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RESEARCH ARTICLE

Physiological responses and adjustments of corals to strong seasonal temperature variations (20–28°C)

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ABSTRACT

Temperature is a key driver of metabolic rates. So far, we know little about potential physiological adjustments of subtropical corals to seasonal temperature changes (>8°C) that substantially exceed temperature fluctuation experienced by their counterparts in the tropics. This study investigated the effect of temperature reductions on *Montastraea cavernosa* and *Porites astreoides* in Bermuda (32°N; sea surface temperature ∼19–29°C) over 5 weeks, applying the following treatments: (i) constant control temperature at 28°C, and (ii) temperature reduction (0.5°C day^{-1}) followed by constant temperature (20 days; acclimatization period) at 24°C and (iii) at 20°C. Both species decreased photosynthesis and respiration during temperature reduction as expected, which continued to decrease during the acclimatization period, indicating adjustment to a low energy turnover rather than thermal compensation. Trajectories of physiological adjustments and level of thermal compensation, however, differed between species. *Montastraea cavernosa* zooxanthellae metrics showed a strong initial response to temperature reduction, followed by a return to close to control values during the acclimatization period, reflecting a high physiological flexibility and low thermal compensation. *Porites astreoides* zooxanthellae, in contrast, showed no initial response, but an increase in pigment concentration per zooxanthellae and similar photosynthesis rates at 24°C and 20°C at the end of the experiment, indicating low acute thermal sensitivity and the ability for thermal compensation at the lowest temperature. Respiration decreased more strongly than photosynthesis, leading to significant build-up of biomass in both species (energy reserves). Results are important in the light of potential poleward migration of corals and of potential latitudinal and species-specific differences in coral thermal tolerance.

KEY WORDS: Seasonal temperature variability, Coral, *Symbiodinium*, Physiological adjustment, Temperature reduction, Subtropics

INTRODUCTION

Temperature is a core driver of various metabolic processes owing to its tight relationship with biochemical reactions. The reaction rates in enzyme kinetics are expressed as a gradual increase of enzyme activity with increasing temperature until reaching an optimum. Beyond the optimum, further rise in temperature causes a sharp decrease of enzyme activity, often due to enzyme denaturation. This right-skewed optimum curve is reflected in many metabolic processes of ectotherms and autotrophs (e.g. respiration and photosynthesis rate; De Long et al., 2017; Silbiger et al., 2019). However, on broader temporal (e.g. seasons) or spatial (e.g. distinct thermal habitats, across latitudes) scales, organisms can compensate for temperature-induced reductions of enzyme activity. These adjustments may include, for example, changes or differences in enzyme structure featuring a lower activation energy or increases in enzyme concentrations (Somero, 2004; Kirk, 2010; Heidarvand et al., 2017). In temperature acclimatization experiments, this would be reflected in a change of metabolic rate efficiency over time. Primary producers, in addition, display mechanisms that adjust the capture and utilization of light by changing the concentrations of photo-harvesting and photo-protective pigments in response to temperature changes (Ensminger et al., 2006). Generally, adjusting mechanisms do not allow for a complete temperature compensation (Clarke and Fraser, 2004), but enough to cover the energetic requirements that are often lower at decreased temperatures as well (Somero, 2004). Although these temperature-compensating mechanisms are fairly well studied across large latitudinal ranges (e.g. comparisons between tropical and polar fish species; Somero, 2004) or from regions with strong seasonality (Ensminger et al., 2006), they are not well characterized in narrower temperature regimes. However, low to medium seasonal temperature variability as occurs from the tropics to the subtropics can still have substantial implications on species performance, such as in reef-building shallow water corals.

Warm water corals – found in the tropics and subtropics – are filter-feeding animals that live in symbiosis with unicellular endosymbiotic algae, termed zooxanthellae (dinoflagellates of the family Symbiodiniaceae). Zooxanthellae cover the majority of the energy and carbon requirement of corals through photosynthesis, allowing corals to thrive in nutrient poor waters (Falkowski et al., 1984). Although the highest diversity of corals (and symbionts) is found in the warm waters of the tropics (between ∼27°C and 31°C), there are also a number of tropical species thriving in subtropical regions, where temperature can vary between ∼16°C and 29°C (∼20–33°N and S; e.g. Florida, Bermuda, Northern Red Sea, Japan, Lord Howe Island, Hong Kong and Persian/Arabian Gulf; Kleypas et al., 1999; Abrego et al., 2021). As a consequence of increasing sea surface temperature (SST), it has been proposed that corals (like other taxa) may undergo a poleward migration (Yamano et al., 2011; Baird et al., 2012; McIlroy et al., 2019), which would allow corals to escape high summer temperatures in the tropics. At the same time, however, corals would also be exposed to increasing temperature variability and decreasing temperature minima, as well as lower winter light intensities, generally lower pH and higher nutrient conditions (Abrego et al., 2021). The impact of changing light on corals is understood fairly well as mechanisms of photo-acclimation

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are not only relevant for seasonal adjustments, but also for adjustment to different light regimes within a reef (e.g. along a depth gradient). Under low light conditions, corals can maximize light harvesting to sustain high photosynthetic rates by increasing zooxanthellae cell density and/or concentration of light-harvesting pigments (e.g. chlorophyll *a* and peridinin). Moreover, zooxanthellae reduce the investment in mechanisms that protect from high irradiance by decreasing the concentration of photo-protective pigments (e.g. xanthophylls; Falkowski and Dubinsky, 1981; Mass et al., 2007; Stambler et al., 2008; Sawall et al., 2014). The response of corals to seasonal temperature changes has been far less studied than that to light, and there is a lack of understanding of how corals adjust to cool winter temperatures in sub-tropical seas (Abrego et al., 2021).

Previous studies that investigated seasonal patterns of coral performance and tissue composition have not allowed the identification of potential temperature-specific mechanisms of physiological adjustment. It is unknown whether observed changes in, for example, zooxanthellae densities, pigmentation, photosynthetic efficiency (Mass et al., 2007; Ulstrup et al., 2008; Sawall et al., 2014), feeding mode (autotrophy versus heterotrophy; Ferrier-Pagès et al., 2011; Hinrichs et al., 2013; Rossi et al., 2020) and zooxanthellae clade composition (Ziegler et al., 2015) are due to changes in light, temperature or nutrients, or a combination of all. However, a recent study by Jurriaans and Hoogenboom (2020) investigated the sensitivity of corals to temperature variations in summer and winter and found a higher temperature sensitivity of corals in winter than in summer as determined by temperature performance curves (Central Great Barrier Reef, Δ5°C). This finding is further supported by a heat exposure experiment conducted by Scheufen et al. (2017), who found a higher sensitivity of 'winter corals' than 'summer corals' when exposed to the same relative change in temperature (Δ4°C; Mexican Caribbean). Although these studies suggest that physiological adjustment to seasonal temperature changes occurs in corals, the underlying mechanisms are still largely unidentified.

Temperature is a highly critical parameter affecting all major metabolic processes of corals, which include photosynthesis (the coral's main source of energy and carbon), respiration (the major ATP generating process), calcification (coral growth), biomass growth, cell maintenance and mucus production (Edmunds and Davies, 1986; Riegl and Branch, 1995; Muller-Parker and Davy, 2001; Davy et al., 2012; Tremblay et al., 2012). Therefore, understanding the coral's capacity and mechanisms for physiological adjustment to temperature changes is of paramount importance. Advancing this knowledge would be beneficial not just to forecast the consequences of potential poleward migration of corals, but also to gain further understanding about the relationship between species thermal history and thermal tolerance towards events of abnormal temperature.

