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BIOCHEMICAL COMPOSITION OF OVARIES AND BODY TISSUE DURING OVARIAN MATURATION IN THE BARNACLE, *MEGABALANUS TINTINNABULUM* LINNAEUS, 1758 (CIRRIPEDIA: BALANIDAE)

Shaheena Niaz* Ali and Zarrien Ayub

Centre of Excellence in Marine Biology University of Karachi, Karachi-75270, Pakistan
*Corresponding author; e-mail: shaheenaniazali@gmail.com

ABSTRACT

The concentrations of protein, carbohydrate and lipid were measured in the ovaries and body tissue of the barnacles, *Megabalanus tintinnabulum* Linnaeus, 1758 during the ovarian maturation stages that is, immature, growing and mature. The protein, carbohydrate and lipid concentrations increased significantly in ovarian tissue with the advancement of maturation stages. The same was the case with the body tissue, that is, the biochemical constituents in tissue increased with the advancement of maturation stages. The concentrations of protein, carbohydrate and lipids in ovaries reached maximum values in winter which is the brooding period of barnacles. In the winter season, the body tissue also contained the maximum concentrations of protein and lipid indicating that the body tissue served as a storage site in barnacles instead of hepatopancreas in other crustaceans.

Key words: barnacles, gonadal maturation, proximate composition, ovaries, soft tissue

INTRODUCTION

The reproductive changes in marine invertebrates can be determined by variations in the biochemical composition of the body tissue, ovaries and other organs during different stages of gonad development (Giese, 1959, 1969; Giese and Pearse, 1974). As compared to vast literature available on other crustacean species, the study of biochemical components in tissue and individual organ systems of barnacles is meagre. For example, the biochemical constituents, such as, carbohydrate, protein, lipid, etc. have been determined during the embryonic development in barnacle species, including *Tetraclita squamosa rufotincta*, *Balanus perforatus*, *B. balanoides*, *Chthamalus dentatus*, *C. stellatus*, *Euraphia depressa*, *Octomeris angulosa*, *Pollicipes cornucopia* (Achituv and Barnes, 1976, 1978; Achituv *et al*., 1980; Achituv, 1981; Achituv and Wortzlavski, 1983; Mizrahi and Achituv, 1991). These biochemical studies showed that proteins and lipids contributed most of the energy used during development of fertilized eggs. The body weight of barnacle, *C. stellatus* showed seasonal changes during three-year period of study but the concentrations of carbohydrate, lipid and protein in the body tissue showed no significant variations with the seasons (Barnes, 1972). The increase in body weight was related to the increase in total lipids, carbohydrates and proteins in the body tissue, however, after breeding the biochemical components of body tissue decreased in two species, *C. stellatus* and *E. depressa* from the Mediterranean coast of Israel (Mizrahi and Achituv, 1990, 1991).

Studies on biochemical changes in gonads and tissue in relation to maturity in crustaceans, such as, shrimps and crabs are meagre in Pakistan (Nisa and Sultana, 2010; Fatima *et al*., 2013; Fatima, 2013). In the present study the biochemical constituents, that is, protein, lipid and carbohydrate have been estimated in the body tissue and ovaries of *Megabalanus tintinnabulum* (Linnaeus, 1758) with the progress of maturation. The seasonal variations in the biochemical composition in the ovaries and body tissue have also been studied in this species.

MATERIAL AND METHODS

The biochemical analysis was done on the samples of barnacles *M. tintinnabulum* collected each month from January 2012 to December 2013 on the Buleji rocky shore. The ovarian development stages of the barnacles were determined through the macroscopic observations based on the descriptions of the gonad, such as size, colour, texture, etc. (Barnes, 1963; Hines, 1978; Lewis and Chia, 1981; Burrows *et al*., 1992; O'Riordan *et al*., 1995). Based on the above mentioned characteristics the ovaries were divided into immature, growing and mature stages. From each barnacle the soft body tissue and their respective ovaries were removed and washed with phosphate buffer of pH 7 and homogenized in same buffer. The total protein was estimated as per the Folin-Ciocalteu method (Lowry *et al*., 1951) with bovine serum albumin (BSA) as the standard. The estimation of carbohydrate was carried out by Phenol Trichloroacetic acid method (Dubois *et al*., 1956). The lipids were quantitatively determined by Sulpho-

