

Biochemical studies on the live feed polychaete, *Nereis* sp., in relation to maturity stages

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Biochemical composition (protein, lipids, carbohydrates and moisture content) and fatty acid profile of the *Nereis* sp. was analyzed on dry weight basis in relation to maturity stages. Hatchery bred polychaetes (*Nereis* sp.) were procured and segregated into four size groups based on the oocyte diameter as immature (4.0 - 9.0 cm), early maturing (9.1-13.0 cm), late maturing (13.1-16.0 cm) and matured (16.0 cm and above). With the increase in maturity stages/size groups, the oocyte diameter was found to increase in size with a minimum of 9.48 μm (immature) to a maximum of 325.82 μm (matured). The amount of protein was found to be maximum (74.47 $\pm$ 1.08%) in immature group and minimum (49.3 $\pm$ 0.85%) in matured. Percentage of lipid gradually increased from immature to late maturing group but in matured group it was considerably reduced. Carbohydrate composition was almost same in all the groups ranging from 2.16 $\pm$ 0.07 to 2.12 $\pm$ 0.06%. Interestingly, fatty acid profile of *Nereis* sp. revealed significant differences among the various size groups. MUFA (monounsaturated fatty acid) was the most abundant fatty acid constituting about 47% to 53% of the total fatty acid. Saturated fatty acid (SFA) content was found to be increased with the increase in size group. Among PUFAs (polyunsaturated fatty acid), arachidonic acid was the most dominant fatty acid and with the increase in maturity the concentration of PUFA declined abruptly. Present study led to an understanding of why polychaetes and especially *Nereis* sp. is one of the most important food sources in aquaculture sector worldwide.

[Keywords] *Nereis* sp.; Proximate composition; Fatty acid profile; polyunsaturated fatty acids; oocytes]

Introduction

Knowledge on the bio-chemical composition of marine benthic organisms is essential for the assessment of nutritional value and for the evaluation of potential sources of protein, carbohydrate and lipid for commercial use¹. Researchers worldwide evince keen interest in this line in view of importance of its biochemical make up which are important for the aquatic organisms as well as human health and nutrition². Among the various species of polychaetes, *Nereis* sp. (Muller 1776) lives in a U-shaped burrow in close contact with sediments³. It provides key linkages between primary producers and higher trophic levels in the marine food chains⁴. Nereid worms are valued by the industry as an excellent source of polyunsaturated fatty acids (PUFAs), and they have the potential to supplement fish oil as sources of essential lipid components of feeds^{5,6} and hence

they are known as omega worms⁷. Bioturbation caused by the rag worm greatly affects the biogeochemical cycle of both nutrients and contaminants^{8,9}. The quest for *Nereis* sp. are increasing rapidly, predominantly due to its important role in nutrient stimulation, gonad maturation and spawning in hatchery-reared fin and shellfish species. This feeding regime resulted in an increased number of eggs per spawning event for each female and increased egg viability and larval survival in shrimps¹⁰ besides more rapid maturation in cultured prawns^{11,12}. This enhanced increase in reproductive performance is known as '*Nereis* effect'¹³, which is anticipated to be caused by the high amount of PUFA in nereids. As a result, the food regimes containing nereids potentially improve the reproductive fitness of cultured animals. Therefore, polychaetes and especially

nereids are extensively used as a live feed item in aquaculture industry¹⁴.

During the period of sexual immaturity and sufficient food availability, several marine invertebrates, including polychaetes, accumulate lipid stores, which are then exhausted during maturation and/or starvation. Hence, in the physiology of *Nereis* sp. lipid plays a vital role during the periods of poor feeding and negative energy balance, sexual development and breeding¹⁵. During the past decades, the importance of lipids and especially the fatty acids has been realized well as they are of great importance for the food web. Fatty acids can be used as biological markers and diet indicators in marine ecosystems¹⁶. Fatty acids from marine organisms contain 14 to 24 carbon atoms and have varying degrees of unsaturation¹⁷. Therefore, taking cognizance of the viewpoints stated above, the present investigation was carried out with a view to study the variations in oocyte diameter, proximate composition, and fatty acid profile of *Nereis* sp. in relation to maturity stages.

Materials and Methods

Among the various species, *Nereis* sp. (Muller 1776) is an important species in the soft bottom benthic community since it forms an important prey item in the higher trophic level consumers e.g. fish¹⁸. This species shows high physiological tolerance to extreme variations in environmental factors, and can grow and reproduce in different sediment types and in stressed environments^{3,19}. This species is characterized by its elongated body with numerous segments; head with a distinct prostomium and peristomium²⁰.

