CHAPTER III

RESULTS

Sperm Quality of Pond-Reared *P. monodon* at Different Sizes.

A. Artificial Insemination.

Spermatophores collection technique is very useful for artificial insemination of the closed-thelycum penaeid shrimp. The present study shows that the optimal parameters of the electroejaculation in male *P. monodon* are 160 per sec in frequency, 0.5 sec in width (intensity ration) and 10-12.5 volts in amplitude (duration of width voltage intensity). Before ejaculation, prawn was first necessarily secured on a wooden platform with wet towel soaked with seawater and firmly fastened by cloth belts, to prevent flexion of abdomen and thorax. Afterwards, the male was induced to ejaculate by attaching the anode to the right side and the cathode to the left side of the inner area of coxae of the fifth pereiopods or reversed for 1-3 sec. The spermatophores extruded smoothly and passed through the gonopores. If it is necessary, the electroejaculation could be repeated again. However, the electroejaculation could not be repeated more than 3 times, otherwise the prawn would be seriously injuried.

Sperm quality of pond-reared *P. monodon* at different sizes was investigated by the artificial insemination technique. Evaluation for sperm quality using the following criteria; percentages of morphological normality of fertilized eggs, total fertilized eggs,

morphological normality of live nauplii, morphological normality of nauplii, and total hatched nauplii. The results could be summarized in Table 1 and data are shown in Appendix I.

Table 1. Results of electroejaculated spermatophore transplantation of pond-reared males *P. monodon.*

Variables (%)	N	Min.	Max.	Mean	Std. Dev.
Normal fertilized eggs	51	0	98	48	36
Total fertilized eggs	51	0	98	51	36
Normal live nauplii	40	0	100	86	26
Normal nauplii	40	0	100	90	23
Hatched nauplii	51	0	100	41	35

The percentages of morphological normality of fertilized eggs, total fertilized eggs, morphological normality of total nauplii, morphological normality of live nauplii and total hatched nauplii were positively affected by male size ($P < 0.05$). The expression of these relations are shown in Figures 4-8 and presented by linear regression models;

PNFE = 0.91*MBW, $r = 0.84$, $N = 51$, $P < 0.05$,

PFE = 0.95*MBW, $r = 0.84$, $N = 51$, $P < 0.05$,

PLNN = 39.64 + 0.84*MBW, $r = 0.40$, $N = 40$, $P < 0.05$,

PNN = 38.52 + 0.94*MBW, $r = 0.50$, $N = 40$, $P < 0.05$,

PH = 0.78*MBW, $r = 0.80$, $N = 51$, $P < 0.05$,

where

PNFE = percentage of morphological normality of fertilized eggs,

PFE = percentage of total fertilized eggs,

PLNN = percentage of morphological normality of live nauplii,

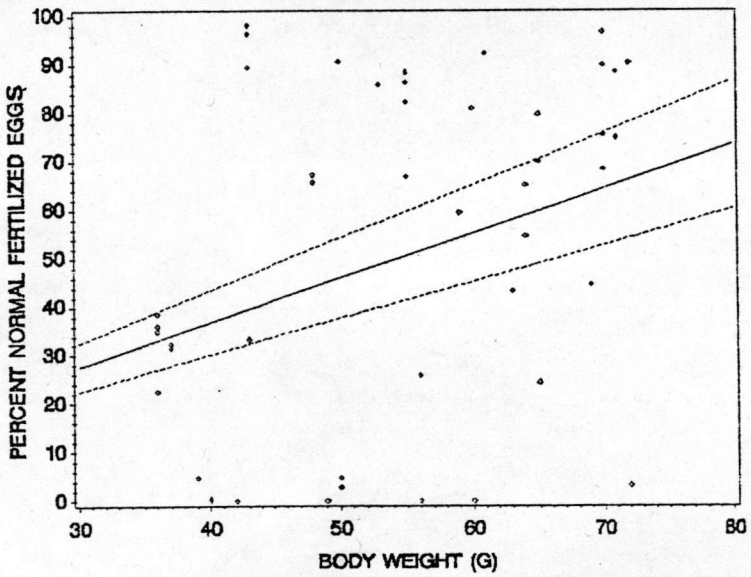

Figure 4. Relationship between the percentage of morphological normality of fertilized eggs and body weight of male *P. monodon*.

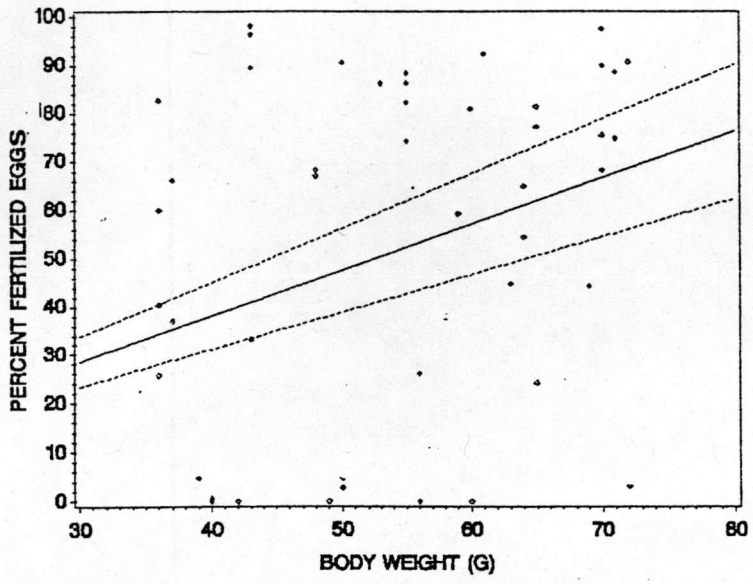

Figure 5. Relationship between the percentage of total fertilized eggs and body weight of male *P. monodon*.

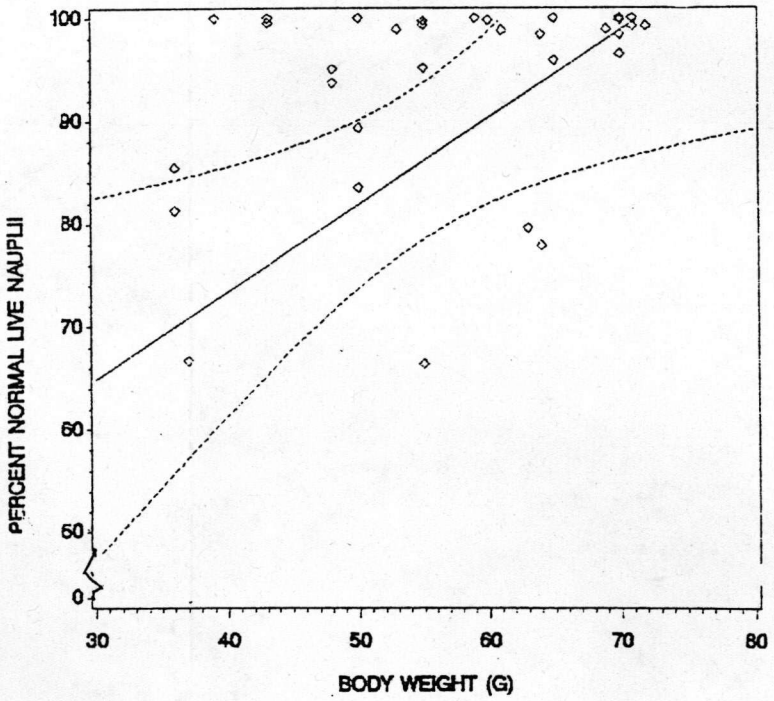

Figure 6. Relationship between the percentage of morphological normality of live nauplii and body weight of male *P. monodon*.

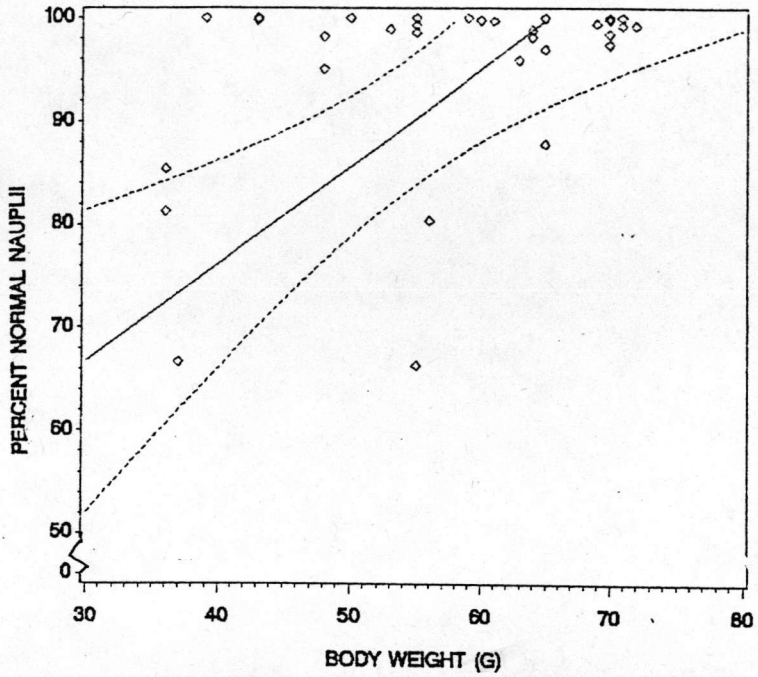

Figure 7. Relationship between the percentage of morphological normality of total nauplii and body weight of male *P. monodon*.

