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The Effects of Temperature, Light, and Feeding on the Physiology of *Pocillopora damicornis*, *Stylophora pistillata*, and *Turbinaria reniformis* Corals

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Abstract: Evidence has shown that individually feeding or reduced light can mitigate the negative effects of elevated temperature on coral physiology. We aimed to evaluate if simultaneous low light and feeding would mitigate, minimize, or exacerbate negative effects of elevated temperature on coral physiology and carbon budgets. *Pocillopora damicornis*, *Stylophora pistillata*, and *Turbinaria reniformis* were grown for 28 days under a fully factorial experiment including two seawater temperatures (ambient temperature of 25 °C, elevated temperature of 30 °C), two light levels (high light of 300 µmol photons m^{-2} s^{-1}, low light of 150 µmol photons m^{-2} s^{-1}), and either fed (*Artemia* nauplii) or unfed. Coral physiology was significantly affected by temperature in all species, but the way in which low light and feeding altered their physiological responses was species-specific. All three species photo-acclimated to low light by increasing chlorophyll *a*. *Pocillopora damicornis* required feeding to meet metabolic demand irrespective of temperature but was unable to maintain calcification under low light when fed. In *T. reniformis*, low light mitigated the negative effect of elevated temperature on total lipids, while feeding mitigated the negative effects of elevated temperature on metabolic demand. In *S. pistillata*, low light compounded the negative effects of elevated temperature on metabolic demand, while feeding minimized this negative effect but was not sufficient to provide 100% metabolic demand. Overall, low light and feeding did not act synergistically, nor additively, to mitigate the negative effects of elevated temperature on *P. damicornis*, *S. pistillata*, or *T. reniformis*. However, feeding alone was critical to the maintenance of metabolic demand at elevated temperature, suggesting that sufficient supply of heterotrophic food sources is likely essential for corals during thermal stress (bleaching) events.

Keywords: coral physiology; heterotrophy; carbon budget; energy reserves; calcification; light; temperature; feeding

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1. Introduction

Under predicted future ocean conditions, mean global ocean temperature is estimated to increase 1–4 °C by 2100 [1]. As corals already live close to their upper thermal maximum, this baseline temperature shift is predicted to increase the frequency and severity of mass bleaching events [2,3]. Increasing sea surface temperature can cause the coral host-algal symbiosis to break down, resulting in coral bleaching as the endosymbiotic algae (Symbiodiniaceae) and/or algal pigments are lost from the coral tissue (e.g., [4–6]).

Within a healthy coral holobiont, endosymbiotic algae photosynthesize in excess of their daily metabolic needs and translocate fixed carbon to the coral host, often providing more than 100% of daily metabolic requirements (e.g., [7–9]), while heterotrophy can provide between ≈5 and 46% of daily metabolic requirements (e.g., [7,10,11]). However, under thermal stress, the loss of the endosymbiotic algae and/or photosynthetic pigments causes photosynthesis to decline (e.g., [12–14]), and corals are no longer able to meet metabolic demand through photosynthesis alone [7]. To compensate for this decrease in photosynthetic capacity, coral can undertake one or more of the following strategies to aid in the recovery and maintenance of metabolic demand: (1) increase heterotrophy (e.g., [7,15–17]), (2) catabolize energy reserves (e.g., [7,18,19]), (3) decrease respiration (e.g., [14,20,21]), and (4) decrease calcification rates (e.g., [22–24]). Heterotrophy can also mitigate bleaching damage to algal endosymbionts by stimulating photosynthesis and the re-establishment of host–algal symbioses [20,25,26], and by providing fixed carbon for tissue building and lipid synthesis [17,27,28].

Increases in photosynthetically active radiation (PAR) can inhibit the photosystems of endosymbiotic algae (photoinhibition) [29], which can be exacerbated with increased temperature (e.g., [30–32]). Therefore, reduced PAR may decrease damage from, or mitigate the negative effects of, elevated temperature stress on algal endosymbionts [33], so decreasing the thermal sensitivity of the holobiont [34–38]. Furthermore, Lesser [39] determined that the production of reactive oxygen species (ROS) by Symbiodiniaceae is greater under high light and thermal stress, and that low light could prevent, or decrease, the production of ROS that occurs under elevated temperature. Such evidence suggests that shading of corals (i.e., naturally on cloudy days or artificially via manipulation with shade-cloths) could provide a mechanism for protecting corals from elevated temperature stress. However, as photosynthetic capacity also decreases under low light, corals must photo-acclimate (i.e., increasing algal and/or pigment densities) [40–42] and/or increase heterotrophy to compensate [43–46].

Much work has been carried out on the individual effects of increased temperature, light, and feeding on coral physiology (e.g., [20,47,48]), but only one has tested the multi-stressor effects of all three simultaneously [49]. This study found feeding and low light levels to mitigate the negative effects of elevated temperature on chlorophyll *a* concentrations and photosynthetic rates in *Turbinaria reniformis* and *Stylophora pistillata*. However, no experiment to date has examined the interactive effects of all three variables on the overall coral holobiont (i.e., coral + endosymbiotic algae) physiology (i.e., calcification, biomass, lipids, chlorophyll *a*, and biogeochemistry) and carbon budgets of multiple species.

Here, we experimentally assess the individual and interactive multi-stressor effects of elevated temperature, reduced light, and feeding on the physiology, biogeochemistry, and carbon budget of three aquarium-cultured Red Sea coral: *Pocillopora damicornis*, *Stylophora pistillata*, and *Turbinaria reniformis*. These species were chosen as they represent a range of morphologies (fine branching, thick branching, and foliose, respectively), are ubiquitous throughout the tropical Pacific and Indian oceans, and have been well studied [9,16,49–55]. We hypothesize that reduced light and feeding will act synergistically to mitigate or minimize negative effects of elevated temperature on coral holobiont physiology and carbon budgets.

2. Materials and Methods

2.1. Experimental Design

Full metadata associated with this study is given in Table S1 [56]. Five colonies of *S. pistillata* and *T. reniformis* (originally sourced from the Red Sea) were collected from the Centre Scientifique de Monaco where they had been reared for >5 years in Mediterranean flow-through seawater tanks at 24.5 °C, 200 µmol photons m^{-2} s^{-1} with a 12:12 h day/night diurnal light cycle and fed two-day-old *Artemia* nauplii twice weekly. All colonies were assumed to be distinct genets. Additionally, in November 2012, five colonies of *P. damicornis* (originally sourced from the Red Sea) were acquired from five different mariculture facilities (Centre Scientifique de Monaco, Cap d'Agde, Oceanopolis, Nausicaa, and Marineland-Antibes), where they were reared under similar conditions for prolonged periods. Acquiring the *P. damicornis* from the 5 facilities increased the probability that colonies were distinct genets.

In March 2013, each colony of *P. damicornis*, *S. pistillata*, and *T. reniformis* was divided into 8 large and 8 small ramets, totaling 40 large (≈5 cm^2) and 40 small (≈3 cm^2) ramets per species. All ramets recovered under ambient seawater conditions (25 °C flow-through, filtered, oligotrophic Mediterranean seawater, 200 µmol photons m^{-2} s^{-1}, and fed two-day-old *Artemia* nauplii twice weekly) for one month. On 12 April 2013, ramets were distributed randomly and evenly among eight 17 L experimental tanks such that each tank contained one large and one small ramet from each coral colony of each species (N = 30 per tank). *P. damicornis* and *T. reniformis* ramets, each with a clay epoxy base engraved with a unique identifier, were placed on the tank bottom. *S. pistillata* ramets were suspended mid-tank by nylon thread with unique identifiers. Ramets acclimated for two additional weeks under ambient conditions as described above.

