

## Biological Aspects of *Diopatra claparedii* Grube, 1878 (Onuphidae, Polychaeta) Maintained at Different Salinity Levels

Eko Setio Wibowo<sup>1\*</sup>, Atang<sup>1</sup>, Eko Setiyono<sup>1</sup>, Hana<sup>1</sup>, Sorta Basar Ida Simanjuntak<sup>1</sup>,  
Untung Susilo<sup>1</sup>, Phuping Sucharitakul<sup>2</sup>, Yuni Apriyanti<sup>3</sup>, Joko Pamungkas<sup>4</sup>

<sup>1</sup>Faculty of Biology, Universitas Jenderal Soedirman

Jl. Profesor DR. HR Boenyamin No.708, Purwokerto Jawa Tengah 53122 Indonesia

<sup>2</sup>School of Environment, Tsinghua University  
Beijing 100084, China

<sup>3</sup>Directorate of Scientific Collection Management, National Research and Innovation Agency

<sup>4</sup>Research Center for Biosystematics and Evolution, National Research and Innovation Agency  
Jl. Raya Bogor KM. 46. Cibinong Jawa Barat 16911 Indonesia  
Email: tio\_eko@yahoo.co.id

### Abstract

*Diopatra claparedii* Grube, 1878, is a tubicolous polychaete species widely used as fishing bait by local anglers in Cilacap, Central Java, Indonesia. Little is known about its biological characteristics despite its ecological and economic importance. This study aimed to examine the survival rate, the growth, the metabolic rate and the osmoregulatory capacity of *D. claparedii* under different salinity conditions. The experiment was conducted using plastic containers (20 × 30 × 25 cm) filled with muddy substrate and water at four salinity levels, i.e. 10, 15, 20, and 25 ppt, with each treatment was replicated five times. The results showed that the survival rate of *D. claparedii* ranged from 87% to 93%, while its growth varied between 0.17 and 0.58 g. The metabolic rate, measured in the form of oxygen consumption, ranged from 0.201 to 0.467 mg·g<sup>-1</sup>·h<sup>-1</sup>. The osmoregulatory capacity of the worms varied between 1.26 and 1.54 mOsm·kg<sup>-1</sup> solvent. Statistical analysis indicates that salinity did not significantly affect survival ( $p > 0.05$ ). However, it significantly influenced growth, metabolic rate and osmoregulatory capacity ( $p < 0.05$ ). The results suggested that a 15–25 ppt salinity range was more favorable for the species, with 20 ppt being the most favorable for its maintenance and growth. This study highlights the potential for cultivating *D. claparedii* under controlled conditions, providing a sustainable alternative to wild harvesting. As polychaete farming has been successfully implemented in several countries, similar efforts could be applied in Indonesia to support local fisheries while conserving natural populations.

**Keywords:** Annelida, *Diopatra claparedii*, mariculture, marine worms

### Introduction

*Diopatra claparedii* Grube, 1878, is an estuarine polychaete species that belongs to the family Onuphidae, class Polychaeta (bristle worms), phylum Annelida (segmented worms). The species commonly occurs in Southeast Asian countries such as Malaysia, Singapore, Thailand and the Philippines (Grube, 1878; Paxton, 2002; Idris and Arshad, 2013). The first record of this species in Indonesia was reported by Pamungkas *et al.* (2023) based on the specimens obtained from Donan Creek, Cilacap, Central Java Province. All the local specimens examined had distinct pectinate chaetae with funnel-like combs, which are unique to *D. claparedii* (Grube, 1878; Paxton, 2002; Idris and Arshad, 2013).

Rather similar to the worms *Diopatra neapolitana* Delle Chiaje, 1841, that are massively harvested in Europe (e.g. Cunha *et al.*, 2005; Berke,

2022), the worms *D. claparedii* are known to have economic importance for use as fishing baits particularly by the locals of Cilacap (Pamungkas *et al.*, 2023). Each day, about 300–400 individuals of *D. claparedii* inhabiting Donan and Sapuregel Creeks in Cilacap are harvested by a few locals – how they catch the worms was documented by Pamungkas (2023). This worm-catching activity is generally considered unsustainable as it may lead to the depletion of natural resources.

