The diversity and species composition of epiphytes on *Eucheuma denticulatum* (Rhodophyceae) cultivated on horizontal net

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Abstract. *Eucheuma denticulatum* is one of the species of seaweed cultivated in Indonesia. On its development, there were problems regarding epiphytes attaching to the seaweed thallus and cultivation tools. This research aims to analyze the diversity and species composition of the epiphytes attach on thallus of *E. denticulatum*. The cultivation of *E. denticulatum* utilizes horizontal net cages in order to avoid herbivorous graze on samples. The epiphyte samples (sized >1 mm) were randomly obtained from the thalli of *E. denticulatum*. The species composition of the attained epiphyte consisted of 14 species, including 2 species of the Phaeophyceae class, 4 species of the Clorophyceae class, and 8 species of Rhodophyceae. Several species were very dominant on thallus of *E. denticulatum*, such as *Neosiphonia* sp., *Polysiphonia* sp. and *Ulva clathrata*. The percentage of the presence of *Neosiphonia* sp. was 57-95%, *Polysiphonia* sp. ranged from 6 to 19% and *U. clathrata* was 42%. Water parameter measurement results showed temperatures between 25 and 26ºC, 30-32‰ salinity levels, and a current velocity between 0.0175 and 0.0676 m s^{-1}. The nitrate levels measured were between 0.0142-0.0296 mg L^{-1} inside the cages. Phosphate levels measured inside the cages was 0.0011-0.0086 mg L^{-1}. There was no correlation evidenced between abundance of epiphyte and environmental factors.

Key Words: seaweed, cultivation, production, growth rate, macroepiphyte.

Introduction. *Eucheuma denticulatum* is a genus of Eucheuma that is widely cultivated in tropical waters. This type is unique and contains carrageenan which is a raw material for various industries in the world. The utilization of seaweed is currently quite extensive in daily life, both as a source of food, medicine and raw materials for the food, cosmetics and pharmaceutical industries (Kasim et al 2016; Kasim & Mustafa 2017). The amount of market demand for seaweed is spurring the development of seaweed cultivation. One of the success parameters of seaweed cultivation is growth; hence growth is one of the biological aspects that must be considered. The growth of Eucheuma is influenced by internal and external factors (Kasim et al 2017). Internal factors that influence the growth of seaweed include species, strain, thallus section and age, while external factors include physical environmental conditions, such as water chemistry, pest attacks, diseases and technological management. Seaweed cultivation continues to develop in most countries in Southeast Asia (Luxton 1993; Lirasan & Twide 1993; Luxton et al 1987; Bindu 2011; Mollion & Braud 1993; Ohno et al 1996; Hayashi et al 2007; Ask & Azanza 2002). The development of methods provides a dynamic production value in each region. Several Southeast Asian countries use the same methods, namely the longline and the raft methods (Azanza-Corales 1990). Some countries with shallow waters and a sandy beach base use the method of long rope tie or base stocking. Different cultivation methods will produce different production values. Each different method also impacts the biological response that appears at the cultivation site. One biological response is the attachment of epiphytes on each seaweed thallus. The epiphyte attachment process can happen very quickly because epiphyte is a macroalgae group with fairly fast growth. The fast attachment of epiphytes is an apparent problem in the world of seaweed cultivation.

Epiphytic attachment is not a new phenomenon and has been known since cultivation activities began (Doty & Alvarez 1975). Epiphytes are organisms that live attached to other plants. They are classified according to size: mesoepiphytes which are <1 mm in size, while macroepiphytes have a size of >1 mm. Some examples of epiphyte species are *Chaetomorpha* sp., *Ulva* sp. and *Hydroclathrus* sp. (Pelinggon & Tito 2009). The attachment of epiphytes in host plants is thought to limit sunlight penetration, which will disrupt the process of photosynthesis in the host plants and will lead to stunted growth, thin thalli and low daily growth rates (Susanto 2005). This study attempts to reveal information on the diversity of the epiphytes attach on the thalli of *E. denticulatum*.

