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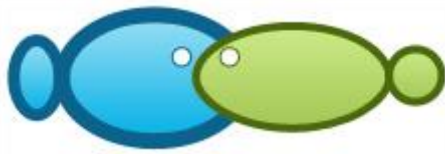
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Deep sea phytoplankton community of the Sangihe-Talaud Islands waters

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Abstract. As a photosynthetic organism, phytoplankton faces unfavorable conditions at great depths, especially limited light availability. Nevertheless, earlier studies have found the existence of chlorophyll containing microorganisms in the aphotic zone. The region off the Sangihe-Talaud Islands is a pivotal region for fisheries resources and the Indonesian Throughflow (ITF). Until today, the deep-sea phytoplankton in the Sangihe-Talaud waters is poorly studied. The present study investigates the composition of species and abundance of phytoplankton in the deep sea (600 m) of the Sangihe-Talaud Islands waters. Plankton samples were collected using the Rosette Sampler (10 L) during the EWIN field campaign with the Indonesian RV Baruna Jaya VIII research vessel in October 2018. Environmental parameters like temperature, salinity, and fluorescence were also measured. The results showed that diatoms predominated with 54.2% of phytoplankton composition, with the most dominant species being *Thalassiosira punctigera*, *Chaetoceros peruvianus*, and *Chaetoceros borealis*. The highest phytoplankton abundance was observed at the sampling stations near islands. Temperature and salinity measurements exhibited nearly uniform values of $6.86 \pm 0.25^\circ\text{C}$ and $34.53 \pm 0.01\text{‰}$ in the study area. Furthermore, fluorescence values ranged from 0.03 to 0.04 mg/m^3 , indicating there is no photosynthetic activity at 600 m. According to Principal Component Analysis (PCA), we suggest salinity is the main factor that influenced the phytoplankton community in the depth of 600 m. Temperature profile shows a weak influence on phytoplankton community in the study area.

Key Words: deep sea phytoplankton, abundance, fluorescent, Sangihe-Talaud Islands.

Introduction. Sangihe and Talaud Islands are situated in northeastern Indonesia and are comprised of 105 islands (Setiawan et al 2016). Ocean region off the Sangihe-Talaud Islands is characterized by steep bathymetry and is a pivotal region for commercial fishes (Satria et al 2012). Also, the region provides one of the main gates for the Indonesian Throughflow (ITF) (Sprintall et al 2014). Ocean conditions off the Sangihe-Talaud Islands is regulated by the Australian-Indonesian Monsoon (AIM) system (Susanto et al 2006; Setiawan et al 2020). During the Southeast Monsoon season (June-August), southeasterly winds blow from Australia, and carry warm and dry air to the Maritime Continent (Gordon 2005). These winds commonly induce upwelling in the Indonesian Seas (Setiawan et al 2019; Setiawan et al 2020; Wirasatriya et al 2019). Whereas during the Northwest Monsoon season (December-February), northwesterly winds blow from the Eurasian Continent, bringing warm and moist air to Indonesia (Gordon 2005; Qu et al 2005). Generally during the Northwest and Transition (March-April and October-November) monsoons, ocean surface condition in the Indonesian Seas is qualified as oligotrophic, due to the sea surface dynamics being dominated by the downwelling process (Setiawan & Habibi 2010; Setiawan et al 2019; Setiawan et al 2020).

Phytoplankton is a primary producer and via photosynthesis serves as the nutritional foundation for marine and freshwater trophic levels. Phytoplankton and

zooplankton communities are distributed horizontally and vertically in the oceans and both can be used as an indicator of ecological changes due to their sensitivity to environmental stressors (Paerl et al 2007; Liu et al 2003; Dutkiewicz et al 2019). According to Millero (2005), phytoplankton has limited mobility. Nutrients availability, ocean current, and light determine their spatial distribution (Millero 2005; Firdaus et al 2020). It is also widely known that vertical distribution of phytoplankton is influenced by those parameters (Brierley 2017). As the ocean depth increases, there will be significant changes in environmental conditions, which in turn affect the phytoplankton life. Ocean depth of 200 m is suggested as the limit for ongoing primary productivity, due to significant reduction of sunlight penetration (Suthers & Rissik 2008; Triyulianti et al 2017). Thus, deep ocean condition becomes a limiting factor for phytoplankton life. An interesting fact about this condition is how phytoplankton, as photosynthetic organisms, can be found below 200 m.

