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Microplastic exposure represses the growth of endosymbiotic dinoflagellate Cladocopium goreaui in culture through affecting its apoptosis and metabolism

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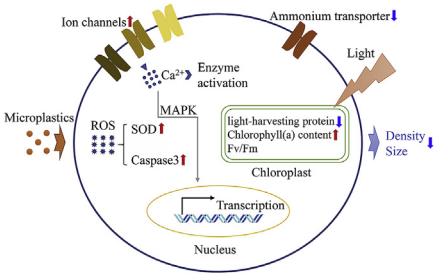
HIGHLIGHTS

- The growth and density of algal cells were repressed by microplastic exposure.
- Microplastics declined detoxification activity, nutrient uptake, and photosynthesis.
- Microplastics raised oxidative stress, apoptosis level and ion transport.

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastics are widespread emerging marine pollutants that have been found in the coral reef ecosystem. In the present study, using *Cladocopium goreaui* as a symbiont representative, we investigated cytological, physiological, and molecular responses of a Symbiodiniaceae species to weeklong microplastic exposure (Polystyrene, diameter 1.0 μm, 9.0×10^9 particles L^{-1}). The density and size of algal cells decreased significantly at 7 d and 6–7 d of microplastic exposure, respectively. Chlorophyll *a* content increased significantly at 7 d of exposure, whereas Fv/Fm did not change significantly during the entire exposure period. We observed significant increases in superoxide dismutase activity and caspase3 activation level, significant decrease in glutathione S-transferase activity, but no change in catalase activity during the whole exposure period. Transcriptomic analysis revealed 191 significantly upregulated and 71 significantly downregulated genes at 7 d after microplastic exposure. Fifteen GO terms were overrepresented for these significantly upregulated genes, which were grouped into four categories including transmembrane ion transport, substrate-specific transmembrane transporter activity, calcium ion binding, and calcium-dependent cysteine-type endopeptidase activity. Thirteen of the significantly upregulated genes encode metal ion transporter and ammonium transporter, and five light-harvesting protein genes were among the significantly downregulated genes. These results demonstrate that microplastics can act as an exogenous stressor, suppress detoxification activity, nutrient uptake, and photosynthesis, elevate oxidative stress, and raise the apoptosis level through upregulating ion transport

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and apoptotic enzymes to repress the growth of *C. goreaui*. These effects have implications in negative impacts of microplastics on coral-Symbiodiniaceae symbiosis that involves *C. goreaui*.

1. Introduction

Microplastics are defined as plastic particles or fragments smaller than 5 mm, and have been considered a potential threat to marine ecosystems globally (Galloway et al., 2017). Microplastic debris has also been observed in the seawater, sediment, and organisms in many marine ecosystems, including coral reef and deep-sea ecosystems (Woodall et al., 2014; Sharma and Chatterjee, 2017). Microplastic are ingested at multiple trophic levels to enter into the marine food web (Setala et al., 2014; Sun et al., 2017; Rotjan et al., 2019), and may be bioaccumulated. In organisms microplastics can cause adverse effects through the blockage of alimentary tract, owing to the inability of the organisms to degrade them or the toxicity of mixed additives and adsorbed marine pollutants on them. Different types of additives such as bisphenol A, phthalates and flame retardants can be mixed into plastic monomers during the manufacturing process, while microplastics with large surface area-to-volume ratio can easily adsorb marine pollutants such as trace metal and persistent organic pollutants (Ivar do Sul and Costa, 2014; Tsang et al., 2017; Guo and Wang, 2019). Adsorbed additives and pollutants have severe toxic effects on the consumer organisms, and can also function as environmental hormones to negatively modulate physiological activities of marine organisms (Panti et al., 2015; Jeong et al., 2017).

Marine phytoplankton and protists are among the victims of microplastic pollution in the ocean. It has been reported that microplastics can be hetero-aggregated with diatom *Chaetoceros neogracile* and single-cell green alga *Chlamydomonas reinhardtii* (Lagarde et al., 2016; Long et al., 2017), adhered to *Skeletonema marinoi* and dinoflagellate *Lingulodinium polyedrum* (Casabianca et al., 2019), and ingested by the heterotrophic dinoflagellate *Oxyrrhis marina* (Cole et al., 2013). These microplastics have different effects on the growth of different phytoplankton species. For example, exposure to microplastics seems to enhance growth of the microalga *Raphidocelis subcapitata* (Canniff and Hoang, 2018) yet depress that of *C. reinhardtii* (Lagarde et al., 2016). In addition, it has been suggested that microplastics has a negative effect on the photosynthesis activity of phytoplankton through reducing chlorophyll content and photochemical efficiency, yet it increases the expression level of genes involved in the sugar biosynthesis pathways during a long-term exposure (Zhang et al., 2017; Mao et al., 2018; Prata et al., 2019; Wu et al., 2019). Overall, the mechanism underlying the contradicting effects of microplastics on phytoplankton is not well understood.

