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High- and low-temperature stress responses of *Porites lutea* from the relatively high-latitude region of the South China Sea

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ABSTRACT

Global climate change has led to more frequent extreme temperature (extreme heat and cold) events, posing a serious threat to coral reef ecosystems. Higher latitudes are considered potential refuges for reef-building corals, but their response to extreme temperature stress in these regions remain unclear. This study, indoor simulated stress experiments ranging on *Porites lutea* from Weizhou Island in the northern part of the South China Sea, simulating suitable (26 °C) to extreme high (34 °C) and extreme low (12 °C) temperatures. Physiological, biochemical, and transcriptional responses, were analysed. Results showed *P. lutea*'s tentacles contracted, and symbiotic relationships broke down at both high and low temperatures; leading to oxidative stress, and a higher risk of disease. The coral host's response to temperature stress was positively regulated, mainly through apoptosis and metabolic inhibition pathways, whereas Symbiodiniaceae C15 showed no significant response to either high- or low-temperature stress. The coral host played a dominant role in the holobiont's stress response, using similar mechanisms for both high- and low-temperatures with some differences in the details. This study enhances understanding the temperature response mechanisms of the dominant coral species, *P. lutea* in the relatively high-latitude regions of the South China Sea.

1. Introduction

Global climate change has led to more frequent extreme temperature events (high and low) (Sanches et al., 2023; Stott, 2016; Sung et al., 2021; Vautard et al., 2024), which has contributed to the degradation of coral reefs. Corals lose their color through the expulsion of Symbiodiniaceae or degradation of photosynthetic pigments, a process known as coral bleaching (Lesser, 1997). Persistent bleaching ultimately leads to coral death (Baker et al., 2008; Glynn, 1996). Coral reefs form the foundations of reef ecosystems, and mass bleaching and death of corals have devastating impacts on all marine organisms. Thermal coral bleaching was first reported in the 1930s (Cziesielski et al., 2019). Sea surface temperature (SST) induced bleaching events have a major influence on reef coral cover degradation (Glynn, 1996; Sully et al., 2019); between 2014 and 2017, mass coral bleaching occurred worldwide due to record-breaking high temperatures (Eakin et al., 2019; Skirving et al., 2019). Low temperatures have also been suggested to cause coral bleaching. A study of coral reef profiles in the northern South China Sea found that anomalous winter cooling led to at least nine historical cold bleaching events (Yu et al., 2004 b). Not coincidentally, extremely cold winter of 2010 in Florida Keys, USA led to extensive coral bleaching (Colella et al., 2012). In summary, extremely high and low temperature events pose a serious threat to coral reef ecosystems.

Understanding the coral response mechanisms to high- and low-temperature stress is crucial for predicting their future survival. Research indicates that high-temperature stress affects coral physiology, including processes such as the rupture of symbiotic relationships, antioxidant expression, and pro-regulatory death (Cziesielski et al., 2019). For example, during a 2009 marine heat wave in Hawaii, corals exhibited significant metabolic inhibition and acid-base destabilization (Innis et al., 2021). Low-temperature stress similarly affects coral physiology (Chen et al., 2016; Li et al., 2009; Nielsen et al., 2020; Pontasch et al., 2017; Roth and Deheyn, 2013; Saxby et al., 2003).

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Nomenclature

National Oceanic Atmospheric Administration's (NOAA's)
Sea Surface Temperature (SST)
superoxide dismutase (SOD)
catalase (CAT)
peroxidase (POD)
glutathione (GSH)
Gene Ontology (GO)
Kyoto Encyclopedia of Genes and Genomes (KEGG)
Non-redundant (NR)
Clusters of Orthologous Groups (COG)
Transcripts Per Million reads (TPM)
Principal component analysis (PCA)
Differentially expressed genes (DEGs)
Least significant difference (LSD)
Tukey s-b (K)
Waller–Duncan (W)
Reactive oxygen species (ROS)

Acropora valida exhibited albinism and tissue decay at a low temperature of 14 °C (Tsang and Ang, 2015). It is therefore clear that both high- and low-temperature stresses significantly affect the physiology of corals, whether these response mechanisms are linked remains unknown. Previous studies suggest that corals may respond similarly to high- and low-temperature stress, both showing elevated antioxidant enzyme activities (Marangoni et al., 2021). However, some studies have observed different responses, such as the relatively favorable physiological status of *Acropora millepora* in the cold-treated group compared with that in the heat-treated group (Nielsen et al., 2020). In addition, cold stress was found to be more threatening to *Astrangia poculata* (Wuitchik et al., 2021), and *Acropora yongei* was found to be more severely affected by short-term lower temperatures than by high temperatures, with *A. yongei* acclimating to low temperatures and gradually improving its physiological conditions after longer periods of stress (Roth et al., 2012). These differences may be related to coral species and habitats. Therefore, further research on the mechanisms of coral temperature stress responses in other regions is required to help understand these phenomena.

