



# Variation in Protein, Carbohydrate and Lipid Content in the Body Wall of Commercial Sea Cucumber *Holothuria spinifera* (Theel, 1886) in Relation to Reproductive Stages

P. S. Asha\* and P. Muthiah

Tuticorin Research Centre of CMFR Institute, South Beach Road, Karapad, Tuticorin - 628 001, India

## Abstract

The dry body wall from sea cucumbers, is 'beche-de-mer', is an important item of trade in the developing countries in the Indo-Pacific region. A study was conducted to assess the variation in biochemical constituents of the body wall of commercial sea cucumber *Holothuria spinifera* concerning the reproductive stages. The body wall of sea cucumbers belong to eight reproductive stages were pooled and dried in hot air oven at 80°C for 48 h. Standard procedures estimated the protein, total carbohydrate and total lipids. The mean values of proteins, carbohydrate and lipid content in the body wall of *H. spinifera* were 17.2, 2.8 and 1.3%, respectively. The highest concentration of these organic constituents during the maturing stages, decline during the post-spawning periods indicating their role as the source of energy during gametogenesis. The one-way ANOVA on the differences in the mean protein, carbohydrate and lipid content in the body wall to the reproductive stages indicated a high level of significance ( $p < 0.001$ ).

**Keywords:** 'Beche-de-mer', *Holothuria spinifera*, protein, carbohydrate, lipid

## Introduction

Holothurians are exploited commercially, for the raw body-wall or viscera, but mostly for processed dry product called 'beche-de-mer', which is an important item of trade in the developing countries in the Indo-Pacific region. The edible part of the sea cucumber body wall is the loose connective tissue

unlike the muscular tissues of vertebrates (Tanikawa & Yoshitani, 1955; Danis, 2012). Knowledge about biochemical constituents and their concentration provide information related to the physiological and nutritional value of organisms (Barbarino & Lourenço, 2009). From a nutritional standpoint, sea cucumbers are of great value because of its high protein content, and low-fat percentage. It is also rich in vitamins like the vitamins A, B1 (thiamine), B2 (riboflavin), B3 (niacin) and minerals like calcium, magnesium, iron and zinc and forms an important food item in many South-East Asian countries for centuries (Fredalina et al., 1999; Chen 2003).

Information is available on the proximate chemical composition of sea cucumbers like *Stichopus japonicus* (Tanikawa et al., 1955; Tanikawa & Yoshitani, 1955); *Parastichopus californicus* (Giese, 1966); *Actinopyga agassizi*, *Synaptula hydriformis*, *Pentacta pygmaea* and *Parastichopus californicus* (Prim et al., 1976); *Benthogone rosea*, *Paelopatides gigantean* and *Psychropotes longicauda* (Sibuet & Lawrence, 1981); *Parastichopus* spp (Chang-Lee et al., 1989); *Holothuria atra* and *Holothuria arenicola* (Salarzadeh et al., 2012); *H. atra* and *Actinopyga mauritiana* (Haider et al., 2015) and *Isostichopus* sp. (Vergare & Rodriguez., 2016).

Commercially important sea cucumber species occur in Gulf of Mannar, Palk Bay Lakshadweep and Andaman & Nicobar Islands along the Indian coast. Though sea cucumbers were not popular as an edible commodity in India, seven commercial species were exploited for 'beche-de-mer' preparation and constituted a significant livelihood resource especially along South-East coast of India, until the ban enforced by the Ministry of Environment, Forests and Climate Change, Government of India since 2003. *H. spinifera* was a medium valued and widely utilised sea cucumber species for 'beche-de-mer' preparation along Gulf of Mannar and Palk Bay

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\* E-mail: [ashasanil@gmail.com](mailto:ashasanil@gmail.com)

coast (Chellaram et al., 2003; Asha & Muthiah, 2005). James & Badrudeen (1997) estimated annual landings of 460 t of fresh *H. spinifera* along the South-East coast of India. Apart from the Gulf of Mannar and Palk Bay off the Indian coast, *H. spinifera* is reported to occur in the Red Sea, Persian Gulf, Sri Lanka, northern Australia, Philippines, China and Tanzania (James 1995; Purcell et al., 2012).