* Corresponding author: Shaheena Niaz Ali; E-mail: shaheenaniazali0509@gmail.com

phospho vanillin method (Barnes and Blackstock, 1973). Every month for each maturation stage, two samples in triplicate were used for the analysis. For the statistical analysis, one-way ANOVA ($P<0.05$) was used to test the biochemical composition of ovaries and soft bodies with maturation stages as factor followed by the Tukey multiple comparison test. The values of protein, carbohydrate and lipid of ovaries and soft tissue in different seasons were compared through one-way ANOVA ($P<0.05$) followed by Tukey test. For statistical analysis SPSS 14.0 software was used. Seasons were divided into spring (March to April), summer (May-September), autumn (October) and winter (November-February).

RESULTS

Biochemical composition of the ovaries and soft tissues during gonadal maturation

The concentration of protein in the ovaries of *M. tintinnabulum* varied from 24.0 to 62.0 mg. g^{-1}, that of carbohydrate from 5.0 to 11.0 mg. g^{-1} and lipid from 11.0 to 22.5 mg. g^{-1} (Table 1). The protein, carbohydrate and lipid concentrations in the ovaries increased significantly as the ovary maturation advanced ($P < 0.05$) (Fig. 1).

The concentration of protein, carbohydrate and lipid in the body tissue of barnacle varied from 15.0 to 45.0 mg. g^{-1}, 4.5 to 10.0 mg. g^{-1} and 6.8 to 15.0 mg. g^{-1}, respectively (Table 2). The protein, carbohydrate and lipid concentrations in the tissue increased significantly as the ovary maturation advanced ($P < 0.05$) (Fig. 1).

Seasonal variations in biochemical composition of the ovaries and soft tissues

The protein concentrations in ovaries are shown in Table 3. The highest concentrations ($P<0.05$) of protein were observed in winter, whereas in summer the concentrations of protein were lower and significantly different from winter. The concentrations of carbohydrate and lipids in ovaries reached maximum values in winter and autumn ($P<0.05$) and the lowest values were recorded in summer (Table 3). The concentrations of total proteins ($P < 0.05$) in body tissues showed significant seasonal variation being highest in winter. No significant variation was found in the concentration of carbohydrate but the lipid concentrations in tissues were significantly higher in winter and autumn (Table 3).

Table 1. The minimum, maximum, average with standard deviation concentrations of protein, carbohydrate and lipid in the ovaries during the gonadal maturation stages of *Megabalanus tintinnabulum* L.

Mean concentrations (mg g^{-1}) in ovaries

Stage	Protein			Carbohydrate			Lipid		
	Min	Max	Av ± STD	Min	Max	Av ± STD	Min	Max	Av ± STD
Immature	24.0	32.0	27.5 ± 2.4	5.0	6.5	5.5 ± 0.5	11.0	14.0	12.3 ± 0.9
Growing	33.0	45.0	36.8 ± 3.0	5.0	8.0	6.9 ± 1.0	10.5	16.0	13.5 ± 1.5
Mature	45.0	62.0	51.0 ± 5.4	5.9	11.0	7.8 ± 1.2	17.0	22.5	19.2 ± 1.6

Table 2. The minimum, maximum, average (with standard deviation) concentrations of protein, carbohydrate and lipid in the tissue during the gonadal maturation stages of *Megabalanus tintinnabulum* L.

Mean concentrations (mg g^{-1}) in tissue

Stage	Protein			Carbohydrate			Lipid		
	Min	Max	Av ± STD	Min	Max	Av ± STD	Min	Max	Av ± STD
Immature	15.0	21.0	17.2 ± 1.3	4.5	7.0	5.6 ± 0.7	6.8	9.4	7.8 ± 0.8
Growing	17.0	28.0	22.9 ± 2.8	5.0	8.0	7.0 ± 0.8	7.0	12.0	10.2 ± 1.6
Mature	35.0	45.0	38.3 ± 2.4	7.0	10.0	8.3 ± 0.6	10.0	15.0	12.6 ± 1.2

