Competitive effects of the macroalga *Caulerpa taxifolia* on key physiological processes in the scleratinian coral *Turbinaria peltata* under thermal stress (#85054)

First revision

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Competitive effects of the macroalga *Caulerpa taxifolia* on key physiological processes in the scleratinian coral *Turbinaria peltata* under thermal stress

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An increased abundance of macroalgae has been observed in coral reefs damaged by climate change and local environmental stressors. Macroalgae have a sublethal effect on corals that includes the inhibition of their growth, development, and reproduction. Thus, this study explored the effects of the macroalga, *Caulerpa taxifolia,* on the massive coral, *Turbinaria peltate,* under thermal stress. We compared the responses of the corals' water-meditated interaction with algae (the co-occurrence group) and those in direct contact with algae at two temperatures. The results show that after co-culturing with *C. taxifolia* for 30 days, the density and chlorophyll *a* content of the endosymbionts was not influenced by the presence of *C. taxifolia* at ambient temperature (27 °C). The protein content of *T. peltata* decreased by 37.2% in the co-occurrence group and 49.0% in the direct contact group compared to the control group. Meanwhile, the growth rate of *T. peltata* decreased by 57.7% in the co-occurrence group and 65.5% in the direct contact group compared to the control group. The activity of the antioxidant enzymes significantly increased, and there was a stronger effect of direct coral contact with *C. taxifolia* than the co-occurrence group. A temperature elevation of 3 °C significantly decreased the endosymbiont density, chlorophyll *a* content, and growth rate of *T. peltata* compared to the control temperature; the same pattern was seen in the increase in antioxidant enzyme activity. Additionally, when the coral was co-cultured with macroalgae at 30 °C, there was no significant decrease in the density or chlorophyll *a* content of the endosymbiont compared to the control temperature. However, the interaction of macroalgae and thermal stress was evident in the feeding rate, growth rate, superoxide dismutase (SOD), and catalase activity (CAT) compared to the control group. The direct contact of the coral with alga had a greater impact than water-meditated interactions. However, the competition between corals and macroalgae may be more intense under thermal stress.

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Abstract

An increased abundance of macroalgae has been observed in coral reefs damaged by climate change and local environmental stressors. Macroalgae have a sublethal effect on corals that includes the inhibition of their growth, development, and reproduction. Thus, this study explored the effects of the macroalga, *Caulerpa taxifolia,* on the massive coral, *Turbinaria peltate,* under thermal stress. We compared the responses of the corals' water-meditated interaction with algae (the co-occurrence group) and those in direct contact with algae at two temperatures. The results show that after co-culturing with *C. taxifolia* for 30 days, the density and chlorophyll *a* content of the endosymbionts was not influenced by the presence of *C. taxifolia* at ambient temperature (27 °C). The protein content of *T. peltata* decreased by 37.2% in the co-occurrence group and 49.0% in the direct contact group compared to the control group. Meanwhile, the growth rate of *T. peltata* decreased by 57.7% in the co-occurrence group and 65.5% in the direct contact group compared to the control group. The activity of the antioxidant enzymes significantly increased, and there was a stronger effect of direct coral contact with *C. taxifolia* than the co-occurrence group. A temperature elevation of 3 °C significantly decreased the endosymbiont density, chlorophyll *a* content, and growth rate of *T. peltata* compared to the control temperature; the same pattern was seen in the increase in antioxidant enzyme activity. Additionally, when the coral was co-cultured with macroalgae at 30 °C, there was no significant decrease in the density or chlorophyll *a* content of the endosymbiont compared to the control temperature. However, the interaction of macroalgae

and thermal stress was evident in the feeding rate, growth rate, superoxide dismutase (SOD), and catalase activity (CAT) compared to the control group. The direct contact of the coral with alga had a greater impact than water-meditated interactions. However, the competition between corals and macroalgae may be more intense under thermal stress.

Introduction

The recent effects of climate change and other anthropogenic impacts have caused the severe degradation of coral reefs worldwide (Leggat et al., 2022). The first mass coral bleaching event was observed in 1998 and it killed approximately 8% of the world's coral; an additional 14% of corals were lost between 2009 and 2018 (Souter 2021).

Many studies have asserted that ocean warming is a major factor in the reduction of coral cover (Hughes et al., 2017, 2019; Lough et al., 2018; Leggat et al., 2022). For example, the successive bleaching events in 2016-2017 devastated Australia's Great Barrier Reef and resulted in an 89% decline in larval recruitment in 2018 compared to historical levels (Hughes et al., 2017,2019; Lough et al., 2018). During this period, 31% of reefs experienced 8–16 degree heating weeks (DHWs, °C-weeks). A decline in coral cover may lead to an increase in the cover of other benthic organisms in the reefs, such as macroalgae (Fulton et al., 2019). The impact of herbivorous fishes is mostly ignored, although some studies assert that overfishing and nutrient pollution is the main cause of phase shifts towards macroalgae (Barott et al., 2012). Research had shown that prior to 2011, the estimated global average cover of algae at the global scale was low (~16%) and stable for 30 years. Since 2011, the amount of algae on the world's coral reefs has increased by about 20% (Souter, 2021). Thus, the coral reef ecosystem is undergoing an ecological phase transition to that of a large algal bed.