Material and Method. The present research was conducted in June-August 2016 at Lakeba Beach, Baubau City, Southeast Sulawesi Province. It was followed by the sample observation at the Laboratory of the Faculty of Fishery and Marine Science, Halu Oleo University, Kendari, Indonesia. *E. denticulatum* was cultivated for 40 days using the horizontal net method with the size of 210 x 80 x 60 cm in order to protect against herbivorous fish (Kasim et al 2018; Budiyanto et al 2019). All outer parts of cage were covered by nets of 1 cm mesh size. During the field research, several horizontal net were used for repetitive observation. Each horizontal net was filled with 40 thalli of *E. denticulatum* with the initial weight of 100 g. The horizontal nets were placed in four different locations. The cultivated *E. denticulatum* was not cleaned of the epiphytes to explore the number and species composition of the attached epiphytes during the field study.

Collection of epiphyte samples. Epiphyte samples were randomly collected from thallus of *E. denticulatum* and only those sized <1 mm (microepiphyte) were considered. The frequency of epiphyte sampling was conducted every 10 days with 5 thalli in each cage, with a total of 20 thalli. Epiphytes were taken from each *E. denticulatum* thallus using tweezers. The epiphytes attached to the thalli and horizontal nets were identified according to their species level. Each epiphyte species was documented by a macro zoom digital camera to make identification easier. The dominating epiphyte sample such as *Chaetomorpha crassa* was weighed using 0.1 g accuracy analytical scales. The epiphyte samples were identified using the seaweed identification manual according to Setyobudiandi et al (2009) and through online identification according www.algaebase.com and http://biogeodb.stri.si.edu/pacificalgae/list.

Water physio-chemical parameter measurements. The collection of water physio-chemical parameter data (temperature, salinity, water transparency, and current speed) was conducted through measurement and direct observation on location, while nitrate and phosphate were analyzed at the laboratory. The nitrate and phosphate samples were specifically collected inside and outside the horizontal nets. The physio-chemical parameter samplings were simultaneously taken with the epiphyte samples at 10 day intervals.

The epiphyte data was tabulated and analyzed quantitatively, while the water physico-chemical parameter measurement results were tabulated and then analyzed descriptively.

Results and Discussion. The epiphytes attached to thallus of *E. denticulatum* were different in each sample collection time. *Dictyota dichotoma* appeared continuously on day 10, 20 and 30, while *Acrosorium ciliolatum* only appeared on day 30 (Table 1). Epiphyte species composition consisted of 12 types, dominated by *Neosiphonia* sp., *Polysiphonia* sp. and *U. clathrata*. The *Neosiphonia* sp. occurrence percentage was between 57 and 95%, *Polysiphonia* sp. between 6 and 19% and *U. clathrata* 42% (Table 1).

Table 1

Epiphyte species composition on thallus of *Eucheuma denticulatum*

No.	Species	Day			
		t10 (%)	t20 (%)	t30 (%)	t40 (%)
1	*Acanthophora muscoides*	0.6	2.5	0.9	0.6
2	*Acanthophora spicifera*	-	-	-	0.1
3	*Acrosorium ciliolatum*	-	-	-	0.05
4	*Ceramium* sp.	0.05	-	0.5	0.3
5	*Dictyota dichotoma*	0.27	0.25	0.2	0.4
6	*Gracilaria brevis*	-	-	-	0.06
7	*Hypnea nidulans*	-	-	0.7	0.1
8	*Neosiphonia* sp.	56.7	94.8	90	78.3
19	*Polysiphonia* sp.	-	-	6.3	19.3
10	*Sargassum cristaefolium*	0.12	0.29	-	-
11	*Ulva lactuca*	-	1.1	0.8	0.4
12	*Ulva clathrata*	42.3	-	0.1	-

t10 - day 10, t20 - day 20, t30 - day 30, t40 - day 40.

The diversity, uniformity and dominance index of the epiphytes seems to varied at each station. However, some species of epiphyte were seen to be dominant on day 20, 30 and 40 (Figure 1).

