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# Culture Technique, Growth and Comparative Nutritional Analysis of *Perinereis nuntia* in Southeast Region of Bangladesh

**Methila Chowdhury[1], Shanjida Islam Shikha[2], Shahin Miah[3], Md. Faisal[2] and Helena Khatoon[1*]**

[1]Department of Aquaculture, [2]Department of Fishing and Post-Harvest Technology, and [3]Department of Fish Biology and Biotechnology, Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University, Chattogram 4225, Bangladesh.

***Corresponding author:** Helena Khatoon; E-mail: helena@cvasu.ac.bd

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**ABSTRACT**

The culture methods and nutritional analysis of *Perinereis nuntia*, a polychaete species commonly used as feed for carnivorous aquatic animals, including fish and marine shrimp, were thoroughly examined. *Perineiris nuntia* is an endogenous polychaete species of Thailand that is commercially cultured in different hatcheries for rearing shrimp brood stock in Bangladesh. Larvae are reared in a direct saline water supply from the Bay of Bengal in three different stages. The cultivation process includes various stages, including egg collection, hatching, larval rearing, adult nurturing, brood stock management, and harvest. *P. nuntia* exhibited distinct developmental phases (monotrochophore, trochophore, metatrochophore, and nectochaete) each with specific feeding requirements. The polychaetes had segmented bodies with parapodia; the females carried green eggs, while the males carried white sperm. Adults were fed at 200–300 microns, whereas larvae were fed at 5–50 microns. Brood death after gamete discharge was a characteristic of tidal-influenced reproductive activity. It was noted that during fertilization, the male to female sex ratio was maintained at 1:5. The total development of a polychaete required seven to eight months. The culture method yields 3-4 kg of polychaete per square meter area of the culture tank or 77 kg from each culture tank. According to proximate composition study, juvenile polychaetes had higher nutrient content than adults (63.58% protein, 10.03% lipid, 8.97% ash, and 1.49% moisture). The result suggests that the polychaete juveniles of three to four months contain a greater nutritional value or there is a significant difference in protein and lipid content Farmers can short the culture period for supplying feed to the shrimp broodstock to fulfill protein demand. In order to provide useful information for both scientific research and commercial applications, this study was to clarify the viability and difficulties related to *P. nuntia* growing.

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## Introduction

Polychaetes, belonging to the phylum Annelida, are a key component of benthic ecosystems. In marine habitats, they are known for their great diversity, high population density, and vast biomass at different depths. Polychaetes usually live in fresh, brackish, and marine habitats as well as in the coast, estuaries and flooded areas. Alternatives for maintaining polychaete stock have emerged due to the aquaculture industry's desire for these animals (Pombo et al., 2018). Beginning in the United Kingdom and the Netherlands, early polychaete farming spread throughout Europe before continuing to grow globally (Costa et al., 2003). Among benthic organisms, polychaetes constitute a dominating group (Alvarez-Aguilar et al., 2022; Hutchings, 1998). Their each body segment has paddle-shaped appendages that extend from the side, making polychaetes easy to identify. Several species use the small bristles that cover these structures to help move about and create water movements for feeding. This abundance of bristles is what gives polychaetes the term, which is a combination of the Greek words "poly" (many) and "chaeta" (hairs) (Verdonschot, 2015). The majority of polychaetes are less than 100 mm long and have fewer than 100 segments, however some can grow up to 3 meters long and have thousands of segments. The longest specimen ever documented was 6 meters long and was discovered at Port Jackson, Australia. Smaller species usually survive for 12 to 18 months or give birth to several generations each year. They can have ragged bodies with noticeable parapodia and chaetae or cylindrical, earthworm-like morphologies. While tubicolous worms are cylindrical, microscopic species are slender, and parasitic worms can be disc-shaped, organisms that live freely often have a flattened ventral side and a convex dorsal side (Fauchald, 2001).