Unlike some Javanese nereidid species (the worms were mostly identified as *Nereis* sp. yet the animals are most likely to be *Dendronereis* sp. due to the presence of distinctive branchiae) that have been extensively studied by a number of local researchers (e.g. Wibowo *et al.*, 2019; Rachmad and Yuwono, 2000; Yuwono, 2005; Wibowo *et al.*, 2020a,b; Wibowo *et al.*, 2021), little attention has been paid to *D. claparedii*. Several biological aspects of *D.*

*claparedii* collected from Cilacap, however, have been initially studied by Wibowo *et al.* (2022; 2023a,b) – the worms were at the time identified as *Diopatra* sp. by the authors, and as the European species *D. neapolitana* by Purwati *et al.* (2020). The aspects that were investigated included the segment number-body weight relationship, the sex ratios, the sexual maturity levels and the macronutrient contents of the worms. Other biological aspects such as the survival rate, the growth, the metabolic rate and the osmoregulatory capacity of the animals have not been studied. The aim of this research was thus to study these biological aspects in *D. claparedii* maintained at different salinity levels. As the growth of polychaete worms is greatly influenced by salinity (e.g. Freitas *et al.*, 2015), the best salinity level for supporting the species' life needs to be investigated as a basis for future polychaete farming.

## Materials and methods

### Sample collection and identification

The sexually immature worms *D. claparedii* were collected from Donan and Sapuregel Creeks, Cilacap Regency, Central Java Province. These immature animals showed no gametes in their body cavity and therefore cannot be sexually distinguished. Both creeks are situated in the eastern part of the Segara Anakan Lagoon (SAL), and have been identified by the locals as the areas with the most individuals of *D. claparedii*. In addition, the substrate and the sea water used in this work were obtained from Donan Creek. The worms obtained from the field

were first identified under both stereo and compound microscopes using the identification keys of Fauchald (1977) and Budaeva and Fauchald (2011). This identification step was essential to ensure if the worms obtained were *D. claparedii* (Figure 1).

### Experimental design and statistical analyses

The substrate was placed in 20 containers, each of which measured 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; each treatment with five replications. All the containers containing substrate and salt water were then aerated for one week to stabilize the salinity levels. In each container, five individuals of sexually immature *D. claparedii* were placed and acclimatized for one week. Before treatment, the worms were weighed then fed with D-O fish food at the rate of 2.5% of their wet body weight once a week over the next two months. During the treatment, either saline or freshwater was added to maintain the salinity.

Survival rate and growth parameters were calculated based on the formula of Hariyadi and Yuwono (1998). By counting the number of experimental animals,  $SR = (N_t/N_0) \times 100\%$ , where SR is the survival rate,  $N_t$  is the number of experimental animals that survive until the end of study, and  $N_0$  is the number of experimental animals maintained at the beginning of study.  $WG = B_t - B_0$ , where WG is the body weight gained,  $B_t$  is the wet weight of experimental animals at the end of study,



**Figure 1.** Living *Diopatra claparedii* collected from Donan Creek, Cilacap, Central Java. Notes: bra= branchiae; ma= median antenna; rla= right lateral antenna; rpal= right palp; lla= left lateral antenna; lpal= left palp.

and BO is the wet weight of experimental animals at the beginning of study. A digital scale was used to measure the animals' weight.

To study the metabolic rate of the animals, oxygen consumption was measured using the formula of Fidhiyany (1999).  $VO_2 = (cO_{2i} - cO_{2f}) \times V \times H^{-1} \times W^{-1}$ , where  $VO_2$  is the oxygen consumption ( $mg \cdot g^{-1} \cdot h^{-1}$ ),  $cO_{2i}$  is the initial dissolved oxygen ( $mg \cdot L^{-1}$ ),  $cO_{2f}$  is the final dissolved oxygen ( $mg \cdot L^{-1}$ ),  $V$  is the volume of the tube after subtracting the worm (L),  $H$  is the time interval between the initial and the final oxygen measurements (hours), and  $W$  is the weight of the worms (g). Dissolved oxygen was calculated using the formula of APHA (2005).  $O_{tae} = (1000/100) \times p \times q \times 8$ , where  $O_{tae}$  is the initial dissolved oxygen ( $mg \cdot L^{-1}$ ), 1000 is the volume of one liter of water, 100 is the volume of water in the Erlenmeyer flask,  $p$  is the volume of 0.025N  $Na_2S_2O_3$  solution used,  $q$  is the normality of 0.025N  $Na_2S_2O_3$  solution, and 8 is the weight equivalent to oxygen. Technically, the measurement was done using titration by the Winkler method.

