Journal of Experimental Marine Biology and Ecology 516 (2019) 140–149

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

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

Photosynthesis by symbiotic sponges enhances their ability to erode calcium carbonate

Michelle Achlatis[a,b,c,*], Christine H.L. Schönberg[d], Rene M. van der Zande[a,b,c], Todd C. LaJeunesse[e], Ove Hoegh-Guldberg[a,b,c], Sophie Dove[a,b]

[a] The University of Queensland, School of Biological Sciences, Coral Reef Ecosystems Laboratory, St. Lucia, QLD 4072, Australia
[b] The University of Queensland, Australian Research Council Centre of Excellence for Coral Reef Studies, St. Lucia, QLD 4072, Australia
[c] The University of Queensland, Global Change Institute, St. Lucia, QLD 4072, Australia
[d] The University of Western Australia, Oceans Graduate School and UWA Oceans Institute, Crawley, WA 6009, Australia
[e] The Pennsylvania State University, Department of Biology, University Park, PA 16802, USA

ARTICLE INFO

Dataset link: Research data related to this submission: 10.17632/9g8sp5fsvt.1

Keywords:
Bioerosion
Cliona
Diuron
Gerakladium
Photosynthesis
Symbiodiniaceae

ABSTRACT

Photosynthesis is an important driver of calcium carbonate deposition on tropical coral reefs largely due to the symbiosis of numerous invertebrates with photosynthetic dinoflagellates in the family Symbiodiniaceae. In bioeroding sponges, however, similar symbioses appear to support the decalcification of carbonate substrates. Compared to its role in calcification, the relative importance of photosynthesis to decalcification processes is less known. Here, the daytime and night-time chemical bioerosion rates of the common Indo-Pacific sponge *Cliona orientalis* were examined under varying levels of photosynthetic activity and/or varying abundances of intracellular dinoflagellates. Photosynthesis was manipulated either by preconditioning the sponges with the photosynthetic inhibitor diuron (DCMU), or by exposing them to short-term heat stress to achieve bleaching (loss of symbionts). DCMU reduced symbiont numbers and diminished their ability to evolve oxygen. Thermal stress caused a significantly greater loss of symbionts, but photosynthesis was less inhibited. In both cases, decreases in photosynthetic activity and symbiont densities led to proportionately lowered daytime chemical bioerosion rates. Moreover, increased rates of night-time bioerosion were linked to greater daytime rates of photosynthesis, rather than to the night-time respiration of the sponge holobiont. Our findings support the conclusion that photosynthetic products (photosynthates) and/or by-products (oxygen) stimulate sponge bioerosion. This work further reveals the importance of symbionts in the ecology of such sponges and in their ability to sustain high bioerosion activity in otherwise nutrient-poor ecosystems.

1. Introduction

Tropical coral reefs thrive in shallow clear waters where sunlight powers productive and diverse food chains. Symbioses between invertebrates and a variety of photosynthetic microorganisms enable high rates of productivity on reefs despite low environmental concentrations of inorganic nutrients (Muscatine and Porter, 1977; Seckbach, 2004; Yellowlees et al., 2008). The evolutionary success that results from hosting phototrophic microbes such as dinoflagellates, diatoms, chlorophytes, rhodophytes, or cyanobacteria is seen in a wide range of diverse invertebrates belonging to taxa such as Cnidaria, Mollusca, Foraminifera, Platyhelminthes, and Porifera (Trench, 1993; Stat et al., 2006; Lipps and Stanley, 2016).

Presently almost 200 sponge species (Porifera) are known to host microbial photosynthetic symbionts ("photosymbiotic" sponges), which may contribute to the nutrition and health of the sponge (e.g. Wilkinson, 1987; Hill et al., 2011; Thacker and Freeman, 2012). Sponge species that receive substantial contributions in this way are often named "phototrophic" after the nutritional mode of their symbionts, or "mixotrophic" given that they also depend on filter-feeding by specialized food-trapping sponge cells. The nutrition of sponges is often further supplemented by inputs from intimate associations formed with heterotrophic bacterial populations of varying density and diversity (e.g. Hentschel et al., 2003; Taylor et al., 2007).

Most sponge-inhabiting photosymbionts are cyanobacteria (Erwin and Thacker, 2007; Diaz et al., 2007; Simister et al., 2012) although

Abbreviations: DCMU, diuron or N′-(3,4-dichlorophenyl)-*N*,*N*-dimethylurea; PAM, pulse amplitude modulation

* Corresponding author at: The University of Queensland, School of Biological Sciences, Coral Reef Ecosystems Laboratory, St. Lucia, QLD 4072, Australia.
E-mail addresses: michelle.achlatis@uq.net.au (M. Achlatis), christine.schonberg@uwa.edu.au (C.H.L. Schönberg), rene.vanderzande@uq.net.au (R.M. van der Zande), tcl3@psu.edu (T.C. LaJeunesse), oveh@uq.edu.au (O. Hoegh-Guldberg), sophie@uq.edu.au (S. Dove).

https://doi.org/10.1016/j.jembe.2019.04.010
Received 3 December 2018; Received in revised form 5 April 2019; Accepted 30 April 2019

many bioeroding sponges in the family Clionaidae are unique due to their association with photosynthetic dinoflagellates (family Symbiodiniaceae). Invertebrates that host these dinoflagellates generally contribute to the deposition of calcium carbonate ($CaCO_3$) and reef growth, as exemplified by reef-building corals (e.g. Trench et al., 1981; Allemand et al., 2011; Al-Horani, 2016). In contrast, bioeroding sponges penetrate $CaCO_3$ substrates by manipulating acidity to chemically dissolve calcareous frameworks by excavating small $CaCO_3$ chips that are then expelled mechanically (Pomponi, 1980; Zundelevich et al., 2007; Webb et al., 2019). Not all bioeroding sponges host dinoflagellates, yet the Symbiodiniaceae-hosting "*Cliona viridis* species complex" is one of the groups accounting for substantial erosion of coral reef frameworks globally, often eroding > 10 kg per metre sponge area and year (Schönberg et al., 2017). This remarkable excavating capacity is commonly attributed to energetic inputs provided by a dense intracellular dinoflagellate population (Vacelet, 1981; Rützler, 1990; Weisz et al., 2010; Fang et al., 2014; Achlatis et al., 2018). Understanding this complex symbiosis is crucial at the ecosystem level given the role of bioeroding sponges in shaping the structural complexity and carbonate budgets of reef systems, tipping the balance towards carbonate dissolution in certain localities (e.g. Nava and Carballo, 2008; Schönberg et al., 2017).

Theoretically, photosynthesis by dinoflagellate symbionts could increase the local pH inside the sponge due to CO_2 uptake, making $CaCO_3$ dissolution less favourable (Garcia-Pichel, 2006). In contrast to this possibility, photosynthesis and enhanced dissolution by excavating sponges take place simultaneously (Fang et al., 2016). This suggests that the coexistence of the two processes is made possible, to a certain extent, by the spatial rather than temporal separation of the two processes: potentially the tight insertion of the thin cellular extensions of the eroding cells into the etching fissures creates a chemically controllable microenvironment which is void of dinoflagellates (Rützler and Rieger, 1973; Pomponi, 1980; Schönberg et al., 2017). Besides, most of the dinoflagellate cells migrate to the surface layer of the sponge by day, while very few of them remain deeper and nearer to the endolithic cell front where bioerosion takes place (Schönberg and Suwa, 2007; M. Achlatis, pers. obs.). Not only do photosynthesis and dissolution co-occur in photosymbiotic sponges, but in fact light enhances bioerosion rates (Rosell and Uriz, 1992; Hill, 1996; Schönberg, 2006), and the symbiosis is crucial to the overall health and excavation capacity of the host (Fang et al., 2014; Achlatis et al., 2017; Ramsby et al., 2018a). However, studies disentangling the direct effect of photosynthesis from other diurnal effects and establishing the relation between photosynthesis and erosion on a diurnal basis that respects endogenous circadian rhythms are lacking. It remains unclear which exact process enhances chemical bioerosion: (a) photosynthesis of the symbiont, supplying carbon-rich photosynthetic compounds and/or a highly oxygenated environment to drive sponge mitochondria, and/or (b) respiration of the symbiont, providing a more acidified environment through net CO_2 production alongside host respiration.

The present study explores bioerosion by *Cliona orientalis* Thiele, 1900 in response to manipulating the population density of its resident symbionts and their photosynthetic activity. *C. orientalis* belongs to the "*Cliona viridis* species complex", it is especially abundant on the Great Barrier Reef (Schönberg, 2001; Schönberg and Ortiz, 2008; Ramsby et al., 2017a), and it forms a specific and co-evolved relationship with dinoflagellates in the genus *Gerakladium* LaJeunesse (formerly the metazoan lineage of *Symbiodinium* Clade G) (Schönberg and Loh, 2005; Schönberg et al., 2005; Hill et al., 2011; Pochon et al., 2014; Ramsby et al., 2017b; LaJeunesse et al., 2018). The rate of chemical bioerosion by individual sponges with different photosynthetic activities and/or densities of *Gerakladium* were compared in the light (by day) and in the dark (by night). To achieve photosynthetic inhibition and/or reduction of symbiont populations, the sponges were preconditioned in two ways: a) exposure to the herbicide/algaecide DCMU [N′-(3,4-dichlorophenyl)-*N*,*N*-dimethylurea or diuron, the use of which has not been previously reported in the study of sponge-dinoflagellate associations] that reversibly binds to the D1 protein of photosystem II and blocks the electron transport across the chloroplast thylakoid membrane (Krause and Weis, 1991), without negatively affecting the host (e.g. Negri et al., 2005; Watanabe et al., 2006; Cantin et al., 2007), and b) exposure to elevated seawater temperature that triggered symbiont loss related to heat stress (Achlatis et al., 2017).

