Kronologi pengajuan naskah "Spiny lobster feeding grounds- an eDNA Metabarcoding Assessment reveals a high level of Plankton biodiversity in Tawang Bay, Indonesia			
No.	Tanggal	Agenda	Halaman Bukti
1.	12 Maret 2023	**Pengajuan naskah:** Penulis mengajukan naskah artikel yang berjudul " *eDNA Metabarcoding Assessment on Plankton Diversity and Abundance in Tawang Bay,settlement habitat of spiny lobster in East Java, Indonesia*" pertama kali pada *Jurnal Biodiversity*	1-26
2.	18 April 2023	**Revisi** Reviewer I dan II menyatakan bahwa naskah perlu adanya revisi minor	27
3.	28 April 2023	**Submit Naskah Revisi:** Penulis mengirimkan hasil revisi ke pihak jurnal melalui submit di portal jurnal Biodiversity	28-61
4	1 Mei 2023	**Accepted** Artikel telah dinyatakan diterima untuk publikasi	62-72

1. Pengajuan naskah
12 Maret 2023

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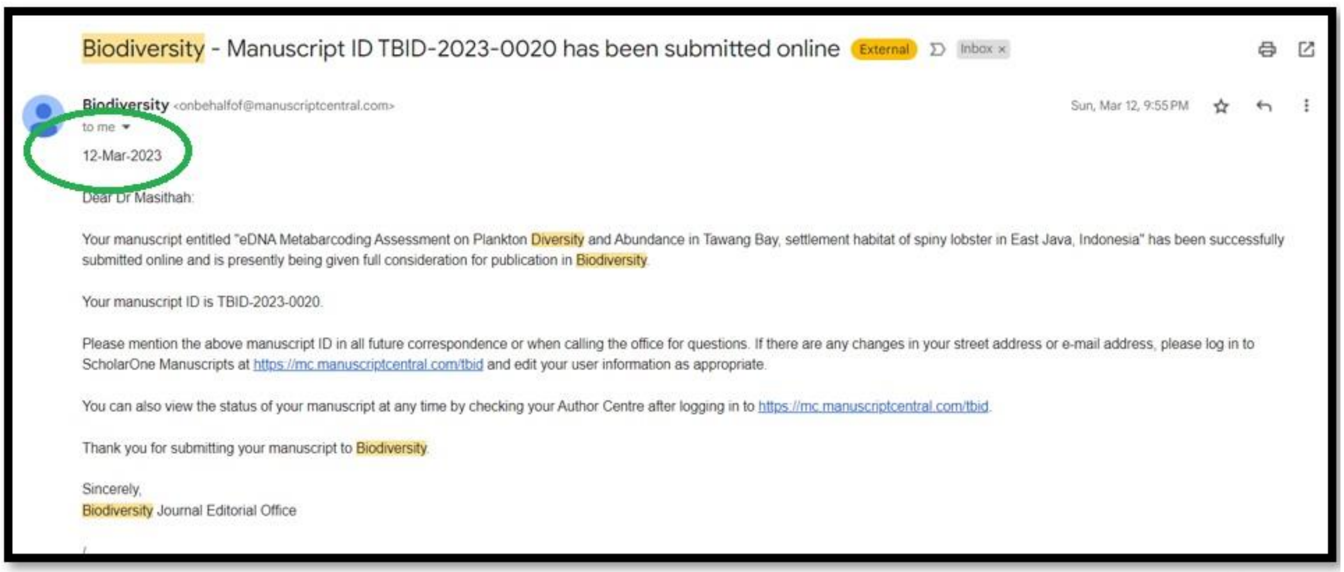

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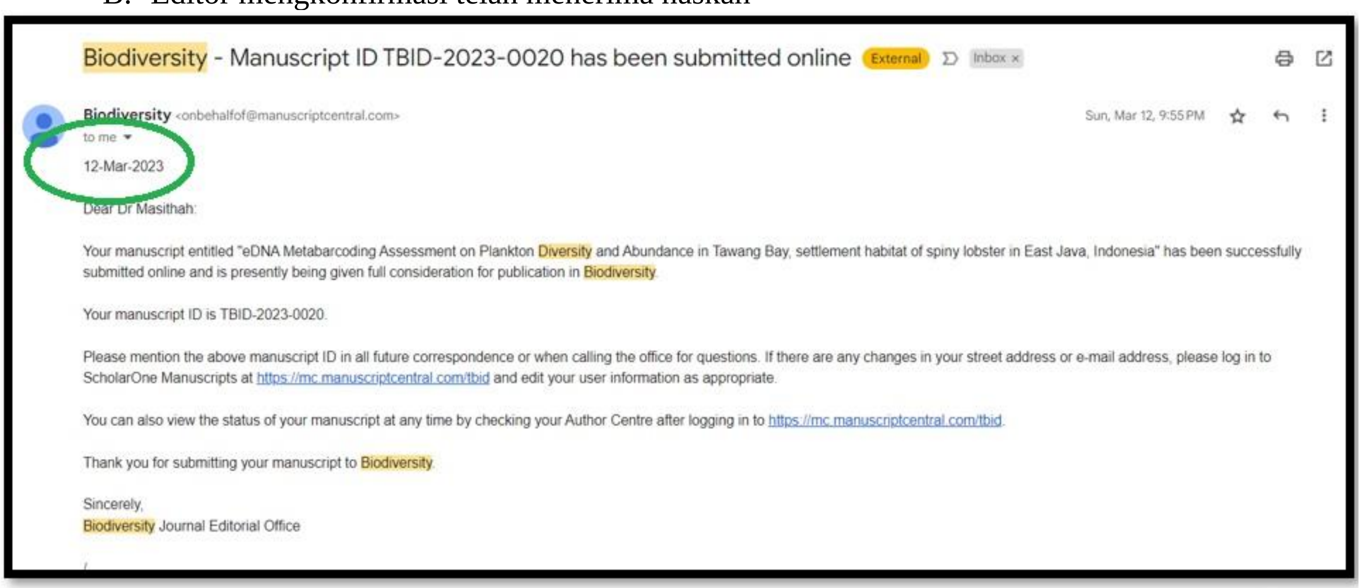

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C. Naskah awal yang dikirim

Spiny lobster feeding grounds- an eDNA Metabarcoding Assessment reveals a high level of Plankton biodiversity inTawang Bay, Indonesia

Journal:	*Biodiversity*
Manuscript ID	TBID-2023-0020.R1
Manuscript Type:	Article
Keywords:	diversity, eDNA, plankton, feeding_grounds, lobster

Spiny lobster feeding grounds- an eDNA Metabarcoding Assessment reveals a high level of Plankton biodiversity in Tawang Bay, Indonesia

Plankton has been considered an important live diet for various marine aquatic species including spiny lobster larvae. Thus, studying the diversity and abundance of plankton taxa in the natural settlement habitat of spiny lobster larvae might reveal alternative live diets for the spiny lobster larvae. The study was conducted in Tawang Bay, an important habitat for spiny lobsters in East Java, Indonesia. Plankton samples were collected using a plankton net and analyzed using eDNA metabarcoding and high-throughput sequencing. Of the collected samples, 26 phyla, 40 classes, 54 orders, 75 families, 85 genera, and 97 species were identified. Further analysis indicated that the diversity index was considered moderate as the value was 2.16. While the uniformity and dominant indices were 0.22, and 0.24 respectively, which indicate that there is no dominant plankton species in Tawang Bay. Among the identified taxa, at least 18 species including *Acartia bispinosa*, *Oithona simplex*, *Oithona* sp., *Pseudodiaptomus euryhalinus*, *Calocalanus pavo*, and *Calocalanus minutus* are potential live diets for larval of aquaculture species including lobster larvae andtherefore should be further studied.

Keywords: dominance; diversity; eDNA_metabarcoding; plankton; uniformity

Introduction

Environmental conditions including physical, chemical, and biological factors in natural habitats highly determine the recruitment rates of lobster larvae (Keulder 2005; Wahle and Incze 1997; Wahle and Steneck 1991). Several authors have previously reported the physical and chemical characteristics of the natural settlement areas of lobster larvae (Amin et al. 2022a; Boudreau et al. 1992; Lillis and Snelgrove 2010). In addition, studies viewing biological aspects of settlement habitat in natural environment lobster larvae have also been done in several studies. For instance, Amin et al. (2022b) reported plankton communities in Gerupuk Bay, one of the most common settlement habitats of spiny lobster in West-Nusa Tenggara Indonesia. Similarly, Mazur et al. (2020), revealed the type and abundance of plankton in the settlement area of American lobster in the Northeast US Shelf, USA. These studies have been conducted to understand plankton availability and biological factors that effect the settlement habitat of lobster larvae in the wild and to find candidates for live diets when lobster are grown in captivity.

Numerous studies have shown that biological factors such as plankton availability are important for the natural diet of lobster larvae (O'Rorke et al. 2014) and marine plankton are important for most marine species including molluscs and fishes (Pan et al. 2022). For instance, the plankton *Oithona* sp. which has been previously reported to be abundance in marine environments (Amin et al. 2022b), has been documented as a potential live diet for seabass, *Lates calcarifers*, larvae (Santhanam and Perumal 2012), and white leg shrimp, *Litopenaeus vannamei* (Dinesh Kumar et al. 2017). Thus, a study of plankton availability in a specific locations may be tied to the presence of certain aquatic species in that location. Many of the past studies have been performed using a conventional approach, with microscope observation and phenotypic identification. According to Falciatore et al. (2020) phenotypic identification might misidentify or inaccurately calculate the abundance of plankton in a certain location. Acknowledging the importance of plankton as a live diet for various marine species, this study focuses on a molecular approach to cleary idenitify the genetic diversity of plankton species and their abundance.

Thisstudy specifically focused on assessing plankton diversity, uniformity, and dominant indices of plankton in Tawang Bay, an important natural settlement habitat for spiny lobster larvae in East Java, Indonesia. It appears that the cnditions in Tawang Bay are suitable environmental conditions as well as good diet availability for lobster larvae.

In addition, the present study used environmental DNA (eDNA) metabarcoding to increase result accuracy.

Materials and Methods

Sample collection

Water samples were collected from Tawang Bay, in the East Java Province of Indonesia. More details on the sample site can be found in Amin et al. (2022d). For this study, water samples were collected at three sample points (L1, L2, and L3) with four different depths: 0-0.30 m (Surface), 2.5 m, 5m, and 20 m using a water sampler. The water samples were then mixed, filtered using a 355 µm mesh plankton net, and transported to the Microbiology Laboratory, Faculty of Fisheries and Marine Science, Universitas Airlangga in a cold box. Thereafter, the homogenized water samples were filtered using Whatman no 5-filter paper and stored in a -20°C freezer until further analysis.

DNA Extraction

DNA of plankton was extracted using ZymoBIOMICS™ DNA Miniprep Kit (D4300T) according to Amin et al. (2022c) with slight modifications. The filter paper was cut into sections with sterile scisors and added to ZRbashingBead™ Lysis Tubes (0.1 and 0.5 mm) followed by the addition of 750 µl ZymoBIOMICS™ Lysis solution. While the rest of the steps were performed according to the instruction manual of the ZymoBIOMICS™ DNA Miniprep Kit. Then, the quality of the DNA extract was measured using a nanodrop and afterward was adjusted at 30ng/µl before sending for sequencing. The DNA samples were sent to a service provider (1st Base) for amplification and next-generation sequencing. Bioinformatics analysis was performed as previously described by Amin et al. (2022d).

Data analysis

The type, abundance, diversity, uniformity, and dominance index of plankton species identified in the water samples were calculated according to these formulas:

$$H' = -\sum \mathrm{Pi}\ln \mathrm{Pi},\ where\ \mathrm{Pi} = \frac{ni}{N}$$ (Fachrul, 2016)

$$\mathrm{E} = \frac{\mathrm{H'}}{\mathrm{Ln\,s}}$$ (Odum 1971)

$$\mathrm{d} = \frac{\mathrm{Nmax}}{\mathrm{N}}$$ (Berger dan Parker, 1970)

Where: H’ is the Diversity index, H’is Shannon Wiener Diversity Index, ni is the number of individuals of the i-th species, “n” is the number of species, N is the total individual number, and Pi is the number of individuals of the i-th species, “E” is uniformity index, H’: Shannon Wiener Diversity Index, “S” is the Total number of species. d is the Simpson Dominance Index, “Nmax” is the most abundant number of individual species, and N is the total individual number.

RESULTS

Overview and taxa detected

The eDNA metabarcoding results showed that a total of 45,978 raw paired-end readings were obtained from three pooled water samples and became 42,135 readings after filtering, and 34,127 rarified reads. The 34,127 readings were assigned to 330 amplicon sequence variants (ASVs). Using the National Center for Biotechnology Information (NCBI) database, the 330 ASVs were assigned to 26 phyla, 40 classes, 54 orders, 75 families, 85 genera, and 97 species, Figure 1.

