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Biochemical changes during the reproductive activity in a female sand lobster *Thenus orientalis*

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ABSTRACT

There is a considerable importance on the changes in the biochemicals in the different tissues in the aquatic animals in accordance with the reproductive activities. In the present study the sand lobster *Thenus orientalis* is taken as the testing animal and the biochemicals like protein, carbohydrate, lipid and the enzymes like LDH, NSE, ACP and ALP were estimated.

Key words: *Thenus orientalis*, protein, carbohydrate, lipid, lactate dehydrogenase, non specific esterase, acid phosphatase and alkaline phosphatase.

INTRODUCTION

In recent years crustacean reproduction has taken a maximum gain. Orton (1970) and many research workers on crustacean reproduction confirmed that temperature is the main influencing factor and playing an important role in the reproductive physiology. There are considerable attentions focused on the fluctuations in the biochemical composition of different reproductive tissues in fresh as well as marine molluscan forms in relation to the reproductive activity (Giese, 1969; Webber, 1970). Similar work has also been done on crustaceans particularly in brachyurans (Adiyodi, 1968; Diwan, and Nagabushanam, 1974)

In a marine penaeid prawn *Parapenaeopsis*, Kulkarni and Nagabushanam (1979) have identified the chemical mobilization of organic reserves during the ovarian development. They have further suggested that there is a continuous decline in the protein, glycogen and fat concentrations hepatopancreas during the ovarian development in the estuarine hermit crab. Ajmal khan and Natarajan (1982) have identified the quantitative variation in protein, carbohydrate and fat contents in hepatopancreas, muscle and ovary during ovarian cycle of *Clibinarius longitarsis*. Fingerman (1985) revealed that hepatopancreas is the main storage organ of protein; glycogen and lipids.

Esterase activity during vitellogenesis has been reported by Ezhilarasi and Subromonium (1984) in *Scylla serrata* and suggested that esterase activity reaches its maximum peak during high lipid mobilization in ovary. The accumulation of esterase within the oocytes is suggested to reflect their

storage for future utilization during embryogenesis. Jayalectumie and Subromonium (1987) reported on the biochemical composition with special reference to lactate dehydrogenase activity in the different reproductive tissues of field crab *Paratelphusa hydrodromous* and showed that there are fluctuations in all the tissues at different stages. Manjuladevi *et al.,* (1993) reported the lactate dehydrogenase activity in the hepatopancreas and muscle of a field crab *Uca pugilator* after exposing the crabs to heavy metals, cadmium. Chenak-zhou (1994) reported the presence of alkaline phosphatase in the tissue of *Scylla serrata* a green mud crab.

Shyamasundari (1991) reported on histochemical investigations of male reproductive system in a sand lobster *Thenus orientalis*. Rahman *et al,* (1987) studied on the egg developmental stages of sand lobster *Thenus orientalis* and suggested that the stages based on the colour changes are due to the biochemical changes occurring during the development. He also suggested that protein and lipid contents decreased as the egg development advanced and increase in carbohydrates till the final stage of development.

In the present study an attempt has been made on various aspects of biochemical analysis on various tissues during the reproductive activity of female sand lobster *Thenus orientalis.*

MATERIAL AND METHODS

For the present study live specimens of sand lobster, *Thenus orientalis* were collected from the commercial catches of Royapuram Fishing Harbour. They are appears to be available from May to April in all seasons throughout the year. They are brought immediately to the laboratory in a plastic bucket with sea water and maintained in the laboratory for biochemical analysis.

The biochemical analysis of proteins, carbohydrates and total lipids were estimated in the ovary , spermatheca , hepatopancreas ,muscle and haemolymph of stageI and stage II of only female *Thenus orientalis*, and also enzyme activity such as lactate dehydrogenase (LDH) non specific esterases, acid phosphatases, and alkaline phosphatase activity were studied.

Protein

The protein content of the tissue extracts was estimated by the method of Lowery *et al*; (1951).

Carbohydrate

The carbohydrate content of the tissue and haemolymph extracts was estimated by the method of Roe (1955).

Lipid

The lipid content was estimated by phosphovanillin method of Barnes and Blackstock (1973).

Lactate dehydrogenase

The lactate dehydrogenase activity was estimated by the method of Jhonson (1972).

Non specific esterase activity

The activity of Non specific esterases was estimated as per the method of Van Asperson (1962). The reaction mixture containing substrate, buffer and enzyme was prepared as per the method of Gomori (1951)

Acid phosphatase activity

The activity of acid phosphatase activity was estimated as per the method of Wotten (1964).