We hypothesized that if the symbiont invests photosynthetic (by) products into bioerosion, then bioerosion rates should be boosted during the day in control sponges. Alternatively, there might be a time lag if the sponges store the translocated (by)products and utilize them to erode at night. Generally, pH conditions are more favourable for $CaCO_3$ dissolution at night due to CO_2 accumulated by host respiration and by respiration of the symbionts that now reside slightly deeper in the tissue, coinciding with naturally lower water flow rates through the aquiferous system (Strehlow et al., 2016). We also hypothesized that the reduction in O_2 evolved by the symbionts in the preconditioned sponges would translate to a loss of bioerosion potential. In particular, we expected that the DCMU exposure would have a relatively greater negative impact on bioerosion by day than by night (relative to control). On the other hand, we expected that the loss of symbionts due to elevated temperature would drive a reduction in the rate of erosion via reduced photosynthetic contributions by day, and potentially via reduced respiratory CO_2 contributions by night.

2. Materials and methods

2.1. Sponge collection

In January 2015, encrusting "beta" morphology *Cliona orientalis* sponges (Schönberg et al., 2017) inhabiting dead massive corals were obtained by SCUBA (5–8 m depth) at "Harry's Bommie" (151.9357°E, 23.4675°S; Heron Island, southern Great Barrier Reef). A set of eight cylinders 35 mm in diameter was cored out of three different sponges using a pneumatic drill and hole-saw down to a depth of 15 mm. Each core contained sponge-penetrated substrate plus an underlying ca. 3 mm thin disc of non-infested $CaCO_3$ with a bulk density and porosity of 1.52 ± 0.05 g cm^{-3} and 30.85 ± 2.08% respectively (mean ± SEM of 4 substrate cores per sampled sponge). To heal and acclimate before the preconditioning phase, the experimental cores were kept in outdoor aquaria (40 L) receiving flow-through seawater for 2 weeks. The aquaria were initially shaded with neutral density shade cloths (~70% light reduction), followed by Marine Blue 131 light filters that mimic the typical light quality and quantity of the collection depth (~40% light reduction, Lee Filters, Andover, UK; Sampayo et al., 2016).

2.2. Preconditioning phase

In the preconditioning phase, 24 sponge cores (8 from each sponge genotype) were randomly assigned to one of three conditions (per condition $n = 2$ aquaria × 4 cores, Fig. 1A, Section 2.5):

(a) Exposure to DCMU in a closed water system at ambient temperature (100 μg/L; Falkowski and Raven, 2007; Parrin et al., 2017), hereafter collectively termed "DCMU exposure".
(b) Exposure to elevated temperature in a flow-through regime [peak of +3.1 °C above the local maximum monthly mean (MMM; 50-km pixel satellite data, http://coralreefwatch.noaa.gov)].
(c) Control conditions in an ambient temperature flow-through regime without DCMU.

The sponge cores were maintained under their respective conditions for 9 days from the 12th to 21st of February 2015 (Fig. 1B, see Table S1A for a summary of seawater conditions). All seawater used was filtered through a 10 μm sand filter, thus still containing the main fraction of common diets in the Clionaidae (Lynch and Phlips, 2000; Maldonado

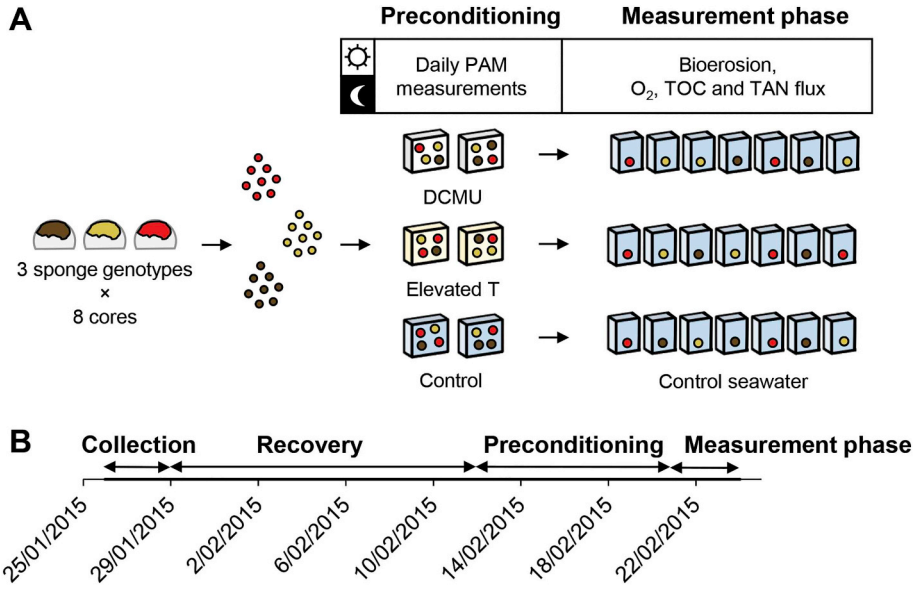

Fig. 1. Schematic illustration of (A) experimental design and (B) timeline. Three different sponges inhabiting dead massive corals were sampled, and cores were randomly distributed over three experimental conditions: addition of the herbicide DCMU in a closed water system, elevated temperature in a flow-through regime, and control conditions. During the preconditioning phase, the photosynthetic efficiency of the symbiotic community of the sponge was monitored daily (day and night-time PAM measurements, see Supplementary material). In the measurement phase, seven out of eight sponges were returned to ambient conditions and individual incubations were performed to measure bioerosion rates as well as oxygen, total organic carbon (TOC) and total ammonia nitrogen (TAN) fluxes. One core per condition was preserved for other purposes. Schematics are not to scale.

et al., 2012; Mueller et al., 2014). Duplicate chambers/aquaria were used for every condition to lessen the amount of DCMU contamination managed at the offshore site (Tolosa et al., 2011; Tremblay et al., 2016).

DCMU-exposed sponges were kept in independent 3L chambers equipped with a small wave maker (Hydor, Sacramento, USA) and partially submerged in 40L aquaria for ambient temperature regulation. To ensure nutrient replenishment, chamber water was changed every morning without exposing the sponges to air, and DCMU dissolved in ethanol was added daily (final DCMU concentration 100 µg/L as established in the pilot study described in the Supplementary Material) (Negri et al., 2005; Underwood et al., 2006; Suggett et al., 2011). The small quantity of ethanol added did not influence the sponges (Supplementary Material). Despite water replenishment, the pH in the DCMU chambers was lower than in the two flow-through conditions due to build-up of respiratory CO_2 (Table S1A). Although necessary to avoid DCMU contamination at the offshore site, seawater confinement could theoretically act as a confounding factor in the preconditioning phase of our experiment. However, carry-over effects of the seawater confinement during the preconditioning phase to the measurement phase were expected to be negligible given the duration of the exposures, the volume and replenishment rate of the water, naturally occurring pH variations, and the tolerance of *C. orientalis* to larger pH decreases than the one observed here (Fang et al., 2013a; Wisshak et al., 2012).

Control sponges and sponges exposed to elevated temperature were kept in 40 L aquaria that received seawater via the ocean warming and acidification simulation system (Dove et al., 2013; Achlatis et al., 2017). For the control simulation, this system reproduced diurnally-variable seawater temperature based on in situ measurements on the reef. For the heated sponges, the system was set to warm the flow-through water by 3.5 °C above the control simulation.

During the preconditioning phase, daily observations of the tissue colour of the sponges were made in combination with daily assessment of chlorophyll fluorescence by pulse-amplitude modulated (PAM) fluorometry (Genty et al., 1989). These measurements indicated that after 9 days the photosynthetic capacity was impaired in the DCMU- and heat-exposed sponges, determining the onset of the measurement phase (Supplementary Material).

2.3. Measurement phase

After the preconditioning phase, we measured daytime and night-time chemical bioerosion rates and other physiological parameters of the sponges that had been subjected to the DCMU exposure, elevated temperature or control conditions (see below). Measurements were made in incubation chambers that received the same DCMU-free ambient seawater (Fig. 1A, Table S1B). In this way, physiological effects of the preconditioning could be isolated without the need for a negative control for the direct effects of DCMU or temperature elevation on seawater chemistry during the incubations of the measurement phase. One hour prior to daytime measurements, DCMU-conditioned sponges were moved from DCMU exposure to ambient running seawater. Since the effects of DCMU on symbiont photosystems are at least partially reversible (Jones et al., 2003), sponges were re-exposed to DCMU after completion of the first round of incubations in preparation for the final incubations of the next day. Heated sponges were moved to ambient running seawater on the evening prior to the first day of incubations to allow them to adjust to the lower temperature.

2.3.1. Oxygen flux

Actual photosynthetic CO_2 fixation was estimated through photosynthetic/respiratory oxygen flux measurements made during the measurement phase in the day (14:00, realized CO_2 fixation) and at night (21:00 h) as described previously (Fang et al., 2014; Achlatis et al., 2017). Incubations were conducted on seven randomly chosen sponge cores per condition to match the maximum capacity of the respirometry setup (the eighth core of each condition was preserved for other purposes). To measure maximum net photosynthesis (P_{net} in µmol O_2 cm^{-2} h^{-1}) of the photosymbionts during the day, and starting at an oxygen saturation of 60%, the sponges were subjected to 30 min of light with an intensity of 450 µmol quanta m^{-2} s^{-1} at 25 °C, with oxygen levels logged every 15 s. Consecutively, the light was turned off and oxygen levels were logged for an additional 15 min to measure light-enhanced dark respiration (LEDR). At night, incubations started at an oxygen saturation of 80% (an average value for forereefs at night; Adey and Steneck, 1985), and oxygen depletion due to dark respiration (DR) was logged every 15 s over 20 min.

2.3.2. Chemical bioerosion, heterotrophy and ammonium flux

Chemical bioerosion rates, as well as the use of total organic carbon and ammonium by the sponges, were determined at the end of the entire study by conducting 4 h incubations by day and by night (11:00 h to 15:00 h and 20:00 h to 24:00 h respectively, using the same seven sponge replicates per condition). Total organic carbon served as a measure of heterotrophic feeding rates in relation to bioerosion rates.

