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

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

JianRong Fu [1], **Jie Zhou** [1], **JiaLi Zhou** [1], **YanPing Zhang** [1], **Li Liu** [Corresp. 1]

[1] Fisheries College, Guangdong Ocean University, Zhanjiang, Guangdong, China

Corresponding Author: Li Liu
Email address: zjouliuli@163.com

With the degradation of coral reefs induced by climate change and local environmental stressors, an increasing abundance of macroalgae is observed. The sublethal damage caused by macroalgae on corals includes inhibiting growth, development, and reproduction. Thus, this study explored the effects of the macroalga *Caulerpa taxifolia* on the massive coral *Turbinaria peltata* under thermal stress. We compared the responses of corals' water-meditated interaction with algae and those in direct contact with algae. The results show that at ambient temperature (27°C), after coculture with *C. taxifolia* for 30 days, the density and chlorophyll *a* content of the endosymbiont were not influenced by the presence of *C. taxifolia*. With algae, the protein content of *T. peltata* decreased by 37.2% in water-meditated interaction group and 49.0% in direct contact group compared to the control group at 27°C. Meanwhile, the growth rate of *T. peltata* decreased by 57.7% in water-meditated interaction group and 65.5% in direct contact group compared to the control group. The activities of antioxidant enzymes were significantly increased, and the direct coral contact with *C. taxifolia* had a much stronger impact than under a water-meditated interaction. When the temperature was increased by 3°C, the endosymbiont density, chlorophyll *a* content, and growth rate of *T. peltata* significantly decreased compared to the control temperature, whereas the same pattern was seen in the increase in antioxidant enzyme activity. Additionally, when the coral interacted with macroalgae at 30°C, there was no significant decrease in both, the density and chlorophyll *a* content of 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. Overall, a direct contact of the coral with the alga was much more evident with stronger impacts than water-meditated interactions. ~~These results indicate that the negative effects of the macroalga *C. taxifolia* on the coral *T. peltata* are comparable to those of ocean warming.~~ Yet, the competition between corals and macroalgae may be more intense under thermal

stress.

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Jian Rong Fu[1], Jie Zhou[1], Jia Li Zhou[1], Yan Ping Zhang[1]

1 Fisheries College, Guangdong Ocean University, Zhanjiang, China, 524088;

Corresponding author:

Li Liu[1]

Haida road, Zhanjiang, Guangdong Province, China

Email address: corresponding_Li Liu_zjouliuli@163.com

Abstract

With the degradation of coral reefs induced by climate change and local environmental stressors, an increasing abundance of macroalgae is observed. The sublethal damage caused by macroalgae on corals includes inhibiting growth, development, and reproduction. Thus, this study explored the effects of the macroalga *Caulerpa taxifolia* on the massive coral *Turbinaria peltata* under thermal stress. We compared the responses of corals' water-meditated interaction with algae and those in direct contact with algae. The results show that at ambient temperature (27°C), after coculture with *C. taxifolia* for 30 days, the density and chlorophyll *a* content of the endosymbiont were not influenced by the presence of *C. taxifolia*. With algae, the protein content of *T. peltata* decreased by 37.2% in water-meditated interaction group and 49.0% in direct contact group compared to the control group at 27°C. Meanwhile, the growth rate of *T. peltata* decreased by 57.7% in water-meditated interaction group and 65.5% in direct contact group compared to the control group. The activities of antioxidant enzymes were significantly increased, and the direct coral contact with *C. taxifolia* had a much stronger impact than under a water-meditated interaction. When the temperature was increased by 3°C, the endosymbiont density, chlorophyll *a* content, and growth rate of *T. peltata* significantly decreased compared to the control temperature, whereas the same pattern was seen in the increase in antioxidant enzyme activity. Additionally, when the coral interacted with macroalgae at 30°C, there was no significant decrease in both, the density and chlorophyll *a* content of 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. Overall, a direct contact of the coral with the alga was much more evident with stronger impacts than water-meditated interactions. These results indicate that the negative effects of the macroalga *C. taxifolia* on the coral *T. peltata* are comparable to those of ocean warming. Yet, the competition between corals and macroalgae may be more intense under thermal stress.

Keywords: coral-macroalgal interaction, *T. peltata, C. taxifolia,* thermal stress, key physiological processes

Introduction

In recent years, with the combined effects of climate change and anthropogenic impacts, ~~the~~ coral reefs worldwide ~~has been~~ undergoing severe degradation (Leggat et al. 2022). Due to the global change, the first mass coral bleaching event was monitored in 1998 which killed approximately 8% of the world's coral, and ~~the amount of coral has progressive loss~~ 14% between 2009 and 2018 (Souter 2021).

