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LETTER

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DMSP biosynthesis by an animal and its role in coral thermal stress response

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Globally, reef-building corals are the most prolific producers of dimethylsulphoniopropionate (DMSP)[1,2], a central molecule in the marine sulphur cycle and precursor of the climate-active gas dimethylsulphide[3,4]. At present, DMSP production by corals is attributed entirely to their algal endosymbiont, *Symbiodinium*[2]. Combining chemical, genomic and molecular approaches, we show that coral juveniles produce DMSP in the absence of algal symbionts. DMSP levels increased up to 54% over time in newly settled coral juveniles lacking algal endosymbionts, and further increases, up to 76%, were recorded when juveniles were subjected to thermal stress. We uncovered coral orthologues of two algal genes recently identified in DMSP biosynthesis, strongly indicating that corals possess the enzymatic machinery necessary for DMSP production. Our results overturn the paradigm that photosynthetic organisms are the sole biological source of DMSP, and highlight the double jeopardy represented by worldwide declining coral cover, as the potential to alleviate thermal stress through coral-produced DMSP declines correspondingly.

It is widely accepted that the production of dimethylsulphoniopropionate (DMSP), a central molecule in the marine sulphur cycle, is restricted to marine algae and a few species of intertidal plants[5]. Marine bacteria subsequently use DMSP as a source of sulphur and carbon and can metabolize this compound into the volatile gas dimethylsulphide (DMS)[6], by which the largest natural flux of sulphur enters the atmosphere where it exerts considerable influence on atmospheric chemistry[7]. Despite recent controversy regarding the impact of DMS emissions on global climate[8], DMS is probably involved in local climate regulation through its oxidation into aerosol particles that induce the formation of clouds and increase their reflectivity, thereby reducing light levels and water temperatures in the marine environment[3,4].

Concentrations of DMSP and DMS found in reef-building corals are among the highest recorded in the environment[1,2], but it has been assumed that DMSP production derives solely from the coral's endosymbiotic microalgae *Symbiodinium*. Evidence that the total amounts of DMSP recorded in corals are consistently higher than levels present in *Symbiodinium* cells alone[9,10] raises the possibility of a cryptic source of DMSP in reef-building corals. A clear understanding of the sources of DMSP on reefs and the possible effects that global warming may have on DMSP production is paramount, given the influence that coral-reef-derived sulphur emissions may have on local climates[11]. Corals in the genus *Acropora* are the most abundant reef-building organisms in the Indo-Pacific region[12] and, as broadcast spawners, they acquire *Symbiodinium* from their surrounding environment after larval development. The *Symbiodinium*-free larvae of this genus provide a unique opportunity to investigate *Symbiodinium*-independent production of DMSP in corals. Results presented here demonstrate that coral hosts (kingdom: Animalia) are capable of biosynthesizing DMSP in high concentrations in the absence of symbiotic microalgae, refuting the current paradigm that photosynthetic organism are the only DMSP sources in the environment.

DMSP levels were quantified in juveniles lacking photosynthetic symbionts for two common coral species, *Acropora millepora* and *A. tenuis*, using nuclear magnetic resonance (NMR) spectroscopy[13]. Juveniles were raised in the dark in 0.2-μm-filtered seawater to ensure they remained algae-free, and the absence of any photosynthetic organisms in these juveniles was confirmed using five different DNA markers, ranging from *Symbiodinium*-specific to universal 23S rRNA plastids primers, targeting all eukaryotic algae and cyanobacteria. A lack of any detectable amplification was observed for all markers (Extended Data Tables 1 and 5). Despite the complete absence of any photosynthetic microalgae, high concentrations of DMSP, two orders of magnitude greater than concentrations reported for benthic algae from the Great Barrier Reef[2], were recorded in all coral juvenile samples (Fig. 1). Repeated sampling over a 6-day period after larval settlement revealed that the initially high DMSP concentrations in juveniles increased significantly over time; specifically, DMSP increased by 44% (that is, 1.1 nmol mm^{-2}) in *A. millepora* and by 54% (that is, 1.7 nmol mm^{-2}) in *A. tenuis* (ANOVA, $n = 6$, $P < 0.005$; Fig. 1 and Extended Data Table 2). These results demonstrate unambiguously that the DMSP levels measured were not inherited from parent colonies but instead were produced by coral juveniles growing in the absence of photosynthetic symbionts.

Increases in DMSP concentrations over time were even more pronounced when coral juveniles experienced thermal stress (32 °C), with *A. tenuis* and *A. millepora* exhibiting 65% and 76% increases over the 6-day experimental period, respectively (ANOVA, $n = 6$, $P < 0.005$; Fig. 1 and Extended Data Table 2). Conversely, concentrations of the DMSP breakdown product acrylate decreased in both species, with thermally stressed juveniles containing 33% (*A. millepora*) and 61% (*A. tenuis*) less acrylate after 6 days than juveniles at ambient temperature (Fig. 1c, d). Similar declines in acrylate in marine algae subjected to stressful conditions[14] have been related to its antioxidant properties; acrylate and DMS are both extremely efficient scavengers of hydroxyl radicals and other reactive oxygen species (ROS)[14]. During thermal stress, the production of ROS by coral mitochondria (and *Symbiodinium*, when present) increases, damaging coral cells[15]. The observed decrease in acrylate concentrations in thermally stressed coral juveniles probably reflects its involvement in ROS detoxification, implicating DMSP and its breakdown products as functionally important in coral stress responses.

Further experimental studies involving adult corals, the building blocks of coral reefs and the most ecologically relevant life history stage in terms of DMSP production, confirm the importance of this

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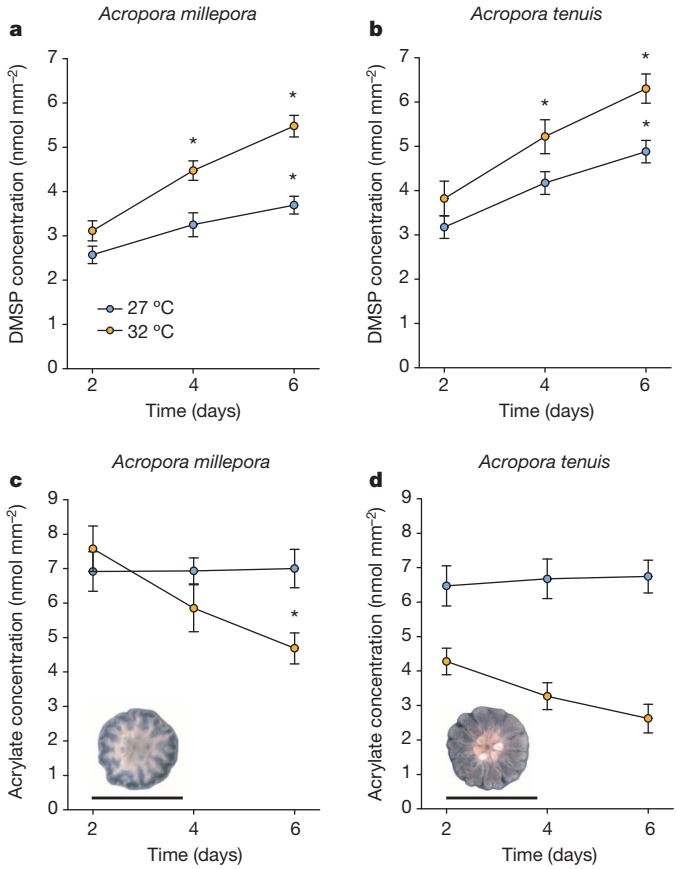