The marine ecology depends on polychaetes as a food supply for fish, crabs, and shorebirds (Pamungkas and Glasby, 2015). Because of their high nutritional content, particularly for broodstock, polychaetes are becoming more and more popular as fish and crustacean feed. They aid in gonad growth and are a great source of vital fatty acids. Particularly successful are cold water, high-lipid species like as *Glycera* spp. and *Nereis* spp. Europe has a modest but thriving aquaculture business, where species like Nereis virens, Alitta succinea, and A. acutifolia are cultivated in response to the increasing demand for polychaetes as bait in recreational and commercial fishing (e Costa et al., 2003). To evaluate the state of benthic ecosystems in coastal marine habitats, the polychaete *Perinereis nuntia* has been employed as an indicator species (Rhee and Won, 2012). From Thailand, China, and Hong Kong to Australia (north of 27°S) and Fiji, the species is frequently recorded throughout the tropical Indo-Pacific; nevertheless, many of these records require serious re-evaluation (Wilson & Glasby, 1993). The intertidal zone of the Sitakunda coast in Chittagong, Bangladesh, yielded seven species of polychaetous annelids, five of which were new to the nation. Many of the worms in this ecoregion are known to be tolerant of freshwater and they are impacted by the outflows of the Irrawaddy, Ganges, Hooghly and Mahanadi Rivers (Muir and Hossain, 2014).

Shrimp brood stocks are fed live sandworms (polychaetes) to enhance gonadal development (Wouters et al., 2001). This is due to the high levels of hormones and fatty acids found in worms (Meunpolet al., 2007). *Perinereis nuntia* is utilized as live feed in hatcheries throughout Thailand to promote reproductive development prior to mating. According to a nutritional analysis, polychaetes have less fiber than commercial pellets but more protein, fat, and important fatty acids (eicosapentaenoic and arachidonic acids). Growth and survival rates were higher in groups fed polychaetes, according to a four-week feeding trial. In comparison to the pellet-fed animals, they also showed increased spermatophore weight and total sperm count at Weeks 3 and 4, whereas at Week 4, the percentage of defective sperm was lower and the percentage of acrosome reactions was higher (Leelatanawit et al., 2014). When polychaete worms were fed red algae, their levels of fatty acids (SFA) were 35.88 ± 0.01, (MUFA) were 43.82 ± 0.01, and (PUFA) were 108.98 ± 0.01 (mg g-1). *Perinereis nuntia* included a large number of fatty acids, including C20:0, C20:1(n-9), C20:5(n-3) and C22:6(n-3) (Asghari et al., 2017). In recent years, the possible use of polychaetes for aquaculture sludge bioremediation has gained more attention. Numerous polychaete species have shown that they may lower the amount of organic matter and nutrients in various aquaculture wastes (Wang et al., 2019; Angladeet al., 2023). Some polychaete species, including Hediste diversicolor, Abarenicola pusilla, and Perinereis nuntia vallata, have been shown in several studies to increase their lipid content, assimilate nitrogen, and organic carbon when fed particular kinds of aquaculture sludge (Honda and Kikuchi, 2002; Marques et al., 2018; Gomez et al., 2019).

A large-scale harvest from the wild may severely reduce the natural supply of polychaete. *Perinereis nuntia* is endemic to Thailand which can be cultivated by artificial fertilization of eggs and sperm releasing them to sand bed. The polychaetes have had ecological role as they are bottom dweller and contributes to the aquatic food chain (Poltana et al., 2007). Commercial polychaete culture might help to lessen certain ecological problems caused by capturing them from the wild (Fidalgo et al., 2000). Sustainable aquaculture methods, particularly for shrimp, depend on polychaete research to increase production efficiency and expand industrial expertise. Sustainable polychaete aquaculture is made possible by these culture techniques, which can satisfy the demand for live shrimp and other crustacean feed. The goal of this study is to optimize Perinereis nuntia culture methods in southeast Bangladesh. It compares its nutritional profile and assesses growth performance in various scenarios. The research aims to improve sustainable production practices. The results will validate its possible application in the feed and aquaculture sectors.

## Materials and method

### Polychaete culture technique

#### *Water and sand treatment*

Water and sand treatment are most important for the successful production system. Sand of several sizes like small and large stones were collected and mixed. Sand sieving was done to eliminate very small sand from most of the sand to use in the culture of polychaete in the system. Normally, sea earthworm larvae released in high densities, and this will take time to culture for a long time. This would cause the sand to become compacted during farming and cause a $H_2S$ gas condition if we did not remove very small sands, which could cause damage to polychaetes. Sands were initially soaked with much diluted HCl solution and then washed with water to remove impurities. Water supply system includes collection tank, filtration tank, reservoir and overhead tank. In filtration tank, first filter bed was made of large stone and large sand. Where second filter bed was facilitated with small stone and large coal. Finally, third filter was made of small stone, carbon and sand. The size of whole filtration tank was 20ft × 10ft × 14ft. The reservoir tank acted as an intermediate water storage point. The tank had a suitable size of 50ft × 20ft × 12ft. The culture of the polychaete species was accompanied by measurements of a few environmental factors, such as salinity, temperature and pH. A digital portable equipment (HANNA 10pH; pH - ºC Meter) was used to measure the pH and temperature of the water in the field, and a calibrated Beckman Induction Salinometer was used to determine the salinity (Model RS-7C). The salinity range was maintained between 25 to 32 ppt with a pH of 8.3 and temperature between 27-30 °C.

