



# Effects of bleaching on oxygen dynamics and energy metabolism of two Caribbean coral species

L.B. Linsmayer<sup>a</sup>, S.K. Noel<sup>a</sup>, M. Leray<sup>c</sup>, D. Wangpraseurt<sup>a,b</sup>, C. Hassibi<sup>a</sup>, D.I. Kline<sup>a,c</sup>, M. Tresguerres<sup>a,\*</sup>

<sup>a</sup> Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA 92093, USA

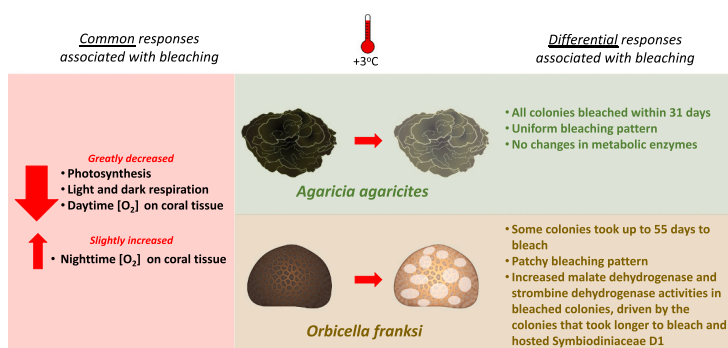
<sup>b</sup> Department of Nanoengineering, University of California San Diego, La Jolla, CA 92093, USA

<sup>c</sup> Smithsonian Tropical Research Institute, Apartado Postal 0843-03092, Panamá, Panama

## HIGHLIGHTS

- *Agaricia agaricites* bleached at a faster rate than *Orbicella franksi*.
- Photosynthesis and respiration rates decreased as bleaching progressed.
- O<sub>2</sub> dynamics in the diffusive boundary layer of bleached corals was altered.
- *O. franksi* colonies that took longer to bleach contained Symbiodiniaceae D1.
- Bleaching-resilient colonies also had upregulated metabolic enzymatic activities.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

Editor: Julian Blasco

### Keywords:

Reef  
Strombine  
Hypoxia  
Warburg  
*Orbicella*  
*Agaricia*  
Bocas del Toro

## ABSTRACT

As mass coral bleaching events become more frequent, it is increasingly important to elucidate the factors underlying coral susceptibility and survival. We measured photosynthesis, respiration, and O<sub>2</sub> concentration at the coral tissue surface, Symbiodiniaceae genotypes, and energy metabolic enzyme activities in *Agaricia agaricites* and *Orbicella franksi* throughout experimentally-induced thermal bleaching (+3 °C). *A. agaricites* colonies started to bleach two days into the thermal treatment and were fully bleached between Days 19–31. In contrast, *O. franksi* colonies only started to bleach on Day 12 and five colonies fully bleached between Days 24–38 while the remaining three colonies took up to 55 days. Both species experienced decreased photosynthesis and respiration rates as bleaching progressed. As a result, daytime O<sub>2</sub> concentration at the coral surface shifted from hyperoxia in unbleached corals to normoxia in partially bleached corals, and to near hypoxia in fully bleached corals. Additionally, nighttime tissue surface O<sub>2</sub> concentration shifted from hypoxia to normoxia, likely resulting from decreased symbiotic algae density, respiration, and photosynthates that fuel coral aerobic respiration. Genetic profiling of internal transcribed spacer 2 (ITS2) revealed differences in Symbiodiniaceae clade proportions between control and bleached colonies. Activity levels of energy metabolic enzymes did not significantly vary between control and bleached *A. agaricites*, but malate dehydrogenase and strombine dehydrogenase activities were significantly higher in bleached *O. franksi* colonies compared to controls. These differences were driven by

\* Corresponding author.

E-mail address: [mtresguerres@ucsd.edu](mailto:mtresguerres@ucsd.edu) (M. Tresguerres).

<https://doi.org/10.1016/j.scitotenv.2024.170753>

Received 5 June 2023; Received in revised form 2 February 2024; Accepted 4 February 2024

Available online 13 February 2024

0048-9697/© 2024 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC license (<http://creativecommons.org/licenses/by-nc/4.0/>).

the three *O. franksi* colonies that took the longest to bleach and contained >98 % *Durussidinium* sp. D1. The shifts in O<sub>2</sub> dynamics within the microhabitat of bleached corals may have important implications for the metabolism of the coral holobiont while the changes in Symbiodiniaceae ITS2 profile and the upregulation of energy metabolic enzymes identify a potential factor contributing to bleaching dynamics.

## 1. Introduction

Coral bleaching refers to the loss of coral pigmentation due to the massive expulsion of their endosymbiotic Symbiodineaceae dinoflagellate algae (Hoegh-Guldberg, 1999). The most common causes of bleaching are elevated seawater temperature and solar irradiance, which can act alone (Gleason and Wellington, 1993; Jokiel and Coles, 1990) or synergistically (Coles and Jokiel, 1978; Lesser et al., 1990). In addition, regional coral bleaching events have been attributed to eutrophication (Wiedenmann et al., 2013; Lesser, 2021) and hypoxia (Altieri et al., 2017). Since corals meet the majority of their organic carbon needs from their photosymbiotic algae (Muscattine et al., 1981), a bleached coral is in a vulnerable state and experiences increased susceptibility to disease and mortality (Lewis et al., 2017; Thurber et al., 2017; Baird and Marshall, 2002). As bleaching events increase in frequency and severity due to ocean warming and anthropogenic activities, global reefs are at risk of severe degradation by the end of this century (Grottoli et al., 2014). In the Caribbean, coral reefs have been hard-hit by major bleaching events in 1998, 2005, 2010–11, 2015 and 2017 (Muñiz-Castillo et al., 2019), with up to 40 % coral mortality in 2005 (Eakin et al., 2010). Although coral bleaching is widely recognized as the largest threat to the future of reefs worldwide, many physiological aspects of coral bleaching remain poorly understood.

Bleaching dramatically reduces photosynthate supply to host cells resulting in an energy deficit (Lesser, 1997; Warner et al., 1996; Porter et al., 1989). However, not all coral colonies or species are equally sensitive to bleaching, and some species are able to survive in a bleached state longer than others (Neal et al., 2017; Loya et al., 2001; Hueerkamp et al., 2001) thus increasing the probability of symbiont repopulation and recovery from bleaching. The mechanisms that determine susceptibility to bleaching are not entirely clear, but have been suggested to relate to coral nutrient reserves (Porter et al., 1989; Fitt et al., 1993; Grottoli et al., 2006; Fitt et al., 2000; Grottoli et al., 2004), heterotrophic feeding rates (Grottoli et al., 2006; Cunning et al., 2017; Houlbrèque and Ferrier-Pagès, 2009), Symbiodiniaceae strain identity (Silverstein et al., 2017), the capacity to cope with deoxygenation (Alderdice et al., 2021), as well as tissue thickness (Wangpraseurt et al., 2012a), colony shape (Loya et al., 2001), and coral optical properties (Wangpraseurt et al., 2017a).

In addition to providing nutritional compounds, photosynthesis by the endosymbiotic algae produces O<sub>2</sub> that create hyperoxic conditions in coral tissues during the day and significantly boosts aerobic respiration (Linsmayer et al., 2020; Köhl et al., 1995; Shick, 1990). Bleached corals demonstrate reduced rates of photosynthetic O<sub>2</sub> production (Porter et al., 1989; Wangpraseurt et al., 2017b; Rodrigues and Grottoli, 2007); however, the effects of bleaching on O<sub>2</sub> levels in the coral microenvironment have not been thoroughly characterized. Similarly, respiration by the coral holobiont at night can result in hypoxia (or even anoxia) in healthy corals (Linsmayer et al., 2020; Köhl et al., 1995; Hughes et al., 2020; Shashar et al., 1993; Wangpraseurt et al., 2012b), but this aspect has not been investigated in bleached corals. In the presence of enough O<sub>2</sub>, organisms can sustain mitochondrial aerobic respiration; however, when O<sub>2</sub> is scarce, organisms become more dependent on fermentation. Since the former is much more efficient at producing ATP from food sources, O<sub>2</sub> availability critically affects energy budgets (Hochachka and Somero, 2002), making O<sub>2</sub> dynamics an overlooked aspect of coral bleaching with potential important implications for survival. Understanding aerobic and anaerobic coral energy metabolism gains additional significance when we consider reports of increasing hypoxic

conditions of coral reefs worldwide resulting from ocean warming (Pezner et al., 2023) and expansion of ‘dead zones’ (Altieri et al., 2017).

With this in mind, we induced thermal bleaching of two important Caribbean reef building coral species, *Agaricia agaricites* and *Orbicella franksi*; the latter is generally more resilient to bleaching and tends to recover faster than other Caribbean species. Coral colonies were taken to a flow-through outdoor aquarium and subjected to an increase in temperature of 3 °C to match conditions experienced during the 2010 bleaching event (Neal et al., 2017). Using O<sub>2</sub> microsensors, we measured photosynthesis and respiration rates as well as O<sub>2</sub> levels within the diffusive boundary layer (DBL) of control colonies kept at ambient temperature, and unbleached, partially bleached, and fully bleached colonies sampled throughout exposure to increased temperature. Control and fully bleached corals were then sampled for genetic profiling of Symbiodineaceae and for enzymatic activities of citrate synthase (CS), malate dehydrogenase (MDH), lactate dehydrogenase (LDH), and strombine dehydrogenase (SDH) as proxies for aerobic and fermentative energy metabolic capacity (Linsmayer et al., 2020).

## 2. Methods

### 2.1. Coral collection and experimental aquarium

In January 2017, 20 colonies each of *A. agaricites* and *O. franksi* (about 10 cm diameter) were collected on SCUBA using a hammer and chisel under their dead bases at 6–8-m depth at Punta Caracol, Bahia del Almirante, Bocas Del Toro, Panama (9.363°N, 82.282°W). Care was taken to collect *O. franksi* with a dark brown coloration and *A. agaricites* with a dark green coloration, indicative of a healthy state for each species. The collection and research permit was provided by the Ministerio de Ambiente de Panamá (SE/AO-8-16). Coral colonies were immediately transported to the Smithsonian Tropical Research Institute Bocas Research Station (STRI-Bocas) in seawater-containing buckets and placed in the flow-through outdoor aquarium supplied with seawater from Bahia del Almirante. The overall experimental design and sampling schedule is shown in FigS1.