Figure 1 | Changes in DMSP and acrylate concentrations (mean ± s.e.) in coral juveniles lacking photosynthetic symbionts ($n = 6$; 40 juveniles per replicate) through a 6-day period after coral settlement. **a**, **b**, Patterns are compared for two thermal regimes: ambient (27 °C, blue) and thermal stress (32 °C, orange), and for the coral species *Acropora millepora* (**a**) and *Acropora tenuis* (**b**). DMSP concentrations increase significantly through time in the juveniles exposed to ambient temperature (ANOVA simple main effect test, $F_{2,20} = 6.30$, $^*P < 0.01$ for *A. millepora* and $F_{2,20} = 7.51$, $^*P < 0.005$ for *A. tenuis*). Furthermore, DMSP concentrations in coral juveniles exposed to elevated temperature (32 °C) for 6 days were significantly higher than controls kept at ambient temperature (27 °C) (simple main effect test, $F_{1,10} = 27.68$, $^*P < 0.0005$ for *A. millepora* and $F_{1,10} = 10.44$, $^*P < 0.01$ for *A. tenuis*). **c**, **d**, Conversely, acrylate concentrations decreased significantly in juveniles of the corals *A. millepora* (**c**) and *A. tenuis* (**d**) when exposed to elevated temperature (simple main effect test, $F_{1,10} = 5.58$, $^*P < 0.05$ for *A. millepora* and $F_{1,10} = 61.68$, $^*P < 0.0005$ for *A. tenuis*). Scale bar, 1 mm.

compound in coral stress responses and the production of high concentrations of DMSP by the coral host. Colonies of *A. millepora* exposed to 32 °C for 10 days showed an 84% reduction in their *Symbiodinium* cell densities (Extended Data Fig. 1a and Fig. 2a–c), thus photosynthetic symbionts were unlikely to contribute significantly to DMSP production. Furthermore, transmission electron microscopy showed that 100% of the remaining *Symbiodinium* cells examined within coral tissues ($n = 272$ cells) were structurally compromised (Extended Data Fig. 2). In particular, thylakoid membranes within chloroplasts were completely disrupted and general cell structure was disordered (Extended Data Fig. 2), characteristics typical of advanced necrosis[16]. Consistent with these observations of dysfunctional cell structures, pulse amplitude modulation (PAM) fluorometry measurements confirmed significant reductions in their photochemical efficiency (ANOVA, $n = 12$, $P < 0.005$; Extended Data Fig. 1b and Extended Data Table 3). In further support of our conclusion that *Symbiodinium* were not contributing significantly to DMSP production, previous experiments on *Symbiodinium* cultures have shown that DMSP content per cell volume decreases under thermal stress[17].

Despite the severe degradation and depletion of algal symbionts from coral tissues, thermally stressed corals contained 68% more DMSP (that is, 9 nmol mm^{-2}) and concordantly 36% less acrylate than control colonies (Fig. 2). These results are in close accordance with those we found for coral juveniles. Taken together, our results provide conclusive evidence that the observed increases in DMSP concentrations in thermally stressed corals cannot be attributed to the activity of *Symbiodinium* cells. This implies that DMSP production is not restricted to juvenile life stages, but also occurs at high levels in adult reef-building corals subjected to thermal stress. In addition to its antioxidant role, DMSP is involved in several key cellular and ecological processes in reef-building corals, notably as an osmoprotectant during salinity fluctuations, but also as a signal molecule attracting specific bacteria which form microbial communities that are integrally associated with corals and underpin their health[18]. The multiple functions of this molecule might explain the ecological advantage of producing it directly.

To further support our conclusion that DMSP is produced by the coral host, we examined potential molecular mechanisms underlying their DMSP biosynthetic capabilities. In some marine algae, DMSP is produced from methionine via a pathway that involves the successive action of four different enzymes[19], but until recently, very little was known about genes involved in its biosynthesis. A recent study identified candidate genes for each of the four steps of the biosynthesis pathway in the diatom *Fragilariopsis cylindrus*[20]. We searched for potential orthologues of these genes in the comprehensive molecular resources available for two coral species, the *A. millepora* transcriptome[21] and the *Acropora digitifera* genome[22], and found that two genes identified in diatoms have clear orthologues in corals. These genes encode a NADPH-reductase and an AdoMet-dependant methyltransferase, which mediate the second and third steps of the biosynthesis process, respectively (Fig. 3a). The orthologous relationship between the corals' and diatom's genes is supported by best reciprocal blast hits and their mapping to the same OrthoMCL clusters[23] (Extended Data Table 4). We also identified two *Symbiodinium* sequences (from a comprehensive transcriptome assembly[24]) belonging to the same OrthoMCL clusters as the reductase and methyltransferase identified in diatoms and corals (Extended Data Table 4). This indicates that the function of these enzymes in DMSP biosynthesis may be conserved between diatoms, alveolates, green plants and corals.

Results derived from the *A. millepora* transcriptome[21] enabled us to estimate the level of expression of these two genes throughout five life stages, from embryos to adults. We found the gene encoding the reductase, a reversible step in the DMSP biosynthesis process[5], to be highly expressed throughout all life-history stages (Fig. 3b). The phylogenetic distribution of its OrthoMCL cluster revealed 45 orthologues spread throughout all kingdoms (Extended Data Fig. 3), in line with the widespread presence of this enzyme among plants that do not produce DMSP[5]. In contrast, the methyltransferase mediates a non-reversible step regulating intracellular DMSP levels[25] and is believed to be specific to this pathway[5]. The gene encoding the methyltransferase was highly expressed in early coral life stages, but its expression decreased after settlement and remained relatively low in adult corals (Fig. 3c). This expression pattern correlates with the establishment of symbiosis with DMSP-producing *Symbiodinium* around the time of settlement. The OrthoMCL cluster corresponding to the methyltransferase has an unusually sparse phyletic pattern with only nine orthologues, including seven in photosynthetic organisms and two in other eukaryotes (Extended Data Fig. 3), indicating that corals are likely to be the exception, rather than the rule, in terms of DMSP production by marine invertebrates harbouring photosynthetic symbionts[26].