The proximate chemical composition of the sea cucumber body wall depends on the reproductive stages, feeding and environmental conditions (Tanikawa et al., 1955; Diniz et al., 2014). Generally, among invertebrates, the energy is stored for the process of gametogenesis in the form of glycogen, lipid, and protein which plays an essential role in the physiology and reproduction of the animals (Dridi et al., 2007; Yan et al., 2007). The knowledge on the qualitative and quantitative buildup of biochemical reserve in the body wall of sea cucumbers and its mobilization during spawning stages is significant for the scientific broodstock management in the hatchery operations as well as for the rational exploitation and management of sea cucumber stocks in wild. Attempts are being made to standardize the hatchery technology of *H. spinifera* to aid conservation of this species (Asha & Muthiah, 2002; 2007).

Except for the elaborated account of the seasonal changes in the body wall constituents of *Holothuria scabra* with reproductive stages (Krishnaswamy & Krishnan, 1967), not much research has been conducted on nutritional evaluation especially in relation to reproductive stages of other cultivable sea cucumber species from Indian water. Because of the paucity of such information on *H. spinifera*, the present work was carried out for further research in this field.

## Material and Methods

Monthly, 18–25 specimens of *H. spinifera* of length ranging from 110 to 403 mm were collected from the commercial catch of trawlers, in the vicinity of Koswari (8°51'52"N lat.;78°12'14"E) and Van Island (8°49'50"N la.;78°12'013"E) off Tuticorin. The collection was carried out during the period from September 2000 to October 2001. The samples were brought to the laboratory in the live condition in plastic containers filled with sea water at 28° to 30°. Once they were killed, internal fluids were drained, and body weight was recorded with a precision scale. Animals were cut longitudinally from the

ventral portion, after which the viscera and the muscle bands were extracted.

The body wall of individuals of *H. spinifera* in each reproductive stage was pooled for biochemical analysis. Food processor homogenised the tissue and moisture content of 5 g of homogenised sample was determined by drying the sample in the oven at 105°C until a constant mass was obtained. The dried and labelled tissues were packed in aluminium foils and kept in desiccators. The fractions of the dried tissues were homogenised using a mortar and pestle and used for estimations in triplicates.

Folin Ciocalteu method (Lowry et al., 1951) was followed for protein estimation. The supernatant obtained after the precipitation of protein was used for the estimation of total carbohydrate following the phenol sulphuric acid method of Dubois et al., (1956). The total lipids were quantitatively estimated by following the sulphur vanillin method of Barnes & Blackstock (1973). All the results were calculated as percentage dry weight.

The mean values of the protein, carbohydrate and lipid in the body wall concerning the reproductive stages were used in the one-way analysis of variance (ANOVA) using SPSS version 20 software. Since the ANOVA yielded a significant result ( $p < 0.05$ ), the multiple comparison – the Duncan test was applied to determine the pair wise comparisons.

## Results and Discussion

The protein content was comparatively higher in male specimens than the female and the highest mean value was noticed in the maturing stages of both the sexes. In maturing males, the average protein content was  $18.0 \pm 0.07\%$ , and in the female, it was  $17.2 \pm 0.02\%$ . The mature and spent stages of male reported the mean value of  $17.7 \pm 0.02\%$  and  $16.6 \pm 0.07\%$ , respectively. The mature and spent females had  $16.5 \pm 0.07\%$  and  $16.7 \pm 13\%$ , respectively. In the indeterminate stages I and II, the protein content was  $17.3 \pm 0.08\%$  and  $17.4 \pm 0.1\%$ , respectively, much better than the female counter parts. (Fig. 1). The one way ANOVA on the differences in the mean protein content in the body wall concerning reproductive stages showed high levels of significance ( $p < 0.001$ ). In Duncan's multiple comparison tests, a significant difference was noticed in the protein content between Indeterminate-I and maturing male; mature female and

spent male. Indeterminate-II indicated a significant difference between mature female and spent male. The maturing male has significant difference between mature female, spent male and spent female, whereas the maturing female indicated a significant difference between mature female and spent male. The estimated protein content of the body wall of *H. spinifera* is more or less similar to 16.64% reported for *Thelenota pineapple* and higher than 12.94% reported for *Acaudina molpadioides* (Chen, 2003).