Dinoflagellates of the family Symbiodiniaceae are the most prevalent photosynthetic symbionts in tropical and subtropical coral reef ecosystems. The mutualisms of Symbiodiniaceae with scleractinian corals are fundamental to the existence of coral reef ecosystems worldwide, because they supply their coral hosts with photosynthates that can meet up to 95% of the corals' energy requirements (Houlbreque and Ferrier-Pages, 2009; Gonzalez-Pech et al., 2019). Symbiodiniaceae has evolved into diverse lineages of symbionts of corals and other invertebrates, including genera of *Symbiodinium* (Clade A), *Breviolum* (Clade B), *Cladocopium* (Clade C), *Durusdinium* (Clade D) and *Fugacium* (Clade F) (LaJeunesse et al., 2018). In the coral-Symbiodiniaceae relationship, Symbiodiniaceae cells are recognized by scleractinian corals presumably through the interaction between coral lectin and algal glycan, and the flagellated motile form of Symbiodiniaceae cells will be transformed into the nonmotile coccoid form (equivalent to the symbiotic stage) (Jimbo et al., 2010). The community composition and density of symbiotic Symbiodiniaceae in scleractinian corals can change dynamically in response to changes in the internal and external environment (Cooper et al., 2011).

In this study, we used *Cladocopium goreaui* as a representative species to gain understanding on microplastic effects on Symbiodiniaceae and underlying mechanisms. *C. goreaui* belongs to clade C, the most abundant clade of Symbiodiniaceae in the coral reef ecosystem in the Pacific. To understand the potential effects of microplastic exposure on survival and reproduction of this species, growth and photosynthesis parameters, crucial enzyme activities, and transcriptomic profiles of *C. goreaui* were investigated after an exposure to microplastics. Our results provide insights for further understanding the response mechanisms of Symbiodiniaceae to microplastic pollution and its potential influence on scleractinian coral hosts.

2. Materials and methods

2.1. Algal culture and microplastic exposure

C. goreaui strain CCMP 2466 originally isolated from the anemone *Discosoma sancti-thomae* was provided by the National Center for Marine Algae and Microbiota. Cells were cultured in sterilized oceanic seawater, which was filtered through 0.22 μm membranes, enriched with the full nutrient regime of the L1 medium. The cultures were incubated at 26 °C and under a 14:10 h light:night cycle, with a photons of 110 μE m^{-2} s^{-1}.

Polystyrene microplastics (diameter 1.0 μm, density 1.05 g cm^{-3}, Saierqun, China) with smooth surface were used in the microplastic exposure experiment. Algal cells were cultured until its density exceeded 1.0×10^5 cells mL^{-1}, and the culture was divided equally into ten culture bottles, each one containing 250 mL of L1 medium. Microplastics were added into five cultures at the final concentration of 5.0 mg L^{-1} (9.0×10^9 particles L^{-1}), while the other five cultures received no microplastic additionserve as the control group. Twenty-seven milliliters of algal cells were sampled daily from each culture to determine cell density, cell size, chlorophyll content, photochemical efficiency, and major enzyme activities. When the cell density in the microplastic group changed significantly (cultured for a week), 50 mL of algal cells from each culture were harvested by centrifugation at 5000 *g* 4 °C for 10 min and resuspended in 1 mL TRI-Reagent (Molecular Research Center, Inc., Cincinnati, OH, USA) and stored at −80 °C for subsequent RNA extraction.

2.2. Measurement of cell density and size

One mL samples were fixed in Lugol's solution, and cell density was determined by directed counting three times using a Sedgwick-Rafter counting chamber under the microscope, and the growth curves were plotted (Lin et al., 2012). The average cell size was measured as equivalent spherical diameter using Z2-Coulter Particle Counter (Beckman Coulter, USA). The mean cell diameter

was calculated with a particle count and size analyzer ranging from 5 μm to 15 μm (Li et al., 2016).

2.3. Determination of chlorophyll content and photochemical efficiency

Five mL samples were filtered onto a 25 mm GF/F glass microfiber filter (Whatman, USA) to obtain the cells. The algae-containing filters were each immersed into 90% acetone separately and kept in the dark for 48 h at 4 °C to extract chlorophyll *a*, which was determined using Turner Trilogy (Turner Designs fluorometer, USA) following the non-acidification method (EJ and GB, 1997) and averaged to content per cell. Photochemical efficiency was measured using Xe-PAM (Walz, Germany) after 30 min of incubation in the dark.

