

## BIOCHEMICAL COMPOSITION OF GONAD AND SOFT BODIES DURING GONADAL MATURATION IN THE LIMPETS, *CELLANA KARACHIENSIS* (GASTROPODA: PATELLIDAE)

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### ABSTRACT

The present study measured the concentration of protein, carbohydrate and lipid in the gonad and soft bodies of male and female limpets, *Cellana karachiensis* during the gonadal maturation stages that is, developing, ripe and spawning. The protein concentrations increased significantly in gonadal tissue with the advancement of maturation stages in both males and females of *C. karachiensis*. The concentrations of carbohydrate in the developing, ripe and spawning gonads were similar in both sexes. However, the lipid concentration in the gonads of male and female limpets was significantly higher in ripe gonads. The concentrations of protein, carbohydrate and lipid in the soft bodies of males and females did not vary significantly during maturation stages, however, the total lipid and carbohydrate concentrations showed significant difference in the soft bodies being higher in females than males.

**Key words:** Limpets, gonadal maturation, proximate composition, gonad, soft bodies

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### INTRODUCTION

Reproductive changes in marine invertebrates are often related with translocation of some of the biochemical constituents between body tissue and reproductive organs (Giese, 1959, 1969; Giese and Pearse, 1974). The annual reproductive cycles in these animals can be determined by variations in the biochemical composition of the body tissue and individual organ systems during different stages of gonad development (Giese and Pearse, 1974). In prosobranch molluscs, a very high concentration of lipid and low concentration of polysaccharide in the gonad and high concentration of polysaccharide and low concentration of lipid in the muscular tissue, particularly in the foot has been reported (Najmudeen, 2007). As compared to vast literature available on oysters (Galtsoff, 1964) and other bivalves, biochemical studies on gastropods are meagre. Some studies have explained the various aspects of the biochemical composition in gastropods including limpets (Barry and Munday 1959; Blackmore 1969; Suryanarayanan and Nair, 1976; Patil and Mane, 1982) snails (Giese 1967; Oates *et al.*, 1990; Carasco *et al.*, 2006) and abalone (Mercer *et al.* 1993; Knauer *et al.*, 1994; Chiou *et al.*, 2001, 2002; Litaay and De Silva, 2003; Najmudeen, 2007).

In Pakistan studies on seasonal biochemical changes have been undertaken on bivalves such as, oysters, clam and mussels (Qasim *et al.*, 1985 Fatima *et al.*, 1986; Aftab, 1987; Aftab and Qasim, 1993; Perveen *et al.*, 1994; Jahangir *et al.*, 2010). No work has been carried out on biochemical changes in gastropods with relation to seasons or maturity. In the present study the protein, lipid and carbohydrate concentrations have been measured in the body tissue and gonad of *C. karachiensis* (Winkworth) to determine the changes associated with the maturity of gonads.

### MATERIAL AND METHODS

The biochemical analysis was performed on the developing, ripe and spawning males and females of *C. karachiensis*. For this purpose the samples of limpets were collected from the Buleji rocky shore in October 2009. The sex and gonadal stages of the limpets were determined by detaching the foot from the visceral mass and the shell. The gonad was examined for the color, size and texture following the methodology described by earlier workers (Orton *et al.*, 1956; Rao, 1973; Creese and Ballantine, 1983). Based on the above mentioned characteristics the gonads were divided into developing, ripe and spawning stages. After that the soft bodies and their respective gonads were removed and washed with phosphate buffer (pH 7) and homogenized in same buffer (pH 7). The total protein was estimated as per the Folin-Ciocalteu method (Lowry *et al.*, 1951) and the estimation of carbohydrate was carried out by Phenol Trichloroacetic acid method (Dubois *et al.*, 1956). The lipids were quantitatively

determined by Sulpho-phospho vanillin method (Barnes and Blackstock, 1973). For each maturation stage 6 samples in triplicate were used for the analysis. Two-factor ANOVA was used to test for differences in biochemical composition of gonad and soft bodies with sex and maturation stages as factors. To test which means were significantly ( $P < 0.05$ ) different, Tukey test (multiple comparison test) was performed. The SPSS 14.0 software was used to analyze the data.