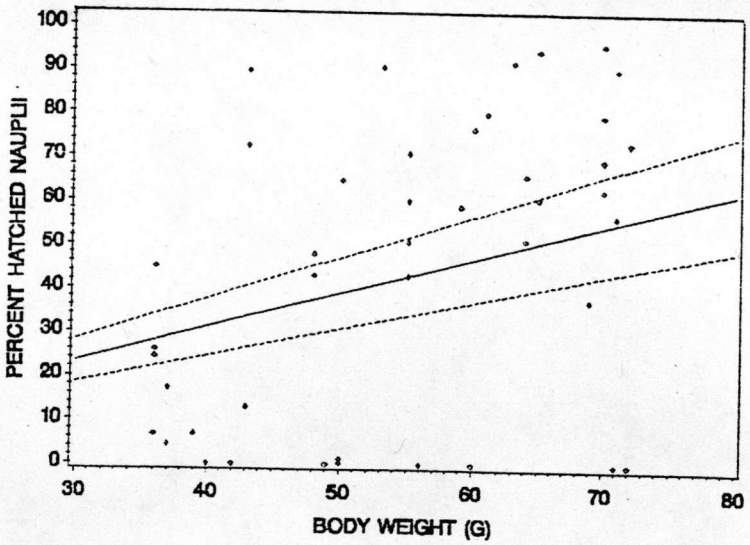

Figure 8. Relationship between the percentage of total hatched nauplii and body weight of male *P. monodon*.

PNN = percentage of morphological normality of total nauplii,

PH = percentage of total hatched nauplii, and

MBW = body weight of male (g).

In contrast, increasing female size per spermatophore weight had significant negatively effect on percentages of morphological normality of fertilized eggs, total fertilized eggs, and total hatched nauplii ($P < 0.05$). These relations are shown in Figures 9-11 and presented by linear regression models;

$\ln PNFE = 11.12 - 1.01 * \ln SPFCL$, $r = 0.53$, $N = 51$, $P < 0.05$,

$\ln PFE = 10.57 - 0.93 * \ln SPFCL$, $r = 0.49$, $N = 51$, $P < 0.05$,

$\ln PH = 10.18 - 0.90 * \ln SPFCL$, $r = 0.44$, $N = 51$, $P < 0.05$,

where

lnPNFE = natural logarithm of percentage of morphological normality of fertilized eggs,

lnPFE = natural logarithm of percentage of total fertilized eggs,

lnPH = natural logarithm of percentage of total hatched nauplii, and

lnSPFCL = natural logarithm of carapace length of female per spermatophore weight.

Meanwhile, the percentages of morphological normality of fertilized eggs, total fertilized eggs, and total hatched nauplii were positively affected by spermatophore size ($P < 0.05$). The relations are shown in Figures 12-14 and presented by linear regression models;

$\ln PNFE = 6.97 + 1.00 * \ln SPWT$, $r = 0.53$, $N = 51$, $P < 0.05$,

$\ln PFE = 6.74 + 0.92 * \ln SPWT$, $r = 0.48$, $N = 51$, $P < 0.05$,

$PH = 846.67 * SPWT$, $r = 0.81$, $N = 51$, $P < 0.05$,

where

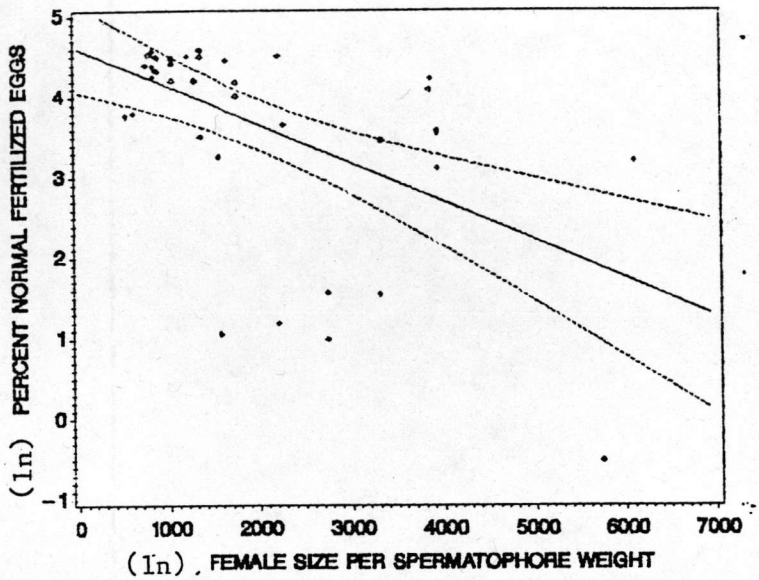

Figure 9. Relationship between the percentage of morphological normality of fertilized eggs and carapace length of female per spermatophore wight.

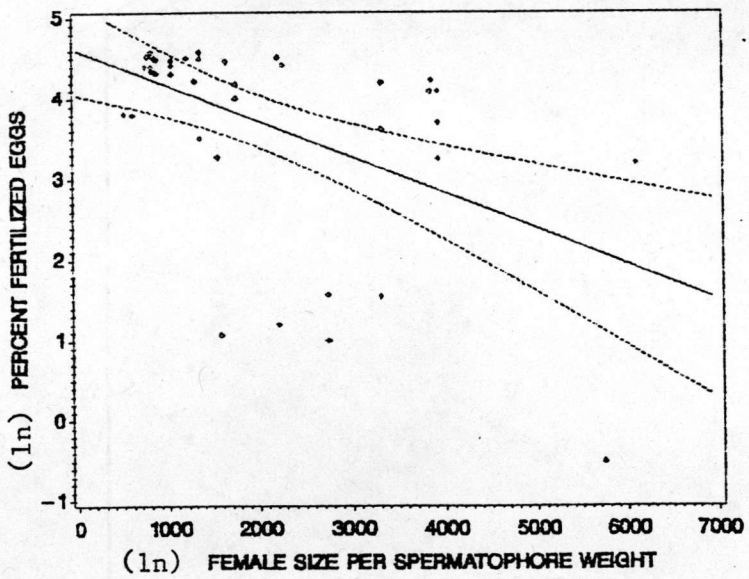

Figure 10. Relationship between the percentage of total fertilized eggs and carapace length of female per spermatophore weight.

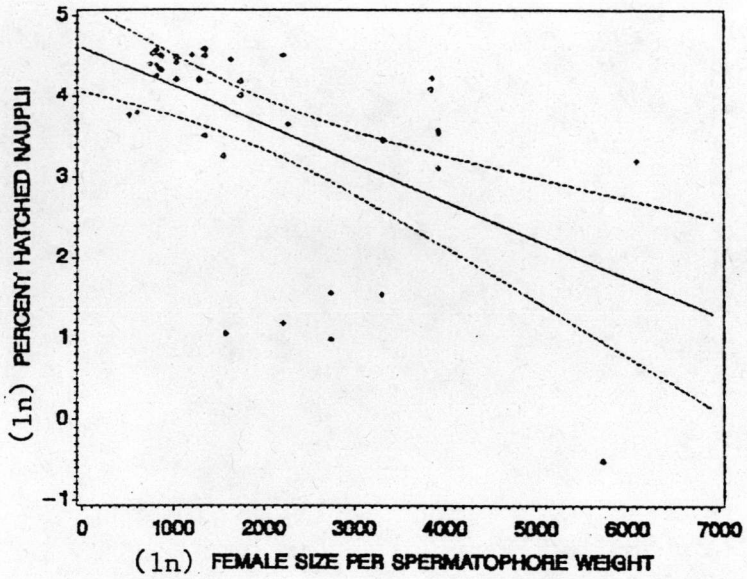

Figure 11. Relationship between the percentage of total hatched nauplii and carapace length of female per spermatophore weight.

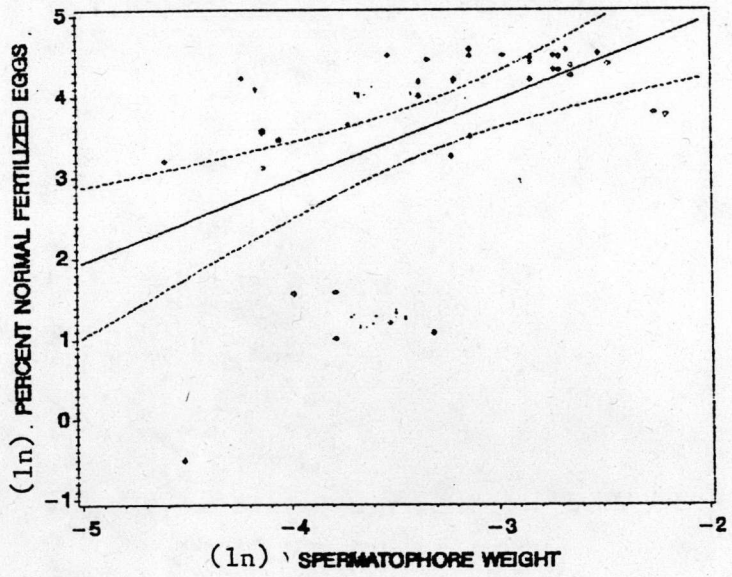

Figure 12. Relationship between the percentage of morphological normality of fertilized eggs and spermatophore weight of male *P. monodon*.