Macroalgae are important functional communities that are important for stabilizing reef structure (Fulton et al., 2019), generating primary productivity (Fulton et al., 2014; 2019), maintaining nutrient cycling in reef areas, and providing food sources for herbivores (Dubinsky and Stambler, 2011). However, there is competition between macroalgae and corals. Macroalgae may affect the physiological responses of corals in different ways. ~~The~~ ~~micro~~algae ~~compete~~ through direct contact (Coyer et al., 1993; Manikandan et al., 2021) and allelopathy (Roberta et al., 2014; Fong et al., 2020), weakening the photosynthe~~sis~~ performance of ~~corals~~ (Rasher et al., 2011), causing the retraction of polyps (Jompa and Mccook, 2003), increasing the number of pathogenic microorganisms (Clements et al., 2020; Rasher and Hay, 2010), triggering coral

bleaching (Roberta et al., 2014), and resulting in the reduced calcification ~~of~~ coral growth, fecundity, survival rate, and settlement rate (Fong et al., 2020; Jason and Tanner, 1995; Leong et al., 2018; Rasher and Hay, 2010). Specifically, macroalgae affect coral feeding, endosymbiont function, tissue recovery, and oxidative stress. Morrow and Carpenter (2008) found that *Dictyopteris undulata* weakened the particle capture rates of *Corynactis californica* by redirecting particles around polyps and causing contraction of the feeding tentacles. The dissolved organic carbon (DOC) and terpenoids released by macroalgae decreased photosynthesis and the density of endosymbionts (Rasher et al., 2011; Smith et al., 2006; Diaz-Pulido and Barrón, 2020). Bender (2012) asserted that the green filamentous macroalga, *Chlorodesmis fastigiate,* significantly reduced tissue recovery in *Acropora pulchra* and led to the infection of *A. pulchra* with ciliates. High levels of reactive oxygen species (ROS, a byproduct of biological aerobic metabolism) could cause damage to cells and the gene structure (Blanckaert et al., 2021). Shearer et al. (2012) found that the oxidative stress of *Acropora millepora* was activated in response to ROS by altering the transcription factors after ~~coming into~~ contact with the macroalga *Chlorodesmis fastigiata* ~~thalli~~ and ~~their~~ hydrophobic extract over a short-term period (1 h and 24 h).

In addition, the combined effects of ocean warming, acidification, and macroalgae contact could significantly alter the physiological response of corals (Chadwick et al., 2011; Kornder et al., 2018; Brown et al., 2019; Rölfer et al., 2021). Rölfer et al. (2021) have shown that ~~light~~ calcification rates of *Porites lobata* were negatively affected ~~by the interaction of~~ *Chlorodesmis fastigiata* ~~contact~~ in ~~the~~ ocean warming and acidification scenario, compared to coral under ambient conditions. Typically, the competitive ability of macroalgae, which determines the outcome of coral-algal competition, is related to seasonal and temporal cycles. These, in turn, may be related to the abundance, biomass, and composition of macroalgae, as well as the seasonal dynamics of temperature, pCO_2, and light intensity ~~*in situ*~~ (Brown et al., 2019; 2020). The sensitivity of various macroalgae to environmental stressors is also different. For example, intermediate levels of ocean warming could enhance the growth and production of *Laurencia* sp. and *Lobophora sp.*, which was not the case for *Sargassum sp.* (Fulton et al., 2014; Hernández et al., 2018). Additionally, overfishing and eutrophication have been shown to lead to an increased growth rate of some kinds of macroalgae (Lapointe and Bedford, 2010), which may indirectly enhance the competitive ability of macroalgae. Therefore, to better understand the resilience of coral reef ecosystems in the future, it is necessary to determine how coral-algal interactions ~~fluctuate~~ under global and local stressors.

According to the China Ocean Climate Monitoring bulletin (www.oceanguide.org.cn), the average seawater temperature in the Xuwen Sea area is 27-30 °C from May to September. When compared with the DHW in the coral reef, it is plausible that the physiological responses of corals in the Xuwen Coral Reef National Nature Reserve of China may be affected by thermal stress (Spady et al., 2022). During thermal stress, coral feeding rates are drastically reduced and more energy is needed for decomposing tissue proteins to maintain biological processes (DNA repair etc.) to resist heat stress (Ferrier-Pagès et al., 2010; Chakravarti et al., 2020). Triggered by thermal stress, ROS, a substance that damages cells by accumulation, may be produced by the endosymbiont mainly due to PS II dysfunction caused by damage to the D1 protein (Warner, 1999) ~~or~ host cells (Nii and Muscatine, 1997). The increase in the production of ROS is a stress signaling mechanism that can potentially trigger an oxidative stress and apoptotic cascade in coral cells (Hensley et al., 2000; Drury et al., 2022).

The relationship between macroalgae and corals under climate change conditions remains inconclusive. To investigate the effects of macroalgae on hermatypic coral under ocean warming, the massive coral, *Turbinaria peltate,* and macroalga, *Caulerpa taxifolia,* which are common species with frequent interactions in the Xuwen Sea, were selected as study species. *C. taxifolia* is a multinucleate siphonous green alga and is known to have great invasive potential worldwide (Zubia et al., 2020). Furthermore, it has been found that *C. taxifolia* can produce potential allelochemicals, such as monoterpenes and sesquiterpenes (Guerriero et al., 1992, 1993). Given that *C. taxifolia* usually grows on various hard substrates ~~that contain~ large numbers of live coral colonies, the physical and chemical impacts ~~cannot be underestimated.~ ~~Thus,~ to evaluate the effect of its chemical and physical effects, ~~the~ indirect ~~interaction~ group was used to investigate chemical effects, and the direct-contact group was used to explore the combined effects of physical and chemical processes. In this study, we show that increased temperature, representing the ocean-warming range projected for this century (Spady et al., 2022), enhances the ability of seaweeds to impact the physiology of corals, potentially shifting the competitive interaction between corals and seaweeds in favour of seaweeds. It provides a reliable basis for the evolution of competition between corals and macroalgae under future global changes.

Materials & Methods

~~Portions~ of this text were previously published as part of a preprint (https://www.researchsquare.com/article/rs-1878684/v1).

Sample collection

T. peltata (with a skeleton size of length:width:height = 20:18:12) and *C. taxifolia* (5 kg) were collected from the Xuwen Coral Reef National Nature Reserve (109° 55' E, 20° 16' N) at a depth of approximately 4 m. The samples were transported to the laboratory and cultured in two 200-L tanks at a temperature of 26.5 °C, pH of 8.0, salinity of 33, and 200 μmol photons $m^{-2}s^{-1}$ with a 12: 12 h light/dark cycle for 3 months. Blue lighting was provided by a photo-synthetically active radiation (PAR) lamp (Aqua Knight M029). An illuminometer (UNI-T UT383) was used for light measurement. After 3 months of acclimation, the corals from the same colony were cut into 54 pieces, approximately 4 cm in diameter, and were fixed on a ceramic base with aqua rubber. The sampled were then placed in another 200-L tank for one week until the tentacles grew.

