Nitrogen Concentration In Coral Reef: The Contribution of Coral Forms and Nitrogen Fixing Bacteria In Karimunjawa Islands

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Nitrogen Concentration In Coral Reef: The Contribution of Coral Forms and Nitrogen Fixing Bacteria In Karimunjawa Islands

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Abstract.
Coral is an ecosystem in the coastal area which plays an important role in controlling nutrient cycle in the sea. This research aimed to study the the nitrogen concentration in coral and to analyze the influence of nitrogen fixing bacteria on the concentration of nitrogen in coral. The research was carried out by field observation, by taking coral reef samples and analyzed in the laboratory for its nitrogen concentration and number of nitrogen fixing bacteria. Data analysis was carried out by anova and regression. The analysis resulted that there was a significant difference of nitrogen concentration in massive and branching corals. However, there is no significant correlation between the sampling location and types of coral on the density of nitrogen fixing bacteria, both *Nitrosomonas* sp. and *Nitrobacter* sp. Regression analysis showed that the concentration of nitrogen in coral tissue was not significantly related to the density of nitrogen fixing bacteria. The result of this research implies that the concentration of nitrogen in the coral tissue may be affected by many other factors.

Keywords: bacteria, coral, fixation, nitrogen, tissue

Introduction

As the development of coastal area increase, the problems related to the coastal ecosystem arises. This includes the coral reef ecosystem which has the most important role in supporting marine biodiversity. Coral also provides micro-biome which shows the relationship among organisms in the coral surface. Various processes occur in the micro-biome, such as sulphur cycling, nitrogen fixing, as well as the it defensive system (Krediet, Ritchie, Paul, & Teplitski, 2013).

Coral is a rocky organism that lives in the shallow marine ecosystem ranging from the tropical to sub-tropical area. Ecologically, shallow coral reefs are considered as the huge source of benthic nitrogen fixation (Diaz, 2017). The biodiversity in coral reef ecosystem is supported by the coral associated organisms, such as sulphur recycling bacteria or nitrogen fixing bacteria. All of them existed in the coral ecosystem and provide defense toward various threats.

As a living organism, coral absorbs various minerals to grow. One of the important nutrient for coral is nitrogen. Nitrogen for coral is mainly provided by zooxanthelae which which lives mutalistically with coral polyp (Krediet et al., 2013). Coral polyp is where various ecological processes occur. Various physiological response due to the arising stress in the environment is detected and responded in the polyp (Hohn & Merico, 2012).

Symbiodinium is the algae species which has mutualistic association with coral. However, the micro-biome in coral is just recognized recently. The micro-biome is the system which provide support to the organization of coral ecoystems which involved various microorganisms (Rädecker, Pogoreutz, Voolstra, Wiedenmann, & Wild, 2015). Among the functions the microbiome provides, nitrogen cycling is considered as the most important process for coral sustainability.

There are several studies wich found that coral polyp also contain nitrogen fixing bacteria. Nitrogen is an important element required by any plant to grow. However, in some cases the availability of nitrogen in the coral reef ecosystem is limited. In this condition, nitrogen fixing is the main source for coral to support its growth(Putnam, Barott, Ainsworth, & Gates, 2017). The nitrogen fixing bacteria are frequently found in coral mucus (Lema, Willis, & Bourne, 2012).

The nitrogen fixing bacteria is commonly called diazotrophs. Nitrogen fixing bacteria are mostly found in a reef building symbiotic coral as the source of nitrogen for coral's growth (Wang et al., 2015). The bacteria favors the nitrogen supply to the coral organism directly through bacterivory, through polyp and zooxanthelae (Diaz, 2017). The species of nitrogen fixing bacteria is varied among coral species and locations. At least there are 23 species of nitrogen fixing bacteria. Nitrosomonas and nitrobacter are frequently used as bioremediator for aquaculture. It is mostly functioned as the remediator for ammonia and nitrite (Barik, Ram, Haldar, & Vardia, 2018).