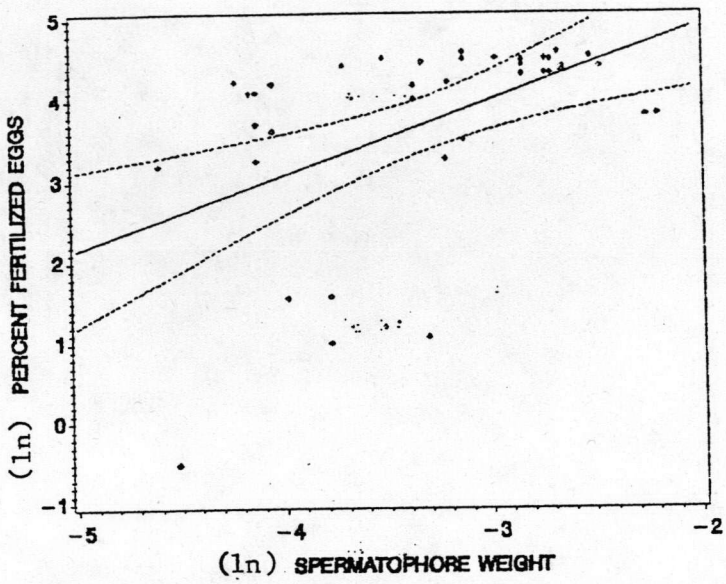

Figure 13. Relationship between the percentage of total fertilized eggs and spermatophore weight of male *P. monodon*.

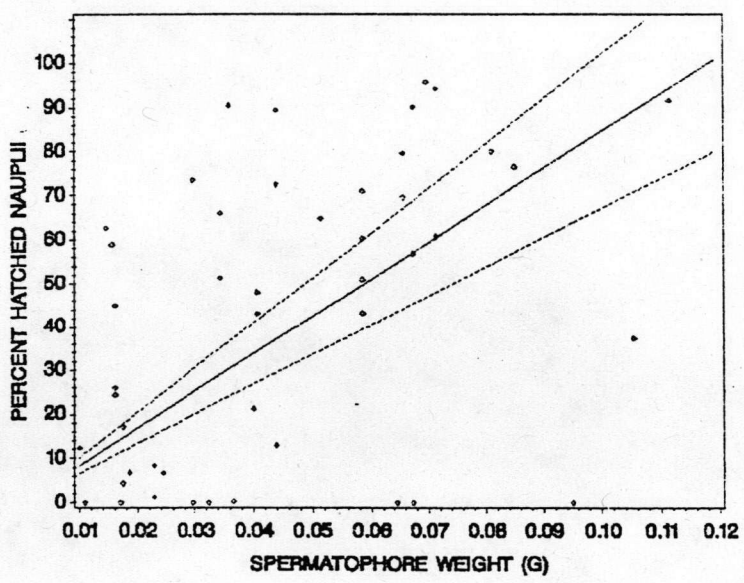

Figure 14. Relationship between the percentage of total hatched nauplii and spermatophore weight of male *P. monodon*.

lnPNFE = natural log of percentage of morphological normality of fertilized eggs,

lnPFE = natural log of percentage of total fertilized eggs,

PH = percentage of total hatched nauplii, and

lnSPWT = natural log of spermatophore weight (g).

The duration of sperm storage in female thelycum had also significantly negatively effect on the percentage of total hatched nauplii. This relationship is expressed in Figure 15 according to the linear regression model,

$PH = 65.28 - 2.34*TDAY, \quad r = 0.31, N = 51, P < 0.05,$

where

PH = percentage of total hatched nauplii, and

TDAY = duration of sperm storage in thelycum (days).

The results also clearly indicated that an increased duration of sperm storage in the thelycum and/or sequential spawning resulted in decreasing production of artificial insemination technique.

In order to understand combined effects on production of artificial insemination, the multiple regression analysis was used. The relations of percentages of morphological normality of fertilized eggs, total fertilized eggs, morphological normality of live nauplii, morphological normality of nauplii, and total hatched nauplii to body weight of male, carapace length of female per spermatophore weight, duration of male captivity, and duration of sperm storage in thelycum could be expressed as;

PNFE = 0.86*MBW + 0.54*DAY - 0.01*SPFCL,

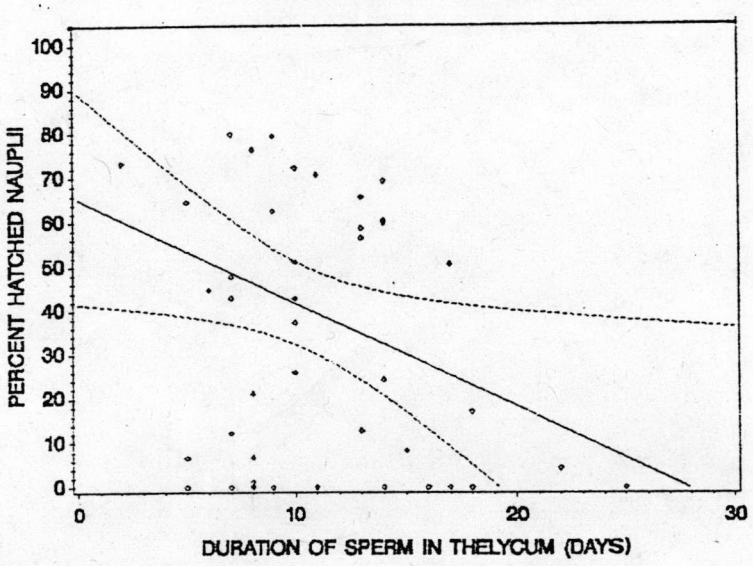

Figure 15. Relationship between the percentage of total hatched nauplii and duration of sperm in thelycum, *P. monodon*.

$r = 0.87$, $N = 51$, $P < 0.05$,

$PEE = 1.16*MBW - 0.01*SPFCL$,

$r = 0.85$, $N = 51$, $P < 0.05$,

$PLNN = 9.03*MBW + 0.71*DAY$,

$r = 0.97$, $N = 51$, $P < 0.05$,

$PNN = 39.25 + 0.78*MBW - 2.23*TDAY - 0.01*SPFCL$,

$r = 0.60$, $N = 51$, $P < 0.05$,

$PH = 1.29*MBW - 1.71*TDAY - 0.01*SPFCL$,

$r = 0.84$, $N = 51$, $P < 0.05$,

Where

PNFE = percentage of morphological normality of fertilized eggs,

PFE = percentage of total fertilized eggs,

PLNN = percentage of morphological normality of live nauplii,

PNN = percentage of morphological normality of total nauplii,

PH = percentage of total hatched nauplii,

MBW = body weight of male (g),

SPFCL = carapace length of female per spermatophore weight,

DAY = duration of male captivity (days), and

TDAY = duration of sperm storage in thelycum (days).

All correlations were significant at $P < 0.05$. The models implied that percentage of morphological normality of fertilized eggs was significant affected by male size, female size per spermatophore weight, and duration of male captivity. The percentage of total fertilized eggs was significant affected by male size and female size per spermatophore weight. The percentage of morphological normality of live nauplii was significantly affected by male size and duration of male captivity. Meanwhile, the percentages of morphological normality of total nauplii and total hatched nauplii were

significantly affected by male size, female size per spermatophore weight, and duration of sperm storage in thelycum.

In order to understand size effect of the male on productions with artificial insemination technique, four interval sizes of male were determined by body weight. These were size A (36-40 g), size B (41-50 g), size C (51-60 g) and size D (61-72 g).

The results of this study showed in Table 2 and Figures 16-20, the larger males, the better percentages of morphological normality of fertilized eggs, total fertilized eggs, morphological normality of live nauplii and morphological normality of total nauplii, and total hatched nauplii could be obtained. Males, smaller than 40 g body weight always showed lower productions. The results clearly indicated that prawns in size classes C and D or body weight over 50 g could be used for artificial insemination and possibly enable to use as broodstock.

Table 2. Comparative results of electroejaculated spermatophore transplantation of pond-reared males at interval sizes in uncopulated soft thelycum *P. monodon*. Values are Mean ± standard deviation.

Male sizes (g)	Percentages of				
	Normal fertilized eggs	Total fertilized eggs	Normal live nauplii	Normal nauplii	Hatched nauplii
36-40	b 22.3±16.1	b 35.3±30.0	b 53.7±40.8	b 55.8±37.8	c 14.5±15.0
41-50	b 37.7±42.1	b 36.9±42.3	a 96.1±5.8	a 99.3±1.6	bc 29.8±37.6
51-60	a 57.6±35.7	a 58.5±36.0	a 89.4±17.6	a 92.9±12.7	ab 47.2±31.2
61-72	a 66.9±25.7	a 67.6±25.9	a 91.7±17.6	a 98.0±3.1	a 60.1±31.2

Note: a,b, and c denoted significant difference in column. Mean with the same letter are not significant different ($P < 0.05$).

