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Nitrogen Preference for Growth Rate of *Ulva reticulata* cultivated in Eutrophied Coastal Waters: A Seaweed Laboratorium Testing Experiment

Andi Sompaa^{1,3}, Ambo Tuwo^{2,4}, Muhammad Lukman^{2,3}, Inayah Yasir^{2,3,4}

¹Integrated Coastal Resources Management Postgraduate Program, Faculty of Marine Science and Fisheries, Universitas Hasanuddin, Makassar-90245

²Department of Marine Science, Faculty of Marine Science and Fisheries, Universitas Hasanuddin, Makassar-90245

³Center of Excellence for Development and Utilization of Seaweed, Universitas Hasanuddin, Makassar-90245

⁴Multitrophic Research Group, Universitas Hasanuddin, Makassar-90245

*Email: andisompa07@gmail.com

Abstract— This study aims to evaluate seaweed *Ulva reticulata* preference for available nitrogen forms of eutrophic coastal waters for its growth rate. Simple experiment was developed for laboratorium testing of *U. reticulata* preference for NH_3 , NO_2 , NO_3 for 20 days. Levels of those nitrogen species and their composition were provided naturally from filtered eutrophic coastal waters of western coast of south Sulawesi Indonesia, without any exchange. The composition of NH_3 : NO_2 : NO_3 in the eutrophic water of the experiment was 1.0 : 2.4 : 3.3. The results showed that the NO_2 was the most preferred form of nitrogen for the growth. The average amount of uptake of NO_2 , NH_3 , and NO_3 was respectively $4.58 \pm 1.71 \mu\text{g/l/day}$, $2.70 \pm 0.17 \mu\text{g/l/day}$, and $1.98 \pm 1.19 \mu\text{g/l/day}$. The average growth rate of *U. reticulata* was $15.40 \pm 3.12 \% \text{ day}^{-1}$.

Keywords— Nitrogen preference, *Ulva reticulata*, Seaweed, Eutrophic coastal waters.

I. INTRODUCTION

Seawater multicellular macroalga, seaweed aquaculture in Indonesia becomes one of main economic revenues from the coastal and marine sectors, contributing 54.46 % or US\$ 129.92 million of Indonesia (Central Bureau of Statistics 2018) Coastal water of south Sulawesi, Indonesia is one of the seaweed aquaculture foci, with production reaches 13 million tones per annum. Instead of providing revenue and livelihood for coastal community, the seaweed aquaculture plays a significant role in abating coastal eutrophication resulting from fish aquaculture, as one of the targets of SDG 14. The use of aquaculture seaweed (or even multitropic model) for coastal eutrophication abatement has increased scientific attention over the last two decades (Troell & Berg, 1997; Bates et al., 2001). It lies on the capacity of seaweed to readily uptake nutrient (Choi et al., 2009; Luo et al., 2012; Martinez and Rico, 2012; Bulboa, 2014; Rabie et al., 2014; Brundu and Chindris, 2018). Seaweed uses nutrients (N and P) as a food source for growth and development. Nitrogen is assimilated into amino acid constituent, while phosphorus is a major source of chemical energy for photosynthesis (Graham & Wilcox,

2000). This capacity to absorb nutrients is then sought to be an alternative mitigation measures of eutrophication in the waters.

The health of ecosystems and their use in coastal areas is an indicator of water quality, especially in the field of marine conservation and various activities in other fisheries sectors. Aquaculture and agricultural effluents are a major challenge in maintaining the quality of waters in coastal areas, where increased concentrations of nutrients i.e. nitrogen, phosphorus and silicon from these activities can cause eutrophication of waters which have a negative impact on organisms. This condition has been found in the waters off the west coast of South Sulawesi where nutrient enrichment is quite large (Lukman et al., 2014; Nasir et al., 2015) and symptoms of eutrophication have been identified (Nurfadillah, 2016).

Waste discharges from aquaculture, especially shrimp and fish ponds, and agriculture have been recognized as are the main sources of nutrient enrichment causing cultural eutrophication and impacting the coastal ecosystems in the western coast of South Sulawesi (Nasir et al., 2016; Hopkins et al., 1995). The availability of excessive nutrients cause changes in the composition of community

structures in marine ecosystems such as microorganisms (Kegler et al., 2017), plankton (Nasir et al., 2015) and coral reef ecosystems (Teichberg et al., 2018; Edinger et al., 1998). Therefore efforts to overcome eutrophication or nutrient pollution need to get serious attention. Increased nutrient in coastal waters can be mitigated by increasing nutrient partition coefficient in the aquatic compartments which absorb or consume these nutrients (Carpenter, 2008). Absorption by seaweed can accumulate and store organic materials such as nitrogen in thallus cells (Boyajian & Carrieria 1997). Organic waste stored in seaweed thallus cells will be degraded through photosynthetic assimilated sunlight to produce energy used for growth (Boyajian & Carrieria 1997).