Many studies asserted that ocean warming was a major factor which cause ~~of~~ coral cover ~~decrease~~ (Hughes et al. 2017,2019; Lough et al. 2018; Leggat et al. 2022). For example, the successive bleaching events in 2016-2017, during which 31% of reefs experienced 8–16 degree heating weeks (DHWs, °C-weeks), 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). A decline ~~in~~ coral cover may lead to an increase in the cover of other benthic organisms in the reefs, macroalgae ~~is one of them~~ (Fulton et al. 2019). Research showed that prior to 2011, the estimated global average cover of algae 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 succession of a large algal bed.

Macroalgae are important functional communities that play important roles in 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). But~~,~~ there is ~~underlying~~ competition between macroalgae and corals. Macroalgae may affect physiological responses of corals in different ways. The macroalgae competitive mechanisms include direct contact (Coyer et al. 1993; Manikandan et al. 2021) and

allelopathy (Roberta et al. 2014; Fong et al. 2020), weakening photosynthesis performance (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 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 affected coral feeding, endosymbiont function, tissues recovery, and oxidative stress. Morrow and Carpenter (2008) found that *Dictyopteris undulata* weakened 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 density ~~on~~ endosymbiont (Rasher et al. 2011; Smith et al. 2006; Diaz-Pulido and Barrón 2020). Bender (2012) asserted that the green filamentous macroalga *Chlorodesmis fastigiata* significantly reduced tissue recovery in *Acropora pulchra* and led to the infection of *A. pulchra* with ciliates. And high levels of reactive oxygen species (ROS, which is a byproduct of biological aerobic metabolism) could cause damage to cells and gene structure (Blanckaert et al. 2021). Shearer et al. (2012) found that after contacting with macroalga *Chlorodesmis fastigiata* thalli and their hydrophobic extract for short-term (1h and 24h), *Acropora millepora* oxidative stress were activated in response to ROS increase by alteration of transcription factors.

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, have a relationship with seasonal cycles and temporal cycles, which can 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). Moreover, the sensitivity of various macroalgae to environmental stressors is 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 China Ocean Climate Monitoring Bulletin (www.oceanguide.org.cn), the average seawater temperature in the Xuwen Sea area from May to September is 27-30 °C. Thus, Xuwen Coral Reef National Nature Reserve of China ~~was undergoing the influences of ocean warming~~ and the physiological responses of corals in this area might be affected by thermal stress. During thermal stress, corals feeding rate showed a vivid decrease and need more energy by decomposing tissue proteins to ~~activate~~ biological processes (DNA repair etc.) to resist heat stress (Ferrier-Pagès et al. 2010; Chakravarti et al. 2020). Triggered by thermal stress, ROS may be produced by endosymbiont mainly due to PS II dysfunction caused by damage to the D1 protein (Warner 1999) or host cells (Nii and Muscatine 1997). The increasing in ROS production as stress signaling mechanisms which potentially triggering an oxidative stress and apoptotic cascade (Hensley et al. 2000; Drury et al. 2022).

~~Thus,~~ to investigate the effects of macroalgae on hermatypic coral under ocean warming, the massive coral *Turbinaria peltata* and macroalga *Caulerpa taxifolia,* which are common species and interactions between each are frequently observed in the Xuwen, 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 chemical and physical effects, the water-mediated interaction group was set up to investigate the chemical effects, and the direct-contact group can be used to explore the combined effects of physical and chemical processes. It provides a reliable basis for the evolution of competition between corals and macroalgae under future global changes.

Materials & Methods

2.1 Sample collection

T. peltata and *C. taxifolia* were collected from Xuwen Coral Reef National Nature Reserve (109° 55' E, 20° 16' N) at a depth of approximately 4 m. They 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. After 3 months acclimation, the

corals were cut into 54 pieces of approximately 4 cm in diameter, fixed on ~~the~~ ceramic base with aqua rubber, and placed in another 200-L tank for one week until the corals elongated normally.

