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Laboratory Culture and Biochemical Characterization of the Calanoid Copepod, *Acartia southwelli* Sewell, 1914 and *Acartia centrura* Giesbrecht, 1889

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Abstract: This work reports on the successful production of calanoid copepod *Acartia southwelli* and *A. centrura*. The generation time under optimal conditions is about 12-15 days. Algal culture was separately maintained for feeding copepods and the stock culture of *Chlorella marina, Nannochloropsis salina* and Isochrysis *galbana*. During copepod culture, the system produced average of 41,603 org./l. from the 2 months culture period. Among these, nauplii density was 24,123 org./l, copepodids 10,564 org./l and adults 6,916 org./l. biochemical composition (%) of the calanoid copepod, *Acartia southwelli*, in dry weight basis, biochemical constituents like moisture, protein, lipid, carbohydrates and ash were (82.94±0.53, 63.85±0.24, 16.92±0.26, 10.56±0.24 and 5.12±0.19) and *Acartia centrura* (82.75±0.60, 63.98±0.69, 17.06±0.62, 10.48±0.55 and 5.36±0.49) respectively. The *A. southwelli* and *A. centrura* was found to be a rich source of PUFAs and HUFAs.

Key words: Marine Calanoid copepod · Laboratory culture and biochemical composition

INTRODUCTION

Marine copepods are a potential source of live feed for marine fish larviculture. However, the technology to mass culture the marine copepods, at a low-cost technology is still being developed [1]. Calanoid copepod, *Acartia* spp. are being promoted as a food-organism in some marine hatcheries. Marine copepods are natural feed, which can act as alternatives or supplements to *Artemia* nauplii, of doubtful nutritional suitability [2]. Nutrition-wise, the copepods, *Acartia* spp. are found to be rich in highly unsaturated fatty acids, especially eicosapentaenoic acid and docosahexaenoic acid [2]. Moreover, they provide a wide size range of food organisms for hatchery use (6 nauplii stages and 6 copepodid stages).

Various workers have attempted to mass culture different species of copepods especially in temperate waters. [3] was the first researcher to attempt on planktonic copepods culture. [4] conducted culture experiments on *Oithona* spp. And [5] have experimented the culture of brackishwater cyclopoids for their use in mariculture hatchery system. Mass culture of *Acartia* spp. have been described by [6-8]. Recently, marine copepod culture was successfully maintained by [9-12]. In India, [5] have experimented the culture of brackishwater cyclopoids and used them in mariculture hatchery system and very recently, [13, 14] have successfully cultured the cyclopoid copepods, *Oithona rigida, O. bervicarnis* and calanoid copepods *Acartia clausi*, *A. erythraea* in laboratory condition.

Studies on biochemical composition of tropical copepods particularly from Indian waters are seemed to be very limited and restricted with the works of [15-18]. Determination of biochemical composition of cultured copepod has become important to understand their physiological functions, metabolism and nutritive value, which are relevant to marine ecosystem for its energy transfer and secondary production.

The marine copepods are considered to be "nutritionally superior live feeds" for commercially important cultivable species, as they are a valuable source of protein, lipid (especially HUFA, 20:5 *n*-3 and 22:6 *n*-3), carbohydrates and enzymes (amylase, protease, exonuclease and esterase), which are essential for larval survival, growth, digestion and metamorphosis [19-21]. Nauplii of cyclopoids (*Oithona* sp.) and calanoids (mainly of *Paracalanus*) are numerically the most important food items in the gut contents of the larvae of anchovy *Engraulis japonicus* found in Toyama Bay, Japan [22].

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The copepods *Temora longicornis*, *Eurytemora* sp. and *Calanus finmarchicus* have frequently been used as live feed for fish larvae of marine cold water species in Norway, particularly Atlantic halibut (*Hippoglossus hippoglossus*) and cod (*Gadus morhua*). Larval pigmentation, growth and survival are normally satisfactory when copepods are used as live feed, and the fatty acid composition in the live feed organisms are considered to be important in this respect [9]. Nutrition-wise, the copepods, *Acartia* spp. are found to be rich in highly unsaturated fatty acids, especially eicosapentaenoic acid and docosahexaenoic acid [2]. Moreover, they provide a wide size range of food organisms for hatchery use (6 naupliar stages and 6 copepodid stages). [10, 23-30] have documented the high nutritional quality of cultured copepods from different parts of the world and only limited information is available for our marine copepods.