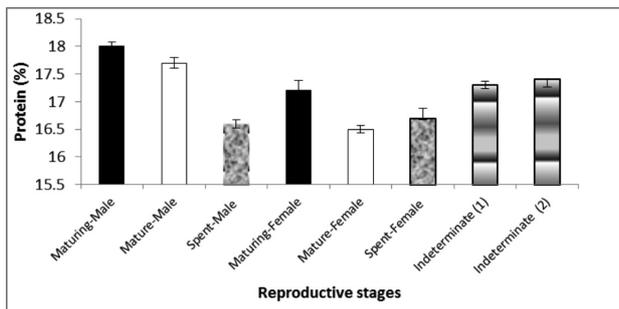


Fig. 1. Mean ( $\pm$  SE, n=24) values of the protein content in the body wall of *Holothuria spinifera* at different reproductive stage

Unlike the protein content, the carbohydrate content was comparatively better in female specimens than the males. In both the sexes, the mean value was highest in the maturing specimens. The maturing stages of female had the highest average value of  $3.6 \pm 0.7\%$ , whereas the male had only  $3 \pm 0.04\%$ . The mature stages of female reported a higher value of  $2.9 \pm 0.2\%$  than the counterparts in males ( $2.5 \pm 1.1\%$ ). The spent stages of male reported a higher value of  $2.4 \pm 0.4\%$  than the females ( $1.9 \pm 0.2\%$ ). Comparatively a better value of carbohydrate content was reported in both the indeterminate stages I ( $2.6 \pm 0.02\%$ ) and II ( $3.2 \pm 0.09\%$ ), than the mature and spent stages of both sexes (Fig. 2). The one-way ANOVA on the differences in the mean carbohydrate content in the body wall concerning reproductive stages also indicated statistical significance ( $p < 0.01$ ). In the multiple comparison tests, a significant difference in carbohydrate content was noticed only between indeterminate I and maturing female.

The lipid content was comparatively better in male specimens. The lipid content was highest of  $1.8 \pm 0.2\%$  for maturing male specimens, whereas the maturing females had only  $1.3 \pm 0.16\%$ . The lipid content of the mature and spent stages of the male

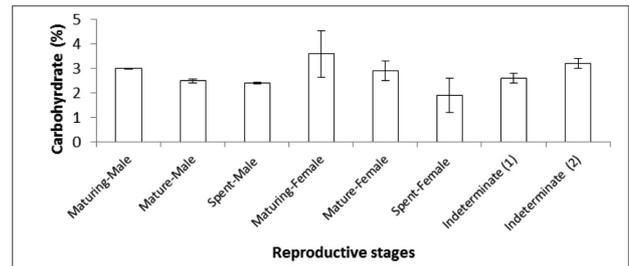


Fig. 2. Mean ( $\pm$  SE, N=24) values of the carbohydrate content in the body wall of *Holothuria spinifera* at different reproductive stages

were  $0.9 \pm 0.007\%$  and  $1.7 \pm 0.01\%$ , respectively, whereas the values were  $0.8 \pm 0.02\%$  and  $0.9 \pm 0.04\%$ , respectively for the mature and spent stages of the female. The total lipid content was comparatively better in the intermediate stages. It was  $1.7 \pm 0.2\%$  and  $1.0 \pm 0.04\%$  for the indeterminate stages I and II, respectively (Fig. 3). The one-way ANOVA indicated highly significant differences in the mean lipid content in the body wall with respect to the reproductive stages ( $p < 0.001$ ). In Duncan's multiple comparison tests, a significant difference was noticed in the lipid content between indeterminate II and mature female and spent male. The mature male indicated a significant difference between mature female; spent male and spent female. The mature female had a significant difference between spent male and spent female. The spent male showed a significant difference in the lipid content only between spent female.

