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Quantitative Protein Profile of Three *Macrobrachium* Species during Reproductive Cycle

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ABSTRACT

The total protein content of Hepatopancreas, hemolymph and ovary of adult freshwater prawns *Macrobrachium malcomsonii* (H. Milne Edwards) *Macrobrachium rosenbergii*, (H. Milne Edwards) *Macrobrachium lammarei* (H. Milne Edwards) was studied in relationship with gonadosomatic index during different stages (early-mature, mature and spent) of reproductive cycle. The gonadosomatic index increased drastically during maturation in all three species, when compared to the respective early-mature ovaries. The protein content in different organs was estimated by Bradford method. The protein content in hepatopancreas and hemolymph recorded a gradual decline during maturation stages while a marked increase was noted in ovary during maturation and at the spent stage, protein content was highest in hepatopancreas, whereas very lowest in hemolymph and ovary during the reproductive cycle in all three freshwater prawn species.

Key words: Gonadosomatic index, hepatopancreas, hemolymph, ovary, *Macrobrachium* species, protein concentration

INTRODUCTION

The freshwater prawns *M. rosenbergii*, *M. malcolmsonii* and *M. lammarei* constitute important fishery resource in India. In crustaceans, Gonadosomatic index is a gross quantitative indicator of gonad condition and represents the simplest way to measure changes in size and weight of organ in relation to total weight of the organism (Rodriguez-Gonzalez *et al*., 2006). In fish species such as *Liza parsia*, high fecundity is highly correlated with gonadosomatic index (Rheman *et al*., 2002). Quantitative changes in protein content provide more accurate information about gonadal performance during gametogenesis (Sagi *et al*., 1996). Biochemical changes during molting, reproduction in the gonads, hepatopancreas and muscle have been examined in a number of crustacean species (Rosa and Nunes, 2002). Knowledge of biochemistry and reproductive processes that occur during the reproductive cycle are essential for complete understanding of crustacean reproduction. In many species, vitellogenin, the precursor molecule to vitellin, is transported through hemolymph to developing oocytes, where it is sequestered and modified with addition of polysaccharides and lipids into vitellin (Tsukimura, 2001). Synthesis of several proteins including enzymes, peptide hormones and egg yolk proteins are essentially important in maturation and reproduction (Yehezkel *et al*., 2000). Vitellogenin is synthesized in the hepatopancreas of freshwater prawn *M. malcolmsonii* (Shanju and Geraldine, 2009). In *M. rosenbergii*, there is correlation between gonadosomatic index, ovarian protein content and vitelhn levels during ovarian maturation (Lee *et al*., 1997). In *Cherax quadricarinatus* higher protein content in the gonad was

observed in ripe oocytes, as a result of active mobilization of energy reserves from exogenous sources to the gonad and their introduction to oocyte (Abdu *et al*., 2000). There is lack of correlation between protein content in hepatopancreas and development of gonad in *C. quadricarinatus* (Rodriguez-Gonzalez *et al*., 2006). Similar results were observed in *M. rosenbergii* protein content in hepatopancreas and hemolymph remain low and is constant during different stages of ovarian maturation (Lee *et al*., 1997). This information would provide useful indicators of oocyte growth, quality and more reliable methods for large scale hatchery production of juveniles of *M. malcolmsonii M. lammarei* and *M. rosenbergii*. The aim of the present study was to study the changes in protein concentration in the hepatopancreas, hemolymph and ovary of *M. malcolmsonii*, *M. rosenbergii* and *M. lammarei* during the reproductive cycle in correlation with gondosomatic index and analyze the role of hepatopancreas in protein synthesis in all three freshwater prawn species.

MATERIALS AND METHODS

Experimental animals: Adult prawns, *M. malcolmsonii*, (Length 240-320 mm, Weight 15-20 g) *M. rosenbergii* (Length 310-320 mm, Weight 15-20 g) and *M. lammarei* (Length 49-60 mm, Weight 1.2 g) were collected from different local sites such as pond cultures in aquaculture farms and dam. Adult *M. malcolmsonii* was collected from the lower anicut of river Cauvery while *M. rosenbergii* was collected from prawn farms in Tanjore. *M. lammarei* were collected from fresh water pond in Gundur, Tiruchirappalli which is the natural habitat of this prawn. They were brought to the laboratory in oxygen filled polyethylene bag and put in to cement tanks of 36×12 m maintained at a temperature of 32°C. They were fed with *ad libitum*. Prawns were acclimatized to the laboratory conditions for about 15 days and used for experimental purposes. The project was conducted from 2006 to 2007 at the Department of Animal Sciences, Bharathidasan University, Tiruchirapalli, Tamilnadu, India. Female prawns were identified by their smaller size than the males and were categorized based on the nature of developing ovary. The ovary of females was classified into 3 stages on the basis of the size, gonadosomatic index according to (Shanju and Geraldine, 2005). The ovaries in stage 1 is referred as the early maturing (Pale yellow) color which occupies 1/4th of carapace, stage 2 as mature stage (Yellowish orange) color which occupies 3/4th of carapace, the stage 3 is spent stage (transparent) occupied only a small portion.

