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REPORT

Effects of coral-derived organic matter on the growth of bacterioplankton and heterotrophic nanoflagellates

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Abstract Exudates derived from hermatypic corals were incubated with <2 µm filtered seawater containing heterotrophic bacteria and <10 µm filtered seawater containing bacteria and nanoflagellates (HNF) under dark conditions for 96 h to quantify the growth of both bacteria and HNF in response to coral-derived dissolved organic matter (DOM). The addition of coral-derived DOM caused significantly higher growth rates and production of bacteria and HNF compared to those in control seawater without coral exudates. During the incubation, HNF exhibited their peak in abundance 24–48 h after the peak abundance of bacteria. The growth efficiencies of both bacteria and HNF were significantly higher with coral-derived DOM, suggesting higher transfer efficiency from bacteria that is fueled by coral organic matter to HNF. Therefore, trophic transfer of coral-derived DOM from bacteria to HNF can contribute to efficient carbon flow through the microbial food web.

Keywords Bacteria · Coral mucus · DOC · Microbial food web · Heterotrophic nanoflagellates (HNF)

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Introduction

In coral reef ecosystems, hermatypic corals continuously release organic matter, a complex mixture of carbohydrates, lipids and protein (Bailey and Robertson 1982; Wild et al. 2010; Nelson et al. 2013), into the seawater as a result of metabolic activity (Davies 1984; Tanaka et al. 2009, 2010) and as a defense mechanism against stressors such as desiccation, sedimentation and pathogens (Brown and Bythell 2005). More than half (ca. 60–90%) of the organic matter derived from corals is released in the form of dissolved organic matter (DOM) that passes through glass fiber filters (e.g., Whatman GF/F, a nominal pore size 0.7 µm) (Wild et al. 2004; Tanaka et al. 2008b; Nakajima et al. 2009, 2010; Naumann et al. 2010). Organic matter enrichment due to exudation of DOM by corals can result in significantly increased growth of heterotrophic bacteria (hereafter "bacteria") (Tanaka et al. 2008a; Haas et al. 2011, 2013; Nelson et al. 2013).

During the last three decades, a number of studies have evaluated the uptake of coral-derived DOM by both planktonic and benthic bacteria (Moriarty et al. 1985; Herndl and Velimirov 1986; Linley and Koop 1986; Paul et al. 1986; Ferrier-Pagès et al. 2000; Wild et al. 2004; Allers et al. 2008; Nakajima et al. 2009, 2015; Tanaka et al. 2011). As a result, coral-derived DOM has been experimentally demonstrated to induce a rapid increase in bacterial abundance (Allers et al. 2008; Nakajima et al. 2015) and can sustain a considerable portion of bacterial growth even at low concentrations (Ferrier-Pagès and Furla 2001; Taniguchi et al. 2014). However, little is known about the productivity of higher microbial trophic levels such as bacterivorous protists resulting from increased bacterial growth on coral-derived DOM. While bacterial growth efficiency from coral-derived DOM has been evaluated

(van Duyl and Gast 2001; Tanaka et al. 2008b; Nakajima et al. 2015), quantitative studies investigating the growth efficiency of bacterivorous protists from feeding on bacteria fueled by coral-derived DOM remain unclear. A previous mesocosm study investigating the growth of bacteria and flagellates in seawater enriched with coral exudates reported that both auto- and heterotrophic bacteria and autotrophic flagellates increased, but heterotrophic flagellate growth was not detected, though this may be due to their short incubation time (10 h) (Ferrier-Pagès et al. 2000).

Here, we tested the hypothesis that bacterial growth fueled by coral-derived DOM could subsequently lead to the emergence of bacterivorous protists (e.g., heterotrophic nanoflagellates, HNF), which are capable of transferring carbon to higher trophic levels through the microbial food web (Azam et al. 1983; Caron et al. 1991; Sanders et al. 1992; Nakano 2000). We experimentally investigated the growth of bacteria and the subsequent growth of HNF in seawater with and without coral-derived DOM for 96 h.

Materials and methods

Coral collection and incubation

This study took place at Sesoko Island, Okinawa, Japan (26°37–39′N, 127°51–52′E). All experiments and preparation of samples were carried out in the facilities of the Sesoko Research Station, Tropical Biosphere Research Center, University of the Ryukyus. The experiments were conducted during two summers (August 2014 and July 2015) using hermatypic branching corals *Acropora muricata* (experiment I) or *A. intermedia* (experiment II), respectively. Colonies of branching corals, *A. muricata* or *A. intermedia,* were collected from the reefs of Sesoko Island. One 5- to 10-cm branch was cut from each of 14 coral colonies. The cut branches were transferred to the prepared maintenance outdoor aquarium (inner volume: 187 L) and suspended right side up using nylon lines. The corals were acclimated in the outdoor aquarium for 7–20 d before experiments. Natural reef seawater was supplied to the maintenance tank at a rate of ca. 10 L min^{-1} at in situ water temperature during the acclimation periods. Temperature in the maintenance tank was monitored every 10 min with a data logger (U22 Water Temp Pro v2, HOBO). The aquarium used shaded natural light to simulate the irradiance in the natural reef habitat (0.5–1 m water depth; average midday photosynthetically active radiation, ca. 600 µmol photons m^{-2} s^{-1}).

