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RESEARCH

The Dynamics of Symbiodiniaceae and Photosynthetic Bacteria Under High-Temperature Conditions

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Abstract

Coral thermal tolerance is intimately linked to their symbiotic relationships with photosynthetic microorganisms. However, the potential compensatory role of symbiotic photosynthetic bacteria in supporting Symbiodiniaceae photosynthesis under extreme summer temperatures remains largely unexplored. Here, we examined the seasonal variations in Symbiodiniaceae and photosynthetic bacterial community structures in *Pavona decussata* corals from Weizhou Island, Beibu Gulf, China, with particular emphasis on the role of photosynthetic bacteria under elevated temperature conditions. Our results revealed that Symbiodiniaceae density and Chlorophyll *a* concentration were lowest during the summer and highest in the winter. Notably, the summer bacterial community was predominately composed of the proteorhodopsin bacterium BD 1–7 _clade, alongside a significant increase in Cyanobacteria, particularly *Synechococcus*_CC9902 and *Cyanobium*_PCC-6307, which represented 61.85% and 31.48% of the total Cyanobacterial community, respectively. In vitro experiments demonstrated that Cyanobacteria significantly enhanced Symbiodiniaceae photosynthetic efficiency under high-temperature conditions. These findings suggest that the increased abundance of photosynthetic bacteria during summer may mitigate the adverse physiological effects of reduced Symbiodiniaceae density, thereby contributing to coral stability. Our study highlights a potential synergistic interaction between Symbiodiniaceae and photosynthetic bacteria, emphasizing the importance of understanding these dynamic interactions in sustaining coral resilience against environmental stress, although further research is necessary to establish their role in preventing coral bleaching.

Keywords Coral reef · Photosynthetic symbionts · Synergistic effect · Seasonal variation

Introduction

Coral reefs are complex ecosystems composed of coral hosts and a diverse community of associated microorganisms, including Symbiodiniaceae, bacteria, archaea, fungi, protists, viruses, and others [1]. These microorganisms play crucial and intricate roles in the coral's response to thermal stress [2]. Among these symbiotic partners, photosynthetic microorganisms are essential components of coral holobionts, contributing significantly to coral growth, development, and the intricate ecological interactions within coral reef ecosystems [3]. For instance, Symbiodiniaceae, as faithful partners of corals, transfer most photosynthetic products to corals, providing energy for coral calcification [4]. Cyanobacteria, as the predominant photosynthetic bacterial group within corals [5], actively participate in carbon and nitrogen cycling, potentially aiding corals in maintaining homeostasis under environmental stress [6].

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Despite the well-studied relationship between corals and Symbiodiniaceae, the roles of the photosynthetic bacteria within corals remain largely unexplored[7, 8]. The breakdown of coral-Symbiodiniaceae symbiosis due to rising sea surface temperature has led to global coral reef degradation [9, 10]. Within coral holobionts, Symbiodiniaceae and bacteria engage in metabolic exchanges essential for element cycling [11]. For instance, Symbiodiniaceae growth is enhanced through metabolic interactions with associated bacteria[12]. The co-localization of bacteria and Symbiodiniaceae within coral tissues suggests a mutualistic relationship suggests a mutualistic relationship, essential for metabolic cycling [13]. Cyanobacteria were observed adjacent to Symbiodiniaceae and are often in high densities throughout the gastrodermal tissues[6, 14]. Given this close association, it is likely that Symbiodiniaceae preferentially uptake the products from Cyanobacteria over those from host tissues [15].

Understanding the interactions between Symbiodiniaceae and photosynthetic bacteria is critical for advancing coral reef recovery efforts [16]. Photosynthetic bacteria may compensate for the photosynthesis of Symbiodiniaceae, and there may be synergistic effects between them, which were impacted by variations in temperature [17], light [18], pH [19], or other environmental stresses [20]. However, mechanisms for such synergistic effects' environmental impact were poorly understood. To gain a deeper understanding of the role of photosynthetic bacteria in coral adaptation to seasonal environmental fluctuations, especially in the context of high-temperature conditions during the summer, we focused on *Pavona decussata* in Weizhou Island, Beibu Gulf. Weizhou Island exhibits clear seasonality, particularly during the summer when the maximum sea water temperature exceeds 32 °C [21].

We aimed to verify the auxiliary role of photosynthetic bacteria in supporting the photosynthesis of Symbiodiniaceae under high-temperature conditions. The results initially revealed the compensatory effect of symbiotic photosynthetic bacteria on algae photosynthetic efficiency, which should help us better understand the ecological resilience and survival strategies of coral holobionts.

Material and Methods

Coral Sample Collection and Environmental Data

Coral samples of *P. decussata* were collected on Weizhou Island (21°N8.27′, 109°E12.6′) in July and October 2019 and in January and April 2020, respectively (Fig. 1a). A total of 20 samples were collected across four seasons, with 5 coral fragments collected in each season. Sampling was conducted at a depth range of 6—7 m. To avoid duplicate sampling and the collection of individuals involved in coral asexual reproduction, the distance between sampling points for the same specimen exceeded 6 m [22]. To minimize contamination by exogenous microorganisms, each coral fragment was thoroughly rinsed three times with sterile seawater immediately after retrieval. Subsequently, the coral samples were immediately placed in sterile, airtight bags, flash-frozen in liquid nitrogen, and then transported back to the laboratory for further experimentation.

Remote sensing data for the Weizhou Island coral reef area from July 2019 to July 2020 were obtained using the Giovanni Ocean Color tool (https://giovanni.gsfc.nasa.gov/giovanni). The data included Sea Surface Temperature (SST; °C), Photosynthetically Active Radiation (PAR; E m^{-2} d^{-1}), Chlorophyll *a* concentration (mg m^{-3}), 490 nm diffuse attenuation coefficient (Kd490; Kd m^{-1}), and Particulate Organic Carbon (POC; mg m^{-3}).

Determination of Symbiodiniaceae Density and Chlorophyll a Concentration

The determination of Symbiodiniaceae density followed the standard procedure [23]. To separate Symbiodiniaceae from coral skeletons and tissues, we utilized a high-pressure water jet (WaterPik irrigator) powered by sterile filtered seawater. All rinse water was collected and measured. A 1 mL of rinse water was centrifuged at 4000 × g for 10 min, and the supernatant was discarded. The remaining cells were resuspended in 1 mL of 4% formaldehyde fixative. This suspension was placed on a hemocytometer, covered with a glass slide, and counted under the optical microscope (Nikon, DS-Ri2). The washed coral skeletons were dried in an oven at 60 °C for 72 h to remove moisture. Each coral skeleton fragment was fully wrapped in aluminum foil. A 5 cm × 5 cm piece of aluminum foil was weighed to determine the coral surface area based on the weight-to-area ratio [24]. The density of Symbiodiniaceae per unit area of coral was calculated as described in the previous study [25]. To determine Chlorophyll *a* concentration, coral fragments were soaked in 90% acetone and extracted in the dark at 4 °C for 24 h. The absorbance of 664 nm, 647 nm, and 630 nm was measured using a spectrophotometer (Thermo Fisher), and the concentration was calculated using the equations recommended by Jeffery and Humphrey [26].

DNA Extraction, High Throughout Sequencing and Bioinformatics Analysis

A fragment weighing about 90 mg, including calcium carbonate skeleton, tissue, and mucous components, was taken from each coral sample to extract genomic DNA. DNA extraction was performed using the Marine Animal Genomic DNA Extraction Kit (TIANGEN, DP324), following the