Incubation chambers were temperature-regulated and contained one sponge replicate each, 3 L of seawater, and a small wave maker (Hydor, Bassano del Grappa, Italy). Water chemistry was monitored over the 4 h to control appropriate oxygen saturation, temperature and pH. Chemical sponge bioerosion was quantified by measuring changes in total alkalinity (A_T) of the incubation seawater from $t = 0$ h to $t = 4$ h (Zundelevich et al., 2007). A_T was measured with a minimum precision of ± 3 μmol kg^{-1} with Gran titration after Kline et al. (2012) using an automated titrator (T50, Mettler Toledo, Langacher, Switzerland) calibrated daily with pH NBS scale buffers (Radiometer Analytical, Lyon, France) and Dickson's Standards (University of California, San Diego, USA). Total ammonia nitrogen values (TAN = NH_3 + NH_4^+) served to correct the alkalinity signal for potential ammonia protonation (see below). Quantification of nitrogen oxides and phosphate fluctuations can further improve the alkalinity precision (De Bakker et al., 2018), but these were not considered here. The change in alkalinity due to $CaCO_3$ precipitation or dissolution ($\Delta A_{T(CaCO3)}$) and the associated chemical sponge bioerosion were calculated based on Eqs. (1) and (2):

$$\Delta A_{T(CaCO3)} = \Delta A_{T(inc)} - \Delta A_{T(TAN)} \quad (1)$$

$$W_{CaCO3} = (\Delta A_{T(CaCO3)}/2) \times MW_{CaCO3} \times V_{sw} \times d_{sw} \quad (2)$$

where $\Delta A_{T(inc)}$ is the total alkalinity change of the incubation, $\Delta A_{T(TAN)}$ is the contribution to alkalinity by TAN, W_{CaCO3} is the weight of the dissolved $CaCO_3$, $\Delta A_{T(CaCO3)}$ is divided by 2 to match the molar value of dissolved $CaCO_3$, MW_{CaCO3} is the molecular weight of $CaCO_3$ (100 g mol^{-1}), V_{sw} is the incubation volume, and d_{sw} is the seawater density (modified from Schneider et al., 2011 and Zundelevich et al., 2007). Chemical bioerosion was expressed as substrate weight loss per area of sponge tissue per hour.

Water samples for total organic carbon quantification (dissolved and particulate organic carbon) were analysed at the Advanced Water Management Centre at the University of Queensland as detailed by Achlatis et al. (2017) using a Total Organic Carbon Analyzer (TOC-L CSH with TNM-L TN unit, Shimadzu). Total ammonia nitrogen was photometrically quantified from seawater samples of the same incubations (Parsons et al., 1984).

2.4. Density and phylogeny of Gerakladium

Once all incubations were completed, the sponge cores were snap-frozen in liquid nitrogen and transferred at −80 °C to the St. Lucia Campus of The University of Queensland. Upper surface areas of the cores were quantified using a standard aluminium foil technique (Marsh, 1970), and subsamples for symbiont DNA extraction (from 4 cores per condition) were preserved in high-salt, 20% DMSO, 0.25 M EDTA buffer (Seutin et al., 1991). To isolate the sponge tissue from the $CaCO_3$ substrate of the remaining core, an acid decalcification method was used and symbionts cells were extracted and quantified from seven cores per condition (as described in detail by Fang et al., 2013a, 2013b). The cell concentrations were then normalized to the surface areas of the sponge cores (cells cm^{-2}, where applicable corrected for the subsampled area). Apart from *Gerakladium*, *C. orientalis* hosts a low abundance of other microorganisms such as alpha-, delta- and gammaproteobacteria and a limited cyanobacterial population (low microbial abundance sponge; Pineda et al., 2016; Ramsby et al., 2018b). Although responses were overall addressed at the holobiont level, microbial analyses focused on *Gerakladium* since it forms the vastly dominant symbiotic community in *C. orientalis* in terms of biomass, and is the primary uptake site of inorganic carbon and nitrogen in this holobiont (Achlatis et al., 2018).

To determine the identity and stability of endosymbiont *Gerakladium* in control and preconditioned sponges, nucleotide sequences of diagnostic genetic markers were obtained. Genomic DNA was extracted following the methods described by LaJeunesse et al. (2003). Chloroplast large-subunit rDNA (cp23S, amplified based on conditions described by Zhang et al., 2000) and the partial coding and entire noncoding region of the chloroplast *psbA* (*psbA*^{ncr}, amplified as described by LaJeunesse and Thornhill, 2011) were sequenced. DNA amplification and product sequencing were performed as detailed by Ramsby et al. (2017b) at the Pennsylvania State University Genomics Core Facility, using an Applied Biosciences sequencer (Applied Biosciences, Foster City, CA, USA). Sequences of these genes obtained from control and preconditioned samples were deposited at Mendeley Data (http://dx.doi.org/10.17632/9g8sp5fsvt.1). Aligned sequences were analysed via maximum parsimony and distance using the software PAUP* v.4.0d151 (Swofford, 2014). Bootstrap analyses were conducted based upon 1000 replicates.

2.5. Statistical analysis

Statistical analyses were done after testing the assumptions of normality with a Shapiro-Wilk test and homogeneity of variances with a Levene's test. Preliminary analysis showed no significant effect of genotype or aquaria (nested within the experimental conditions) for any of the variables, therefore data from duplicate aquaria were pooled according to standard methods (Tremblay et al., 2016; Underwood, 1997). All oxygen flux datasets and the *Gerakladium* density dataset were analysed by means of one-way ANOVAs followed by Tukey's post hoc tests (Table 1). “Preconditioning” was the between-subjects factor with 3 levels, namely DCMU exposure, elevated temperature, and control. Chemical bioerosion rates, ammonium uptake and carbon uptake were analysed by means of 2-way split-plot ANOVAs followed by post hoc comparisons (Table 1). “Preconditioning” was the between-subjects factor of the split-plot ANOVA with levels as above, and “Time” was the within-subjects or repeated-measures factor (2 levels: day and night). These three datasets were additionally tested for equality of covariances using a Box's test. Simple linear regression models were used between chemical bioerosion and physiological parameters of interest, after assessing normality and homoscedascity of residuals. Statistical tests were evaluated at a 0.05 level of significance using Statistica 13 (StatSoft, Tulsa, USA). Means and standard errors of means are reported throughout the results (mean ± SEM).

3. Results

3.1. Oxygen flux

No areas of mortality were observed in any of the sponges during the short experimental period, and sponges in all three experimental conditions displayed open and pumping oscula. By day 10, after 9 days of preconditioning, autotrophic feeding rates differed between the groups. Control sponges were daytime net producers of photosynthetically-derived oxygen, whereas heat-preconditioned sponges respired as much as they produced, and DCMU-preconditioned sponges were net consumers of oxygen (4.06 ± 0.13, 0.01 ± 0.20 and − 1.02 ± 0.05 μmol O_2 cm^{-2} h^{-1} respectively, ANOVA, $F_{(2,18)} = 355.08$, $p < .0001$ and Tukey's post hoc, $p < .001$ for both comparisons, Fig. 2A, Table 1). Accordingly, light-enhanced dark respiration measured in the day was strongest in the control sponges (LEDR, ANOVA, $F_{(2,18)} = 9.66$, $p = .001$ and Tukey's post hoc, $p < .02$ for both comparisons). Although overall weaker than LEDR, night-time consumption of oxygen by the holobiont was also strongest in the control sponges (−2.19 ± 0.14 in controls as opposed to −1.54 ± 012 and − 1.20 ± 0.04 μmol O_2 cm^{-2} h^{-1} in the DCMU- and heat-preconditioned sponges respectively; ANOVA, $F_{(2,18)} = 20.09$, $p < .0001$ and Tukey's post hoc, both $p < .002$, Fig. 2A, Table 1). Dark respiration did not differ between the other two groups ($p = .112$).

Table 1
Results of analysis of variance and follow-up tests where applicable for the key parameters of the experiment. The between-subjects factor for all one-way analyses was "Preconditioning", with 3 levels: DCMU exposure, elevated temperature (abbreviated as "T"), and control ("Ctrl"). In the two-way split-plot analyses the within-subjects factor "Time" was added, with levels "Day" and "Night". Note that post hoc comparisons may refer to negative values (i.e. not absolute values). Significant results (evaluated at a 0.05 level) are highlighted in boldface. * Dataset transformed based on log(x + 1).

	Analysis of variance							Post hoc comparisons	
	ANOVA	Source of variation	DF	SS	MS	F	P	Conclusions	P
Oxygen flux (μmol O_2 $cm^{-2}h^{-1}$)									
Net Photosynthesis	1-way	Preconditioning	**2**	**101.13**	**50.567**	**355.08**	**< 0.0001**	**DCMU < T**	**< 0.001**
		Error	18	2.563	0.142			**DCMU < Ctrl**	**< 0.001**
								T < Ctrl	**< 0.001**
Dark respiration (DR)	1-way	Preconditioning	**2**	**3.545**	**1.772**	**20.09**	**< 0.0001**	DCMU – T	0.112
		Error	18	1.588	0.088			**DCMU > Ctrl**	**< 0.002**
								T > Ctrl	**< 0.001**
Light-enhanced DR	1-way	Preconditioning	**2**	**2.594**	**1.296**	**9.66**	**0.001**	DCMU – T	0.495
		Error	18	2.416	0.134			**DCMU > Ctrl**	**0.016**
								T > Ctrl	**0.001**
Organic carbon uptake (mg C $cm^{-2}h^{-1}$)									
	Split-plot	Preconditioning	2	0.0001	0.00006	1.51	0.247		
		Error	18	0.0007	0.00004				
		Time	**1**	**0.0004**	**0.0004**	**9.59**	**0.006**	**Day > Night**	
		Preconditioning × Time	2	< 0.0001	0.00001	0.356	0.705		
		Error	18	0.0008	0.00004				
Bioerosion rates (mg $CaCO_3$ $cm^{-2}h^{-1}$)									
Chemical*	Split-plot	Preconditioning	**2**	**0.005**	**0.003**	**22.52**	**< 0.0001**	**DCMU < T**	**0.021**
		Error	18	0.002	0.0001			**DCMU < Ctrl**	**< 0.001**
		Time	**1**	**0.001**	**0.001**	**12.16**	**0.003**	**T < Ctrl**	**0.004**
		Preconditioning × Time	2	0.0001	0.0002	2.46	0.114	**Day < Night**	
		Error	18						
Ammonium uptake (μmol $cm^{-2}h^{-1}$)									
	split-plot	Preconditioning	**2**	**0.026**	**0.013**	**7.25**	**0.005**	DCMU – T	0.227
		Error	18	0.033	0.002			**DCMU < Ctrl**	**0.004**
		Time	1	0.002	0.002	0.77	0.390	T – Ctrl	0.120
		Preconditioning × Time	2	0.003	0.001	0.45	0.639		
		Error	18	0.053	0.003				
Gerakladium density (10^6 cells cm^{-2})									
	1-way	Preconditioning	**2**	**1.477**	**0.738**	**49.92**	**< 0.0001**	**DCMU > T**	**0.002**
		Error	15	0.222	0.015			**DCMU < Ctrl**	**< 0.001**
								T < Ctrl	**< 0.001**

3.2. Heterotrophy

Regarding heterotrophic feeding, the total organic carbon used by the sponges was highly variable and did not differ between the three experimental conditions. Total organic carbon uptake was greater by day than by night, with sponges tending to acquire organic carbon by day, but to reduce that uptake or to release carbon into the environment by night (uptake of on average 4.8 ± 0.7 μg C $cm^{-2}h^{-1}$ by day and release of 1.4 ± 1.7 μg C $cm^{-2}h^{-1}$ by night, split-plot ANOVA, diurnal effect, $F_{(1,18)} = 9.59$, $p = .006$, Table 1).