Coral branches were incubated in a closed system for several hours to obtain the seawater containing coral exudates. Two transparent polycarbonate bottles (inner volume: 20 L) were used: one for coral incubation and the other for control (without coral). The bottles were cleaned with acid and alkali before use. Each bottle contained 16 L of filtered seawater, which was prepared by filtering natural reef seawater through 0.45-µm filters (Omnipore membrane, Millipore). The coral branches were quickly transferred into one of the bottles, suspended by nylon lines and fully submerged under the water. Fouling algae attached on the nylon lines were removed before transferring into the bottle. The two bottles, each with or without corals, were incubated for 6 h (0900–1500 hrs for experiment I) or 7 h (0800–1500 hrs for experiment II) in an outdoor aquarium. The aquarium was continuously supplied with fresh reef seawater, effectively serving as a water bath. The seawater in the bottles was gently stirred using submersible magnetic stirrers (Octopus CS-1, AsOne). The average (mean ± SD) water temperature was 29.0 ± 1.0 °C in experiment I and 29.7 ± 0.5 °C in experiment II. The average irradiance during the 6- to 7-h incubations was 601.9 ± 499.6 µmol photons m^{-2} s^{-1} for experiment I and 447.0 ± 409.5 µmol photons m^{-2} s^{-1} for experiment II, which was monitored every 10 min with a data logger (DEFI-L, JFE).

After 6- to 7-h incubation, the coral branches were taken out of the bottle. The total surface area of branches was measured by the aluminum foil method (Marsh 1970). The incubated water of each bottle was thoroughly homogenized, and then all water (16 L) was filtered through combusted (500 °C, 5 h) GF/F filters (2 inch, Whatman). Triplicate subsamples (10 mL each) from the filtrate were sealed into pre-combusted (500 °C, 5 h) amber glass ampoules for the measurement of dissolved organic carbon (DOC). The remaining filtrates were immediately used for the subsequent microbial incubation experiments.

Water sampling

Natural reef seawater was collected at a reef close by the research station to prepare the seawater containing bacteria or HNF. The seawater was collected with an acid- and alkali-cleaned bucket (inner volume: 10 L). The collected seawater was immediately filtered through 10-µm mesh by reverse filtration to remove predators of nanoflagellates (<10 µm filtered seawater (FSW) containing bacteria and HNF). Then, 5 L of the <10 µm FSW was gravity filtered through 2-µm membrane filters (Nuclepore, Whatman) to remove predators of bacteria (<2 µm FSW containing bacteria). We used six filtering apparatus for gravity filtration to complete the filtration procedure within 2 h of water collection. The prepared filtered seawater was used for microbial incubation experiments.

Microorganism incubation with and without coral exudates

For microorganism incubations, the seawater incubated with corals (coral SW) or control water without coral (control SW) was mixed with either <2 μm FSW (containing bacteria) or <10 μm FSW (containing bacteria and HNF) in the following four treatments: (A) control water + <2 μm FSW; (B) coral incubation water + <2 μm FSW; (C) control water + <10 μm FSW; and (D) coral incubation water + <10 μm FSW (Fig. 1). Triplicate polycarbonate bottles (inner volume, 2.4 L) were prepared for each treatment (12 bottles in total). First, we added 1.5 L of either control or coral incubation water into the bottles, followed by 0.8 L addition of either <2 μm or <10 μm FSW. In total, 2.3 L of mixed water was added to each bottle.

All bottles were incubated for 96 h in a temperature-controlled room at 28 ± 0.5 °C and kept in the dark to prevent growth of autotrophic organisms. The medium was continuously stirred during the experiments by means of magnetic stirrer for experiment I or modular roller apparatus (Model II, Wheaton Instruments) for experiment II. The incubation media were sampled every 24 h, and three separate samples were taken for bacterial counts (15 mL), HNF counts (200 mL) and DOC measurements (10 mL). The samples for bacterial counts were transferred into sterilized Corning tubes, fixed with buffered formalin (1% final concentration) and stored at −20 °C until analysis. Samples for HNF counts were transferred into dark polycarbonate bottles, fixed in 1% glutaraldehyde seawater and stored at 5 °C until analysis. The samples for DOC measurements were filtered through pre-combusted (500 °C, 5 h) GF/F filters using a glass syringe (inner volume, 50 mL) and filter holder (Swinnex, Millipore). Filtered seawater samples were sealed into pre-combusted (500 °C, 5 h) amber glass ampoules and stored at –20 °C until analysis. The glass syringes and filter holders were acid- and alkali-cleaned and then rinsed with Milli-Q water (Millipore) before use. Before each sampling, the filtration apparatus was rinsed twice with 10 mL of the respective sample water. DOC was measured by the high-temperature catalytic oxidation method using a total organic C analyzer (TOC-L CSH J100, Shimadzu) following Ogawa et al. (2003).

