Article

Effects of *Caulerpa Taxifolia* on Physiological Processes and Gene Expression of *Acroproa Hyacinthus* during Thermal Stress

Jian Rong Fu[1], Jie Zhou[1], Yan Ping Zhang[1], Li Liu[1,2*]

[1] Fisheries College, Guangdong Ocean University, Zhanjiang, China, 524088;
[2] Guangdong Laboratory of Southern Ocean Science and Engineering, Zhanjiang, China, 524025;
* Correspondence: Correspondence: E-mail: zjouliuli@163.com

Abstract: An increasing ecological phase-shift from coral dominated reefs to macroalgae dominated reefs as a result of anthropogenic impacts, such as eutrophication, sedimentation, and overfishing, has been observed in many reef systems around the world. Ocean warming is a universal threat to both corals and macroalgae, which may alter the outcome of competition between them. Therefore, in order to explore the effects of indirect and direct exposure to macroalgae on the physiological, biochemical, and genetic expression of corals at elevated temperature, the coral Acroproa hyacinthus and highly invasive green algae Caulerpa taxifolia have been chosen. Physiologically, the results exhibited that distinguish from control and direct contact treatments, the density and chlorophyll a content of zooxanthella decreased by 53.1% and 71.2% respectively, when coral indirect contacted with algae at ambient temperature (27°C). Besides, enzyme activities of superoxide dismutase (SOD) and catalase (CAT) in coral tissue were enhanced by interacting with algae. After an increase of 3°C, the density and chlorophyll a content of zooxanthella reduced by 84.4% and 93.8% respectively, whereas the enzmy activities of SOD and CAT increased by 2.3 and 3.1-fold. However, only the zooxanthellae density and pigment content decreased when C.taxifolia co-culture with A.hyacinthus at 30°C. Molecularly, different from the control group, the differentially expressed genes (DEGs) such as Rab family, ATG family and Casp7 were significantly enriched in endocytosis, autophagy and apoptosis pathways , regardless whether A.hyacinthus was indirect or indirect exposure to C.taxifolia at 27°C. Under thermal stress without algae interaction, the DEGs were significantly enriched in microbial immune signal transduction pathways, such as Toll-like receptor signaling pathway and TNF signaling pathway, while multiple cellular immunity (IFI47, TRAF family) and oxidative stress (CAT, SODC, HSP70) genes were up-regulated. Inversely, compared with corals without interaction with algae at 30°C, the DEGs of corals interacted with C.taxifolia at 30°C, were remarkably enriched in apoptosis, NOD-like receptor signaling pathway, including the transcription factors such as Casp family, TRAF family. In conclusion, the density and chlorophyll a content of zooxanthella remained a fading tendency induced by macroalgae at ambient temperature. The oxidative stress and immune response levels of coral has been elevated at 30°C, but macroalgae alleviated the negative effects triggered by thermal stress.

Keywords:Acropora hyacinthus; Caulerpa taxifolia; thermal stress; physiological processes; gene expression

1. Introduction

Coral reefs are the rainforests of marine ecosystems [1]. However, human activities have been contributing to global warming, leading to a continuous deterioration of the coral coverage in recent years [2]. A subsequent phenomenon appeared that the coral reef succession to algal bed [3]. Facutally, macroalgae are critial communities that play an important roles in stabilizing reef structure, generating primary productivity in reef areas [4]. Nevertheless, niche competition between partial macroalgae and hermatypic coral is

 doi:10.20944/preprints202211.0223.v1

drastic. Competition mechanisms in macroalgae are primarily through the retraction of polyps caused by physical contact and injury by pathogenic microorganisms or lipid-soluble compounds [5-7], resulting in reduced calcification of coral growth, fecundity, survival and settlement rate [8-12]. However, the extent of the impact induced by macroalgae on corals varied between species and exposure conditions[13].Some studies suggested that competition among macroalgae becomes more manifest in the context of coral reef degradation, perhaps because weakened corals do not have adequate energy to compete for space as they need to maintain various functions [14,15]. As a result, coral reef ecosystems are facing severe challenges from the invasion of macroalgae.

A transcriptome is a subset of genes expressed by an organism that induces gene transcription at specific circumstances to alter different molecular mechanisms [16,17]. However, the impact of ambient pressure on corals is generally determined only by apparent physiological damage [18]. Literally, regulation of gene expression is prevalent before the injury [19]. For example, the influences of thermal stress on coral growth plasticity, bleaching tolerance, and skeletal properties may be related to zooxanthellae communities, microbiome composition, and coral gene expression patterns [20-22]. Hermatypic coral adapt to the rising temperature through positive regulation of innate immune responses, protein ubiquitination and apoptosis [23]. At present, it is universally admitted that transcriptome sequencing technology is beneficial for enlightening and evaluating how ecological factors affect scleractinian corals. Davies S W et al. [24] discovered ascended transcription of the H^+ transporter gene in corals under stress from ocean acidification, confirming the role of proton transport in promoting calcium production in the presence of strong pCO_2.

