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APPENDICES

APPENDIX A

Paraffin method for ovarian tissues for *Penaeus monodon*

(Modified from : Baker, J.R. 1958; Bell, T.A and Lightner, D.V. 1988; Humason, G.L. 1967; Gray, P. 1958; and Willey, R.L. 1971)

1. Fixation

Cell death leads to rapid breakdown of cell contituents as a result of enzymes and action of external agents such as bacteria. The aim of tissue preparation for microscopic investigation is to the preserve cells in a state as close to the living condition as possible. Tissue fixation is necessary to prevent changes in tissue cells. Selected tissues must be placed in fixative solution as soon as possible after death. Fixatives or fixing solution should be selected for; (1) rapid penetration, (2) separation of protoplasmic from the aquous phase. This is done to convert cell parts into materials that will remain insoluable during subsequent treatment, in their original position, (3) protection of tissues against distortion and shrinkage, (4) properties enabling cells to be seen through a light microscope by changing the refractive index of the cell organelles, or by making them stainable.

Primary fixatives solutions of single chemicals seldom have all the properties of a good fixative. Some fixative are, however, adequate for routine work. One such fixative is formalin.

Formalin is a non-coagulent fixative which reacts with protein to form linkage between adjacent protein chains. It has a moderate penetration rate. Its action is slow, and somewhat incomplete unless tissues are left in it for some time. Formalin is a good fixative for lipids, since it does not dissolved lipids or fats. It does not fix soluable carbohydrate, but it does dissolve some glycogen and urea. Formalin reacts most efficiently in a buffered solution at about pH 7.5 to 8.0.

Gonadal tissue of *Penaeus monodon* were fixed in buffered formalin. Each tissue section was covered by at least ten times its own volume. After fixation was accomplished (>7 days), excess formalin must be washed from the tissue with running water for at least 18 hrs. After washing, the tissue was transfered to 50% alcohol for 1 hr, and then to 70% alcohol. Gonadal tissues were stored for several weeks or months in 70% ethyl alcohol, but it is always desireable to dehydrate and embed as soon as possible. Storage in alcohol for long periods of time (a year or longer) tends to reduce the staining capacity of tissues.

Buffered Formalin solution (Humerson,1967)

- 10% Formalin (10 volumes of formalin or 40% formaldehyde saturated aqueous solution, to 90 volumes of distilled water) 1,000 ml.
- Sodium acid phosphate, $NaH_2 PO_4.H_2O$ .....4.0 g.
- Anhydrous disodium phosphate, $Na_2 HPO_4$ .....6.5 g.

Note that tissue may remain in fixative indefinitely. Wash in water to remove formalin.

2. Dehydration

Before embedding tissue in paraffin, all water must be removed. Dehydration is usually achieved in a series of gradually increasing percentages of alcohol in water. Gradual changing through 30, 50, 70, 80, 95% to absolute alcohol is said to reduce some shrinkage or distortion. If time does not permit such a series, the 30% and 80% steps, and even the 50% change may be eliminated without great tissue damage. Ethyl alcohol dilution is usually accomplished using 95% alcohol diluted with distilled water.

3. Clearing

Clearing is a necessary intermedietly step between dehydration and infiltration, since alcohol used for dehydration will not dissolved or mix with paraffin. Some fluid able to mix with both alcohol and paraffin must therefore be used before infiltration can take place. Toluene and xylene are two reagent commonly used for this purpose. Toluene is probably the safest to use; and it does not harden as much as xylene.

4. Infiltration with paraffin or paraplast

Paraplast is an excellent embedding medium, consisting of a rigidly controlled mixture of paraffin and several plastic polymers of defined molecular weight. Paraplast is smooth and cuts with less compression than paraffin.

After ovarian tissue was well cleared with toluene, it is transferred to saturated toluene with paraplast or 1:1 ratio of toluene and paraplast. The tissue should remain in this solution for 30 to 60 mins. The tissues is then removed with a warm spatula and

transferred to melted paraplast in a heated oven. The oven temperature should be heat enough to maintain the paraffin in a melted stated, no higher. After 30 to 60 mins in the first bath, the tissue is transferred to a second container of paraplast for a similar length of time. Two changes of paraplast or paraffin are sufficient for most normal requirments (Humerson, 1967).

