CHAPTER III

RESULTS

Experiment 1 : Effects of Broodstock Sources and Sizes on Ovarian Maturity and Spawning of *P. monodon*.

Ovarian Maturity.

The results on ovarian maturity of giant tiger prawns of different sources and sizes reared in the closed recirculating seawater system for 60 days are shown in Table 4. The percentage of gravid females (stage IV ovarian maturity), indicated that the groups of large prawns, regardless the sources, could undergo stage IV ovarian maturity more than the small prawn groups. Prawns from the two sources , i.e. wild caught and pond reared, were seemed to undergo comparable maturity. Some females could remature repeatedly, especially the large females which rematured more frequently than the small prawns.

The elapsed time between the eye-stalk ablation and the first stage IV ovarian maturity of the four groups of prawns did not differ significantly ($P > 0.05$). These elapsed times averaged at 20 days. Furthermore, the elapsed times for the other consecutive stage IV ovarian maturities were significantly shorter and did not differ among the four groups ($P > 0.05$). These elapsed times averaged at 9 days.

Table 4. Ovarian maturity of giant tiger prawns of the two sources (wild-caught and pond-reared) and different sizes (small and large) during the 60 days experiment.

Prawn groups	Stage IV ovarian maturity		Elapsed time (days)[2]		
	No.of gravid females	Percent gravid females	Average	Minimum	Maximum
Small pond-reared					
Ab-1[1]	9	30.0	16.2^{a}	7	32
2	2	6.7	14.5^{b}	14	15
Large pond-reared					
Ab-1	14	46.7	21.1^{a}	12	38
2	5	16.7	12.6^{b}	6	19
3	2	6.7	3.0^{b}	3	3
4	2	6.7	9.5	6	13
Small wild-caught					
Ab-1	8	28.6	25.0^{a}	5	39
2	3	10.7	13.7^{b}	11	16
3	1	3.6	4.0^{b}	4	4
Large wild-caught					
Ab-1	14	45.2	16.9^{a}	2	38
2	4	12.9	5.8^{b}	3	14
3	3	9.7	7.3^{b}	3	4
4	1	3.2	2.0	2	2

(1) Ab = Eye-stalk ablation; numbers are consecutive ovarian maturities.

(2) Time in days after the eye-stalk ablation and the consecutive stage IV ovarian maturities.

Significant differences ($P \leq 0.05$) of the average elapsed time are indicated by different letters, i.e. a and b within the column.

Spawning.

The results on the spawning of giant tiger prawns of different sources and sizes are shown in Table 5. Similar to ovarian maturity results, the large prawn groups produced more spawners than the small prawn groups. The two sources of prawns showed comparable spawning success. Some females, especially in the large prawn groups, spawned repeatedly.

The elapsed time between the eye-stalk ablation and the first spawning of the four groups of prawns did not differ significantly ($P > 0.05$). These elapsed time averaged at 17 days. Furthermore, the elapsed times for the other consecutive spawnings were somewhat shorter and averaged at 9 days.

Egg Quantity and Quality.

The results on egg quantity and quality are summarized in Table 6. The analysis of variance for the factorial design experiment revealed that the average number of egg spawned per female of the large size prawns was significantly higher than that of the small ones ($P \leqslant 0.05$). The large size prawns of different sources spawned comparable amount of eggs. The eggs spawned per female averaged at 191,986 eggs. The smaller prawns of different sources also showed a similar pattern as the large prawns with an average of 41,627 eggs. The amount of eggs of different sources and sizes did not show an interaction.

Table 5. Spawning of giant tiger prawns of the two sources (wild-caught and pond-reared) and different sizes (small and large) during the 60 days experiment.

Prawn groups	Spawning		Elapsed time (days)[2]		
	No. of spawners	Percent	Average	Minimum	Maximum
Small pond-reared					
Ab-1[1]	5	16.7	15.8[a]	8	22
Large pond-reared					
Ab-1	9	30.0	20.2[a]	3	28
2	5	16.7	13.0[b]	6	21
3	1	3.3	3.0	3	3
4	1	3.3	13.0	13	13
Small wild-caught					
Ab-1	3	10.7	23.3[a]	6	36
2	1	3.6	6.0[b]	6	6
Large wild-caught					
Ab-1	12	38.7	14.6[a]	3	36
2	3	9.7	3.3[b]	3	4
3	2	6.4	3.0	3	3
4	1	3.2	2.0	2	2

(1) Ab = Eye-stalk ablation; numbers are consecutive spawnings.

(2) Time in days after the eye-stalk ablation and the consecutive spawnings

Significant differences ($P \leqslant 0.05$) of the average elapsed time are indicated by different letters, i.e. a and b within the column.