## RESULTS

### Biochemical composition of the gonad

The concentration of protein in the male gonadal tissue of *C. karachiensis* varied from 22.4 to 75.3 mg g<sup>-1</sup>, that of carbohydrate from 4.2 to 7.6 mg g<sup>-1</sup> and lipid from 2.2 to 8.8 mg g<sup>-1</sup> (Table 1). In females the concentration of protein in the gonads varied from 22 to 82 mg g<sup>-1</sup>, that of carbohydrate from 4.0 to 9.6 mg g<sup>-1</sup> and lipid from 2.4 to 18.6 mg g<sup>-1</sup> (Table 2).

In male and female *C. karachiensis* significant difference in concentrations of protein and lipid in the gonadal tissue was observed (Table 3), whereas, the concentration of carbohydrate was not significantly different in the gonads of two sexes. The protein concentrations increased significantly with the maturation of gonadal tissue. The concentrations of carbohydrate in the developing, ripe and spawning gonads were similar in both sexes. The lipid concentration in the gonad of male and female *C. karachiensis* was significantly higher in ripe gonad ( $P < 0.05$ ) (Figure 1).

Table 1. The minimum, maximum and average (with standard deviation) concentration of protein, carbohydrate and lipid in the male gonad during the gonadal maturation stages of *Cellana Karachiensis* at Buleji.

Stage	Mean concentration (mg g <sup>-1</sup> ) in male gonad								
	Protein			Carbohydrate			Lipid		
	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average
Develoing	22.4	35	28.0±4.4	4.2	7.1	5.5±1.0	4.0	6.5	5.1±0.8
Mature	60.8	75.3	67.7±5.3	5.6	7.6	6.5±0.8	4.0	8.8	6.1±1.9
Spawning	35.3	48.3	40.8±4.6	4.2	7.5	5.9±1.1	2.2	4.4	3.3±0.9

Table 2. The minimum, maximum and average (with standard deviation) concentration of protein, carbohydrate and lipid in the female gonad during the gonadal maturation stages of *Cellana Karachiensis* at Buleji.

Stage	Mean concentration (mg g <sup>-1</sup> ) in male gonad								
	Protein			Carbohydrate			Lipid		
	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average
Develoing	22.0	40.2	32.0±7.5	4.0	6.6	5.5±1.1	5.2	10.8	7.5±1.9
Mature	66.4	82.0	75.9±5.6	5.0	9.6	7.1±1.6	12.2	18.6	15.8±2.5
Spawning	38.0	59.0	48.5±7.6	4.6	8.4	6.4±1.3	2.4	9.8	6.8±2.7

### Biochemical composition of soft bodies

The concentration of protein, carbohydrate and lipid in the soft body of male limpets varied from 28.6 to 70 mg g<sup>-1</sup>, 2.9 to 8.2 mg g<sup>-1</sup> and 2.2 to 7.2 mg g<sup>-1</sup>, respectively (Table 4). In females of *C. karachiensis* the concentration of protein in the soft body was 32 to 78 mg g<sup>-1</sup>, carbohydrate 2.5 to 8.6 mg g<sup>-1</sup> and lipid 2.8 to 8.5 mg g<sup>-1</sup> (Table 5.).

The total carbohydrate and lipid concentrations in the soft bodies showed significant difference in male and female *C. karachiensis* (Table 6), while the concentration of protein was not significantly different in the soft bodies of two sexes. However, during gonadal maturation the concentrations of protein, carbohydrate and lipid in the soft bodies of males and females did not vary significantly.

Table 3. Two way analysis of variance to test differences in biochemical concentration in the gonad of male and female *C. karachiensis* during maturation stages. Significant at  $P < 0.05$  level; NS = not significant.