Critical concern on the microbiological role on the coral reef ecosystem arises the question wether the associated microorganism also affect the nutrien uptake by coral reef. Least information is known regarding the processes and impact of the nitrogen fixing bacteria to the livelihood of coral community. This research aimed to study the nitrogen concentration in coral and to analyze the influence of nitrogen fixing bacteria on the concentration of nitrogen in coral.

Methods

The research was carried out in June – July 2019 in the area of Karimunjawa National Park. The research was carried out by field observation. The research stations were located in Karimunjawa island, Menjangan Kecil island, and Cemara Kecil island, while laboratory analysis was in the *Tropical Marine Biotechnology (TMB), Faculty of Fisheries and Marine Science Diponegoro University Semarang*.

There were three sampling stations occupied in the research. Each station consisted of three sampling spots. The objects of the research were including the concentration of Nitrogen in coral organisms and the density of nitrogen fixing bacteria. Coral samples were obtained from two groups of coral organisms, including the massive and branching corals. Thus, the research design applied 3 x 2 factorial treatment.

Data collection was carried out through field sampling followed by laboratory measurement and identification. The coral was sampled with the length of 10 cm. The coral samples were stored in a cool box an identified in the laboratory for its density of nitrogen fixing bacteria and the concentration of nitrogen. The bacteria were isolated from the coral and placed in the enrichment media (nutrient agar). The bacteria collected as the object of the research was *Nitrosomonas* sp. and *Nitrobacter*sp.

Data analysis was carried out with anova and regression. Multivariate anova was carried out to analyze the difference of nitrogen concentration and the abundance of nitrogen fixing bacteria. Regression was carried out to analyze the influence of nitrogen fixing bacteria on the concentration of nitrogen in the coral. The regression analysis was carried out through multiple regression to analyze the simultaneous effect of nitrogen fixing bacteria, and curve estimation to analyze the effect of nitrogen fixing bacteria separately. Analysis was carried out by SPSS.

Results

The result of the laboratory analysis showed that there was variation on the concentration of nitrogen in coral samples. Generally, the concentration of nitrogen obtained from massive coral was lower than that in the branching coral. The concentration of nitrogen in Cemara Kecil island was also higher than in Karimunjawa and Menjangan Kecil for respective types of coral. Detailed result of laboratory analysis and the statistical analysis for nitrogen concentration is presented in Table 1.

Table 1. Average concentration of nitrogen in coral (mg/kg) from each sample

No.	**Location**	**Types of Coral**		***Total by Location***
		Massive	**Branching**	
1.	Karimunjawa	61.380*a	231.527⹋a	*146.453‡p*
2.	Menjangan Kecil	73.662*a	197.390‡a	*135.526‡p*
3.	Cemara Kecil	160.223‡a	221.486⹋a	*190.855‡p*
	Total by Types of Coral	*98.422*x*	*216.801⹋y*	

Notation: * = very low
‡ = low
⹋ = moderate
Cells, coloumn (total by types of coral), and rows (total by location) with similar letter indicates insignificant difference at α = 0.05

Table 1 shows that the average concentration of nitrogen in the coral was ranging from very low to moderate. However, moderate nitrogen concentration was obtained from branching coral, while very low concentration was obtained from massive coral. The concentration of nitrogen is considered as very low within the range < 0.1 %, low within the range 0.1 – 0.2 and moderate within the range 0,2

– 0.5 (Wahyuni, Kusnadi, & Honorita, 2017). Thus, it can be noted that massive coral has a lower accumulation of nitrogen.

Statistical data analysis showed that the nitrogen concentration was affected by the types of coral. Massive coral tended to have lower nitrogen concentration than the branching coral. However, other treatments did not show any significant effect on the nitrogen concentration. However, the difference in nitrogen concentration affected by location, and types of coral altogether was noticeable. Data analysis resulted F value for location was 2.311 (p = 0.142), types of coral showed the value of 28.285 (p = 0.000), and combination of both 2.009 (p = 0.251).

Assessment of the abundance of nitrifying bacteria *Nitrosomonas* sp. showed that massive coral had higher average density. The collected data as presented in Table 2 showed that higher density of *Nitrosomonas* sp. in massive coral was obtained from all locations. Furthermore, the density of bacteria from the coral sample obtained from Menjangan Kecil island was the highest, while Karimunjawa provided the lowest density.

