Influence of thermal stress and bleaching on heterotrophic feeding of two scleractinian corals on pico-nanoplankton

Influence of Thermal Stress and Bleaching on Heterotrophic Feeding of Two Scleractinian Corals on Pico-Nanoplankton

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Abstract

The feeding strategies of *Montipora digitata* and *Porites lutea*, two dominant corals in the Okinawan reefs, were investigated. The focus was on pico- and nanoplankton feeding efficiencies, using 6-h incubations. Although healthy *M. digitata* consumed from 72% to 87% more pico-nanoplankton cells than *P. lutea*, feeding rates of bleached corals of both species were similarly low at heat stress (33 °C). Heterotrophic carbon acquisition with respect to dark respiration varied from 3% to 65% in *M. digitata* and from 7% to 68% in *P. lutea*. A decrease in the feeding efficiency of bleached *M. digitata* under heat stress shows its vulnerability to water heating events. Feeding rates of *P. lutea* were low under all conditions and treatments; therefore, this species is less vulnerable to heat stress due to the strategy of meeting metabolic costs by using translocated organic matter from endoliths and selecting pico-nanoplankton cells with a high C/N ratio.

Keywords: coral feeding; pico-nanoplankton; bleaching; thermal stress; *Montipora digitata*; *Porites lutea*

1. Introduction

Reef-building corals form symbiotic relationships with dinoflagellate algae of the family Symbiodiniaceae and other microorganisms. Collectively, these consortia form the so-called “coral holobiont” (Bourne et al., 2009; Koren and Rosenberg, 2006; Sangsawang et al., 2017; Thompson et al., 2015). Autotrophy is the main source of photosynthate acquisition (about 90 %) in scleractinian corals. Organic molecules are translocated to the coral host from Symbiodiniaceae endosymbionts (Muscatine et al., 1981; Baumann et al., 2014) in the form of sugars, glycerol, and amino acids (Tremblay et al., 2014). In addition, dissolved organic matter from the surrounding waters is acquired by osmotrophy (Wild et al., 2004; Laflamme et al., 2009; Naumann et al., 2010; Goldberg, 2018).

To build new tissue, the corals must supplement their phototrophically sourced carbon-rich diets with nitrogen- and phosphorus-rich material (Titlyanov et al., 2001; Palardy et al., 2006). To accomplish this, corals feed heterotrophically on suspended particulate matter (Anthony and Fabricius, 2000), including bacterioplankton, phytoplankton, zooplankton, detritus (Borell et al., 2008; Ferrier-Pagès et al., 2003; Sorokin, 1973), and dissolved organic matter (Al-Moghrabi et al., 1993; Suzuki and Casareto, 2011). Heterotrophic behavior may increase under certain conditions. In shaded reef areas, corals obtain > 60 % of their energy requirements from heterotrophic feeding (Falkowski et al., 1984). Bleached and recovered *Montipora capitata* can meet more than 100% of their daily metabolic energy requirement by increasing their feeding rates (Grottoli et al., 2006). Moreover, feeding on zooplankton helps the corals maintain photosynthetic activity and reduce pigmentation loss under elevated temperature stress (Borell et al., 2008). It is well known that scleractinian corals feed on mesozooplankton, which they trap by using their tentacles (Sebens et al., 1996; Ferrier-Pagès et al., 2003 Houlbrèque et al., 2004; Goldberg, 2018). The ability to trap pico- and nanoplankton has also been studied in several coral species (Houlbrèque et al., 2004; Picciano and Ferrier-Pagès, 2007; Goldberg, 2018). Picoplankton (0.2-2 μm, comprising heterotrophic bacteria, picocyanobacteria, and picoflagellates) and nanoplankton (2-20μm, comprising mainly nanoflagellates) maintain high

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primary production rates and constitute the most important biomass fractions in the ocean, (Li, 1994; Worden et al., 2004; Grob et al., 2007; Richardson and Jackson, 2007). Pico-nanoplankton supply a substantial proportion of the metabolic requirements of most scleractinian corals (Goldberg, 2018), and represent a continuously available source of biomass in the reefs (Tremblay et al., 2012). Corals trap them by secreting mucus nets, which are then resorbed after being enriched with pico-nanoplankton (Goldberg, 2018). The first observation of coral feeding on bacterioplankton was obtained by using labeled bacteria (Sorokin, 1973). Among pico-nanoplankton groups, the azooxanthellate coral *Tubastrea aurea* feeds on bacteria, picocyanobacteria, picoflagellates, and nanoflagellates. In contrast, the zooxanthellate corals *Galaxea fascicularis* and *Stylophora pistillata* feed mainly on nanoflagellates (80% and 50% of total ingested carbon and nitrogen, respectively), whereas bacteria, picocyanobacteria, and picoflagellates constitute only 1–7% of total ingested carbon (Houlbrèque et al., 2004). The red coral *Corallium rubrum* also preys on pico-nanoplankton and flagellates, which together comprise its major food source (43–70% of C and N intake) (Picciano and Ferrier-Pagès, 2007). The diet of the Mediterranean coral *Cladocora caespitosa* is highly heterogeneous (0.2-200 μm cell diameter), and this coral can survive by heterotrophy alone (Tremblay et al., 2011). Furthermore, size differences in the polyps affect plankton capture. The picoplankton capture efficiency of *Stylophora pistillata* was greater than that of *Montipora stellata* due to its larger polyp size (Wang et al., 2012). Other studies have found that particle removal efficiency may depend on the polyp size (Houlbrèque et al., 2004). The studies mentioned above provide important information about the organic matter and food that corals incorporate by heterotrophy when they are not under environmental stress. However, the relative importance of heterotrophy versus autotrophy may vary in relation to environmental conditions.

Recent environmental changes include elevated surface seawater temperature and increased solar irradiance (Lesser et al., 1990; Lesser and Farrell, 2004), increased frequency and intensity of marine heatwaves (Heron et al., 2016; Le Nohaïc et al., 2017; Oliver et al., 2018; Eakin et al., 2019), diminishing water quality due to nutrients input (Fabricius, 2005; Møller et al., 2014), contamination from microplastics (Moore, 2008), and metal pollution (Prouty et al., 2013). These factors have increased the incidence and severity of coral bleaching worldwide (Coles and Brown, 2003; Heron et al., 2015; Hughes et al., 2017, 2018). Bleaching occurs when corals lose their algal symbiont (Symbiodiniaceae), and/or when the latter experience a reduction or loss of photosynthetic pigments (Glynn, 1996; Hoegh-Guldberg, 1999; D'Croz et al., 2001; Baumann et al., 2014; Suzuki et al., 2015), meaning that relatively fewer photosynthates are translocated to the corals. Consequently, the corals undergo a decline in metabolic energy levels, alterations in physiology, and reduced survivorship (Grottoli et al., 2006; Rodrigues and Grottoli, 2007; Hughes et al., 2010; Baumann et al., 2014). Symptoms of coral bleaching include reductions in calcification (Leder et al., 1991; Rodrigues and Grottoli, 2006), increases in the catabolism of reserved materials (Rodrigues and Grottoli, 2007; Schoepf et al. 2015), and enhanced heterotrophy (Grottoli et al., 2006; Anthony et al., 2009; Tremblay et al., 2016; Mies et al., 2018; Marangoni et al., 2019). Previous studies have shown increased feeding rates in bleached corals (Wooldridge 2014, and the references therein); however, feeding rates might be affected when bleached corals are exposed to prolonged heat stress. In *Porites lutea*, the association with endolithic communities represents an alternative energy source with translocation of organic matter (Sangsawang et al., 2017). Based on data regarding ^{13}C atom percentages (Sangsawang et al., 2017), up to 8% of the total carbon fixed by endoliths in healthy corals and 6% in bleached corals can be translocated to the coral tissue. This input may help to offset metabolic costs during bleaching events.

