Sexual Maturity and Macronutrient Contents in *Diopatra* sp. (Onuphidae, Polychaeta) Maintained at Different Salinity Levels

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Abstract

Diopatra sp. (Onuphidae, Polychaeta) is an economically important polychaete species commonly used as a fishing bait by local anglers in Cilacap, Central Java Province. Some biological aspects of this local species, however, have not been studied yet. The aim of this research was to see the effect of salinity levels on the sexual maturity and the macronutrient contents of the animals. Diopatra specimens in the present study were collected from the intertidal mangrove habitats of Jeruklegi Village, Cilacap, in sexually immature condition. The worms were then transported to the laboratory and were maintained in containers with four different salinity levels, i.e. 10, 15, 20 and 25 ppt over the next two months. The sexual maturity of the animals were observed based on the presence or absence of coelomic gametes. Proximate analysis was performed to determine macronutrient contents of the animals. The effect of salinity levels on the animals' sexual maturity and macronutrient contents was analyzed using one-way ANOVA. The results showed that salinity levels did not significantly affect the sexual maturity level of Diopatra worms ($p > 0.05$). Similarly, they did not affect the protein and fat contents, but impacted the carbohydrate content of the animals ($p < 0.05$). The protein and the fat contents in Diopatra sp., i.e. 41–43% and 6–9%, respectively, met the amount required to be used as shrimp broodstock and fish feeds. While the species has the potential for use in local aquaculture, the annual reproductive cycle as well as the identity of the species, however, require further research.

Keywords: *Annelida, aquaculture, proximate analysis, reproduction*

Introduction

Diopatra (Audouin and Edwards, 1833) is the only genus of the family Onuphidae whose members possess spiral branchiae (Budaeva and Fauchald, 2011). Members of this genus occur worldwide in both temperate and tropical regions. The animals, dwelling in a solitary tube, typically inhabit intertidal and subtidal areas of estuaries with soft sediment despite some species being reported to occur at greater depths (Berke, 2022).

Some *Diopatra* species have high economic importance as they are commercially used as fishing baits by anglers. The most popular and exploited one is the European species *Diopatra neapolitana* Delle-Chiaje, 1841. Other Asian species include *D. aciculata* (Knox and Cameron, 1971) and *D. sugokai* (Izuka, 1907), in Japan, as well as *D. claparedii* Grube, 1878, in Malaysia. In Cilacap, Central Java Province, Indonesia, *Diopatra* worms are also used as fishing bait by local anglers (Wibowo *et al*., 2022) – the animals are called '*lur umah*' in the local language, which means a worm with housing. The worms were initially identified as *D. neapolitana* by Purwati *et al*. (2020), but the occurrence of this European species in Indonesian waters is highly unlikely considering the long distance between the two geographic regions. In such case, a detailed taxonomic work will reveal the identity of the species; for example, the worms *D. neapolitana* in Malaysia were later identified as *D. claparedii* by Idris and Arshad (2013).

Diopatra worms were obtained generally rely on digging from their natural habitat. For instance, approximately 4.3 million individuals of *D. neapolitana* may be annually harvested by worm diggers in Portugal's Canal de Mira (Ria de Aveiro estuary) with an economic value of more than EUR

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https://ejournal.undip.ac.id/index.php/ijms
DOI: 10.14710/ik.ijms.28.1.49-56
Received : 26-09-2022
Accepted : 13-02-2023

325,000 (Cunha *et al*., 2005). In Cilacap, an experienced worm digger may collect about 100 individuals of *Diopatra* sp. within a few hours of work. The digger exclusively trades the animals in his house – not in a traditional market – IDR 1000 per individual (pers. obs.). These activities are concerning because they may threaten the sustainability of the species with potentially detrimental effects on the natural environment, therefore mariculture can be proposed to be the solution. Despite some biological aspects of *Diopatra* sp. that have been studied by Wibowo *et al*. (2022), initial information regarding reproductive aspects and macronutrient contents of this species is still lacking. The aim of this research was to study the sexual maturity levels and the macronutrient contents of *Diopatra* sp. maintained at different salinity levels.

Materials and Methods

Diopatra worms, as well as the substrate and the seawater were collected from the intertidal mangrove habitats of Jeruklegi Village, Cilacap Regency, Central Java Province, during a low tide. All the worms obtained were sexually immature. The type of the substrate was clay, with a composition of 3–6% sand, 15–18% silt and 74–79% clay, with 4–8% C-organic content and 8–14% organic matter.