Lastly, osmoregulatory capacity was determined based on the ratio of the osmolality of the animal's body fluid to the osmolality of the medium (Lignot et al., 2000). The body fluid of *D. clapedredii* (60  $\mu$ L) was taken to calculate its osmolality ( $mOsm \cdot kg^{-1}$  solvent) using an SMC-30D osmometer. The osmolality of the medium was measured using the same method. The data obtained were analyzed statistically using ANOVA followed by the Tukey test using MINITAB 16.

## Results and Discussion

### Survival rate

The present study showed that the survival rate of *D. clapedredii* maintained at different salinity levels was not significantly different ( $p > 0.05$ ) (Table 1), indicating that *D. clapedredii* is generally able to live in a salinity range of 10 to 25 ppt. These results are in accordance with the study by Hakkim (1975) reporting that the estuarine species of *Diopatra* are generally capable of tolerating a wide range of salinity levels from 15 to 40 ppt. This range explains the lower survival rate of the animals at the salinity of 10 ppt, i.e. about 87% (Table 1); 15 ppt therefore appears to be the lower limit of salinity levels favorable for the animals to live. Apparently, this is also the reason why the animals have never been reported to occur in the western part of the SAL – because the area has relatively low salinity levels (between 2.8 and 10.2 ppt) due to the presence of both Cibeureum and Citanduy Rivers (Nordhaus et al., 2009; Pamungkas, 2013). This explains why local worm catchers always hunt the animals in the eastern part of the lagoon, i.e.

in Donan and Sapuregel Creeks (Pamungkas et al., 2023), as the salinity levels in this area are relatively higher, i.e. between 7.0 and 34.9 ppt (Pamungkas, 2013).

The salinity range tolerance of *D. clapedredii* in the present work seems to be in line with that of *D. variabilis* (Krishnamoorthi, 1963) as the survival rate of both species was found to be higher in the environment with relatively high salinities. However, it differs from that of *D. neapolitana*. While *D. clapedredii* can tolerate a salinity range of 10 to 25 ppt, the mortality rate of *D. neapolitana* maintained at salinities 14, 21 and 42 ppt was found to be higher than those maintained at salinities 28 and 35 ppt (Freitas et al., 2015). By contrast, the Javanese nereidids were reported to have a higher survival rate at salinities 5–15 ppt (Wibowo et al., 2019). This generally indicates that the salinity range tolerance of polychaetes appears to be species-specific.

### Growth

The results of the present study suggested that salinities did affect the ability of *D. clapedredii* to grow ( $p < 0.05$ ) (Table 1). The animals maintained at salinities 15, 20 and 25 ppt gained more weight than those maintained at salinity 10 ppt (Table 1). These can be attributed to *D. clapedredii* using less energy for physiological and biochemical processes at higher salinities, resulting in the better growth. These findings are similar to those of Wibowo et al. (2019) in which the Javanese nereidids collected from Jeruklegi Village, Cilacap – the worms were identified as *Nereis* sp. yet the identification requires further verification – demonstrated better growth in higher salinities (15 and 25 ppt) than a lower salinity (5 ppt). Bigger and more polychaete worms were found in the eastern part of the SAL where the salinity was generally higher than that of the western part of the lagoon (Pamungkas, 2013).

