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Biochemical Characterisation of Different Male Morphotypes of *Macrobrachium rosenbergii* (De Man)

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Protein, carbohydrate, lipid, DNA and RNA contents were studied in the muscle tissue, hepatopancreas and gonads of male morphotypes of *M. rosenbergii* viz. small males (SM), orange clawed males (OC) and blue clawed males (BC) and their transitional stages, weak orange clawed (WOC) and strong orange clawed (SOC). Significant difference could be seen in protein, DNA and RNA contents of muscle tissue, carbohydrate and RNA contents in hepatopancreas and DNA and RNA contents of the gonads of various male morphotypes. Highest protein content in muscle was recorded for SOC (19.10%) and minimum for WBC (17.71%) and OBC (17.52%). Highest values of RNA (1.020 mg/g) and DNA (0.68mg/g) of muscle tissue was also recorded in SOC. Carbohydrate content (2.37%) and RNA (0.744 mg/g) content of hepatopancreas were also found to be high in SOC when compared to other male morphotypes. In gonads highest DNA content (0.891 mg/g) was observed in OBC whereas RNA content was maximum in SBC (1.159 mg/g). Results of the present study would be useful in providing biochemical evidence for the existence of morphotypes in male populations of *M. rosenbergii*.

Key words: ***Macrobrachium rosenbergii*, Male Morphotypes, Biochemical Characterization.**

Sexually mature male population of *Macrobrachium rosenbergii* belonging to same age group has been differentiated into three distinct morphotypes, small males (SM), orange clawed males (OC) and blue clawed males (BC), (Brody *et al.*, 1980; Cohen *et al.*, 1981). Two transitional stages of both OC and BC are also distinguishable from the fully differentiated males. (Sagi & Ra'anan, 1988; Harikrishnan & Kurup, 1997). Review of literature showed that no attempt has so far been made to evaluate the biochemical variations, if any, taking place in these morphotypes. Therefore an attempt was made to study the biochemical basis of morphotypic differentiation seen in male population of *M. rosenbergii*.

Materials Methods

Prawn samples for the present study were collected from Abhangura Aqua farms, a polder based monoculture grow out of *M. rosenbergii* situated adjoining to the Vembanad lake, Kerala, South India. The sampling was done on the day of total harvesting of the pond with a view to study male morphotypic composition of the harvested population (Brody *et al.*, 1980; Cohen *et al.*, 1981; Sagi & Ra'anan, 1988; Harikrishnan & Kurup, 1997) and also to ensure that the morphotype collected for biochemical characterisation belong to the same age group. The samples were transported to the laboratory in live condition and segregated into different morphotypes (Kuris *et al.*, 1987; Harikrishnan &

Kurup, 1997). Length and weight were measured and thereafter specimens were sacrificed and muscle and hepatopancreas were taken. Each sample of gonad comprised of testes collected from three specimens. SM belong to a length group of 88 to 121 mm with an average of 107 mm (11.10g) were considered for analysis during the present study. Orange clawed males viz., WOC, SOC and pre-transforming strong orange clawed males (t-SOC) with average lengths of 165.5 mm (46.87g), 201.92 mm (89.90g) and 199.22 mm (82.57g) respectively were used for various biochemical analyses. The mean length of WBC, SBC and OBC used for the present study were 186.5 mm, (72.00g), 249.55 mm (186.24g) and 271.50 mm (217.47g) respectively. A weighed portion of the sample was kept in a hot air oven at 60°C and dried to constant weight in order to determine the moisture content. Protein (Micro-Kjeldahl's method), carbohydrates and lipids were estimated following AOAC (1976), Heath & Barnes (1970) and Folch *et al.*, (1957) respectively. RNA and DNA were extracted from the tissues and estimated following the procedure of Schneider (1957). 0.5 g sample was homogenised in 2.5 ml 10% TCA and centrifuged and the residue so obtained was washed once with 2.5 ml TCA. The final residue after removal of acid soluble compounds was extracted twice with 5 ml 95% ethanol and centrifuged. The lipid free residue was suspended in 1.33 ml of water and 1.3 ml of 10% TCA and the mixture was heated for 15 min at 90°C and centrifuged. In order to estimate DNA, 1 ml of the nucleic acid extract was mixed with 2 ml diphenyl amine reagent and heated for 10 min in boiling water bath and absorbency was read at 600 nm along with standard (Calf thymus DNA) (Schneider, 1957). In the case of RNA 0.5 ml of nucleic acid extract was diluted to 5 ml and heated with 5 ml orcinol reagent and absorbency was read at 660 nm (Schneider, 1957). The results of the biochemical studies were analysed statistically using ANOVA and t-test (Snedecor & Cochran, 1962).

