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Study on LDH and Esterase Activity of Different Tissues in a Fiddler Crab *U Ca Triangularis Bengali* of Pulicat Lake Tamilnadu

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Biochemical analysis was carried out in tissues of the male crabs belonging to different maturity stages were collected from pulicat lake and the biochemical changes studied correlated with the nutritional aspects during the reproductive activity and developmemtal changes correlated with nutritional aspects occurring in the gonads and other organs like hepatopancreas and muscles have monitored with regard to different types of isoenzymes such as LDH , and esterase activity taking place in the different tissues during maturation have been studied and quantified.The trends in the fluctuation of metabolites indicates that the transactions takes place from the chief synthetic and storage organ. During the reproductive activity, the levels began to increase at certain stage and high levels were maintained during higher developing stages with growth and these levels decreased during prespawning , ecdysis and molt cycles and correspondingly maturation stages.It showed high levels of changes which were not in advance in the immature and developing stages . A considerable importance on the changes in different tissues in the aquatic animals in accordance with the reproductive activities has been noted in the present study of the crabs of *Uca triangularis Bengali.*of pulicatlake.

Key words:*Ucatriangularis Bengali,Pulicat lake,* lactate dehydrogenase, Non specific esterase, Reproductive tissues , Isoenzymes, Hepatopancreas and vasdeferens.

The reproductive activities are based on the biochemical changes in vitellogenesis have revealed that the biochemical components of the tissues arise by autosynthesis . Studies on 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. The biological significance of the occurrence of enzymes in multiple forms is a problem which has greatly interested biochemists during the past decade .Although there is volume of information regarding the genetic determination of these enzymes , the possible physiological functions of the heteropolymorphic isoenzymes are yet to be precisely determined . Among a number of isoenzyme the possible role of lactate dehydrogenase (LDH) isoenzymes as regulators of oxidative and glycolytic process has been obtained from tissue culture and chick embryo studies under aerobic and anaerobic conditions .(Good friend and Kaplan 1964)

Previous information in isoenzymes is mostly from the studies on vertebrate tissues .Isoenzymes of invertebrate tissues have received very little attention .The work of Wilson *et al* (1964) in *Orconectus limoses* showed only one tetrameric L-LDH with no tissue specific isoenzymes . Gleason *et al* (1971) studied in detail the isoenzymes of crustaceans . A decapod

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crustacean *Emerita asiatica* has been studied for its LDH pattern during different stages of moult cycle by Dhandayuthapani *et al* (1982) using disc gel electrophoresis ,they have observed that the muscle muscle tissues from the post and inter moult stages contain a single fraction whereas in the premoult stage three fractions have been obtained .In the haemolymph ,a slow moving and a fast moving band have been observed in all moult stages . from hepatopancreas two fast moving fractions appear to be tissue specific .

In other crustaceans like P. hydrodromous the LDH isoenzymes have been studied on reproductive tissues .The work of Jayalectumie and Subramoniam (1987) showed activity in the spermatophores .Electrophoretic separation of LDH isoenzymes of spermatophores demonstrated the occurrence of six fractions of which most fractions resolve in between LDH3 and LDH4 .They found that these fractions are homologous to the mammalian sperm specific LDH fraction . The foregoing observations clearly illustrate that LDH of different tissues of invertebrates show remarkable variations with reference to their forms and functions .Evidences relating to the systematic position of animals have been brought forward by the characterization of isoenzymes in different tissues .Manjula devi et al ., (1993) have reported the lactate dehydrogenase activity in the hepatopancreas and the abdominal muscle in the fiddler crab Uca pugilator after exposing the crabs to heavy metal ,cadmium.

