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Ecological Studies of Epiphytic Diatom on *Eucheuma denticulatum* (Rhodophyta) thallus Cultivated in Horizontal Floating Cage

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ABSTRACT

Epiphytic diatoms are phytoplankton groups commonly attached to substrates such as macroalgae. This study analyzes the community structure and diversity of epiphyte diatoms attached to *Eucheuma denticulatum* cultivated using the horizontal floating cage method. Six thalli of *E. denticulatum* propagules were taken randomly from each station. The samples were analyzed and identified in the laboratory. The research results clarified that epiphytic diatom composition consisted of 3 classes, two orders, 38 genera, and 79 species. The three classes that have been found include class Coscinodiscophyceae (10 genera) with percentages ranging between 8-34%, class Fragillaryophyceae (12 genera) with percentages ranging between 34-46%, and class Bacillariophyceae (16 genera) with percentages ranging between 26-46%. The uniformity index was 0.8287-0.9286, which is considered relatively high. The dominance species index was 0.0644-0.2055, categorized as low. Fluctuations in water's physical and chemical factors have no significant effect on the growth of diatom epiphytes in the thallus of *E. denticulatum*.

1. Introduction

Eucheuma is a species of macroalgae that is widely cultivated and spread very widely in the shallow coastal waters of Indonesia (Budiyanto *et al.* 2019; Kasim *et al.* 2016, 2018). The genera *Eucheuma* belongs to the Family Solieriaceae of the order Gigartinales. The thallus of *Eucheuma* is very cartilaginous, usually facing downward. Each branch is cylindrical. From each thallus springs a small thallus of thorns. The surface is relatively rough and serrated (Trono 1992). Although it has a small thallus on which it is difficult for dirt to attach, the small protruding thorn on the entire thallus is a perfect place for epiphyte diatom to attach firmly (Kasim *et al.* 2017).

Epiphytic diatoms are benthic diatoms mostly belonging to the Bacillariophyceae class (Kasim and Mukai 2006). This group is a phytoplankton component that most commonly appears in the water column. Diatoms are at the bottom of the food chain that utilizes photosynthesis to convert sun energy into chemical energy (Perry 2003). Diatoms are divided into two groups; planktonic diatoms and benthic diatoms. Planktonic diatoms live in the water column and are significantly affected by water current, while benthic diatoms live attached to unanimated objects or creatures in the water. Benthic diatoms attached to a plant are categorized as epiphytic diatoms (Kasim and Mukai 2006). The essential characteristic of a diatom is its protoplasm, which is covered by a silicate cell wall called frustule (De Stefano *et al.* 2009). Diatom epiphyte characteristics on different seaweed are almost the same (Tanaka 1986). Epiphytes are an important component in several marine ecosystems, including seagrass beds and seaweeds, one of the most productive coastal ecosystems.

Moreover, they take up the largest share of the epiphytic biomass (Jacobs and Noten 1980). The presence of epiphytes on underwater plants can reach 40% of the total underwater plant communities (Penhale 1977). Epiphytic algal populations' production often exceeds seagrass production (Moncreiff *et al.* 1992). Frequently, diatom densities in seagrass beds or on macroalgae are greater than

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those attached to rocks. Besides being attached to plants or rocks, epiphyte diatoms are often found in the intestines of fish rather than algal species in the mud, suggesting that these epiphytic algae are important for feeding fish and animals in shallow seas (Phillips and McRoy 1990).

Studies on epiphytic diatoms attached to *E. denticulatum* in diversity and species composition have never been done. However, much research has also been done on the attachment of epiphytes to macroalgae (Comte and Cazaubon 2002; Snoeijs 1994). The most commonly reported is epiphytic diatoms attached to seagrass (Frankovich and Zieman 1994). The present research is critical as the seaweed *Eucheuma* sp. is very popular cultivated macroalgae in Indonesia and epiphytic diatoms are a recurrent problem in its cultivation.

2. Materials and Methods

This research was conducted in July–August. The sampling location is at Lakeba coast, Baubau City, South East Sulawesi, Indonesia (05° 27' 24.5'' SL and 122° 36' 43.5'' EL). This study used four stations in areas with similar environmental conditions. Epiphytic diatom identification and chemical-physical analysis were conducted at the Faculty of Fishery and Marine Science Laboratory, Halu Oleo University.