Conspicuously, transcriptome sequencing has been used in corals, while expression regulation of genes at various temperatures has not been reported in exploring spatial interactions of macroalgae-coral. To investigate it,the branch-like coral *A.hyacinthus* and macroalgae *C.taxifolia* were selected as study species. In order to evaluate the physical and chemical infections of seaweed, the direct-contact group and indirect-contact (exposed) group was set up. Through a comprehensive analysis of physiological manifestations combined with gene expression in *A. hyacinthus*, the results can be used to explore the potential ecological impact of macroalgae on reef-forming corals and provide basic data and references for understanding the relationship between corals and macroalgae.

2. Materials and Methods

2.1. Expreimental animals

A.hyacinthus and *C. taxifolia* were collected from Xuwen Coral Reef National Nature Reserve (109° 55' E, 20° 16' N) , transported to the laboratory. The coral was segmentted into approximately 4 cm nubbins. Then the nubbins were epoxied on the ceramic bases, 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-2s-1 with a 12: 12 h light/dark cycle for 3 months.

2.2. Experimental design

Prior to the experiment, 54 coral nubbins were randomly allocated into 18 aquariums (10L). Two temperature treatments were set by increasing from ambient temperature (27°C, L) to stress temperature (30°C, H) at a rate of 1°C per day [25]. Equal amounts of macroalgae (25 g) were interacted with the coral nubbins in the following three ways: (1) No algae were added to the culture system, i.e., the control group (D, Fig. 1A). (2) The aquariums, in which the corals were exposed to the aquaculture water that flowed through external algae box, were referred to indirect contact group (H, Fig. 1B). (3) The algae and corals were cultured in the same aquarium, in contact with each other but at the same height. The treatment is set as the direct contact group (M, Fig. 1C). Therefore, there were 6 treatments in this experiment, namely control group with ambient temperature-

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LD, indirect contact group with ambient temperature-LH, direct contact group with ambient temperature-LM, control group with higher temperature-HD, indirect contact group with higher temperature-HH, direct contact group with higher temperature-HM. Three coral nubbins were placed in each aquarium and each treatment sets up three aquariums. Half of seawater in each aquarium was replaced every 3 days. Coral samples were collected after four-week incubation.

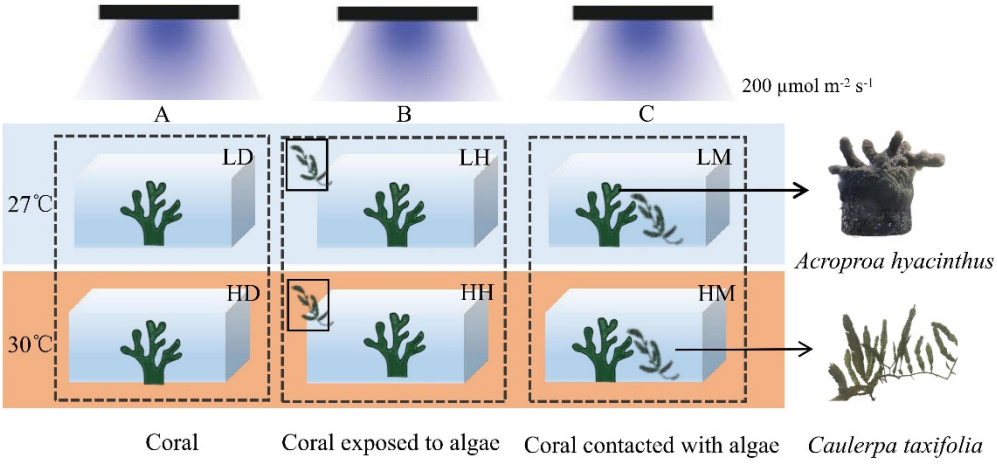

Fig. 1 Experimental design. (A) Control group, **(B)** Indirect contact group, **(C)** Direct contact group. The orange and bule color represent different temperature treatments (27°C and 30°C). The conditions in the treatments are presented in Supplementary Tab. Three aquariums were set for each treatment, n=3.

2.3. Determination of physiological and biochemical indexes

2.3.1. Samples collection

After 4-week incubation, 3 nubbins were collected from each group and rinsed with 4°C sterilized seawater. The slurry was homogenized into 6 portions of 12 mL and centrifuged at 4000 rpm for 10 min at 4°C. The precipitate was used to determine the density and chl *a* content of zooxanthellae. After drying, the skeletal surface area is determined by the aluminium foil technique [26].

2.3.2. Zooxanthellae density and Chl a content

Three parts of the pellet was suspended in 5 mL formaldehyde to count the zooxanthellae density using the microscope with a blood counting plate. Another portions was resuspended in 8 mL methanol. The pigments were extracted at 4°C for 24h. The extract was centrifuged (4000 rpm min-1, 4°C, 10 min), and Chl a was determined according to UV-visible spectrophotometer [27]. Data were normalized to skeletal surface.

2.3.3. Growth rate

Methods of weights were referenced on a technique where the nubbins were put on the bottom of the beaker contained 1 L of filtered seawater (27°C, salinity 32) for the growth rate measurements. The measurements were repeated every 7 d in mg $cm^{-2}d^{-1}$ [28].