Table 2. Average density of nitrogen fixing bacteria *Nitrosomonas* sp. (cfu/g) from each sample

No.	Location	Types of Coral		
		Massive	**Branching**	***Total***
1.	Karimunjawa	$58.27x10^{5(a)}$	$39.93x10^{5(a)}$	*$49.10x10^{5(p)}$*
2.	Menjangan Kecil	$11.30x10^{6(a)}$	$15.00x10^{6(a)}$	*$13.15x10^{6(p)}$*
3.	Cemara Kecil	$11.07x10^{6(a)}$	$64.33x10^{5(a)}$	*$87.50x10^{5(p)}$*
	Total	*$93.98x10^{5(x)}$*	*$84.76x10^{5(x)}$*	

Notation: Cells, column (total by types of coral), and rows (total by location) with similar letter indicates insignificant difference at α = 0.05

Statistical analysis showed that there was no significant effect of the source of coral samples and the types of coral to the density of *Nitrosomonas* sp. Data analysis with univariate anova showed that the F value obtained from the location, types of coral and combination of both were 2.847 (p = 0.097), 0.107 (p = 0.749) and 0.753 (p = 0.492) respectively. Thus, it can be suggested that massive coral provided better support to the nitrogen fixing bacteria. The environmental condition of Menjangan Kecil island was also more supportive than the other two islands.

The average density of nitrogen fixing bacteria *Nirobacter* sp. is the las parameter observed in this research. Assessment on the average density of *Nitrobacter* sp. showed that there was slight difference between the massive and branching corals. However, branching coral had higher *Nitrobacter* sp. density even though the difference was not significant. Noticeable difference was obtained from the bacterial density in Karimunjawa island. The difference was obtained not only from the total average density, but also in the density of respective massive and branching coral types. Table 3 presents the detailed*Nitrobacter* sp. density of the samples obtained from the research location.

Table 3. Average density of nitrogen fixing bacteria *Nitrobacter* sp. (cfu/gr) from each sample

No.	Location	Types of Coral		
		Massive	**Branching**	***Total***
1.	Karimunjawa	$96.57x10^{5(a)}$	$59.43x10^{5(a)}$	*$78.00x10^{5(p)}$*
2.	Menjangan Kecil	$15.00x10^{6(a)}$	$19.67x10^{6(a)}$	*$17.33x10^{6(p)}$*
3.	Cemara Kecil	$15.33x10^{6(a)}$	$16.90x10^{6(a)}$	*$16.12x10^{6(p)}$*
	Total	*$13.33x10^{6(x)}$*	*$14.17x10^{6(x)}$*	

Notation: Cells, column (total by types of coral), and rows (total by location) with similar letter indicates insignificant difference at α = 0.05

Table 3 shows that the density of *Nitrobacter* sp. in Karimunjawa National Park was higher than the *Nitrosomonas* sp. However, there was no significant difference in the density of nitrogen fixing bacteraia Nitrobacter sp. both partially and simultaneously. Statistical analysis with anova showed the F value of coral source, types of coral and combination of both were as much as 1.877 (p = 0.195), 0.037 (p = 0.851) and 0.313 (p = 0.737) respectively.

Among the results from the assessment of nitrogen concentration as well as the density of *Nitrosomonas* sp. and *Nitrobacter* sp., it can be suggested that Karimunjawa always had the lowest value. This could be due to the environmental condition of the island. However, further indepth research is needed to study the possible causes.

Statistical analysis to assess the influence of nitrogen fixing bacteria showed that the concentration of nitrogen in the coral was not related to the density of *Nitrosomonas* sp. nor *Nitrobacter* sp. Both multiple regression and curve estimation analysis did not show the significance of the relationship. Thus, it can be concluded that the concentration of nitrogen in coral is affected by some other factors, or involves some other factors as moderator or as cooperating factor.