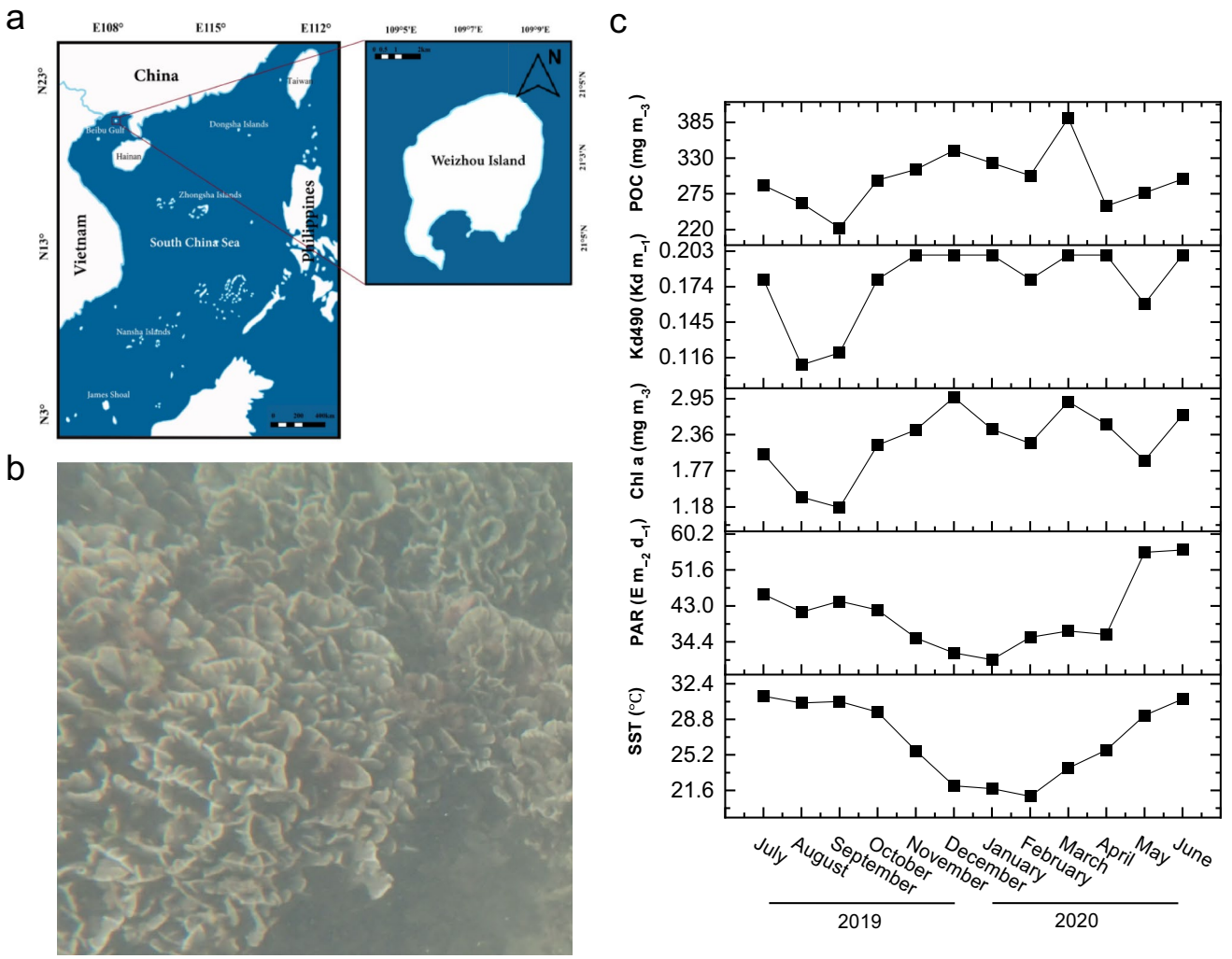

Fig. 1 The collection of coral samples and environmental data. (**a**) Sampling site in the coral reef area of Weizhou Island; (**b**) State of *P. decussata* in summer; (**c**) Environmental parameters of Weizhou Island coral reef area each month from 2019 to 2020

manufacturer's instructions. After extraction, DNA quality was checked using agarose gel electrophoresis and UV A260/A280 absorbance measurements to ensure it met the required standards. Using high-quality DNA as a template, the ITS2 region of Symbiodiniaceae and the V3-V4 region of the bacterial 16S gene were amplified separately. The PCR reactions were set up, and conditions followed as described [17]. Qualified PCR products were sent on dry ice to Shanghai Meiji Biomedical Technology Co., Ltd. for MiSeq library construction and high-throughput sequencing.

To ensure the quality of the sequencing data, raw reads from the Illumina MiSeq Platform were processed using Trimmonmatic to filter out low-quality bases. High-quality reads were assembled and trimmed using PEAR, and chimeras were detected and removed through MOTHUR. Primer sequences were trimmed using CUTADAPT. The data were cleaned using DADA2 and Deblur to correct sequencing errors, remove chimeric sequences, filter out low-quality reads, and trim potential contaminants. This process produced accurate ASV representative sequences and abundance tables.

To analyze Symbiodiniaceae community composition, we used a refined, non-duplicate ITS2 database to avoid replicate sequences. This database was created by compiling several published datasets and processing them with the CD-HIT Suite at a 100% sequence identity cut-off, as described in our previous study [27]. This method allowed us to accurately identify representative sequences and generate abundance tables for Symbiodiniaceae subclades.

Isolation and Cultivation of Coral-Associated Photosynthetic Microorganisms

To facilitate the subsequent isolation of photosynthetic microorganisms, the collected *P. decussata* were transported to the Coral Reef Research Center at Guangxi University and placed in an aquarium with stable conditions: 600 mm × 600 mm × 600 mm (length × width × height), water flow at 6,500 L/h, color temperature at 15,000 K (12 h of light daily from 6:00 to 18:00), salinity of 35 g/L, pH between 7.5–8.0, Ca^{2+} concentration of 380–450 ppm, Mg^{2+} concentration of 1,200–1,320 ppm, and water temperature at 25 °C.

All subsequent microbial isolations were from *P. decussata*. Once the coral's tentacles had fully extended, a healthy 2 cm × 2 cm fragment was excised and placed in sterile cryovials. The fragment was rinsed three times with sterile seawater to remove contaminants, then transferred to a sterile shaker with grinding beads (Shanghai Jingxin, JXFSTPRP-24) and 3 mL of sterile seawater. The shaker was set to 50 Hz for 1 min to disrupt the coral polyps and release the Symbiodiniaceae cells.

The cultivation of Symbiodiniaceae was as previously described [28]. Briefly, the Symbiodiniaceae were isolated from coral fragments using micro-strainer filtration and density gradient centrifugation, then cultured in 96-well plates with L1 medium. Single cells were isolated by dilution to extinction, and species and phylogenetic relationships were identified using ITS2 sequences. Culturing was conducted in a light incubator (Yanghui Instruments, RDN-500D-CO2) under the following conditions: 25 °C, 45 ± 5 µmol photons m^{-2} s^{-1}, and a 14:10 h light–dark cycle for two weeks.

Cyanobacteria were released and isolated similarly. Cyanobacteria were cultured on BG-11 plates under the same conditions as the Symbiodiniaceae. Colonies with significant morphological differences were selected for isolation and purification. Morphological characteristics were observed using optical and scanning electron microscopes, as described previously [29].

Temperature Experiment of Coral-Associated Photosynthetic Microorganisms

The temperature stress experiments were designed based on remote sensing data from the Weizhou Island coral reef area. High, ambient, and low temperatures were set at 32 °C, 25 °C, and 20 °C, respectively, representing the highest summer SST, the average spring and autumn SST, and the lowest winter SST (Fig. 1c).

The experiments included four parts: individual cultivations of *Cladocopium* C1 and *Cyanobium*_PCC-6307 and co-cultivation with a density ratio 100:1. Photosynthetic microorganisms were synchronously cultured under different temperature conditions for 7 days. The temperature experiment used isoclonal cultures, with three biological replicates for each condition, and each biological replicate included at least three technical replicates. After the temperature experiment, the following photosynthetic physiological parameters were measured. (1) Fv/Fm measurement: Fv/Fm was measured using Pulse-Amplitude Modulation (Walz Heinz GmbH, Effeltrich, Germany) to determine the PSII photochemical efficiency of photosynthetic microorganisms 20 min after turning off the light. (2) Density measurement: Symbiodiniaceae density was counted using a hemocytometer under an optical microscope [30]. The density of individually cultured cyanobacteria was measured by reading the absorbance value at 730 nm to estimate the number of cells [31]. The density of cyanobacteria in co-cultures was measured using Real-Time Quantitative PCRs. (3) Chlorophyll *a* measurement: Chlorophyll *a* was extracted using 90% acetone at 4 °C in the dark for 24 h. Absorbance values were measured at 664 nm, 647 nm, and 630 nm using a spectrophotometer (Varioskan LUX, Thermos Fisher Scientific, USA), and the concentration was calculated using the equations by Jeffery and Humphrey [26]. (4) Reducing sugar measurement: Reducing sugar content was determined using the phenol–sulfuric acid method[32].

Real-Time Quantitative PCR

To quantify changes in Cyanobacterial abundance in the co-culture experiment, the primers BD16SF (5' CACACT GGGACTGAGACAC −3') and BD16SR (5' CTGCTGGCA CGGAGTTAG −3') were selected as Cyanobacteria-specific primers for real-time quantitative PCR [33]. The reactions mixture, with a total volume of 10 µL, consisted of 5 µL SYBR Premix EX Taq™, 0.2 µL of each primer (10 pmol/µL), 1 µL DNA template, and 3.6 µL ddH_2O. The reactions were run on the QuantStudio™ 5 Real-Time PCR Instrument (Thermos Fisher Scientific, USA), with the following conditions: an initial denaturation at 95 °C for 30 s, followed by 35 cycles of 95 °C for 30 s, 56 °C for 30 s, 72 °C for 45 s, with a final extension at 72 °C for 10 min.

Statistical Analysis and Data Visualization

For data following a normal distribution, a one-way analysis of variance (ANOVA) was conducted to assess significant differences, followed by Tukey's Honestly Significant Difference (HSD) post-hoc test for pairwise comparisons. The non-parametric Kruskal–Wallis test was applied for data not following a normal distribution, followed by pairwise comparisons with the Bonferroni-Dunn adjustment for multiple comparisons. Statistical analyses were performed using

SPSS 27.0. Data visualization was carried out using Origin Pro 2023 and R 4.3.3.