Seaweed is cultivated with a horizontal floating cage method to eliminate herbivorous attacks (Kasim and Mustafa 2017). The thalli of *E. denticulatum* used in this experiment were cleaned up first using a soft brush. Each thallus sample is scattered in a horizontal floating cage without being tied. The initial weight of *E. denticulatum* thalli was 100 grams. There is one horizontal floating cage at each station, so the total horizontal floating cages in this study were 4. Each horizontal floating cage was occupied by 20 thalli of *E. denticulatum*. Every ten days, a collection of 5 thalli was carried out. Sampling was carried out four times a month. Sampling took a month as the harvest period of *E. denticulatum* in the cultivation area was 40 days. Epiphytic diatom samples were taken from the thallus of *E. denticulatum*. The thallus was cut 5 cm long on every edge. Each thallus sample was scraped using a soft brush with a flat surface. All diatoms attached to the thallus were washed so that all diatom samples were collected in 10 ml of filtered ocean water in sample bottles. Those samples were identified further in the laboratory using a microscope. The Epiphytic diatoms were identified and counted to the lowest taxonomic level as far as possible. The taxonomic identification of phytoplankton was carried out according to Yamaji (1977), Kawamura and Hirano (1989), Mizuno and Saito (1990), Kato *et al.* (1977), Sawai and Nagumo (2003), and the following online publications: https://planktonnet.awi.de/, http://www.marbot.gu.se/sss/diatoms, and http://protist.i.hosei.ac.jp/index.html.

The environmental parameters measurements were done simultaneously with the diatom epiphytes' sample pickup. Physical parameters measured in the field included temperature, transparency, and water current speed. The measured chemical parameters included salinity, nitrate, orthophosphate, and silicate. The chemical parameter samples for nitrate and orthophosphate were measured using spectrophotometry.

2.1. Data Analysis

The diversity represents species heterogeneity in a community. Shannon diversity is a widely used index for comparing diversity between various habitats (Clarke and Warwick 2001). It was calculated to know the species diversity in different habitats (Hutchinson 1970). Dominance index according to Simpson Dominance Index and Community similarity coefficient were calculated according to the formula in Krebs (1989).

2.2. Statistical Analysis

Correlation coefficients between diversity, similarity, and dominance with environmental factors were calculated using simple linear models (Pearson's r). Statistical analyses were performed using the SPSS package 24.

3. Results

3.1. Epiphytic Diatom Diversity

The diatom epiphytes that have been found attached to *E. denticulatum* included three classes, two orders, 38 genera, and 79 species that consisted of Coscinodiscophyceae class (10 genera), which belonged to the Centrales order, Fragillaryophyceae class (12 genera), and Bacillariophyceae (16 genera), which both belonged to Pennales order (Table 1).

Table 1. Occurrence of epiphyte diatom found attached on the thallus of *Eucheuma denticulatum*