Alkaline phosphatase activity

The activity of alkaline phosphate was estimated by P-NPP method of Comb and Bowis (1972).

Statistical analysis for the biochemical composition for all the tissues of stage I and stage II was analysed according to Fisher and Yates (1948).

RESULTS AND DISCUSSION

Ovarian morphology

The females are classified into two different stages based on their size and weight .The females at stageI shows the maximum weight of 90 gms to 230 gms .During the stage II the weight increases to 500 to 700 gms .The weight of the animal increases correspondingly to the carapace length and width and the maturation in both the males and

females reaches to the high peak with increase in weight of the animal .In general females appear to be larger in size than males.

At stage I, the ovary is thin and fragile, transparent, flaccid and white in colour. The hepatopancreas is yellow in colour. In stage II the ovary is light orange in colour. Ovarian lobes are largely spaced and extend profusely near the carapace region into the abdomen the hepatopancreas is dark yellow in colour.

Protein

The results obtained in the stage I and stage II show very interesting information. In stage I the protein content of the ovary, spermatheca, hepatopancreas, muscle and haemolymph are 18.13, 17.38, 20.53, 20.05 and 9.13 respectively. The protein content of hepatopancreas is more which was followed by muscle haemolymph, ovary and spermatheca. In stage II, the protein content of ovary, spermatheca, hepatopancreas, muscle and haemolymph are 22.75, 23.68, 12.54, 22.47 and 9.84 respectively. Among these values the protein content is high in spermatheca followed by ovary, muscle, hepatopancreas and haemolymph as shown in (Table 1). The statistical analysis performed on the protein content of different tissues in these two stages showed the following results. There is significant (P<0.01) increase in the protein content of stage II spermatheca, ovary ,muscle and hepatopancreas ,when compared to stage I. Similarly the haemolymph showed a significant increase of (P<0.05) when compared to stage I.

Carbohydrate

In the stage I, the carbohydrate content of ovary and spermatheca are 7.70, 8.78 hepatopancreas, muscle and haemolymph were 10.77, 10.66 and 7.76 respectively .These results showed that the carbohydrate content in the hepatopancreas is high, followed by the muscle, spermatheca, haemolymph and ovary. In the stage II the carbohydrate content of ovary and spermatheca are 10.74 and 11.43; hepatopancreas, muscle and haemolymph were 6.70, 9.93 and 7.82 respectively. From the results it is evident that the carbohydrate level rises till the final stage of the ovary and the carbohydrate level was high in the spermatheca, ovary, muscle, haemolymph followed by hepatopancreas as given in the (Table 2).

The statistical analysis performed on carbohydrate contents of various tissues of these two stages showed following results. A significant (P<0.01) increase in carbohydrate content of stage II ovary, spermatheca and hepatopancreas are noticed .Similarly a significant (P>0.05) increase in the carbohydrate level of stage III muscle and a significant (P>0.05) increase of carbohydrate level of stage II haemolymph when compared to stage I.

Table 1: Quantitative estimation of Protein content in different tissues of Stage I and Stage II of female *T. orientalis*

Tissues	Stage – I	Stage – II
Ovary	18.13 ± 0.621	22.75 ± 0.467**
Spermatheca	17.38 ± 0.670	23.68 ± 0.477**
Hepatopancreas	20.53 ± 0.682	12.54 ± 1.109**
Muscle	20.05 ± 0.492	22.47 ± 0.432**
Haemolymph	9.13 ± 0.329	9.84 ± 0.278*

Each value is mean = SEM of 12 samples, expressed as mg/gm wet tissue and mg/ml haemolymph.
Note: *p<0.05 denotes significant at 5% level.
**p<0.01 denotes significant at 1% level.

Table 2: Quantitative estimation of Carbohydrate level in different tissues of Stage I and Stage II of female *T. orientalis*

Tissues	Stage – I	Stage – II
Ovary	7.70 ± 0.412	10.74 ± 0.414**
Spermatheca	8.78 ± 0.367	11.43 ± 0.502**
Hepatopancreas	10.77 ± 0.419	6.70 ± 0.447**
Muscle	10.66 ± 0.382	9.93 ± 0.324*
Haemolymph	7.76 ± 0.380	7.82± 0.381 NS

Each value is mean = SEM of 12 samples, expressed as mg/gm wet tissue and mg/ml haemolymph.
Note: *p<0.05 denotes significant at 5% level.
**p<0.01 denotes significant at 1% level.
NS p<0.05 denotes not significant.