Experimental design

The laboratory work was conducted between January and March 2022. The substrate was placed in 12 containers, each of which measures 20 x 30 x 25 cm. The containers were then grouped into four different water salinity treatments, i.e. 10, 15, 20 and 25 ppt (freshwater was used to dilute the seawater obtained from the field). The containers containing substrate and saline water were then aerated and left for one week to stabilize the salinity. Three individuals of *Diopatra* sp. were put and maintained in each container (the worms were acclimatized for one week and weighed prior to the treatment) and were fed with D0 ornamental fish feed (2% of the worm's body weight) once a week over the following two months. This treatment was replicated five times.

Sexual maturity observation

The sexual maturity of *Diopatra* worms were observed based on the presence or absence of coelomic gametes. The gametes were taken out of the coelomic cavity by cutting off the anterior part of the animals. The part was then pressed on an object glass to release the gametes (if the gametes present). The gametes were dripped using saline water taken from the animals' container, and were observed under a compound microscope with 100x magnification. The diameter of the worms' oocytes was measured using a calibrated micrometer. The proportion between the numbers of sexually mature and immature worms per salinity treatment was calculated by using the following formula:

Percentage (%) =(number of sexually mature worms per salinity/number of total worms per salinity) x 100%

Macronutrient content measurement

To measure the macronutrient contents, wet *Diopatra* worms were weighed using a balance with an accuracy of 0.0001 g. The animals were then dried in an oven at a temperature of 80 °C. After a constant weight was achieved, the animals were mashed and stored at a room temperature for a further proximate analysis (Sudarmadji *et al*., 1998).

Statistical analysis

The significance of salinity effects towards the proportion between sexually mature and immature *Diopatra* worms, as well as the macronutrient contents of the animals, was analyzed using one-way ANOVA. The analysis was performed using *Minitab*.

Results and Discussion

Sexual maturity

Our experiment demonstrated that after two months 40 and 33% of *Diopatra* worms maintained in the containers with salinity levels of 10 and 15 ppt, respectively, were sexually mature, whereas 66 and 73% of the animals maintained in the containers with salinity levels of 20 and 25 ppt were sexually mature. The rest of the animals were undetermined (no gametes were observed in the coelom) and considered sexually immature. In this stage, no morphological differences were noticed between males and females, which is in line with the study by Pires *et al*. (2012) on *D. neapolitana*.

This study found that several female *Diopatra* worms maintained in the containers with salinity levels of 10 and 20 ppt were in sexually submature and mature stages. The oocytes' shape was rounded, and the diameter of the oocytes in the sexually submature worms ranged between 200 and 210 μm with an average of 205.8 μm, whereas sexually mature worms had oocytes with a diameter ranging between 220 and 230 μm with an average of 225.4 μm (Figure 1A.). These findings are in agreement with the study of Purwati *et al.* (2020) in which *Diopatra* worms collected from Cilacap showed different levels of sexual maturity based on the diameter of the oocytes. The oocyte diameters of the sexually submature and mature female worms were below

200 μm and 208–240 μm, respectively, and the cells did not appear to have nurse cells. The shape and the diameter range of *Diopatra*'s oocytes in the present study were also similar to the larger oocytes of *D. neapolitana* investigated by Pires *et al.* (2012), i.e. rounded with a diameter of 140–240 μm. The study also clarified that nurse cells were only observed in smaller oocytes with a diameter of up to 160 μm; larger cells did not have nurse cells attached. Having a diameter of around 200 μm, oocytes should be released from the coelom into the water column (Dağli *et al.* 2005).

The male gametes of *Diopatra* worms were much smaller than the oocytes and were in the cluster and tetrad stages (Figure 1B.). In the former stage, the spermatid cells were fused and were inactive, while in the latter stage the cells were fused in a group of four and started to move actively. Mature sperms of the animals, which were characterized as having a long tail attached to a spherical, short and rounded head in *D. neapolitana* (Conti *et al*., 2005; Pires *et al*., 2012), were not found in this study.

Unlike *Diopatra* sp. from Jeruklegi, the reproductive cycle in the European *D. neapolitana* has been well-studied. Annually, the beginning of gametogenesis took place in spring, i.e. March or April, while the spawning period typically occurred from the end of spring in May to the end of summer in August – the amount of mature oocytes during this period were peaked, and the mature sperms had the highest mobility. The gametogenic inactivity period, thereafter, lasted from the end of autumn in November to the end of winter in February (Pires *et al*., 2012). The reproductive cycle of the Javanese *Diopatra* sp. in the present work may be different considering that Indonesia only has two different seasons, i.e. rainy and dry. A monthly sampling over one year is hence necessary to see the reproductive pattern of this local species.