Lipids are particularly important in fish nutrition not only for supplying calorific energy but also for providing the essential polyunsaturated fatty acids (PUFAs) required for normal cell membrane function [29]. In the case of marine fish, these PUFAs are the highly unsaturated fatty acids (HUFAs) of the (n-3) series, eicosapentaenoic acid [20:5(n-3); EPA] and docosahexaenoic acid [22:6(n-3); DHA] [31]. The (n-3) HUFA requirement of juvenile marine fish is ~0.5-1.0% of the dry weight of their diet, but the requirement during the early developmental stages of larvae is likely to be greater because of their rapid growth and the critical early development of specialized cells and tissues. Several investigators have studied the (n-3) HUFA requirements of a number of marine fish species [32-35] and they suggest that DHA is more efficacious than EPA in promoting larval health and survival.

DHA and EPA are essential fatty acids (EFAs) for marine fish species because they do not have or have only a very low 5-desaturate activity, which is necessary for the conversion of C-18 PUFA to long-chain HUFA [36]. In addition, although these fish may be capable of converting EPA to DHA, the rate at which they do so may be too low to satisfy the high DHA requirement during early larval growth [31]. DHA, which is abundant in all fish tissues [37] tends to accumulate in terrestrial vertebrates in neural and reproductive tissues, suggesting a specific functional role for DHA in particular cell membranes of these tissues [38, 39]. However, neural membranes of fish are also especially rich in DHA [40 and 41] suggested that the same specialized function(s) exists for DHA in fish as in terrestrial vertebrates. Studies with larval sea bass (*Dicentrarchus labrax)* and herring (*Clupea harengus*) fed DHA-deficient diets established a deficit of didocosahexaenoyl phospholipids in the eyes of the fish, which was correlated with impaired visual performance in the latter species [42]. In addition, normal dorsal pigmentation in flatfish larvae (post-metamorphosis) is influenced by dietary HUFA, and it has been suggested that albinism may be linked with impaired larval neural function [35].

Hence in the present study, an attempt was made to estimate the biochemical composition, *viz*. protein, lipid, carbohydrate, moisture, ash and fatty acid profiles of cultured copepod, *A. southwelli* and *A. centrura.*

MATERIALS AND METHODS

Transparent fiberglass tank of 25-Litre capacity was used for culturing copepod. Rearing containers were covered with nylon cloth to prevent excessive evaporation. Seawater from the natural water source where the animals occur in the field was used. It was filtered through a membrane filter (pore size greater than 1µm) and the contamination of the rearing tank was reduced by frequent water exchange.

Zooplankton samples were collected using a 158-µm mesh (0.35 m mouth diameter) at early morning from the Vellar estuary during full moon time. The samples were immediately transported to laboratory and thoroughly rinsed to reduce the contamination from other zooplankters. The collected copepods were identified under the microscope using the keys of [43].

After the collection, the zooplanktons were screened to isolate the size fraction containing dominantly adult copepods and later stage of copepodites of the species, *Acartia southwelli and Acartia centrura.* The zooplankton samples were first screened coarsely through a 500 µm mesh sieve to remove fish and prawn larvae. Then they were rinsed for two hours in a zooplankton washer [11] fitted with a 190-µm-mesh screen to remove smaller zooplankters such as rotifers, copepod nauplii and barnacle nauplii. The mesh size of the zooplankton washer was chosen depending upon the length and width of the adult *A. southwelli and A. centrura.* The vigorous rinsing was made continuously for two hours, rotifer contamination was always decreased and the rinsing was done periodically to reduce rotifer numbers. After rinsing, the remaining adult copepods and larger copepodites were used to start the stock culture.