The low levels of lipids in *H. spinifera* were similar to the observation in *H. atra* by Lawrence (1972) and the observation made in several sea cucumber species from Malaysia by (Ridzwan et al., 2014).

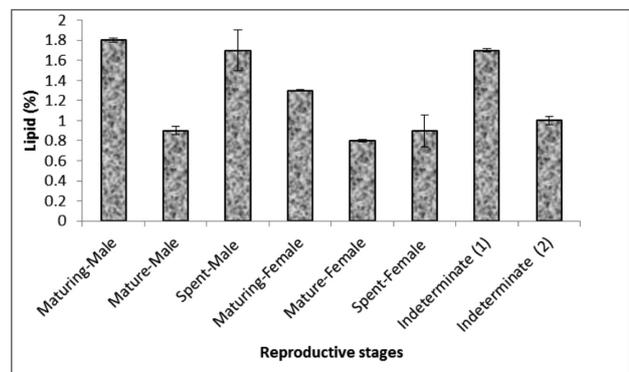


Fig. 3. Mean ( $\pm$  SE, n=24) values of the lipid content in the body wall of *Holothuria spinifera* at different reproductive stages

There is an apparent change in the lipid value in the body-wall of *H. spinifera* concerning the reproductive stages. Similarly, in the histochemical studies, Krishnan (1968) also pointed out that though the accumulation of lipids with the advancement of maturity occurs in both sexes, there seems to be a sex-related change in lipid synthesis in different tissues during different phases of the reproductive cycle. Similarly, Vergara & Rodríguez, (2016) indicated that the lipid concentration is likely to affect the reproductive potential of *Isostichopus* sp., which previously mobilise its lipid reserves for reproduction and development of gamete.

The mean values of protein, carbohydrate and lipid content in the body wall of *H. spinifera* were  $17.2\pm 0.19\%$ ,  $2.8\pm 0.18\%$ , and  $1.3\pm 0.15\%$ , respectively. Similarly, Prim et al. (1976) observed 18.6% protein in *Actinopyga agassizi* and 18.2% in *Parastichopus californicus* and 2.4% carbohydrate in *Synapta hydriformis*. However, low values of protein and lipid content ( $8.22\pm 0.43\%$  and  $0.27\pm 0.05\%$ ) have been reported in *H. spinifera* from Sri Lankan water (Ketharani & Sivashanthini, 2016). The higher values of organic compounds in the body wall of holothurians than in other echinoderms might have been due to the lack of dense calcification in the body wall of the sea cucumbers as envisaged by Prim et al. (1976).

In *H. spinifera*, the highest values of protein, carbohydrate and lipid were noticed in the maturing stages of both sexes. The increased nutrient content during the reproductive stages of sea cucumbers might be due to the mobilization of nutrients for the breeding season and also for the subsequent release of gametes and hatching rates (Audelo & Rodríguez, 2015). Similar observation of high nutrient content especially the protein during the previtellogenic stages was reported in the body wall of *Isostichopus* sp. (Vergara & Rodríguez (2016).

The differences in the values of protein, carbohydrate and lipid between the reproductive stages of both female and male were observed highly significant. Tanikawa et al., (1955) suggested the possible role of these organic constituents as an alternative source of energy during gametogenesis and the general decline in their levels during the post spawning periods. The differences in the protein and lipid were highly significant ( $p < 0.001$ ) than the values of carbohydrate during the reproductive stages. Similarly based on histochemical

studies, Krishnan (1968) opined that protein and lipids appear to be the first and second most important compounds varying in quantity during the different months of the reproductive cycle of *H. scabra* and carbohydrate do not seem to play a significant role in its reproductive cycle.

The results obtained in the present study are consistent with the nutritional properties of other species of sea cucumbers. The data gathered also warrants the need for further research on elaborate chemical and pharmaceutical applications of sea cucumber species from Indian waters.

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