CHAPTER III

RESULTS

Osmotic Study of Subadult *Penaeus monodon*

1. Osmoregulation Type

Intermolt stages of subadult *P. monodon* were used for studying salinity effects on osmoregulation. Table 3 shows means and standard deviation of osmotic concentration of prawn haemolymph after being 7 days acclimated in each salinity. It was found that *Penaeus monodon* held in salinity 20 ^{o}/oo to 45 ^{o}/oo can regulate their haemolymph osmolality as hypo-hyperosmoregulator.

The osmotic response of the prawn to a change of salinity can evolve in different ways as a function of medium osmotic pressure. Three different types of osmoregulation are illustrated in Figure 5. The isosmotic point of subadult prawn was about 28 ^{o}/oo or 789 $\pm$ 40 mmol/kg. When salinity was lower than isosmotic point, the osmolality of haemolymph is greater than the medium surrounding and **hyper-osmoregulation** occurs. On the other hand, in hypersalinity, 30 ^{o}/oo to 45 ^{o}/oo in which salinity is higher than isosmotic point, osmolality or osmotic pressure of body fluid are less than seawater, the result is call **hypo-osmoregulation.**

The analysis of covariance indicated that osmoregulation of these prawns were not only affected by salinity but also by eyestalk

Table 3 Mean and standard deviation (SD) of haemolymph osmotic concentration of subadult *Penaeus monodon* at various salinities.

Group	Salinity (o/oo)	Blood OP±SD (mmol/kg) [1]	n (Blood) [2]	Seawater OP±SD (mmol/kg) [1]	n (Seawater) [2]
Ablated prawn	20	716.8 ± 12.19	5	559.4 ± 3.39	5
	25	750.3 ± 36.26	6	719.6 ±11.65	6
	28	789.7 ± 40.16	10	786.3 ± 5.86	3
	30	812.6 ± 38.51	22	931.6 ±59.15	12
	35	924.8 ± 39.53	4	1102.6 ±11.08	5
	40	999.1 ± 68.18	19	1298.9 ±81.33	10
	45	1100.7 ±112.00	3	1343.4 ± 8.85	5
Unablated prawn	20	688.8 ± 12.95	6	559.4 ± 3.39	5
	30	779.3 ± 21.86	18	892.3 ±44.90	12
	35	844.8 ± 42.52	4	1102.6 ±11.08	5
	40	921.5 ± 58.49	11	1258.7 ±66.66	10
	45	817.3 ± 89.36	3	1343.4 ± 8.85	5

[1] OP = average osmotic pressure (mmol/kg)

[2] = sample size

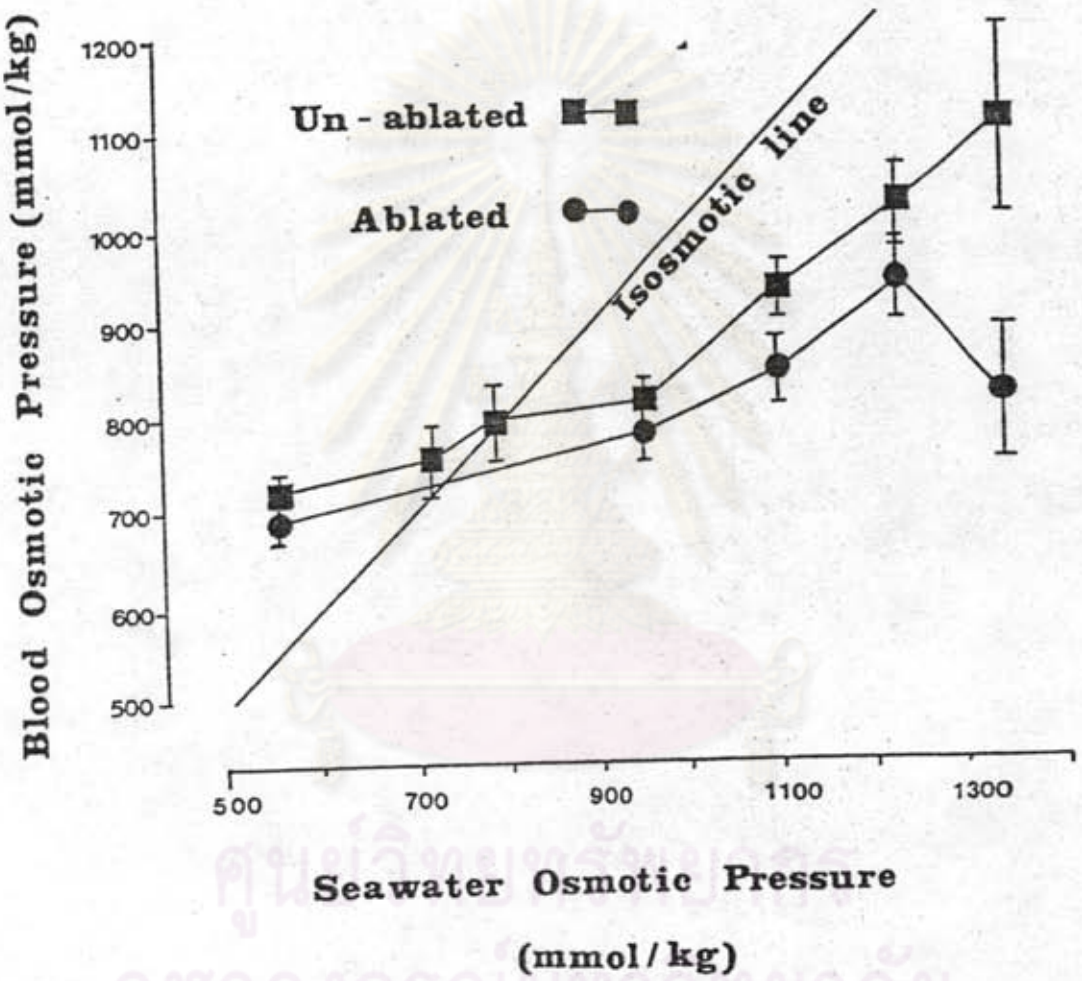

Figure 5 Relationship between osmotic pressure and osmotic pressure of seawater for subadult female *Penaeus monodon* with and without eyestalk ablation.

ablation (Table 4, P<0.01). In salinity range 20 °/oo to 45 °/oo, haemolymph concentration of ablated prawns was lower than that of unablated one. However, tukey HSD showed that haemolymph concentration of ablated prawns was only significantly lower (Table 5, P<0.05) than at salinity 45 °/oo. In addition, Figure 5 and Table 3 indicated that the deviation of osmolality from iso-osmotic line increase after acclimation at high salinities, particularly in ablated prawns.

2. Relationship between Osmoregulation and Hypersaline Acclimation Time

Unablated prawns *Penaeus monodon* were acclimated in salinity 30 °/oo and 40 °/oo for 1, 2, 7, 15, 30 and 45 days. Table 6 shows means and standard deviation of haemolymph osmotic pressure and osmotic gradient of subadult prawn at each time. Hypo-osmoregulation occurred at both salinities. After 24 hours exposure to hypersalinity, the prawn reached the steady state of complete acclimation,the haemolymph osmotic pressure was significantly (ANCOVA: P>0.01) unaltered by the time (Figure 6 and Table 7). However, physiological stress may continue and cause some specimens died.