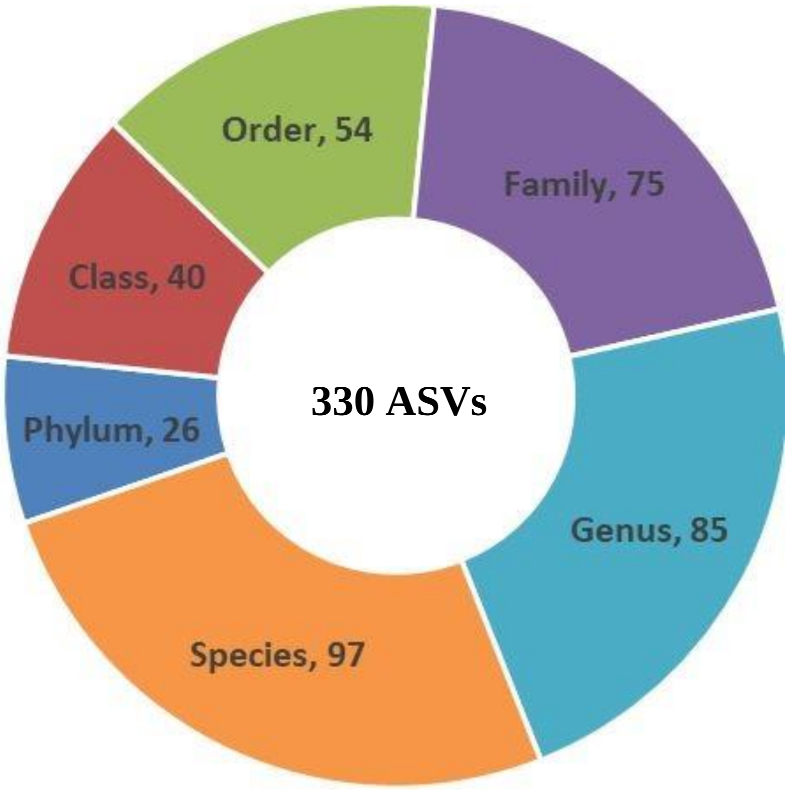


Figure 1. The number of Phyla, Classes, Orders, Families, Genera, and species from the natural settlement habitat of lobster larvae identified using eDNA metabarcoding.

Phylum composition

In terms of numbers, a total of 26 phyla were identified from water samples collected from Tawang Bay, East Java Indonesia. The five most abundant phyla were Arthropoda, Radiozoa, Myzozoa, Basidiomycota, and Cnidaria. Phylum Arthropoda was counted for 3,042 sequence reads (21.64%), followed by Radiozoa with 2,160 reads (15.37%), Myzozoa with 1,853 reads (13.18%), Basidiomycota with 788 reads (5.61%), and Cnidaria with 765 reads (5.61%). The other 21 phyla including were Chlorophyta, Bryozoa, Mollusca, Haptophyta, Cercozoa, Discosea, Foraminifera, Chordata, Cryptophyta, Ochrophyta, Chrorophyta, Endomyxa, Loukozoa, Heterolobosea, Amoebozoa, Porifera, Ascomycota, Platyhelminthes, Tracheophyta, Annelida, and

Chaetognatha counted for 3,838 reads (27.31%). While 3,838 reads were assigned to unclassified phyla or unknown, Figure 2.

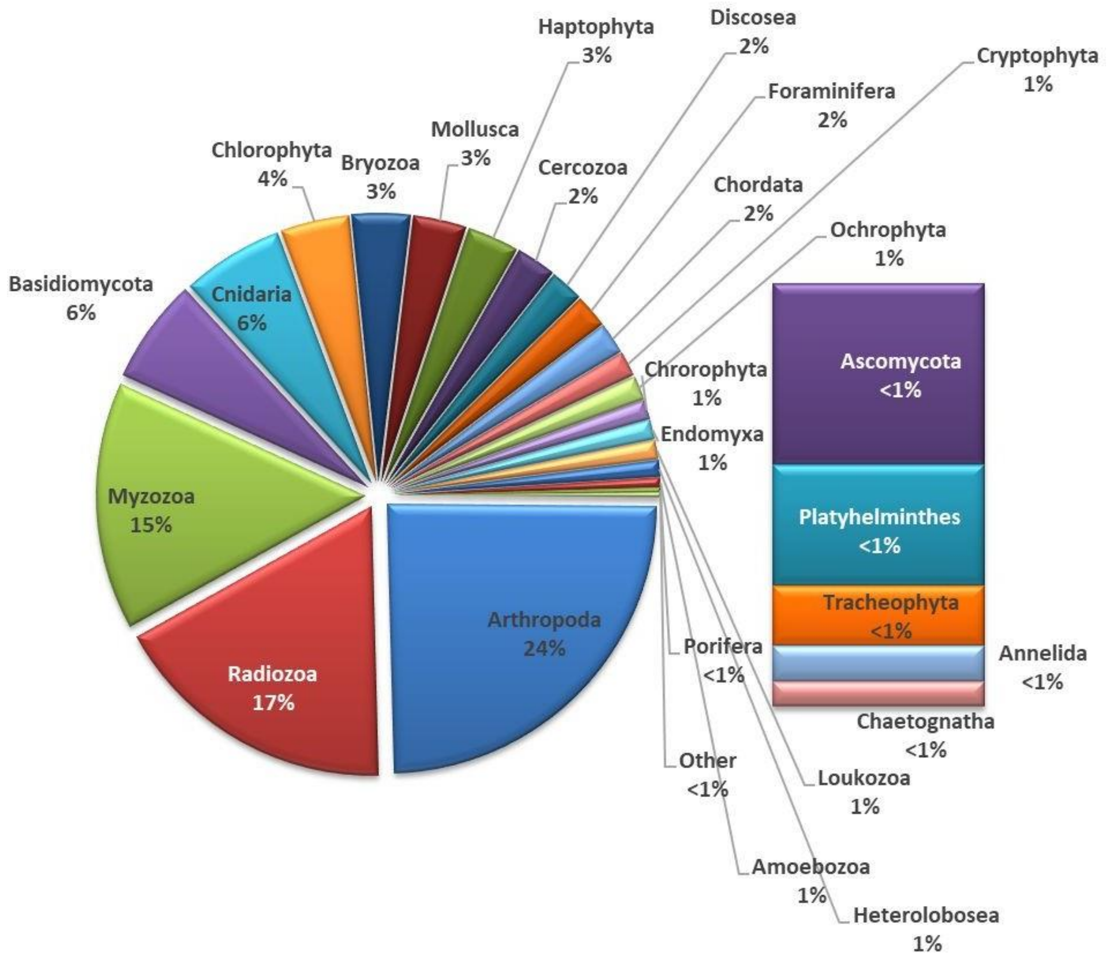


Figure 2. Composition and relative abundance of planktons at phylum level identified from Tawang Bay, a natural settlement habitat of spiny lobster larvae.

Ordo composition

The relative abundance of taxa identified in the sample showed that Calanoida, Spumellaria, Sliphonophorae, Cylopoida, and syndiniales were the five most abundant orders found in the samples, Figure 3. The Calanoida was counted for 2,014 reads (14.33%), followed by Spumellaria with 1,947 reads (13.85%), Siphonophorae with 981 reads (6.98%), Cylopoida with 789 reads (5.61%), and syndiniales with 487 reads (3.46%). The other orders and abundance were presented in Figure 3. In addition, the present study also obtained at least 1,753 reads or 12.47 % which were assigned into unclassified or unknown orders.

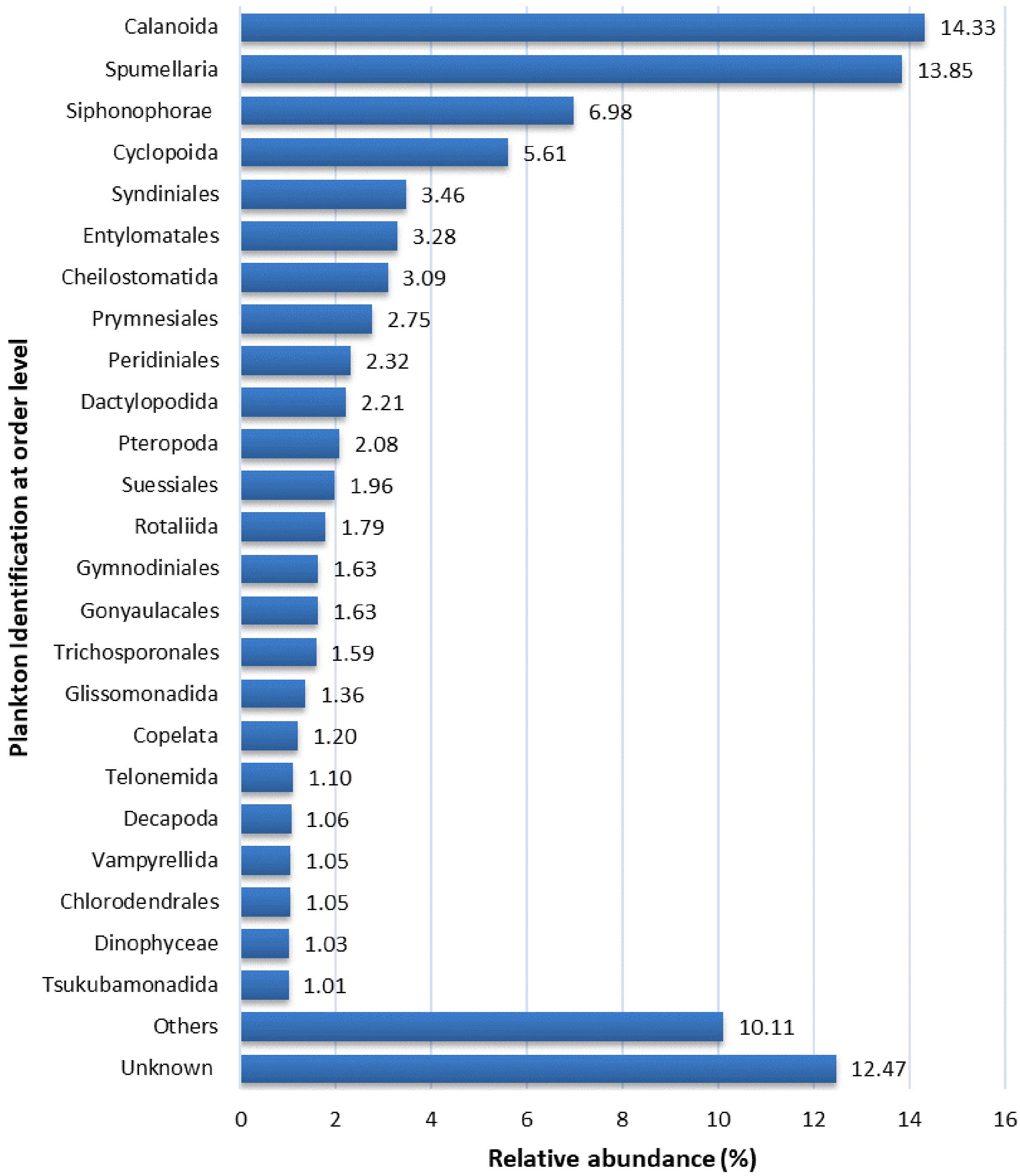
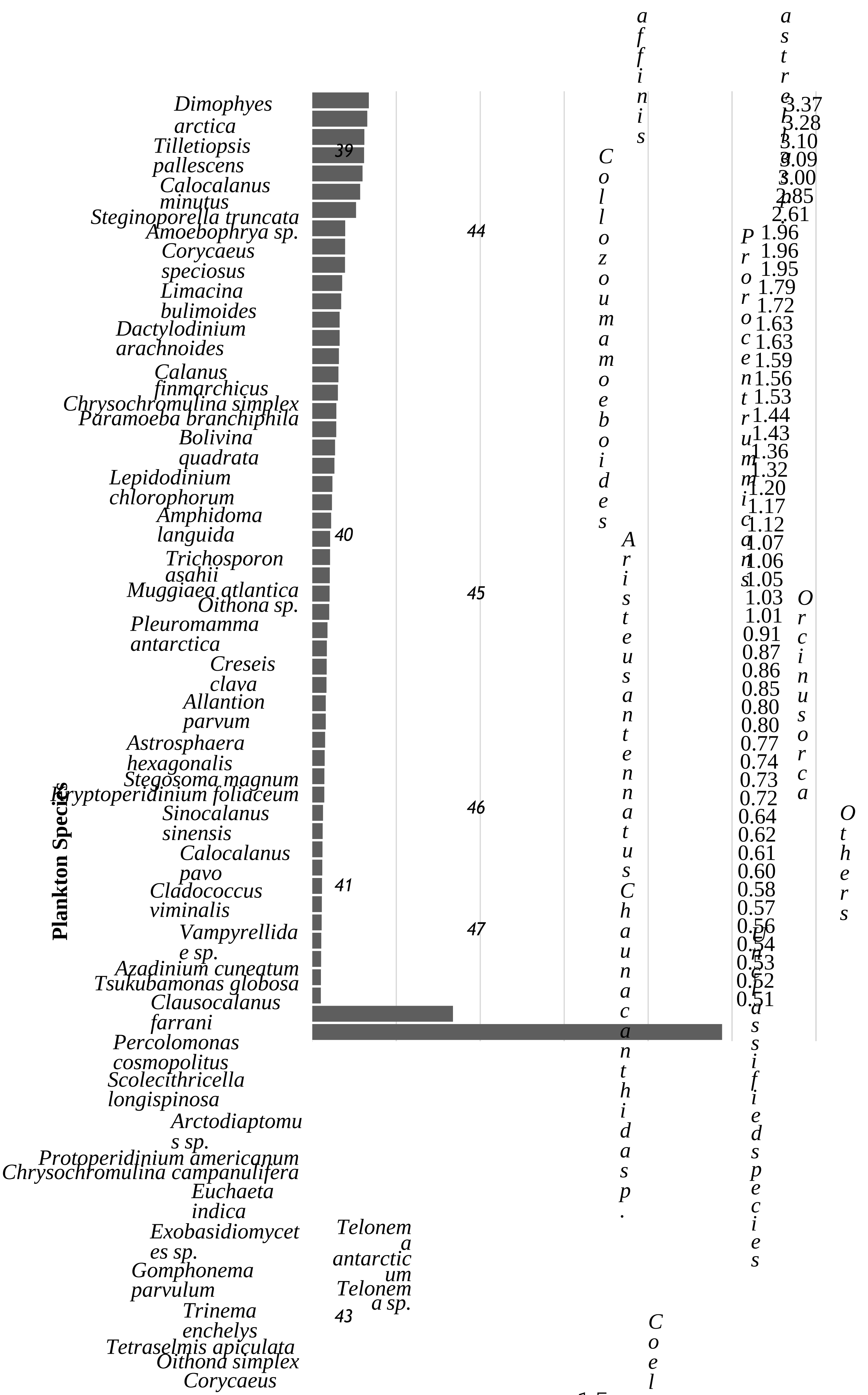


Figure 3. Relative abundance of Planktons identified from Tawang Bay, a natural

satellement habitat of spiny lobster larvae in East Java Indonesia, presented at the order level.