Results

Seasonal Variation of Environmental Parameters at Weizhou Island

The environmental parameters in the Weizhou Island coral reef area show significant seasonal fluctuations, with the most extreme values occurring in either summer or winter. SST and PAR follow similar trends, with their lowest values in winter and highest in summer and late spring. During the sampling period, the highest values for SST and PAR were 31.15 °C and 45.78 E m^{-2} d^{-1}, respectively, occurring in summer (July 2019). The lowest values were 20.99 °C and 30.15 E m^{-2} d^{-1}, respectively, in winter (July 2019, February, and January 2020). The two parameters have a strong positive correlation (Pearson correlation coefficient, $r = 0.789$, $P < 0.01$, Table S1) and exhibit consistent, gradual seasonal changes.

Chl *a* Kd490, and POC exhibit extreme values in either summer or winter. The highest values for Chl *a*, Kd490, and POC were 2.9 mg m^{-3}, 0.201 Kd m^{-1}, 391.783 mg m^{-3}, respectively, in the summer (July 2019). The lowest values were 1.17 mg m^{-3}, 0.116 Kd m^{-1}, 222.738 mg m^{-3} in autumn (December 2019) and winter (March 2020). These three environmental parameters do not show a strong correlation with seasonal changes. Overall, within the sampling time frame, Weizhou Island exhibits experienced high temperatures and strong sunlight in summer, as well as low temperatures and weak sunlight in winter. Notably, *P. decussata* showed no signs of bleaching (Fig. 1b).

Seasonal Variation of Coral-Associated Photosynthetic Physiological State

The density of Symbiodiniaceae varied across the four seasons, ranging from 0.32×10^6 cells·cm^{-2} to 1.47×10^6 cells·cm^{-2} (Fig. 2a). The lowest density was in summer (0.51×10^6 cells·cm^{-2}), followed by spring and autumn. The highest was in winter (0.84×10^6 cells·cm^{-2}), about 1.64 times higher than in summer. Chlorophyll *a* concentration ranged from 3.48 μg·cm^{-2} to 12.45 μg·cm^{-2} (Fig. 2b). Similar to Symbiodiniaceae density, Chlorophyll *a* concentration was lowest in summer (5.20 ± 0.38 μg·cm^{-2}), and highest in winter (8.93 ± 1.79 μg·cm^{-2}), approximately 1.72 times that of summer. Statistic analysis showed that Symbiodiniaceae density and Chlorophyll *a* concentration were significantly higher in winter than in summer.

Molecular marker analysis based on ITS2 was performed on *P. decussata* samples. After quality control and filtering, 117 ASVs were detected. All identified Symbiodiniaceae belonged to the genus *Cladocopium*, with 115 subclade sequences. At the subclade level, the Symbiodiniaceae community composition showed no significant variation across the seasons. The dominant subclade was consistently C1, with relative abundances ranging from 81.63% to 82.33%. Other subclades, such as C1ca, C1p, C72, and Cspc, had relative abundances ranging from 2.57% to 3.06%, 3.30% to 3.11%, 2.45% to 2.77%, and 1.98% to 2.18%, respectively. Overall, the Symbiodiniaceae community remained stable throughout the four seasons.

Seasonal Variation of Coral-Associated *Bacteria*

At the phylum level, Proteobacteria and Bacteroidota dominated the bacterial communities across all four seasons. Proteobacteria had the highest relative abundance, ranging from 65.21% to 88.52%. Bacteroidota was the second most abundant phylum, with relative abundances from 1.48% to 21.07% (Fig. 3a). At the genus level, the dominant genera were the BD1-7_clade and *Endozoicomonas* of the phylum Proteobacteria. BD1-7_clade had relative abundances of 50.59%, 45.70%, 34.67%, and 21.25% in summer, autumn, winter, and spring, respectively. *Endozoicomonas* had relative abundances of 34.76%, 28.16%, and 24.84% in autumn, winter, and spring but dropped to 0.13% in summer (Fig. 3b).

Principal Coordinates Analysis (PCoA) based on the Bray–Curtis algorithm at the ASV level showed significant differences in the bacterial community structure among the seasons (PERMANOVA, $R^2 = 0.26$, $P < 0.05$, Fig. 3c). While there was some overlap in the bacterial communities across all seasons, the structure in summer was significantly different from the others. The relative abundance of the photosynthetic bacterium BD1-7_clade peaked in summer at 50.59%, compared to 21.25%—45.70% in other seasons. In contrast, the relative abundance of *Endozoicomonas* in summer dropped to 0.13% (Fig. 3b), much lower than that in the other three seasons (24.84%—34.76%). This shift indicates that BD1-7_clade dominates the bacterial community composition in summer, while in other seasons, both BD1-7_clade and *Endozoicomonas* co-dominate.

Seasonal Variation of Coral-Associated Photosynthetic *Bacteria*

Among the top 20 species at the genus level by relative abundance, we identified three photosynthetic bacteria: the proteorhodopsin bacterium BD1-7_clade, *Synechococcus*_CC9902, and *Cyanobium*_PCC-6307. The relative abundance of all three photosynthetic bacteria peaked in the summer. BD1-7_clade, the dominant genus in all four seasons, had a relative abundance of 50.59% in summer, 45.70%

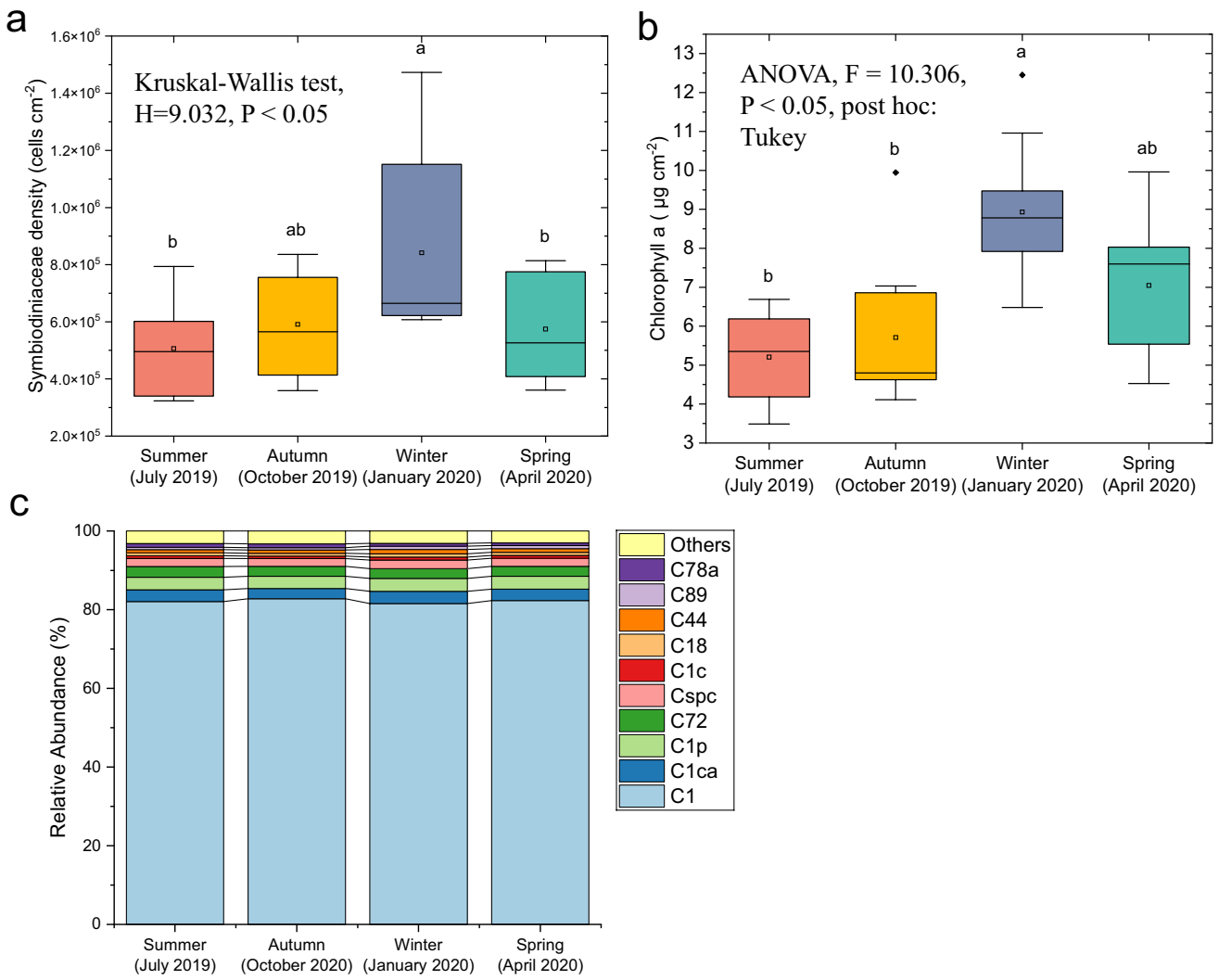

Fig. 2 Seasonal variation differences in the photosynthetic physiological state of *P. decussata*. (a) Seasonal variation differences in Symbiodiniaceae density of *P. decussata*; (b) Seasonal variation differences in Chlorophyll *a* concentration of *P. decussata*. (c) Seasonal variation differences in Symbiodiniaceae community composition of *P. decussata*. Data in Fig. 2c are mean values, and the number of samples for each season is 5

in autumn, 34.67% in winter, and 21.25% in spring. Despite showing no significant seasonal differences in relative abundance, BD1-7_clade reached its highest levels in summer (Figs. 3b and 4c). The relative abundance of *Synechococcus*_CC9902 was 1.08% in summer, 0.60% in autumn, 0.05% in winter, and 0.27% in spring, showing significant differences across the seasons. Similarly, the relative abundance of *Cyanobium_PCC-6307* was 0.55% in summer, 0.17% in autumn, 0.009% in winter, and 0.01% in spring, demonstrating significant seasonal variation (Fig. 4a).