2.2 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-macroalgae interaction in coral reefs, the same amount of macroalgae was interacted with the coral samples in the tanks as following ways: (1) No algae were added to the tank, i.e., the control group (Fig. 1a). (2) Algae were cultured in external algae boxes, and there was no direct contact between the algae and the coral samples, which are referred to as water-mediated interaction group (Fig. 1b). (3) the algae and corals were co-cultured in the same tank with direct contact, and the height of the algae was set parallel to the coral samples, which is referred to as the direct contact group (Fig. 1c). And to explore the coral-macroalgae interaction variation under thermal stress, the experimental tanks were subjected to ambient conditions(27°C) and the shared socioeconomic paths (SSPs) scenario SSP2-4.5 (30°C) conditions(Zhongming et al. 2021), which is also the typical temperature range that the corals experienced at Xuwen Coral Reef Nature Reserve (www.oceanguide.org.cn). Thus, there were totally 6 treatments in this study, i.e. 2 temperature treatments ~~vs~~ 3 algae treatments. Each treatment contained 3 repelicate tanks, within which 3 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. ~~The spoiled~~ algae were replaced each day to ensure that the experimental group had 25 g (0.0025g cm^{-3}) of fresh algae, which is the amount of algae with the density of 0.0022 g cm^{-3} surveyed in inshore reef of Xuwen Coral Reef Nature Reserve. Fifty percent of the seawater was replaced every three days for each tank. Organisms were kept under treatment conditions for a period of 28 days and physiological measurements were performed subsequently.

2.3 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 uniformly. An aliquot of the slurry was taken and centrifuged (4000 rpm min^{-1}, 4 °C, 10 min) ~~to remove the supernatant.~~ Part of the pellet was suspended in 5 mL formaldehyde to count the endosymbiont density under the microscope with a blood counting plate. Another portion was resuspended in 8 mL methanol. The pigments were extracted at 4 °C for 24 h. The extract was centrifuged (4000 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 (Marsh 1970).

2.4 Feeding rate

~~Prior to measurement,~~ nubbins were moved into the feeding tanks (1L) 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 ~~to~~ polyp. The measurement was performed once a week and conducted at 11:00 - 12:00 a.m. The number of polyps in each nubbins were visually counted before experiment.

2.5 Growth rate

Weights of coral nubbins were measured on a balance (accuracy=0.01g) using the buoyant weight technique (Davies 1989). A glass beaker contained 1 L of filtered seawater (27°C, salinity 32), and the nubbins were placed on the bottom of the beaker for the growth rate measurements. Before each measurement, the surface of the coral ceramic base was lightly brushed with a toothbrush to remove algae. The growth rate (mg d^{-1}) was calculated as $\left(M_{ti} - M_{t0}/T_i\right) \times 1000$, where M_{t0} represent 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 by the aluminium foil technique (Marsh 1970).

2.6 SOD and CAT

The homogenized coral tissue slurry in 2.3 was centrifuged using a ~~freezing~~ centrifuge (4000 rpm min^{-1}, 10 min, 4 °C), and the ~~quantitative~~ supernatant was collected to measure the SOD and CAT activities, which were determined in the dilution using kits (A001-1-1, A007-1-1, Nanjing Jicheng, China). ~~And~~ 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}.

2.7 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 normality of 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 was used to identify significant differences between

temperature treatments. A post hoc Fisher's least significant difference (LSD) test was used to determine differences between algal treatments. The data was analysed and graphed using GraphPad Prism 8.0 and $p<0.05$ was considered a significant difference.

Results

3.1 Seawater chemistry monitoring

As shown in Table 1, temperature, pH and salinity values were averaged during the experiment and measured continuously during the experiment (28 d). The high standard deviation is due to daily variation.

3.2 Endosymbiont density and Chl *a* content

Fig. 2a and 2b showed that the density and pigment content of endosymbiont were significantly influenced by temperature. In the absence of macroalgae, thermal stress resulted in a average 44.6% (from 1.3±0.1 to 0.72±0.07 $\times 10^6$ 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 ($p<0.01$). However, the negative effects caused by thermal stress on endosymbiont density and chl *a* content~~,~~ were absent in algae treatments, which indicated the antagonistic effect between temperature and algae treatments($p<0.05$). Compared with control group ~~in~~ ambient temperature, the corals interacted with macroalgae in both temperature treatments showed lower mean values of endosymbiont density and chl *a* content, which were not significantly different between water-mediated interaction group and direct contact group.

3.3 Feeding rate

The results of the feeding rate are displayed in Fig. 3a. Primarily, thermal stress had a significantly detrimental effect on the feeding rate among three algae treatments ($p<0.01$). However, there was no obvious~~ly~~ difference ~~among~~ three algae treatments at ambient temperature. ~~Under~~ 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 macroalgae (F=4.7, $p=0.04$, Table 2).