Lipid

In the stage I, the lipid content of the ovary, spermatheca, hepatopancreas, muscle and haemolymph were 16.97, 18.15, 21.47, 17.84 and 17.24 respectively. The lipid content of hepatopancreas is more followed by spermatheca, muscle, haemolymph and ovary.

In the stage II the lipid content of the ovary, spermatheca, hepatopancreas, muscle and haemolymph are 23.80, 21.80, 14.26, 18.75 and 12.21 respectively. Among these values the lipid content of the ovary is high followed by spermatheca, muscle, hepatopancreas and haemolymph presented in the Table 3. The statistical analysis performed on the lipid content of different tissues in these two stages showed following results. There is significant (P<0.01) increase in the lipid content of stage II ovary, spermatheca, hepatopancreas and haemolymph, when compared to the stage I. Similarly a significant (P>0.05) increase in the stage II muscle is noticed when compared to stage I.

Study of lactate dehydrogenase activity

In stage I, the ovary, spermatheca, hepatopancreas, muscle and haemolymph the lactate dehydrogenase activity is found to be 9.66, 9.38, 8.61, 9.97 and 8.43 respectively, whereas in the stage II the lactate dehydrogenase activity is 8.42, 8.54, 7.78, 7.69 and 7.49 in ovary, spermatheca, hepatopancreas, muscle and haemolymph respectively, as given in Table 4. The statistical analysis performed on the lactate dehydrogenase activity of various tissues in the two stages, stage I and stage II revealed a significant increase of (P<0.01) in spermatheca, hepatopancreas and haemolymph in stage II when compared to the stage I. Similarly (P<0.05) an increase in lactate dehydrogenase activity in the ovary and muscle of stage II are found when compared to the stage I tissues.

Study of non-specific esterase activity

The non specific esterase activity in the stage I and stage II tissues of female *T.orientalis* revealed interesting reports. In the stage I the non specific esterase activity of the ovary, spermatheca, hepatopancreas, muscle and haemolymph are 8.18, 7.90, 7.92, 8.35 and 8.54 respectively. On the other hand in the stage II esterase activity of the ovary (7.28) spermatheca (7.39) and hepatopancreas, muscle and haemolymph are 6.57, 8.77 and 5.42 respectively. The values of stage I and stage II showed that esterase activity is high in the ovary, muscle and haemolymph of stage I followed by hepatopancreas and spermatheca. Similarly the esterase activity is high in the stage II muscle followed by spermatheca, ovary, hepatopancreas and haemolymph as given in (Table 5). Statistical analysis performed on the non specific esterase activity of these two stages showed a significant (P<0.05) increase in the stage II ovary, spermatheca and hepatopancreas when compared to the stage

Table 3: Quantitative estimation of Lipid level in different tissues of Stage I and Stage II of female *T. orientalis*

Tissues	Stage – I	Stage – II
Ovary	16.97 ± 0.573	23.08 ± 0.472**
Spermatheca	18.15 ± 0.546	21.80 ± 0.260**
Hepatopancreas	21.47 ± 0.435	14.26 ± 0.645**
Muscle	17.84 ± 0.881	18.75 ± 0.739 NS
Haemolymph	17.24 ± 0.619	12.21 ± 0.740**

Each value is mean = SEM of 12 samples, expressed as mg/gm wet tissue and mg/ml haemolymph.

Note: *p<0.05 denotes significant at 1% level.

NS p<0.05 denotes not significant.

Table 4: Lactate dehydrogenase activity in the Stage I and Stage II of female *T. orientalis*

Tissues	Stage – I	Stage – II
Ovary	9.66 ± 0.290	8.42 ± 0.273**
Spermatheca	9.38 ± 0.461	8.54 ± 0.291*
Hepatopancreas	8.61 ± 0.487	7.78 ± 0.319*
Muscle	9.97 ± 0.453	7.69 ± 0.328**
Haemolymph	8.43 ± 0.440	7.49 ± 0.313*

Each value is mean = SEM of 12 samples, expressed as u/gm wet tissue and u/ml haemolymph.

Note: *p<0.05 denotes significant at 5% level.

*p<0.01 denotes significant at 1% level.

I and a significant increase of (P<0.01) of the stage II haemolymph when compared to stage I and a significant (P>0.05) increase in the muscle of the stage II when compared to stage I.