Although higher salinity levels seem to result in more sexually mature worms, statistical analysis shows that salinities did not significantly affect the sexual maturity level of the animals ($P > 0.05$) (Figure 2A.). This finding is in line with the study by Wibowo *et al.* (2021) in which variations in salinities did not significantly affect the proportion between sexually mature and immature *Nereis* worms (family Nereididae). While sexually immature *Diopatra* sp. possibly had not naturally entered the reproductive period, the result of this study was also biased from wounded worms, which were found in all the experimental containers. These worms typically lost the posterior part of their body and during the study were found to be regenerating this part – the regenerating area was pink and whitish yellow (Figure 1C). Regeneration of lost body parts can adversely impact reproduction as the worms tend to use their energy for regenerating their parts rather than producing gametes (e.g. Pires *et al*., 2015).

Macronutrient contents

Our proximate analysis revealed that *Diopatra* worms collected from Jeruklegi mostly comprised protein (41–43%) followed by carbohydrate (24–29%), ash (16–21%), fat (6–9%) and fiber (1–3%) as presented in Table 1. The protein content of the animals (41–43%) was almost similar to the Malaysian *Diopatra*, i.e. around 49% (Selvam, 2021) – the worms were identified as *D. neapolitana* by the author, yet the identification needs to be rechecked as the occurrence of this species in Asian waters is highly unlikely due to the long distance between European and Malaysian waters (e.g. Idris and Arshad, 2013; Berke, 2022). Compared to some local polychaete species, the protein content of *Diopatra* worms in this study was rather lower than that of *Nereis* sp. (family Nereididae) collected from Randusanga Village in Brebes, i.e. about 52% (Rachmad and Yuwono, 2000), and Jeruklegi, i.e. 60–66% (Wibowo *et al*., 2019) and 47–51% (Wibowo *et al.,* 2020[a]). It was also lower than that of female epitokes of *wawo* worms (family Eunicidae) collected from Ambon, i.e. about 54% (Pamungkas, 2015). The protein content of *Diopatra* sp. was nonetheless almost similar to that of *Dendronereis pinnaticirris* Grube, 1878 (family Nereididae), i.e. 32–43% (Wibowo *et al.,* 2020[b]).

The fat content of *Diopatra* sp. from Jeruklegi (6–9%) was three to four times lower than that of the Malaysian *Diopatra* (approximately 24%) (Selvam, 2021). It was also much lower than that of some local species including *Nereis* sp. collected from Randusanga, i.e. nearly 30% (Rachmad and Yuwono, 2000), and Jeruklegi, i.e. 12–22% (Wibowo *et al*., 2020[a]), as well as the female epitokes of *wawo* worms, i.e. about 11% (Pamungkas, 2015). The fat content of *Diopatra* worms from Jeruklegi was nevertheless rather similar to that of *Nereis* sp. collected from the same area by Wibowo *et al.* (2019), i.e. 6–11%, and *D. pinnaticirris*, i.e. 2–9% (Wibowo *et al.,* 2020[b]).

The carbohydrate content of *Diopatra* sp. from Jeruklegi (24–29% in the form of non-nitrogen extract matter) was similar to that of the Malaysian *Diopatra*, i.e. about 24% (Selvam, 2021), but much lower than that of *D. pinnaticirris*, i.e. 39–50% (Wibowo *et al.,* 2020[b]). The content was however higher than that of *Nereis* sp. studied by Wibowo *et al*. (2019, 2020[a]), i.e. 14–22% and 1–10%, respectively, and the female epitokes of *wawo* worms, i.e. about 12% (Pamungkas, 2015).

This study suggested that the levels of protein and fat in *Diopatra* worms from Jeruklegi may be suitable for using as shrimp broodstock and fish feeds since both feeds typically have more than 40% of protein and about 10% of fat. A number of polychaete species, especially members of the genera *Nereis* and *Glycera*, have been used for feeding crustaceans and fishes as the use of these worms ensures sufficient nutrition for reared broodstock as well as significantly stimulates the development of the animals' gonads (e-Costa *et al*., 2003). Several European species such as *Arenicola marina* (Linnaeus, 1758) and *A. defodiens* Cadman and Nelson-Smith, 1993, from the family Arenicolidae, as well as *Alitta virens* (Sars, 1835) and *Hediste diversicolor* (Müller, 1776) from the family Nereididae – both species were previously known as *Nereis virens* and *N. diversicolor*, respectively – are recognized as commercial species and have been cultivated to meet the market demand (e-Costa *et al*., 2003).