Based on 16S amplicon sequencing data, the community composition and ASVs of Cyanobacteria at the phylum level were obtained for each sample. 381 Cyanobacteria ASVs were detected across the coral samples in all four seasons, belonging to 3 orders, 11 families, 21 genera, and 36 species (Table S2). In summer and autumn, *Synechococcus*_CC9902 and *Cyanobium*_PCC-6307 were dominant, with their relative abundance peaking in summer, at 61.85% and 31.48%, respectively (Fig. 4b).

Effect of High Temperatures on Coral-Associated Photosynthetic Microorganisms

We successfully isolated *Cladocopium* C1 and *Cyanobium*_PCC-6307 from *P. decussata,* but could not isolate BD1-7_clade and *Synechococcus*_CC9902. Symbiodiniaceae cultures are known to harbor their own microbiomes, which may include *Synechococcus* and *Cyanobium* [34]. To account for this, we sequenced the in vitro microbiomes of the isolated *Cladocopium* C1 cultures. The results showed

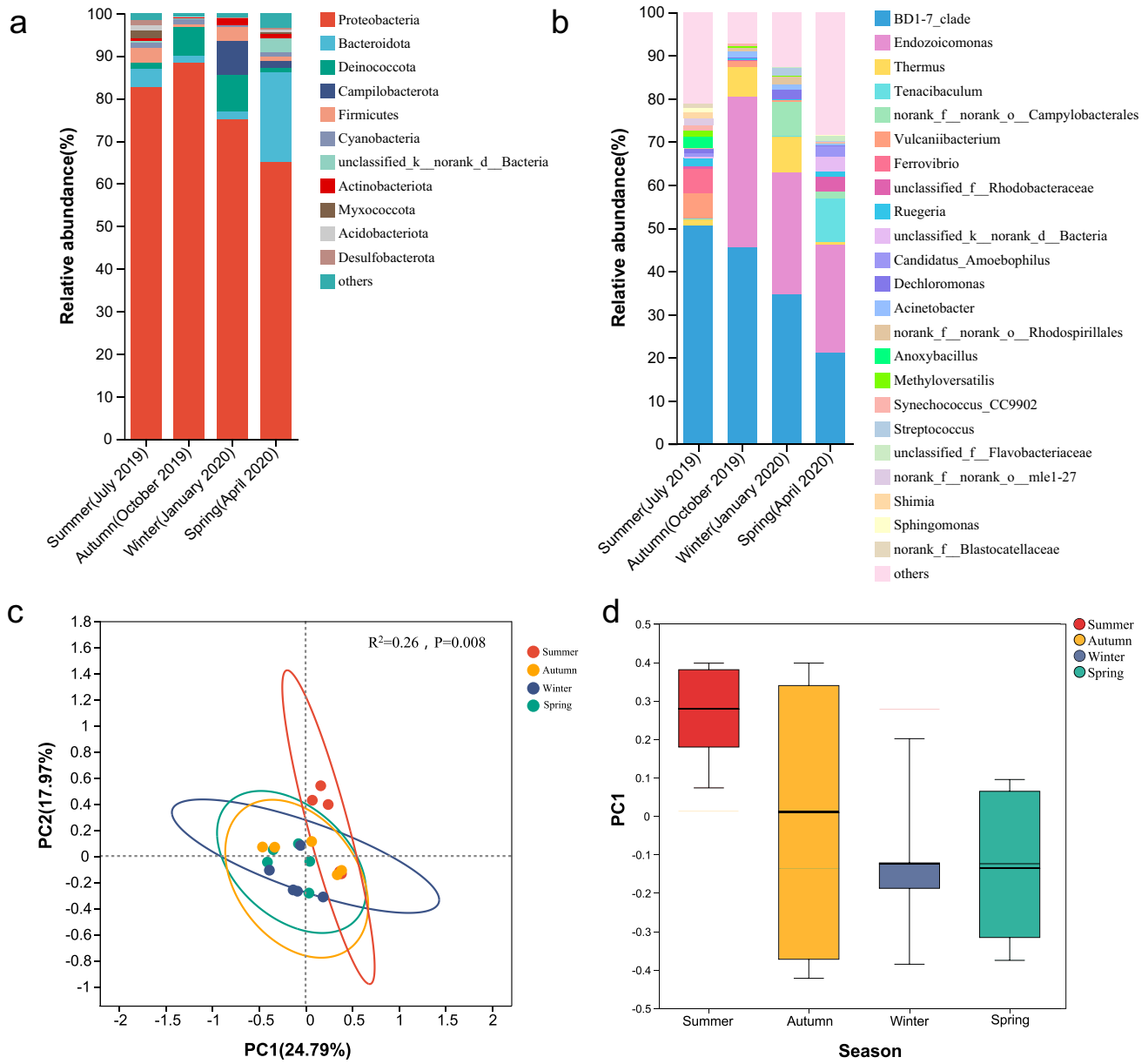

Fig. 3 Seasonal variation differences in the bacterial community of *P. decussata*. (a) The bacterial community composition at the phylum level; (b) The bacterial community composition at the genus level; (c) Principal Coordinates Analysis (PCoA) of the bray–curtis distance matrix representing differences in community structure at the ASV level; (d) The distribution dispersion of samples on the PC1 axis under different seasons. Data in the Fig. 3 are mean values, and the number of samples for each season is 5

that the associated bacterial community of *Cladocopium* C1 did not contain any Cyanobacteria (Table S3 and Fig. S2).

Under different temperature conditions, the photosynthetic physiological indicators of *Cladocopium* C1 changed significantly. At 20 °C, 25 °C, and 32 °C, the Symbiodiniaceae density was $(1.20 \pm 0.05) \times 10^6$ cells cm^2, $(3.25 \pm 0.22) \times 10^6$ cells cm^2, and $(1.15 \pm 0.12) \times 10^6$ cells cm^2, respectively (Fig. 5c). At 20 °C, 25 °C, and 32 °C, the Fv/Fm was 0.57 ± 0.05, 0.68 ± 0.04, and 0.46 ± 0.03, respectively (Fig. 5d). The Chlorophyll *a* concentrations at 20 °C, 25 °C and 32 °C were 0.50 ± 0.04 μg/mL, 1.33 ± 0.23 μg/mL, and 0.42 ± 0.03 μg/mL, respectively (Fig. 5e). Statistic analysis showed that both high and low temperatures led to a significant decrease in cell density, Fv/Fm, and Chlorophyll *a* concentration, with a more pronounced reduction at high temperatures.

In contrast, the photosynthetic physiological indicators of the *Cyanobium*_PCC-6307 improved with increasing temperature. At 32 °C, OD_{730} was 1.36 ± 0.07, representing a significant increase of 25.93% and 49.45% compared to 25 °C and