Unfavorable salinity levels, therefore, seem to be a major limiting factor for polychaetes to survive. According to Freitas et al. (2015), *D. neapolitana* exposed to unfavorable salinity levels demonstrated higher mortality rates, required more days to regenerate missing body regions, and regenerated fewer chaetigers. Lower glycogen and protein contents were also reported in such case as the worms used considerably more energy for physiological and biochemical processes.

### Metabolic rate

Salinity levels were found to affect the metabolic rate of *D. clapedredii* ( $p < 0.05$ ). The oxygen consumption rate of the animals was highest at salinity 10 ppt, i.e.  $0.467 mg \cdot g^{-1} \cdot h^{-1}$ , and lower at

**Table 1.** Survival rate, body weight gain and oxygen consumption of *D. clapedredii* maintained at different salinities

No.	Salinity (ppt)	Survival rate (%)	Body weight gain (g)	Oxygen consumption rate (mg.g <sup>-1</sup> .h <sup>-1</sup> )
1	10	87 ± 11.55 <sup>a</sup>	0.17 ± 0.061 <sup>a</sup>	0.467 ± 0.179 <sup>a</sup>
2	15	93 ± 11.55 <sup>a</sup>	0.51 ± 0.070 <sup>b</sup>	0.284 ± 0.089 <sup>b</sup>
3	20	93 ± 11.55 <sup>a</sup>	0.58 ± 0.156 <sup>b</sup>	0.201 ± 0.055 <sup>b</sup>
4	25	93 ± 11.55 <sup>a</sup>	0.52 ± 0.032 <sup>b</sup>	0.262 ± 0.046 <sup>b</sup>

Note: Numbers followed by different letters within the same column indicate significant differences between treatments ( $p < 0.05$ )

**Table 2.** Body and average media osmolalities, and osmoregulatory capacity of *D. clapedredii* maintained at different salinities

No.	Salinity (ppt)	Body osmolality (mOsm.kg <sup>-1</sup> solvent)	Average medium osmolality (mOsm.kg <sup>-1</sup> solvent)	Osmoregulatory capacity (mOsm.kg <sup>-1</sup> solvent)
1	10	489.20±24.79 <sup>a</sup>	318.60±0.55 <sup>a</sup>	1.54±0.08 <sup>a</sup>
2	15	615.00±12.25 <sup>b</sup>	473.80±1.10 <sup>b</sup>	1.30±0.02 <sup>b</sup>
3	20	820.40±29.35 <sup>c</sup>	610.80±4.02 <sup>c</sup>	1.34±0.04 <sup>b</sup>
4	25	949.60±9.24 <sup>d</sup>	756.40±8.41 <sup>d</sup>	1.26±0.02 <sup>b</sup>

Note: Numbers followed by different letters within the same column indicate significant differences between treatments ( $p < 0.05$ )

salinities 15–25 ppt, i.e. about 0.2 mg.g<sup>-1</sup>.h<sup>-1</sup> (Table 1). A higher oxygen consumption rate generally indicates higher energy used for metabolic activities (Schmidt-Nielsen, 1990). In this case, a lower salinity may have resulted in *D. clapedredii* using more energy for physiological and biochemical processes rather than for growth. A lower oxygen consumption rate, in turn, reflects lower energy allocated for both processes, and as a result the animals gained more body weight as shown in Table 1. These results are different from those of Wibowo *et al.* (2019) in which the Javanese nereidids showed a higher metabolic rate at salinity 25 ppt than at salinities 5–15 ppt.

### Osmoregulatory capacity

The present study indicated that salinity levels affected the body osmolality of *D. clapedredii* ( $p < 0.05$ ). The body osmolality gradually increased with the increase of salinities, i.e. from 489.20 mOsm.kg<sup>-1</sup> solvent at salinity 10 ppt to 949.60 mOsm.kg<sup>-1</sup> solvent at salinity 25 ppt. The body osmolality of *D. clapedredii* was generally higher than the medium osmolality, which ranged between 318.60 mOsm.kg<sup>-1</sup> and 756.40 mOsm.kg<sup>-1</sup> solvent at salinities 10 and 25 ppt, respectively (Table 2). These findings are in line with those of Rivera-Ingraham and Lignot (2017) reporting that most marine invertebrates (including polychaetes) do not invest energy in transport mechanisms when exposed to salinity changes. Rather, they regulate the osmolality of their internal cells following the osmolality of the environment – this is because polychaetes are generally osmoconformers (Freitas *et al.*, 2015). The cellular osmolality regulation process typically includes: (1) increasing or decreasing the concentrations of

osmotically active solutes, and (2) modifying membrane-bound transporters (e.g. Gilles, 1987; Kirschner, 1991; Péqueux, 1995).

