Journal of Experimental Marine Biology and Ecology 504 (2018) 53–60

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Release of dissolved and particulate organic matter by the soft coral *Lobophytum* and subsequent microbial degradation

Ryota Nakajima[a,b,*], Andreas F. Haas[c], Cynthia B. Silveira[d], Emily L.A. Kelly[b], Jennifer E. Smith[b], Stuart Sandin[b], Linda W. Kelly[d], Forest Rohwer[d], Nobuyuki Nakatomi[e], Haruko Kurihara[f]

[a] Department of Marine Biodiversity Research, Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Yokosuka, Kanagawa 237-0061, Japan
[b] Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA 92083, USA
[c] NIOZ Royal Institute for Sea Research and Utrecht University, Texel, The Netherlands
[d] San Diego State University, San Diego, CA 92182, USA
[e] Faculty of Science and Engineering, Soka University, Tokyo 192-8577, Japan
[f] Faculty of Science, University of the Ryukyus, Okinawa 903-0213, Japan

ARTICLE INFO

Keywords:
DOC
POC
Coral mucus
C:N ratio
Bacteria
Soft corals

ABSTRACT

Understanding the release and remineralization of organic matter by benthic macroorganisms provides insight into nutrient cycling and microbial metabolism in coral reef environments. The release rate of particulate (POC) and dissolved organic carbon (DOC) by the soft coral *Lobophytum crassum* was quantified and subsequent bacterial growth rates determined in response to this resource, and compared with results from those of the common hard coral *Acropora intermedia*. The results of this study show that the soft coral released more DOC than POC into the surrounding seawater, similar to what was measured for the hard coral species. However, the soft coral-derived organic matter fostered a lower microbial growth rate with a lower growth efficiency compared to DOC and POC of hard corals, likely due to the lower C:N ratio of the organic matter derived from soft corals. These results suggest that soft coral exudates are relatively refractory compared to the mucus of hard corals. Possible phase shifts from hard to soft corals on degraded reefs may represent very different changes in microbial community dynamics and metabolism as compared to the widely studied coral-algal phase shifts.

1. Introduction

Understanding the release and remineralization of organic matter by benthic macroorganisms provides insight into biogeochemical cycling in coral reef environments. For example, hermatypic hard corals continuously release organic matter to the surrounding seawater in the form of both particulate (POM) and dissolved organic matter (DOM) (Nakajima et al., 2010; Tanaka et al., 2008), where they act to transfer energy or trap particles, thus contributing to coral reef biogeochemical cycling (Naumann et al., 2009; Wild et al., 2004).

While much research has focused on understanding the organic matter release and associated microbial degradation from hard corals and benthic algae (Haas et al., 2016, 2011, 2010; Naumann et al., 2010; Tanaka et al., 2008), much less is known about other types of benthic reef organisms. Specifically, soft corals (*Alcyonacea*) can be one of the most abundant components of non-reef-building benthic communities on coral reefs, and in some areas their density can equal or exceed that of hard corals (Dinesen, 1983; Inoue et al., 2013; Pratchett, 2010). Soft corals may represent an underappreciated source of organic matter contributing to biogeochemical cycling in coral reef communities where they are abundant.

At present, very little is known about the release rate of organic matter by soft corals. To the best of our knowledge, quantitative measurement of POM and DOM release by soft corals has only been done for *Xenia* sp. from the northern Red Sea, which showed no organic matter release under natural environmental conditions (Bednarz et al., 2012). Although qualitative, however, there is also a report that the soft coral *Briarium asbestinum* releases organic matter (mucus) under natural conditions (Rublee et al., 1980). These patterns of organic matter release by soft corals are not unequivocal, requiring further investigations of organic matter release by soft corals. Similar to hard corals, many soft corals species have symbiotic zooxanthellae in their tissue (Fabricius and Klumpp, 1995). Thus they may release a fraction of their primary production as organic matter (DOM and POM) into the surrounding seawater (Meikle et al., 1988).