Collection of hemolymph: Hemolymph was withdrawn from the pericardial sinus of the prawn by inserting a needle fitted with a 2 mL sterile plastic syringe (Prerinsed with hemolymph anticoagulant medium 10 mM EDTA, 450 mM NaCl 10 mM KCl, 10 Mm HEPES (pH-7.8). The hemolymph was placed in sterile centrifuge tubes and diluted with HAM. The diluted hemolymph was centrifuged at 7500 X g for 10 min at 4°C to remove hemocytes and other cell debris. The clear supernatant obtained was stored at -20°C for further analysis.

Collection of hepatopancreas: The entire hepatopancreas obtained from the abdominal regions of the prawn was placed in a petridish, washed thoroughly with cold saline solution (NaCl 35%) and excess of the solution was removed with blotting paper.

Collection of ovary: The prawns were cut opened in the mid-dorsal line and the adjoining tissues were removed. The reproductive system was transferred to a petridish, washed thoroughly with cold saline solution and the ovaries were isolated.

Determination of gonadosomatic index: Gonadosomatic index was calculated as gonad weight/body weight×100 at different stages of development of the ovary. The adult prawn was wiped well with a thin cloth and filter paper to remove the water. Then the weight was taken in a top loading mono-pan electronic balance accurately. The entire gonad removed from the abdomen was placed on a blotting paper to remove the excess hemolymph and weighed accurately with the help of the balance. The early mature ovary occupies 1/4th of the carapace and mature ovaries were found to occupy 3/4th of the carapace, respectively whereas the spent ovary occupied only a small portion of the carapace.

Biochemical studies

Estimation of protein: The protein estimation was determined according to Bradford (1976) with bovine serum albumin as standard. The Optical Density (OD) was measured at 595 nm in a spectrophotometer (Systronics UV-Vis. Spectrophotometer).

Statistical analysis: Duncan's Multiple Range Test was performed to assess the significance of differences in the GSI. The student't' test was employed to determine the significance of differences in the mean total protein concentrations using SPSS statistical package SPSS (1990).

RESULTS

Gonadosomatic index: During the reproductive cycle, GSI of the ovary increased from 1.6±0.01 in early mature stage to 13.4±0.1 in the mature stage and decreased to 0.76±0.01 in the spent stage in *M. malcolmsonii*. So also in *M. rosenbergii* GSI was found to increase from 1.4±0.01 in early mature stage to 12.8±0.1 in mature stage and decreased relatively to 0.66±0.01 in spent stage. However, in *M. lamarrei*, the changes in GSI was found to be less pronounced for it increased from 0.94±0.01 in early mature stage to 5.8±0.2 in mature stage and to decrease to 0.76±0.01 in the spent stage (Table 1).

Protein estimation studies: In *M. malcolmsonii* the total protein concentration of the hepatopancreas exhibited a tendency to fluctuate depending on the stage of reproductive period at which tested. The total protein content in the hepatopancreas at early mature stage was found to be 78.43±0.77 mg g^{-1} tissue, whereas it was lower (51.48±1.20 mg g^{-1}) at mature stage. The highest concentration of protein in the hepatopancreas (88.46±.69 mg g^{-1}) was noted at the spent stage. These differences in protein content were found to be significant ($p<0.05$) (Table 2).

A different pattern of protein concentrations, depending on the stage of reproductive cycle, was noted in hemolymph of *M. malcolmsonii* The total protein concentration in the hemolymph of *M. malcolmsonii* was highest at the early mature stage (162.71±1.4 mg mL^{-1}), lower at mature stage (154.61±2.4 mg mL^{-1}) least at spent (144.13±3.5 mg mL^{-1}). The difference between the protein concentrations in hemolymph at mature and spent stages was found to be significant ($t = 5.51$; $p<0.05$).