Algal Culture: Algal culture was separately maintained for feeding copepods and the stock culture of *Chlorella marina, Nannochloropsis salina* and Isochrysis *galbana.* The cultures of these species were maintained in controlled conditions in the laboratory. Stock cultures were maintained in 250, 500 and 1000 ml conical flasks containing filtered seawater at 28°C temperature, with 32‰ salinity and 2100 W tube lamps were used to provide artificial illumination and fertilized with Guillard's medium.

Copepod Culture: Stock culture of *A. southwelli and A. centrura* was maintained in a rectangular, flat-bottomed fiberglass tank (0.23m di ameter, 0.4mm length) filled with 25 litre-chlorinated seawater and vigorous aeration was given. The salinity and temperature were maintained in ranged of: 32-37‰ and 28-30°C, respectively. The copepods were cultured and fed with *Chlorella marina*, *Nannochloropsis salina* and *Isochrysis galbana* in the ratio of 2:2:2: (20,000: 20,000: 20,000 cells/ml) respectively.

The stock cultures were harvested at every 14 days by gentle siphoning from the culture tank. Zooplankton was washed and rinsed with seawater for 2hrs from the reservoir. Finally, the adults and late-stage copepodites remaining in the zooplankton washer were used to restart the culture or scale up production producing more live feed for the fish larvae.

Biochemical Composition: A known quantity of sample was taken and the excess moisture was removed using a filter paper [44]. Then the sample was dried in a hot air oven at a constant temperature of 60°C until the wet sample was dried completely. The dried samples were used for estimation of protein [45], carbohydrate [46], lipid [47] and the ash content was determined by burning oven-dried sample in a muffle furnace at 550°C [48].

Fatty Acid Estimation: Fatty acids were saponified and methylated using 2% NaOH in methanol, 14% BF_3/methanol and heptane. The fatty acid methyl esters (FAME) were determined on a Hewlett Packard HP 5890 gas chromatograph equipped with a flame ionization detector. The sample was injected at 190 °C onto a J and W Scientific DB23 fused silica capillary column (30 m x 0.25 mm i.d., 0.25-µm film thicknesses) with hydrogen as the carrier gas. The column was operated isothermally at an oven temperature of 180 °C and a detector temperature of 210 °C. Fatty acids were identified by comparing with authentic standards.

RESULTS

The generation time under optimal conditions is about 12-15 days at 24-26°C. *A. southwelli* having 6 naupliar stages and 6 copepodite stages (including the adult); the newly hatched nauplii (N1) measured 65 × 65 × 95 and the copepodites C6 measured 162 × 175 × 850 µm. Before harvesting the copepods, the biomass and carrying capacity of the population must be calculated. To achieve these three samples of 2 ml was taken daily and the different development stages were counted under a binocular microscope. With these data the required harvest volume was therefore be estimated. N1 can be collected from the culture medium on a 37µm sieve and separated from the other nauplii using a 75µm sieve and the copepodites were concentrated on a 100µm screen.

Population Density of Copepods: Over 15 days operation, the system, *A. southwelli* produced an average of 1,880 nauplii, 1160 copepodid and 626 adults per litre. The maximum density was recorded at 4,185 nauplii, 2,145 copepodid and 1,285 adults per litre. For the entire culture period (2 months), the total production was 55,103 org/l. Of these, 28,206 nauplii, 17,403 copepodid and 9,404 adults per litre were produced and A. *centrura*, produced an average of 1,608 nauplii, 704 copepodids and 461 adults per litre. The maximum density was recorded at 3,547 nauplii, 1,714 copepodids and 1,142 adults per litre. For the entire culture period (2 months), the total production was 41,603 org/l. Of these, 24,123 nauplii, 10,564 copepodids and 6,916 adults per litre were produced.

In the present study, 15 days of culture has been found to be optimal under laboratory condition. The candidate species of *A. southwelli and A. centrura had* a generation period of 12 to 15 days, which depended upon the temperature and the availability of food. Experiment in laboratory culture indicated that a 15 days culture was sufficient for obtaining maximum copepods density. The maximum production of nauplii was attained on 12th day of culture and it was noticed that the nauplii production decreased from the 13 th day onwards. The daily and total production of copepods is shown in Figs. 1-2.