It is clear from an extensive body of work that hard coral-derived

* Corresponding author at: Department of Marine Biodiversity Research, Japan Agency for Marine-Earth Science and Technology (JAMSTEC), 2-15 Natsushima, Yokosuka, Kanagawa 237-0061, Japan.
E-mail address: nakajimar@jamstec.go.jp (R. Nakajima).

https://doi.org/10.1016/j.jembe.2018.02.008
Received 24 April 2017; Received in revised form 25 January 2018; Accepted 25 February 2018
Available online 22 April 2018
0022-0981/

organic matter induces a rapid increase in bacterial abundance and can sustain high bacterial production in coral reef ecosystems (Tanaka and Nakajima, 2018). In contrast, however, none of the previous studies have confirmed bacterial utilization of organic matter derived from soft corals, though bacterial aggregation on soft coral mucus was previously found (Rublee et al., 1980). It has been reported that the organic matter released by soft corals was predominantly protein and had a lower percentage of combined carbohydrate as compared to organic matter released by hard corals (Meikle et al., 1988). This result suggests that soft corals might release organic matter with relatively lower C:N ratio into the surrounding seawater, which might not facilitate bacterial growth rates and growth efficiencies as high as those observed in the organic matter released by hard corals (Haas and Wild, 2010; Nakajima et al., 2017; Naumann et al., 2010).

Here, we tested the hypothesis that soft corals release organic matter (DOM and POM), but that soft coral-derived organic matter could subsequently lead to lower bacterial growth as compared to hard coral-derived organic matter. For this purpose we examined the chemical composition and the release rate of organic matter by a soft coral species (*Lobophytum crassum*) and subsequent microbial growth on a Japanese coral reef, and compared results with those from a hard coral species (*Acropora intermedia*) of the same reef.

2. Methods

2.1. Coral collection

This study was conducted on Sesoko Island (26°37.4N, 127°51.4E), Okinawa, Japan in July 2015. Soft corals are widely distributed around Sesoko Island, with relatively high cover (10–50%) in the southeast coast of the island (Okinawa-Prefectural-Government, 2010), of which *Lobophytum* (*Alcyoniidae*) is the most dominant genus occupying 90% of total soft coral cover (S. Inoue, personal communication, Euglena Co. Ltd.). Colonies of the soft coral *Lobophytum crassum* and a commonly found branching hard coral *Acropora intermedia* (*Acroporidae*) were collected from the reefs of Sesoko Island.

In collecting soft corals, 5–8 cm diameter colonies were broken off at the lower basal hard section using a chisel and hammer to avoid mechanical damage to the soft living tissue. For hard corals, ca. 10 cm long branches were cut from coral colonies with bone cutters. All experiments and preparation of samples occurred at the Sesoko Research Station, Tropical Biosphere Research Center, University of the Ryukyus.

2.2. Rate of organic matter release

The amount of organic matter released into the surrounding seawater was analyzed for both soft (*L. crassum*) and hard coral (*A. intermedia*) specimens. Each of the six experimental branches of hard corals and six colonies of soft corals were transferred to individual aquariums (inner volume: 187 L each) located outside. Hard coral branches were suspended in seawater in tanks using nylon lines. Soft coral colonies were placed on the bottom of the tank. Both soft and hard coral specimens were acclimated to the aquarium for > 7 d before the beginning of the experiment. Outdoor tanks had flow-through seawater pumped from the adjacent reef habitat at in situ temperature (28.3 ± 0.6 °C), Seawater was pre-filtered through carbonate sands at a rate of ca. 10 L min^{-1}. Tanks were shaded to mimic light conditions on the reef at 0.5–1 m depth (average mid-day photosynthetically active radiation (PAR), ca. 600 μmol photons $m^{-2} s^{-1}$).