Species composition

At the species level, a total of 97 plankton species were identified from the Tawang Bay habitat, of which the ten most abundant species were *Dimophyes arctica* with 474 reads (3.37%), *Tilletiopsis pallescens* with 461 reads (3.28%), *Calocalanus minutus* with 463 reads (3.10%), *Steginoporella truncate* with 434 reads (3.09%), *Amoebophrya* sp. with 421 reads (3.00%), *Corycaeus speciosus* with 401 reads (2.85%), *Limacina bulimoides* with 367 reads (2.61%), *Dactylodinium arachnoides* with 276 reads (1.96%), *Calanus finmarchicus* with 275 reads (1.96%), and *Chrysochromulina simplex* with 251 reads (1.95%). The other 87 species with their relative abundances were presented in Figure 4. The present result also indicated that a total of 3.431 reads (24.41%) were assigned into unclassified species.


Plankton Species
Dimophyes arctica
Tilletiopsis pallescens
Calocalanus minutus
Steginoporella truncata
Amoebophrya sp.
Corycaeus speciosus
Limacina bulimoides
Dactylodinium arachnoides
Calanus finmarchicus
Chrysochromulina simplex
Paramoeba branchiphila
Bolivina quadrata
Lepidodinium chlorophorum
Amphidoma languida
Trichosporon asahii
Muggiaea atlantica
Oithona sp.
Pleuromamma antarctica
Creseis clava
Allantion parvum
Astrosphaera hexagonalis
Stegosoma magnum
Kryptoperidinium foliaceum
Sinocalanus sinensis
Calocalanus pavo
Cladococcus viminalis
Vampyrellidae sp.
Azadinium cuneatum
Tsukubamonas globosa
Clausocalanus farrani
Percolomonas cosmopolitus
Scolecithricella longispinosa
Arctodiaptomus sp.
Protoperidinium americanum
Chrysochromulina campanulifera
Euchaeta indica
Exobasidiomycetes sp.
Gomphonema parvulum
Trinema enchelys
Tetraselmis apiculata
Oithona simplex
Corycaeus
Telonema antarcticum
Telonema sp.
Collozoum amoeboides
Aristeus antennatus
Chaunacanthida sp.
Procentrum micans
Orcinus orca
Others
Unclassified species
3.37
3.28
3.10
3.09
3.00
2.85
2.61
1.96
1.96
1.95
1.79
1.72
1.63
1.63
1.59
1.56
1.53
1.44
1.43
1.36
1.32
1.20
1.17
1.12
1.07
1.06
1.05
1.03
1.01
0.91
0.87
0.86
0.85
0.80
0.80
0.77
0.74
0.73
0.72
0.64
0.62
0.61
0.60
0.58
0.57
0.56
0.54
0.53
0.52
0.51

8.38

24.41

0.00 5.00 10.00 15.00 20.00 25.00 30.00

Relative Abundance (%)

Figure 4. Relative abundance of Plankton species identified at Tawang Bay, a natural

settlement habitat of spiny lobster larvae presented at the species level.

Diversity, uniformity, and dominant indices in terms of species

The plankton diversity index was 1.65 and considered moderate since the value was between 1 and 3. While the plankton uniformity index calculated from the sample was 0.22 and categorized as low uniformity since the value is less than 0.4. In addition, the dominant index was 0.24 and considered a low as well because the value was less than 0.5, Figure 5.

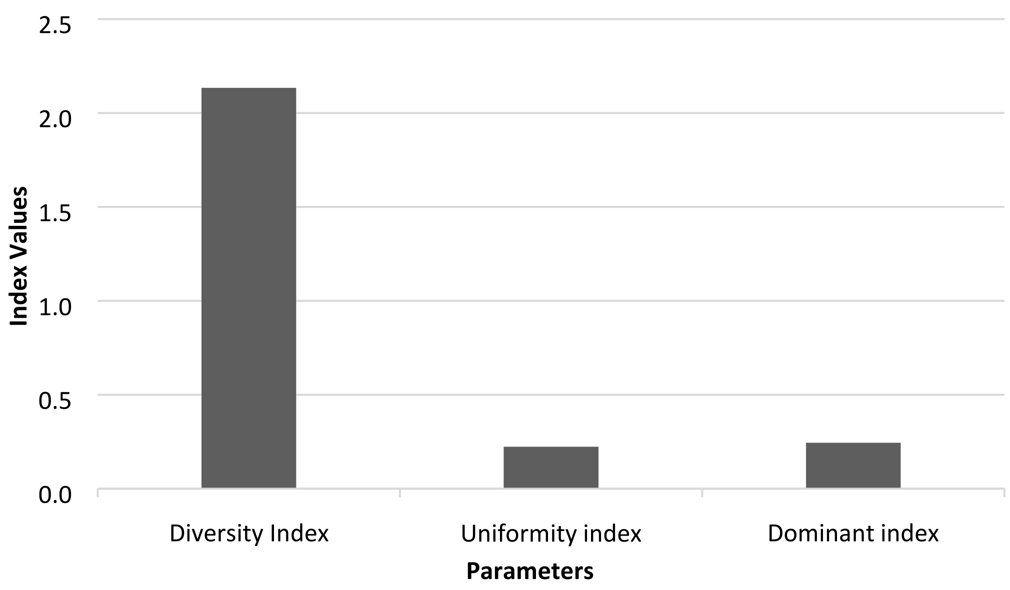


Figure 5. Diversity, uniformity, and dominant indices of plankton species identified using eDNA metabarcoding in the Tawang Bay habitat.

Discussion

Indonesia is well known for its high biodiversity including the plankton community (Amin et al. 2022b; Borbee et al. 2022). This study reported the type, abundance, diversity, uniformity, and dominant indices of plankton in Tawang Bay, one of the most common settlement habitats for spiny lobster in East Java, Indonesia to find potential live prey for aquatic organisms including lobster larvae. The result showed that there were 26 phyla, 40 classes, 54 orders, 75 families, 85 genera, and 97 plankton species.

Calculated based on species data, the diversity index of plankton at Tawang Bay was at a moderate level (Awwaluddin et al. 2017). This result suggests that the numbers of species in the selected study were quite varied, which is in line with 97 species identified from the present study. In addition, Plankton uniformity and dominant indices in the Tawang Bay habitat were 0.22, and 0.24 respectively, which are considered low (Fachrul, 2016). These results suggest plankton distribution in Tawang Bay was quite diverse and no dominant plankton species are present in the water habitat (Berger and Parker 1970). Among the identified taxa, phylum Arthropoda was in the greatest abundance, followed by Radiozoa, Myzozoa, Basidiomycota, and Cnidaria. Many members of Arthropoda are considered economically important aquatic commodities including spiny lobsters (*Panulirus* spp.), marine crabs (*Portunus* spp.), and marine shrimps (*Penaeus* spp.) (Amelia et al. 2021; Utama et al. 2021; Wiloso et al. 2022). In fact, these marine commodities are among the most economically important species for Indonesian and are valued as superior export commodities. In addition, many studies also previously reported that diverse members of Arthropoda have been revealed to play critical roles ecologically in certain habitats including as pollution bioindicators and live diets for aquaculture species (Amador-Marrero et al. 2023; Amin et al. 2022d; Hirai et al. 2021).

Thisstudy identified at least 23 plankton species that belonged to the Phylum Arthropoda, of which 18 species have been documented as live prey for aquaculture species in their natural habitats. This high level of plankton biodiversity was not expected in this region but may be likely for spiny lobster larvae. Three species of marine Arthropoda (*Acartia bispinosa*, *Oithona simplex*, *Oithona* sp., *Pseudodiaptomus euryhalinus*) have been found in the stomach contents of the larvae of ornate spiny lobster, *Panulirus ornatus* (Amin et al. 2022d), which suggest that these species are live prey for the ornate spiny lobster. In addition, *Acartia* sp. has been reported as a live diet for seabass larvae, *Lates calcarifer* (Rajkumar 2006), fat snook, *Centropomus parallelus* (Barroso et al. 2013), and many other aquatic larvae (Sarkisian et al. 2019). Some studies also confirmed that these plankton species were identified in the stomach content of lobster larvae. *Acartia* sp. has also been documented to be a good live diet for aquatic larvae such as seabass, *Lates calcarifer* (Rajkumar 2006), and fat snook, *Centropomus parallelus* (Barroso et al. 2013). *Acartia clausi* has been described to have a higher protein (63.12%) and lipid content (16.65%) and is also richer in omega − 3 fatty acids (33.94%) compared to *Artemia nauplii* and rotifers (Rajkumar, 2006). In addition, a member of Acartia (*Acartia tonsa*) has previously been documented to provide an important nutritional benefit to fat snook larvae undergoing metamorphosis (Vanacor-Barroso et al. 2017).

Furthermore, *Oithona* sp., which is a marine calanoid copepod has been documented to have a high protein content, ~59.33% (Santanumurti et al. 2021). Additionally, *Oithona* sp. has been described as having a high content of fatty acid profiles including polyunsaturated fatty acids (26.47%) and omega-3 fatty acids (36.30%), which are higher than a commercial live diet such as *Artemia* sp. (Magouz et al. 2021b). Therefore, the marine copepod has been frequently used as a live diet for fish or mollusc larvae. For instance, *Oithona* sp. has been documented as a live diet of shrimp larvae (Dinesh Kumar et al. 2017), and European seabass (*Dicentrarchus labrax*) postlarvae (Magouz et al. 2021a). *Oithona* sp. has been reported from the stomach content of spiny lobsters at the early life stage (Amin et al. 2022d; Khvorov et al. 2012). Furthermore, the plankton species had also been identified in the stomach contents of the spiny lobster larvae (Amin et al. 2022b; Amin et al. 2022d).

Additionally, other plankton species detected in the study have also been reported as live prey for several marine aquatic larvae. For example, *Pseudodiaptomus* *euryhalinus* had been documented as live prey for the larvae of Pacific red snapper, *Lutjanus peru* (Amador-Marrero et al. 2023). *Calanus finmarchicus* has high lipid content (0 to 190 μg individual^{-1}) (Jónasdóttir et al. 2019). Other copepods such as *Calocalanus pavo*, and *Calocalanus minutus* had been reported as small marine copepod that were important prey for fish larvae in the Kuroshio region off southern Japan (Hirai et al. 2021). *Corycaeus affinis* as a live prey for Japanese larval and juvenile sardine, *Sardinops melanostictus*, and anchovy, *Engraulis japonicus*, in the western North Pacific (Okazaki et al. 2019), and Japanese jack mackerel (*Trachurus japonicus*) juveniles in the East China Sea (Sassa et al. 2019). Similarly, *Sinocalanus sinensis* was reported as a live prey of pipefish, which was revealed by fecal eDNA metabarcoding (Ntshudisane et al. 2021). All this information suggests that many potential live prey can be used for aquatic species, especially at larval stages. Therefore it is recommended that further studies explore the effects of isolation and culturing of this plankton with high nutrient content.

Other plankton species found in thisstudy were *Clausocalanus farrani*, *Pleuromamma antarctica*, *Scolecithricella longispinosa*, and *Corycaeus speciosus*. *Euchaeta indica and Lucicutia ovaliformis* have not been documented as live prey or components of the diets of aquaculture species. However, these species have been frequently reported in marine water including in the Western Indian Ocean and the China Seas (Al-Aidaroos et al. 2019; Shih et al. 2022). In addition, the cellular body sizes and their close relation to the other species that have been previously described might suggest that these plankton species are also potential live prey for the larvae of aquatic species including spiny lobsters. Further study is required to confirm this preliminary assumption.

Conclusion

This study identified at least 330 ASVs from water samples of Tawang bay which were assigned to 26 phyla, 40 classes, 54 orders, 75 families, 85 genera, and 97 species. Among the identified taxa, 18 species have been documented as live prey for aquaculture species in their natural habitat including *Acartia bispinosa*, *Oithona simplex*, *Oithona* sp., and *Pseudodiaptomus euryhalinus*, *Calocalanus pavo*, and *Calocalanus minutus*. The biodiversity of plankton species has often been overlooked in studies but is an important component of marine larval diets and should be considered in aquaculture. Given the high level of plankton diversity in this region, it is recommended that Indonesia should recognize the natural value of these areas.