In this study, we investigated the effect of gradual temperature changes on key coral metabolic rates and tissue metrics in order to understand the coral's ability to adjust to seasonal temperature variation. For this, we conducted a 5-week laboratory-based temperature manipulation study with two widely distributed Atlantic coral species collected from a high-latitude reef in Bermuda (32°N), where seasonal temperature variation is >10°C. Corals were collected in summer at the annual peak of SST (28°C) and were gradually acclimatized to 24°C and 20°C. The treatment included an 8-day (24°C) and a 16-day (20°C) temperature reduction period of 0.5°C/day that was followed by a 20-day acclimatization period (constant temperature). We predicted that metabolic rates (photosynthesis and respiration per surface area) would strongly decrease during the temperature reduction period given the effect of reduced temperature on enzyme activity. During the experimental acclimatization period, however, we predicted that metabolic rates would increase again to an 'intermediate level' as a result of thermal compensation mechanisms in the zooxanthellae and the coral host. Thermal compensation mechanisms may be evident in (i) an increase of biomass (zooxanthellae density and/or coral host biomass) and (ii) an increase of metabolic rate efficiency (which in turn would indicate changes in enzyme concentration or sensitivity – not directly measured in this study). At the same time, zooxanthellae are expected (iii) to either reduce the concentration of photo-harvesting pigments (chlorophyll *a*, peridinin) or (iv) to enhance the concentration of photo-protective pigments (xanthophyll) to avoid potential photo-damage caused by reduced capacity for light processing (e.g. owing to reduced enzyme activity of the photosynthetic apparatus).

MATERIALS AND METHODS

Study species and collection site

The two coral species *Montastraea cavernosa* (Linnaeus 1767) and *Porites astreoides* Lamarck 1816 were chosen for this study, as they are common species in Bermuda as well as in many other regions of the tropical and subtropical Western Atlantic (Caribbean, Brazil; Nunes et al., 2011). Both species are highly abundant on fore-reef slopes (Manzello et al., 2015), with *M. cavernosa* featuring a greater depth distribution (~1–80 m deep) than *P. astreoides* (1–50 m deep; Fricke and Meischner, 1985). Furthermore, *M. cavernosa* is generally considered less bleaching resistant than *P. astreoides* (Cook et al., 1990; Wagner et al., 2010; Smith et al., 2013). *Montastraea cavernosa* usually harbors a diverse zooxanthellae (*Symbiodinium* spp.) community of clades A, C and D, with clade C being the dominant clade, while *P. astreoides* harbors a consortium of clade A types (Hauff et al., 2016). The two species differ in their means of reproduction, as *M. cavernosa* is a gonochoric broadcast spawner reproducing in late August, and *P. astreoides* is a brooder active from January to September (Szmant, 1986).

In June 2019, eight coral colonies per species (~15 cm in diameter, >5 m between colonies) were collected with a hammer and chisel at 5 m depth from Sea Venture Shoals, Bermuda (32°22′53″N, 64°38′11″W), an exposed reef featuring a typical Bermuda reef community with a high coral cover (>30%, Y.S. personal observation). Bermuda's coral reefs are high-latitude subtropical reefs featuring pronounced seasonality in SST ranging from ~19°C in March to ~29°C in August (Steinberg et al., 2001; Gould et al., 2021). Water temperature during coral collection was close to 28°C. Corals were transported in coolers filled with seawater to the wetlab at the Bermuda Institute of Ocean Sciences (BIOS) within 1 h of collection. At BIOS, they were kept in outdoor seawater tanks supplied with water from the adjacent Ferry Reach channel under light levels adjusted to *in situ* conditions. Corals were collected under license no. 201906005 provided by the Bermuda Government – Department of Environment and Natural Resources.

Experimental design

Prior to the experiment, each coral colony was cut with a diamond saw into seven fragments, which were then placed in an array of indoor aquaria. Each aquarium had a constant supply of fresh seawater and a small aquarium pump to ensure water mixing. Temperature was controlled using chillers (TECO TK500) and aquarium heaters (Accu-therm, Cobalt Aquatics) connected to temperature controllers (BTC201, Bayite). Light was supplied with

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aquarium lamps (PAR ~210–240 µmol photons m^{-2} s^{-1}; indoor grow light, Sun Blaze T5 Fluorescent 44) from 06:00 to 20:00 h. Coral fragments were left to recover and acclimatize to laboratory conditions for a period of 2 weeks. A total of nine aquaria were used, which were split between the three treatments (temperature levels). The fragments were randomly distributed between the three aquaria of the same treatment: (i) three of the seven fragments per colony were placed in a control aquarium where corals were kept at 28°C throughout the experiment (control), (ii) two of the seven fragments per colony were placed in an aquarium where the temperature was gradually decreased from 28°C to 24°C (24°C treatment), and (iii) the remaining two of the seven fragments per colony were placed in an aquarium where the temperature was gradually decreased from 28°C to 20°C (20°C treatment). The temperature was reduced by 0.5°C day^{-1} starting on day 1 of the experiment in the 20°C treatment and on day 8 in the 24°C treatment in order to reach target temperature on the same day (day 16). After the temperature reduction period, corals were kept at target temperature for 20 days (acclimatization period).

Measurements of net photosynthesis, respiration and gross photosynthesis

Net photosynthesis (NP) and respiration (R) were determined via respirometry (incubations) of one fragment per colony and treatment every 4 days, except between day 24 and 36 of the experiment, when no measurements were conducted. Incubations were conducted using the same coral fragments throughout the duration of the experiment. Non-coral organisms (e.g. algae) growing on exposed skeletal parts of the coral fragments were gently removed prior to incubations. Incubations were conducted as described in Sawall et al. (2020). Briefly, each fragment was placed separately in a 1 liter glass incubation chamber equipped with an oxygen sensor spot (PyroScience, Germany), a magnetic stirring bar, and a grid for coral placement above the stirring bar. The temperature inside the chambers was kept constant (same as in treatment aquaria) by placing the chambers inside a tub of water placed on top of the magnetic stirring plate. Light during incubations was equal to the experimental light intensity (PAR: 220–240 µE m^{-2} s^{-1}). The incubation setup allowed measuring 12 fragments in parallel, meaning that four rounds of incubations were necessary to incubate all desired fragments (48). For NP measurements, fragments were incubated for 30 min in the light, which was followed by a 30-min dark incubation for R measurements. Oxygen concentration was measured in the beginning and at the end of each incubation period (FireSting, PyroScience, Germany), and the change in oxygen concentration was used to calculate NP and R. NP and R rates were standardized to surface area and gross photosynthesis (GP) was calculated by adding R to NP. Surface area was determined by photography and subsequent picture analysis using ImageJ (fragments had a rather flat surface because both study species have a massive growth form).