The presence of pigmented cells of phytoplankton in the aphotic zone has been reported by previous studies (Kimball et al 1963; Platt et al 1983; Agusti et al 2015; Guo et al 2018). Kimball et al (1963) found chlorophyll containing phytoplankton at 4000 m in the Atlantic and the Pacific Oceans. They cultured it in enriched seawater using light cabinet, and they evidenced that the phytoplankton was allochthonous. Platt et al (1983) took water samples from a depth of 1000 m using a Niskin bottle and found pigmented cells there. Platt et al (1983) also cultured it and suggested these cells can photosynthesize. Agusti et al (2015) has shown that phytoplankton collected from depths between 2000-4000 m using a bottle net contained well-preserved cells of phytoplankton, which was predominated by Bacillariophyceae.

The presence of healthy phytoplankton pigmented cells at deep-sea is an interesting phenomenon, particularly the mechanism that supports them to reach this depth. In general, phytoplankton distribution is influenced by environmental factors such as nutrients availability, ocean current, temperature, salinity, pH, and light (Brierley 2017). In the deep-sea environment, phytoplankton faces unfavorable conditions like low temperature, relatively higher salinity, and lack or absence of light. Ocean depth of 200 m is suggested as the limit for ongoing primary productivity due to a significant reduction of sunlight penetration (Suthers & Rissik 2008; Triyulianti et al 2017). This means photosynthesis cannot occur to support the life of phytoplankton below 200 m.

Phytoplankton distribution in the Sangihe-Talaud waters have been investigated by earlier studies (Thoha & Fitriya 2010; Sriwijayanti et al 2019a; Sriwijayanti et al 2019b). Thoha and Fitriya (2010) studied phytoplankton distribution at depths between 100 and 200 m using the Kitahara net. They found diatoms (*Chaetoceros* spp., *Nitzschia* spp., and *Rhizosolenia* spp.) were the dominant phytoplankton community. Sriwijayanti et al (2019a; 2019b) took phytoplankton samples using the Rosette sampler at the surface (5 m) and thermocline layers and found phytoplankton community at the surface was dominated by diatoms and Cyanophyceae, whereas at the thermocline layers were dominated by diatoms. Although there have been studies of phytoplankton distribution in the Sangihe-Talaud Islands waters, the region of interest has historically lacked *in situ* measurement of deep-sea phytoplankton. Therefore, unravelling spatial distribution of deep-sea phytoplankton in the Sangihe-Talaud Islands waters is of utmost priority.

Material and Method

Description of the study site. The Sangihe and Talaud Islands are located in the northern Indonesian Seas and are connected to the Pacific Ocean in the northeast and east, the Maluku Sea in the south, and the Sulawesi Sea in the west (Figure 1). The region of interest is influenced by the Australian-Indonesian monsoon system. Generally, this monsoon system affects ocean surface circulation and productivity in the Indonesian Seas (Setiawan et al 2019; Setiawan et al 2020; Wirasatriya et al 2019). Presently, the ocean region off the Sangihe-Talaud Islands is the pivotal link between the Pacific Ocean and the Indian Ocean as it transports Pacific Ocean water into the Indian Ocean through the ITF (Gordon 2005; Sprintall et al 2014).

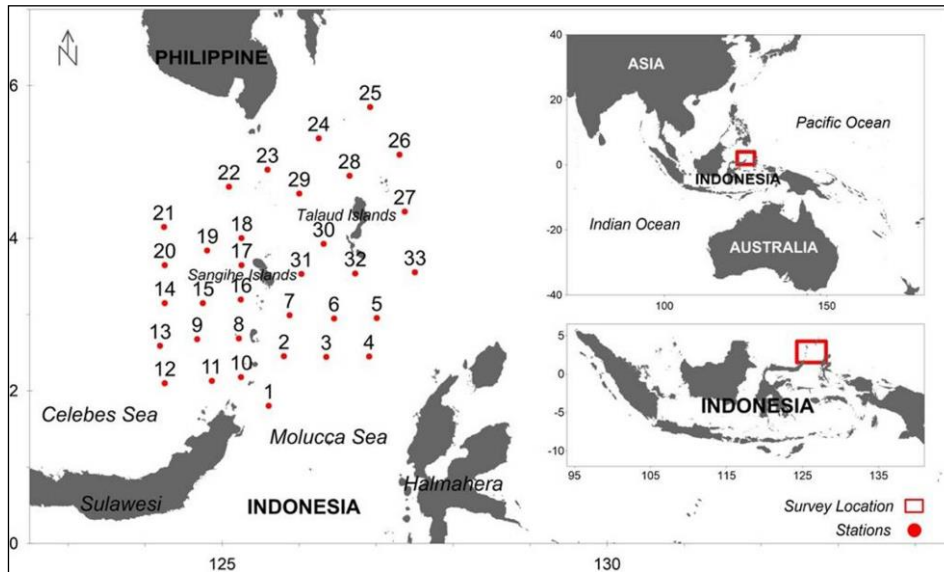


Figure1. Map of research stations off the Sangihe-Talaud Islands.