On 2 May 2013, a fully factorial experiment with eight treatments was initiated (Figure 1), including two temperatures (ambient temperature of 25 °C, elevated temperature of 30 °C), two light levels (high light of 300 µmol photons m^{-2} s^{-1}, low light of 150 µmol photons m^{-2} s^{-1}), and either fed (ad libitum twice a week for approximately 70 min with two-day old *Artemia* nauplii at an average concentration of 12.3 *Artemia* nauplii mL^{-1}) or unfed (Figure 1). While individual treatment replication was not possible due to equipment and space constraints within the experimental facility, there were four tanks of each treatment type rendering statistical analyses robust at that level (Figure 1). Any interpretation of effects between individual tanks was done with this caveat in mind. Coral ramets under ambient temperature, high light, and unfed served as the control within the experiment in order to test the effect of each treatment. Temperature conditions in each of the elevated temperature tanks was increased by 0.5 °C per day, until the target temperature of 30 °C was reached after 11 days. Temperature was continuously monitored and regulated within ±0.2 °C using temperature controllers, and it was recorded almost daily starting on 6 May 2013 (Figure 2). The ambient temperature of 25 °C is the long-term average temperature that these aquaria-reared corals have experienced for the past several years, while the average elevated temperature of 30 °C was employed because it has been shown that Red Sea species require a much larger temperature increase to experience temperature stress [16,57–59]. Previous studies have generally considered low light to be <300 µmol photons m^{-2} s^{-1} (e.g., [60–64]). The photosystem II of each species is saturated at 300 µmol photons m^{-2} s^{-1} (*Stylophora pistillata* [49], *Pocillopora damicornis* [64], and *Turbinaria reniformis* [65]). In addition, the Mediterranean seawater supplied to the tanks was filtered, but it still contained dissolved and particulate organic carbon, which is a known source of heterotrophic carbon to corals [66–70]. The fed corals were supplied *Artemia* nauplii twice a week at the same time and on the same days as they were accustomed to for the past several years, while the unfed corals were not. Coral ramets were subjected to experimental conditions (ramping plus target conditions) for 28 days, on a 12:12 h day/night diurnal light cycle, with an average flow rate of 14.4 L hr^{-1}. Ramet positions within each tank were rotated three times per week to remove any positional effects within the tanks, and tanks were cleaned once a week to minimize filamentous algal growth.

2.2. Host and Symbiont Physiology

Each large ramet was buoyant weighed at the start (3–4 May 2013) and end (27–29 May 2013) of the experiment [71]. During the last 3 days of the experiment (27 May–29 May 2013), the following measurements were also made on a subset (N = 3) of the small ramets: maximum photosynthetic rate (P_{max}) [72], light-enhanced dark respiration (R) [72] and feeding rate capacity [16]. Additionally, large and small ramets were photographed underwater. Once these measurements were made, all ramets were frozen on 30 May 2013, shipped, and stored at −80 °C at the Ohio State University until further analysis.

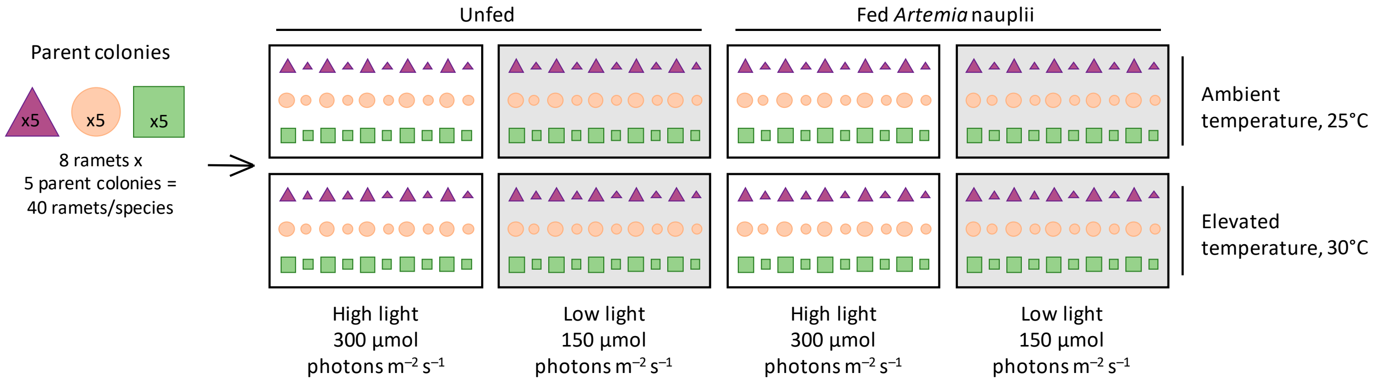

Figure 1. Experimental design schematic. Five parent colonies of *Pocillopora damicornis* ▲, *Stylophora pistillata* ●, and *Turbinaria reniformis* 🟩 were fragmented into eight large and eight small ramets and distributed randomly and evenly among eight treatments of a fully factorial experimental design: two seawater temperatures (ambient temperature of 25 °C, elevated temperature of 30 °C), two light levels (high light of 300 µmol photons m^{-2} s^{-1}, low light of 150 µmol photons m^{-2} s^{-1}), and either fed (*Artemia* nauplii) or unfed.

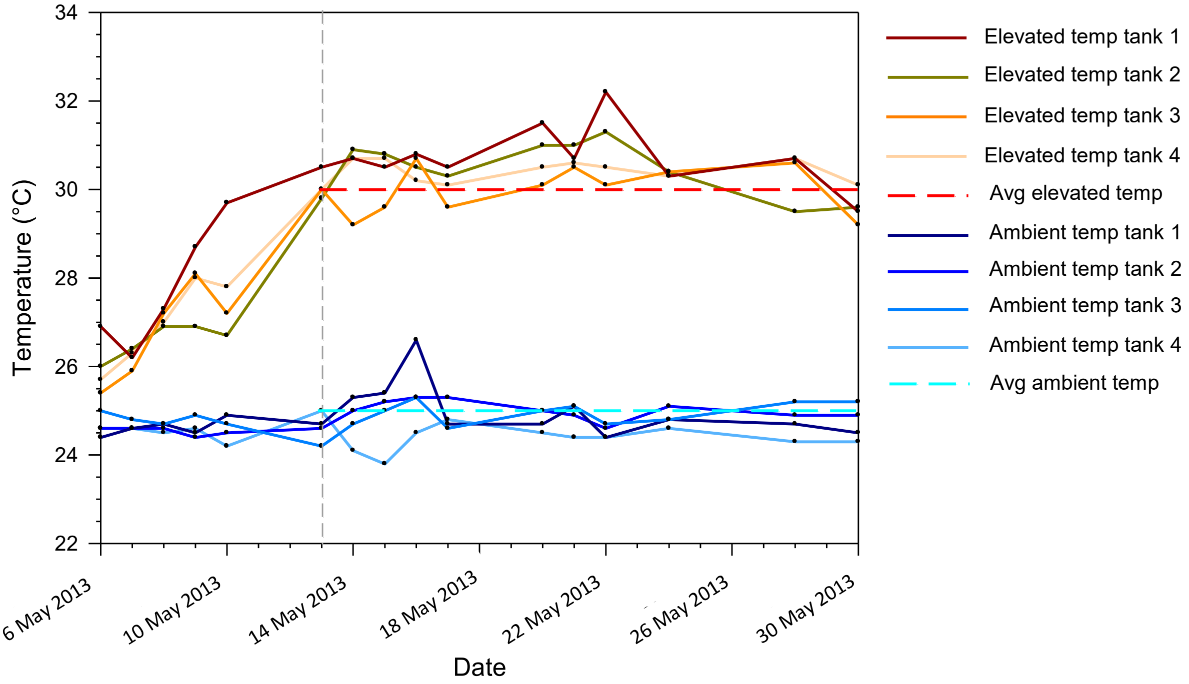

Figure 2. Temperature (°C) in each of the eight tanks over the duration of the study. The temperature ramp ended on 13 May 2013 as indicated by the vertical dashed gray line. Average temperature of the four ambient temperature tanks was 25 °C ± 0.5 (1SD), while average temperature for the elevated temperature tanks was 30 °C ± 0.6 (1SD). Average temperatures were calculated from the end of the ramping period on 13 May 2013 until the end of the experiment on 30 May 2013.

Surface area of large and small ramets were determined using the wax dipping technique [73] for *P. damicornis* and the foil technique [74] for *S. pistillata* and *T. reniformis*. Each small ramet of each species was airbrushed, and the tissue slurry was collected for isotopic analyses. Due to the delicate nature of *P. damicornis* ramets, air-brushed slurry was prepared for further downstream analyses of this species for small and large ramets. The large ramets of *S. pistillata* and *T. reniformis* were ground into a homogenous paste using a mortar and pestle. Chlorophyll *a* content was measured according to methods in Jeffrey and Humphrey [75], calcification using the buoyant weight technique of Jokiel et al. [71], and biomass according to methods in McLachlan et al. [76]. Photosynthesis (P), respiration (R), feeding rate, chlorophyll *a*, calcification, and biomass were standardized to surface area. Total lipids were extracted according to methods in McLachlan et al. [77], normalized to tissue biomass (g g^{-1} ash-free dry weight), and converted into Joules [78]. Total lipids were not measured on *P. damicornis* due to the limited sample available.