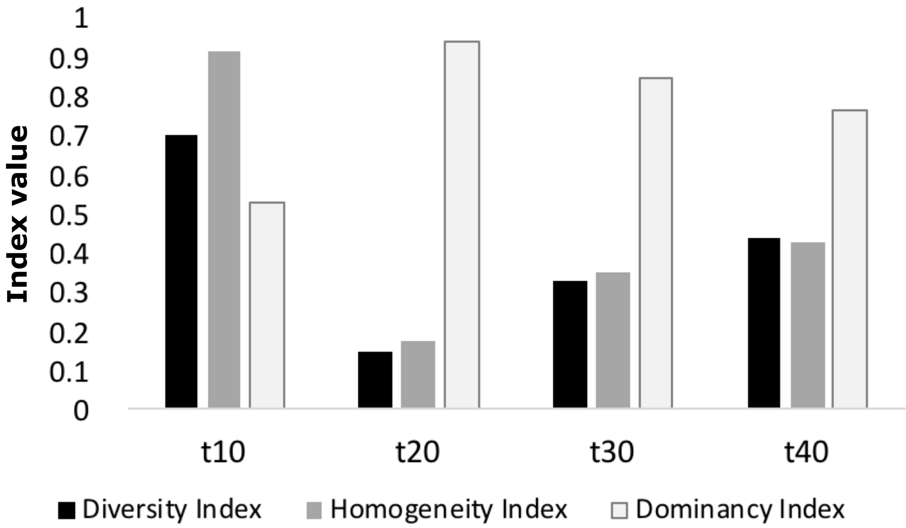

Figure 1. The diversity, uniformity and dominance index of epiphytes attached to *Eucheuma denticulatum* thallus.

Based on the present research, there were 14 epiphytes found. The epiphytes consisted of two species belonging to Phaeophyceae class (*Padina australis* and *S. cristaefolium*); three species belonging to Chlorophyceae class (*U. lactuca*, *C. crassa* and *U. clathrata*); and eight species belonging to Rhodophyceae class (*Neosiphonia* sp., *H. nidulans*, *A. muscoides*, *A. spicifera*, *Polysiphonia* sp., *A. ciliolatum*, *G. brevis*). Yulianto et al (1990) stated that there were a total of 14 epiphyte species found in Pulau Arar, Sorong, Indonesia. There are four species belonging to Rhodophyta, four species to Clorophyta, and six species to Phaeophyta class. Rombe et al (2013) exploring the Banteang Regency coastal line, South Sulawesi Province, Indonesia, reported 10 macroepiphyte species, two species belonging to Clorophyta and eight species to Rhodophyta group. Munoz & Fotedar (2009) found 24 species of epiphytes attached to *Gracilaria cliftonii* thallus. There were 21 species belonging to Rhodophyta and 3 species belonging to Chlorophyta phylum. *Ceramiales* is one of the dominant orders that including four species belongs to *Ceramium* genus and two species belonging to Pinnulariales. Soares & Fujii (2012) found that there are 48 species of epiphyte at Boa Viagen Beach, Brazil. The species were consisting of 20 species belonging to Chlorophyta, 27 species belonging to Rhodophyta, and one species belonging to Phaeophyta. These epiphytes were distributed into 11 orders and 16 families. The difference between the amount and epiphyte species attached to macroalgae is caused by the structure and shape of its host. Schmidt & Scheibling (2006), explain that the difference of macroalgae shape will affect the epiphyte species attach on the thallus. Small sized algae are not enough to protect epiphytes from predation and damage caused by tidal waves and cannot gather adequate foods. Algae with a complex and wide surface areas of thallus have more epiphyte species attached. The highest composition of the epiphyte species during the present research was the *Neosiphonia* sp. This species is able to reach 57-59% dominancy on thallus of *E. denticulatum*. *Polysiphonia* sp. is epiphyte with low dominance on thallus of *E. denticulatum* with 6-19% occurrence. The high percentage of *Neosiphonia* sp. and *Polysiphonia* sp. is related to the low current velocity. In our study site, current velocity was ranged 0.0175-0.0676 m s^{-1}. Devi (2020) reported that epiphyte attached to the seaweed species such as *Neosiphonia* sp. and *Polysiphonia* sp. were influenced by low current velocity. The occurrence of Neosiphonia *sp.* has been found more near seaweed cultivation areas. Vairappan (2006) stated that *Melanothamnus savatieri* is initially shaped as a little black spot on the thallus and became larger. Therefore, the epiphyte has been living attached to the thallus since the beginning of cultivation in larger scale. *Neosiphonia* sp. and *Polysiphonia* sp. were found in high percentages in cultivation areas. Borowitzka (1972), explain that epiphyte species such as *Ulva* sp. has high reproduction ability and can survive in dynamic water conditions, even in polluted water. Callow (1986) states that *Ulva* sp. has a high tolerance towards water quality changes and high reproduction ability, hence it vastly spreads in the water and also attaches to macroalgae. Neushul et al (1976) describes that other algae such as *Chondria* sp. and *Polysiphonia* sp. also have a high ability for recruitment and survival growth.

