ILMU KELAUTAN: Indonesian Journal of Marine Sciences *September 2019 Vol 24(3):113-120* ISSN 0853-7291

Metabolism and Nutritional Content of Polychaeta *Nereis* sp. with Maintenance Salinity and Different Types of Feed

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Abstract

Nereis sp. is an invertebrate member of Familia Nereidae, Classis Polychaeta that lives in the estuarin ecosystem as benthic. Nereis sp. is one of the natural foods that can trigger the maturation of shrimp gamete cells up to 70% because they contain high levels of amino acids and unsaturated fatty acids, but the fulfillment of Nereis sp. still rely from nature. That condition encourages the cultivation of Nereis sp., but there is not much information about it. This study aims to determine the metabolic rate and nutritional content of Nereis sp. with different maintenance salinity and feed. This reasearch used immature Nereis sp with two different type of feed, i.e. with vegetable protein and animal protein. They were maintained in three different salinity i.e. 5, 15, and 25 ppt. The results showed that oxygen consumption rate of Nereis sp was affected by salinity of the medium, but was not influenced by the type of feed given. The highest oxygen consumption was observed in Nereis sp. that maintained at 25 ppt. The body protein content is influenced by the salinity and the type of feed given, while the fat content is not affected by the salinity and the feed. The highest protein, fat and carbohydrate content of the body was detected in salinity of 15 ppt and fed with vegetable protein foods

Keywords: *feed, oxygen consumption, body protein, body fat*

Introduction

Polychaeta can be found in areas with sediments that contain high organic matter, but some of them live with tubes attached to mangrove roots (Beesley *et al.*, 2000). Polychaeta is also found in deep seas such as the Straits of Flores, Lamakera, and Alor (Widianwari and Widianingsih, 2011). *Nereis* sp. is one of Polychaeta members, Familia Nereididae, which live in the Estuarin ecosystem, living as benthic but also actively swimming in the waters when reproduction (Wallace *et al.,* 1991). According to Wibowo *et al*. (2017), *Nereis* sp. from the Jeruklegi Cilacap region was found in the salinity of of 9-30 ppt. Further According to Yuwono *et al*. (2002) *Nereis* worms in nature able to live on salinity 14–30 ppt, and have a survival and growth rate high at 15 ppt.

Nereis sp. can digest the remains of plants and animals by swallowing sedimentary surfaces in the form of organic matter resulting from degradation of aerobic and anaerobic microbial processes in the form of proteins, cellulose, and lignin (Kristensen, 2001). Nereis worms fed zooplankton show faster growth than phytoplankton, due to the better nutritional value of zooplankton. Zooplankton contains animal protein which is more easily digest, thus not much energy is needed for digestion and metabolism (Yuwono, 2003).

Polychaeta includes *Nereis* sp. can be used to feed shrimp because it contains amino acids and high unsaturated fatty acids, which are needed to improve the quality of gamete cells in the mother shrimp (Yuwono *et al*., 2002). Polycheta *Nereis* sp. that found in the aquaculture area of Jeruklegi village, Cilacap district has a protein content of 42.06-51.68%, fat 12.93-22% (Wibowo *et al*., 2017). According to Yuwono (2005) *Nereis* sp. given as feed, both in fresh and in the form of pellets increase the growth and survival of shrimp and fish.

The availability of natural food in aquaculture is very important. Therefore Nereis worm cultivation is necessary to provide natural food and to reduce dependence on nature. Polychaeta worms have the potential to substitute fishmeal as raw material for shrimp feed (Rachmad and Yuwono, 2000; Yuwono *et al.,* 2002), so it is necessary to conduct a study of metabolism and nutritional content to support sustainable *Nereis* sp. worm cultivation. This study provides information on environmental conditions (maintenance media salinity) and suitable feed to support *Nereis* sp. Therefore, to produce optimum growth and reproduction and produce the body's nutrients that suitable to meet gonad maturation

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ijms.undip.ac.id
DOI: 10.14710/ik.ijms.24.3.113-120
Received : 10-12-2018
Accepted : 18-03-2019

needs of the parent fish and shrimp to produce fish and shrimp seeds optimally.