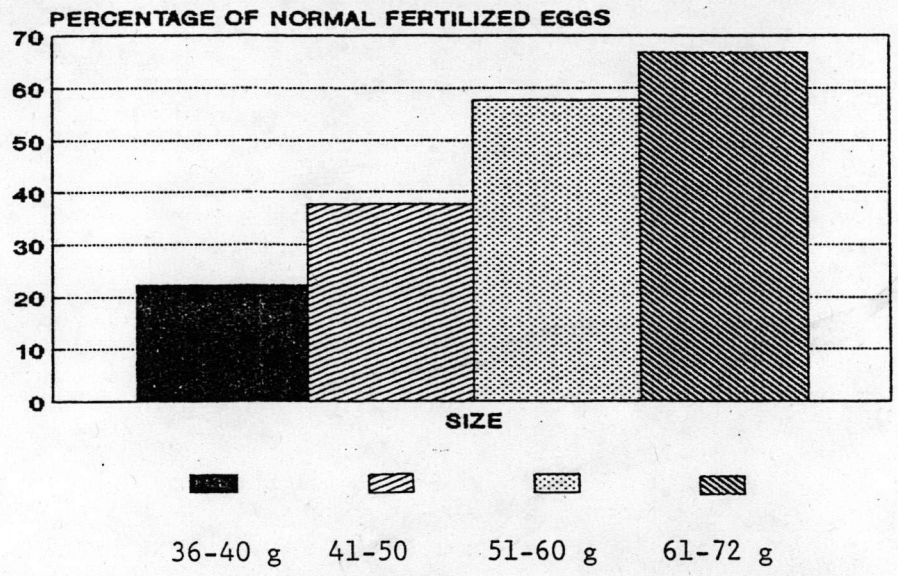

Figure 16. Comparison of the mean percentage of morphological normality of fertilized eggs at interval sizes of pond-reared male *P. monodon*.

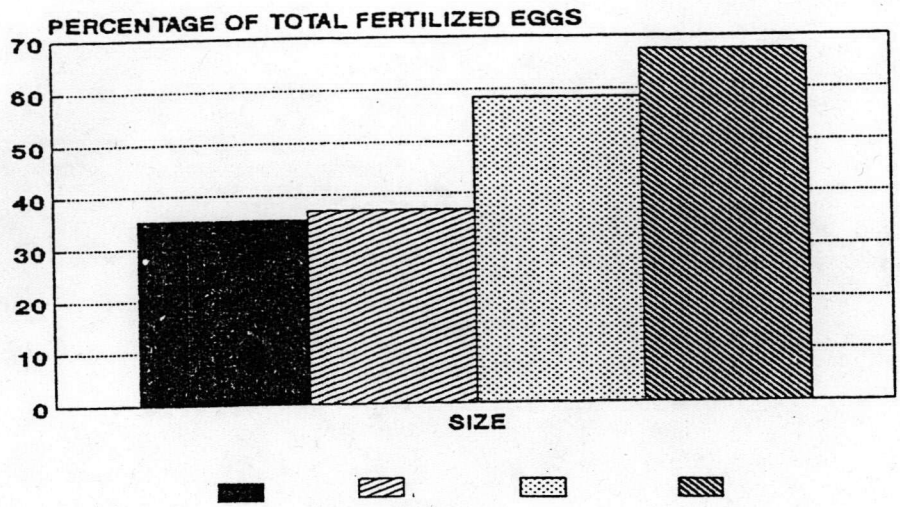

Figure 17. Comparison of the mean percentage of total fertilized eggs at interval sizes of pond-reared male *P. monodon*.

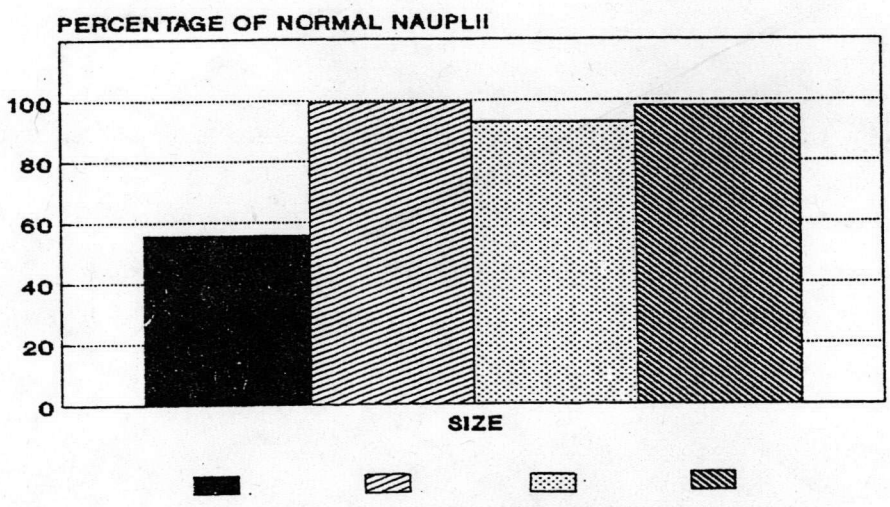

Figure 18. Comparison of the mean percentage of morphological normality of live nauplii at interval sizes of pond-reared male *P. monodon*.

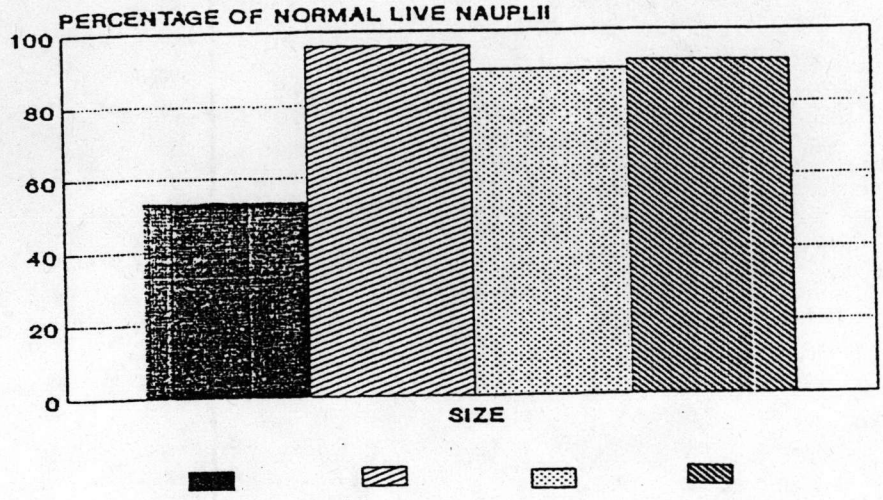

Figure 19. Comparison of the mean percentage of morphological normality of nauplii at interval sizes of pond-reared male *P. monodon*.

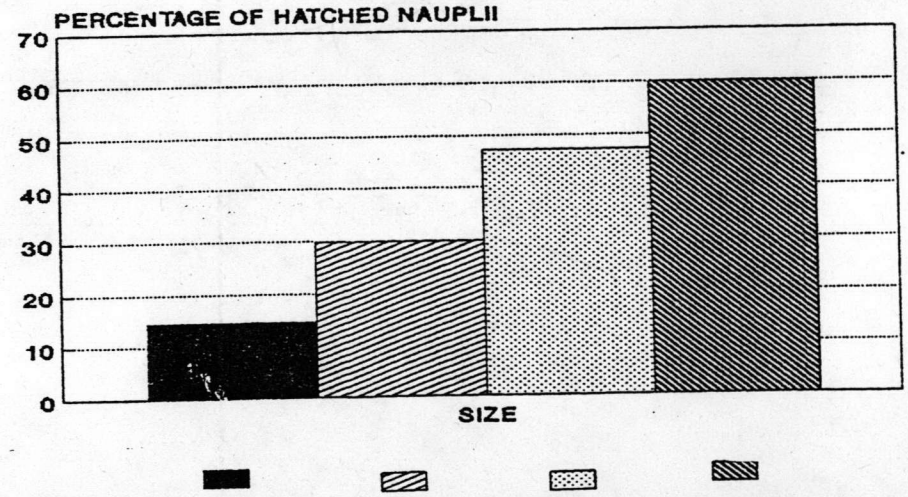

Figure 20. Comparison of the mean percentage of total hatched nauplii at interval sizes of pond-reared male *P. monodon*.

B. Sperm Examination.

Sperm quality of pond-reared *P. monodon* at sizes ranging from 22 to 90 g body weight from 446 observations were evaluated as followed: (1) spermatophore weight (2) percentage of morphological normality of sperm, and (3) percentage of active sperm. The results could be summarized in Table 3 and data are shown in Appendix 2.

Table 3. Results of spermatophore weight, number of sperm per spermatophore, percentages of morphological normality of sperm and active sperm from pond-reared *P. monodon* at different sizes.

Variables	N	Min.	Max.	Mean	Std. Dev.
Spermatophore weight	434	0.0012	0.1410	0.0307	0.0217
Number of sperm (*1,000)	318	0	20,300	1,173	2,504
% Normal sperm	302	0	100	21.3	18.8
% Active sperm	267	0	39	3.7	7.1

The morphological characteristics of normal sperm were a round posterior main body (head) and central cap connected to the long anterior spike (Figure 21). The morphologically abnormal sperm in *P. monodon* consisted of round body shape with missing spike (Figure 22a), round body with short or bent spike (Figure 22b), malformed head (Figure 22c) and several deformity (Figure 22d). The abnormality of sperm could be easily determined under light microscope with magnification (*400).