Table 6. Egg quantity and quality of different groups of giant tiger prawns. (ranges are in parentheses)

Egg quantity and quality	Pond-reared		Wild-caught	
	small size	Large size	Small size	Large size
Ave. no. of eggs per female	30382^{a}	191029^{b}	53671^{a}	192913^{b}
Ave. no. of eggs per spawner	182292	636762	500933	498358
Ave. % fertility	49.9 (17.4-76.9)	30.6 (0-88.6)	50.9 (0-94.3)	35.0 (0-90.6)
Ave. % hatching rate	8.81 (0-37.9)	8.0 (0-75.9)	6.2 (0-25.7)	4.5 (0-69.3)
Ave. % metamorphosis from egg to protozoea stage	5.3 (0-24.6)	5.1 (0-44.5)	4.6 (0-18.3)	2.9 (0-50.1)

Note : Significant differences ($P<0.05$) are indicated by different letters, i.e. a and b.

The average number of eggs spawned per spawner of the four groups of prawns did not differ significantly. The number of eggs per spawner averaged at 454,586 eggs.

The amount of eggs spawned in the different consecutive spawnings did not differ significantly and was not affected by broodstock sources and sizes ($P > 0.05$).

Egg fertility (fertilized eggs), hatching rate and metamorphosis rate (from egg to protozoea stage) varied greatly. The average percent fertility of total eggs spawned of the four prawn groups did not differ significantly ($P > 0.05$). The average percent fertility in each consecutive spawning of the four prawn groups also did not differ significantly ($P > 0.05$). These fertility rates averaged at 35.2%.

The average hatching rates were much lower than the fertility rate. Similar to the fertility rates, the average hatching rate of total eggs spawned of the four prawn groups did not differ significantly ($P > 0.05$). The average hatching rate in each consecutive spawning of the four prawn groups also did not differ significantly ($P > 0.05$). These hatching rates averaged at 6.6%.

The percent metamorphosis from eggs to the first protozoea stage also showed the same pattern as the hatching rates. These metamorphosis rates averaged at 4.2%.

Moulting.

The moulting pattern of female prawns are shown in Table 7. The period between eyestalk ablation and the first moulting was significantly shorter than the elapsed time between the following consecutive moultings ($P \leqslant 0.05$). The period between eye-stalk ablation and the first moulting of the large prawns, regardless the sources, was significantly longer than that of the small ones ($P \leqslant 0.05$). The period for the large prawns averaged at 12 days while the period for the small spawns averaged at 10 days.

The elapsed time between two consecutive moultings of the four groups also showed the similar pattern; this elapsed time for the large prawns averaged at 18 days while the elapsed time for the small prawns averaged at 15 days.

Mortality.

The analysis of covariance revealed that the overall mortality of the pond-reared prawns, regardless the sizes, was significantly higher than that of the wild-caught prawns (Figure 6). Mortality of the large females, regardless the sources, was significantly higher than that of the small ones (Figure 7). Mortality of the females, regardless the sources and sizes, was significantly higher than that of the males (Figure 8).

Most prawns had orange-pink gills. Spores of fungus, *Fusarium* sp. were found in their gills but no infectious bacteria were found.

Table 7. Average elapsed time (days) between consecutive moultings for giant tiger prawn broodstocks.

Prawn groups	Sequential moulting		
	1	2	3
Small pond-reared	$10.8^{a} \pm 4.7$	$15.6^{c} \pm 4.8$	$13.5^{c} \pm 6.4$
Large pond-reared	$12.4^{b} \pm 6.5$	$16.2^{d} \pm 3.4$	$17.8^{d} \pm 3.3$
Small wild-caught	$8.4^{a} \pm 4.8$	$15.5^{c} \pm 4.1$	$12.3^{c} \pm 14.9$
Large wild-caught	$11.14^{b} \pm 7.0$	$19.5^{d} \pm 3.4$	$19.7^{d} \pm 0.6$

Note : Significant differences ($P \leq 0.05$) are indicated by different letters, i.e. a, b, c, and d.