Experimental design

After acclimation, 54 coral nubbins were randomly allocated into 10 L experimental tanks, with three coral nubbins per tank. To mimic the coral-macroalgal interaction in coral reefs, the same amount of macroalgae was co-cultured with the coral samples in the following ways: (1) no algae were added to the tank, i.e., the control group (Fig. 1A). (2) Algae (25 g) were cultured in external algae boxes with no direct contact between the algae and the coral samples. The allelopathic substances produced by algae ~~were carried by flowing water~~ to determine whether the algae had a water-mediated interaction in the co-occurrence group (Fig. 1B). (3) The algae and corals were co-cultured in the same tank with direct contact, and polymethyl methacrylate fixation was used to fix the height of the algae parallel to the coral samples; this was referred to as the direct contact group (Fig. 1C). The experimental tanks were subjected to ambient conditions (27 °C) and the shared socioeconomic paths (SSPs) scenario SSP2-4.5 (30 °C) (Zhongming et al., 2021), to mimic the typical temperature range that the corals experienced at Xuwen Coral Reef Nature Reserve (www.oceanguide.org.cn) in order to explore the coral-macroalgae ~~interaction variation~~ under thermal stress. There was a total of six treatments in this study, i.e. two temperature treatments, and four algae treatments. Each treatment contained three replicate tanks, within which three coral nubbins were placed.

The temperature in each experimental group was increased to the set temperature by 1 °C per day. The first three days were the temperature adjustment period. Some algae tips decomposed due to a faster life cycle or blue light intolerance. Therefore, the algae were checked and replaced each day to ensure that the experimental group had 25 g (0.0025 g cm^{-3}) of fresh algae, which is the amount of algae with the density of 0.0022 g per cubic meter of water surveyed from the inshore reef of Xuwen Coral Reef Nature Reserve. Fifty percent of the seawater was replaced

every three days in each tank. Organisms were kept under treatment conditions for a period of 28 days and physiological measurements were subsequently performed.

Endosymbiont density and Chl *a* content

At the end of the experiment, coral tissue were removed from the nubbins using a waterpick (0.45 µm filtered seawater), and the slurry was homogenized. Six 15 ml samples were taken from the slurry, centrifuged (4,000 rpm min^{-1}, 4 °C, 10 min), and the supernatant was removed. Part of the pellet was suspended in 5 mL formaldehyde to count the endosymbiont density under an inverted microscope (DMI 6001B, magnification eyepiece × magnification objective: 10×20) with a blood counting plate (CKSLAB CB30). Another portion was resuspended in 8 mL methanol. The pigments were extracted at 4 °C for 24 h. The extract was centrifuged (4,000 rpm min^{-1}, 4 °C, 10 min), and Chl *a* was determined according to the method described by Ritchie (2006). Data were normalized to skeletal surface with the aluminium foil technique (Marsh 1970).

Feeding rate

A total of 1ml of *Artemia* solution was added to 1 L of water and a plankton counter was used to measure the mixed solution and to invert the concentration of the *Artemia* solution. Nubbins were moved into the feeding tanks (1 L) with an *Artemia* concentration of ~ 2 ind mL^{-1}, while one tank served as a control (without coral). After an incubation period of 1 h, the coral nubbins were rinsed with seawater and returned to their respective positions in the experimental tanks. The feeding rate was calculated as the decline in *Artemia* in the feeding tanks and normalized per polyp. The measurement was performed once a week between 11:00-12:00 a.m. The number of polyps in each nubbins were visually counted before experiment.

Growth rate

The coral nubbins were weighed on a balance (UW 2200H, accuracy = 0.01 g) using the buoyant weight technique (Davies, 1989). Before each measurement, the surface of the coral ceramic base was lightly brushed with a toothbrush to remove algae. A glass beaker was filled with 1 L of filtered seawater (27 °C, salinity 32) ~~and zeroed.~~ Then the nubbins were placed on the bottom of the beaker ~~for reading weight.~~ The growth rate (g d^{-1}) was calculated as $\left(M_{ti} - M_{t0}/T_i\right)$, where M_{t0} represents the nubbin weight at the beginning of experiment, and T_i represents the duration in days. The measurement was repeated every 7 d. Data were normalized to skeletal surface area determined with the aluminium foil technique (Marsh, 1970).

SOD and CAT

The homogenized coral tissue slurry that was used to measure the endosymbiont density and Chl

a content was centrifuged (4,000 rpm min^{-1}, 10 min, 4 °C), and the supernatant was collected to measure the SOD and CAT activities. These measurements were determined in the dilution using kits (A001-1-1, A007-1-1, Nanjing Jicheng, China). A BCA kit was used to determine the protein concentration (A045-3-1, Nanjing Jicheng, China). The enzyme activities were normalized to total protein content as U mg $prot^{-1}$.

Data analysis

The results are presented as the means ± standard deviations. Data were tested for homogeneity of variance (visual inspection of residuals vs. fitted values), and the normality of the residuals was tested using the Shapiro–Wilk normality test. All response data of corals were tested using a two-factor analysis of variance (ANOVA) with "temperature" and "algae" as fixed factors, including the interaction term. Tukey's test and a *post-hoc* Fisher's least significant difference (LSD) test were used to identify significant differences in all treatments. The data was analysed and graphed using GraphPad Prism 8.0 and $p < 0.05$ was considered a significant difference.

Results

Seawater chemistry monitoring

The temperature, pH, and salinity values were measured continuously during the experiment (28 d) (Figure 2). The standard deviation is due to daily variations.

