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Effect of Ecdysial Cycle on the Semen and Spermatophore production of the Indian White Prawn (*Penaeus indicus* H.M. Edwards)

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Adult male *Penaeus Indicus* have a prolonged premolt phase and short inter and postmolt phases in its ecdysial cycle. Vas deferens factor and spermatophore weight of male prawns show a declining tendency from post to premolt phases with a successive reduction in the biochemical composition of vas deferens and terminal ampoule. The correlation analysis indicate differential intensity of coefficients between biochemical and morphometrical relationships of semen and spermatophore production of *P. Indicus*.

Key words: ***P. Indicus*, Ecdysial cycle, Semen spermatophore, morphometric relations.**

Several studies have been carried out recently on the electro - ejaculation of spermatophores and artificial insemination in prawns (Muthu & Laxminarayana, 1984; Lin, 1986; Main & Fulks, 1990) and much useful information have been collected on the mating behaviour of penaeid prawns (yane *et al.*, 1988). A knowledge of the biochemistry and physiology of the spermatophore and semen is a prerequisite for artificial insemination in prawns. This report describes the ecdysis related changes in the morphometric variations of the male reproductive tract, spermatophore and biochemical constitution of the parts of the tract of *P. indicus*.

Materials and Methods

Adult male *P. indicus* collected from kadalundi estuary, kozhikode, kerala during April 1983, were used for the present investigation. The prawns were stocked at the rate of 4 to 5 per square meter in 1x1x1 m size rectangular cement tanks. Water was maintained at 0.6m level and was changed twice a week while debris and molted shells were removed daily. The prawns were fed *ad lib* with boiled pieces of egg white at the rate of 12 percentage of biomass: equally divided and fed twice a day. The weight and carapace length of each prawn wa noted followed by identification of molt stage. By observing the inner uropodal setae under microscope, the molting stages were divided into postmolt (B), intermolt (C) and premolt (D). The molt stage of prawns was calculated in predetermined individual samples for a full molt cycle through daily microscopical observation. From each of the excised male reproductive tracts, the vas deferens and terminal ampoule were separated and weighed before biochemical analysis. The vas deferens factor (Vdf) in relation to molting stage was calculated using the formula of Aiken & Waddy (1981) as

$$\frac{\text{Weight of the vas deferens in milligrams}}{\text{Cube of the carapace length in centimeters}} \times 100$$

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The spermatopphores were mechanically removed from the excised terminal ampoule and weighed separately. The proteins (Lowry *et al.*, 1951), lipids (Vogel, 1959), oligosaccharides and polysaccharides (Johnston & Davies, 1972; Dubois *et al.*, 1956) and total free aino acids (Lee & Takahashi, 1966) were estimated in the vas deferens and terminal ampoule in relation to molting stages. The correlation coefficient analysis of vdf with that of vas deferns biochemical constituents and spermatophore weight with that of terminal ampoule constituents were carried out for all samples.

Results and Discussion

The duration between two ecdysis of adult male *P. indicus* was 29. 3±3.6 days (n=32) on an average with a range of 27 to 35 days. The prawn remains in B stage for 4 to 6 days, C stage for 5 to 6 days and D stage for about 16 to 24 days. The molt related change was observed in the Vdf with peak intensity at b stage. The bulky terminal ampoule and large spermatophores were more conspicuous in the B stage than any other stage with a tendency to decline form B to D stages in morphometry (Table 1). In relation to successive stages of molting a successive reduction of biochemical composition of vas deferens and terminal ampoule of *P. indicus* occurred with exceptions of lipids of vas deferens and proteins of terminal ampoule which have peaks at D and C stages respectively (Tables 2 and 3). The

Tabel 1. Ecdysis related changes in Vdf, terminal ampoule and spermatophore

	Stages of ecdysial cycle (mg)		
	Postmolt (B)	Intermolt (C)	Premolt (D)
Vdf mg	56.716± 4.337 (5)	46.132± 3.469 (5)	44.829± 3.876 (5)
Terminal ampoule Weight mg	18.2± 3.2 (5)	17.8± 2.6 (4)	9.5± 2.9 (4)
Spermatophore weight mg	9.2± 1.8 (5)	8.9± 1.6 (4)	4.7± 2.0 (5)

Significant at 0.05 level. Numbers in parenthesis indicate the number of animals used.