High latitudes are considered future coral refuges under climate change, as they experience lower thermal stress and may be more suitable for coral survival (Beger et al., 2014). However, the response mechanisms of corals surviving at relatively high latitudes to high- and low-temperature stresses are unknown, and understanding these may be beneficial for predicting the future course of coral reefs and enabling their better protection and management. Weizhou Island (109°10′ E, 21°07′ N) located in the northern part of the South China Sea, lies at a high latitude (Yu et al., 2019). Between 1983 and 2020, 112 marine heat waves occurred in the Weizhou Island area, causing significant coral bleaching (Feng et al., 2022). Between 1960 and 2001, the lowest monthly mean SST was 17.3 °C (Yu et al., 2004 a). SST below 18 °C in winter were found to severely impact corals (Veron and Minchin, 1992). Frequent high- and low-temperature stress events on Weizhou Island have been shown to affect coral growth rates (Chen et al., 2013). In addition, long-term seasonal temperature fluctuations and anthropogenic disturbances have led to changes in the composition of coral species on Weizhou Island, and *Porites lutea* has become the dominant coral species in this region (Yang et al., 2021). The good growth ability of *P. lutea* in different environments may be attributed to its unique response strategy, therefore, *P. lutea* is an excellent coral species for studying the high- and low-temperature stress response mechanisms of corals from Weizhou Island. The reciprocal symbiotic relationship between coral hosts and Symbiodiniaceae is the key to the existence of coral reef ecosystems, and the genetic adaptations of both enable corals to cope with extreme temperatures (Howells et al., 2016). Further exploration of the response of coral host-symbiotic algae to temperature stress will help us understand the role of Symbiodiniaceae in the response of holobionts.

Therefore, this study aimed to investigate the phenological changes, physiological and biochemical indices (such as antioxidant enzyme activities) and transcriptional changes in *P. lutea* and Symbiodiniaceae. To achieve this (1) the commonalities and characteristics of the response strategies of *P. lutea* were analysed under high- and low-temperature stress, and (2) the responses of Symbiodiniaceae in the host's body analysed under extreme temperatures.

2. Materials and methods

2.1. Research sites and materials

In May 2020, *P. lutea* samples were collected from Weizhou Island (109°10′ E, 21°07′ N) by scuba diving at a depth of 4–10 m. After collection, the *P. lutea* samples were quickly sent to the Coral Reef Research Center of Guangxi University (China). *P. lutea* samples were placed in a 250 L recirculating mariculture tank at a water temperature of 26 °C for 2 weeks for recovery. SST data were obtained from Coral Reef Watch (https://coralreefwatch.noaa.gov) (Fig. 1a).

2.2. Experimental design

By analysing the satellite SST data from 1985 to 2019 (Fig. 1a), we found that the SST of Weizhou Island was generally about 30 °C in summer and 19 °C in winter. In the past few decades, The SST around Weizhou Island has risen at a rate of 0.33 °C/10yr (Yu et al., 2004 a). Therefore, we set the SST as 34 °C to simulate the possible future seawater temperature (Huang et al., 2024). For the SST analysis of Weizhou Island from 1960 to 2009, there was an extreme low SST of 13.2 °C near the island, while in Daya Bay in the northern part of the South China Sea, there was a low water temperature for 6 consecutive days (12.3 °C) in 2008. (Chen et al., 2009, 2013; Zhou et al., 2010). Therefore, stress experiments at 12 °C were conducted to simulate certain extreme weather conditions.

2.2.1. Temperature stress experiment

After the recovering for 2 weeks, 5 *P. lutea* samples were selected and each was divided into 6 fragments with a surface area of approximately 15 cm^2 to obtain a total 30 fragments. The *P. lutea* fragments were placed in three 250 L mariculture tanks with 10 fragments in each tank as the control, high-temperature, and low-temperature groups, respectively. In each tank, 250 Watt metal halide lamps and four T5HO lamps were employed to simulate sunlight, and set to 12 h:12 h (light:dark) each day. Seawater is prepared manually (Synthetic seawater was manually prepared by dissolving a mixture of salts and minerals into reverse osmosis water, simulating natural seawater) and the water flow was maintained by a pump. Biological and mechanical filtration were performed using live-rock and protein skimmers. The temperature was regulated using a heating rod and a circulating water cooler. Initially, the temperature of the three tanks was first set at 26 °C and temporarily held for 2 weeks to mitigate the effects caused by breakage. The water quality parameters were adjusted to the following: pH 8.0–8.1, salinity 34–35 parts per thousand, calcium (Ca^{2+}) 380–400 ppm, magnesium (Mg^{2+}) 1330–1350 ppm, and carbonate hardness 7–7.5 dKH. Subsequently, the water from the three tanks was mixed with each other in a 3-day cycle, and the seawater was replaced once a week and tested for consistency of the above parameters.

In the stress experiments, the control tanks were maintained at 26 °C until the end of the experiment. The high-temperature stress tanks were heated at a rate of 1 °C/d and maintained at 30 °C (summer temperature) for 3 days to allow sufficient time for the coral to adapt to the