5. Embedding

As soon as the tissue was throughly infiltrated with paraplast or paraffin, it is ready for embeding provided that the paraffin has solidify around and within the gonad tissues. Place the tissue in a small container filled with melted paraplast (58 to 62 C). Before transferring the tissues, warm the transfer instruments to prevent paraffin congealing on metal surface. Handle the tissue as rapidly as possible to prevent paraffin from solidifying before the tissue is positioned.

Embedding boxes were made from cast-metal lead L's, placed on a small flat metal (5x5 cm^2) sheet. The box can be adjusted to several size for embedding, and being metal, they cool the paraffin or paraplast more quickly than glass.

Schedule for Paraffin Method

(For gonadal tissues blocks 10 mm in size)

The schedule for ethyl alcohol use is shown below:

1. Fix in *bufferred formalin* overnight or longer.
2. Wash in runing tap water,for 6 to 8 hrs or overnight.
3. Transfer to 50% alcohol: 1 hr
4. Transfer to 70% alcohol: 1 hr

5. Transfer to 90% alcohol: 1/2 hour
6. Transfer to 95% alcohol: 1/2 hour
7. Transfer to absolute ethyl alcohol #1:1/2 hour
8. Transfer to absolute ethyl alcohol #2:1/2 hour
9. Transfer to absolute Toluene #1:15 min
10. Transfer to absolute Toluene #2:15 min
11. Transfer to saturated paraplast in toluene:1/2 hour
12. Transfer to melted paraplast # 1:1/2 hour
13. Transfer to melted paraplast # 2:1/2 hour
14. Embed

6. Sectioning

1. Embedded blocks are trimmed into squares or rectangles. The side edges need not be parallel.

2. Wooden blocks are covered with a layer of paraffin, and tissue blocks are pressed firmly into the molten paraffin before it cools. After the block was cools, it is ready for sectioning.

3. Clamp the wooden block in the microtome carrier.

4. Cut sections on the microtome with 10 microns thickness. while cutting, place the ribbon sections on sheets of clean paper in clean boxes with covers.

7.Fixing and Mounting

Cut sections are attached to slide with egg albumen and water. It is essential that slides be absolutely clean to insure adherence of the sections throughout the staining procedure. Dilute a few drops of albumen fixative with about 10 ml of distilled water, and float the sections on the warm solution. Albumen acts as a surface tension

depressant and aids in closer attraction of sections to slides. A water bath at 40 to 45 C was used to help maintain a warm temperature during spreading (albumen was also added to the water bath).

When removing cut sections from the microtome knife, or from the box, stretch them as flat as possible placing them slide surfaces. After they have warmed, they can be pulled more easily to their original shape with dissecting needles. Dip the slide under them, and with a needle hold them against the slide while removing them from the water bath.

Preparation of Egg Albumen Fixative (Humerson, 1967)

1. Egg of white .....50 ml
2. Glycerol .....50 ml
3. Formalin (40%) .....a few drops

First, mix egg white with a few drops of glacial acetic acid. Pour into cylinder and let stand until the air brings suspended material to the surface (overnight). Pour off liquid from the cylinder bottom and added an equal volume of glycerol. A few drops of formalin (1:100) prevents mold growth.

8. Stains and Staining

Unless tissues are stained, all organelled lack sufficient contrast to be easily distinguished by the human eye. Two stains of contrasting colors (stain and counterstain) are used to differentiate between nucleus and cytoplasm.

8.1 Stains: Hematoxylin and Mordant

Hematoxylin is a natural dye, extracted from the heartwood of logwood trees (*Hematoxylin campechiawum*). Its color must be allowed to develops after oxidation to hematin. Oxidation may be accomplished in either of two way; artificially by the use of an oxidizing agent, or naturally by a slow process of air exposure for 3 to 6 weeks (as in Heidenhain's hematoxylin). Used alone, hematin is a weak and diffuse dye with little affinity for tissues. A weak acid will not combine with nuclear elements in sufficient quantity to produce efficient staining. Some form of mordanting is therefore required to form a base for this dye, which will then stain the acidic nuclear element. The most commonly use mordants are alum salts of aluminum, potassium, or iron. When mordant and dye are used seperately, regressive staining usually is more effective. In regressive staining, the section are overstained, and excessive amounts are removed by use of excess mordant.

When excess free mordant is present outside the tissue, the tissure mordant-dye complex is mobilized, and since the amount of mordant in the tissue is smaller than that in the differentiating fluid, the dye moves from the tissue and the fluid. Nuclei hold considerably more dye than the cytoplasm. When the correct dye intensity remains in the nuclei, remove the slides from the mordant and wash then throughly, to remove excess mordant. Traces of remaining mordant can cause the stain to fade in time.