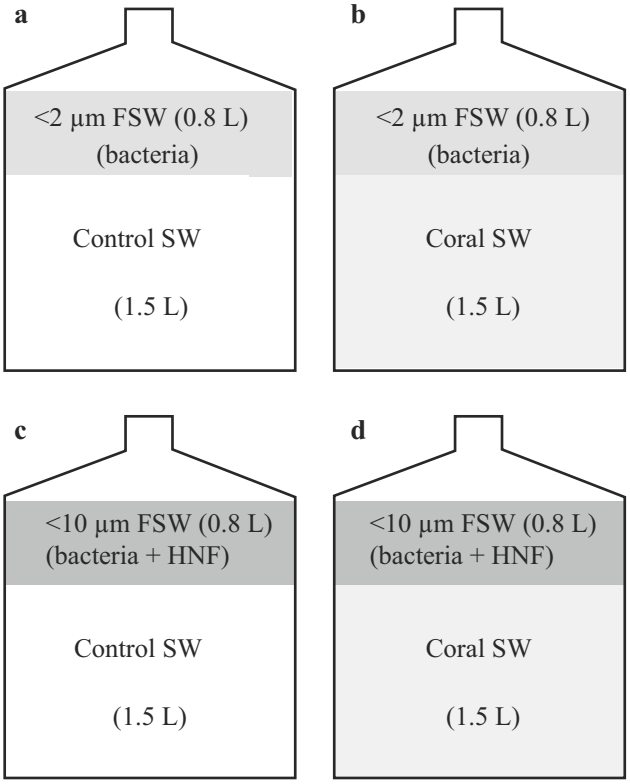

Fig. 1 Schematic diagram of experimental design. Seawater (SW) incubated with corals (coral SW) or without coral (control SW) was mixed with <2 μm filtered seawater (FSW) containing bacteria or <10 μm FSW containing bacteria and heterotrophic nanoflagellates (HNF), for preparing four treatments: **a** control SW + <2 μm FSW (treatment A), **b** coral SW + <2 μm FSW (treatment B), **c** control SW + <10 μm FSW (treatment C) and **d** coral SW + <10 μm FSW (treatment D). Both coral SW and control SW were pre-filtered through Whatman GF/F glass fiber filters before mixing with either <2 or <10 μm FSW

Microbial counts

To enumerate bacteria, 1.6–2.0 mL of the formalin-fixed sample was filtered onto a 0.2-μm black membrane filter (Isopore, Millipore) and stained with SYBR Gold (Molecular Probes) following Shibata et al. (2006). For HNF, 100 mL of glutaraldehyde-fixed sample was filtered onto a 0.8-μm black membrane filter (Isopore, Millipore), and the filter was stained with primulin (Sigma) following Sherr et al. (1993). Bacteria and HNF were counted with an epifluorescence microscope (Axioskop 2 plus, Zeiss) using blue and green excitations at ×1000 magnification. Autotrophic flagellates were distinguished from non-pigmented HNF by autofluorescence signals. For bacteria, at least 400 cells were counted per filter. Twenty microscope fields per filter were scanned for HNF. Microbial counts were made within 10 d of sampling for HNF and within 30 d for bacteria.

Growth rates and efficiency

Specific growth rates (μ, d^{-1}) of bacteria were obtained on the basis of results from the <2 μm fraction (treatments A and B), and those of the HNF were calculated from <10 μm fractions (treatments C and D) according to the equation:

$$\mu = \left(\ln N_f - \ln N_i\right) / \left(T_f - T_i\right)$$

where N_f and N_i are cell numbers (cells mL^{-1}) at the beginning (T_i) and a certain time (T_f) of the incubation period. The specific times were set for the maximal abundance during incubation.