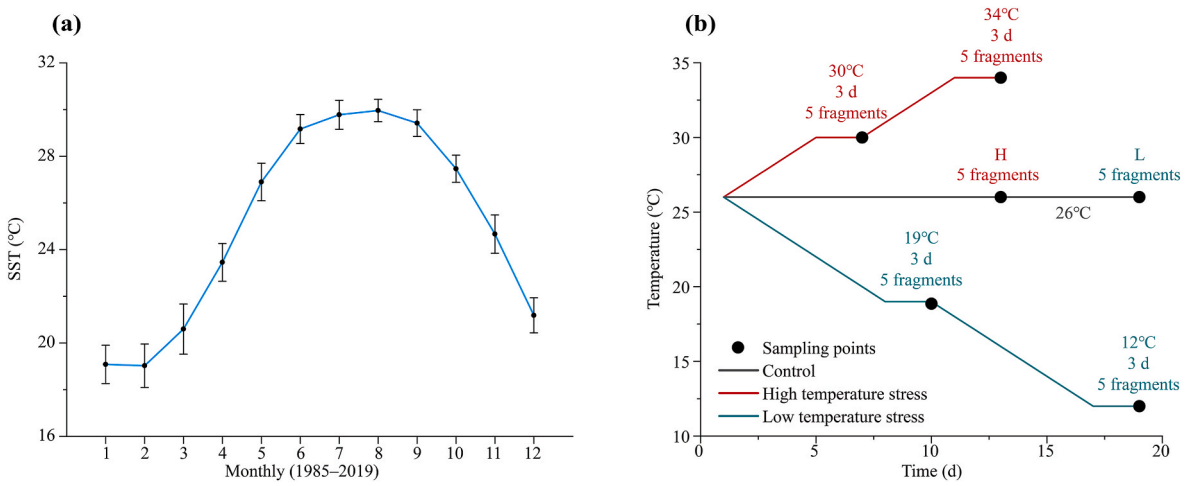

Fig. 1. SST records and experimental design. (a) Monthly mean and standard deviation of SST at sampling sites. (b) Design of high- and low-temperature stress experiments and number of samples collected at sampling points. SST: sea surface temperature.

temperature change. The temperature was then raised to 34 °C (extremely high temperature) at the same rate for 3 days to provide extreme temperature stress. On the seventh day of the experiment, five fragments of the sample at 30 °C were collected. By the thirteenth day, another set of five fragments were collected from both the 26 °C and 34 °C samples. Concurrently, the low-temperature tank was cooled at a rate of 1 °C per day, reaching 19 °C (winter temperature), which was maintained for three days, before being further cooled to 12 °C (extreme low temperature) and held for another three days. On the tenth day of the experiment, five fragments from the 19 °C sample were collected. By the nineteenth day, five fragments from both the 26 °C and 12 °C samples were collected respectively (Fig. 1b).

All samples were snap-frozen in liquid nitrogen and stored at −80 °C in a freezer prior to analysing the coral physiological and biochemical indexes and conducting a sequencing analysis. Five biological replicates were used as physiological and biochemical indicators. Three biological replicates were used to detect the composition of symbiotic algae. Due to the limitations of the cost of RNA sequencing and the difficulty of RNA extraction, three samples with high RNA quality were selected from each sample at 12 °C, 26 °C and 34 °C for analysis, and named L 1–3, C 1–3 and H 1–3, respectively.

2.3. Physiological and biochemical indicators

At 15:00 daily, we observed the coral to determine any phenotypic changes such as tentacle extension and bleaching. Underwater photographs were taken to record the condition of the tentacles and degree of bleaching (Tough TG-5, Olympus, OM Digital Solutions Corporation, Tokyo, Japan) (Figs. S1 and S2). With reference to previous methods, the maximum photon yield (*Fv/Fm*) of the corals was determined using Diving-PAM (Heinz Walz GmbH, Effeltrich, Germany) (Higuchi et al., 2015; Warner et al., 1996).

In subsequent measurements, a coral tissue homogenate is required. This involves filtering seawater through a 0.45 μm filter membrane, then injecting the filtered seawater into a dental irrigator to flush the sample surface, thereby separating the coral tissue from the skeletal structure. The volume of the rinsing solution was measured and the samples were collected and preserved separately for subsequent testing. The surface area of the coral was measured using the aluminum foil technique (cm^2) after the skeleton was dried (Marsh Jr, 1970). According to a previous method (Higuchi et al., 2008; Jeffrey and Humphrey, 1975), a suitable amount of rinsate was taken and centrifuged to measure the density of Symbiodiniaceae (cells/cm^2) using a haemocytometer plate (XB-K-25, QIUJING, Shanghai QIUJING Biochemical Reagent & Instrument Co, Shanghai, China), and the chlorophyll *a* content (Chl *a*, μg/cm^2) was measured through acetone extraction and spectrophotometry (UV-2700, SHIMADZU, Shimadzu Corporation, Tokyo, Japan). A small amount of the high-concentration rinse solution was centrifuged at 6000 *g* for 15 min, and the protein concentration in the supernatant was measured using a BCA protein analysis kit (Shanghai Sangong Biological Engineering Co., Ltd., Shanghai, China). The antioxidant enzyme activities and contents of the supernatants (A001 superoxide dismutase (SOD), A007 catalase (CAT), A084 peroxidase (POD), and A006 reduced glutathione (GSH) were measured using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions.