Endosymbiont density and Chl *a* content

Figures 3A and 3B show that the density and pigment content of the endosymbiont were significantly influenced by temperature. Without algae, thermal stress resulted in an average 44.6% (from 1.3 ± 0.1 to 0.72 ± 0.07 ×10^6 cells cm^{-2}) decrease in endosymbiont density ($p < 0.05$) and a average 58% (from 17.1 ± 1.5 to 7.2 ± 2.6 µg cm^{-2}) decrease in Chl *a* content ($p < 0.01$). Compared with the control group at ambient temperature, corals that interacted with macroalgae in both temperature treatments showed lower mean values of endosymbiont density and chl *a* content. However, these were not significantly different between the co-occurrence and direct contact groups. The negative effects caused by thermal stress on endosymbiont density and chl *a* content were absent in algae treatments, which indicated the antagonistic effect of temperature and algae treatments (endosymbiont, F = 9.1, $p = 0.01$; chl *a*, F = 7.3, $p = 0.02$, Table 1).

Feeding rate

The results of the feeding rate are displayed in Fig. 4A. Primarily, thermal stress had a significantly detrimental effect on the feeding rate among the algae treatments ($p < 0.01$, Table 1).

There was no obvious difference between the three algae treatments at ambient temperature. After a 3 °C increase in temperature, direct contact with algae caused the coral feeding rate to fall to a minimum of 12.8 ± 1.1 ind polyp^{-1} h^{-1}, which reflected the synergistic effect of thermal stress and presence of macroalgae (F = 4.7, $p = 0.04$, Table 1).

Protein content

The protein content was mainly impacted by macroalgae ($p < 0.01$, Figure 4B, Table 1). At ambient temperature, exposure to algae caused coral to lose 37.2% protein content in the co-occurrence group ($p = 0.01$) and 49.0% protein content in the contacted interaction group ($p < 0.01$). Although contact with algae made the mean of protein content ~~even lower,~~ the difference was not obvious with the co-occurrence group. As the temperature increased, the direct interaction with algae resulted in the lowest protein content of 1.2 ± 0.3 mg cm^{-2} ($p < 0.01$) compared with the control and co-occurrence groups. No significant difference was observed between the temperature treatments in the presence or absence of algae (p = 0.11, Table 1). There was no significant interaction between algae and the temperature treatment (F = 2.86, p = 0.12, Table 1).

Growth rate

As indicated by the change in buoyant weight, the growth rate (Fig. 4C) was affected by both algae and thermal stress. At 27 °C, the growth rate of corals in the control group was highest, with a mean value of 4.1 ± 1.4 mg cm^{-2} d^{-1}. Compared with control group, the coculture with macroalgae decreased the growth rate of coral by 57.7% in the co-occurrence group ($p = 0.06$) and 65.5% in the direct contact group ($p = 0.03$). Thermal stress had an inhibitory effect on the growth rate in the coral culture system without algae. The growth rate in this group was 83.4% lower than the control group at ambient temperature ($p < 0.01$). The elevated temperature combined with direct contact~~ed~~ algae resulted in the lowest coral growth rate, with a value of 0.57 ± 0.45 mg cm^{-2} d^{-1}. However, the differences among algae treatments at an elevated temperature were not significant. The interaction between temperature and algae was ~~obvious~~ (F = 28, $p < 0.01$, Table 1).

SOD and CAT

As shown in Fig. 5A, macroalgae treatments increased the antioxidant capacity of corals under both temperature conditions. At 27 °C, co-occurrence with algae increased the SOD activity of coral 1.85-fold compared with control group ($p = 0.03$). Moreover, the SOD activity was higher for the direct contact group (288.1 ± 16.6 U mgprot^{-1}) than control groups ($p < 0.01$) and co-occurrence group ($p = 0.03$). At 30 °C, the mean SOD activity of coral without the presence of algae increased by 1.77-fold compared with its counterpart at ambient temperature ($p = 0.03$). In

the direct contact group under thermal stress, SOD activity increased to the highest level of 354.3 ± 59.56 U mg prot^{-1} compared with control group ($p < 0.01$). However, in the coculture system, there was no significant difference caused by thermal stress, indicating that both factors did not interact ($F = 2.37$, $p = 0.16$, Table 1).

The CAT activity also increased after algae interaction and thermal stress, as shown in Fig. 5B. At ambient temperature, co-occurrence with algae caused the CAT activity in coral tissue to rise ~~by~~ 5.3-fold ($p < 0.05$), which is comparable to the level of CAT activity in the contact interaction group. ~~As the temperature increased~~, the CAT activity in the ~~pure coral system~~ also increased by 7.1-fold ($p < 0.01$). Moreover, when cultured in contact with the algae, the CAT activity further doubled compared with the control group ($p = 0.03$). The combined effect of temperature and macroalgae was significant ($F = 5.13$, $p = 0.04$, Table 1).

Discussion

This study explored the crucial issue of how physiology and the oxidative stress response of a hermatypic coral are affected by macroalgae under elevated temperatures. We set up three treatments of the macroalga *C. taxifolia* (direct contact, indirect, water-mediated presence, no alga) to act on the coral *T. peltata* under ambient temperatures (27 °C) and a temperature increase of +3 °C. The results demonstrated that algal presence altered the physiology of coral as well as increasing the antioxidant activity. In addition, combined with rising temperature, there was a remarkably synergistic effect that impacted the physiology and further increased the oxidative stress of coral, in which contact with algae had a more severe effect than indirect interaction.

Effects of *C. taxifolia* on endosymbiont of *T. peltata*

Algae was found to influence the average ~~value of~~ endosymbiont density and chl *a* content of *T. peltata*, however, no bleaching occurred. A number of studies have reported that coral's photosynthetic efficiency decreased (Fv/Fm), or bleaching occurred, when there was direct or indirect contact~~ed~~ with macroalgae. However, not all coral species are equally susceptible to algae and not all algae will have deleterious effects on corals (Smith et al., 2006; Rasher et al., 2010; Fong et al., 2020). Rasher et al. (2010) suggested that *Padina perindusiata* and *Sargassum sp.* did not inhibit photosynthetic efficiency or induce bleaching of *Porites porites*, which might be explained by the fact that the 20-day interaction period was too short to impact the coral health. Additionally, *T. peltata* is a massive coral that could resist environmental pressure by increasing

its basic metabolism (Loya et al., 2001). This may explain why there was no significant effects of macroalgal interactions on endosymbiont density.