Tissue metrics: analysis of zooxanthellae pigments and densities, and biomass

In order to investigate the underlying mechanism of potential changes in GP and R rates, we examined zooxanthellae density, light-harvesting and photo-protecting pigment concentration, and biomass of the coral host and the holobiont (host+zooxanthellae). For this, coral fragments were harvested at three time points throughout the experiment: on days 1, 17 and 37. On day 1, one fragment per colony was removed from the 28°C treatment, which served as a baseline for all subsequent tissue metric analyses. On days 17 and 37, one fragment per colony was removed from the 28°C, 24°C and 20°C treatments, with the corals harvested on day 37 being those used for incubations throughout the experiment. Coral tissue was removed from the skeleton immediately after removal from the treatment aquarium with an airbrush and filtered seawater (15–30 ml). The resulting tissue slurry was homogenized for 30 s using an UltraTurrax homogenizer, the total volume of the slurry was measured and then distributed between aliquots. Aliquots were stored at −20°C for later zooxanthellae density and biomass analyses and at −80°C for later pigment analyses.

Pigment concentrations were measured by means of reverse-phase high performance liquid chromatography (HPLC; Agilent 1100) following an adapted method of Wright et al. (1991). For pigment extraction, 350 µl of tissue slurry was combined with 3 ml of 100% acetone, mixed with an UltraTurrax homogenizer, and then placed in a freezer (−20°C, 18–20 h). The sample was briefly homogenized again and then centrifuged to remove cell debris from the extract (7460 ***g***, 10 min). The extract was filtered through a 0.2 µm PTFE membrane filter (Pall Life Sciences) and 1 ml of the filtered extract was transferred to an amber glass HPLC vial and diluted for injection with 300 µl water. A gradient elution was used which included 80:20 methanol:ammonium acetate, 90:10 acetonitrile:water and 100% ethyl acetate at a flow rate of 1 ml min^{-1} for 38 min. A Waters C18 Spherisorb column (5 µm particle size, 250×4.6 mm) was used in addition to a Waters C18 Spherisorb guard column (5 µm particle size, 10×4.6 mm). Pigment peaks were detected via a diode array detector measuring absorbance at 436 nm and pigment species was identified using retention time comparison to previously analyzed commercial reference standards (DHI). To quantify pigment concentration, peak areas were integrated manually in Agilent ChemStation 2 software. The light-harvesting pigments chlorophyll *a*, chlorophyll c_2 and peridinin, and the photoprotective pigments diadinoxanthin and diatoxanthin were measured and standardized to the coral surface area.

Zooxanthellae density was measured after defrosting the tissue slurry and briefly homogenizing the sample again. A drop of slurry was placed on a hemocytometer and zooxanthellae cells were counted under a microscope (e.g. Sawall et al., 2014). An average of six replicate counts (six drops) was used to calculate zooxanthellae density standardized to surface area.

Biomass of the coral host and zooxanthellae were determined separately. For this, tissue slurry was thawed and homogenized, and 1.5 ml of slurry was placed in a dry and pre-weighed 2-ml centrifuge tube. The slurry was centrifuged (7460 ***g***, 10 min) to separate coral zooxanthellae from host tissue. The supernatant (host tissue) was filtered through a dry and pre-weighed GF/F filter (Whatman) using a syringe and filter holder. Another 1.5 ml of slurry was placed in the same centrifuge tube and centrifuged again. The supernatant was discarded and the zooxanthellae pellet, as well as the filter with the host biomass, was dried at 60°C until reaching constant mass. Dry mass (biomass) of host and zooxanthellae were determined, and host and holobiont biomass were standardized to surface area. Host biomass was used as an indicator for potential changes in energy reserves and holobiont biomass as an additional approach to standardize R rates.

Data analysis

Data are presented as the relative difference (Δ) in percent between the 28°C control treatment and the 24°C and 20°C treatments, respectively, at any given time point, as well as in absolute values (Figs S1–S4). Delta values were applied to gain a clearer picture of

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temperature-driven trends over time, as some parameters also showed a slight change over time in the control treatment (28°C; see Figs S1–S4). The relative differences in gross photosynthesis (ΔGP) and respiration (ΔR) were plotted against time (days), and a polynomial curve fit was conducted to visualize changes over time. Data outside the cooling period of the 24°C treatment (first 8 days of experiment) were excluded from the curve fit, because no change in metabolic rates was expected at constant 28°C. All other response parameters were plotted against time (days) as well, but no curve fitting was conducted owing to the low number of repeat measurements (three). Data are presented as means±s.e.m., and the mean is the average of six to eight biological replicates. The original replication is eight, but as some fragments died throughout the experiment, replication decreased by one to two coral fragments over time in some treatments.

Generalized linear mixed models (GLMMs) were applied to each response parameter to statistically test the effect of temperature over time. The analysis was carried out in the R environment (version 4.0.5; https://www.r-project.org/) using the R package lme4 (version 1.1-27.1; Bates et al., 2015). As the interest was in the effect of temperature reduction over time, the fixed effects were temperature and time. Because the parameters were measured on the same individuals throughout the duration of the experiment, the replicates as well as the experimental units (aquaria) were considered as random effects. The family applied was gamma with log link and the models used were either polynomial function of the first or second order, depending on the best fit based on Akaike's information criterion (AIC).

RESULTS

Temperature

Temperature was 28.2±0.4°C (mean±s.d.) throughout the experiment in the control treatment, and 24.2±0.2°C and 20.2±0.5°C after reaching target temperature in the 24°C and 20°C treatments (Fig. 1).

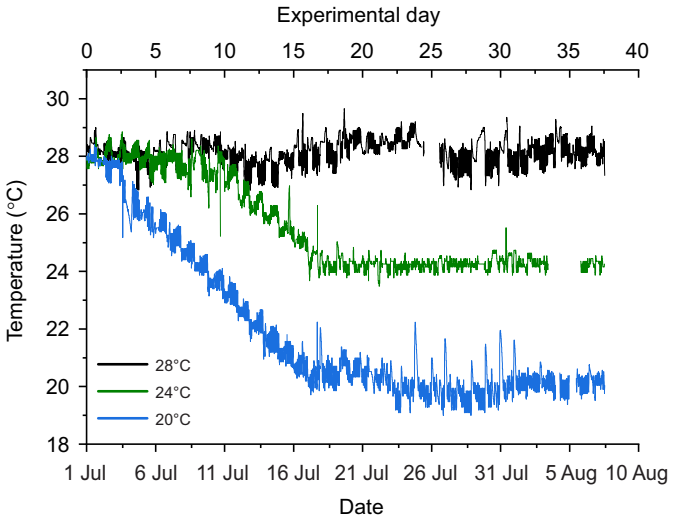

Fig. 1. Temperature measured in one aquarium of each treatment group throughout the duration of the experiment. Gaps in the data set indicate missing data.

Metabolic rates

Both GP (ΔGP) and R (ΔR) decreased substantially and significantly with temperature over time, and the magnitude of decrease varied significantly between treatment levels (24°C versus 20°C) in both species (Fig. 2, Table 1). Specifically, the GP of *M. cavernosa* exposed to 24°C decreased to 77±9% of the control value at the end of the temperature reduction period (day 17) and to 69±6% at the end of the acclimatization period (day 37). This was more pronounced at 20°C, where the GP of *M. cavernosa* decreased to 49±8% and further to 33±4%, respectively (Fig. 2A). The GP of *P. astreoides* initially decreased less than that of *M. cavernosa*, in particular at 24°C, remaining at 81±13% of the control value at the end of temperature reduction period. However, this was followed by a strong decrease of GP during the acclimatization period, resulting in only 43±8% on day 37 (Fig. 2B). At 20°C, the GP of *P. astreoides* reached 57±12% at the end of temperature reduction period and 47±9% at the end of the acclimatization period (Fig. 2B).