2.4. DNA and RNA extraction and sequencing

2.4.1. DNA extraction and identification of symbiodiniaceae

A portion of coral tissue from each sampling site was placed into a 2 mL centrifuge tube, frozen in liquid nitrogen for 15 min, and then stored in a refrigerator at −80 °C until required for use. Genomic DNA was extracted from the coral symbionts according to the instructions of the DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) and TIANamp Marine Animals DNA Kit (Tiangen Biotech, Beijing, China). A mixture of plant and animal DNA was used as a template, and the primers ITS-intfor2 (5′-GAATTGCAGA ACTCCGTG-3′) (LaJeunesse and Trench, 2000) and ITS2-Reverse (5' -GGGATCCATA TGCTTAAGTT CAGCGGGT-3′) (Coleman et al., 1994) were used for PCR amplification of the ITS2 region of the rDNA of Symbiodiniaceae. The data were analysed on the online Majorbio Cloud Platform (www.majorbio.com) using an Illumina MiSeq instrument (Illumina, San Diego, CA, USA) for high-throughput sequencing in 2 × 300 bp paired-end mode. All raw data were submitted to the NCBI for Biotechnology Information Sequence Read Archive (SRA) database (BioProject: PRJNA1026307).

2.4.2. RNA extraction and platform sequencing

Total RNA was extracted from coral samples using Trizol reagent and purified using a Micro Elute ® RNA Clean Up Kit (Omega Biotech, Guangzhou, China) according to the manufacturer's instructions. An Illumina TruseqTM RNA sample prep kit (Illumina, San Diego, CA, USA) was used for library construction, and sequencing was performed using the Illumina Novaseq 6000 sequencing platform (Illumina, San Diego, CA, USA). Data were analysed using the online Majorbio Cloud Platform (www.majorbio.com). All raw data are available from the NCBI SRA database (BioProject PRJNA1025981).

After quality control, the data were sequentially aligned to the reference genomes of *P. lutea* and C15 sub-clade using TopHat2 (http ://tophat. cbcb. umd. edu) (Kim et al., 2013; Robbins et al., 2019).

The matched genes were then aligned using BLAST on Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), Non-redundant (NR), Swiss-Prot, Pfam, and Clusters of Orthologous Groups (COG) databases for annotation. The overall gene expression levels were quantified using RNA-Seq with Expectation-Maximisation RSEM (http://deweylab.github.io/RSEM/) (Li and Dewey, 2011) software, and the quantitative metric was determined using Transcripts Per Million reads (TPM). Principal component analysis (PCA) was performed to determine the relationships and magnitudes of variation among the nine samples. DESeq2 (Ver-sion1.24.0) (Love et al., 2014) was used for differential expression analysis, and genes were differentially expressed genes (DEGs) when *Padjust*< 0.05 & |log2FC|≥1 (*Padjust* is the corrected *Pvalue*). To investigate which functional and metabolic pathways the DEGs were mainly involved in, GO and KEGG database enrichment analyses were performed. The enrichment of this pathway was considered significant at *Padjust*< 0.05.

2.5. Statistical analysis

Data obtained from the physiological and biochemical indicators were analysed by one-way ANOVA using IBM SPSS Statistics 26. The least significant difference (LSD), Tukey's s-b (K), and Waller–Duncan (W) tests were used for comparison. This selection was based on the confirmation that each group was normally distributed, and chi-squaredness, with the level of significance set at $p < 0.05$, indicated that $p < 0.05$ was considered statistically significant. The data were plotted using Origin 2023 software. Software Adobe Illustrator CC 2019 was used to organise and type-set all the images.