Effects of *C. taxifolia* and thermal stress on the physiology of *T. peltata*

In this study, there were no significant effects of *C. taxifolia* (both direct and indirect interaction) on the feeding rate of *T. peltata* at ambient temperature. However, the feeding rate was affected by thermal stress. Johannes and Tepley (1974) also found that the feeding rate of coral decreased in heat stress because of the polyp contraction or a loss of nematocyst function. Our results suggested that a decrease in chl *a* content and endosymbiont density was the reason why the feeding rate was impacted in thermal stress. Endosymbionts provide photosynthate to host cells (van Oppen and Blackall, 2019). A decrease in the endosymbiont density at high temperatures may result in reduced energy expenditure to maintain normal physiological functions and reduce resistance to predation. This study showed that contact with *C. taxifolia* led to decreased feeding rates at 30 °C. In summary, thermal stress was a crucial factor affecting the predation of *T. peltate*, which became more severe when in contact with macroalgae.

Microalgae can induce reduced protein content in corals. Damage to coral tissue by contact with macroalgae has been documented in many studies. Bender et al. (2012) asserted that *Acropora sp.* lost tissue and decreased its growth rate due to allelopathy mechanisms after coming into contact with *Chlorodesmis fastigiata*. In fact, macroalgae may transfer many allelopathic substances to corals, altering the structure of the microbial community and ~~injuring~~ the physiological processes of corals (Fong et al., 2020). This damage may ultimately ~~give rise to losses in~~ protein content. Under stress, massive corals with thicker tissues may overcome the effects of endosymbiont loss through catabolism (DeCarlo and Harrison, 2019). Macroalgae may affect corals tissue by creating anoxic zones. Barott et al. (2009) demonstrated that after the interactions between corals (*Pocillopora verrucosa*, *Montipora sp.*) and some species of macroalgae (e.g. *Gracilaria sp.*, *Bryopsis sp.*, and various turf algae), the characteristic patterning of coral pigments and polyps was altered and the tissue appeared damaged.

The growth rate of coral was altered by the interaction of macroalgae and temperature; these results were consistent with previous studies (Tanner 1995; Rölfer et al., 2021; Rebecca et al., 2012; Vermeij et al., 2009). Brown et al. (2019) also demonstrated that coral growth was reduced or even negative at 30 °C when in contact with algae. Longo et al. (2015) determined that corals weakened at 30 °C, and contact with *Halimeda heteromorpha* further contributed to a decreased growth rate and increased mortality rate. These results may be due to the simultaneous decline in

the autotrophic and heterotrophic activities of corals under the impacts of thermal stress or macroalgae, resulting in a drop of protein content and ultimately affecting the growth rate.

Effects of *C. taxifolia* and thermal stress on oxidative stress of *T. peltata*

Corals under thermal stress may produce ROS (Blanckaert et al., 2021). Downs (2002) documented that when exploring the varied oxidative stress response of coral under seasonal change, the SOD in summer was three times higher than that in winter. This study determined that SOD in corals was higher when macroalgae was present, the temperature increased, or there was the synergistic effect of both. Thus, weakened corals were found to be more vulnerable to competition from algae, which was also supported by the results of Diaz-Pulido et al (2010). The level of both antioxidant enzyme activities was similar to thermal stress alone when *C. taxifolia* indirectly contacted *T. peltata*. These results indicate that the stress triggered by macroalgal allelochemicals on coral was equivalent to that induced by increasing temperature. The temperature effect was more obvious in CAT activity compared with SOD activity under direct contact, which may be related to the reduced protein content in coral tissues caused by rising temperature under the direct contact treatment. Due to the evident decrease in the protein content of coral tissues in the direct contact group, the amount of antioxidant enzymes produced by coral is not enough to resist the damage of ROS.

Conclusions

The shift from coral dominance to algal dominance that has been observed in many reefs due to global climate change. Coral dominance is sensitive to key algal groups and other benthic groups, and shifts in ecosystem phases have a noticeable impact (Tebbett et al., 2023). The results of this study showed that *C. taxifolia* negatively affected the growth rate and protein content of *T. peltate* and increased the antioxidant activity at 27 °C. The combination of thermal stress and macroalgae interactions may further exacerbate the adverse effects on corals. Future studies are needed to explore the interactions of multiple coral-macroalgal species under climate change. Because of the vulnerability and sensitivity of coral reef ecosystem, relevant entities should take urgent steps to prevent CO_2 emissions that exceed the goals of the Paris Climate Agreement. Herbivorous fish populations should also be restored to improve macroalgae management in reefs.

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348 **References**

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Figure 1

Experimental operating system

System control (grey) with a feedback loop to adjust the conditions. Seawater at 27 °C (blue) and 30 °C (red) was heated in different collection sumps (36 L) and then fed into the each tanks (10 L). The control group had a separate sump, and the algae treatment group shared a sump. Macroalgae treatments were applied (A) *T. peltata* (B) *T. peltata* indirect interaction with *C. taxifolia*. Water flowed first into the algae box (yellow) and then into the tanks to deliver the allelopathic substances secreted by the algae. (C) *T. peltata* contact with *C. taxifolia*. Polymethyl methacrylate fixation was used to fix the height of the algae parallel to the coral samples. Each treatment contained three replicate tanks, within which three coral nubbins were placed.