Recurring coral bleaching events were recently observed in the Okinawan coral reefs (Bachok et al., 2006; Kayanne, 2017; Kayanne et al., 2017). Here, already bleached corals are exposed to prolonged periods of thermal stress in shallow lagoons during the summer (Kayanne et al., 2017), resulting in a drastic decrease in coral cover (Hongo and Yamano, 2013). The goal of the present study was to understand the feeding strategies of two dominant scleractinian coral species in the Okinawan reef, the branching *Montipora digitata* and the massive *Porites lutea*. It is

already known that heterotrophy is of crucial importance for recovery from bleaching events (Grottoli et al., 2006). However, there are no data available on coral heterotrophy for this region. We focused our study on pico-nanoplankton feeding efficiency, since these size fractions are the most abundant in reef lagoons (Casareto et al., 2000; Ferrier-Pagès and Gattuso, 1998; Tada et al., 2003), and hence are the primary organic matter sources for scleractinian corals (Houlbrèque et al., 2004; Picciano and Ferrier-Pagès, 2007; Tremblay et al., 2012). We attempted to answer the following questions: (1) What are the feeding rates, prey preferences, and organic matter assimilation efficiencies of these coral species? We also wanted to know if differences in polyp size influence feeding efficiency. (2) How much does feeding efficiency change when bleached corals are exposed to extended thermal stress in Okinawan shallow lagoons? In order to understand the consumption of energy reserves during our experiment, we measured fluxes of the two main autotrophic products (glycerol and glucose) and compared these values with assimilated organic carbon by heterotrophy. (3) How important was heterotrophy for overcoming the metabolic cost under the conditions and treatments of our experiment? We hypothesized that *M. digitata* and *P. lutea* would show different feeding strategies due to the differences in their polyp size and density. Moreover, the presence of an endolithic community in *P. lutea* (Sangsawang et al., 2017) could greatly influence the feeding behavior of this coral.

2. Materials and Methods

2.1 Coral sampling and experimental design

2.1.1 Coral sampling and acclimatization

The scleractinian corals *Montipora digitata* and *Porites lutea* were sampled at Sesoko Reef, Okinawa, Japan (26°39′ N, 127°51′ E) at low tide (depth 0.5-1 m) during the summer season of 2017. Seawater temperature in the Sesoko reef lagoon varied from 30.0 °C to 33.3 °C (Multiparameter Mini Sonde, OTT, Hydrolab MS5, Ireland) during the sampling period (September 21, 2017, from 12:00 to 16:00), conducted at the low ebb of a spring tide (Supplementary material: Table S1). During September 2017, the degree of heating weeks (DHW) was 15.8 °C-week, and the bleaching threshold was 29.5 °C and the area was under bleaching alert level 2 (NOAA Coral Reef Watch Virtual Station in Northern Ryukyu Island, Japan). Coral sampling was conducted under Permit No. 28-75, which was obtained from the Okinawa Prefectural Government. An incubation experiment was designed to test the feeding rates of these two coral species. Three mother colonies of healthy *M. digitata* were sampled and divided into three branches each (3 cm long). The same sampling procedure was followed for bleached *M. digitata* from three mother colonies. Healthy fragments of *P. lutea* were obtained from three mother colonies and bleached fragments from the other three colonies. Three fragments of 3 cm^2 were obtained from each. Some extra fragments from the same colonies were also taken for measurements of biological parameters and symbiont density. Bleached conditions for the two coral species were determined on the basis of the Coral Watch Coral Health Chart; http://interpreter.ne.jp/umibe/. The experiment consisted of three steps as follows: acclimatization after sampling (nine days for fragments exposed to normal temperature and three days for fragments exposed to thermal stress); temperature ramp-up (six days for fragments exposed to thermal stress only); and a feeding experiment (6 h). The initial time corresponds to the 0 h of the feeding incubation experiment. For acclimatization, coral fragments were attached to a polyethylene net in an aquarium with natural running seawater (flow-rate of 10 mL min^{-1}), with a temperature that fluctuated between 26.8 °C and 27.4 °C, and attenuated natural illumination with maximum 280 µmol cm^{-2} sec^{-1}. Coral polyps were active and showed expanded tentacles at night after the three days' acclimatization period. After this period, some of the healthy and bleached fragments were transferred to other aquaria, where the seawater temperature was gradually increased at a rate of 1 °C per day until it reached 33 °C (temperature ramp-up) which coincides with the maximum temperature

measured in the sampling area (Supplementary material: Table S1). Subsequently, the feeding experiment (described in Section 2.1.2) was performed in dark conditions to enhance heterotrophic feeding by synchronizing the starting time with the dark period during acclimatization.

2.1.2 Feeding experiment design

After acclimatization and temperature ramp-up period (for those fragments exposed to 33 °C), healthy and bleached coral fragments of the two species were incubated in 800-mL glass bottles with a closed running seawater system flowing through at 6 mL min^{-1}. One coral fragment per bottle was set up in the center of the bottle using a polyethylene net. To enhance water movement, stirrers were placed in each bottle. The seawater temperature was set to 27 °C or 33 °C. Both coral species were tested in a 2×2 factorial design (two temperatures [27 °C or 33 °C] and two coral conditions [healthy or bleached]) with three replicates per treatment. Controls (incubation bottles with only seawater) were also set up (three replicates per each temperature) to estimate natural planktonic variations. Thirty incubation bottles were kept in a water bath at normal seawater temperature (27 °C; 15 bottles) and higher seawater temperature (33 °C; 15 bottles) for 6 h. Temperatures were maintained using thermostats (IC thermostat DX-003, GEX International Corporation, Osaka, Japan, 300W) set up in the incubation system; monitoring and continuous recording were carried out with in situ sensors (MDS-MkV/T, Alec Electronics, Kobe, Japan).

2.2. Coral feeding experiment

2.2.1 Preparation of seawater for incubation

Seawater was sampled at the same site from which the coral was collected before starting the incubation. The seawater was pre-filtered with a 100-µm plankton net to remove microplankton. Pico-nanoplankton were concentrated with a tangential flow filtration system (Vivaflow 50 Crossflow Cassettes, 100K MWCO RC; membrane pore diameter 0.2 µm; Sartorius AG, Göttingen, Germany) to minimize cell damage. The plankton were concentrated in order to improve the measurement efficiency of feeding rates over a short time. The cell concentration ranges were 5.4-10.0 $\times 10^5$ cells mL^{-1} in the natural seawater and 27.3-54.5 $\times 10^5$ cells mL^{-1} in the concentrated seawater.

2.2.2 Coral feeding rates

The plankton in the running seawater, which included bacteria (BA), picocyanobacteria (PCY), picoflagellates (PF), and nanoflagellates (NF), were measured and recorded before and after the 6 h incubation period, and for each incubation condition. The feeding rates were calculated according to Ribes et al., (1998), Frost, (1972), and Houlbrèque et al., (2004). Feeding rates were determined according to plankton growth rates in the control and coral chamber and then normalized to the protein concentration in the coral tissues. Briefly, the Feeding Rate (I = prey ingested $colony^{-1}$ h^{-1}) was calculated as:

$$\mathbf{I = F \times C}$$

Where **F** is the filtration rate (cells h^{-1})

$$\mathbf{F = V \times g/N}$$

V = the volume of seawater in the chamber,

g = the feeding coefficient (h^{-1})

N = the number of colonies in the chamber

Where **C** is the average prey concentration (cells ml^{-1}) during the experiment

$$\mathbf{C = C_0[e^{(k-g)(T_t-T_0)}-1]/(k-g)(T_t-T_0)}$$

157 k = the growth rate of the prey

158 $k = \ln(C_t/C_0)/T_t\text{-}T_0$

159 where C_0 = the prey concentrations in the chambers (cell ml^{-1}) at the initial time (T_0)

160 C_t = the prey concentrations in the chambers (cell ml^{-1}) at the final time (T_t)

161 g = feeding coefficient (h^{-1}) where

162 $g = k_c - k_g$

163 where k_c = the growth rate in the control chamber (h^{-1})

164 k_g = the growth in the coral chambers (h^{-1})

165 #### 2.2.3 Enumeration of the pico-nanoplankton and calculation of biomass from biovolumes

166 To enumerate pico-nanoplankton, samples were transferred from the incubation chambers into sterile 50-mL tubes,
167 fixed with 25% w/v glutaraldehyde (1% final concentration), and kept at 4 °C until analysis. For BA, PCY, PF, and
168 NF, the samples were stained with DAPI (4', 6-diamidino-2-phenylindole); (Porter and Feig 1980). The 3 mL aliquots
169 of each of these samples were filtered through 0.2-µm black polycarbonate filters to count BA and PCY. The 30 mL
170 aliquots were filtered through 0.8-µm black polycarbonate filters to count PF and NF. The filters were mounted onto
171 glass slides, and the cells were enumerated under an epifluorescence microscope (Eclipse/E600; Nikon Corp., Tokyo,
172 Japan). Approximately 10-15 fields per sample were counted to reach counts of around 500 cells. The biomasses of
173 the various pico-nanoplankton groups were calculated from the biovolume-to-biomass relationships. The average cell
174 dimensions of the pico-nanoplankton were measured from groups >30 cells. Biovolumes were determined from the
175 average cell dimensions assuming the most similar geometrical shape, according to the method of Sun and Liu (2003).
176 Carbon biomasses were calculated using the biovolume–to-biomass conversion factors and nitrogen using C/N ratio
177 as BA = 30.2 fg C $cell^{-1}$ and 5.8 fg N $cell^{-1}$ (Fukuda et al. 1998), PCY = 700 fg C μm^{-3}, and 50 fg N μm^{-3} and PF or
178 NF = 220 fg C μm^{-3} and 26 fg N μm^{-3} (See Houlbrèque et al., 2004 and references therein). The C/N ratio of each of
179 the plankton groups was calculated according to Fukuda et al., (1998) for BA and Houlbrèque et al., (2004 and
180 references therein) for the other groups.