As such the informations with regard to esterase activity is also very less . Few workers have shown that esterase activity, also shows variations in all the tissues of both male and female reproductive tissues especially in the hepatopancreas .In an isopod Doyle et al ., (1959) found that the freshly laid eggs contain a significant quantity of esterases which show a gradual increase during embryogenesis suggesting , the possibility of the embryonic synthesis of this enzyme in addition to the stored esterase in the eggs.The esterase activity has been reported by Ezhilarasi and Subramoniam(1984) in Scylla serrata where they have estimated quantitatively and qualitatively the esterase activity in ovary , haemolymph and hepatopancreas during various stages of ovarian maturation . In the hepatopancreas carboxyl esterase activity reaches its maximum during high lipid mobilization , whereas in the ovary the maximum esterase activity coincides with active vitellogenesis . The accumulation of esterase within the oocytes is suggested to reflect their storage for future utilization during embry Hence the aim of the present investigation deals with the study of two isoenzymes such as lactate dehydrogenase (LDH) and esterase activity sof various tissues such as hepatopancreas , haemolymph , muscle and reproductive tissues of both male and female of two particular stages in an crustacean fiddler crab Uca triangularis bengali of pulicat lake , Tamilnadu.

MATERIALS AND METHODS

The specimens of fiddler crab ,*Uca triangularis bengali* (crane ,1975) were collected from pulicat lake of Tamilnadu .It is quite opposite to the state fisheries department and near the Madras Christian college laboratory.This is very common species of fiddler crabs of the pulicat lake .The specimens were collected by hand picking and also by digging the mud below upto a depth of one meter with with the help of a shovel . The specimens were washed immediately and thus cleaned of the mud and adhering particles .They were brought immediately to the laboratory in a plastic bucket and maintained in the laboratory and they were transferred to plastic troughs and,the specimens were carefully examined and the males and females were separated for studying the anatomy of reproductive system , and from this the degree of reproductive maturation of gonads has been noted .As they exhibit a linear relationship between carapace length and width with regard to there size and weight of the animals the males were classified into immature and mature stages and similarly females were classified into stage I and stage III , with regard to there morphological characters .

In each , specimen carapace was dissected and removed with a fine scissors and the viscera were exposed ,and examined under dissecting binocular microscope with the help of a fine needle and forceps and by sprinkling 1% neutral buffered for hardening the entire reproductive system of both female and male was studied as shown in the.Further the tissues like hepatopancreas , ovary , muscle from female of

stage I and stage III was collected for further analysis with regard to the biochemical aspect on (LDH) isoenzymes as lactate dehydrogenase , and esterase activity. Similarly males of immature and mature stages were selected ,and the tissues like testis , proximal vasdeferens (PVD), mid vasdeferens (MVD), distal vasdeferens (DVD) ,muscle and hepatopancreas. Before sacrificing the animals ,haemolymph is collected using a sterilized syringe and needle and before collecting the haemolymph the needle was rinsed in 0.2% EDTA to avoid coagulation (Subhashini and Ravindranath ,1980).The different tissues were stored in ice boxes until for further bio-chemical analysis .

The LDH enzyme activity was assayed according to the method of (King 1965)

Reagents

Glycine buffer

(0.1 M) 7.5 of glycine and 5.85 of sodium chloride were dissolved in 1 ltr of distilled water **.**

Buffered substrate

2.78 of lithium lactate was dissolved in 0.1 N sodium hydroxide solution .This was prepared just before use .

2,4 dinitro phenyl hydrazine reagent (DNPH) 200 mg of DNPH was dissolved in 1 litre of 1.0 N hydrochloric acid

Standard phenyl solution

11.2 mg of sodium phenyl was dissolved in 100 ml of buffer solution .

Procedure

To a set of tubes ,1.0 ml , of the buffered substrates and 0.1 ml of samples were added and the tubes were incubated at 37°C for 15 minutes .After adding 0.2 ml of NAD solution , the incubation was continued for another 15 minutes . The reaction was then arrested by adding 1.0 ml , of DNPH reagent and the tubes were incubated for another 15 mins at 37°C ,0.1 ml of sample was added to blank tubes after arresting the reaction with DNPH 7.0 ml of 0.4 N sodium hydroxide solution was added and colour developed was measured at 420 nm in a Shimadzu spectrophotometer ,Suitable aliquots of the standard were analysed by the same procedure.