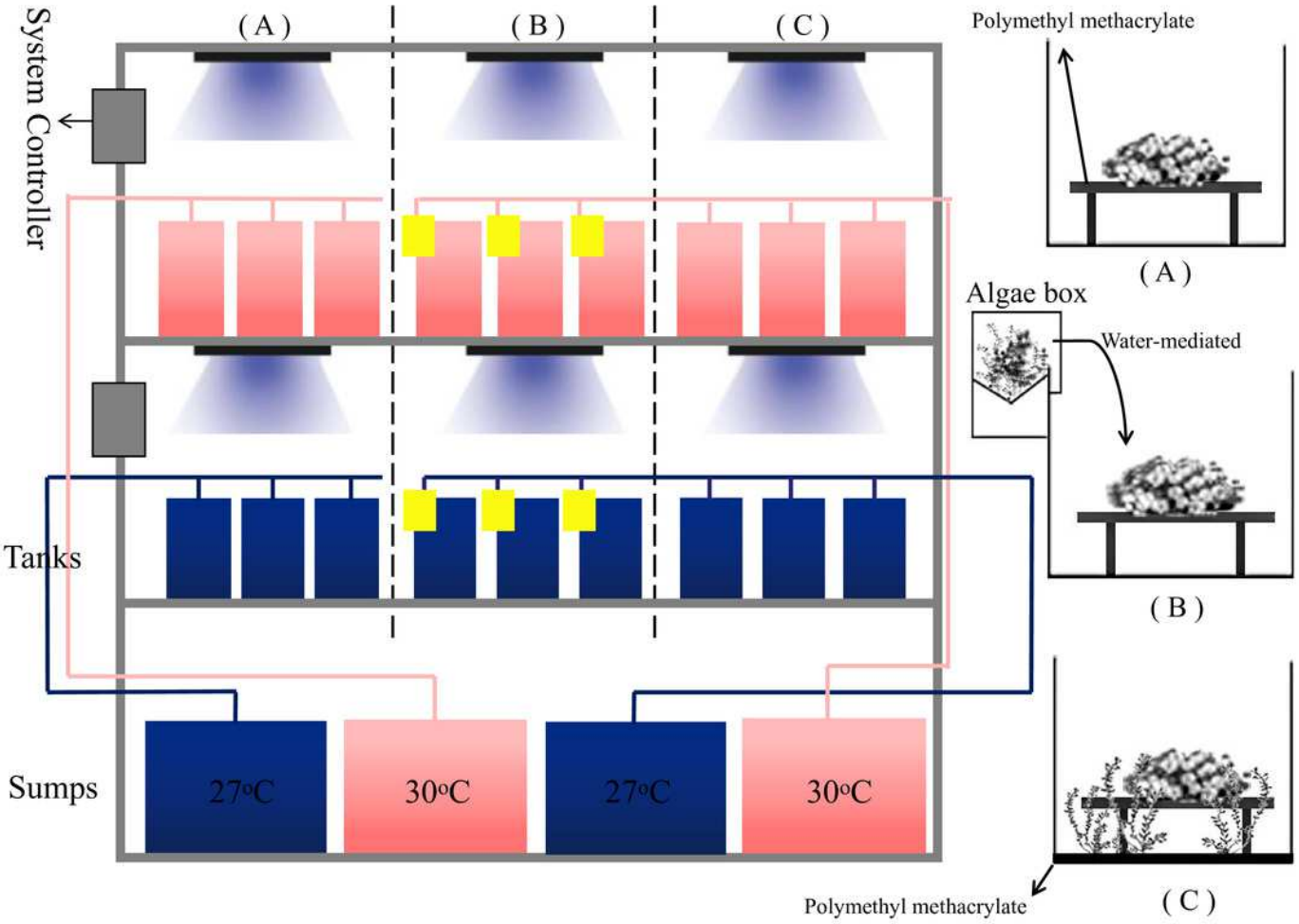
System Controller
(A)
(B)
(C)
Tanks
Sumps
27°C
30°C
27°C
30°C
Polymethyl methacrylate
(A)
Algae box
Water-mediated
(B)
(C)
Polymethyl methacrylate

Figure 2

The variability in the temperature, pH, and salinity over the course of the 4 weeks experiment.

Seawater at different temperatures of 27 °C (blue) and 30 °C (red). Without algae (A), (C), (E). Co-cultured with algae (B), (D), (F). The dotted lines represent deviation values for direct and co-occurrence groups of environmental factors.