3.3. Chemical bioerosion

Sponge-generated chemical bioerosion was driven by independent diurnal and preconditioning effects (split-plot ANOVA, $F_{(1,18)} = 12.16$, $p = .003$ and $F_{(2,18)} = 22.52$, $p < .0001$ respectively, Fig. 2B, Table 1). Chemical erosion rates were overall higher by night than by day (Tukey's post hoc, $p = .003$), although this diurnal effect tended to be minimal in the control condition where the variation between the night-time measurements was notably high (Fig. 2B). Net erosion rates gradually decreased from control to heat-preconditioned to DCMU-preconditioned sponges (Tukey's post hoc, all three comparisons $p < .022$).

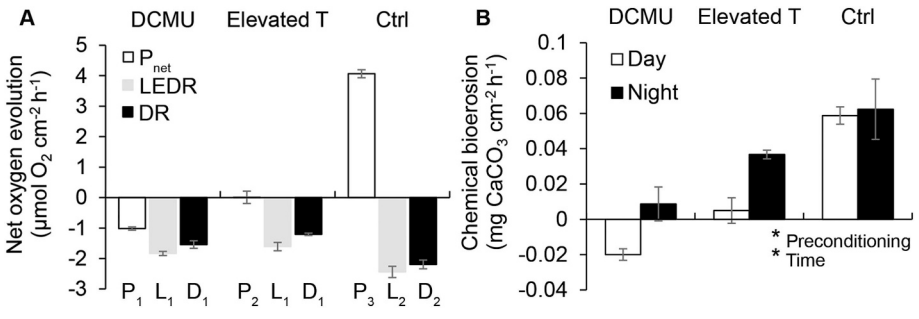

Fig. 2. (A) Oxygen flux of the bioeroding sponge holobiont *Cliona orientalis* in the day (net photosynthesis, P_{net} and light-enhanced dark respiration, LEDR) and in the night (dark respiration, DR) under three different conditions: (1) exposure to the herbicide DCMU, (2) symbiont loss due to elevated temperature (T), and (3) control condition (Ctrl). Significant differences between P_{net} (P) across the three conditions are indicated by the different number subscripts (e.g. $P_1 \neq P_2 \neq P_3$). Likewise for LEDR (L) and DR (D). (B) Chemical bioerosion rates of the sponge in the day and in the night under the three conditions. Asterisks (*) indicate significant main effects. Bars display means ± SEM.

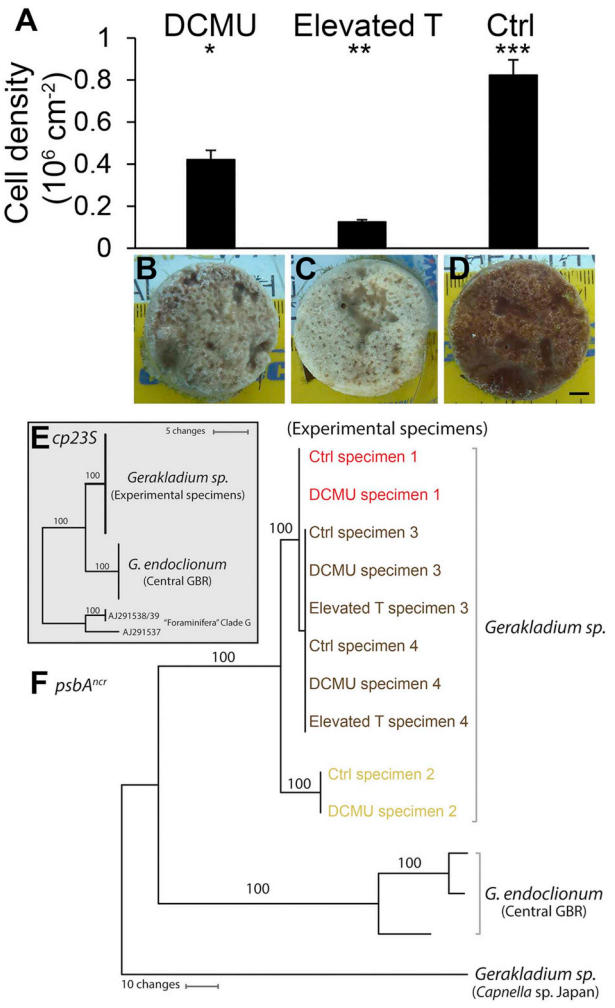

Fig. 3. Density of *Gerakladium* cells (A, mean ± SEM) and tissue colour (B–D) of the *Cliona orientalis* sponge holobiont under the three experimental conditions with photographs taken by day: (B) 9-day exposure to the herbicide DCMU, (C) symbiont loss due to elevated temperature, and (D) control condition. Asterisks indicate significant differences between groups. The scale bar in (D) equals 0.5 cm. (E) *cp23S* and (F) *psbA*^{ncr} phylogenies identified a single species of *Gerakladium* sp. (formerly the metazoan lineage of *Symbiodinium* Clade G) in the control samples and comprising the remnant populations from DCMU- and heat-preconditioned sponges. Font colours correspond to the sponge genotypes in Fig. 1A. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.4. Ammonium flux

Ammonium use by the sponges did not follow a diel pattern, but a preconditioning effect was detected (split-plot ANOVA, $F_{(2,18)} = 7.25$, $p = .005$, Table 1). Ammonium was absorbed by the sponge holobionts in the control condition (0.024 ± 0.015 µmol NH_4^+ $cm^{-2}\,h^{-1}$) in contrast to the DCMU-preconditioned sponges, which released ammonium (-0.037 ± 0.014 µmol NH_4^+ $cm^{-2}\,h^{-1}$, Tukey's post hoc, $p = .004$). Use of ammonium in heat-preconditioned sponges did not differ from the other two groups (-0.009 ± 0.007 µmol NH_4^+ $cm^{-2}\,h^{-1}$).

3.5. Density and phylogeny of Gerakladium

Overall, exposure of the sponge cores to the chemical and thermal preconditioning led to visible and statistically significant changes in the symbiotic community of the sponges (ANOVA, $F_{(2,15)} = 49.91$, $p < .0001$, Fig. 3A–D, Table 1). DCMU exposure over 9 days resulted in moderate paling of the surface tissue of the sponges (viewed by day), and the *Gerakladium* population decreased by approximately 50% compared to control sponges (Tukey's post hoc, $p < .001$). Exposure to elevated temperature caused a stronger yet still incomplete loss of symbionts (85% loss, Tukey's post hoc, $p < .001$). Sequencing of cp23S and *psbA*^{ncr} indicated that the resident *Gerakladium* was identical among control samples and remaining populations in DCMU and heat-preconditioned specimens (Fig. 3E and F). Moreover, phylogenetic analyses indicated that the sponges contained a new species of *Gerakladium* sp. different from *G. endoclionum*, which was recently described as the symbiont common to *C. orientalis* from Okinawa, Japan, and the central Great Barrier Reef (Ramsby et al., 2017b). The *psbA*^{ncr} further indicated that each of the three sponge genotypes (Fig. 1A) contained a distinct genotype, or strain, of this new species of endosymbiont (Fig. 3F). Per symbiont cell, the net photosynthetic oxygen flux amounted to -2.5 ± 0.2 pmol O_2 h^{-1} in the sponges pre-exposed to DCMU, and -0.5 ± 2.1 pmol O_2 h^{-1} in those exposed to elevated temperature, as opposed to 5.1 ± 0.3 pmol O_2 h^{-1} in the control cells (ANOVA, $F_{(2,15)} = 9.956$, $p = .002$ and Tukey's post hoc, $p < .05$ for control versus remaining conditions).

3.6. Chemical bioerosion in relation to oxygen flux

Simple regression analysis showed a significant (positive) correlation between net daytime photosynthesis and daytime as well as night-time chemical bioerosion activity in *C. orientalis* ($F_{(1,19)} = 80.21$, $p < .001$, Fig. 4A, and $F_{(1,19)} = 9.28$, $p = .007$, Fig. 4B, respectively). The proportion of variance of daytime chemical bioerosion rates that could be accounted for by net photosynthesis equalled 81%. The equivalent proportion for night-time bioerosion rates was 33% (Fig. 4). Night-time respiration explained an additional 8% of the variance in night-time bioerosion rates, but by itself, this correlation was not significant (not shown in Fig. 4).

