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# Spiny lobster feeding grounds: an eDNA metabarcoding assessment reveals a high level of plankton biodiversity in Tawang Bay, Indonesia

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**ABSTRACT**

Plankton has been considered an important live diet for various marine 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 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 analysed 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 dominance indices were 0.22 and 0.24, respectively, which indicates 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 larvae of aquaculture species including lobster and therefore should be further studied.

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## 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, Simard, and Bourget 1992; Lillis and Snelgrove 2010). In addition, biological aspects of settlement habitat in the natural environment of lobster larvae have also been examined 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) studied the type and abundance of plankton in the settlement area of American lobster in the Northeast US Shelf, USA. These studies were conducted to understand plankton availability and biological factors that affect 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, Souissi, and Jepsen 2022). For instance, the plankton *Oithona* sp., which has been previously reported to be abundant 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 were 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 focusses on a molecular approach to clearly idenitify the genetic diversity of plankton species and their abundance.

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This study specifically focussed on assessing the diversity, uniformity, and dominance indices of plankton in Tawang Bay, an important natural settlement habitat for spiny lobster larvae in East Java, Indonesia. It appears that the conditions in Tawang Bay provide a suitable environment as well as good diet availability for lobster larvae. In addition, the present study used environmental DNA (eDNA) metabarcoding to increase the accuracy of the results.

## 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) and four depths (0–0.30 m (surface), 2.5 m, 5 m, 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 scissors and added to ZRbashingBead™ Lysis Tubes (0.1 and 0.5 mm) followed by the addition of 750 μL ZymoBIOMICS™ Lysis solution. The rest of the steps were performed according to the instruction manual for the ZymoBIOMICS™ DNA Miniprep Kit. Then, the quality of the DNA extract was measured using a nanodrop and afterward was adjusted at 30 ng/μL before sending for sequencing. The DNA samples were
1 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 the following equations:

$H' = -\sum \mathrm{PilnPi}$, *where* $\mathrm{Pi} = \frac{ni}{N}$ (Fachrul et al. 2016)

$\mathrm{E} = \frac{\mathrm{H'}}{\mathrm{Lns}}$ (Odum 1971)

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

where H' is the Shannon-Wiener diversity index, ni is the number of individuals of the ith species, 'n' is the number of species, N is the total number of individuals Pi is the number of individuals of the ith species, 'E' is the uniformity index, 'S' is the total number of species, d is the Simpson dominance index, and 'Nmax' is the most abundant number of individual species.

## Results

### Overview and taxa detected

The eDNA metabarcoding results showed that a total of 45,978 raw paired-end readings were obtained from the 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).

### Phylum composition

In terms of numbers, a total of 26 phyla were identified from the 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 3042 sequence reads (21.64%), followed by Radiozoa with 2160 reads (15.37%), Myzozoa with 1853 reads (13.18%), Basidiomycota with 788 reads (5.61%), and Cnidaria with 765 reads (5.61%). The other 21 phyla were Chlorophyta, Bryozoa, Mollusca, Haptophyta, Cercozoa, Discosea, Foraminifera, Chordata, Cryptophyta, Ochrophyta, Chrorophyta, Endomyxa, Loukozoa, Heterolobosea, Amoebozoa, Porifera, Ascomycota, Platyhelminthes, Tracheophyta, Annelida, and Chaetognatha, which collectively accounted for 3838 reads (27.31%). Finally, 3838 reads were assigned to unclassified phyla or unknown (Figure 2).