We show that the unparalleled levels of DMSP present in reef-building corals are not attributable solely to their endosymbiotic algae; instead, a significant fraction is produced by the coral animal. DMSP concentrations measured in coral juveniles devoid of symbiotic algae represented approximately half of those present in symbiont-bearing adult corals, indicating that the coral animal contributes extensively to

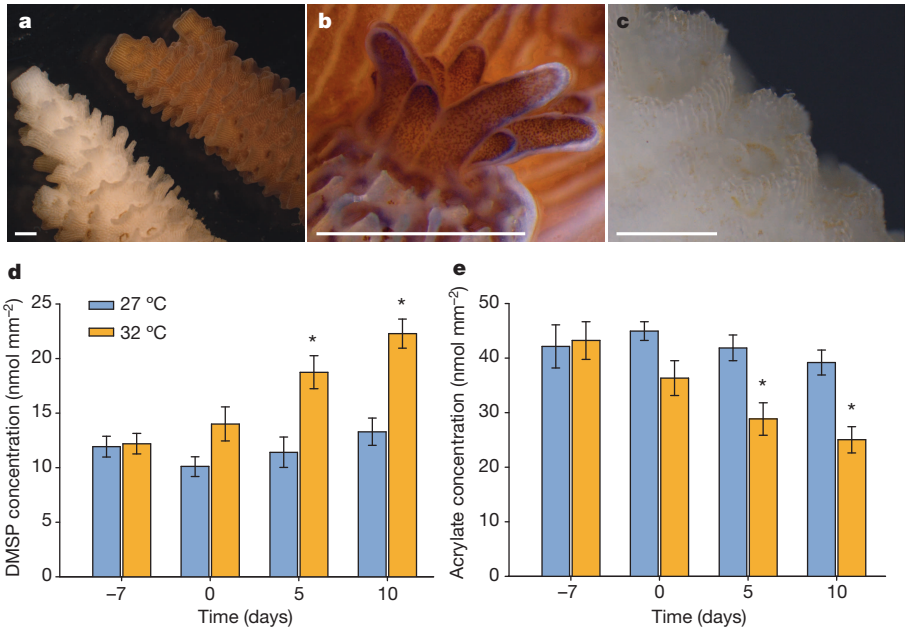

Figure 2 | Effects of thermal stress on adult colonies of the coral *Acropora millepora*. Temperature was raised gradually over 7 days to 32 °C, mimicking a realistic thermal stress scenario, therefore thermal stress commenced on day 0. The total duration of the experiment was 17 days. **a**, Micrograph of representative coral nubbins showing visual differences in the density of *Symbiodinium* cells present in the tissues of *A. millepora* maintained under control (27 °C, brown, right) or thermal stress (32 °C, white, left) conditions for 10 days. **b**, **c**, Higher magnification micrographs of coral polyps showing visual differences in tissue colour as intracellular *Symbiodinium* densities decline between 27 °C (**b**) and 32 °C (**c**) (scale bars, 1 mm). **d**, Changes in DMSP concentration (mean ± s.e.) in adult corals ($n = 12$) exposed to control (27 °C) and thermal stress (32 °C) treatments (ANOVA simple main effect test, $F_{1,22} = 10.79$, $^*P < 0.005$). **e**, Corresponding changes in acrylate concentration (mean ± s.e.) in adult corals ($n = 12$) exposed to control (27 °C) and thermal stress (32 °C) treatments (ANOVA simple main effect test, $F_{1,22} = 8.4$, $^*P < 0.01$).

the DMSP pool produced on coral reefs. Surveys over Australia identified the Great Barrier Reef as a significant hotspot for the emission of sulphur aerosol particles[27,28]. The Great Barrier Reef is the largest biological structure on the planet and the release of these particles along its 2,600-km length could constitute a major source of cloud condensation nuclei. Coral-reef-derived sulphur aerosol emission might therefore have a central role in cloud formation in areas of the world with high coral densities, such as the Great Barrier Reef and the Coral Triangle[11,29]. Considering declining trends in coral cover[30] and predicted increases in coral mortality worldwide caused by anthropogenic stressors, the associated decline in sulphur aerosol production from coral reefs may further destabilize local climate regulation and accelerate degradation of this globally important and diverse ecosystem.

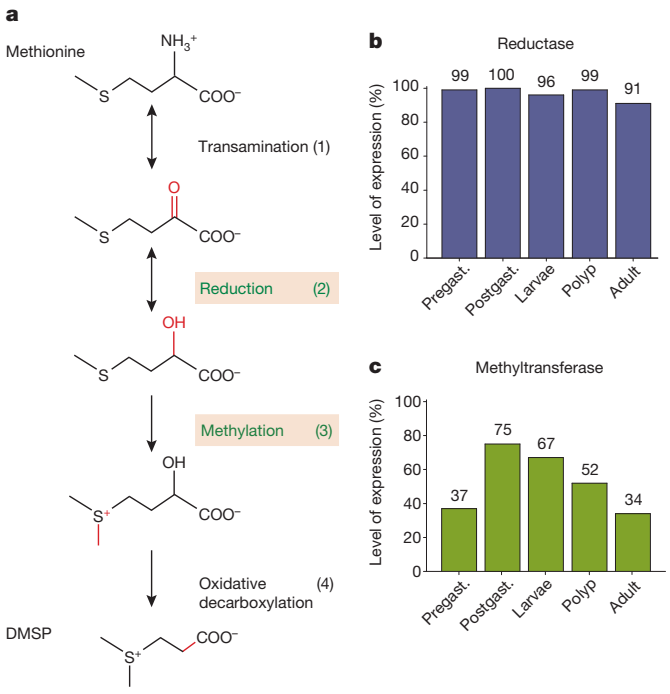

Figure 3 | Putative DMSP biosynthesis pathway and gene expression of coral orthologues. a, Pathway of DMSP biosynthesis present in marine algae[19] (the first two steps are reversible). The proposed diatom genes encoding the second and third steps (in green) have orthologues in *Acropora millepora* and *Acropora digitifera* genomes (see Extended Data Table 4). **b**, **c**, Gene expression percentiles of the two putative genes involved in DMSP biosynthesis in *A. millepora* through five coral life stages, from embryos (pregast., pregastrula; postgast., postgastrula) to adults, based on transcriptomic data from ref. 21.