2.3.4. SOD and CAT

50ml of homogeneous solution 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 determined in the dilution using kits (A001-1-1, A007-1-1, Nanjing Jicheng, China) and finally a BCA kit to determine the protein concentration (A045-3-1,

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Nanjing Jicheng, China). The total protein content of each sample was determined, and the activity unit of the two enzymes was standardized as U mg $prot^{-1}$.

2.4. Transcriptome sequencing and analysis of A.hyacinthus

2.4.1. RNA-Seq data analysis

At the end of the experiment, 3 corals were quickly taken from each group, slightly shaken in PBS, and stored in liquid nitrogen for RNA extraction and transcriptome sequencing. Total RNA from each tissue was extracted by Trizol method [29]. After detecting the quality of the RNA samples by agarose gel electrophoresis, 18 eligible samples were constructed and sequenced by Beijing Baimike Biotechnology.

Primely, raw reads were filtered, and clean reads were obtained for splicing and assembly analysis. Secondly, Trinity (v2.5.1) was used to perform de novo assembly on clean reads to obtain transcripts and remove redundancy. The longest transcript in each transcript cluster was selected as a single gene (unigene) for subsequent analysis. Eventually, unigene was compared with SwissProt, COG, KOG, GO, KEGG, Pfam and eggNOG databases, and the E-value was < 1e-5.

All gene-matched transcripts from the 18 libraries were normalized and the differential gene expression analysis was performed using the software Deseq2. Differential groups were selected as LH vs LD, LM vs LD, HD vs LD, HH vs HD, HM vs HD. DEGs screening threshold was false discovery rate (FDR) < 0.001 and $\log_2$Fold Change ≥ 2. ClusterProfiler software was used for enrichment analysis of differential genes.

2.4.2. Quantitative PCR for mRNA expression

Twelve genes were selected from DEGs of each treatment for quantitative verification (Supplementary Tab for primers). The reaction system was 15μL, including 7.5μL 2×Power Green qPCR Mix, 0.3μL upstream and downstream primers (10μmol/L), 1.5μL cDNA, and 5.4μL ddH2O. Each sample was repeated three times and the reactions were run on a Roche LightCycler 96. The reaction procedure was as follows: 94°C for 3min; 94°C for 15s, 58°C for 15s, 72°C for 20s (fluorescence collection), 40 cycles. The specificity of the PCR product was detected by melting curve analysis. β-actin was used as the reference gene, and the expression level of target gene was statistically analyzed by the $2^{-\Delta\Delta Ct}$ method.

2.5. 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 diff erences between temperature treatments. A post hoc Fisher's least significant difference (LSD) test was used to determine differences between algal treatments. The data were analysed and graphed using GraphPad Prism 8.0. $p < 0.05$ was considered a significant difference. Pictures were also drawn by Rstudio and chiplot (https://www.chiplot.online/).

3. Results

3.1. Results of macroalgae on phsiological processes

As shown in Figs. 2A and B, at ambient temperature, LH corals experienced a 53.1% and 71.2% decline in density and chl *a* of zooxanthellae ($p=0.046$, $p< 0.01$) than LD corals. There was no significant discrepancy between treatments LH and LM. With the rising temper, *A.hyacinthus* in HD decreased by 84.4% and 93.8% respectively compared with LD ($p< 0.01$), at which point the addition of *C. taxifolia* recovered the density and pigment

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content in the HH and HM groups. Hence, there was an interaction between algae and temperature treatment (F= 6.9, *p= 0.02*, F= 10.8, *p< 0.01*).