As such , similarly the esterase activity was also estimated using the method of Van Asperson (1962) .

Enzyme source

Dilute aqueous tissue homogenate were prepared in chilled distilled water and centrifuged at 200 rpm for 10 minutes , to collect clear supernatant after removing the lipid was used as the enzyme source .

Enzyme assay (esterase activity)

1 – Napthol liberated from 1 – napthy acetate by the enzyme activity of the sample was measured to quantify the esterase activity . To estimate esterase activity M/15 phosphate buffer from pH 6 to 9 at an interval of 0.5 was used . The reaction mixture containing substrate , buffer and enzyme was prepared as per the method of Gomori (1951) .After 30 minutes of incubation at 37°C , the enzymatic reaction was arrested by the addition of freshly prepared reagent containing two parts of 1% (w/v) sodium laryl sulphate .

Non enzymatic hydrolysis (control) of 1- napthyl acetate was checked by incubating the buffered substrate for 30 mins and then arresting the reaction as per the other experimental tubes of that particular pH (Kaplin and Ahmed ,1980) .Initial zero readings (control 2) were also noted immediately after the addition of the enzyme to the buffer / substrate mixture . Both control 1 and 2 value were subtracted from the corresponding experimental readings .All the samples of the controls were read against the blanks at 590nm in a Bausch and Lomb spectronic 21 UV spectrophotometer.

The data obtained from the biochemical analysis were statistically analysed and expressed as mean ± SEM (standard error of mean). The SEM was calculated by the method of Ostle (1966)

$$SEM = \frac{\Sigma x2 - \frac{(\Sigma x)2}{n}}{n\,(\,n-1\,)}$$

Where .x = individual observation and n = number of observations

Students 't' test was used to compare the mean values of two groups and 't' values were calculated by using the formulae .

$$t = \frac{\Sigma x1 - \Sigma x2}{S\sqrt{1/n1 + 1/n2}}$$

Where 'S ' is the standard deviation ,which was calculated using the formula

$$S = \sqrt{\frac{\Sigma x1^2 \frac{(\Sigma x1)^2 + (\Sigma x^2)^2}{n1} - \frac{(\Sigma x^2)^2}{n2}}{(n1 + n2) - 2}}$$

n1 and n2 denotes the number of observations in the two classes being compared .The values of probability was obtained from the degree of freedom by using the standard table given by Fisher and Yates (1948).If the calculated value was more than the table value ,it is significant at the probability levels of $p < 0.01$, $p < 0.05$ and $p > 0.05$.

RESULTS

Gonadial morphology

The ovaries in *Uca triangularis Bengali* are paired and "H" shaped .They occupy the anterior-lateral regions beneath the carapace and asymmetrical in nature which the right and left limbs of the ovary are connected by the ovarian bridge. They are situated above the alimentary canal and intermingled with hepatopancreas. They contain many ovarian lobes which are uneven in shape particularly in mature stages . The ovaries exhibit different colours depending upon the stage of maturation. The general account of the ovarian morphology is almost the same as explained by Williamson(1904) and pearson (1908) in *Cancer pagurus*. The ovaries are classified into the following two maturity stages depending upon the colour, nature and the size of the oocytes.

Stage- I

In the stage I , ovary is immature, white to cream in colour thin, transparent, fragile, flaccid with sparse light pigmentation over the surface. The hepatopancreas in this stage is pale white in colour

Stage-II

In stage III. ovary is light yellow and conspicuous over the surface of the ovary in stage I is very much reduced. The hepatopancreas is pale yellow in colour.

The male reproductive system of *Uca triangularis Bengali* resembles the "H". This is similar to that of other crustacean forms. The testis have two lobes which are interconnected by a stout commissure called testicular bridge. They are located in the body cavity above the alimentary canal and extended in the cephalothoracic region. The commisures which is situated at the anterior end divides each testis into short thick horn like pre-commisural limb and long thin post commissural limb. The hepatopancreas lies between these two lobes. The male reproductive tract namely vasdeferens from the middle portion of each testis and extends into the musculature. based on the morphological characters the vasdeferens is classified into three regions namely proximal, middle and distal vasdeferens which finally ends in a narrow tube called ejaculatory duct.In an immature male, there are two thin elongated testis occupying smaller region of the cephalothorax and interconnected by a thin testicular bridge. The testis is very small and thin and creamy white in appearance. The testis are interconnected by a testicular bridge at the anterior end.