Species	Class	Days of observation				Species	Class	Days of observation			
		10	20	30	40			10	20	30	40
Actinocyclus actonarius	C	-	-	√	√	*Synedra gracilis*	F	-	√	√	√
Biddulphiopsis	C	-	√	√	√	*Synedra pulchella*	F	√	√	√	√
membranacea						*Synedra ulna*	F	√	√	√	√
Biddulphia alternans	C	√	√	√	-	*Synedra rumpens*	F	-	-	√	√
Cyclotella sp.	C	√	√	√	√	*Striatella unipunctata*	F	√	√	√	√
Coscinodiscus sp.	C	√	√	√	√	*Tabellaria* sp.	F	-	-	-	√
Coscinodiscus radiatus	C	-	√	-	√	*Tabellaria flocculosa*	F	√	√	√	√
Coscinodiscus wailesii	C	√	√	√	√	*Tabellaria fenestrate*	F	√	√	√	√
Coscinodiscus	C	-	-	√	-	*Thalassionema*	F	-	√	-	√
concinnus						*nitzschioides*					
Coscinodiscus	C	-	-	√	√	*Rhabdonema*	F	-	-	-	√
commutatus						*adriaticum*					
Coscinodiscus gigas	C	-	-	-	√	*Ardissonia fulgens*	B	-	√	√	√
Leptocylindrus danicus	C	-	-	√	-	*Amphora* sp.	B	-	√	-	-
Leptocylindrus	C	-	-	√	√	*Amphora inflexa*	B	-	-	√	√
minimus						*Amphora hyaline*	B	-	-	√	-
Melosira granulate	C	-	√	√	√	*Bacillaria paxillifer*	B	√	√	√	√
Odontella aurita	C	-	-	√	-	*Cymbella* sp.	B	-	√	√	-
Thalassiosira	C	-	√	√	√	*Cocconeis* sp.	B	-	√	-	-
punctigera						*Diploneis* sp.	B	√	√	√	√
Thalassiosira hendeyi	C	-	√	-	√	*Diploneis bombus*	B	-	√	-	-
Triceratium junctum	C	-	-	√	-	*Diploneis crabro*	B	-	√	√	-
Asterionellopsis	F	-	√	√	√	*Donkinia pecta*	B	-	√	-	√
glacialis						*Eunotia* sp.	B	-	√	√	-
Climacosphenia	F	√	√	√	√	*Eunotia bilunaris*	B	√	-	-	√
moniligera						*Gyrosigma* sp.	B	√	√	√	-
Fragillaria sp.	F	-	√	√	√	*Gyrosigma obtusatum*	B	-	-	√	-
Fragillaria islandica	F	-	-	√	√	*Mastogloia* sp.	B	-	√	-	√
Grammatophora	F	-	-	-	√	*Nitzschia* sp.	B	√	√	√	√
oceanic						*Nitzschia palea*	B	√	√	√	√
Lioloma sp.	F	-	-	√	-	*Nitzschia sigma*	B	√	-	-	-
Licmophora sp.	F	√	-	-	-	*Nitzschia sigmaformis*	B	-	√	√	√
Licmophora flabellata	F	√	√	√	√	*Nitzschia longissima*	B	√	√	-	√
Licmophora juergensii	F	-	√	-	-	*Nitzschia distans*	B	-	√	-	√
Licmophora dalmatica	F	√	-	√	√	*Nitzschia closterium*	B	-	√	√	-
Licmophora ehrenbergii	F	-	√	-	√	*Navicula* sp.	B	√	√	-	√
Licmophora splendid	F	√	√	-	√	*Navicula transitans*	B	√	√	√	√
Licmophora ovulum	F	-	-	-	√	*Navicula gregaria*	B	√	√	√	√
Licmophora paradoxa	F	√	-	√	√	*Pinnularia* sp.	B	√	√	√	√
Licmophora gracilis	F	-	√	√	√	*P. major*	B	√	-	-	-
Licmophora lyngbyei	F	-	√	√	√	*Pleurosigma* sp.	B	√	√	√	-
Podocystis sphatulata	F	√	√	√	√	*Pleurosigma normanii*	B	-	-	-	√
Synedra sp.	F	√	√	√	√	*Surirela* sp.	B	-	-	-	√
Synedra Formosa	F	-	√	√	√	*Trachyneis aspera*	B	-	√	-	-
Synedra acus	F	√	√	√	√						

C: Coscinodiscophyceae, B: Bacillariophyceae, F: Fragillaryophyceae, (√): Occurs, (-): non occurrence

Diatom epiphyte species composition during the research has been found to include Bacillariophyceae class at the highest percentage around 26-46%, Fragillaryophyceae class 34-45%, and Coscinodiscophyceae class 8-34% (Figure 1). The average value of the diversity index on day 10 ranged from 1.81 to 2.14, with the highest value in Station 4 and the lowest at Station 1 (Figure 2). The average value of the similarities index on day 10 ranged between 0.83 and 0.91, with the highest value in Station 3 and the lowest in Station 1 (Figure 3).

The average value of the dominance index on day 10 ranged between 0.14-0.21, with the highest value in Station 1 and the lowest in Station 4 (Figure 4).