181 ## 2.3 Coral biological characteristics

182 #### 2.3.1 Polyp size and density

183 Polyp diameters were measured under a stereomicroscope (C-DSS115, Nikon Corp., Tokyo, Japan). The averages of
184 30–40 measurements were recorded. Polyp density was determined from polyp counts within an area of 1 cm^2.

185 #### 2.3.2 Symbiodiniaceae density

186 Coral tissues were separated from the skeleton using a Waterpik® (Ricoh Elemex Corporation, Aichi, Japan) filled
187 with 3.5% w/v NaCl solution (Johanes and Wiebe, 1970). The extracted tissues were homogenized with a glass
188 homogenizer and centrifuged at 3,000*g* for 15 min. The supernatants were removed, and Symbiodiniaceae pellets
189 were resuspended in the 3.5% w/v NaCl solution. This procedure was repeated three times to remove the remaining
190 coral tissue. Symbiodiniaceae were counted with a Neubauer-line hemocytometer (Erma Inc., Tokyo, Japan) under an
191 ECLIPSE 80i microscope (Nikon Corp., Tokyo, Japan). The data were normalized to the coral surface area (cm^2).
192 The surface areas of the coral nubbins were determined by the aluminum foil method (Marsh, 1970).

193 2.3.3 Protein, glycerol, and glucose concentration

194 A small portion of coral fragment of about 1 cm^2 was ground in a mortar containing 2 mL of 50 mM Tris-HCl buffer
195 at pH 7.5, then centrifuged at 10,000 g for 3 min to remove skeletons and insoluble components, including broken
196 membranes from the Symbiodiniaceae cells. The extracted supernatants were separated into 3 fractions and used for
197 measurement of protein, glycerol, and glucose concentrations. Protein content in the supernatant was determined by
198 the method of Palmer et al., (2009); triplicate 25- μ L supernatant aliquots were loaded into 96-well microtiter plates
199 and quantified with a PierceTM BCA protein assay kit (Thermo Fisher Scientific, Rockford, IL, USA) by absorbance
200 measurement at 562 nm with a multi-detection microplate reader (BioTek Synergy HT; BioTek Instruments, Inc.,
201 Winooski, VT, USA). Glycerol and glucose were measured in duplicate samples with Glycerol Colorimetric Assay
202 Kit (Cayman Chemical, Ellsworth Rd, Ann Arbor, MI, USA) and Glucose Colorimetric Assay Kit (Cayman
203 Chemical) following the manufacturer's protocol. For glycerol measurement, the 10 µl aliquots of extracted
204 supernatants and diluted glycerol standard solution were added in each well in triplicates of a 96 well microtiter plate,
205 and 150 µl of the diluted enzyme buffer solution was added to each well to initiate the reaction. The plate was kept at
206 room temperature for 15 minutes, and absorbance at 540 nm was measured using a microplate reader. For glucose
207 measurement, the 15 µl aliquots of extracted supernatants and diluted glucose standard solution were added to each
208 well in triplicates of a 96 well microtiter plate, and 85 µl of the diluted assay buffer and 100 µl of the enzyme solution
209 was added to each well to initiate the reaction. The plate was incubated for 10 min at 37 °C and absorbance at 510 nm
210 was measured using a microplate reader. Concentrations of glycerol and glucose were determined using the stabilized
211 glucose standard supplied by the manufacturer and normalized to protein concentration unit.

212 2.4 Dissolved organic carbon (DOC)

213 Thirty milliliters of seawater were collected from each incubation bottle using a glass syringe directly connected to a
214 25 mm diameter glass fiber filter set (Whatman GF/F; Toyo Roshi Kaisha Ltd., Bunkyo-ku, Tokyo, Japan). The
215 seawater samples were filtered and directly dispensed into brown glass vials (Shinomura et al., 2005). They were
216 preserved at -20 °C until analysis. The glass fiber filters were pre-combusted at 500 °C for 4 h. The DOC
217 concentrations were measured with a TOC-LCPH (Shimadzu Corp., Kyoto, Japan) by the high-temperature
218 combustion catalytic oxidation method (Suzuki et al. 1992). The analytical precision of the DOC measurement was
219 within 0.95%. The intercept and slope of the calibration curves were determined for the total blank. An international
220 certified reference material (deep Sargasso Sea water; Hansell Lab, University of Miami, Coral Gables, FL, USA)
221 was used as reference material for the DOC measurements. The DOC concentrations were calculated by subtracting
222 the value of the intercept and dividing the difference by the slope of the calibration curve (Shinomura et al., 2005).

223 2.5 Estimation of organic carbon fluxes

224 In order to estimate consumption of photosynthates during the 6 h incubations, we measured concentrations of the
225 two most important products from Symbiodiniaceae that are translocated to coral tissues, glycerol and glucose (Figure
226 1) (Battey and Patton, 1984; Burriesci et al., 2012; Suescún-Bolívar et al., 2016; Molina et al., 2017). On the basis of
227 the molecular weights and the atomic carbon weights of glycerol and glucose, we calculated the organic carbon fluxes
228 of these two products. We further compared the results with carbon incorporation via heterotrophic feeding (Figure 7).
229 Fluxes were calculated as the difference between final and initial concentrations (positive values for increased and

negative values for decreased concentrations). Carbon calculations were performed using glycerol ($C_3H_5(OH)_3$: 36/92) and glucose ($C_6H_{12}O_6$: 72/180) as references.

2.6 Statistical analysis

Data were checked for normality using the Kolmogorov-Smirnov test; square root transformation was performed when data were not normally distributed. One-way analysis of variance (ANOVA) was used to test significant differences between coral conditions, two-way ANOVA was used to test significant differences between seawater temperature (normal 27 °C vs. high 33 °C; two levels) and coral conditions (healthy vs. bleached; two levels), and three-way ANOVA was used to identify differences among coral conditions, temperature treatments, and time (0 h vs. 6 h incubation). Pairwise comparisons with post hoc Tukey's test were used to test level of significant differences ($p < 0.001$, $p < 0.01$, and $p < 0.05$). A p value < 0.05 was considered significant. Minitab v. 14 (Minitab Inc., State College, PA, USA) was used for all statistical analyses.

3. Results

3.1 Morphological and biological characteristics of healthy and bleached *Montipora digitata* and *Porites lutea*

In comparison with *Montipora digitata,* the polyps of *Porites lutea* were twice as large, with concomitantly lower density. At the initial stage (before starting the feeding experiment, 0 h), the protein concentrations in *P. lutea* were 2.24 mg cm^{-2} (healthy) and 1.55 mg cm^{-2} (bleached); these were 5 and 4.4 times higher than in *M. digitata*, respectively. Symbiodiniaceae densities in *P. lutea* were also high, with 3.5 (healthy) to 3.8 (bleached) times higher densities than in *M. digitata* (Table 1).

Variations of the glycerol concentrations (µg mg^{-1} protein) during the incubations are shown in Figure 1. The initial glycerol concentrations in healthy *M. digitata* were 1.5 times (37.9 ± 8.5 µg mg^{-1} protein, $p < 0.001$) higher than those in the bleached fragments. Under thermal stress (33 °C), the glycerol concentrations significantly decreased (19.79 ± 6.2 µg mg^{-1} protein, $p < 0.001$) compared to the initial levels. Bleached nubbins showed fluctuations during incubation, but no statistically significant changes were observed. In *P. lutea,* the initial concentration of glycerol was 2 times higher than in the bleached nubbins. For both healthy and bleached coral, a slight increase in glycerol was observed after 6 h incubation at 27 °C, and a slight decrease under 33 °C, but no statistical difference was observed.

At the initial stage of the experiment, the glucose concentrations of healthy *M. digitata* were 1.4 times higher than those of the bleached coral. During incubation, glucose concentrations decreased in relation to initial values in all treatments, but no significant differences were noted (Figure 1C). Conversely, glucose in *P. lutea* increased during incubation, particularly in healthy corals under thermal stress ($p < 0.05$; Figure 1D).

Table 1. Morphological and biological characteristics of healthy and bleached corals before incubation (means ± SD).