Ovarian Maturation Experiments of *Penaeus monodon*

1. Adult Prawn Maturation in Hypersalinity

Adult female prawns, collected from Chantaburi province (Oct., 1988), were used to examine the effect of hypersaline acclimation duration. The comparison of ovarian index and condition index were measured at salinity 30 °/oo and 40 °/oo, during 2.5 months acclimation without eyestalk ablation. The regression analysis

Table 4 Analysis of covariance for effect of eyestalk ablation and salinity on haemolymph osmotic pressure (BLOOD) of subadult female *Penaeus monodon*

ANALYSIS OF COVARIANCE

DEPENDENT VAR: HAEMOLYMPH OSMOTIC PRESSURE (BLOOD)

N : 95 MULTIPLE R: 0.859 SQUARED MULTIPLE R: 0.737

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
EYE	111478.038	1	111478.038	30.963	0.0001
SAL	769364.764	1	769364.764	213.693	0.0001
ERROR	331230.357	92	3600.330		

SAL = salinity (30 and 40 $^{o}/_{oo}$)

EYE = eyestalk manipulation (uni-ablated eyestalk and normal eyes or unablated eyestalks)

Table 5 Matrix of pairwise comparison (tukey HSD multiple comparisons) probabilities of haemolymph concentration of subadult intermolt *Penaeus monodon* in salinity between 20 °/oo and 45 °/oo

TUKEY HSD MULTIPLE COMPARISONS
MATRIX OF PAIRWISE COMPARISON PROBABILITIES

	1	2	3	4	5	6	7	8	9	10
1	1.000									
2	0.995	1.000								
3	0.004	0.001	1.000							
4	0.179	0.014	0.524	1.000						
5	0.001	0.001	0.017	0.001	1.000					
6	0.005	0.001	0.975	0.325	0.527	1.000				
7	0.001	0.001	0.001	0.001	0.401	0.001	1.000			
8	0.001	0.001	0.006	0.001	1.000	0.476	0.200	1.000		
9	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	1.000	
10	0.133	0.022	1.000	0.963	0.212	0.999	0.001	0.173	0.001	1.000

Note: Groups definition;

1 = 20 o/oo ; un-ablated prawn
2 = 20 o/oo ; ablated prawn
3 = 30 o/oo ; un-ablated prawn
4 = 30 o/oo ; ablated prawn
5 = 35 o/oo ; un-ablated prawn
6 = 35 o/oo ; ablated prawn
7 = 40 o/oo ; un-ablated prawn
8 = 40 o/oo ; ablated prawn
9 = 45 o/oo ; un-ablated prawn
10 = 45 o/oo ; ablated prawn

Table 6 Mean values and standard deviation (SD) of osmotic concentration of haemolymph, seawater and osmotic gradient between seawater and blood of *Penaeus monodon* at salinity 30 °/oo and 40 °/oo

Salinity (o/oo)	Acclimation Time (days)	n 1/	Osmotic Pressure (mmol/kg)				Osmotic Gradient (mmol/kg)
			Blood (mmol/kg)		Seawater (mmol/kg)		
			$\bar{X}$	SD	$\bar{X}$	SD	
30	1	5	813.2	38.97	881.25	25.41	-68.1 ± 64.38
	2	5	842.8	18.86	897.80	29.15	-55.0 ± 48.01
	7	7	821.3	15.42	922.00	23.99	-100.7 ± 39.41
	15	5	827.4	52.08	973.50	15.78	-146.4 ± 67.86
	30	3	842.7	6.66	952.70	7.37	-100.0 ± 14.03
	45	5	850.6	39.86	971.75	16.60	-121.2 ± 39.86
40	1	4	974.8	27.61	1247.30	44.19	-272.5 ± 71.80
	2	4	935.0	60.49	1272.30	47.77	-337.3 ±108.26
	7	6	937.5	78.89	1244.40	15.44	-270.9 ± 94.33
	15	5	931.4	37.43	1235.30	14.50	-303.9 ± 51.93
	30	5	931.0	37.73	1237.80	8.84	-306.8 ± 46.57
	45	3	971.3	11.06	1264.70	17.04	-293.4 ± 28.10

1/ = sample size

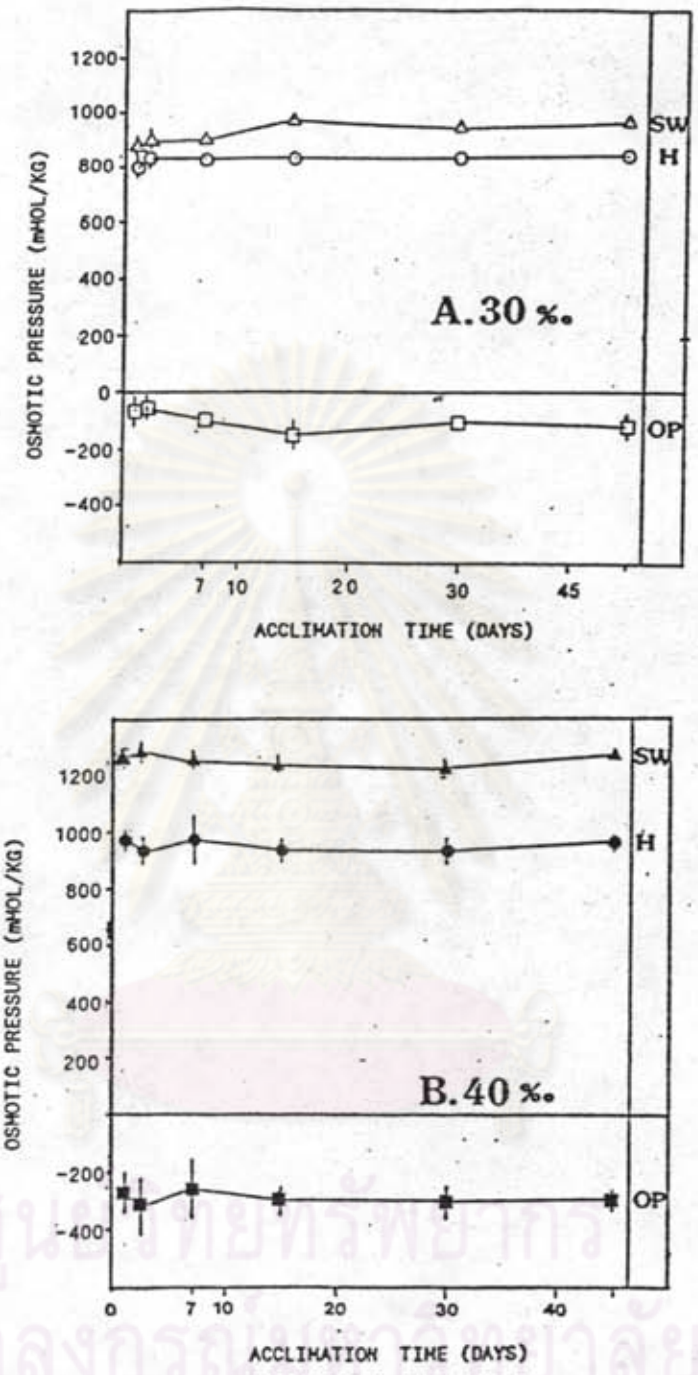

Figure 6 Prawn haemolymph osmotic pressure (H)(o , ●), seawater osmotic pressure (SW) (△,▲), and the difference between the H and SW (□,■) during acclimation in hypersalinity.