Contrary to both body and medium osmolalities, the osmoregulatory capacities of *D. clapedredii* generally decreased with the increase of salinities. A higher osmoregulatory capacity (1.54 mOsm.kg<sup>-1</sup> solvent) was found in the animals maintained at salinity 10 ppt, whereas lower osmoregulatory capacities (between 1.26 and 1.34 mOsm.kg<sup>-1</sup> solvent) were found in the animals maintained at salinities 15–25 ppt (Table 2). A higher osmoregulatory capacity in *D. clapedredii* was apparently associated with a higher metabolic rate, indicating that at salinity 10 ppt the animals used more energy for physiological and biochemical processes, resulting in lower survival rate and body weight gained (Table 1).

### Conclusion

The present study demonstrated that *D. clapedredii* was generally able to live in the environment with a salinity range of 10–25 ppt. However, a salinity range of 15–25 ppt appeared to be more favorable for the worms to live. In such range, the animals showed a higher survival rate (93%), a better growth (between 0.51 and 0.58 g), and lower oxygen consumption rate (about 0.2 mg.g<sup>-1</sup>.h<sup>-1</sup>) as well as osmoregulatory capacity (between 1.26 and 1.34 mOsm.kg<sup>-1</sup> solvent) compared to the animals maintained at salinity 10 ppt. The best salinity level for supporting the life of *D. clapedredii* was 20 ppt.

## Acknowledgement

We would like to thank Muji Pramono for assisting us with the worms collection. This research was funded by DIPA BLU UNSOED (Basic Research Scheme of Jenderal Soedirman University No. 1218/UN23/PT.01.02 /2023). This research was conducted in collaboration with the Research Center for Biosystematics and Evolution, National Research and Innovation Agency (MoU No. 92/V/KS/ 08/2022 and T/502/UN23.6/ HK.06.00/2022).

