

Nitrogen dynamics in symbiotic relationships in corals

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Abstract. Symbiotic relationships in corals are found to be complex and diverse. The system supports the ecosystem in coral reefs with nitrogen cycles in usually oligotrophic environments. By introducing stable isotopes as carbon and nitrogen into seawater simultaneously, we found that uptake ratios of carbon and nitrogen fluctuate, possibly caused by variation in internal resource availability to the symbiotic system. In this study, empirical data have demonstrated imbalance of nitrogen budget that contributed in the symbiotic relationships with measurement such as nutrients in seawater and nitrogen isotope in corals. The study demonstrated mathematical model based on the findings in the incubation experiment. The model revealed that isotope method overestimate uptake rate by coral symbiosis as only uptake but not excrete rates is considered in the symbiotic relationship. Here, we propose possible method to evaluate internal and external nitrogen cycles in symbiotic system in corals. In the future, the mathematical model is going to be updated as a tool to predict symbiotic complex relationship in corals using new sampling techniques.

Key words: Nutrients, symbiosis, modeling

Introduction

Symbiotic relationships in/on corals enable corals to survive in nutrient-poor environments by exchanging metabolites and photosynthates that contain C, N, and P. Uptake and exchange of nutrients from seawater by zooxanthellae in the coral symbiotic relationships has been studied in the past (D'Elia and Cook 1988; Swanson and Hoegh-Guldberg 1998; Tanaka et al. 2001; Grover et al. 2002; Dugdale and Wilkerson 1986). Recently, the importance of internal cycling was suggested as not only based on the uptake rate but also the release rate for understanding of symbiotic mechanisms in corals (Falkowski et al. 1993).

The objectives of this study are to identify the N resource to symbiotic complex relationship between corals and zooxanthellae, to construct a preliminary mathematical model based on empirical data, and to understand an important process of internal cycling of nutrients as a critical mechanism for abundance of zooxanthellae. Until now, there is very few data on the nutrients concentrations in cnidarians (Fitt et al. 1995). In this paper, we would like to report the first data of nutrient concentration in the internal coral compared with those in seawater. We also intend to propose a new preliminary mathematical model for symbiotic complex relationships between host corals and zooxanthellae.

Material and Methods

Preparation of coral nubbins

Nubbins of 1-2 cm length of *Montipora digitata* and *Galaxea sp.* were collected from shallow water (< 1 m) at Bise, Okinawa, Japan. Nubbins were kept in a tank with running water for at least one week before experimentation to minimize impacts of sampling and transportation on nubbins. All containers and instruments were washed with Extran and HCl before they were used for experiments.

Incubation experiment

A nubbin was kept in each 500 ml incubation container. Light intensity was controlled with metal halide lamps. $^{15}\text{NO}_3^-$ or $^{15}\text{NH}_4^+$, and $\text{H}^{13}\text{CO}_3^-$ were added to the seawater adjusting the final concentration to 0.07 μM , 0.05 μM , and 0.23 mM, respectively. After 6 hours of incubation period (starting between 5:00 to 7:00 under light), incubation media was sampled for nutrient measurement. Nutrients, NO_3^- , NO_2^- , PO_4^{3-} , and NH_4^+ were measured with TRAACS 2000 (BRAN+Luebbe). Nubbins were removed from the incubation container and tissues on nubbins were obtained using a WaterPik® (Johannes and Wiebe 1970). The tissue slurry was homogenized, and filtered with 47 mm GF/F filter (Whatman). The filter was acidified, dried, and analyzed for isotope ratios in carbon and nitrogen ($^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$).

Nutrient concentrations in coral nubbins

To study nutrient availability in the symbiotic complex in corals, nubbins were ground with a grinder, and 35% NaCl solution in Milli-Q water

(NaClaq.) was poured in the grinder. The ground coral skeleton and solution were transferred to a tube. The grinder was rinsed thoroughly with NaClaq. and combined with the solution in the tube. NaClaq. was added to the tube until the volume reached 50 ml. The tube was shaken well, and the supernatant of the tube was filtered with 0.22 μm filter unit (Millipore). Filtrate was stored as a frozen sample for nutrients measurement.

Results

Incubation experiments

The isotope ratio changes in POC and PON from nubbins were directly used to estimate uptake rate from the incubation seawater to symbiosis in corals (Dugdale and Wilkerson 1986, Grover et al. 2003). The isotope ratios of C and N in corals changed after incubation treatment. With $^{15}\text{NO}_3^-$ and $^{15}\text{NH}_4^+$ treatment, uptake rates were $6.76 \times 10^{-8} \text{ M cm}^{-2}\text{h}^{-1}$ and $6.66 \times 10^{-8} \text{ M cm}^{-2}\text{h}^{-1}$, respectively. C/N in taken up by coral nubbins, which was calculated from stable isotope measurement, differed among samples. Enriched with $^{15}\text{NO}_3^-$ had higher variability in C/N taken up compared with the treatment enriched with $^{15}\text{NH}_4^+$.