Procedures. Field campaign in the Sangihe-Talaud Islands waters was conducted from 6-26 October 2018 using the Indonesian RV Baruna Jaya VIII research vessel. Figure 1 shows the sampling stations (33 stations) of plankton collection and measurement of environmental parameters. Samples of phytoplankton were taken from the depth of 600 m using 12 bottles of 10-liter Rosette sampler. Samples were then filtered using a hand-plankton-net (mesh size of 20 μm) and were preserved with Lugol 1% solution. Phytoplankton enumeration was performed using the Sedgewick-Rafter Counting Chamber (SRCC) under microscope at the Laboratory of Plankton and Primary Productivity, Indonesian Institute of Sciences. Identification and differentiation of phytoplankton were carried out by morphological observations based on several references such as Omura (2012), Yamaji (1979), Wickstead (1965), Shirota et al (1966) and Newell et al (1963). Measurements of temperature, salinity, and fluorescence were conducted using the SBE 911 CTD (Conductivity Temperature Depth) device, which was attached to the Rosette sampler.

Data Analysis. Plankton identification was carried out at the Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada. Plankton abundance was expressed as individuals per m^3 and calculated using the following equation (1):

$$D = \frac{N_f \times V_p}{V} \quad (1)$$

where D is the plankton abundance (individuals/ m^3), N_f is the number of plankton per 1 ml, V_p is the dilution volume, and V is the filtered water volume (m^3).

Univariate analysis is used as an ecological indicator through a diversity index. The indicator is the diversity index of the identified plankton species. The plankton diversity index is calculated the following equation (2) that is developed by Spellerberg and Fedor (2003):

$$H' = - \sum P_i \ln P_i \quad (2)$$

where H' is the diversity index, P_i is the species proportion ($P_i = n_i/N$), n_i is the individual sum of i-th species, and N is the number of total individuals of all species. The diversity is categorized as follows: $H' < 1.0$ indicates low diversity, $1 < H' < 3.322$ indicates medium diversity, and $H' > 3.322$ indicates high diversity (Krebs 1989).

The evenness index is calculated using the formula of Pielou (1966) as shown in the following equation (3):

$$J' = \frac{H'}{H_{max}} \quad (3)$$

where J' is the Pielou's evenness index, H' is the diversity index, and H_{max} is the maximum value of H' . Evenness index (J') ranges from 0 to 1. If the value of J' is close to 1, then the distribution of species is evenly distributed. Whereas if the value of J' is close to 0, then the distribution of species is classified as uneven or there is a particular dominant group (Odum & Barrett 2005).

The most dominating type of phytoplankton in a community is calculated using the Simpson formula (Odum & Barrett 2005) showed in the following equation (4):

$$C = \sum \left(\frac{n_i}{N} \right)^2 \quad (4)$$

where n_i is the total number of individuals of the i -th type, N is total individuals of all types, and C is the Simpson dominance scale. Simpson's formula produced two categories of dominance i.e., if the value approaches 0, then dominance is classified as low, which is usually followed by a large J' value (Evenness Index). If the value is close to 1 means the dominance is classified as high and J' value is low.

The diagram of temperature-salinity was generated using Ocean Data View (ODV) ver. 5.1.7. The Principal Component Analysis (PCA) was processed using PAST Ver. 4.0.2.

Results

Composition, type, and abundance of phytoplankton. Results of phytoplankton identification revealed there were 48 species that could be classified into three classes, Bacillariophyceae (26 species), Dinophyceae (19 species), and Raphidophyceae (3 species). According to Figure 2, the class of Bacillariophyceae (54.2 %) dominated the type and abundance of phytoplankton in the study area. Closer examination of phytoplankton species of each station showed that there were three most dominant species originating from the diatom Bacillariophyceae class (Figure 3) i.e., *Thalassiosira* sp. (619,206 cells/m³), *Chaetoceros peruvianus* (190,667 cells/m³), and *Chaetoceros borealis* (173,889 cells/m³). Whereas the other 45 species exhibit low abundance with values <10×10⁴ cells/m³. Figure 4 shows phytoplankton abundance in the region of interest ranging from 2,667 to 297,778 cells/m³. The highest phytoplankton abundance was observed at stations close to islands (Stations 1, 8, 30, and 32).