In Indonesia, initial studies on the potential of polychaete worms for aquaculture use have been limited to the Javanese *Nereis* sp. collected from several estuarine habitats in Central Java Province. The worms were used to feed two species of shrimps, including the giant freshwater prawn *Macrobrachium rosenbergii* (De Man, 1879) and the giant tiger prawn *Penaeus monodon* Fabricius, 1798. The results of the studies showed that the shrimps fed with feed containing the worms had better growth and higher survival rates than those fed with other feed (Yuwono *et al*., 1993, 1994, 1995; Rachmad and Yuwono, 2000; Yuwono, 2005). This is due to the fact that the worms contained high essential amino and fatty acids required by the shrimps to grow (Rachmad and Yuwono, 2000; Yuwono, 2005; Yuwono *et al*., 2005).

Besides *Nereis* sp., the Javanese *Eunice* sp. from the family Eunicidae (Yuwono *et al*., 2005) and *Diopatra* sp. (present study) also have the potential for use as feed in aquaculture due to their relatively high protein and fat contents. The latter two species, however, are still poorly studied despite an initial research on some biological aspects of *Diopatra* by Wibowo *et al.* (2022). The identity of these three Javanese species also remains unknown to date that further taxonomic investigations are required; they may represent new records or even new species considering that polychaete taxonomic studies in the southern coast of Java have been limited (Pamungkas and Glasby, 2019).

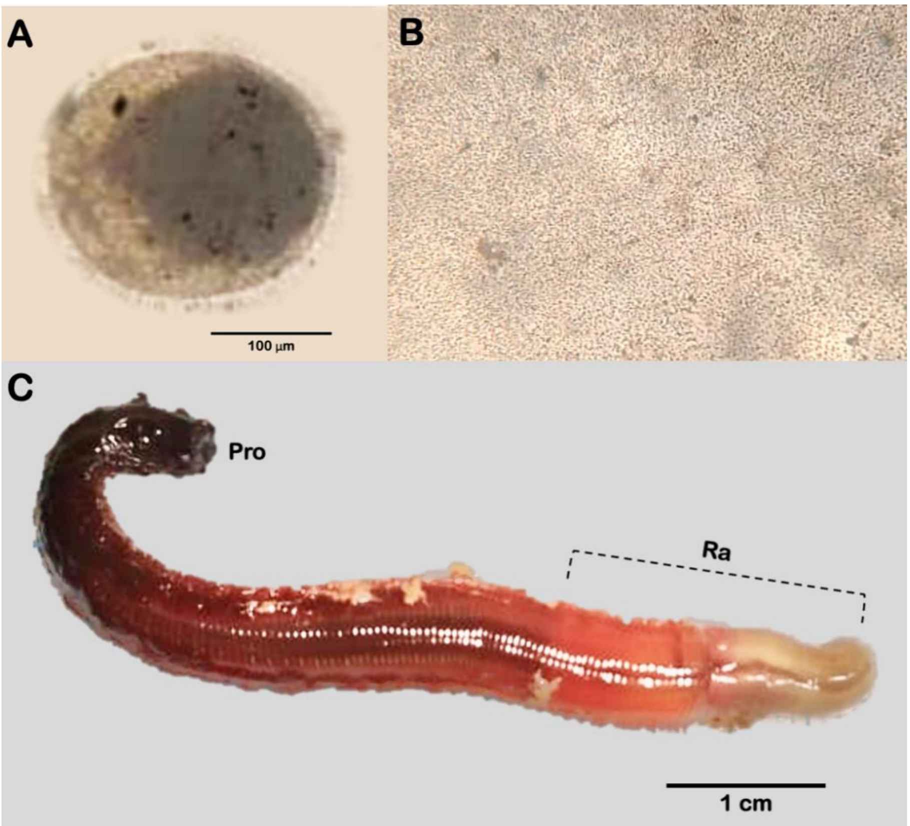

Figure 1. A mature oocyte (ovum) of *Diopatra* sp. from Jeruklegi, Cilacap (A), spermatids of the animals in the tetrad stage (B), *Diopatra* sp. regenerating the anterior part of the body (C). Pro, prostomium; Ra, regenerating area

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Table 1. Results of the proximate analysis of *Diopatra* sp. from Jeruklegi Village, Cilacap

Salinity	Dry weight (%)	Water (%)	Protein*	Fat*	Carbohydrate*	Fiber*	Ash*
10	89.91	10.09	41.74	6.02	28.59	2.72	20.92
15	90.40	9.60	42.61	8.26	26.70	1.40	21.03
20	90.78	9.22	43.42	9.84	24.24	1.83	20.67
25	89.10	10.90	42.22	9.42	29.11	3.24	16.00