### 2.2. Induction of thermal bleaching and terminal sampling

After a 11-day acclimatization to the aquarium system, 10 coral colonies of each species were randomly distributed in one of two 76-L tanks supplied with flow-through seawater and equipped with aquarium pumps (1287 L hr<sup>-1</sup>, Hydor Koralia Nano 3.5 W Aquarium Circulation Pumps, USA) to promote seawater circulation. One tank was maintained at ambient temperature (“Control”; 28.5 ± 0.1 °C) and the other tank was warmed +3 °C above the Control tank (“Thermal treatment”; 31.3 ± 0.2 °C) using two 500 W titanium aquarium heaters (Finnex HMX 500 W Titanium Heater, USA). Seawater temperature was raised 1 °C per day over three days. Salinity (32.3 ± 0.2 ppt) and temperature were recorded throughout the experiment using a YSI probe (YSI Pro2030, Xylem Brand, Ohio, USA).

The coral colonies were repositioned weekly throughout the tanks to minimize positional effects. Coral colonies were inspected daily and bleaching progression was assessed approximately weekly using the CoralWatch Coral Health Chart (Siebeck et al., 2008). Each colony was assigned into one of three categories: Unbleached (Level 6 in the chart), partially bleached (Levels 5.5–3.5), or fully bleached (Level ≤ 3). Colonies showing an heterogeneous color pattern were assigned a score that reflected the average color of the colony.

All colonies started at state 6 (B6 for *A. agaricites*, E6 for *O. franksi*), and control colonies remained at that state throughout the experiment. Terminal sampling took place around 6 p.m. Each colony was quickly drip-dried to remove seawater and mucus and snap frozen in liquid N<sub>2</sub>; the frozen samples were stored in a dry shipper (−150 °C) until homogenization for the various assays listed below. All control corals were sampled on Day 34; however, the coral colonies bleached at different rates so terminal sampling of *A. agaricites* occurred between Day 24–38 and of *O. franksi* colonies on Days 38 and 55. In the thermal treatment, sudden tissue sloughing occurred in one *A. agaricites* colony at Day 2 and in two *O. franksi* colonies at Day 35; these colonies were discarded and, as a result, the number of replicates for the experiments that used terminal samples was  $n = 9$  for *A. agaricites* and  $n = 8$  for *O. franksi*.

### 2.3. O<sub>2</sub> microsensor measurements

O<sub>2</sub> microsensor measurements were taken on “unbleached corals” during the nine days prior to inducing thermal stress, on “partially bleached corals” halfway through thermal stress (Day 13–27), and on “fully bleached corals” (Day 33–55). Measurements on corals from the control temperature treatment (“control corals”) were taken between Days 31–34. Coral colonies were transported to an indoor 76-L tank with flow-through seawater that was heated to the same temperature as the treatment or control tank. LED lights (XR15wPro EchoTech Radion, Pennsylvania, USA) suspended above the tank provided a downwelling light intensity of  $215 \pm 1 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  (measured using an Odyssey PAR logger at the top of the colonies). O<sub>2</sub> microsensor measurements were performed in a flow chamber that delivered an inflow velocity of 1 L/min, mimicking the flow environment of the main tanks during the bleaching experiment. The O<sub>2</sub> microsensors were connected to a multimeter operated using the SensorTrace Logger software (Unisense, Aarhus, Denmark) and mounted on a manually-operated micro-manipulator (World Precision Instruments, USA) at a 20° angle relative to the incident vertical light (Kühl et al., 1995). Photosynthesis and respiration rates were determined using Clark-type O<sub>2</sub> microsensors with a 90 % response time of <0.5 s and a tip diameter of 25  $\mu\text{m}$  (Ox25-fast, Unisense), which were calibrated daily against air-saturated seawater (100 % at experimental temperature and salinity) and anoxic water (0.1 M sodium ascorbate +0.1 M NaOH).

Before O<sub>2</sub> measurements, colonies were allowed to reach steady state in the light or dark, which typically took between 5 and 10 min. O<sub>2</sub> microsensor profiles were performed from the coral tissue surface in 0.5 mm steps through the diffusive boundary layer (DBL) into the ambient water column. O<sub>2</sub> profiles were exclusively performed on coenosarc tissue to minimize potential interference from polyp movement (Kühl et al., 1995). Two replicate sets of photosynthesis (P) and respiration (R) measurements were made on each coral colony on coenosarc tissue spots at least 1 cm away, which were averaged prior to statistical analysis. On thermally stressed *O. franksi* that displayed patchy pigmentation, the O<sub>2</sub> microsensors were placed on areas with average color. Net photosynthesis (P<sub>N</sub>) and dark respiration (R<sub>D</sub>) were estimated as the diffusive O<sub>2</sub> flux from steady state O<sub>2</sub> microsensor profiles in light and dark, respectively, using Fick's first law of diffusion:

$$J = -D_0 \cdot dC/dz,$$

where  $D_0$  is the molecular diffusion coefficient of O<sub>2</sub> ( $D_0 = 2.5781 \times 10^{-5} \text{ cm}^2 \text{s}^{-1}$ ) and  $dC/dz$  is the measured slope of the O<sub>2</sub> concentration profile in the linear part of the diffusive boundary layer (DBL).

Volumetric gross photosynthesis (P<sub>G</sub>) (nmol O<sub>2</sub> cm<sup>−3</sup> s<sup>−1</sup>) was estimated using the light-dark shift technique (Revsbech and Jørgensen, 1983; Glud et al., 1992). To obtain areal P<sub>G</sub> rates, the volumetric rates were depth-integrated over the living photic tissue and assuming homogenous photosynthetic rates across coenosarc tissue thickness (*A. agaricites* =  $227 \pm 43 \mu\text{m}$ , *O. franksi* =  $200 \pm 29 \mu\text{m}$  for *O. franksi*, measured using a needle attached to a micromanipulator). Light

respiration (R<sub>L</sub>) was calculated (Kühl et al., 1995) as the difference between areal P<sub>G</sub> and P<sub>N</sub> ( $R_L = P_G - P_N$ ). After the P and R measurements, steady-state O<sub>2</sub> concentration profiles were measured from the coral tissue surface through the DBL.

### 2.4. Coral surface area, chlorophyll a concentration, and Symbiodiniaceae density

Tissue was removed from the flash frozen corals with an airbrush (Szmant and Gassman, 1990) and homogenization buffer (100 mM Tris, pH 7.5). Tissue slurries were homogenized further on ice using a T8.10 Ultra-Turrax Werke handheld electronic tissue grinder (IKA Dispergierstation, Germany) set at 25,000 rpm and immediately re-frozen in liquid N<sub>2</sub>. The coral tissue homogenates were stored at −80 °C until Symbiodiniaceae, chlorophyll a (Chl a), and enzymatic activity analyses. The surface area of bare skeletons was measured using the single-dip paraffin wax method (Veal et al., 2010).

Coral tissue homogenates were thawed and spun down (15,500 xg, 10 min); the pellets were washed twice and resuspended in homogenization buffer and aliquots were saved for Symbiodiniaceae cell counts, another pellet aliquot was preserved in 95 % ethanol at −20 °C for genotyping. The remaining sample was spun down again and the pellet was resuspended in 90 % acetone and sonicated in the dark (four pulses of 10 s on ice; 50 s rest in between pulses). The acetone extract was spun down as before; the supernatant was measured on a spectrophotometer (UV-1280, Shimadzu Scientific Instruments, Maryland, USA) at 630 nm and 663 nm and the readings were used to calculate Chl a content (Jeffrey and Humphrey, 1975). Chl a content was normalized to surface area and to Symbiodiniaceae density.

Symbiodiniaceae cells were counted on coral tissue homogenates on a hemocytometer (Hausser Phase Contrast Hemacytometer, Pennsylvania, USA) at 40× magnification on a binocular stereo dissecting microscope. Values from two replicates per colony were averaged and normalized to surface area.

### 2.5. Enzyme activity assays

CS, MDH, SDH, and LDH activities were measured in total tissue homogenates using methods optimized for corals (Linsmayer et al., 2020). Enzyme assays were conducted at 28 °C on a Shimadzu UV-1280 spectrophotometer. Coral tissue homogenates were combined with CS assay buffer (2.0 mM MgCl<sub>2</sub>, 0.1 mM 5,5'-dithio-bis-[2-nitrobenzoic acid], 0.1 mM acetyl CoA, 0.5 mM oxaloacetate, 80 mM Tris pH 8.0), MDH buffer (100 mM KCl, 0.3 mM oxaloacetate, 0.15 mM NADH in 80 mM imidazole-HCl buffer pH 7.0), SDH assay (100 mM glycine, 0.3 mM Na<sup>+</sup>-pyruvate, 0.3 mM NADH in 100 mM Tris-HCl pH 7.0), or LDH buffer (100 mM KCl, 1.0 mM Na<sup>+</sup>-pyruvate, 0.15 mM NADH in 50 mM imidazole buffer pH 7.0) (1,15 v:v homogenate:buffer ratio). Activity rates were calculated from the decrease in absorbance over the linear range of the reaction (typically the first minute) at 412 nm (CS) or 340 nm (MDH, SDH and LDH). Enzymatic activities were standardized to total soluble protein concentration determined in matching coral tissue homogenates by the Bradford method (Bradford, 1976) (Bio-Rad Protein Assay kit, Life Science Group, USA) and expressed as nmol substrate mg<sub>prot</sub><sup>−1</sup> min<sup>−1</sup>. A few enzyme activity values were slightly negative after blank subtraction and were considered as zero.