Discussion

The accumulation of nitrogen in massive and branching coral is suggested as the effect of growth rate between the two. Nitrogen is required for coral metabolism. However, the environmental condition significantly affect the concentration of nitrogen. Shaded environment tends to cause the increase of the C/N ratio in coral. Moreover, the concentration of nitrogen is decreased along with the increasing depth (Alamaru, Loya, Brokovich, Yam, & Shemesh, 2009).

The size of coral is related to its capability to tolerate environmental stress (Edmunds & Burgess, 2016). However, each coral species has its defensive method to overcome the environmental stress. The response of coral toward environmental disturbance also involved the metabolic system provided by coral's microstructure (Delgadillo-Nuño, Liñán-Cabello, Reyes-Gómez, & Soriano-Santiago, 2014).

The sustainability of coral organisms relies on the nutrient suply. The symbiotic between coral and the symbiodinium is highly reliable to the availability of nitrogen(Rädecker et al., 2015). The significant difference between nitrogen concentration in massive and branching coral resulted in this research can be caused by the absorption rate rather than the nitrification process. Higher nitrogen concentration in the branching corals may be caused by polyp abundance. Branching coral tended to have larger surface due to its three dimensional form, while the massive coral only has the top surface. Thus, since the sample was collected in the same size, the abundance of coral polyp in the branching coral was more than the massive coral. Thus, it affected the nitrogen absorption.

The abundance of nitrogen fixing bacteria indicates its nitrification rate provided by the community (Zulfarina, Rusmana, Mubarik, & Santosa, 2017). It also indicates the potential nitrogen produced from the nitrification process. However, the research found that there was no significant effect of the density of *Nitrosomonas* sp. and *Nitrobacter* sp. to the concentration of nitrogen in coral tissue. The nitrification process occurred in the polyp surface, but it mainly affects the nitrogen availability in the environment. Unfortunately, the high nitrification rate is not followed by higher nitrogen uptake by coral organism.

The difference in the density of nitrogen fixing bacteria is dominantly caused by the environmental condition. One of the affecting factors of nitrifying bacteria is the depth of which the bacteria sampled. The abundance of nitrifying bacteria is higher in the shallow water (Smith, Damashek, Chavez, & Francis, 2016). Unfortunately, measurement on the depth of the sampling location was not carried out.

The bacteria is sensitive to the change of environmental condition, such a temperature (Diaz, 2017). At the research location, Karimunjawa island is the most exploited area. Thus, anthropologic aspects may play a role in changing the coral waters. However, this should be assured by investigating the condition of coral reef ecosystem. Any environmental stress may break the symbiosis of coral and algae (zooxanthellae) (Wooldridge, 2013). This may affect the other associated microorganisms in the polyp.

Nitrosomonas and Nitrobacter are frequently used as bioremediator in aquaculture. However, the preferences of the two is not much understood. Related to the nitrogen fixing process in Karumunjawa National Park, Nitrosomonas and Nitrobacter might have an important role in remediating coastal environment. The density of both bacterias may be related to the high input of ammonia since Karimunjawa island has been much exploited for tourism activities. Nitrosomonas and Nitrobacter mainly act as bioremediator of both pollutant (Barik et al., 2018).

Even though nitrogen fixing bacteria exists in the coral mucus or tissue, but it's suggested that the bacteria are associated with Symbiodinium rather than directly to the coral (Lema et al., 2012).

This explains why the Nitrosomonas sp. and Nitrobacter sp. did not influence the concentration of nitrogen significantly.

The role of nitrogen fixing bacteria is obvious in the nitrogen limited environment (Putnam et al., 2017). However, since the effect of Nitrosomonas sp. and Nitrobacter sp. in Karimunjawa National Park remains insignificant, it can be concluded that the source of nitrogen is sufficient. Or otherwise, since the coral growth requires nitrogen, the coral can not accumulate the nitrogen it consumed.

Conclusion

Based on the analysis result, the concentration of nitrogen is higher in branching coral as the impact of its higher growth rate. However, the concentration of nitrogen in coral tissue is not affected by the abundance of nitrogen fixing bacteria.

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