A:30 °/oo ; B:40 °/oo

Table 7 Analysis of covariance between acclimation time and haemolymph osmotic pressure (BLOOD) of intermolt subadult prawn, *Penaeus monodon*

ANALYSIS OF COVARIANCE

DEPENDENT VARIABLE : BLOOD (HAEMOLYMPH OSMOTIC PRESSURE)

N: 57 MULTIPLE R: 0.824 SQUARED MULTIPLE R: 0.679

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
SAL	206188.241	1	206188.241	113.474	0.001
TIME	794.635	1	794.635	0.437	0.511
ERROR	98120.584	54	1817.048		

SAL = salinity (30 and 40 $^{o}/oo$)

TIME = acclimation time (1, 2, 7, 15, 30, and 45 days)

shows that there is a significant relationship between prawn ovarian index or gonad index (GI) and acclimation duration in both 30 °/oo and 40 °/oo (Appendix B: Table 12). The relationships are expressed in the following formulas:

$$\% GI_{30} = 0.266 + 0.039 \text{ Time}, (P=0.0001, R^2=0.411)$$

and

$$\% GI_{40} = 0.393 + 0.029 \text{ Time}, (P=0.0001, R^2=0.302),$$

when GI_{30} is ovarian index of prawn acclimated at salinity 30 °/oo, GI_{40} is ovarian index of prawn acclimated at 40 °/oo and Time is acclimation duration in days. The r^2 value indicated that only 41.1% and 30.2% could be described by the equations at 30 and 40 °/oo, respectively. The relationship between ovarian index and acclimation duration in both salinity are shown in Figure 7.

The data demonstrate that ovarian index of prawn was affected by hypersaline acclimation. Over the 2.5 month acclimation period , ovarian index increases either at 30 °/oo or 40 °/oo compared to initial group (Appendix B: B-1). However, there was no significant difference between the gonad index increasing rates at the two salinities (Table 8, P>0.05).

During acclimation period, prawn condition index (fatness) was not seemed to be change with time (Appendix B: Table 16, P>0.05). An ANCOVA indicates that there was no significant effect of salinity on the relationship of condition index and acclimation time (Appendix B: Table 17, P>0.05). Furthermore, there was no interaction between condition index and gonad index (P>0.05) (Appendix B: Table 18).

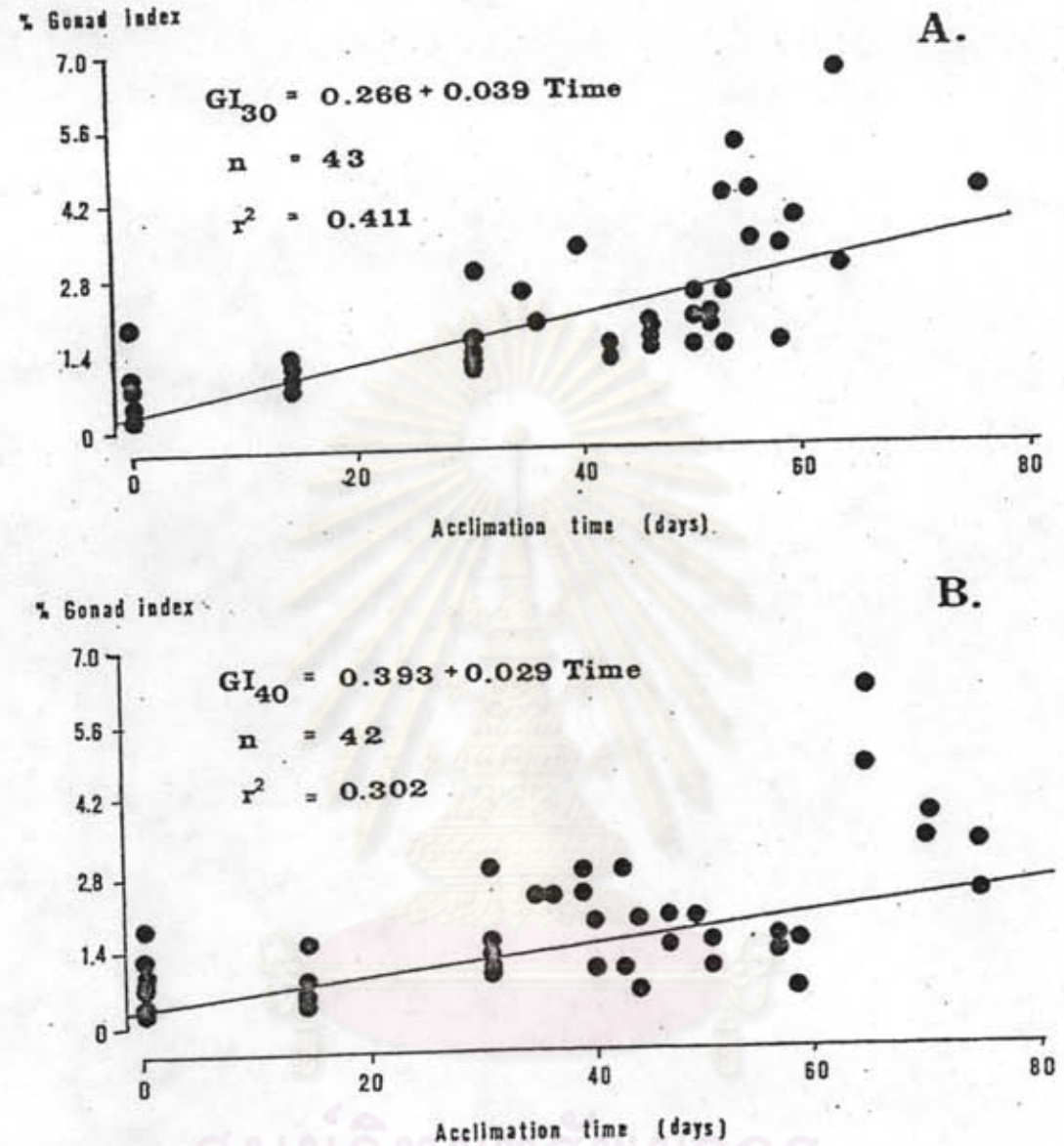

Figure 7 Relationship between gonad (ovarian) index of adult female prawns, *Penaeus monodon* and acclimation duration under hypersaline condition. A: 30 °/oo ; B: 40 °/oo

Table 8 The Analysis of Covariance of the hypersalinity effect on relationship of ovarian index (GI) and acclimation duration (TIME) of adult female prawns, *Penaeus monodon*.

ANALYSIS OF COVARIANCE

DEPENDENT VARIABLE: GI(OVARIAN INDEX)

N: 85 MULTIPLE R: 0.600 SQUARED MULTIPLE R: 0.360

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
SAL	1.375	1	1.375	1.307	0.256
TIME	47.607	1	47.607	45.257	0.001
ERROR	86.258	82	1.052		

SAL = salinity (30 and 40 $^{o}/_{oo}$)

TIME = acclimation time (covariate data)

2. Prawn Size, Hypersaline Acclimation and Eyestalk Ablation Effect on Maturation of Subadult *Penaeus monodon*

The result of this experiment was obtained from two size groups of subadult female prawns; 5-month old, in closed recirculating water system. Mean values and standard deviation of body weight, carapace length, condition index, ovarian index, and mortality rate are shown in Table 9.