Biochemical composition	Source of variation	df	F ratio	P value
Protein	Sex	1	11.080	0.002
	Maturation stage	2	150.935	0.001
	Sex * maturation stage	2	0.441	NS
Carbohydrate	Sex	1	1.037	NS
	Maturation stage	2	3.893	0.031
	Sex * maturation stage	2	0.265	NS
Lipid	Sex	1	67.746	0.001
	Maturation stage	2	32.180	0.001
	Sex * maturation stage	2	13.009	0.001

Table 4. The minimum, maximum and average (with standard deviation) concentration of protein, carbohydrate and lipid in the male soft tissue during the gonadal maturation stages of *Cellana Karachiensis* at Buleji.

Stage	Mean concentration (mg g <sup>-1</sup> ) in male gonad								
	Protein			Carbohydrate			Lipid		
	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average
Develoing	28.6	48.0	38.6±6.7	2.9	4.3	3.7±0.5	2.4	5.3	3.8±1.1
Mature	50.0	70.0	58.2±12.8	3.3	5.3	4.3±0.9	2.2	7.2	4.3±1.8
Spawning	32.3	52.6	43.1±7.8	4.6	8.2	6.2±1.3	3.5	5.6	4.3±0.8

Table 5. The minimum, maximum and average (with standard deviation) concentration of protein, carbohydrate and lipid in the female soft tissue during the gonadal maturation stages of *Cellana Karachiensis* at Buleji.

Stage	Mean concentration (mg g <sup>-1</sup> ) in male gonad								
	Protein			Carbohydrate			Lipid		
	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average
Develoing	32.0	54.0	414±8.9	2.5	5.6	4.1±1.3	2.8	5.6	4.3±1.1
Mature	54.2	78.0	64.9±9.6	4.6	4.6	5.8±0.9	3.6	8.5	5.5±1.8
Spawning	36.0	58.6	42.8±8.1	6.6	6.6	7.5±0.8	4.4	7.2	5.5±1.0

**Difference in biochemical composition of soft body and gonad in males and females**

The concentrations of protein, carbohydrate and lipid in the soft bodies of male and female limpets are shown in Table 7. Total protein concentrations in the soft bodies of males and females did not vary significantly ( $F = 2.036$ ;  $df = 1$ ;  $p = 0.162$ ), while the total carbohydrate ( $F = 6.319$ ;  $df = 1$ ;  $p = 0.016$ ) and lipid ( $F = 4.780$ ;  $df = 1$ ;  $p = 0.035$ ) concentrations in the soft bodies was significantly higher in females than in males.

The concentrations of protein, carbohydrate and lipid in the gonad of male and female limpets are shown in Table 8. Analysis of variance showed no significant differences in total protein and carbohydrate concentrations in the gonad of male and female limpets, while the total lipid ( $F = 19.134$ ;  $df = 1$ ;  $p = 0.001$ ) concentrations in the gonad was significantly higher in females than in males.

Table 6. Two way analysis of variance to test differences in biochemical concentration in the soft tissue of male and female *C. karachiensis* during maturation stages. Significant at  $P < 0.05$  level; NS = not significant.

Biochemical composition	Source of variation	df	F ratio	P value
Protein	Sex	1	2.192	NS
	Maturation stage	2	1.755	NS
	Sex * maturation stage	2	1.539	NS
Carbohydrate	Sex	1	7.966	0.008
	Maturation stage	2	4.950	0.014
	Sex * maturation stage	2	1.476	NS
Lipid	Sex	1	4.754	0.037
	Maturation stage	2	1.601	NS
	Sex * maturation stage	2	0.274	NS

Table 7. The minimum, maximum and average (with standard deviation) concentration of protein, carbohydrate and lipid in the male and female soft tissue of *Cellana Karachiensis* at Buleji.