METHODS SUMMARY

Coral juvenile sampling. Coral larvae were raised for 12 days in 0.5-µm-filtered seawater (FSW), subsequently washed three times in 0.2-µm FSW and settled in sterile 6-well plates (8 plates per species, 40 larvae per well; each well filled with 10 ml of 0.2-µm FSW). Eight hours after settlement, the plates were separated between two treatments: 4 plates per species were incubated at 27 °C (control temperature), and the other 4 plates were ramped to 32 °C over 5 h (thermal stress treatment). All plates were kept in the dark (to prevent the growth of potential photosynthetic organisms) and 6 random wells were sampled every 2 days.

DNA extractions and PCR amplification. At each time point, the contents of two wells were scraped into a 2-ml tube using a scalpel blade, snap frozen with liquid nitrogen, and used for total DNA extraction. Multiple sets of primers were subsequently used to target the *Symbiodinium*, coral and other photosynthetic organisms (Extended Data Tables 1 and 5).

Identification of candidate genes. Orthology between coral, *Symbiodinium* and diatom genes was inferred based on best reciprocal BLAST hits. Orthologues in other species were identified using release 5 (March 2011) of the OrthoMCL database[3]. Database for *A. digitifera* genome: http://marinegenomics.oist.jp/genomes/download?project_id=3 annotation version 1.0. Database for *A. millepora* transcriptome: NCBI Transcriptome Shotgun Assembly (TSA)[21].

Adult thermal stress experiment. Ten coral colonies were split to give a total of 24 fragments, each comprising approximately 25 branches (nubbins). Fragments were arranged in eight indoor tanks in a randomized block design. After 2 weeks of acclimatization, the temperature in four tanks was ramped-up slowly to 32 °C, whereas the other four tanks were maintained at 27 °C. Each temperature treatment contained a total of 12 fragments.

qNMR analysis. Adult and juvenile corals were extracted and analysed in accordance with the qNMR technique described previously[13].

Online Content Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Contributions J.-B.R. and A.L. designed the experiments. J.-B.R., C.A.M., D.A. and D.M.T. performed the juvenile experiment. J.-B.R., C.A.M., A.L., F.O.S. and D.M.T. performed the adult experiment. J.-B.R., P.L.C., C.A.M. and D.M.T. analysed the results. S.F. and J.-B.R. identified the candidate genes. J.-B.R. and B.L.W. wrote the manuscript. All authors edited the manuscript before submission.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to J.-B.R. (j.raina@aims.gov.au).

METHODS

Adult corals

Thermal stress experiment. *Acropora millepora* colonies ($n = 10$) were collected from Pelorus Island, Great Barrier Reef, Australia (18°33′ S/146°29′ E) and transferred to the Australian Institute of Marine Science. Coral colonies were fragmented to give a total of 24 fragments, each comprising approximately 25 branches (nubbins). Fragments were arranged in eight indoor tanks in a randomized block design, resulting in the allocation of 12 coral fragments to each of the control and thermal stress temperature treatments (27 °C and 32 °C, respectively). All tanks were continuously supplied with fresh 1-µm filtered seawater (FSW), which was maintained at 27 °C (± 0.1 °C) via computer control using a flow-through system at a rate of 1.5 l min^{-1}. Ultraviolet-filtered lights were mounted above each tank and provided an average underwater light intensity of 350 µE over a 12/12 h light/dark cycle (400 W metal halide lamps, BLV), typical of light intensities recorded at the collection site. The fragments were acclimatized for 2 weeks before starting the experiment. Seawater temperatures in four tanks were slowly and continuously ramped to 32 °C (± 0.05 °C) over a 7-day period, via computer control, while the remaining four control tanks were maintained at 27 °C for the entire duration of the experiment.

Coral nubbins were sampled four times during the experiment: before any temperature changes when both treatments were at 27 °C ($t = -7$); once the 32 °C target temperature had been reached in the thermal stress treatment ($t = 0$); after 5 days at 32 °C, when the first physiological effects of temperature stress were visible ($t = 5$); and after 10 days at 32 °C when all colonies in the 32 °C treatment were completely bleached ($t = 10$). At each time point, one coral nubbin (approximately 50 mm in length) was collected from each coral fragment ($n = 24$) and immediately transferred to a tube containing 2 ml of HPLC-grade methanol for quantitative nuclear magnetic resonance (qNMR) analysis. Another coral nubbin was collected from each coral fragment ($n = 24$) to evaluate *Symbiodinium* densities. In addition, one coral nubbin was collected at each time point from four different coral fragments, transferred directly into fixative (1.25% glutaraldehyde + 0.5% paraformaldehyde in 0.2-µm FSW) and stored at 4 °C until processed for structural investigations by transmission electron microscopy (TEM).

Quantitative NMR analysis. The coral nubbins were extracted in methanol for 2 h with sonication followed by a second extraction with an additional 1 ml of HPLC-grade methanol for 10 min. The two extracts were pooled and dried using a vacuum-centrifuge then re-suspended in a mixture of deuterium oxide (D_2O, D 99.8%, 250 µl) and deuterated methanol (CD_3OD, D 99.8%, 750 µl) (Cambridge Isotope Laboratories). A 700 µl aliquot of the particulate-free extract was transferred into a 5-mm Norell 509-UP-7 NMR tube (Norell Inc.) and analysed immediately by ^1H NMR.

^1H NMR spectra were recorded on a Bruker Avance 600 MHz NMR spectrometer with TXI 5-mm probe and quantification performed using the ERETIC method[13]. This technique generates an internal electronic reference signal, calibrated using commercial stock solutions of 4 mM acrylate and DMSP. The concentrations of DMSP and acrylate were determined by integration of their respective signals in a 0.10 p.p.m. window[13].

***Symbiodinium* densities.** Freshly collected coral nubbins were airbrushed (80 lb in^{-2}) in individual plastic bags in 4 ml of 0.2-µm FSW. The slurry was homogenized to breakdown aggregates and centrifuged at 3,000 r.c.f. (relative centrifugal force). The supernatant was removed and the pellet re-suspended in 1 ml of 10% formalin. Homogeneous extracts were placed on a hemocytometer (depth 0.1 mm) and *Symbiodinium* cells were counted under a light microscope (eight technical replicates were averaged per sample).

***Symbiodinium* genotype.** *In hospite Symbiodinium* populations of each experimental coral fragment were characterized on the basis of sequence differences in the nuclear ribosomal DNA internal transcribed spacer 1 region using single-strand conformation polymorphism (SSCP) analysis[31]. Total DNA was extracted using a modified protocol[32]. *Symbiodinium* ITS1 region was amplified with fluorescently labelled Sym ITS1 PCR primers. Genotype was determined using SSCP with known reference samples running alongside experimental samples and scored manually using gel images. All colonies contained only *Symbiodinium* type C2 (GenBank accession AF380552) SSCP profiles from all samples were single bands identical to the reference).

Surface-area calculation. Coral skeletons remaining after samples were extracted for qNMR analyses and *Symbiodinium* densities were lyophilized overnight and their surface area determined using a wax dipping technique[33]. The surface area of each individual nubbin was used to normalize the qNMR and *Symbiodinium* data.