### 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). Calanoida was counted for 2014 reads (14.33%), followed by

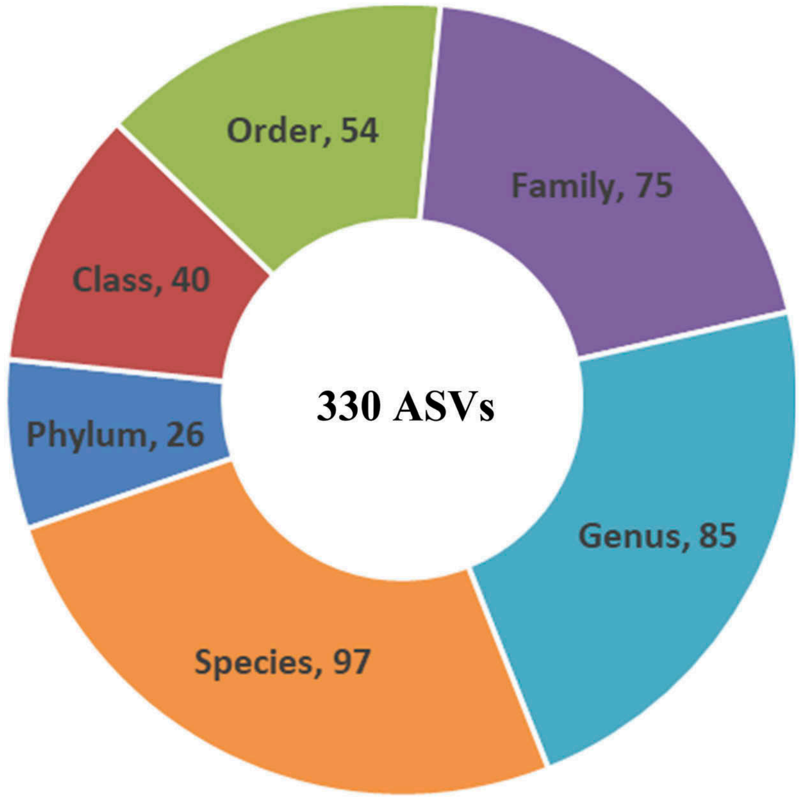


Figure 1. The number of phyla, classes, orders, families, genera, and species from the natural settlement habitat of lobster larvae identified using eDNA metabarcoding.

Spumellaria with 1947 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 their abundance are presented in Figure 3. In addition, the present study also obtained at least 1753 reads or 12.47% which were assigned to unclassified or unknown orders.

### Species composition

At the species level, a total of 97 plankton species were identified from the Tawang Bay habitat, of which the 10 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 truncata* 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, along with their relative abundances, are presented in Figure 4. Also, a total of 3.431 reads (24.41%) were assigned to unclassified species.

### Diversity, uniformity, and dominance indices in terms of species

The plankton diversity index was 1.65 and is considered moderate since the value is between 1 and 3. The plankton uniformity index calculated from the sample was 0.22; this is categorized as low uniformity since the value is less than 0.4. Finally, the dominance index was 0.24; this is considered low as well because the value is less than 0.5 (Figure 5).

## Discussion

Indonesia is well known for its high biodiversity, including of the plankton community (Amin et al. 2022b; Borbee et al. 2022). This study reported the type, abundance, diversity, uniformity, and dominance 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 results 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, Suwarso, and