In our study site, during days 30 and 40, there were appearance of several epiphytes that belongs to Rhodophyceae class. The species were consisted of *Polysiphonia* sp., *H. nidulans*, *Ceramium* sp., *A. muscoides*, *A. spicifera*, *A. ciliolatum*, *G. brevis*. Atmadja et al (1999), explain that there are community succession of epiphyte which occurs in Pari Island, Southeast Sulawesi Indonesia. Several green algae were growth in the period of two weeks. *A. spicifera*, *Hypnea* sp. and *Rhosera* sp. occurred in weeks III and IV. Rhodophyceae class was low in recruited samples compared to Chlorophyceae and Phaeophyceae class. Romimohtarto & Juwana (2009) stated that one interesting characteristic of red algae growth is the absence of spore or vibrated feather swimming gamete. This deviates from the living body growth habit occurring inside the water medium. This creates spread and intimate encounters between growth cells dependent towards the current. The attachment epiphyte on *E. denticulatum* thallus consists of *P. australis* and *S. cristaefolium* that belongs to Phaeophyceae group. Soares & Fujii (2012) also found that from 48 species of epiphytes that belonged to Phaeophyceae class on Boa Viagem beach, Brazil.

Epiphytes on the horizontal net. During the present research, there were 11 species of epiphytes attached on horizontal nets. Four species belonged to Phaeophyceae (*Turbinaria ornata*, *D. dichotoma*, *P. australis*, *Sargassum cristaefolium*) three species to Clorophyceae (*Ulva intenstinalis*, *U. lactuca*, *C. crassa*), four species to Rhodophyceae (*A. muscoides*, *Hypnea valentiae*, *Hypnea esperi*, *A. spicifera*) (Figure 2).

Hamsia (2014) explains that several species found in seaweed farming tools include *Acanthophora* sp., *C. crassa*, *D. dichotoma*, *Hypnea* sp., *Padina* sp. and *Ulva* sp. Soenardjo (2011) explain that several species such as *Ulva* sp., *Padina* sp., and *Chaetomorpha* sp. were abundant on seaweed cultivation tools (floating cage) in Bantarpanjang, Pulau Nusakambangan, Indonesia. The epiphytes live and grow in the horizontal nets even though they has negative impact on seaweed growth, especially in terms of uptake of nutrients, nitrate, and phosphate concentration inside the cage.

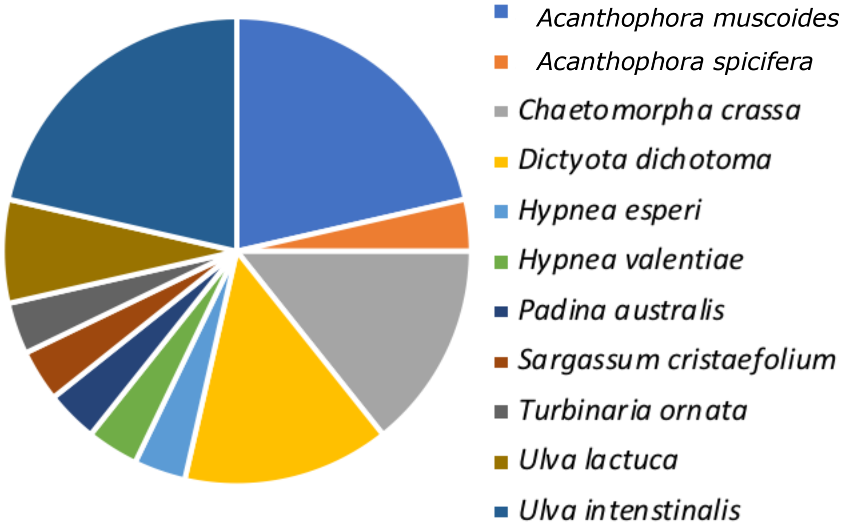

Figure 2. List of species attached to the horizontal nets.