2.4. Activity assay of antioxidases and detoxification enzyme

Cells were harvested from a 10 mL sample from each culture using centrifugation at 5000 *g* 4 °C for 10 min. Cell pellets were resuspended in PBS (phosphate buffered saline, PH 7.4) and homogenized using 0.5 mm diameter ceramic beads and a bead-beater (FastPrep® −24 Sample Preparation System, MP Biomedicals, USA). The homogenate was centrifuged at 12000 *g* 4 °C for 10 min to obtain the supernatant (crude protein extract). The activities of superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST) in the supernatants were measured using commercial kits (A001, A007 and A004, JIANCHENG, China), following the manufacturer's recommendations. Then, the concentration of total protein in the supernatant was quantified with BCA Protein Assay kit (Sangon Biotech, China), and used to normalize the measured activities of SOD, CAT and GST to U mg^{-1} protein.

2.5. Activity assay of caspase3

Caspase3 activity in the protein extract was measured using Caspase-3 Colorimetric Assay Kit (KeyGEN BioTECH) according to the manufacturer's protocol. Briefly, 50 μL reaction buffer and 5 μL substrate were added to 50 μL protein extract, which was then incubated for 4 h in the dark at 37 °C. Next, the activity of caspase3 was measured spectrophotometrically at 405 nm (A_{405}), and the activation level of caspase3 in algal cells was defined as the ratio of A_{405} in samples to that of the control group at 1 d.

2.6. RNA extraction and transcriptome sequencing

RNA extraction was carried out using TRI-Reagent combined with the Qiagen RNeasy Mini kit (Qiagen) following a previously reported protocol (Lin et al., 2010). The RNA concentrations of samples were measured using NanoDrop ND-2000 spectrophotometer (Thermo Scientific), and the RNA quality was evaluated using the RNA 6000 Nano LabChip Kit (Agilent 2100 Bioanalyzer, Agilent Technologies, Australia). The extracted RNA from the microplastics-treated and the control, 3 replicates in each, was used for RNA-seq (transcriptome sequencing). Six paired-end fragment libraries (2 × 100 bp) were constructed and sequenced on the BGISEQ-500 platform (BGI, Shenzhen, China). The generated raw sequencing reads were deposited at the NCBI (National Center for Biotechnology Information) Short Read Archive under BioProject **No. PRJNA510646**.

2.7. Reads mapping and identification of differentially expressed genes (DEGs)

The genome sequences and annotation of *C. goreaui* (http://symbs.reefgenomics.org/) (Liu et al., 2018) were used as reference. The alignment of the paired-end reads to the reference was performed using the HISAT2 software (Pertea et al., 2016). StringTie and DESeq2 software were used to estimate transcript abundances and identify DEGs between the microplastic and control groups (Love et al., 2014). FDR <0.05 was used to call statistically significant DEGs.

2.8. Functional annotation of differentially expressed genes

After the identification of significantly upregulated and downregulated DEGs, their protein sequences were retrieved from the reference database mentioned above to align to Non-redundant protein database (Nr) using the online BLASTP program (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Furthermore, the domains of these proteins were further predicted through Simple Modular Architecture Research Toll 8.0 (SMART, http://smart.embl-heidelberg.de/). The functions of the DEGs were annotated based on the functions of the best-hit homologous proteins and domains.

2.9. GO overrepresentation of differentially expressed genes

For GO analysis, sequences of all 35,913 Symbiodiniaceae proteins were aligned using local BLASTP search to the Uniport-sprot database with evalue <0.001, and analyzed using InterProScan software to predict their domains. The obtained results were parsed by Blast2GO software (https://www.blast2go.com/) for assigning GO terms. GO overrepresentation analysis was implemented via the hypergeometric test with filter value of 0.05. The significantly upregulated or downregulated DEGs were selected as test set, while all genes were used as the reference set. The BiNGO tool was employed to calculate the overrepresented GO terms and display the network of significant GO terms (Maere et al., 2005).

2.10. Statistical analysis

All data were presented as means ± standard deviation (SD) obtained from all replicates for each experimental condition. All of the data were subjected to two-way analysis of variance (two-way ANOVA) followed by multiple comparisons (S-N-K and Duncan) using SPSS v22.0 to determine significant differences between the microplastic treatment group and the control group. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Algal cell density and cell size after microplastic exposure

As shown in Fig. 1, cell density increased slowly in both the microplastic and control groups. No significant difference was observed between these two groups during 1–6 d after microplastic exposure, but on the 7th d, the cell density in the microplastic group ($2.37 \pm 0.10 \times 10^5$ cells mL^{-1}, $p < 0.05$) was significantly lower than that in the control group ($2.83 \pm 0.16 \times 10^5$ cells mL^{-1}). Similarly, cell size in the microplastic group was significantly smaller than that in the control group during 6–7 d after exposure, reaching the lowest level at 7 d (6.43 ± 0.18 μm) (Fig. 1B).