Carbon Budgets. P_G and R were used to calculate the Contribution of Zooxanthellae (Symbiodiniaceae) to Animal Respiration (CZAR) [8], while feeding rate capacity was used to calculate Contribution of Heterotrophy to Animal Respiration (CHAR) from feeding on *Artemia* nauplii using equations modified from Grottoli et al. [7]:

$$\text{CHAR} = \frac{H_C}{R_C} \times 100\% \tag{1}$$

where R_C is total daily respiration (gC day^{-1} gdw^{-1}), and H_C is the contribution of heterotrophy to daily metabolic demand (gC day^{-1} gdw^{-1}). H_C was modified and calculated as follows:

$$H_C = (F_R \times 3.65) \times C_Z \times M_i P_i \times 1.18 \tag{2}$$

where F_R is the feeding rate capacity (*Artemia* nauplii hour^{-1} gdw^{-1}), C_Z is the average proportion of carbon in *Artemia* nauplii (30%), M_i is the average ash-free dry weight of a single *Artemia* nauplius (3.04 × 10^{-6} g), P_i is the proportion of the diet that the plankton type contributes, 1.18 is the number of hours corals were allowed to feed on feeding days, and 3.65 is the proportionality constant that equates measured feeding rate capacity at the concentration of food available during feeding trials to the concentration of food available during the experiment, as the feeding capacity is related to food concentration [79–81]. The constant 3.65 was determined as follows:

$$\frac{B_E}{B_F} = 3.65 \tag{3}$$

where B_E is the average concentration of *Artemia* nauplii per ml available for consumption during the experiment, considering tank volume and flow rate (12.25 *Artemia* nauplii mL^{-1}), and B_F is the average concentration of *Artemia* nauplii per ml available for consumption during feeding trials (3.36 *Artemia* nauplii mL^{-1}). As coral were fed *Artemia* nauplii only two days per week, we assumed that on days they were not fed, the CHAR contribution was 0%. In addition, we did not measure the DOC and POC fluxes of the corals and so were unable to calculate CHAR from those potential heterotrophic sources. Thus, our CHAR calculations are very conservative as they only account for feeding on *Artemia* two days a week and do not include contributions from DOC or POC. To assess total carbon budget, CZAR and CHAR were summed to yield Contribution of Total fixed carbon to Animal Respiration (CTAR) [23]. Thus, the calculated CTAR values of fed coral only consider feeding days.

Host δ^{13}C ($\delta^{13}C_h$) isotopes. *Stylophora pistillata* were prepared for isotopic analyses according to methods modified from Hughes and Grottoli [17]. A subsample of coral tissue slurry was homogenized, sonicated (probe sonicator: 20% amplitude for 60 s total, 1:1 cycle), and 35 mg mL^{-1} NaCl added. The subsample was filtered through 20 µm nitex mesh to remove skeletal material, the algal endosymbiont isolated onto GF/F filters, and the host tissue filtrate dried down in 9 mm × 10 mm tin capsules on a heat plate (60 °C) with ultra-pure nitrogen gas (this step does not affect sample nitrogen isotope values [82]. *Pocillopora damicornis* and *T. reniformis* were prepared for isotopic analyses according to methods modified from Hughes and Grottoli [17] and described in Price et al. [83]. All *P. damicornis* and *T. reniformis* host fractions were combusted in an Elementar Vario EL Cube/Micro Cube elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer at the stable isotope facility at University of California—Davis. Repeated measurements of an internal standard have produced the long-term standard deviation of ±0.02‰ for δ^{13}C. *Stylophora pistillata* host fractions were combusted in a Costech elemental analyzer stable isotope ratio mass spectrometer (EA-IRMS), and the resulting C gas was automatically analyzed with a Thermo Finnigan Delta Plus isotope ratio mass spectrometer via a ConFlow open split interface in the Grottoli Stable Isotope Biogeochemistry Lab at Ohio State University. Repeated measurements of internal standards had a standard deviation of ±0.10‰ for $\delta^{13}C$. All $\delta^{13}C$ values are reported as the permil deviation of the ratio of stable carbon isotopes ^{13}C:^{12}C relative to Vienna PeeDee Belemnite (v-PDB). Approximately 10% of samples were run in duplicate, with an average standard deviation of ±0.16‰.

Artemia nauplii fed to the corals during this experiment have previously been measured to have $\delta^{13}C$ values of −12‰ [54].

2.3. Statistical Analyses

A Euclidean distance-based resemblance matrix was constructed to test the effect of treatment on overall coral holobiont physiological profile using normalized data of the primary physiological variables: chlorophyll *a*, $\delta^{13}C_h$, calcification, biomass, and (for *S. pistillata* and *T. reniformis* only) total lipids. Photosynthesis, respiration, and feeding rate were not included, as these variables were only measured on a subset of ramets within each species (N = 3). Collinearity amongst physiological response variables was investigated using Draftsman's plots and Pearson's correlation coefficient. No variables were strongly correlated (i.e., $|r| < 0.70$ [84]) and thus were treated as independent. Homogeneity of variance in the multivariate dispersions (PERMDISP) was tested, before three-way permutational multivariate analysis of variance (PERMANOVA) was used to evaluate the effects of temperature, light, and feeding on the overall physiology of each species [85]. Multivariate analyses were conducted using Primer v6 [85].

ANOVA analyses were also performed on each variable independently. Each variable dataset was tested for normality using Shapiro–Wilk's test, and homogeneity of variance was assessed with plots of expected vs. residual values. Calcification in *P. damicornis*, total lipids in *S. pistillata*, and calcification and chlorophyll *a* in *T. reniformis* were log-transformed to meet assumptions of normal distribution prior to further analysis. Normal distribution was not achievable for CZAR, CTAR, and $\delta^{13}C_h$ in *P. damicornis*, even with multiple transformation attempts. Cook's Distance was used to identify outliers [86], but outlier removal did not improve normality. Since ANOVA is robust to data with a non-normal distribution [87], ANOVA were still performed on the non-normal datasets, and any interpretations were made with this caveat in mind. Univariate Type III four-way analysis of variance (ANOVA) was used to test the effects of temperature, light, feeding, and genet on each measured variable for each species. Temperature was fixed with 2 levels (25 °C, 30 °C), light was fixed with 2 levels (high light at 300 μmol photons m^{-2} s^{-1}, low light at 150 μmol photons m^{-2} s^{-1}), feeding was fixed with 2 levels (fed *Artemia* nauplii, unfed), and genotype was included as a random effect. As no genotypes were found to be systematically different from all others, we concluded that the colonies represented natural variation in the population, genotype was removed from the models, and three-way type III ANOVAs were run (temperature, light, feeding) and used in interpretation of the data. The lack of any consistent genotype effect agrees with findings of previous similarly designed experiments [14,24,88]. Bonferroni corrections were not employed so as to not enhance Type II error [89]. For significant single effects, post-hoc Tukey tests were run to determine which means were significantly different. Finally, post-hoc slice tests were run to test the effect of the temperature within each light and feeding treatment for each variable. The use of replicate genotypes across all treatments reduced the overall variation between treatments. Since all coral ramets were reared under the same conditions except treatment, any differences between treatments and controls for any variable were due to the treatment effects alone. Univariate parametric statistics were generated using SAS® software (SAS Institute, Cary, NC, USA) version 9.3 for Windows. $p \leq 0.05$ were considered significantly different.

3. Results

3.1. *Pocillopora damicornis*

All *P. damicornis* ramets survived the experiment. The *P. damicornis* holobiont physiological profile was significantly affected by temperature and feeding (Table S2). Chlorophyll *a* content was lower under elevated temperature compared to ambient temperature and under high light compared to low light (Figure 3a; Table S3). No significant differences were observed in $\delta^{13}C_h$ (Figure 3b; Table S3). Calcification was lower in the low light + fed corals compared to all other treatments, irrespective of temperature (Figure 3c; Table S3), while no significant differences were observed in biomass (Figure 3d; Table S3).