The magnitude of decline was stronger for R than for GP. The R of *M. cavernosa* exposed to 24°C decreased to 56±29% of the control value during the temperature reduction period and further to 46±14% at the end of the acclimatization period. At 20°C, the R of *M. cavernosa* decreased more severely than at 24°C, resulting in only 32±8% on day 17 and 8±3% on day 37, with the latter being the overall lowest metabolic activity measured throughout the experiment (Fig. 2C). The R of *P. astreoides* decreased to a lesser degree than in *M. cavernosa*, but still substantially, following a similar pattern as the GP of *P. astreoides* (24°C: 87±16% on day 17 and 43±8% on day 37; 20°C: 58±13% and additional 40±19%; Fig. 2D). Because R declined stronger than GP, the GP/R ratios increased significantly with decreasing temperature over time, and the magnitude of increase was significantly different between treatment levels (Table 1). This was more pronounced in *M. cavernosa*, where GP/R increased almost 5-fold by the end of the experiment in the 20°C treatment, while in *P. astreoides*, GP/R increased only approximately 3-fold (Fig. 2E,F).

Zooxanthellae metrics

In general, zooxanthellae metrics of *M. cavernosa* changed significantly over time, although the effects of the temperature levels did not differ (Table 1). The zooxanthellae density deviated from the control values more strongly on day 17 (after the temperature reduction period) than on day 37 (after the acclimatization period; Fig. 3). Specifically, zooxanthellae density of *M. cavernosa* increased to 224±17% (24°C) and to 163±29% (20°C) of the control value, light-harvesting pigments increased to 175±32% (24°C) and 180±29% (20°C) and photoprotective pigments increased to 196±56% (24°C) and 210±54% (20°C) at the end of the temperature reduction period. All metrics returned close to control values at the end of the acclimatization period (Fig. 3A,C,E,G). In contrast, the response of *P. astreoides* to temperature was much less pronounced (Fig. 3B,D,F,H, Table 1), and the strongest deviations from control conditions were found on day 37, with an increase in light-harvesting pigments to 158±38% (Fig. 3D).

As a product of a simultaneous decrease of areal GP (GP cm^{-2}; Fig. 2) and increase of pigment concentration (Fig. 3), pigment-specific GP rate or photosynthetic efficiency (GP light-harvesting $pigments^{-1}$; Fig. 3I) of *M. cavernosa* decreased significantly by day 17 (Table 1), resulting in GP light-harvesting $pigments^{-1}$ of 58±22% at 24°C and 35±12% at 20°C; Fig. 3I). During the acclimatization period, GP light-harvesting $pigments^{-1}$ remained rather stable.

In *P. astreoides*, GP light-harvesting $pigments^{-1}$ responded less strongly than in *M. cavernosa* during the temperature reduction period as GP cm^{-2} and zooxanthellae metrics changed less

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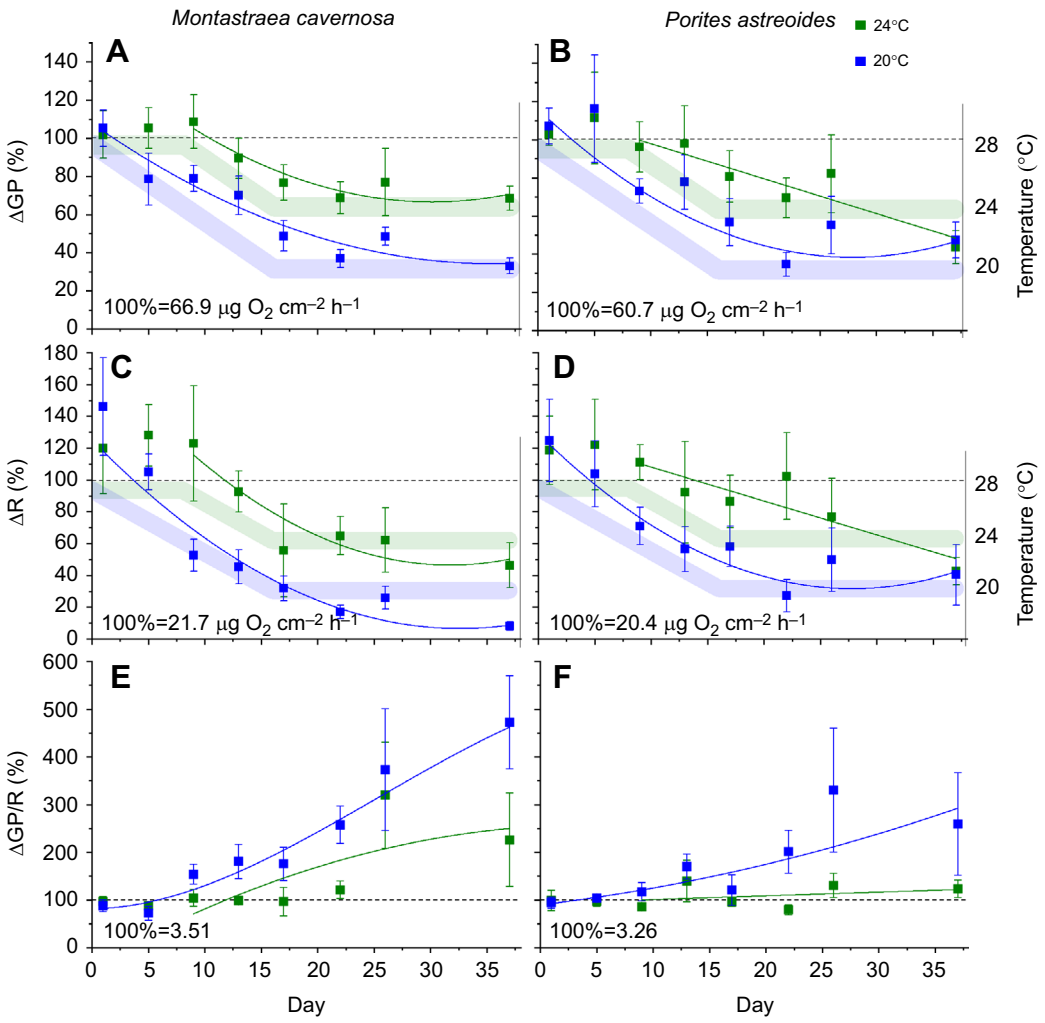

Fig. 2. Metabolic rates of *Montastraea cavernosa* (left) and *Porites astreoides* (right) over the experimental period of 37 days. (A–D) Difference (Δ) in gross photosynthesis (GP) and respiration (R) rates between 28°C and 24°C (green) and between 28°C and 20°C (blue). Mean±s.e.m., *n*=6–8. Curve fittings follow first- or second-order polynomial functions. (E,F) Difference (Δ) in GP/R ratios between 28°C and 24°C (green) and between 28°C and 20°C (blue). For reference, values inside each graph indicate the mean value measured at 28°C on day 1 of the experiment. Temperature treatments are shown as thick semi-transparent lines. Black dashed lines are reference lines at 100%.

strongly as well. Hence, GP light-harvesting pigments^{-1} increased to 123±17% at 24°C and decreased to 70±20% at 20°C (Fig. 3J). At the end of the acclimatization period, however, all rates decreased significantly, reaching between 50 and 60% of the control values in both temperature treatments (Fig. 3J, Table 1).

