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# Biochemical Profile of Zooplankton, *Daphnia galeata*

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***Abstract:***
*Zooplankton with good source of protein, amino acid, lipids, fatty acid, minerals, carbohydrate and enzymes could be an inexpensive ingredient to replace expensive fishmeal. Few studies have been made on the chemical composition of zooplankton although such information is vital to evaluate a species and its suitability as feed, in aquaculture. The present study also deals with analysis of biochemical composition of Daphnia galeata (Cladocera), which cultured in laboratory. In present study Daphnia galeata contended 14.1 % total lipid; 63.3 % protein and 15.68 % glycogen.*

***Key Word****:Zooplankton, Daphnia galeata, Biochemical composition.*

## Introduction

The production of planktonic organisms in good nutritional condition to feed fish larvae and fingerlings is a basic requirement in fish culture. In a vast majority of fish farms in India, it is a common practice to add organic and chemical fertilizers into the hatchery ponds (Sá-Junior, 1994). Although this procedure ensures a quick response in terms of algal biomass increase, both zooplankton composition and nutritional condition change abruptly, causing low fish larvae survival rates, due to the bad quality of food (Santeiro and Pinto-Coelho, 2000). An adequate plankton biochemical composition ensures the nutritional requirements for fish larvae, especially during their initial developmental stages. The living food improvement may decrease the high fish larvae mortality rate, a common problem in fish farms (Coutteau and Sorgeloos, 1997).

Zooplankton are considered to be ''living capsules of nutrition'' for commercially important cultivable and ornamental species, as they are valuable sources of proteins, lipids, carbohydrates, vitamins, minerals, amino acids, fatty acids and carotenoids (New, 1998; Hernandez Molejon and Alvarez- Lajonchere, 2003; Rajkumar et al., 2008; Pronob*et al.,* 2012). In the natural food web, they play a major role as diet for several invertebrates and vertebrate organisms and it is generally believed that the calorific value of zooplankton can meet the nutritional requirements of fish (Evjemo Ove *et al.,* 2003). In aquaculture practices, live food is difficult to sustain and requires considerable space and expense, on the other hand micro diets are easier to maintain and usually have lower
production costs (Jones *et al.,* 1993; Person *et al.,* 1993). In spite of the difficulties found in practicing live feed culture, Wang *et al.* (2005) found that the survival was significantly higher in larvae fed with live food than in larvae fed the three formulated diets. Introduction of live zooplankton is therefore being investigated as an alternate to pond fertilization for increasing fish yields while avoiding water quality deterioration (Jha *et al.,* 2007).

Studies on the biochemical composition and energy content of zooplankton are important to assess the energy available to plankton feeders (Bhat and Wagh, 1992). Such information is of much importance in estimating the energy available to higher tropic levels which in turn can be used to estimate harvestable fishery resources. Much of the available information about the biochemical composition and nutritive value of zooplankton is from estuarine, coastal, inshore and off share waters of India (Krishna Kumari and Goswami, 1993; Nageswara and Ratna Kumari, 2002; Jagadeesan*et al*., 2009).

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Proteins are the most abundant macromolecules and constitute over half of the dry weight of most organisms. Proteins are extremely complex nitrogen containing molecules, which play important role in nearly all biological processes as structural components, biocatalysts, hormones and repositories of genetic information. They also help in storage, transport, mechanical support, control of growth and differentiation (Kale, 2002). Carbohydrates play vital and central role in cellular biochemistry, in addition to their functions as structural units and food reserves (Rao and Murthy, 1980). Carbohydrate metabolism in the animal is to meet the energy demands by the organs and systems for proper functioning. In the animal the chief carbohydrate of the tissue is glycogen, while glucose is of the haemocoelomic (blood) and other body fluids (Holden, 1972). Glycogen, a storage polysaccharide is reversibly converted to glucose. The equilibrium between the glycogen and glucose conversion tends to maintain blood glucose in a steady state. The equilibrium between glycogenesis and glycogenolysis is governed by the extrinsic and intrinsic environmental factor that governs the physiology of organs (Pickering *et al.*, 1983). Lipids are heterogeneous group of water insoluble (hydrophobic) organic molecules are not only a major source of energy but also provide the hydrophobic barrier that Live feed Culture, nutritional potential and biochemical composition permits partitioning of the aqueous contents of cells and sub cellular structures (Villalan*et al.*, 1990).