Results and Discussion

a) Muscle tissue

The mean values of protein, carbohydrates, lipids, DNA, RNA and RNA/DNA ratio in different tissues of the various morphotypes of *M. rosenbergii* are given in Table 1. Results of analysis of variance showed that there was significant difference ($P<0.01$) in protein, DNA and RNA contents of muscle among various male morphotypes (Table 2). Highest values of DNA and RNA were recorded in SOC and t-SOC and the lowest, in WBC and SM. Results of pair-wise analysis showed that muscle protein and DNA contents of SOC and its transitional stage, t-SOC showed significant difference from that of SBC and its transitional stages, WBC and OBC (Table 3). No significant differences could be seen in the muscle protein content of SM when compared with other morphotypes (Table 3).

b) Hepatopancreas

In general, the moisture and protein content were found to be lower in hepatopancreas than in muscle, but the lipid content was higher (Table 1). Results of ANOVA showed that there was significant difference ($P<0.05$) in carbohydrate and RNA contents of hepatopancreas in various morphotypes (Table 2). Carbohydrate levels in the hapatopancreas of SM showed significant

Table 1. Biochemical composition of different tissues of various male morphotypes of *M. rosenbergii*

Morphotypes	Moisture (%)	Protein (%)	Carbohydrates (%)	Lipids (%)	DNA (mg/g)	RNA (mg/g)	RNA/DNA Ratio
Muscle							
SM	76.12±1.50 (9)	18.50±.0.97 (9)	1.04±0.15 (9)	1.62±0.22 (9)	0.306±0.05 (8)	0.412±0.11 (8)	1.334±0.18 (8)
WOC	75.19±1.09 (10)	18.99±0.95 (10)	1.15±0.29 (10)	1.66±0.31 (10)	0.440±0.02 (4)	0.510±0.05 (4)	1.166±0.17 (4)
SOC	75.20±1.14 (11)	19.10±1.20 (11)	1.25±0.25 (11)	1.75±0.26 (11)	0.688±0.02 (6)	1.020±0.18 (6)	1.489±0.29 (6)
t-SOC	75.20±0.73 (9)	18.62±0.50 (9)	1.30±0.35 (9)	1.79±0.31 (9)	0.652±0.12 (14)	0.977±0.30 (14)	1.492±0.34 (14)
WBC	76.01±0.95 (12)	17.71±0.71 (12)	1.13±0.16 (12)	1.72±0.28 (12)	0.362±0.09 (8)	0.441±0.09 (8)	1.292±0.27 (8)
SBC	75.91±0.99 (11)	17.83±0.81 (11)	1.21±0.16 (11)	1.89±0.34 (11)	0.407±0.07 (18)	0.641±0.10 (18)	1.626±0.38 (18)