The comparison of ovarian index and condition index was made at salinity 30 °/oo and 40 °/oo. The effects of size groups of subadult prawn were determined and found that size of subadult broodstock could influence the ovarian development under hypersaline condition with eyestalk ablation. However, eyestalk ablation could accelerate ovarian development only in large size subadult prawns. (Appendix C: Table 19). Eyestalk ablation did not affect merely the ovarian index but also mortality. Percent mortality of ablated female, prawns were higher than unablated prawns (Table 9).

Acclimation for 30 days at salinity 30 °/oo and 40 °/oo had no significant effect on ovarian development of subadult prawns in both size groups but resulted in the improvement of condition index especially in the small size subadult prawns (Appendix C: Tables 20 and 21). The condition index of small size prawn was less than the large group.

However, data in Table 9 indicated that acclimation at 30 °/oo was probably better for ovarian maturation, survival, and condition index than at 40 °/oo.

According to the analysis of these data it may be concluded that maturation process of subadult *Penaeus monodon* could be

Table 9 Ovarian index, condition index, mortality rate of two size groups subadult, *Penaeus monodon*

Size Group	Salinity (o/oo)	Time (days)	Eye Stalk	Body Weight(g)		Carapace Lenght(cm)		Condition Index		% Ovarian Index				Mortality (%)
				X	SD	X	SD	X	SD	X	SD	n	max	
Small Size Subadult	20	0	N	28.722	1.106	3.700	0.085	7.762	0.219	0.349	0.083	6	0.485	-
	30	30	N	32.554	2.935	3.707	0.148	8.782	0.692	1.263	1.558	6	4.434	40
	30	30	A	31.402	4.027	3.773	0.170	8.304	0.746	1.167	0.891	5	2.732	50
	40	30	N	32.338	2.270	3.797	0.071	8.514	0.545	0.491	0.443	7	1.486	30
	40	30	A	35.327	4.228	4.309	0.254	8.724	0.559	0.415	0.113	4	0.559	60
Large Size Subadult	25	0	N	52.374	9.524	4.532	0.352	11.533	1.652	0.543	0.312	10	1.049	-
	30	30	N	56.200	5.428	4.736	0.260	11.859	0.644	0.783	0.508	8	1.839	20
	30	30	A	56.165	6.156	4.765	0.373	11.770	0.551	1.205	0.619	6	1.996	40
	40	30	N	55.108	8.698	4.595	0.071	10.935	0.593	0.652	0.373	7	1.388	30
	40	30	A	56.253	5.399	4.893	0.227	11.487	0.821	1.649	0.954	6	3.381	40

induced by using hypersaline condition with or without eyestalk ablation. However, large size subadult was more effective for maturation induction at hypersalinity and eyestalk manipulation than the small size group.

3. Manipulation Techniques to Induce Ovarian Maturation of *Subadult Penaeus monodon*

Table 10 shows mean values and standard deviation of prawn size (body weight and carapace length), condition index and ovarian index of subadult prawns. Furthermore, the explanation of operational techniques (TRM) for induced maturation is also shown in Table 10 and Table 2 in methodology.

The experiment was performed using for large size of subadult female *Penaeus monodon*, 5-month-old. These prawns were collected from intensive farms.

Effects of short-term acclimation (15, 30, and 45 days), hypersalinity (30 ^{o}/oo and 40 ^{o}/oo), eyestalk manipulation, and the combination were mainly studied on ovarian development in closed recirculating water system.

With acclimated duration, the ovarian index of prawns seemed to be improved signally either at 30 ^{o}/oo or 40 ^{o}/oo (Table 10), but there was no statistical significance ($P>0.05$). Ovarian index of ablated and un-ablated prawn were compared at 30 and 40 ^{o}/oo. The result confirmed that ovarian maturation was dependeny on eyestalk ablation. That is, ovarian index of ablated prawn was significantly ($P<0.05$) higher than the unablated ones (Appendix D: Tables 22 and 23). An analysis of variance and tukey HSD test indicated that combination of hypersaline acclimation and eyestalk manipulation

Table 10 Mean values (X) and standard deviation (SD) of body weight, carapace length, condition index and ovarian index of subadult *Penaeus monodon* that acclimated by various manipulation techniques (TRM).

Code Treatment (TRM)	Salinity (o/oo)	1/ Eye	First hypersaline acclimation time (days)	Second hypersaline Acclimation			Body Weight (g)		Carapace Length (cm)		Condition Index		x OvarianIndex			
				Salinity (o/oo)	1/ Eye	Acclimation Time (days)	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	Max	n
1	30	N	15	-	-	-	59.999	10.739	4.631	0.305	12.451	1.445	0.914	0.480	1.879	12
1	30	N	30	-	-	-	54.168	5.030	4.533	0.178	11.946	0.927	1.380	1.373	4.461	8
1	30	N	45	-	-	-	59.306	10.753	4.786	0.297	12.428	2.436	0.680	0.275	1.059	6
2	30	A	15	-	-	-	58.696	6.004	4.664	0.224	12.283	0.703	1.581	1.332	4.136	9
2	30	A	30	-	-	-	51.332	2.950	4.445	0.074	11.549	0.648	2.305	1.297	4.853	9
2	30	A	45	-	-	-	62.234	1.306	4.915	0.109	12.662	0.080	1.005	0.159	1.189	3
3	40	N	15	-	-	-	55.853	9.404	4.536	0.185	11.528	1.075	0.719	0.376	1.529	21
3	40	N	30	-	-	-	55.087	6.336	4.722	0.183	11.638	0.962	0.927	0.393	1.590	13
3	40	N	45	-	-	-	-	-	-	-	-	-	-	-	-	*
4	40	A	15	-	-	-	57.440	8.608	4.653	0.272	11.865	1.452	2.204	1.977	5.807	18
4	40	A	30	-	-	-	56.179	7.135	4.717	0.222	11.895	1.209	1.450	0.472	2.014	7
4	40	A	45	-	-	-	-	-	-	-	-	-	-	-	-	*
5	30	N	30	30	A	15	55.946	5.147	4.795	0.341	11.660	0.508	1.533	0.443	2.059	4
6	30	N	30	40	N	15	54.935	8.349	4.716	0.143	11.629	1.529	0.606	0.099	0.735	5
7	40	N	30	30	N	15	56.151	5.466	4.735	0.211	11.840	0.656	0.873	0.473	1.296	4
8	30	N	30	40	A	15	55.992	3.908	4.677	0.154	11.967	0.633	1.405	0.613	2.469	6
9	40	N	30	30	A	15	57.286	7.858	4.706	0.159	12.146	1.267	1.810	1.206	3.165	5
10	30	A	30	40	-	15	52.701	5.905	4.744	0.203	11.092	0.898	1.920	1.028	4.151	7
11	40	A	30	30	-	15	58.541	6.019	4.685	0.267	12.539	1.572	3.332	1.583	6.887	8
12	25-28	N	0	-	-	-	53.978	8.089	4.648	0.312	11.591	1.342	0.627	0.317	1.054	24

1/ Eyestalk manipulation ; N=normal eyes; A=uni-ablated eye

significantly (Tables 11 and 12 ,$P<0.01$) influenced the ovarian index. The eyestalk ablation coupled with two steps hypersaline acclimation as in TRM-11 method; ovary would greatly develop and oocyte maturation occurred. Ovarian index of prawns by such methods were the highest one. The maximum ovarian index was 6.887%

By paraffin sectioning, it appeared that the largest ovary dominantly contained mature ova and some degenerated ova (Figures 11 and 12). The oocyte size and shaped did not differ from general model of Bell and Lightner (1988). This result confirmed that induced maturation technique did not affected oocyte morphology and development.