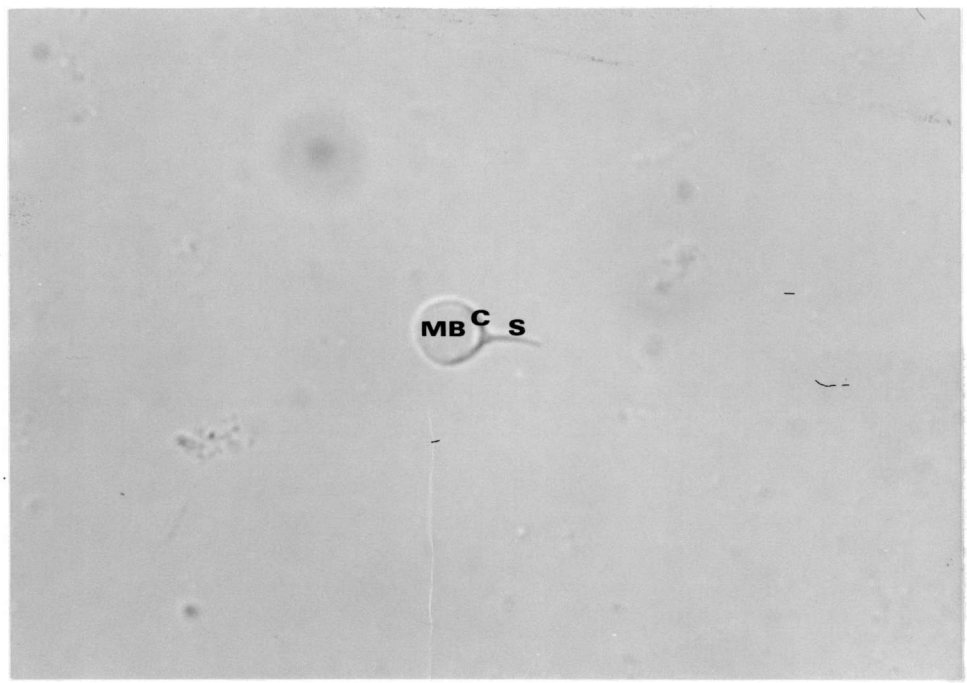

Figure 21. Light micrograph representation of the surface topography of a morphological normality of sperm, *P. monodon* illustrating the main body (MB), cap (C), and spike (S) (*1200).

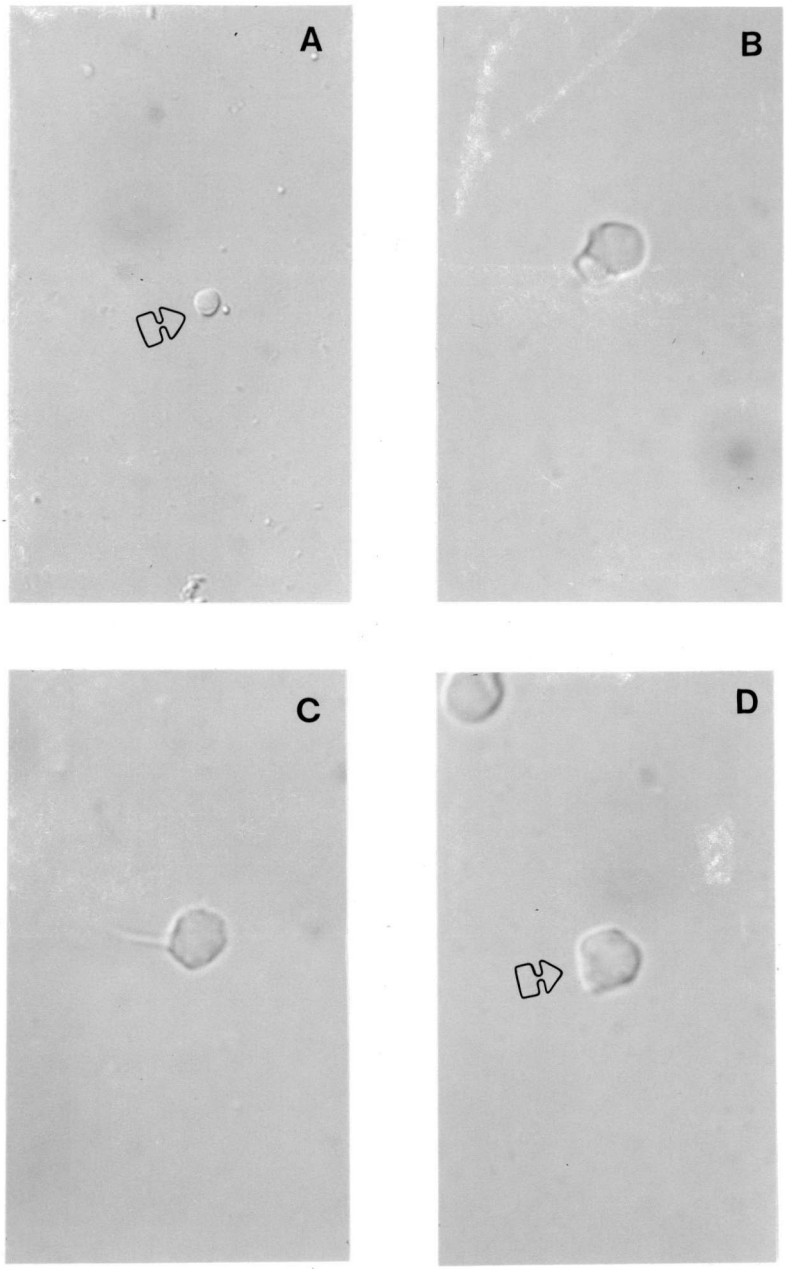

Figure 22. Light micrograph representation of the characteristics of morphological abnormalities of sperms, *P. monodon.*

a: round body with missing spike (*470),

b: round body with bent spike (*1200),

c: malformed head (*1200),

d: malformed head and missing spike (*1200).

Besides, the method of assessing sperm quality applied by Griffin *et al.* (1987) was used. The technique evaluating sperm quality by observing changes in acrosomal reaction which could be induced *in vitro* using egg water. Active sperm showed morphological changes underwent the acrosome reaction *in vitro*. The sperm rapidly react, by the rapid retraction of spike. Suddenly, the cap begins swelling, followed by dehiscence of pentalaminar membrane in the final stage. The process could be observed by light microscope as shown in Figures 23a, b, c and d.

1. Effect of Male Size on Spermatophore Size.

Over 49 days of experiment, most of 446 males exhibited whitish colour and excellent turgidity of terminal ampoules and spermatophores. Only 20 males were flaccid and less white, and merely 5 males failed to produced spermatophore.

The relationship between spermatophore weight and male weight is shown in Figures 24. The relation is expressed according to the linear regression model;

$$AS = -0.0326 + 0.0012*BW, \quad r = 0.73, \; N = 434, \; P < 0.05,$$

where

AS = average spermatophore weight (g),

BW = body weight of male (g),

The spermatophore weight was significantly affected by male weight. The larger male size, the larger spermatophore weight could be got.

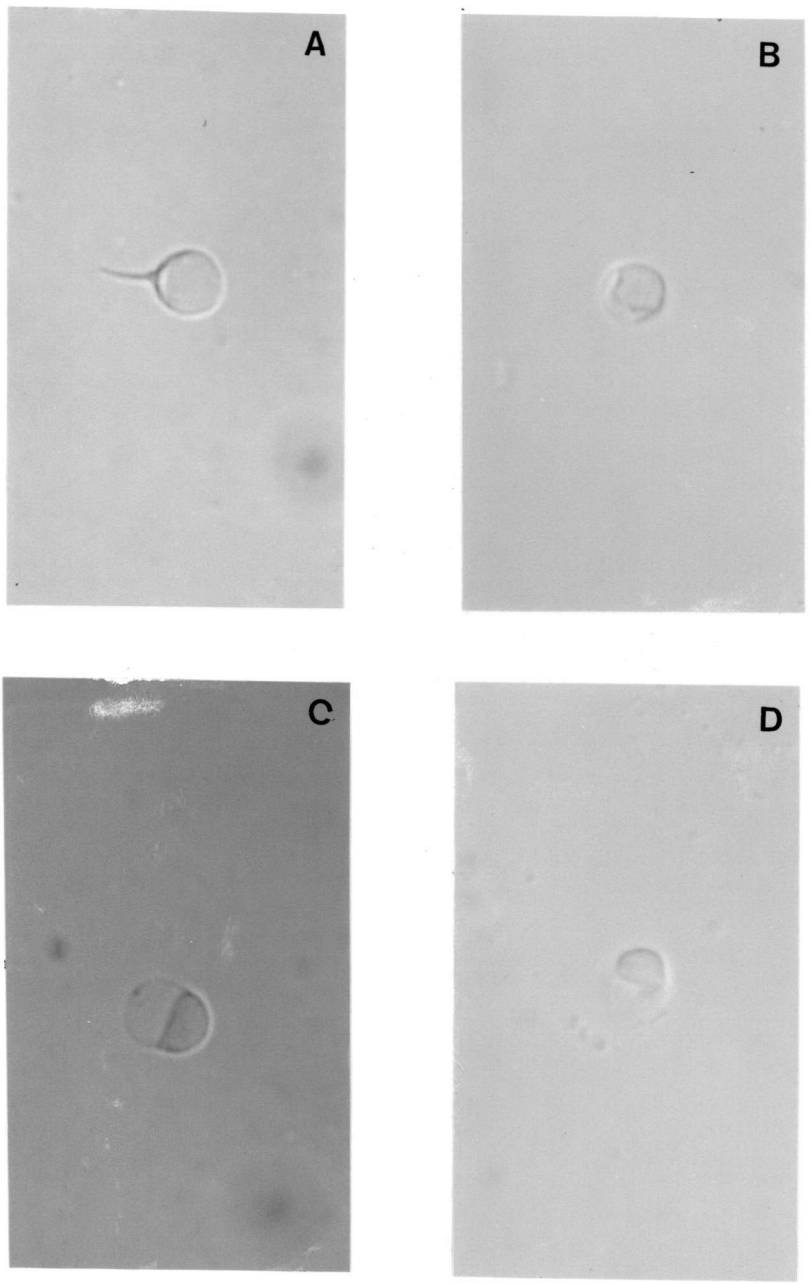

Figure 23. Light micrograph representation of morphological change in active sperm, *P. monodon* (*1200).