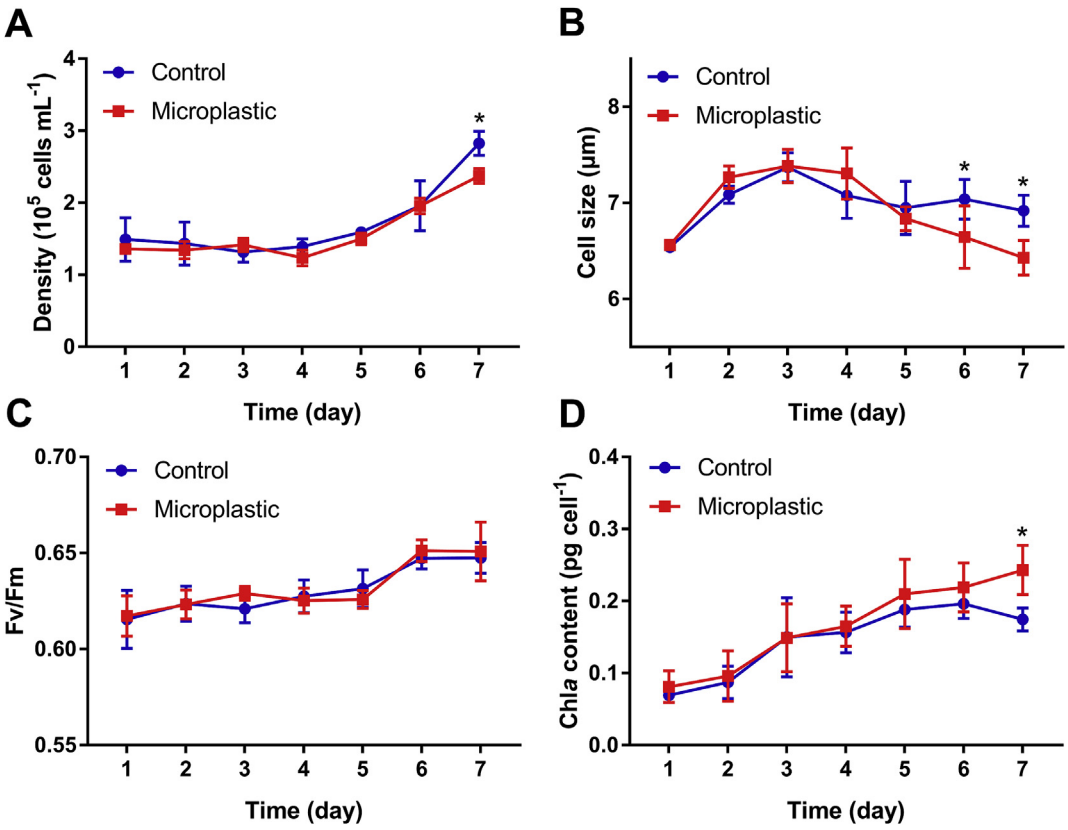

Fig. 1. Variations of cell density (A), cell size (B), photochemical efficiency Fv/Fm (C), and chlorophyll *a* content (D) in cultured *Cladocopium goreaui* after microplastic exposure. Data points represent means and error bars represent standard deviations (N = 5). Asterisks depict significant differences between the microplastic and control groups ($p < 0.05$).

3.2. Photochemical efficiency and chlorophyll a content after microplastic exposure

There was no significant difference in the photochemical efficiency Fv/Fm between the microplastic and the control groups for the entire duration of the experiment (Fig. 1C). In contrast, chlorophyll *a* content increased in the microplastic exposure group, and became significantly higher at 7 d of the exposure (0.17 ± 0.02 pg cell^{-1}, $p < 0.05$) than that in the control group (Fig. 1D).

3.3. Activity alterations of SOD, CAT, and GST after microplastic exposure

SOD, CAT, and GST activities in the algal cells were determined after microplastic exposure. The SOD activity began to increase significantly at 1 d of exposure (97.12 ± 13.01 U mg^{-1}, $p < 0.05$), in comparison to the control group. After reaching the peak at 2 d exposure (114.99 ± 19.37 U mg^{-1}, $p < 0.05$), the SOD activity in the microplastic group returned to the control level (Fig. 2A). No significant difference was observed in CAT activity between the microplastic and control groups during the whole period of microplastic exposure (Fig. 2B). For the GST activity, there was no significant difference throughout the experiment except at 4 d exposure, when the activity (1024.62 ± 260.71 U mg^{-1}) in the microplastic group was lower than that in the control group ($p < 0.05$) (Fig. 2C).