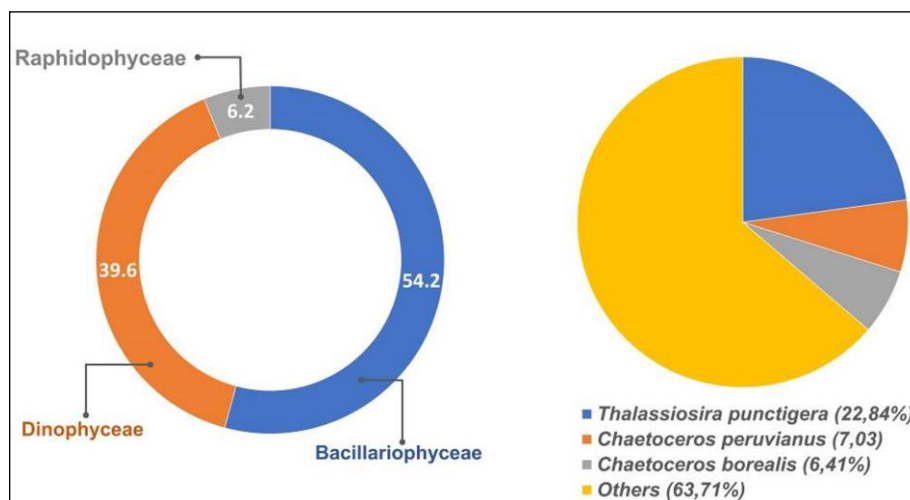


Figure 2. Phytoplankton class composition (left panel) and the most dominant phytoplankton (right panel) at a depth of 600 m.

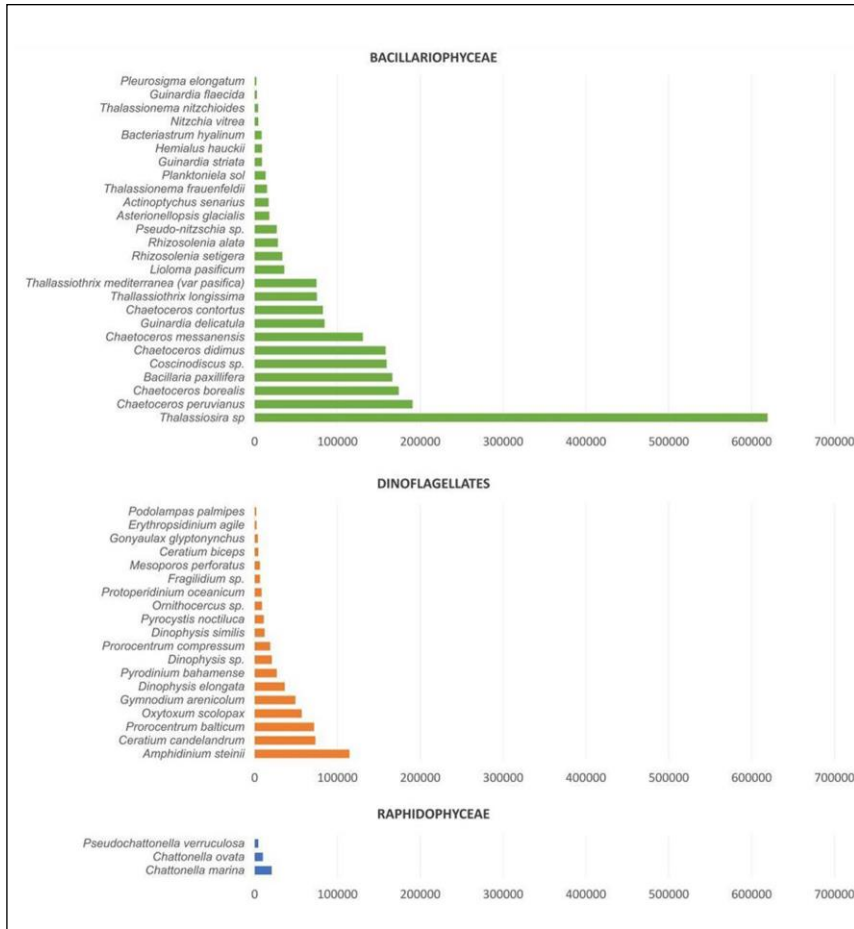


Figure 3. The list and abundance of phytoplankton at a depth of 600 m in the Sangihe-Talaud Islands waters during the study period.