Heidenhain's Iron Hematoxylin (regressive method)

Heidenhain's iron hematoxylin is a two step hematoxylin method. Tissues are first exposed to mordant, and are then placed in

stain solution. This method creates sharp contrasts between cell parts, and works well with almost any fixative.

Double solutions are never mixed before use;

Solution A:

- Ferric alum, $FeNH_4(SO_4)_2.12H_2O$.....3.0 g
- Distilled water .....100.0 ml

Keep in refrigerator to prevent precipitation on sides of bottle.

Solution B

- Hematoxylin .....2.5 g
- 95% Ethyl alcohol ..... 25.0 ml

Add 25.0 ml of this stock solution to 500 ml of distilled water. This gives a practically aqueous solution.

Differentiator: 1.5% ferric alum solution in water.

Counterstains (Plasma Stains)

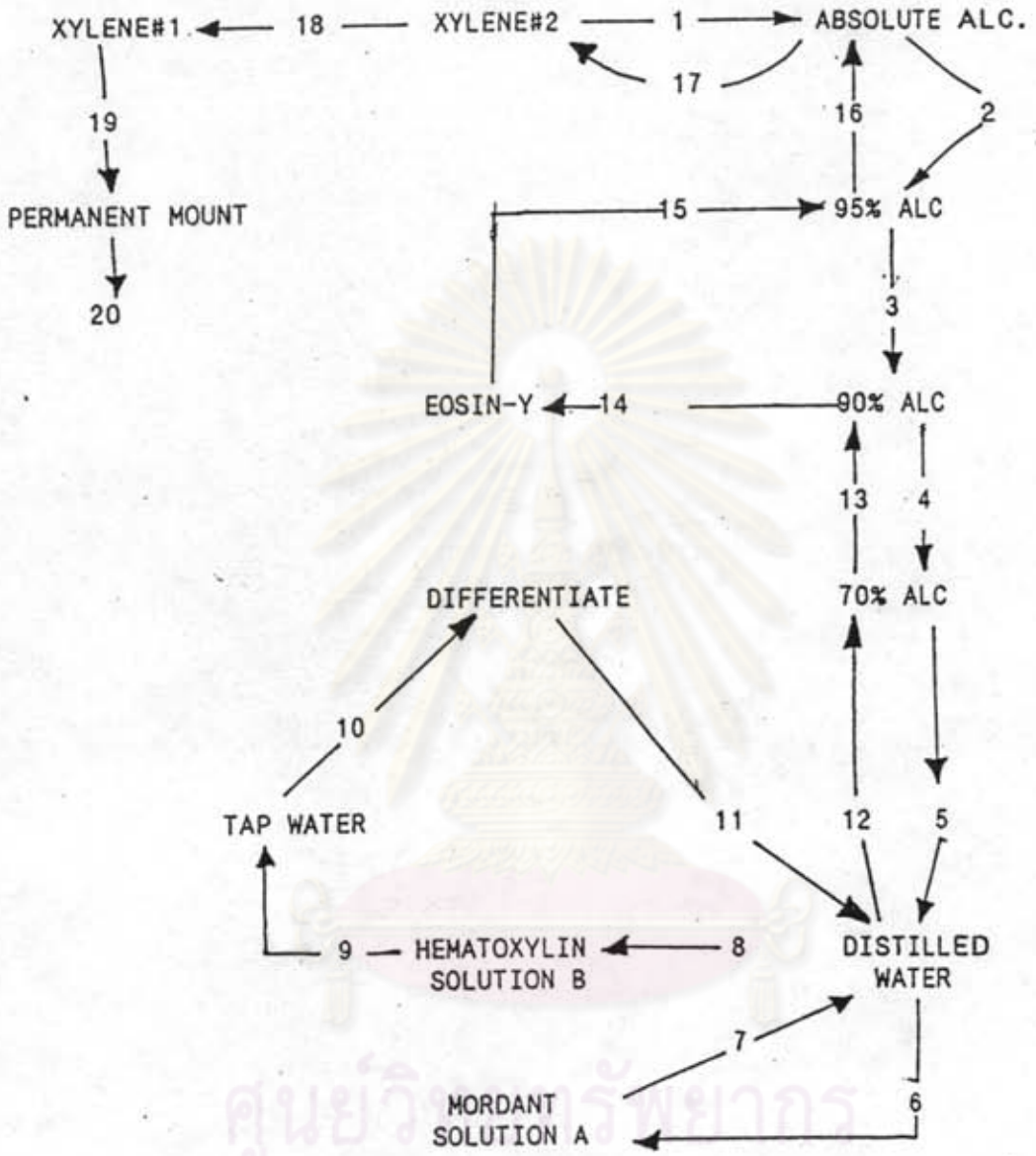
Eosin: (Willey, 1971)