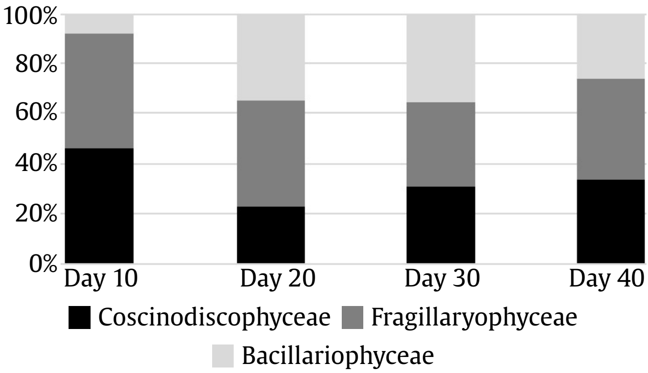

Figure 1. Percentage composition of epiphytic diatoms on *Eucheuma denticulatum*

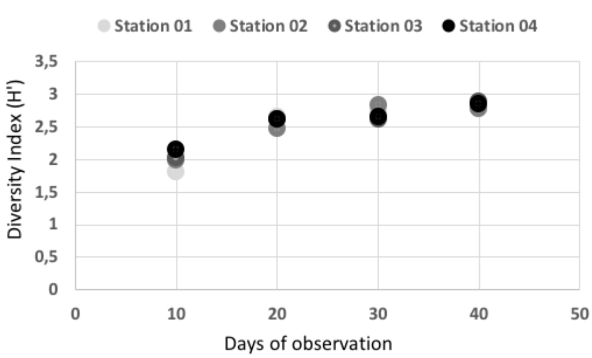

Figure 2. Diversity index of epiphytic diatoms in every station (station 1 = A, station 2 = B, station 3 = C, station 4 = D)

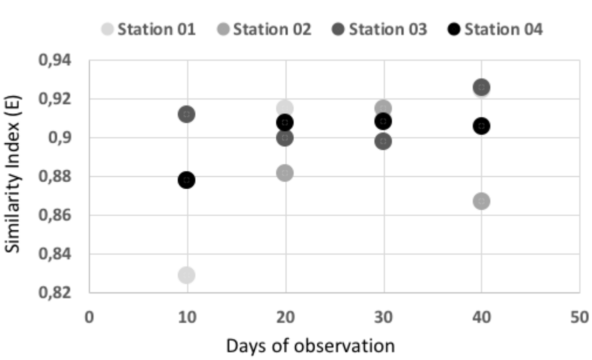

Figure 3. Similarites index of epiphytic diatoms in every station (station 1 = A, station 2 = B, station 3 = C, Station 4 = D)

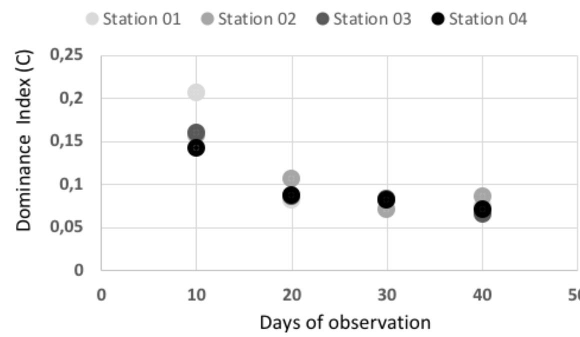

Figure 4. Domiance index of epiphytic diatoms in every station (station 1 = A, station 2 = B, station 3 = C, station 4 = D)

3.2. Water Quality Parameters

The average temperature value measurement result during the research ranged between 25-26°C. The temperature level in sampling sites from the beginning until the end of measurement showed no significant difference. However, it decreased on day 40 to 25°C. The transparency level measurement result during the research is 100%. During the research, current velocity level measurement results ranged between 0.02-0.07 m/sec. Salinity level measurement results on horizontal floating cage during the research range between 30-32 ppt. The average value of nitrate concentration in all stations during the research ranged from 0.01 to 0.03 mg/L. The average value of orthophosphate concentration in all stations during the research ranged from 0.001 to 0.009 mg/L. The average value of silicate in all stations during the research ranged from 0.08 to 0.36 mg/L. These values fluctuated significantly (Figure 5). Pearson analysis results showed that diversity, similarity, and dominancy, do not correlate with all environmental factors. However, nitrate negatively correlates to temperature and salinity with a confidence level of 0.05 (Table 2).