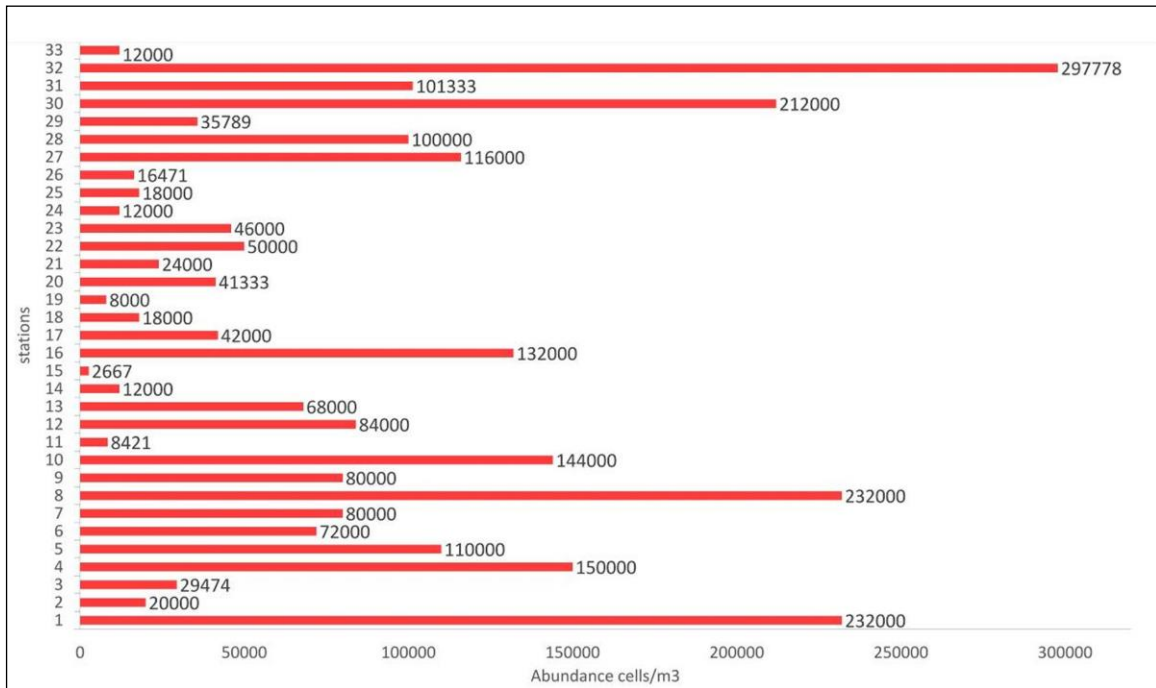


Figure 4. Phytoplankton abundance at a depth of 600 m during the study period.

Diversity, dominance, and evenness indices of phytoplankton. The value of the Diversity Index (H') ranged from 0.39 to 2.04 with an average value of 1.208 (Figure 5 and Figure 6). The Dominance Index (C) value ranged from 0.16 to 0.80 with average value of 0.41. Meanwhile, the Evenness Index (J') ranged from 0.4 to 1 with average value of 0.79.

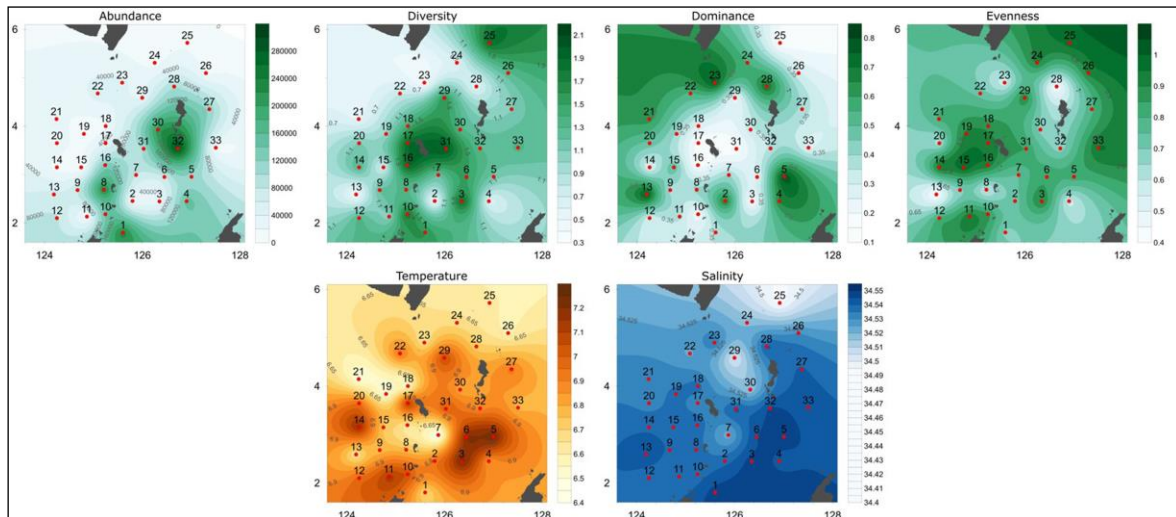


Figure 5. Spatial distribution of phytoplankton community structure and environmental parameters at a depth of 600 m.