Organic matter released by the corals was measured for each specimen using the method described by Herndl and Velimirov (1986) with some modifications. Briefly, each specimen was placed in a 2 L transparent plastic container and incubated in this closed system for 6 h. Eighteen containers were used for incubations: 6 for hard corals, 6 for soft corals and 6 for controls (without corals). All containers were cleaned with HCl (1 N) and alkali detergent (Extran M01, Merck Millipore, 2%) and rinsed with Milli-Q water (Millipore) before use. Each container had 1300 mL of filtered seawater, which was prepared by filtering natural reef seawater through GF/F filters (Whatman). Hard and soft coral specimens were transferred directly from the outdoor holding tank into containers and were fully submerged under the water. Hard coral specimens were suspended using the nylon lines as in the holding tank and soft corals placed directly on the bottom of containers. Fouling algae either attached on the nylon lines for hard corals or attached on the basal section of soft corals were removed by wiping or brushing 2 h before transferring into the container. The 18 experimental containers were placed in a large outdoor aquarium with flow-through seawater to act as a water bath. Experimental containers were then incubated for 5 h (1000–1500 h). Containers were covered with transparent cellophane film during the incubation to avoid input of airborne particles, with small side openings for air exchange (Naumann et al., 2010). Seawater in the containers was not stirred during the incubation. Mean (± SD) irradiance and water temperature were 608 ± 461 μmol photons $m^{-2} s^{-1}$ and 29.7 ± 0.2 °C, respectively, during the 5 h incubations, which were monitored every 10 min with data loggers (DEFI-L, JFE and U22 Water Temp Pro v2, HOBO). Although we did not conduct a direct assessment of coral health in the experimental tanks (such as dissolved oxygen concentration), no visible signals of stress (such as bleaching) were found in the corals during and after the experiment.

After the 5 h incubation, soft and hard coral specimens were removed from containers, and the incubated water of each container was thoroughly homogenized. Each of the experimental containers was subsampled in duplicate (500 mL each) for particulate organic carbon (POC) and nitrogen (PON) and in duplicate (10 mL each) for dissolved organic carbon (DOC) measurement. Subsamples for the POC and PON analysis were filtered onto pre-combusted (500 °C, 4 h) GF/F filters (1 in., Whatman). Filters were rinsed with HCl (1 N) to remove carbonate particles and washed with Milli Q water (Millipore). Filters were then dried and stored in a desiccator until analysis. Subsamples for DOC measurements were filtered through pre-combusted GF/F filters (1 in., Whatman) using a 50 mL glass syringe and a filter holder (Swinnex, Millipore), and the filtrate was sealed into pre-combusted (500 °C, 4 h) amber glass ampoules and stored at −20 °C until analysis. The glass syringe and filter holder were acid- and alkaline-cleaned before use. Upon sampling, the inner wall of the syringe and the filter holder with filter were rinsed twice with 10 mL of the sample water.

To measure the surface area of the incubated coral specimens, each specimen was wrapped in either aluminum foil (hard coral) or cellophane film (soft coral). Photographs were taken with a digital camera of the opened foil or film with a scale for calibration. The opened foil or film area was estimated by tracing its outline on digital photographs using Image J (National Institutes of Health freeware) to calculate coral surface area.

2.3. Mucus collection

Mucus from both soft (*L. crassum*) and hard (*A. intermedia*) corals was collected for chemical composition measurements and for experiments on mucus degradation. To collect mucus, soft and hard coral specimens were removed from water, exposed to air, and inverted under real sunlight for 5 to 10 min. Within seconds of being inverted in air, both soft and hard coral specimens released gel-like mucus, which was separately collected in sterilized 50 mL Corning tubes. Collected mucus was immediately refrigerated at 5 °C. Refrigerated mucus was first analyzed for chemical composition and then used in mucus degradation experiments within 0.5 h of sampling.

2.4. Chemical composition of mucus

To determine the chemical composition of mucus from *L. crassum* and *A. intermedia*, triplicate subsamples were taken from the collected