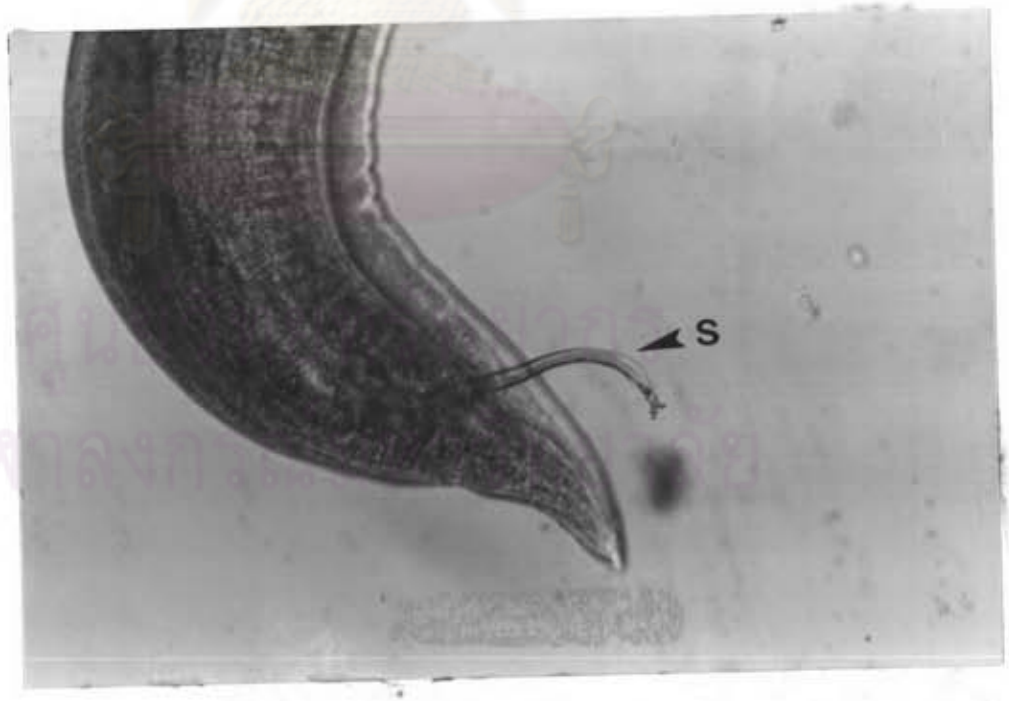


Figure 31 : Posterior end of the male nematode (Family Oxyuridae) showing the spicule [S] (40X)

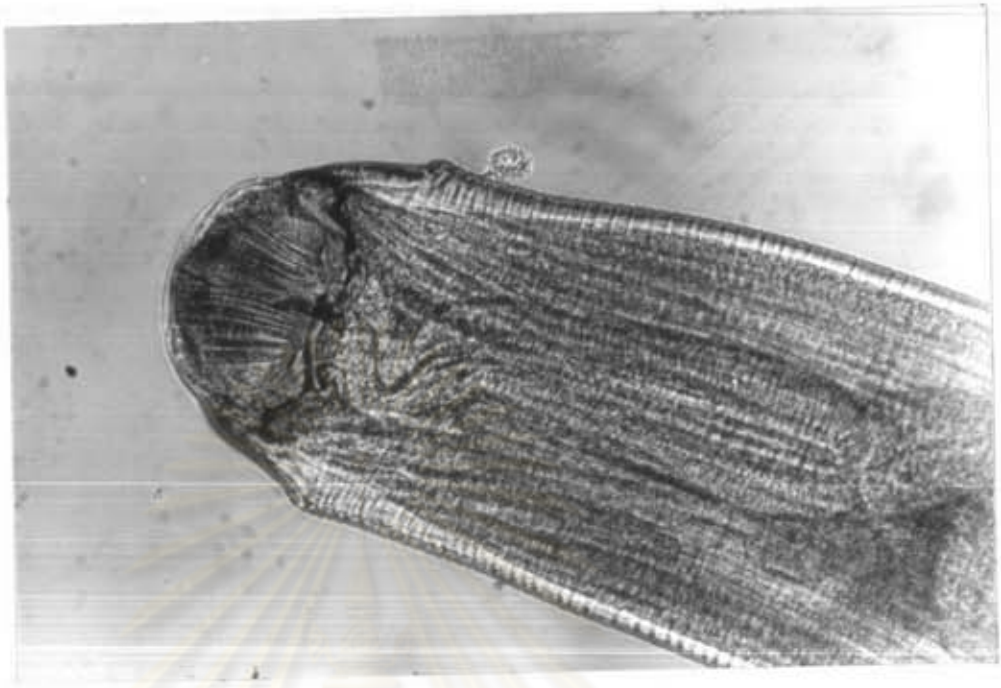


Figure 32 : A Nematode in the Family Rhabditidae found in the stomachs, small and large intestines of *M. subtrijuga* showing the anterior end (40X)

Figure 33 : Mid portion of the female nematode (Family Rhabditidae) showing eggs in the uterus (20X)

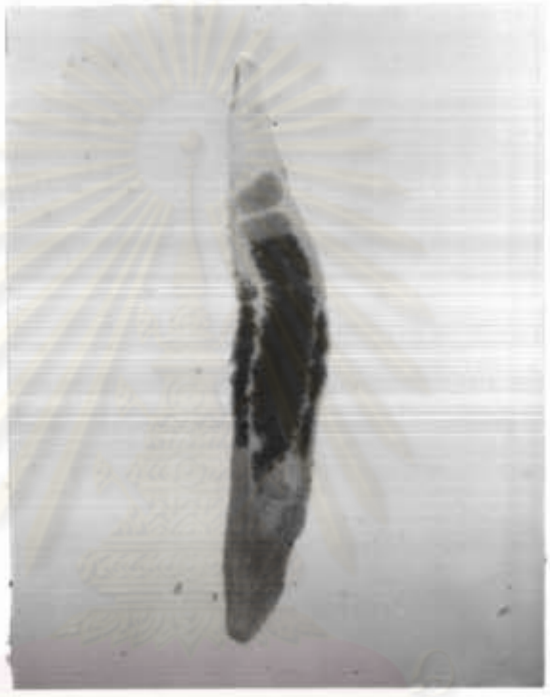


Figure 34 : A fluke in the Suborder Prosostomata found in the stomachs, small and large intestines of *M. subtrijuga* (10X)