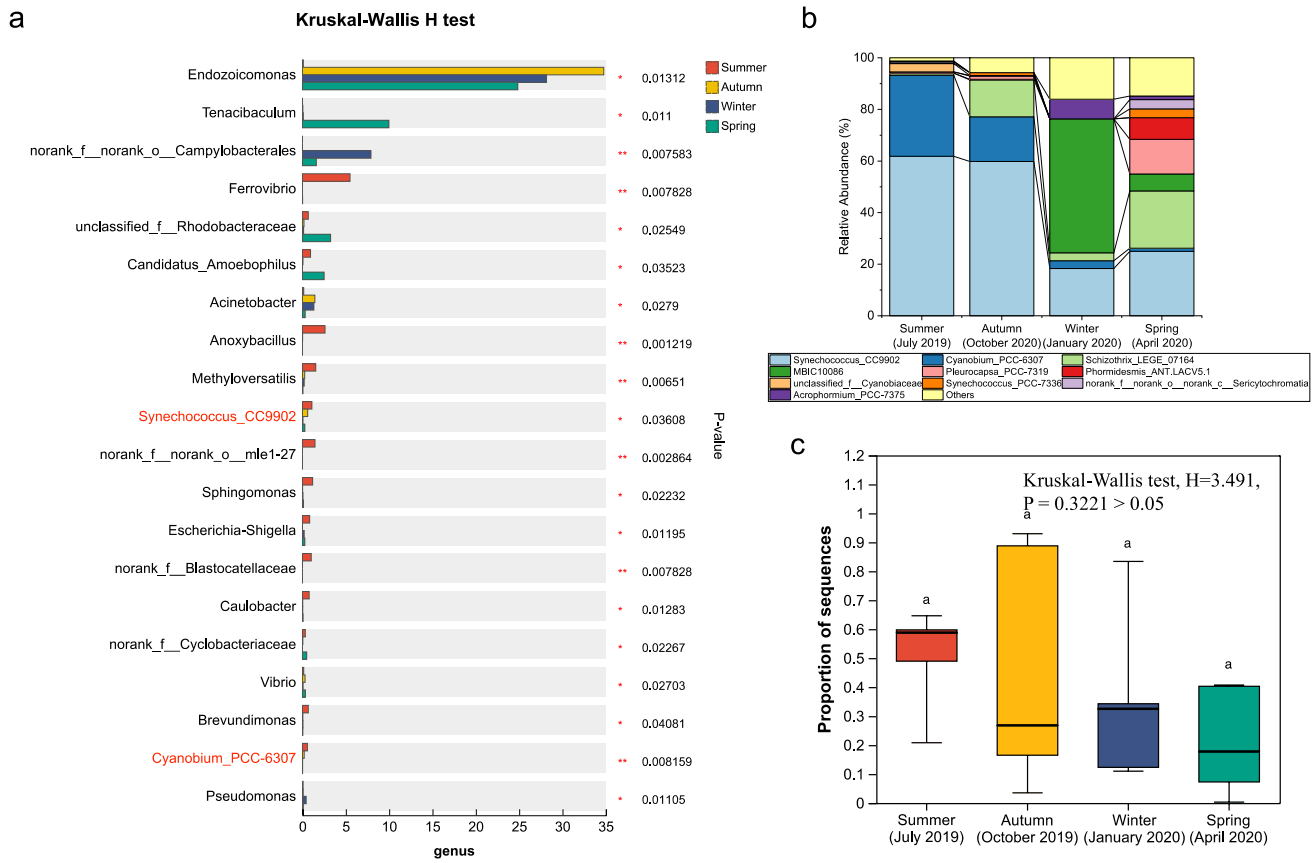

Fig. 4 Changes of photosynthetic bacteria in different seasons. (**a**) Multiple comparisons of the top 20 species with significant differences in relative abundance at the genus level. * $0.01 < P \leq 0.05$, ** $0.001 < P \leq 0.01$, *** $P \leq 0.001$. (**b**) Seasonal variation differences in Cyanobacteria community composition of *P. decussata* on genus level (**c**) The proportion of BD 1–7 clade in four seasons. Data in the Fig. 4 are mean values, and the number of samples for each season is five

20 °C, respectively (Fig. 5h). Similarly, at 32 °C, Fv/Fm was 0.52 ± 0.15, showing a significant increase of 10.64% and 44.44% compared to 25 °C and 20 °C, respectively (Fig. 5i).

The photosynthetic indicators exhibited distinct patterns under different temperature conditions in *Cladocopium* C1 and *Cyanobium*_PCC-6307 co-culture. The density of *Cladocopium* C1 and *Cyanobium*_PCC-6307 at 25 °C were significantly higher than at 20 °C and 32 °C (Figs. 6a and 6b). The density of *Cladocopium* C1 at 25 °C was $(8.39 \pm 2.22) \times 10^5$ cells/mL. At 20 °C and 32 °C, it decreased to $(4.61 \pm 1.19) \times 10^5$ cells/mL and $(4.78 \pm 1.33) \times 10^5$ cells/mL, respectively. The density of *Cyanobium*_PCC-6307 at 25 °C was $(1.53 \pm 0.39) \times 10^7$ copies/mL. At 20 °C and 32 °C, it decreased to $(1.06 \pm 0.43) \times 10^7$ copies/mL and $(0.65 \pm 0.54) \times 10^7$ copies/mL. However, Fv/Fm increased significantly with rising temperature, reaching 0.64 ± 0.02, 0.70 ± 0.02, 0.72 ± 0.03 at 20 °C, 25 °C, and 32 °C, respectively (Fig. 6c). Additionally, the concentration of reducing sugar and Chlorophyll *a* did not show significant differences with temperature changes. The reducing sugar concentration at 20 °C, 25 °C, and 32 °C were 0.81 ± 0.07 μg/mL, 0.81 ± 0.03 μg/mL, and 0.79 ± 0.04 μg/mL, respectively. The Chlorophyll *a* concentration at 20 °C, 25 °C, and 32 °C were 0.42 ± 0.06 μg/mL, 0.43 ± 0.05 μg/mL, and 0.39 ± 0.02 μg/mL, respectively.

Discussion

Seasonal Variation Pattern of Coral-Associated Photosynthetic Physiological State

Symbiodiniaceae plays a crucial role in the health and resilience of corals, with its density and community composition commonly used to characterize coral photosynthetic performance and assess environmental adaptability [35]. Higher Symbiodiniaceae density is generally associated with better stress resistance in corals [23]. Corals can also adjust the relative abundance of symbiotic Symbiodiniaceae or acquire new types from the environment through processes known as reshuffling or replacement to adapt to changing environmental conditions [36].

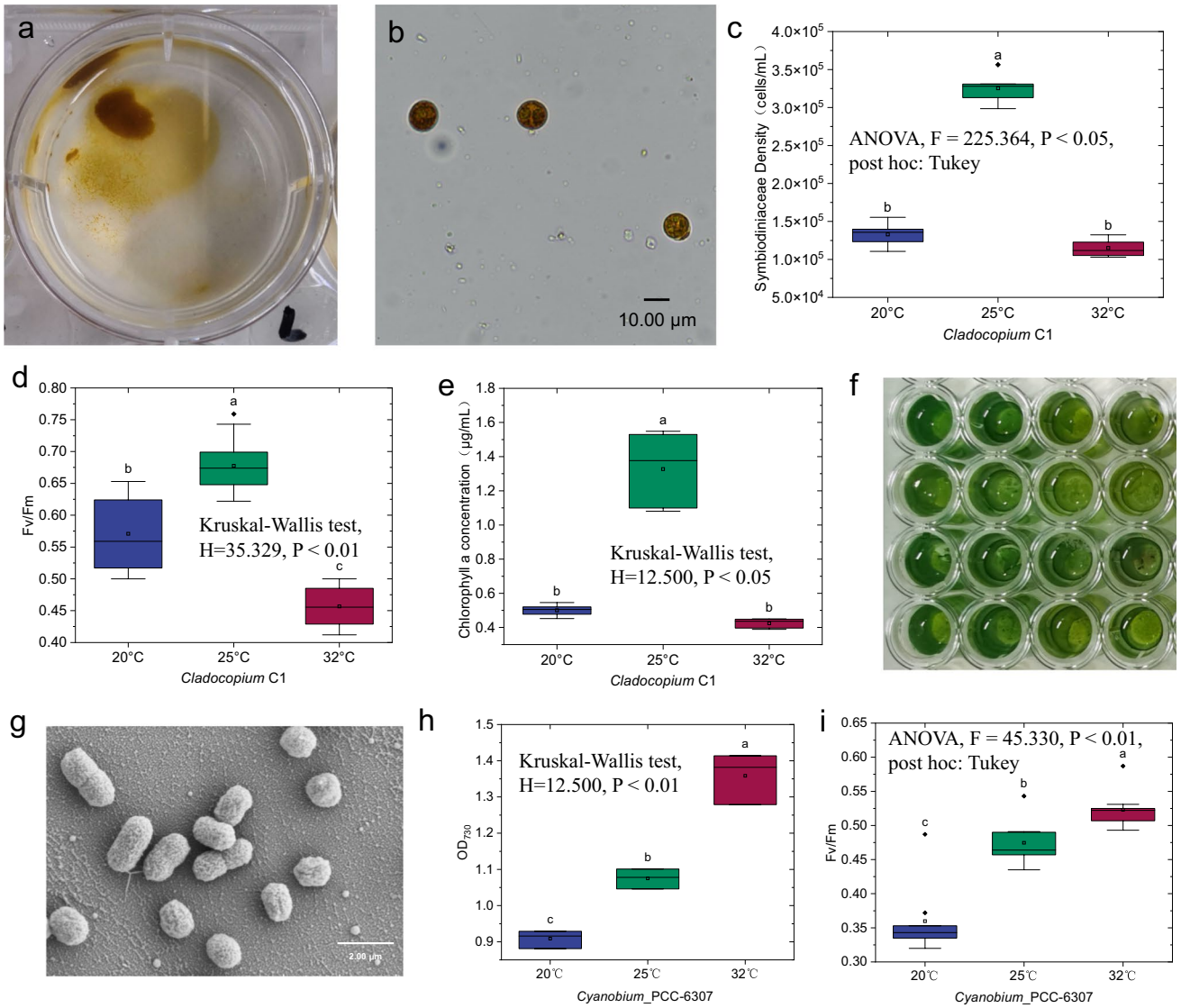

Fig. 5 The photophysical status of coral-associated photosynthetic microorganisms under different temperature conditions. (**a**) L1 medium showing growth of *Cladocopium* C1. (**b**) Morphological characteristic of *Cladocopium* C1 in vitro under optical microscopy. (**c**) The density of *Cladocopium* C1 under different temperatures. (**d**) The Fv/Fm of *Cladocopium* C1 under different temperatures. (**e**) The Chlorophyll *a* concentration of *Cladocopium* C1 under different temperatures. (**f**) BG-11 medium showing growth of *Cyanobium*_PCC-6307. (**g**) Morphological characteristic of *Cyanobium*_PCC-6307 in vitro under scanning electron microscopy. (**h**) Changes in OD_{730} of *Cyanobium*_PCC-6307 under different temperatures. (**i**) Changes in Fv/Fm of *Cyanobium*_PCC-6307 under different temperatures