**Table 1.** Tank measurements and parameters of water supply system

| Tank name | Salinity (ppt) | | pH | Temperature (°C) | Length (feet) | Width (feet) | Depth (feet) |
|---|---|---|---|---|---|---|---|
| | Sunny day | Rainy day | | | | | |
| Water collection tank | 32 | 25 | 8.3 | 24-30 | 20 | 10 | 14 |
| Filtration tank | 32 | 25 | 8.3 | 24-30 | 20 | 10 | 14 |
| Reservoir tank | 32 | 25 | 8.3 | 24-30 | 50 | 20 | 12 |

***Collection and rearing of P. nuntia***

Supplying fresh saline water of 35 ppt from the culture tank, sexually matured male and female polychaetes were separated and placed in a box. The sex ratio of males and females was maintained at 5:1. 50 to 75 thousand eggs were collected from each box. Male and female broods had white sperm and green eggs, respectively in their coelomic cavity, which was the easiest way of identifying sexually mature male and female polychaete. Fertilized oocytes were transferred to an indoor closed system equipped with black cylindrical fiber tanks and kept there for two days. Salinity level was carefully controlled at 31-32 ppt and their black color absorbed heat and represented a good hatching condition. Hatching was also dependent on lunar and tidal calendars. Each tank contained around eight lakhs of eggs to become fertilized. Fertilized oocytes were transformed into the nectochaete stage within 48 hours in the tanks. Each egg developmental stages were observed and recorded to understand their transformation. Once the polychaete eggs were hatched and grown to a suitable size, they were harvested using traditional methods and collected the hatched eggs to introduce in the larval rearing tank. This involved sieving by scoop net and placing them in bowls containing water. A minimum of 80-90 thousand hatched eggs were introduced in each tray. Before releasing them, trays were filled with filtered seawater with the correct salinity of 32 ppt, and aeration was provided to match the environment similar to the hatching tank from where they were collected. Metamorphosis of larvae was prominent in this stage with different quantities of feeding supplements till 45 days. Collection of polychaetes from larvae rearing tank was performed after 45 days. Then they were left in the culture tank until sexual maturity which obtained within 6-7 months. There was a total of 48 such tanks of almost 20 square meter each, providing around 70 kg polychates, 3-4 kg per square meter.

**Table 2.** Tank setup and feeding strategies of *P. nuntia*

| Stages | Tank setup | | Feeding | | |
|---|---|---|---|---|---|
| | Tank size | Sediment (inch) | Period | Quantity (g) | Size (micron) |
| larval | 6ft × 2ft × 8inch | 2-3 inches (mixture of small and large stone) | 1-7 days | 2g | 5-50 micron |
| | | | 8-14 days | 12g | mix of 50 and 100 micron |
| | | | 15-45 days | 25g | mix of 100 and 200 micron |
| Adult | 20 $m^2$ | 6-12 inches (mixture of small and large stone) | $1^{st}$ month | 25g | 100-200 micron |
| | | | 2-6 months | 50g | 200-300 micron |

Offering a variety of feeds helped to ensure a well-rounded diet. To enhance their nutritional profile, vitamin-enriched liquid marine supplements (cod liver oil) were incorporated into their diet to maintain their health and growth. These could provide essential vitamins and minerals. Feeding was practiced twice daily in the morning and evening. The feeding regimen was adjusted as needed based on their appetite and the tank's cleanliness. *P. nuntia* had variations in their feeding behavior and dietary preferences depending on environmental factors and reproductive cycles, and their diet was adjusted accordingly.