Furthermore, from an economic standpoint, the Indonesian government encourages increased seaweed aquaculture and industrialization as a response to increasing demand for seaweed commodities both on a national and international scale (Radiarta et al., 2016). The potential and quality of Indonesian seaweed attract demands from various countries in the world. There are more than 550 types of seaweed in Indonesia and most of the products from seaweed has been exported as dried seaweed as well as processed forms. These various opportunities owned by one of the biological resources in Indonesia make seaweed can be used as a superior export product (Ditjen PEN, 2013).

However, a comprehensive study of the preference of any particular seaweed, for instance *Ulva reticulata*, to all forms of available nitrogen – ammonia (NH_3), nitrite (NO_2), and nitrate (NO_3) - as nutrients in waters in the context of eutrophication is still relatively lacking and even non-existent, especially in the South Sulawesi region. Therefore, research on the ability to nutrient uptake seaweed is important, to optimize seaweed aquaculture and eutrophication mitigation effort. This is also to developing application of ecosystem-based seaweed farming, a sustainable cultivation system that is a world trend (FAO, 2011).

II. RESEARCH METHOD

This study used *Ulva reticulata* taken from the Lae-lae Island, and cultivated in the laboratory under their natural condition. As much as 72 grams of the The decreasing rate (or equivalent to nutrient uptake) of NO_2 , NH_3 , and NO_3 was $4.58 \pm 1.71 \mu\text{g/l/day}$, $2.70 \pm 0.17 \mu\text{g/l/day}$, and

$1.98 \pm 1.19 \mu\text{g/l/day}$, respectively. It indicated that the NO_2 become *Ulva reticulata* preference nutrient uptake in the eutrophic coastal waters. Compared to other *Ulva reticulata* in this experiment took up nutrient less than other experiments (Table 1). It is most likely due to that there was no nutrient additional inputs into the water systems, so the available nutrient in the experimental aquarium gradually lessen was prepared for each aquarium filled with filtered 42-liter eutropic coastal waters taken from the western coast of south Sulawesi, Indonesia, without any exchange for 20-day period of experiment. Moreover, each of the aquarium was light-enriched with 1,561 lux, and small pump was introduced in the aquarium to obtain slow movement of water. The experiment was undertaken in triplicates, with additional control experiment in also triplicates. Concentration measurements of NH_3 , NO_2 , and NO_3 were carried out every 10 days, using APHA method (1989) with trichlorometric spectroscopy. Seaweed weights was measured for growth. Seaweed growth was calculated using the method adopted by Balina et al (2017). Water quality was checked in situ for parameters: temperature, pH, salinity and dissolved oxygen. Absorption nutrients by seaweed were calculated from the rate of decrease in nutrients in the water column. With the formula, the rate of decline = $(T1-T2) / t$. Where t is the monitoring period, T1 is the initial nutrient concentration and T2 is the final nutrient concentration.

III. RESULT AND DISCUSSION

3.1. Decreased nutrient concentration (N)

The initial concentrations of NH_3 , NO_2 , and NO_3 were respectively 0,05 mg/l, 0,120 mg/l, and 0.163 mg/l. All the nutrient experienced decreasing in concentration during the experimental period (Fig 1). Significant decreased in nitrogen availability compared to the control experiments indicates nutrient uptake by the *Ulva reticulata*. Changes in this composition showed that NO_2 experienced higher decrease in the concentration than the other NH_3 and NO_3 species. NO_2 decreased 1.8% from the initial concentration. While, NO_3 was decreased by around 0.20% and NH_3 around 0.13% during the trial period. The decrease in concentration of the three forms of nitrogen (NO_2 , NO_3 , and NH_4) shows that seaweed is very requires nitrogen in nutrients the period of its growth (Wahyudi, et. al., 2018).

