Microbial Activity and Dissolved Organic Matter in Sesoko reef, Okinawa

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Abstract. Dissolved organic carbon and nitrogen (DOC and DON respectively) in coral reef waters play a key role in supporting the microbial food web. However, the relationship between DOC, DON, microbial activity, and macro-organisms in the reef environment is not well understood. Temporal patterns in DOC, DON, and microbial abundances were studied on the fringing reef of Sesoko Island (Okinawa, Japan) by comparing seawater samples collected in the morning, afternoon, and evening. Concentrations of DOC and DON increased between the afternoon and evening (68.5 μM C to 74.1 μM C; 3.8 μM N to 4.4 μM N) and decreased to minima in the morning (to 66.6 μM C and 3.4 μM N). Concurrently, microbial activity increased in the morning, as shown by increases in bacterial abundance throughout the afternoon and evening to peaks in the following morning samples, potentially due to consumption of organic material derived from reef organisms such as corals. Detectable amino acid concentrations also increased throughout the day (from 140 nM to 150 nM), showing a positive correlation with microbial abundance. These results indicate a link between microbial activity and DOC and DON in reef waters. We suggest that the measurement of DOC and DON in shallow reef water will be an important indicator to detect interactions between microbial activity and coral stress.

Key words: DOC, DON, Microbial Activity

Introduction

Coral reefs in tropical and sub-tropical oceans are biologically diverse ecosystems with high gross primary production (Sorokin, 1995). This high productivity in oligotrophic areas with very low nutrient supply may be due to the rapid recycling of organic matter to inorganic forms (Suzuki et al., 2000). Dissolved organic matter (DOM), either as dissolved organic carbon (DOC) or dissolved organic nitrogen (DON), plays an important role because it is rapidly consumed and re-mineralized by the microbial community in reef waters.

Several studies have suggested that DOC derived from coral mucus must sustain the high production rates that occur in coral reef waters by promoting microbial growth (van Duyl and Gast 2001, Ferrier-Pages et al., 2000, Kline et al. 2006). The relationships between DOC, DON, microbial activity, and macro-organisms in reef environments are not well understood due to limited field data. Therefore further studies are necessary to understand DOC and DON in relation to microbial activity. This will be an important step towards understanding mechanisms of nutrient-cycling related to microbial interactions in the water column.

This paper reports temporal patterns in the abundance of DOC, DON, and planktonic microbial communities on the fringing reef of Sesoko Island (Okinawa, Japan).

Material and Methods

Study site and water sampling

Sesoko reef is located on the west coast of Sesoko Island, Okinawa, Japan (26°38'N, 27°51'E). The reef is characterized by a shallow lagoon (0.2 – 6.5 m depth) and a well-developed reef crest, located 150–200 m from the shore. A mass bleaching event in 1998 decreased the number of coral species on Sesoko reef by 61%, and percentage cover of corals was reduced from 70% in 1997 to less than ~ 5% in 1999 (Loya et al., 2001). Presently, however, the reef is undergoing recovery, with recent recruitment of new coral colonies (Nakano et al., unpublished data). The live coral community is composed of about 44% *Goniastrea aspera*, 21% *Montipora digitata*, 7.4% *Porites sp.*, and 2.2% *Acropora digitifera.*

Seawater samples from Sesoko reef were collected from the surface along transects at two locations: Station A in the middle of the lagoon, and Station B located near the entrance of the lagoon (Fig. 1). These

two stations (two transects along near shore to reef crest) were selected for the water sampling with the objective of showing the relationships between DOC, DON, microbial activity, and macro-organisms in reef environments. Coral cover was relatively high between near shore and middle of the lagoon compared to reef crest.