Transmission electron microscopy. Fixed coral nubbins were decalcified in a formic acid:fixative mixture (1:3), with the solution changed every 12 h until complete dissolution of the skeleton. Three individual polyps per sample were post-fixed in osmium and subsequently dehydrated with increasing concentrations of ethanol followed by dry acetone. Dehydrated samples were infiltrated in increasing concentrations of Araldite resin before being cured for 24 h at 60 °C. Longitudinal sections 90-nm thick were collected on copper grids and imaged at 120 kV in a JEOL 2100 TEM.

Pulse amplitude modulation (PAM) fluorometry measurements. Photosystem II (PSII) photochemical efficiency was measured with a Diving-PAM (Walz) on three random nubbins per coral fragment. Minimum and maximum fluorescence (F_O and F_M) were recorded daily, 2 h before the start of the light cycle. PS II photochemical efficiency was expressed as maximum quantum yields $((F_M - F_O)/F_M) = (F_V/F_M)$.

Data analyses. All data were square root transformed and no significant tank effect was detected for the DMSP, acrylate and PAM fluorometry data (nested ANOVA, $P > 0.05$) (Statistica 7, Statsoft). The sample size was chosen based on results from a pilot study. Repeated measures ANOVA were carried out on the time series data (data met all assumptions of the test). Simple main effect tests[34] were used to compare the results between the two temperature treatments, and between temperatures at each time point (Extended Data Table 3). This statistical technique was used to minimize the number of multiple comparisons (focusing only on comparisons of interest), decreasing the likelihood of type I error.

Coral juveniles

Sample collections. Colonies of *A. millepora* ($n = 10$) and *A. tenuis* ($n = 3$) were collected from Orpheus Island, Great Barrier Reef, Australia (18°34′ S/146°30′ E) and transferred to the Australian Institute of Marine Science outdoor aquarium facility 4 days before the predicted spawning event in November 2011. One hour before spawning, the colonies were isolated in 70 l tanks with 1-µm FSW. Gametes were collected from the surface of these tanks and fertilized in separate 70-l tanks with FSW. After fertilization, embryos were gently rinsed three times by transferring to new containers and were subsequently transferred to 500-l tanks (containing 0.5-µm FSW) where they were kept through larval development. After 12 days, *Symbiodinium*-free coral larvae were collected using a 1 µm mesh net and washed three times in 0.2-µm FSW. Larvae were subsequently settled in sterile 6-well plates (8 plates per species, 40 larvae per well; each well filled with 10 ml of 0.2-µm FSW). Eight hours after settlement, the plates were separated between two temperature regimes: 4 plates per species were incubated at 27 °C (control temperature), and the other 4 plates were ramped to 32 °C over 5 h (thermal stress treatment); all plates were maintained in the dark (to prevent the growth of potential photosynthetic organisms). Settled juveniles were incubated at their respective temperature treatments and six random wells were sampled every 2 days. The size of the sampled juveniles was measured using a motorized stereomicroscope (Leica MZ16A, Leica Microsystems); sizes did not vary significantly between day 2 and day 6 with an average size of 0.79 mm^2 (±0.05) for *A. millepora* juveniles and of 0.82 mm^2 (±0.07) for *A. tenuis* juveniles.

qNMR analysis. After the required incubation time, seawater was removed from each well with a pipette, and a sterile cotton bud was used to soak up any residual seawater, taking care not to disturb the settled juvenile corals. Juveniles in six wells were extracted by adding 300 µl of deuterated methanol (CD_3OD) to each well, followed by 30s of gentle shaking; 200 µl of this extract was transferred into a 3-mm Bruker MATCH NMR tube and analysed immediately. In addition, negative control wells without settled juveniles were extracted following the same procedure. The concentrations of DMSP and acrylate were normalized initially to the number of settled coral juveniles in the respective well. They were then normalized to the averaged surface area of the juveniles (note that juveniles were approximated to perfect circles).

DNA extractions and PCR amplification. At each time point, the contents of two wells were scraped into a 2-ml tube using a scalpel blade, snap frozen with liquid nitrogen, and used for total DNA extraction, according to methods in ref. 35. Multiple sets of primers were subsequently used to target different taxonomic groups: *Symbiodinium*[31], coral[36] and general primers for the algal 23S rDNA plastid[37] and chloroplast DNA[38] (Extended Data Table 1). Furthermore, three other DNA markers were used to target microbes: Bacteria[39], Archaea[40] and Fungi[41] (Extended Data Table 1).

The PCR consisted of 1 µl of DNA template (dilution series from 1 to 10^{-5} of the original concentrations), 10 µl of buffer containing dNTP and $MgCl_2$ (Bioline), 1.5 µl of each primer (10 µM), and 0.5 µl of *Taq* polymerase (Bioline), adjusted to a final volume of 50 µl with sterile MilliQ water. Amplified PCR products were visualized by electrophoresis on 1% agarose gel stained with ethidium bromide.

Clone libraries construction. PCR products from bacterial 16S ribosomal RNA gene were purified using a QIAquick gel extraction kit (Qiagen) according to the manufacturer's instructions. Purified DNA was ligated into a TOPO-TA cloning vector (Invitrogen) and competent cells were transformed following the manufacturer's instructions. Four samples, derived from the last experimental time point (6 days), were analysed (*A. millepora* at 27 °C; *A. millepora* at 32 °C; *A. tenuis* at 27 °C; and *A. tenuis* at 32 °C). A total of 48 clones were sequenced per sample (Macrogen Inc.) and chimaeric sequences were removed from subsequent analysis.

The nucleotide sequences obtained have been deposited in GenBank database under the accession numbers (KF619251 to KF619442).

Identification of candidate genes. Orthology between coral and diatom genes was inferred on the basis of best reciprocal BLAST hits[42]. Orthologues in other species were identified using release 5 (March 2011) of the OrthoMCL database[23]. Database for *A. digitifera* genome: http://marinegenomics.oist.jp/genomes/download?project_id=3 annotation version 1.0; for *A. millepora* transcriptome: NCBI Transcriptome Shotgun Assembly (TSA)[21].

Data analyses. Repeated measure ANOVA were performed on the normalized DMSP and acrylate concentrations (data met all assumptions of the test, except the acrylate data from *A. tenuis* that violated sphericity assumption; in that unique case, Greenhouse–Geisser correction was applied to the degrees of freedom). Simple main effect tests[34] were then used to compare the results from the temperature treatment within species (Extended Data Table 2).