***Feed formulation technique for P. nuntia***

Fish meal, soybean meal, rice bran, shrimp meal, and clam meal were among the large feed materials that were put into the first mixer to mix them uniformly. The following step involved adding palm oil, cod liver oil, and vitamin B, and after a few minutes, finer materials (wheat, mold, and binders) were added for blending (Table 2). These supplements improved the feed's nutritional content. The produced mixture was placed in a steam cooker following the mixing phases. The mixture's texture was improved by the use of steam cooking. In addition to improving texture and possibly lowering anti-nutritional elements in the foods, cooking also sterilized the food. After cooking, the feed was moved to a hopper and placed in a dehydrator to reduce moisture, which was necessary for pellet formation and stable storage over time. A shifter was used to eventually release the conditioned mixture as pellets, which were then left to cool. To ensure that the meal was the precise micron size required for larval rearing, cooled pellets were ground into finer powders.

**Table 2.** Feed ingredients quantity for polychaete feed formulation as an example

| Ingredients | Amount | Ingredients | Amount |
|---|---|---|---|
| Wheat | 190 kg | Enzyme | 1 kg |
| Soya bean meal | 250 kg | Vitamin (A, D) | 500 ml |
| Fish meal | 250 kg | Vitamin C | 500 g |
| Shrimp meal | 200 kg | D.C.P | 1 kg |
| Rape seed | 100 kg | Mold | 2 kg |
| Clam meal | 10 kg | Binder | 6 kg |
| Fish oil | 10 kg | | |

***Embryonic and larval development***

The development of polychaetes was seen under a light microscope at predetermined intervals: the first cleavage took place in 1-2 hours, the second cleavage took place in 3-4 hours, and there were further phases at 24, 30, 36, and 48 hours. Size and length of polychaetes were measured as morphometric characteristics are important for knowing the growth rate and life cycle of polychaetes.

***Proximate analysis***

Standard procedures were used to determine the polychaete samples' approximate composition (AOAC, 2005). The Kjeldahl machine (DK 20/26, Italy) was used to determine the protein content of 0.3g of the sample. Using a Soxhlet device (FOOD ALYTRD40, Germany), the lipid content was measured, where solvent was used to extract fats from 2g of the sample. Using a muffle furnace (Nabertherm-L9/13/13, Germany) set to 550°C for six hours to extract residues, 3g of each sample was added to crucibles to quantify the amount of ash present. To find the moisture content, 3g of each sample was dried overnight at 105°C in a hot air oven (BINDER-ED115, Germany).

**Figure 1.** Culture technique of *P. nuntia.* a. sediment treatment, b. collection of egg from broods, c. hatching tank setup, d. tank preparation for eggs, e. collection of nectochaete from hatching tank, f. larval rearing tank setup, g. formulated polychaete feed, h. culture tank setup for adult polychaete, i. polychaete harvesting by sediment shifting, j. harvested polychaete

### Statistical analysis

Data analysis of proximate composition was done by MS Excel software, and bar chart was created.

## Results

### Embryonic development

The embryonic development of polychaete began with the first cleavage, which occurred inside the protective vitelline membrane within 1-2 hours. Three to four hours later, the embryo was further split and formed by the second cleavage. After 24 hours, a significant result was obtained when the embryo developed a ring of cilia that allowed for active movement, like a monotrochophore. The polychaete's trunk started to elongate at 30 hours, marking the embryo's entry into the trochophore stage. During this crucial phase of growth, three pairs of chaetigerous sacs emerged. After thirty-six hours, the trochophore transformed into the metatrochophore, which was more mobile and could swim freely in the water. The arrival at the nectochaete stage at 48 hours was indicated by the growth of three distinct chaetigerous segments, a crucial stage toward maturity. About 50% of the fertilized eggs reached this stage, indicating the effectiveness of the culture environment and pointing to promising possibilities for polychaetes' continued development.

During their developmental stage, the larvae transformed and began to resemble young worms. To promote burrowing behavior, a suitable substrate was supplied in the rearing container. As transformation began, the larvae's ability to swim progressively deteriorated. They changed morphologically to become more benthic. There have been noticeable morphological alterations throughout the metamorphosis. The distinctive chaetae or bristles on the larvae's body segments, which were essential for mobility and burrowing in the substrate, started to form. In the culture tank, the settled larvae which were subsequently regarded as juvenile worms kept developing and maturing. They continued to differentiate their organs and developed more body segments over time.