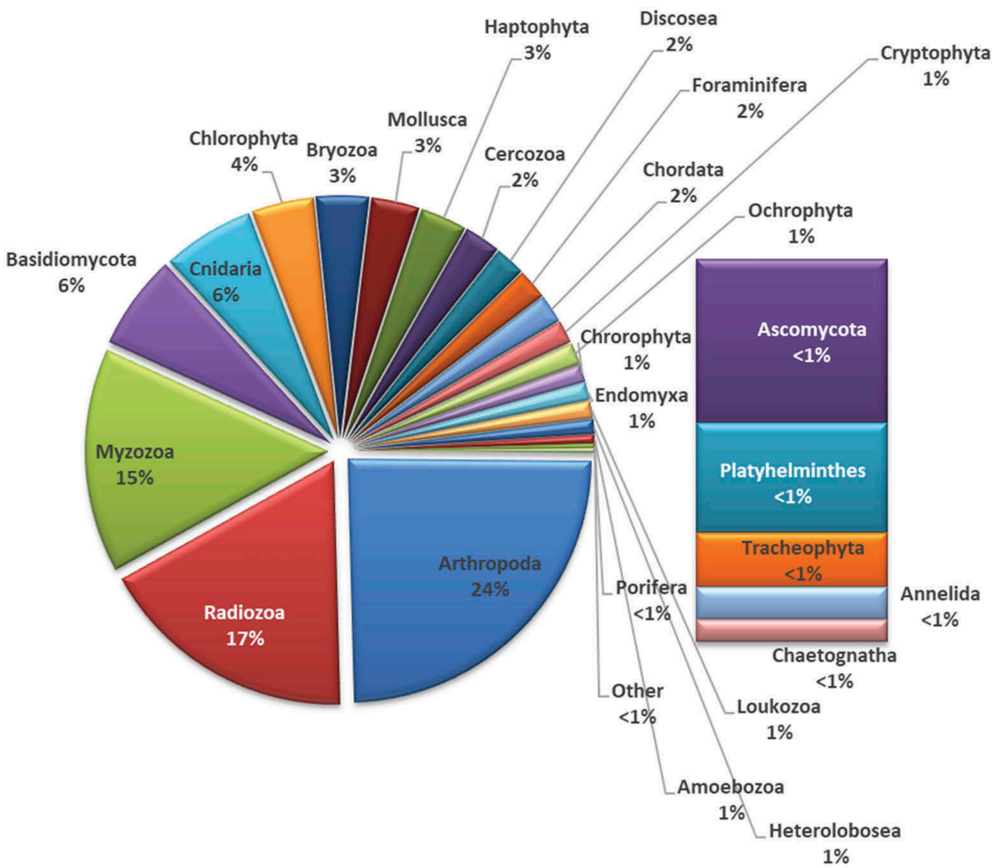


Figure 2. Phylum-level composition and relative abundance of plankton identified from Tawang Bay, a natural settlement habitat of spiny lobster larvae.

Setiawan 2017). This result suggests that the numbers of species in the selected study were quite varied, which is in line with the 97 species identified from the present study. In addition, plankton uniformity and dominance index values in the Tawang Bay habitat were 0.22 and 0.24, respectively, which are considered low (Fachrul et al. 2016). These results suggest that plankton distribution in Tawang Bay is quite diverse and no dominant plankton species are present in the water habitat (Berger and Parker 1970). Among the identified taxa, phylum Arthropoda showed 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, Yustiati, and Andriani 2021; Utama, Yustiati, and Rostika 2021; Wiloso et al. 2022). In fact, these marine commodities are among the most economically important species for Indonesia and are valued as superior export commodities. In addition, many earlier studies 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 study identified at least 23 plankton species belonging to the phylum Arthropoda, of which 18 species have been documented as live prey for aquaculture species in their natural habitats. Such a high level of plankton biodiversity was not expected in this region but may be likely around 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 suggests 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 other studies also identified these plankton species in the stomach content of lobster larvae. *Acartia* sp. has also been documented as 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