Nitrate and phosphate levels, outside of the horizontal net are higher than inside the cage. The decrease in nutrients in the horizontal net is due to nutrients absorbed by the epiphyte in the horizontal net during the photosynthesis process and covering the surface of the nets and preventing nutrient absorption in the horizontal nets. Indriani & Sumiarsih (1997) stated that the attaching epiphytes on the seaweed or nets can hinder light penetration and disturb nutrient levels. Rombe et al (2013) also arguing that epiphyte can be a competitor for algae in absorbing the nutrients and occupy space for seaweeds growth. Furthermore, epiphytes with high density could deprive cultivated seaweed to receive sunlight. The occurrence of epiphyte on the horizontal nets does not only prevent nutrients from entering the horizontal net but also damages its structure. Some epiphyte such as *Ulva*, *Hypnea*, *Chaetomorpha*, and *Enteromorpha* often tangles the plants and cultivation structure and damages it, hence the cultivation media must often be cleaned and well maintained. Algae epiphyte has an important role in the water benthic community (Longtin et al 2009). Epiphytes play a positive role as a food source and supply habitat for water organism (Karez et al 2000; Viejo & Aberg 2003), while its negative impact, will reduce the growth of the macroalgae (Buschmann & Gomez 1993; Kraberg & Norton 2007). Macroepiphytes that attach to *E. denticulatum* thallus can become a competitor in obtaining space, nutrients, and sunlight absorbance. The characteristics of epiphytes that attach to the seaweed thallus surface is that it can hinder the process of sunlight and nutrient absorption and also damage the thallus tissue. This condition is also found in *Gracillaria* spp. where it is attached by epiphytes; Buschmann & Gomez (1993) argue that the main problem for the productivity and quality of *Gracillaria* spp. is that the epiphytes reduce the growth of its host and its biomass will decrease due to direct competition with the algae host for space, nutrients, and inorganic carbon from the water column. This is also supported by Pelinggon & Tito (2009) stating that the causes of cultivated seaweed biomass reduction are ice-ice disease, epiphyte parasitism, and predators. *Neosiphonia* sp. and *Polysiphonia* sp. macroepiphyte parasitism on seaweed thallus could damage the thallus itself, thus making it prone to ice-ice disease. The spores of *Polysiphonia* sp. and *Neosiphonia* sp. epiphyte will penetrate the seaweed thallus pores to the surface of the thallus. When the macroepiphyte dies, it will leave scars large enough for bacteria like Vibro-Aeromonas and Cytophaga-Flavobacterium to easily infect it. Bacteria will process erosion towards the epidermal cells and damage the chloroplast, causing the thallus to lose its color pigment and become white; this disease is called ice-ice. Munoz & Fotedor (2009) also stated that epiphytes generally attach to its host surface, but some types such as *Polysiphonia* sp. and *Ceramium* sp. can also penetrate its host through threads and affect the growth of *G. cliftonii*. Horizontal net utilization has its advantages, such as easy cleaning of macroepiphyte on the thallus and epiphyte algae on cultivation media, minimizing macroepiphyte attaching and thallus falls. This cage also avoids herbivore fishes such as *Siganus* sp. This cage as well simplifies the processes of cultivation and harvest because seaweed grown in horizontal net only need to be lifted out and stored on the boat.

Physio-chemical parameters. During the present research, temperature ranged between 25-26°C, while nitrate (inside and outside of confinement) ranged from 0.0141 to 0.0474 mg L^{-1} and phosphate from 0.0011 to 0.0086 mg L^{-1} (Table 2).