Production rates (P, μmol C L^{-1} d^{-1}) of bacteria (BP) and HNF ($HNFP$) were calculated based on their increased biomass (B, μmol C L^{-1}) during a certain incubation period. Biomass (B) was estimated based on its cell density (A, cells L^{-1}) and cell carbon weight (CW, μmol $cell^{-1}$): $B = A \times CW$. Bacterial cell carbon weight was assumed to be 20 fg C per bacterial cell (Lee and Fuhrman 1987). Cell volumes (μm^3) of HNF were converted to carbon units using a conversion factor of 183 fg C μm^{-3} (Caron et al. 1995). Cell volumes of HNF were calculated from the length and width measured by image analysis software (AxioVision, Zeiss) and a digital camera (Zeiss AxioCam MRc5, Zeiss) mounted on the microscope.

Bacterial growth efficiency (BGE, %) was calculated as: $BGE = BP\ (BP + BR)^{-1} \times 100$ (Kirchman 2012), where BP is bacterial production and BR is bacterial respiration. In this study, BP and BR were assumed to an increased bacterial C biomass and decreased DOC during the 24-h incubation. The growth efficiency of HNF (FGE, %) was calculated as the proportion of $HNFP$ to BP: $FGE = HNFP\ (BP)^{-1} \times 100$ (Grujčić et al. 2015).

Statistical analysis

Statistical differences in the concentration of DOC, abundance, growth rates and efficiency in either bacteria or HNF between the coral incubation and control waters were determined using two-sided Mann–Whitney's U test. The U test was conducted for data combined with results from experiments I and II (n = 6 for each treatment).

Results

Changes in DOC concentration

The average (mean ± SD) initial DOC concentrations in the controls (treatment A, control water + <2 μm filtered seawater (FSW); and treatment C, control water + <10 μm FSW) were 73.0 ± 3.4 and 73.9 ± 4.4 μmol L^{-1} in experiment I and 69.8 ± 3.0 and 70.7 ± 3.8 μmol L^{-1} in experiment II, respectively (Fig. 2). In contrast, the initial DOC concentrations in the coral incubation water (treatment B, coral incubation water + <2 μm FSW; and treatment D, coral incubation water + <10 μm FSW) were 92.0 ± 0.8 and 92.8 ± 0.8 μmol L^{-1} in experiment I and 93.7 ± 5.3 and 94.7 ± 3.7 μmol L^{-1} in experiment II, respectively. Therefore, the addition of coral-derived DOC was 18.8–18.9 μmol L^{-1} in experiment I and 23.8–24.0 μmol L^{-1} in experiment II. During the first 24-h incubation, the concentration of DOC decreased from 6.5 to 12.0 μmol L^{-1} in the coral incubation water, while it decreased from 6.9 to 9.4 μmol L^{-1} in the control waters (Fig. 2).

Development of bacterial abundance

The bacterial abundance in all treatments showed their peaks after 24 h, except for controls in experiment I (treatments A and C) which showed abundance maxima after 48 h (Fig. 3). Increased bacterial abundance during the 24 h in coral incubation water was 1.4- to 2.3-fold higher than those in control waters (Table 1). The calculated bacterial production (increased bacterial biomass during the 24 h) in the coral incubation (treatment B, <2 μm fraction) was significantly higher than those in the control waters (treatment A, <2 μm fraction) when results from experiments I and II were combined (U test, $P = 0.0065$) (Table 1). The difference between the treatments C and D (<10 μm fraction) was not significant ($P = 0.17$).

The average bacterial growth rates (d^{-1}) in the coral incubation water (treatment B) during the 24 h were 1.35 ± 0.17 d^{-1} in experiment I and 1.74 ± 0.08 d^{-1} in experiment II (Table 1). These growth rates in coral incubation water were 1.6- to 2.6-fold higher than those in the control water (treatment A, 0.51 ± 0.16 d^{-1} in experiment I; 1.11 ± 0.18 d^{-1} in experiment II) with a significant difference with results from experiments I and II combined (U test, $P = 0.011$). The averages of BGE (%) in the coral incubation water (treatment B, 11.3 ± 1.1% in experiment I; 19.9 ± 4.3% in experiment II) were 1.5 to 1.6 times higher than those in controls (treatment A, 7.4 ± 3.5% in experiment I; 12.7 ± 5.7% in experiment II) (Table 1), with a significant difference with combined results from experiments I and II (U test, $P = 0.037$).

Development of HNF abundance

HNF showed their peaks in abundance 24–48 h later than the peaks of bacterial abundance: 72 h in experiment I and 48 h in experiment II (Fig. 4). The increased HNF abundance in the coral incubation water (treatment D) was 3.9-fold (experiment I) and 2.2-fold (experiment II) higher than those in the control water (treatment C) (Table 2). The calculated HNF production (i.e., increased HNF biomass during either the 72 h for experiment I or 48 h for experiment II) in the coral incubation was significantly higher