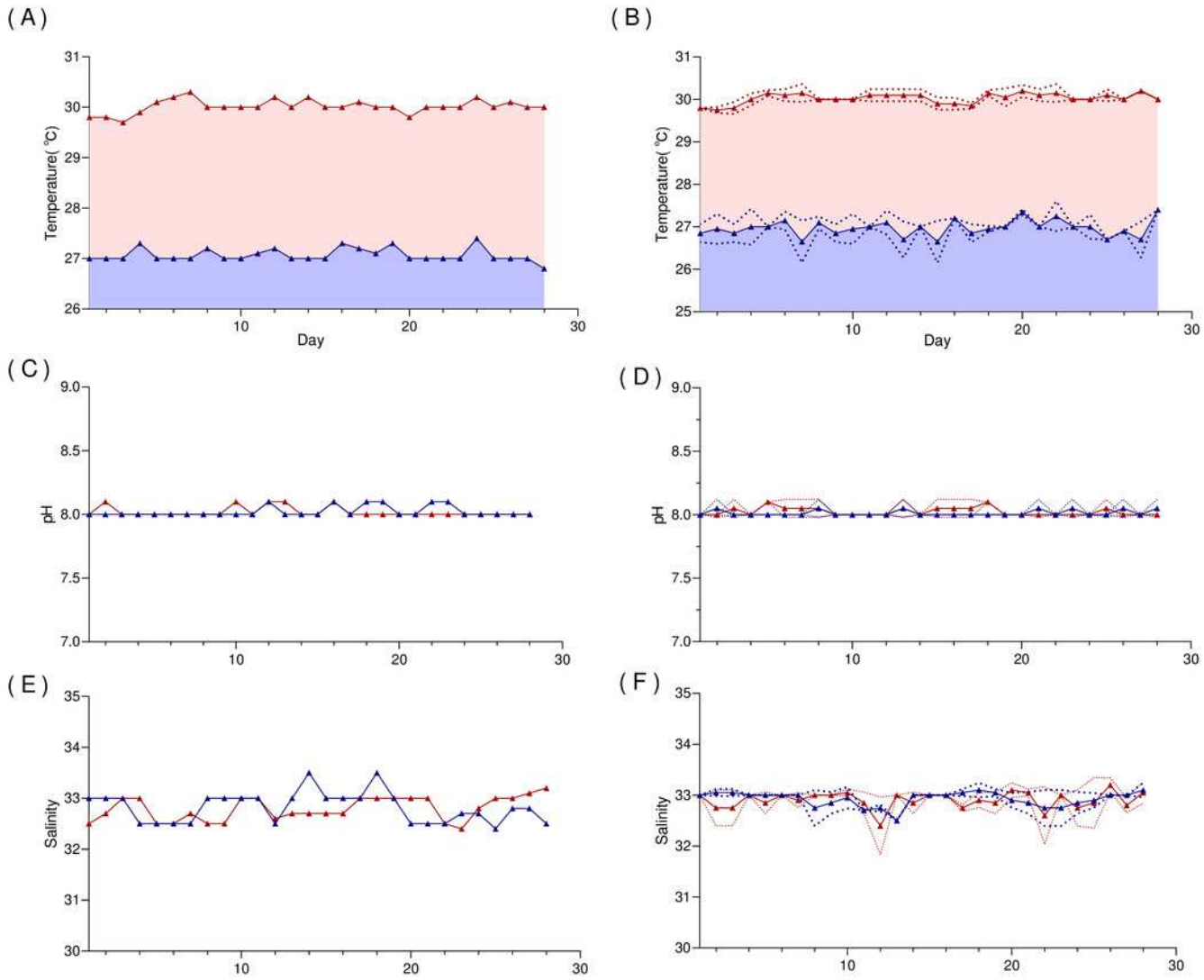

Figure 3

The effect of 27 °C (grey) and 30 °C (blue) temperatures on the (A) endosymbiont and (B) Chl a of corals treated by macroalgae after 4 weeks.

Upper case letters represent differences between temperatures, lower case letters represent differences between algal treatments at the same temperature, and different letters represent significant differences ($p<0.05$). Data are expressed in terms of the mean ± standard deviation, n=3.

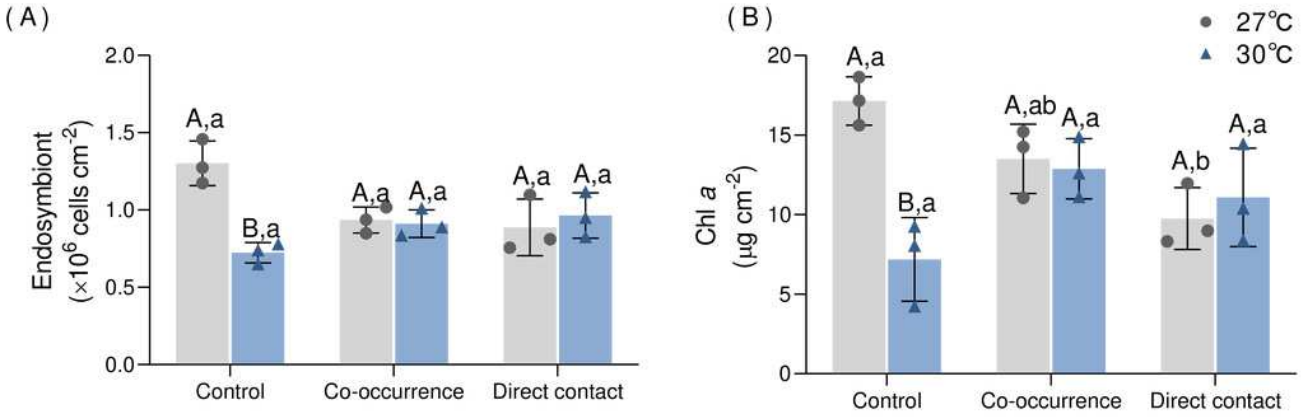

Figure 4

The effect of 27 °C (grey) and 30 °C (blue) temperatures on the (A) feeding rate, (B) protein and (C) growth rate of corals treated by macroalgae after 4 weeks of the experiment.

Upper case letters represent differences between temperatures, lower case letters represent differences between algal treatments at the same temperature, and different letters represent significant differences ($p<0.05$). Data are expressed in terms of the mean ± standard deviation, n=3.