Table 2. Ecdysis related changes in the biochemical composition of the vas deferens of *P. Indicus*

Biochemical constituents	Stages of ecdysial cycle		
	B	C	D
Proteins	7.998± 0.441 (5)	7.831± 0.569 (5)	7.635± 0.0699 (5)
Lipids	3.794± 0.168 (5)	3.064± 0.241 (5)	3.441± 0.138 (5)
oligosa-charides	1.520± 0.152 (5)	1.236± 0.095 (5)	1.099± 0.097 (5)
Polysa-ccharides	3.903± 0.094 (5)	3.648± 0.121 (5)	3.140± 0.376 (5)E
Free amino acid	0.462± 0.021 (5)	0.420± 0.031 (5)	0.320± 0.027 (5)E

Significant at 0.05 level and E significant at 0.01 level. Number mentioned in paranthesis indicate the number of animals used

Table 3. Ecdysis related changes in the biochemical composition of the terminal ampoule of *P. indicus*

Biochemical constituents	Stages of ecdysial cycle		
	B	C	D
Protines	10.035± 0.271 (5)	10.656± 0.147 (5)	9.653± 0.035 (5)
Lipids	4.239± 0.615 (4)	3.708± 0.120 (4)	2.905± 0.342 (5)
Oligosa-ccharides	2.612± 0.358 (5)	2.560± 0.159 (5)	2.063± 0.072 (5)$
polysa-ccharides	2.495± 0.230 (5)	2.339± 0.469 (5)	2.037± 0.314 (5)
Free amino acids	0.410± 0.025 (5)	0.358± 0.012 (5)	0.267± 0.010 (5)$

Significant at 0.05 level and $ significant at 0.01 level. value in parenthesis indicate the number of animals used.

correlation analysis show a higher coefficient value with that of proteins and free amino acids. Comparative decrease of vdf with decrease of vaspolysaccharides is less, while in the terminal ampoule the polysaccharide level decrease is comparable with that of spermatophore weight reduction. The oligosaccharide fraction in both parts of the male reproductive tract show a contrasting coefficient to that of polysacharides on correlation analysis (Table 4).

Table 4. Correlation coefficients between the different biochemical components of vas deferens (A) and terminal ampoule (B)

Biochemical constituents	"r" of	
	A	B
Proteines	0.865	0.824
Oligosaccharides	0.769	0.368
Polysaccharides	0.309	0.643
Free amino acids	0.859	0.842

n1 + n2 - 2 = 28

Reproduction and ecdysis in natantian decapods are synergistic processes. In prawns sixty percent of the ecdysial duration is occupied by D stage (Wickins, 1976). Similarly the males of *P. indicus* show a predominant D phase (50 to 80 percent) in their total ecdysial duration. Chamberlain & Lawrence (1981) indicated that all male penaeids are not necessarily active in reproduction. To differentiate the specific molt stage of *P. indicus* where the peak activity occur in male reproduction tract, the vdf of Aiken & waddy (1981) was utilized and the peak activity was observed at B stage. In the freshwater prawn, Macrobrachium Idella (Sreekumar, 1987) that peak male reproductive tract activity was observed at C stage, while in *P. indicus* the quantum is higher at B stage specifically with that of spermatophore and terminal ampoule weights. In prawns, the molt hormone titre in the haemolymph is affected by unilateral eyestalk ablation. The eye stack further increases the spermatozoan count and spermatophore weight in *P. vannamei* (Loung Truijillo & Lawrence, 1985). Our observations indicate that in male *P. indicus*, a prolonged D phase may not help more in the formation of full sprematophores but only the formation hypertrophied spermadophores. The laboratory rearing of male *P. setiferus* by Leung - Truijille & Lawrence (1987) upto 7 weeks led to less of spermatophore weight with an increase in spermatozoan mortality. The freshly ejaculated spermatophore of *P. indicus* has a sperm sac and foliaceous wings (Sasikala & Subramoniam, 1987) similar to other closed thelycum penaeids (Chow *et al.*, 1990). Hudinaga's (1942) observation in *P. japonicus* suggests that mating occurs in the kuruma prawn between hardmales and soft females, which is confirmed in *P. indicus* too. The fully grown up spermatophore is naturally programmed to release at B stage and possibly at early C stage too. The lipid levels of terminal ampoule of *P. indicus* reaches its peak level at B stage only. Seminal lipids of *P. indicus* maily composed of cholesterol and triglycerides (Muthuraman & Adiyodi, 1985), the former being a close relative of the molting hormone 'ecdysone' reaches its peak level in the haemolymph of the crab *Carcinus maenas* at D stage, Eventhough it is not essential for spermiation (Arvy *et al.*, 1956), the esterified sterols dominate the testis of *P. japonicus* at B and D stages after injection of B - Cistosterol (Kanazawa *et al.*, 1976). The presence of polysaccharides at high