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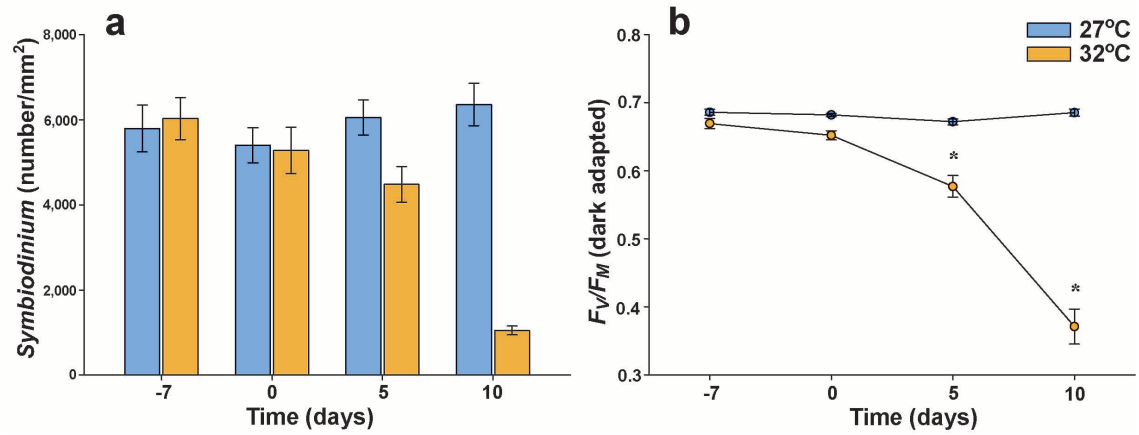
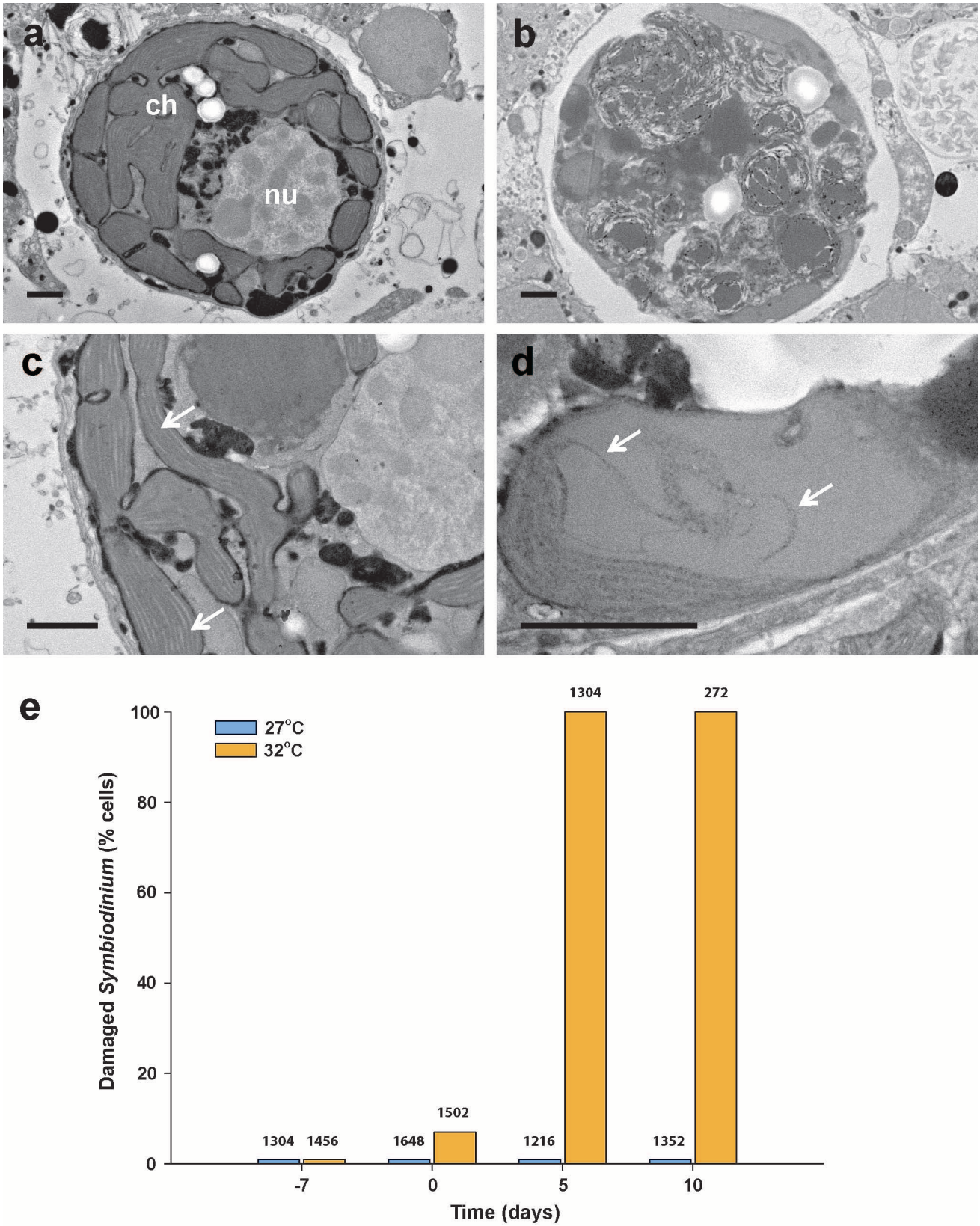
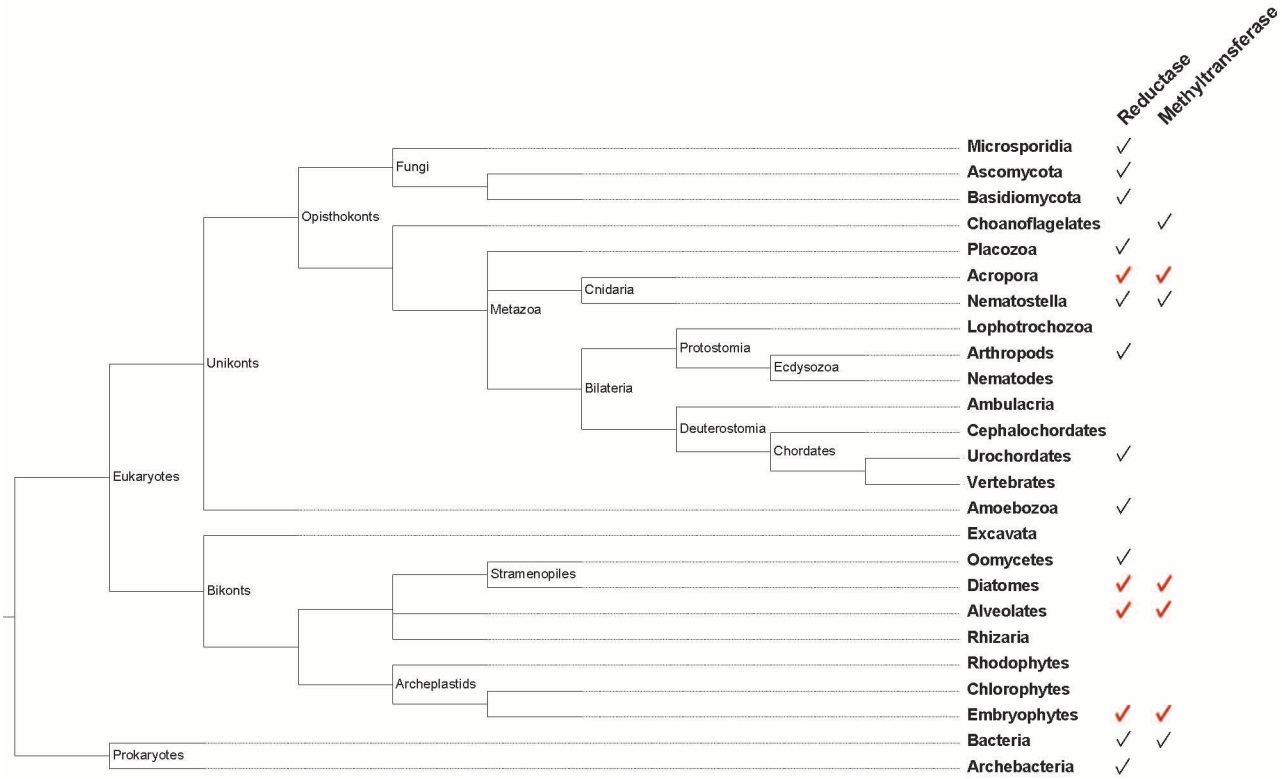