Table 1: Gonadosomatic index of three different species of freshwater prawns at different stages of the reproductive cycle

Stages	*M. malcolmsonii*	*M. rosenbergii*	*M. lamarrei*
Early mature	1.60±0.01^{b}	1.40±0.01^{b}	0.94±0.01^{b}
Mature	13.40±0.01^{a}	12.80±0.10^{a}	5.80±0.20^{a}
Spent	0.76±0.01^{bc}	0.66±0.01^{bc}	0.76±0. 01^{bc}

Means within a column followed by the same superscripts are not significant ($p>0.05$)

Yet another pattern of variations in total protein concentrations depending on the stage of reproductive cycle at which tested, was noted in the ovaries, of *M. malcolmsonii* that is, peak concentration was observed at mature stage (397.00±1.20 mg g^{-1}). The total protein concentration was lower at early mature stage (52.26±1.03 mg g^{-1}) at the spent stage. The difference between concentrations at the early mature stage and at the mature stage was found to be significant ($t = -727.66$; $p<0.05$).

The varying patterns of total protein concentration observed in the organs of *M. malcolmsonii* depending on the stage of the reproductive cycle, were also observed in the corresponding organs of *M. rosenbergii* The total protein concentration of hepatopancreas showed a decrease from 65.9±2.61 mg g^{-1} in early mature stage to 44.5±2.75 mg g^{-1} in mature stage and recorded a significant increase ($t = 14.3$; $p<0.05$) to 72.5±2.6 mg g^{-1} at the spent stage.

The hemolymph protein concentration of *M.rosenbergii* was highest (161.95±1.4mg mL^{-1}) at early mature stage, less (155.76±2.6 mg mL^{-1}) at the mature stage and least (142.05±1.28 mg mL^{-1}) at the spent stage.

The total protein concentration in ovary of *M. rosenbergii* showed a ten fold increase ($p<0.05$) from 42.1±1.34 mg g^{-1} tissue at the early mature stage to 417.05±7.14 mg g^{-1} at mature stage and then almost 200 fold decrease to 2.14±0.13 mg mL^{-1} at spent stage.

In *M. lamarrei*, total concentration of protein in the hepatopancreas was higher (54.65±2.4 mg g^{-1}) in the early mature stage than in the mature stage (46.65±1.6 mg g^{-1}). However, the protein concentration in the hepatopancreas was highest (64.69±2.4 mg g^{-1}) in the spent stage. In the hemolymph of *M. lamarrei* the total protein concentration was highest (145.50±3.4 mg mL^{-1}) at early mature stage, less (132.21±1.18 mg mL^{-1}) at the mature stage and least (126.05±2.87 mg mL^{-1}) at the spent stage. The total protein concentration in the ovary of *M.lamarrei* showed a significant increase from 8.30±63 mg g^{-1} in early mature stage to 62.3±3.8 mg g^{-1} in mature stage. During the spent stage, the ovarian protein concentration drastically decreased to 2.61±0.34 mg g^{-1} tissue (Table 2).

The quantitative analysis of total protein concentrations in the hepatopancreas, hemolymph and ovary suggested a similar pattern in all three *Macrobrachium* species depending on the stage of the reproductive cycle. The total protein concentrations in the hepatopancreas and hemolymph appeared to gradual decline from the early mature stage to mature stage, whereas a marked

Table 2: Protein concentration (Mean±SD; n = 6) in the hepatopancreas, hemolymph and ovary at different stages of the reproductive cycle in *Macrobrachium* species

Stages of reproductive cycle	Hepatopancreas (mg g^{-1})	Hemolymph (mg g^{-1})	Ovaryv (mg g^{-1})
M. malcolmsonii			
Early mature	78.43±0.77	162.71±1.40	52.26±1.03
Mature	51.48±1.20	154.60±2.40	397.00±1.20
Spent	88.46±0.69	144.13±3.50	3.36±0.25
M. rosenbergii			
Early mature	65.90±2.61	161.95±1.40	42.10±1.34
Mature	44.50±2.75	155.76±2.60	417.05±7.41
Spent	72.50±2.60	142.05±1.28	2.14±0.13
M.lamarrei			
Early mature	54.65±2.4	145.50±3.40	8.30±0.63
Mature	46.65±1.6	132.21±1.18	62.30±3.80
Spent	64.69±2.4	125.05±2.87	2.61±0.33

Values within a column differ significantly ($p<0.05$) form each other, The values are Mean±SD of 6 females each ($p<0.50$ highly significant) significance is based on 't' test. ;t- test is carried for observations between early mature, mature and spent

increase was noted in ovary during maturation. At the spent stage, the total protein concentration was at its peak in hepatopancreas, but was at its lowest level in the hemolymph and ovary.