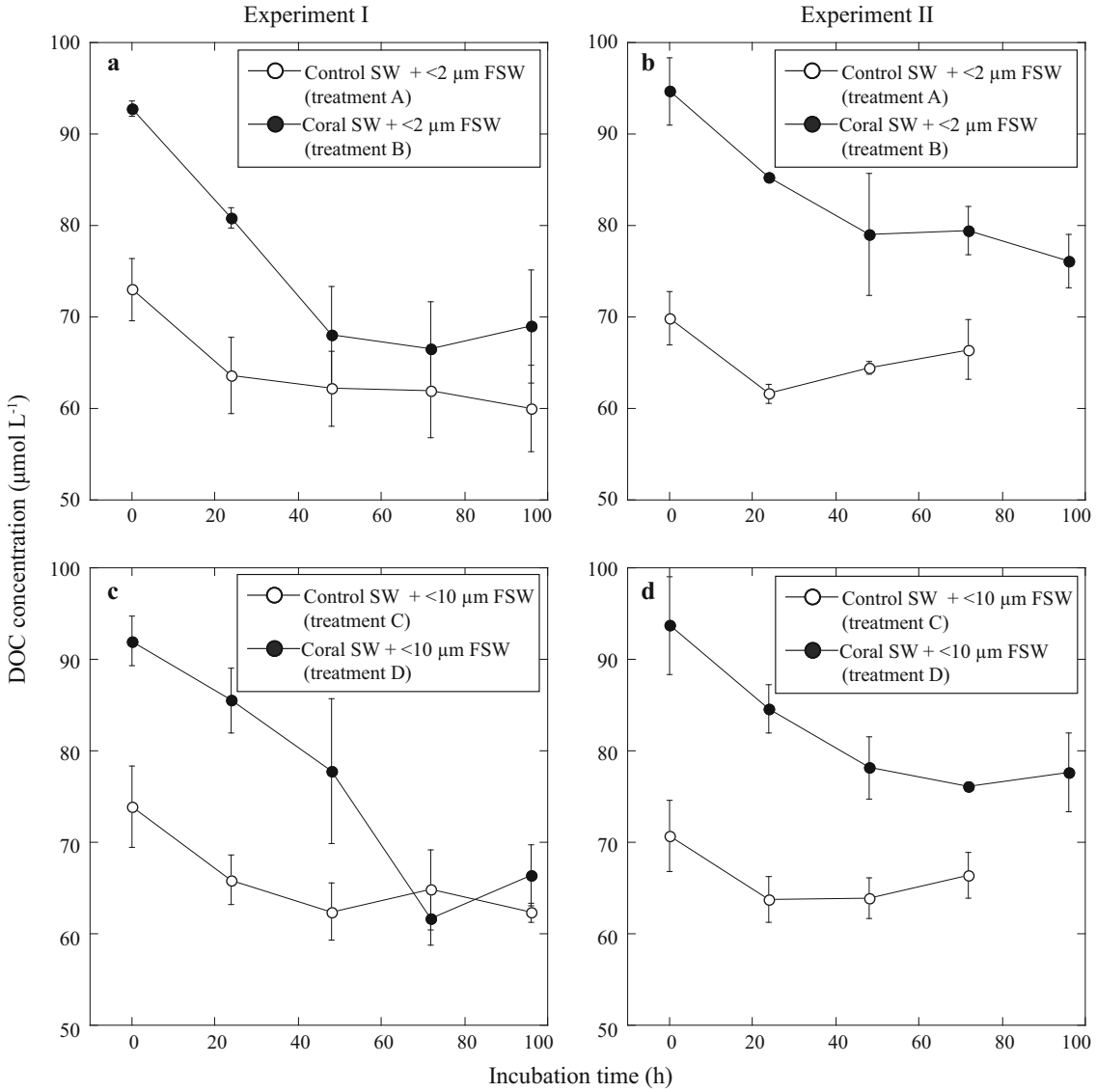

Fig. 2 Changes in dissolved organic carbon (DOC) concentration during incubation in either control seawater or coral seawater in experiment I (**a**, **c**) and experiment II (**b**, **d**). Treatments A–D correspond to those in the schematic diagram in Fig. 1. *Error bars* are standard deviations from triplicate bottles. No data at 96-h incubation for the control runs (treatments A and C) in experiment II

than those in the control waters with results from experiments I and II combined (*U* test, $P = 0.016$) (Table 2).

The growth rates (d^{-1}) of HNF in the coral incubation water (treatment D) in experiment I (0.46 ± 0.14 d^{-1}) and experiment II (1.85 ± 0.25 d^{-1}) were 1.5- to 2.3-fold higher than those in the controls (treatment C, 0.20 ± 0.11 d^{-1} in experiment I; 1.22 ± 0.25 d^{-1} in experiment II, Table 2) with a significant difference (*U* test, $P = 0.049$ for both experiments). Growth efficiency (%) of HNF (i.e., the proportion of HNF production to bacterial production in <10 µm fraction) in the coral incubation water ($25.1 \pm 14.2\%$ in experiment I; $21.1 \pm 2.4\%$ in experiment II; Table 2) was 1.2- to 2.5-fold higher than in control water ($10.0 \pm 7.7\%$ in experiment I; $17.3 \pm 4.4\%$ in experiment II) with a significant difference (*U* test, $P = 0.037$) with results from experiments I and II combined.