Biochemical analysis

In marine and fresh water, the attention has been focused on the biochemical composition of reproductive organs (Giese, 1969: and Webber, 1970) and also many crustacean workers reported on the analysis of biochemical components in various tissuses. (Adiyodi, 1968 a, b: Nagabhushanam and kulkarni, 1977; Ajmalkhan and Natarajan,1982) and (Jeyalectumie and Subramoniam, 1991.

Study of lactate dehydrogenase activity in the tissuses of stage I and stage III of female. *Uca triangularis Bengali*

In the stage I , the ovary , hepatopancreas, muscle and haemolymph the lactate dehydrogenase activity is found to be 9.66 , 8.61 , 9.97 , 8.43 respectively .whereas in stage III the lactate dehydrogenase activity is 8.42 , 7.78 , 7.69 and 7.49 in ovary, hepatopancreas , muscle and haemolymph respectively as shown in the (Tab I & Fig I)

The statistical analysis performed on lactate dehydrogenase activity of various tissuses

in two stages, stage I and stage III revealed a significiant increase of ($p<0.01$) in hepatopancreas and haemolymph in stage III when compared to the stage I. Similarly ($p<0.05$) an increase in lactate dehydrogenase activity in the ovary and muscle of stage III are found when compared to the stage I tissuses.

Study of lactate dehydrogenase activity in the tissuses of immature and mature of male . *Uca Triangularis Bengali*

The lactate dehydrogenase activity is estimated in the different tissuss of immature stage of testis , PVD, MVD, DVD., hepatopancreas, muscle and haemolymph. They are as follows 12.89, 15.43, 16.99, 20.38, 20.47, 10.04 and 7.66. The lactate dehydrogenase activity in the hepatopancreas is high followed by DVD, MVD , PVD, testis , muscle and haemolymph. Whereas in the mature male the lactate dehydrogenase activity in the testis is 18.04 followed by PVD, MVD , DVD, hepatopancreas, muscle and haemolymph (15.98, 16.95, 15.84, 15.18, 16.98 and 16.97). The lactate dehydrogenase activity in the mature male of testis seems to be high and gradually, there is decrease in the muscle followed by haemolymph, MVD, PVD and hepatopancreas as shown in the (Tab II & Fig II).

The statistical analysis performed on the lactate hydrogenase activity of different tissuses are as follows. There is significiant ($P<0.01$) increase in lactate dehydrogenase activity in the testis DVD., hepatopancreas , muscle and haemolymph in the mature males when compared to immature males. The lactate dehydrogenase activity in the regions of PVD. and MVD is increased significiantly ($P>0.05$) in the mature males when compared to the immature males.

Study of esterase activity in the tissues of stage I and stage III of female *Uca triangularis bengali*

The esterase activity in the stage I and stage III tissues of female *Uca triangularis*

Table 1. Lactate dehydrogenase activity in the stage - I & stage - III of female *Uca triangularis bengali*

Tissuses	Stage - I	Stage - III	t-valve
Ovary	9.66 ± 0.290	8.42 ± 0.273**	3.10
Hepatopancreas	8.61 ± 0.487	7.78 ± 0.319*	1.42
Muscle	9.97 ± 0.453	7.69 ± 0.328**	4.07
Haemolymph	8.43 ± 0.440	7.49 ± 0.313*	1.74

Each value is mean ± SEM of 12 samples , expressed as u / mg tissue and u / ml haemolymph
Note : *P<0.05 denotes significiant at 5% level.
**P<0.01 denotes significiant at 1% level.