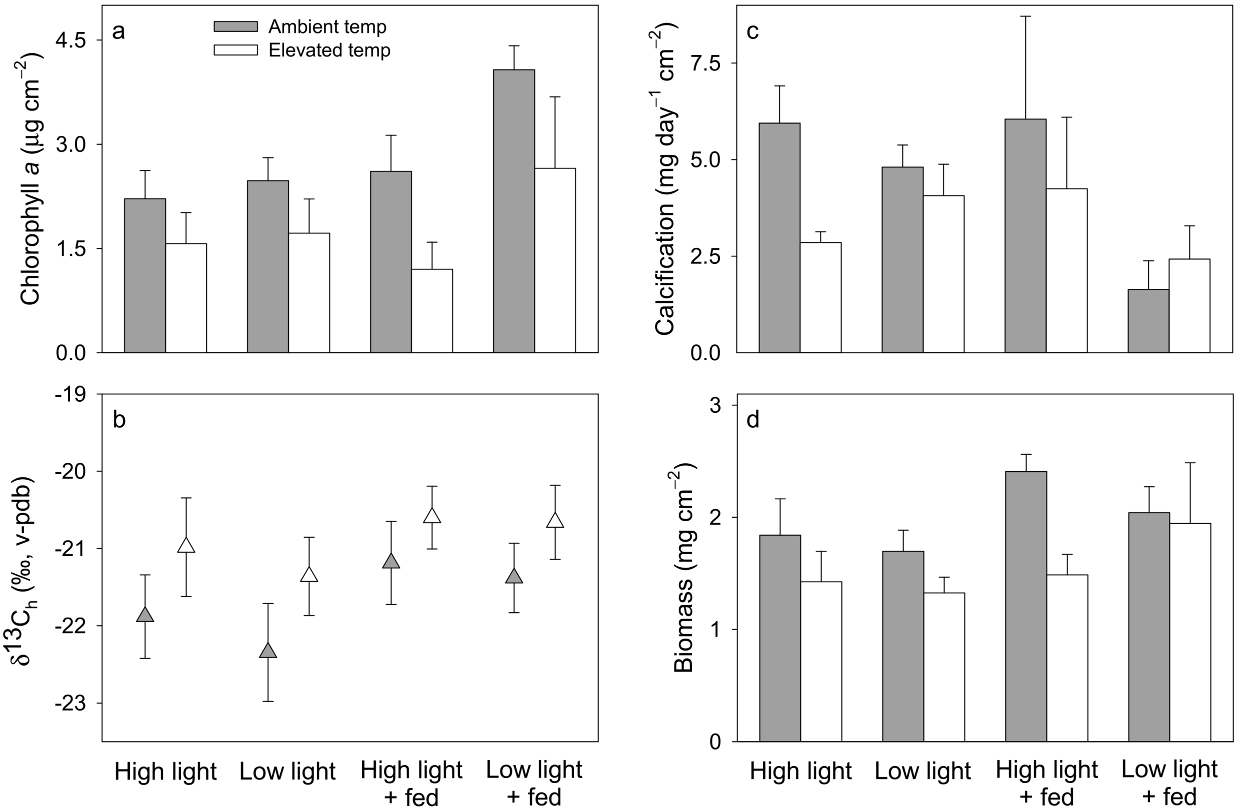

Figure 3. *Pocillopora damicornis* average (±1 SE) (**a**) chlorophyll *a*, (**b**) $\delta^{13}C_h$, (**c**) calcification, and (**d**) biomass, under ambient temperature (25 °C, solid bars and symbols) or elevated temperature (30 °C, open bars and symbols) within high light (300 µmol photons m^{-2} s^{-1}, unfed), low light (150 µmol photons m^{-2} s^{-1}, unfed), high light + fed (300 µmol photons m^{-2} s^{-1}, fed), and low light + fed (150 µmol photons m^{-2} s^{-1}, fed) treatments. N = 3–4 for all averages. Significant effects from corresponding ANOVAs indicated in Table S3.

No significant differences were observed in photosynthesis, respiration, feeding rate, CZAR, CHAR, or CTAR (Figure 4a–e; Table S4). However, corals were only able to meet 100% CTAR when fed at ambient temperature (Figure 4e).

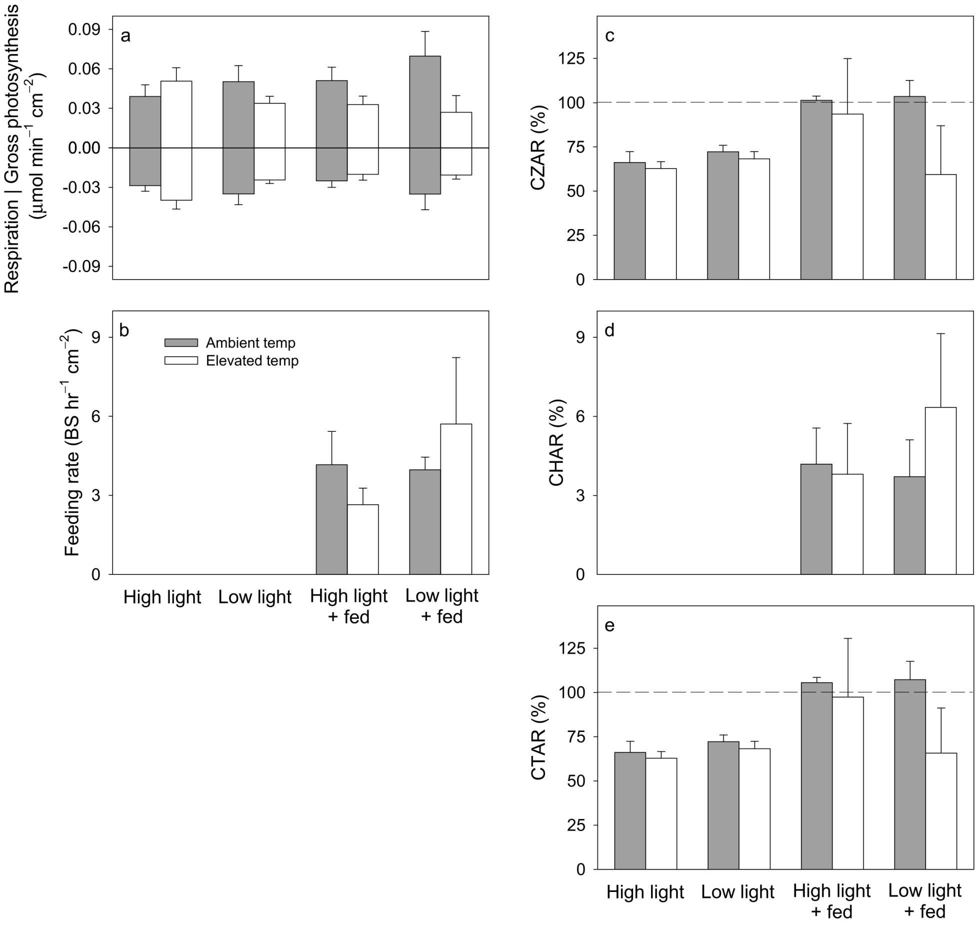

Figure 4. *Pocillopora damicornis* average (±1 SE) (**a**) photosynthesis and respiration, (**b**) feeding rate, (**c**) Contribution of Zooxanthellae (Symbiodiniaceae) to Animal Respiration (CZAR), (**d**) Contribution of Heterotrophy to Animal Respiration (CHAR) from feeding on *Artemia* nauplii, and (**e**) Contribution of Total acquired fixed carbon relative to Animal Respiration (CTAR = CZAR + CHAR), under ambient temperature (25 °C, solid bars) and elevated temperature (30 °C, open bars) within high light (300 μmol photons m^{-2} s^{-1}, unfed), low light (150 μmol photons m^{-2} s^{-1}, unfed), high light + fed (300 μmol photons m^{-2} s^{-1}, fed), and low light + fed (150 μmol photons m^{-2} s^{-1}, fed) treatments. N = 3 for all averages. Significant effects from corresponding ANOVAs in Table S4.

3.2. *Stylophora pistillata*

All *S. pistillata* ramets survived the experiment. The *S. pistillata* holobiont physiological profile was affected by temperature, light, and feeding (Table S5). Chlorophyll *a* content was higher in corals under low light than in corals under high light and in fed corals compared to unfed corals (Figure 5a; Table S6). Under high light, $\delta^{13}C_h$ was more enriched in corals at elevated temperature than at ambient temperature (Figure 5b; Table S6). No significant effects were observed in calcification, biomass, or total lipids (Figure 5c–e; Table S6). However, while overall ANOVA models were not significant, a significant temperature effect in calcification ($p = 0.0162$) and total lipids ($p = 0.0321$) suggest a trend of lower calcification and total lipids in corals at elevated temperature compared to those at ambient temperature (Figure 5c,e; Table S6).