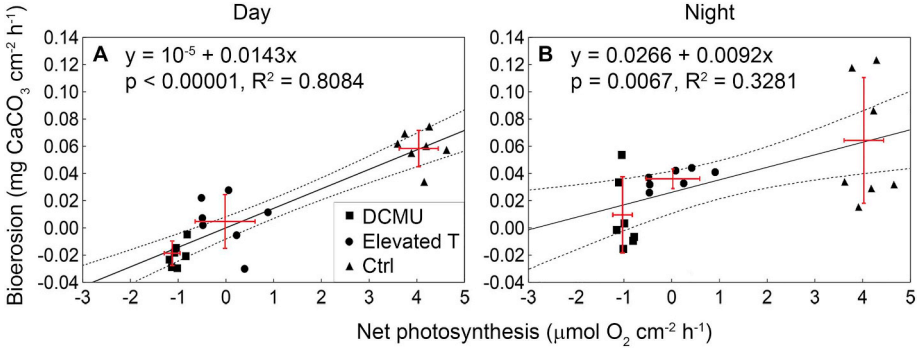

Fig. 4. Regression analysis between net daytime photosynthesis and chemical bioerosion rates of the bioeroding sponge holobiont *Cliona orientalis* by day (A) and by night (B). Net daytime photosynthesis is strongly related to daytime chemical bioerosion rates, but also to night-time chemical bioerosion rates. For each group, the mean and standard deviations for each axis are shown in red. Note the relatively high standard deviation in the night-time erosion rates of the control group. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

Exposure of the photosymbiotic bioeroding sponge *Cliona orientalis* to the herbicide DCMU or to elevated temperatures resulted in a decrease in the photosynthetic activity and density of *Gerakladium* sp. (Symbiodiniaceae) hosted by the sponge. Chemical bioerosion by the sponge decreased steeply as photosynthetic activity declined, demonstrating the presence of a strong and direct link between photosynthetic (by)products and bioerosion. Surprisingly, night-time respiration of the host alone proved insufficient to support high levels of bioerosion despite the favourable water chemistry, revealing that day-time photosynthesis also supports night-time erosion activity. These findings confirm that photosymbioses on coral reefs is not only the energetic basis for how some organisms build reef frameworks (Muscatine, 1990; Gattuso et al., 1999), but also how bioeroding organisms such as *C. orientalis* can erode them.

4.1. Photosynthesis and bioerosion rates

The absolute bioerosion rates we report approximate the upper range of earlier rates for the same species, although variable substrate density and other methodological issues introduce data variability (Achlatis et al., 2017; De Bakker et al., 2018a). Exposure-driven decreases in photosynthetic rates coincided with decreases in bioerosion rates, with DCMU-preconditioned sponges eroding the least. In other words, photosynthetic rates explained 81% of the variability found in chemical bioerosion during the day, and 33% by night. While correlations do not imply causation necessarily, and the existence of other non-photosynthetic preconditioning effects on bioerosion were not observed but cannot be excluded, this link between photosynthesis and bioerosion is consistent with previous studies, and may result from photosynthate transfer to and/or boosted oxygen supply to the mitochondria of cells undertaking the etching process. Depressed bioerosion rates in *C. orientalis* were previously found in shaded specimens (Rosell and Uriz, 1992; Hill, 1996; Schönberg, 2006) and after short- and long-term bleaching, when the sponges eventually suffered from an unsustainable carbon budget that resulted in starvation (Fang et al., 2014, 2018a; Achlatis et al., 2017). Furthermore, photosynthetic compounds fixed by dinoflagellates are shared with hosting sponge cells in the outer symbiont-rich and light-exposed layer of *C. orientalis* and other photosymbiotic sponges (Weisz et al., 2010; Achlatis et al., 2018), and translocation of these photoassimilates to deeper etching cells requires further study.

Assuming that photosynthetic products would directly boost sponge bioerosion, we would have expected more intense bioerosion during the day compared to during the night under ambient conditions. However, day and night rates appeared to be similar. We found no significant interaction between preconditioning and sampling time, and therefore we could not statistically compare day and night rates within each condition. A previous study suggested that diurnal changes in ambient seawater acidity are of less importance in regulating the bioerosion of healthy *C. orientalis* than the input of photosynthetic energy during the day, leading to higher chemical erosion rates by day than by night (8 h autumn incubations; Fang et al., 2016). This has been observed in other photosymbiotic *Cliona* species (Webb et al., 2017; De Bakker et al., 2018), but no diurnal variability was found in a dinoflagellate-hosting *Pione* species (possibly *Pione mussae*; Zundelevich et al., 2007). According to our observations both seawater chemistry and photosynthesis play a role, but their proportional contributions can shift with the condition of the photosymbionts. In our experiment (4 h post-exposure incubations under summer conditions), daytime bioerosion rates of the control sponges did not surpass the highly-variable night-time rates, but photosynthetic products appeared to enable daytime bioerosion to nearly meet night-time levels when water chemistry is generally more favourable for dissolution. Photosynthetic loss under DCMU exposure or heating removed this effect and coincided with a trend of higher bioerosion by night.

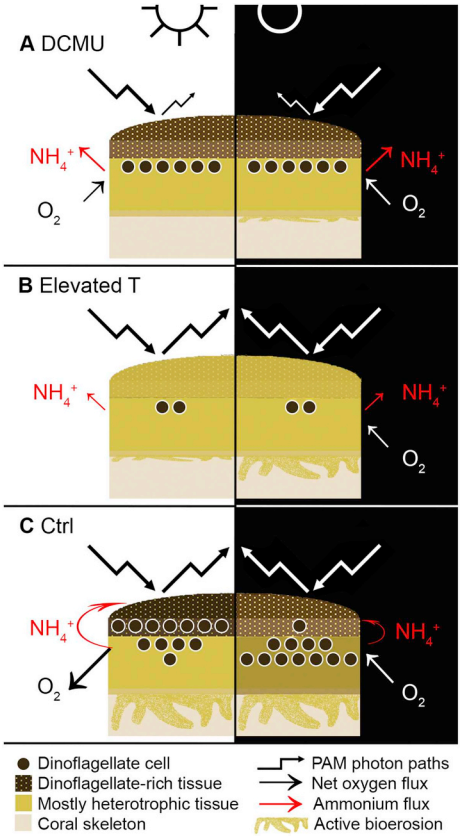

Fig. 5. Schematic representation summarizing the main physiological responses monitored in experimental cores of *Cliona orientalis* by day (left panels, in white) and by night (right panels, in black) after (A) exposure to the herbicide DCMU and (B) exposure to elevated temperature (T) over 9 days relative to control sponges (C). Cross sections of sponge cores display a heterogeneous distribution of dinoflagellate densities (varying colours representing the corresponding *Gerakladium* sp. abundance; while colouration suggests zones, there is no clear separation, but one merges into the other). The abundance of *Gerakladium* cells (not to scale), the saturating pulse of light and resulting yields of Photosystem II (incoming and outgoing PAM Photon paths respectively, pulse- amplitude modulated fluorometry), the net oxygen and ammonium flux, and the chemical bioerosion rates are schematically represented by day and by night. The size of the symbols displays the approximate intensity of the corresponding function. For example, in DCMU-preconditioned sponges (A): (i) The saturating pulse of light during the PAM measurement (incoming Photon path) results in low chlorophyll fluorescence (outgoing Photon path) indicative of poor photosynthetic potential. (ii) Since photosynthesis is inhibited, the sponge holobiont is a net consumer of O_2 both by day and by night. (iii) The symbiont population is halved compared to the control and kept lower in the sponge body by day for protection from light and herbicide. (iv) Ammonium is released by the host as a metabolic waste product without consumption by *Gerakladium* (v) Bioerosion takes place only by night, and at reduced rates compared to the control sponges.

When the dinoflagellate population was diminished, or when net daytime O_2 production was significantly impeded by preconditioning effects, bioerosion rates decreased as compared to those of control sponges by day as well as by night (schematic summary in Fig. 5). The decrease by night indicates that, in intact symbioses, photosynthetic (by)products are potentially stored for night-time use. In favour of the metabolic or oxygenic spill-over from day to night is the observation that under long-term darkness, bioerosion rates of *C. orientalis* decrease (Pineda et al., 2016; Fang et al., 2017). Reductions in the contribution and acidifying effect of respired CO_2, potentially due to reductions in symbiont biomass or symbiont derived carbohydrates, may contribute to the observed reduction in night-time chemical erosion for preconditioned compared to control sponges. In particular, healthy

dinoflagellates undergo chlororespiration at night which adds to total respiration (e.g. Hill and Ralph, 2008), but since DCMU does not allow the plastoquinone pool to accept electrons, this respiration and any bioerosion it may have facilitated would likely be diminished.

Interestingly, the alkalinity of the water decreased during the daytime incubation of the DCMU-preconditioned sponge holobionts. In combination with the lowering of the chemical bioerosion rate, the decrease in alkalinity may be explained by the release of protons due to enhanced microbial calcification or other metabolic processes (e.g. Riding, 2000; Reyes-Nivia et al., 2013). Similar decreases in alkalinity may have been masked by the erosion signal in the heated or control sponges. Alkalinity shifts due to shifts in the composition of the microbiome of the preconditioned *C. orientalis* cores are plausible, since the microbiome community changes in response to different light and temperature conditions (Pineda et al., 2016; Ramsby et al., 2018b). Further research is necessary to fully explain this decrease in alkalinity.

4.2. Other holobiont responses

The observed differences in autotrophic activity and hence bioerosion between the sponge groups can be explained by photostress that resulted either in reductions of the photosymbiont density (expulsion, or even digestion of photosymbionts by the host; Rosell, 1993; Hill and Hill, 2012), or reductions of their photosynthetic efficiencies, or both (Fig. 5). The DCMU exposure allowed the persistence of a large yet photochemically inactive population of resident symbionts. The few symbionts that persisted under elevated temperature remained photosynthetically active (oxygen production per cell almost met respiratory costs), perhaps taking advantage of the larger availability of light or nutrients in the bleached tissue (Enríquez et al., 2005; Hoogenboom et al., 2010).

When applied at high concentrations and over several days, DCMU causes photochemical changes that lead to the near-complete bleaching of scleractinian corals (e.g. Jones, 2004; Negri et al., 2005; Underwood et al., 2006). In the sponges studied here, just over half of the dinoflagellate population remained in place, despite the saturating DCMU concentration and despite photosynthetic impairment. In the heat-stressed sponges (MMM + 3.1 °C), there was no gradual decline in photosynthetic potential of individual symbionts as typically seen at the onset of coral bleaching (Jones et al., 1998; Jones, 2004). Instead, symptoms were delayed until a sudden collapse occurred, presumably when not enough functional symbionts remained (Supplementary Material). These observations indicate that partnerships between clionaid sponges and dinoflagellates respond differently to photosynthetic stress as compared to the coral- dinoflagellate mutualisms (Hill et al., 2011; Schönberg et al., 2008; Schönberg and Wisshak, 2012). Host physiological attributes such as symbiont migration, active pumping and regulation of the lipid bilayer composition of cell membranes may moderate light and/or heat stress (Schönberg and Suwa, 2007; Schönberg et al., 2017; Bennett et al., 2018), enabling the sponge to better cope with certain levels of stress prior to the eventual disassociation of the symbiosis (see also Achlatis et al., 2017; Fang et al., 2018a,b; Ramsby et al., 2018a).