a: nonreacted normal sperm,

b: spike depolymerization,

c: membrane pouch swell, and

d: dihiscence of pentalaminar membrane.

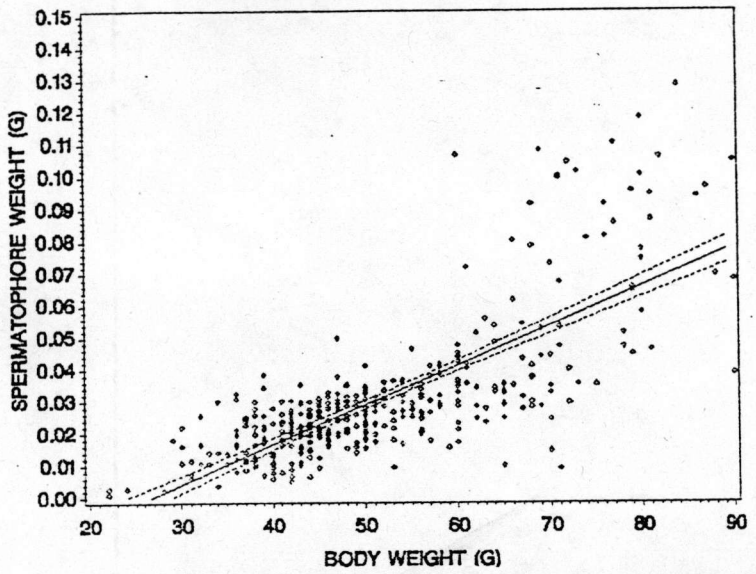

Figure 24. Relationship between average spermatophore weight and body weight of male *P. monodon*.

The multiple regression analysis indicated that the relationship among spermatophore weight, male body weight, and duration of male captivity could be explained;

AS = -0.0258 + 0.0012*BW - 0.0003*DAY, r = 0.78, N = 434, P< 0.05,

where

AS = average spermatophore weight (g),

BW = body weight of male (g), and

DAY = duration of male captivity (days).

The model implied that increasing the duration of male captivity negatively affected on spermatophore weight.

2. Effect of Spermatophore Size and Male Size on Number of Sperm per Spermatophore.

The relationships among number of sperm per spermatophore, male size, and spermatophore size of *P. monodon* are shown in Figures 25 and 26. These relationships could be expressed as regression models;

TNS = 23.99*BW, r = 0.44, N = 318, P < 0.05,

TNS = 34,967.95*AS, r = 0.46, N = 318, P < 0.05,

where

TNS = number of sperm per spermatophore ($*10^3$),

AS = average spermatophore weight (g), and

BW = body weight of male (g).

Number of sperm per spermatophore increased either with an increasing male weight or spermatophore weight.

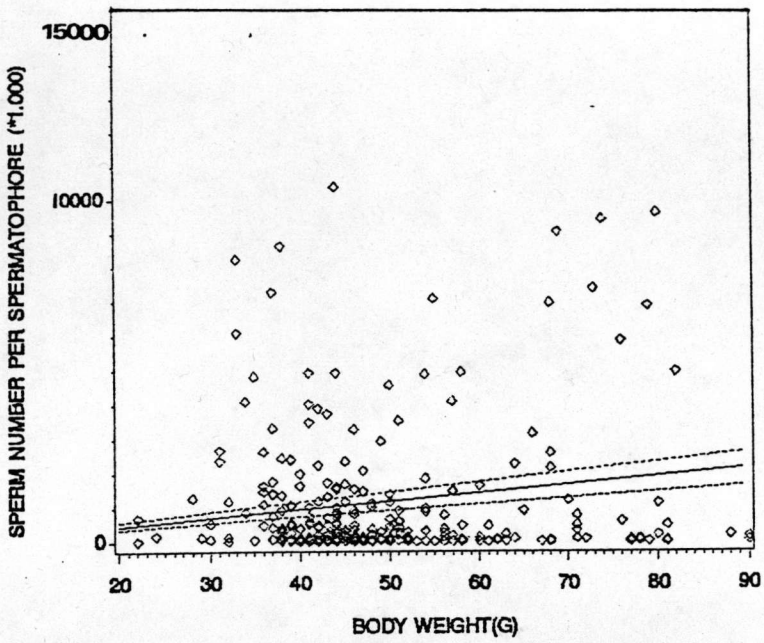

Figure 25. Relationship between number of sperm per spermatophore and body weight of male *P. monodon*.

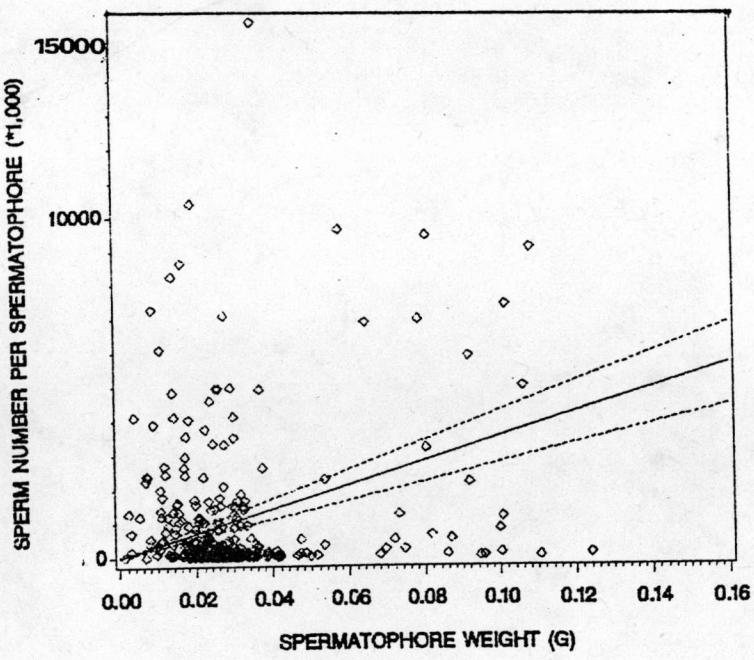

Figure 26. Relationship between number of sperm per spermatophore and average spermatophore weight.

3. Effect of Male Size and Number of Sperm on Morphological Normality of Sperm.

The relationship between the percentage of morphological normality of sperm, male size, and number of sperm per spermatophore, could be explained by a multiple regression model;

$PN = 29.98 - 0.21*BW + 0.001*TNS$, $r = 0.22$, $N = 302$, $P < 0.05$,

where

PN = percentage of morphological normality of sperm,

BW = body weight of male (g), and

TNS = number of sperm per spermatophore ($*10^3$).

The equation showed that the percentage of morphological normality of sperm decreased with male size, but increased with number of sperm.

4. Effect of Male Size and Morphological Normality of Sperm on Active Sperm.

Generally, the percentage of active sperm of pond-reared males *P. monodon* was relatively low. The present study show the active sperm ranged 0 to 39 percent of total sperm. The percentage of active sperm was positively affected by the percentage of morphological normality of sperm. This relation is shown in Figure 27, and could be expressed by a linear regression model;

$PAC = 0.16*PN$, $r = 0.57$, $N = 267$, $P < 0.05$,

where

PAC = percentage of active sperm, and

PN = percentage of morphological normality of sperm.