3.4. Activation level change of caspase3 after microplastic exposure

The activation level of caspase3 was measured in the algal cells after the exposure to microplastics. Its activation level in the microplastic group increased significantly during 2–3 d exposure, compared to those in the control group. Furthermore, the highest activation level was observed at 3 d of the exposure (1.71 ± 0.29-fold, $p < 0.05$), and then it decreased to the control level (Fig. 2D).

3.5. Gene expression of C. goreaui after microplastic exposure

A total of six paired-end (2 × 100 bp) transcriptome libraries were constructed, including three libraries in the control group and three libraries in the microplastic exposure group, and sequenced to saturated level to compare the transcript abundances and characterize transcriptomic response to microplastic exposure. A total of 210,756,476 high-quality reads were obtained after quality control, comprising 33,886,745, 32,531,926 and 35,860,663 reads from the three control libraries, and 35,342,075, 34,305,438 and 38,829,629 reads from the three microplastic libraries, respectively (Table 1).

The high-quality clean paired-end reads obtained from all six transcriptome libraries were mapped to the reference genome of *C. goreaui* using HISAT2 software, and the mapping rates and detected genes were very consistent among the six libraries, ranging from 87.08% to 87.69% and from 22,332 to 22,449, respectively (Table 1). StringTie software was employed to analyze all gene expression levels and export the counts of mapped reads for each gene to be fed into the DESeq2 software to identify DEGs (Supplementary Table S1). After library calibration, the expression levels of all algal genes were compared between the microplastic and control groups. A total 262 DEGs were obtained, which accounted for 0.73% of total number of the algal genes, comprising 191 significantly upregulated and 71 significantly downregulated DEGs (Supplementary Table S2).

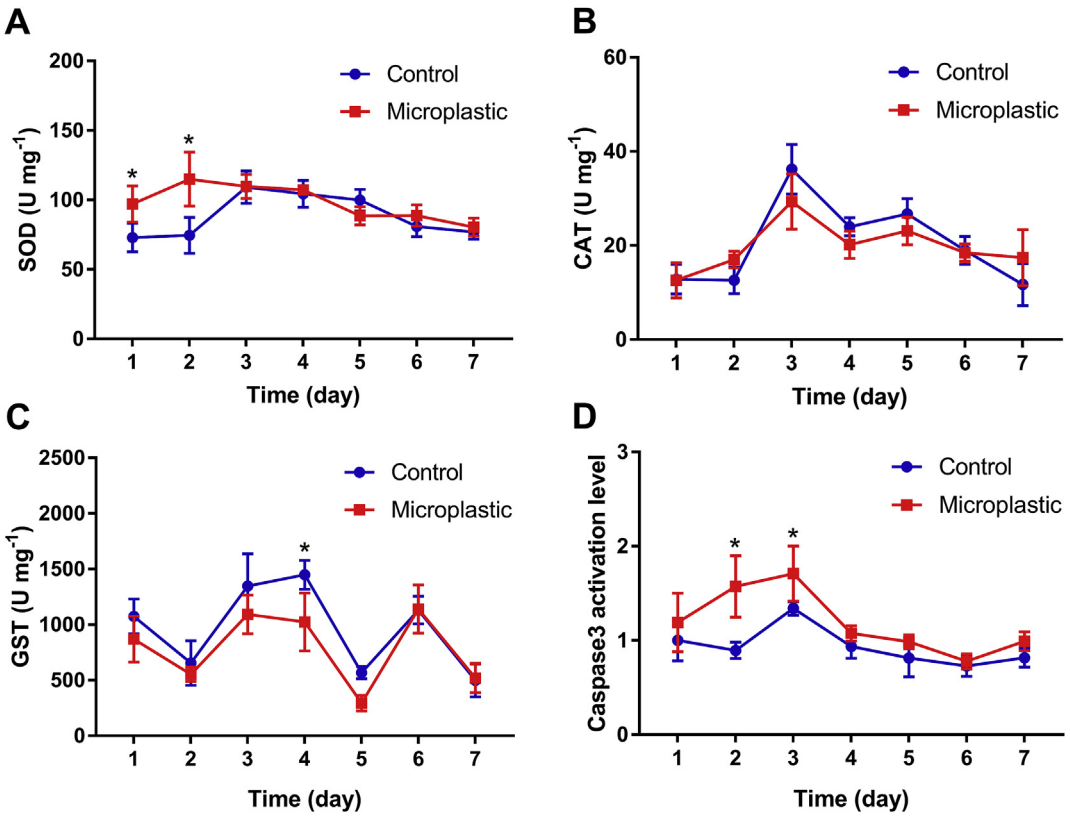

Fig. 2. Temporal patterns of SOD, CAT, GST activities, and caspase3 activation level in cultured *Cladocopium goreaui* after microplastic exposure. (A) Superoxide dismutase (SOD). (B) Catalase (CAT). (C) Glutathione S-transferase (GST). (D) Caspase3 activation level. Data points represent means and error bars represent standard deviations (N = 5). Asterisks depict significant differences between the microplastic and control groups ($p < 0.05$).