Proximate Composition: In the present study biochemical composition (%) of the calanoid copepod, *Acartia southwelli*, in dry weight basis, biochemical constituents like moisture, protein, lipid,

Table 1: Fatty acids composition of, *A. southwelli* and *A. centrura*[a]

Fatty acid	*Acartia southwelli*	*Acartia centrura*
12:00	0.13	0.14
14:00	6.89	6.79
14:01	0.77	0.87
15:00	1.81	1.84
16:00	12.87	12.91
16:01	4.63	4.67
17:01	6.75	6.77
18:00	12.84	12.93
18:01	1.85	1.87
18:01 n-9	8.54	8.61
18:2 n-6	1.05	1.07
18:3 n-6	-	-
18:3 n-3	8.77	8.79
18:4 n-3	2.86	2.87
20:00	-	-
20:01	1.85	1.88
20:02	-	-
20:2 n-6	1.84	1.85
20:4 n-6	-	-
20:4 n-3	0.75	0.76
20:3 n-3	1.99	2.01
20:5 n-3	10.05	10.07
21:00	0.08	0.11
22:00	0.25	0.27
22:01	0.07	0.08
22:5 n-6	0.34	0.35
22:5 n-3	0.78	0.79
22:6 n-3	9.58	9.61
Saturates[b]	34.87	34.99
Monounsaturates[c]	15.92	16.14
PUFA[d]	38.01	46.78
Total n-3[e]	34.78	34.9
Total n-6[f]	9.23	3.27

-not detected.

[a] Fatty acid values (percentage of total fatty acid methyl esters) were adjusted to express a percent of the total area identified in the chromatograms, unidentified peaks were not considered in the computations.

[b] Saturates: 12:0, 14:0, 15:0; 16:0, 18:0, 20:0, 21:0; 22:0.

[c] Monounsaturates: 14:1, 16:1, 17:1; 18:1, 20:1, 22:1.

[d] PUFA and HUFA: 16:2, 16:3, 16:4, 18:2 n-6, 18:3 n-6, 18:3 n-3, 18:4 n-3, 20:2 n-6, 20: 3 n-6, 20:4 n-6, 20:3 n-3, 20:5 n-3, 22:2, 22:3, 22:4, 22:5 n-3, 22:5 n-6; 22:6 n-3.

[e] Total n-3: 18:3 n-3, 18:4 n-3, 20:3 n-3, 20:5 n-3, 22:5 n-3, 22:6 n-3.

[f] Total n-6: 18:2 n-6, 18:3 n-6, 20:2 n-6, 20:3 n-6, 20:4 n-6.

carbohydrates and ash were (82.94±0.53, 63.85±0.24, 16.92±0.26, 10.56±0.24 and 5.12±0.19) and *Acartia centrura* (82.75±0.60, 63.98±0.69, 17.06±0.62, 10.48±0.55 and 5.36±0.49) respectively.

Fatty Acid Profile: The present study reveals fatty acid profiles of the calanoid copepod, *A. southwelli* (Table 1) is a rich sources of PUFAs, HUFAs and n-3 fatty acids. The total n-3 fatty acid content of *A. southwelli* was 34.78%, Total Saturated fatty acid is 34.87 %, Total monounsaturated fatty acid is 15.92 %, total PUFA and HUFA is 38.01 % and the total n-6 fatty acid is 9.23 % and *A. centrura* was 34.9 %, total Saturated fatty acid was 34.99 %, total monounsaturated fatty acid was 16.14 %, total PUFA and HUFA was 46.78 % and the total n-6 fatty acid was 3.27 %.

DISCUSSION

The nutritional superiority of copepods (for marine fish larvae) to traditional live feed like the rotifers *Brachionus plicatilis* and *Artemia* nauplii is well-established [9, 49,50]. Besides being the natural live pray for marine larvae fish, copepods are a rich source of phospholipids, of essential high unsaturated fatty acid (HUFA) and of normal antioxidants [49].