Condition index of subadult prawn in these experiments were unaltered by acclimation time and induction techniques. An ANOVA showed no significant affect of eyestalk ablation and hypersaline acclimation as well as their combination on condition index ($P<0.05$) as showed in Table 24 (Appendix D).

Molting of Broodstock *Penaeus monodon*

There were several indications of an impending molt. By observation, the prawn stopped feeding approximately 48 hours before molting. They usually inhabited unactively at the bottom of the rearing tank. After molting, the animals did not feed regularly until approximately 24 hours. The old exuvium was generally eaten, but the harder carapace was always left. Molting interval could be recorded from the tagging number on which carapace. Most of prawns molted at night, but some could molted at day time in the dark tank with low light intensity.

Table 11 Analysis of variance for effects of manipulation techniques (TRM) on ovarian index (GI) of subadult *Penaeus monodon*

DEPENDENT VARIABLE : GI(OVARIAN INDEX)

N : 171 MULTIPLE R : 0.570 SQUARED MULTIPLE R : 0.325

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
TRM	79.354	11	7.214	6.960	0.0001
ERROR	164.815	159	1.037		

Note : SOURCE = source of variation

DF = degree of freedom

TRM = manipulation techniques (The definition of manipulation techniques (TRM) is showed in Tables 2 and 9

Table 12 Computation output of tukey HSD test for manipulation techniques (TRM) effect on ovarian index (GI) of subadult *Penaeus monodon*

TUKEY HSD MULTIPLE COMPARISONS

MATRIX OF PAIRWISE COMPARISON PROBABILITIES

	1	2	3	4	5	6	7	8	9	10	11	12
1	1.000											
2	0.921	1.000										
3	1.000	0.014	1.000									
4	0.954	1.000	0.535	1.000								
5	0.996	0.999	0.193	1.000	1.000							
6	1.000	0.311	1.000	0.616	0.687	1.000						
7	1.000	0.884	1.000	0.925	0.989	1.000	1.000					
8	1.000	0.980	0.544	0.996	1.000	0.864	0.998	1.000				
9	0.985	1.000	0.641	1.000	1.000	0.726	0.969	1.000	1.000			
10	0.931	1.000	0.250	1.000	0.999	0.463	0.894	0.991	1.000	1.000		
11	0.001	0.001	0.003	0.377	0.001	0.003	0.001	0.001	0.094	0.054	1.000	
12	1.000	0.182	1.000	0.557	0.550	1.000	1.000	0.784	0.667	0.371	0.001	1.000

Note : The TRM definition is showed in Tables 2 and 9

The effects of body weight, hypersaline acclimation, and eyestalk ablation on molting interval of *Penaeus monodon* were performed for subadult (5-month-old) and adult (8-month-old) prawns at salinity at 30 °/oo and 40 °/oo. Mean values and standard deviation (SD) of molting interval were summarized in Table 13. For subadult prawn, the result indicated that molting interval of *Penaeus monodon* was not affected by body weight, salinity, and eyestalk ablation ($P>0.05$)(Appendix E: Table 25).

In case of adult prawn, molting interval was independent of sex ($P>0.05$) and salinity acclimation (Appendix E: Table 26). In contrast, there was a significantly difference ($P<0.01$) molting interval of prawn with age-size group (Appendix E: Tables 27 and 28). The subadult prawns showed shorter molting interval period than that of the adult ones.

Water Quality

1. Nitrogenous wastes

Ammonia, nitrite and nitrate were lower than that of the controlled limits as mentioned in methodology.

NH_4^+ was 0.0 - 0.50 mg/l (ppm)

NO_2^- was 0.0 - 0.15 mg/l (ppm)

NO_3^- was 0.0 - 50.0 mg/l(ppm).

2. Calcium Determination

Calcium (free ions) concentration of rearing water in recirculating water system was determined by a complexometric titration (Kremling, 1976). The result is showed in Table 14. The

Table 13 Molting interval of *Penaeus monodon* under laboratory condition, hypersaline acclimation and eyestalk ablation.

Group	Eyestalk manipulation[1]	Salinity (o/oo)	Sex	Body Weight (g)		Carapace length (cm)		Molting Interval(days)		n[2]
				X	SD	X	SD	X	SD	
Subadult 5-month old	N	25	F	52.19	3.20	4.45	0.11	15.50	1.72	10
	N	30	F	52.97	6.10	4.59	0.22	15.40	2.41	34
	N	40	F	56.86	6.00	4.58	0.19	15.90	2.68	25
	A	30	F	53.94	6.20	4.70	0.20	15.68	1.89	22
	A	40	F	54.92	4.60	4.35	0.10	15.67	2.60	24
Adult 5-month old	N	30	F	95.71	8.3	5.54	0.28	20.8	3.79	12
	N	30	M	86.25	3.5	5.01	0.15	23.8	3.2	4
	N	40	F	98.4	6.1	5.07	0.47	23.2	5.21	5
	N	40	M	86.6	7.1	4.89	0.29	21.3	3.55	7

1/ N=normal eyes; A=uni-ablated eye

2/ sample size

Table 14 Calcium ions concentration in closed recirculating water system.

Treatment		Volumn of Sample (ml)	EGTA (ml)	Concentration of Calcium (g/l)	Calcium : Salinity (X 100)
Salinity (o/oo)	Acclimation Time (days)				
35 *	-	-	-	0.4236	1.210
30(a)	0	10	4.90	0.1921	0.640
30(a)	15	10	8.80	0.3450	1.150
30(a)	30	10	10.92	0.4280	1.427
30(b)	0	10	5.15	0.2020	0.673
30(b)	15	10	7.85	0.3080	1.027
40(a)	0	10	9.40	0.3680	0.920
40(a)	30	10	15.37	0.6020	1.506
40(b)	0	10	10.25	0.4018	1.005
40(b)	15	10	14.55	0.5704	1.426

(a) =Seawater was prepared from brine seawater stock 70 ppt
(b) =Seawater was prepared from brine seawater stock 55 ppt
* = Standard sea water value

brine water from salt farm having salinity between 55 °/oo to 70 °/oo was found to have Ca:S °/oo ratio lower than normal seawater. After diluting the brine water with tap water and recirculating it through the filter bed the Ca:S °/oo ratio started to increase up to the normal ratio. However, the increase in calcium concentration depended on the duration the brine water or diluted brine water was recirculated through the filter bed.

Diluted water (30 °/oo and 40 °/oo) which was prepared from brine water with salinity of 55 °/oo had higher calcium concentration and Ca:S °/oo ratio than the water prepared from brine water with higher salinity (70 °/oo).

Great variation of calcium concentration in tap water that was used for dilution was found. Therefore, it was one of the important factors that caused variation in calcium concentration of diluted water.

In conclusion, calcium concentration in this rearing system was dependent on four factors; salinity of diluted water and brine water; calcium concentration of tap water; and subsand filtration rate.