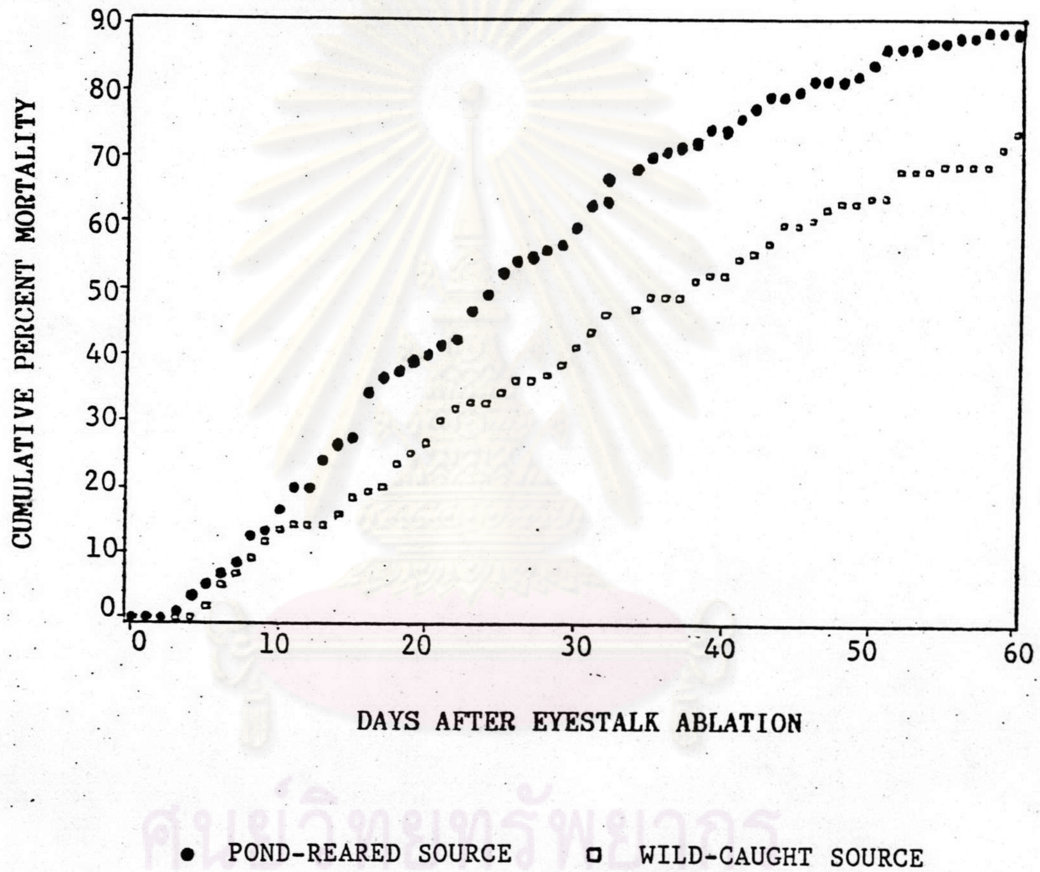

Figure 6. Cumulative percent mortality of giant tiger prawns of different sources (Experiment 1).

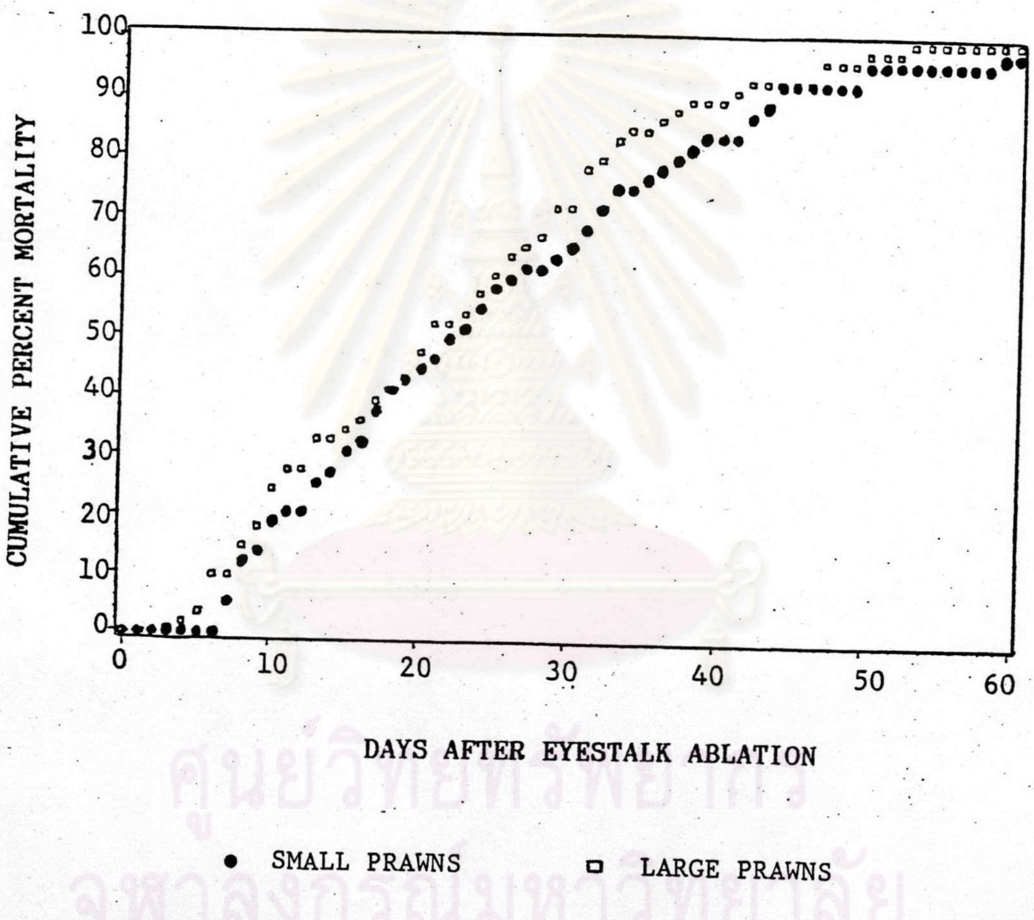

Figure 7. Cumulative percent mortality of female giant tiger prawns with different sizes (Experiment 1).