Materials and Methods

Materials used were substrate of Jeruklegi Cilacap farming, sea water, fresh water, ornamental fish food (contain 39.53% vegetable protein and 31.93% animal protein), alcohol 75% and ice cubes. Other materials were catalyst (boric acid 3%, NaOH 40 %, phenolphthalein 1%, HCl 0.1N, fat solvent I (chloroform and methanol with the ratio of 2:1), fat solvent II (chloroform, aquadest and methanol with a ratio of 47:48:3), 0.88% NaCl, 1.25% sulfuric acid 3.25% sodium hydroxide, dilute sulfuric acid, sodium hydroxide, methanol, 20% boron trifluoride solution, saturated sodium chloride, 85% phosphoric acid, standard ferrous sulfate solution 1.0 N, indicator of barium diphenylamine sulfonate 0.16%, $MnSO_4$ solution, KOHKI solution, concentrated H_2SO_4 solution, 0.025 N $Na_2S_2O_3$ solution, starch indicator, and distilled water.

Experimental design

The study was carried out experimentally using a randomized block design (RBD) method with treatment: S5PN= *Nereis* sp. with a medium of maintenance salinity of 5 ppt and feed with the main content of vegetable protein (39,53%). S5PH= *Nereis* sp. with a medium of maintenance salinity of 5 ppt and feed with the main content of animal protein (31,93%). S15PN= *Nereis* sp. with a medium of maintenance salinity of 15 ppt with the main content of vegetable protein (39,53%). S15PH= *Nereis* sp. with a medium of maintenance salinity of 15 ppt and feed with the main content of animal protein (31,93%). S25PN= *Nereis* sp. with media of maintenance salinity of 25 ppt and feed with the main content of vegetable protein (39,53%). S25PH= *Nereis* sp. with media of maintenance salinity of 25 ppt and feed with the main content of animal protein (31,93%). Each treatment provided with three units as replications.

Preparation of culture media, the substrate was taken from aquaculture area, then were dried for 2x24 h. Sludge inserted in a trial container (20x30 cm) with a thickness of 5 cm. Each treatment container is filled with a substrate with water salinity of maintenance media 5, 15 and 25 ppt with a water level of 15 cm with a water level of 15 cm, and aerated them for 1 (one) week.

Nereis sp. (2.27-3.57 g) were taken from ponds in Jeruklegi Village, Cilacap, Central Java. Samples used were immature Nereis sp (the growth period when male and female morphology cannot be distinguished, namely brownish red). Ten worms were placed in a container after acclimation for two weeks. Initially, the worms were weighted and the number of segments was calculated. The study was carried out for two months, and each treatment was given once a week by ad libitum

Metabolism (oxygen consumption)

Observation of oxygen consumption parameters was carried out at the beginning and the end of the study. Oxygen consumption was measured by respirometer using the Winkler model Fry (1971) method *in* Brougher *et al.* (2005). The formula calculates initial dissolved oxygen according to APHA (2005). Oxygen consumption ($mg.g^{-1}.hour^{1}$) of worm measured using Fidhiany method (1999). Measurement of oxygen consumption is carried out at a water temperature of 25^{o}C.

The composition *Nereis* sp. was measured in wet conditions weighted with an accuracy of 0.0001 g after it dried in an oven at a temperature of 80^{o}C to dry (constant weight). After drying, the sample was mashed and stored at room temperature to further measurement of composition of the macromolecule (Sudarmadji *et al*., 1998). Data were statistically analyzed by two-way (factorial) variance analysis followed by Tukeys test for the smallest significant difference. The analysis was carried out using the MINITAB 16 software program.

Results and Discussion

Metabolism

The results showed that salinity had significant effect on the rate of oxygen consumption. While the feed and, interaction between salinity and feed did not influence the rate of oxygen consumption. See Table 1 and Figure 2. According to Schmidt-Nielsen (1990), oxygen consumption is an important part of bioenergetic balance because it describes direct energy use in metabolic work including metabolism for the main needs of life, eating, and activity. Furthermore, bioenergetic models can be used to predict growth potential in different environmental conditions (Karim, 2007). These results confirm that the metabolic work of *Nereis* sp. influenced by environmental salinity, in this case, the related with the energy needs to do osmoregulation in maintaining ionic homeostasis in the body.