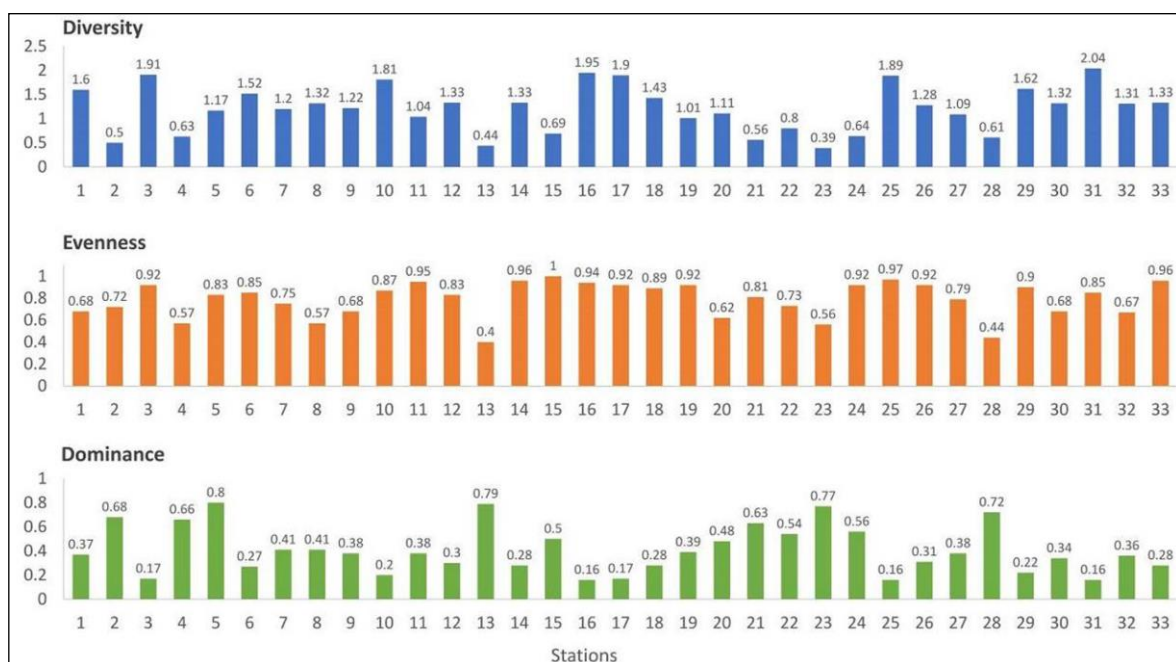


Figure 6. The diversity, dominance, and evenness indices of phytoplankton community at a depth of 600 m.

Environmental conditions. Temperature and salinity at a depth of 600 m exhibited similar values for all stations (Figure 7). Temperature ranged from 6.42°C to 7.33°C with an average of $6.86 \pm 0.25^\circ\text{C}$. Salinity ranged from 34.49‰ to 34.55‰ with a mean value of 34.53 ± 0.01 ‰.

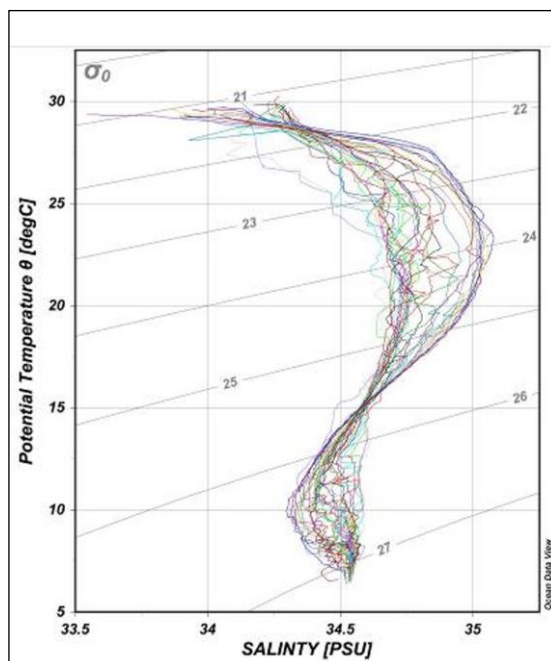


Figure 7. Profile of temperature and salinity at a depth of 600 m.