Set number (10 animals/group) of hatchery bred polychaetes (*Nereis* sp.) were collected and measurements were made based on the length of L3 (length of prostomium plus peristomium and 1st setiger) according to the method described by Gillet^{21,22}. After measurement, polychaetes were divided into four different size groups based on their size namely size group I (4.0 to 9.0 cm); size group II (9.1 to 13.0 cm); size group III (13.1 to 16.0 cm) and size group IV (16.0 cm and above). Further, the polychaetes were cut into pieces and ground well by using tissue homogenizer and centrifuged at 3000 rpm for 30 min. The settled oocytes were then observed under compound microscope (Leica

DFC 290 Germany) and the measurement was done by using the software Leica Application Suite (Version 2.8.1).

The crude extract from the dried polychaetes was prepared using the homogenization buffer (50 mM Tris hydrochloride, 120 mM sodium chloride, 5 mM potassium chloride, 1 mM magnesium chloride and 2 mM calcium chloride). 10 mg of dried polychaetes from each size group were taken and homogenized with 2 ml of homogenization buffer in a manual tissue homogenizer. Mixture was centrifuged at 14,000 rpm for 10 min. at 4°C and the supernatant (considered as crude extract) was retained and stored at -20° C for further use²³.

Total protein content was estimated by following the method of Lowry *et al.*²⁴. Carbohydrate content was estimated by Phenol-sulfuric acid method²⁵ and lipid by following the method of Folch *et al.*²⁶. Moisture content was estimated according to the method proposed by Association of Official Analytical Chemists²⁷.

The preparation of fatty acid methyl esters (FAMES) from polychaete samples were performed according to the method described by Anon *et al.*²⁸ and Sahin *et al.*²⁹. FAMES were analyzed in GC-MS-QP (2010) (Shimadzu). Helium was used as carrier gas. Identification of the FAMES was based on comparison of retention times to those of reference compounds. The amount of individual fatty acids is given as percentage of the total amount of all fatty acids determined.

Results

For each size group, a minimum of fifteen numbers of oocytes were observed and measured. After keen observation of the oocyte diameter of each size group, it was found that the size of the oocyte increased with increase in size of the animal (*i.e.* along with maturity stages) (Table 1 and Fig. 1).

Among the four groups, maximum protein (74.47±1.08%) was recorded in group I and minimum (49.3±0.85%) in group IV (Table 2); lipid content showed the maximum (19.46±0.69%) in group III while the minimum (14.53±1.21%) in group I. There was not much variation in the carbohydrate level among the four groups of *Nereis* sp., even though maximum (2.16±0.07%) carbohydrate content was recorded in group I and minimum (2.12±0.06%) in group IV. Likewise,

maximum ($83.19 \pm 1.27\%$) moisture content was recorded in group I and minimum ($75.36 \pm 1.02\%$) in group IV.

Among the saturated fatty acids (SFA), palmitic acid (C16:0) was found to be the most dominant one, with an occurrence of 17.97%, 17.64%, 24.44% and 21.91% in groups I, II, III and IV respectively (Fig. 2). The other groups of SFA were found in meager level.

With respect to monounsaturated fatty acids (MUFA), C20:1 ω -5 was found to be the dominant with 8.35, 8.37, 8.38 and 9.58% in groups I, II, III and IV respectively, followed by C16:1 ω -7 (11.21, 16.29, 15.01 and 3.72% in groups I, II, III and IV respectively) and C18:1, cis-6 (6.43, 3.23, 6.04, 6.05 in groups I, II, III and IV respectively). As found in SFA, the other groups of MUFA were recorded in lesser amount (Fig. 2). The contribution of vaccenic acid was 3.56%, 3.44%, 3.34% and 5.56% in groups I, II, III and IV respectively.

Among PUFA, arachidonic acid was found to be the most abundant polyunsaturated fatty acid in all the size groups (Fig. 2). The contribution of α -linolenic acid (ALA, C18:3 (ω -3)) was 7.01%, 7.56%, 6.24% and 2.50% in groups I, II, III and IV respectively. Eicosapentaenoic acid (EPA, C20:5 (ω -3)) was found almost in similar proportions in the samples but, interestingly the presence of EPA was not found in size group I. Docosahexaenoic acid (DHA, C22:6 (ω -3)), which is essential for gonad maturation both in fin and shellfishes, was found only in size group I (7.04%), while it was conspicuous by their absence in other groups. Maximum amount of omega-3 fatty acid was found in size group I, which gradually decreased with the progress of maturity stages as shown in Fig. 3. Omega-6 fatty acid showed maximum in size group III (12.39%) and minimum in group IV (10.48%).