Copepod nauplii have been shown to be a highly advantageous food source for larval fish and hence the culture copepods and harvest their nauplii [9, 51, 52]. When larval ornamentals first begin to feed, the larvae are small and consumption of a suitable food is critical for healthy development. Copepod nauplii are small enough for first feeding, and they offer a higher nutritional content to ornamental finfish larvae [53]. They have a preponderance of phospholipids rather than

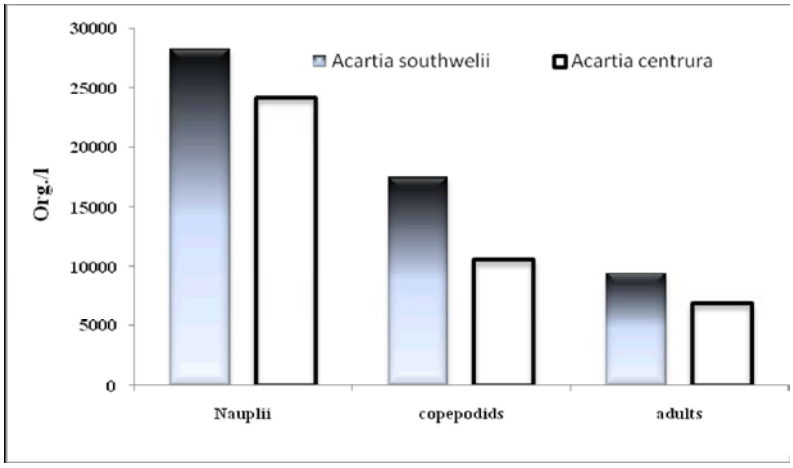

Fig. 1: Daily production of copepod, *A. southwelli* and *A. centrura*

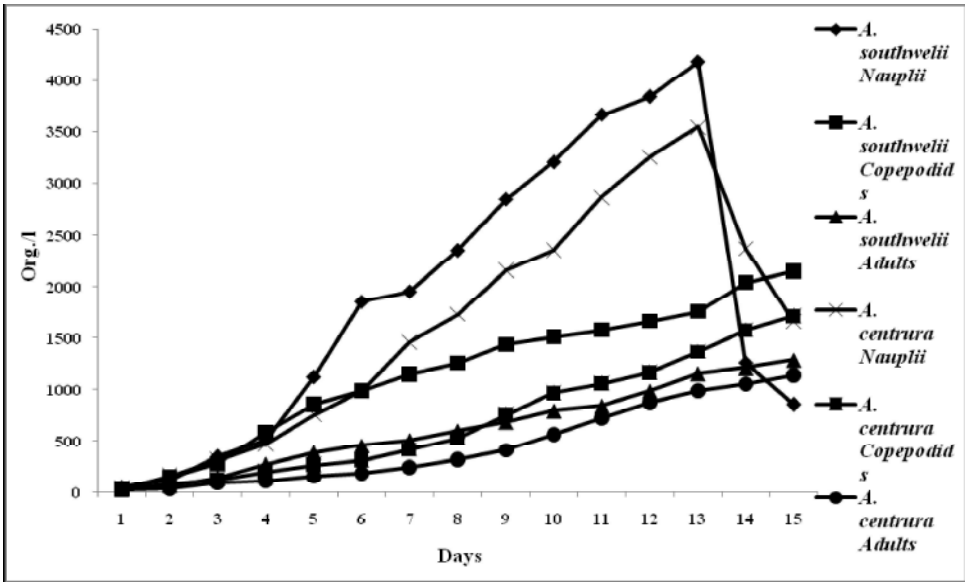

Fig. 2: Total production of copepod, *A. southwelli* and *A. centrura*

triacylglycerols, as well as ratios of fatty acids that more closely approximate the natural diet of marine finfish larvae [2, 54, 55]. *Acartia southwelli* and *A. centrura* has demonstrated high potential as a food source because of their small size. Calanoids that are less then 1 mm frequently produce nauplii that are less then 100 µm in the first and second stages of development. The initial stages of nauplius development are believed to be critical because ornamentals prefer a food size of 50 to 100 µm [53].