Species	Polyp size (mm)	Polyp density (cm^{-2})	Protein (mg cm^{-2})		Symbiodiniaceae (cells cm^{-2})	
			Healthy	Bleached	Healthy	Bleached
M. digitata	0.51 ± 0.0	63.64 ± 4.7	0.43 ± 0.1	0.35 ± 0.0	0.69 ± 0.1 × 10^6	0.37 ± 0.1 × 10^6
P. lutea	1.17 ± 0.2	49.09 ± 3.7	2.24 ± 0.1	1.55 ± 0.4	2.41 ± 0.0 × 10^6	1.40 ± 0.7 × 10^6
Replicates (n)	30-40	10-11	9	9	6	6

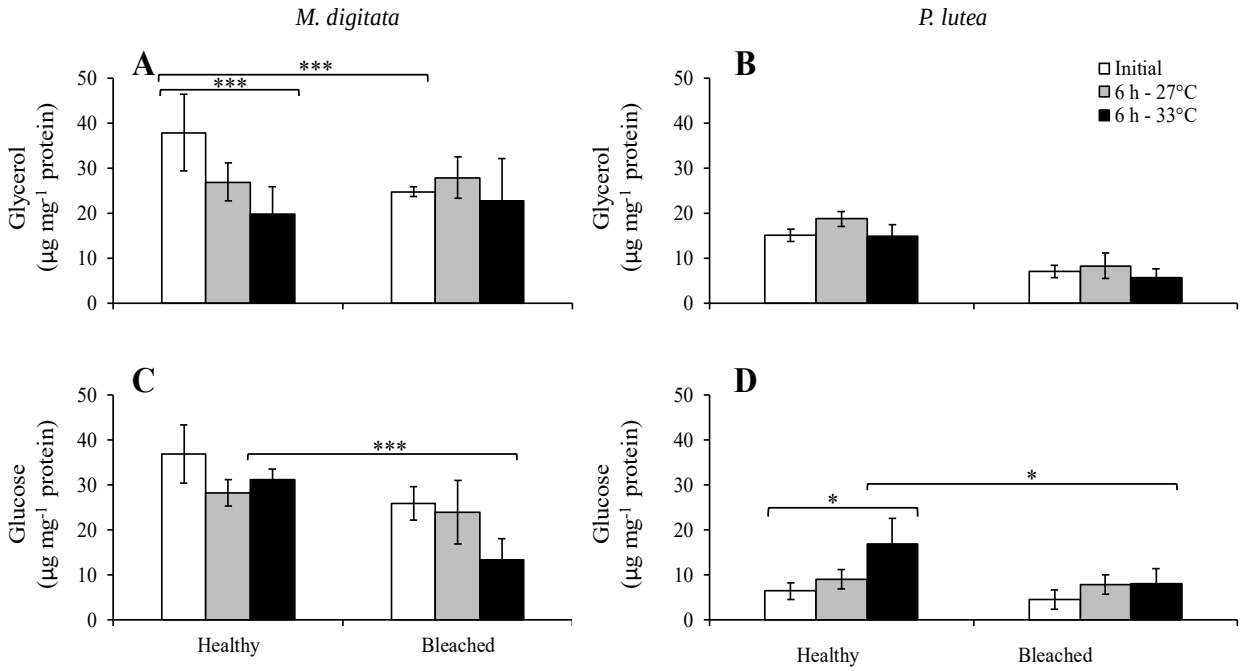

Figure 1. Glycerol (A, B) and glucose (C, D) concentrations in the healthy and bleached *M. digitata* and *P. lutea* at initial (0 h) and after 6 h incubations at 27 °C and 33 °C of seawater temperature. Data are the means ± SD, n = 6 for each treatment. Three-way ANOVA and post hoc Tukey's test were applied to identify the differences among coral conditions, the temperature treatments, and incubation time * $p < 0.05$; *** $p < 0.001$.

3.2 Feeding rates

3.2.1 Cell capture and consumption rates

The feeding rates on different groups of pico-nanoplankton (cells per coral tissue protein per hour) are shown in (Figure 2). Healthy *Montipora digitata* at 27 °C preferentially consumed NF, followed by PCY and BA. Bleached fragments showed a greater preference for BA than healthy fragments ($p < 0.01$), followed by NF. In the seawater in which bleached corals were incubated, the PCY and PF concentrations increased (negative values), indicating that the rate of consumption by coral was lower than that of plankton growth (Figure 2A). At 33 °C, the healthy nubbins consumed more BA and NF than the bleached fragments ($p < 0.01$), while the bleached nubbins consumed relatively more PCY than the healthy fragments (Figure 2C). At 27 °C, *Porites lutea* consumed all food sources provided; consumption rates of bleached corals were higher than those of healthy corals (Figure 2B). At 33 °C, both healthy and bleached *P. lutea* consumed food sources in similar quantities, and both preferred PCY (Figure 2D).

Figure 3 shows the total feeding rates (number of pico-nanoplankton cells), and summarizes the above results with higher feeding rates in *M. digitata* than in *P. lutea* in all treatments except the bleached fragments at 33 °C, which displayed similar feeding rates. Under normal temperature (27 °C), feeding rates of bleached corals were higher than those of healthy fragments ($p < 0.01$). However, at 33 °C, feeding rates of bleached fragments were lower than those of healthy fragments ($p<0.001$ in *M digitata* but non-significant in *P. lutea*).

Supplementary material Table S2 shows the cell capture per polyp in *M. digitata* and *P. lutea*. High cell capture was found in *P. lutea* in all treatments except the healthy condition at 27 °C in which *M. digitata* showed high cell capture. However, none of these data showed statistically significant differences.

3.2.2 Assimilation of organic matter

Assimilation of organic carbon (ng C mg^{-1} protein h^{-1}) is shown in Figure 4. The most important organic carbon source under all conditions for both coral species was NF, with the exception of bleached *P. lutea* at 33 °C, for which PCY was the most important carbon source. In particular, bleached *M. digitata* incorporated significantly ($p < 0.01$) more carbon from NF than their healthy counterparts under normal temperature; conversely, healthy corals incorporated more carbon from NF than the bleached ones at 33 °C ($p < 0.001$). Bleached *P. lutea* corals incorporated more carbon than the healthy ones from all available food sources at 27 °C. Consumption of BA did not significantly contribute to the incorporation of organic carbon in either coral species.

The total organic carbon (Figure 5A-B) and nitrogen (Figure 5C-D) assimilated by *M. digitata* was higher than that taken up by *P. lutea*. Despite the comparatively lower C and N incorporation by the bleached nubbins, *M. digitata* incorporated ~50% and ~63% (C and N, respectively) more than *P. lutea* under thermal stress. ~~A~~t 27 °C, bleached nubbins assimilated higher C and N than the healthy ones, but differences were not statistically significant. For *P. lutea*, the patterns were similar, but the differences between treatments and conditions were not statistically significant. The C/N ratios reflected the coral feeding preferences under each condition: under thermal stress, healthy *M. digitata* only consumed NF, and thereby represents a comparatively low C/N ratio. However, bleached nubbins under thermal stress consumed low amounts of food but also incorporated PCY, which represents a comparatively high C/N food source (Table 2). Under thermal stress, bleached *P. lutea* consumed mainly PCY, which are characterized by a relatively high C/N ratio. Therefore, both corals under bleached conditions and thermal stress preferred higher C/N-ratio foods.

Supplementary material Table S2 shows the carbon assimilation per polyp in *M. digitata* and *P. lutea*. High carbon assimilation was found in *P. lutea* in all treatments (bleached at 27 °C $p<0.001$; bleached at 33 °C $p<0.01$) except the healthy condition at 27 °C in which *M. digitata* showed high carbon assimilation ($p<0.001$).