### 2.6. Symbiodiniaceae genotype profiling

Prior to DNA isolation, tubes containing Symbiodiniaceae were centrifuged at 12,000 g for 8 min and ethanol carefully removed by pipetting. We used the protocol of the Qiagen Powersoil DNA extraction kit with three modifications to increase DNA yield: (1) extended bead beating step to 10 min on a vortex adaptor at maximum speed during homogenization, (2) additional 10 min incubation at 56 °C, (3) DNA was eluted in 50  $\mu\text{L}$ . A two-step Polymerase Chain Reaction (PCR) strategy

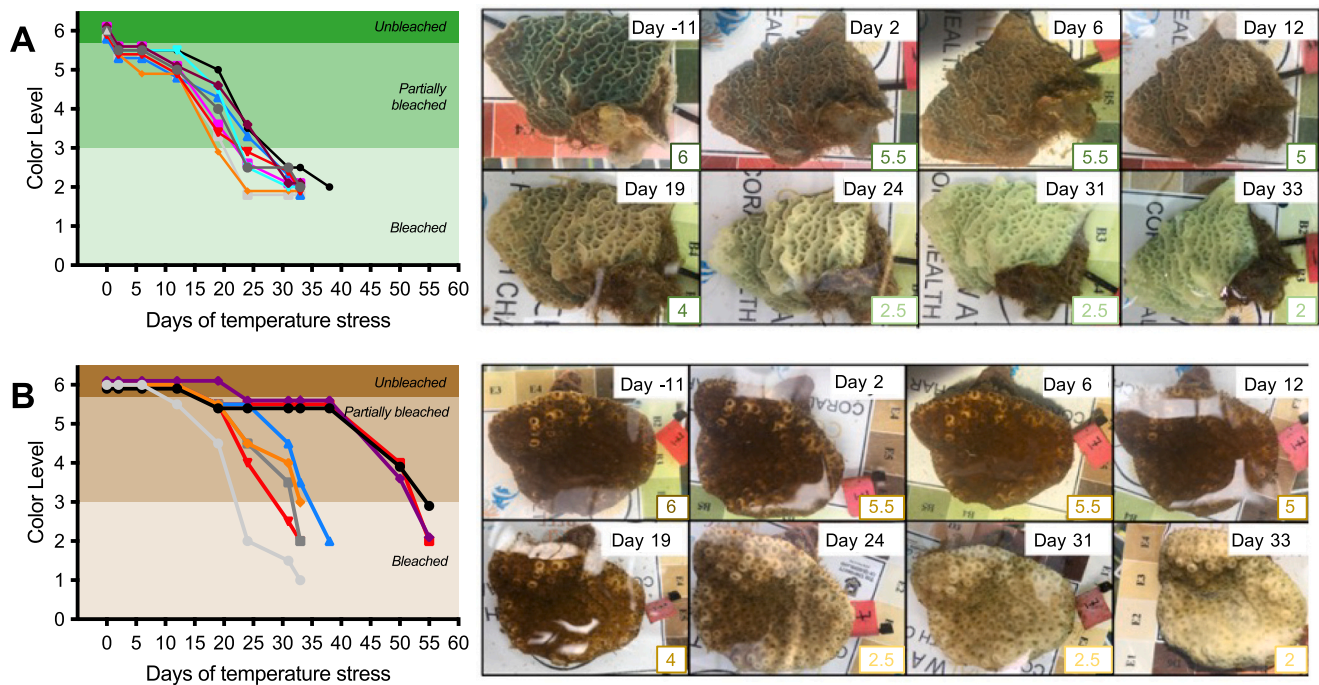


**Fig. 1.** Progression of coral bleaching during + 3 °C thermal stress. (A) *A. agaricites* colonies ( $n = 9$ ) bleached at similar rates and within 31 days. (B) *O. franksi* colonies ( $n = 8$ ) bleached at different rates, and three colonies took 55 days to bleach. Each line (each drawn in a different color for clarity) represents observations of one coral colony. The side panels show one colony of each species that bleached at comparable rates. The color levels are shown in the bottom right corner of each image. “Day -11” = on the day of collection from the field, 11 days before starting the thermal treatment. See FigS2 for photos of all the coral colonies throughout the experiment.

was used for amplicon library preparation (Bradford, 1976). The first PCR (amplicon PCR) used primers specific to the internal transcribed spacer 2 (ITS2) region of the nuclear ribosomal cistron [“ITS-DINO” forward (5'-GTGAATTGCAGAACTCCGTG-3') and “ITS2Rev2” reverse (5'-CCTCCGCTTACTTATATGCTT-3') (Lasker et al., 1984; Jones, 1997)] to which Illumina overhang sequences were appended. The second PCR (index PCR) incorporated barcodes and Illumina index adapters. Amplicon PCRs were conducted in 11  $\mu$ L reactions containing 5.1  $\mu$ L ultrapure water (Invitrogen), 5.0  $\mu$ L of Platinum™ II Hot-Start PCR MasterMix (2 $\times$ ) (ThermoFisher), 0.2  $\mu$ L of 10  $\mu$ M forward and reverse primers and 1  $\mu$ L of DNA 2–10 ng). The following cycling conditions were run on an Applied Biosystems thermal cycler (Thermo Fisher): 94 °C for 3 min; 40 cycles of 94 °C for 30 s, 52 °C for 30 s and 72 °C for 90 s; 72 °C for 7 min. Three independent PCRs were conducted for each sample, checked on a 1.5 % TAE agarose gel and triplicate PCRs were pooled per sample. Pooled PCR reactions were purified using solid-phase reversible immobilization KAPA HyperPure beads (Roche) with a ratio bead:sample of 1.6:1, quantified using a dsDNA Qubit Fluorometer (Invitrogen) and concentrations were standardized for each sample to 5 ng/ $\mu$ L. Index PCRs were performed in 10  $\mu$ L reactions, with 1.0  $\mu$ L (5 ng) of purified PCR, 1.0  $\mu$ L of each index primer (10  $\mu$ M), 2.0  $\mu$ L of ultrapure water, 5.0  $\mu$ L of Platinum II™ Hot-Start PCR Master Mix (2X) (Invitrogen) using the following cycling conditions: 94 °C for 3 min; 6 cycles of 94 °C for 45 s, 50 °C for 60 s, 72 °C for 90 s; and finally 72 °C for 2 min.

The product of all index PCRs was pooled and purified twice consecutively using KAPA HyperPure Beads (Roche) with a ratio bead: sample of 1:1. Clean index PCR product was quantified using the dsDNA Qubit Fluorometer (Invitrogen) and the concentration was standardized by dilution to 10 ng/ $\mu$ L solution. Paired-end sequencing was performed on an Illumina MiSeq platform at the Smithsonian Tropical Research Institute (Panama) using a 2  $\times$  250 v2 kit following the manufacturer's protocol. Raw sequence data in FASTQ format were sorted by sample based on unique index sequence combinations and uploaded to SymPortal (Le Tissier and Brown, 1996). The absolute and relative count

tables obtained from SymPortal were imported into R (Falkowski et al., 1984) for downstream analyses and visualizations using the vegan (Muscatine et al., 1984), phyloseq (Muscatine et al., 1984), and ggplot2 (Patterson et al., 1991) packages. To explore potential regional differences in Symbiodiniaceae genotype profiling, subsamples of four control *A. agaricites* colonies, four bleached *A. agaricites* colonies, five control *O. franksi* colonies and four bleached *O. franksi* colonies were analyzed.

## 2.7. Statistical analyses

Data is presented as mean  $\pm$  SEM and analyzed using two-way analysis of variance (2-way ANOVA) followed by Tukey's or Šidák's multiple comparisons tests when appropriate as described in the figure captions. CS and SDH data were square root transformed and MDH and LDH were log transformed prior to 2-way ANOVA. All statistical analyses were done in Prism 9 for macOS (GraphPad Software, La Jolla, CA).

## 3. Results and discussion

### 3.1. Progression of coral bleaching

Matching previous reports (Neal et al., 2017; Lasker et al., 1984), the progression of thermal bleaching was faster in *A. agaricites* than in *O. franksi*. All *A. agaricites* colonies experienced color loss within two days of the start of the thermal treatment although temperature had only been ramped up 2 °C. The loss in color in *A. agaricites* was homogenous throughout each colony, and all colonies were fully bleached within 19–31 days of thermal stress (Fig. 1A). In contrast, the first color loss in an *O. franksi* colony was observed on Day 12, the other colonies did not lose any color until Day 19–24, the discoloration pattern was patchy, and many bleached colonies retained brown pigmentation in the polyp mouth area (Fig. 1B). Furthermore, *O. franksi* colonies took longer to fully bleach: five colonies reached Level  $\leq 3$  by Day 38 but the three remaining colonies were at stage 5.5 at that time. These colonies were

**Table 1**  
Symbiodiniaceae density and chlorophyll *a* content in control and fully bleached corals. Sym = Symbiodiniaceae. The number of replicates for each determination is in parentheses. <sup>†</sup>Colonies with undetectable numbers of Sym were omitted from this analysis.

|                      |          | Sym density 10 <sup>6</sup> Sym cm <sup>-2</sup> | Chl <i>a</i> content / Sympg Chl <i>a</i> Sym <sup>-1</sup> |
|----------------------|----------|--------------------------------------------------|-------------------------------------------------------------|
| <i>A. agaricites</i> | Control  | 0.292 ± 0.063 (9)                                | 15.0 ± 2.68 (9)                                             |
|                      | Bleached | 0.010 ± 0.005 (9)                                | 83.2 ± 27.7 (4 <sup>†</sup> )                               |
| <i>O. franksi</i>    | Control  | 0.490 ± 0.010 (9)                                | 12.7 ± 2.43 (9)                                             |
|                      | Bleached | 0.033 ± 0.014 (8)                                | 58.2 ± 11.8 (7 <sup>†</sup> )                               |

kept under thermal stress and were sampled on Day 55 when they were at Level 2–3.

Post-mortem analyses confirmed significantly decreased Symbiodiniaceae density in bleached colonies of both *A. agaricites* and *O. franksi*. Additionally, Symbiodiniaceae from bleached colonies had significantly increased chlorophyll content, a phenomenon previously reported in corals that had bleached in the wild (Jones, 1997; Le Tissier and Brown, 1996) (Table 1).