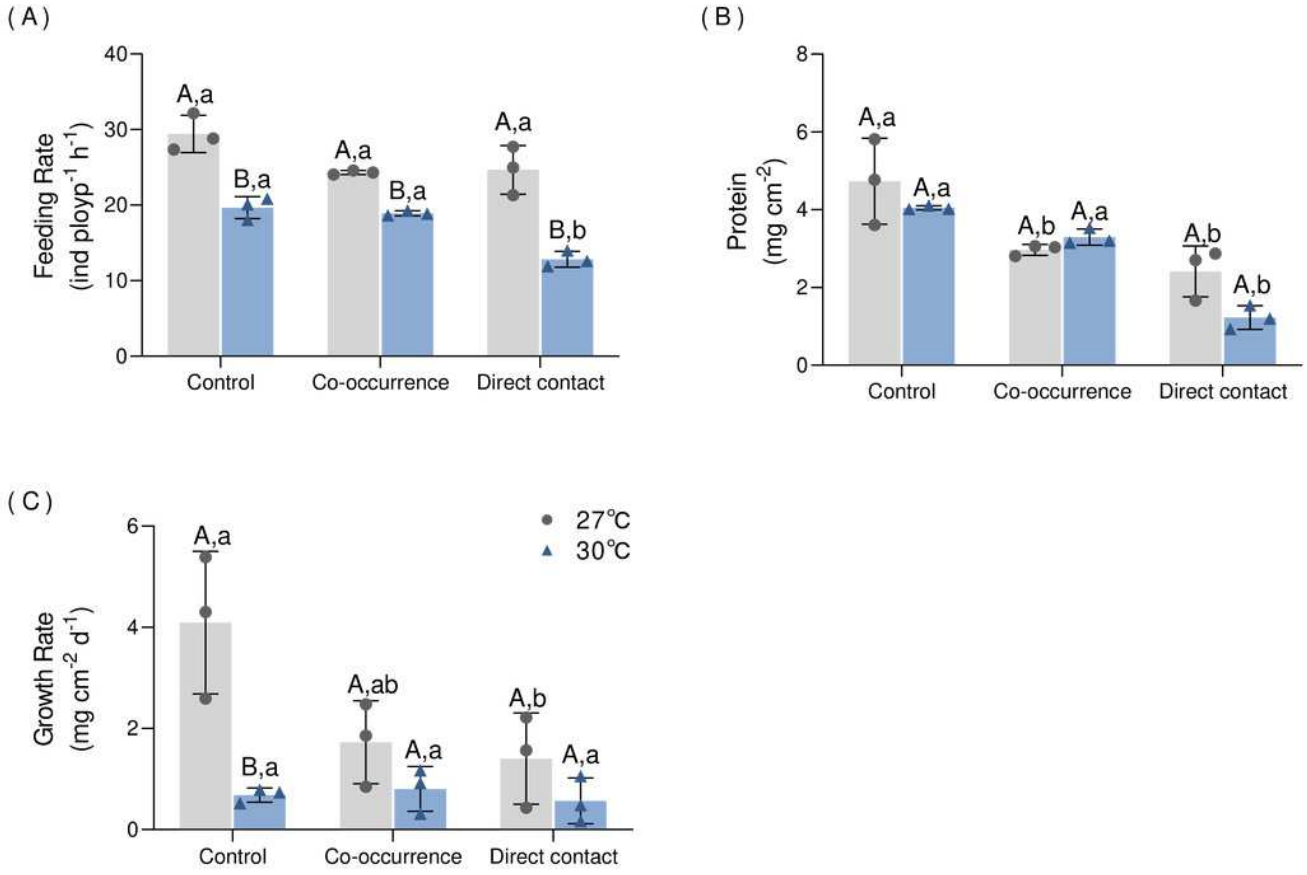

Figure 5

The effect of 27 °C (grey) and 30 °C (blue) temperatures on the (A) SOD, (B) CAT of corals treated by macroalgae after 4 weeks.

Upper case letters represent differences between temperatures, lower case letters represent differences between algal treatments at the same temperature, and different letters represent significant differences ($p<0.05$). Data are expressed in terms of the mean ± standard deviation, n=3.

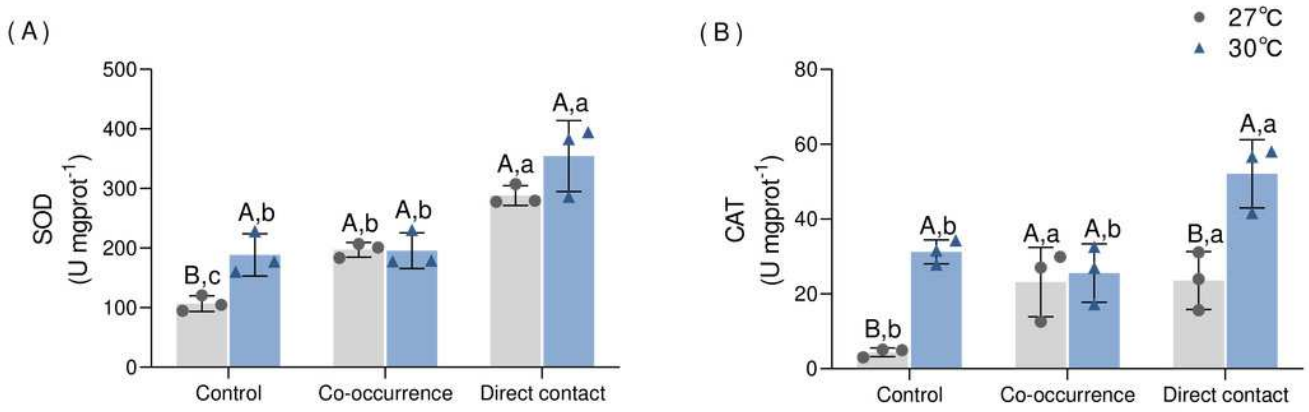

Table 1(on next page)

Two-way ANOVA output of different variables for *T. peltate*.

The bold values indicate the significant effects on the variable. F=F value; p =p value (significant <0.05).

Variable	Source of variation	F	p
Growth Rate	Algae	F(2,8) = 28.80	**<0.01**
	Temperature	F(1,4) = 7.78	**0.049**
	Interaction	F(2,8) = 28.00	**<0.01**
Feeding Rate	Algae	F(2,8) = 14.57	**<0.01**
	Temperature	F(1,4) = 119.80	**<0.01**
	Interaction	F(2,8) = 4.70	**0.04**
Endosymbiont density	Algae	F(2,8) = 0.75	0.5
	Temperature	F(1,4) = 21.94	**0.01**
	Interaction	F(2,8) = 9.05	**0.01**
Chl *a*	Algae	F(2,8) = 1.58	0.27
	Temperature	F(1,4) = 90.92	**<0.01**
	Interaction	F(2,8) = 7.29	**0.02**
Protein	Algae	F(2,8) = 31.87	**<0.01**
	Temperature	F(1,4) = 4.28	0.11
	Interaction	F(2,8) = 2.86	0.12
SOD	Algae	F(2,8) = 38.81	**<0.01**
	Temperature	F(1,4) = 16.01	**0.02**
	Interaction	F(2,8) = 2.37	0.16
CAT	Algae	F(2,8) = 10.01	**<0.01**
	Temperature	F(1,4) = 64.48	**<0.01**
	Interaction	F(2,8) = 5.13	**0.04**

1