Acknowledgments

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Conflict of Interest

Authors declare no conflict of interest

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2. Revisi (18 April 2023)

Reviewer mengirim saran yang harus diperbaiki

Dear Dr Masithah:

Your manuscript entitled "eDNA Metabarcoding Assessment on Plankton Diversity and Abundance in Tawang Bay, settlement habitat of spiny lobster in East Java, Indonesia", which you submitted to Biodiversity, has been reviewed. The reviewer comments are included in the attached file.

The reviews are in general favourable and suggest that, subject to minor revisions, your paper could be suitable for publication. Please consider these suggestions. I look forward to receiving your revised manuscript.

When you revise your manuscript please highlight the changes you make in the manuscript by using the track changes mode in MS Word or by using bold or coloured text.

To start the revision, please click on the link below:

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This will direct you to the first page of your revised manuscript. Please enter your responses to the comments made by the reviewer(s) in the space provided. You can use this space to document any changes you made to the original manuscript. Please be as specific as possible in your response to the reviewer(s).

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IMPORTANT: Your original files are available to you when you upload your revised manuscript. Please delete any redundant files before completing the submission.

Because we are trying to facilitate timely publication of manuscripts submitted to Biodiversity, your revised manuscript should be uploaded by 02-May-2023. If it is not possible for you to submit your revision by this date, we may have to consider your paper as a new submission.

Once again, thank you for submitting your manuscript to Biodiversity and I look forward to receiving your revision.

Sincerely,
Dr Trueman
Managing Editor, Biodiversity Journal

Reviewer(s)' Comments to Author:

Reviewer: 1

Comments to the Author
paper is well written with slight grammar errors

Reviewer: 2
Please see comments in the attached file.

Date Sent: 18-Apr-2023

File 1: spiny-lobster.doc

3. Submit Naskah Revisi (28 April 2023)

A. Author menjawab saran reviewer melalui system. Untuk detail revisi yang telah diperbaiki terdapat pada point B.

ACTION	STATUS	ID	TITLE	SUBMITTED	DECISIONED
	ME: Trueman, Rebecca • Accept (01-May-2023) view decision letter Contact Journal	TBID-2023-0020.R1	Spiny lobster feedinggrounds-an eDNA Metabarcoding Assessment revealsa high level of Plankton biodiversityin Tawang Bay, Indonesia View Submission	28-Apr-2023	01-May-2023

B. Artikel yang sudah direvisi

eDNA Metabarcoding Assessment on Plankton Diversity and Abundance in Tawang Bay, settlement habitat of spiny lobster in EastJava, Indonesia

Endang D. Masithah[a]*, Muhamad Amin[b], Muhammad G. Fadhilah[c], LailaMusdalifah[d], Hussein Taha[e], and Takaomi Arai[e]

[a]*Department of Marine, Faculty of Fisheries and Marine, Universitas Airlangga, Campus C UNAIR Mulyorejo, Jl. Mulyorejo Surabaya 60115 Indonesia The* [b]*Aquaculture Study Program, Department of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Campus C UNAIR Mulyorejo, Jl. Mulyorejo Surabaya 60115 Indonesia.*

[c]*Department of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga,Campus C UNAIR Mulyorejo, Jl. Mulyorejo Surabaya 60115 Indonesia.*

[d]*Research Center for Fishery, National Research and Innovation Agency, Republic ofIndonesia, Jakarta, Indonesia.*

[e]*Environmental and Life Sciences Program, Faculty of Science, Universiti BruneiDarussalam, Jalan Tungku Link, Gadong BE1410, Brunei Darussalam*

*corresponding author: endang.masithah@fpk.unair.ac.id

Provide short biographical notes on all contributors here if the journal requires them.

Spiny lobster feeding grounds- an eDNA Metabarcoding reveals ~~Assessment~~ a high level of ~~on~~ Plankton bio~~D~~diversity ~~and Abundance~~ in Tawang Bay, ~~settlement habitat of spiny lobster in East Java,~~ Indonesia

Plankton has been considered an important live diets for various marine aquatic species including spiny lobster larvae. Thus, studying the diversity and abundance of plankton taxa in the natural settlement habitat of spiny lobster larvae might reveal alternative live diets for the spiny lobster larvae. The ~~present~~ study was conducted in Tawang Bay, an important ~~one of the most common settlements~~ habitat for~~of~~ spiny lobsters in East Java, Indonesia. Plankton samples were collected using a plankton net and analyzed using eDNA metabarcoding and high-throughput sequencing. Of the collected samples, 26 phyla, 40 classes, 54 orders, 75 families, 85 genera, and 97 species were identified. Further analysis indicated that the diversity index was considered moderate as the value was 2.16. While the uniformity and dominant indices were 0.22, and 0.24 respectively, which indicate that there is no dominant plankton species in Tawang Bay.

Among the identified taxa, at least 18 species including *Acartia bispinosa*, *Oithona simplex*, *Oithona* sp., *Pseudodiaptomus euryhalinus*, *Calocalanus pavo*, and *Calocalanus minutus* are potential live diets for larval of aquaculture species including lobster larvae and, therefore should be further studied.

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Keywords: dominance; diversity;

eDNA_metabarcoding; plankton; uniformity Subject

classification codes: include these here if the journal

requires them

Introduction

Environmental conditions including physical, chemical, and biological factors in natural habitats highly determine the recruitment rates of lobster larvae (Keulder 2005; Wahle and Incze 1997; Wahle and Steneck 1991). Several authors have previously reported the physical and chemical characteristics of the natural settlement areas of lobster larvae (Amin et al. 2022a; Boudreau et al. 1992; Lillis and Snelgrove 2010). In addition, studies viewing biological aspects of settlement habitat in natural environment lobster larvae have also been done in several studies. For instance, Amin et al. (2022b) reported plankton communities in Gerupuk Bay, one of the most common settlement habitats of spiny lobster in West-Nusa Tenggara Indonesia. Similarly, Mazur et al. (2020), revealed the type and abundance of plankton in the settlement area of American lobster in the Northeast US Shelf, USA. These studies have been are conducted to get an overview ofunderstand plankton availability andor biological factors that effe habitat of lobster larvae in the wild and to find a potential candidat when lobster are grown in captivity.

Numerous Many studies have shown conclude that biological plankton availability are can be important information onfor the nat larvae (O'Rorke et al. 2014) and . Mmarine planktons are importan all most marine aquatic species including molluscs and fishes (Pan instance, the plankton *Oithona* sp. which has been previously repor in marine environments (Amin et al. 2022b), has been documented as a potential live diet for seabass, *Lates calcarifers*, larvae (Santhanam and Perumal 2012), and white leg shrimp, *Litopenaeus vannamei* (Dinesh Kumar et al. 2017). Thus, a study of planktons

that are availabilityle in a specific certain locations may be tied to the presence of reveal potential live diets for certain aquatic species which are available in thate location. Many of the past studies have been However, these studies were performed using a conventional approach, with microscope observation and phenotypic identification. According to Falciatore et al. (2020) phenotypic identification might miss identifyied or inaccurately calculated in the abundance of plankton communities that are available in a certain location. Acknowledging the importance of plankton as a live diet for various marine species, this study focuses on a a better method such as a molecular approach to cleary idenitify the genetic diversity of plankton species and their abundance.~~should be performed to get more accurate results.~~

Th~~e present~~is study ~~aimed at~~ specifically focused on assessing plankton diversity, uniformity, and dominant indices of plankton int Tawang Bay, ~~one of the most common~~ an important natural settlement habitat~~s of~~ for spiny lobster larvae in East Java, Indonesia. It ~~is assumed that~~ appears that the cnditions in Tawang Bay are ~~having~~ suitable environmental conditions as well as good diet availability for lobster larvae. In addition, the present study used environmental DNA (eDNA) metabarcoding to increase result accuracy.~~Paragraph: use this for the first paragraph in a section, or to continue after an extract.~~

Materials and Methods

Sample collection

Water samples were collected from Tawang Bay, ~~one of the most common settlement habitats of lobster larvae~~ in the East Java Province of Indonesia. More details on the sample site can be found in ~~as previously described by~~ Amin et al. (2022d). For this study, ~~In brief,~~ water samples were collected at three sampl~~ing~~e points (L1, L2, and L3) with four different depths: 0-0.30 m (Surface), 2.5 m, 5m, and 20 m using a water sampler. ~~Then, t~~The water samples were then mixed, filtered using a 355 µm mesh plankton net, and transported to the Microbiology Laboratory, Faculty of Fisheries and

Marine Science, in a cold box. Thereafter, the homogenized water samples were filteredusing Whatman no 5-filter paper and stored in a -20°C freezer until further analysis.

Commented [RT2]: At what university? Universitas Airlangga?

DNA Extraction

DNA of plankton was extracted using ZymoBIOMICS™ DNA Miniprep Kit (D4300T)according to Amin et al. (2022c) with slight modifications. ~~In brief,~~ The filter paper was cut into sections~~pieces~~ with ~~a~~ sterile scisors, and added to ZRbashingBead™ Lysis Tubes (0.1 and 0.5 mm) followed by the addition of 750 µl ZymoBIOMICS™ Lysis solution. While the rest of the steps were performed according to the instruction manual of the ZymoBIOMICS™ DNA Miniprep Kit. Then, the quality of the DNA extract was measured using a nanodrop and afterward was adjusted at 30ng/µl before sending for sequencing. The DNA samples were sent to a service provider (1st Base) for amplification and next-generation sequencing. Bioinformatics analysis was performed as previously described by Amin et al. (2022d).

Data analysis

The type, abundance, diversity, uniformity, and dominance index of plankton species identified in the water samples were calculated according to these formulas:

$$H' = -\sum Pi \ln Pi, \quad where\ Pi = \frac{ni}{N}$$ (Fachrul, 2007)

$$E = \frac{H'}{Ln s}$$ (Odum 1971)

$$d = \frac{Nmax}{N}$$ (Berger dan Parker, 19

Where: H' is the Diversity index, H'is Shannon Wiener Diversity Index, ni is the number of individuals of the i-th species, "n" is the number of species, N is the total individual number, and Pi is the number of individuals of the i-th species, "E" is uniformity index, H': Shannon Wiener Diversity Index, "S" is the Total number of species. d is the Simpson Dominance Index, "Nmax" is the most abundant number of individual species, and N is the total individual number.

RESULTS

Overview and taxa detected

The According to the results with eDNA metabarcoding results showed that , a total of 45,978 raw paired-end readings were obtained from three pooled water samples and became 42,135 readings after filtering, and 34,127 rarified reads. The 34,127 readings were assigned to 330 amplicon sequence variants (ASVs). Using the National Center for Biotechnology Information (NCBI) database, the 330 ASVs were assigned to 26 phyla, 40 classes, 54 orders, 75 families, 85 genera, and 97 species, Figure 1.

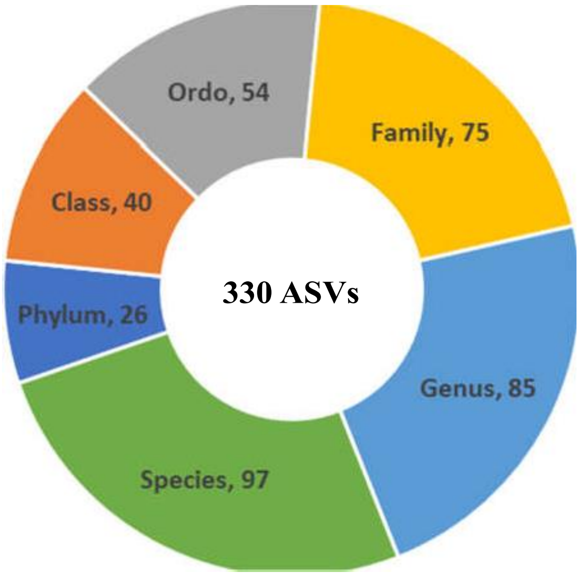

Figure 1. The number of Phyla, Classes, Orders, Families, Genera, and species from the natural settlement habitat of lobster larvae identified using eDNA metabarcoding.

Phylum composition

In terms of numbers, a total of 26 phyla were identified from water samples collected from Tawang Bay, East Java Indonesia. The five most abundant phyla were Arthropoda, Radiozoa, Myzozoa, Basidiomycota, and Cnidaria. Phylum Arthropoda was counted for 3,042 sequence reads (21.64%), followed by Radiozoa with 2,160 reads (15.37%),

Myzozoa with 1,853 reads (13.18%), Basidiomycota with 788 reads (5.61%), and Cnidaria with 765 reads (5.61%). The other 21 phyla including were Chlorophyta, Bryozoa, Mollusca, Haptophyta, Cercozoa, Discosea, Foraminifera, Chordata, Cryptophyta, Ochrophyta, Chrorophyta, Endomyxa, Loukozoa, Heterolobosea, Amoebozoa, Porifera, Ascomycota, Platyhelminthes, Tracheophyta, Annelida, and

Chaetognatha counted for 3,838 reads (27.31%). While 3,838 reads were assigned tounclassified phyla or unknown, Figure 2.