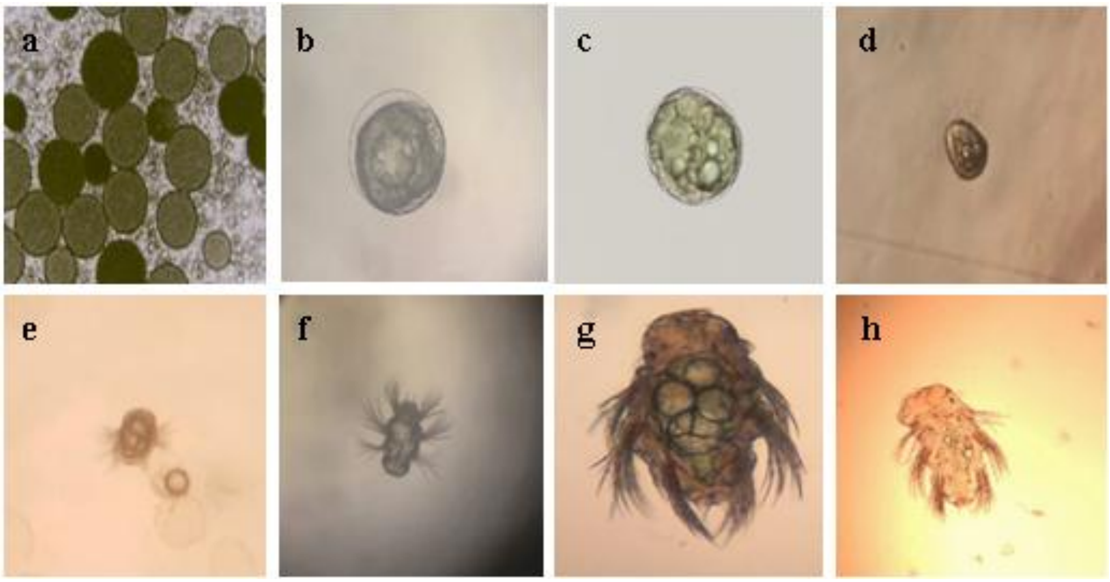


**Figure 2.** Developmental stages of *P. nuntia* polychaete. a. maturing egg, b. fertilized egg before cell cleavage, c. 8- to 64-cell embryo, d. at 24 hours, d. the monotrochophore displayed an equatorial ring of cilia (arrows), e. trochophore displaying three sets of arrows like chaetigerous sacs, f. Metatrochophore, g. Nectochaete, h. 3-segmented juvenile

**Larval development**

Long individuals with minimal variation in length across developmental stages were the defining feature of this species' population. The worms had length from 6.5- 12 cm, with an average of 9 cm estimated from 10 samples. Body length and weight were measured at different stages of growth; the weight ranged from 0.31 g to 1.66 g. Body weight was measured using an analytical balance to track development and survival rates, and information about each individual was recorded at specific times.

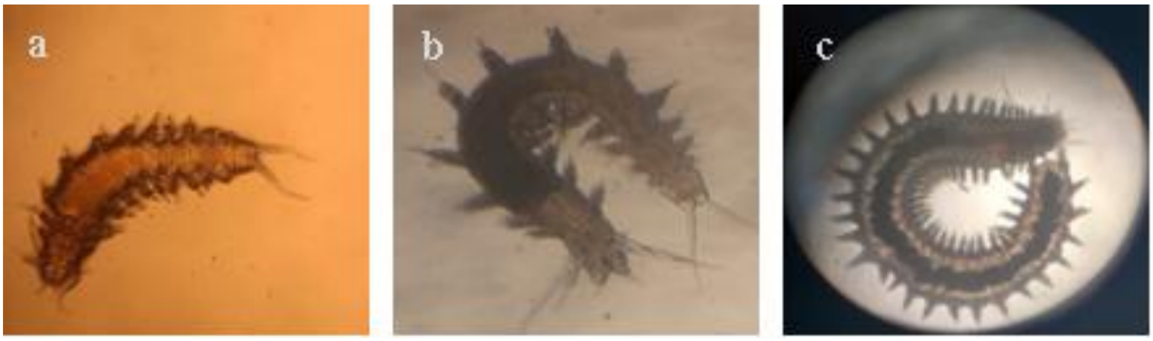


**Figure 3.** Larval development of polychaete. a. 30 days old polychaete, b. 45 days old polychaete, c. 60 days old polychaete