Table 2. Lactate dehydrogenase activity in the different tissues of immature and mature male *Uca Triangularis bengali*

Tissuses	Immature	Mature	t-value
Testis	12.89 ± 0.879	18.04 ± 0.407**	5.32
Proximalvasdeferens	15.43 ± 0.706	15.98 ± 0.689NS	0.55
Mid vasdeferens	16.99 ± 0.505	16.95 ± 0.763NS	0.05
Distal vasdeferens	20.38 ± 0.997	15.84 ± 0.916**	3.35
Hepatopancreas	20.47 ± 1.213	15.18 ± 0.750**	3.70
Muscle	10.04 ± 0.710	16.98 ± 0.580**	7.56
Haemolymph	7.66 ± 0.652	16.87 ± 0.659**	10.04

Each value is mean ± SEM of 12 samples expressed as u/mg wet tissue and u/ml of haemolymph
Note : **P<0.01 denotes significiant at 1% level
NS P>0.05 denotes not significiance

bengali revealed interesting reports .In the stage I, the esterase activity of the ovary, hepatopancreas , muscle and haemolymph are 8.18 , 7.92 , 8.35 and 8.45 respectively On the otherhand in stage III the esterase activity of the ovary was 7.28 and in the hepatopancreas, muscle and haemolymph are 6.57 , 8.77 and 5.42 respectively . The values of stage I and stage III showed that the esterase activity is high in the ovary , muscle and haemolymph of stage I followed by hepatopancreas . Similarly the esterase activity is high in the stage II of muscle followed by ovary, hepatopancreas and haemolymph as shown in (Table –III & Fig –III

Statistical analysis performed on the esterase activity of these two stages showed a significant (P<0.05) increase in the stage III ovary and hepatopancreas when compared to the stage I and a significant increase of (P<0.01) of the stage III haemolymph when compared to stage I and a significant (P>0.05) increase in muscle of the stage III when compared to stage I .(Table 3 & Fig 3)

Study of esterase activity in the tissues of immature and mature male *Uca triangularis bengal*

The esterase activity in the immature male testis , PVD , and MVD are 8.15 , 14.63 , 14.59 , followed by DVD , hepatopancreas , muscle and haemolymph are 14.31 , 12.05 , 12.10 , and 16.69 . On the other hand in mature male the esterase activity in the testis , PVD , MVD , DVD , hepatopancreas , muscle , and haemolymph are 15.28 , 17.15 , 16.93 , 15.16 , 15.63 , 13.58 and 12.28 . Among these values the esterase activity seems to be increased in the haemolymph , PVD , MVD , DVD , muscle , hepatopancreas and the testis of the immature males showed that in the mature male there is a gradual increase of esterase activity in the PVD and MVD and decreases in the other tissues as shown in the (Table 4 & Fig – 1)

Table 3. Esterase activity in the stage I and stage-III female *Uca triangularis bengali*

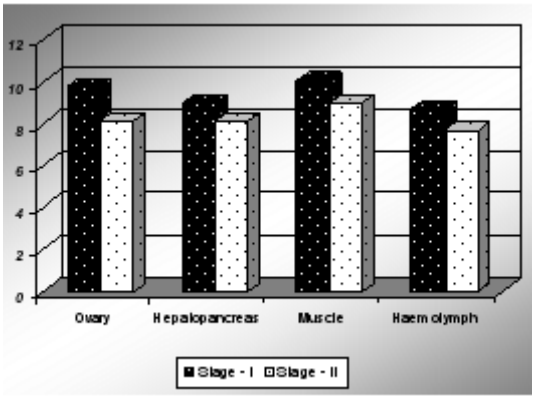
Tissuses	Stage - I	Stage - III	t-valve
Ovary	8.18 ± 0.354	7.28 ± 0.505*	1.47
Hepatopancreas	7.92 ± 0.468	6.57 ± 0.454*	2.07
Muscle	8.35 ± 0.420	8.77 ± 0.445NS	0.68
Haemolymph	8.54 ± 0.393	5.42 ± 0.341*	6.01