Table 1
Transcriptome mapping statistics.

Library	Total reads	Mapped rate	Detected genes
Control_1	33,886,745	87.23%	22,332
Control_2	32,531,926	87.08%	22,362
Control_3	35,860,663	87.48%	22,338
Microplastic_1	35,342,075	87.58%	22,338
Microplastic_2	34,305,438	87.31%	22,365
Microplastic_3	38,829,629	87.69%	22,449

Table 2
Domain statistics (three or more) of proteins encoded by significantly upregulated genes of *Cladocopium goreaui* after microplastic exposure.

ID	Domain Name	Domain Number	Gene Number
1	ANK	28	6
2	Efh	23	9
3	WD40	19	3
4	Ion_trans	14	13
5	CysPc	4	4
6	EZ_HEAT	3	1

3.6. Functional annotation of DEGs after microplastic exposure

For the 191 significantly upregulated and 71 significantly downregulated DEGs identified from 7 d after exposure, functional annotation was carried out through homologous searching and domain prediction. Furthermore, GO overrepresentation analysis was conducted at multiple GO levels in the whole categories. The amino acid sequences encoded by the significantly upregulated DEGs were aligned to the Nr and SMART database, and the information of the returned homologous molecules and domains were retrieved (Supplementary Table S3). Predicted domains that appeared more than three times included ANK (28 times), Efh (23), WD40 (19), Ion_trans (14), CysPc (4), and EZ_HEAT (3), which were contained in 6, 9, 3, 13, 4, and 1 genes, respectively (Table 2). Furthermore, a total of 15 major GO terms were overrepresented for the significantly upregulated DEGs (Fig. 3A, Supplementary Table S4). According to the dependency relationship, these GO terms were classified into four groups: transmembrane ion transport, substrate-specific transmembrane transporter activity, calcium-dependent cysteine-type endopeptidase activity, and calcium ion binding (Fig. 3B).

The homologous molecules and protein domains of 71 significantly downregulated DEGs are shown in Table S4. Eight domains appeared three times or more, including LRR (22 times, in 2 genes), Chloroa_b-bind (9 times, 5 genes), WD40 (8 times, 1 gene), HMG (6 times, 1 gene), VCBS (4 times, 2 genes), Pumilio (4 times, 1 gene), ANK (3 times, 1 gene) and Ammonium_transp (3 times, 1 gene) (Table 3). These significantly downregulated DEGs did not particularly enrich any GO terms.

4. Discussion

Microplastic pollution poses a serious threat to marine ecosystems such as coral reef (Avio et al., 2017), and has toxic effects on at least some marine microalgae (Zhang et al., 2017). Little information is available about effects and their molecular underpinnings of microplastic exposure in Symbiodiniaceae, the endosymbionts of scleractinian corals. In the present study, we found that microplastic exposure caused significant increase in SOD activity and