Stage	Mean concentration (mg g <sup>-1</sup> ) in male gonad								
	Protein			Carbohydrate			Lipid		
	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average
male	28.6	70.0	44.9±10.1	2.8	5.3	4.0±0.07	2.2	7.2	4.2±1.24
female	32.0	58.0	40.7±7.48	2.5	8.5	5.0±1.56	2.8	8.5	5.1±1.39

Table 8. The minimum, maximum and average (with standard deviation) concentration of protein, carbohydrate and lipid in the male and female gonad of *Cellana Karachiensis* at Buleji.

Stage	Mean concentration (mg g <sup>-1</sup> ) in male gonad								
	Protein			Carbohydrate			Lipid		
	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average
male	22.4	75.3	45.5±17.6	4.2	7.6	5.9±1.0	2.2	8.8	4.8±1.7
female	22.0	82.0	52.1±19.7	4.0	9.6	6.3±1.5	2.4	18.6	10.0±4.8

## DISCUSSION

Reproductive changes in marine invertebrates are often related with translocation of the biochemical constituents between body tissue and reproductive organs (Giese, 1959, 1969). In the present study protein concentrations increased significantly in gonadal tissue with the advancement of maturation stages in both males and females of *C. karachiensis*. The concentrations of carbohydrate in the developing, ripe and spawning gonads were similar in both sexes. However, the lipid concentrations in the gonads of male and female limpets were significantly higher in ripe gonads. A similar observation was made by Najmudeen (2007) that the ovarian lipid content increased as maturation advanced and declined in the spent stage of abalone, *Haliotis varia*.

The concentration of carbohydrate in the soft bodies and in the gonads did not vary significantly during different stages of gonadal maturation in *C. karachiensis*. This is similar to the analysis by Santini and Chelazzi (1995) who found that the glycogen concentrations in the foot of two limpets, *P. rustica* and *P. caerulea* showed no

difference in the immature and developed gonads. On the contrary Barry and Munday (1959) and Blackmore (1969) reported that glycogen play an important role in the development of gonads of limpet, *P. vulgata*. In this species the glycogen concentration of the hepatopancreas and foot reached to a maximum value in summer, when the limpets were fully matured and then it decreased sharply in winter, when the gonads were either in spent or immature stage. Similarly, Davies *et al.* (1990) reported that in *P. vulgata* that carbohydrate concentration in the pedal mucus reached the maximum with the onset of spawning and was minimum at the end of the spawning season. The total lipid concentrations in the gonad of *C. karachiensis* were significantly higher in females than in males in the present study. The lipid concentrations of gonad in the present study seems to follow the pattern described for other molluscan broadcast fertilisers in which the levels of female gonad lipid were twice the male gonad lipid (Giese, 1967; Litaay and De Silva, 2003).

In the present study the protein concentrations in the soft bodies showed no difference in developing, mature and spawning *C. karachiensis*. Contrary to this other authors have reported an increase in protein concentration in body tissues when the breeding activity were high in *P. vulgata* (Davies *et al.*, 1990), *C. rota* (Patil and Mane, 1982) and *C. radiata* (Suryanarayanan and Nair, 1976). The concentrations of total carbohydrate and lipid in the soft bodies showed significant difference and were higher in females than males of *C. karachiensis* while the protein concentration was same in both the sexes. The higher concentrations of carbohydrate and lipid in soft bodies of female showed that these are supplied to the gonad for maturation process. A similar result has been reported by Patil and Mane (1982) in *C. rota* in which the concentration of glycogen, fat and protein in females was slightly higher than in males. Storage of energy in the foot muscle and digestive gland and their transfer of nutrients to the gonad has been reported in other mollusks (Ansell, 1974; Gabbott 1975; Le Pennec *et al.* 1991; Pazos *et al.* 1997; Berthelin *et al.* 2000; Litaay and De Silva, 2003). A further investigation is required on variations in the biochemical composition in various tissues of limpets during different stages of gonad development in order to understand the annual reproductive cycles in these animals.

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