* in % of dry weight

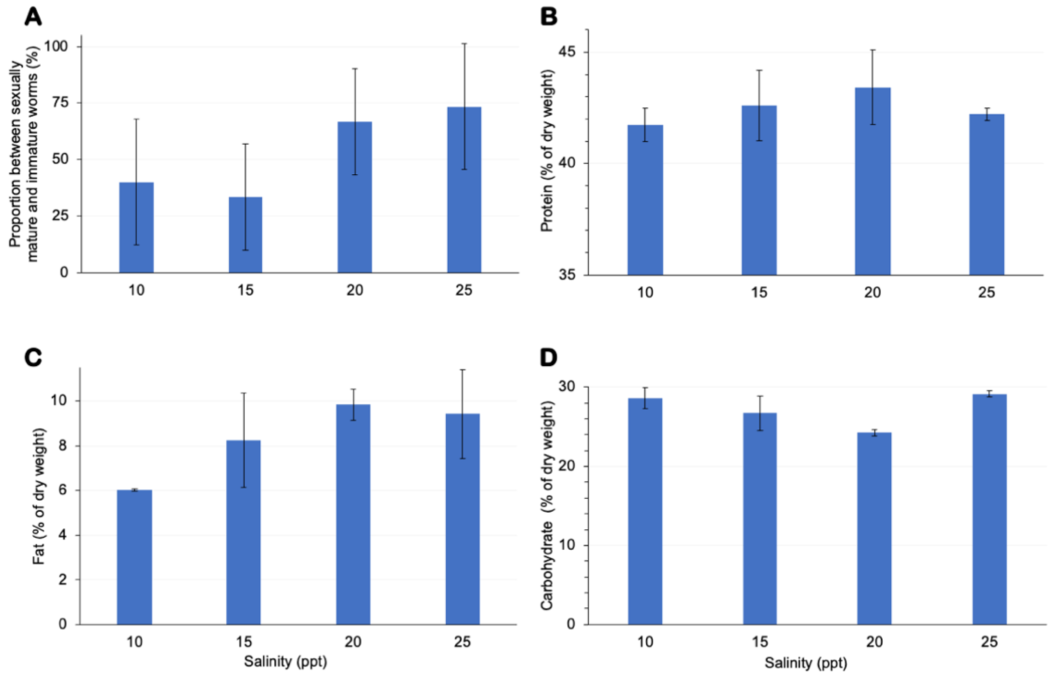

Figure 2. Proportion between sexually mature and immature worms (A), average protein (B), fat (C) and carbohydrate contents (D) of *Diopatra* worms maintained at four different salinity levels.

Statistical analysis indicates that salinity levels did not affect the protein and fat contents ($P > 0.05$; Figure 1B & C), but affected the carbohydrate content of the animals ($P < 0.05$; Figure 1D.). Both the lowest and the highest carbohydrate contents were found in the worms maintained at the salinity levels of 20 and 25 ppt, respectively (Figure 1D.). Despite this, carbohydrates generally do not appear to be essential for shrimp broodstock diets (Meena *et al.,* 2013). The water salinity was reported to have more impact on the regenerative capacity of the worms; it was the most responsible environmental factor influencing the regenerative capacity of *D. neapolitana* compared to pH and temperature (Freitas *et al*., 2015; Pires *et al*., 2015). Macronutrient contents of polychaetes generally depend on various factors such as the species (the contents vary between species), the diet (what they eat), the sexual maturity level (sexually mature worms tend to have a higher protein content due to the presence of gametes in their body cavity) and the method of proximate analysis used (e.g. Kjeldahl vs Biuret methods for protein analysis and the determination of carbohydrate content) – see a comparison of the macronutrient contents of various commercially important polychaete species by Selvam (2021). A particular range of salinity levels (between 15 and 40 ppt), however, are required for *Diopatra* worms to live (Hakkim, 1975).

Conclusion

Salinity levels did not significantly affect the sexual maturity level of *Diopatra* worms collected from Jeruklegi. They also did not affect the protein and fat contents, but impacted the carbohydrate content of the animals. The levels of protein and fat in *Diopatra* sp. in the present work, i.e. 41–43% and 6–9%, respectively, were suitable for using as shrimp broodstock and fish feeds. *Diopatra* sp. has the potential use in local aquaculture. The annual reproductive cycle and the identity of the species, however, requires further research.

Acknowledgement

We would like to thank everyone involved in this study. This research was funded by DIPA BLU UNSOED.

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