4. Discussion

4.1. Epiphytic Diatoms Diversity

Diatom epiphytes found were three classes, 2 orders, 38 genera, and 79 species that consisted of Coscinodiscophyceae class (10 genera). These were

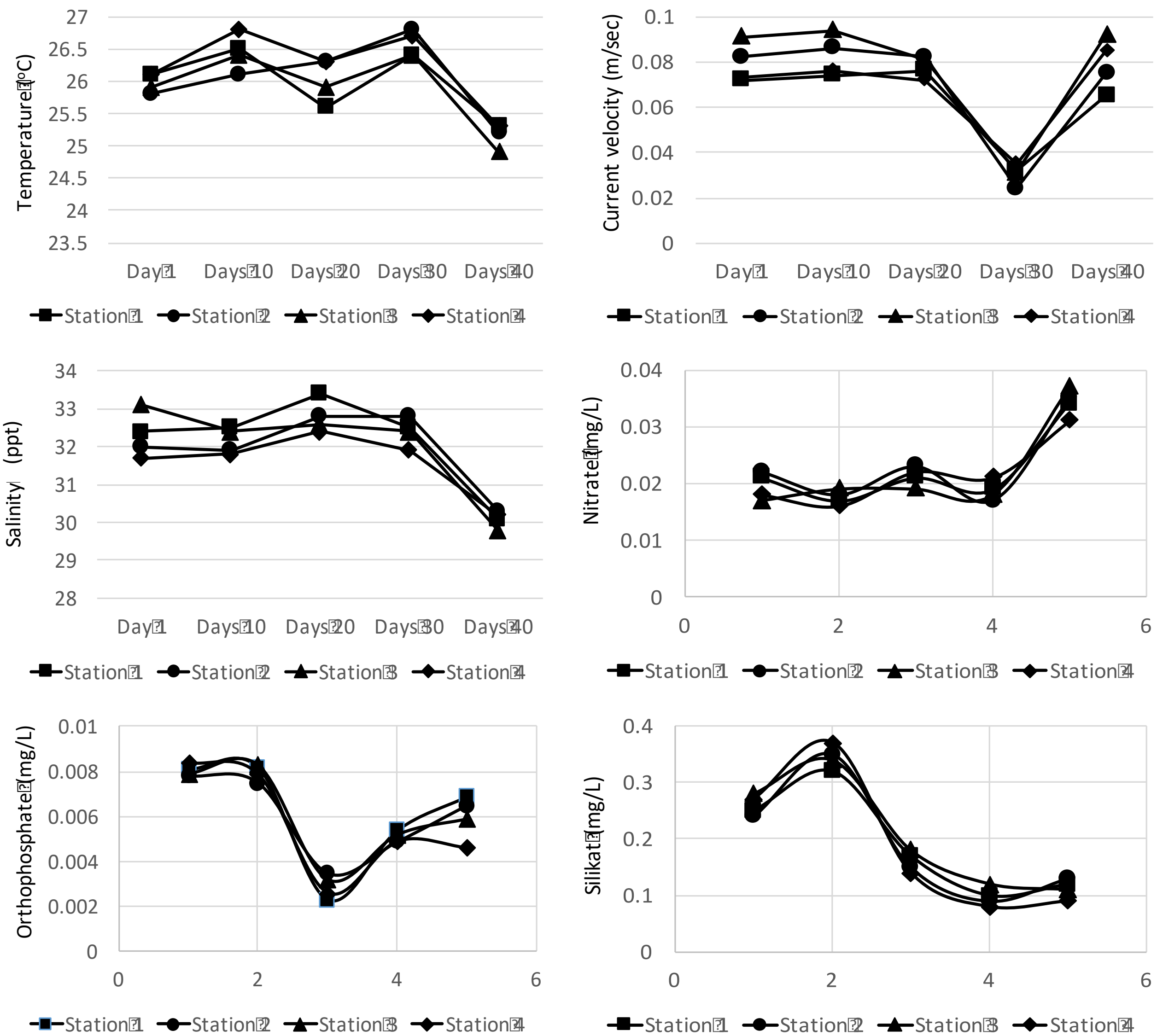

Figure 5. Variation of temperature, salinity, current velocity, nitrate, orthophosphate, silicate concentration in every station

Table 2. Pearson correlation analysis between diversity, similarity, and dominancy with environmental factors