3.4 Protein content

As shown in Fig. 3b, the protein content was mainly impacted by macroalgae ($p<0.01$). At ambient temperature, exposure to algae caused coral to lose 37.2% protein content in water-mediated interaction group ($p=0.01$) and 49.0% protein content in contacted interaction group ($p<0.01$). Although contact with algae made the mean protein content of coral even lower, the difference was not obvious. As the temperature increased, compared with the control group and

water-mediated interaction group, the direct interaction with algae resulted in the lowest protein content of 1.2±0.3 mg cm^{-2} ($p<0.01$). Actually, no significant difference was observed between temperature treatments whatever in the presence or absence of algae (p=0.11). Therefore, there was no interaction between algae and temperature treatment (F=2.86, p=0.12, Table 2).

3.5 Growth rate

As indicated by the change in buoyant weight, the growth rate (Fig. 3c) was affected by both algae and thermal stress. At 27°C, growth rate of corals in the control group was highest, with a mean value of 4.1±1.4 mg cm^{-2} d^{-1}. ~~And~~ the coculture with macroalgae decreased the growth rate of coral by 57.7% in water-mediated interaction group ($p=0.06$) and 65.5% in ~~contacted interaction~~ group ($p=0.03$). Thermal stress had a obvious~~ly~~ inhibitory effect on the growth rate in the coral culture system without algae interaction, which resulted in an 83.4% decline ($p<0.01$). The elevated temperature combined with direct contacted 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 in elevated temperature were not significant. ~~It turned out that~~ the interaction between temperature and algae was obvious (F=28, $p<0.01$, Table 2).

3.6 SOD and CAT

As shown in Fig. 4a, macroalgae treatments ~~enhanced~~ the antioxidant capacity of corals under both temperature conditions. At 27 °C, the water-mediated interation with algae increased the SOD activity of coral by 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 other groups ($p=0.03$, $p<0.01$). At 30 °C, the mean SOD activity of coral, without interacted with algae, increased by 1.77-fold compared with conterpart at ambient temperature ($p=0.03$). In direct contacted interaction group under thermal stress, SOD activity increased to the highest level of 354.3±59.56 U mg $prot^{-1}$ ($p=0.03$, $p<0.01$). However, in the coculture system, there was no prominent discrepancy caused by thermal stress, indicating that both factors did not interact (F=2.37, p=0.16, Table 2).

Similarly, the CAT activity ~~was also enhanced by~~ algae interaction and thermal stress in Fig. 4b. At ambient temperature, the water-mediated interaction with algae has caused CAT activity in coral tissure to rise by 5.3-fold ($p<0.05$), which is comparable to the level of CAT activity in contacted interaction group. With the temperature increased, the CAT activity in the pure coral system enhanced by 7.1-fold ($p<0.01$). Moreover, when cultured in contact with the algae, the CAT activity further doubled ($p=0.03$, $p<0.01$). The combined effect of temperature and

macroalgae was significant (F=5.13, *p=0.04*, Table 2).

Discussion

This study explored the crucial issue of how physiology and the oxidative stress response of ~~the~~ hermatypic coral are affected by macroalgae under elevated temperatures. We set up three treatments of the macroalga *C. taxifolia* to act on the coral *T. peltata* under ambient temperatures (27°C) and an increase of +3°C, ~~including water-meditaed and contacted interaction.~~ The results demonstrated that ~~the coculture with macroalgae prominently~~ altered the physiology of coral as well as ~~enhancing~~ the antioxidant activity. In addition, combined with rising temperature, there was a remarkable synergistic effect on impacting the physiology and further increasing oxidative stress of coral, in which contact with algae is much more severe than water-mediated interaction.

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

In this study, it was found that there was no significant effects of *C. taxifolia* interactions (both water-mediated and indirect-contacted interactions) on endosymbiont density and chl *a* content of *T. peltata* at either ambient or elevated temperature . A number of studies have reported that the ~~coral came into decreasing in chlorophyll photochemical efficiency~~ (Fv/Fm) or bleaching when interaction with macroalgae, but not all coral species are equally susceptible to algal mediated mortality and that 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* ~~or~~ *Sargassum sp.* did not inhibit photosynthetic efficiency or induce bleaching of *Porites porites* ~~whether it's the physical or chemical effects of macroalgae, and it might be related to~~ that the 20-day interaction period was too short to produce impacts on coral health. In addition to that, as a massive coral, *T. ~~P~~eltata* could resist environmental pressure by increasing its basic metabolism (Loya et al. 2001). The reduction ~~of~~ protein content in this study might be related to prevent the decrease of endosymbiont density, which might be the reason why there was no significant effects of macroalgal interactions on endosymbiont density.