- Eosin Y,C.I. 45380..... 1.0 g
- 95 % ethyl alcohol .....100.0 ml
- Glacial acetic acid .....5.0 ml

8.2 Staining procedure:

The staining schedule is shown in Figure.

Staining produces blue black to black color in nuclei, and grey or orange color in cytoplasm.



(1) = De-paraffinize FOR >2 min.
(2)-(6) = Hydrate the slides in water.
(7) = Mordant in 3% iron alum for 15 min.
(8),(12) = Wash rapidly in distilled water.
(9) = Stain in hematoxylin 15 min. Section should be jet black.
(10) = Rinse in tap water.
(11) = Differentiate in 1.5% aqueous iron alum .
(13),(14) = Dehydrate to 90% alcohol.
(15) = Counterstain for 2 t0 3 min.
(16) = Wash rapidly in 95 % ethyl alcohol.
(17)-(19) = Dehydrate for 1-2 min.
(20) = Permanent mount

Figure 15. Scedule for routine staining of ovarian tissue

APPENDIX B

Data available for ovarian maturation experiments of *Penaeus monodon* : Adult prawn maturation in hypersalinity

Appendix B-1 Data available for ovarian maturation experiments of *Penaeus monodon*: Adult prawn maturation in hypersalinity

Salinity o/oo	Acclimation time (days)	Body Weight(g)	Carapace lenght(cm)	Gonad Weight(g)	% Gonad index	Condition index
30	0	103.680	5.830	1.508	1.454	17.784
30	0	100.200	5.800	0.669	0.668	17.276
30	0	100.700	5.190	0.848	0.842	19.403
30	0	103.500	5.605	0.214	0.207	18.466
30	0	103.500	5.850	0.632	0.611	17.692
30	0	98.700	5.155	0.553	0.560	19.146
30	0	88.600	4.975	0.772	0.871	17.809
30	0	111.200	6.240	0.251	0.226	17.821
30	30	104.700	5.480	0.198	0.189	19.106
30	30	83.925	-	2.056	2.450	-
30	30	103.954	-	0.896	0.862	-
30	30	78.844	-	0.997	1.265	-
30	30	90.700	5.675	0.131	0.145	15.982
30	30	101.340	5.550	0.882	0.871	18.259
30	30	106.600	6.310	0.830	0.779	16.894
30	30	103.200	6.105	0.990	0.959	16.904
30	35	90.000	5.520	2.008	2.231	16.304
30	36	98.000	5.820	1.392	1.420	16.838
30	40	112.000	5.960	3.218	2.873	18.792
30	42	108.000	5.850	1.469	1.360	18.462
30	43	103.000	5.850	1.345	1.305	17.607
30	46	90.000	5.435	1.257	1.397	16.559
30	47	98.000	5.510	1.311	1.338	17.786
30	50	100.000	5.830	1.571	1.571	17.153
30	50	100.000	5.920	2.070	2.070	16.892
30	51	105.000	5.930	1.197	1.140	17.707
30	52	83.000	5.380	1.270	1.530	15.428
30	52	97.000	5.580	1.736	1.790	17.384
30	53	110.000	5.915	4.161	3.782	18.597
30	53	103.000	5.870	1.981	1.923	17.547
30	53	105.000	5.890	1.462	1.392	17.827
30	55	115.000	5.985	5.498	4.781	19.215
30	56	95.000	5.910	3.880	4.084	16.074
30	56	115.000	6.255	0.450	0.391	18.385
30	58	110.000	5.675	3.572	3.247	19.383
30	59	118.000	6.050	1.385	1.174	19.504
30	60	90.500	6.015	3.234	3.573	15.046
30	64	105.500	5.830	6.512	6.172	18.096
30	64	85.500	5.520	2.192	2.564	15.489
30	76	93.500	5.760	3.600	3.850	16.233

Appendix B -1 (continue)