### 3.2. Effects of bleaching on photosynthesis

Unbleached and control corals had robust P<sub>G</sub>, volumetric P<sub>G</sub>, and P<sub>N</sub> rates, all of which decreased as bleaching progressed (Fig. 2A–C). In *O. franksi*, control colonies had a higher P<sub>G</sub> than unbleached colonies

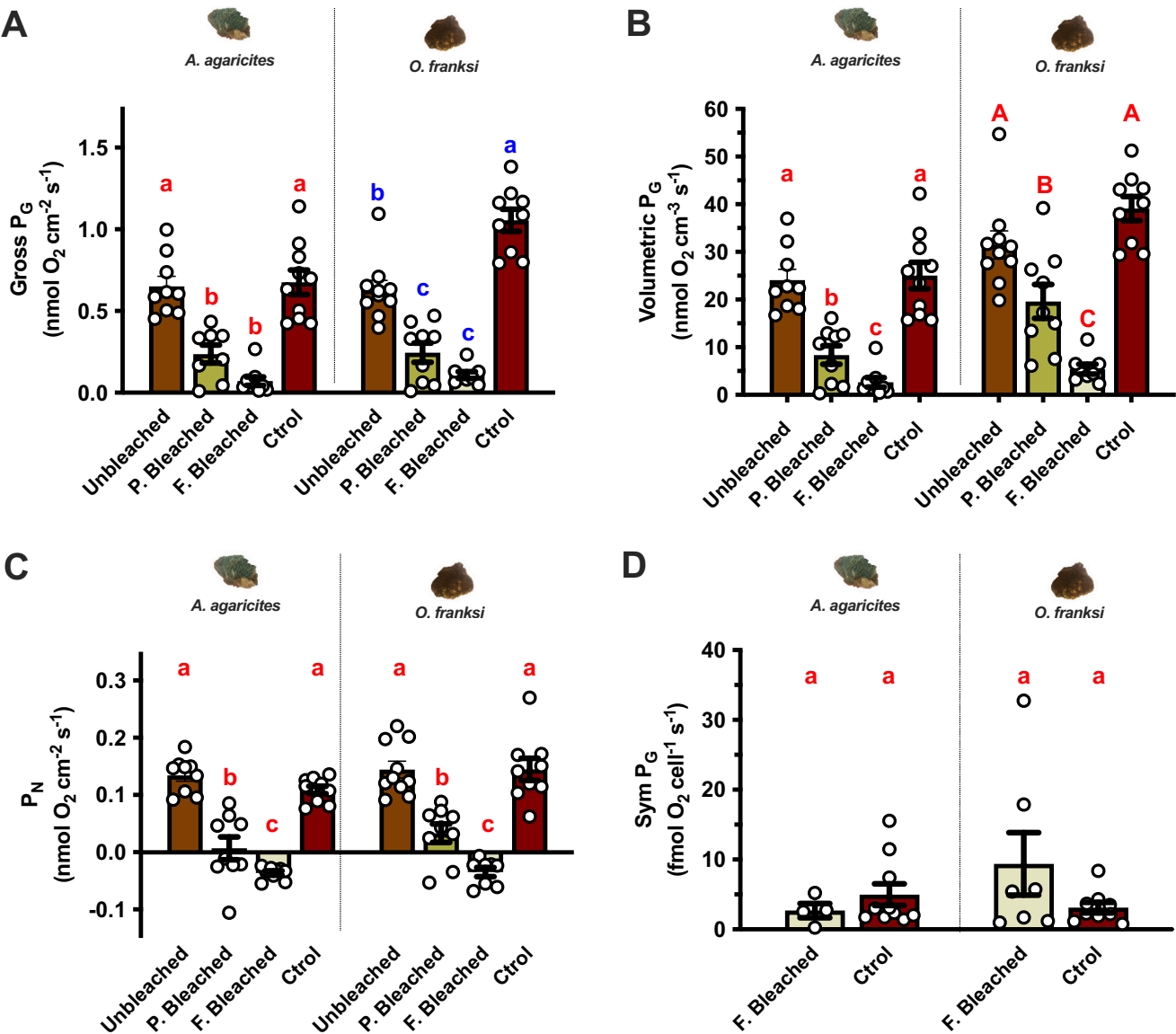


**Fig. 2.** Photosynthesis rates during progression of thermal induced bleaching. Rates of A) Gross photosynthesis (P<sub>G</sub>); B) Volumetric P<sub>G</sub>; C) Net photosynthesis (P<sub>N</sub>); D) Symbiodiniaceae (Sym) cell-specific P<sub>G</sub>. The letters indicate statistical significance based on 2-way ANOVA (factors: “bleaching status” and “coral species”). P<sub>G</sub> (shown in A) demonstrated a significant effect of both factors as well as their interaction; differences within each coral species (red or blue letters) were dissected using Šidák's test. Volumetric P<sub>G</sub> (shown in B) had no significant interaction between factors and *O. franksi* values were significantly greater than those of *A. agaricites* (indicated with lowercase vs. capitalized letters); differences between “bleaching status” were dissected using Tukey's test. P<sub>N</sub> (shown in C) had no significant interaction between factors and no effect of coral species; differences between “bleaching status” were dissected using Tukey's test. There were no statistical differences in Sym P<sub>G</sub> between factors and there was no interaction. The level of significance ( $\alpha$ ) in the post-hoc tests was corrected for multiple comparisons. Calculation of Sym cell-specific P<sub>G</sub> requires terminal sampling and thus it was only possible in F. Bleached with detectable Sym numbers and Ctrol colonies. See Table S1 for summary values and number of replicates ( $N = 8-10$ ).

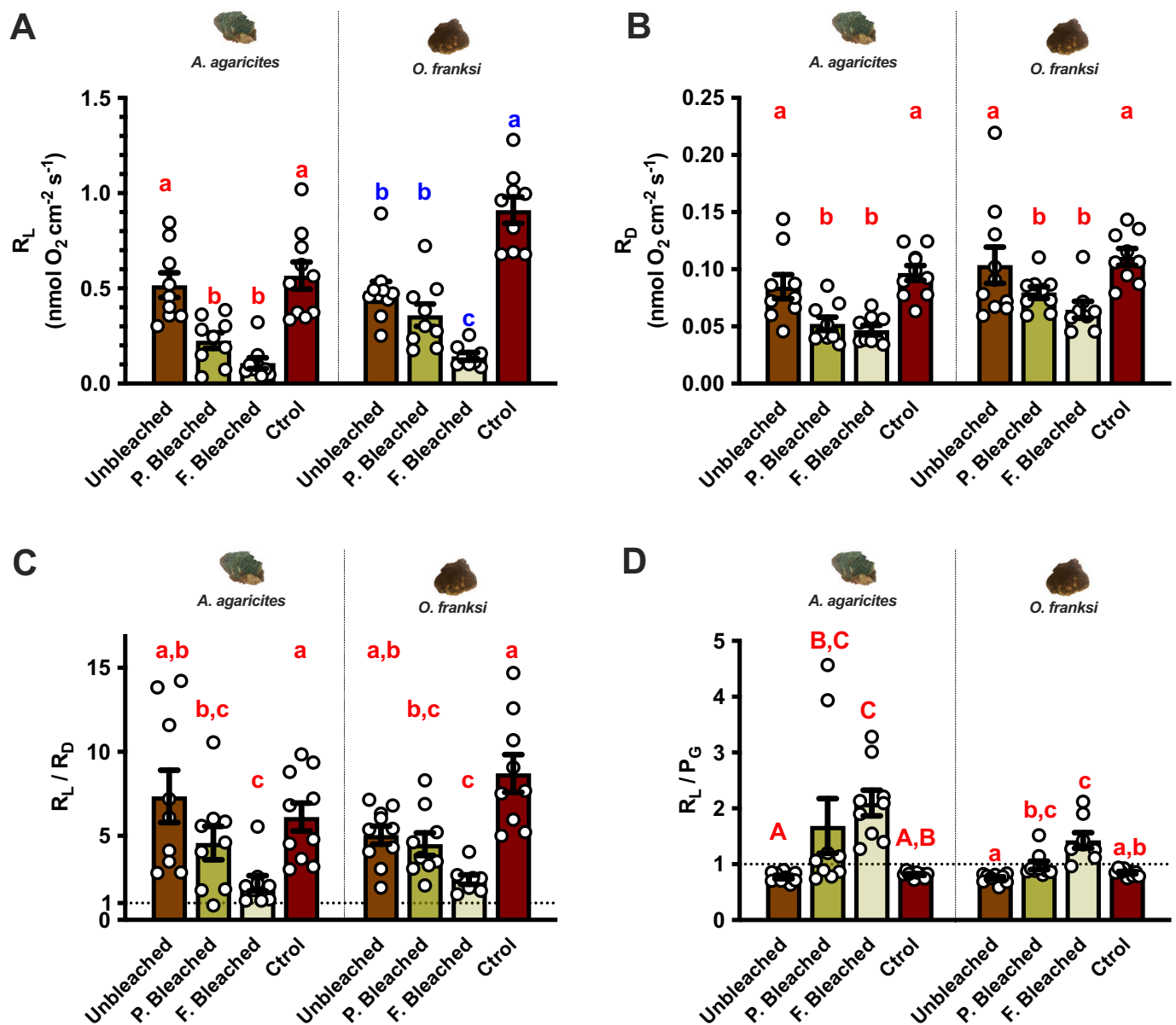


**Fig. 3.** Respiration rates and ratios during progression of thermal induced bleaching. Rates of A) Light respiration ( $R_L$ ); B) dark respiration ( $R_D$ ); C)  $R_L/R_D$  ratio; D)  $R_L/P_G$  ratio. The letters indicate statistical significance based on 2-way ANOVA (factors: “bleaching status” and “coral species”).  $R_L$  (shown in A) demonstrated a significant effect of both factors as well as their interaction; differences within each coral species (red or blue letters) were dissected using Sidák’s test.  $R_D$  (shown in B) and  $R_L/R_D$  (shown in C) had no significant interaction between factors and no effect of coral species; differences between “bleaching status” were dissected using Tukey’s test.  $R_L/P_G$  (shown in D) had no significant interaction between factors and *A. agaricites* values were significantly greater than those of *O. franksi* (indicated with lowercase vs. capitalized letters); differences between “bleaching status” were dissected using Tukey’s test. The level of significance ( $\alpha$ ) in the post-hoc tests was corrected for multiple comparisons. The dotted lines in C and D indicate a value of 1; i.e.  $R_L = R_D$  and  $R_L = P_G$ , respectively. See Table S2 for summary values and number of replicates ( $N = 8-10$ ).

(Fig. 2A), which perhaps could be due to putative differences in Symbiodiniaceae density or to having been measured at different time points > 1 month from each other (SFig. 1). Regardless, the two coral species demonstrated the same general response to warming whereby  $P_G$  in partially bleached corals was reduced to about half compared to unbleached corals (i.e. pre-heat stress), while fully bleached corals still sustained some  $P_G$  but at only about 10 % of the original values (Fig. 2A). Volumetric  $P_G$  followed the same pattern (Fig. 2B), although *O. franksi* rates were significantly higher due to their thicker tissue. In addition,  $P_N$  was virtually zero in partially bleached corals and reached negative values in fully bleached corals (Fig. 2C), indicating that photosynthetic O<sub>2</sub> production matched respiration in the former while the coral holobiont was net respiring during daytime. This indicates that

bleached corals must have taken up O<sub>2</sub> from the seawater to support light respiration. Despite the higher per cell chlorophyll content in the Symbiodiniaceae that remained in bleached colonies (Table 1), their cell-specific  $P_G$  was not significantly different from that of the Symbiodiniaceae in control colonies (Fig. 2D).