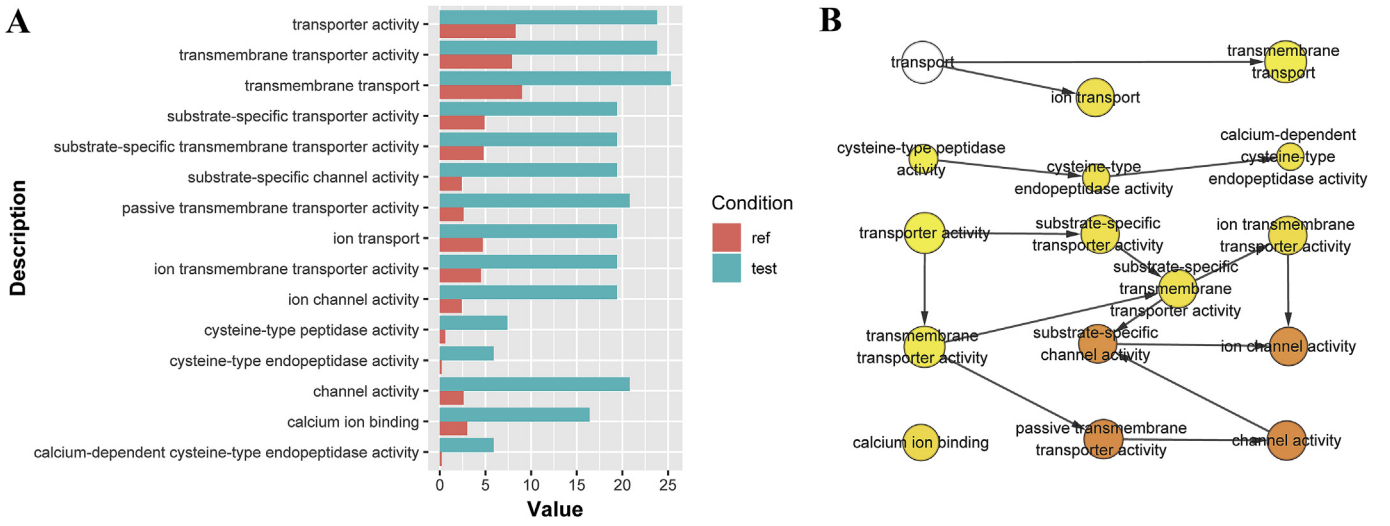

Fig. 3. Overrepresented GO terms of the significantly upregulated DEGs of cultured *Cladocopium goreaui* in the microplastic group at 7 d after microplastic exposure. (A) Fifteen GO terms were found overrepresented for these significantly upregulated DEGs. "Ref" refers to the proportion of genes functionally assigned a GO term out of all genes existing in the reference genome, while "test" refers to the proportion of genes assigned to that GO term out of the significantly upregulated DGEs. (B) grouping of fifteen overrepresented GO terms after microplastic exposure.

Table 3
Domain statistics (three or more) of proteins encoded by significantly down-regulated genes of *Cladocopium goreaui* after microplastic exposure.

ID	Domain Name	Domain Number	Gene Number
1	LRR	22	2
2	Chloroa_b-bind	9	5
3	WD40	8	1
4	HMG	6	1
5	VCBS	4	2
6	Pumilio	4	1
7	ANK	3	1
8	Ammonium_transp	3	1

caspase3 activation level in the coral endosymbiont *C. goreaui*, and significant decrease in GST activity at the early period of exposure. At extended exposure, *C. goreaui* cell concentration and size both exhibited a significant decrease, whereas the content of chlorophyll *a* increased significantly. Transcriptomic analysis further revealed the regulation of ion transport, calcium-dependent cysteine-type endopeptidase activity and light-harvesting protein, suggesting elevated apoptosis and depressed photosynthesis.

4.1. The suppression of algal growth by microplastic exposure

In order to understand the response of Symbiodiniaceae to microplastic exposure, growth and cell size of *C. goreaui* was determined under microplastic and control conditions. Both cell yield and cell size in the microplastic group significantly decreased relative to the control group at 7 d and during 6–7 d of microplastic exposure, respectively. This demonstrates that the microplastic exposure could suppress the growth and cell size of cultured (aposymbiotic) *C. goreaui*. Similar negative effects have previously been observed in the microalgae *C. reinhardtii* and *Skeletonema costatum* after microplastic exposure, and the negative effects were mainly attributed to the hetero-aggregation between microplastics and algal cells (Lagarde et al., 2016; Zhang et al., 2017). This might not apply to our study, however, because self-aggregation of *C. goreaui* also occurred in the control culture. The suppressing effect on cell yield and cell size of cultured *C. goreaui* might have derived from ingestion of microplastics or effects of toxic substances released by microplastics. It has been reported that the dinoflagellate *O. marina* could ingest microplastics during 1 h of exposure (Cole et al., 2013). The decrease in cell size and yield might have resulted from the apoptosis promoting or proliferation and nutrient uptake inhibiting effects of microplastics. This is consistent with the results of our transcriptomic analysis, which are elaborated below.

4.2. The induction of apoptosis and impacts on photosynthesis and ion transport in C. goreaui by microplastic exposure

We monitored the activities of several enzymes in cultured *C. goreaui* to characterize physiological responses of *C. goreaui* to microplastic exposure. Caspase3 is a key enzyme associated with apoptosis, and increase in its activation level observed in cultured *C. goreaui* during 2–3 d after microplastic exposure indicated that microplastic exposure induced the apoptosis level of algal cells. Meanwhile, we observed a rise of SOD activity during 1–2 d and a decline of GST activity at 4 d after the exposure, suggesting the induction of ROS in cultured *C. goreaui* by microplastic exposure in the early stage. Both SOD and CAT are main functional antioxidants in dinoflagellates (Leitao et al., 2003). The increase of SOD activity demonstrates that microplastics activates the stress response of the algal cells. GST is a crucial phase II metabolic enzymes in the detoxification process of all eukaryotes (Nicosia et al., 2014), and the decrease of GST activity indicates that detoxification response can be suppressed by microplastics, which together with the activation of stress response can bring about increasing apoptosis. These results suggest in concert that microplastics as an exogenous stressor could induce oxidative stress and repress the detoxification response, possibly raising the apoptosis level of Symbiodiniaceae and leading to the decline of their growth.