Salinity o/oo	Acclimation time (days)	Body Weight(g)	Carapace lenght(cm)	Gonad Weight(g)	% Gonad index	Condition index
40	0	103.680	5.830	1.508	1.454	17.784
40	0	100.200	5.800	0.669	0.668	17.276
40	0	100.700	5.190	0.848	0.847	19.403
40	0	103.500	5.605	0.214	0.207	18.466
40	0	103.500	5.850	0.632	0.611	17.692
40	0	98.700	5.155	0.553	0.560	19.146
40	0	88.600	4.975	0.772	0.871	17.809
40	0	111.200	6.240	0.251	0.226	17.821
40	30	104.700	5.245	0.198	0.189	19.962
40	30	75.110	-	0.643	0.856	-
40	30	90.700	5.130	0.131	0.145	17.680
40	30	79.135	-	0.795	1.005	-
40	30	101.340	5.230	0.882	0.871	19.377
40	30	79.212	-	1.914	2.416	-
40	30	106.600	5.960	0.830	0.779	17.886
40	30	78.957	-	0.754	0.954	-
40	30	103.200	5.715	0.990	0.959	18.058
40	34	97.800	5.840	2.099	2.146	16.747
40	36	95.500	5.570	1.866	1.953	17.145
40	38	80.500	5.540	1.799	2.235	14.531
40	38	101.000	5.785	2.678	2.651	17.459
40	40	80.000	5.290	0.712	0.890	15.123
40	42	90.000	5.440	1.341	1.490	16.544
40	42	75.000	5.450	2.095	2.793	13.761
40	42	98.000	5.630	0.470	0.480	17.407
40	44	80.500	5.365	0.178	0.221	15.005
40	44	100.000	5.420	1.680	1.680	18.450
40	46	100.000	5.640	1.460	1.460	17.730
40	47	95.500	5.725	1.261	1.320	16.681
40	49	95.000	5.655	1.349	1.420	16.799
40	51	100.000	5.810	0.851	0.851	17.212
40	51	87.500	5.425	0.864	0.987	16.129
40	57	103.000	5.620	1.357	1.317	18.327
40	57	85.000	6.060	1.080	1.270	14.026
40	59	118.000	6.540	0.496	0.420	18.043
40	59	85.000	5.370	0.799	0.940	15.829
40	65	115.000	5.980	6.452	5.610	19.231
40	65	100.000	5.800	4.541	4.541	17.241
40	70	118.000	6.105	4.052	3.434	19.328
40	70	115.500	6.350	3.593	3.111	18.189
40	75	110.500	6.210	2.155	1.950	17.794
40	75	118.000	6.055	3.533	2.994	19.488

Table 15 Linear regresion analysis for relationship between ovarian index (GI) and hypersaline acclimation time (TIME) of adult prawn, *Penaeus monodon*

(a) SALINITY = 30 °/oo

DEPENDENT VARIABLE : GI(OVARIAN INDEX)
N : 43 MULTIPLE R : 0.641 SQUARED MULTIPLE R : 0.411
ADJUSTED SQUARED MULTIPLE R: 0.396 STANDARD ERROR OF ESTIMATE : 1.048

VARIABLE	COEFFICIENT	STD ERROR	STD COEF	T	P(2 TAIL)
CONSTANT	0.266	0.308	0.000	0.864	0.3920
TIME	0.039	0.007	0.641	5.345	0.0001

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
REGRESSION	31.377	1	31.377	28.569	0.0001
RESIDUAL	45.029	41	1.098		

(b) SALINITY = 40 °/oo

DEPENDENT VARIABLE : GI(OVARIAN INDEX)
N : 42 MULTIPLE R : 0.549 SQUARED MULTIPLE R : 0.302
ADJUSTED SQUARED MULTIPLE R: 0.284 STANDARD ERROR OF ESTIMATE: 1.001

VARIABLE	COEFFICIENT	STD ERROR	STD COEF	T	P(2 TAIL)
CONSTANT	0.393	0.302	0.000	1.301	0.2010
TIME	0.029	0.007	0.549	4.159	0.0001

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
REGRESSION	17.345	1	17.345	17.296	0.0001
RESIDUAL	40.113	40	1.003		

Table 16 Linear regression analysis on effect of hypersalinity (SAL) on condition index (CI)of adult prawn, *Penaeus monodon*.