### 3.3. Effects of bleaching on respiration

Similar to  $P_G$  (Fig. 2A), the  $R_L$  of control *O. franksi* colonies was greater than that of unbleached *O. franksi* colonies and while the causes are unknown we speculate they are due differences in Symbiodiniaceae density. Regardless, both *A. agaricites* and *O. franksi* colonies experienced progressive decreases in  $R_L$  (Fig. 3A) and  $R_D$  (Fig. 3B) as bleaching

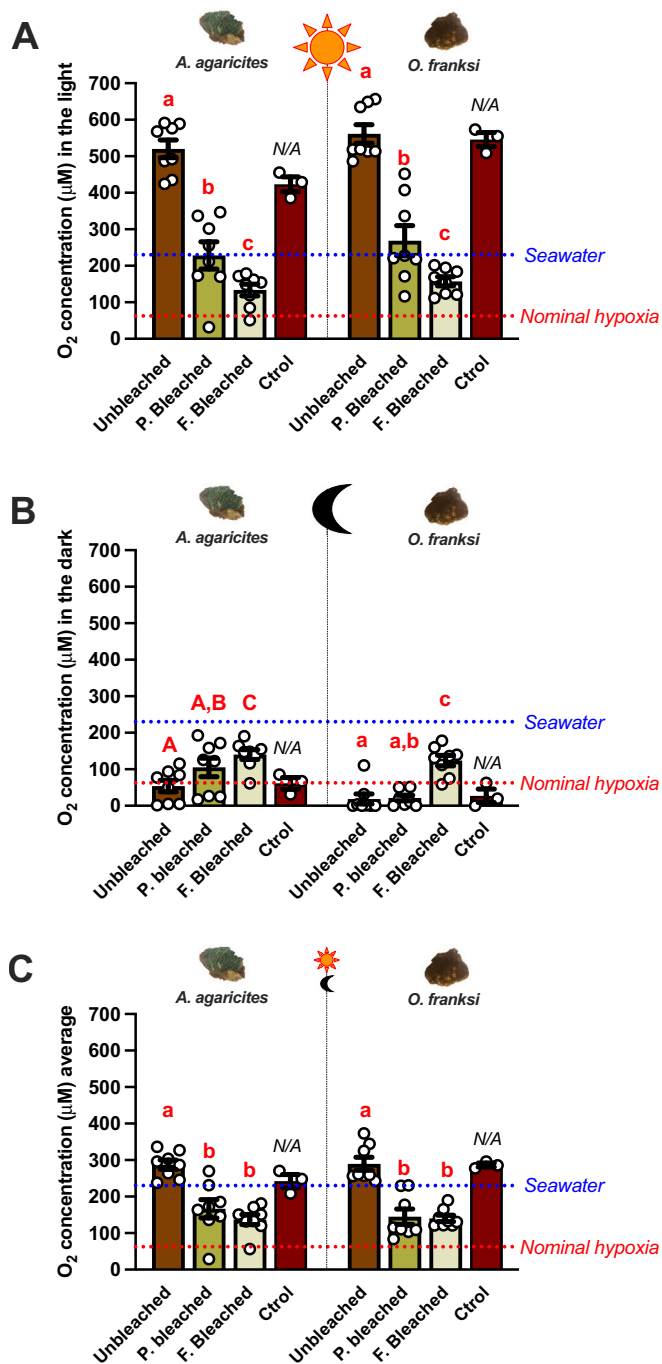


**Fig. 4.** O<sub>2</sub> concentration at the coral tissue surface during progression of thermal induced bleaching. (A) Light conditions; (B) dark conditions; (C) average light/dark. The letters indicate statistical significance based on 2-way ANOVA (factors: “bleaching status” and “coral species”). “O<sub>2</sub> in the light” (shown in A) and “Average O<sub>2</sub>” (shown in C) had no significant interaction between factors and no effect of coral species; differences between “bleaching status” were dissected using Tukey’s test. “O<sub>2</sub> in the dark” (shown in B) had no significant interaction between factors and *A. agaricites* values were significantly greater than those of *O. franksi* (indicated with lowercase vs. capitalized letters); differences between “bleaching status” were dissected using Tukey’s test. The level of significance ( $\alpha$ ) in the post-hoc tests was corrected for multiple comparisons. NA = this treatment was not included in the statistical analysis because only three colonies were measured due to time constraints. The dotted red line corresponds to nominal hypoxia (62.5  $\mu$ M) and the dotted blue line corresponds to average O<sub>2</sub> concentrations in the tank (Seawater) in light (229  $\mu$ M) and dark (226  $\mu$ M). See Table S3 for summary values.

progressed, which were associated with a marked reduction in the  $R_L/R_D$  ratio (a measurement for the relative aerobic respiration rate in light vs. dark) (Fig. 3C). The decrease in  $R_L$  is linked to the lower photosynthetic rates due to the losses of symbiotic algae and photosynthetic O<sub>2</sub> production (Fig. 2), likely coupled with decreased photosynthetic sugar supply (Grottoli et al., 2006; Falkowski et al., 1984; Muscatine et al., 1984; Patterson et al., 1991) that fuels mitochondrial aerobic respiration. In the presence of photosynthetic activity,  $R_L$  of bleached *A. agaricites* was about 20 % and  $R_L$  of bleached *O. franksi* was about 33 % of their respective control and unbleached colonies. In the dark -in the absence of photosynthesis-  $R_D$  of bleached colonies of both species was approximately halved compared to control and unbleached colonies, which reflects the lower density of symbiotic algae (which also respire), and perhaps coupled with decreased aerobic metabolism of coral host cells. The  $R_L/R_D$  ratio of unbleached and control colonies was about 7 (which aligns well with previous reports on *Favia* sp. (Kühl et al., 1995)) but it was only about 2 in bleached corals reflecting the greater effect of bleaching on  $R_L$  than on  $R_D$ . Interestingly, this metric also suggests that bleached corals sustain higher aerobic rates in the light than in the dark, which could be enabled by photosynthetic O<sub>2</sub> and sugar supply of the symbiotic algae that remain in the coral tissues, increased activity that consumes ATP, or a combination. We also calculated the  $R_L/P_G$  ratio (Fig. 3D) to quantify the relative availability of photosymbiont produced O<sub>2</sub> ( $P_G$ ) to support  $R_L$ . Unbleached and control colonies had a  $R_L/P_G$  ratio of about 0.8, indicating that photosynthetic O<sub>2</sub> production was in excess of aerobic respiration rate. In contrast, partially and fully bleached colonies had a  $R_L/P_G$  ratio  $> 1$ , suggesting the need of taking up additional O<sub>2</sub> from the water column to sustain aerobic metabolism (even though  $R_L$  of bleached colonies was about 5-fold lower than that of unbleached colonies). This identifies a potential vulnerability of bleached corals during environmental hypoxia that might be associated with high temperature events, eutrophication and expansion of “dead zones” (Altieri et al., 2017). Interestingly, the  $R_L/P_G$  ratio of bleached *A. agaricites* (2.1) was greater than that of bleached *O. franksi* (1.4). Interestingly, *A. lamarcki* (which is closely related to *A. agaricites*) has been reported to be especially vulnerable to hypoxia in terms of both bleaching and survival (Altieri et al., 2017), which perhaps could be explained by a greater reliance on environmental O<sub>2</sub> to support aerobic processes compared to other coral species.

### 3.4. Effects of bleaching on the coral tissue O<sub>2</sub> microenvironment

The concentration of O<sub>2</sub> at the coral surface of unbleached and control colonies from both species was  $>400$   $\mu$ M O<sub>2</sub> during exposure to light (Fig. 4A), which resulted from endosymbiont photosynthesis and was hyperoxic compared to the surrounding seawater (about 220  $\mu$ M O<sub>2</sub>) as previously reported for multiple corals species (Linsmayer et al., 2020; Kühl et al., 1995; Shashar et al., 1993). As bleaching progressed, O<sub>2</sub> at the coral surface decreased, reaching values in partially bleached colonies that were indistinguishable from the water column, and below that in fully bleached colonies (about 140  $\mu$ M O<sub>2</sub>). The pattern was reversed at night (Fig. 4B). In the dark, the combined respiration of coral, symbiotic algae, and other holobiont-associated organisms in the absence of photosynthesis drew the average O<sub>2</sub> concentration at the coral surface of unbleached and control colonies down to  $\leq 60$   $\mu$ M O<sub>2</sub> in *A. agaricites* and to  $<30$   $\mu$ M O<sub>2</sub> in *O. franksi*. These are hypoxic conditions compared to the commonly used threshold of 62.5  $\mu$ M O<sub>2</sub> (Hofmann et al., 2011). The decrease in  $R_D$  that occurred as the colonies bleached (Fig. 3B) resulted in an increase in O<sub>2</sub> concentration that was significantly higher in fully bleached corals; however, O<sub>2</sub> levels remained below those found in the water column (Fig. 4B). Thus, despite partial alleviation of hypoxia in the dark compared to control corals in the same condition, fully bleached corals experienced constant near-hypoxia as well as the disruption of the diel hyperoxia/hypoxia cycle observed in control corals. We also estimated average daily coral surface O<sub>2</sub> levels from the light and the dark O<sub>2</sub> microsensor measurements