Table 2

Water physico-chemical parameters around study sites

No.	*Parameter*	*Measurement time*				
		t1	*t10*	*t20*	*t30*	*t40*
1.	Physical parameters					
	- Brightness (m)	2.71	2.83	2.78	2.44	1.85
	- Temperature (°C)	26	26	26	26	25
	- Current velocity (m s^{-1})	0.0676	0.0671	0.0175	0.0352	0.0676
2.	Chemical parameters					
	Nitrate (mg L^{-1})					
	- Inside the cage	0.0164	0.0145	0.0150	0.0142	0.0296
	- Outside the cage	0.0175	0.0141	0.0136	0.0191	0.0474
	Phosphate (mg L^{-1})					
	- Inside the cage	0.0086	0.0084	0.0011	0.0055	0.0059
	- Outside the cage	0.0069	0.0087	0.0012	0.0059	0.0060
	Salinity	32	32	32	32	30

t1 - day 1, t10 - day 10, t20 - day 2, t30 - day 30, t40 - day 40.

During the research, there were no fluctuations in physical and chemical factors. However, based on Pearson correlation analysis, there are positive correlation between physic-chemical factors and the occurrence of epiphytes (Table 3).

Water current velocity is very important for seaweed growth. Water current supports nutrient supply for most species of macroalga and plays an important role in macroalga spore distribution. Parenrengi et al (2010) states that current speed is a supporting factor for nutrient absorption because water movement functions to supply nutrients and clean the dirt that attaches to the seaweed. The current velocity in Lakeba Beach coast area, Southeast Sulawesi Indonesia was categorized as very slow during research with range of 0.0175-0.0676 m s^{-1}. Welch (1980) categorized current speed in to fast (<1 m s^{-1}), intermediate (0.25-0.50 m s^{-1}), slow (0.10-0.25 m s^{-1}), and very slow (<0.10 m s^{-1}). Parenrengi et al (2010) states that, in low speed, current cannot fulfill *E. denticulatum* growth, the current speed that is good for cultivation is between 0.20 and 0.40 cm s^{-1}. Susanto (2005) explain that a relatively calm current water condition causes rapid growth in macroepiphytes, causing covering of the thallus surface which disturbs the growth of the seaweed. Low current speed will trigger macroepiphyte growth on seaweed and horizontal nets. Low current speed causes good epiphyte fouling growth and will dominate the light absorbance, space, and food from cultivated alga. Sunlight will be absorbed better by the epiphyte than the cultivated alga. In contrast, a current speed that is too high will also give a negative impact for the seaweed cultivation (Rombe et al 2013).

Table 3

Pearson correlation between diversity (H'), uniformity (E), dominancy (D) and environmental factors

Parameter	*Current ($m\ s^{-1}$)*	*Nitrate inside ($mg\ L^{-1}$)*	*Nitrate outside ($mg\ L^{-1}$)*	*Phosphate inside ($mg\ L^{-1}$)*	*Phosphate outside ($mg\ L^{-1}$)*	*H'*	*E*	*D*
Temperature (^oC)	-.559	-.850	-.988*	-.148	.602	-.101	-.726	.019
Current ($m\ s^{-1}$)		.818	.542	.871	.294	.881	.141	-.831
Nitrate inside ($mg\ L^{-1}$)			.885	.609	-.117	.487	.683	-.380
Nitrate outside ($mg\ L^{-1}$)				.177	-.567	.083	.815	.015
Phosphate inside ($mg\ L^{-1}$)					.700	.950*	-.028	-.903
Phosphate outside ($mg\ L^{-1}$)						.687	-.532	-.706

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

Kasim et al (2019) stated that high current velocity with big waves could possibly damage *Kappahycus alvarezii*. Moreover, nutrient absorbance will be obscured and will be difficult to be absorbed by the seaweed thallus. The water current speed at our study sites was very slow and affected the nutrient elements (nitrate and phosphate) in the water. Parenrengi et al (2010) stated that current speed is one of the supporting factors in nutrient absorbance, whereas water movement functions as a nutrient supply.