Discussion

This study quantitatively investigated the effect of coral-derived organic matter on the growth and production of bacterioplankton and HNF assemblages. This is the first study to describe the growth efficiency of HNF as a result of increased bacterial growth on coral-derived DOM. Although the extent differed between experiments, addition of coral-derived DOM increased the growth efficiency of

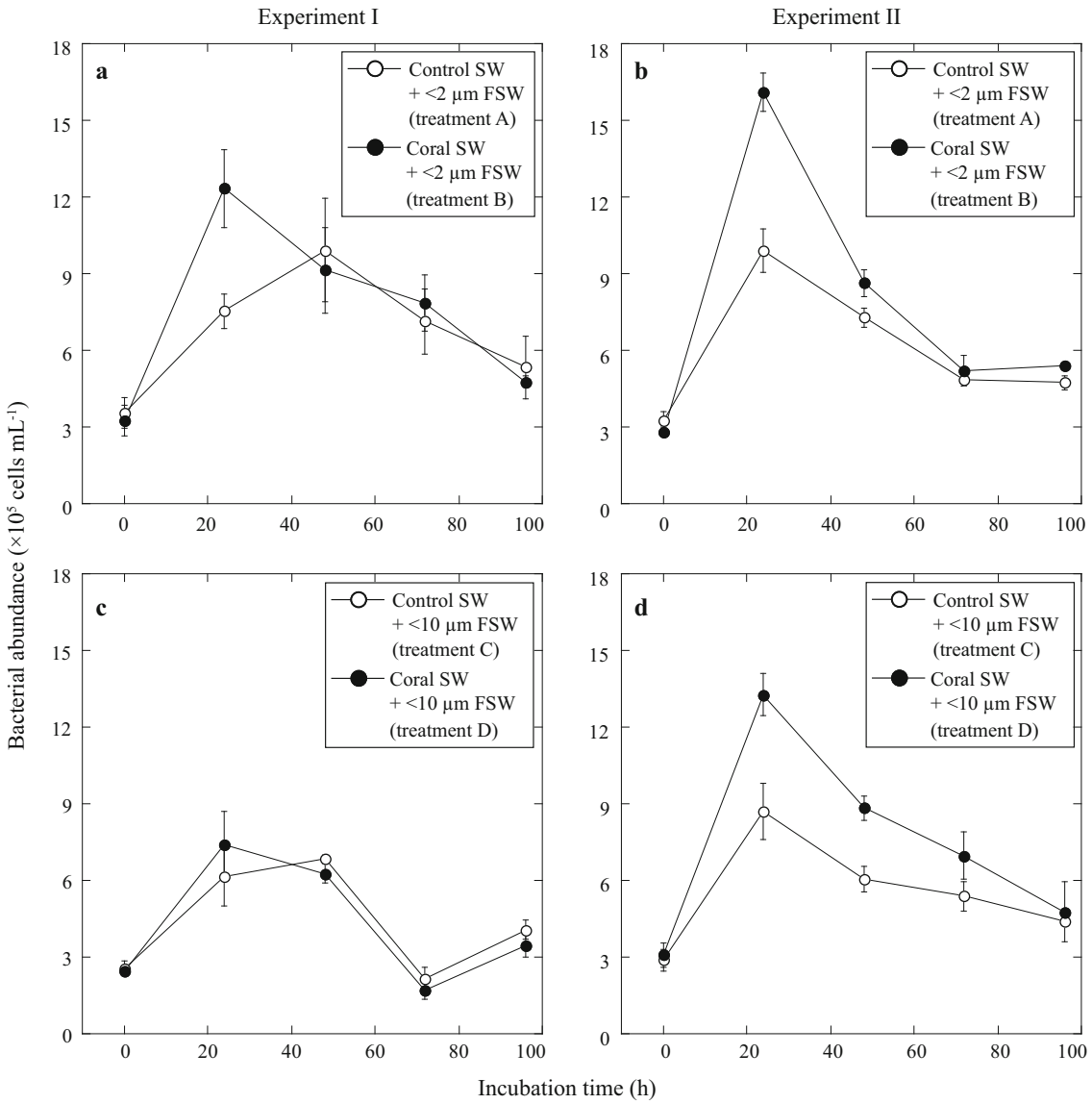

Fig. 3 Changes in bacterial abundance during incubation in either control seawater or coral seawater in experiment I (**a**, **c**) and experiment II (**b**, **d**). Treatments A–D correspond to those in the schematic diagram in Fig. 1. *Error bars* are standard deviations from triplicate bottles

bacteria and HNF, and growth and production rates were significantly higher in the coral incubation water than those in the control seawater without corals.