Our results showed that *Cladocopium* C1 stably symbioses with *P. decussata*, remaining the dominant species (81.63%~82.33%), with a stable Symbiodiniaceae community composition (Fig. 2c). The Symbiodiniaceae density and Chlorophyll *a* concentration followed a seasonal pattern, being lowest in summer and highest in winter (Figs. 2a and 2b), consistent with previous studies [37]. In summer, high temperatures and intense light can damage the photosystem II of Symbiodiniaceae chloroplasts [38], reducing photosynthetic efficiency, generating excess electrons, leading to the overproduction of reactive oxygen species, which can damage coral tissue and DNA, ultimately causing Symbiodiniaceae cell death or expulsion from the coral host [39]. Consequently, in the high-temperature, intense-light environment of summer, Symbiodiniaceae density and Chlorophyll *a* concentration significantly decrease, photosynthetic efficiency drops, and the ability to fix carbon may decrease [40], leading to a significant reduction in organic carbon transferred to the coral host. This suggests that the energy supply of *P. decussata* may be compromised. However, field observations in summer showed that *P. decussata* on Weizhou Island did not exhibit apparent bleaching (Fig. 1b), indicating that other microorganisms may help maintain the stability of *P. decussata*.

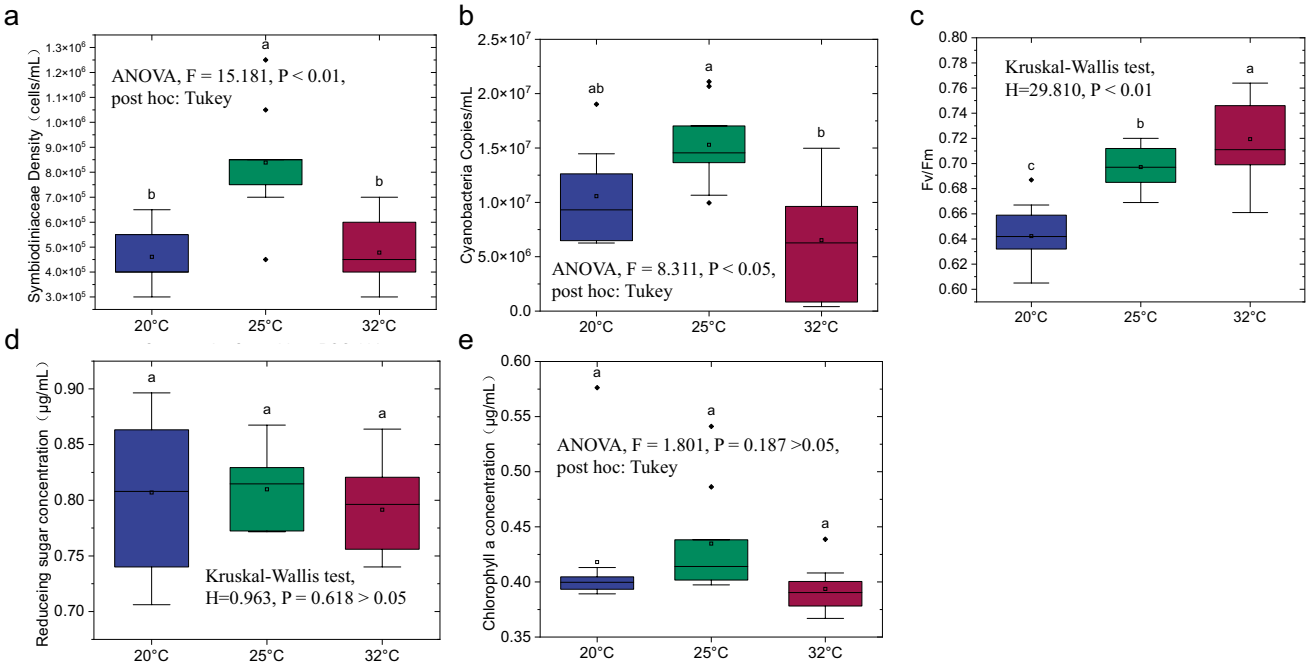

Fig. 6 The photophysical status of *Cladocopium* C1 and *Cyanobium*_PCC-6307 co-cultured under different temperatures. (**a**) The density of co-cultured *Cladocopium* C1 under different temperatures. (**b**) The copies of co-cultured *Cyanobium*_PCC-6307 under different temperatures. (**c**) The Fv/Fm of *Cladocopium* C1 and *Cyanobium*_PCC-6307 co-cultured under different temperatures. (**d**) The reducing sugar concentration of *Cladocopium* C1 and *Cyanobium*_PCC-6307 co-cultured under different temperatures. (**e**) The Chlorophyll *a* concentration of *Cladocopium* C1 and *Cyanobium*_PCC-6307, was co-cultured under different temperatures

Seasonal Variation Pattern of Coral-Associated Photosynthetic Microorganisms

Symbiotic bacteria are integral to coral adaptation to environmental changes, playing critical roles in nutrient cycling, energy provision, and immune responses [41]. Our results showed that the bacterial structure during the summer significantly differed from other seasons, shifting from the co-dominance of BD1-7_clade and *Endozoicomonas* to the sole dominance of BD1-7_clade. Additionally, the relative abundance of *Synechococcus*_CC9902 and *Cyanobium*_PCC-6307 peaked during the summer.

BD1-7_clade is an oligotrophic bacteria adapted to extreme carbon source limitation and nutrient depletion conditions [42]. Its genome contains genes encoding rhodopsin, carotenoids, and polyketide synthase, allowing it to perform photosynthesis via proteorhodopsin even in environments with low organic matter [43]. This reduces the energy demands on *P. decussata* and likely explains the peak abundance of BD1-7_clade in summer. BD1-7_clade's ability to perform photoheterotrophy through rhodopsins may also help mitigate the impact of high light conditions on *P. decussata* and offset the reduced photosynthesis by Symbiodiniaceae.

Similarly, Cyanobacteria displayed a comparable seasonal variation pattern. *Synechococcus*_CC9902 and *Cyanobium*_PCC-6307 had the highest abundance in summer under high temperatures and intense light, while they were lowest in winter under low temperatures and weak light (Fig. 4b). These Cyanobacteria are small, distributed in marine environments globally [44], and have evolved highly efficient photosynthetic apparatuses [45]. They also tolerate high temperatures and can uptake nutrients at low concentrations [46, 47]. For instance, *Synechococcus*_WH7803 prefers high temperatures, with an optimal growth temperature of up to 33 °C, and maintains high photosynthetic efficiency even at elevated temperatures [48, 49]. Synechococcales are well adapted to oligotrophic environments, with cyanobacterial densities reaching up to 4×10^5 cells/mL [50]. Given their high photosynthetic efficiency under low nutrient concentration, *Synechococcus*_CC9902 and *Cyanobium*_PCC-6307 may potentially supply photosynthetic products to Symbiodiniaceae during summer.

Response Pattern of Cultured Coral-Associated Photosynthetic Microorganisms to High-temperature Conditions

The in vitro cultivation of coral-associated microorganisms is crucial for understanding how coral responds to

environmental changes [51]. To explore whether photosynthetic bacteria can compensate for the decreased photosynthetic efficiency of Symbiodiniaceae under high-temperature conditions, we studied *Cladocopium* C1 and *Cyanobium*_PCC-6307, both individually and in co-culture, under different temperature conditions.

After 7 days of single culturing, the physiological indicators of *Cladocopium* C1 declined under high and low temperatures. Fv/Fm and Chlorophyll *a* concentration, which reflect the photosynthetic efficiency of Symbiodiniaceae[52], showed significant decreases. This suggests that extreme temperatures, particularly high temperatures, impair the photosynthetic efficiency of Symbiodiniaceae, supporting previous research indicating that *Cladocopium* C1 is thermally sensitive [53]. Abnormal temperatures can lead to the accumulation of reactive oxygen species (ROS) [54], damaging to the photosystem that absorbs excitation energy [55]. In contrast, *Cyanobium*_PCC-6307 demonstrated strong adaptability to high temperatures. After 7 days of 32℃, the Fv/Fm of and OD_{730} of *Cyanobium*_PCC-6307 significantly increased compared to routine and low-temperature conditions (Figs. 5h and 5i), indicating enhanced photosynthetic efficiency under high-temperature conditions. This is consistent with previous reports that certain Cyanobacterial strains prefer high temperatures, showing maximal growth rates, cell density, and Fv/Fm [48].