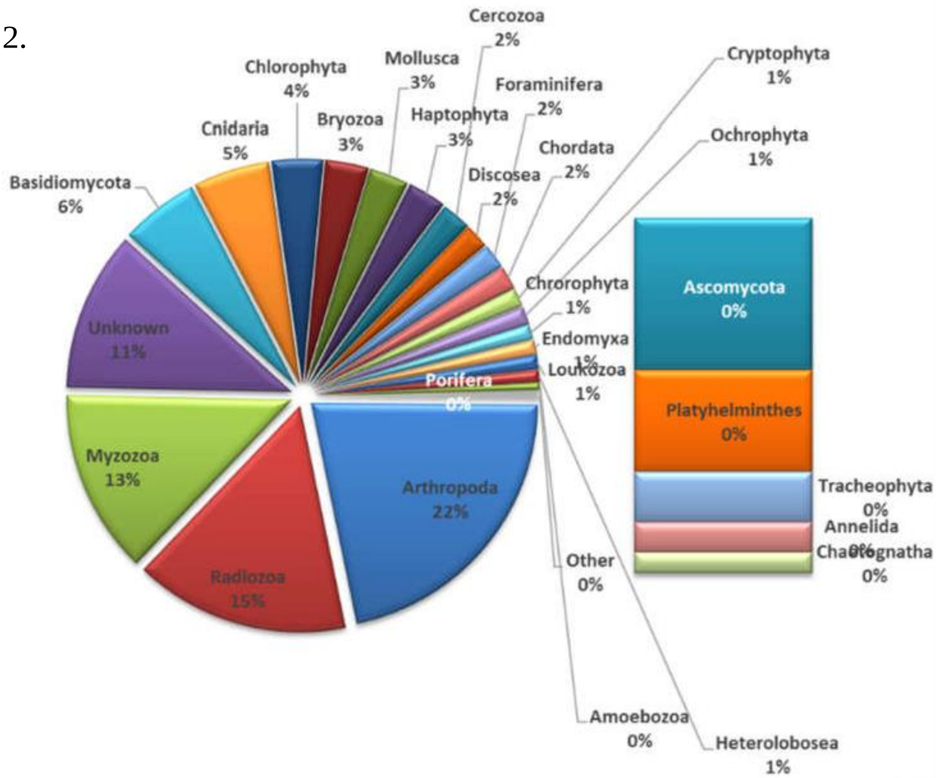

Figure 2. Composition and relative abundance of planktons at phylum level identifiedfrom Tawang Bay, a natural settlement habitat of spiny lobster larvae.

Ordo composition

The relative abundance of taxa identified in the sample showed that Calanoida, Spumellaria, Sliphonophorae, Cylopoida, and syndiniales were the five most abundant orders found in the samples, Figure 3. The Calanoida was counted for 2,014 reads (14.33%), followed by Spumellaria with 1,947 reads (13.85%), Siphonophorae with 981

reads (6.98%), Cylopoida with 789 reads (5.61%), and syndiniales with 487 reads

(3.46%). The other orders and abundance were presented in Figure 3. In addition, the present study also obtained at least 1,753 reads or 12.47 % which were assigned into unclassified or unknown orders.

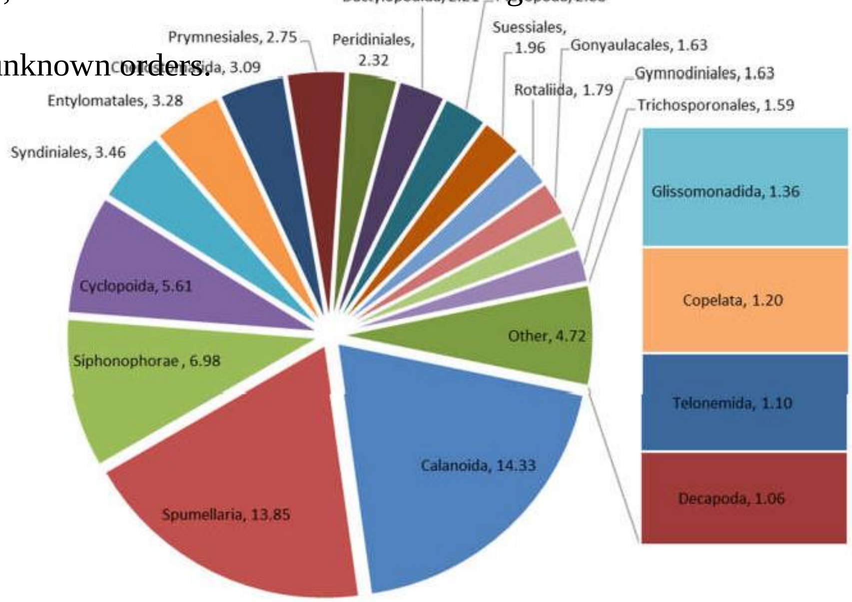

Figure 3. Relative abundance of Planktons identified from Tawang Bay, a natural satellement habitat of spiny lobster larvae in East Java Indonesia, presented at the order level.

Species composition

At the species level, a total of 97 plankton species were identified from the Tawang Bay habitat, of which the ten most abundant species were *Dimophyes arctica* with 474 reads (3.37%), *Tilletiopsis pallescens* with 461 reads (3.28%), *Calocalanus minutus* with 463 reads (3.10%), *Steginoporella truncate* with 434 reads (3.09%), *Amoebophrya* sp. with 421 reads (3.00%), *Corycaeus speciosus* with 401 reads (2.85%), *Limacina bulimoides*

with 367 reads (2.61%), *Dactylodinium arachnoides* with 276 reads (1.96%), *Calanus* *finmarchicus* with 275 reads (1.96%), and *Chrysochromulina simplex* with 251 reads (1.95%). The other 87 species with their relative abundances were presented in Figure 4. The present result also indicated that a total of 3.431 reads (24.41%) were assigned into unclassified species.

Plankton Species

Dimophyes arctica
Tilletiopsis pallescens
Calocalanus minutus
Steginoporella truncata
Amoebophrya sp.
Corycaeus speciosus
Limacina bulimoides
Dactylodinium arachnoides
Calanus finmarchicus
Chrysochromulina simplex
Paramoeba branchiphila
Bolivina quadrata
Lepidodinium chlorophorum
Amphidoma languida
Trichosporon asahii
Muggiaea atlantica
Oithona sp.
Pleuromamma antarctica
Reseisclava
Allantion parvum
Astrosphaer
Grahexagonalis
Stegosoma magnum
Kryptoperidinium foliaceum
Sinocalanus sinensis
Calocalanus pavo
Cladococcus viminalis
Vampyrellidae sp.
Azadinium cuneatum
Tsukubamonas globosa
Clausocalanus farrani
Percolomonas cosmopolitus
Scolecithricella longispinosa
Arctodiaptomus sp.
Protoperidinium americanum
Chrysochromulina campanulifera
Euchaeta indica
Exobasidiomycetes sp.
Gomphonema parvulum
Trinema enchelys
Tetraselmis apiculata
Oithona simplex
Corycaeus affinis
Collozoum amoeboides
Aristeus antennatus
Chaunacanthida sp.
Telonema antarcticum
Telonema sp.
Coelastrella sp.
Prorocentrum micans
Orcinus orca
Others
Unclassified species

8.
38

3.37
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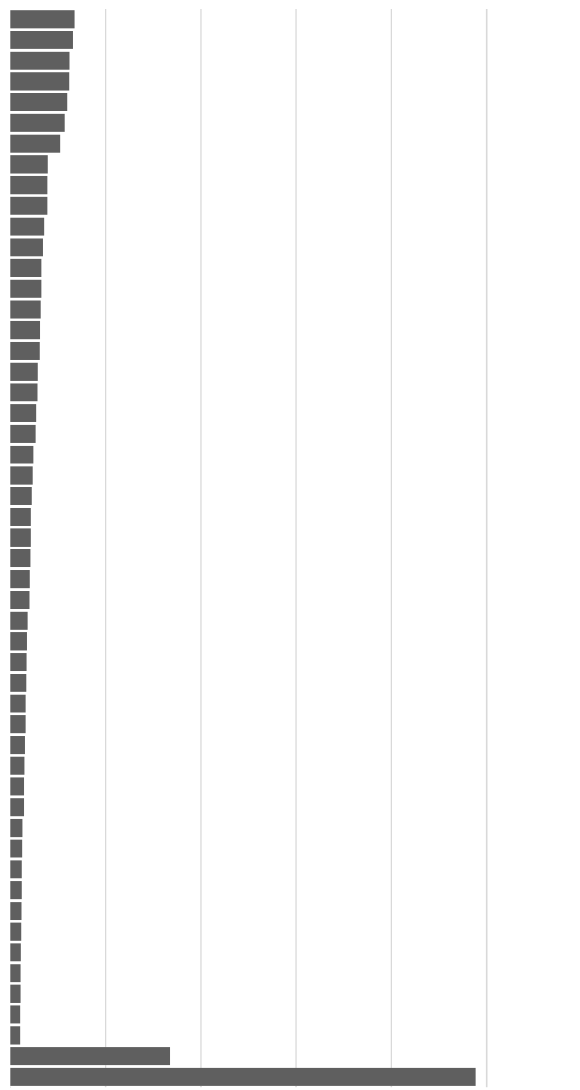
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Relative Abundance (%)

Figure 4. Relative abundance of Plankton speciess identified at Tav settlement habitat of spiny lobster larvae presented at the species le

Diversity, uniformity, and dominant indices in terms of species

The plankton diversity index was 1.65 and considered moderate since the value was between 1 and 3. While the plankton uniformity index calculated from the sample was 0.22 and categorized as low uniformity since the value is less than 0.4. In addition, the dominant index was 0.24 and considered a low as well because the value was less than 0.5, Figure 5.

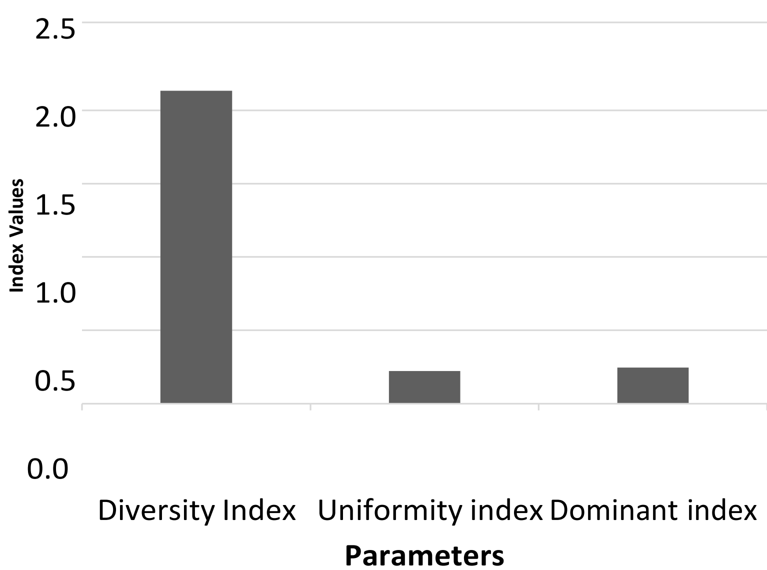

Figure 5. Diversity, uniformity, and dominant indices of plankton species identified using eDNA metabarcoding in the Tawang Bay habitat.

Discussion

Indonesia is well known for its high biodiversity including the plankton community (Amin et al. 2022b; Borbee et al. 2022). This~~e present~~ study reported the type, abundance, diversity, uniformity, and dominant indices of plankton in~~at~~ Tawang Bay, one of the most common settlement habitats for spiny lobster in Eas

find potential live prey for aquatic organisms including lobster larvae. The result showed that there were 26 phyla, 40 classes, 54 orders, 75 families, 85 genera, and 97 plankton species. Calculated based on species data, the diversity index of plankton at Tawang Bay was at a moderate level (Awwaluddin et al. 2017). This result suggests that the numbers of species in the selected study were quite varied, which is in line with 97 species identified from the present study. In addition, Plankton uniformity and dominant indices in the Tawang Bay habitat were 0.22, and 0.24 respectively, which are considered low (Fachrul, 2007). These results suggest plankton distribution in Tawang Bay was quite diverse and no dominant plankton species are present in the water habitat (Berger and Parker 1970). Among the identified taxa, phylum Arthropoda was in the

~~highest~~ greatest abundance, followed by Radiozoa, Myzozoa, Basid

Cnidaria. Many members of Arthropoda are considered economically important aquatic commodities including spiny lobsters (*Panulirus* spp.), marine crabs (*Portunus* spp.), and marine shrimps (*Penaeus* spp.) (Amelia et al. 2021; Utama et al. 2021; Wiloso et al. 2022). In fact, these marine commodities are among the most economically important species ~~and become~~for

Indonesian and are valued as superior export commodities. In addition, many studies also previously reported that diverse members of Arthropoda have been revealed to play critical roles ecologically in certain habitats including as pollution bioindicators and live diets for aquaculture species (Amador-Marrero et al. 2023; Amin et al. 2022d; Hirai et al. 2021).