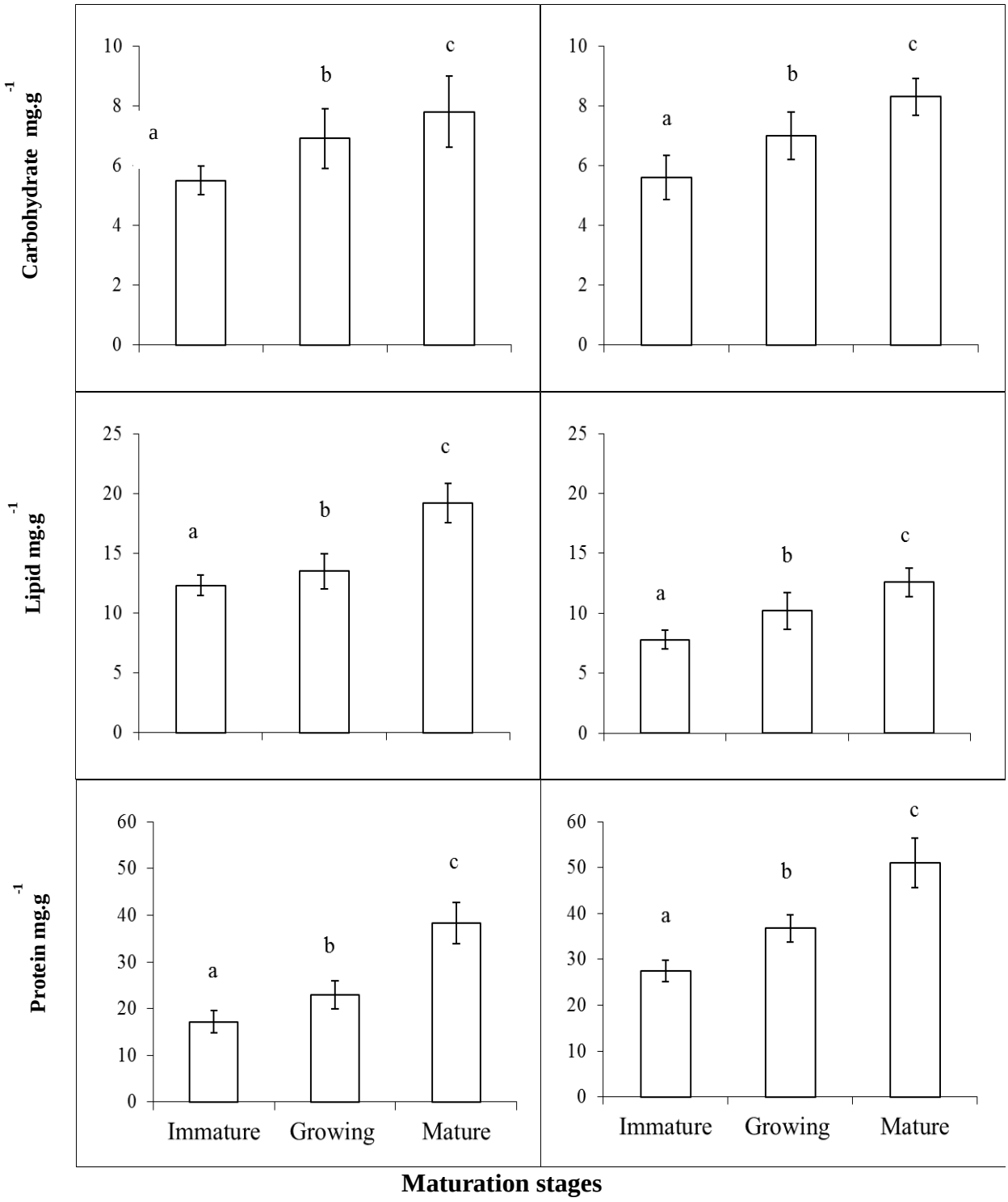

Fig. 1. Mean (± standard deviation) of protein, lipid and carbohydrate in the gonad and tissue of *Megabalanus tintinnabulum* at different stages of maturation. Means with different letters are significantly different at P< 0.05 level.

Table 3. Seasonal concentrations of protein, carbohydrate and lipid in the ovaries and tissues of *Megabalanus tintinnabulum* L.