(a) SALINITY = 30 o/oo

6 CASES DELETED DUE TO MISSING DATA.

DEPENDENT VARIABLE : CI (CONDITION INDEX)
N: 37 MULTIPLE R: 0.260 SQUARED MULTIPLE R: 0.067
ADJUSTED SQUARED MULTIPLE R: 0.041 STANDARD ERROR OF ESTIMATE: 1.159

VARIABLE	COEFFICIENT	STD ERROR	STD COEF	T	P(2 TAIL)
CONSTANT	18.102	0.374	0.000	48.445	0.0001
TIME	-0.014	0.008	-0.260	1.591	0.1210

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
REGRESSION	3.404	1	3.404	2.532	0.121
RESIDUAL	47.045	35	1.344		

(b) SALINITY = 40 o/oo

4 CASES DELETED DUE TO MISSING DATA.
DEPENDENT VARIABLE : CI (CONDITION INDEX)
N: 38 MULTIPLE R: 0.037 SQUARED MULTIPLE R: 0.019
ADJUSTED SQUARED MULTIPLE R: 0.000 STANDARD ERROR OF ESTIMATE: 1.519

VARIABLE	COEFFICIENT	STD ERROR	STD COEF	T	P(2 TAIL)
CONSTANT	17.771	0.473	0.000	37.578	0.0001
TIME	-0.009	0.01	-0.137	-0.830	0.4120

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
REGRESSION	1.590	1	1.590	0.689	0.412
RESIDUAL	83.086	36	2.308		

TIME = acclimation time (days)

Table 17 Analysis of covariance of hypersalinity effect (SAL) on relationship of condition index (CI) and acclimation time (TIME) of adult shrimp, *Penaeus monodon*.

DEPENDET VARIABLE : CI (CONDITION INDEX)

N: 75 MULTIPLE R: 0.196 SQUARED MULTIPLE R: 0.039

ANALYSIS OF COVARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
TIME	4.779	1	4.779	2.640	0.109
SAL	0.437	1	0.437	0.242	0.625
ERROR	130.346	72	1.810		

Note : Source = source of variation
DF = degree of freedom
CI = condition index
SAL = salinity (30 and 40 $^{o}/oo$)
TIME = acclimation time (days)

Table 18 Analysis of Covariance of the hypersalinity effect (SAL) on relationship of ovarian index (GI) and condition index (CI)

DEPENDENT VARIABLE : GI (OVARIAN INDEX)
N: 75 MULTIPLE R: 0.108 SQUARED MULTIPLE R: 0.012

ANALYSIS OF COVARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
SAL	1.478	1	1.478	0.835	0.364
CI	0.061	1	0.061	0.035	0.853
ERROR	127.349	72	1.769		

Note : Source = source of variation
DF = degree of freedom
GI = gonad index
SAL = salinty (30 and 40 o/oo)
CI = condition index

APPENDIX C

Data available for ovarian maturation experiments of *Penaeus monodon* : Prawn size, hypersaline acclimation and eyestalk ablation effects on maturation of subadult *Penaeus monodon*

Table 19 Analysis of variance on effect of hypersalinity (30 °/oo and 40 °/oo) and eyestalk ablation on gonad index of subadult *Penaeus monodon*

(a) SIZE = SMALL SIZE SUBADULT PRAWN

DEPENDENT VARIABLE : GI(OVARIAN INDEX)

N = 22 MULTIPLE R: 0.402 SQUARED MULTIPLE R: 0.161

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
EYE	0.039	1	0.039	0.042	0.839
SAL	3.055	1	3.055	3.326	0.085
EYE* SAL	0.000	1	0.000	0.001	0.982
ERROR	16.532	18	0.918		

(b) SIZE = LARGE SIZE SUBADULT

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
EYE	3.351	1	3.351	8.461	0.008
SAL	0.163	1	0.163	0.412	0.527
EYE* SAL	0.552	1	0.552	1.395	0.250
ERROR	9.110	23	0.396		

Note : Source = source of variation

DF = degree of freedom

EYE = eyestalk abaltion

SAL = salinity

Table 20 Analysis of variance for effect salinity (30 °/oo 40 °/oo) and acclimation time on ovarian index of subadult prawn, *Penaeus monodon*

(a) SIZE = SMALL SIZE SUBADULT PRAWN

DEPENDENT VARIABLE : GI (OVARIAN INDEX)
N : 25 MULTIPLE R ; 0.454 SQURED MULTIPLE R : 0.206

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
SAL	0.925	1	0.925	1.452	0.242
T	1.737	1	1.737	2.724	0.114
SAL* T	0.925	1	0.925	1.452	0.242
ERROR	13.387	21	0.637		