Ovarian Development

1. Hypersalinity and Eyestalk Ablation Effects on Mature Oocyte Size and Morphology

Oocyte sizes were measured from subadult and adult prawns at different maturation stage. It appeared that hypersalinity (30 and 40 °/oo) and eyestalk ablation had no influence on oocytes morphology.

Moreover, the sizes of mature oocytes of subadult and adult prawns; ablated and unablated, were not different by salinity acclimation (30 °/oo and 40 °/oo).

2. Descriptive Histological Studied on Ovaries development of *Penaeus monodon*

The female reproductive system of *Penaeus monodon* conforms to general decapod plan (Bell and Lightner, 1988; Motoh, 1981). The paraffin histological method can be used to assess oocyte developmental stage of *Penaeus monodon*. The ovary condition of *P. monodon* is classified into six stages as undeveloped, developing, nearly gravid, mature or gravid, inactive, and redeveloping. The stages of ovarian development are based on external appearance. Depending on oocyte size, yolk deposition and contact with the follicle cells, ovaries are classified into the following six developmental phases:

2.1 Undeveloped Stage

This stage is typically found in newly procured and control group of prawns. The ovaries are small, rod-like in appearance, occupied a small part of the cephalothoracic cavity, with white color, which is translucent and invisible through the exoskeleton (Figure 8). The reproductive cells or gametes can be seen by sectioning the germinal layer or zone of peripheration where they are typically found. Within this zone oogonia are also found which upon cell division produce primary and secondary oocytes. The oocytes migrate away from the germinal layer toward the periphery of the central ovarian cavity. Oocytes are found in nodules or cysts,

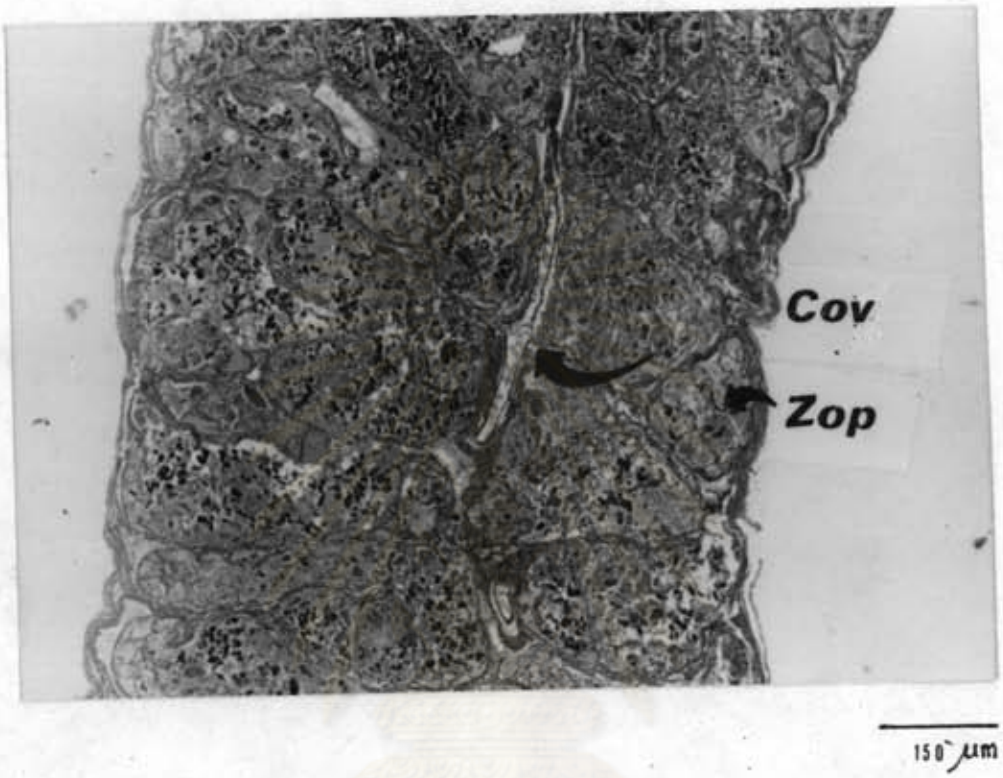

Figure 8 Overall view of an immature ovaries. Primary oocyte or previtellogenesis cells are produced from germinal layer of oogonia, or zone of periferation (Zop). Oocytes are very small and located in cysts. The cytes are separated by hemal sinuses and connected with the central ovarian cavity (Cov).

surrounded by follicle cells, and the cysts are separated by hemal sinuses. Oocytes are very small, rounded cells with large nucleus. Early nucleolar cells stained deep violet with haematoxylin. The immature ova or primary oocyte are uniform in size with a diameter of about 16.47 to 33.56 μm. Follicle cells surrounding the oocytes become apparent at this stage. The ovarian indices range 0.1 to 1.8%

2.2 Developing Stage

Ovarian color during this stage of ovaries is semi-transparent, or milky to gray. Developing ovaries are easily distinguished from other tissues. Subsequently, the ovaries became enlarged and more convoluted. Ovaries extend anteriorly to just behind the eyes and posteriorly to the abdominal segment during the late developing stage. Ovaries contain oogonia cells, follicle cells and various size of oocytes (Figure 9). Vitellogenesis, or yolk deposition begins in primary developing phase. Oocytes are almost in late perinucleolar stage and contain early yolk vesicle cells. Oocyles sizes range from 18.89 to 89.89 μm diameter. In the later developing stage, oocytes begin to lose their affinity for haematoxylin stained and contain yolk granules in cytoplasm. The cytoplasm now stained pink with eosin. Follicle cells surround the developing ova, and the ova radiate towards the ovarian wall.

2.3 Nearly gravid Stage

Ovaries are large and turgid. Their overall color range from gray to green-gray and they are visible through the prawn's exoskeleton. The ovaries contain previtellogenic and vitellogenic ova (Figure 10). Young oocytes, in previtellogenesis, line the central ovarian, cavity. Early yolk vesicle oocytes make up