OBC	76.48±1.20 (6)	17.52±0.58 (6)	1.46±0.41 (6)	1.94±0.44 (6)	0.371±0.05 (4)	0.482±0.06 (4)	1.298±0.01 (4)
Hepatopancreas							
SM	53.86±2.28 (9)	9.22±1.64 (9)	1.89±0.34 (9)	29.45±1.87 (9)	0.371±0.13 (8)	0.532±0.21 (8)	1.453±0.26 (8)
WOC	53.22±2.55 (10)	8.90±2.34 (10)	2.00±0.39 (10)	30.22±1.90 (10)	0.438±0.08 (4)	0.530±0.03 (4)	1.243±0.16 (4)
SOC	53.04±2.73 (11)	9.34±1.67 (11)	2.37±0.35 (11)	31.06±2.12 (11)	0.537±0.10 (6)	0.744±0.18 (6)	1.381±0.17 (6)
t-SOC	52.91±3.24 (9)	9.21±1.41 (9)	2.28±0.35 (9)	29.73±2.36 (9)	0.536±0.17 (14)	0.716±0.14 (14)	1.555±0.76 (14)
WBC	53.58±2.12 (12)	9.88±1.66 (12)	2.11±0.37 (12)	28.80±2.49 (12)	0.351±0.10 (8)	0.414±0.06 (8)	1.281±0.35 (8)
SBC	53.29±3.04 (11)	8.77±2.04 (11)	1.93±0.23 (11)	29.73±2.87 (11)	0.385±0.13 (14)	0.468±0.19 (18)	1.355±0.31 (14)
OBC	54.37±2.81 (6)	9.67±1.95 (6)	2.06±0.44 (6)	28.89±2.30 (6)	0.395±0.04 (4)	0.497±0.12 (4)	1.252±0.18 (4)
Gonads							
SM	75.08±1.18 (9)	18.36±1.12 (6)	1.06±0.47 (10)	3.50±0.38 (6)	0.439±0.01 (4)	0.493±0.03 (4)	1.126±0.11 (4)
WOC	75.19±1.03 (10)	17.01±0.52 (10)	1.37±0.38 (12)	3.58±0.28 (6)	0.534±0.01 (4)	0.610±0.03 (4)	1.141±0.02 (4)
SOC	74.71±0.68 (11)	18.77±0.56 (14)	1.69±0.36 (14)	3.68±0.28 (10)	0.530±0.09 (6)	0.697±0.12 (6)	1.322±0.12 (6)
t-SOC	74.70±1.15 (9)	18.52±0.92 (18)	1.76±0.47 (10)	3.80±0.10 (8)	0.635±0.11 (14)	0.942±0.17 (14)	1.495±0.21 (14)
WBC	74.45±0.93 (12)	18.35±1.14 (8)	1.45±0.38 (12)	3.72±0.24 (6)	0.751±0.09 (8)	1.065±0.11 (8)	1.567±0.25 (8)
SBC	73.95±0.73 (11)	18.95±0.95 (10)	1.58±0.40 (12)	3.81±0.29 (4)	0.643±0.16 (18)	1.159±0.31 (18)	1.800±0.81 (18)
OBC	74.38±1.06 (6)	18.07±1.32 (8)	1.22±0.48 (8)	3.77±0.39 (6)	0.891±0.07 (4)	0.916±0.07 (4)	1.029±0.01 (4)

Values are presented as AVG±SD; Values in parenthesis denote the number of samples

SM - Small males
WOC - Weak orange clawed male
SOC - Strong orange clawed males
tSOC - Pretransforming strong orange clawed males
WBC - Weak blue clawed males
SBC - Strong blue clawed males
OBC - Old blue clawed males

Table 2. Comparison of various biochemical constituents in different male morphotypes of *M. rosenbergii*