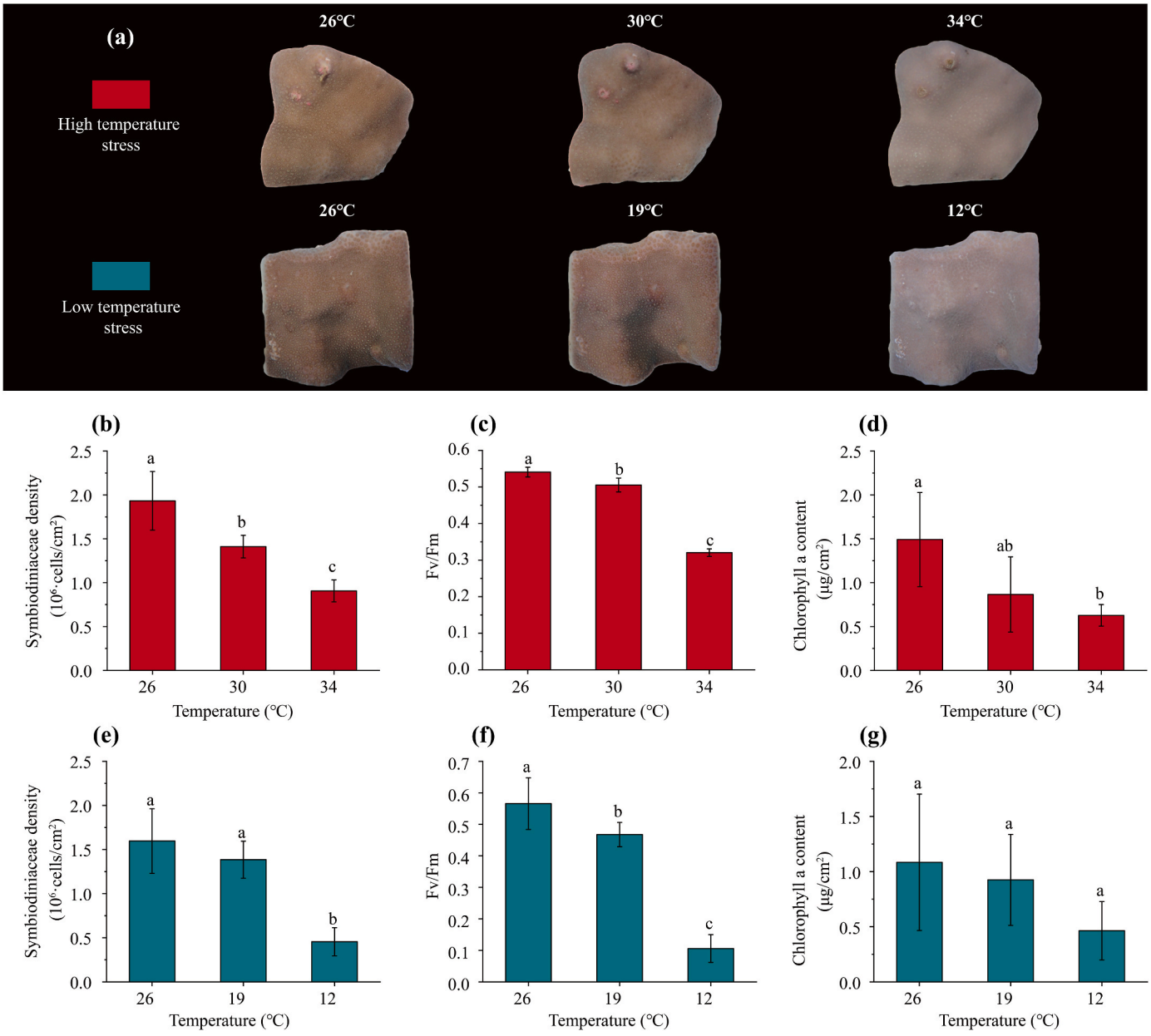

Fig. 2. Physiological changes in symbiotic functionaries. (a) Phenotypic changes in *P. lutea*. Changes in (b) Symbiodiniaceae density, (c) *Fv/Fm*, and (d) chlorophyll *a* content under high-temperature stress. Changes in (e) Symbiodiniaceae density, (f) *Fv/Fm* and (g) chlorophyll *a* content under low-temperature stress. The error line is the standard deviation (mean ± SD) of the mean obtained from multiple replications, and different letter labels indicate significant differences ($p < 0.05$) in parameters by one-way ANOVA.

3. Results

3.1. Changes in physiological indicators

During the experiment, *P. lutea* maintained healthy physiology in the control group, while the high- and low-temperature experimental groups, it exhibited tentacle contraction and color fading as the temperature changes. Coral survival under high-temperature stress appeared to be lower compared to low-temperature oblique stress (Fig. 2a, S1, S2). The density of Symbiodiniaceae associated with *P. lutea* significantly decreased at extrme temperatures (34 °C, 12 °C) compared to that of the control group (Fig. 2b and e, $p < 0.05$). *Fv/Fm* showed a significant decrease at both limiting temperatures (34 °C, 12 °C) (Fig. 2c and f, $p < 0.05$). The Chl *a* content showed a decreasing trend in both high- and low-temperature treatments.

3.2. Antioxidant enzyme activity

Compared to the control group, the activities or contents of several enzymes, except POD, tended to first increase and then decrease under the high-temperature treatment. Except for CAT, which increased significantly, changes in the activities or contents of the other enzymes were not significant ($p > 0.05$). In contrast, under the low-temperature treatment, the activities of the three enzymes, CAT, SOD, and POD, as well as the GSH content, significantly increased (Fig. 3, $p < 0.05$), and the activities or contents decreased when the temperature was extremely low.

3.3. Coral host sequencing results and analysis

Nine cDNA libraries were constructed for *P. lutea* from all temperature groups, with three biological replicates per group. A transcriptome analysis of the nine coral hosts was performed and a total of 63.3 Gb of clean data were obtained. There are more than 5.62 Gb of clean data for each sample, with a Q30 base percentage of more than 93.83%, GC content of 42.61–45.99%, and the ratio of clean reads to reference genes for each sample ranged from 46.66 to 66.58% (Table S1). In the high temperature group, PC1 and PC2 accounted for 37.92% and 23.68% of the total variance respectively, with samples at 34 °C and 26 °C roughly classified into two different regions (Fig. 4a). Although H1 was slightly closer to the control group, all sample data were retained for further analysis. In the low-temperature group, PC1 and PC2 accounted for 58.10% and 17.17% of the total variance, clearly distinguishing the control and experimental groups (Fig. 4b), and indicating consistency among the replicates in each group.

3.3.1. GO enrichment analysis of upregulated and downregulated DEGs from P. lutea in high- and low-temperature treatments

A Venn analysis of the DEG sets of *P. lutea* was performed under different temperature treatments (Fig. 4d), and *P. lutea* coped with both high- and low-temperature stress by co-upregulating 281 genes and co-downregulating 331 genes. Subsequently, GO enrichment analysis revealed that the co-upregulated DEGs were significantly enriched in netrin receptor activity, which is mainly associated with protease activity. Co-downregulated DEGs were mainly associated with antioxidant activity and transmembrane transport, including responses to oxidative stress, antioxidant activity, peroxidase activity and solute: sodium symporter activity, neurotransmitter: sodium symporter activity, solute: cation symporter activity and amidinotransferase activity (Table S2).