Meanwhile, several physiological parameters related to the assimilation metabolism were measured to further understand the repression of Symbiodiniaceae growth after microplastic exposure. With respect to photosynthesis, the content of chlorophyll *a* increased significantly at 7 d of microplastic exposure, whereas the photochemical efficiency showed no significant change during the entire exposure period. The results seem to be inconsistent with the growth depressing and stress inducing effects discussed above and

with other reports in *Chlorella pyrenoidosa* and *S. costatum* that microplastics could reduce algal photosynthesis through decreasing chlorophyll content and photochemical efficiency (Zhang et al., 2017; Mao et al., 2018). However, five light-harvesting protein genes were observed to be among the 71 significantly downregulated DEGs in the present study, corresponding to 9 Chloroa_b-bind domains. These results together suggest that microplastics can depress photosynthesis of cultured *C. goreaui* through decreasing the formation of light-harvesting complex without decreasing chlorophyll content and photochemical efficiency. The increase of chlorophyll *a* might have been a response to the shading of microplastics, because increased chlorophyll content is a common response in algae to reduction of light availability (Falkowski and Owens, 1980). The inhibited photosynthesis would cause the reduction of photosynthate and the lack of energy in *C. goreaui* after microplastic exposure. In addition, we also documented the decreased expression of ammonium transporter gene, indicative of decreased ability of cultured *C. goreaui* to take up ammonium as a result of microplastic exposure. Ammonium is the main nitrogen nutrient for symbiotic Symbiodiniaceae (Pernice et al., 2012; Tanaka et al., 2018), but the L1 medium used in this study contains nitrate instead of ammonium as the source of nitrogen nutrient. The decline in the expression of ammonium transporter is likely to signal a lowered requirement of nitrogen as a result of decreased photosynthesis. Therefore, microplastic exposure seemed to exert inhibitory effects on assimilation metabolism of carbon dioxide and inorganic nitrogen, which could result in the lack of organic carbon and nitrogen nutrients in algal cells. This might have contributed to the induction of apoptosis and the repression of growth.

The transcriptomes of cultured *C. goreaui* at 7 d of microplastic exposure further reveals the molecular response mechanism of Symbiodiniaceae to microplastic. The significantly upregulated DEGs under microplastic exposure were mainly related to ion transport and utilization. Among all 191 significantly upregulated DEGs, 13 genes encode ion channel proteins for potassium, sodium and calcium. This suggests that microplastic exposure activates these ion channels. To our knowledge, no similar results have been reported in other organisms. The transmembrane ion flux has been reported to be associated with the change of cell state and cell cycle in dinoflagellates (Gordeeva et al., 2004; Yeung et al., 2006; Smith et al., 2011), and therefore the increased expression of these ion channel genes might be evidence of heightened cellular response to microplastic exposure. Furthermore, the calcium flux might have induced the transcriptome alteration in *C. goreaui* after exposing to microplastics, such as the decreased expression of light-harvesting proteins, because calcium channels are implicated in calcium-based signaling in photosynthetic eukaryotes (Verret et al., 2010). Calcium ion is both essential secondary messenger and the substrate of calcification, and its influx into the symbiotic *C. goreaui* in coral association might further affect the form of host carbonate exoskeleton. In sum, our results presented in this paper suggest that microplastics as an exogenous stressor could repress detoxification activity and assimilatory metabolism, trigger ion transport and raise apoptosis level resulting in the decline of growth of the endosymbiotic dinoflagellate *C. goreaui*. Previous studies have documented contradicting effects of microplastics on phytoplankton, which could have resulted from the disaccordance of tested physiological variables and the lack of molecular analysis. In this study, concerted effects of microplastic exposure on stress response, detoxification, apoptosis and ion transport were observed through biochemical and molecular methods, underscoring the value of the combined methods in studying Symbiodiniaceae response to environmental stress.

Declaration of interest statement

This manuscript is original and it has not been previously published or submitted in part or whole. All authors have agreed to be listed and have seen the manuscript, and approved the submission to *Chemosphere*. This manuscript has no conflicts of interest. Our study does not involve human subjects.

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Appendix A. Supplementary data

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