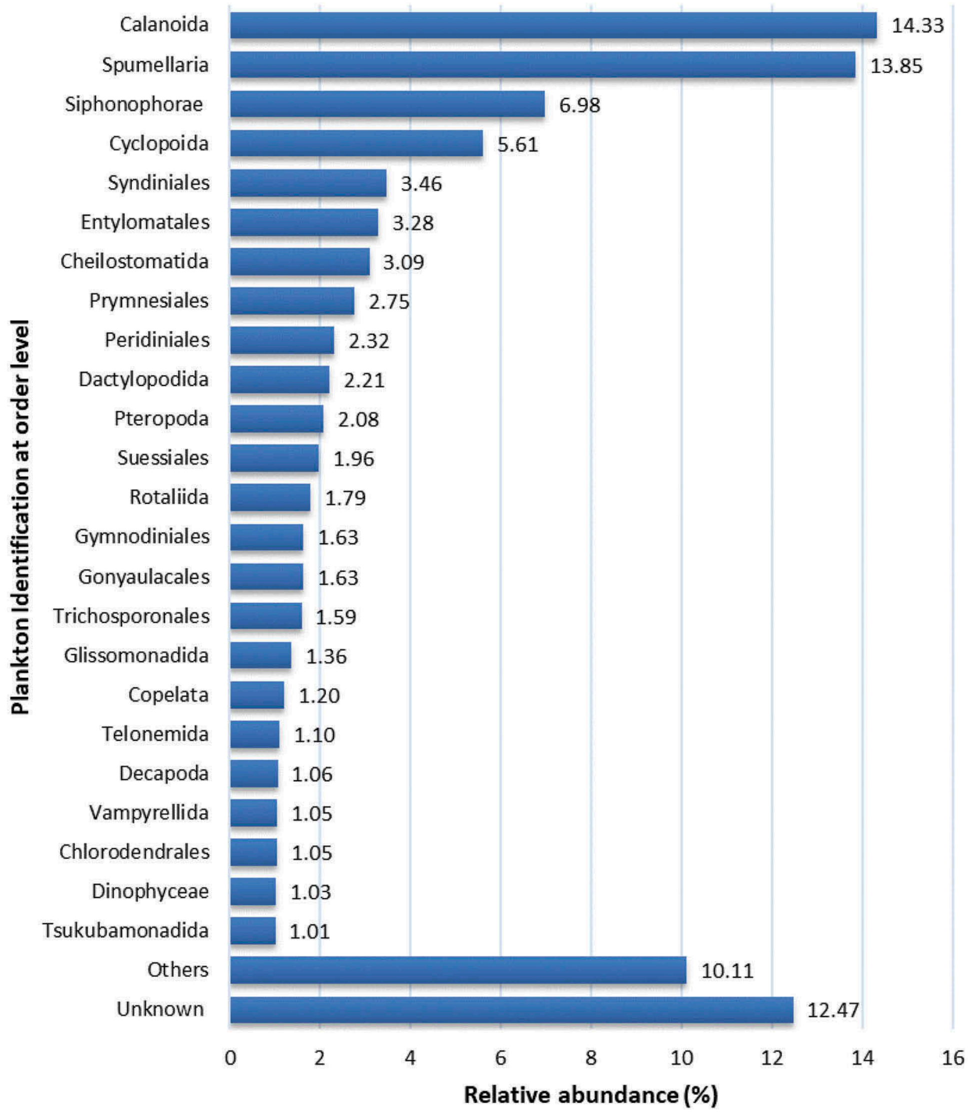


Figure 3. Relative abundance of plankton identified from Tawang Bay, a natural settlement habitat of spiny lobster larvae in East Java, Indonesia, presented at the order level.

(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., 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 high fatty acid contents including polyunsaturated fatty acids (26.47%) and omega-3 fatty acids (36.30%), which are higher than found in 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 contents of spiny lobsters at the early life stage (Amin et al. 2022d; Khvorov, Piontkovski, and Popova 2012). Furthermore, this plankton species had also been identified in the stomach contents of spiny lobster larvae (Amin et al. 2022b, 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 a high lipid content (0 to 190 µg individual$^{-1}$) (Jónasdóttir et al. 2019). Other copepods such as *Calocalanus pavo*


Figure 4. Relative abundance of plankton species identified at Tawang Bay, a natural settlement habitat of spiny lobster larvae.

and *Calocalanus minutus* have been reported as important prey for fish larvae in the Kuroshio region off southern Japan (Hirai et al. 2021). *Corycaeus affinis* has been reported 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 for 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

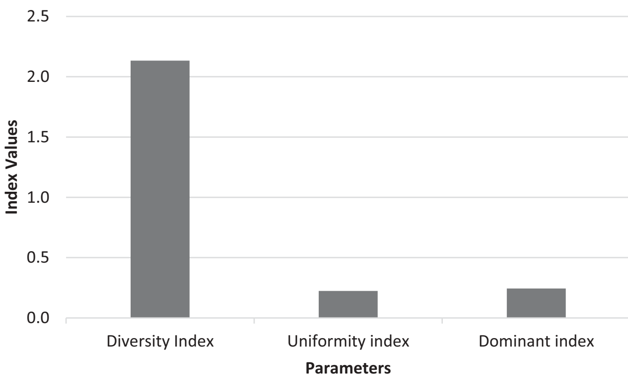


**Figure 5.** Diversity, uniformity, and dominant indices of plankton species identified using eDNA metabarcoding in the Tawang Bay habitat.

is recommended that further studies explore the effects of isolation and culturing of this plankton with high nutrient content.

Other plankton species found in this study 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 waters including in the Western Indian Ocean and the China Seas (Al-Aidaroos, El-Sherbiny, and Mantha 2019; Shih et al. 2022). In addition, the cellular body sizes and their close relationship to 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., *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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## Disclosure statement

No potential conflict of interest was reported by the authors.

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