## References

- American Public Health Association (APHA). 2005. Standard Methods for the Examination of Water and Waste Water 12th ed. American Public Health Association Inc.
- Berke, S.K. 2022. A review of *Diopatra* Ecology: Current knowledge, open questions, and future threats for an ecosystem engineering polychaete. *Biology*, 11(10): p.1485. <https://doi.org/10.3390/biology11101485>.
- Budaeva, N. & Fauchald, K. 2011. Phylogeny of the *Diopatra* generic complex with a revision of *Paradiopatra* Ehlers, 1887 (Polychaeta: Onuphidae). *Zool. J. Linn. Soc.*, 163(2): 319–436. <https://doi.org/10.1111/j.10963642.2011.00701.x>.
- Cunha, T., Hall, A. & Queiroga, H. 2005. Estimation of the *Diopatra* neapolitana annual harvest resulting from digging activity in Canal de Mira, Ria de Aveiro. *Fish. Res.*, 76(1): 56–66. <https://doi.org/10.1016/j.fishres.2005.05.008>.
- Fauchald, K. 1977. The Polychaete Worms: Definitions and Keys to the Orders, Families and Genera. *Natural History Museum of Los Angeles County, Science Series*, 28: 1–188.
- Fidhiany, L. 1999. Pengaruh Umur dan Suhu Air pada Metabolisme Ikan Air Tawar *Cichlasoma nigrofasciatum*. *J. Ilmu-Ilmu Perairan dan Perikanan Indonesia*, 1: 29–47.
- Freitas, R., Coelho, D., Pires, A., Soares, A.M.V.M., Figueira, E. & Nunes, B. 2015. Preliminary evaluation of *Diopatra* neapolitana regenerative capacity as a biomarker for paracetamol exposure. *Environ. Sci. Pollut. Res.*, 22(17): 13382–13392. <https://doi.org/10.1007/s11356-015-4589-1>.
- Gilles, R. 1987. Volume regulation in cells of euryhaline invertebrates. *Curr. Top. Membr. Trans.*, 30: 205–247. [https://doi.org/10.1016/S0070-2161\(08\)60372-X](https://doi.org/10.1016/S0070-2161(08)60372-X).
- Grube, A.E. 1878. Annulata Semperiana. Beiträge zur Kenntniss der Annelidenfauna der Philippinen nach den von Herrn Prof. Semper mitgebrachten Sammlungen. *Mémoires l'Académie Impériale des Sciences de St. Pétersbourg (série 7)*, 25(8): 1–300. <https://doi.org/10.5962/bhl.title.85345>.
- Hakkim, V.M. 1975. Salinity tolerance of *Diopatra* neapolitana Delle Chiaje: Annelida-Polychaeta. *Indian J. Mar. Sci.*, 4: 99–101.
- Hariyadi, H. & Yuwono, E. 1998. Penelitian pendahuluan kelulushidupan dan pertumbuhan juvenile cacing lur (*Nereis* sp.) yang dipelihara dalam media dengan salinitas berbeda. *Biosfera*, 11: 17–22.
- Idris, I. & Arshad, A. 2013. Checklist of polychaetous annelids in Malaysia with redescription of two commercially exploited species. *Asian J. Anim. Vet. Adv.*, 8: 409–436. <https://doi.org/10.3923/ajava.2013.409.436>.
- Kirschner, L.B. 1991. Water and ions. In *Comparative Animal Physiology: Environmental and Metabolic Animal Physiology* (ed C.L. Prosser), pp. 13–107. New York: Wiley Liss.
- Krishnamoorthi, B. 1963. Volume regulation in eggs, larvae and adults of a brackish-water polychaete *Diopatra variabilis* (Southern). *Proc. Ind. Aca. Sci.*, 57: 276–289.
- Lignot, J.H., Pierot, C.S. & Charmantier, G. 2000. Osmoregulatory capacity as a tool in monitoring the physiological condition and the effect of stress in crustaceans. *Aquacult.*, 191: 209–245.
- Nordhaus, I., Hadipudjana, F. A., Janssen, R. & Pamungkas, J. 2009. Spatio-temporal variation of macrobenthic communities in the mangrove-fringed Segara Anakan lagoon, Indonesia, affected by anthropogenic activities. *Reg. Environ. Change*, 9: 291–313.
- Pamungkas, J. 2013. Polychaete community composition of the Segara Anakan mangrove forest, Cilacap, Central Java, Indonesia. Universität Bremen, Bremen.
- Pamungkas, J. 2023. First record of *Diopatra clapedii* Grube, 1878 (Onuphidae, Polychaeta) from Indonesian waters (video). <https://www.youtube.com/watch?v=1cBQx1yDQQU&t=52s>.
- Pamungkas, J., Atang & Wibowo, E.S. 2023. First record of *Diopatra clapedii* Grube, 1878 (Onuphidae, Polychaeta) from Indonesian