The results showed that oxygen consumption of *Nereis* sp. higher at 25 ppt salinity, whereas at 5 and 15 ppt salinity shows a low level of oxygen consumption. This phenomenon occurs due to the osmoregulation process which is influenced by the salinity of the media, thus influencing the level of energy use in metabolism. Salinity is related to the osmotic pressure of water, the further difference in the osmotic pressure between the body and the environment, the more metabolic energy is needed to do osmoregulation as an adaptation effort (Fujaya 2004). If there are a sudden change of salinity in large range, it will need a complex osmoregulation arrangements for aquatic animals in their body that not an easy way to do (Rachmawati *et al*., 2012). It requires energy derived from burning protein, fat and carbohydrates so that it can reduce the amount of energy, reduce the rate of growth and cause death. These results confirm that *Nereis* sp. requires relatively smaller energy when living in media salinity 5-15 ppt, compared to if it lives at 25 ppt salinity. This condition indicates that the salinity of 5-15 ppt is the optimum salinity to support the growth of *Nereis* sp. This result is by Detwiler *et al.* (2002) which shows that *N. succinea* worms have been able to adapt to the increase in salinity, but cannot stand if the salinity exceeds the osmoregulation limit.

Oxygen consumption of *Nereis* sp. who given different feeds showed that the administration of plant-based protein feed had a relatively slightly higher oxygen consumption/metabolic rate compared to those fed animal feed. This relatively higher oxygen consumption is one of the features of the body's high metabolic processes in digesting vegetable protein food because it has more fiber than animal protein food. According to Darmadi *et al.* (2003), metabolic activities of animals cannot be separate from food consumed as an energy source. Costa *et al.* (2006), showed that animal food can be absorbed more quickly than vegetable materials that are rich in fiber. Furthermore, according to Ranjhan (1993) in Darmadi *et al*. (2003), fibrous foods will cause increased energy needed in the digestive process, so that energy that can be used to increase

Table 1. Oxygen consumption rate ($mg.g^{-1}.hour^{-1}$) of *Nereis* sp. which maintained with different salinity and feed

No.	Treatment	Range	Average
1	S5PN	0,03051-0,04576	0,03559±0,0088[a]
2	S5PH	0,01834-0,05501	0,03668±0,0183[a]
3	S15PN	0,03051-0,04576	0,04657±0,0081[a]
4	S15PH	0,01720-0,03440	0,02293±0,0099[a]
5	S25PN	0,15394-0,17593	0,16860±0,0127[b]
6	S25PH	0,12367-0,18550	0,15802±0,0315[b]

Note: Numbers followed by different letters in the same column show significant differences between treatments (*P*<0.05).

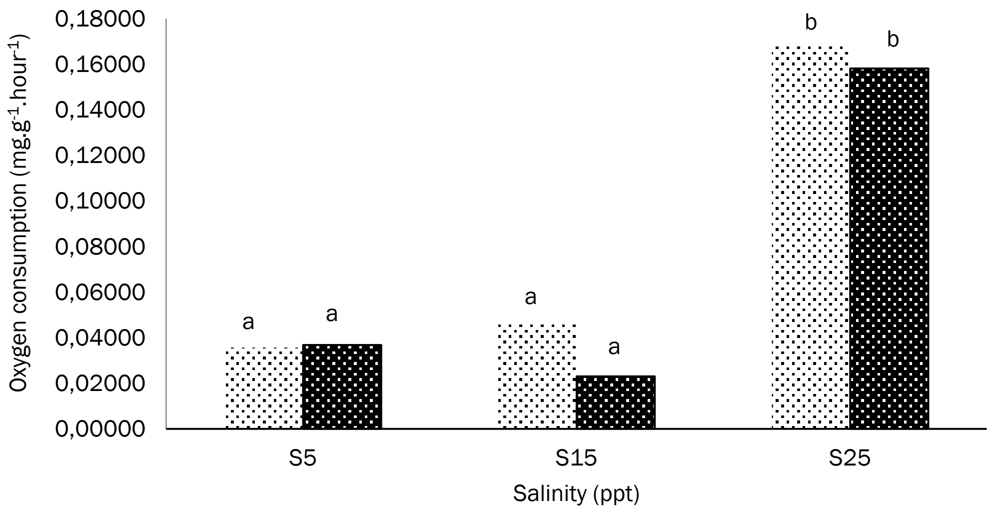

Figure 2. Oxygen consumption rate (± SD) *Nereis* sp. which maintained with different salinity and feed
Note : ▫ = vegetable feed, ▪ = animal feed. Different letters on the bar chart show significant differences between treatments (*P*<0.05).

body tissue released for the process of digesting fibrous feed.