***Proximate composition***

The results of the protein analyses showed that 63.73% of the dry weight was composed of protein in the juvenile, and the adult polychaete was composed of 59.60%. The mean moisture content of dry juvenile (3 to 4 months old) *Perinereis nuntia* is 1.47%. The moisture content of juveniles on a wet weight basis is 79.96% of 25 samples. The mean moisture content of adult polychaete was 1.58%, whereas on a wet weight basis, the moisture content of adult polychaete is 75.02% of 25 samples. The ash percentage of juvenile *Perinereis nuntia* was 8.95% and adult polychaete was 7.94%. The lipid content of juvenile polychaete was 10.03%, and the lipid content of adult Polychaete was 13.54%. Finally, the carbohydrate content on the dry wet basis in juvenile and adult polychaete is 15.58% and 17.34 %, respectively

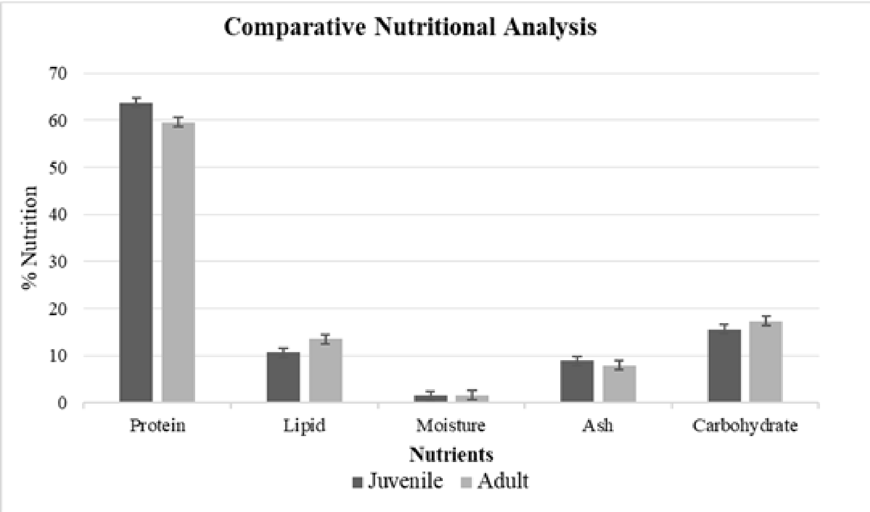


**Figure 4.** Comparative proximate analysis of juvenile and adult polychaetes

## Discussion

**Embryonic development**

The culture method described in this article may be one of the culture methods that could be adopted for the large-scale production of this polychaete species. This method needs some modification according to the cultural area. The survival rate was higher, but with the flow of aeration. Mortality occurred due to lower oxygen in the fertilized egg. Mortality was observed in sexually mature eggs after releasing both egg and sperm. The findings of this article also agree that both male and female polychaetes mature sexually at the same time, which is a requirement for mating success. Further research is required for egg preservation and transportation methods. The cellular structure and developmental stages described here are is to the previous study (Poltana et al., 2007). After embryonic development, larval rearing ensured the appropriate growth of polychaete adults. The number of eggs found in this study ranged from 50,000 to 75,000 per female, which was higher than the maximum of 52,426 eggs per female found by Hamdy et al. (2020) and significantly higher than the 30000 eggs per female observed by Zheng (2002). However, it was lower than the 393245 eggs per female recorded by Darya et al. (2016).

**Larval development**

*P. nuntia* showed a size range of not less than 6.5 cm and weight was greater than 0.3g showing similarity with the results of Hamdy et al. (2020) where *P. nuntia* lengths varied from 5.4 to 9.5 cm for males, 5.2 to 19.9 cm for females, and 3.4 to 16.2 cm for immature worms. Males range in weight from 0.13 to 0.448 g, females range from 0.11 to 2.141 g, and immature forms range from 0.031 to 1.582 g. While other species of polychaete, in the case of *Platynereis dumerilii*, the immature stages of this worm ranged in length from 1.05 to 5.0 cm, females ranged from 2.1 to 6.5 cm, and males ranged from 3.1 to 3.5 cm. The weight ranges from 0.028 to 0.268 g for the female, 0.038 to 0.201 g for the male, and 0.003 to 0.138 g for the immature forms. According to Magurran et al. (2013), the body size of a species may be a reflection of its evolutionary history and the ecological conditions surrounding it, whereas Dorgham et al. (2014) found that it is influenced by changes in pH and salinity.