(b) SIZE = LARGE SIZE SUBADULT

N : 35 MULTIPLE R : 0.268 SQUARED MULTIPLE R : 0.072

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
SAL	0.037	1	0.037	0.261	0.613
T	0.260	1	0.260	1.837	0.185
SAL* T	0.037	1	0.037	0.261	0.613
ERROR	4.390	31	0.142		

Note : Source = source of variation

DF = degree of freedom

SAL = salinity

TIME = accclimation tion (days)

Table 21 Analysis of variance for effect salinity (30 °/oo 40 °/oo) and acclimation time on condition index of subadult prawn, *Penaeus monodon*

(a) SIZE = SMALL SIZE SUBADULT PRAWN

DEPENDENT VARIABLE : CI(CONDITION INDEX)

N = 25 MULTIPLE R: 0.720 SQUARED MULTIPLE R: 0.519

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
SAL	0.112	1	0.112	0.505	0.485
T	4.883	1	4.883	22.038	0.001
SAL* T	0.112	1	0.112	0.505	0.485
ERROR	4.653	21	0.222		

(b) SIZE = LARGE SIZE SUBADULT PRAWN

N : 32 MULTIPLE R : 0.201 SQUARED MULTIPLE R : 0.040

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
SAL	1.452	1	1.452	0.766	0.389
T	0.137	1	0.137	0.072	0.790
SAL* T	1.452	1	1.452	0.766	0.389
ERROR	53.089	28	1.896		

Note : Source = source of variation

DF = degree of freedom

SAL = salinity

TIME = accclimation time(days)

APPENDIX D

Data available for ovarian maturation experiments of *Penaeus monodon* : Manipulation techniques to induce ovarian maturation of subadult *Penaeus monodon*

Table 22 Analysis of variance for salinity and eyestalk ablation effects on ovarian index (GI) of subadult prawn *Penaeus monodon*

DEPENDENT VARIABLE : GI (OVARIAN INDEX)
N : 137 MULTIPLE R ; 0.416 SQURED MULTIPLE R : 0.173

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
SAL	0.138	1	0.138	0.128	0.721
EYE	27.747	1	27.747	25.770	0.0001
SAL* EYE	1.579	1	1.579	1.467	0.228
ERROR	143.204	133	1.077		

Note : Source = source of variation
DF = degree of freedom
SAL = salinity (30 °/oo and 40 °/oo)
EYE = eyestalk ablation

Table 23 Matrix of pairwise comparison (tukey HSD multiple comparisons) among probabilities for eyestalk ablation and salinity effects on ovarian index (GI) of subadult prawn *Penaeus monodon*

TUKEY HSD MULTIPLE COMPARISONS
MATRIX OF PAIRWISE COMPARISON PROBABILITIES

	1	2	3	4
1	1.000			
2	0.021	1.000		
3	0.930	0.006	1.000	
4	0.000	0.688	0.000	1.000

Group definition: 1 = 30 o/oo , normal eyes shrimp
2 = 30 o/oo , uni-ablation shrimp
3 = 40 o/oo , normal eyes shrimp
4 = 40 o/oo , uni-ablation shrimp

Table 24 Analysis of variance for effects of condition index (CI) of subadult prawn *Penaeus monodon*

EFFECTS OF TRM GROUPING ON CONDITION INDEX

BARTLETT TEST FOR HOMOGENEITY OF GROUP VARIANCES = 39.038

APPROXIMATE F = 2.166 DF = 17, 4525 PROBABILITY = 0.007

ANALYSIS OF VARIANCE

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F	PROBABILITY
BETWEEN GROUPS	22.775	17	1.340	0.891	0.585
WITHIN GROUPS	211.935	141	1.503		

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

APPENDIX E

Data available for molting of broodstock *Penaeus monodon* in closed recirculating water system

Table 25 Analysis of variance of body weight, salinity, and eyestalk ablation effects on molting interval of subadult prawn, *Penaeus monodon*

THE FOLLOWING RESULTS ARE FOR SUBADULT SHRIMP

DEPENDENT VARIABLE : MOL1 (INTERMOLT PERIOD I)
N: 105 MULTIPLE R: 0.121 SQUARED MULTIPLE R: 0.015

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
BWO	3.918	1	3.918	0.662	0.418
SAL	1.509	1	1.509	0.255	0.615
EYE	0.275	1	0.275	0.046	0.830
SAL* EYE	2.188	1	2.188	0.370	0.545
ERROR	591.793	100	5.918		