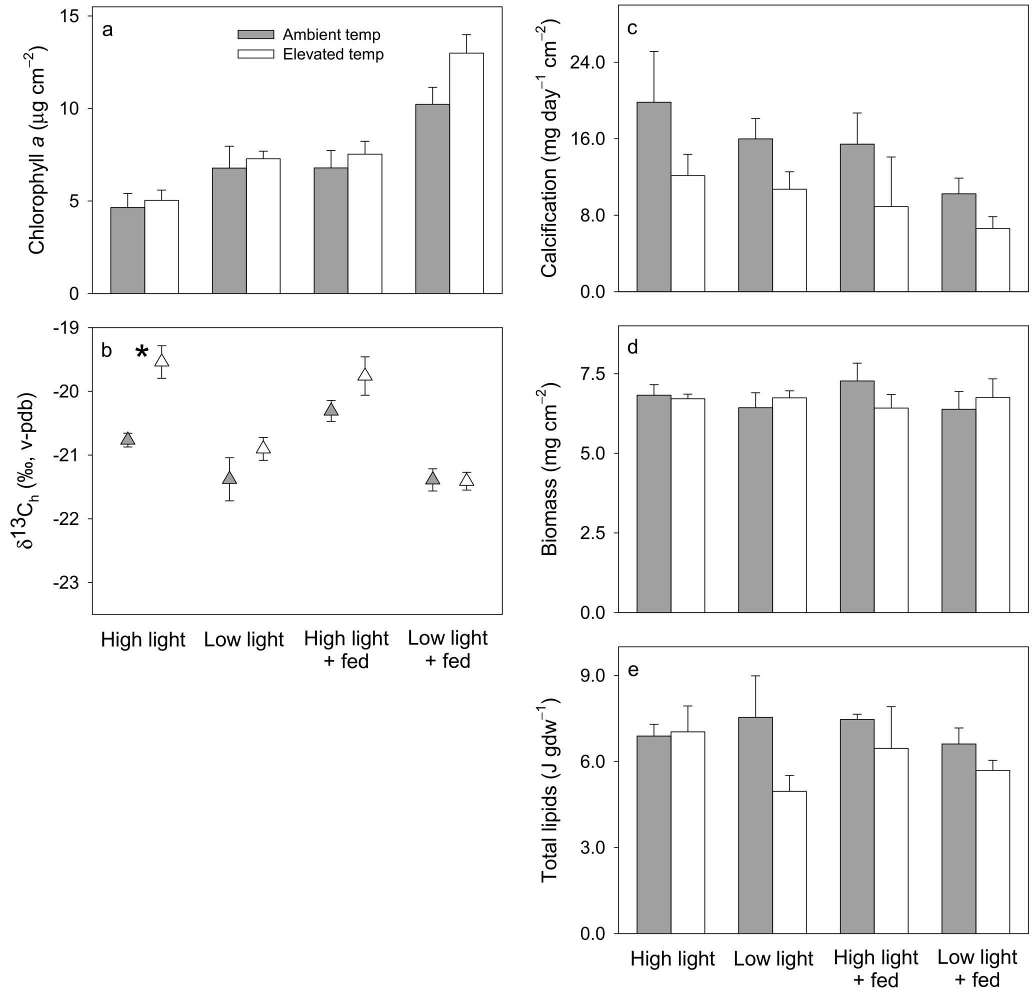

Figure 5. *Stylophora pistillata* average (±1 SE) (**a**) chlorophyll *a*, (**b**) $\delta^{13}C_h$, (**c**) calcification, (**d**) biomass, and (**e**) total lipids, under ambient temperature (25 °C, solid bars) and elevated temperature (30 °C, open bars) within high light (300 µmol photons m^{-2} s^{-1}, unfed), low light (150 µmol photons m^{-2} s^{-1}, unfed), high light + fed (300 µmol photons m^{-2} s^{-1}, fed), and low light + fed (150 µmol photons m^{-2} s^{-1}, fed) treatments. Asterisks indicate significant differences between ambient and elevated temperature treatments within each light and feeding treatment combination. N = 5 for all averages. Significant effects from corresponding ANOVAs in Table S6.

Photosynthesis and respiration were lower in corals under elevated temperature compared to ambient temperature when fed (Figure 6a; Table S7). Under low light, CZAR was lower in corals under elevated temperature compared to those under ambient temperature (Figure 6c; Table S7). No significant effects were detected in feeding rate or CHAR (Figure 6b,d; Table S7). Unfed corals in low light had lower CTAR at elevated temperature than at ambient temperature (Figure 6e; Table S7). Interestingly, *S. pistillata* was not able to meet 100% CTAR under any of the treatment conditions.

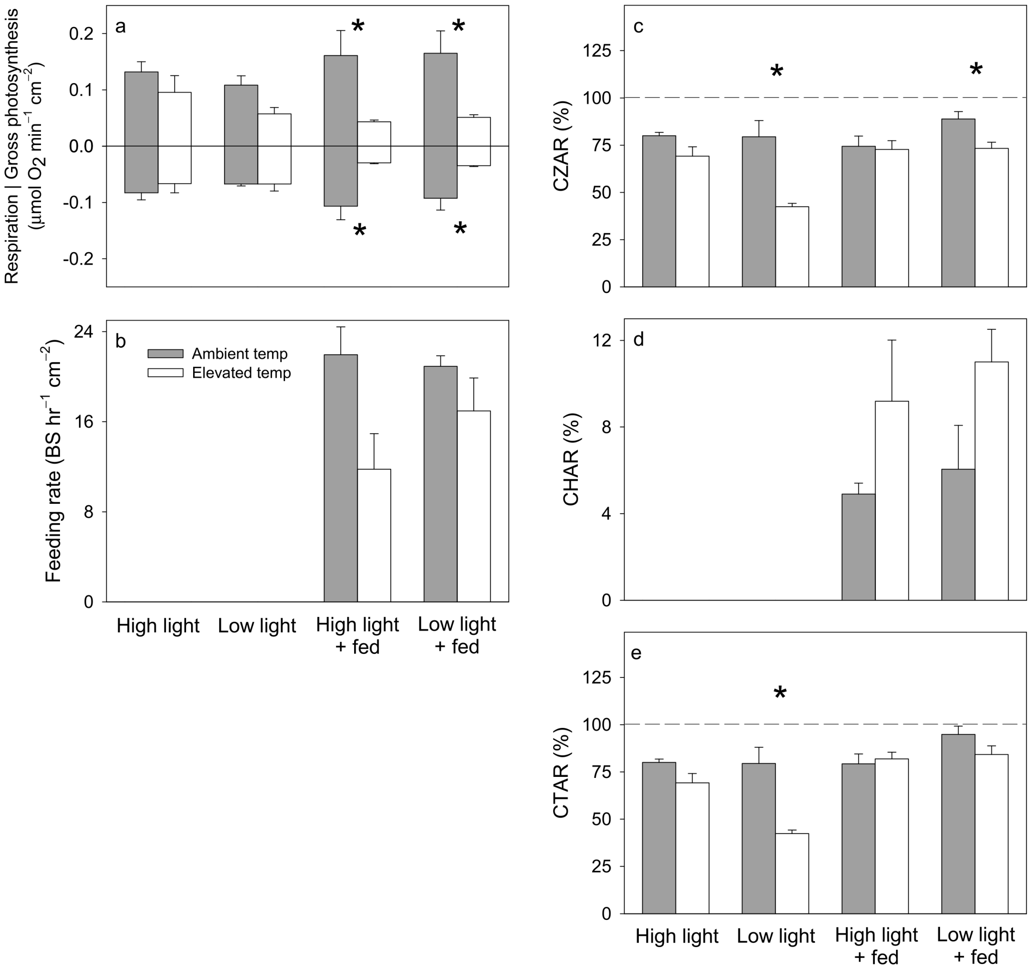

Figure 6. *Stylophora pistillata* average (±1 SE) (**a**) photosynthesis and respiration, (**b**) feeding rate, (**c**) Contribution of Zooxanthellae (Symbiodiniaceae) to Animal Respiration (CZAR), (**d**) Contribution of Heterotrophy to Animal Respiration (CHAR) from feeding on *Artemia* nauplii, and (**e**) Contribution of Total acquired fixed carbon relative to Animal Respiration (CTAR), under ambient temperature (25 °C, solid bars) and elevated temperature (30 °C, open bars) within high light (300 µmol photons m^{-2} s^{-1}, unfed), low light (150 µmol photons m^{-2} s^{-1}, unfed), high light + fed (300 µmol photons m^{-2} s^{-1}, fed), and low light + fed (150 µmol photons m^{-2} s^{-1}, fed) treatments. Asterisks indicate significant differences between ambient and elevated temperature treatments within each light and feeding treatment combination. N = 3 for all averages. Significant effects from the corresponding ANOVAs in Table S7.