3.3.2. Differential expression analysis of P. lutea in high temperature

Seven hundred five DEGs (*Padjust*< 0.05 & |log2FC|≥1) were identified in the high temperature treatment group, of which, 342 and 363 genes were significantly up- and down-regulated, respectively (Fig. 4c). KEGG enrichment analysis was performed on the DEGs, and the TOP 20 pathways enriched with upregulated and downregulated genes were analysed. The results indicated that the upregulated genes were mainly associated with cellular processes, signaling and bacterial and viral infectious diseases, such as apoptosis–multiple species, PI3K-Akt signaling pathway, MAPK signaling pathway, Hepatitis B, Herpes simplex virus 1 infection, *Staphylococcus aureus* infection, Kaposi sarcoma-associated herpesvirus infection, etc. (Fig. 5a). The enriched pathways of the downregulated genes were mainly involved in environmental information processing, metabolism, and organic systems, for example, Wnt signaling pathway, drug metabolism-cytochrome P450, porphyrin and chlorophyll metabolism, arginine and proline metabolism, nitrogen

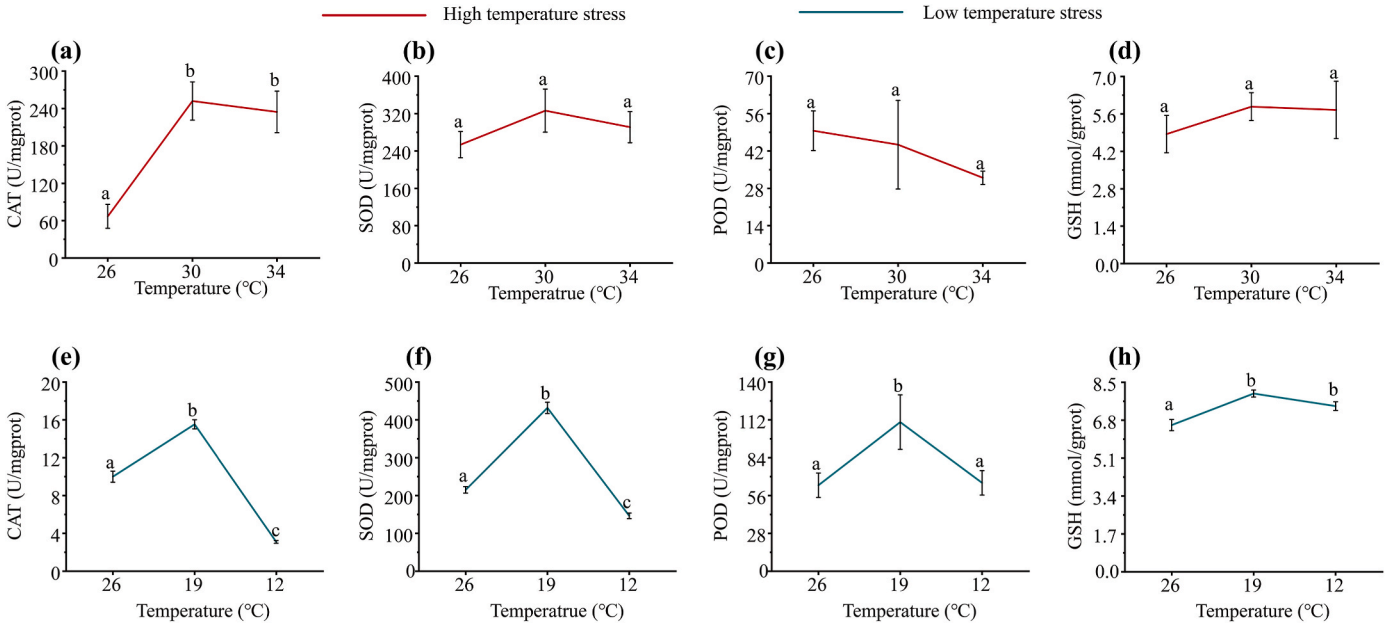

Fig. 3. Enzyme activities. Changes in (a) CAT activity, (b) SOD activity, (c) POD activity, and (d) reduced GSH content at high-temperature stress. Changes in (e) CAT, (f) SOD activity, (g) POD activity, and (h) reduced GSH content under low-temperature stress. The error line is the standard error (mean ± SE) of the mean obtained from multiple replications, and different letter labels indicate significant differences ($p < 0.05$) in parameters by one-way ANOVA.