Coral host metrics

Coral host biomass remained close to control values during the temperature reduction period, but increased significantly during the acclimatization period in both species, which was more pronounced in the 20°C treatment compared with the 24°C treatment (Fig. 4A,B, Table 2). Hence, final biomass of *M. cavernosa* was 133±12% at 24°C and 181±6% at 20°C (Fig. 4A), and 173±34% at 24°C and 229±23% at 20°C in *P. astreoides* (Fig. 4B). Biomass-specific R rate (holobiont biomass; R biomass^{-1}) declined significantly in both species over time (Table 2) with a steeper decline of R biomass^{-1} during the temperature reduction period in *M. cavernosa* and during the acclimatization period in *P. astreoides* (Fig. 4C,D). The combination of increased biomass and reduced R cm^{-2} resulted in particularly low R biomass^{-1} rates at the end of the experiment especially in the 20°C treatment [24°C: 45±17% (*M. cavernosa*), 30±13% (*P. astreoides*); 20°C: 7±4% (*M. cavernosa*), 7±1% (*P. astreoides*); Fig. 4C,D, Table 2].

Synthesis of results

Fig. 5 represents a synthesis of the results that allows direct comparison of the most important response parameters. The following patterns become apparent. (i) Parameters related to metabolic rates (GP and R; solid symbols in Fig. 5) decrease with temperature during the temperature reduction period and either decrease further or remain rather stable during the experimental acclimatization period. (ii) Parameters representing zooxanthellae tissue metrics either increase with decreasing temperature during the temperature reduction period and return close to control values during the acclimatization period (*M. cavernosa*) or do not change during the temperature reduction period and alter only little during the acclimatization period (*P. astreoides*). (iii) Host biomass remains stable during the temperature reduction period, but increases substantially during the acclimatization period. (iv) Temperature effects are generally stronger in the 20°C than in the 24°C treatment. (v) *Montastraea cavernosa* appears to have a faster and stronger temperature response than *P. astreoides*.

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Table 1. GLMM results of each metabolic rate and zooxanthellae metric response variable

		Montastraea cavernosa				*Porites astreoides*			
Response variable	Fixed effects	Estimate	s.e.	*t*	Pr(>|z|)	Estimate	s.e.	*t*	Pr(>|z|)
Gross photosynthesis (GP)	(Intercept)	4.086	0.082	49.71	**<0.001**	4.168	0.113	36.85	**<0.001**
	poly(Time)1	−4.267	0.464	−9.19	**<0.001**	−3.684	0.660	−5.58	**<0.001**
	poly(Time)2	–	–	–	–	1.325	0.653	2.03	0.042
	Temp	0.334	0.060	5.57	**<0.001**	0.242	0.082	2.94	0.003
	poly(Time)1:Temp	2.559	0.658	3.89	**<0.001**	0.838	0.897	0.93	0.350
	poly(Time)2:Temp	–	–	–	–	−1.914	0.913	−2.10	0.036
Respiration (R)	(Intercept)	3.667	0.119	30.86	**<0.001**	4.034	0.149	27.06	**<0.001**
	poly(Time)1	−9.519	0.881	−10.81	**<0.001**	−4.817	1.027	−4.69	**<0.001**
	poly(Time)2	–	–	–	–	1.875	0.982	1.91	0.056
	Temp	0.738	0.113	6.51	**<0.001**	0.477	0.125	3.82	**<0.001**
	poly(Time)1:Temp	5.850	1.259	4.65	**<0.001**	1.316	1.351	0.98	0.330
	poly(Time)2:Temp	–	–	–	–	−2.746	1.354	−2.03	0.043
GP/R	(Intercept)	5.276	0.092	57.21	**<0.001**	5.082	0.103	49.33	**<0.001**
	poly(Time)	6.380	0.799	7.99	**<0.001**	4.106	0.759	5.41	**<0.001**
	Temp24	−0.374	0.096	−3.89	**<0.001**	−0.457	0.094	−4.85	**<0.001**
	poly(Tme):Temp	−2.383	1.122	−2.12	**0.033**	−3.159	1.035	−3.05	0.002
Zooxanthellae density	(Intercept)	4.838	0.057	84.87	**<0.001**	4.558	0.094	48.68	**<0.001**
	poly(Time)1	0.934	0.347	2.70	**0.007**	−0.367	0.385	−0.95	0.341
	poly(Time)2	−0.870	0.367	−2.37	**0.018**	–	–	–	–
	Temp	0.025	0.086	0.30	0.768	−0.005	0.085	−0.06	0.956
	poly(Time)1:Temp	−0.401	0.565	−0.71	0.478	−0.044	0.534	−0.08	0.934
	poly(Time)2:Temp	−1.011	0.492	−2.06	**0.040**	–	–	–	–
Light-harvesting (LH) pigments	(Intercept)	4.814	0.065	73.54	**<0.001**	4.740	0.103	45.92	**<0.001**
	poly(Time)1	0.555	0.373	1.49	0.137	0.885	0.439	2.02	0.044
	poly(Time)2	−1.287	0.373	−3.45	**0.001**	–	–	–	–
	Temp	−0.066	0.087	−0.76	0.445	−0.189	0.096	−1.96	0.050
	poly(Time)1:Temp	−0.473	0.549	−0.86	0.389	−0.767	0.594	−1.29	0.197
	poly(Time)2:Temp	−0.129	0.506	−0.25	0.800	–	–	–	–
Photoprotective pigments	(Intercept)	4.775	0.101	47.22	**<0.001**	4.540	0.088	51.60	**<0.001**
	poly(Time)1	0.138	0.410	0.34	0.736	−0.442	0.477	−0.93	0.355
	poly(Time)2	−1.589	0.414	−3.84	**<0.001**	–	–	–	–
	Temp	0.064	0.095	0.67	0.500	−0.061	0.102	−0.60	0.550
	poly(Time)1:Temp	0.605	0.598	1.01	0.312	−0.119	0.643	−0.19	0.853
	poly(Time)2:Temp	0.395	0.548	0.72	0.471	–	–	–	–
LH pigments zooxanthellae^{-1}	(Intercept)	4.575	0.077	59.41	**<0.001**	131.060	12.630	10.38	**<0.001**
	poly(Time)1	−0.658	0.473	−1.39	0.164	179.030	61.320	2.92	0.007
	poly(Time)2	−0.869	0.483	−1.80	0.072	–	–	–	–
	Temp	0.044	0.108	0.41	0.685	−20.680	13.750	−1.50	0.146
	poly(Time)1:Temp	1.221	0.722	1.69	0.091	−81.250	85.670	−0.95	0.353
	poly(Time)2:Temp	1.744	0.622	2.80	**0.005**	–	–	–	–
GP LH pigments^{-1}	(Intercept)	3.847	0.157	24.49	**<0.001**	4.141	0.140	29.66	**<0.001**
	poly(Time)1	−3.108	1.009	−3.08	**0.002**	−3.552	0.651	−5.46	**<0.001**
	poly(Time)2	3.336	1.157	2.89	**0.004**	0.357	0.741	0.48	0.630
	Temp	0.322	0.191	1.69	0.092	0.444	0.131	3.39	**<0.001**
	poly(Time)1:Temp	2.095	1.330	1.58	0.115	0.542	0.852	0.64	0.525
	poly(Time)2:Temp	−1.615	1.528	−1.06	0.290	−2.838	0.969	−2.93	0.003

The numbers 1 and 2 after '(Time)' of fixed effects refer to the order of the polynomial function (poly) applied. In case of a second-order polynomial function, two *P*-values are given: the first for the first component of the function (bx) and the second for the second component of the function (cx^2). Temp, temperature. GLMM results of the random effects are provided in Table S1. Bold indicates a statistically significant effect.