Zooplanktons are an important food source for many species of fish and flavour texture of fish is also improved with zooplankton as feed. Goswami *et al.*, (1981) studied biochemical contents of marine copepods. Bhat and Wagh (1983) reported biochemical composition and calorific value of marine rotifers. Sreepada*et al.*, (1992) observed biochemical composition of zooplankton from Arabian Sea. Tiwari and Nair, (1993) studied protein composition of rotifers. Kumari *et al.*, (1993) studied biochemical composition of zooplankton from the offshore oil field of Bombay. Nageshwara and Rathnakumari (2002) studied biochemical composition of zooplankton from east coast of India. Aman and Altaff, (2004) studied the biochemical profile of copepod *Heliodiaptomusviduus, Sinodiaptomus (Rhinediaptomus) indicus, and Mesocyclopsaspericornis*and their dietary evaluation for postlarvae of *Macrobachiumkistnensis.* Ishizaki (1968) studied the ostracod, *Xestoleberishanaii*by culturing it under controlled laboratory conditions for five generations, its life history including oogenesis, ovulation, oviposition, embryogenesis mating behaviour and ontogeny.

This work deals with laboratory culture of *Daphnia galeata (Cladocera)*, using *Chlorella* algae as supplement. The present study also deals with analysis of biochemical composition of *Daphnia galeata (*Cladocera).

## Material And Methods

**Biochemical analysis:**

The samples of *Daphnia galeata were* collected from laboratory monoculture circular glass tank with the help of plankton net (60 µm mesh size) as well as dropper in 25 ml beaker. The collected samples were washed with distilled water. The partially wet sample was kept on filter paper for surface drying. After the weight of sample is measured it was transferred into glass petridish and kept into oven at 700c for drying. The dried sample was used for estimation of protein, lipid and carbohydrate. Water content was determined by determining difference between initial wet weight and final dry weight.

**Estimation of lipid (Lehtonen 1996):**

The analysis was performed following the method described by Lehtonen (1996). Approximately 15 mg of dried material was weighed and homogenized in 0.5 ml of chloroform: methanol (2:1) solution, and then centrifuged for 30 minutes. The precipitate was washed with 0.5 ml chloroform: methanol (2:1) and centrifuged again for 30 seconds. Twenty per cent volumes (0.02 ml) of 0.9 % NaCl solution were added to the chloroform: methanol (2:1) solution for both washes, and centrifuged. The chloroform phase containing the dissolved lipids was placed into tarred cups, and the solvent evaporated. The cups were then weighed, and the weight of the lipids calculated from triplicate sub samples.

**Estimation of total proteins (Lowry *et al.* 1951):**

Oven dried material was homogenized in the proportion of 0.5 mg to 3 ml of pure water (Micropur) into 10 ml test tubes. The water-soluble protein content was analysed (n = 5-6 sub samples) using the method described by Lowry *et al*. (1951), as modified by Fernandes *et al*. (1994). 0.1ml of the aliquot was transferred into a test tube and 4 ml of alkaline copper sulphate reagent was added, followed by 0.4 ml of diluted commercial Folins reagent. The optical density of the blue colour developed was read at 540 µm after 30 minutes of addition of the Folins reagent using

UV-VIS spectrophotometer (Model Digispec 200 GL). Bovine serum albumin was used as a standard. The protein content was expressed as mg/100 mg wet weight of the tissue. Live feed Culture, nutritional potential and biochemical composition.

**Estimation of glycogen (DeZwaan and Zandee 1972):**

Samples were separated for analysis, following essentially the same procedure as for proteins. The homogenates were analyzed (n = 4-5 sub samples) with the method of DeZwaan and Zandee (1972). The homogenate mixture was kept in boiling water bath for 3 to 5 minute to dissolve the tissue and then cooled. Before centrifugation 2 ml of 96% ethyl alcohol was added and the mixture was kept overnight in refrigerator. Next day this mixture was centrifuged at 3000 rpm for 15 minutes. The glycogen cake settled down on the bottom was collected and 2 ml of distilled water was added to the cake and mixed well. This mixture was kept at 700C for 5 minutes in a hot water bath. 0.1 ml of the aliquot was mixed with 0.9 ml of distilled water and 5 ml of anthrone reagent was added. This mixture was kept in hot water bath for 10 minutes. The optical density was read at 610 µm against blank using UV-VIS spectrophotometer. Glycogen content is expressed in terms of mg glucose / 100 mg wet weight of tissue (Glycogen conversion is factor 0.927).