Metabolic activities that are increasingly high in cells of an animal according to Hochachka (1991) cause their oxygen demand also to increase which is followed by an increase in oxygen consumption by these animals. According to Cook *et al.* (2000); Zimmermann and Kunzmann (2001), oxygen consumption will be influenced by changes in digestibility towards feed. Oxygen consumption also influenced by several factors, including age, reproductive status, feed in the intestine, physiological stress, activity, season or environmental temperature and body size (Yuwono, 2008). The rate of metabolism animal poikiloterm depends on body size, activity, temperature and some other factors such as nutrient status, salinity, hormones, race (Hurkat and Mathur, 1976). According to Suadicani *et al.* (1991), oxygen consumption influenced by internal factors such as type, size, reproductive status, and daily activities, besides that, it is also influenced by external factors, such as temperature, salinity and dissolved gases content.

Body chemical content

Results of proximate analysis of the body of *Nereis* sp. at the end of the study obtained the nutritional content of the highest to lowest protein in the treatment of S15PN, S25PH, S5PH, S15PH, S5PN and S25PN, while the highest fat content in the treatment was S15PN, S15PH, S5PH, S5PN, S25PN and S25PH the lowest. See Table 2.

The average protein content of all treatments ranged from 60.88-66.53%. This result is greater than the protein content at Nereis sp. from Randusanga Brebes (Rachmad and Yuwono, 2000), namely 52.26%, while Polycheta *Nereis* sp. from nature in the Jeruklegi village aquaculture area, Cilacap district has a protein content of 42.06-51.68%, (Wibowo *et al.,* 2017). The results of Wu *et al.* (1985), the protein content of *N. diversicolor* 61.44%, while *N. virens* 63.91%. Dorgham *et al.* (2015), reported the protein content of the Nereid body *Perinereis cultrifera* from Mediterranean coast of Egypt affected by the season, which the highest in winter (59.9%) and slightly lower in other seasons (spring: 55.1 %, summer: 55.6%, autumn: 57.3%).

The results of body protein content at the end of the experiment showed that salinity of the maintenance media and feed type affected the body's protein content (P <0.05) (Figure 3). The highest body protein content was obtained at maintenance with 15 ppt media salinity and vegetable protein feed (66.53%), while the lowest protein content obtained at salinity 25 ppt and vegetable protein feed (60.88%). These results indicate that salinity affects the conversion of feed proteins given to body proteins. The optimum salinity for *Nereis* sp. sustainability was found on 15 ppt salinity that make the energy used for osmoregulation process become lower and protein obtained from feed are converted into body protein. This result was confirmed by high metabolism at 25 ppt salinity. Salinity is related to the osmotic pressure of water, the higher difference in osmotic pressure between the body and environment, the more metabolic energy needed to do osmoregulation as an adaptation effort (Fujaya, 2004).

The body protein content in all treatments was relatively higher when compared to the feed protein content given (39.53% vegetable protein feed and 31.74% animal protein feed). The nutritional composition of feed strongly influences the protein and body fat content; these results indicate that *Nereis* sp. able to convert the given feed protein into body protein. According to Lovell (1989), protein on feed will be absorbed and used to build or repair damaged body cells and very efficient for energy. According to Buwono (2000), if the protein content in the feed is too high, only part of it is absorbed (retention) and used to form or repair damaged body cells, while the rest of it wil converted into energy.

Table 2. The chemical content of the body (%) of *Nereis* sp. which maintained with different salinity and feed.

Sample	Water %	Dry Weight (%)	Crude Fibre (%)				
			Protein	Fat	Fiber	Ash	Non-Nitrogen Extract Matter
S5PN	16,49	83,51	62,34	7,70	0,53	6,88	22,55
S5PH	9,96	90,04	63,36	8,48	0,48	8,73	18,94
S15 PN	13,46	86,54	66,53	11,04	0,29	7,71	14,43
S15PH	14,96	85,04	63,31	10,06	0,19	10,42	16,02
S25PN	13,07	86,93	60,88	7,19	0,47	13,72	17,75
S25PH	14,85	85,15	65,18	6,67	0,59	12,53	15,03