Extended Data Figure 1 | Density and photosynthetic efficiency (mean ± s.e.) of *Symbiodinium* cells within adult colonies of the coral *Acropora millepora* maintained under control (27 °C) or thermal stress (32 °C) conditions for 10 days. **a**, Density of *Symbiodinium* cells in the same coral fragments through time. **b**, Comparison of photosystem II photochemical efficiency (maximum quantum yields: F_V/F_M) through time (repeated measure ANOVA, $*P < 0.001$; post-hoc simple main effect test, $*P < 0.01$). See also Extended Data Table 3.



Extended Data Figure 2 | Representative transmission electron micrographs showing the effects of thermal stress on the internal structure of endosymbiotic *Symbiodinium* cells associated with the coral *Acropora millepora*. **a**, **c**, *Symbiodinium* cells after 10 days at 27 °C, showing intact cell structures (**a**) and intact thylakoid membranes of chloroplasts (arrows), the photosynthetic centre of cells (**c**). **b**, **d**, *Symbiodinium* cells after 10 days at 32 °C, showing structurally degraded cells (**b**) with highly disrupted thylakoid membranes (arrows) (**d**). Scale bars, 1 µm. ch, chloroplast; nu, nucleus. **e**, Percentage of structurally damaged *Symbiodinium* cells within adult tissue throughout the thermal stress experiment. The numbers above the bars refer to the total number of *Symbiodinium* cells observed.



Extended Data Figure 3 | Phylogenetic distribution of the reductase and methyltransferase orthologues (OrthoMCL groups OG5_131390 and OG5_156314, respectively). Note the unusually sparse distribution of OG5_156314. In red: co-occurrence of these two enzymes occurs predominantly in DMSP-producing organisms. The only species of bacteria in the OrthoMCL database where these two enzymes occur simultaneously is the marine cyanobacterium *Synechococcus*.

Extended Data Table 1 | Primer pairs used to target the genomic DNA of various microorganisms possibly responsible for DMSP production to assess presence in coral juveniles

Target organisms	Target region	Primer name	Amplicon size	Annealing temperature	Number of cycles	Reference	Amplification
Coral	mtDNA	RNS2/GR	700 bp	54°C	30	36	Yes
Symbiodinium	ITS1	ITSFP/ITSRP	350 bp	59°C	30	31	No
Algae	23S rDNA plastid	p23SrVf1/Vr1	410 bp	Touchdown PCR (66 to 58°C)	35	37	No
Photosynthetic organisms	cpDNA	a/b	Variable	55°C	35	38	No
Photosynthetic organisms	cpDNA	c/d	Variable	55°C	35	38	No
Photosynthetic organisms	cpDNA	e/f	Variable	55°C	35	38	No
Bacteria	16S rRNA	63f/1387r	1300 bp	55°C	30	39	Yes
Fungi	ITS1	ITS1f/ITS4	Variable	55°C	30	41	No
Archaea	16 rRNA	349f/806r	457 bp	59°C	30	40	No

mtDNA, mitochondrial DNA; cpDNA, chloroplast DNA; ITS, internal transcribed spacer; bp, base pairs.

Extended Data Table 2 | Sums of squares (SS), mean squares (MS) and significance levels for ANOVAs of the *Symbiodinium*-free juvenile experiment

a

DMSP in *Acropora millepora*

Effect	SS	df	MS	F	*p*
Intercept	132.6612	1	132.6612	6959.515	0.000000
Temperature	0.8075	1	0.8075	42.3610	0.000068
Time 2	0.0788	1	0.0788	4.1343	0.069420
Time 4	0.3015	1	0.3015	15.8185	0.002612
Time 6	0.5277	1	0.5277	27.6862	0.000367
Error	0.1906	10	0.0191		
Time	1.2373	2	0.6186	24.7230	0.000004
Time × Temperature	0.1007	2	0.0504	2.0120	0.159840
27°C	0.3160	2	0.1580	6.3099	0.007500
32°C	1.0220	2	0.5110	20.4073	0.000015
Error	0.5005	20	0.0250		

DMSP in *Acropora tenuis*

Effect	SS	df	MS	F	*p*
Intercept	162.4001	1	162.4001	6250.471	0.000000
Temperature	0.5032	1	0.5032	19.368	0.001333
Time 2	0.0858	1	0.0858	3.3033	0.099181
Time 4	0.1720	1	0.1720	6.6189	0.027760
Time 6	0.2715	1	0.2715	10.4499	0.008981
Error	0.2598	10	0.0260		
Time	1.5042	2	0.7521	19.8690	0.000018
Time × Temperature	0.0261	2	0.0130	0.3440	0.712926
27°C	0.5677	2	0.2839	7.5112	0.003688
32°C	0.9625	2	0.4812	12.7343	0.000271
Error	0.7571	20	0.0378		

b

Acrylate in *Acropora millepora*

Effect	SS	df	MS	F	*p*
Intercept	230.3004	1	230.3004	1830.657	0.000000
Temperature	0.3525	1	0.3525	2.8020	0.125088
Time 2	0.0433	1	0.0433	0.3442	0.570427
Time 4	0.1583	1	0.1583	1.2583	0.288179
Time 6	0.7030	1	0.7030	5.5882	0.039685
Error	1.2580	10	0.1258		
Time	0.4889	2	0.2444	5.4270	0.013110
Time × Temperature	0.5521	2	0.2760	6.1290	0.008394
27°C	0.0010	2	0.0005	0.0111	0.988964
32°C	1.0400	2	0.5200	11.5479	0.000463
Error	0.9008	20	0.0450		