**Statistical analysis:**

The results of biochemical analysis were expressed as mean of three replicates and data were analyzed statistically by using student 't' test (Mungikar, 2003).

## I. Result

**Table1:** Biochemical composition of live feed zooplankton *Daphnia galeata*

| **Parameters** / **Zooplanton** | **Lipid %** | **Protein %** | **Glycogen%** | **Water %** |
|---|---|---|---|---|
| ***Daphnia galeata*** | 14.1 | 63.3 | 15.68 | 89.7 |

## II. Discussion

Live feeds are being utilized as nursery/weaning/maturation diets and they also improve energy balance which results in maturation, quick growth, coloration and physiological conditions (Mitchell, 1991; Munuswamy et al., 1997; Velu and Munuswamy, 2007). Estimation of biochemical composition of zooplankton is important in understanding their physiological function, metabolic rate, nutritive value and energy transfer (Jha et al., 2007). Assessments of biochemical constituents like lipid, protein and glycogen in *Daphnia galeata*is important for better understanding of the organic production, cycling of biogeochemical elements and its nutritive potential. In present study *Daphnia galeata*contentained 14.1 % total lipid. Earlier Watanabe *et al.* (1983) reported 23.1 % lipid in *Branchionusplicatilis. Moinamacracopa*contained 8.94 % total lipid. Earlier Krishnakumari*et al.* (1993) recorded 45.65 % lipid in another ostracod *Xestoleberis nitida*. Higher values of lipid in different zooplankton species have been reported earlier by many workers (Maruthanayagam and Subramanian, 1999; Goswami *et al.*, 2000; Prabhu *et.al.*, 2005; Rajkumar *et al.*, 2008).

In the present study *Daphnia galeata had* 63.3 % protein. Higher protein contents in copepods *Acartiaspinicuda*and *Acartiasimilis*from costal water of Parangipettai have been reported by Rajkumar *et al.*, (2008) and Rajkumar and Santhanad (2009). The protein may function as metabolic reserve in zooplankton. Guisande*et al.*, (2000) made Comparison between the amino acid composition of females, eggs and food to determine the relative importance of food quantity and food quality on copepod reproduction.

In the present study *Daphnia galeata contained* 15.68 % glycogen. Watanabe *et al.* (1983) recorded comparable quantity (16.68 %) of glycogen in *Branchionusplicatilis. Moinamacracopa*contained maximum glycogen (19.64 %). Lower values of glycogen have also been reported earlier by many workers (Maruthanayagan and Subramanian, 1999; Rao and Krupanidhi, 2001; Prabhu *et al*., 2005*;* Rajkumar *et al.*, 2008) in different group of zooplankton. Maruthanayagan and Subramanian, (1999) felt that the glycogen might be oxidized directly by zooplankton and that fats might be oxidized on need or stored as principal reserve food. In general, low glycogen content in zooplankton led to contemplation on the functional role of other biochemical fractions in their metabolism. The fluctuations in glycogen content of animals generally depend upon their feeding activities (Rao and Krupanidhi, 2001).The present observation of low glycogen content may be attributed to the fact that glycogen is the usual storage carbohydrate in many animals.

In present study the water content (89.7 %) was found in *Daphnia galeata* .Watanabe *et al.* (1983) also recorded 87.9 % water in *Branchionusplicatilis*confirming the present result. *Moinamacracopa*contained 86.8 % water. Blazka (1966) reported 92.9 % water in *Daphnia pulicaria.* Yurkowaski and Tabachek (1979) found 94% water in *Daphnia pulex*whereas Tay *et al* (1991) reported 87.9 % water in *Moinamicrura.* These findings support the present result. Earlier Krishnakumari*et al.* (1993) recorded 30 % water in another ostracod *Xestoleberis nitida*. Simhachalam*et al.* (2015) reported that zooplanktons are rich in protein, lipids, essential amino acids and fatty acids.

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