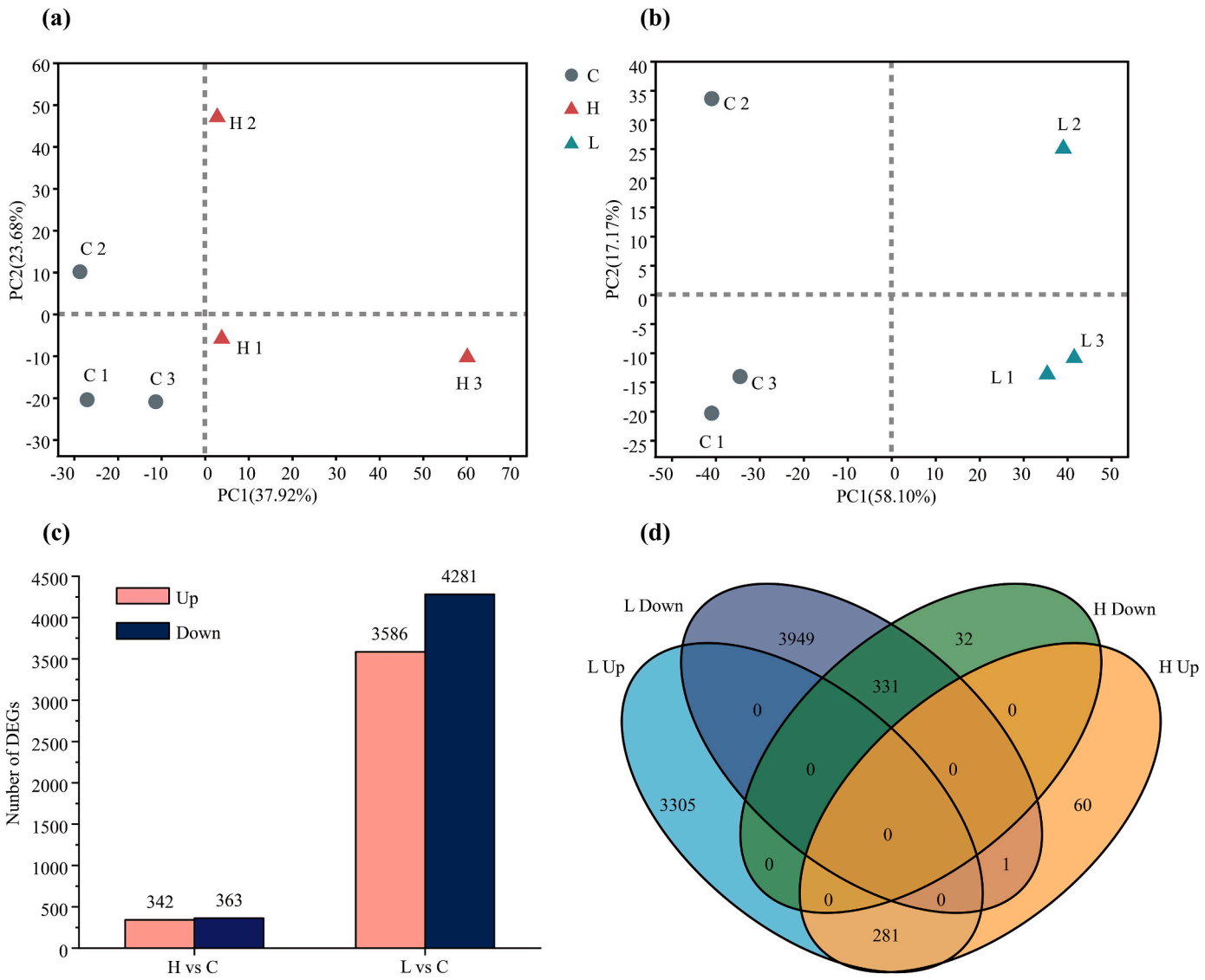

Fig. 4. Inter-sample PCA and differential expression analysis. (a) High-temperature PCA, (b) low-temperature PCA, (c) number of differentially expressed genes, and (d) gene set Venn analysis. PCA: principal component analysis; H: high-temperature; L: low-temperature; C: control temperature.

metabolism, salivary secretion (Fig. 5b).

3.3.3. *Differential expression analysis of P. lutea in low temperature*

Low temperature treatment significantly affected *P. lutea* gene expression (*Padjust*< 0.05 & |log2FC| ≥ 1), identifying 7867 DEGs, with 3586 upregulated and 4281 downregulated genes (Fig. 4c). KEGG enrichment analysis was conducted on the DEGs to explore the TOP 20 pathways. The upregulated genes were mainly involved in cellular processes, signaling, translation, folding, and sorting and degradation. It was also involved in human diseases, such as apoptosis–multiple species, necroptosis, MAPK signaling pathway, PI3K-Akt signaling pathway, protein processing in endoplasmic reticulum, transcriptional misregulation in cancer, chronic myeloid leukemia, proteoglycans in cancer, endometrial cancer, etc. (Fig. 5c). Downregulated genes were mainly associated with cellular processes, replication and repair, and metabolism, including cell cycle, DNA replication, arginine and proline metabolism, glycolysis/gluconeogenesis, tryptophan metabolism, glutathione metabolism, pyrimidine metabolism, arachidonic acid metabolism etc. (Fig. 5d).

3.4. *Symbiodiniaceae composition and sequencing analysis*

In this experiment, the dominant genus of Symbiodiniaceae associated *P. lutea* was *Cladocopium*, with C15 being the dominant type, showing a relative abundance of >92.5% (Fig. 6a). In the transcriptional sequencing analysis, nine samples of C15, like the host, yielded 22.8 Gb of clean data, and the clean data of all the samples reached more than 1.68 Gb. The percentage of Q30 bases was more than 93.35%, the GC content ranged from 42.8% to 49.27%, and the comparison rate with the reference genes ranged from 4.33% to 26.57% (Table S3). The inter-sample PCA showed no significant differences in C15 across the temperature treatments ($p > 0.05$; Fig. 6b and c). There were no DEGs in the high-temperature treatment group compared to those in the control group. There were 89 DEGs in the low temperature treatment group, of which 23 were upregulated and 63 were downregulated (Fig. 6d). However, there were no significantly enriched terms in the subsequent KEGG enrichment analysis for either the upregulated or downregulated DEGs (Table S4).