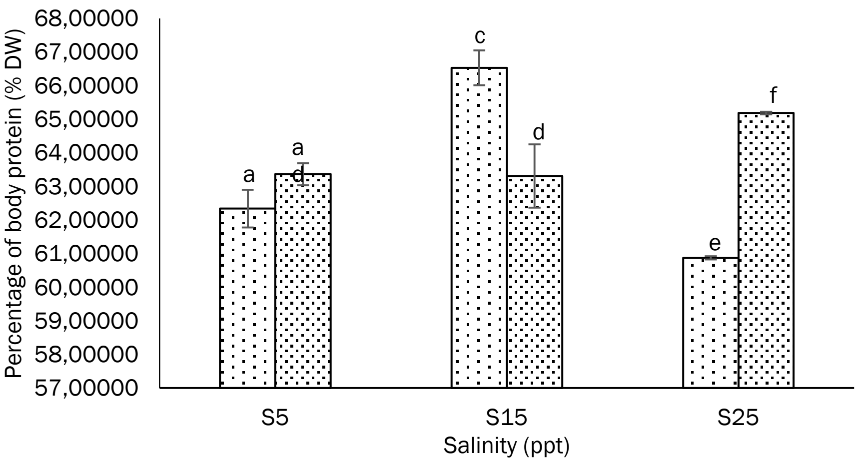

Figure 3. Average of body protein content (± SD) *Nereis* sp. which maintained with different salinity and feed
Note: ⬚ = vegetable feed, ▦ = animal feed. Different letters on the bar chart show significant differences between treatments (P <0.05).

The body protein content in all treatments was relatively higher when compared to the feed protein content given (39.53% vegetable protein feed and 31.74% animal protein feed). The nutritional composition of feed strongly influences the protein and body fat content; these results indicate that *Nereis* sp. able to convert the given feed protein into body protein. According to Lovell (1989), protein on feed will be absorbed and used to build or repair damaged body cells and very efficient for energy. According to Buwono (2000), if the protein content in the feed is too high, only part of it is absorbed (retention) and used to form or repair damaged body cells, while the rest of it wil converted into energy.

The average fat content of all treatments ranged from 6.67-11.06%. This result is lower than the fat content of *Nereis* sp. from Randusanga Brebes which was obtained by Rachmad and Yuwono (2000), which was 29.83%. Polycheta *Nereis* sp. that live in the Jeruklegi village aquaculture area, Cilacap district has a fat content of 12.93-22% (Wibowo *et al*., 2017). The results of Wu *et al*. (1985), fat from *N. diversicolor* 15.16%, while *N. virens* 7.0%. The study Dorgham *et al.* (2015), shows that the total lipids of Nereid *Perinereis cultrifera* on the Mediterranean coast of Egypt are varies according to the seasons ranging from 11.6-13.4%, the lowest value discovered in winter and the highest total lipid discovered in summer.

The results of body fat content at the end of the experiment influenced by salinity and type of feed (P <0.05) (Figure 4). the highest body fat content of Nereis sp was obtained at 15 ppt of salinity and fed by vegetable protein (11,04%), while the lowest fat content obtained at 25 ppt of salinity and different type of feed. Salinity in this case, affect energy use for osmoregulation process and make the retention of fat is reduced because it used for metabolism.

The results also showed that the nutritional composition of feed strongly influenced body fat content. According to Watanabe (1988), fat as an energy source in feed can help the effectiveness of using feed proteins, fat acts as a protein sparring effect. According to Haryati *et al.* (2010), fat is a source of energy, phospholipids, and essential fatty acids. The higher fat in feed means the higher contribution of essential fatty acids. These results are obtained by Luis and Passos (1995), which shows that the composition of food is a determining factor in determining the composition of fatty acids *N. diversicolor*.

Carbohydrate content at the end of the experiment ranged between 14.43-22.55%. This result is higher than the Abida (2012) study, which shows the carbohydrate content of *Nereis* sp. in Kwayar Bangkalan coastal waters of 13.46-18.73% and Elayaraja *et al.* (2011), showing the carbohydrate content in *Perinereis cultrifera* with amylase supplementation in feed ranged from 10.2-9.6 $g.kg^{-1}$.

Carbohydrate content of body *Nereis* sp. is influenced by salinity and type of feed given (*P* <0.05) (Figure 5.). The lowest carbohydrate content obtains at a salinity treatment of 15 ppt with vegetable protein feed and the highest at a salinity of 5 ppt with vegetable protein feed. According to Yuwono (2008), carbohydrate reserves are deposits that made from a very complex formation and