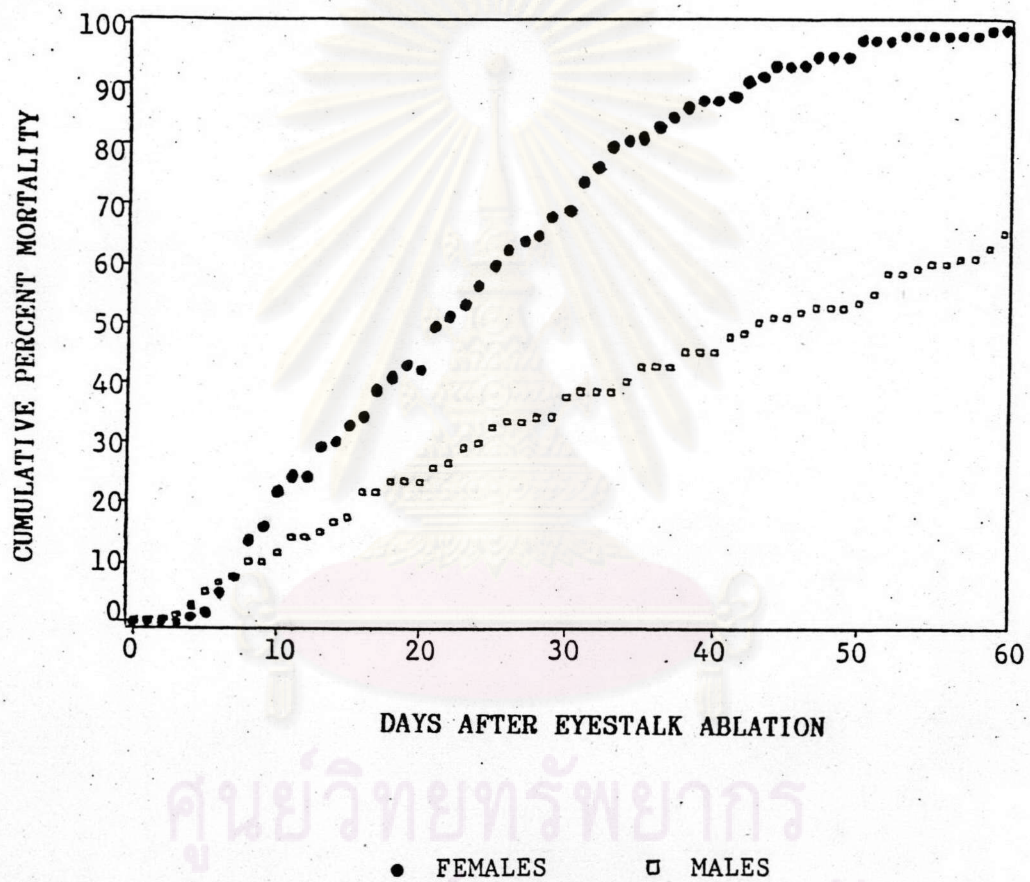

Figure 8. Cumulative percent mortality of male and female giant tiger prawns (Experiment 1).

Water Quality.

Water quality data are summarized in Table 8. No significant difference in water quality between two maturation tanks was observed. Dissolved oxygen, pH and salinity variations were very small. Temperature fluctuated widely between 24 - 30 ^{o}C with an average at 26.5 ^{o}C. The ammonium-N and nitrite-N concentrations were very low and were in acceptable level for penaeid hatchery. Nitrate-N concentration increase was noted in both two maturation tanks. This was a normal characteristic of the closed recirculating water systems. Phosphate-P concentrations also showed the same pattern as the nitrate-N concentrations.

Nutritional Values of the Diets.

Nutritional values of the diets used in this study are shown in Table 9. Crude protein (50.80%) and crude fat (11.41%) of the maturation diet were relatively higher as compared to the commercial diet. Whereas, crude fiber, crude ash and moisture contents of the maturation diet were slightly lower when compared with the commercial diet.

Table 8. Dissolved oxygen (D.O.), pH, temperature, salinity, ammonium-N (NH_4^+), nitrite-N (NO_2^-), nitrate-N (NO_3^-) and phosphate-P(PO_4^{3-}) in each maturation tank during Experiment 1. The average, standard deviations and ranges of each parameter are shown.