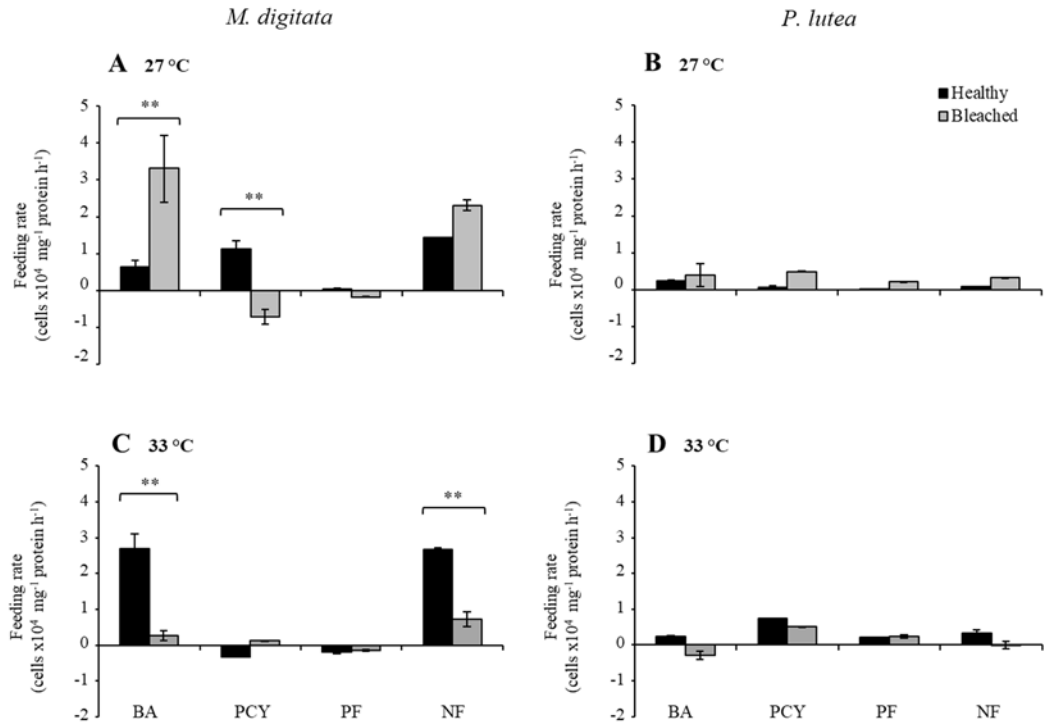

Figure 2. Feeding rates expressed in number of cells (pico-nanoplankton) consumed per mg protein per hour. (A, C) Healthy and bleached *M. digitata*: (A) 27 °C and (B) 33 °C. (B, D) Healthy and bleached *P. lutea*: (B) 27 °C, (D) 33 °C. Data are mean ± SD, n = 3, for each incubation condition. BA = bacteria, PCY = picocyanobacteria, PF = picoflagellates, and NF = nanoflagellates. One-way ANOVA and post hoc Tukey's test were applied to identify differences between coral conditions; ** $p < 0.01$.

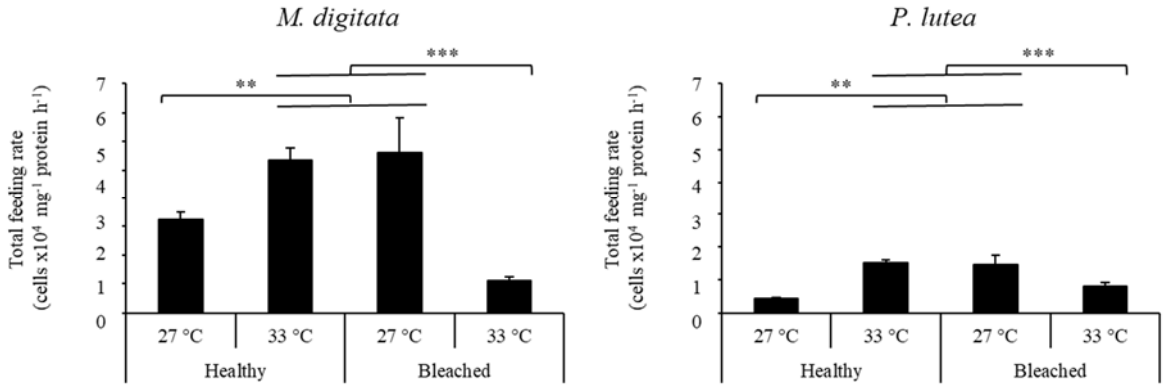

Figure 3. Feeding rates expressed as total number of cells (pico-nanoplankton) consumed per mg protein per hour in healthy and bleached corals: *M. digitata* and *P. lutea* at 27 °C and 33 °C. Data are mean ± SD, n = 3 for each incubation condition. Two-way ANOVA and post hoc Tukey's test were applied to identify differences among coral conditions and incubation temperatures; ** $p < 0.01$ and *** $p < 0.001$.

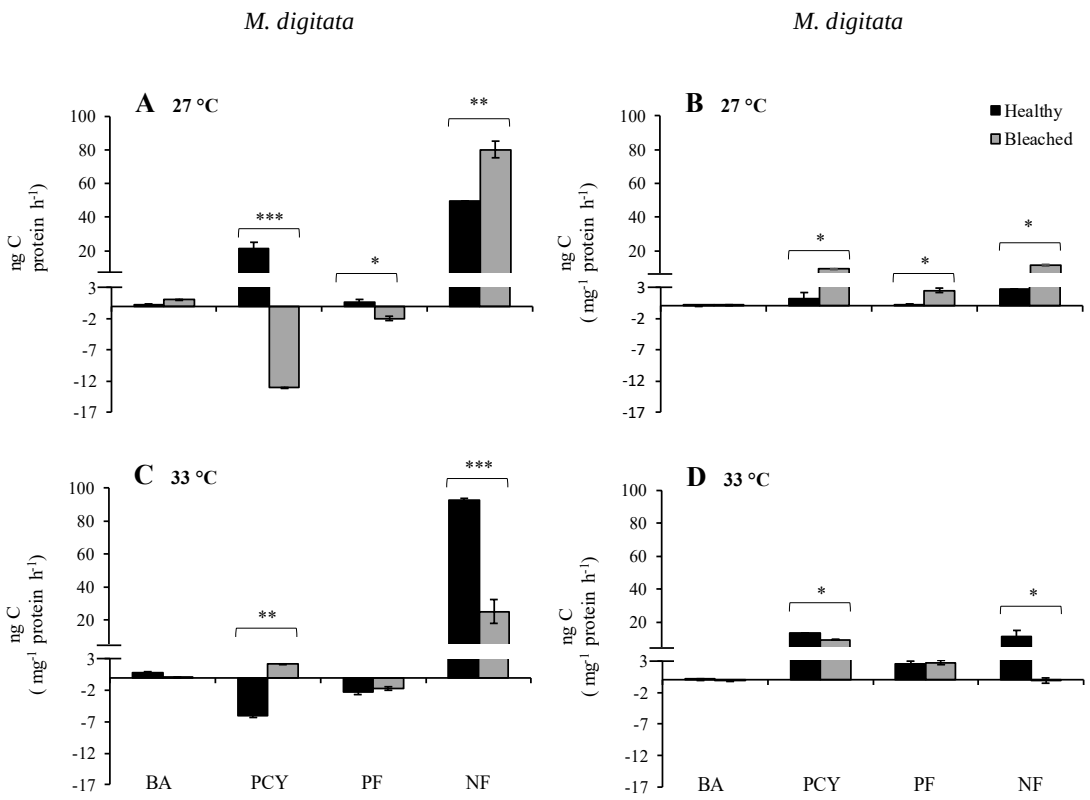

Figure 4. Feeding rates in ng C per mg protein per hour of healthy or bleached *M. digitata* at (A) 27 °C and (C) 33 °C and healthy or bleached *P. lutea* at (B) 27 °C and (D) 33 °C. Data are mean ± SD, n = 3 for each incubation condition. BA = bacteria, PCY = picocyanobacteria, PF = picoflagellates, and NF = nanoflagellates. One-way ANOVA and post hoc Tukey's test were applied to identify differences between coral conditions; * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

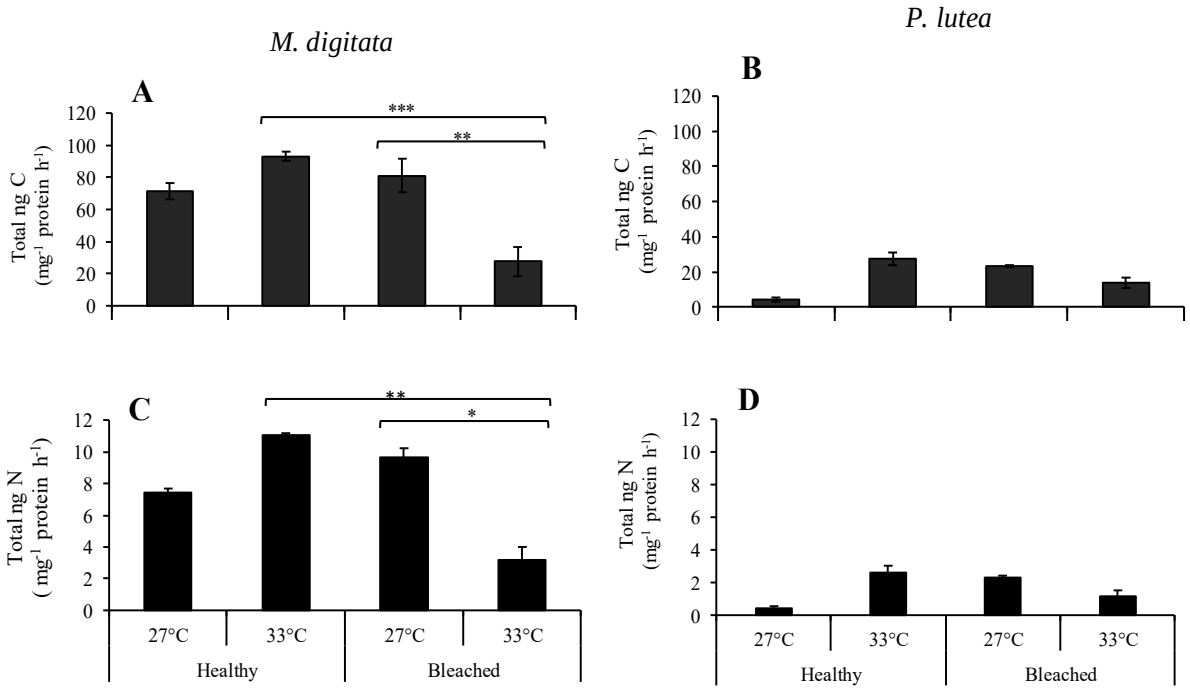

Figure 5. Feeding rates in total ng C and N per mg protein per hour incorporated by healthy and bleached corals: (A, C) *M. digitata* and (B, D) *P. lutea* at 27 °C and at 33 °C. Data are mean ± SD, n = 3 for each incubation condition. Two-way ANOVA and post hoc Tukey's test were applied to identify the differences among coral conditions and incubation temperatures; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 2. C:N ratio of incorporated organic matter by *Montipora digitata* and *Porites lutea* (mean ± SD, n = 3).