Each value is mean ± SEM of 12 samples , expressed as u/mg wet tissue and u/ml of haemolymph.
Note : * P<0.05 denotes significiant at 5% level;
**P<0.01 denotes significiant at 1% level.
NS P>0.05 denotes not significiant

Table 4. Esterase activity in the different tissuses of immature and mature male *Uca Triangularis bengali*

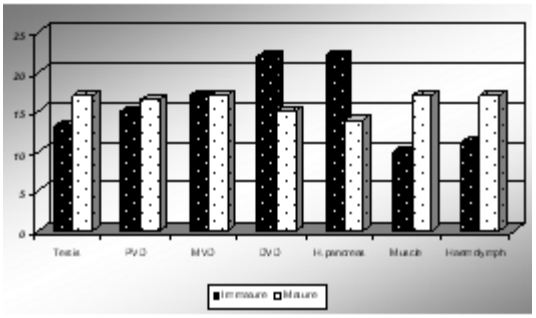
Tissuses	Immature	Mature	t-value
Testis	8.15 ± 0.752	15.28 ± 0.813**	6.44
Proximalvasdeferens	14.63 ± 0.714	17.15 ± 0.645*	2.61
Mid vasdeferens	14.59 ± 0.937	16.93 ± 0.864 NS	1.83
Distal vasdeferens	14.31 ± 1.082	15.16 ± 0.982 NS	0.58
Hepatopancreas	12.05 ± 1.005	15.63 ± 1.049*	2.47
Muscle	12.10 ± 1.040	13.58 ± 1.680 NS	0.75
Haemolymph	16.69 ± 0.726	12.28 ± 1.099 NS	3.35

Each value is mean ± SEM of 12 samples expressed as u/mg wet tissue and u/ml of haemolymph
Note : **P<0.01 denotes significiant at 1% level
NS P>0.05 denotes not significiance



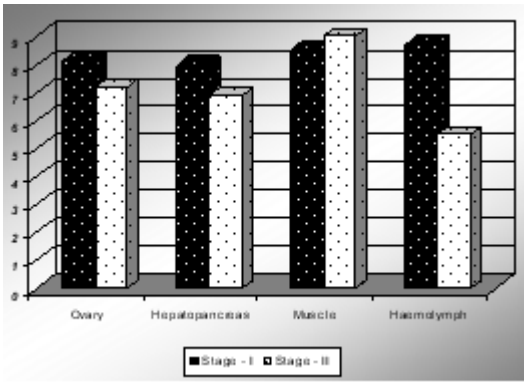
Each value is mean ± SEM of 12

Fig. 1. Showing the enzyme analysis of Lactate dehydrogenase in stage I &III of female *Uca triangularis bengali*



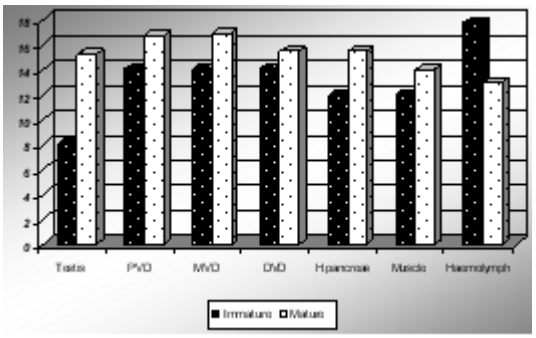
Each value is mean ± SEM of 12

Fig. 2. Showing the enzyme analysis of lactate dehydrogenase activity in the immature and mature male of *Uca triangularis bengali*



Each value is mean ± SEM of 12

Fig. 3. Showing the analysis of esterase activity in the stage – I and stage – III of female *Uca triangularis bengali*



Each value is mean ± SEM of 12

Fig. 4. Showing the analysis of esterase activity in the immature and mature males of *Uca triangularis Bengali*

Statistical analysis performed on the esterase activity of different tissues of immature and mature males show that there is a significant increase of ($P<0.01$) in the mature male of testis when compared to the immature male and a significant of ($P<0.05$) increase in PVD and hepatopancreas in the mature male when compared to immature male and a significant of ($P>0.05$) increase in the MVD ,DVD , muscle , and haemolymph of mature male when compared to immature male .