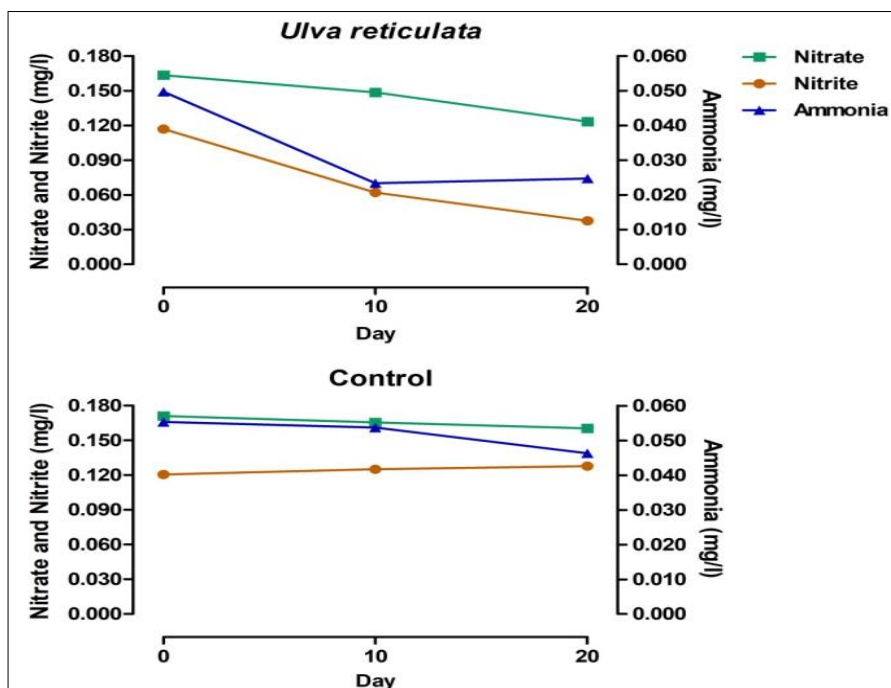


Fig . Decreased in NH₃, NO₂, NO₃ in the experiment aquariums relative to the control ones

3.2. Nutrient Uptake

The decreasing rate (or equivalent to nutrient uptake) of NO₂, NH₃, and NO₃ was 4.58 ± .71 µg/l/day, 2.70 ± 0.17 µg/l/day, and 1.98 ± 1.19 µg/l/day, respectively. It indicated that the NO₂ become *Ulva* preference nutrient uptake in the eutrophic coastal waters. Compared to other

Ulva in this experiment took up nutrient less than other experiments (Table 1). It is most likely due to that there was no nutrient additional inputs into the water systems, so the available nutrient in the experimental aquarium gradually lessen.

Table . Comparison of nutrient uptake of various species of ulva seaweed

No.	Seaweed	Nutrient Uptake of Nitrogen Species			References
		NO ₃	NO ₂	NH ₃	
1	<i>Ulva reticulata</i>	1.98 ± 1.19 µg/l/day	4.58 ± 1.71 µg/l/day	2.70 ± 0.17 µg/l/day	This study
2	<i>Ulva lactuca</i>	1.89 µmol/l/day	-	0.092 µmol/l/day	Bulboa, 2014
3	<i>Ulva plorifera</i>	125.24 mol g-1DM h-1	-	284.60 mol g-1DM h ⁻¹	Luo et al., 2012
4	<i>Ulva linza</i>	109.13 mol g-1DM h ⁻¹	-	250.25 mol g-1DM h ⁻¹	
5	<i>Ulva reticulata</i>	0.33 mg/l/12 day	1 mg/l/day	1 mg/l/day	Rabie et al., 2014
6	<i>Ulva lactuca</i>	12.09 mg/l/day	0.68 mg/l/day	0.71 mg/l/day	Brundu & Chindris, 2018
7	<i>Ulva pertusa</i>	0.984 µM/g/h	-	-	Choi et al., 2009
8	<i>Ulva intestinalis</i>	1.25 µmol/g/h	1.07 µmol/g/h	3.67 µmol/g/h	Martinez & Rico, 2012

Note: h= hour

3.3. Effect of seaweed growth on nutrients (N)

The growth rate of *Ulva reticulata* is linear until the end of the trial (20 days). The average growth rate is 15.40 ± 3.12% day⁻¹. Fig. 1 reflects the effect of each species of

nitrogen on the rate of growth. The negative impact on growth is only shown by NH₃ with a slope of -1780 (R² = 0.880) . Positive trends in growth are shown by NO₂ and NO₃. with slope of 146.4 (R² = 0.718) and 68.66 (R² =

0.148). The highest decrease in NO₂ concentration in the absorption process by *Ulva reticulata* was also found in a study conducted by Rabie, 2014. The relationship of NO₂ absorption to *Ulva reticulata* seaweed growth was

explained by Marinho-soriano, et al., 2011 that the high ability of *Ulva reticulata* to absorb NO₂ is a strategy to get a large amount nitrogen which is useful for growth.