DEPENDENT VARIABLE: MOL1 (INTERMOLT PERIOD I)
N: 105 MULTIPLE R: 0.089 SQUARED MULTIPLE R: 0.008

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
SAL	1.946	1	1.946	0.330	0.567
EYE	0.036	1	0.036	0.006	0.937
SAL* EYE	2.165	1	2.165	0.367	0.546
ERROR	595.711	101	5.898		

Note: MOL1 = time elaped (days) between two consecutive ecdysis
BWO = body weight (gm)
SAL = salinity (30 and 40 o/oo)
EYE = eyestalk manipulation (normal eyes and uni-ablation)

Table 26 Analysis of variance of sex and salinity effects on molting interval of adult prawn, *Penaeus monodon*

EFFECTS OF SEX ON MOLTING OF P.MONODON

DEPENDENT VARIABLE: MOL1 (INTERMOLT PERIOD I)
N: 28 MULTIPLE R: 0.311 SQUARED MULTIPLE R: 0.097

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
SAL	0.000	1	0.000	0.000	0.997
SEX	1.743	1	1.743	0.112	0.741
SAL* SEX	35.715	1	35.715	2.297	0.143
ERROR	373.229	24	15.551		

Note: MOL1 = time elaped (days) between two consecutive ecdysis

SAL = salinity (30 and 40 o/oo)

SEX = sex of adult shrimp

Table 27 Analysis of variance of age and salinity effects on molting interval of *Penaeus monodon*

DEPENDENT VARIABLE: MOL1 (INTERMOLT PERIOD I)
N: 87 MULTIPLE R: 0.696 SQUARED MULTIPLE R: 0.484

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
AGE	704.061	1	704.061	75.449	0.0001
SAL	6.148	1	6.148	0.659	0.419
AGE* SAL	0.001	1	0.001	0.000	0.991
ERROR	774.521	83	9.332		

Note: MOL1 = time elaped(days) between two consecutive ecdysis or intermolt period I

AGE = age of broodstock shrimp (5-month shrimp and 8-month adult shrimp)

SAL = salinity (30 and 40 o/oo)

Table 28 Computation output of tukey HSD multiple comparison among probabilities of molting interval of subadult and adult prawn, *Penaeus monodon*

DEPENDENT VARIABLE: MOL1 (INTERMOLT PERIOD I)

BARTLETT TEST FOR HOMOGENEITY OF GROUP VARIANCES
CHI-SQUARE = 8.228 DF= 3 PROBABILITY = 0.050

GROUPS: 1 = 30 o/oo SUBADULT (5-month-old)
2 = 40 o/oo SUBADULT (5-month-old)
3 = 30 o/oo ADULT (8-month-old)
4 = 40 o/oo ADULT (8-month-old)

ANALYSIS OF VARIANCE

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F	PROBABILITY
BETWEEN GROUPS	726.743	3	242.248	25.960	0.0001
WITHIN GROUPS	774.521	83	9.332		

MATRIX OF PAIRWISE ABSOLUTE MEAN DIFFERENCES

	1	2	3	4
1	0.000			
2	0.567	0.000		
3	6.147	5.580	0.000	
4	6.730	6.163	0.583	0.000

TUKEY HSD MULTIPLE COMPARISONS
MATRIX OF PAIRWISE COMPARISON PROBABILITIES

	1	2	3	4
1	1.000			
2	0.895	1.000		
3	0.000	0.000	1.000	
4	0.000	0.000	0.959	1.000

BIOGRAPHY

Miss Theeranuj Sapayasant was born on 22nd October 1965 in Bangkok. She graduated with a B.Sc in Marine Biology and Fisheries from Marine Science Department, Chulalongkorn University in 1987. She jointed student activities in Faculty of Science during her undergraduate. During 21 August to 4 September 1986, she was awarded a fellowship to attend an International Inter-University Exchange Seminar from the Association of International Education, Japan.

She had an occasion to be a student chief of Marine Science student in 1987. She was nominated as an outstanding marine science graduated student of 1987 from Professor Dr. Tab Nilanidhi Foundation, Chulalongkorn University.

She had received as a research assistant during 1988-1989 from Sichange Marine Science Research and Training Center (SMART) to work in Science and Technology Development Board (STDB) project, Grant No. DSN 87A-1-06-085.