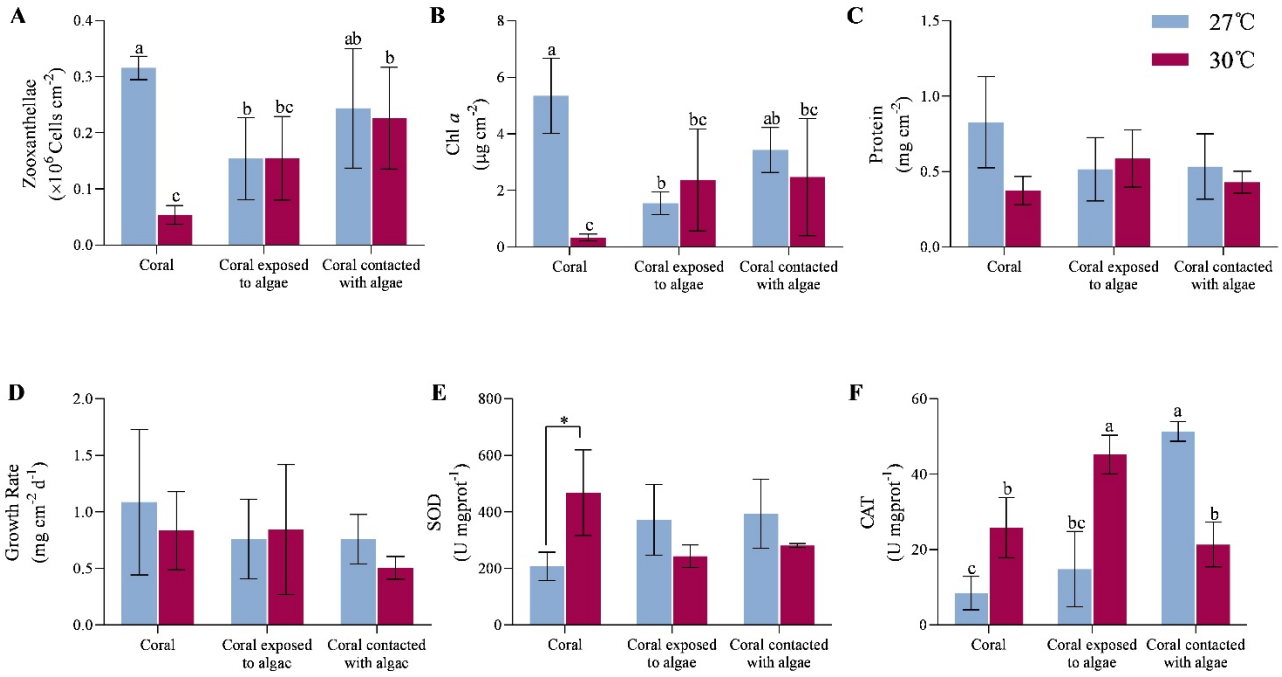

Fig. 2 Summary of physiological indicators. The effect of different temperatures of 27 °C (blue) and 30 °C (red) on the **(A)** zooxanthellae, **(B)** Chl *a*, **(C)** protein content, **(D)** growth rate, **(E)** SOD and **(F)** CAT of macroalgae-treated corals after 4 weeks of the experiment. Different 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. The source data and two-factor analysis of variance (ANOVA) data are provided as a source data file in Supplementary Tab.

The constant high temper or algae treatment elicited no response in protein and growth rates (Figs. 2C and D). After 4 weeks of *A.hyacinthus* cocultured with *C.taxifolia* at 27°C, the protein content of tissue in LH and LM nubbins had a 37% reduction when compared with the LD nubbins (*p>0.05*). At 30°C, the increasing temper visiblely had a 55% inhibitory impact on the tissue protein in HD nubbins (*p>0.05*), but did not provoke distinct variations in both HH and HM nubbins.

As indicated by the change in buoyant weight, the growth rate of corals in LD treatment was highest at 27 °C, with a mean value of 1.1±0.6 mg cm^{-2} d^{-1}. And coculture with macroalgae (whether contacted or not) decreased the growth rate of coral by 31% (*p>0.05*). During upgraded temper pressure, there was a slightly inhibitory influence on the growth rate in HD corals (*P>0.05*). What's more, elevating temper resulted in LM corals coming into growing at the lowest rate, with a value of 0.5±0.1 mg cm^{-2} d^{-1}, but there was no difference among the treatments.

As shown in Figs. 2E and F, macroalgae treatments increased the antioxidant capacity of corals in all conditions. At a surrounding temperature (27°C), SOD active increased from 207.3±50.6 U $mgprot^{-1}$ in LH system to 371.5±125.8 U $mgprot^{-1}$ in LD system, while that ceaselessly promoted to 393.3±121.7 U $mgprot^{-1}$ in LM system（*p*>0.05）. Moreover, with the temperature climbing to 30°C, the mean of SOD in HD system increased 2.3-fold compared to LD system (*p=0.02*). While the level in LH and LM systems mildly rebounded 40%-48.1% compared to HD system（*p>0.05*）, indicating that both factors did interact (F=6.3, *p=0.02*).

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Simultaneously, at a surrounding temperature, active of CAT increased from 8.4±4.5 U mgprot^{-1} in LD system to 14.8±10 U mgprot^{-1} in LH system, while that continued to increase to 51.4±2.6 U mgprot^{-1} in LM system（$p< 0.01$）. Among the treatments at rising conditions, active of CAT in HH system attained the highest levels at 45.2±5.1 U mgprot^{-1} ($p< 0.05$) and LM system is comparable to LD system. However, the CAT responses of coral in thremal was dramatic, which increased 3.1-fold when HH system versus to LH system and in the HM system was 58.5% less than LM system($p< 0.05$). The combined effect of temperature and macroalgae affected the coral ($F= 43.5$, $p< 0.01$).

3.2. Results of transcriptome analysis of A.hyacinthus

After removing the sequencing adaptor and low-quality data, a total of 126 587 106 clean reads were obtained, and the Trinity mapping yielded 53 676 unigenes with an average assembly rate of 66.6%, of which 33 263 were longer than 1000 bp and 4 011 bp were of N50 length. SwissProt, COG, KOG, GO, KEGG, Pfam, eggNOG databases were used to annotate the above unigene and 14 559, 10 483, 18 073, 27 495, 22 743, 28 571, 22 532 unigene were annotated (Supplementary Tab). Vivdly depicted in Supplementary Fig. s1. was a noteworthy scenario in which distinguish from HD group the LD, HH, and HM treatments as converge to a single community as LH and LM groups.