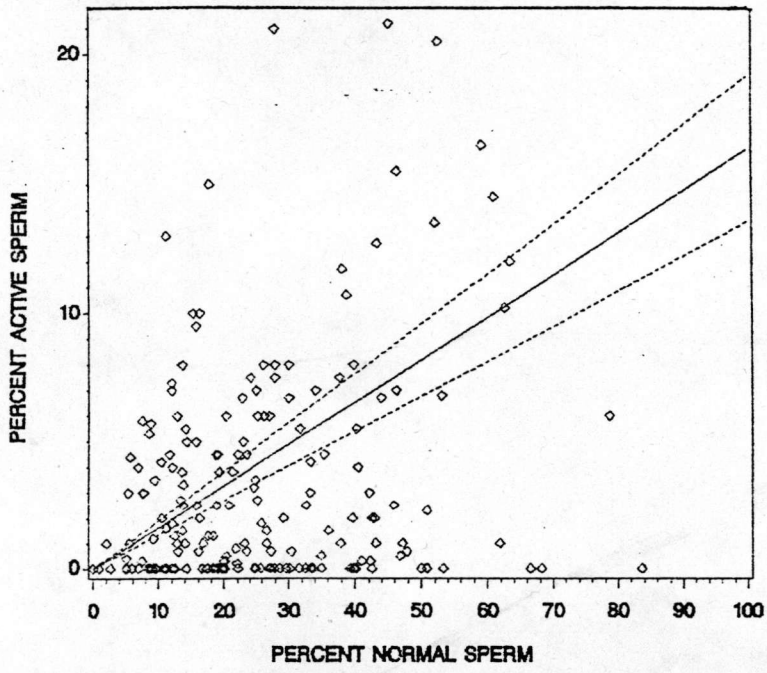

Figure 27. Relationship between percentage of active sperm and percentage of morphological normality of sperm.

The relationship among the male weight, the percentage of morphological normality of sperm, and the percentage of active sperm could be expressed by a multiple regression model;

$PAC = 5.50 - 0.10*BW + 0.14*PN$, $r = 0.40$, $N = 267$, $P < 0.05$,

where

PAC = percentage of active sperm,

PN = percentage of morphological normality of sperm, and

BW = body weight of male (g).

The equation showed that the percentage of active sperm decreased with male size, but increased with the percentage of morphological normality of sperm.

5. Effect of Interval Size on Sperm Quality.

To compare results of interval male sizes on sperm quality, analysis of variance and Duncan's new multiple range test were used. Four interval male sizes were determined by body weight; size A (22-40 g), size B (41-50 g), size C (51-60 g), and size D (61-90 g), respectively. The result was summarized in Table 4.

Clearly, the largest prawn had largest spermatophore weight (size D > size C > size B > size A, $P < 0.05$). Similarly, the number of sperm per spermatophore in large size (D) was significantly higher than that of others size classes ($P < 0.05$). In contrast, the small prawns (size A) showed the highest percentages of morphological normality of sperm and active sperm.

Table 4. Comparison of spermatophore weight, number of sperm per spermatophore, the percentages of morphological normality of sperm and active sperm among interval sizes of males, *P. monodon*. Values are Mean ± Standard Deviation.

Size (g)	Spermatophore weight (g)	Number of sperm per spermatophore ($*10^3$)	percentages of normal sperm	percentages of active sperm
22-40	0.0165 ± 0.0083 d	1,399.4 ± 1,970.4 b	32.0 ± 19.6 a	6.7 ± 10.7 a
41-50	0.0227 ± 0.0067 c	796.8 ± 2,071.1 b	17.1 ± 15.6 b	2.7 ± 5.7 b
51-60	0.0306 ± 0.0114 b	725.1 ± 1,441.2 b	20.1 ± 17.8 b	3.0 ± 4.9 b
61-90	0.0566 ± 0.0300 a	2,497.3 ± 4,225.2 a	21.0 ± 22.2 b	1.5 ± 2.5 b

Note: a,b, and c denote significant differences in column. The same letter indicates no significant different ($P < 0.05$).

Comparison of Sperm Quality between Normal and Ablated Pond-Reared *P. monodon*.

In this experiment, spermatophore weight, number of sperm per spermatophore, the percentages of morphological normality of sperm and active sperm were obtained. Spermatophore ejaculation was done sequentially during a period of 7 to 81 days in captivity. The results could be summarized in Table 5 and data are presented in Appendix 3.

The results indicated that eyestalk ablation significantly positive effect on the percentage of morphological normality of sperm. The expression of this relationship is shown in Figure 28, and could be represented by a linear regression model;

$$PN = 11.47*EXT, \quad r = 0.77, \quad N = 48, \quad P < 0.05,$$

where

PN = percentage of morphological normality of sperm, and

EXT = spermatophore ejaculation frequency.

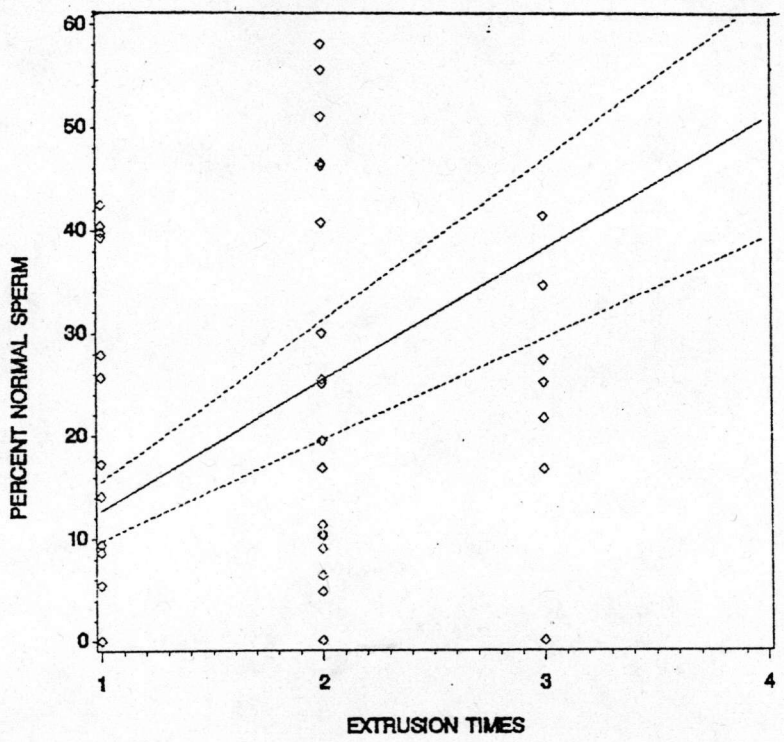

Figure 28. Relationship between the percentage of the morphological normality of sperm and ejaculated spermatophore frequency in ablated pond-reared *P. monodon*.

During 2-3 months in captivity, male prawns showed very rare syndrome of deterioration and discoloration of terminal ampoules and spermatophores. However, the eyestalk ablated males showed lower survival rate than the normal males.

Table 5. Results of spermatophore weight, number of sperm per spermatophore, the percentages of morphological normality of sperm and active sperm of normal and ablated pond-reared *P. monodon*.

Group	Variables	N	Min.	Max.	Mean	Std. Dev.
Normal	Spermatophore weight	50	0.0102	0.0418	0.0242	0.0075
	Number of sperm (* 1,000)	53	0	2,611	328	503
	% Normal sperm	49	0	72	15	16
	% Active sperm	49	0	26	3	5
	% Survival	-	-	-	30	-
Ablation	Spermatophore weight	51	0.0098	0.0576	0.0262	0.0090
	Number of sperm (* 1,000)	54	0	7,188	462	1,210
	% Normal sperm	48	0	64	21	19
	% Active sperm	48	0	21	3	5
	% Survival	-	-	-	5	-

Comparing results between groups analyzed by Student's t-test, showed no significant difference among spermatophore weight, number of sperm per spermatophore, percentages of morphological normality of sperm and active sperm in eyestalk ablated group and normal group.

Comparison of Sperm Quality between Wild and Pond-Reared *P. monodon*.

Spermatophore weight, number of sperm per spermatophore, the percentages of morphological normality of sperm and active sperm of wild and pond-reared stocks are summarized in Table 6. These data obtained from 68 ejaculations of 56 wild males with 46 g to 138 g body weight, and 202 ejaculations of 182 pond-reared males with 50 g to 90 g body weight. The data are shown in Appendix 4.

Table 7 summarizing data indicates that spermatophore weight, number of sperm per spermatophore, the percentages of morphological normality of sperm and active sperm in the wild males showed higher values than that of the pond-reared male. However, only spermatophore weight showed significant difference ($P < 0.05$).

Throughout the experimental period, very few males exhibited deterioration and discoloration of terminal ampoules and spermatophores. However, the wild males did show more deterioration of terminal ampoules and spermatophores than that of the pond-reared males at the same period in captivity.

Table 6. Results of spermatophore weight, number of sperm per spermatophore, the percentages of morphological normality of sperm and active sperm of wild and pond-reared *P. monodon*.

Source	Variables	N	Min.	Max.	Mean	Std. Dev.
Pond	Spermatophore weight	202	0.0094	0.1410	0.0428	0.0259
	Number of sperm (* 1,000)	120	0	16,483	1,463	3,040
	% Normal sperm	115	0	100	20	19
	% Active sperm	84	0	26	3	4
Wild	Spermatophore weight	68	0.0218	0.1102	0.0566	0.0191
	Number of sperm (* 1,000)	69	0	47,247	2,677	6,572
	% Normal sperm	57	0	64	21	18
	% Active sperm	49	0	26	4	6

Table 7. Comparison of spermatophore weight, number of sperm per spermatophore, the percentages of morphological normality of sperm and active sperm between wild and pond-reared males *P. monodon*. Values are Mean ± Standard Deviation.