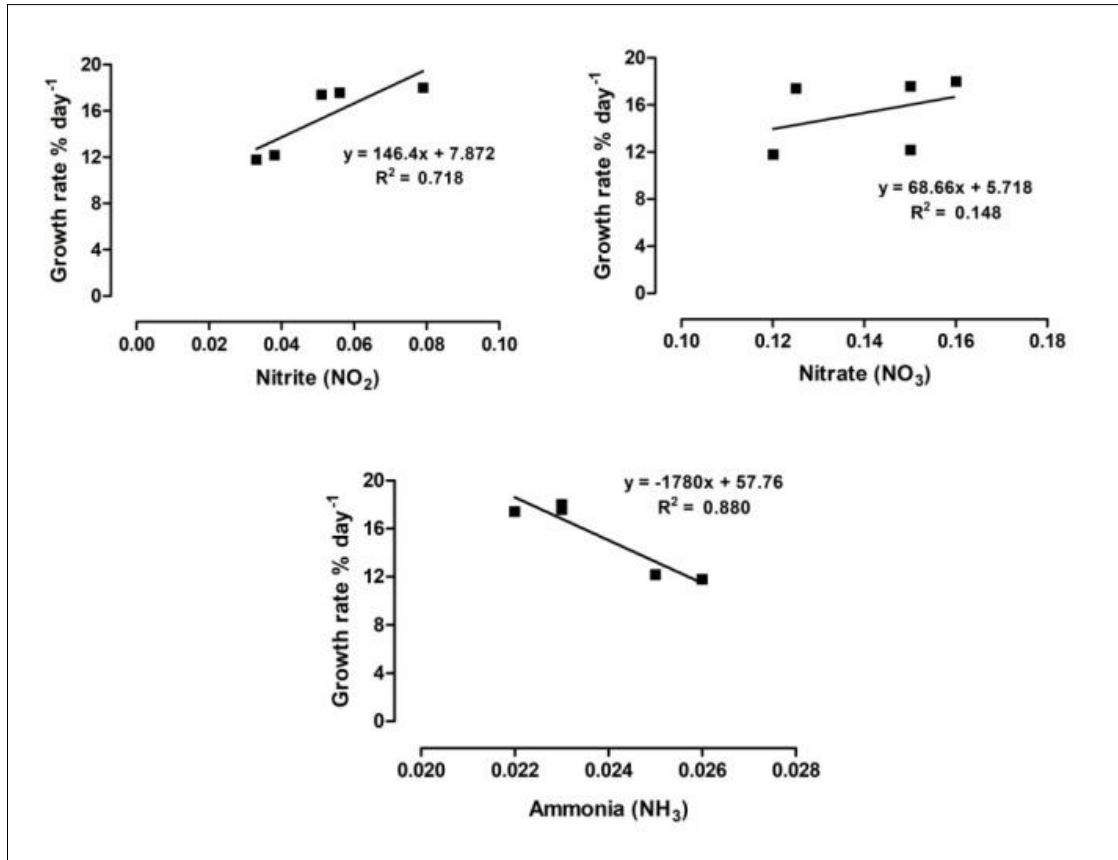


Fig . Growth of *Ulva reticulata* under no water exchange during lab experiment

3.4. Water Quality Condition

Experimental water quality provided sufficient condition for seaweed cultivation and nutrient preference experiments. The water quality was maintained almost stable during the period of experiment. ANOVA test

results for water quality parameters ($p > 0.05$; Table 2). The salinity remained in the average of 30 - 31 ‰. pH varied between 7.8 and 8.10. Water temperature was between 29 – 30 °C, and dissolved oxygen (DO) was in the range of 5.63 to 6.38 mg/l.

Table . Water quality condition during the period of experiment (mean ± standard deviation)

Parametes	Species	Number of day		
		0	10	20
Salinity (‰)	<i>Ulva reticulata</i>	31 ± 1.0	30 ± 0.43	30 ± 0.60
	Control	31 ± 0.58	30 ± 0.19	30 ± 0.69
pH	<i>Ulva reticulata</i>	7.48 ± 0.07	7.71 ± 0.23	8.10 ± 0.07
	Control	7.48 ± 0.07	7.70 ± 0.26	8.18 ± 0.05
Temp (°C)	<i>Ulva reticulata</i>	29 ± 0.58	30 ± 0.43	30 ± 0.43
	Control	29 ± 1.0	30 ± 0.43	29 ± 0.43
DO (mg/l)	<i>Ulva reticulata</i>	5.63 ± 0.32	6.07 ± 0.49	6.38 ± 0.20
	Control	4.83 ± 0.55	6.05 ± 0.60	6.66 ± 0.31

IV. CONCLUSION

Without exchanging of water meaning no additional nutrient in the experiment, the preference of *Ulva reticulata* in eutrophic coastal waters of western coast of south Sulawesi, Indonesia was to NO₂. The uptake of the NO₂ was $4.58 \pm 1.71 \mu\text{g/l/day}$, and the growth rate of the seaweed was $15.40 \pm 3.12 \% \text{ day}^{-1}$. The experimental condition was stable indicating water quality had no significant impact to the growth.

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