Ovaries	Winter	Spring	Summer	Autumn
Protein mg g^{-1}	42.8 ± 2.1^{a}	36.9 ± 1.9^{b}	37.1 ± 2.2^{b}	40.2 ± 1.2^{ab}
Carbohydrate mg g^{-1}	7.8 ± 0.3^{a}	7.1 ± 0.1^{b}	6.9 ± 0.2^{b}	7.9 ± 0.3^{a}
Lipid mg g^{-1}	16.2 ± 0.9^{a}	14.3 ± 0.1^{b}	14.0 ± 0.7^{b}	15.8 ± 0.4^{a}
Tissues				
Protein mg g^{-1}	27.6 ± 1.6^{a}	24.7 ± 1.5^{b}	25.4 ± 0.7^{b}	26.0 ± 0.0^{ab}
Carbohydrate mg g^{-1}	7.2 ± 0.4	7.3 ± 0.2	6.7 ± 0.5	7.1 ± 0.0
Lipid mg g^{-1}	10.8 ± 0.8^{a}	9.2 ± 0.6^{b}	9.1 ± 0.7^{b}	10.8 ± 0.0^{a}

Values in a row with different superscripts are significantly different ($P<0.05$). Values without a superscript are not significantly different.

DISCUSSION

The concentrations of proteins, carbohydrates and lipids in an animal reflect its adaptive nature in an environment. A number of biotic and abiotic factors including reproduction, food availability temperature, humidity, salinity, dissolved oxygen may affect the biochemistry and physiology of crustaceans (Rosa and Nunes, 2003a, b; Oliveira *et al*., 2004; Vinagre *et al*., 2007).

In the present study protein, carbohydrate and lipid concentrations increased significantly in ovaries of barnacles *M. tintinnabulum* with the advancement of ovarian maturation stages. The concentrations of protein, carbohydrate and lipid were lowest in immature ovaries and highest in mature ovaries. This result is similar to the studies in other crustaceans where an increase in the biochemical constituents of the ovaries during maturation has been reported (Pillay and Nair, 1973; Read and Caulton, 1980, Rosa and Nunes, 2003a, b; Fatima *et al*., 2013; Fatima, 2013). The increase in protein, carbohydrate and lipid concentrations was estimated in the body tissue of *M. tintinnabulum* when their respective ovaries showed advancement in maturation. This is similar to the studies reported in other crustaceans, for example, during the reproductive period it has been found that not only protein increased in gonads, but the proteins reserves in hepatopancreas and muscle may move to gonads (Rosa and Nunes, 2003a, b). The hepatopancreas are considered as the main lipid storage site in crustaceans (Garcia *et al*., 2002), but lipids can be stored in muscle tissue and female gonad (Komatsu and Ando, 1992). In barnacles the digestive gland or hepatopancreas is absent; therefore, it appeared that tissue serves as a storage site. The concentrations of carbohydrate in the soft tissue and ovaries varied significantly during different stages of ovarian maturation in barnacles *M. tintinnabulum* being highest in mature ovaries and their respective tissues. This indicated that carbohydrate along with protein and lipid play an important role in the development of ovaries of barnacles. Glucose levels in hemolymph are required for proper functioning of the various systems in crustaceans and may be stored in the form of glycogen in the hepatopancreas and muscles (Vinagre and Da Silva, 2002; Oliveira *et al*., 2003).

The decrease in protein, carbohydrate and lipid contents in the ovaries and tissue of barnacles, *M. tintinnabulum* in summer after winter, that is, the brooding period, which is similar to the studies in which it has been reported that after breeding the biochemical components of body tissue decreased in two barnacle species, *C. stellatus* and *E. depressa* from the Mediterranean coast of Israel (Mizrahi and Achituv, 1990, 1991).

Proteins are the principal components of the eggs (García-Guerrero *et al*., 2003) as is the case in present study, the protein concentrations were higher in autumn and winter which is the brooding period in barnacles. The brooding eggs required more protein for its development which is provided by increased concentrations of protein in gonads and body tissue. Similarly lipid concentrations were higher in the gonad and body tissue in autumn and winter when the brooding barnacles were found as lipid is considered as an important source of energy during reproduction in other crustaceans (Antunes *et al*., 2010). In summer, the concentrations of lipid were decreased in the tissue of barnacles which indicated that these lipids are used as a source of energy. It is usually in summer that the animals reduced their activity and the availability of food is also less.

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