As shown in Figs. 3A and B, there were 12 034 and 12 473 DEGs in LH and LM treatments compared with LD treatment, among which several vesicle transport (Rab family), autophagy (ATG 5 and ATG10), apoptosis (Casp7 and Casp8) related genes were identified. Making a distinction between LD and HD treatments had 1 601 DEGs, which associated with calcium balance (CalM and Galaxin), oxidative stress (CAT, SODC and HSP family) and immune response (CYP3A, TLR2, TRAF3, MYD88 and IFI47). Respectively, HH and HM treatments had 2 877 and 328 DEGs versus to HD treatment. DEGs in HH treatment were related to stress immunity (SODC, HSP70, CYP3A, IFI47 and TRAF5) and apoptosis (FADD, HIRA2, Casp3 and Casp7) and HM identified partial immune-related DEGs (TNFRSF14 and GPX). The qPCR results of 12 DEGs were consistent with the transcriptome analysis results, indicating the reliability of the transcriptome results of this experiment (Figs. 3C and D).

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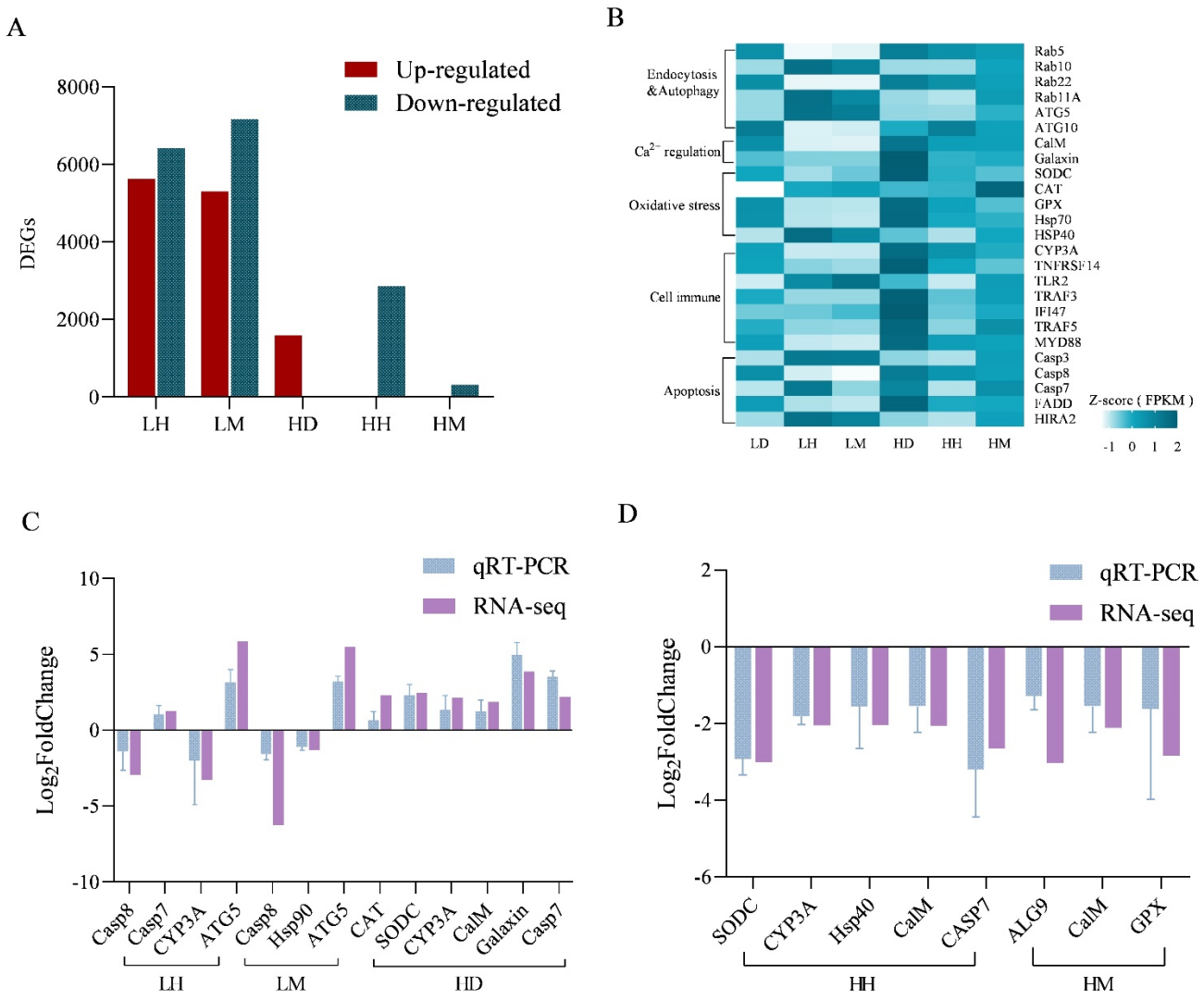

Fig. 3 Gene expression dynamics of *A. hyacinthusl* after 4 weeks of the experiment. (A) DEGs statistics in all treatments. Select FDR < 0.001 and shown as LH, LM and HD groups vs LD group, HH and HM groups vs HD group. **(B)** FPKM expression map of different biological processes regulated by DEGs **(C)** Compared with LD group, the DEGs verification of LH, LM and HD groups **(D)** Compared with HD group, the DEGs verification of HH and HM. The source data are provided as a source data file in Supplementary Tab.