	Between samples		within samples		F ratio	Prob.
	df	MSS	df	MSS		
Muscle						
Moisture	6	2.457	61	1.320	1.8614	P>0.05
Protein	6	3.963	61	0.829	4.7805	P<0.01
Carbohydrates	6	0.141	61	0.074	1.9054	P>0.05
Lipids	6	0.110	61	0.101	1.0891	P>0.05
DNA	6	0.239	55	0.007	34.1429	P<0.01
RNA	6	0.545	55	0.032	17.0313	P<0.01
RNA/DNA ratio	6	0.221	55	0.102	2.1667	P>0.05
Hepatopancreas						
Moisture	6	1.786	61	7.918	0.2256	P>0.05
Protein	6	1.607	61	3.693	0.4351	P>0.05
Carbohydrates	6	0.333	61	0.149	2.2349	P<0.05
Lipids	6	6.448	61	5.829	1.1062	P>0.05
DNA	6	0.049	51	0.024	2.0417	P>0.05
RNA	6	0.160	55	0.023	6.9565	P<0.01
RNA/DNA ratio	6	0.197	51	0.209	0.9426	P>0.05
Gonads						
Moisture	6	1.805	61	0.999	1.8068	P>0.05
Protein	6	0.961	67	0.682	1.4090	P>0.05
Carbohydrates		0.381	71	0.196	1.9439	P>0.05
Lipids	6	0.080	39	0.095	0.8421	P>0.05
DNA	6	0.070	51	0.015	4.6667	P<0.05
RNA	6	0.319	51	0.032	9.9687	P<0.01
RNA/DNA ratio	6	0.366	51	0.259	1.4131	P>0.05

SM - small males
SOC - strong clawed males
WBC - weak blue clawed males
OBC - old blue clawed males
WOC - weak orange clawed males
t-SOC - pretransforming strong orange clawed males
SBC - strong blue clawed males.

difference from that of SOC, t-SOC and WBC and similar differences could be seen between SOC and SBC as well as t-SOC and SBC (Table 3). t-SOC also showed significant difference in RNA content of the hepatopancreas with that of SM and SBC. Similarly SOC and SBC were also found to differ significantly in carbohydrate content of hepatopancreas (Table 3).

c) Gonads

Higher values of protein and lipids were recorded in SBC while the carbohydrates value were high in t-SOC (Table 1). RNA content in gonads of WBC and SBC was very high and in SM, WOC and SOC it was lower. Results of the ANOVA revealed that DNA and RNA content of the gonads of male morphotypes showed significant difference (P<0.01) (Table 2). RNA content of gonad of SM was significantly different from that of other morphotypes (Table 3). Similarly RNA content of gonads of WOC also differed significantly from all other morphotype except SOC. RNA content of gonads of SOC showed significant difference with all the three BC morphotypes, whereas t-SOC showing significant difference only with WBC (Table 3).

Table 3. Results of pairwise analysis using t-test on various biochemical components showing variation in different morphotypes of *M. rosenbergii*