After 7 days of co-culturing, Fv/Fm increased significantly with rising temperature, reaching the highest value at 32 °C, which was higher than that observed in the single cultures of *Cladocopium* C1 and *Cyanobium*_PCC-6307, reflecting a synergistic effect. However, further studies are needed to quantify the individual contributions to overall photosynthetic performance. The concentrations of reducing sugar and Chlorophyll *a* were unaffected by temperature, showing no significant differences across the temperature gradients. Surprisingly, *Cladocopium* C1 and *Cyanobium*_PCC-6307 exhibited the same responses to temperature changes. The Symbiodiniaceae density in the co-culture system was highest at 25 °C and significantly decreased at 20 °C and 32 °C, consistent with the results from the single cultures. However, the Cyanobacteria copies were lowest at 32 °C, contrary to the results observed in the single cultures. This discrepancy may be due to interactions between the symbiotic bacterial community of Symbiodiniaceae and Cyanobacteria [16]. Despite this, the Cyanobacteria density still reached 10^6 copies/mL, sufficient to maintain the photosynthesis efficiency of the co-culture system. Results from the co-culture experiment indicated that high temperatures had minimal impact on the photosynthesis efficiency of *Cladocopium* C1 and *Cyanobium*_PCC-6307 co-culture system. Previous research has shown that Symbiodiniaceae can benefit from metabolic exchanges with bacteria. For example, the co-culture of Symbiodiniaceae and *Ruegeria* sp. thrives in nitrogen-free conditions, with ^{15}N-stable isotope probing-single cell Raman spectroscopy verifying *Ruegeria* sp.'s role as a nitrogen supplier to Symbiodiniaceae [56]. Furthermore, *Labrenzia alexandrii* and *Marinobacter* sp. synthesize indole-3-acetic acid (IAA), a phytohormone that enhances the growth of Symbiodiniaceae [12].

Our results show that *Cyanobium*_PCC-6307 can help Symbiodiniaceae improve photosynthetic efficiency and may offer metabolic benefits under high-temperature conditions. Further research is needed to establish a direct link between these findings and the prevention of coral bleaching *in hospite*. Overall, our results highlight the significant role of photosynthetic bacteria, particularly *Cyanobium*_PCC-6307, in enhancing the photosynthetic efficiency of Symbiodiniaceae under high-temperature conditions. By boosting Symbiodiniaceae photosynthesis, *Cyanobium*_PCC-6307 indirectly contributes to maintaining coral health and aiding adaptation to environmental changes. This knowledge provides new insights into the potential role of photosynthetic bacteria within the coral holobiont and is essential for developing strategies to protect and restore coral reefs.

Conclusion

We employed a comprehensive approach utilizing amplicon sequencing, microbiological isolation, and cultivation to analyze the dynamic changes in symbiotic Symbiodiniaceae and photosynthetic bacteria in *P. decussata* across four seasons on Weizhou Island. Additionally, we examined the physiological states of *Cladocopium* C1, *Cyanobium_PCC-6307*, and their co-culture under varying temperature conditions in the laboratory. Our findings suggest that symbiotic Symbiodiniaceae and photosynthetic bacteria may collectively contribute to the energy supply of *P. decussata* symbiotic consortia in response to seasonal changes. Furthermore, the results indicate a potential compensatory effect of photosynthetic bacteria on the photosynthesis of Symbiodiniaceae under high-temperature conditions. However, while our results highlight the possible synergistic interaction between photosynthetic microorganisms in maintaining coral photosynthetic physiology under high-temperature conditions, the direct impact of these interactions on the coral host, particularly in maintaining healthy photosynthate translocation, requires further investigation. These findings underscore the potential role of photosynthetic bacteria in supporting coral adaptation to high-temperature environments, particularly during the summer. It provides valuable insights into the environmental adaptability of coral symbiotic consortia.

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Data Availability Sequence data supporting this study's findings have been deposited in the NCBI SRA database (Accession number: PRJNA880748).

Declarations

Competing interests The authors declare no competing interests.

References

1. Voolstra CR, Suggett DJ, Peixoto RS et al (2021) Extending the natural adaptive capacity of coral holobionts. Nat Rev Earth Environ 2:747–762. https://doi.org/10.1038/s43017-021-00214-3
2. Voolstra CR, Raina J-B, Dörr M et al (2024) The coral microbiome in sickness, in health and in a changing world. Nat Rev Microbiol 1–16. https://doi.org/10.1038/s41579-024-01015-3
3. van Oppen MJH, Blackall LL (2019) Coral microbiome dynamics, functions and design in a changing world. Nat Rev Microbiol 17:557–567. https://doi.org/10.1038/s41579-019-0223-4
4. Jacobovitz MR, Hambleton EA, Guse A (2023) Unlocking the Complex Cell Biology of Coral-Dinoflagellate Symbiosis: A Model Systems Approach. Annu Rev Genet 57:411–434. https://doi.org/10.1146/annurev-genet-072320-125436
5. Huggett MJ, Apprill A (2019) Coral microbiome database: Integration of sequences reveals high diversity and relatedness of coral-associated microbes. Environ Microbiol Rep 11:372–385. https://doi.org/10.1111/1758-2229.12686
6. Lesser MP, Mazel CH, Gorbunov MY, Falkowski PG (2004) Discovery of symbiotic nitrogen-fixing cyanobacteria in corals. Science 305:997–1000. https://doi.org/10.1126/science.1099128
7. Ainsworth TD, Fordyce AJ, Camp EF (2017) The Other Microeukaryotes of the Coral Reef Microbiome. Trends Microbiol 25:980–991. https://doi.org/10.1016/j.tim.2017.06.007
8. Davy SK, Allemand D, Weis VM (2012) Cell Biology of Cnidarian-Dinoflagellate Symbiosis. Microbiol Mol Biol Rev 76:229–261. https://doi.org/10.1128/MMBR.05014-11
9. Yu K (2012) Coral reefs in the South China Sea: Their response to and records on past environmental changes. Sci China Earth Sci 55:1217–1229. https://doi.org/10.1007/s11430-012-4449-5
10. Morris LA, Voolstra CR, Quigley KM et al (2019) Nutrient Availability and Metabolism Affect the Stability of Coral-Symbiodiniaceae Symbioses. Trends Microbiol 27:678–689. https://doi.org/10.1016/j.tim.2019.03.004
11. Matthews JL, Raina J-B, Kahlke T et al (2020) Symbiodiniaceae-bacteria interactions: rethinking metabolite exchange in reef-building corals as multi-partner metabolic networks. Environ Microbiol 22:1675–1687. https://doi.org/10.1111/1462-2920.14918
12. Matthews JL, Khalil A, Siboni N et al (2023) Coral endosymbiont growth is enhanced by metabolic interactions with bacteria. Nat Commun 14:6864. https://doi.org/10.1038/s41467-023-42663-y
13. Ainsworth TD, Krause L, Bridge T et al (2015) The coral core microbiome identifies rare bacterial taxa as ubiquitous endosymbionts. ISME J 9:2261–2274. https://doi.org/10.1038/ismej.2015.39
14. Kvennefors E, Roff G (2009) Evidence of cyanobacteria-like endosymbionts in Acroporid corals from the Great Barrier Reef. Coral Reefs 28:547–547. https://doi.org/10.1007/s00338-009-0473-0
15. Lesser MP, Falcón LI, Rodríguez-Román A et al (2007) Nitrogen fixation by symbiotic cyanobacteria provides a source of nitrogen for the scleractinian coral Montastraea cavernosa. Mar Ecol Prog Ser 346:143–152. https://doi.org/10.3354/meps07008
16. Camp EF, Kahlke T, Nitschke MR et al (2020) Revealing changes in the microbiome of Symbiodiniaceae under thermal stress. Environ Microbiol 22:1294–1309. https://doi.org/10.1111/1462-2920.14935
17. Liang J, Deng C, Yu K et al (2021) Cross-Linked Regulation of Coral-Associated Dinoflagellates and Bacteria in Pocillopora sp. during High-Temperature Stress and Recovery. Microorganisms 9:1972. https://doi.org/10.3390/microorganisms9091972
18. Matthews JL, Hoch L, Raina J-B et al (2023) Symbiodiniaceae photophysiology and stress resilience is enhanced by microbial associations. Sci Rep 13:20724. https://doi.org/10.1038/s41598-023-48020-9
19. Ge R, Liang J, Yu K et al (2021) Regulation of the Coral-Associated Bacteria and Symbiodiniaceae in Acropora valida Under Ocean Acidification. Front Microbiol 12:3745. https://doi.org/10.3389/fmicb.2021.767174
20. Claar DC, McDevitt-Irwin JM, Garren M et al (2020) Increased diversity and concordant shifts in community structure of coral-associated Symbiodiniaceae and bacteria subjected to chronic human disturbance. Mol Ecol 29:2477–2491. https://doi.org/10.1111/mec.15494
21. Yu X, Yu K, Liao Z et al (2021) Seasonal fluctuations in symbiotic bacteria and their role in environmental adaptation of the scleractinian coral Acropora pruinosa in high-latitude coral reef area of the South China Sea. Sci Total Environ 792:148438. https://doi.org/10.1016/j.scitotenv.2021.148438
22. Pettay DT, LaJeunesse TC (2013) Long-Range Dispersal and High-Latitude Environments Influence the Population Structure