Tank	D.O. (ppm)	pH	Temperature (°C)	Salinity (ppt)	NH_4^+-N (mg/l)	NO_2^--N (mg/l)	NO_3^--N (mg/l)	PO_4^{3-}-P (mg/l)
Pond-reared prawn tank	7.5 ± 0.4 (6.6-8.1)	7.4 ± 0.3 (6.9-8.2)	26.8 ± 1.3 (24.8-29.9)	29.4 ± 1.0 (27.5-31.0)	0.0006 ± 0.0003 (0.0001-0.0010)	0.0017 ± 0.0019 (0.0004-0.0068)	16.1 ± 13.0 (0.7 - 35.4)	0.34 ± 0.04 (0.27 - 0.39)
Wild-caught prawn tank	7.5 ± 0.5 (6.5-8.2)	7.4 ± 0.2 (7.0-8.0)	26.3 ± 1.2 (24.3-29.0)	29.5 ± 1.0 (27.5-31.0)	0.0008 ± 0.0002 (0.0004-0.0011)	0.0016 ± 0.0007 (0.0004-0.0027)	18.6 ± 12.6 (0.7 - 33.3)	0.35 ± 0.04 (0.27 - 0.40)

Table 9. Proximate analysis of artificial diets used in Experiment 1 and Experiment 2.(values are in %)

Diet types	Moisture content	Crude protein	Crude fat	Crude fiber	Ash
Maturation diet (1)	5.51	50.80	11.41	3.48	14.91
Maturation diet (2)	5.39	51.15	11.87	3.57	15.46
Commercial diet	9.80	42.45	4.74	3.54	18.10

(1) The diet for maturation test in Experiment 1.

(2) The diet for maturation test in Experiment 2.

Experiment 2 : Effects of Diets on Ovarian Maturity and Spawning of Large Size Pond-reared *P. monodon*.

Ovarian Maturity.

The results on ovarian maturity of the eye-stalk ablated giant tiger prawns fed with different diets in the semi-flow-through seawater systems for 60 days are shown in Table 10 - 12. The percentage of gravid females, indicated that the prawns fed with fresh natural diets (Treatment 1) and the prawns fed with combined diets (Treatment 2) were seemed to undergo comparable stage IV. ovarian maturity, whereas the prawns fed with artificial diet (Treatment 3) underwent stage IV ovarian maturity lower than the two formers. Some females could remature repeatedly, especially those in Treatment 1 and Treatment 2. The rematuration of these two treatments were more frequently than Treatment 3.

The elapsed time between the eye-stalk ablation and the first stage IV ovarian maturity of the prawn of the three treatments did not differ significantly ($P > 0.05$). These elapsed times averaged at 25 days. Furthermore, the elapsed times of the other two consecutive stage IV ovarian maturities were significantly shorter ($P \leqslant 0.05$) and did not differ among the three treatments ($P > 0.05$). These elapsed times averaged at 3 days.

Table 10. Ovarian maturity of giant tiger prawns fed with fresh natural diets during the 60 days experiment.

Consecutive ovarian maturities	Stage IV ovarian maturity		Elapsed time (days) (2)		
	No. of gravid female	Percent gravid females	Average	Minimum	Maximum
Ab-1 (1)	17	67.9	26.5 [a]	6	53
2	15	59.9	5.8 [b]	3	21
3	13	52.2	3.7 [b]	3	9
4	9	35.9	6.0 [b]	3	18
5	8	32.0	6.4 [b]	3	15
6	2	8.0	9.0 [b]	6	12
7	1	4.2	3.0	3	3
8	1	4.2	3.0	3	3

(1) Ab = Eye-stalk ablation.

(2) Time in days after the eye-stalk ablation and the consecutive stage IV ovarian maturities.

Significant differences ($P \leq 0.05$) of the average elapsed time are indicated by different letters, i.e. a and b within the column.

Table 11. Ovarian maturity of giant tiger prawns fed with combined diets during the 60 days experiment.

Consecutive ovarian maturities	Stage IV ovarian maturity		Elapsed time (days)[(2)]		
	No. of gravid female	Percent gravid females	Average	Minimum	Maximum
Ab-1[(1)]	15	66.9	26.8^{a}	8	54
2	11	48.1	7.9^{b}	3	21
3	11	48.1	8.5^{b}	3	21
4	8	35.4	4.1^{b}	3	9
5	3	12.7	6.0^{b}	3	12
6	2	8.8	3.0^{b}	3	3
7	1	5.0	3.0	3	3
8	1	5.0	3.0	3	3
9	1	5.0	6.0	6	6
10	1	5.0	3.0	3	3
11	1	5.0	3.0	3	3

(1) Ab = Eye-stalk ablation.

(2) Time in days after the eye-stalk ablation and the consecutive stage IV ovarian maturities.

Significant differences ($P \leq 0.05$) of the average elapsed time are indicated by different letters, i.e. a and b within the column.

Table 12. Ovarian maturity of giant tiger prawns fed with artificial diet during the 60 days experiment.

Consecutive ovarian maturities	Stage IV ovarian maturity		Elapsed time (days) (2)		
	No. of gravid female	Percent gravid females	Average	Minimum	Maximum
(1) Ab-1	5	20.9	16.0^{a}	5	47
2	3	13.2	4.5^{b}	3	9
3	2	7.7	3.0^{b}	3	3
4	2	7.7	3.0^{b}	3	3

(1) Ab = Eye-stalk ablation.