Combination	Muscle Protein		Muscle DNA		Muscle RNA		Hepatopancreas Carbohydrates		Hepatopancreas RNA		Gonad DNA		Gonad RNA	
	df	t	df	t	df	t	df	t	df	t	df	t	df	t
1 SM X WOC	17	1.0392 +	10	4.7153 **	10	1.1614 +	17	0.7262 +	10	1.4190 +	6	8.9805 **	6	4.7539 +
2 SM X SOC	18	1.1668 +	12	22.4763 **	12	7.3644 **	18	2.6182 *	12	3.5925 **	8	1.7747 +	8	2.9502 +
3 SM X TSOC	16	0.3155 +	20	7.5512 **	20	4.9376 **	16	2.3475 *	20	4.5752 **	16	3.2813 **	16	4.8679 *
4 SM X WBC	19	2.0611 +	14	1.2364 +	14	0.5050 +	19	1.4404 +	14	1.0356 +	10	6.3096 **	10	10.8084 *
5 SM X SBC	18	1.5971 +	24	3.5927 **	24	5.2732 **	18	0.4032 +	24	0.1524 +	20	2.3561 *	20	3.8120 **
6 SM X OBC	13	2.0810 +	10	1.9475 +	10	1.1231 +	13	0.8564 +	10	0.5955 +	6	7.1500 **	6	9.4342 *
7 WOC X SOC	19	0.2340 +	8	21.0484 **	8	4.9655 **	19	1.9113 +	8	2.0954 +	8	0.0880 +	8	1.2753 +
8 WOC X TSOC	17	0.9686 +	16	3.3848 **	16	2.9369 **	17	1.5469 +	16	2.5531 *	16	1.6866 +	16	3.6077 *
9 WOC X WBC	20	3.4516 **	10	1.3120 +	10	1.3056 +	20	0.6424 +	10	3.2870 **	10	4.3909 **	10	9.0003 **
10 WOC X SBC	19	2.8634 **	20	0.9286 +	20	2.5395 *	19	0.5098 +	20	0.6133 +	20	1.2211 +	20	2.8905 +
11 WOC X OBC	14	3.2205 **	6	2.2385 +	6	0.6289 +	14	0.2524 +	6	0.4844 +	6	4.9801 **	6	7.1387 +
12 SOC X TSOC	18	1.0668 +	18	0.9917 +	18	0.3087 +	18	0.4184 +	18	0.3603 +	20	1.9224 +	20	2.9887 +
13 SOC X WBC	21	3.3229 **	12	9.6390 **	12	7.2913 **	21	1.4463 +	12	4.4628 **	12	4.2080 **	12	6.8972 **
14 SOC X SBC	20	2.8204 **	22	13.3791 **	22	6.3437 **	20	2.7665 *	22	2.9592 **	22	1.4905 +	22	2.6022 +
15 SOC X OBC	15	2.8945 *	8	16.9830 **	8	5.1982 **	15	1.2913 +	8	2.1724 +	8	3.6135 **	8	2.9343 +
16 TSOC X WBC	19	3.1432 **	20	5.4062 **	20	4.7365 **	19	1.0225 +	20	5.6710 **	20	2.3914 *	20	3.0451 +
17 TSOC X SBC	18	2.4244 *	30	7.1541 **	30	4.3560 **	18	2.5309 *	30	3.9632 **	30	0.0347 +	30	0.4285 +
18 TSOC X OBC	13	5.1838 **	16	6.2009 **	16	4.4030 **	13	1.4255 +	16	3.9131 **	16	2.5805 *	16	0.3846 +
19 WBC X SBC	21	0.3696 +	24	1.2257 +	24	4.8416 **	21	1.3463 +	24	0.7506 +	24	1.8241 +	24	1.9737 +
20 WBC X OBC	16	0.5373 +	10	0.1574 +	10	0.7653 +	16	0.2485 +	10	1.4938 +	10	0.0200 +	10	3.6718 +
21 SBC X OBC	15	0.7849 +	20	0.9239 +	20	3.0669 **	15	0.7523 +	20	0.2734 +	20	1.3221 +	20	0.4646 +

\+ Not Significant
* Significant at 5% level (P>0.05)
** Significant at 1% level (P>0.01)

SM - Small Males
WOC - Weak Orange Clawed Males
SOC - Strong Orange Clawed Males
TSOC - Pretransforming Strong Orange Clawed Males
WBC - Weak Blue Clawed Males
SBC - Strong Blue Clawed Males
OBC - Old Blue Clawed Males

In the present study, the protein content in the muscle of different morphotypes was found varying from 16.22 to 20.3%. Significant differences in protein, DNA and RNA content of muscle in various morphotypes may serve as an index of difference in cellular activity (Lemmens, 1995) which would manifest the possibilities of heterogeneous growth rates in various morphotypes of *M. rosenbergii* (Kuris *et al.*, 1987). RNA content can be directly related to the protein synthesis (Ikeda, 1989). High protein and RNA values were observed in the muscle tissue of SOC and this indicates higher rate of protein synthesis. The same has been recorded in the case of juvenile spider crab, *Hyas araneus* (Anger & Hirche, 1990). Similarly, muscle DNA content was also high in SOC and t-SOC. Bulow (1970) and Anger & Hirche (1990) reported that increase of DNA can be taken as an indication of faster growth rate of fishes and crustaceans. Among the various morphotypes, SOC and its transitional stages are reported to be the fastest growing animals (Sagi & Ra'anan, 1988; Kurup *et al.* (unpublished). The highest levels of protein, RNA and DNA

were recorded in the body muscles of SOC and this explains the biochemical basis of the above biological manifestation in this morphotype.

