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Sex-related biochemical investigation of the diaptomid, *Heliodiaptomus viduus* Gurney (Crustacea: Copepoda)

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Abstract. The freshwater diaptomid, *Heliodiaptomus viduus* was subjected to biochemical analysis to study its sex-related differences. Its chemical composition was similar to that of other copepods, the concentration of the components decreased in the order of protein > lipid > carbohydrate. The ovigerous females show higher dry weight and consequently the protein, lipid and carbohydrate concentrations are greater than non-ovigerous females and males. There are also considerable qualitative and quantitative differences in the free amino acid composition.

Keywords. *Heliodiaptomus viduus*; sex-related biochemical composition; freshwater tropical copepod.

1. Introduction

Although estimation of biochemical composition of freshwater micro- and macro-crustaceans was attempted in the past (e.g. Michael and Chandran 1967; Rajendran 1973), following the method of Birge and Juday (1922) on freshwater plankton to understand their metabolism, no efforts were made to distinguish males from females and ovigerous females from non-ovigerous females. Altaff and Chandran (1988) carried out an electrophoretic analysis of the body homogenate of *Heliodiaptomus viduus* to distinguish the protein fractions of males, females and ovigerous females. The present study is an attempt to find out the sex-related differences in protein, carbohydrate, lipid and free amino acid composition of the body homogenate of *H. viduus*. The copepod was chosen for detailed investigation as it forms an important constituent of the biota of most of the freshwater bodies of south India (Rajendran 1973).

2. Materials and methods

Zooplankton were collected from the Chetput pond of the Hydrobiological Station, Tamil Nadu State Fisheries Department, Madras, using bolten silk plankton net and maintained in filtered pond water in the laboratory. In the present study biochemical composition of males, females and ovigerous females of *H. viduus* has been determined using 5 sets of samples. For each analysis about 100 mg of 24 h starved males, females and ovigerous females were separated under a binocular dissection microscope and pooled. They were then transferred on to a tarred bolten silk piece and the excess moisture removed using a filter paper as suggested by Rajendran (1973). The moisture-free animals were weighed in a monopan balance. This procedure was uniformly followed for all the samples.

To estimate water and dry tissue content, the samples were dried in a hot air oven at 60°C to a constant weight. The colorimetric procedure of Lowry *et al* (1951) was suitably adopted for protein estimation and the colour intensity of the solution was measured at 520 nm. Different concentrations of bovine serum albumin served as the standard.

Qualitative analysis of free amino acids was carried out following the method of Smith (1968). Descending double dimension chromatogram was run using solvent I (butanol, glacial acetic acid and distilled water in the ratio of 12:3:5) for the first run and solvent II (160 g phenol, 40 ml distilled water and 1 ml of 25% ammoniacal solution) for the second run. Ninhydrin (0·2%) was used to detect the amino acids. A standard chart of amino acid was prepared using commercially available amino acids. For quantitative estimation of free amino acids the volume of aqueous layer of free amino acid was measured and 100 μl of this was spotted on the chromatographic paper. To prepare the standard, 1 mg of each commercially available amino acid was weighed and dissolved in 12 ml of 80% ethanol and 20 μl of this was spotted and double dimension chromatogram was run. Immediately after staining, the amino acid bands were eluted in 3 ml of 50% acetone and the optical density measured at 420 nm for proline and at 530 nm for all other amino acids.

Carbohydrates were estimated following the method of Roe (1955). Anthrone reagent was used and the colour developed was measured at 620 nm. Glucose was used as the standard.

Lipid was extracted according to the method of Folch *et al* (1957) and the estimation was followed as described by Barnes and Blackstock (1973). Sulpho-phosphovanillin was used as reagent and the colour developed was read at 520 nm (Spectronic 21 model of Bausch and Loumb). Statistical analysis of the data was performed using the Student's *t* test.

3. Results and discussion

The mean values of biochemical components of males, females and ovigerous females of *H. viduus* were found to be 65·08% of protein, 14·42% of lipid and 4·55% of carbohydrate. These values conform to those of other copepods in general (Birge and Juday 1922; Raymont *et al* 1964; Rajendran 1973). However, the males, females and ovigerous females of *H. viduus* show considerable variation in their biochemical composition. The mean protein, lipid and carbohydrate levels in males, females and ovigerous females are presented in figure 1.

Statistical analysis for the test of significance with reference to water content, protein, lipid and carbohydrate in males and non-ovigerous females indicates that the difference is not significant. However there is a significant difference ($P < 0{\cdot}001$) in protein and lipid content of males as well as non-ovigerous females when compared to the ovigerous females. The ovigerous females exhibit significant differences with respect to water and carbohydrate content also ($P < 0{\cdot}01$) when compared with that of males and non-ovigerous females (table 1). The lower water content (1%) and higher values of protein, lipid and carbohydrate of ovigerous females may be attributed to the reproductive phase of the ovigerous females in general and to the eggs in particular. It is known that the yolk material of the egg is rich in protein, lipid and carbohydrate in many crustaceans (Barnes 1965), which qualifies it to serve as a reserve food material for embryo development. The higher

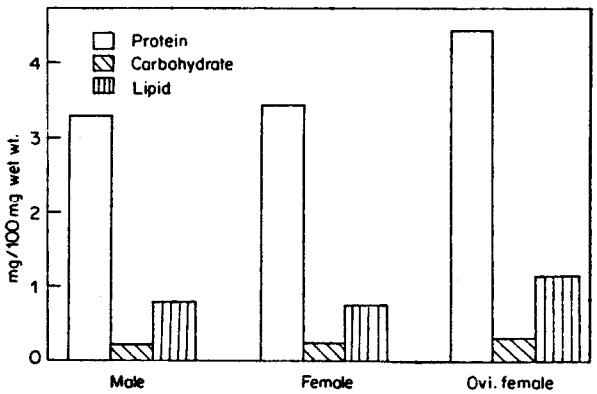

Figure 1. Variation in the biochemical components of male, female and ovigerous female of *H. viduus*.