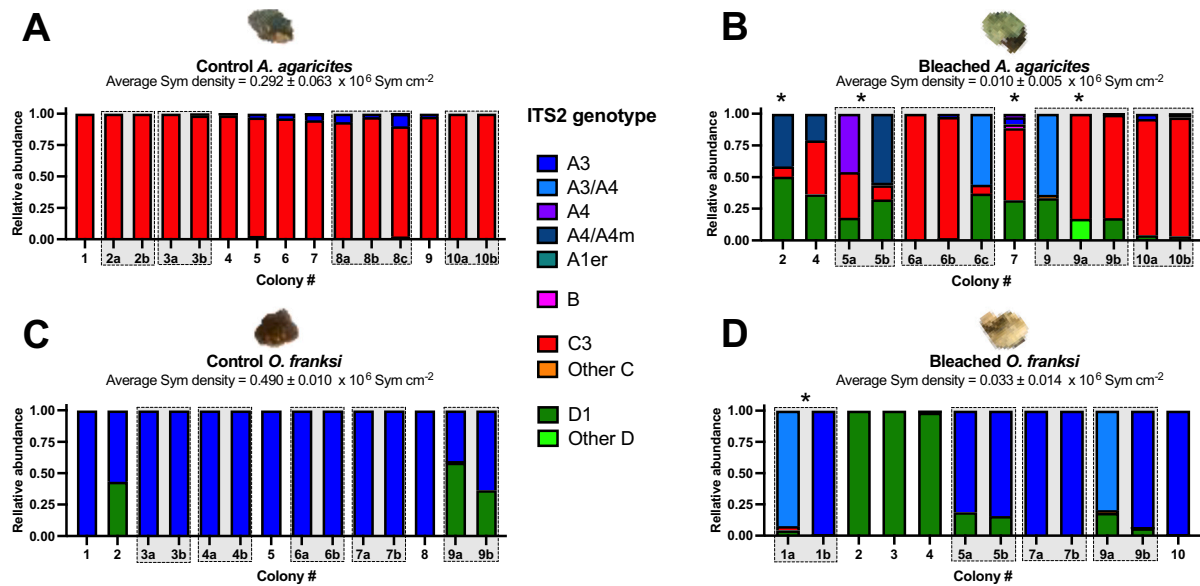


**Fig. 5.** Relative abundance of Symbiodiniaceae ITS2 type profiles in control and bleached coral colonies. A) Control *A. agaricites* colonies predominantly hosted C3 Symbiodiniaceae with very minor proportions of A3 (colonies #3, 5, 6, 7, 8, 9), A4 (colonies #4, 7, 8) and D1 (colonies #5 and 8). B) Bleached *A. agaricites* colonies had much more heterogenous Symbiodiniaceae ITS2 patterns both between colonies and within a same colony, which included C3 as well as several A, B, C and D genotypes. C) Control *O. franksi* colonies predominantly hosted A3 Symbiodiniaceae; however, colonies #2 and 9 had similar proportions of A3 and D1. D) Bleached *O. franksi* colonies had more heterogeneous Symbiodiniaceae ITS2 patterns that were dominated by A genotypes together with minor proportions of D1 and very minor proportions of C3 and other C genotypes (colonies #1, 5, 7, 9 and 10). However, colonies #2, 3, and 4 exclusively hosted the heat-stress resistant genotype D1 (with the exception of a very minor proportion of A3 in colony #4). Other C = C3an, C3b, C3gb, C42.2. Other D = D4, D5. The grey boxes indicate genotyping from different sections of a same colony (Control *A. agaricites* #2, 3, 8, 10; Bleached *A. agaricites* #5, 6, 9, 10; Control *O. franksi* #3, 4, 6, 7, 9; Bleached *O. franksi* #1, 5, 7, 9). The asterisks indicate colonies that had undetectable numbers of Symbiodiniaceae in hemocytometer determinations. As explained throughout the text and in SFig 1, all measurements come from different colonies (i.e. “control” colonies are different from “bleached” colonies, so we did not perform repeated sampling of the same colonies throughout the progression of bleaching). See Supplemental Materials for a complete list of ITS2 genotypes.

(Fig. 4C). Although  $O_2$  dynamics in the coral reef environment are bound to be more complex due to clouding, suspended sediments, currents, and presence of other organisms, our average microsensor  $O_2$  measurements can serve as a proxy for differences between unbleached and bleached corals in the wild. We found that the DBL  $O_2$  levels of unbleached corals was on average slightly hyperoxic, but that of bleached corals was in the normoxic range and approaching hypoxia (Fig. 4C), suggesting bleached corals were operating, on average, on an  $O_2$  debt.

### 3.5. Symbiodiniaceae genotyping

Symbiodiniaceae ITS2 profiling in control *A. agaricites* colonies matched previous studies that reported a dominance of ITS2 majority sequence C3 (LaJeunesse, 2002) (*Cladocopium thermophilum*) with very minor proportions of A3 (*Symbiodinium tridacnidorum*), A4 (*Symbiodinium*), B19 (*Breviolum*), B1 (*Breviolum*) and D1 (*Durudinium glynnii*) (Fig. 5A). While C3 remained the dominant ITS2 genotype in three of the bleached *A. agaricites* colonies, the other colonies were dominated by A and D1 ITS2 types. Furthermore, subsamples from some of the bleached colonies (Aa 5 and 6) had rather different ITS2 profiles (Fig. 5B), which may indicate regional heterogeneity in the distribution of Symbiodiniaceae.

Previous studies reported that *Orbicella* (formerly *Montastraea*) may simultaneously host combinations of *Symbiodinium*, *Breviolum*, *Cladocopium*, *Durudinium* and *Effrenium* (Toller et al., 2001; Rowan and Knowlton, 1995) (former clades A-E [65]). However, ITS2 profiling revealed that most of our control *O. franksi* colonies exclusively hosted Symbiodiniaceae type A3 and two colonies hosted A3 and D1 in similar proportions (Fig. 5C). Most bleached *O. franksi* colonies also hosted A3 or A3/A4 as the dominant Symbiodiniaceae type with a minor proportion of D1 type. Strikingly, the three *O. franksi* colonies that took longer

to bleach (Of 2–4) exclusively hosted Symbiodiniaceae type D1 (D1.D4, D4c.D1c.D2.D1k), which have been reported to be more resistant to bleaching than other strains (Silverstein et al., 2017; Rowan, 2004) (Fig. 5C). These results suggest that the coral colonies that took longer to bleach may have originally hosted greater proportions of type D1 Symbiodiniaceae that preferentially remained in symbiosis during thermal stress (as seen for control *O. franksi* colonies 2 and 9). However, de novo acquisition of Symbiodiniaceae from the water column cannot be ruled out. Importantly, five of the bleached colonies that were subjected to ITS2 profiling did not have any detectable Symbiodiniaceae algae by hemocytometry (indicated with asterisks in Fig. 5). While this is possible because PCR can amplify very scarce DNA, the ITS2 profile of these severely depleted symbiont communities might be functionally inert and might overestimate ITS2 profile heterogeneity. Noteworthy, all three *O. franksi* colonies that took longer to bleach (and hosted D1) had detectable Symbiodiniaceae in hemocytometry counts ( $0.073 \pm 0.018 \times 10^6$  Sym  $cm^{-2}$ ), thus suggesting that the ITS2 profiles of these colonies are biologically meaningful.

### 3.6. Metabolic energy enzymes

Lastly, we measured the activity of the enzymes CS, MDH, SDH and LDH to gain insights into the energy metabolism of control and bleached colonies (Fig. 6). CS is a rate-limiting enzyme of the citric acid cycle in mitochondria and thus CS activity is a proxy for aerobic capacity. MDH is a multifunctional enzyme that is often used as a proxy for total metabolic capacity. SDH is a dehydrogenase enzyme that oxidatively condensates pyruvate with glycine to form the opine compound strombine in the terminal step of glycolysis. SDH activity is coupled with the reduction of  $NAD^+$  into NADH thus enabling continuous ATP production under anaerobic conditions in many marine invertebrates. In addition, corals use SDH even during aerobic conditions using the abundant

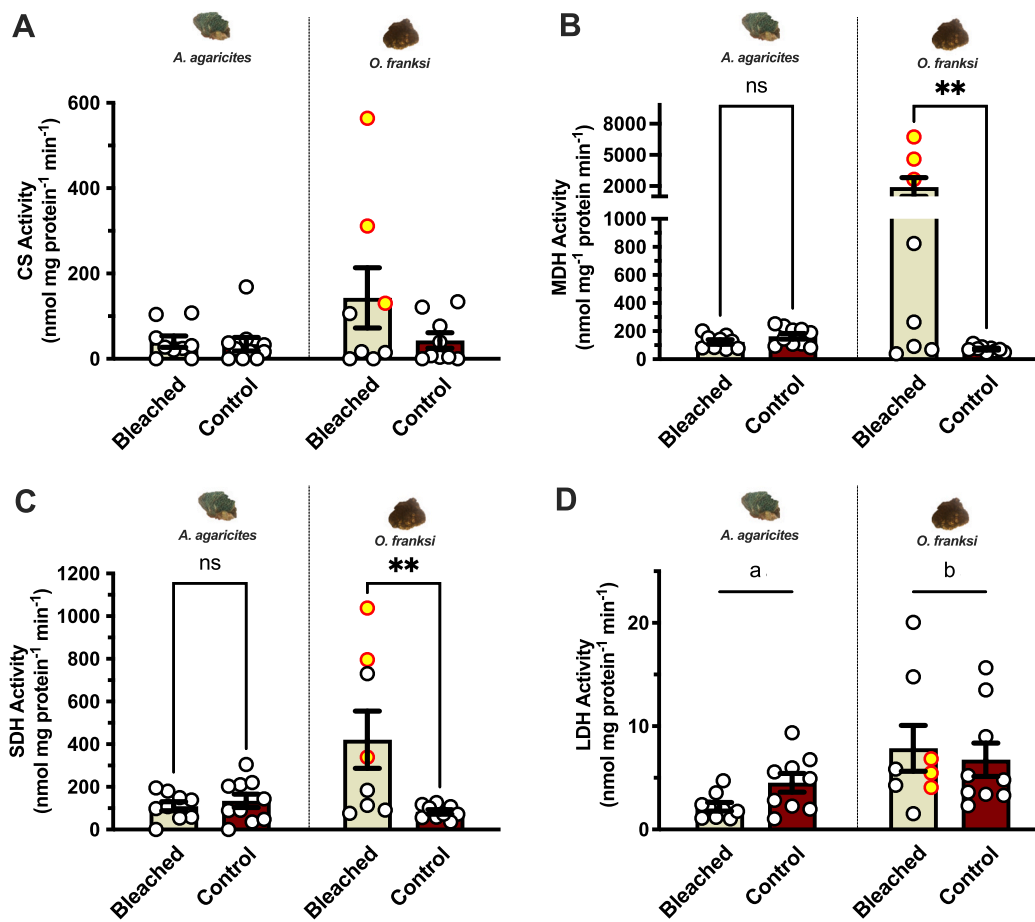


**Fig. 6.** Metabolic energy enzyme activities in control and bleached corals. (A) Citrate synthase (CS), (B) malate dehydrogenase (MDH), (C) stroma dehydrogenase (SDH), (D) lactate dehydrogenase (LDH). CS and SDH data were square root-transformed and MDH and LDH data were log-transformed prior to 2-way ANOVA (factors: “bleaching status” and “coral species”). CS (shown in A) had no significant differences in either factor, and no significant interaction. MDH and SDH (shown in B, C) had no significant effect of “coral species” but a significant effect of “bleaching status” and interaction. Differences within each species were dissected using Šídák test, indicated with \*\* ( $p < 0.005$ ). LDH (shown in D) had a significant effect of “coral species” (indicated with letters) but no significant differences between “bleaching status” or significant interaction. Values from the three *O. franksi* colonies that took longer to bleach are indicated by yellow-red circles. See Table S4 for summary values and number of replicates ( $N=8-10$ ).

sugars produced by algal photosynthesis as metabolic fuel in a process known as aerobic glycolysis (or the Warburg effect) (Linsmayer et al., 2020). LDH is the glycolytic terminal dehydrogenase in vertebrates and many invertebrates, and while we did not detect LDH activity in *Acropora yongei* (Linsmayer et al., 2020), it was present in both *A. agaricites* and *O. franksi*.