DEGs were conspicuously enriched in 257, 273, 175, 193 and 64 KEGG pathways in LH, LM, HD, HH and HM groups. Among them, *C.taxifolia* mainly affected FoxO, mTOR signaling pathway, endocytosis, autophagy, apoptosis etc. in LH or LM (Figs. 4A and B). Thermal principally affected microbial immune response pathway of *A.hyacinthusl*, such as Cytosolic DNA-sensing pathway, Herpes simplex virus 1 infection, Toll-like receptor signaling pathway (Fig. 4C). At high temper, DEGs gathered in NOD-like receptor, TNF cell immune signal transduction and apoptosis pathway when *C.taxifolia* indirect contact with *A.hyacinthusl* (HH) (Fig. 4D), while DEGs gathered in the immune pathway like Cytokine-cytokine receptor interaction when *C.taxifolia* direct contact with *A.hyacinthusl* (Fig. 4D).

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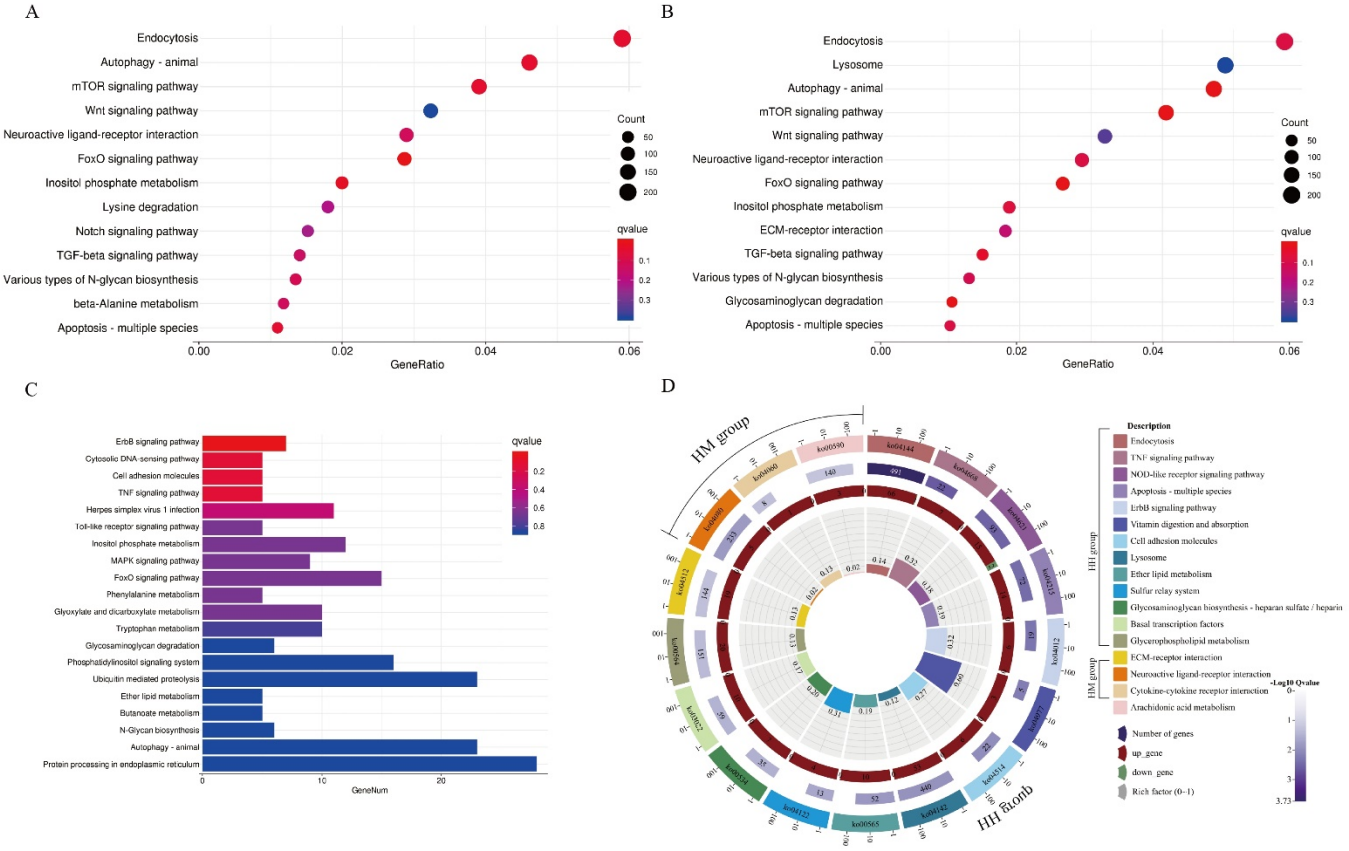

Fig.4 Molecular plasticity explains effects of macroalgae on corals. Distribution of gene ontologies which were signifificantly enriched in KEGG pathways of **(A)** LH and **(B)** LM **(C)** HD treatments. **(D)** HH and HM treatments KEGG enrichment pathway ($p < 0.05$). Color represent KEGG pathway description and genes description (up or down). Rich factor represents DEGs divided by total genes in this pathway. The source data are provided as a source data file in Supplementary Tab.