The persistence of Symbiodiniaceae cells within stressed corals may be related to differences in tolerance among different dinoflagellates (Berkelmans and Van Oppen, 2006; Sampayo et al., 2008; Wang et al., 2017). In the sponge however, high resolution phylogenetic analysis of remnant *Gerakladium* found no differences between the symbionts in control specimens and those with reduced populations from chemical and thermal preconditioning (Fig. 3) (see also Ramsby et al., 2018a). Therefore, variation in the persistence of symbionts under DCMU or heat most likely reflects physiological heterogeneity of the symbiont population affected by location in the sponge tissue (Achlatis et al., 2018). Symbionts in the uppermost, sunlit layer are more readily exposed to herbicide, thermal and light stressors and are likely to be physiologically compromised and, therefore, are more vulnerable to expulsion from the host (Jones et al., 2003; Jones, 2004).

Cnidaria that are symbiotic with dinoflagellates may compensate for the loss of photosynthetically-derived nutrients by depending more on heterotrophy (Grottoli et al., 2006; Dove et al., 2013), but such modulation in behaviour is undocumented in sponges (Fang et al., 2014, 2017, 2018a; Achlatis et al., 2017; Pineda et al., 2017). In our experiment, uptake of organic carbon was generally higher by day than by night regardless of the preconditioning undergone, which is in accordance with naturally higher pumping rates of *C. orientalis* by day (Strehlow et al., 2016). Since heterotrophic rates did not differ between the preconditioned groups, our experiment was not suitable for testing the impact of heterotrophic feeding on bioerosion rates, although previous studies have established a strong link between the two (e.g. Holmes et al., 2009; Achlatis et al., 2017; Webb et al., 2017).

Ultimately, the reduction of photosynthesis through chemical inhibition or symbiont cell loss in *C. orientalis* allowed examination of nutrient cycles that are linked to the presence of photosymbionts. Ammonium is commonly excreted as a metabolic waste product in sponges that host a low abundance of microbes (Hentschel et al., 2012; Maldonado et al., 2012), but the *Gerakladium* community of *C. orientalis* rapidly reassimilates ammonium (Achlatis et al., 2018). This can also be seen in *Cliona varians* and in various Cnidarian hosts (Corredor et al., 1988; Wang and Douglas, 1998; Rädecker et al., 2015). Our control sponges demonstrated net uptake of ammonium, likely through the costly glutamine synthetase/glutamate synthase (GOGAT) pathway of dinoflagellates (Muscatine and D'Elia, 1978; Rahav et al., 1989; Foyer et al., 2003). Accordingly, with the loss of *Gerakladium* cells and/or their photosynthetic ability, ammonium was no longer assimilated during the day or night, and a net excretion was measured. Similar observations have been made in bleached or DCMU-exposed symbiotic corals (e.g. Muscatine et al., 1979; Rahav et al., 1989; Falkowski et al., 1993).

4.3. Conclusions

Photosynthetic activity is a driver of bioerosion rates in photosymbiotic clionaid sponges, and symbiont presence is integral to the functional ecology of these sponges on a diurnal basis. Further investigations of the photo-bleaching behaviour and thresholds of these understudied bioeroders can be performed, as demonstrated, with the aid of the photosystem II herbicide DCMU, and will advance our understanding of their susceptibility to warming waters. With photosynthesis being crucial to the metabolic needs of photosymbiotic clionaids, their ecological prevalence may depend on the continued maintenance of a stable mutualism with co-evolved symbionts (Achlatis et al., 2017; Fang et al., 2018a; Ramsby et al., 2018a). Animal-photosymbiont associations on tropical coral reefs have exploited solar energy to power decalcification as well as calcification for millions of years, yet this energetic benefit comes with the price of an increased vulnerability to the abrupt environmental changes accompanying current and projected shifts in global climate.

Funding

This work was supported by the Australian Research Council (ARC) and NOAA Coral Reef Watch, the ARC Laureate grant (FL120100066 to O.H.G.), the ARC Centre of Excellence for Coral Reef Studies (CE0561435 to O.H.G. and S.D.), the Australian Government Research Training Program Scholarship (to M.A.) and a Holsworth Wildlife Research Endowment – Ecological Society of Australia (to M.A.). Research on characterizing the sponge symbionts was supported by the USA National Science Foundation (IOS-1258058 and OCE-1636022 to T.C.L.).

Declarations of interest

None.

Acknowledgements

We thank D. Bender-Champ, A. Kubicek and K. Brown as well as the Heron Island Research Station staff for assistance. An anonymous reader is thanked for their constructive suggestions during manuscript preparation. Two reviewers are also thanked for their valuable input during manuscript revision. All research was conducted under sample permit nr. G14/37212.1 issued by the Great Barrier Reef Marine Park Authority.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jembe.2019.04.010.

References

Achlatis, M., van der Zande, R.M., Schönberg, C.H.L., Fang, J.K.H., Hoegh-Guldberg, O., Dove, S., 2017. Sponge bioerosion on changing reefs: ocean warming poses physiological constraints to the success of a photosymbiotic excavating sponge. Sci. Rep. 7, 10705.

Achlatis, M., Pernice, M., Green, K., Guagliardo, P., Kilburn, M.R., Hoegh-Guldberg, O., Dove, S., 2018. Single-cell measurement of ammonium and bicarbonate uptake within a photosymbiotic bioeroding sponge. ISME J. 12, 1308.

Adey, W.H., Steneck, R.S., 1985. Highly productive eastern Caribbean reefs: synergistic effects of biological, chemical, physical and geological factors. Ecol. Coral Reefs 3, 163–187.

Al-Horani, F.A., 2016. Physiology of skeletogenesis in scleractinian coral. In: Woodley, C.M., Downs, C.A., Bruckner, A.W., Porter, J.W. (Eds.), Diseases of Coral. John Wiley & Sons, New Jersey.

Allemand, D., Tambutté, É., Zoccola, D., Tambutté, S., 2011. Coral calcification, cells to reefs. In: Coral Reefs: An Ecosystem in Transition. Springer, pp. 119–150.

Bennett, H., Bell, J.J., Davy, S.K., Webster, N.S., Francis, D.S., 2018. Elucidating the sponge stress response; lipids and fatty acids can facilitate survival under future climate scenarios. Glob. Chang. Biol. 24, 3130–3144.

Berkelmans, R., Van Oppen, M.J., 2006. The role of zooxanthellae in the thermal tolerance of corals: a 'nugget of hope'for coral reefs in an era of climate change. Proc. R. Soc. B 273, 2305–2312.

Cantin, N.E., Negri, A.P., Willis, B.L., 2007. Photoinhibition from chronic herbicide exposure reduces reproductive output of reef-building corals. Mar. Ecol. Prog. Ser. 344, 81–93.

Corredor, J.E., Wilkinson, C.R., Vicente, V.P., Morell, J.M., Otero, E., 1988. Nitrate release by Caribbean reef sponges. Limnol. Oceanogr. 33, 114–120.

De Bakker, D.M., Webb, A.E., van den Bogaart, L.A., Van Heuven, S.M.A.C., Meesters, E.H., van Duyl, F.C., 2018. Quantification of chemical and mechanical bioerosion rates of six Caribbean excavating sponge species found on the coral reefs of Curaçao. PLoS One 13, 1–22.

Diaz, M.C., Thacker, R.W., Rützler, K., Piantoni, C., 2007. Two new haplosclerid sponges from Caribbean Panama with symbiotic filamentous cyanobacteria, and an overview of spongecyanobacteria associations. Porifera Res. Biodivers. Innov. Sustain. 31–39.

Dove, S.G., Kline, D.I., Pantos, O., Angly, F.E., Tyson, G.W., Hoegh-Guldberg, O., 2013. Future reef decalcification under a business-as-usual CO_2 emission scenario. Proc. Natl. Acad. Sci. U. S. A. 110, 15342–15347.

Enríquez, S., Méndez, E.R., Iglesias-Prieto, R., 2005. Multiple scattering on coral skeletons enhances light absoprtion by symbiotic algae. Limnol. Oceanogr. 50, 1025–1032.

Erwin, P.M., Thacker, R.W., 2007. Incidence and identity of photosynthetic symbionts in Caribbean coral reef sponge assemblages. J. Mar. Biol. Assoc. UK 87, 1683–1692.

Falkowski, P.G., Raven, J.A., 2007. Aquatic Photosynthesis, 2nd ed. Princeton University Press, Princeton.

Falkowski, P.G., Dubinsky, Z., Muscatine, L., Mccloskey, L., 1993. Population control in symbiotic corals. Bioscience 43, 606–611.

Fang, J.K.H., Mello-Athayde, M.A., Schönberg, C.H.L., Kline, D.I., Hoegh-Guldberg, O., Dove, S., 2013a. Sponge biomass and bioerosion rates increase under ocean warming and acidification. Glob. Chang. Biol. 19, 3581–3591.

Fang, J.K.H., Schönberg, C.H.L., Kline, D.I., Hoegh-Guldberg, O., Dove, S., 2013b. Methods to quantify components of the excavating sponge *Cliona orientalis* Thiele, 1900. Mar. Ecol. 34, 193–206.

Fang, J.K.H., Schönberg, C.H.L., Mello-Athayde, M.A., Hoegh-Guldberg, O., Dove, S., 2014. Effects of ocean warming and acidification on the energy budget of an excavating sponge. Glob. Chang. Biol. 20, 1043–1054.

Fang, J.K.H., Schönberg, C.H.L., Hoegh-Guldberg, O., Dove, S., 2016. Day-night ecophysiology of the photosymbiotic bioeroding sponge *Cliona orientalis* Thiele, 1900. Mar. Biol. 163, 1–12.