Coral species	Healthy		Bleached	
	27 ºC	33 ºC	27 ºC	33 ºC
M. digitata	9.6 ± 0.2	8.4 ± 0.01	8.4 ± 0.02	9.4 ± 0.4
P. lutea	9.3 ± 0.8	10.6 ± 0.4	10.0 ± 0.1	11.8 ± 0.7

3.3 Net fluxes of dissolved organic carbon (DOC) during incubations

The dissolved organic carbon (DOC) concentrations at the initial stage (seawater), after 6 h incubation (final), in the control chambers (only seawater), or in the coral chambers and DOC fluxes (ΔDOC or **δ**DOC) are shown in Table 3. DOC fluxes in the control chambers represent the contributions of the planktonic community to the incubated seawater. DOC fluxes in the coral incubations represent the contributions of both the corals and the planktonic community to the incubated seawater. Finally, **δ**DOC represents the contributions of the corals only to the incubated seawater. The DOC contributions of healthy *Montipora digitata* were similar at both 27 °C and 33 °C (9.2 ± 0.8 µmol L^{-1} and 10.0 ± 1.2 µmol L^{-1}, respectively). The highest DOC contribution (17.1 ± 1.0 µmol L^{-1}) was observed for bleached nubbins at 27 °C. Nevertheless, a negative flux, which represents osmotrophy (-18.9 ± 2.0 µmol L^{-1}), was observed for the bleached nubbins at 33 °C; therefore, these corals were taking up DOC from the surrounding seawater. Significant statistical differences were found between temperature treatments on bleached corals ($p < 0.01$) and between coral health conditions at 33 °C treatment ($p < 0.01$). For *Porites lutea,* higher DOC contributions were observed in the bleached coral compared with the healthy coral, at both 27 °C and 33 °C. The healthy nubbins at 33 °C contributed less DOC (5.4 ± 4.9 µmol L^{-1}) than those at 27 °C (12.3 ± 7.4 µmol L^{-1}), but no statistical differences were found.

To test for a possible association between DOC release (as mucus) and pico-nanoplankton trapping efficiency, the correlations between feeding rates (cells × 10^5 mg^{-1} protein) and the **δ**DOC flux (µmol mg^{-1} protein) are presented in Figure 6. A positive significant correlation was observed for *M. digitata* (r = 0.887, n = 12, $p < 0.05$). However, no correlation was found for *P. lutea* (r = 0.100, n = 12).

Table 3. Concentrations of dissolved organic carbon (DOC; µmol L^{-1}) for the initial seawater samples and those incubated (control or coral incubation), for healthy and bleached corals at normal (27 °C) and high (33 °C) seawater temperatures (means ± SD, n = 3). Differences between coral conditions and temperatures were tested by two-way ANOVA and post hoc Tukey's test. **a** indicates statistical difference with **b** at $p < 0.01$

Corals and condition	initial DOC (seawater)	final DOC (seawater) Control	final DOC (coral)	ΔDOC seawater (control-initial)	ΔDOC coral (coral-initial)	δDOC (ΔDOC coral - ΔDOC seawater)
M. digitata						
27 °C Healthy	90.2 ± 3.9	135.6 ± 1.9	144.8 ± 2.2	45.3 ± 2.1	54.6 ± 1.9	9.2 ± 0.8
27 °C Bleached	99.8 ± 2.0	127.5 ± 5.0	144.7 ± 5.7	27.8 ± 3.0	44.9 ± 3.8	17.1 ± 1.0[a]
33 °C Healthy	106.7 ± 2.1	134.8 ± 3.1	144.8 ± 2.1	28.1 ± 3.2	38.1 ± 2.9	10.0 ± 1.2[a]
33 °C Bleached	99.8 ± 2.0	140.2 ± 1.2	121.2 ± 1.0	40.4 ± 2.0	21.4 ± 3.0	-18.9 ± 2.0[b]
P. lutea						
27 °C Healthy	76.0 ± 2.3	93.5 ± 0.7	105.9 ± 7.9	17.6 ± 2.5	29.9 ± 9.7	12.3 ± 7.4
27 °C Bleached	78.3 ± 2.1	141.1 ± 1.9	163.4 ± 13.4	62.8 ± 3.1	85.1 ± 14.4	22.2 ± 15.0
33 °C Healthy	76.0 ± 2.3	103.3 ± 4.3	108.8 ± 8.5	27.4 ± 4.5	32.8 ± 9.3	5.4 ± 4.9
33 °C Bleached	78.3 ± 2.1	114.4 ± 4.1	136.9 ± 3.4	36.1 ± 3.2	58.6 ± 2.5	22.5 ± 5.6

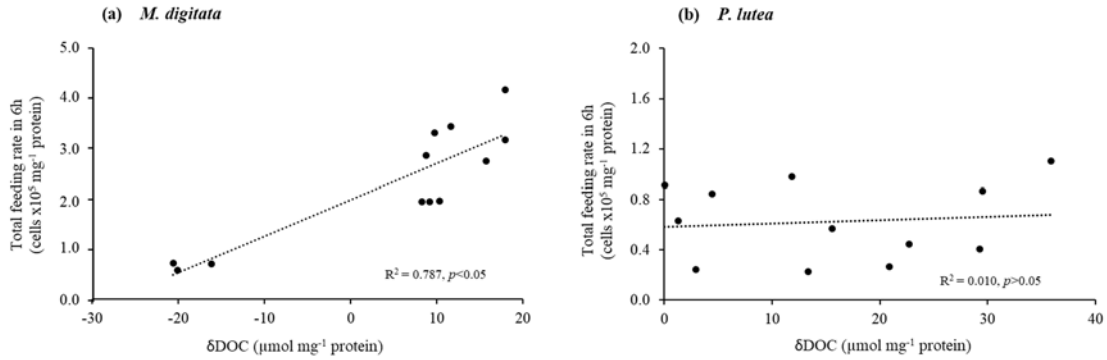

Figure 6. Correlations between total feeding rate and δ-dissolved organic carbon (DOC) for (a) *M. digitata* and (b) *P. lutea* (n = 12 for each species).

3.4 Net organic carbon flux from autotrophy and carbon acquisition from heterotrophic feeding.

In healthy *Montipora digitata*, fluxes of organic carbon from glycerol were negative (consumption) and more pronounced (1.4 times higher) for corals under thermal stress. However, in bleached nubbins, positive fluxes of carbon from glycerol were observed at 27 °C (Figure 7A). Carbon from glucose was consumed under both temperature treatments and for both coral conditions. In *Porites lutea*, the pattern was quite different, with positive fluxes of carbon from glucose in all treatments, and consumption of glycerol for both coral conditions under thermal stress (Figure 7B).

In *M. digitata*, the carbon acquisition by heterotrophic feeding (%), with respect to consumption of carbon from autotrophic products (Table 4) was high (65%) for bleached fragments at 27 °C but very low (3%) under thermal stress. The carbon contribution from heterotrophic feeding was similar (6%) in healthy corals at both seawater temperatures. In thermally stressed *P. lutea,* carbon acquisition from heterotrophic feeding was 68% in healthy corals, but only 7% in bleached corals. However, under normal temperature, fluxes of autotrophic products were not detected in healthy and bleached *P. lutea* within our incubation time.