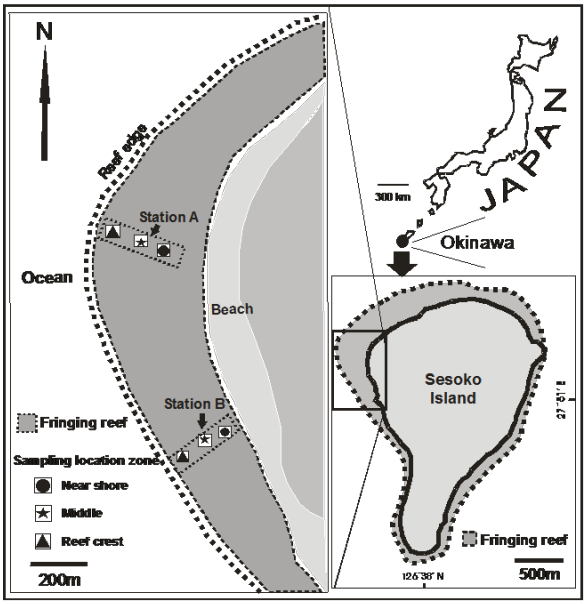

Figure 1: Map of Sesoko coral reef showing water sampling points at Station A; A1 (nearshore), A2 (middle of the lagoon) and A3 (at the reef crest, i.e. nearest the ocean); and at Station B; B1 (near shore), B2 (middle) and B3 (reef crest).

Sesoko reef water condition

Light and temperature were measured along transects at the two stations (Fig. 1) using *in situ* light and temperature sensors (Alec electronics).

Samples of surface waters above reefs at the nearshore, middle lagoon and reef crest sites along transects at Stations A (Stn. A) and B (Stn. B) were collected on 9-10 May 2008 (14:30, 17:30 and at 06:00, except for the reef crest sample at Station A, which was not collected at 06:00) into 5 liter Nalgene® clean bottles. These reef water samples were used for chemical analysis and for determination of microbial abundance.

Samples for chemical analysis and microbial abundance

One subsample was collected from each sample for measurement of DOC, DON, dissolved inorganic nitrogen (DIN, including nitrate, nitrite, and ammonium) and total hydrolyzed amino acids (THAA). Measurement of THAA within the DON pool could be an important indicator of biological production mainly from bacteria, particularly at near shore waters where dilution is limited. Therefore one sample from the nearshore point from each of the two stations was analyzed for THAA and considered as a preliminary study. Subsamples were collected by filtering seawater into 40ml cleaned, pre-combusted amber glass vials, after passing through pre-combusted 25mm GF/F filter (Whatman). The sample vials were covered with Teflon-lined caps and stored at -20°C until analysis of DOC, DON, DIN and THAA. All bottles (Nalgene®) and glass vials used for sampling and sample storage were washed and rinsed with 10% HCL and Milli-Q water before use.

Another 50 ml sub-sample was collected into bacteria-free (sterile) tubes for microscopic analysis of microbial abundance after preserving in glutaraldehyde.

Analytical methods for chemical parameters

DOC and total dissolved nitrogen (TDN) were determined using a total organic carbon analyzer equipped with a total nitrogen unit (TOC V, Shimadzu), following the methods of Suzuki et al., (1993) and UNESCO (1994). DIN was determined with an autoanalyzer (TRAACS-2000) following the methods of Hansen and Koroleff (1999). DON concentration was calculated by subtracting the total dissolved inorganic nitrogen ($[NO_2 + NO_3 + NH_4]$) or DIN from the concentrations of the total dissolved nitrogen (TDN).

THAA concentrations were measured for sub-samples collected from nearshore points after analysis of other parameters, i.e., DOC, DON, and DIN for Stn. A and Stn. B. (high performance liquid chromatography [HPLC, Agilent]) according to the methods Lindorth and Mopper (1979).

Microbial abundance

Microbial abundance was measured after staining samples (preserved in glutaraldehyde) using DAPI (4', 6-diamino-2-phenylindole) under an epi-florescence microscope (Nikon-Eclipse) following the method of Porter and Feig (1980). Measurements of microbial abundance indicated with mean of ten direct counts.

Results

Sesoko reef water condition

The maximum light intensity during the day was 2024 µM $cm^{-2}s^{-1}$, with temperature recorded at 26.8°C. The average temperature recorded during the sampling period was 25.5°C±2°C, with a diurnal variation of ±2 °C.

Variation in the levels of DOC, DON and DIN was found, with respect to both sampling location and sampling time (Fig. 2). Values of these measurements at Stn. B were consistently higher than those at Stn. A.

The highest DOC concentrations were recorded in the evening at nearshore sites A1 and B1 (70.5 µMC and 77.8 µMC, respectively). Lowest values were recorded in the morning, also for nearshore sites, as 63.11 µMC for A1 and 68.9 µMC for B1 (Fig 2a).

Similarly, highest DON concentrations were recorded in the evening at nearshore A1 and B1 sites, and were 4.8 µMN and 5.1 µMN, respectively. Lowest DON concentrations were recorded in the morning, also from nearshore sites, i.e., 3.0 µMN at A1 and 3.6 µMN at B1 (Fig 2b).