The species of the genus, *Acatia* are the most commonly occurring calanoid copepods, especially the *A. southwelli* and *A. centrura* is commonly occurring in estuaries and it has the capacity to grow fast and breed continuously with high reproductive capacity [30]. The adult male and female copepods of the species, *A. southwelli and A. centrura* were stocked in culture tanks and the copulation occurred between pairs and after that the eggs were released and nauplii hatched within 24 hours. The present study is similar to the work of [30]. In the present study the maximum daily production was: *A. southwelli* 4,185 nauplii, 2,145 copepodits and 1,285 adults per litre and *A. centrura* 3,547 nauplii, 1,714 copepodids and 1,142 adults per litre. They reported the maximum densities in the *A. tsuensis* cultured in outdoor tanks, with 1,136 nauplii, 588 copepodid and 280 adults per liter. In the present study is similar to the reports of [11].

In the present experiment, egg production by copepods is influenced by several factors including temperature and food concentration [56, 57]. The growth and biomass increased when the temperature was maintained in a certain limit with the range of thermal tolerance. The biomass of copepod *A. southwelli* and *A. cenrura* increased when the temperature was maintained in the range of 28 to 30°C, as [11] have proved in the case of calanoid copepod. Similar results were also reported by different researchers [7, 58-60]. Salinity acts as prime factor, which seemed to influence the production of copepod. This is an agreement with the earlier works of [5] in *Oithona hebes,* [6] in *Acartia tonsa* and [13] in *Oithona rigida.* Similar observations were earlier reported by [61, 62]. The dissolved oxygen concentration was maintained in the optimum level of 5-6.8 ml/l was found to promote the better growth of *A. southwelli and A.centrura* Aeration may not be needed when animals are reared in a large amount of water or low densities of copepods. Therefore in the present study, the oxygen concentration was kept to near the saturation level. [3] reported a higher biomass at optimum level of dissolved oxygen concentration and observed mass mortality in low O_2 level in the calanoid copepods.

The algal diet is very important for copepod culture as it support the growth and density of copepods. The nutritional value of algal diets maybe evaluated based on ingestion rates and ranked according to growth performance, survival, protein content, fatty acid composition and metamorphosis [63]. Food concentration may have acted as a limiting factor in our cultures. [64] also examined the influence of food concentration on developmental time of *C. finmarchicus*. An increase in food concentrations would also suggest a better balance

in the sex ratio as a low food regime is known to cause a predominance of females [65]. Recent discussions concerning the nutritional value of microalgae as copepod food and the possible deleterious effects of algae and diatom on copepod reproduction have suggested that dinoflagellates or flagellates in the diet, results in higher egg production and survival rates in experiments [66-68]. Copepods feeding on the cryptophyte, *Rhodomonas salina*, which is rich in LCn-3 PUFAs, exhibited high egg production efficiency and naupliar growth rate [69]. The abundance of nauplii, copepodits and adults of *A. southwelli and A. centrura* increased in proportion to the increased food concentration and also combination. In the present study, the successful rearing of copepods, *A. southwelli and A. centrura* has been accomplished by providing mixture of feeds such as *Chlorella marina* and *Nannochloropsis salina* with high concentration, which resulted the higher growth and biomass. Similar results were earlier obtained by [70-73].

Also the haptophyte, *Isochrysis* sp. is a suitable food for all naupliar stages and small copepodid stages, which is rich in the highly unsaturated fatty acid, docosahexaenoic acid (DHA) that has been proven beneficial to the growth, development and survival of marine fish larvae (Watanabe, 1991). High DHA levels may also have a positive effect on the long-term productivity of copepod populations [75]. *Isochrysis* sp. contained higher levels of DHA as compared to EPA. *Isochrysis* gave the best growth response in copepods. *I. galbana* lipid has large amounts of 22:6n-3 and small amounts of 20:5n-3. For fish cultivation, the HUFA content of their diet can be manipulated through the food chain. The live-food organisms used to feed the fish can have their HUFA content increased by feeding them with micro algae containing the desirable HUFA's [76].