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

In this study, there were no effects of *C. taxifolia* (both direct and indirect interaction) on feeding rate of *T. peltata* at ambient temperature. However, ~~it~~ was affected by thermal stress. Johannes and Tepley (1974) also found that the feeding rate of coral was decreased in heat stress because of the polyp contraction or a loss of nematocyst function. ~~And~~ our results ~~showed~~ that decrease in chl *a* content ~~or~~ endosymbiont density ~~might be~~ the reason why feeding rate ~~would be~~

impacted in thermal stress. Endosymbiont provide photosynthate to host cells (van Oppen and Blackall 2019). The decrease of endosymbiont density at high temperature might result in the reduction of energy to maintain the normal physiological function, and thus leading to the decline of the ability of predation. Moreover, this study showed that contacted with *C. taxifolia* led to the decrease in feeding rate at 30°C. In summary, thermal stress was a crucial factor affecting the pradation of *T. peltata* and it would be much more severe when contacted with macroalgae.

The results also indicated that the impacts of macroalgae can induce the reduction of the protein content of corals. Damage to coral tissue by contact with macroalgae has been documented in many studies. Bender et al. (2012) asserted that *Acropora sp.* lost the tissue and decreased growth rate of bone due to allelopathy mechanisms after contacted with *Chlorodesmis fastigiata.* In fact, macroalgae may transfer many allelopathic substances when corals approach it, which will alter the structure of the microbial community, thus injure the physiological process of corals (Fong et al. 2020), and ultimately give rise to protein content loss. Under stress, massive corals with thicker tissues may overcome the effects of endosymbiont loss through catabolism (DeCarlo and Harrison. 2019). In addition, 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.

It was investigated that the growth rate of coral was altered by the interaction of macroalgae and temperature, which was 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) discovered that corals were weakened at 30 °C, and contaction 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 exacerbating the growth rate.

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

Corals under thermal stress would produce ROS (Blanckaert et al. 2021). Downs (2002) documented that when exploring the varied oxidative stress response of coral under seasonal change, the SOD activity in summer was 3 times higher than that in winter. Furthermore, this study

discovered that SOD in corals was more active under interaction with macroalgae and increasing temperature or the synergistic effect of both. In other words, it is illustrated that weakened corals are more vulnerable to competition from algae, which ~~is the same as~~ in Diaz-Pulido et al (2010). The results suggested that the level of both antioxidant enzyme activities when *C. taxifolia* indirectly contacted ~~with~~ *T. peltata* was ~~equal~~ to that under thermal stress alone, which indicate~~d~~ that the stress triggered by macroalgal allelochemicals on coral was equivalent to that induced by increasing temperature. Additionally, this study indicated that under water-mediated interaction scenarios, there was no significant difference in the oxidative stress response of corals between the two temperature groups. The temperature effect was more obvious in CAT activity compared with SOD activity under direct contact treatment, which may be related to the decrease in protein content in coral tissues caused by rising temperature under 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 transformation of coral reef ecosystems into algae beds~~ is becoming more apparent due to the global climate change driven by human activities. The results of this study showed that, firstly, at 27°C *C. taxifolia* ~~mainly~~ affected the growth rate and protein content of *T. peltata* as well as enhancing the antioxidant activity. Secondly, thermal stress adversely affected all paramaters of coral in this study. Thirdly, this study provides an opinion of the combination of thermal stress and macroalgae interaction may further exacerbate the adverse effects on corals. Further ~~researches~~ 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 departments should urgently take necessary actions to prevent CO_2 emissions out line with the goals of the Paris Climate Agreement, and moderate clean-up activities can be undertaken in areas where *C. taxifolia* blooms for coral reef management.

Conflict interest

We declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authorship contributions

Jian Rong Fu: Investigation, Conceptualization, Methodology, Data analysis and visualization, Writing - original draft, Writing - review & editing. Jie Zhou: Investigation,

Conceptualization, Methodology, Data verification and analysis, Writing - review & editing, Funding acquisition. Jia li Zhou: Investigation, Software, Methodology. Hui ting Yang: Investigation and conceptualization. Yan Ping Zhang: Investigation, Conceptualization, Methodology. Li Liu: Resources, Funding acquisition, Project administration, Supervision, Writing - review & editing.

Consent for publication

There is no conflict of interest to report.

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Figure 1

Experimental operating system.