of a "Stress-Tolerant" Dinoflagellate Endosymbiont. PLoS ONE 8:e79208. https://doi.org/10.1371/journal.pone.0079208
23. Qin Z, Yu K, Wang Y et al (2019) Spatial and intergeneric variation in physiological indicators of corals in the south china sea: insights into their current state and their adaptability to environmental stress. J Geophys Res: Oceans 124:3317–3332. https://doi.org/10.1029/2018JC014648
24. Shu LI, Kefu YU, Tianran C et al (2011) Seasonal patterns of densities of symbiotic zooxanthellae in scleractinian corals from Daya Bay, northern South China Sea, and relation to coral bleaching. J Trop Oceanogr 30:39–45. https://doi.org/10.11978/j.issn.1009-5470.2011.02.039
25. Xu L, Yu K, Li S et al (2017) Interseasonal and interspecies diversities of Symbiodinium density and effective photochemical efficiency in five dominant reef coral species from Luhuitou fringing reef, northern South China Sea. Coral Reefs 36:477–487. https://doi.org/10.1007/s00338-016-1532-y
26. Jeffrey SW, Humphrey GF (1975) New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. Biochem Physiol Pflanz 167:191–194. https://doi.org/10.1016/S0015-3796(17)30778-3
27. Chen B, Yu K, Liang J et al (2019) Latitudinal Variation in the Molecular Diversity and Community Composition of Symbiodiniaceae in Coral From the South China Sea. Front Microbiol 10:1278. https://doi.org/10.3389/fmicb.2019.01278
28. Qin L, Xu Y, Chen J et al (2023) Optimization of in vitro culture method for zooxanthellae associated with reef-building corals. Acta Microbiologica Sinica 63:1658–1671. https://doi.org/10.13343/j.cnki.wsxb.20220656
29. Lyndby NH, Rädecker N, Bessette S et al (2020) Amoebocytes facilitate efficient carbon and nitrogen assimilation in the Cassiopea-Symbiodiniaceae symbiosis. Proceed Royal Soc B: Biol Scie 287:20202393. https://doi.org/10.1098/rspb.2020.2393
30. Liang Y, Yu K, Pan Z et al (2021) Intergeneric and geomorphological variations in Symbiodiniaceae densities of reef-building corals in an isolated atoll, central South China Sea. Mar Pollut Bull 163:111946. https://doi.org/10.1016/j.marpolbul.2020.111946
31. Korosh TC, Dutcher A, Pfleger BF, McMahon KD (2018) Inhibition of Cyanobacterial Growth on a Municipal Wastewater Sidestream Is Impacted by Temperature. mSphere 3:https://doi.org/10.1128/msphere.00538-17
32. Nielsen SS (2010) Phenol-Sulfuric Acid Method for Total Carbohydrates. In: Nielsen SS (ed) Food Analysis Laboratory Manual. Springer, US, Boston, MA, pp 47–53
33. Pinto F, Pacheco CC, Ferreira D et al (2012) Selection of Suitable Reference Genes for RT-qPCR Analyses in Cyanobacteria. PLoS ONE 7:e34983. https://doi.org/10.1371/journal.pone.0034983
34. Maire J, Girvan SK, Barkla SE et al (2021) Intracellular bacteria are common and taxonomically diverse in cultured and in hospite algal endosymbionts of coral reefs. ISME J 15:2028–2042. https://doi.org/10.1038/s41396-021-00902-4
35. Hinrichs S, Patten NL, Waite AM (2013) Temporal Variations in Metabolic and Autotrophic Indices for Acropora digitifera and Acropora spicifera – Implications for Monitoring Projects. PLoS ONE 8:e63693. https://doi.org/10.1371/journal.pone.0063693
36. Baker AC (2003) Flexibility and Specificity in Coral-Algal Symbiosis: Diversity, Ecology, and Biogeography of Symbiodinium. Annu Rev Ecol Evol Syst 34:661–689. https://doi.org/10.1146/annurev.ecolsys.34.011802.132417
37. Xu Y, Chen B, Qin L et al (2023) Seasonal Variation Characteristics of the Symbiodiniaceae Community Associated with Acropora pruinosa from Weizhou Island. Guangdong Agric Sci 50:164–172. https://doi.org/10.16768/j.issn.1004-874X.2023.07.017
38. Warner ME, Fitt WK, Schmidt GW (1999) Damage to photosystem II in symbiotic dinoflagellates: A determinant of coral bleaching. PNAS 96:8007–8012. https://doi.org/10.1073/pnas.96.14.8007
39. Lesser MP (1997) Oxidative stress causes coral bleaching during exposure to elevated temperatures. Coral Reefs 16:187–192. https://doi.org/10.1007/s003380050073
40. Grottoli AG, Rodrigues LJ, Palardy JE (2006) Heterotrophic plasticity and resilience in bleached corals. Nature 440:1186–1189. https://doi.org/10.1038/nature04565
41. Rosenberg E, Koren O, Reshef L et al (2007) The role of microorganisms in coral health, disease and evolution. Nat Rev Microbiol 5:355–362. https://doi.org/10.1038/nrmicro1635
42. Cho J-C, Giovannoni SJ (2004) Cultivation and Growth Characteristics of a Diverse Group of Oligotrophic Marine Gammaproteobacteria. Appl Environ Microbiol 70:432–440. https://doi.org/10.1128/AEM.70.1.432-440.2004
43. Oh H-M, Kang I, Ferriera S et al (2010) Genome Sequence of the Oligotrophic Marine Gammaproteobacterium HTCC2143, Isolated from the Oregon Coast. J Bacteriol 192:4530–4531. https://doi.org/10.1128/JB.00683-10
44. Waterbury JB, Watson SW, Guillard RRL, Brand LE (1979) Widespread occurrence of a unicellular, marine, planktonic, cyanobacterium. Nature 277:293–294. https://doi.org/10.1038/277293a0
45. Huokko T, Ni T, Dykes GF et al (2021) Probing the biogenesis pathway and dynamics of thylakoid membranes. Nat Commun 12:3475. https://doi.org/10.1038/s41467-021-23680-1
46. Agawin NSR, Duarte CM, Agustí S (2000) Nutrient and temperature control of the contribution of picoplankton to phytoplankton biomass and production. Limnol Oceanogr 45:591–600. https://doi.org/10.4319/lo.2000.45.3.0591
47. Otero-Ferrer JL, Cermeño P, Bode A et al (2018) Factors controlling the community structure of picoplankton in contrasting marine environments. Biogeosciences 15:6199–6220. https://doi.org/10.5194/bg-15-6199-2018
48. Pittera J, Humily F, Thorel M et al (2014) Connecting thermal physiology and latitudinal niche partitioning in marine Synechococcus. ISME J 8:1221–1236. https://doi.org/10.1038/ismej.2013.228
49. Pittera J, Jouhet J, Breton S et al (2018) Thermoacclimation and genome adaptation of the membrane lipidome in marine Synechococcus. Environ Microbiol 20:612–631. https://doi.org/10.1111/1462-2920.13985
50. Latasa M, Scharek R, Morán XAG et al (2022) Dynamics of phytoplankton groups in three contrasting situations of the open NW Mediterranean Sea revealed by pigment, microscopy, and flow cytometry analyses. Prog Oceanogr 201:102737. https://doi.org/10.1016/j.pocean.2021.102737
51. Schultz J, Modolon F, Rosado AS, et al (2022) Methods and strategies to uncover coral-associated microbial dark matter. mSystems 7:e00367-22. https://doi.org/10.1128/msystems.00367-22
52. Xiao L, Johansson S, Rughöft S et al (2022) Photophysiological response of Symbiodiniaceae single cells to temperature stress. ISME J 1–5. https://doi.org/10.1038/s41396-022-01243-6
53. LaJeunesse TC, Parkinson JE, Gabrielson PW et al (2018) Systematic Revision of symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts. Current Biology 28:2570-+. https://doi.org/10.1016/j.cub.2018.07.008
54. Lesser MP (1996) Elevated temperatures and ultraviolet radiation cause oxidative stress and inhibit photosynthesis in symbiotic dinoflagellates. Limnol Oceanogr 41:271–283. https://doi.org/10.4319/lo.1996.41.2.0271
55. Takahashi S, Whitney S, Itoh S et al (2008) Heat stress causes inhibition of the de novo synthesis of antenna proteins and photobleaching in cultured Symbiodinium. PNAS 105:4203–4208. https://doi.org/10.1073/pnas.0708554105
56. Liu Y, Wu H, Shu Y et al (2024) Symbiodiniaceae and Ruegeria sp. Co-Cultivation to Enhance Nutrient Exchanges in Coral Holobiont. Microorganisms 12:1217. https://doi.org/10.3390/microorganisms12061217