(2) Time in days after the eye-stalk ablation and the consecutive stage IV ovarian maturities.

Significant differences ($P \leq 0.05$) of the average elapsed time are indicated by different letters, i.e. a and b within the column.

Spawning.

The results on spawning of giant tiger prawns fed with different diets are shown in Table 13 - 15. Similar to ovarian maturity results, the prawns fed with fresh natural diets (Treatment 1) and the prawns fed with combined diets (Treatment 2) were seemed to undergo comparable spawning success and produced more spawners than the prawns fed with artificial diet (Treatment 3). Some females, especially those in Treatment 1 and Treatment 2 spawned more frequently than those in Treatment 3.

The elapsed time between the eye-stalk ablation and the first spawning of the prawns of the three treatments did not differ significantly ($P > 0.05$). These elapsed times averaged at 26 days. Furthermore, the elapsed times for the other two consecutive spawnings were significantly shorter and did not differ among the three treatments ($P > 0.05$). These elapsed times averaged at 6 days.

Egg Quantity and Quality.

The results on egg quantity and quality are summarized in Table 16. The statistical analysis revealed that the average number of eggs spawned per female of the prawns of Treatment 1 was significantly higher than that of Treatment 3, while the prawns in Treatment 2 spawned an intermediate quantity of eggs.

The average number of eggs spawned per spawner of the three treatments did not differ significantly ($P > 0.05$). The number of eggs spawned per spawner averaged at 981,675 eggs.

Table 13. Spawning success of giant tiger prawns fed with fresh natural diets during the 60 days experiment.

Consecutive spawnings	Spawning success		Elapsed time (days)[2]		
	No. of spawners	Percent	Average	Minimum	Maximum
Ab-1[1]	17	68.0	26.3[a]	7	64
2	14	56.1	5.1[b]	2	22
3	13	52.2	3.9[b]	3	10
4	8	32.0	6.4[b]	3	18
5	7	28.2	6.4[b]	3	15
6	2	8.0	8.5[b]	6	11
7	1	4.2	3.0	3	3
8	1	4.2	3.0	3	3

(1) Ab = Eye-stalk ablation.

(2) Time in days after the eye-stalk ablation and the consecutive spawning success.

Significant differences ($P \leq 0.05$) of the average elapsed time are indicated by different letters, i.e. a and b within the column.

Table 14. Spawning success of giant tiger prawns fed with combined diets during the 60 days experiment.

Consecutive spawnings	Spawning success		Elapsed time (days)[2]		
	No. of spawners	Percent	Average	Minimum	Maximum
Ab-1[1]	14	61.9	27.8^{a}	9	49
2	11	48.1	10.6^{b}	3	36
3	8	35.4	5.0^{b}	2	18
4	6	26.5	4.0^{b}	3	9
5	2	8.8	8.0^{b}	3	13
6	2	8.8	3.0^{b}	3	3
7	1	5.0	3.0	3	3
8	1	5.0	3.0	3	3
9	1	5.0	6.0	6	6
10	1	5.0	3.0	3	3
11	1	5.0	3.0	3	3

(1) Ab = Eye-stalk ablation.

(2) Time in days after the eye-stalk ablation and the consecutive spawning success.

Significant differences ($P \leq 0.05$) of the average elapsed time are indicated by different letters, i.e. a and b within the column.

Table 15. Spawning success of giant tiger prawns fed with artificial diet during the 60 days experiment.

Consecutive spawnings	Spawning success		Elapsed time (days) (2)		
	No. of spawners	Percent	Average	Minimum	Maximum
Ab-1 (1)	4	17.1	13.5^{a}	7	36
2	3	13.2	4.5^{b}	3	9
3	2	7.7	3.0^{b}	3	3
4	2	7.7	3.0^{b}	3	3

(1) Ab = Eye-stalk ablation.

(2) Time in days after the eye-stalk ablation and the consecutive spawning success.

Significant differences ($P \leq 0.05$) of the average elapsed time are indicated by different letters, i.e. a and b within the column.

Table 16. Egg quantity and quality of the groups of giant tiger prawns fed with three types of diet.

Egg quantity and quality	Fresh natural diets	Conbined diets	Artifical diet
Ave. no. of eggs per female	1002300 [a]	848478 [ab]	140227 [b]
Ave. no. of eggs per spawner	1473970	1393929	77125
Ave. % fertility	11.1 (0-69.3)	10.6 (0-86.8)	11.0 (0-48.2)
Ave. % hatching	2.8 (0-42.2)	2.1 (0-33.1)	0.2 (0-2.3)
Ave. % metamorphosis from egg to protozoea stage	1.9 (0-36.6)	1.2 (0-29.4)	0.1 (0-1.4)

Note : Significant differences ($P \leq 0.05$) are indicated by different letters, i.e. a and b.