Similarity	Similarity	Dominancy	Temperature	Current	Salinity	Nitrate	Phosphate	Silicate
Diversity	-0.426	1.000**	0.41	0.365	0.797	-0.632	-0.23	0.485
Similarity		-0.411	-0.208	-0.458	-0.329	0.497	-0.74	-0.846
Dominance			0.397	0.37	0.79	-0.617	-0.247	0.471
Temperature				-0.546	0.81	-.931*	0.054	0.33
Current					-0.237	0.286	0.234	0.518
Salinity						-.913*	-0.206	0.295
Nitrate							-0.154	-0.523
Phosphate								0.693
Silicate								

*Correlation is significant at the 0.05 level
**Correlation is significant at the 0.01 level

the same species found in Tanaka (1986) research that the diatom epiphyte species composition on different natural seaweed included *Amphora* sp., *Achanthes* sp., *B. paxilifer, Cocconeis* sp., *Cocconeis scutellum, Gomphonema* sp., *Licmophora* sp., *Licmophora flabellata, Licmophora paradoxa, Nitzschia* sp., *Nitzschia closterium, Nitzschia hybrida, Navicula* sp., *Skeletonema costatum, Synedra tabulata*, and *Synedra fulgens*. Diatom epiphyte species are relatively similar to those in the water column and sediment surface. Kasim and Mukai (2006) explained that diatom epiphyte species composition is similar to the diatom groups in water columns and sediment surfaces. Benthic diatom species composition in sediment surface are *Amphora* sp., *Amphora ventricosa, Bacillaria paradoxa* var paxilifer, *Cocconeis costata, C. scutellum, Nitzschia rhynchocephala, Navicula* sp., *Navicula sigma, Paralia sulcata, Rhabdonema arcuatum, Rhoicosphenia curvata, Synedra ulna, Melosira juergensi*, and *Thalassiosira* sp. From the research conducted on the Southern Coast of Korea, 81 diatom species were found (2 orders, Centrales and Pennales, three suborders, eight families, 26 genera) (Winesti 2014).

Seaweeds give more space to attach epiphytic diatom colonies. The attachment of the epiphyte diatom to the thallus is the link to the surface of the host thallus' substrate, which will give the diatom a chance to stick. Colonization and adhesion of epiphyte diatoms occurred at the 12th week at sea. The attachment is preceded by the attachment of some abiotic material that pollutes the surface (Jacobi and Langevin 1996). Epiphytic diatoms attach to host tissues such as seaweed thallus with loose or attached components. Differences in diversity and community structure appear to result from an accumulation of seasons and water conditions, which support the attachment of the diatom epiphyte. However, the reduction of epiphytic biomass in the thallus does not differ from that of the hosting site at the same age and exposure. It is caused by the insufficient time of colonization rather than the inhibition by seaweed (Cattaneo and Kalff 1978).

All species found in every station during our observation were different. In the present study, the increase in species number occurred on day 40. It was caused by an increase in the nutrient content such as nitrate (0.03 mg/L), orthophosphate (0.006 mg/L), and silicate (0.11 mg/L). Nutrient increase pattern causes the phytoplankton population's improvement, and the reduction of the nutrient also affects the phytoplankton population's decrease (Kasim and Mukai 2006, 2009).

Based on the observation during this research, the percentage of the highest species composition comes from Bacillariophyceae class ranging from 26-46% and Fragillaryophyceae class ranging from 33-45%. Bacillariophyceae and Fragillaryophyceae classes are considered pennate diatoms that usually live as benthic diatoms. Bacillariophyceae has a morphological structure called raphe, used for attachment and movement on surfaces. According to Geim *et al.* (2003), the raphe diatom is the biggest diatom group in term of species number and are easily recognized by a dividing system for the occurrence of the paired raphe along with a long frustula. Coscinodiscophyceae class found during the research ranged between 8-33%. This value is lower compared to Bacillariophyceae class and Fragillaryophyceae class. Coscinodiscophyceae class or centrales order lives in planktonic form in the water column. However, it can also be found living as an epiphytic organism. Diatom community in sediment is categorized as pelagic species. Many pelagic diatomic cells can be found on sediment surfaces at specific times (Kasim and Mukai 2006). The epiphytic microalgae community attached to seaweed consists of various diatom species, which belong to the class of Rhodophyceae, Phaeophyceae, Chlorophyceae, and Cyanophyceae (Frankovich and Zieman 1994). The dynamics, abundance, and structure of epiphytic microalgae communities are influenced by biotic factors such as leaf age, host seasonal cycles, grazing pressure by herbivores, and the movement of light, temperature, nutrition, and water (Mabrouk *et al.* 2011; Prado *et al.* 2007).