Fang, J.K.H., Schönberg, C.H.L., Hoegh-Guldberg, O., Dove, S., 2017. Symbiotic plasticity of *Symbiodinium* in a common excavating sponge. Mar. Biol. 164, 104.

Fang, J.K.H., Schönberg, C.H., Mello-Athayde, M.A., Achlatis, M., Hoegh-Guldberg, O., Dove, S., 2018a. Bleaching and mortality of a photosymbiotic bioeroding sponge under future carbon dioxide emission scenarios. Oecologia 187, 25–35.

Fang, J.K.H., Schönberg, C.H., Ormond, R.F., 2018b. Are some photosymbiotic bioeroding sponges more bleaching-tolerant than hard corals? J. Mar. Biol. Oceanogr. 07, 2017–2019.

Foyer, C.H., Parry, M., Noctor, G., 2003. Markers and Signals Associated with Nitrogen Assimilation in Higher Plants. vol. 54. pp. 585–593.

Garcia-Pichel, F., 2006. Plausible mechanisms for the boring on carbonates by microbial phototrophs. Sediment. Geol. 185, 205–213.

Gattuso, J., Allemand, D., Frankignoulle, M., 1999. Photosynthesis and calcification at cellular, organismal and community levels in coral reefs: a review on interactions and control by carbonate chemistry. Am. Zool. 39, 160–183.

Genty, B., Briantais, J.-M., Baker, N.R., 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim. Biophys. Acta 990, 87–92.

Grottoli, A.G., Rodrigues, L.J., Palardy, J.E., 2006. Heterotrophic plasticity and resilience in bleached corals. Nature 440, 1186–1189.

Hentschel, U., Fieseler, L., Wehrl, M., Gernert, C., Steinert, M., Hacker, J., Horn, M., 2003. Microbial diversity of marine sponges. In: Sponges (Porifera). Springer, pp. 59–88.

Hentschel, U., Piel, J., Degnan, S.M., Taylor, M.W., 2012. Genomic insights into the marine sponge microbiome. Nat. Rev. Microbiol. 10, 641–654.

Hill, M., 1996. Symbiotic zooxanthellae enhance boring and growth rates of the tropical sponge *Anthosigmella varians* forma *varians*. Mar. Biol. 125, 649–654.

Hill, M., Hill, A., 2012. The magnesium inhibition and arrested phagosome hypotheses: new perspectives on the evolution and ecology of *Symbiodinium* symbioses. Biol. Rev. 87, 804–821.

Hill, R., Ralph, P.J., 2008. Dark-induced reduction of the plastoquinone pool in zooxanthellae of scleractinian corals and implications for measurements of chlorophyll a fluorescence. Symbiosis 46, 45–56.

Hill, M., Allenby, A., Ramsby, B., Schönberg, C., Hill, A., 2011. *Symbiodinium* diversity among host clionaid sponges from Caribbean and Pacific reefs: evidence of heteroplasmy and putative host-specific symbiont lineages. Mol. Phylogenet. Evol. 59, 81–88.

Holmes, G., Ortiz, J.-C., Schönberg, C.H.L., 2009. Bioerosion rates of the sponge *Cliona orientalis* Thiele, 1900: spatial variation over short distances. Facies 55, 203–211.

Hoogenboom, M., Beraud, E., Ferrier-Pagès, C., 2010. Relationship between symbiont density and photosynthetic carbon acquisition in the temperate coral *Cladocora caespitosa*. Coral Reefs 29, 21–29.

Jones, R.J., 2004. Testing the 'photoinhibition' model of coral bleaching using chemical inhibitors. Mar. Ecol. Prog. Ser. 284, 133–145.

Jones, R.J., Hoegh-Guldberg, O., Larkum, A.W.D., Schreiber, U., 1998. Temperature-induced bleaching of corals begins with impairment of the CO_2 fixation mechanism in zooxanthellae. Plant Cell Environ. 21, 1219–1230.

Jones, R.J., Muller, J., Haynes, D., Schreiber, U., 2003. Effects of herbicides diuron and atrazine on corals of the Great Barrier Reef, Australia. Mar. Ecol. Prog. Ser. 251, 153–167.

Kline, D.I., Teneva, L., Schneider, K., Miard, T., Chai, A., Marker, M., Headley, K., Opdyke, B., Nash, M., Valetich, M., Caves, J.K., Russell, B.D., Connell, S.D., Kirkwood, B.J., Brewer, P., Peltzer, E., Silverman, J., Caldeira, K., Dunbar, R.B., Koseff, J.R., Monismith, S.G., Mitchell, B.G., Dove, S., Hoegh-Guldberg, O., 2012. A short-term in situ CO_2 enrichment experiment on Heron Island (GBR). Sci. Rep. 2, 1–9.

Krause, G.H., Weis, E., 1991. Chlorophyll fluorescence and photosynthesis: the basics. Annu. Rev. Plant Physiol. Plant Mol. Biol. 42, 313–349.

LaJeunesse, T.C., Thornhill, D.J., 2011. Improved resolution of reef-coral endosymbiont (*Symbiodinium*) species diversity, ecology, and evolution through *psbA* non-coding region genotyping. PLoS One 6, e29013.

LaJeunesse, T.C., Loh, W.K.W., van Woesik, R., Hoegh-Guldberg, O., Schmidt, G.W., Fitt, W.K., 2003. Low symbiont diversity in southern Great Barrier Reef corals, relative to those of the Caribbean. Limnol. Oceanogr. 48, 2046–2054.

LaJeunesse, T.C., Parkinson, J.E., Gabrielson, P.W., Jeong, H.J., Reimer, J.D., Voolstra, C.R., Santos, S.R., 2018. Systematic revision of Symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts. Curr. Biol. 28, 2570–2580.

Lipps, J.H., Stanley, G.D., 2016. Photosymbiosis in Past and Present Reefs. Springer, Dordrecht, pp. 47–68.

Lynch, T.C., Phlips, E.J., 2000. Filtration of the bloom-forming cyanobacteria *Synechococcus* by three sponge species from Florida Bay, USA. Bull. Mar. Sci. 67, 923–936.

Maldonado, M., Ribes, M., van Duyl, F.C., 2012. Nutrient fluxes through sponges: biology, budgets, and ecological implications. Adv. Mar. Biol. 62, 113.

Marsh, J.A., 1970. Primary productivity of reef-building calcareous red algae. Ecology 51, 255–263.

Mueller, B., de Goeij, J.M., Vermeij, M.J.A., Mulders, Y., van der Ent, E., Ribes, M., van Duyl, F.C., 2014. Natural diet of coral-excavating sponges consists mainly of dissolved organic carbon (DOC). PLoS One 9, e90152.

Muscatine, L., 1990. The role of symbiotic algae in carbon and energy flux in reef corals. Ecosyst. World 25, 75–87.

Muscatine, L., D'Elia, C., 1978. The uptake, retention, and release of ammonium by reef corals. Limnol. Oceanogr. 23, 725–734.

Muscatine, L., Porter, J.W., 1977. Reef corals: mutualistic symbioses adapted to nutrient-poor environments. Bioscience 27, 454–460.

Muscatine, L., Masuda, H., Burnap, R., 1979. Ammonium uptake by symbiotic and aposymbiotic reef corals. Bull. Mar. Sci. 29, 572–575.

Nava, H., Carballo, J.L., 2008. Chemical and mechanical bioerosion of boring sponges from Mexican Pacific coral reefs. J. Exp. Biol. 211, 2827–2831.