Discussion

In the present study, the results revealed that with the progress in maturity stages/size groups, the size of the oocyte diameter was also found to increase. Variations in overall oocyte growth-rates may occur not only among females from a given polychaete population, but also among the oocytes of a single female³⁰. Oocyte recorded presently ranged from a minimum of 9.48 μ m to a maximum of 325.82 μ m, which is in close agreement with the findings of Kupriyanova³¹ who

recorded the similar range in serpulid polychaetes, and in some errant polychaetes^{32,33,34}. The classification made in the present study based on oocyte diameter as immature, early maturing, late maturing and matured is similar to the ones made by Olive *et al.*³⁵. Oocytes of size group I occurred sparsely in the coelomic fluid around the year. This might be attributed to the rapid growth of oocytes at this stage which was noticed earlier in *Nereis virens*³⁶. On the contrary, the number of oocytes was more in size group III as a result of long developmental period that is necessary for oocytes of this stage. This long duration helps the sperms and eggs attain their full maturation simultaneously in both the sexes³³.

With the increase in size and progress of maturation, protein content was found to decrease which is in accordance to the fact that *Nereis* sp. is a semelparous organism and a substantial proportion (>50%) of total energy is being diverted to germinal tissues during maturation although some amount of energy is used to support general metabolism during this phase of life cycle the animals do not feed. Similarly, the lipid content gradually increased from size group I (immature) to size group III (late maturing), but in the size group IV (mature) it abruptly got declined, indicating that these lipid reserves are metabolized during the final stages of sexual maturation. This drop in lipid level at later stages is likely to be associated with the reduction in the foraging activity that is associated with the onset of sexual maturation in this species and the consequent requirement of nutrients by the fasting animal Olive *et al.*³⁴. There was no significant difference in the concentration of carbohydrate in relation to size groups, indicating the non-relatedness of the carbohydrate level with respect to maturity stages.

The requirements of essential fatty acids for both marine fishes and freshwater counterparts have been extensively studied over the last 20 years and are known to vary both qualitatively and quantitatively^{38,39,40}. Among the saturated fatty acids (SFA), the major fatty acid recorded was palmitic acid (C16:0) followed by margaric acid (C17:0) and stearic acid (C18:0). Similar results were reported earlier by Luis and Pass¹². Palmitic acid (16:0) was found at high concentration in all the samples indicating that this saturated fatty acid may be an important item in 'fatty' diets. Not only is that, palmitic acid is the first metabolite of fatty acid synthetase and the initial product of *de novo*

lipogenesis, and a precursor of many kinds of molecules with physiological relevance such as membrane lipids, fats and waxes⁴¹. In oligochaete, *Eisenia fetida*, palmitic acid, octadecanoic acid and oleic acid were identified as major fatty acids with maximum concentrations detected during the sub-mature stage⁴². Concentration of palmitic acid was found abundantly, which is in agreement with the observations made by Garcia *et al.*⁴³. Among PUFAs, arachidonic acid (C20:4 ω -6) was found to be the most dominant in all the size groups. Similar dominance of arachidonic acid was reported in the earthworm, *Lumbricus terrestris*⁴⁴. The concentration of the PUFA was found to have decreased with the increase in size as has been reported by Bonnet *et al.*⁴⁵. High level of PUFA especially C20:4 ω -6 in fish might be due to lower oxygen solubility in warmer waters which can also be correlated with *Nereis* sp⁴⁶. Eicosapentaenoic acid was represented as the next abundant fatty acid in the size groups experimented. It is one of the major components of fish oil, a precursor of prostaglandins and thromboxane. The ω 3/ ω 6 ratio is a better index in comparing relative nutritional value of fish⁴⁷. Ratio of 1:1 is considered to be optimal for nutritional purposes⁴⁸ and in the present study ω 3/ ω 6 ratio decreased gradually from size group I (1.35%) to group IV (0.55%) and only size group I and II showed value higher than 1. Recently, polychaete fatty acids was evaluated as potential inhibitor against human glioblastoma multiforme⁴⁹.

Conclusion

Present investigation was made with the prime objective of estimating the proximate composition and the fatty acid profile of hatchery bred *Nereis* sp. in relation to maturity stages. The results obtained from the present study led to an understanding of why polychaetes and especially *Nereis* sp. is one of the most important live feed sources worldwide and substantiate the immense value in commercial culture of important marine organisms. Further, it is undoubtedly clear that immature and matured groups of (size groups I and II) polychaetes could be much better live feed than the late mature (size group III and IV) ones with respect to biochemical compositions. Presence of highly unsaturated fatty acids such as DHA and EPA greatly enhanced the value of *Nereis* sp. for being a major component of fish or shrimp diets. However, the knowledge of nutritional

requirements of culture fish species is essential to improve the productive development. Therefore, a proper evaluation of the dietary requirement of the culture species as well as proper understanding of the complex interactions between saturated, monounsaturated and polyunsaturated fatty acids quantitatively is need of the hour. Even though the polychaete, *Nereis* sp. signaled its utility and its potential as an excellent live feed, further studies on the structure and function of the microbial community in the gut of polychaete worms is required to know the interactions between polychaete and their gut microbiota, which ultimately influence ecosystem level function such as organic matter decomposition and nutrient cycling.

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