Mean values of protein, DNA and RNA content in muscle tissue were found to be lower in SM, which occupies the initial stage of morphogenesis pathway. Growth of SM is also reported to be very slow as they convert a large part of their energy for mating attempts using a sneak mating behavior (Teleckey, 1984; Ra'anan & Sagi, 1985). In BC morphotypes and its transformation stages, protein, DNA and RNA values were found to be increasing gradually. Ghosh & Ray (1992) observed 1.242 mg/g and 0.51 mg/g RNA and DNA respectively in muscle tissue of *M. rosenbergii* and this, in comparison with the present study, is on the higher side. The difference may be attributed to the stages of maturity of the animal used for the study. Anger & Hirche (1990) also observed a decrease in RNA-DNA ratio with the advancement of developmental stages in crabs. However, in the present study RNA-DNA ratio in the muscle of male morphotypes did not show any specific pattern commensurate with the stages of morphogensis.

Though the lipid content in muscle, hepatopancreas and gonads does not show remarkable difference among the morphotypes, the values were low in SM and thereafter a gradually increasing trend, commensurate with the advancement of development pathway could be discernible. An exception to this trend was WBC, in which lipid was found to be less when compared to t-SOC and SBC, and this finding would lend support to the possibility of an alternative transformation pathway from SM to WBC. SM showed changes in colouration similar to that of BC when it was maintained with females in the absence of a dominant BC male (Sureshkumar & Kurup, 1996) and this finding will also support the above inference. Sherief *et al.* (1992) reported lower values of lipids in stunted males and this finding fully conform to the present observation.

Hepatopancreas plays a significant role in the food assimilation and mobilisation of energy during moulting, pigmentation, gluconeogenesis and carbohydrate storage (Dall & Moriarty, 1983; Skinner, 1985; Ghidalia, 1985). Significant variations ($P<0.05$) in carbohydrate level and RNA content of the hepatopancreas observed in the present study support the differences in growth, pigmentation and relative size of hepatopancreas among various male morphotypes. (Cohen *et al.*, 1981; Kuris *et al.*, 1987; Sagi & Ra'anan, 1988). Juinio *et al.* (1992) reported a rapid increase in total DNA and RNA in post-moult *Homarus americanus* immediately after ecdysis with an increased availability of food. The findings of the present study suggest the possibility of better food assimilation and carbohydrate storage and corresponding activity of hepatopancreas of SOC which in turn contribute to the higher somatic growth (Dall & Moriarty, 1983; Sagi & Ra'anan, 1988). Carbohydrate content of hepatopancreas of SOC and t-SOC also showed significant variation when compared with SBC and this finding would also supports the structural and functional variations of hepatopancreas between OC and BC males (Ra'anan & Sagi 1988).

In the present study a gradual increase in RNA content of the gonad could be observed from SM to SBC, which occupies the initial and penultimate stages respectively of male morphogenesis of *M. rosenbergii* (Table 1). However, a decrease in RNA content of gonad of OBC is noteworthy. This observation is in agreement with that of Ra'anan & Sagi (1985) who reported that OC have a reduced reproductive ability when compared to BC, which is reproductively very active. RNA content of SM was apparently the lowest among all the morphotypes studied and this finding may not be conforming to their high reproductive activity. Although the RNA content in the gonad of WOC and SOC were found to be relatively higher than that of SM, these are reproductively submissive (Ra'anan & Sagi, 1985). It would thus appear that the gonads of OC males are also endowed with high cellular activity identical with that of SM. RNA synthesis in gonads was found to be controlled by androgenic hormones before meiotic prophase (Pochon-Masson, 1983). The significant difference in the RNA content in gonads of male morphotypes observed in the present study may be due to the varying activity of androgenic glands (Sagi 1988).