Negri, A., Vollhardt, C., Humphrey, C., Heyward, A., Jones, R., Eaglesham, G., Fabricius, K., 2005. Effects of the herbicide diuron on the early life history stages of coral. Mar. Pollut. Bull. 51, 370–383.
Parrin, A.P., Somova, E.L., Kern, P.M., Millet, T.A., Bross, L.S., Blackstone, N.W., 2017. The use of *in vivo* microscopy to image the cnidarian stress response in three octocoral species. Invertebr. Biol. 136, 330–344.
Parsons, T.R., Maita, Y., Lalli, C.M., 1984. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, Oxford, New York, Sydney.
Pineda, M.C., Strehlow, B., Duckworth, A., Doyle, J., Jones, R., Webster, N.S., 2016. Effects of light attenuation on the sponge holobiont- implications for dredging management. Sci. Rep. 6, 39038.
Pineda, M.C., Strehlow, B., Sternel, M., Duckworth, A., Webster, N.S., 2017. Effects of suspended sediments on the sponge holobiont with implications for dredging management. Sci. Rep. 7, 4925.
Pochon, X., Putnam, H.M., Gates, R.D., 2014. Multi-gene analysis of *Symbiodinium* dinoflagellates: a perspective on rarity, symbiosis, and evolution. Peer J. 2, e394.
Pomponi, S.A., 1980. Cytological mechanisms of calcium carbonate excavation by boring sponges. Int. Rev. Cytol. 65, 301–319.
Rädecker, N., Pogoreutz, C., Voolstra, C.R., Wiedenmann, J., Wild, C., 2015. Nitrogen cycling in corals: the key to understanding holobiont functioning? Trends Microbiol. 23, 490–497.
Rahav, O., Dubinsky, Z., Archituv, Y., 1989. Ammonium metabolism in the zooxanthellate coral *Stylophora pistillata*. Proc. R. Soc. B 337, 325–337.
Ramsby, B.D., Hill, M.S., Thornhill, D.J., Steenhuizen, S.F., Achlatis, M., Lewis, A.M., Lajeunesse, T.C., 2017a. Sibling species of mutualistic *Symbiodinium* clade G from bioeroding sponges in the western Pacific and western Atlantic oceans. J. Phycol. 53, 951–960.
Ramsby, B.D., Hoogenboom, M.O., Whalan, S., Webster, N.S., Thompson, A., 2017b. A decadal analysis of bioeroding sponge cover on the inshore Great Barrier Reef. Sci. Rep. 7, 2706.
Ramsby, B.D., Hoogenboom, M.O., Smith, H.A., Whalan, S., Webster, N.S., 2018a. The bioeroding sponge *Cliona orientalis* will not tolerate future projected ocean warming. Sci. Rep. 8, 1–13.
Ramsby, B.D., Hoogenboom, M.O., Whalan, S., Webster, N.S., 2018b. Elevated seawater temperature disrupts the microbiome of an ecologically important bioeroding sponge. Mol. Ecol. 27, 2124–2137.
Reyes-Nivia, C., Diaz-Pulido, G., Kline, D., Guldberg, O.-H.H., Dove, S., 2013. Ocean acidification and warming scenarios increase microbioerosion of coral skeletons. Glob. Chang. Biol. 19, 1919–1929.
Riding, R., 2000. Microbial carbonates: the geological record of calcified bacterial–algal mats and biofilms. Sedimentology 47, 179–214.
Rosell, D., 1993. Effects of reproduction in *Clionia viridis* (Hadromerida) on zooxanthellae. Sci. Mar. 57, 405–413.
Rosell, D., Uriz, M.J., 1992. Do associated zooxanthellae and the nature of the substratum affect survival, attachment and growth of *Cliona viridis* (Porifera: Hadromerida)? An experimental approach. Mar. Biol. 114, 503–507.
Rützler, K., 1990. Associations between Caribbean sponges and photosynthetic organisms. In: Rützler, K. (Ed.), New Perspectives in Sponge Biology. Smithsonian Institution Press, Washington, DC, pp. 455–466.
Rützler, K., Rieger, G., 1973. Sponge burrowing: fine structure of *Cliona lampa* penetrating calcareous substrata. Mar. Biol. 21, 144–162.
Sampayo, E.M., Ridgway, T., Bongaerts, P., Hoegh-Guldberg, O., 2008. Bleaching susceptibility and mortality of corals are determined by fine-scale differences in symbiont type. Proc. Natl. Acad. Sci. U. S. A. 105, 10444–10449.
Sampayo, E.M., Ridgway, T., Franceschinis, L., Roff, G., Hoegh-Guldberg, O., Dove, S., 2016. Coral symbioses under prolonged environmental change: living near tolerance range limits. Sci. Rep. 6, 36271.
Schneider, K., Silverman, J., Woolsey, E., Eriksson, H., Byrne, M., Caldeira, K., 2011. Potential influence of sea cucumbers on coral reef $CaCO_3$ budget: a case study at One Tree Reef. J. Geophys. Res. Biogeosci. 116, 2–7.
Schönberg, C.H.L., 2001. Small-scale distribution of Great Barrier Reef bioeroding sponges in shallow water. Ophelia (1), 39–54.
Schönberg, C.H.L., 2006. Growth and erosion of the zooxanthellate Australian bioeroding sponge *Cliona orientalis* are enhanced in light. In: Proc. 10th Int. Coral Reef Symp., Okinawa, pp. 168–174.
Schönberg, C.H.L., Loh, W.K.W., 2005. Molecular identity of the unique symbiotic dinoflagellates found in the bioeroding demosponge *Cliona orientalis*. Mar. Ecol. Prog. Ser. 299, 157–166.
Schönberg, C.H.L., Ortiz, J.C., 2008. Is sponge bioerosion increasing. In: Proc 11th Int Coral Reef Symp, Ft. Lauderdale, pp. 520–523.
Schönberg, C.H.L., Suwa, R., 2007. Why bioeroding sponges may be better hosts for symbiotic dinoflagellates than many corals. Porifera Res. Biodivers. Innov. Sustain. Mus. Nac Rio Janeiro 569–580.
Schönberg, C.H.L., Wisshak, M., 2012. The perks of being endolithic. Aquat. Biol. 17, 1–5.
Schönberg, C.H.L., De Beer, D., Lawton, A., 2005. Oxygen microsensor studies on zooxanthellate clionaid sponges from the Costa Brava, Mediterranean Sea. J. Phycol. 41, 774–779.
Schönberg, C.H.L., Suwa, R., Hidaka, M., Loh, W.K.W., 2008. Sponge and coral zooxanthellae in heat and light: preliminary results of photochemical efficiency monitored with pulse amplitude modulated fluorometry. Mar. Ecol. 29, 247–258.
Schönberg, C.H.L., Fang, J.K.H., Carballo, J.L., 2017. Bioeroding sponges and the future of coral reefs. In: Bell, J.J., J, C. (Eds.), Climate Change, Ocean Acidification and Sponges. Springer, Berlin – Heidelberg, pp. 179–372.
Seckbach, J., 2004. Symbiosis: Mechanisms and Model Systems. Kluwer Academic Publishers, Dordrecht, The Netherlands.
Seutin, G., White, B.N., Boag, P.T., 1991. Preservation of avian blood and tissue samples for DNA analyses. Can. J. Zool. 69, 82–90.
Simister, R.L., Deines, P., Botté, E.S., Webster, N.S., Taylor, M.W., 2012. Sponge-specific clusters revisited: a comprehensive phylogeny of sponge-associated microorganisms. Environ. Microbiol. 14, 517–524.
Stat, M., Carter, D., Hoegh-Guldberg, O., 2006. The evolutionary history of Symbiodinium and scleractinian hosts—symbiosis, diversity, and the effect of climate change. Perspectives in Plant Ecology, Evolution and Systematics 8 (1), 23–43.
Strehlow, B.W., Jorgensen, D., Webster, N.S., Pineda, M.-C., Duckworth, A., 2016. Integrating a thermistor flowmeter and time lapse imagery to monitor sponge (Porifera) behaviour. PeerJ 4, e2761.
Suggett, D.J., Prašil, O., Borowitzka, M.A., 2011. Chlorophyll a Fluorescence in Aquatic Sciences: Methods and Applications. Springer, Dordrecht, The Netherlands.
Swofford, D.L., 2014. PAUP* Phylogenetic Analysis Using Parsimony (*and Other Methods). 4.0d146 ed. Sinauer Associates, Sunderland, Massachusetts.
Taylor, M.W., Radax, R., Steger, D., Wagner, M., 2007. Sponge-associated microorganisms : evolution, ecology, and biotechnological potential. Microbiol. Mol. Biol. Rev. 71, 295–347.
Thacker, R.W., Freeman, C.J., 2012. Sponge-microbe symbioses. Recent advances and new directions. Recent advances and new directions. In: Advances in Marine Biology. vol. 62. Academic Press. Elsevier, pp. 57–111.
Tolosa, I., Treignier, C., Grover, R., Ferrier-Pagès, C., 2011. Impact of feeding and short-term temperature stress on the content and isotopic signature of fatty acids, sterols, and alcohols in the scleractinian coral *Turbinaria reniformis*. Coral Reefs 30, 763.
Tremblay, P., Gori, A., Maguer, J.F., Hoogenboom, M., Ferrier-Pagès, C., 2016. Heterotrophy promotes the re-establishment of photosynthate translocation in a symbiotic coral after heat stress. Sci. Rep. 6, 38112.
Trench, R., 1993. Microalgal-invertebrate symbioses-a review. Endocytobiosis Cell Res. 9, 135–175.
Trench, R., Wethey, D.S., Porter, J.W., 1981. Observations on the symbiosis with zooxanthellae among the Tridacnidae (Mollusca, Bivalvia). Biol. Bull. 161, 180–198.
Underwood, A.J., 1997. Experiments in Ecology, their Logical Design and Interpretation Using Analysis of Variance. Cambridge University Press, Cambridge.
Underwood, J.N., Souter, P.B., Ballment, E.R., Lutz, A.H., Van Oppen, M.J.H., 2006. Development of 10 polymorphic microsatellite markers from herbicide-bleached tissues of the brooding pocilloporid coral *Seriatopora hystrix*. Mol. Ecol. Resour. 6, 176–178.
Vacelet, J., 1981. Algal-sponge symbioses in the coral reefs of New Caledonia: a morphological study. In: Proc. 4th Int. Coral Reef Symp., Manila, pp. 713–719.
Wang, J., Douglas, A.E., 1998. Nitrogen recycling or nitrogen conservation in an alga-invertebrate symbiosis? J. Exp. Biol. 201, 2445–2453.
Wang, J.-T., Keshavmurthy, S., Chu, T.-Y., Chen, C.A., 2017. Diverse responses of *Symbiodinium* types to menthol and DCMU treatment. PeerJ 5, e3843.
Watanabe, T., Yuyama, I., Yasumura, S., 2006. Toxicological effects of biocides on symbiotic and aposymbiotic juveniles of the hermatypic coral *Acropora tenuis*. J. Exp. Mar. Biol. Ecol. 339, 177–188.
Webb, A.E., van Heuven, S.M.A.C., de Bakker, D.M., van Duyl, F.C., Reichart, G.-J., de Nooijer, L.J., 2017. Combined effects of experimental acidification and eutrophication on reef sponge bioerosion rates. Front. Mar. Sci. 4, 1–15.
Webb, A.E., Pomponi, S.A., van Duyl, F.C., Reichart, G.-J., de Nooijer, L.J., 2019. pH regulation and tissue coordination pathways promote calcium carbonate bioerosion by excavating sponges. Sci. Rep. 9, 1–10.
Weisz, J.B., Massaro, A.J., Ramsby, B.D., Hill, M.S., 2010. Zooxanthellar symbionts shape host sponge trophic status through translocation of carbon. Biol. Bull. 219, 189–197.
Wilkinson, C.R., 1987. Interocean differences in size and nutrition of coral reef sponge populations. Science 236, 1654–1657.
Wisshak, M., Schönberg, C.H.L., Form, A., Freiwald, A., 2012. Ocean acidification accelerates reef bioerosion. PLoS One 7, e45124.
Yellowlees, D., Rees, T.A.V., Leggat, W., 2008. Metabolic interactions between algal symbionts and invertebrate hosts. Plant Cell Environ. 31, 679–694.
Zhang, Z., Green, B.R., Cavalier-Smith, T., 2000. Phylogeny of ultra-rapidly evolving dinoflagellate chloroplast genes: a possible common origin for sporozoan and dinoflagellate plastids. J. Mol. Evol. 51, 26–40.
Zundelevich, A., Lazar, B., Ilan, M., 2007. Chemical versus mechanical bioerosion of coral reefs by boring sponges–lessons from *Pione cf. vastifica*. J. Exp. Biol. 210, 91–96.