Source	Spermatophore weight (g)	Number of sperm per spermatophore ($*10^3$)	percentages of normal sperm	percentages of active sperm
Pond	b 0.0428 ± 0.0191	a 1,463.1 ± 3,040.1	a 19.6 ± 19.2	a 2.5 ± 4.5
Wild	a 0.0566 ± 0.0259	a 2,677.4 ± 6,572.5	a 21.4 ± 18.3	a 4.2 ± 6.2

Note: a and b denote significant difference in column. The same letter are not significantly different ($P < 0.05$).

Minimum Size of the Initial Maturation of Pond-Reared Males *P. monodon*.

In the present study, the initial male maturation was defined as the initiation of morphological normality of sperm in terminal ampoules. Sixty-three pond-reared males *P. monodon* with sizes ranging from 11 to 36 g body weight were used. The results are shown in Appendix 5.

The relationship between the percentage of morphological normality of sperm in terminal ampoules and petasmal length was a linear regression model (Figure 29) which could be expressed as;

$TA = -10.46 + 5.96*PL, \quad r = 0.31, N = 62, P < 0.05,$

where

TA = percentage of morphological normality of sperm in terminal ampoules, and

PL = petasmal length (mm).

The percentage of morphological normality of sperm in terminal ampoules as positively increased with petasmal length.

Under the present study, a positive relationship between petasmal length and carapace length (Figure 30) could be expressed as;

$CL = 23.77 + 2.26*PL, \quad r = 0.78, N = 62, P < 0.05,$

where

PL = petasmal length (mm), and

CL = carapace length (mm).

Also, the relationship between body weight and petasmal length is shown in Figure 31 could be expressed as;

$\ln BW = 2.13 + 0.74* \ln PL, \quad r = 0.81, N = 62, P < 0.05,$

where

lnBW = natural logalithm of body weight (g), and

lnPL = natural logalithm of petasmal length (mm).

From the results above, the regression models estimated that the pond-reared male with size of 28 mm carapace length or 13 g body weight could produce first mature sperm in terminal ampoules.

In addition, this result also showed a significant positive relationship between the percentage of the morphological normality of sperm in terminal ampoules and in vas deferens (Figure 32). This relationship could be explained as;

$TA = 0.40*VD, \quad r = 0.58, \quad N = 43, \quad P < 0.05,$

where

TA = percentage of morphological normality of sperm in terminal ampoules, and

VD = percentage of the morphological normality of sperm in vas deferens.

The equation implied that only about 40 percent of the morphological normality of sperm could be found in vas deferens comparing to a 100 percent in terminal ampoules. It is clear that the sperm in terminal ampoules were more mature than that of ones in vas deferens.

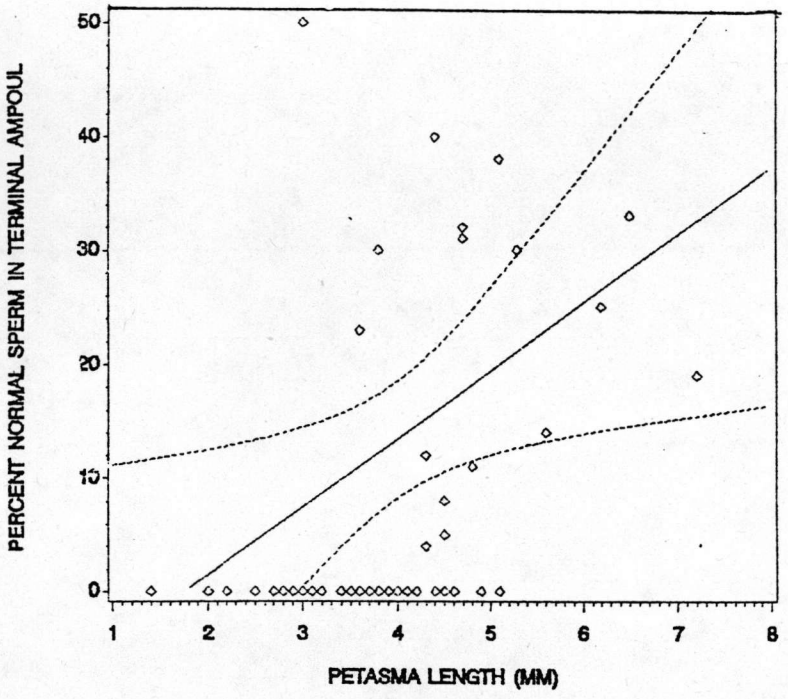

Figure 29. Relationship between the percentage of morphological normality of sperm in terminal ampoules and petasma length of male *P. monodon.*

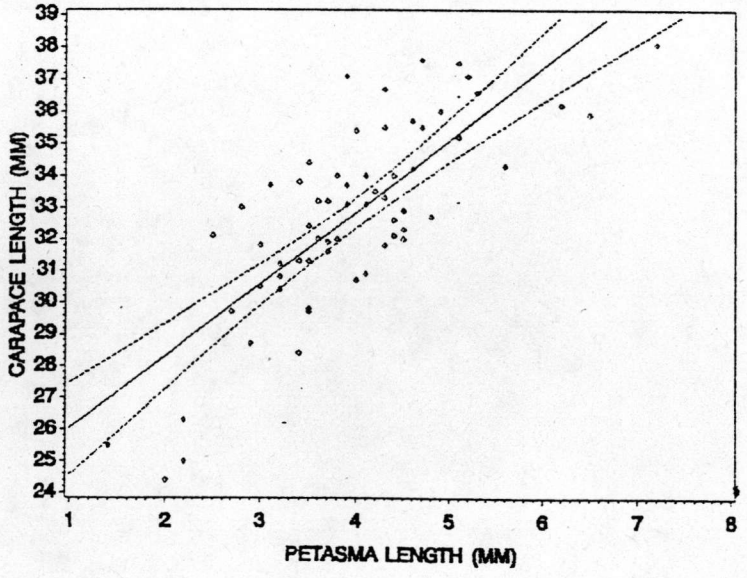

Figure 30. Relationship between petasma length and carapace length of male *P. monodon.*

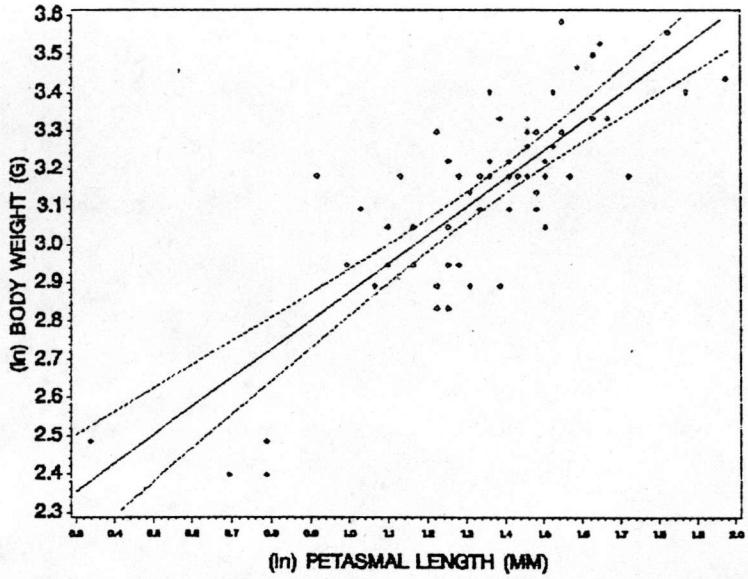

Figure 31. Relationship between petasma length and body weight of male *P. monodon.*

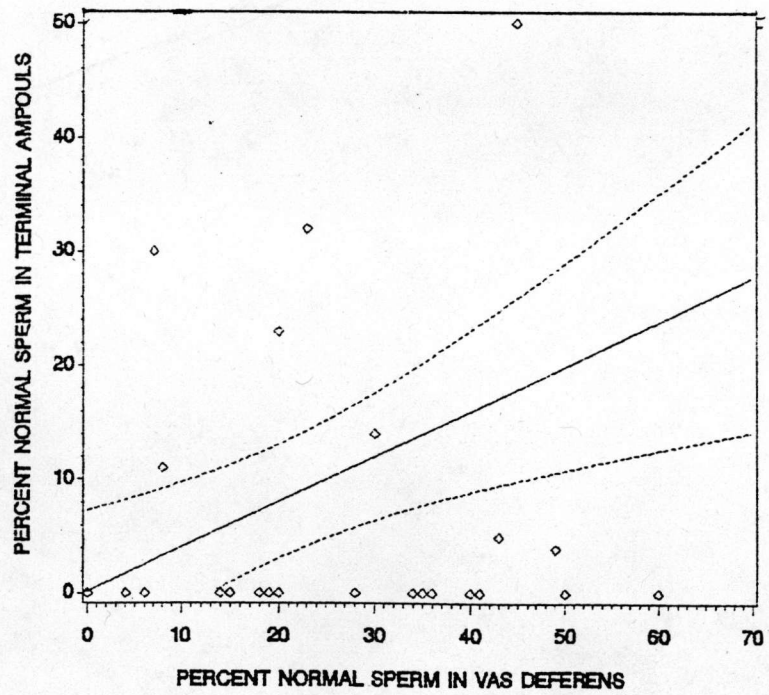

Figure 32. Relationship between the percentage of morphological normality of sperm in terminal ampoules and in vas deferens of male *P. monodon.*