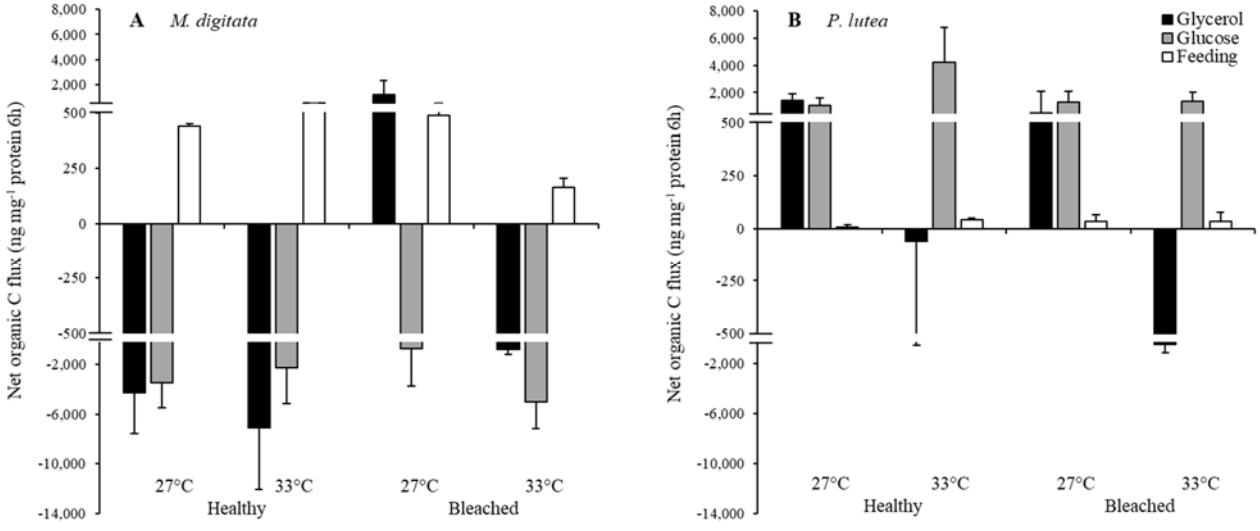

Figure 7. Net fluxes of organic carbon from glycerol and glucose and organic carbon acquisition from heterotrophic feeding during 6 h incubations of *M. digitata* and *P. lutea* at normal (27 °C) and high (33 °C) seawater temperatures. Data are mean ± SD, n = 6 for glucose and glycerol; n = 3 for heterotrophy.

Table 4. Net fluxes of organic carbon from glycerol and glucose and organic carbon acquisition from heterotrophic feeding (% of glycerol and/or glucose consumption) during 6 h incubations of *M. digitata* and *P. lutea* at normal (27 °C) and high (33 °C) seawater temperatures. Data are mean ± SD for each condition, n = 6 for glucose and glycerol; n = 3 for heterotrophy. Negative values indicate consumption.

	M. digitata				*P. lutea*			
ng	Healthy		Bleached		Healthy		Bleached	
mg^{-1} protein 6h	27°C	33°C	27°C	33°C	27°C	33°C	27°C	33°C
C of glycerol	-4,308 ± 3,251	-7,100 ± 4,938	1,209 ± 1,279	-799 ± 417	1,420 ± 464	-60 ± 53	500 ± 1,576	-504 ± 642
C of glucose	-3,493 ± 1,976	-2,267 ± 2,883	-752 ± 3,023	-5,009 ± 2,138	1,057 ± 553	4,233 ± 2,572	1,266 ± 822	1,357 ± 686
C of feeding	439 ± 11	558 ± 8	486 ± 65	165 ± 42	6 ± 1	41 ± 5	34 ± 0.7	35 ± 0.3
% *	6	6	65	3		68		7

*% acquisition respected to consumption of autotrophic products (glycerol and/or glucose)

4. Discussion

Healthy corals living in well-illuminated waters obtain most of their metabolic requirements from autotrophy, but heterotrophy plays a crucial role in supplementing the carbon-rich autotrophic diet with nitrogen and phosphorus (Goldberg, 2018). The survival and recovery of bleached corals may be totally or partially contingent on heterotrophy and even on osmotrophy to compensate for the lack of translocation of photosynthates resulting from the loss of Symbiodiniaceae (Anthony et al., 2009; Hughes and Grottoli, 2013; Schoepf et al., 2015; Tremblay et al., 2016). Heterotrophy may also change according to environmental conditions and coral health (Ferrier-Pagès et al., 2011; Mies et al., 2018; Marangoni et al., 2019). Our experimental configuration enabled us to measure feeding rates in both healthy and bleached coral conditions under normal (27 °C) and stressful (33 °C) seawater temperatures. We used concentrated seawater containing a natural assemblage of pico-nanoplankton. The use of a concentrated plankton assemblage enabled an efficient measurement of feeding rates within a short time frame (6 h). In this manner, changes in coral condition due to chamber-enclosing effects were avoided. Despite the high seawater concentration, the plankton density in our chambers was of a similar order of magnitude to that observed in Okinawan reefs waters (Casareto et al., 2006); therefore, feeding rates were comparable to those found under natural conditions.

Corals produce mucus nets to trap pico- to nanosized particles (Muscatine 1973). Substantial quantities of mucus are released into the seawater, of which 50% to 80% dissolves and serves as food for bacteria. This, in turn, enhances the growth not only of bacteria, but also the pico-nanoflagellates which graze upon them (Wild et al., 2004). Mucus enriched with pico-nanoplankton is re-incorporated by coral polyps. Therefore, we monitored changes in DOC production and fluxes during incubations to determine the role of DOC (as the main component of coral mucus) in coral food capture. Moreover, corals may perform osmotrophy to take up dissolved organic matter from the surrounding seawater to compensate for the reduction in translocation of photosynthates under stressful conditions.

The selected coral species (the branching *Montipora digitata* and the massive *Porites lutea*) show morpho-physiological differences that may reflect their tolerance to environmental stressors, plus food requirements, particle size preferences, and feeding efficiency. Our results showed that *M. digitata* acquired 72-87% more pico-nanoplankton cells and 76-94% more carbon than *P. lutea.* The exception was the bleached fragments under the high-temperature condition, in which both coral species consumed similar quantities of cells. While *M. digitata* consumed mainly BA and NF (the latter is an important organic matter source), *P. lutea* consumed only PCY and PF, which are poor organic matter sources but maintain high C/N ratios. A combination of bleaching and thermal stress reduced the feeding of *M. digitata* dramatically and shifted its food preference mainly to NF. Bleached *P. lutea* incorporated more

carbon-rich foods under both experimental temperatures to overcome the decrease in carbon-rich resources from translocation. In terms of polyp size and density, high grazing efficiency was attributed to corals with big polyps (Anthony and Fabricius, 2000; Marangoni et al., 2019). A comparison between *Stylophora pistillata* (small polyps) and *Montipora stellata* (big polyps with low density) showed that *M. stellata* was less efficient in particle capture (Wang et al., 2012). Our results show that *P. lutea*, with big polyps and low density, incorporated more pico-nanoplankton cells per polyp in all treatments except the healthy corals at 27 °C where *M. digitata* consumed nearly twice as many cells as *P. lutea*, but none of these values were statistically significant. However, in terms of carbon assimilation per polyp, the pattern evidently depends on the coral health condition (Supplementary material: Table S2). As shown in our results, corals change their prey selection preferences according to seawater temperature and their health condition. Therefore, polyp size and density may not be an important parameter for evaluating the coral's efficiency in feeding on pico-nanoplankton. The relationship between coral morphology and ability to acclimate to heat stress and bleaching has previously been discussed by several authors. Massive corals with low growth rates but high respiration rates show better capacities for acclimation to environmental stressors, (Gates and Eomund, 1999; Schlöder and D'Croz, 2004); massive and encrusting corals with thick tissues are less susceptible to bleaching (Loya et al., 2001) as they consume their own tissues (i.e., catabolize) to overcome the loss of symbionts (DeCarlo et al., 2019). Our results also show that *P. lutea,* with massive morphology, is better adapted to high temperatures under bleaching conditions and is able to keep a degree of feeding efficiency under these conditions. This finding shows that coral morphology may be an important parameter when evaluating feeding efficiencies and adaptation capacities. These results answer our first question (1), which concerns the differences in feeding rates in terms of prey selection, organic matter assimilation, and the effect of polyp size and density.