Commercial polychaete feed was prepared using different ingredients to supply nutrients in different stages of its life cycle considering nutrient demand of body which also supported by a finding interpreting that different life stages of polychaetes require different kinds of feed and nutrients and more research should be done on the feed suitability for each stage of life, from the larval to the juvenile to the adult phase (Amran and Mohamad, 2022). Powdered feed was applied for the culture to provide easy access to the polychaetes under the sediments with flowing water which was very important as it is important to choose manufactured commercial feeds wisely based on the feeding ecology of polychaetes, whether they are extruded (floating feeds) or pressure-pelleted (sinking feeds). Sedentary polychaetes stay still and filter-feed at the bottom of the water column, on or in the sediment, whereas errant polychaetes float freely and feed on the surface and within the water column (Pardo, 2004). Among different feed ingredients in feed formulation, fish meal and fish oil were fish-derived which were highly nutritious indicating by a study that high-quality protein, critical minerals including phosphate, vitamins B, and vital fatty acids, and a high energy level are all found in fishmeal. Highly digestible fish lipids found in fish oil supply polyunsaturated fatty acids (PUFA) of the omega-3 and omega-6 fatty acids, which are essential for the nervous system's development in larvae and for the immune system's defense against pathogens (Miles & Chapman, 2006).

**Proximate composition**

For ensuring food security there is thus increasing interest in non-traditional species with potential food value. The present work constitutes a preliminary step as it describes the biochemical composition of the polychaete. *Perinereis nuntia.* It is noteworthy that juvenile *Perinereis nuntia* was recorded as having a mean gross protein content of 64% protein percentage is higher in juveniles than in adults. This finding is like the study of protein in the same species from Thailand (Meunpol et al., 2005). Protein is generally the most expensive basal component of fish feed and finding other protein sources than fish meal stemming from capture fisheries has been in the limelight of research for some years (Devic et al., 2018). Juvenile Polychaetes are actively growing and developing their bodies and reproductive organs. Protein is a critical component of their tissue growth, and they require more protein to support their rapid development. Lipid variation is mainly related to gamete development, with the highest levels of lipids seen during the period when the gonads are ripe (Stabili et al., 2013). My results findings also support, and so total lipid is highest in adult Polychaete.

This polychaete species has a balanced nutritional profile for rearing shrimp broodstock and the described culture method is easy to perform. Since crustaceans are their primary source of nutrition and have a diet that is acceptable for a balanced diet of nutrients, it is possible to determine whether they are suitable for live feed by looking at their nutrient profile. The nutrient value of the polychaete varies, though, depending on its diet, environment, harvest season, and life phases. In practice, worms are integrated into fish artificial feed and utilized as live feed (Salze et al., 2010). The use of live feed contributes to the aquatic species' increased growth and well-being (Cruz-Suárez et al., 2010). Polychaetes typically had higher protein content than other commercial feeds, such as soy bean and wheat bran, when compared to other commercial feeds based on their biochemical composition. In contrast, the minimum lipid content for artificial feed is just 4-5% (Selvam, 2021). Particularly in nereidid species, Dorgham et al. (2015) found that the lipid content of polychaetes varies with their ecological stress and maturation period, while the lipid content of polychaetes employed as live feed requirements is 5% or higher (Meena et al., 2013). It was reported by Balasubramanian et al. (2012) that the physicochemical parameters influence the nutritional content.

## Conclusion

Considering the results achieved from current study that *P. nuntia* has a high nutrient content, suggesting that it could be a viable substitute for fish meal or fish oil in aquaculture, thus lowering the negative environmental effects of aquaculture feed. *P. nuntia* grows during a period of seven to eight months. A well-balanced diet is seen in juvenile polychaetes. The youngsters were between three and four months of age. This is encouraging since it will shorten the culture period and provide wholesome feed for the management of shrimp broodstock. For shrimp farmers in Bangladesh, the culturing procedure is very simple. To determine if *P. nuntia* can be employed as a sole ingredient or as an additional ingredient in aquaculture feed formulation, further research and higher studies are required. The favorable growth rates and excellent survival rates suggest that *P. nuntia* can endure in the cultural sector. The findings suggest that by making better use of the world's finite supply of feed, polychaete farming could improve fish aquaculture's sustainability.

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## Conflict of Interest

Authors declare no competing interests.

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