Table 1. Biochemical components of males, females and ovigerous females of *H. viduus* (X ± SD).

	Water content	Protein (mg/100 mg wet wt)	Carbohydrate (mg/100 mg wet wt)	Lipid (mg/100 mg wet wt)
Male	94·81 ± 0·33	3·27 ± 0·21	0·21 ± 0·04	0·78 ± 0·04
Female	94·73 ± 0·36	3·43 ± 0·16	0·24 ± 0·03	0·76 ± 0·03
Ovigerous female	93·78 ± 0·31*	4·45 ± 0·45**	0·32 ± 0·04*	1·16 ± 0·13**

Values with asterisk are statistically significant.
*$P < 0.01$; **$P < 0.001$.

values of the biochemical components of ovigerous females in the present study conform to the earlier report of glycolipoprotein yolk in *H. viduus* (Altaff and Chandran 1988).

As in other crustaceans, *H. viduus* also shows higher concentrations of free amino acids. However, the concentration of free amino acids in this animal is less when compared to that of *Calanus finmarchicus* (Schoffeniels and Gilles 1970) which may be due to the habitat difference. A noticeable difference is found in the quantity of free amino acids of males, females and ovigerous females (table 2). The difference in the free amino acid content of ovigerous and non-ovigerous females may be due to the higher free amino acid content of the yolk found in the eggs of ovigerous females. Chromatographic separation further reveals the presence of 8 free amino acids in the male and female whereas 12 in the ovigerous female. Out of the 8 amino acids proline, aspartic acid, glycine, iso-leucine and leucine are the 5 amino acids common to both males and females. The other 3 amino acids are lysine, histidine and valine in males whereas serine, threonine and alanine in females. All the 8 amino acids occurring in females are also noticed in the ovigerous females, in addition to these amino acids histidine, lysine, arginine and phenylalanine also occur in the ovigerous females.

Table 2. Free amino acids and their concentration in the male, female and ovigerous female of *H. viduus*.

	μg/100 mg wet weight		
Amino acid	Male	Female	Ovigerous female
Histidine	2·04	—	1·22
Lysine	1·21	—	2·23
Arginine	—	—	2·04
Proline	6·59	6·54	9·63
Aspartic acid	2·72	2·39	12·72
Serine	—	2·72	5·15
Glycine	3·33	9·03	3·63
Threonine	—	1·81	3·03
Alanine	—	1·21	2·72
Phenylalanine	—	—	5·45
Valine	1·12	—	—
Isoleucine	1·18	2·72	5·45
Leucine	3·15	1·36	1·59

From the available data on crustacean metabolism, it may be suggested that the oxidative metabolism in crustacea can differ considerably from the commonly accepted pattern in other organisms. Such data prompted Waterman (1960) to come to the tentative conclusion that though carbohydrate may be the main metabolic substrate in some species, more often a mixture of protein, carbohydrate and lipid is needed. The large amount of free amino acids in crustacean tissues and the variability of its quantity under conditions leading to an energetic adjustment may perhaps be considered as indicating the use of amino acids in the process of energy production.

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References

Altaff K and Chandran M R 1988 Electrophoretic studies on the diaptomid, *Heliodiaptomus viduus* Gurney (Crustacea: Copepoda); *Indian Zool.* **12** 271–274

Barnes H 1965 Studies in the biochemistry of cirripede eggs I. Changes in the general biochemical composition during development of *Balanus balanoides* and *B. balanus*; *J. Mar. Biol. Assoc. UK* **45** 321–339

Barnes H and Blackstock J 1973 Estimation of lipids in marine animals and tissues: Detailed investigation of Sulphophosphovanillin method for total lipids; *J. Exp. Mar. Biol. Ecol.* **12** 103–118

Birge E A and Juday C 1922 The inland lakes of Wisconsin, the plankton, its quality and chemical composition; *Wis. Geol. Nat. Hist. Surv. Bull.* **64** 1–222

Folch J, Lees M and Slvare-Stavely G H 1957 A simple method for the isolation and purification of total lipids from animal tissues; *J. Biol. Chem.* **226** 497–509

Lowry O H, Rosebrough N J, Farr A L and Randall R J 1951 Protein measurement with Folin-phenol reagent; *J. Biol. Chem.* **193** 265–275

Michael R and Chandran M R 1967 On the Biochemical composition of the conchostracan *Caenestheriella annandalai* Daday: *Biol. Zentralbl.* **86** 495–499

Rajendran M 1973 *Studies on fresh water Calanoids*, Ph.D. thesis, Madurai University, Madurai

Raymont J E G, Austin J and Linford E 1964 Biochemical studies on marine Zooplankton. I. The biochemical composition of *Neomysis integer*; *J. Cons. Int. Explor. Mer.* **28** 354–363

Roe J H 1955 The determination of sugar in blood and spinal fluid with anthrone reagent; *J. Biol. Chem.* **212** 335–343

Schoffeniels E and Gilles R 1970 Nitrogenous constituents and nitrogen metabolism in Arthropods; *Chem. Zool.* **5** 199–227

Smith I 1968 *Chromatographic and electrophoretic techniques I* 3rd edition. (London: Wilham Heinemann Medical Book Ltd)

Waterman T H 1960 *The physiology of Crustacea* (New York: Academic Press)