Bacterial growth rates in the coral incubation water in this study (1.4–1.7 d^{-1}) fall within the range of previously reported bacterial growth rates (0.33–3.5 d^{-1}) with experimental addition of coral-derived organic matter (Herndl and Velimirov 1986; van Duyl and Gast 2001; Tanaka et al. 2008b; Nakajima et al. 2009, 2015; Haas et al. 2011; Nelson et al. 2013). BGE in the coral incubation water of this study (11–20%) is also comparable to those reported for the mucus-incubated water of *Acropora pulchra* (12–37%) (Tanaka et al. 2008b). The relatively higher BGE in the coral incubation water than seawater control is likely due to the supply of labile and semi-labile organic carbon (Tanaka et al. 2008a, 2011) and also inorganic nutrients such as phosphate (Nakajima et al. 2015).

HNF abundance started to increase after bacterial growth was observed and showed higher growth rates and production in the coral incubation water than seawater control, suggesting that HNF increased their abundance by grazing on the highly abundant bacteria fueled by coral-derived DOM. The growth rate of HNF in the coral incubation water in this study (1.9 d^{-1} in experiment II) was in the range of previously reported HNF growth rates (1.2–2.2 d^{-1}) in a mesocosm with corals (Ferrier-Pagès and Gattuso 1998). Also, the growth efficiency of HNF was significantly higher in the coral incubation water compared to control seawater, probably due to the higher growth efficiency of bacteria with coral-derived DOM. It should

Table 1 Increased cell number ($\times 10^5$ cells mL^{-1}), calculated production (μmol C L^{-1} d^{-1}), growth rate (d^{-1}) and growth efficiency (%) of heterotrophic bacteria during 24-h incubation in either control seawater (control SW) or coral-incubated seawater (coral SW)

Treatment	Increased cell number ($\times 10^5$ cells mL^{-1})	Production (μmol C L^{-1} d^{-1})	Growth rate (d^{-1})	Growth efficiency (%)
Experiment I				
A (Control SW + <2 μm FSW)	4.0 ± 0.9	0.66 ± 0.14	0.51 ± 0.16	7.4 ± 3.5
B (Coral SW + <2 μm FSW)	9.1 ± 1.3	1.52 ± 0.21	1.35 ± 0.17	11.3 ± 1.1
C (Control SW + <10 μm FSW)	3.6 ± 1.4	0.60 ± 0.24	NC	NC
D (Coral SW + <10 μm FSW)	4.9 ± 1.3	0.82 ± 0.22	NC	NC
Experiment II				
A (Control SW + <2 μm FSW)	6.6 ± 1.1	1.11 ± 0.19	1.11 ± 0.18	12.7 ± 5.7
B (Coral SW + <2 μm FSW)	13.3 ± 0.8	2.21 ± 0.13	1.74 ± 0.08	19.9 ± 4.3
C (Control SW + <10 μm FSW)	5.8 ± 0.7	0.97 ± 0.12	NC	NC
D (Coral SW + <10 μm FSW)	10.2 ± 1.1	1.70 ± 0.19	NC	NC

Treatments A–D correspond to those in the schematic diagram in Fig. 1. Values are shown as mean ± standard deviation of triplicate bottles
FSW filtered seawater, *NC* not calculated