There were no significant differences in the activity levels of any of the four metabolic energy enzymes between control and bleached *A. agaricites* colonies (Fig. 6A-D). Control *O. franksi* colonies had similar CS, MDH and SDH activity levels to *A. agaricites* colonies (Fig. 6A-C) and about 50 % greater levels of LDH activity (Fig. 6D). Moreover, bleached *O. franksi* colonies had significantly greater average MDH (Fig. 6B) and SDH (Fig. 6C) activity levels than control *O. franksi* colonies, but similar LDH activity levels (Fig. 6D). This suggests an upregulation of fermentative metabolism in response to the near hypoxic conditions experienced in the DBL of bleached corals (Fig. 4). Interestingly, these differences were driven by the three *O. franksi* colonies that took longer to bleach (Fig. 1), which also happened to exclusively keep Symbiodiniaceae D1 (Fig. 5D). Since the production of strombine involves the condensation of pyruvate with the amino acid glycine (Fields, 1983; Fields and Quinn, 1981; Fields et al., 1980), the greater SDH activity in the *O. franksi* colonies that took longer to bleach may indicate links between glycine acquisition through heterotrophic feeding and transfer of amino acids (Martinez et al., 2022) or (strombine) to their

Symbiodiniaceae D1 endosymbionts. However, this possibility is highly speculative and requires experimental testing. Additionally, the *O. franksi* colonies that took longer to bleach had much higher CS activity than any other colony of either species, control or bleached. The underlying reasons are also unknown, but this observation highlights the high interindividual variability of coral colonies and the need to analyze individual datapoints rather than relying on average values for the datasets.

#### 4. Conclusions and future directions

The observed decreases in photosynthesis, respiration, and tissue  $O_2$  levels throughout the progression of bleaching have important implications for the coral holobiont, and more broadly, for coral reef ecosystems. It is widely recognized that the loss of symbiotic algae in bleached corals results in a decrease in photosynthate supply and an ensuing energy deficit (Lesser, 1997; Warner et al., 1996; Porter et al., 1989). In addition, Symbiodiniaceae photosynthesis produces an overabundance of  $O_2$  that favors aerobic respiration (Kühl et al., 1995; Shick, 1990); however, the effects of bleaching on  $O_2$  levels on corals, respiration rate, and energy metabolism have received much less attention. Our results confirm bleaching induces a major energy deficit, which in our experimental conditions was evident as a decrease in  $R_L$  to 1/5th and 1/3rd of control values in *A. agaricites* and *O. franksi*, respectively, and a

halving in  $R_D$  in both species. Moreover, our measurements cannot quantify the impact of bleaching on aerobic and anaerobic fermentation (Linsmayer et al., 2020), which are bound to be greatly impacted by the much lower photosynthate supply. Another factor that is often overlooked is that  $O_2$  availability determines the type of metabolic fuel that can be used for producing ATP. Indeed, aerobic conditions critically affect the ability to transfer energy from lipids and amino acids into ATP through  $\beta$ -oxidation and oxidative deamination coupled to the Krebs cycle. For example, while one triglyceride molecule can yield about 400 ATP molecules during aerobic conditions, it only produces 1 ATP during anoxia. Thus, the lower  $O_2$  levels experienced by bleached corals might affect their ability to utilize energy reserves.

Another interesting observation is that healthy corals have pronounced diel swings in tissue  $O_2$  levels and, the greater the number of Symbiodiniaceae, the more pronounced daytime hyperoxia and nighttime hypoxia. How does this affect interpretations of changing  $O_2$  levels on coral reefs? (c.f. (Pezner et al., 2023)). What is the impact on diel changes in microbial community composition? (c.f. (Kelly et al., 2019)). Could microbial nitrogen fixation, which is impaired by  $O_2$ , receive a boost due to the constant near-hypoxic conditions in the coral microhabitat?

Finally, our results provide information about factors that may determine coral resilience to bleaching and survival during bleaching. Our control *A. agaricites* colonies hosted a homogeneous Symbiodiniaceae population mostly comprised of *Cladocopium* (C3), they all bleached within a relatively narrow period (19–31 days), and the coral host did not experience significant changes in energy metabolic enzymes upon bleaching. In contrast, Symbiodiniaceae populations were more heterogeneous in *O. franksi*. While most colonies hosted *Symbiodinium* A3 and A4 some also contained *Durudinium* D1 (the specific type profile D1.D4.D4c.D1c.D2.D1k), which might explain why bleaching took place over a more extended and variable time (24–55 days) compared to *A. agaricites*. Interestingly, the few symbiotic algae that remained associated with the three *O. franksi* colonies that took longer to bleach were all *Durudinium*, which are notoriously heat and bleaching-resistant (Silverstein et al., 2017; Rowan, 2004). Furthermore, these *O. franksi* colonies also demonstrated highly increased levels of MDH and SDH activities, suggesting an ability to upregulate fermentative metabolism that may allow them to better cope with the nutritional and  $O_2$  deficit associated with bleaching. Hypothetically, this intraspecific variability in *O. franksi* could be a selectable trait for survival in future warmer oceans and perhaps also for directed evolution efforts.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2024.170753>.

## CRediT authorship contribution statement

**L.B. Linsmayer:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **S.K. Noel:** Investigation, Writing – review & editing. **M. Leray:** Formal analysis, Funding acquisition, Investigation, Resources, Writing – review & editing. **D. Wangpraseurt:** Data curation, Formal analysis, Writing – review & editing. **C. Hassibi:** Investigation, Writing – review & editing. **D.I. Kline:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing. **M. Tresguerres:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Martin Tresguerres reports financial support was provided by National Science Foundation. David I. Kline reports financial support was

provided by National Science Foundation. Lauren B. Linsmayer reports financial support was provided by Smithsonian Tropical Research Institute. Matthieu Leray reports financial support was provided by Gordon and Betty Moore Foundation.

## Data availability

All data is available in the supplementary material

## Acknowledgements

This work was supported by National Science Foundation Grant OCE 1538495 to DIK and MT. LBL was supported by a STRI predoctoral fellowship hosted by Dr. Nancy Knowlton and Dr. Andrew Altieri. ML was supported by a grant from the Gordon and Betty Moore Foundation GBMF5603 to STRI and UC Davis. We are grateful to the staff from the Bocas del Toro Research station (STRI) for fieldwork support, especially Plinio Góndola, Deyvis González, and Dr. Rachel Collin. We also grateful to Dr. Mark Patterson (Northwestern University) for allowing use of his  $O_2$  multimeter when our instrument succumbed to tropical humidity, and to Dr. Adi Khen (SIO-UCSD) for the coral cartoons used in the graphical abstract and Supplementary Fig. 1.

## References

- Alderice, R., et al., 2021. Divergent expression of hypoxia response systems under deoxygenation in reef-forming corals aligns with bleaching susceptibility. *Glob. Chang. Biol.* 27, 312–326.
- Altieri, A.H., et al., 2017. Tropical dead zones and mass mortalities on coral reefs. *Proc. Natl. Acad. Sci. U. S. A.* 114, 3660–3665.
- Baird, A., Marshall, P., 2002. Mortality, growth and reproduction in scleractinian corals following bleaching on the Great Barrier Reef. *Mar. Ecol. Prog. Ser.* 237, 133–141.
- Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72, 248–254.
- Coles, S.L., Jokiel, P.L., 1978. Synergistic effects of temperature, salinity and light on the hermatypic coral *Montipora verrucosa*. *Mar. Biol.* 49, 187–195.
- Cunning, R., Muller, E.B., Gates, R.D., Nisbet, R.M., 2017. A dynamic bioenergetic model for coral–Symbiodinium symbioses and coral bleaching as an alternate stable state. *J. Theor. Biol.* 431, 49–62.
- Eakin, C.M., et al., 2010. Caribbean corals in crisis: record thermal stress, bleaching, and mortality in 2005. *PLoS One* 5, e13969.
- Falkowski, P.G., Dubinsky, Z., Muscatine, L., Porter, J.W., 1984. Light and bioenergetics of a symbiotic coral. *BioScience* 34, 705–709.
- Fields, J.H.A., 1983. Alternatives to lactic acid: possible advantages. *J. Exp. Zool. Part Ecol. Genet. Physiol.* 228, 445–457.
- Fields, J.H.A., Quinn, J.F., 1981. Some theoretical considerations on cytosolic redox balance during anaerobiosis in marine invertebrates. *J. Theor. Biol.* 88, 35–45.
- Fields, J.H., Eng, A.K., Ramsden, W.D., Hochachka, P.W., Weinstein, B., 1980. Alanopine and strombine are novel imino acids produced by a dehydrogenase found in the adductor muscle of the oyster, *Crassostrea gigas*. *Arch. Biochem. Biophys.* 201, 110–114.
- Fitt, W.K., Spero, H.J., Halas, J., White, M.W., Porter, J.W., 1993. Recovery of the coral *Montastrea annularis* in the Florida Keys after the 1987 Caribbean bleaching event. *Coral Reefs* 12, 57–64.
- Fitt, W.K., McFarland, F.K., Warner, M.E., Chilcoat, G.C., 2000. Seasonal patterns of tissue biomass and densities of symbiotic dinoflagellates in reef corals and relation to coral bleaching. *Limnol. Oceanogr.* 45, 677–685.
- Gleason, D.F., Wellington, G.M., 1993. Ultraviolet radiation and coral bleaching. *Nature* 365, 836–838.
- Glud, R.N., Ramsing, N.B., Revsbech, N.P., 1992. Photosynthesis and photosynthesis-coupled respiration in natural biofilms quantified with oxygen microsenors. *J. Phycol.* 28, 51–60.
- Grottoli, A.G., Rodrigues, L.J., Juarez, C., 2004. Lipids and stable carbon isotopes in two species of Hawaiian corals, *Porites compressa* and *Montipora verrucosa*, following a bleaching event. *Mar. Biol.* 145.
- Grottoli, A.G., Rodrigues, L.J., Palardy, J.E., 2006. Heterotrophic plasticity and resilience in bleached corals. *Nature* 440, 1186–1189.
- Grottoli, A.G., et al., 2014. The cumulative impact of annual coral bleaching can turn some coral species winners into losers. *Glob. Chang. Biol.* 20, 3823–3833.
- Hochachka, P.W., Somero, G.N., 2002. *Biochemical Adaptation*. Oxford University Press, USA.
- Hoegh-Guldberg, O., 1999. Climate change, coral bleaching and the future of the world's coral reefs. *Mar. Freshw. Res.* <https://doi.org/10.1071/MF99078>.
- Hofmann, A.F., Peltzer, E.T., Walz, P.M., Brewer, P.G., 2011. Hypoxia by degrees: establishing definitions for a changing ocean. *Deep Sea Res. Part Oceanogr. Res. Pap.* 58, 1212–1226.