Study of acid phosphatase activity

The acid phosphatase activity in the stage I of ovary, spermatheca, hepatopancreas, muscle and haemolymph are 8.04, 7.69, 9.48, 8.62 and 6.30 respectively. In the hepatopancreas the acid phosphatase activity is high which is followed by muscle, ovary, spermatheca and haemolymph. On the other hand in the stage II of ovarion maturation the acid phosphatase activity is 7.69 in ovary, 10.69 in spermatheca, 7.58 in hepatopancreas, 8.40 in muscle and 7.08 in haemolymph respectively. Among these values the acid phosphatase activity in the spermatheca is high followed by muscle, ovary, hepatopancreas and haemolymph as in (Table 6).

The test of significance performed on the acid phosphatase activity of these two stages showed the following results. There is a significant increase (P<0.05) in the activity of hepatopancreas and haemolymph of stage II when compared to stage I and a significant of (P<0.01) increase of acid phosphatase activity in the spermatheca of stage II when compared to stage I and a significant increase of (P>0.05) in ovary and muscle of stage II when compared to stage I.

Study of alkaline phosphatase activity

The alkaline phosphatase activity in the stage I of ovary, spermatheca, hepatopancreas, muscle and haemolymph are 7.22, 7.64, 9.94, 13.15, and 6.48 respectively. The alkaline phosphatase activity seems to be high in the muscle followed by hepatopancreas, spermatheca, ovary and haemolymph. Whereas in stage II the alkaline phosphatase activity in the ovary is 10.93 followed by spermatheca, hepatopancreas, muscle and haemolymph 11.61, 7.30, 13.40 and 6.15. The alkaline phosphatase activity seems to be high in the muscle at stage I followed by spermatheca, ovary, hepatopancreas and haemolymph as shown in (Table 7). The statistical analysis performed on

Table 5: Non-specific Esterase activity in the Stage I and Stage II of female *T. orientalis*

Tissues	Stage – I	Stage – II
Ovary	8.18 ± 0.354	7.28 ± 0.505*
Spermatheca	7.90 ± 0.392	7.39 ± 0.591*
Hepatopancreas	7.92 ± 0.468	6.57 ± 0.454*
Muscle	8.35 ± 0.420	8.77 ± 0.445 NS
Haemolymph	8.54 ± 0.393	5.42 ± 0.341**

Each value is mean = SEM of 12 samples, expressed as u/gm wet tissue and u/ml haemolymph.
Note: *p<0.05 denotes significant at 5% level.
*p<0.01 denotes significant at 1% level.
NS p<0.05 denotes not significant.

Table 6: Acid phosphatase activity in the Stage I and Stage II of female *T. orientalis*

Tissues	Stage – I	Stage – II
Ovary	8.04 ± 0.297	7.69 ± 0.368 NS
Spermatheca	7.69 ± 0.418	10.69 ± 0.732**
Hepatopancreas	9.48 ± 0.560	7.58 ± 0.411*
Muscle	8.62 ± 0.535	8.40 ± 0.406 NS
Haemolymph	6.30 ± 0.366	7.08 ± 0.344*

Each value is mean = SEM of 12 samples, expressed as u/gm wet tissue and u/ml haemolymph.
Note: *p<0.05 denotes significant at 5% level.
*p<0.01 denotes significant at 1% level.
NS p<0.05 denotes not significant.

Table 7: Alkaline phosphatase activity in the Stage I and Stage II of female *T. orientalis*

Tissues	Stage – I	Stage – II
Ovary	7.22 ± 0.286	10.93 ± 0.293**
Spermatheca	7.64 ± 0.336	11.61 ± 0.638**
Hepatopancreas	9.94 ± 0.418	7.30 ± 0.357**
Muscle	13.15 ± 0.480	13.40 ± 0.567 NS
Haemolymph	6.48 ±0.343	6.15 ± 0.393 NS

Each value is mean = SEM of 12 samples, expressed as u/gm wet tissue and u/ml haemolymph.
Note: **p<0.01 denotes significant at 1% level.
NS p<0.05 denotes not significant.

alkaline phosphatase activity of different tissues in these two stages showed a significant (P<0.01) increase in the content of stage II ovary, spermatheca and hepatopancreas when compared to stage I and a significant increase of (P>0.05) in muscle and haemolymph of the stage II when compared to stage I.

The results obtained in the biochemical analysis of organic components and enzymes in different tissues of female *T. oreintalis* is noted and the values of probability is obtained from the degree of freedom using standard table by Fisher and Yates (1948). The significance level of consolidated of different tissues of stage I & stage II females. If the calculated value is more than table value it is significant at the probability levels of (P<0.01), (P<0.05) and (P>0.05).