This~~e present~~ study identified at least 23 plankton species that belonged to the Phylum Arthropoda, of which 18 species have been documented as live prey for aquaculture species in their natural habitat. This high level of plankton biodiversity was not expected in this region but may be likely for spiny lobster larvae. ~~For instance~~, tThree species of marine Arthropoda (*Acartia bispinosa*, *Oithona si*

Pseudodiaptomus euryhalinus) have been found in the stomach content of ornate spiny lobster, *Panulirus ornatus*, larvae (Amin et al. 2022d), which may surely suggest that these species are live prey for the ornate spiny lobster. In addition, *Acartia* sp. could possess a live diet for seabass larvae, *Lates calcarifer* (Rajkumar 2006), fat snook, *Centropomus parallelus* (Barroso et al. 2013), and many other aquatic larvae (Sarkisian et al. 2019). Some studies also confirmed that these plankton species were identified in the content stomach content of lobster larvae. *Acartia* sp. has also been documented to be a good live diet for aquatic larvae such as seabass larvae, *Lates c*

2006), and fat snook, *Centropomus parallelus* (Barroso et al. 2013). *Acartia clausi* has been described to have a higher protein contents of proteins (63.12%) and lipids content (16.65%) and is also richer in omegan − 3 fatty acids (33.94%) than rotifers (Rajkumar, 2006). In addition, a member of Acartia (*Acarti*

previously d been documented to provide an important nutritional b

larvae undergoing metamorphosis (Vanacor-Barroso et al. 2017).

Furthermore, *Oithona* sp., which is a marine calanoid copepod has been documented to have a high protein content, ~59.33% (Santanumurti et al. 2021). Additionally, *Oithona* sp.

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has been described as having a high content of fatty acid profiles including polyunsaturated fatty acids (26.47%) and omega-3 fatty acids (36.30), which are higher than a commercial live diet such as *Artemia* sp. (Magouz et al. 2021b). Therefore, the marine copepod has been frequently used as a live diet for fish or mollusc larvae. For instance, *Oithona* sp. has been documented as a live diet of shrimp larvae (Dinesh Kumar et al. 2017), and European seabass (*Dicentrarchus labrax*) postlarvae (Magouz et al. 2021a). *Oithona* sp. has been reported from the stomach content of spiny lobsters at the early life stage (Amin et al. 2022d; Khvorov et al. 2012).

Furthermore, the plankton species have also been identified in spiny lobster larvae's stomach content (Amin et al. 2022b; Amin et al. 2022d).

Additionally, other plankton species detected in the present st

been reported as live prey for several marine aquatic larvae. For example, *Pseudodiaptomus euryhalinus* have been documented as live prey of Pacific Red Snapper, *Lutjanus peru*, larvae (Amador-Marrero et al. 2023). *Calanus finmarchicus* has high lipid content (0 to 190 μg individual^{-1}) (Jónasdóttir et al. 2019). Other copepods such as *Calocalanus pavo*, and *Calocalanus minutus* has been reported as small marine copepod which is important prey for fish larvae in the Kuroshio region off southern Japan (Hirai et al. 2021). *Corycaeus affinis* as live prey for Japanese larval and juvenile sardine, *Sardinops melanostictus*, and anchovy, *Engraulis japonicus*, in the western North Pacific (Okazaki et al. 2019), and Japanese jack mackerel (*Trachurus japonicus*) juveniles in the East China Sea (Sassa et al. 2019). Similarly,

Sinocalanus sinensis was reported as live prey of pipefish revealed by fecal eDNA metabarcoding (Ntshudisane et al. 2021). All this information suggestsed that many

potential live preys can be developed used for aquatic species, especially at larval stages. Therefore it is recommended that ~~Thus,~~ further studies explore ~~such as the~~the effects of isolation and culturing~~e~~ of this plankton with high nutrient content.~~ are highly recommended for future studies.~~

Other plankton species found in this~~e present~~ study are *Clausocalanus farrani*, *Pleuromamma antarctica*, *Scolecithricella longispinosa*, and *Corycaeus speciosus*. *Euchaeta indica* and~~,~~ *Lucicutia ovaliformis* have not been documented as live prey or components of the diets of aquaculture species. However, these species have been frequently reported in marine water including in the Western Indian Ocean and the China Seas (Al-Aidaroos et al. 2019; Shih et al. 2022). In addition, the body size and closely related taxa to the previously described species above, might suggest that these plankton species are also potential live prey for larvae of aquatic species including spiny lobsters. Further study is required to Somehow, more studies should be conducted to confirm this preliminary assumption.

Conclusion

The present is study identified at least 330 ASVs from water samples of Tawang bay which were assigned to 26 phyla, 40 classes, 54 orders, 75 families, 85 genera, and 97 species. Among the identified taxa, 18 species have been documented as live prey for aquaculture species in their natural habitat including *Acartia bispinosa*, *Oithona simplex*, *Oithona* sp., and *Pseudodiaptomus euryhalinus*, *Calocalanus pavo*, and *Calocalanus minutus*. Thus, isolation and culture of these species for a live diet of aquatic species including spiny lobster larvae are highly recommended for future studies. The biodiversity of plankton species has often been overlooked in studies but is an important component of marine larval diets and should be considered in aquaculture.

Given the high level of plankton diversity in this region, it is recommended that Indonesia should recognize the natural value of these areas.

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Acknowledgments

The authors thank all colleges at the Faculty of Fisheries and Marine, Universitas Airlangga, who had kindly provided technical advice during the experiment. This research was supported financially by Universitas Airlangga under Grant No.799/UN3.15/PT/2021.

Conflict of Interest

Authors declare no conflict of interest

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4. Accepted (1 Mei 2023)

A. Editor memberi keputusan artikel untuk diterima. Naskah artikel yang telah diterima terdapat terdapat pada point B

Preview (TBID-2023-0020.R1)

From: rtrueman@biodiversityconservancy.org
To: endang_dm@fpk.unair.ac.id
CC: rtrueman@biodiversityconservancy.org
Subject: Biodiversity - Decision on Manuscript ID TBID-2023-0020.R1
Body: 01-May-2023

Dear Dr Masithah:

Ref: Spiny lobster feeding grounds- an eDNA Metabarcoding Assessment reveals a high level of Plankton biodiversity in Tawang Bay, Indonesia

Our reviewers have now considered your paper and have recommended publication in Biodiversity. Congratulations! We are pleased to accept your paper in its current form. Your paper will now be forwarded to the publisher for copy editing and typesetting. The reviewer comments are included at the bottom of this letter.

You will receive proofs for checking, and instructions for transfer of copyright in due course.

The publisher also requests that proofs are checked through the publisher's tracking system and returned within 48 hours of receipt.

Thank you for your contribution to Biodiversity and we look forward to receiving further submissions from you.

Sincerely,
Dr. Trueman
Managing Editor, Biodiversity Journal

Reviewer(s)' Comments to Author:

Date Sent: 01-May-2023

B. Artikel yang telah diterima

Spiny lobster feeding grounds- an eDNA Metabarcoding Assessment reveals a high level of Plankton biodiversity in Tawang Bay, Indonesia

Journal:	*Biodiversity*
Manuscript ID	TBID-2023-0020.R1
Manuscript Type:	Article
Keywords:	diversity, eDNA, plankton, feeding_grounds, lobster

Spiny lobster feeding grounds- an eDNA Metabarcoding Assessment reveals a high level of Plankton biodiversity in Tawang Bay, Indonesia

Plankton has been considered an important live diet for various marine aquatic species including spiny lobster larvae. Thus, studying the diversity and abundance of plankton taxa in the natural settlement habitat of spiny lobster larvae might reveal alternative live diets for the spiny lobster larvae. The study was conducted in Tawang Bay, an important habitat for spiny lobsters in East Java, Indonesia. Plankton samples were collected using a plankton net and analyzed using eDNA metabarcoding and high-throughput sequencing. Of the collected samples, 26 phyla, 40 classes, 54 orders, 75 families, 85 genera, and 97 species were identified. Further analysis indicated that the diversity index was considered moderate as the value was 2.16. While the uniformity and dominant indices were 0.22, and 0.24 respectively, which indicate that there is no dominant plankton species in Tawang Bay. Among the identified taxa, at least 18 species including *Acartia bispinosa*, *Oithona simplex*, *Oithona* sp., *Pseudodiaptomus euryhalinus*, *Calocalanus pavo*, and *Calocalanus minutus* are potential live diets for larval of aquaculture species including lobster larvae andtherefore should be further studied.

Keywords: dominance; diversity; eDNA_metabarcoding; plankton; uniformity

Introduction

Environmental conditions including physical, chemical, and biological factors in natural habitats highly determine the recruitment rates of lobster larvae (Keulder 2005; Wahle and Incze 1997; Wahle and Steneck 1991). Several authors have previously reported the physical and chemical characteristics of the natural settlement areas of lobster larvae (Amin et al. 2022a; Boudreau et al. 1992; Lillis and Snelgrove 2010). In addition, studies viewing biological aspects of settlement habitat in natural environment lobster larvae have also been done in several studies. For instance, Amin et al. (2022b) reported plankton communities in Gerupuk Bay, one of the most common settlement habitats of spiny lobster in West-Nusa Tenggara Indonesia. Similarly, Mazur et al. (2020), revealed the type and abundance of plankton in the settlement area of American lobster in the Northeast US Shelf, USA. These studies have been conducted to understand plankton availability and biological factors that effect the settlement habitat of lobster larvae in the wild and to find candidates for live diets when lobster are grown in captivity.

Numerous studies have shown that biological factors such as plankton availability are important for the natural diet of lobster larvae (O'Rorke et al. 2014) and marine plankton are important for most marine species including molluscs and fishes (Pan et al. 2022). For instance, the plankton *Oithona* sp. which has been previously reported to be abundance in marine environments (Amin et al. 2022b), has been documented as a potential live diet for seabass, *Lates calcarifers*, larvae (Santhanam and Perumal 2012), and white leg shrimp, *Litopenaeus vannamei* (Dinesh Kumar et al. 2017). Thus, a study of plankton availability in a specific locations may be tied to the presence of certain aquatic species in that location. Many of the past studies have been performed using a conventional approach, with microscope observation and phenotypic identification. According to Falciatore et al. (2020) phenotypic identification might misidentify or inaccurately calculate the abundance of plankton in a certain location. Acknowledging the importance of plankton as a live diet for various marine species, this study focuses on a molecular approach to cleary idenitify the genetic diversity of plankton species and their abundance.

Thisstudy specifically focused on assessing plankton diversity, uniformity, and dominant indices of plankton in Tawang Bay, an important natural settlement habitat for spiny lobster larvae in East Java, Indonesia. It appears that the cnditions in Tawang Bay are suitable environmental conditions as well as good diet availability for lobster larvae.

In addition, the present study used environmental DNA (eDNA) metabarcoding to increase result accuracy.

Materials and Methods

Sample collection

Water samples were collected from Tawang Bay, in the East Java Province of Indonesia. More details on the sample site can be found in Amin et al. (2022d). For this study, water samples were collected at three sample points (L1, L2, and L3) with four different depths: 0-0.30 m (Surface), 2.5 m, 5m, and 20 m using a water sampler. The water samples were then mixed, filtered using a 355 µm mesh plankton net, and transported to the Microbiology Laboratory, Faculty of Fisheries and Marine Science, Universitas Airlangga in a cold box. Thereafter, the homogenized water samples were filtered using Whatman no 5-filter paper and stored in a -20°C freezer until further analysis.

DNA Extraction

DNA of plankton was extracted using ZymoBIOMICS™ DNA Miniprep Kit (D4300T) according to Amin et al. (2022c) with slight modifications. The filter paper was cut into sections with sterile scisors and added to ZRbashingBead™ Lysis Tubes (0.1 and 0.5 mm) followed by the addition of 750 µl ZymoBIOMICS™ Lysis solution. While the rest of the steps were performed according to the instruction manual of the ZymoBIOMICS™ DNA Miniprep Kit. Then, the quality of the DNA extract was measured using a nanodrop and afterward was adjusted at 30ng/µl before sending for sequencing. The DNA samples were sent to a service provider (1st Base) for amplification and next-generation sequencing. Bioinformatics analysis was performed as previously described by Amin et al. (2022d).

Data analysis

The type, abundance, diversity, uniformity, and dominance index of plankton species identified in the water samples were calculated according to these formulas:

$$H' = -\sum \text{Pi} \ln \text{Pi}, \; where \; \text{Pi} = \frac{ni}{N}$$ (Fachrul, 2016)

(Odum 1971)

$$\text{E} = \frac{\text{H}'}{\text{Ln s}}$$

$$\text{d} = \frac{\text{Nmax}}{\text{N}}$$ (Berger dan Parker, 1970)

Where: H' is the Diversity index, H'is Shannon Wiener Diversity Index, ni is the number of individuals of the i-th species, "n" is the number of species, N is the total individual number, and Pi is the number of individuals of the i-th species, "E" is uniformity index, H': Shannon Wiener Diversity Index, "S" is the Total number of species. d is the Simpson Dominance Index, "Nmax" is the most abundant number of individual species, and N is the total individual number.

RESULTS

Overview and taxa detected

The eDNA metabarcoding results showed that a total of 45,978 raw paired-end readings were obtained from three pooled water samples and became 42,135 readings after filtering, and 34,127 rarified reads. The 34,127 readings were assigned to 330 amplicon sequence variants (ASVs). Using the National Center for Biotechnology Information (NCBI) database, the 330 ASVs were assigned to 26 phyla, 40 classes, 54 orders, 75 families, 85 genera, and 97 species, Figure 1.