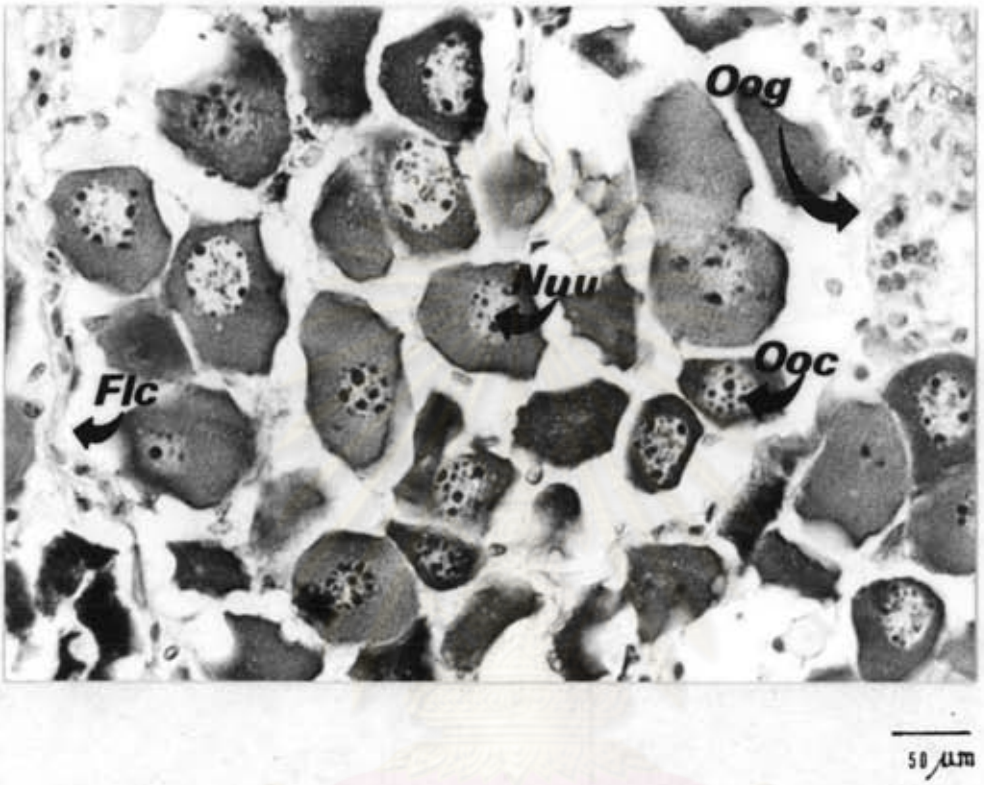

Figure 9 Enlarged view of oocytes (Ooc) in late perinucleolar stage and oogonia cells (Oog). Most of the oocytes begin to contain yolk granules in the cytoplasm. Follicle cells (Flc) are visible surrounded the cyst. Bar length = 50 microns.

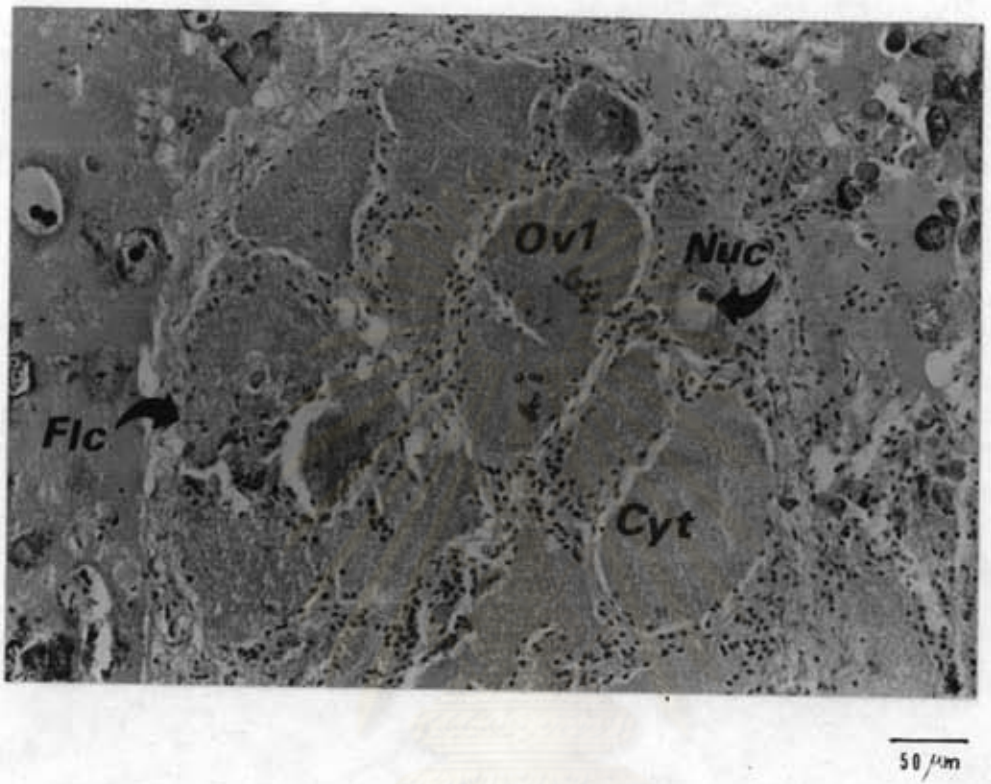

Figure 10 Enlarge view of nearly ripe ovaries. Young oocytes are still developing. Follicle cells (Flc) surround each cyst, and maturing ova (Ovl) make up the cysts wall. Significant increases occur in ova nuclear (Nuc) and cytoplasmic (Cyt) size; and the nuclei or aggregation of chromatin from distinct marginated bodies. Bar length = 50 microns.

most of the ovaries along with a few late yolk vesicle oocytes. Follicle cells surround each maturing ova and make up the cyst wall. Previtellogenic oocytes have an intensely basophilic amorphous cytoplasm and stain hematoxylinophilic, while the cytoplasm of vitellogenic ova are highly vacuolated and acidophilic at the periphery, but basophilic around the nucleus. They stain eosinophilic. In this stage, maturing ova significantly increase their bodies are cytoplasmic and nuclear size. Nuclei dot shaped numerous and distributed around the nucleus. Follicle cells become reduced in size. Oocyte size in primary vitellogenesis is in range 88.50 $\pm$ 22.68 μm diameter and in secondary vitellogenesis is 41.50 to 172.37 μm.

2.4 Mature or Gravid Stage

The ovaries are green-gray in color and more swollen than in the previous stage. They fill virtually all space between other organs. This stage is recognized by the presence of a margin characteristics of peripheral rod-like bodies. Most oocytes are in the cortical vesicle and germinal vesicle stage. A few oocytes are in primary vitellogenesis stage. Mature ova are tightly packed together and distorted in shape. These ova are surrounded by a thin layer of follicle cells. The oocytes in the cortical vesicle stage average 186.32 $\pm$ 29.5 μm in diameter (Figure 11).

2.5 Inactive Stage

Ovarian color of the ovary is generally off-white, while ovarian wall is commonly very thick and convoluted in some areas. Varying degrees of recovery from past ovulations are evident.