3.3. *Turbinaria reniformis*

All *T. reniformis* ramets survived the experiment. The *T. reniformis* holobiont physiological profile was significantly affected by both temperature and light (Table S8). Chlorophyll *a* content was lower in corals at elevated temperature than at ambient temperature, and it was also lower under high light compared to low light (Figure 7a; Table S9). No significant differences were observed in $\delta^{13}C_h$, calcification, or biomass across all treatment conditions (Figure 7b–d; Table S9). Under high light, total lipids were lower in corals at elevated temperature than at ambient temperature (Figure 7e; Table S9). Furthermore, fed corals had lower total lipids under low light than under high light (Figure 7e; Table S9).

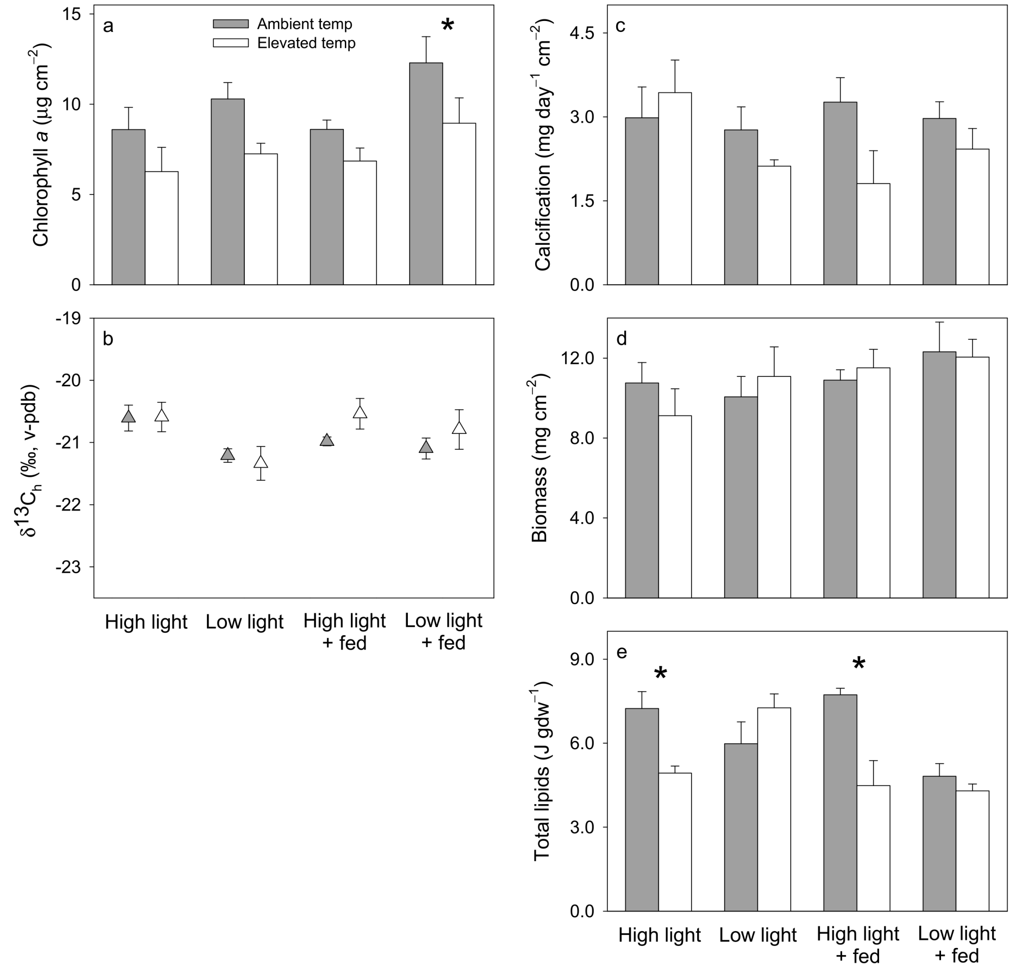

Figure 7. *Turbinaria reniformis* average (±1 SE) (**a**) chlorophyll *a*, (**b**) $\delta^{13}C_h$, (**c**) calcification, (**d**) biomass, and (**e**) total lipids, under ambient temperature (25 °C, solid bars) and elevated temperature (30 °C, open bars) within high light (300 µmol photons m^{-2} s^{-1}, unfed), low light (150 µmol photons m^{-2} s^{-1}, unfed), high light + fed (300 µmol photons m^{-2} s^{-1}, fed), and low light + fed (150 µmol photons m^{-2} s^{-1}, fed) treatments. Asterisks indicate significant differences between ambient and elevated temperature treatments within each light and feeding treatment combination. N = 4–5 for all averages. Significant effects from corresponding ANOVAs in Table S9.

Photosynthesis and respiration were lower in fed corals under elevated temperature (Figure 8a; Table S10). No significant differences were observed in feeding rate, but CHAR was higher in corals under elevated temperature (Figure 8d; Table S10). While the overall ANOVA models were not significant for CZAR or CTAR, a significant temperature by feeding effect on CZAR (p = 0.0347) and CTAR (p = 0.0052) suggest a trend of higher CTAR in corals at ambient temperature compared to elevated temperature when not fed (Figure 8c, e; Table S10). Only fed corals under elevated temperature were able to meet 100% CTAR (Figure 8e).

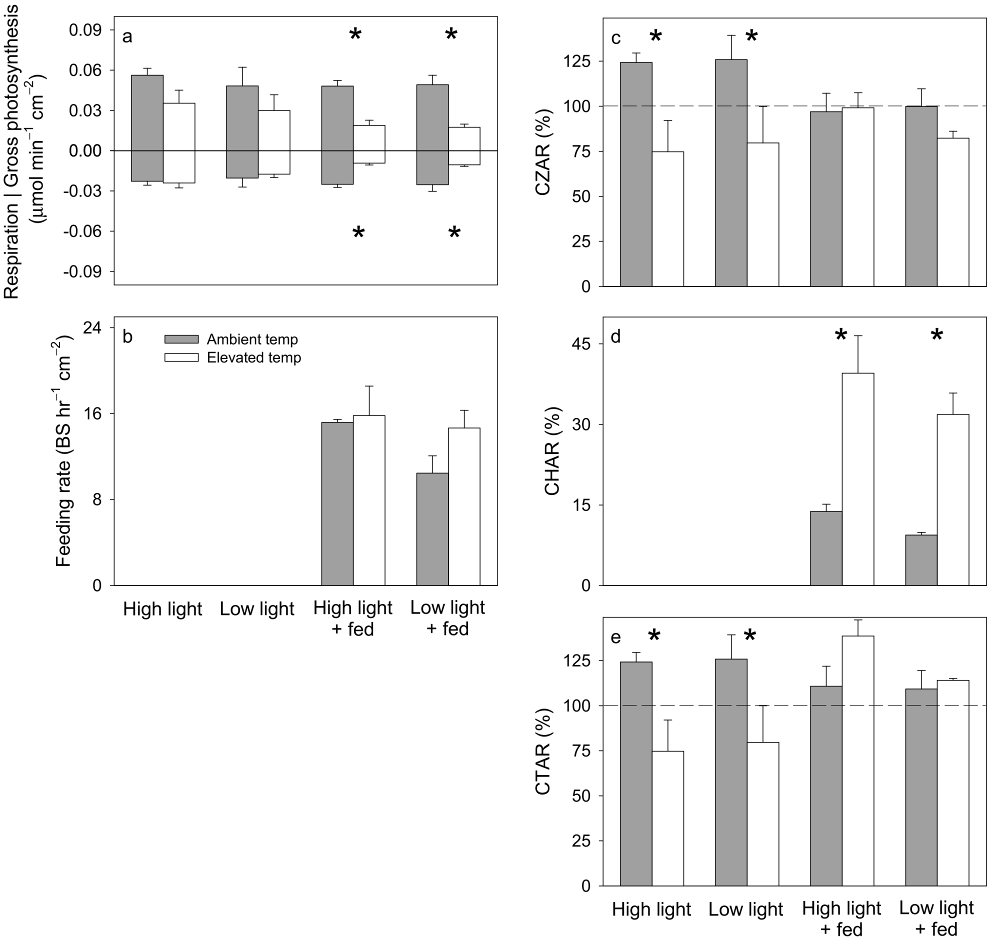

Figure 8. *Turbinaria reniformis* average (±1 SE) (**a**) photosynthesis and respiration, (**b**) feeding rate, (**c**) Contribution of Zooxanthellae (Symbiodiniaceae) to Animal Respiration (CZAR), (**d**) Contribution of Heterotrophy to Animal Respiration (CHAR) from feeding on *Artemia* nauplii, and (**e**) Contribution of Total acquired fixed carbon relative to Animal Respiration (CTAR), under ambient temperature (25 °C, solid bars) and elevated temperature (30 °C, open bars) within high light (300 µmol photons m^{-2} s^{-1}, unfed), low light (150 µmol photons m^{-2} s^{-1}, unfed), high light + fed (300 µmol photons m^{-2} s^{-1}, fed), and low light + fed (150 µmol photons m^{-2} s^{-1}, fed) treatments. Asterisks indicate significant differences between the ambient and elevated temperature treatments within each light and feeding treatment combination. N = 3 for all averages. Significant effects from corresponding ANOVAs in Table S10.