DISCUSSION

The present investigation is aimed at studying the gonadosomatic index and the protein concentration in the ovary, hepatopancreas and hemolymph associated with the reproductive cycle of fresh water decapod crustaceans namely *M. malcolmsonii*, *M. rosenbergii* and *M.lamarrei*. The gonadosomatic index has been used to stage the ovarian development cycle. The gonadosomatic index in all the three species studied, showed increase from early mature to mature stage and decrease in the spent stage. Similarly in banana shrimp *Litopenaeus merguiensis*, GSI increased drastically during ovarian maturation (Auttarat *et al*., 2006). In all three species, the total protein concentration in the ovary was highest at the mature stage, less at the early mature stage and least at the spent stage; very high concentrations were noted in the ovaries of mature stage female probably reflects the fact that ovaries at mature stage contain ripe oocytes. These results are in accordance with Lee *et al*. (1997) that in *M. rosenbergii* the gonadosomatic index, ovarian protein concentrations closely correlated with ovarian stages of development. In the present study, fluctuations in the total protein concentrations in the hepatopancreas and hemolymph at different stages of the reproductive cycle were noted in female prawns of all three *Macrobrachium* species; fluctuations were not as marked as in the case of the protein concentrations in the ovary but definite patterns were discernible. In the hepatopancreas, the protein concentration was lower at the mature stage than at the early mature stage, but was highest at the spent stage. Rosa and Nunes 2002 noted that protein from the hepatopancreas was used for gonadal development in deep sea decapod *Nephrops norvegicus*. Such process may have also occurred in the *Macrobrachium* prawns in the present study and this would explain the decrease in the total protein concentration in the hepatopancreas during ovarian maturation and increase at spent stage (Table 2). With regard to protein concentrations in the hemolymph, in all three species, a gradual decrease was noted from early mature stage through mature stage to spent stage. This finding differs from that of Lee *et al*. (1997) who reported that concentrations of protein in the hemolymph and hepatopancreas remained constant during various stages of the reproductive cycle in *M. rosenbergii* This is due to variation in stages used by the different authors to estimate the protein concentration during the reproductive cycle. In the present study, we had broadly divided into three stages such as early mature, mature and spent stage. Whereas different authors use more than 6-7 stages. It also differs from the observation of Shafir *et al*. (1992) of an increase in total protein concentration in hemolymph consequent to an increase in the size of oocyte of *Penaeus semisulcatus*. In the present study, the decrease in hemolymph protein concentration from early mature to mature stage possibly reflects utilization of the protein by the developing ovary. The decrease in hemolymph protein concentration observed from the mature stage to the spent stage was possibly due to decreased inflow of protein from the hepatopancreas and/or ovary into the hemolymph (leakage of protein from the ovary into the hemolymph has been suggested as a possible mechanism in *P. semisulcatus* (Shafir *et al*., 1992). Rao *et al*. (2007) had reported that in *Litopenaeus vannamei* the protein concentration increased in hepatopancreas, hemolymph and ovary during different stages. The variations in the protein concentration reported by different authors as increase or decrease between different stages may be due to the variations in the stages used by them. In present study we had categorized three different stages in *M. rosenbergii*, whereas Lee *et al*. (1997) had categorized five different stages and reported that protein concentration remained constant

during different stages in hepatopancreas and hemolymph in the same animal. Bello-Olusoji *et al.* (2006) have revealed that prawn *Macrobrachium vollenhoveni* and *Macrobrachium macrobrachion* have similar dietary pattern, irrespective of species, age and environment. Hepatopancreas has been reported as site of vitellin synthesis in freshwater prawn *M. malcolmsonii* by Shanju and Geraldine (2009). Earlier studies by Shanju and Geraldine (2010) have revealed that vitellin of freshwater prawn species are antigenically and biochemically similar in nature. The present study on quantitative analysis of total protein concentrations of hepatopancreas, hemolymph and ovary during reproductive cycle of three different prawn species revealed that similar kind of reproductive mechanism exists and hepatopancreas plays an important role in protein synthesis in all three freshwater prawn species.

CONCLUSION

The present study of total protein concentrations of hepatopancreas, hemolymph and ovary during reproductive cycle of three different prawn species revealed that similar kind of reproductive mechanism exist and hepatopancreas plays an important role in protein synthesis in all three freshwater prawn species.

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