DISCUSSION

The results obtained in the present study reveals that *Uca triangularis bengali* of pulicat lake is a continous breeder and confirms that it breeds throughout the year .Observations on external morphology shows the markings which are true to their mode of life and the habitat in which they live ,and even the colour of their body is matching with the surroundings near by the water bodies which again indicates their actual habitat .

In *Uca triangularis bengali* the morphology shows that the female and male reproductive system in this species is similar to other decapod crustaceans . The ovarian developmental stages was based on the morphological characters .Similarly the development of male reproductive system was also based on the morphological characters with basic similarity to that of other decapodan crustaceans .It is generally believed that male and female reproductive systems of decapod crustaceans has a basic pattern and various regions perform different functions in different species .Since studies relating to biochemical aspect with regard to lactate dehydrogenase activity in different tissues could yield meaningful results and also because of limited documents currently available , *Uca triangularis bengali* a decapodan crustacean of common occurances has been particularly selected for this study with a view to investigate in detail with aspect of LDH activity in different tissues of reproductive systems of male and female species.

The enzyme activity of lactate dehydrogenase in the tissues of ovary ,hepatopancreas , muscle and haemolymph of *Uca triangularis bengali* of stage I female and stage III show fluctuations . There is a significant increase of ($P<0.01$) in ovary and muscle of stage III and ($P<0.05$) increase in hepatopancreas and muscle of stage II when compared to stage I .Similarly the lactate dehydrogenase activity performed in the tissues of immature and mature male *Uca triangularis bengali* reveals significant increase of ($P<0.01$) in the testis , distal vasdeferens , hepatopancreas . muscle and haemolymph of mature male when compared to immature male and a significant increase of ($P>0.05$) in the proximal and mid vasdeferens of mature male when compared to the immature male tissues

The biochemical investigation carried out by Jeyalectumie and Subramoniam (1987) , where two authors have clearly demonstrated the lactate dehydrogenase activity in the reproductive tissues of field crab *Paratelphusa hydrodromous* have suggested the presence of enzyme activity to the occurence 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 the stage

Thus the biochemical analysis of Lactate dehydrogenase activity in the present study of *Uca triangularis Bengali* show variations in stage III of female in hepatopancreas when compared to stage I .This variation is due to the developmental stages leading to growth and maturation and moulting activities and more over hepatopancras is considered as the storage organ hence it is been used for the further development in female and male This similar fluctuations are also noticed in male with immature and mature stages which may be due to organ related or decrease in the enzyme or lack of enzyme in particular organs which are related to growth and maturation process.

The enzyme activity of esterases in the tissues of ovary , hepatopancreas , muscle and haemolymph of *Uca triangularis bengali* of stage I and stage III show fluctuations .There is a significant increase of ($P<0.05$) in the ovary and hepatopancreas of stage III and significant increase of ($P<0.01$) in the haemolymph of stage III and a significant increase of ($P>0.05$) in the muscle of stage III when compared to stage I of tissues .

The esterase activity performed in the different tissues of immature and mature male reveals , a significant increase of ($P<0.01$) in the mature testis and a significant increase of ($P<0.05$) in the PVD and hepatopancreas of mature male and a significant ($P>0.05$) increase in the MVD ., DVD , muscle and haemolymph of mature male when compared to the immature male .

Thus the biochemical analysis with regard to the esterase activity in the present study of *Uca triangularis bengali* of stage I and stage III females and immature and mature males showed variations and fluctuations due to the enzyme activity which may be due to the developmental stages leading to growth and moulting activities and more over ,

hepatopancreas is the storage organ of organic components , hence it has been utilized for the further development in the female and also it may be due to the organ related to enzyme activity or decrease in the enzyme or sometimes lack of the enzyme in particular organs .Further it shows that these fluctuations in both males of immature and mature stages and females of stage I and stage III may be confirmed that it may be due to growth development and maturation processes .

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