4. Disscussion

4.1. Effects of C.taxifolia on the physiology and ecology of A.hyacinthusl

On the physiological regulation hand, it was found that distincted from LD and LM, the indirect contact with *C.taxifolia* (HH) moderately decreased the density and chl *a* content of zooxanthellae in *A.hyacinthusl*, but did not cause coral bleaching. Factually, it was substaintially confirmed that the adverse of allelopathy impacts on zooxanthellae, which migth correlated with dissolved organic carbon (DOC) and terpenoids released by macroalgae [30,31]. The DOC could promote bacterial metabolism on coral surfaces and competition for oxygen [32] and terpenoids might also mediate changes in microbial community structure, both of which result in zooxanthellae injury [11,33].

Corals obtained planktonic zooxanthellae from water through endocytosis and removed senescent and damaged zooxanthellae through endocytosis, autophagy and apoptosis [34]. On the molecular regulation hand, we found that the DEGs that in HH group were extremely enriched in endocytosis, autophagy and apoptosis pathways. For example, the transcription factors PRKCI, Rab11A and Rab10 were up-regulated while Rab5 were down-regulated. In the first place, PKC family proteins might be introduced into vesicle tube clusters through straightforward interaction with the tiny GTPase Rab2 and participate in vesicle transport, thereby facilitating autophagy[35]. Secondly, it was wild acknowledgement that Rab GTP family proteins regulated vesicle trafficking and membrane fusion [36]. Chen et al. [37-39] indicated that Rab5 as an upstream gene regulated

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downstream Rab7 and 11. If Rab5 was absent, antagonism of Rab7 and 11 would be triggered and autophagy of zooxanthellae would be induced. Thus, the decrease of Rab5 expression might be connected to promoto the autophagy process accelerated by PRKCI. Furthermore, ATG family still up-expressed in autophagy. ATG5 factor was deeply involved in several cellular processes, including formation of autophagic vesicles, mitochondrial quality control after oxidative damage, and might promote apoptosis when it overexpressed [40]. Interestingly, the expression of pro-apoptosis genes for example Casp7 was rised as well. In a nutshell, all these processes were responsible for the negative growth of zooxanthellae density.

Additionally, distincting from HH treatment, it was obvious that Rab10 and PRKCI which vesicle transport and autophagy - correlated factors did not rise when *C.taxifolia* direct contact with coral (HM). In the same case, it was evident that FADD were downexpressed and Casp7 apoptosis effector protein was not overexpressed. In all, these processes confirmed that vesicle transport and apoptosis of HM were slower than those of HH indicating that *C.taxifolia* had a assisting effect on the density of symbiotic algae. It may related to the reason macroalgae were able to ensure the free flow keeping the exchange of abundant oxygen due to the coarsening stolon, and avoided the photorespiration process produced by commensal algae because of the foliaceous [41].

4.2. Effects of thermal stress on the physiology and ecology of A.hyacinthusl

In this study, on the physiological regulation side, heat triggered *A.hyacinthusl* in bleaching and SOD and CAT enzymes simultaneously lifted. On the molecular regulation side, as a key factor getting command of cell adhesion molecules pathway, the CalM be inspired by thermal could induce release of Ca^{2+} from the endoplasmic reticulum (ER) storage into the cytoplasmic matrix and activate biological processes such as oxidative stress and immune response [42]. In terms of oxidative stress, several factors involved in the scavenging of free radicals, anti-damage and immunity enhancement were identified, including CAT, SODC, Hsp family, etc.[43]. Commonly, superoxide anion radicals (ROS) would be scavenged by SOD and be converted to H_2O_2 during the oxidative metabolism. However, SOD enzymes be inhibited when an overwhelming majority of H_2O_2 accumulated, which required CAT to decompose H_2O_2 in order to prevent peroxidation. What's more, it was previously warning indicators of coral bleaching that heat shock proteins and molecular chaperones were function in a variety of cellular proceedings, such as protein folding, intracellular protein transported and resistance to protein denaturation [44]. Above all, the most curcial is reflected in the fact that the hermatypic corals prevented thermal stress by excitation of oxidative stress proteins.

At the same time, the immune signaling pathways hinted that microorganisms and viruses were provoked by thermal pressure. Sparkly, the function of Toll-like receptor signaling pathway could mediate antifungal infection semaphore transduction [45]. In this pathway, TLR2 and TRAF3 factors were upregulated. The TLR2 was involved in pathogen recognition and TRAF3 was associated with CD40 signal transduction, all of which had the function of activating immune response [46,47]. Furthermore, this study discovered that TRAF family regulated the downstream expression of IFI47 in TNF signaling pathway by binding to TNF receptor, and the protein encoded by IFI47 might triggere cell resistance to viral and microbial infections. Besides those, CYP450 superfamily were also upregulated among the immune genes, which could catalyze the oxidation of various organic substances, efflux transporters and heavy metal membrane pump exporters, and played a dominant role in detoxification [48]. Meanwhile, the CYP450 family was adjusted by microorganisms as well. For example, *Clostridium butyricum* revised the expression of CYP3A4 in host cell via regulating transcription factors of their own [49]. Hence, the factors Apaf, FADD, Casp3 and Casp7 gained an increasingly upward momentum and ultimately promoted apoptosis, because of an adequate ROS elimination or immunological response to pathogenic bacteria at rising temperature.