4. Discussion

Temperature had a significant effect on the overall coral physiology of each species; light influenced the overall physiology of *P. damicornis* and *S. pistillata*, and feeding influenced the overall physiology of *S. pistillata* and *T. reniformis* (Tables S2, S3 and S8). However, the way in which low light and feeding mitigated or minimized the negative effects of elevated temperature varied by species.

4.1. *Pocillopora damicornis*

Low light minimized the negative effect of elevated temperature on algal endosymbionts (Figure 3a; Table S3) and facilitated the maintenance of photosynthesis across treatments (Figure 4a). While unfed corals were not able to meet 100% of total carbon budget (CTAR), feeding on *Artemia* nauplii was insufficient to fully mitigate the negative effects of elevated temperature on the total carbon budget (CTAR) in low light conditions (Figure 4e).

Photo-acclimation of chlorophyll *a* content in *Pocilloporid* corals to low light conditions has previously been observed [90,91], but this process was not sufficient to maintain calcification here. While calcification typically decreases with light (e.g., [54,92,93]) and increases with feeding in *P. damicornis* (e.g., [65,81,94]), the combination of low light and feeding actually resulted in the lowest calcification rates (Figure 3c). It is possible that less carbon was translocated from the endosymbiont to the host under these conditions (similar to previous observations in *S. pistillata*; [48]). This, coupled with the lack of detectable incorporation of the *Artemia*'s carbon into the host tissue (Figure 3b), suggests that heterotrophically derived carbon was metabolized to aid in maintaining the total carbon budget (CTAR), rather than calcification. Calcification is independent of feeding in *P. damicornis* in the Gulf of Panama [95] and bleached *Montipora capitata* also metabolize heterotrophically derived fixed carbon to maintain energy reserves and endosymbiont population at the expense of calcification [7,28,96]. Feeding rates and contribution of heterotrophy to the total carbon budget (CHAR) were the lowest of the three species and did not significantly change under elevated temperature (Figure 4d). This lack of heterotrophic plasticity in response to temperature stress is consistent with previous findings in Red Sea *P. damicornis* [58,91]. At the same time, *P. damicornis* can linearly increase feeding on suspended particulate organic matter (POM) over a full range of concentrations [79], suggesting that feeding rate and the contribution of heterotrophy to the total carbon budget (CHAR) may have been greater in this study if higher concentrations of *Artemia* nauplii had been employed. Alternatively, this species may prefer to feed on suspended POM rather than *Artemia* nauplii. However, our results are consistent with previous findings that feeding is necessary to maintain metabolic demand by stimulating the contribution of photoautotrophic carbon [97]. While we found no evidence that low light or feeding mitigated the negative effects of elevated temperature on the various coral holobiont physiological variables measured, *P. damicornis* was only able to meet 100% of the total carbon budget (CTAR) when fed, highlighting the fundamental role of heterotrophy for this coral.

4.2. *Stylophora pistillata*

Individually, low light and feeding stimulated chlorophyll *a* content in *S. pistillata* (Figure 5a). At the holobiont scale, the response patterns of total lipids, the contribution of photosynthesis to the total carbon budget (CZAR), and the total carbon budget (CTAR) were similar, suggesting that feeding on *Artemia* nauplii mitigated the compounded negative effects of elevated temperature and low light on total lipids and metabolic demand (Figures 5e and 6c,e). However, the additional fixed carbon available from feeding (Figure 6d) was not sufficient to allow *S. pistillata* to meet 100% of the total carbon budget (CTAR) across treatments (Figure 6e) nor mitigate the negative effect of elevated temperature on calcification (Figure 5c).

Photo-acclimation by increasing chlorophyll *a* content under low light (e.g., [40,90,98]) and when fed (e.g., [49,94,99]) is well documented for *S. pistillata*. However, while a coral may not visibly exhibit paling in response to temperature stress, it can still be physiologically compromised [16,23]. Despite the benefits of low light for algal endosymbiont pigmentation, low light compounded negative effects of elevated temperature on the contribution of photosynthesis to the total carbon budget (CZAR) (Figure 6c), which was minimized in the total carbon budget (CTAR) by feeding (Figure 6e). Similar to findings by Tremblay et al. [26], feeding was not enough to mitigate or minimize the negative effect of elevated temperature on calcification. This species is a voracious feeder [80,81,94], so its inability to meet 100% of the total carbon budget (CTAR) across treatments, or restore calcification, may be an artefact of only being fed twice weekly. However, as feeding minimized the negative effect of elevated temperature stress on total lipids, perhaps feeding more frequently, for longer durations, or with a more nutritious food source (i.e., freshly caught zooplankton) would have resulted in the full mitigation of this coral's total carbon budget (CTAR) and/or calcification under elevated temperature conditions.

4.3. *Turbinaria reniformis*

Low light stimulated *T. reniformis* chlorophyll *a* content, but not enough to avoid bleaching (Figure 7a). However, at the holobiont scale, feeding on *Artemia* nauplii did mitigate the negative effects of elevated temperature on the total carbon budget (CTAR), but at the expense of total lipids (Figures 7e and 8d,e). *T. reniformis* was the only species to meet 100% of the total carbon budget (CTAR) and maintain calcification under elevated temperature, provided it was fed (Figure 8e).

Photo-acclimation to low light by increasing chlorophyll *a* content (Figure 7a) has been previously documented for this genus (Hoogenboom et al. 2009). While *T. reniformis* has previously been observed to catabolize lipids under low light, or when starved [46], our findings suggest that heat-stressed corals under high light may have catabolized lipids to aid in the maintenance of calcification and biomass (Figure 7c,e). In addition, when *T. reniformis* was fed under elevated temperature, photosynthesis and respiration were depressed and the contribution of heterotrophy to the total carbon budget (CHAR) increased, thus allowing the coral to meet 100% of the total carbon budget (CTAR). Therefore, feeding mitigated the negative effects of elevated temperature on the total carbon budget (CTAR) (Figure 8e), which is consistent with previous findings of feeding helping to sustain carbon budgets in bleached *T. reniformis* [16,19].

4.4. Implications

Reduced light and feeding on *Artemia* nauplii did not act synergistically, nor additively, to mitigate nor minimize the negative effects of elevated temperature on coral holobiont physiology and carbon budgets in any of the three coral species examined. The physiological responses of each species to elevated temperature, and the minimizing and/or mitigating effects of low light or feeding were unique to each species. While each species photo-acclimated to the low light conditions by increasing chlorophyll *a* content, this response did not always translate into the maintenance of daily carbon budgets and thus the minimization or mitigation of the negative effects of elevated temperature on holobiont physiology. This may be due to their different morphologies [100], associated Symbiodiniaceae species (*P. damicornis* in symbiosis with *Cladocopium* spp., formerly clade C [101,102]; *S. pistillata* in symbiosis with *Symbiodinium* spp., formerly clade A [101–103]; and *T. reniformis* in symbiosis with *Cladocopium* spp. and *Durusdinium* spp., formerly clades C and D respectively [102,103]), and/or energetic strategies employed, and agrees with previous studies which have shown that chlorophyll *a* content is not a reliable predictor of overall holobiont health [14]. Low light did minimize the negative effects of elevated temperature on total lipids in unfed *T. reniformis* but compounded the negative effects on the total carbon budget (CTAR) in *S. pistillata*. While feeding did not mitigate the negative effects of elevated temperature on the total carbon budget (CTAR) in *P. damicornis*, it did for *T. reniformis*, and it minimized the negative effects of elevated temperature on *S. pistillata* total carbon budget (CTAR) under low light conditions. Furthermore, feeding was required for *P. damicornis* to meet 100% of the total carbon budget (CTAR), irrespective of temperature. The role of feeding in the mitigation of elevated temperature stress within this study is likely conservative, as corals in situ would receive a more nutritious array of natural zooplankton and be exposed to zooplankton daily (rather than *Artemia* nauplii twice weekly). Additional research is needed to quantify dissolved and small particulate organic carbon contributions to the total carbon budget (CTAR), as these heterotrophic sources may bridge the gap for corals to meet 100% of the total carbon budget (CTAR) when heat-stressed [43,66].