For successful aquaculture practices, attempts may also be made to develop intensive culture techniques for other species viz. *Acartia* sp., *Paracalanus* sp., *Eurytemora* sp., *Tigriopus* sp. and *Tisbe* sp. [6, 11, 77-79]. The present culture method detailed here is simple, cost efficient and most importantly, reliable produces large numbers of copepods.

The protein content of copepods showed great variations. These values are comparable with those reported for mixed zooplankton from higher latitudes [16, 80]. In the present estimation, the protein was found to be the major biochemical component in the cultured copepod, *A. southwelli and A. centrura.* It is comparable with the values reported earlier for wild copepods of Laccadive sea [81], Cochin back waters [15], Andaman sea [80], northeastern Arabian Sea [82], northwest Bay of Bengal [83-85] observed the high protein content in copepod, *Calanus finmarchicus*. [86] has reported protein values for the *Euphasia superba*. The protein formed the major fraction compared with lipid and carbohydrate indicating the usefulness as energy reserve [87, 88]. The observed variations in the protein content might be due to the fact that it is utilized as a metabolic substrate [83, 89]. [85] noticed the protein content was positively related with salinity of the water.

In the present observation, the lipid content was slightly higher than that of carbohydrate and lower than that of protein. The continuous supply of phytoplankton would render lipid reserve unnecessary, which might account for the low content. The same opinion was given in the earlier works by [15, 90, 91]. The lipid content of tropical zooplankton when compared to temperate zooplankton is significantly low and it was proved by the findings of [83]. The variations in the lipid content also attributed to its storage and utilization during starved period when it serves as an effective energy reserve. This is proved with information of [89]. The function of protein is an important energy reserve may be true for zooplankton having low lipid content. The present findings are in agreement with the earlier works of [92, 93].

Lipids constitute a significant part of carbon flow through trophic levels [94, 95] and are the major constituents of living organic matter, involved in a variety of cellular function including membrane structure (phospholipids and glycolipids) and energy storage (triacylglycerols and wax esters) [96, 97].

In general, carbohydrate content has been very low in the copepod as compared to protein and lipid. This lower values of carbohydrate of copepods (wild) have been already reported by [80, 84, 89, 93 98]. The low carbohydrate content observed could be due to the fact that glycogen is the useful stored carbohydrate in many marine animals. The utilization of carbohydrate glucose amine during the chitin synthesis in crustaceans may be prone to the decrease of carbohydrate level in the zooplankton.

Long-chain n-3 PUFAs serve important roles in physiological processes [99, 100] and some are considered essential because they cannot be synthesized by organisms other than phytoplankton. However, [101] noted that although EPA is necessary for eicosanoid formation, DHA is likely considered the essential moiety of the n-3 family. In addition to serving as a structural component in cell membranes and the formation of neurological tissue [99], DHA has also been linked to zooplankton growth. [102] reported DHA in seston was strongly correlated with egg production rates in the calanoid copepods *Acartia hudsonica* and *Temora longicornis*.

The copepod, *A. southwelli and A. centrura* is able to produce large amounts of EPA and DHA and it may be due to the desaturating ability of *A. southwelli and A. centrura.* This could be an adaptation to long chain essential fatty acids. Similar trend has been demonstrated for calanoid copepod species, *Acartia* sp. and *Pseudodiaptoms* sp. by [103]. He stated that the quantity and quality of n-3 highly unsaturated fatty acids (n-3 HUFA) is about 2 to 3 times higher than rotifers, as proved by [103]. The fatty acid content of copepod may be influenced by the type of algal species used to feed the copepod [29]. The dietary influence on the lipid production in copepods has also been documented by [104, 105]. The fatty acid content of copepod also influenced by temperature. The PUFA content of copepod was the highest at the minimum temperature of 6° C, when compared to 15 and 20° C [25]. In the present study, Palmitic acid was occupying the highest value in *A. southwelli and A. centrura* as reported earlier by [106-110].

CONCLUSION

During copepod culture, the system produced average of 41,603 org./l. from the 2 months culture period. Among these, nauplii density was 24,123 org./l, copepodids 10,564 org./l and adults 6,916 org./l. The *A. southwelli* and *A. centrura* was found to be a rich source of PUFAs and HUFAs.

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