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4.3. Combined effects of C.taxifolia and thermal stress

Physiologically, it was known from this sutdy that the bleaching rate due to excessive temperature was approximately mitigated or even eliminated when *A.hyacinthusl* interacted with *C.taxifolia*. Besides, different from HD treatments, SOD enzymes in HH group remained a shrinking tendency while CAT enzymes was on the rise. Between HM and HD, SOD and CAT enzymes were on the diminish simultaneously, which might be touch on the damage of coral tissue owing to the physical friction of *C. taxifolia*. It has been thought-provoking reported that the fade in antioxidant enzyme at warming conditions might relevant to DMSP (dimethyl thipropionate) released by zooxanthellae and macroalgae. Raina J B et al. and Hopkins F E et al. [51] have asserted that DMSP could be obtained from the outside or synthesized by *Acropora*, which were translated into DMS (dimethyl sulfur) in the process of scavenging ROS and digestion of oxidative stress [52]. Therefore, DMSP secreted by algae might be one of the reasons for which *A.hyacinthusl* was able to tolerate heat stress.

Molecularlly, in contrast to the HD treatment, DEGs in HH group were predominantly enriched in the NOD-like receptor signaling pathway, which was associated with pathogen recognition and immune response [53]. Immediately posterior, the TRAF family and MYD88 genes in this pathway through falling down expressing regulated several pathways including the Toll-like receptor signaling pathway and TNF signaling pathway, which jointly attenuated the immune stress produced by microorganisms. Yet, apoptosis pathway also getted remission with HIRA2, Casp3, Casp7 factors markedly on the decline. What's more, HM treatment principally acted on Cytokine-cytokine receptor interaction and glutathione metabolism distincted from HD group, which was helpful for promoting healthy immune activity. In conclusion, notwithstanding the ability to alleviate thermal effect in HH seemed to be better than HM, all of these transcription factors conjointly confirmed that *C.taxifolia* assisted *A.hyacinthus* to avoid the invasion of pathogenic microorganisms caused by thermal stress, and to decelerate the processes of oxidative stress and apoptosis.

5. Conclusions

This study explored the crucial issue of how physiology and molecular response of the hermatypic coral *A.hyacinthusl* was affected by foliaceous macoalgae *C. taxifolia* at various tempers. Therefore, the different ways including indirect and direct cocultured with seaweed have been set up at ambient temperatures (27 °C) and an increase of +3 °C. For one thing, the results demonstrated that macroalgae could prominently trigger a drop in the density of zooxanthellae at various temperatures, which has been sincerely associated with the biological processes of vesicle transport, autophagy, and apoptosis regulated by the Rab5, ATG5, and Casp7 transcription factors. For another thing, oxidative stress (CatE, SODC, HPS family) and microbial immune response (IFI47, TRAF family) etc. biological processes were violently aggravated by heat stress resulting in cell apoptosis, at which point *C. taxifolia* alleviated the pressure influences.

 doi:10.20944/preprints202211.0223.v1

CRediT authorship contribution statement

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. Yan Ping Zhang: Investigation, Conceptualization, Methodology, Funding acquisition. Li Liu: Resources, Funding acquisition, Project administration, Supervision, Writing - review & editing.

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.

Ethical approval

The animal use protocol listed below has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC), Fisheries College, Guangdong Ocean University. No.GDOU-IACUC-2021-A1220.

Consent for publication

There is no conflict of interest to report.

Acknowledgments

This work was financially supported by the GuangDong Basic and Applied Basic Research Foundation [Grant No. 2019A1515110225], Guangdong Innovation and Strengthening School Project [Grant No. 230419080], the Program for Scientific Research Start-up Funds of Guangdong Ocean University [Grant No. R18024], and Zhanjiang Science and Technology Planning Project [Grant No. 2019B01005; 2021E05020] which we gratefully acknowledge. We would like to thank Xuwen Coral Reef National Nature Reserve for assistance with fieldwork, as well as anonymous reviewers for providing insightful comments on this manuscript.

Appendix A

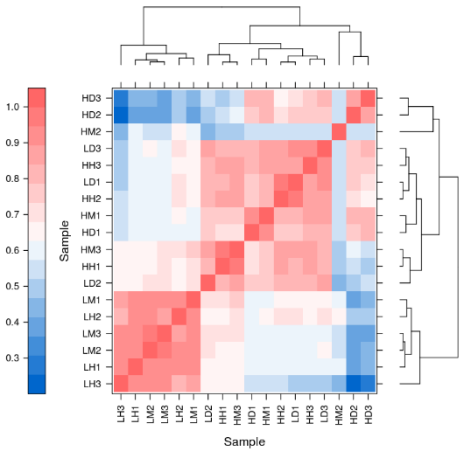

Fig. S1. Correlation analysis chart of *A. hyacinthus*. Based on gene expression of each group of samples.Color represents the correlation between different samples. Line represents the functional relationships between different samples.

 doi:10.20944/preprints202211.0223.v1

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