level at B stage in the male reproductive tract of *P. indicus* possibly indicates that the ejaculation in nature occurs presumably at B or early C stages.

References

Aiken, D.E., & Waddy, S.L. (1981) *Reproductive Biology* (Philipps, C. and C. Cobbs, Eds.) p. 215, In "Biology and management of Lobsters", Academic Press, New York

Arvy, T.l., Echalier, G. & Gabe, M. (1956) *Annuals Science Naturalle Zoologique Biologique Animale* (ii, 18): 263

Chamberlain, G.W. & Lawrence, A.L. (1981) *J.World Mariculture society,* 12: 357

Chow, S., Sandifer, P.A., Dougherty, M.M. & Dougherty. W.J. (1990). In *proceedings of the second Asian Fisheries Forum* (Hirano, S. and I. Hanyu. Eds.) p. 623, Asian Fisheries Society, Manila, Philippines

Dubois, G., Hamilton, K.A., Robers, J.K. & Smith, F. (1956) *Analytical Chemistry,* **28**: 350

Hudinage, M. (1942) *Japancese Journal of Zoology,* **10**: 305

Johnston, M.A. & Davies, P.S. (1972) *Comp. Biochem. and Physiol.* 41B: 443

Kanazawa. A., Guary, J.C.B. & Ceccaldi, H.J. (1976) *Biochem. and Physiol.* 54 B: 205

Lee, Y.P. & Takahasi, T. (1966) *Analytical Chemistry.* **14**: 71

Leung - Truijillo, J.R. & Lawrence, A.L. (1985) *J. World Mariculture Society,* **16**: 258

Leung - Truijillo, J.R. & Lawrence A.l. (1987) *Aquaculture,* **65**: 363

Lin, M.N. (1986) National Science Council/monograph Series Biological Research Centre. Tokyo, Japan, **14**: 209

Lowry, O.H. Rosebrough, N.J., Farr, A.l. & Randall, R.J. (1951) *J. Biolo. Chem.* **193**: 263

Main, K.l. & Fulks, W. (1990) *The culture of cold tolerant Shrimp:* proceedings of an Asian - US Workshop on shrimp culture. The oceanic Institute, Honolulu, Hawaii, USA

Muthu, M.S. & Laxminarayana, A. (1984) *Current Science,* **53**: 1073

Muthuraman, A. & Adiyodi, K.G. (1985) in *"Biology of Marine Benthic Organisms".* (Thompson, M.F., Sarojini, R. and Nagabhushanam, R. Eds.) p. 131, Oxford and IBH publishers Company, New Delhi, India.

Sasikala, S.l. U Subramoniam, T. (1987) *Journal of Experimental Marine Biology and Ecology,* **113**: 145

Sreekumar, S. (1987) *Reproductive physiology of male decapod crustaceans,* Ph.D. Thesis, Calicut University, Kerala India.

Vogel, A.I. (1959) *A text book of practical organic chemistry including qualitative organic analysis.* Spottiswoods, Ballantyne and Company Limited, London

Wickins, J.F. (1976) in *Annual Review of Oceanography and Marine Biology.* (Barnes, H., Eds.) p. 435, Marine Bioloiccal Station, Woodshole, USA

Yano, R.A., Kanna, R.N. & Wyban, J.A. (1988) *Marine Biology.* **97**: 171