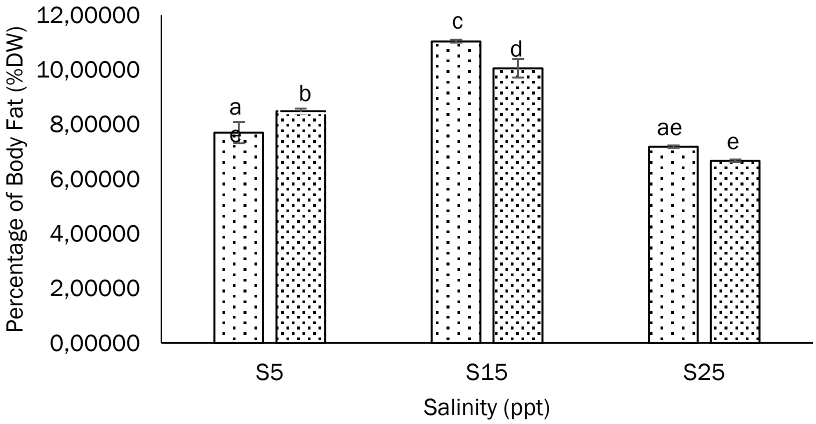

Figure 4. Average of body protein content (± SD) Nereis sp. which maintained with different salinity and feed
Note: ⛚ = vegetable protein feed, ▦ = animal protein feed. Different letters on the bar chart show significant differences between treatments (P<0.05).

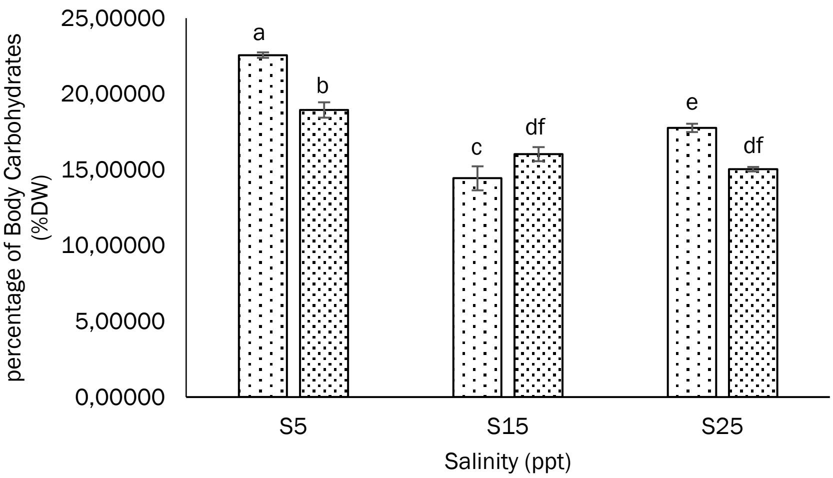

Figure 5. Average of body carbohydrate content (±SD) *Nereis* sp. which maintained with different salinity and feed
Note ⛚ = vegetable protein feed, ▦ = animal protein feed. Different letters on the bar chart show significant differences between treatments (P<0.05).

storage. Meena *et al.* (2013), suspect carbohydrates may not be important for the diet of mature shrimp.

Yuwono (2008), also states that carbohydrates play a small role in fish and aquatic organisms when compared to terrestrial organisms. However, carbohydrates have a role in the accumulation of glycogen in the hepatopancreas and act as binding and nutrient transport in hemolymph (Harrison 1997).

Nutritional content of the body *Nereis* sp. in general, can meet the nutritional needs for shrimp and fish cultivation, where the protein content ranges from 60.88-66, 535%, higher than flour used for shrimp feed ingredients, such as *Lemna gibba* and soy flour with protein 40% (Landesman *et al.,* 2002). According to Yuwono (2005), in addition to the nutrient content and availability in nature, Polychaetha given in the form of flour and fresh can increase the growth and survival of shrimp and fish.

Conclusion

Metabolic rate *Nereis* sp. affected by water salinity with highest metabolism at 25 ppt. The type of feed does not affect the metabolic rate of *Nereis* sp. Salinity and type of feed affect protein content of *Nereis* sp. The highest protein content was at 15 ppt of salinity and fed with vegetable protein. Body fat content *Nereis* sp. was influenced by the salinity and type of feed given. The highest protein content at 15 ppt and fed with vegetable protein. Carbohydrate content *Nereis* sp. influenced by salinity and type of feed. The highest protein content at 15 ppt and fed with vegetable protein. Nutrient content of *Nereis* sp. at all treatments produced a nutritional composition of the body suitable for shrimp and fish feed.

Acknowledgement

This research was funded by DIPA BLU UNSOED. Special thanks to everyone that involved technically and non-technically, for the support and participation in this research.

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