System control (grey) with a feedback loop to adjust the conditions. Seawater at different temperatures of 27 °C (blue) and 30 °C (red) was heated in different collection sumps(36L) and then fed into the each tanks(10L). Macroalgae treatments are applied, (a) *T. peltata* (b) *T. peltata* indirect interaction with C. taxifolia, Algae box (green). (c) *T. peltata* contact with C. taxifolia. Each treatment contained 3 repelicate tanks, within which 3 coral nubbins were placed.

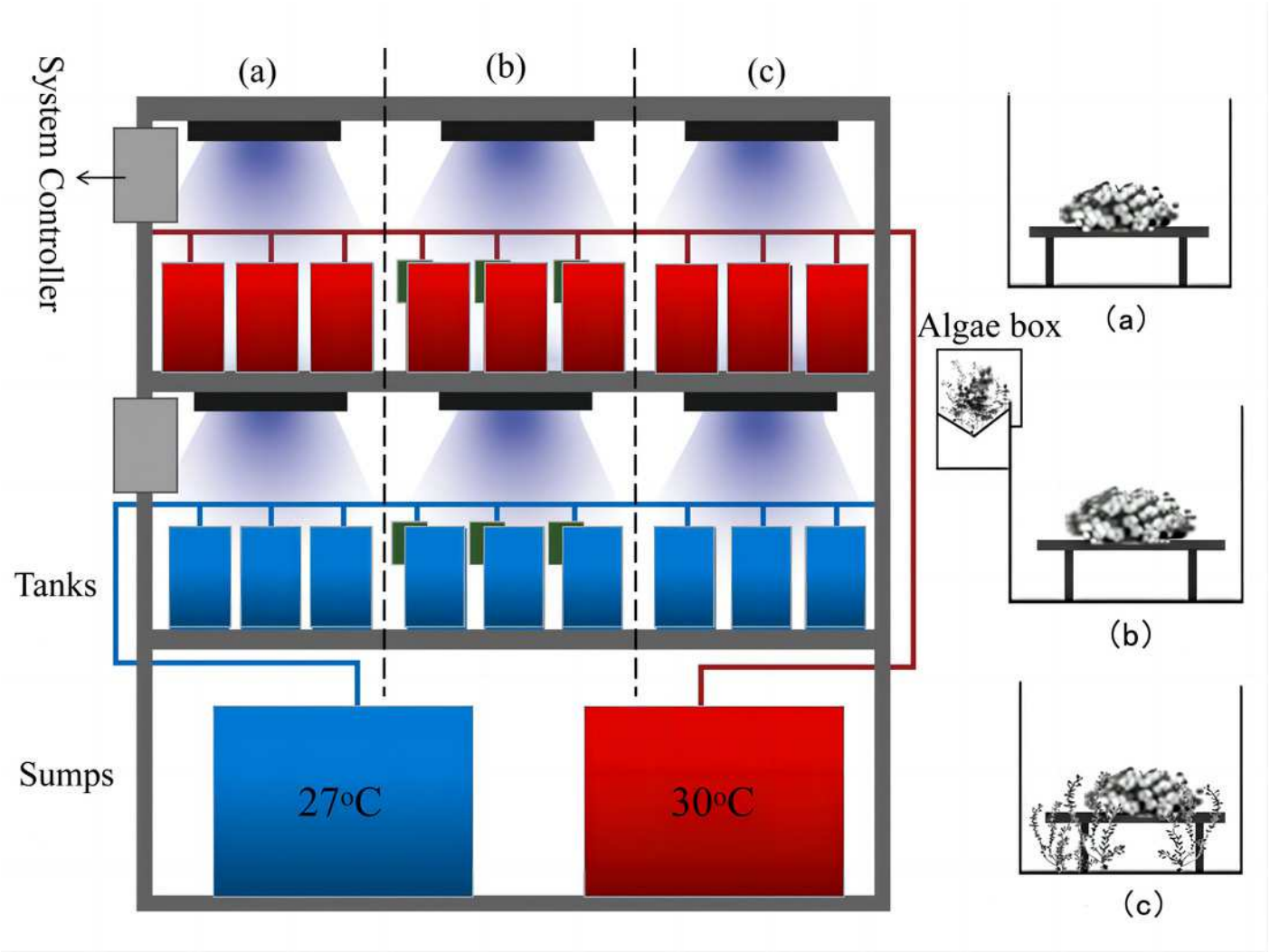

Figure 2

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

Different letters indicate that there are significant differences between macroalgae treatments at the same temperature ($p<0.05$), * indicates that there are significant differences between different temperatures ($p<0.05$). Data are expressed in terms of the mean ± standard deviation, n=3.