The ratio of DOC: DON (C/N) varied from 15 to 18 (Fig 2c), with ratios tending to be lowest in the evenings and highest in the mornings.

DIN concentrations were highest in the evening at both Stn. A and Stn. B. Overall, variation in DIN concentrations was greater at Stn. B than at Stn. A, reaching 3.3 µM at the nearshore B1 site in the evening (Fig. 3).

THAA concentrations were highest at nearshore sites in the morning, reaching 150.5 nm at A1 and 145.6 nm at B1 (Table 1). Contributions from glutamic acid were high compared to other amino acids detected in reef water samples. Other amino acids were methionine, tryptophan, valine and alanine.

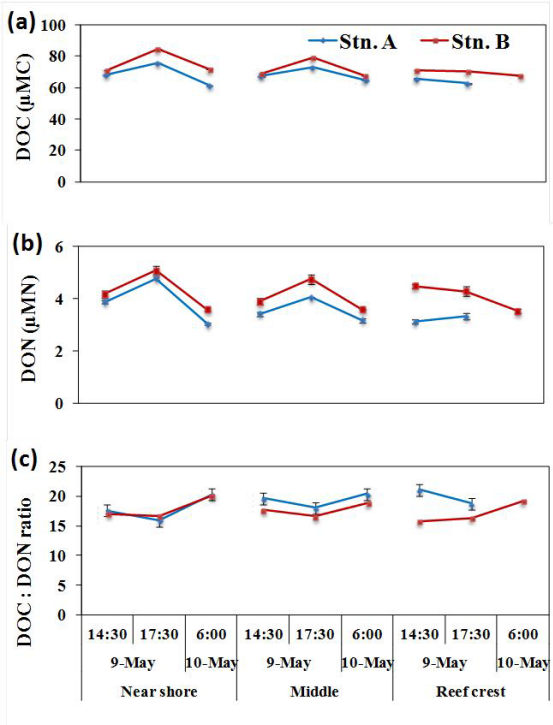

Figure 2: Variation in (a) mean DON and (b) mean DOC concentrations, and (c) the DOC: DON ratio in Sesoko coral reef waters at stations A and B, along three transects (nearshore, middle and reef crest) at 14:00, 17:00 and the following day at 06:00 (except for site A, reef crest) in May 2008. Mean values were calculated from three repeat measurements.

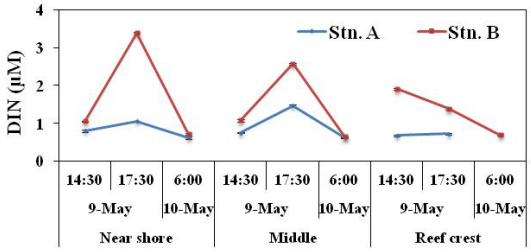

Figure 3: Variation in mean concentration of dissolved inorganic nitrogen from 9th to 10th May 2008 at Sesoko coral reef, Okinawa, at stations A and B along three transects (nearshore, middle and reef crest) at 14:00, 17:00 and the following day at 06:00 (except for site A, reef crest) in May 2008. Mean values were calculated from three repeat measurements.

Microbial abundance

Pico and nano size plankton abundance, namely bacteria, picocyanobacteria and heterotrophic nano flagellates were recorded as cells per milliliter for all sampling times for Sesoko reef seawater (Fig. 4).

Average abundance of bacteria cells ranged from 1.6 X 10^5 ml^{-1} to 18.1 X 10^5 ml^{-1} for Stn. A and Stn. B reef water. Bacterial cell abundance was greatest in the morning compared to afternoon and evening samples at Stn. B and the reef-crest Stn. A site (Fig. 4a).

Table 1: Amino acid (AA) concentrations and total AA for samples with the highest concentrations, i.e., for the nearshore A1 and B1 samples of Sesoko coral reef seawater from 9th to 10th May 2008.