DISCUSSION

The present investigation on biochemical analysis performed on ovary, spermatheca, hepatopancreas, muscle and haemolymph of stage I and stage II female tissues reveals interesting informations. The organic contents such as protein, carbohydrate and lipids showed variations in the levels between stage I and stage II of ovarian maturation .There is gradual increase of protein content in the ovary, spermatheca, and muscle when compared to stage I and decrease in the protein content in the hepatopancreas and haemolymph of stage II when compared to stage I. The carbohydrate content is more in ovary and spermatheca of stage II followed by muscle, haemolymph and hepatopancreas when compared to stage I.

It is interesting to note that these variations in stage I and stage II the protein content, carbohydrate, and lipid is more in the ovary and spermatheca of stage II and less in hepatopancreas, suggesting that the hepatopancreas is considered as the storage organ and hence organic contents are utilized for ensuring the growth, of oocytes and subsequent developmental aspects of the individual through sequestration. Viswanathan (1992) on *Uca triangularis* has suggested that in the protein content of the ovary of the stages II and III are more than stage I in the mature females.

But the present study shows that depending on the maturation of the female ,there is an increase in the concentration of organic substances such as protein, carbohydrates and lipids in the ovary, spermatheca and also in the haemolymph. Whereas in the hepatopancreas, the protein, carbohydrate and lipid contents are less in the stage II than in stage I.

The enzyme activity of lactate dehydrogenase, in the tissues of ovary, spermatheca, hepatopancreas, muscle and haemolymph of *T.orientalis* of female stage I and stage II show fluctuations. There is a significant increase of (P<0.01) in the ovary and muscle of stage II when compared to stage I. Similarly the esterase activity in the different tissues of stage I and stage II shows fluctuations, and there is a significant increase of (P<0.05) in the ovary, spermatheca and hepatopancreas of stage II and a significant increase of (P<0.01) in the haemolymph of stage II and a significant increase of (P>0.05) in the muscle of stage II when compared to stage I of tissues. The results observed in the acid phosphatase activity in the different tissues of stage I and stage II shows fluctuations. There is a significant increase of (P<0.01) in spermatheca of stage II and a significant of (P<0.05) increase in the hepatopancreas and haemolymph of stage II and a significant of (P>0.05) increase in the ovary and the muscle of stage II when compared to stage I. The alkaline phosphatase activity in the different tissues of stage I and stage II of female *T.orientalis* reveals a significant increase of (P<0.01) in the ovary, spermatheca and hepatopancreas of stage II and a significant increase of (P>0.05) in muscle and haemolymph of stage II when compared to stage I.

The biochemical investigation carried out by Jeyalectumie and Subramoniam (1987) where the two authors have clearly demonstrated the lactate dehydrogenase activity in the reproductive tissues of a field crab *Paratelphusa hydrodromous*. They have suggested the presence of enzyme activity to the occurrence of anaerobic metabolism both in male and female crabs, thus indicating the enzyme activity within the spermatozoa and spermatophores and suggested the similarities with those of mammals.

Thebault *et al*, (1981) reported the occurrence of lactate dehydrogenase activity in the crustacean *Palaemon serratus* from the caudal muscle and reported that the LDH activity was similar to vertebrate tissues. Sujatha (1998) reported on the lactate dehydrogenase activity in ovary, spermatheca, hepatopancreas and muscle of female crabs *Uca triangularis* of stage I and stage V and suggested that the lactate dehydrogenase is comparatively higher in all the above mentioned tissues of stage V than in the stage I.

Further she has reported on the survey of esterase activity in the ovary, spermatheca, hepatopancreas and muscle of stage I and stage V and reported that the activity is high in all the tissues of stage V when compared to stage I. Similarly in *Scylla serrata,* Ezhilarasi and Subromonium (1984) reported the maximum esterases activity in the hepatopancreas during high lipid mobilization, whereas in the ovary the maximum esterase activity coincides with active vitellogenesis. They also suggested that the esterase activity is high at stage I and slowly decreases at stage II and III and steeply decreases at stage IV and stage V.

The biochemical analysis in the present study of *T.orientalis* shows clearly that the organic substances vary in stage II of female in hepatopancreas when compared to stage I. This variation is due to developmental stages leading to growth and maturation and moulting activities and more over hepatopancreas is the storage organ of organic components. Hence it is been utilized for further development in the female. With regard to the enzyme activities, similar fluctuations are noticed in the enzyme analysis with stage I and stage II .This may be due to the organ related enzyme activity or decrease in the enzymes or sometimes lack of enzymes in particular organs. Hence such fluctuations are noticed during the maturation of reproductive tissues of the female sand lobster.

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