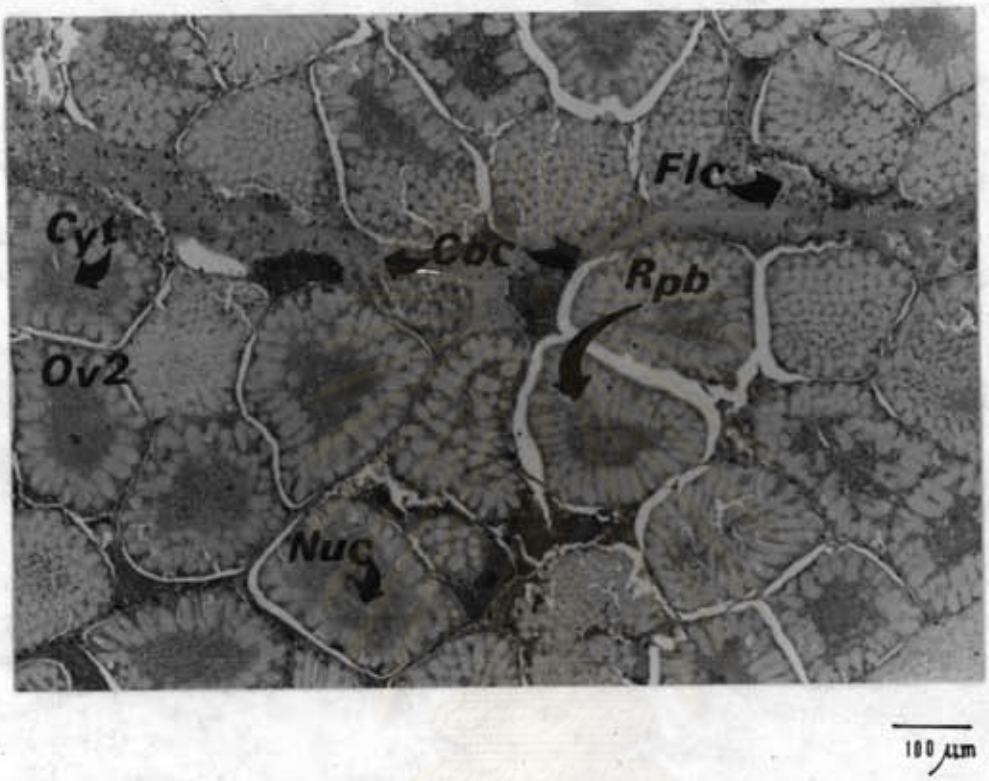

Figure 11 Overall view of mature ovaries from adult *Penaeus monodon*. The ovaries consist primary of mature ova (Ov2), with a few oocytes. The mature ova consist of rod-like periphery bodies (Rpb), yolk globules and nuclei in nucleus (Nuc). Follicle cells (Flc) appear surround the ova and in the central ovaries cavity (Coc). The ovarian index for this specimen was 6.17% . Bar length = 100 microns.

In recovered ovaries, the oocytes occur in previtellogenesis similar to what happens in the undeveloped stage. In some cases, massive reabsorption of ripe un-oviposited ova appears to be occurred (Figure 12). Mature ova (secondary vitellogenesis) of broodstock which reach this ovarian stage without mating, show signs of breakdown, including unsystematic orientation of the rod-like peripheral bodies and followed by reabsorption.

2.6 Redeveloping Stage

Ova characteristics of the developing stage are present, but tend to arise more randomly from the germinal layer. Intersperse is residual ova undergoing various degree of reabsorption (Figure 13). Moreover, oocyte development is in various stages; including perinucleolar, cortical vesicle and germinal breakdown stage.

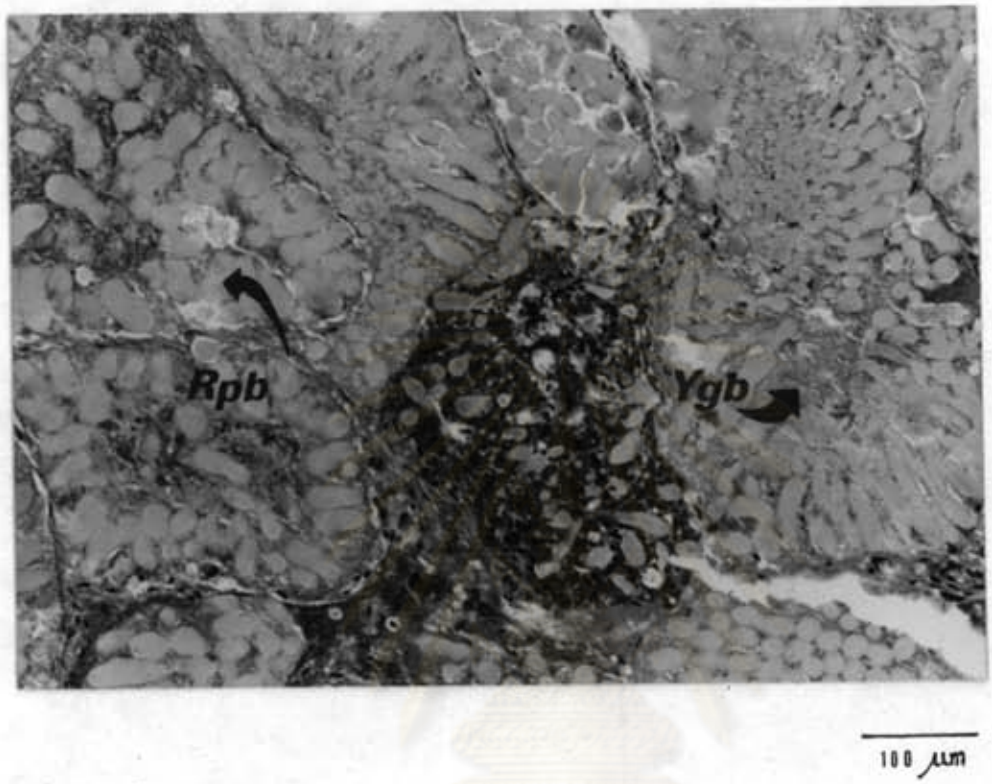

Figure 12 Enlarged view of mature ova undergoing breakdown, to be followed by reabsorption. Some of the cellular component are still recognizable, including rod-like periphery bodies (Rpb), yolk globules (Ygb), and follicle cells (Flc). Bar length = 100 microns.

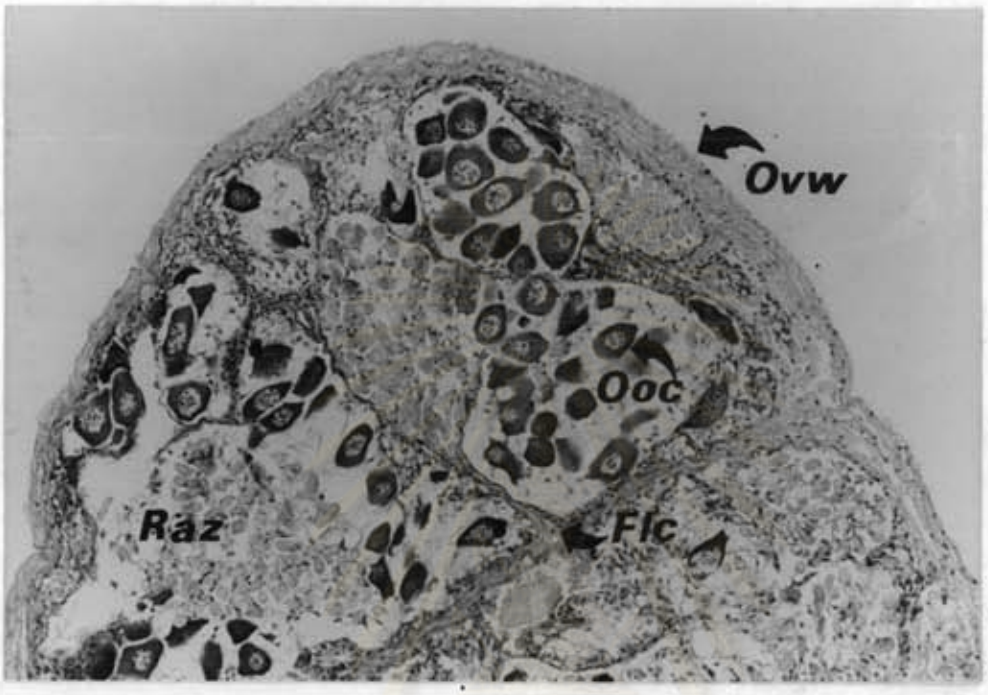

Figure 13 Overall view of an ovaries from subadult 70 grams which had released only a portion of her mature ova without mating. Mature ova are reabsorped. Oocytes (Ooc) were oocytes (Ooc) enlarged and located in each cyst. Follicle cells (Flc) surround each cyst. The ovary wall (Ovw) is thick. Raz is a defined reabsorption zone. Bar length = 100 microns.