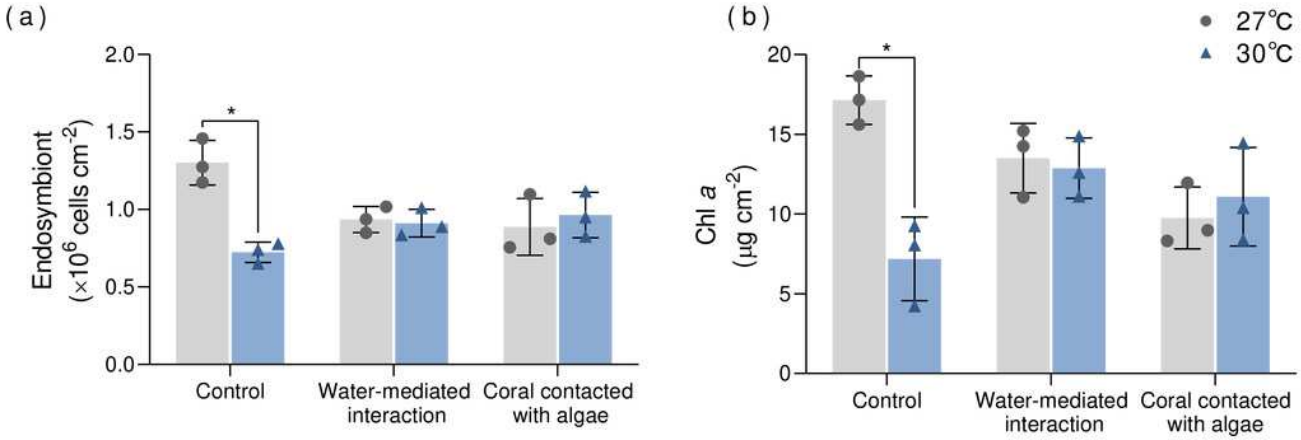

Figure 3

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

Different letters indicate that there are significant differences between macroalgae treatments at the same temperature ($p<0.05$), * indicates that there are significant differences between different temperatures ($p<0.05$). Data are expressed in terms of the mean ± standard deviation, n=3.