Nutrient concentrations in seawater sampled from incubation container decreased in scale of $10^{-2} \mu\text{M}$ over 6-hour-incubation period.

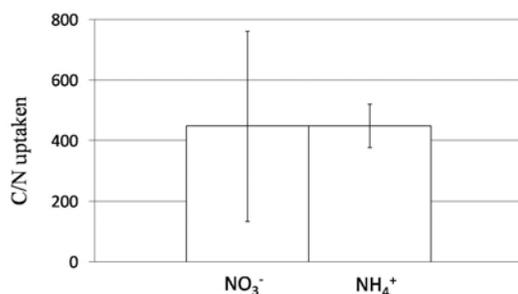


Figure1: C/N taken up by symbiosis in corals over 6 hours. $^{15}\text{NO}_3^-$ treatment had higher variation in C/N than that of $^{15}\text{NH}_4^+$ treatment. ($^{15}\text{NO}_3^-$ treatment (n=5), $^{15}\text{NH}_4^+$ treatment (n=6))

Nutrient concentrations in coral nubbins

Higher concentrations of nutrient compared with those of in seawater were detected in nubbins. Especially in nitrate and ammonium concentration in nubbins were about 100 times higher than those in seawater. The concentration varied among samples. During the treatment, nubbins excreted mucus, but the mucus influence is not subtracted from the data.

Table1: Nutrient concentration in seawater and corals. All species was higher in corals compared with those in seawater.

sample	NO_3^-	NO_2^-	NH_4^+	PO_4^{3-}
	(μM)			
Seawater	0.1	0.1	0.39-0.59	0.05-0.08
Corals	1.35-5.21	0.21-0.76	3.88-103.70	0.64-43.52

Discussion

C/N taken up by the coral symbiotic systems were variable, especially with $^{15}\text{NO}_3^-$ treatment. This implied that the behavior of C and N used by symbiotic systems in corals is not always the same. It seems that the high concentration of nutrients in nubbins supports an internal resource available besides an external resource (seawater). The high nutrient concentration in the coral symbiotic systems implied that nutrients are regenerated in a symbiotic relationship in corals (Lewis and Smith 1971; Falkowski et al. 1993; Wang and Douglas 1998). Especially the ammonium concentration is considered to be low in cells as such chemical species is detrimental to animals' health in general. Although ammonium might be excreted from corals in form of mucus in this method, the data confirmed ammonium availability in coral symbiotic systems. Whether the symbiotic relationships in corals stock nitrogen as in forms of ammonium or not is unknown. Corals may have a system to synthesize ammonium and incidentally excreting it also as mucus content. Incubation experiments with stable isotopes demonstrated that there were exchanges of nitrogen or carbon between the coral and its symbionts and the seawater even though changes of nutrient concentration in seawater appeared almost inexistent (pseudo state of exchange between surrounding sea water and interior coral). Uptake and exchange of materials from seawater to coral and symbionts has been studied in the past (Tanaka et al. 2001; Grover et al. 2002). Also, the uptake rate by symbiosis in corals has been studied (Dugdale and Wilkerson 1986; D'Elia and Cook 1988; Swanson and Hoegh-Guldberg 1998). In this study, since an internal resource was suggested, not only the uptake rate, but also the release rate was studied in symbiotic relationships in corals in order to reveal masked flow of materials in symbiotic relationships in corals (Falkowski et al. 1993).

Model construction

Here, a simplified model is built to describe symbiotic relationship in coral. In the model, to simplify the symbiotic system in corals, only zooxanthellae and

host corals were selected as symbiotic system. Uptake and release rates were given to each. As such high concentration of nutrients was found in coral nubbins, it was assumed that zooxanthellae take-up nutrients also from the host or in symbiotic systems rather than from seawater only (Fig. 2).

In the model, the host coral takes up nutrients from the surrounding water. As demonstrated in the experiment in this study, there is a possibility that host cells contain much higher nutrients than the seawater. Therefore, the uptake of nutrients by zooxanthellae was considered as uptake of nutrients from the host rather than the seawater. From acquired data and mathematical equations, the exchange rates were estimated. Concentration in seawater, host coral, and zooxanthellae were set to be C_{sw} , $Chost$, and C_{zoox} , respectively.