DISCUSSION

The 5-week temperature reduction experiment conducted with corals within their natural temperature range (~19°C to 29°C) revealed pronounced and species-specific responses. Although physiological adjustments to temperature reductions were significant, thermal compensation appeared to be rather limited.

Metabolic rates generally followed the changes in temperature, with decreasing rates during the temperature reduction period and rather consistent or slowly decreasing rates during the constant temperature of the experimental acclimatization period. The decline of metabolic rates during the temperature reduction period meets our expectations, as enzymes involved in photosynthesis and respiration – like most enzymes – are strongly temperature dependent (Kavanau, 1950; Dewar et al., 2001; Hikosaka et al., 2005). However, the fact that R and GP remained low or decreased even further during the acclimatization period is in contrast to our expectation. This is because we predicted that physiological adjustments would at least partly counterbalance temperature-driven enzyme inactivation, which would have been evident in an increase in R and GP during the acclimatization period. Furthermore, this is accompanied by some unexpected patterns in the tissue metrics, most importantly a strong initial response of zooxanthellae densities and/or pigmentation during the temperature reduction period, followed by a return to near-control values during the experimental acclimatization period in *M. cavernosa*. Also, although biomass increased considerably during the acclimatization period in both species, R did not change.

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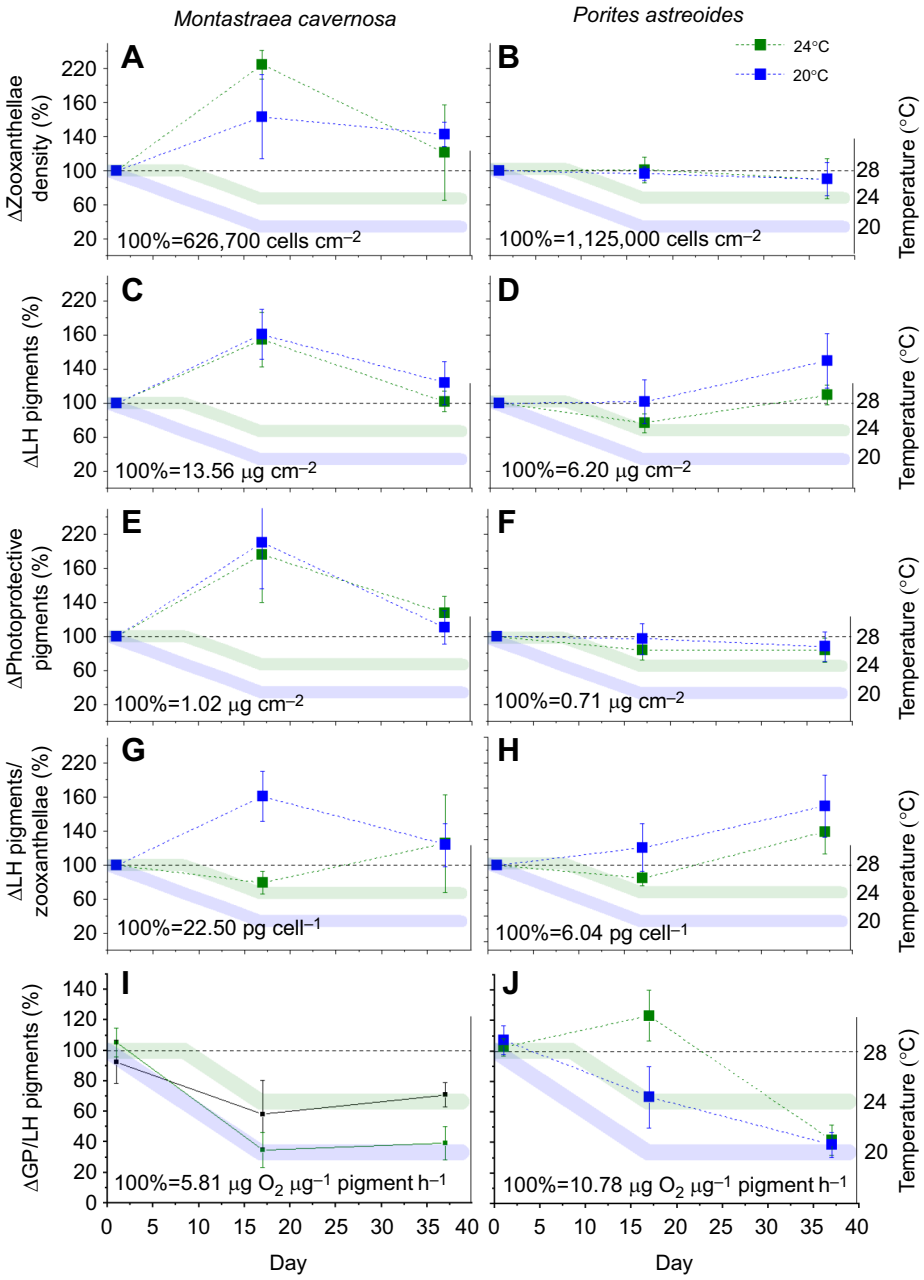

Fig. 3. Zooxanthellae metrics of *Montastraea cavernosa* (left) and *Porites astreoides* (left) over the experimental period of 37 days. (A,B) Difference (Δ) in zooxanthellae density between 28°C and 24°C (green) and between 28°C and 20°C (blue), (C,D) Δ in light-harvesting (LH) pigments, (E,F) Δ in photoprotective pigments, (G,H) Δ in LH pigments per zooxanthellae and (I,J) Δ in GP per µg LH pigments. Mean±s.e.m., n=6–8. Δ values on day 1 equal 100%, since there is differentiation between treatments. For reference, values inside each graph indicate the mean value measured at 28°C on day 1 of the experiment. Temperature treatments are shown as thick semi-transparent lines. Black dashed lines are reference lines at 100%.

The strong initial increase of zooxanthellae densities (more than doubling) in *M. cavernosa* during the temperature reduction period (28°C to 24°C), followed by a decrease in zooxanthellae density during further temperature reduction (24°C to 20°C) and during constant low temperatures (24°C or 20°C) may be explained by an initial ‘over-reaction’. When the coral experiences a change in temperature that exceeds natural short-term temperature fluctuations (e.g. diurnal fluctuations), it counterbalances reductions in photosynthesis by propagating zooxanthellae cells. *Montastraea cavernosa* is typically dominated by *Symbiodinium* clade C (Savage et al., 2002; Hauff et al., 2016), a clade that is common in ‘cooler’ reef waters (LaJeunesse, 2005; Hauff et al., 2016). As in a number of cold-water organisms (Somero, 2004), enzyme activity may show a stronger thermal sensitivity (or responsiveness) in clade C than in other *Symbiodinium* clades, which is also reflected by the strong initial reduction of the photosynthetic efficiency (GP light-harvesting pigments^{-1}) of *M. cavernosa*. During the acclimatization period, however, zooxanthellae densities decreased, light-harvesting pigments per zooxanthella either increased (24°C) or decreased (20°C), approaching near-control conditions, and photosynthetic efficiency (PG light-harvesting pigments^{-1}) remained rather constant. This re-establishment of initial zooxanthellae metrics (density and pigments) may be seen as the zooxanthellae bouncing back from their initial ‘over-reaction’. At the same time, it indicates a lack of temperature compensation, but an adjustment to a new energy equilibrium, namely a lower energy turnover. If temperature compensation had occurred, it would have been expected that photosynthetic efficiency (as well as GP) would have increased during the experimental acclimatization period, as a consequence of adjustments in, for example, enzyme structure or concentration (Somero, 2004; Ensminger et al., 2006; Arcus and Mulholland, 2020). Temperature compensation could have also occurred by changing the genetic composition of the *Symbiodinium* community, towards a community that is more efficient at low