- waters, with some notes on its economic importance. *Treubia*, 50(1): 11–20. <https://doi.org/10.14203/treubia.v50i1.4484>.
- Paxton, H. 2002. Diopatra Audouin and Milne Edwards (Polychaeta: Onuphidae) from Thailand. *Phuket Mar. Biological Center Spec. Publication*, 24: 101–114.
- Péqueux, A. 1995. Osmotic regulation in crustaceans. *J. Crustac. Biol.*, 15: 1–60. <https://doi.org/10.2307/1549010>.
- Purwati, I, Rachmawati, F. N. & Wibowo, E. S. 2020. Aspek-aspek Reproduksi Cacing Diopatra neapolitana Di Cilacap. *BioEksakta: J. Ilmiah Biologi Unsoed*, 2(3): 440–448. <https://doi.org/10.20884/1.bioe.2020.2.3.3494>.
- Rachmad, B. & Yuwono, E. 2000. Pertumbuhan dan Laju Makan serta Efisiensi Protein pada Post Larva Udang Windu yang Diberi Pakan Mengandung Tepung Cacing Lur. *Seminar Nasional Bologi XVI, ITB, Bandung*.
- Rivera-Ingraham, G. A. & Lignot, J. H. 2017. Osmoregulation, bioenergetics and oxidative stress in coastal marine invertebrates: raising the questions for future research. *J. Exp. Biol.*, 220(10): 1749–1760. <https://doi.org/10.1242/jeb.135624>.
- Schmidt-Neilsen, K. 1990. *Animal Physiology: Adaptation and Environment*. 4th Edition. Cambridge University Press, New York.
- Wibowo, E.S., Atang, Palupi, E.S., Setiyono, S. & Susilo, U. 2023a. Potensi Kandungan Makro Nutrisi Polychaeta *Diopatra* sp. dari Kawasan Mangrove Jeruklegi Kabupaten Cilacap Sebagai Pakan Udang. *Bul. Oseanografi Marina*, 12(3): 325–330. <https://doi.org/10.14710/buloma.v12i3.53045>.
- Wibowo, E.S., Pitriani, M.R., Rachmawati, F.N., Susilo, U., Setiyono, E., Atang, A., Sucharitakul, P. & Pamungkas, J. 2023b. Sexual Maturity and Macronutrient Contents in *Diopatra* sp. (Onuphidae, Polychaeta) Maintained at Different Salinity Levels. *Ilmu Kelautan: Indonesian Journal of Marine Sciences*, 28(1): 49–56 <https://doi.org/10.14710/ik.ijms.28.1.49-56>.
- Wibowo, E.S., Palupi, E.S., Puspitasari, I.G.A.A.R. & Atang. 2019. Sintasan dan pertumbuhan cacing Polychaeta *Nereis* sp. dari kawasan pertambakan Desa Jeruklegi Cilacap dengan salinitas media pemeliharaan dan jenis pakan berbeda. *Depik J. Ilmu-Ilmu Perairan, Pesisir dan Perikanan*, 8(2): 67–75. <https://doi.org/10.13170/depik.8.2.12155>.
- Wibowo, E.S., Puspitasari, I.G.A.A.R., Atang & Palupi, E.S. 2021. Pertumbuhan, Retensi Protein, dan Perkembangan Gonad Polychaeta *Nereis* sp. dengan Salinitas dan Pakan Berbeda. *Al-Kauniah: J. Biologi*, 14(2): 304–312. <https://doi.org/10.15408/kauniah.v14i2.17463>.
- Wibowo, E.S., Puspitasari, I.G.A.A.R., Atang, A. & Pamungkas, J. 2022. Biological aspects of *Diopatra* sp. (Onuphidae, Polychaeta) collected from mangrove habitats of Jeruklegi, Cilacap Regency. *Depik J. Ilmu-Ilmu Perairan, Pesisir dan Perikanan*, 11(3): 299–305. <https://doi.org/10.13170/depik.11.3.25787>.
- Wibowo, E.S., Puspitasari, I.G.A.R., Palupi, E.S. & Atang. 2020a. The Potential of *Nereis* sp. (Polychaete) as Prawn Feed at the Coastal Farming of Jeruklegi, Cilacap. *IOP Conf. Ser.: Earth Environ. Sci.*, 550(1): p.012030. <https://doi.org/10.1088/1755-1315/550/1/012030>.
- Wibowo, E.S., Yuwono, E., Sukardi, P. & Siregar, A.A. 2020b. Survival Rate, Growth and Chemical Content of *Dendronereis pinnaticirris* (Polychaeta, Nereidae) in Maintenance with Different Feeds and Substrates. *Ilmu Kelautan: Indonesian Journal of Marine Sciences*, 25(2): 75–84. <https://doi.org/10.14710/ik.ijms.25.2.75-84>.
- Yuwono, E. 2005. Kebutuhan Nutrisi Crustacea dan Potensi Cacing Lur (*Nereis*, Polychaeta) untuk Pakan Udang. *J. Pembangunan Pedesaan*, 5(1): 42–49.