The vertical profiles of fluorescence at stations with the highest (Station 32) and lowest (Station 15) abundance are shown in Figure 8. At Station 32, the chlorophyll fluorescence ranged between 0.0089-0.6532 mg/m³ with an average of 0.0658 mg/m³. Whereas at Station 15, the chlorophyll fluorescence ranged between 0.0075-0.5543 mg/m³ with an average of 0.0785 mg/m³. Furthermore, Figure 8 shows that the depth where chlorophyll was found at Station 32 was deeper than that of Station 15.

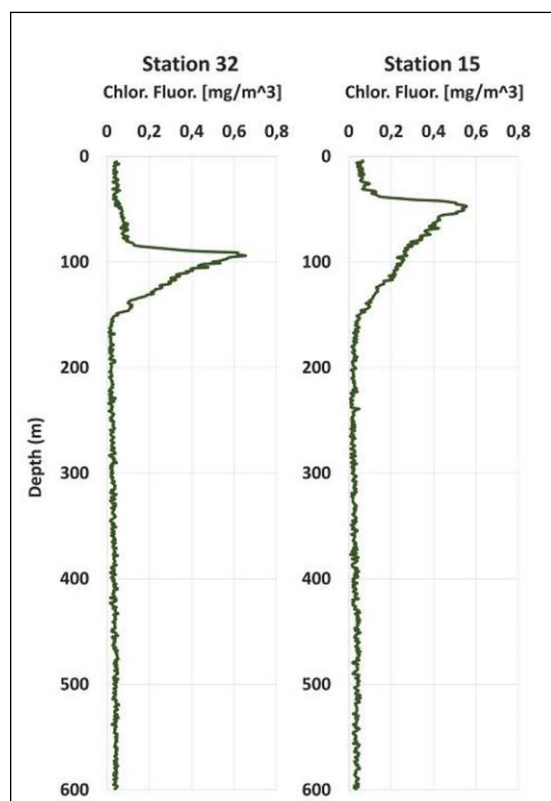


Figure 8. Vertical profile of fluorescence at the stations with highest (Station 32) and lowest (Station 15) abundances.

Discussion. Results of this study showed that Bacillariophyceae dominated phytoplankton community (cell size > 20 μm) in the deep sea (600 m) of the Sangihe-Talaud Islands waters. Bacillariophyceae has high ability to live and survive due to its high competitiveness, endurance, and reproduction rates. For example, Bacillariophyceae has a high ability for nutrients uptake. According to Litchman et al (2006), Bacillariophyceae can absorb phosphate (0.5 μmol/day) three times faster than Dinoflagellates (0.17 μmol/day). Bacillariophyceae can absorb nitrate and ammonium ten times faster than dinoflagellates. Those abilities make Bacillariophyceae a superior competitor, especially at unfavorable conditions like in the deep-sea environment.

Class Bacillariophyceae species that dominated deep-sea phytoplankton community in the Sangihe-Talaud waters is *Thalassiosira pseudonana*. *Thalassiosira pseudonana* can be found in all salinity conditions (2-30 PSU) and can grow at low temperatures (5°C) (Baek et al 2011). *Thalassiosira pseudonana* and *Thalassiosira rotula* have the ability to adapt to euryhaline and eurythermal conditions (Baek et al 2011; Krawiec 1982). Furthermore, the presence of well-preserved phytoplankton cells in the deep-sea has been reported in previous studies since 1960–1970s. Agusti et al (2015) reported the ubiquitous presence of healthy photosynthetic cells, dominated by diatoms, down to 4000 m. Bacillariophyceae is ubiquitously found with high abundance compared to other groups, except in waters with extreme temperatures and salinity (Round et al 1990). It has been suggested that diatoms in all waters encompass 100,000 species (Mann & Vanormelingen 2013) and contributing to 20 % of the total primary production (Falkowski et al 1998). Therefore, we postulate that due to its wide tolerance in salinity and temperature variation, *Thalassiosira* spp. can survive and predominate the deep-sea region of the Sangihe-Talaud Islands waters.

Our hypothesis here is supported by the Principal Component Analysis (PCA). The PCA showed salinity was the strongest factor that influenced phytoplankton community in the deep-sea region of Sangihe-Talaud Islands waters (Figure 9). Salinity exerts a strong positive effect on the abundance and negative effect on the diversity and evenness of the phytoplankton community. Figure 9 shows that salinity and abundance are located in the same quadrant and formed a narrow angle. Temperature shows a weak influence on abundance and evenness, but depicts a strong influence on diversity. Phytoplankton is generally influenced by salinity because most phytoplankton is stenohaline and suffers osmotic stress upon exposure to salinity changes (Potapova 2011; Lionard et al 2005).