The DOC fluxes for *M. digitata* were in accordance with the cell-capture ability of this coral. The bleached nubbins incubated at normal temperature had the highest positive DOC fluxes (net release), followed by the healthy nubbins incubated at 33 °C. This finding is in agreement with Tremblay et al. (2012), who demonstrated that *S. pistillata* under heat stress showed positive POC fluxes relative to the net uptake of control corals. However, in our incubations, a net uptake of DOC was only observed in bleached *M. digitata* incubated at 33 °C. This shows that these fragments needed to perform osmotrophy to compensate for the lower energy resources obtained from autotrophy and feeding. The result is in agreement with previous studies showing that the uptake of DOC by bleached corals may result in a different source of organic carbon (Levas et al., 2013, 2015). The substantial decline in feeding rate from 93.1 ng C mg^{-1} protein h^{-1} in healthy corals to 27.5 ng C mg^{-1} protein h^{-1} in bleached corals under thermal stress suggests that *M. digitata* strongly depends upon mucus production for heterotrophy (Figure 6A), exemplifying the positive correlation between prey capture and DOC fluxes. In contrast, bleached *P. lutea* released higher amounts of DOC than the healthy samples at both temperatures. However, feeding efficiency (both cell capture and carbon incorporation) in bleached *P. lutea* under high temperature was very low.

Moreover, there was no correlation between the feeding rates and DOC fluxes under these conditions, as shown in Figure 6B. Our results suggest that *P. lutea* may not depend upon DOC release for effective prey capture, and it may release mucus under stress for reasons other than predation. Coral mucus quenches harmful reactive oxygen species (Brown and Bythell, 2005), and POC release could be a strategy on the part of the host to avoid photo-inhibition of its symbionts (Wooldridge 2009). Moreover, massive corals may release mucus continuously to avoid desiccation during air exposure during low tide and sedimentation (Wild et al., 2004). These stimuli might also have induced DOC release from bleached *P. lutea* under heat stress conditions. Other authors have also found substantial differences in DOC fluxes among different coral species. *Stylophora pistillata* subjected to heat stress shows a drastic mucus reduction in tissues and a further elevated death rate during recovery in contrast with the high mucus content in tissues of *Porites cylindrica* under heat stress (Fitt et al. 2009). This shows that fluxes of DOC in corals may vary,

and different coral species may show different responses; therefore, DOC fluxes may reflect a species-specific physiological response.

During our incubations, we measured concentrations of glycerol and glucose, which are the two main photosynthetic products from Symbiodiniaceae. We calculated their fluxes (in terms of organic carbon), and compared these with the incorporation of organic carbon from heterotrophy. Glucose is the main autotrophic product of Symbiodiniaceae (Burriesci et al., 2012) and is easily consumed by the host; meanwhile, glycerol is produced by Symbiodiniaceae under osmotic stress (Mayfield and Gates, 2007; Seibt and Schlichter, 2001). Our results showed that glycerol was metabolized during incubation of both healthy and bleached corals subjected to thermal stress; however, glycerol increased in bleached corals under normal temperature conditions. Molina et al., (2017) observed similar patterns and suggested that corals may degrade lipids to glycerol to compensate for the loss of Symbiodiniaceae. Glucose was mainly consumed by *M. digitata* during incubation. In contrast, glucose was incorporated by *P. lutea* under all the experimental conditions. As incubations were performed under dark conditions, the incorporation of glucose by *P. lutea* was not due to translocation from Symbiodineaceae, but probably due to translocation from their endolithic community. In our previous research, we showed that the endolithic community of *P. lutea* could translocate their photoassimilates to coral tissue under both healthy and bleached conditions during extended periods of darkness (Sangsawang et al., 2017). We attributed the huge increase of glucose in *P. lutea* tissues during incubations to endolithic translocation.

In terms of net carbon fluxes in *M. digitata*, substantial consumption of photoassimilates during the dark period was found in healthy corals at any temperature and bleached corals under thermal stress conditions; however, carbon incorporation by heterotrophy did not represent more than 3% to 6% of this consumption. Conversely, bleached corals at normal temperatures consumed lower levels of photoassimilates; therefore, carbon incorporation by heterotrophy appeared to be higher, accounting for 65% of the total. Photoassimilates were consumed by *P. lutea* only under thermal stress conditions, and this consumption was higher in bleached corals. Carbon incorporation by heterotrophy was high in healthy corals (68%); however bleached corals under thermal stress incorporated 7% with respect to glycerol consumption. Respiration of photoassimilates was very high in *M. digitata*; however, the metabolic cost in *P. lutea* was very low owing to glucose incorporation. Other studies have reported comparable contributions from heterotrophy (to daily animal respiration) as 119.8% in *Cladocora caespitosa*, 28.7% in *Oculina patagonica*,15.9% in *Turbinaria reniformis*, and 11% for *S. pistillata* (Tremblay et al., 2011; 2012). In the present study, the relatively high feeding rates of *M. digitata* indicate that this species depends upon heterotrophy to overcome bleaching. However, when bleaching was combined with high seawater temperature, *M. digitata* could not produce enough mucus for particle trapping; therefore, its capture ability was dramatically reduced. Moreover, bleaching with high temperatures forced *M. digitata* to perform osmotrophy (take up DOC from the surrounding seawaters) to compensate for the lack of energy resources. Consideration of the important difference in particle capture and organic matter incorporation shows *P. lutea* to be relatively less dependent on heterotrophy than *M. digitata*. However, *P. lutea* most likely incorporated glucose via translocation from endoliths, which enabled these corals to be relatively independent of heterotrophy. Bleached *P. lutea* continued to secrete mucus, but probably for purposes other than particle capture. These findings answer questions (2) and (3) set out in the introduction, regarding the responses of feeding behaviors in bleached corals that are subjected to thermal stress, and variations in energy allocation under these conditions.

5. Conclusion

The present study revealed different strategies and dependencies of the two selected coral species regarding heterotrophy. *Montipora digitata* appeared to be highly dependent upon heterotrophy. This coral was very efficient at food capturing but, under thermal stresses, the capture efficiency of the bleached coral decreased, forcing uptake of DOC from the surrounding environment to fulfill daily metabolic requirements. *Porites lutea* was comparatively less dependent upon heterotrophy and may utilize organic matter translocated from the endolithic community to produce mucus. This coral was able to maintain heterotrophy (7% to 68% of dark consumption) by selecting higher C/N ratio food items. Therefore, this coral species is relatively more resistant to prolonged bleaching events under elevated seawater temperatures.

In Okinawan waters, the corals *M. digitata* and *P. lutea* represent important components of the reefs; therefore, their survival capacity may have implications for coral reef resilience in this area. While *M. digitata* appears to be more dependent on heterotrophy, *P. lutea* may thrive under high-temperature conditions due to its strategy of meeting metabolic needs through reliance on a potential exogenous supply of organic matter from endolithic communities. In this way, *P. lutea* is relatively more tolerant of bleaching, with greater chances of survival and recovery. Therefore, this species is more likely to be able to colonize decimated coral reef ecosystems.

During 2016–2017, coral reefs in the Ryukyu Island, Japan, suffered from extensive bleaching, with 50% to 80% of corals bleached (Kayanne et al., 2017). The severity of bleaching was well reflected in the NOAA real-time satellite data from 50 km coral bleaching Degree of Heating Weeks (DHW) with the highest value of 12.18 ºC-weeks in 2016 and 15.82 ºC-weeks in 2017. The value of 8 ºC-weeks was considered to be the bleaching threshold for this region (Kayanne et al., 2017). The present research was carried out in the summer of 2017 with corals exposed to these stressful conditions. Therefore, our results can serve to predict responses and tolerance of these coral species in future scenarios when bleached corals are exposed to prolonged periods of thermal stress exceeding the bleaching threshold.

Future research:

Our incubations were set-up under dark conditions to enhance heterotrophy; however, experiments during the day time (light period) need to be performed to compare grazing rates under the two light conditions. Under the light, other important parameters affecting coral physiology, such as the generation of reactive oxygen species (ROS) and their possible effect on grazing capacity must be considered. Evaluation of the supply of photosynthates from autotrophy versus carbon acquisition from heterotrophy in corals under different illumination conditions should be performed in relation to the symbiont genetic identity, to improve understanding of the corals' capacity to adapt to environmental stressors.

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Ethical Statement

Permission to collect samples of *M. digitata* and *P. lutea* was granted by Yuji Onaga, Governor of Okinawa Prefecture (permit no. 28-75) on April 6, 2017. The permit period extended from May 24, 2017 to May 23, 2018. The study was reviewed by the Okinawa Prefectural government following the established protocols.

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Competing Interests

We declare that we have no competing interests.

Authors' Contributions

Kanwara Sangmanee: Conceptualization, Methodology, Software, Writing - Original Draft, Writing - Review & Editing, Visualization. **Beatriz E. Casareto**: Conceptualization, Methodology, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision, Project administration, Funding acquisition. **The Duc Nguyen**: Conceptualization, Methodology, Writing - Original Draft. **Laddawan Sangsawang**: Methodology, Writing - Original Draft. **Keita Toyoda**: Methodology, Writing - Original Draft. **Toshiyuki Suzuki**: Methodology, Software, Writing - Original Draft. **Yoshimi Suzuki**: Conceptualization, Supervision, Project administration, Funding acquisition.

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