Future research with higher tank replication and reef-sourced corals would be desirable to verify these results. Nevertheless, these results suggest that the physiological stress experienced by *P. damicornis* during bleaching events would not be reduced by low light, even in zooplankton-rich reef environments, due to its inability to maintain growth and lack of heterotrophic plasticity. It remains unclear whether low light would benefit *S. pistillata* and *T. reniformis* experiencing temperature stress. However, zooplankton-rich reef environments would likely provide refuge to *S. pistillata* and *T. reniformis* at elevated temperature stress, such as occurs during bleaching events. Therefore, these findings add to the growing body of research highlighting the importance of zooplankton availability on the physiological maintenance and persistence of many coral species in a warming ocean.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/w13152048/s1, Figure S1. Representative images of (A-H) *Pocillopora damicornis,* (I-P) *Stylophora pistillata*, and (Q-X) *Turbinaria reniformis* from each treatment: high light (300 µmol photons m^{-2} s^{-1}, unfed), low light (150 µmol photons m^{-2} s^{-1}, unfed), high light + fed (300 µmol photons m^{-2} s^{-1}, fed), and low light + fed (150 µmol photons m^{-2} s^{-1}, fed), under ambient temperature (25 °C) or elevated temperature (30 °C). These images were recorded on the last day of the experiment, 30 May 2013, prior to removal and preservation. Table S1. Project metadata. Modified from Table 2 in Grottoli et al. [56]. Table S2. *Pocillopora damicornis* results from three-way PERMANOVA, testing for the effects of temperature (Temp), light, and feeding on the coral holobiont physiological profile based on chlorophyll *a*, $\delta^{13}C_h$, calcification, and biomass. Significant effects are in bold. Table S3. *Pocillopora damicornis* results from three-way ANOVAs testing the effect of temperature (Temp), light, and feeding on chlorophyll *a*, $\delta^{13}C_h$, calcification, and biomass. Effects of temperature, light, and feeding were fixed and fully crossed. Significant effects are in bold. Df = degrees of freedom, Mean Sq = mean squares. Tukey test indicates which mean is significantly different from another, for ambient temperature (ambT), elevated temperature (elevT), high light (hiL), low light (lowL), fed (F), and unfed (UF). Table S4. *Pocillopora damicornis* results from three-way ANOVAs testing the effect of temperature (Temp), light, and feeding on photosynthesis, respiration, feeding rate, Contribution of Zooxanthellae (Symbiodiniaceae) to Animal Respiration (CZAR), Contribution of Heterotrophy to Animal Respiration (CHAR), and Contribution of Total acquired fixed carbon relative to Animal Respiration (CTAR). Effects of temperature, light, and feeding were fixed and fully crossed. Significant effects are in bold. Df = degrees of freedom, Mean Sq = mean squares. Table S5. *Stylophora pistillata* results from three-way PERMANOVA, testing for the effect of temperature (Temp), light, and feeding on the coral holobiont physiological profile based on chlorophyll *a*, $\delta^{13}C_h$, calcification, biomass, and total lipids. Significant effects are in bold. Table S6. *Stylophora pistillata* results from three-way ANOVAs testing the effect of temperature (Temp), light, and feeding on chlorophyll *a*, $\delta^{13}C_h$, calcification, biomass, and total lipids. Effects of temperature, light, and feeding were fixed and fully crossed. Significant effects are in bold. Df = degrees of freedom, Mean Sq = mean squares. Tukey test indicates which mean is significantly different from another, for ambient temperature (ambT), elevated temperature (elevT), high light (hiL), low light (lowL), fed (F), and unfed (UF). Table S7. *Stylophora pistillata* results from three-way ANOVAs testing the effect of temperature (Temp), light, and feeding on photosynthesis, respiration, feeding rate, Contribution of Zooxanthellae (Symbiodiniaceae) to Animal Respiration (CZAR), Contribution of Heterotrophy to Animal Respiration (CHAR), and Contribution of Total acquired fixed carbon relative to Animal Respiration (CTAR). Effects of temperature, light, and feeding were fixed and fully crossed. Significant effects are in bold. Df = degrees of freedom, Mean Sq = mean squares. Tukey test indicates which mean is significantly different from another, for ambient temperature (ambT), elevated temperature (elevT), high light (hiL), low light (lowL), fed (F), and unfed (UF). Table S8. *Turbinaria reniformis* results from three-way PERMANOVA, testing for the effect of temperature (Temp), light, and feeding on the coral holobiont physiological profile based on chlorophyll *a*, $\delta^{13}C_h$, calcification, biomass, and total lipid. Significant effects are in bold. Table S9. *Turbinaria reniformis* results from three-way ANOVAs testing the effect of temperature (Temp), light, and feeding on chlorophyll *a*, $\delta^{13}C_h$, calcification, biomass, and total lipids. Effects of temperature, light, and feeding were fixed and fully crossed. Significant effects are in bold. Df = degrees of freedom, Mean Sq = mean squares. Tukey test indicates which mean is significantly different from another, for ambient temperature (ambT), elevated temperature (elevT), high light (hiL), low light (lowL), fed (F), and unfed (UF). Table S10. *Turbinaria reniformis* results from three-way ANOVAs testing the effect of temperature (Temp), light, and feeding on photosynthesis, respiration, feeding rate, Contribution of Zooxanthellae (Symbiodiniaceae) to Animal Respiration (CZAR), Contribution of Heterotrophy to Animal Respiration (CHAR), and Contribution of Total acquired fixed carbon relative to Animal Respiration (CTAR). Effects of temperature, light, and feeding were fixed and fully crossed. Significant effects are in bold. Df = degrees of freedom, Mean Sq = mean squares. Tukey test indicates which mean is significantly different from another, for ambient temperature (ambT), elevated temperature (elevT), high light (hiL), low light (lowL), fed (F), and unfed (UF). Table S11. Raw physiology and biochemistry data: chlorophyll *a* (Chl a), carbon isotope data for host tissue ($\delta^{13}C_h$), calcification (calc), biomass, photosynthesis (P), respiration (R), feeding rate (FR), Contribution of Zooxanthellae to Animal Respiration (CZAR), Contribution of Heterotrophy to Animal Repsiration (CHAR), and Contribution of Total carbon to Animal Respiration (CTAR) for *Pocillopora damicornis* (P), *Stylophora pistillata* (S), and *Turbinaria reniformis* (T). Coral ID consists of species (P, S, or T), genotype (A–E), and treatment (1 = 25 °C, 150 µmol photons m^{-2} s^{-1}, fed; 2 = 30 °C, 150 µmol photons m^{-2} s^{-1}, fed; 3 = 30 °C, 150 µmol photons m^{-2} s^{-1}, unfed; 4 = 25 °C, 150 µmol photons m^{-2} s^{-1}, unfed; 5 = 30 °C, 300 µmol photons m^{-2} s^{-1}, unfed; 6 = 25 °C, 300 µmol photons m^{-2} s^{-1}, unfed; 7 = 30 °C, 300 µmol photons m^{-2} s^{-1}, fed; 8 = 25 °C, 300 µmol photons m^{-2} s^{-1}, fed). Dots indicate missing measurements due to insufficient sample material.

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Institutional Review Board Statement: Corals used in this study have been cultured at the Centre Scientifique de Monaco and were not sampled from the wild. Nevertheless, the study was conducted according to the guidelines of the Declaration of Helsinki.

Informed Consent Statement: Not applicable.

Data Availability Statement: Project metadata can be found in Table S1. The datasets supporting this manuscript can be found in Table S11.

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Conflicts of Interest: The authors declare no conflict of interest exist.

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