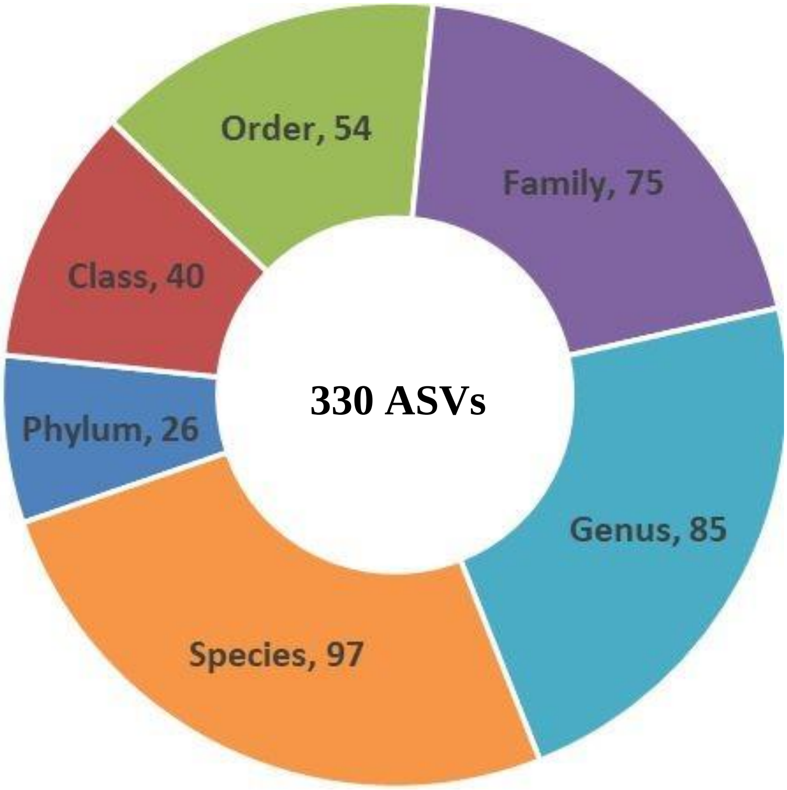


Figure 1. The number of Phyla, Classes, Orders, Families, Genera, and species from the natural settlement habitat of lobster larvae identified using eDNA metabarcoding.

Phylum composition

In terms of numbers, a total of 26 phyla were identified from water samples collected from Tawang Bay, East Java Indonesia. The five most abundant phyla were Arthropoda, Radiozoa, Myzozoa, Basidiomycota, and Cnidaria. Phylum Arthropoda was counted for 3,042 sequence reads (21.64%), followed by Radiozoa with 2,160 reads (15.37%), Myzozoa with 1,853 reads (13.18%), Basidiomycota with 788 reads (5.61%), and Cnidaria with 765 reads (5.61%). The other 21 phyla including were Chlorophyta, Bryozoa, Mollusca, Haptophyta, Cercozoa, Discosea, Foraminifera, Chordata, Cryptophyta, Ochrophyta, Chrorophyta, Endomyxa, Loukozoa, Heterolobosea, Amoebozoa, Porifera, Ascomycota, Platyhelminthes, Tracheophyta, Annelida, and

Chaetognatha counted for 3,838 reads (27.31%). While 3,838 reads were assigned to unclassified phyla or unknown, Figure 2.

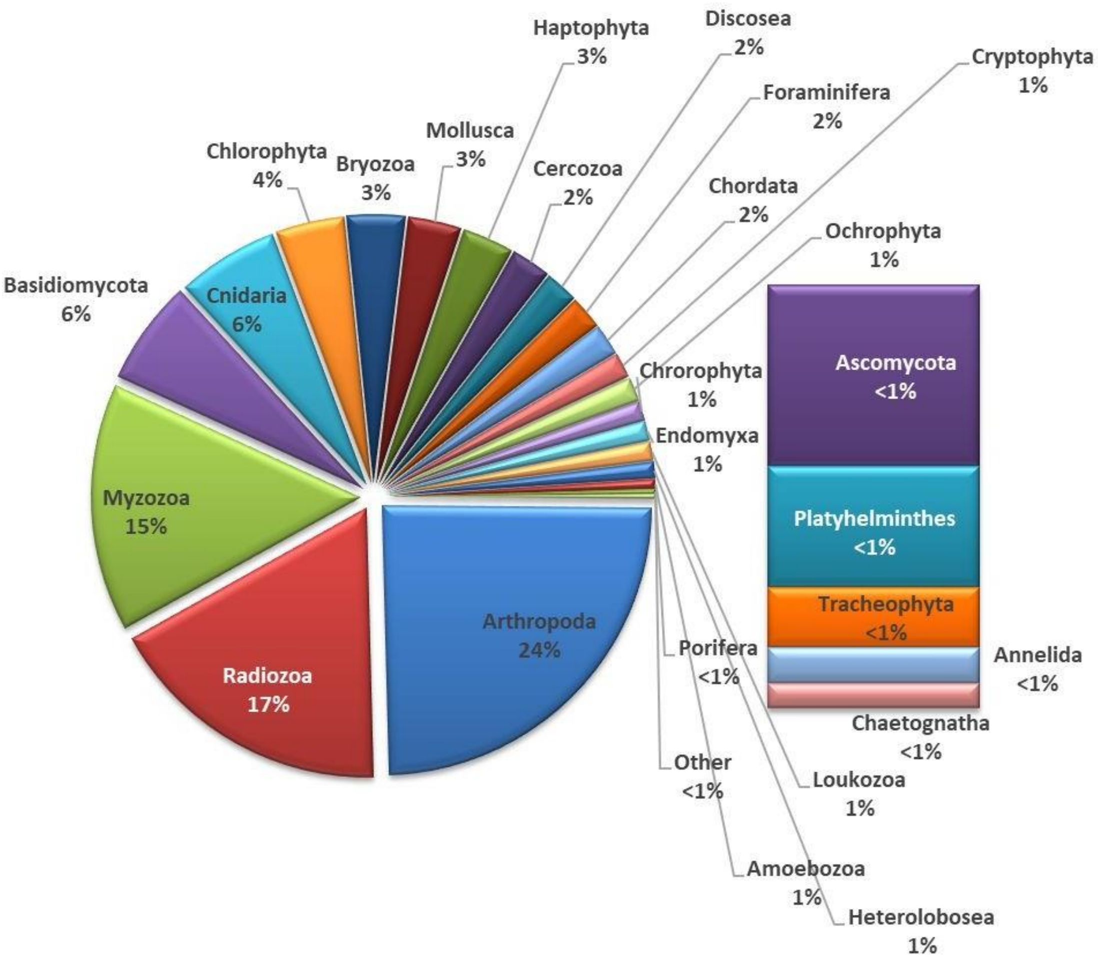


Figure 2. Composition and relative abundance of planktons at phylum level identified from Tawang Bay, a natural settlement habitat of spiny lobster larvae.

Ordo composition

The relative abundance of taxa identified in the sample showed that Calanoida, Spumellaria, Sliphonophorae, Cylopoida, and syndiniales were the five most abundant orders found in the samples, Figure 3. The Calanoida was counted for 2,014 reads

(14.33%), followed by Spumellaria with 1,947 reads (13.85%), Siphonophorae with 981 reads (6.98%), Cylopoida with 789 reads (5.61%), and syndiniales with 487 reads (3.46%). The other orders and abundance were presented in Figure 3. In addition, the present study also obtained at least 1,753 reads or 12.47 % which were assigned into unclassified or unknown orders.

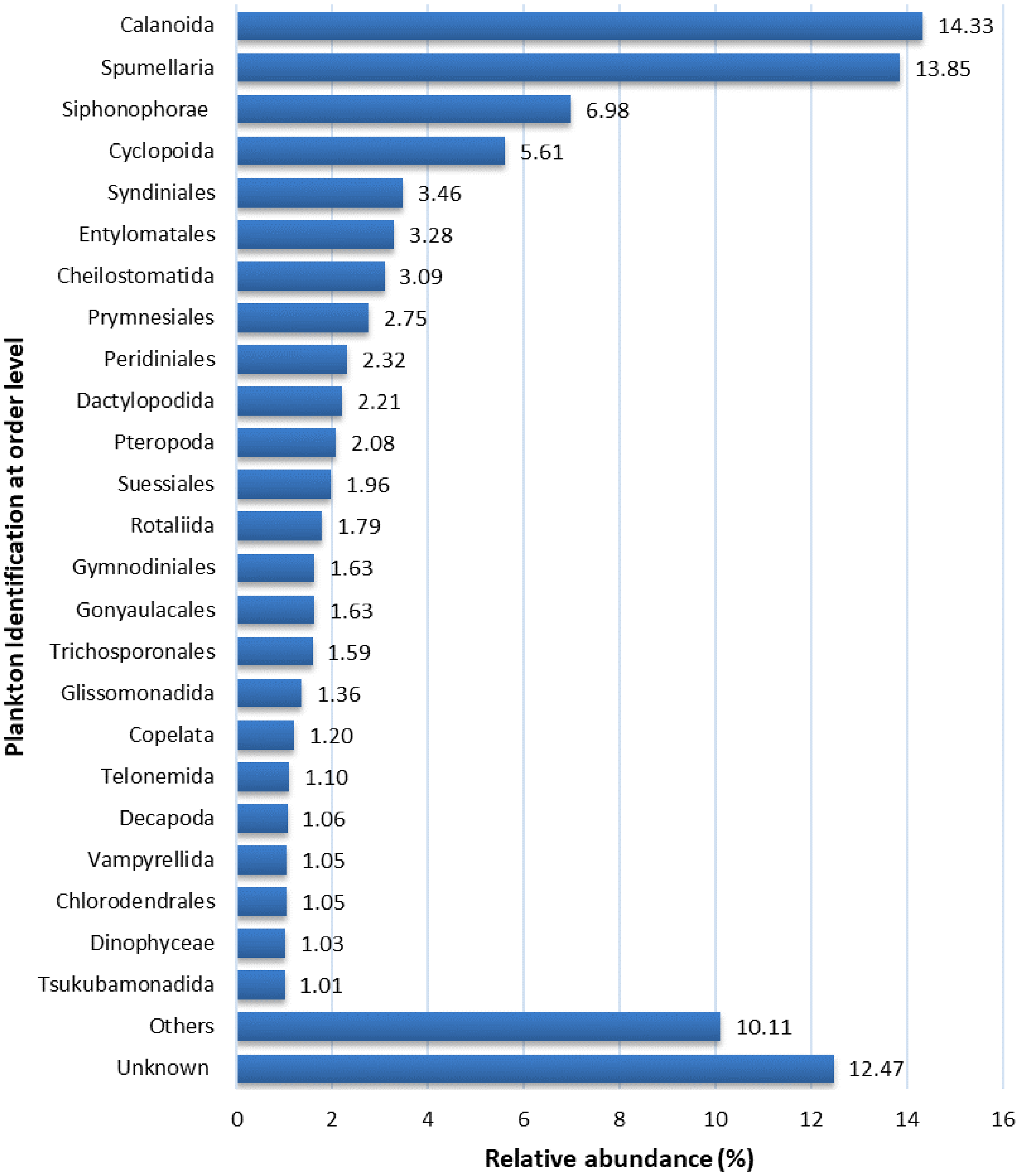
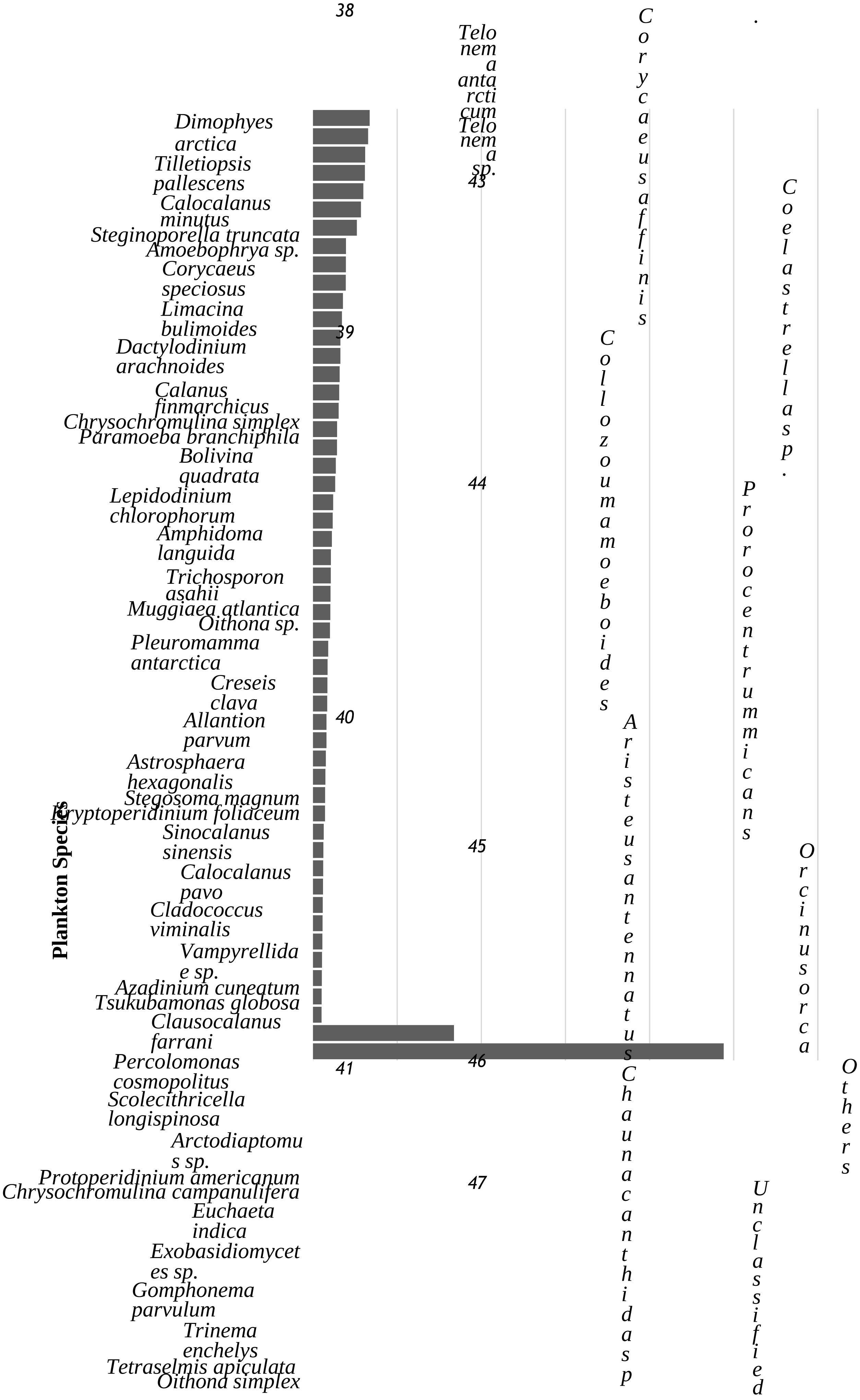


Figure 3. Relative abundance of Planktons identified from Tawang Bay, a natural

satellement habitat of spiny lobster larvae in East Java Indonesia, presented at the order level.