- Houlbrèque, F., Ferrier-Pagès, C., 2009. Heterotrophy in tropical scleractinian corals. *Biol. Rev.* 84, 1–17.
- Hueerkamp, C., Glynn, P.W., DCroz, L., Maté, J.L., Colley, S.B., 2001. Bleaching and recovery of five eastern pacific corals in an El Niño-related temperature experiment. *Bull. Mar. Sci.* 69, 215–236.
- Hughes, D.J., et al., 2020. Coral reef survival under accelerating ocean deoxygenation. *Nat. Clim. Chang.* 10, 296–307.
- Jeffrey, S.W., Humphrey, G.F., 1975. New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. *Biochem. Physiol. Pflanz.* 167, 191–194.
- Jokiel, P.L., Coles, S.L., 1990. Response of Hawaiian and other Indo-Pacific reef corals to elevated temperature. *Coral Reefs* 8, 155–162.
- Jones, R., 1997. Changes in zooxanthellar densities and chlorophyll concentrations in corals during and after a bleaching event. *Mar. Ecol. Prog. Ser.* 158, 51–59.
- Kelly, L.W., et al., 2019. Diel population and functional synchrony of microbial communities on coral reefs. *Nat. Commun.* 10, 1691.
- Kühl, M., Cohen, Y., Dalsgaard, T., Jorgensen, B.B., Revsbech, N.P., 1995. Microenvironment and photosynthesis of zooxanthellae in scleractinian corals studied with microsensors for O<sub>2</sub>, pH and light. *Mar. Ecol. Prog. Ser.* 117, 159–172.
- Lasker, H.R., Peters, E.C., Coffroth, M.A., 1984. Bleaching of reef coelenterates in the San Blas Islands, Panama. *Coral Reefs* 3, 183–190.
- LaJeunesse, T.C., 2002. Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. *Mar. Biol.* 141, 387–400.
- Le Tissier, M., Brown, B., 1996. Dynamics of solar bleaching in the intertidal reef coral *Goniastrea aspera* at Ko Phuket, Thailand. *Mar. Ecol. Prog. Ser.* 136, 235–244.
- Lesser, M.P., 1997. Oxidative stress causes coral bleaching during exposure to elevated temperatures. *Coral Reefs* 16, 187–192.
- Lesser, M.P., 2021. Eutrophication on coral reefs: what is the evidence for phase shifts, nutrient limitation and coral bleaching. *BioScience* 71, 1216–1233.
- Lesser, M.P., Stochaj, W.R., Tapley, D.W., Shick, J.M., 1990. Bleaching in coral reef anthozoans: effects of irradiance, ultraviolet radiation, and temperature on the activities of protective enzymes against active oxygen. *Coral Reefs* 8, 225–232.
- Lewis, C.L., Neely, K.L., Richardson, L.L., Rodriguez-Lanetty, M., 2017. Temporal dynamics of black band disease affecting pillar coral (*Dendrogyra cylindrus*) following two consecutive hyperthermal events on the Florida Reef Tract. *Coral Reefs* 36, 427–431.
- Linsmayer, L.B., Deheyn, D.D., Tomanek, L., Tresguerres, M., 2020. Dynamic regulation of coral energy metabolism throughout the diel cycle. *Sci. Rep.* 10, 19881.
- Loya, Y., et al., 2001. Coral bleaching: the winners and the losers. *Ecol. Lett.* 4, 122–131.
- Martinez, S., Grover, R., Baker, D.M., Ferrier-Pagès, C., 2022. Symbiodiniaceae are the first site of heterotrophic nitrogen assimilation in reef-building corals. *mBio* 13, e01601-22.
- Muñiz-Castillo, A.I., et al., 2019. Three decades of heat stress exposure in Caribbean coral reefs: a new regional delineation to enhance conservation. *Sci. Rep.* 9, 11013.
- Muscatine, L., McCloskey, L., R., Marian, R., E., 1981. Estimating the daily contribution of carbon from zooxanthellae to coral animal respiration1: Muscatine et al. *Limnol. Oceanogr.* 26, 601–611.
- Muscatine, L., Falkowski, P.G., Porter, J.W., Dubinsky, Z., 1984. Fate of the photosynthetic fixed carbon in light- and shade-adapted colonies of the symbiotic coral *Scleractinia*. *Proc. R. Soc. B Biol. Sci.* 222, 181–202.
- Neal, B.P., et al., 2017. Caribbean massive corals not recovering from repeated thermal stress events during 2005–2013. *Ecol. Evol.* 7, 1339–1353.
- Patterson, M.R., Sebens, K.P., Olson, R.R., 1991. In situ measurements of flow effects on primary production and dark respiration in reef corals. *Limnol. Oceanogr.* 36, 936–948.
- Pezner, A.K., et al., 2023. Increasing hypoxia on global coral reefs under ocean warming. *Nat. Clim. Chang.* 13, 403–409.
- Porter, J.W., Fitt, W.K., Spero, H.J., Rogers, C.S., White, M.W., 1989. Bleaching in reef corals: physiological and stable isotopic responses. *Proc. Natl. Acad. Sci.* 86, 9342–9346.
- Revsbech, N.P., Jorgensen, B.B., 1983. Photosynthesis of benthic microflora measured with high spatial resolution by the oxygen microprofile method: capabilities and limitations of the method1. *Limnol. Oceanogr.* 28, 749–756.
- Rodrigues, L.J., Grottoli, A.G., 2007. Energy reserves and metabolism as indicators of coral recovery from bleaching. *Limnol. Oceanogr.* 52, 1874–1882.
- Rowan, R., 2004. Thermal adaptation in reef coral symbionts. *Nature* 430, 742.
- Rowan, R., Knowlton, N., 1995. Intraspecific diversity and ecological zonation in coral-algal symbiosis. *Proc. Natl. Acad. Sci.* 92, 2850–2853.
- Shashar, N., Cohen, Y., Loya, Y., 1993. Extreme diel fluctuations of oxygen in diffusive boundary layers surrounding stony corals. *Biol. Bull.* 185 (455–451).
- Shick, J.M., 1990. Diffusive limitation and hyperoxic enhancement of oxygen consumption in zooxanthellae, sea anemones, zoanthids and corals. *Biol. Bull.* 179, 148–158.
- Siebeck, U.E., Logan, D., Marshall, N.J., 2008. CoralWatch – a flexible coral bleaching monitoring tool for you and your group. In: *Proceedings of the International Coral Reef Symposium*. Ft. Lauderdale, FL.
- Silverstein, R.N., Cunnig, R., Baker, A.C., 2017. Tenacious D: *Symbiodinium* in clade D remain in reef corals at both high and low temperature extremes despite impairment. *J. Exp. Biol.* jeb.148239 <https://doi.org/10.1242/jeb.148239>.
- Szmant, A.M., Gassman, N.J., 1990. The effects of prolonged bleaching on the tissue biomass and reproduction of the reef coral *Montastrea annularis*. *Coral Reefs* 8, 217–224.
- Thurber, R.V., Payet, J.P., Thurber, A.R., Correa, A.M.S., 2017. Virus–host interactions and their roles in coral reef health and disease. *Nat. Rev. Microbiol.* 15, 205–216.
- Toller, W.W., Rowan, R., Knowlton, N., 2001. Zooxanthellae of the *Montastraea annularis* species complex: patterns of distribution of four taxa of *Symbiodinium* on different reefs and across depths. *Biol. Bull.* 201, 348–359.
- Veal, C.J., Carmi, M., Fine, M., Hoegh-Guldberg, O., 2010. Increasing the accuracy of surface area estimation using single wax dipping of coral fragments. *Coral Reefs* 29, 893–897.
- Wangpraseurt, D., Larkum, A.W.D., Ralph, P.J., Kühl, M., 2012a. Light gradients and optical microniches in coral tissues. *Front. Microbiol.* 3.
- Wangpraseurt, D., et al., 2012b. In situ oxygen dynamics in coral-algal interactions. *PLoS One* 7, e31192.
- Wangpraseurt, D., et al., 2017a. In vivo microscale measurements of light and photosynthesis during coral bleaching: evidence for the optical feedback loop? *Front. Microbiol.* 8, 59.
- Wangpraseurt, D., et al., 2017b. In vivo microscale measurements of light and photosynthesis during coral bleaching: evidence for the optical feedback loop? *Front. Microbiol.* 8, 59.
- Warner, M.E., Fitt, W.K., Schmidt, G.W., 1996. The effects of elevated temperature on the photosynthetic efficiency of zooxanthellae in hospite from four different species of reef coral: a novel approach. *Plant Cell Environ.* 19, 291–299.
- Wiedenmann, J., et al., 2013. Nutrient enrichment can increase the susceptibility of reef corals to bleaching. *Nat. Clim. Chang.* 3, 160–164.