The amount of eggs spawned in the different consecutive spawning did not differ significantly and was not affected by the experimental diets ($P > 0.05$).

Egg fertility, hatching rate and metamorphosis rate varied greatly. The average percent fertility of the total eggs spawned of the three treatments did not differ significantly ($P > 0.05$). The average percent fertility in each consecutive spawning of the three treatments also did not differ significantly ($P > 0.05$). These fertility rates averaged at 10.9%.

The average hatching rates were much lower than the fertility rate. Similar to the fertility rates, the average hatching rate of total eggs spawned of the three treatments did not differ significantly. The average hatching rate in each consecutive spawning of the three treatments also did not differ significantly ($P >0.05$). These hatching rates averaged at 2.3%.

The percent metamorphosis from eggs to the first protozoea stage also showed the same pattern as the hatching rates. These metamorphosis rates averaged at 1.5%.

<u>Mortality</u>.

The overall mortality of prawns in Treatment 1 was significantly higher as compared to those in Treatment 2 and Treatment 3 (Figure 9). The overall mortality of females, regardless the treatments, was significantly higher than the males ($P \leqslant 0.05$; Figure 10).

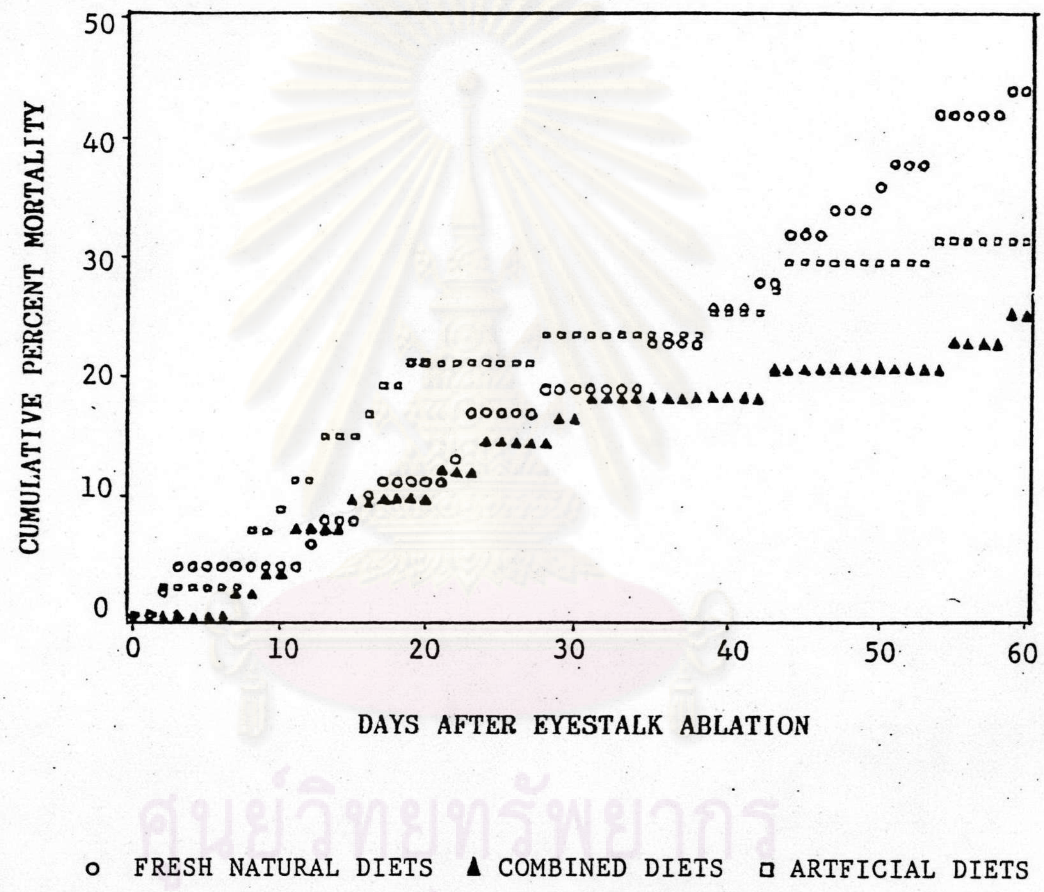

Figure 9. Cumulative percent mortality of giant tiger prawns of different treatments (Experiment 2).