Species composition

At the species level, a total of 97 plankton species were identified from the Tawang Bay habitat, of which the ten most abundant species were *Dimophyes arctica* with 474 reads (3.37%), *Tilletiopsis pallescens* with 461 reads (3.28%), *Calocalanus minutus* with 463 reads (3.10%), *Steginoporella truncate* with 434 reads (3.09%), *Amoebophrya* sp. with 421 reads (3.00%), *Corycaeus speciosus* with 401 reads (2.85%), *Limacina bulimoides* with 367 reads (2.61%), *Dactylodinium arachnoides* with 276 reads (1.96%), *Calanus finmarchicus* with 275 reads (1.96%), and *Chrysochromulina simplex* with 251 reads (1.95%). The other 87 species with their relative abundances were presented in Figure 4. The present result also indicated that a total of 3.431 reads (24.41%) were assigned into unclassified species.


Telonema antarcticum
Telonema sp.
Corycaeus affinis
.
Coelastrella sp.
Collozoum amoeboides
Prorocentrum micans
Aristeus antennatus
Orcinus orca
Chaunacanthida sp
Others
Unclassified
Dimophyes arctica
Tilletiopsis pallescens
Calocalanus minutus
Steginoporella truncata
Amoebophrya sp.
Corycaeus speciosus
Limacina bulimoides
Dactylodinium arachnoides
Calanus finmarchicus
Chrysochromulina simplex
Paramoeba branchiphila
Bolivina quadrata
Lepidodinium chlorophorum
Amphidoma languida
Trichosporon asahii
Muggiaea atlantica
Oithona sp.
Pleuromamma antarctica
Creseis clava
Allantion parvum
Astrosphaera hexagonalis
Stegosoma magnum
Kryptoperidinium foliaceum
Sinocalanus sinensis
Calocalanus pavo
Cladococcus viminalis
Vampyrellidae sp.
Azadinium cuneatum
Tsukubamonas globosa
Clausocalanus farrani
Percolomonas cosmopolitus
Scolecithricella longispinosa
Arctodiaptomus sp.
Protoperidinium americanum
Chrysochromulina campanulifera
Euchaeta indica
Exobasidiomycetes sp.
Gomphonema parvulum
Trinema enchelys
Tetraselmis apiculata
Oithona simplex
Plankton Species

3.37
3.28
3.10
3.09
3.00
2.85
2.61
1.96
1.96
1.95
1.79
1.72
1.63
1.63
1.59
1.56
1.53
1.44
1.43
1.36
1.32
1.20
1.17
1.12
1.07
1.06
1.05
1.03
1.01
0.91
0.87
0.86
0.85
0.80
0.80
0.77
0.74
0.73
0.72
0.64
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0.61
0.60
0.58
0.57
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0.54
0.53
0.52
0.51

8.38

24.
41

0.00 5.00 10.00 15.00 20.00 25.00 30.00

Relative Abundance (%)

Figure 4. Relative abundance of Plankton species identified at Tawang Bay, a natural settlement habitat of spiny lobster larvae presented at the species level.

Diversity, uniformity, and dominant indices in terms of species

The plankton diversity index was 1.65 and considered moderate since the value was between 1 and 3. While the plankton uniformity index calculated from the sample was 0.22 and categorized as low uniformity since the value is less than 0.4. In addition, the dominant index was 0.24 and considered a low as well because the value was less than 0.5, Figure 5.

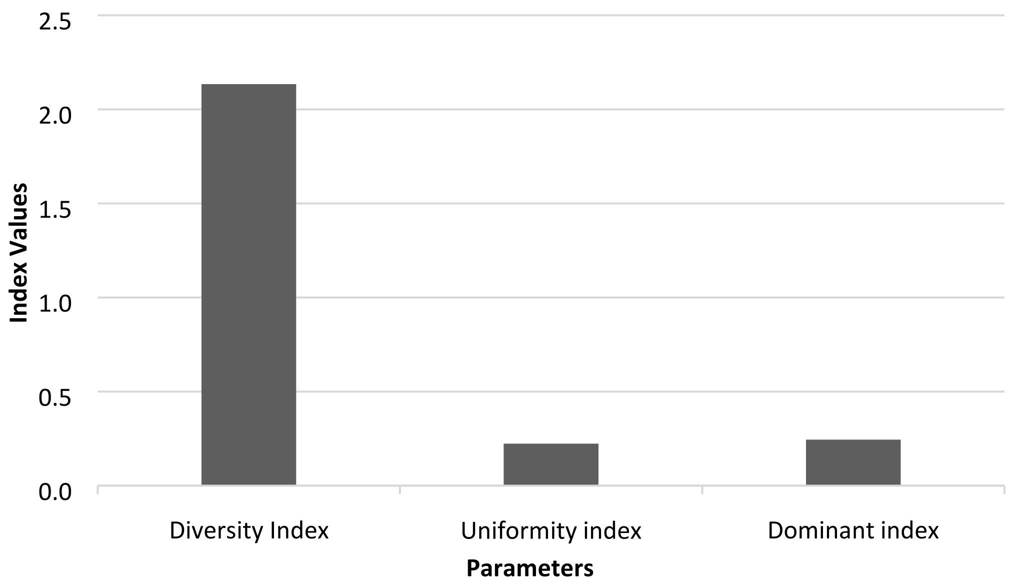

Figure 5. Diversity, uniformity, and dominant indices of plankton species identified using eDNA metabarcoding in the Tawang Bay habitat.

Discussion

Indonesia is well known for its high biodiversity including the plankton community (Amin et al. 2022b; Borbee et al. 2022). This study reported the type, abundance, diversity, uniformity, and dominant indices of plankton in Tawang Bay, one of the most common settlement habitats for spiny lobster in East Java, Indonesia to find potential live prey for aquatic organisms including lobster larvae. The result showed that there were 26 phyla, 40 classes, 54 orders, 75 families, 85 genera, and 97 plankton species.

Calculated based on species data, the diversity index of plankton at Tawang Bay was at a moderate level (Awwaluddin et al. 2017). This result suggests that the numbers of species in the selected study were quite varied, which is in line with 97 species identified from the present study. In addition, Plankton uniformity and dominant indices in the Tawang Bay habitat were 0.22, and 0.24 respectively, which are considered low (Fachrul, 2016). These results suggest plankton distribution in Tawang Bay was quite diverse and no dominant plankton species are present in the water habitat (Berger and Parker 1970). Among the identified taxa, phylum Arthropoda was in the greatest abundance, followed by Radiozoa, Myzozoa, Basidiomycota, and Cnidaria. Many members of Arthropoda are considered economically important aquatic commodities including spiny lobsters (*Panulirus* spp.), marine crabs (*Portunus* spp.), and marine shrimps (*Penaeus* spp.) (Amelia et al. 2021; Utama et al. 2021; Wiloso et al. 2022). In fact, these marine commodities are among the most economically important species for Indonesian and are valued as superior export commodities. In addition, many studies also previously reported that diverse members of Arthropoda have been revealed to play critical roles ecologically in certain habitats including as pollution bioindicators and live diets for aquaculture species (Amador-Marrero et al. 2023; Amin et al. 2022d; Hirai et al. 2021).

Thisstudy identified at least 23 plankton species that belonged to the Phylum Arthropoda, of which 18 species have been documented as live prey for aquaculture species in their natural habitats. This high level of plankton biodiversity was not expected in this region but may be likely for spiny lobster larvae. Three species of marine Arthropoda (*Acartia bispinosa*, *Oithona simplex*, *Oithona* sp., *Pseudodiaptomus euryhalinus*) have been found in the stomach contents of the larvae of ornate spiny lobster, *Panulirus ornatus* (Amin et al. 2022d), which suggest that these species are live prey for the ornate spiny lobster. In addition, *Acartia* sp. has been reported as a live diet for seabass larvae, *Lates calcarifer* (Rajkumar 2006), fat snook, *Centropomus parallelus* (Barroso et al. 2013), and many other aquatic larvae (Sarkisian et al. 2019). Some studies also confirmed that these plankton species were identified in the stomach content of lobster larvae. *Acartia* sp. has also been documented to be a good live diet for aquatic larvae such as seabass, *Lates calcarifer* (Rajkumar 2006), and fat snook, *Centropomus parallelus* (Barroso et al. 2013). *Acartia clausi* has been described to have a higher protein (63.12%) and lipid content (16.65%) and is also richer in omega − 3 fatty acids (33.94%) compared to *Artemia nauplii* and rotifers (Rajkumar, 2006). In addition, a member of Acartia (*Acartia tonsa*) has previously been documented to provide an important nutritional benefit to fat snook larvae undergoing metamorphosis (Vanacor-Barroso et al. 2017).

Furthermore, *Oithona* sp., which is a marine calanoid copepod has been documented to have a high protein content, ~59.33% (Santanumurti et al. 2021). Additionally, *Oithona* sp. has been described as having a high content of fatty acid profiles including polyunsaturated fatty acids (26.47%) and omega-3 fatty acids (36.30%), which are higher than a commercial live diet such as *Artemia* sp. (Magouz et al. 2021b). Therefore, the marine copepod has been frequently used as a live diet for fish or mollusc larvae. For instance, *Oithona* sp. has been documented as a live diet of shrimp larvae (Dinesh Kumar et al. 2017), and European seabass (*Dicentrarchus labrax*) postlarvae (Magouz et al. 2021a). *Oithona* sp. has been reported from the stomach content of spiny lobsters at the early life stage (Amin et al. 2022d; Khvorov et al. 2012). Furthermore, the plankton species had also been identified in the stomach contents of the spiny lobster larvae (Amin et al. 2022b; Amin et al. 2022d).

Additionally, other plankton species detected in the study have also been reported as live prey for several marine aquatic larvae. For example, *Pseudodiaptomus*

euryhalinus had been documented as live prey for the larvae of Pacific red snapper, *Lutjanus peru* (Amador-Marrero et al. 2023). *Calanus finmarchicus* has high lipid content (0 to 190 μg individual^{-1}) (Jónasdóttir et al. 2019). Other copepods such as *Calocalanus pavo*, and *Calocalanus minutus* had been reported as small marine copepod that were important prey for fish larvae in the Kuroshio region off southern Japan (Hirai et al. 2021). *Corycaeus affinis* as a live prey for Japanese larval and juvenile sardine, *Sardinops melanostictus*, and anchovy, *Engraulis japonicus*, in the western North Pacific (Okazaki et al. 2019), and Japanese jack mackerel (*Trachurus japonicus*) juveniles in the East China Sea (Sassa et al. 2019). Similarly, *Sinocalanus sinensis* was reported as a live prey of pipefish, which was revealed by fecal eDNA metabarcoding (Ntshudisane et al. 2021). All this information suggests that many potential live prey can be used for aquatic species, especially at larval stages. Therefore it is recommended that further studies explore the effects of isolation and culturing of this plankton with high nutrient content.

Other plankton species found in thisstudy were *Clausocalanus farrani*, *Pleuromamma antarctica*, *Scolecithricella longispinosa*, and *Corycaeus speciosus*. *Euchaeta indica and Lucicutia ovaliformis* have not been documented as live prey or components of the diets of aquaculture species. However, these species have been frequently reported in marine water including in the Western Indian Ocean and the China Seas (Al-Aidaroos et al. 2019; Shih et al. 2022). In addition, the cellular body sizes and their close relation to the other species that have been previously described might suggest that these plankton species are also potential live prey for the larvae of aquatic species including spiny lobsters. Further study is required to confirm this preliminary assumption.

Conclusion

This study identified at least 330 ASVs from water samples of Tawang bay which were assigned to 26 phyla, 40 classes, 54 orders, 75 families, 85 genera, and 97 species. Among the identified taxa, 18 species have been documented as live prey for aquaculture species in their natural habitat including *Acartia bispinosa*, *Oithona simplex*, *Oithona* sp., and *Pseudodiaptomus euryhalinus*, *Calocalanus pavo*, and *Calocalanus minutus*. The biodiversity of plankton species has often been overlooked in studies but is an important component of marine larval diets and should be considered in aquaculture. Given the high level of plankton diversity in this region, it is recommended that Indonesia should recognize the natural value of these areas.

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Conflict of Interest

Authors declare no conflict of interest

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