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Table 2. GLMM results of the coral host response parameters

		Montastraea cavernosa				*Porites astreoides*			
Response variable	Fixed effects	Estimate	s.e.	*t*	Pr(>|z|)	Estimate	s.e.	*t*	Pr(>|z|)
Host biomass	(Intercept)	4.841	0.070	68.86	**<0.001**	4.921	0.059	84.12	**<0.001**
	poly(Time)1	1.521	0.326	4.67	**<0.001**	2.248	0.216	10.39	**<0.001**
	poly(Time)2	–	–	–	–	0.824	0.204	4.05	**<0.001**
	Temp	−0.103	0.066	−1.55	0.120	−0.154	0.046	−3.37	**<0.001**
	poly(Time)1:Temp	−0.722	0.439	−1.64	0.100	−0.771	0.297	−2.60	0.009
	poly(Time)2:Temp	–	–	–	–	0.115	0.278	0.41	0.680
R biomass^{-1}	(Intercept)	3.474	0.231	15.04	**<0.001**	4.090	0.196	20.88	**<0.001**
	poly(Time)1	−11.872	1.363	−8.71	**<0.001**	−9.020	1.214	−7.43	**<0.001**
	poly(Time)2	–	–	–	–	−3.073	1.317	−2.33	0.020
	Temp	0.745	0.183	4.07	**<0.001**	0.417	0.220	1.90	0.058
	poly(Time)1:Temp	8.052	1.661	4.85	**<0.001**	4.486	1.553	2.89	0.004
	poly(Time)2:Temp	–	–	–	–	0.386	1.702	0.23	0.821

The numbers 1 and 2 after '(Time)' of fixed effects refer to the order of the polynomial function (poly) applied. In case of a second-order polynomial function, two *P*-values are given: the first for the first component of the function (*bx*) and the second for the second component of the function (cx^2). Temp, temperature. GLMM results of the random effects are provided in Table S1. Bold indicates a statistically significant effect.

temperatures. *Montastraea cavernosa* is known for seasonal *Symbiodinium* 'shuffling', meaning that it changes the relative abundance of different *Symbiodinium* clades or types in response to seasonal changes of environmental conditions (Ulstrup and Van Oppen, 2003; Thornhill et al., 2006; Hauff et al., 2016). Our results, however, do not support *Symbiodinium* shuffling during the experiment, indicating that *Symbiodinium* shuffling might be triggered either by another parameter than temperature or by a combination of parameters that vary seasonally.

An increase of zooxanthellae density instead of a change in pigments per cell in response to temperature reduction seems surprising at first, because adjustments on individual components of a cell are typically more cost-effective than propagating entire cells. It was expected that either light-harvesting pigments would be reduced to avoid over-excitation in consequence of a lower capacity for light processing (reduced enzyme activity) at lower temperature, or photoprotective pigments would be increased in order to neutralize excess light energy (Ensminger et al., 2006). A likely reason for changes in cell density rather than on pigment concentrations is therefore based on the need to counterbalance the strong reduction of photosynthetic efficiency by increasing zooxanthellae density, which, at the same time, increases self-shading amongst zooxanthellae, thereby avoiding over-excitation. A change of the internal nutrient dynamics, as suggested by a previous coral cold stress experiment (Saxby et al., 2003), may facilitate the strong increase in zooxanthellae density.

The zooxanthellae community of *P. astreoides* showed a much lower responsiveness to temperature reductions than that of *M. cavernosa*, which is likely related to the predominantly abundant *Symbiodinium* clade A (Hauff et al., 2016; Reich et al., 2017), a clade that is known to be rather thermo-tolerant (Stat et al., 2008; Lesser et al., 2013). More thermo-tolerant clades have previously been found to have a lower temperature responsiveness and a wider thermal breadth, as determined by temperature performance curves (Jurriaans and Hoogenboom, 2020), and supported by the rather low decrease in GP during the temperature reduction period at 24°C in this study. Interestingly, during the experimental acclimatization period, corals at 24°C showed a substantial and stronger decrease in GP and photosynthetic efficiency than at 20°C, while corals at 20°C showed a stronger increase of light-harvesting pigments. The decrease in GP and photosynthetic efficiency points towards an adjustment to a lower energy demand at low temperatures, and a

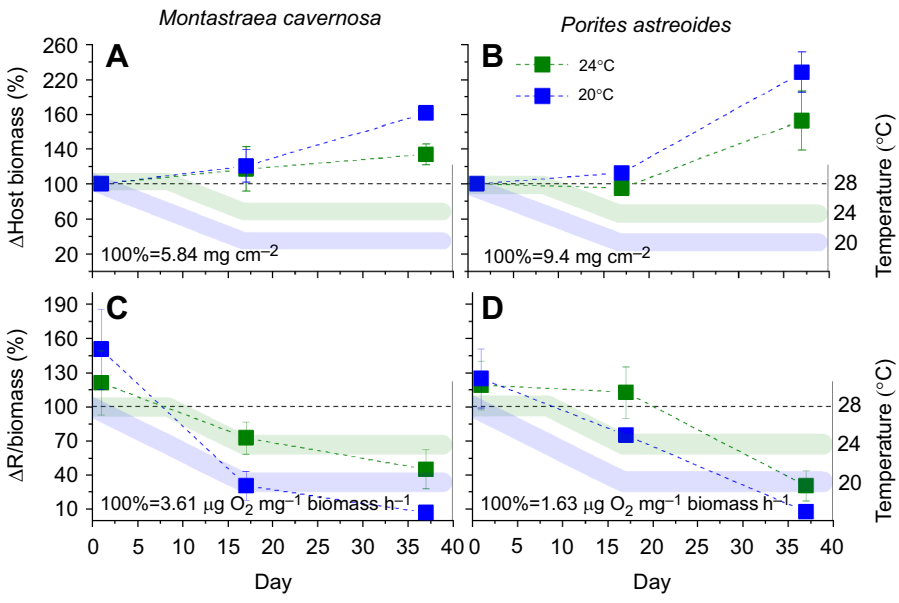

Fig. 4. Coral host characteristics of *Montastraea cavernosa* (left) and *Porites astreoides* (right). (A,B) Difference (Δ) in coral host biomass and (C,D) respiration (R) standardized to total (host+zooxanthellae) biomass between 28°C and 24°C (green) and between 28°C and 20°C (blue). Mean±s.e.m., *n*=6-8. For reference, value inside each graph indicate the mean value measured at 28°C on day 1 of the experiment. Temperature treatments are shown as thick semi-transparent lines. Black dashed lines are reference lines at 100%.