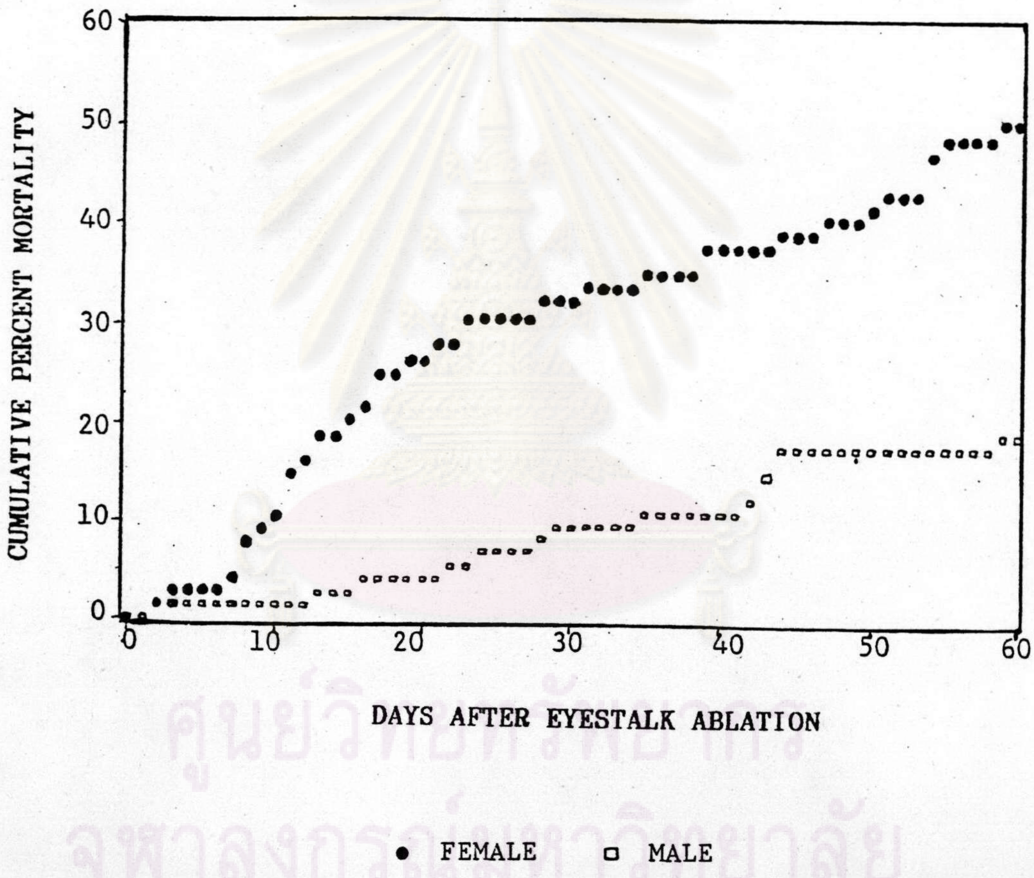

Figure 10. Cumulative percent mortality of male and female giant tiger prawns (Experiment 2).

Water Quality.

Water quality data of the three treatments are summarized in Table 17. No significant differences between Treatments were observed. Each parameter was rather constant and was in acceptable level for penaeid hatchery.

Nutritional Values of the Diets.

The nutritional values of the diets used in this study are summarized in Table 9. Crude protein (51.15%) and crude fat (11.87%) of the maturation diet were relatively higher than the commercial diet. Whereas, crude fiber, crude ash and moisture content of the maturation diet were slightly lower than the commercial diet.

Table 17. Dissolved oxygen (D.O.), pH, temperature, salinity, ammonium-N (NH_4^+), nitrite-N(NO_2^-), nitrate-N(NO_3^-) and phosphate-P(PO_4^{3-}) of the three treatments of Experiment 2.

Treatments	D.O. (ppm)	pH	Temperature (°C)	Salinity (ppt)	NH_4^+-N (mg/l)	NO_2^--N (mg/l)	No_3^--N (mg/l)	PO_4^{3-}-P (mg/l)
Fresh natural diets	6.1 ± 0.6 (5.1 - 7.8)	8.1 ± 0.1 (7.8 - 8.5)	30.5 ± 0.9 (28.5 - 33.1)	33.6 ± 1.3 (30.0 - 35.0)	0.0032 ± 0.0015 (0.0016 - 0.0068)	0.4826 ± 0.1415 (0.3035 - 0.8079)	3.2 ± 0.8 (1.4 - 4.4)	0.33 ± 0.21 (0.16 - 1.02)
Combined diets	6.1 ± 0.5 (5.4 - 7.9)	8.2 ± 0.1 (7.9 - 8.4)	30.5 ± 0.9 (28.4 - 32.5)	33.7 ± 1.3 (30.0 - 35.0)	0.0029 ± 0.0016 (0.0014 - 0.0068)	0.4371 ± 0.1110 (0.2431 - 0.6162)	2.8 ± 0.9 (1.4 - 4.0)	(0.34 ± 0.14 (0.07 - 0.60)
Artificial diet	6.1 ± 0.6 (5.0 - 7.9)	8.2 ± 0.1 (7.8 - 8.4)	30.4 ± 0.9 (27.1 - 33.1)	33.7 ± 1.3 (30.0 - 35.00)	0.0031 ± 0.0012 (0.0015 - 0.0056)	0.4340 ± 0.0975 (0.2630 - 0.5510)	(3.1 ± 1.1 (1.2 - 4.7)	0.38 ± 0.11 (0.29 - 0.55)