During our research, the average value of the diversity index on day 10 was low, and it tended to increase on days 20 and 30. This diversity index can be classified as low to intermediate. On day 10, low diversity was found on every plot because of the dominance of *Coscinodiscus* sp., *Synedra acus*, and *Nitzschia palea* species. However, this dominance does not indicate differences because the diversity level was intermediate the next day. The presence of few species causes the intermediate diversity index. Similarity index average value on day 10 ranged from 0.83 to 0.91, showing that the individual amount of each species is the same. Species dominance index is a different individual amount spread, and there are tendencies for a species to dominate. The dominance index average value on day 10 was high and tended to decrease during days 20 and 30. The values during the research showed a low level; therefore, it could be said that there is no diatom epiphyte species dominance. The epiphyte diatom community structure attached to *E. denticulatum* at research sites was described as relatively stable. This is shown in a diatom epiphyte similarity index, which is relatively low to intermediate; it spread out equal similarity index and dominance index, which is relatively low. The abundance and richness of diatom epiphytes in tropical elevational gradient waters generally increased maximum shape. The species' composition in this area also showed a similar set (Ding *et al.* 2016).

4.2. Water Environment Parameter Condition

During our research, the temperature level ranged between 25 and 26°C. This temperature level is still optimal for diatom epiphyte growth. On day 40, the temperature was reduced to 25°C due to rain. This temperature drop was caused by decreased sunlight intensity and frequent rain. The growth and metabolic processes of a diatom are usually determined by surface temperature (Moisan *et al.* 2002; Raven and Geider 1988) this affects the population size and biomass of the diatom on the surface area (Longhurst 1998; Needoba *et al.* 2007).

Moreover, our results showed that the salinity level decreased on day 40 to 32 ppt caused by rainy weather. Salinity plays a vital role in spreading and presenting epiphytic diatoms in macroalgae (Snoeijs 1994). Current velocity affects the horizontal spread of diatoms in shallow regions. Current velocity will generate turbulence that will lift the diatoms from the bottom to the water's surface (Kasim and Mukai 2009). Nitrate is an essential nutrient needed by diatom epiphytes to support their growth. The availability of nitrate always correlates with phytoplankton growth. The nitrate uptake rate is positively correlated with cell size because larger algae have higher vacuole volume per biomass, where nitrate can be stored (Stolte and Riegman 1996). A low nitrate level in the horizontal floating cage is caused by the competition of diatom epiphyte and the host seaweed to process nitrate. An epiphyte is a major rival for some seaweed to absorb nutrients due to its growth. Epiphyte also does an excellent strategy to offset the seaweed absorbing nutrients in nature (Harrison and Hurd 2001). In addition, the orthophosphate level during the research ranged between 0.001 and 0.009 mg/L. This value is low and less optimal to support diatom epiphyte's growth. Besides, this value becomes the bounding factor for growth because the concentration level is less than 0.02 mg/L. Yazwar (2008) mentioned that orthophosphate is optimal for phytoplankton growth between 0.27-5.51 mg/L. Furthermore, one of the critical nutrients to support the diatom epiphyte growth is silicate. The availability of silicate will impact diatom abundance; however, the low silicate concentration affects diatom density and is related to the availability of nitrate (Gilpin *et al.* 2003; Jezequel *et al.* 2000).

In conclusion, benthic diatoms found in the present study were 97 species that belonged to 3 classes: Bacillariophyceae, Fragillaryophyceae, and Coscinodiscophyceae. The Bacillariophyceae class has a high percentage, which was 26-46%. Benthic diatom populations had a high diversity value and were found attached to the thallus of *Eucheuma denticulatum* on the tenth day of cultivation. Similarity index values are low for all the cultivation periods, and almost no dominance of a benthic diatom species occurred in the *E. denticulatum* thallus. During the study, environmental factors did not correlate with diversity, similarity, and dominancy of benthic epiphytes, with a confidence level of 0.05.

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