Acrylate in *Acropora tenuis*

Effect	SS	df	MS	F	*p*
Intercept	903.6474	1	903.6474	589.2514	0.000000
Temperature	94.6001	1	94.6001	61.6869	0.000014
Time 2	0.6782	1	0.6782	0.4422	0.521095
Time 4	1.8205	1	1.8205	1.1871	0.301475
Time 6	3.0288	1	3.0288	1.9750	0.190211
Error	15.3355	10	1.5355		
Time	2.8944	2	1.4472	1.1180	0.346530
Time × Temperature	5.6668	2	2.8334	2.1888	0.138160
27°C	0.0105	2	0.0052	0.0041	0.995953
32°C	0.6705	2	0.3353	0.2590	0.774379
Error	25.8897	20	1.2945		

DMSP concentrations (**a**) and acrylate concentrations (**b**) are shown. Red lines show significant differences ($P < 0.05$).

Extended Data Table 3 | Sums of square (SS), mean squares (MS) and significance levels for ANOVAs of the thermal stress experiment on adult *Acropora millepora* corals

a

Effect	SS	df	MS	F	*p*
Intercept	1308.7770	1	1308.7770	2314.789	0.000000
Temperature	11.0560	1	11.0560	19.5550	0.000215
Time -7	0.0105	1	0.0105	0.0186	0.893047
Time 0	1.9423	1	1.9423	3.4377	0.077184
Time 5	6.1001	1	6.1001	10.7966	0.003374
Time 10	7.2047	1	7.2047	12.7517	0.001707
Error	12.4390	22	0.5650		
Time	8.7870	3	2.9290	11.2720	0.000005
Time x Temperature	4.2010	3	1.4000	5.3890	0.002226
27°C	1.5627	3	0.5209	2.0035	0.122017
32°C	11.4258	3	3.8086	14.6485	0.000000
Error	17.1510	66	0.2600		

b

Effect	SS	df	MS	F	*p*
Intercept	3531.6680	1	3531.6680	3456.576	0.000000
Temperature	14.4190	1	14.4190	14.1120	0.001090
Time -7	0.0650	1	0.0650	0.0636	0.803235
Time 0	3.0065	1	3.0065	2.9418	0.100373
Time 5	8.5848	1	8.5848	8.4000	0.008338
Time 10	10.1534	1	10.1534	9.9348	0.004626
Error	22.4780	22	1.0220		
Time	11.7760	3	3.9250	7.2940	0.000269
Time x Temperature	7.3910	3	2.4640	4.5780	0.005668
27°C	1.2300	3	0.4100	0.7621	0.519419
32°C	17.9380	3	5.9790	11.1134	0.000005
Error	35.5210	66	0.5380		

c

Effect	SS	df	MS	F	*p*
Intercept	59.3872	1	59.3872	38632.320	0.000000
Temperature	0.1444	1	0.1444	93.9100	0.000000
Time -7	0.0006	1	0.0006	0.3896	0.538929
Time 0	0.0020	1	0.0020	1.3247	0.262124
Time 5	0.0223	1	0.0223	14.4545	0.000977
Time 10	0.2928	1	0.2928	190.1039	0.000000
Error	0.0338	22	0.0015		
Time	0.1689	3	0.0563	103.2300	0.000000
Time x Temperature	0.1733	3	0.0578	105.9100	0.000000
27°C	0.0005	3	0.0002	0.3273	0.805680
32°C	0.3417	3	0.1139	207.0727	0.000000
Error	0.0360	66	0.0005		

DMSP concentrations (**a**), acrylate concentrations (**b**) and PAM data (**c**) are shown. Red lines show significant differences ($P < 0.05$).

Extended Data Table 4 | Description of the reductase and methyltransferase sequences in diatoms, corals and *Symbiodinium*

	Reductase	Methyltransferase
Fragilariopsis cylindrus	jgi|Fracy1|173405|estExt_Genewise1.C_220021	jgi|Fracy1|207357|estExt_Genewise1Plus.C_41074
Acropora digitifera	adi_v1.10730	adi_v1.21031
Acropora millepora	Cluster027405	Cluster022229
Symbiodinium (clade A, strain k8)	kb8_c41244	kb8_rep_c2522
Pfam domain	FMN_red (PF03358.8)	Methyltransf_7 (PF03492.8)
OrthoMCL cluster	OG5_131390	OG5_156314

Extended Data Table 5 | Composition of coral juvenile bacterial communities present in the two different coral species at the two temperature regimes after 6 days

Genus	*A. tenuis* 27°C	*A. tenuis* 32°C	*A. millepora* 27°C	*A. millepora* 32°C	DMSP degradation	DMS degradation
Alteromonas		16.7	6.3	2.1	43	
Endozoicomonas			8.3	10.4	43	
Hyphomonas	4.2		2.1	2.1	44	
Idiomarina	2.1				43	
Labrenzia				4.2	45	
Marinomonas		2.1		4.2	46	
Neptuniibacter	22.9	4.2	8.3	2.1	47	
Oceanicola		2.1	2.1	2.1	48	
Phaeobacter	22.9	4.2	12.5	6.3	49	
Pseudoalteromonas			2.1		44	
Ruegeria	25.0	29.2	33.3	20.8	50	
Sagittula				2.1	46	
Stappia				2.1	48	
Methylophaga	2.1					51
Rhodovulum		12.5	10.4	25.0		52
Bdellovibrio	2.1					
Hellea	2.1					
Krokinobacter	2.1					
Lacimutrix	2.1					
Maricaulis	2.1		2.1			
Mesorhizobium				4.2		
Methylosinus	2.1					
Planifilum			2.1			
Ponticaulis	2.1	10.4	2.1	4.2		
Pseudovibrio		2.4				
Ralstonia	2.1			2.1		
Shimia		6.3	4.2	4.2		
Simiduia		2.1				
Spongiibacter	4.2	4.2	2.1	2.1		
Thalassospira		4.2	2.1			

Data are presented at the genus level in per cent; 48 clones per sample. Bacteria previously implicated in the degradation of DMSP and DMS are shown in green and orange, respectively. Note the proportion of bacteria involved in DMS(P) degradation, representing 79.7% of the bacteria sequenced. References [43–52] are cited in this table.