Sub location	Time and Date	Amino acid concentration (nM)					Total AA
		Glutamic acid	Alanine	Valine	Methionine	Triptophan	
Near shore point (A1) Stn. A	14:30 9th May	107.0	4.0	11.8	4.5	12.9	140.3
	17:30 9th May	109.6	3.7	11.4	3.6	12.9	141.4
	6:00 10th May	108.2	3.6	19.7	5.5	13.2	150.5
Near shore point (B1) Stn. B	14:30 9th May	89.6	4.6	12.3	0	4.0	110.7
	17:30 9th May	117.0	3.0	8.5	4.1	11.8	144.6
	6:00 9th May	112.8	3.7	11.5	5.4	12.0	145.6

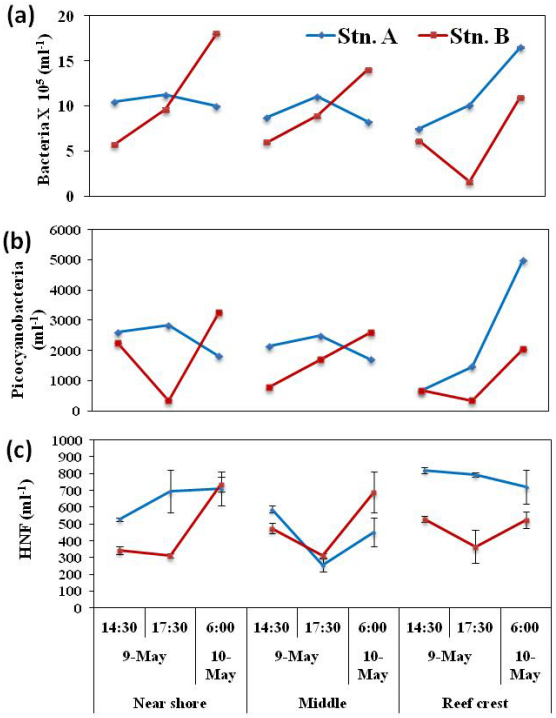

Figure 4: Variation in mean (±SE) abundance of (a) bacteria (b) picocyanobacteria (c) heterotrophic nano flagellates (HNF) in Sesoko coral reef, Okinawa, at site A and site B along three different points (nearshore, middle and reef crest) during 14:00, 17:00 and the following day at 06:00 (except for site A, reef crest) in May 2008.

Pico-cyanobacteria cell counts were similar during day time and evening time at Stn. A and Stn. B reef water, average number of cells were as low as 339 ml^{-1} in the evening at the nearshore Stn. B site, and as high as 4973 ml^{-1} at the reef crest Stn. A site (Fig. 4b).

There were no consistent patterns in the abundance of heterotrophic nano-flagellates temporally or spatially, although abundance tended to be greatest at Station A. The abundance of HNF cells ranged from 256ml^{-1} to 819 ml^{-1}(Fig. 4c).

Discussion

In coral reef ecosystems, carbon and nitrogen are essential elements required to maintain productivity levels and support "micro" and "macro" food webs, however, due to limited data, relationships between DOC, DON and the abundance of microbes and macro-organisms are not well understood. Among the limited number of studies in the area, Wild et al. (2004) showed the importance of coral-derived organic matter in the water column but did not address microbial activity and its relationship to DOC and DON. To date, data on DOC or DON published for coral reef waters have been measured for different purposes and for different reef locations. Few studies have measured DOC (Yoshinaga et al. 1997, Van Duyle & Gast 2001, Hata et al. 2002 and Dinsdale 2008) and DON (Webb et al. 1974, Cox et al. 2006) for coral reef waters. Suzuki et al. (2000) reported concentrations of total organic carbon and total organic nitrogen for Miyako Island, Japan rather than DOC and DON.

The present study compares DOC and DON concentrations with microbial abundances at Sesoko reef, Okinawa, Japan at different times of the day. Results of this study suggest that high bacterial activity in morning samples was fueled by organic material potentially produced by coral reef organisms during the day, which tended to peak in the water column in the evening. Higher bacterial numbers in the morning may be due to higher microbial utilization of dissolved organic matter from the previous day. This connectivity among DOC, DON and microbes was detectable in nearshore areas of the lagoon presumably due to limited physical mixing of water across depths. Links between microbial activity and DOC and DON in reef waters were greatest in nearshore reef samples as it was detectable in some amino acids present in collected water samples. In addition, DIN concentrations were also high at nearshore and middle sampling points. This may be due to cycling of organic nitrogen to inorganic forms at nearshore (internal cycling). These general trends show variations in microbial activity in the water column in different places over reefs where coral cover is high. However more data is needed to establish a clear understanding on how microbial processes are involved in changing water chemistry. This may be useful for improving our understanding of coral-microbe interactions and to better explain how coral reefs survive in oligotrophic waters. We suggest that the measurement of DOC and DON, DIN in shallow reef water will be an important indicator to detect interactions between microbial activity and coral stress.

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