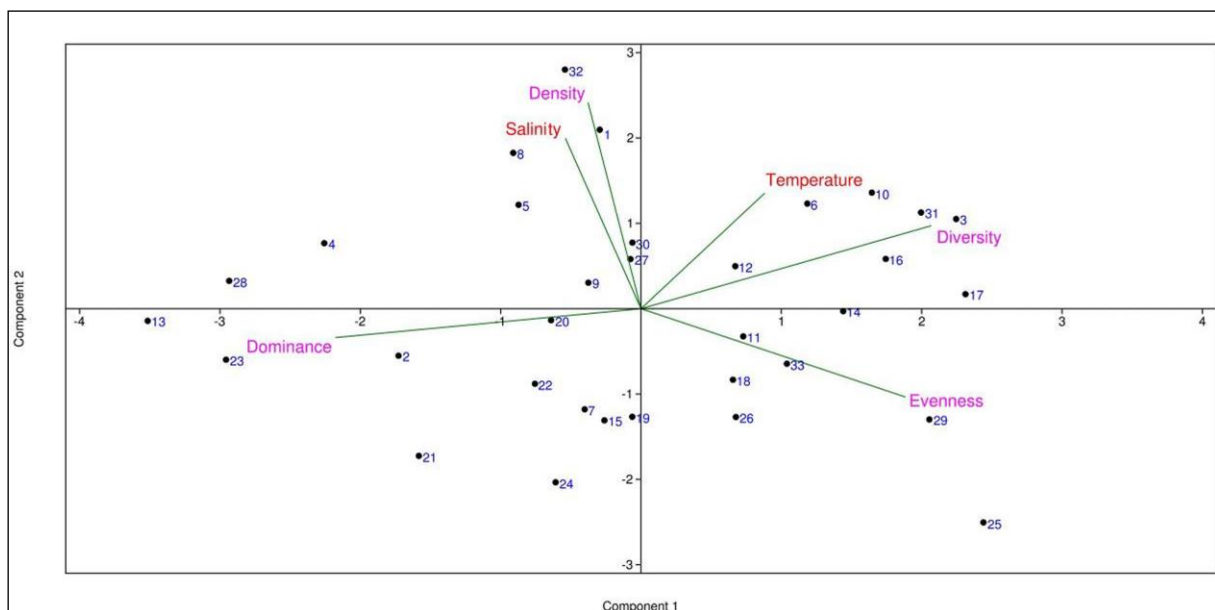


Figure 9. The Principal Component Analysis (PCA) of environmental parameters and community structure of phytoplankton.

According to fluorescence measurements, we found the presence of a low amount of chlorophyll at the depth of 600 m. Nevertheless, we cannot prove the presence of photosynthetic activity there due to limited equipment. Although photosynthesis effectively occurs in the euphotic zone (200 m), it does not mean that there is no photosynthesis below the euphotic zone. Several hundred meters (up to 800 m) below the euphotic zone there is a twilight area called the disphotic zone. In the disphotic zone, there is photosynthetic activity but cannot reach net productivity. Hence, the rate of photosynthesis is lower than the respiration rate. The presence of chlorophyll at deep sea was observed by Platt et al (1983). They took two samples of phytoplankton from 10 m and 1000 m, and found pigmented cells of phytoplankton. In addition, Platt et al (1983) also measured carbon assimilation rates of the samples in a temperature-controlled incubator and found a similar assimilation number, 0.8 mg C/h. Based on this observation, we postulate that the presence of phytoplankton at the depth of 600 m probably due to the fast-sinking rates of aggregates and fecal pellets that transported fresh phytoplankton to deep-sea. The sinking rates of individual phytoplankton are very slow, 1.5 m/day (Bienfang 1980). When individual phytoplankton forms aggregates, the sinking rates increased up to 124–732 m/day (Agusti et al 2015). It means the required times to reach deep-sea ranged from a few days up to a few weeks. Further investigation is needed to gain better understanding on deep-sea phytoplankton and this will be our future study. Overall, the present of study provides novel knowledge about phytoplankton community in the aphotic zone off the Sangihe-Talaud Islands and results of this study can be used as a foundation for future research in the Indonesian Seas.

Conclusions. We have studied phytoplankton community at a depth of 600 m in the region of the Sangihe-Talaud Islands waters. Our study revealed the Bacillariophyceae class was the most dominant class (54.2%) in the studied area. The results showed the most dominant species are *Thalassiosira* sp., *Chaetoceros peruvianus*, and *Chaetoceros borealis*. We hypothesized that the presence of phytoplankton in 600 m induced by biological pumping mechanisms. The sinking rates of the phytoplankton cells accelerated through the forming of fecal pellets and aggregate as a phenomenon of marine snow. Our study confirmed the existence of vertical carbon transport in the waters of Sangihe-Talaud Islands.

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