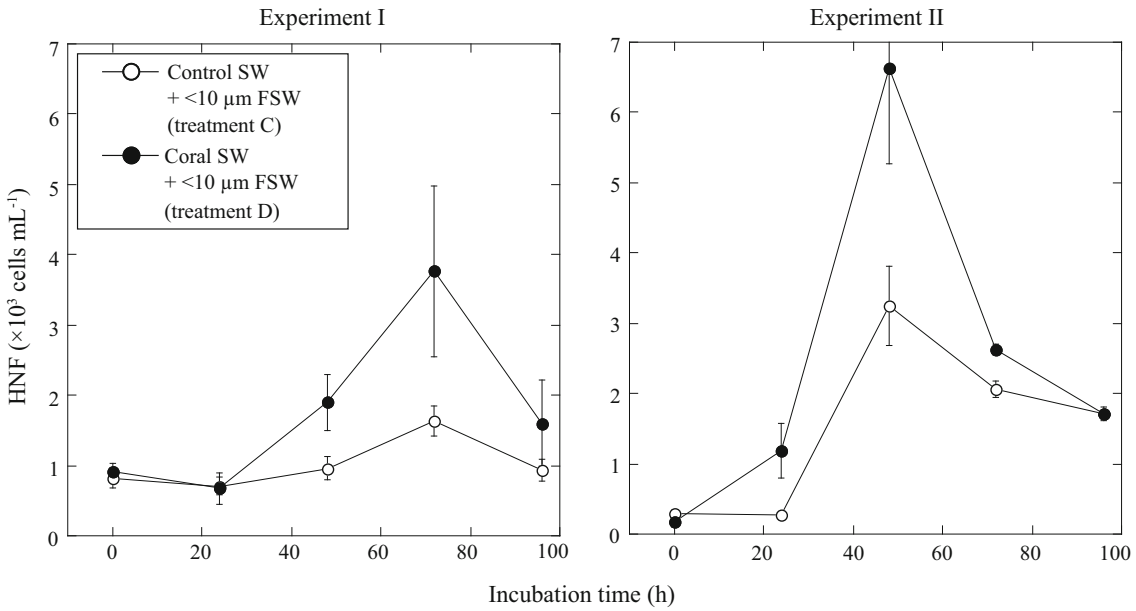

Fig. 4 Changes in heterotrophic nanoflagellates (HNF) abundance during incubation in either control seawater or coral seawater. Treatments C and D correspond to those in the schematic diagram in Fig. 1. *Error bars* are standard deviations from triplicate bottles

also be noted that bacterial cell volume tends to be larger in an enriched environment (Fukuda et al. 1998), and HNF generally show higher selectivity for larger bacterial cells (Jürgens and Massana 2008). Although we did not measure bacterial cell size in this study, larger bacterial cell size as an enrichment effect might also help to explain the higher growth efficiency of HNF in the coral incubation of this study (Jürgens et al. 2000). Higher BGE (and possibly larger cell size) in the coral-incubated seawater forms a plausible explanation for the higher growth efficiency of HNF in the coral-incubated water. This in turn would result in higher carbon transfer efficiency from bacteria to HNF.

Although we did not investigate bacteriophages here, it is reasonable to believe that bacteriophages were present in our experiments, especially in the coral incubation water as coral mucus contains high concentrations of viruses (Leruste et al. 2012). Bacteriophages may be important in controlling bacterial growth and mortality and may have had variable effects on bacterial abundance in this study (Proctor and Fuhrman 1990; Fuhrman and Noble 1995; Hennes and Simon 1995). It is also possible that the experimental design in this study may have introduced pico-sized small flagellates that passed through 2-μm filters and grazed bacteria in the <2 μm fraction treatment

Table 2 Increased cell number (cells mL^{-1}), calculated production (μmol L^{-1} d^{-1}), growth rate (d^{-1}) and growth efficiency (%) of heterotrophic nanoflagellates during incubation in either control seawater (control SW) or coral-incubated seawater (coral SW)

Treatment	Increased cell number (cells mL^{-1})	Production (μmol L^{-1} d^{-1})	Growth rate (d^{-1})	Growth efficiency (%)
Experiment I				
C (Control SW + <10 μm FSW)	737 ± 379	0.05 ± 0.03	0.20 ± 0.11	10.0 ± 7.7
D (Coral SW + <10 μm FSW)	2850 ± 1257	0.20 ± 0.09	0.46 ± 0.14	25.1 ± 14.2
Experiment II				
C (Control SW + <10 μm FSW)	2957 ± 505	0.16 ± 0.03	1.22 ± 0.06	17.3 ± 4.4
D (Coral SW + <10 μm FSW)	6468 ± 1402	0.36 ± 0.08	1.85 ± 0.25	21.1 ± 2.4

Increased cell numbers, production growth rate and efficiency are based on maximum number of HNF during incubation (72 h for experiment I; 48 h for experiment II). Treatments C and D correspond to those in the schematic diagram in Fig. 1. Values are shown as mean ± standard deviation of triplicate bottles. *FSW* filtered seawater

(Ferrier-Pagès and Gattuso 1998), though we did not count flagellates in the <2 μm fraction. These possible factors (phages and small flagellates) may have resulted in the underestimation of bacteria to some degree; thus, the growth rate and efficiency of bacteria may be higher than our estimated value.

This study shows that coral-derived DOM enhances the production of bacteria due to their higher growth efficiency in coral organic matter. Accordingly, we found higher growth efficiency and subsequently higher production of bacterivorous protists. We conclude that coral-derived DOM can contribute to efficient carbon flow through both bacteria and HNF in the microbial food web. Future investigations with shorter sampling intervals (such as 3–6 h) and increased replication are proposed to obtain more detailed analyses of DOC concentration and the abundance of bacteria and HNF. When coupled with modeling, such in-depth investigations could enhance understanding of coral organic matter effects on carbon and energy transfer through microbial food webs.

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