Sunlight is a resource for the photosynthesis process. The photosynthesis process forms the organic material necessary for the growth and development of macroalga. Macroalgae requires high light intensity that penetrates into the water that correlates with water transparency. The light factor is the main resource for giving energy to plants for the photosynthesis process (Wuhlan 2006). In our study sites, water transparency levels were from 1.85 to 2.83 m. Water transparency level was 100%. Sunlight was penetrated until to the bottom of the water. This condition is adequate for seaweed growth because it is able to maximize the photosynthesis process. The water transparency factor that supports seaweed growth is 1.5 m at the minimum. This condition is very supportive for seaweed growth where the photosynthesis process could well occur (Atmadja et al 1999). Temperature plays an important role in seaweed growth. The temperature range is very specific for seaweed growth as supporting enzyme process on seaweed and this will fail in function at condition of excessively cold or overheated. High water temperature could cause death of the seaweed through failure of the photosynthesis process, enzyme damage and unstable membranes (Luning 1990). In contrast, protein membrane and fat could be damaged in low temperatures as a result of the crystallization inside cells, affecting seaweed life. Water temperature is very important for seaweed metabolism because the metabolism speed increases following the increase of water temperature (Thana et al 1993). Nitrate level measurements at the research location had the highest level on day 40 for inside the horizontal net at 0.0296 mg L^{-1} and outside the horizontal net at 0.0474 mg L^{-1}. Lowest nitrate level was recorded on day 20 at 0.0150 mg L^{-1} inside the horizontal net and 0.0136 mg L^{-1} for outside the horizontal net. Nitrate levels in natural water are rarely above 0.1 mg L^{-1} and levels that surpass 5 mg L^{-1} suggest anthropogenic pollution which comes from land. Nitrate levels above 0.2 mg L^{-1} causes water eutrophication which will stimulate rapid algae growth (blooming) and water plant growth (Effendi 2003). The lowest nitrate range for algae growth is 0.3-0.9 mg L^{-1} and 0.91-3.5 mg L^{-1} for optimal growth (Manik 2001). The phosphate in water derives from industrial waste, domestic and farm waste, organic material crumbs, and minerals (Manik 2001). Based on the phosphate level at the research location, the highest phosphate level was recorded on day 10 at 0.0086 mg L^{-1} inside the horizontal net and 0.0087 mg L^{-1} outside the horizontal net. The lowest phosphate level was recorded on day 20 at 0.0011 mg L^{-1} inside the horizontal net and 0.0012 mg L^{-1} outside the horizontal net. The low phosphate content was due to the research location having no rivers or streams carrying phosphate from the land. The lowest level of phosphate concentration for optimal algae growth ranges between 0.18 and 0.90 mg L^{-1}, while the highest level is 8.90-17.8 mg L^{-1} (Patadjai 2007). Therefore according to this argument the phosphate level was still relatively low at the research location. Salinity was between 30 and 33‰, which is still suitable for *E. denticulatum* growth. Kurniastuty et al (2001) stated that the appropriate salinity for seaweed (*E. denticulatum*) is 29-34‰. Anggadiredja et al (2006) adds that good salinity for *Eucheuma* sp. ranges between 28 and 33‰, while Kasim et al (2017) reported that optimal salinity for macroalgae growth is between 33 and 40‰. Atmadja et al (1999) explains that there are algae types which thrive in high saline water, such as *Gelidium* sp. and *Rhodymenia* sp., while some prefer low saline water, such as *Gracilaria* sp. and *Caulerpa* sp. However, several seaweeds are tolerant towards both high and low salinity levels, such as *E. intestinalis* (euryhaline species).

Conclusions. The highest density of epiphytic attachment on the *E. denticulatum* thallus was *Neosiphonia* sp. This type of epiphyte dominated throughout the sampling time on day 10 to 40. The highest uniformity index was found only on the 10^{th} day, while the dominance index occurred on the 20^{th} day. Environmental factors had no positive effect on the attachment of epiphytes on the thallus and cultivation tools. There is significant effect of epiphyte on growth of *E. denticulatum*.

Acknowledgements. We are grateful to the Ministry of Research and Technology/BRIN of the Republic Indonesia, for the full research funding of the present research project. Gratitude for Fishery Laboratory, Faculty of Fishery and Marine Science Halu Oleo University for their assistance in sample analysis. Many thanks for LPPM UHO for facilitation of this research.

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Received: 39 May 2020. Accepted: 21 August 2020. Published online: 30 August 2020.
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How to cite this article:
Kasim M., Balubi A. M., Hamsia, Abadi S. Y., Jalil W., 2020 The diversity and species composition of epiphytes on *Eucheuma denticulatum* (Rhodophyceae) cultivated on horizontal net. AACL Bioflux 13(4):2410-2420.