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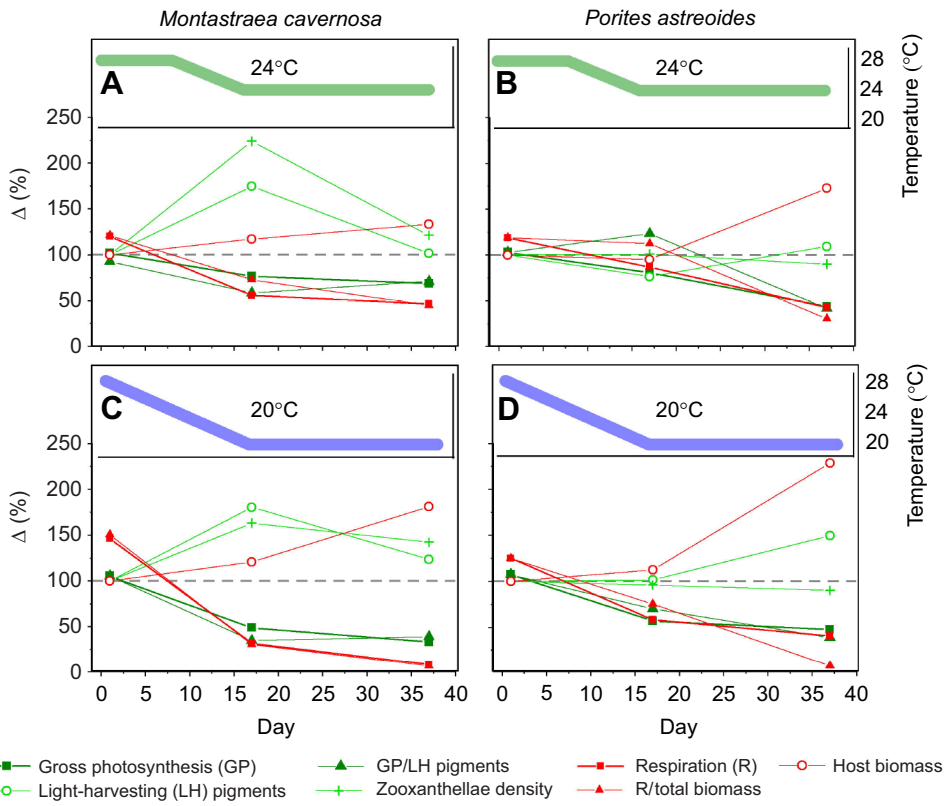

Fig. 5. Synthesis of results. All values are presented as the relative difference (Δ) between the control value (28°C; 100%) and the value at the corresponding temperature treatment (24°C and 20°C) at a given time point. Green, zooxanthellae related parameters; red, coral host related parameters; closed symbols, metabolic rates; open symbols, tissue metrics.

possible underlying mechanism is a decrease in photosynthesis-related enzyme concentrations. In contrast, the increase of light-harvesting pigments, in particular in the 20°C treatment (~50%), shows that temperature compensation is taking place to some degree, keeping GP at 20°C at similar levels as PG at 24°C. It appears that although *P. astreoides* has a rather low immediate sensitivity to temperature changes, it has the ability for thermal compensation, in particular under rather extreme temperature changes (here Δ8°C).

The amount of energy utilized by an organism can be inferred from the respiration rate, as this process transforms fixed organic carbon (through photosynthesis or heterotrophy) into molecular energy (ATP). ATP is required for various cellular processes, including basal metabolic processes of cell maintenance (e.g. protein synthesis and repair, trans-membrane transporters; Clarke and Fraser, 2004), growth (calcification and polyp proliferation), defense and reproduction. Unlike photosynthesis, respiration seems to have a lower capacity to counter temperature-driven changes in metabolic rates resulting in increasing GP/R ratios with decreasing temperature (Dewar et al., 2001). The reason for a seemingly low capacity of mitochondria to counterbalance low temperatures may simply be that there is no need for it. The costs for cell maintenance are reduced under low temperature (Somero, 2004) and lower activity of potential pathogens require less defense capacity. Furthermore, low temperatures are temporary, meaning that corals can shift their main period of growth and reproduction to the warmer months of the year (Sawall et al., 2015; Sawall and Al-Sofyani, 2015), as also observed in Bermuda (Venti et al., 2014; de Putron and Smith, 2011). One may expect that heterotrophy increases under reduced temperature as a consequence of reduced energy supply through photosynthesis, as observed in temperate corals (Ferrier-Pagès et al., 2011). However, our results do not support an increase in heterotrophy as this would be evident in a relative increase of R over GP. In fact, the limited capacity of temperature compensation in mitochondria would also limit the capacity of heterotrophy.

The strong increase of biomass during the experimental acclimatization period (up to 2.2-fold) in our study is most likely the result of increasing GP/R ratios, meaning that more carbon is fixed than released. Photosynthetically derived carbon compounds are poor in nutrients (phosphorous and nitrogen; Dubinsky and Berman-Frank, 2001), and may hence be used as energy stores (e.g. carbohydrates and lipids) rather than to build-up tissue biomass (cells), which complies with the strong decline of R per biomass (here energy stores+tissue biomass) during the acclimatization period. Whether these energy reserves remain high or return to control conditions after an even longer acclimatization period – as observed in some terrestrial plants during long-term temperature manipulation studies (Dewar et al., 2001) – remains to be investigated. It also needs to be kept in mind that under natural (field) conditions, light usually declines before SST, meaning that an equivalent build-up of biomass towards winter under natural conditions is unlikely. This is further supported by a seasonal study conducted in the Red Sea, where biomass was similar in corals at the end of summer (September) and the end of winter (March), when SST differed substantially (Sawall et al., 2015).

Conclusions

The results of this study show that corals in the subtropics strongly reduce their metabolic rates at cooler winter temperatures, and that zooxanthellae adjust to a lower energy demand, which follows species-specific trajectories. The expression of temperature compensation mechanisms, however, seems to be rather limited, which is a likely reason for a decrease in coral diversity with increasing latitude (Sommer et al., 2018; Abrego et al., 2021). The broadcast spawning *M. cavernosa*, harboring a diverse but clade C dominated *Symbiodinium* community, featured a strong initial

temperature response of their zooxanthellae and a subsequent return of zooxanthellae metrics to control conditions, adjusting to a new low-energy equilibrium without (obvious) temperature compensation. In contrast, the brooder *P. astreoides*, predominantly harboring *Symbiodinium* clade A, revealed a rather low temperature sensitivity and hence a delayed adjustment in zooxanthellae metrics to decreased energy demand. Furthermore, *P. astreoides* compensated for very low temperatures, evident in increasing light-harvesting pigments over time at 20°C. Respiration (mitochondria performance) was reduced considerably in both species, but mostly in *M. cavernosa* at 20°C, indicating that instead of temperature compensation, an overall reduction in metabolic performance occurs. This provides a strong indication that the determining factor of whether a species can thrive under subtropical temperature conditions is the coral's energetic requirements. Only corals that are competitive despite reduced growth rates (e.g. effective in defense against space competitors and predators), that are efficient in reproduction, and that excel in performance and are capable of building up energy reserves when conditions are right are able to thrive under subtropical conditions.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: Y.S.; Methodology: Y.S., A.M.N.; Formal analysis: K.M., M.I.; Investigation: Y.S., A.M.N.; Data curation: Y.S., A.M.N.; Writing - original draft: Y.S., K.M.; Writing - review & editing: Y.S., M.I., A.M.N., K.M.; Visualization: Y.S., K.M.; Supervision: Y.S.

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