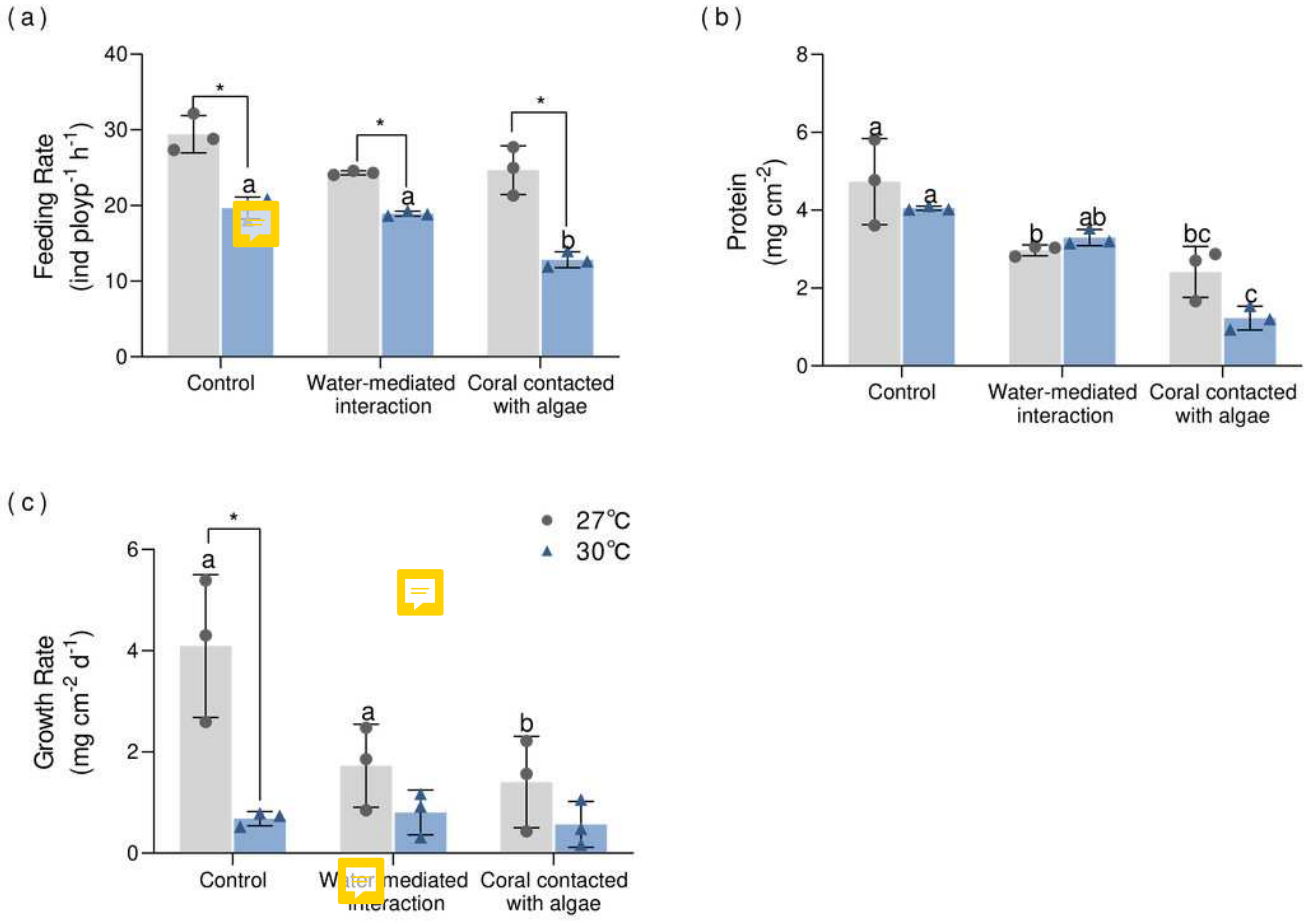

Figure 4

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

Different letters indicate that there are significant differences between macroalgae treatments at the same temperature ($p<0.05$), * indicates that there are significant differences between different temperatures ($p<0.05$). Data are expressed in terms of the mean ± standard deviation, n=3.

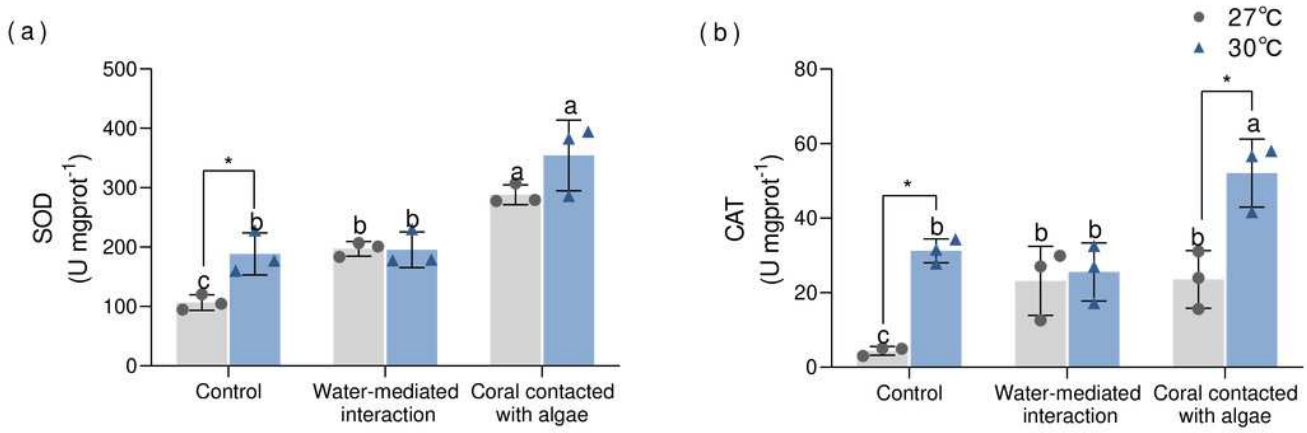

Table 1(on next page)

Summary of values of water chemistry data for all treatments.

	Coral		Water-mediated interaction		Coral contact with algae	
	27°C	30°C	27°C	30°C	27°C	30°C
Temperature [°C]	27.1±0.1	30.0±0.1	26.9± 0.3	30.0 ±0.2	27.0± 0.2	30.0 ±0.1
pH	8.02 ±0.04	8.02± 0.04	8.01± 0.04	8.03± 0.04	8.01 ±0.03	8.02 ±0.04
Salinity	32.1 ±0.6	32.1± 0.5	32.4 ±0.5	32.1 ±0.6	32.2 ±0.4	32.0 ±0.5

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Table 2(on next page)

Two-way ANOVA output of different variables for *T. peltata,* with bold values indicating significant effects on the variable.

F=F value; p =p value (significance <0.05).

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

1