A clear and specific variation in biochemical contents in different male morphotypes of *M. rosenbergii* could be observed in the present study and this manifests the possibility of biochemical characterisation. Differential growth rates exhibited by male morphotypes can be explained with the help of difference noticed in the levels of protein, RNA and DNA in the muscle tissue. Faster somatic growth in SOC can be correlated with the high carbohydrate and RNA contents of hepatopancreas of this group. This shows the physiological adaptation of this group for rapid assimilation of food. There exists an antagonism in energy demand between the somatic growth and reproductive activity in male morphotypes of *M. rosenbergii* (Sagi & Ra'anan, 1988). The results of the present biochemical evaluation showed that this antagonism in energy demand is more perceptible in morphotypes which are reproductively very active.

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Reference

AOAC (1976) *Official Methods of Analysis*, Association of Official Analytical Chemists 12 edn. Washington , DC, USA

Anger, K. & Hirche, H. (1990) *Mar. Biol.* **105,** 403

Brody, T., Cohen, D., Barnes, A. & Spector, A. (1980) *Aquaculture* **12,** 375

Bulow, F.J. (1970) *J. Fish. Res. Bd. Can.* **27,** 2343

Cohen, D., Ra'anan, Z. & Brody, T. (1981) *J. World Maricult. Soc.* **12,** 231

Dall, W. & Moriarty, D.J.W. (1983) In: *The Biology of Crustacea Vol.15, Internal Anatomy and Physiological Regulation*

(Bliss, D.E., & Mantel, L.H., Eds.) pp.215, Academic press Inc. Harcourt Brace Jovanovich, Orlando, Florida

Folch, J., Lees, M. & Sloane-Stanly, G.H. (1957) *J. Biol. Chem.* **26**, 497

Ghidalia, W. (1985) In: *The Biology of Crustacea Vol.9, Integument, pigments and hormonal process* (Bliss, D.E. & Mantel, L.H. eds.) p.301 Academic press Inc., Harcourt Brace Jovanovich, Orlando, Florida

Harikrishnan, M. & Kurup, B.M. (1997) In: *Proc. Ninth Kerala Science Congress*, Thiruvananthapuram, Kerala, India, p.387

Heath, J.R. & Barnes, H. (1970) *J. Expt. Mar. Biol. Ecol.* **5,** 199

Ikeda, T. (1989) *Pro. NIPR Symp. Polar Biol.* Vol.114, 1

Juinio, M.A., Cobb, J.S., Bengston, D. & Johnson, M. (1992) *Mar. Biol.* **114**, 1

Kuris, A.M., Ra'anan, Z., Sagi, A. & Cohen, D. (1987) *J. Crust. Biol.* **7,** 219

Lemmens, J.W.T.J. (1995) *J. Exp. Mar. Biol. Ecol.*, **194,** 143

Pochon-Masson, J. (1983) *Reproductive Biology of invertebrates,* Vol.II Spermatogenesis and Sperm Function (Adiyodi, K.G. & Adiyodi, R.G. eds.) p.407 John Wiley and Sons Ltd.

Ra'anan, Z. & Sagi, A. (1985) *Biol. Bull.* **169**, 592

Sagi, A. (1988) *Israeli J. Aquacult.* **40,** 107

Sagi, A. & Ra'anan, Z. (1988) *J. Crust. Biol.* **8**, 43

Schneider, W.C. (1957) *Method in Enzymology* (Colowick, S.P. & Kalpan, N.O. eds.)p.680 Academic Press, New York

Shereif, K., Mathew, P.M., Sabu Joseph & Alphi Korath (1992) In: *Freshwater Prawns* (Silas, E.G .) p.243 Kerala Agricultural University

Skinner, D.M. (1985) In: *The Biology of Crustacea, Vol.9, Integument, Pigments and hormonal process* (Bliss, D.E. & Mantel, L.H. eds.) p.444, Academic Press Inc. Harcourt Brace Jovanovich, Orlando, Florida

Snedecor, G.W. & Cochran, W.G. (1962) *Statistical Methods.* P.537, Oxford and IBH publishing group

Sureshkumar, S. & Kurup, B.M. (1996). Paper presented in National Seminar on Recent Advancements in Biological Oceanography at NIO Goa 24-26 May 1996 Abs. No.68

Teleckey, J. M. (1984) Pacific Science **38,** 372