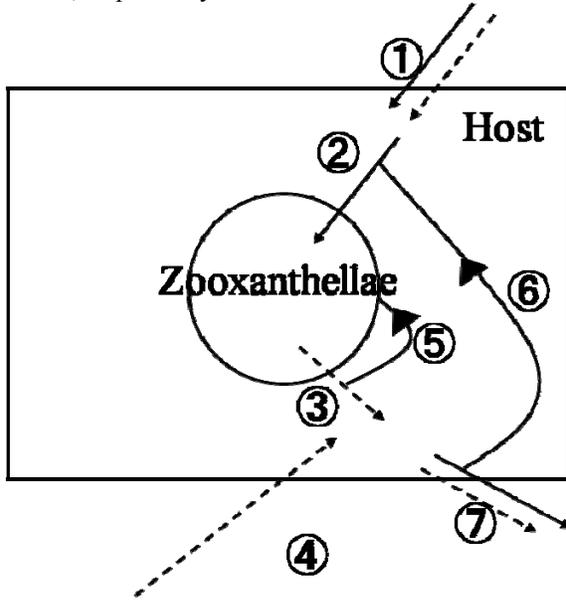


Figure 2: Suggested model of nutrient exchanges in symbiotic complex in corals. Where 1) is uptake of host from seawater, 2) is uptake by zooxanthellae from host tissue, 3) is translocation from zooxanthellae to host, 4) is host uptake of particulate matter from seawater, 5) is allocation of nutrient from zooxanthellae to zooxanthellae, 6) is allocation of nutrient from host to itself, and 7) is release of nutrients and organic matter from host to seawater. Solid arrow is inorganic, and dotted arrow is organic flow.

A mathematical model is constructed to model the nutrient interaction in symbiotic relationship in corals. The model is constructed based on data acquired from the incubation experiment, and from studies in the past. The mathematical model consists of uptake and release. Concentration changes in s over incubation period are rates, V_s ($\Delta C_s/\Delta t$). The changes are integrated from time 0 to time t , then the concentration at time t becomes as the following.

$$Cs(t) = Cs(0) + \int_0^t \{Vs_{uptake}(t) - Vs_{release}(t)\} dt \quad (1)$$

However, the concentration in s is influenced by other factors. For example, concentration in the host is influenced by uptake by zooxanthellae and degradation of particulate matter. The equation for host is

$$Chost(t) = Chost(0) + \int_0^t \{V_{host\ uptake} - V_{host\ release} + V_{host\ deg} - V_{zoox\ uptake}\} dt \quad (2)$$

where $V_{host\ deg}$ is degradation rate, $V_{zoox\ uptake}$ is uptake rate of zooxanthellae from host cell. When the each rate is integrated, $Chost(t)$ becomes

$$Chost(t) = Chost(0) + (\alpha - \beta + \gamma - \delta)e^t \quad (3)$$

where α , β , γ , and δ are coefficients for uptake rate, release rate, and degradation rate of particulate matter of host, and uptake rate by zooxanthellae, respectively. $V_{host\ uptake}$ rate of host was estimated from incubation experiment by using isotope in this study. With this model, major uptake of NH_4^+ from seawater was assumed to be made by host corals (Rees 1987; Miller and Yellowlees 1989). $^{15}NH_4^+$, $V_{host\ uptake}$ was estimated to be 10.9×10^{-9} mol/cm²/h after applying the nutrient concentration dependant model, Michaelis-Menten saturation kinetics (D'Elia et al. 1983). Release rate ($V_{host\ release}$) can be estimated from NH_4^+ changes in seawater and $V_{host\ uptake}$ with assumption that $V_{zoox\ release}$ does not directly impact on NH_4^+ concentration in seawater as described in Figure 2.

$$\frac{\Delta[NH_4^+]_{seawater} \times volume}{area \times \Delta t} = V_{host\ uptake} - V_{host\ release} \quad (4)$$

where $\Delta[NH_4^+]_{seawater}$ is NH_4^+ concentration in seawater, $volume$ is volume of media used for incubation experiment, $area$ is coral surface area, Δt is incubation time. $V_{host\ release}$ is estimated to be 11.32×10^{-9} mol/cm²/h. Here, as uptake and release rates of host are close, isotope method could overestimate uptake rate (Shiroma et al. 2007). From equation (3), by measuring NH_4^+ concentration in host and $V_{zoox\ uptake}$ *in vivo* (Falkowski et al. 1993), $V_{host\ deg}$ can be estimated. This first step in mathematical model is useful to understand symbiotic relationship between host coral and zooxanthellae.

The study is needed to scale down the temporal as well as spatial scale to look into complex symbiotic relationship in host corals. Uptake rate for each components needs to be tracked as nutrient assimilation in dark and light are changing (Rees 1987) implying temporal changes in uptake rate. Regeneration of resource in symbiotic complex in

coral is not known in details yet (Falkowski et al. 1993). Therefore, precise measurement of each component and mathematical model give the allocation rate of nutrient in corals (Swanson and Hoegh-Guldberg 1998). This study limited the symbiotic system by focusing on host coral and zooxanthellae; however, interaction with algae or microbes in symbiotic relationships has been studied today (Knowlton and Rohwer 2003). Empirical data focusing on this scale are needed to develop mathematical model. In addition to degradation of organic matter, the possibility of nitrogen fixation could add some more flow in Fig. 2 (Lesser et al. 2007). Laboratory experiments as well as monitoring *in situ* are required to develop the present model further. Incubation experiment demonstrated that both uptake and release rates need to be measured to estimate nutrient exchange rates in symbiotic relationship in corals. Higher concentrations of nutrients were possibly from coral skeletons, microbes, or symbiotic systems in corals such as mesoglea, stomach, and specific tissues (gastrodermis, ectoderm, or endoderm). New sampling methods will be applied (Agostini et al. 2007) in the future to determine where higher nutrients are distributed or whether the nutrients are directly available to symbiotic complex in corals.

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