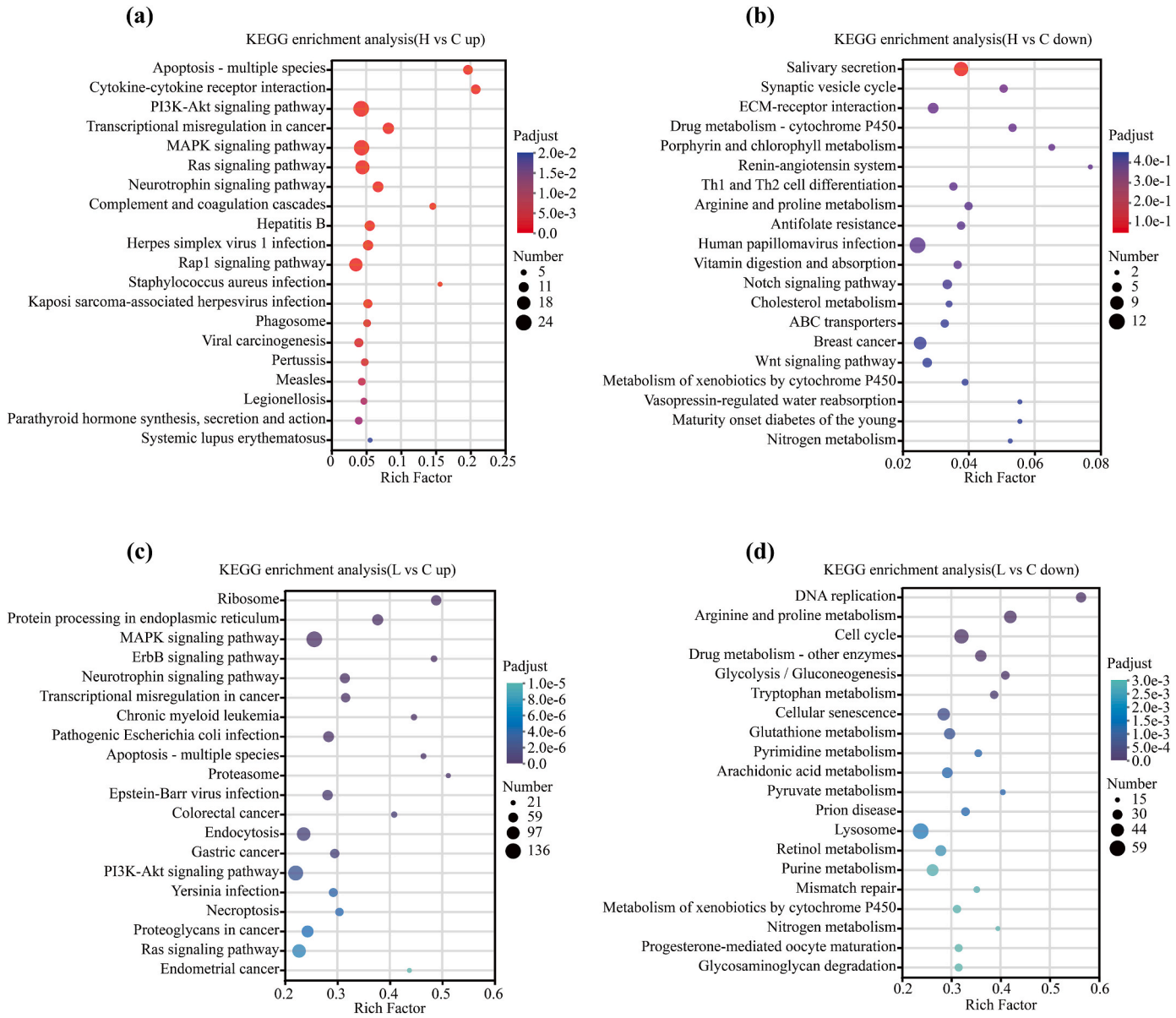

Fig. 5. Analysis of KEGG enrichment of host differential genes. High-temperature stress (a) up-regulated and (b) down-regulated pathways. Low-temperature stress (c) up-regulated and (d) down-regulated pathways. The size of the dots indicates the number of genes in each pathway, while the color of the dots corresponds to different *Padjust* ranges.

4. Discussion

4.1. Physiological status and oxidative stress of P. lutea under high- and low-temperature stresses

In our results, both high and low temperatures affected the physiological state of the coral. These temperatures caused retraction of the tentacles and color fading in *P. lutea*. Tentacle retraction increases reflectivity and prevents photochemical damage or photoinhibition under high irradiance (Brown et al., 1994), which may be a self-protective process that occurs under temperature stress. However, tentacle retraction may also lead to a reduction in the feeding ability of coral (Chen et al., 2016), which has an adverse effect on coral health. The effect of extreme temperatures led to a significant decrease in the density of Symbiodiniaceaes present on *P. lutea*. By disrupting this symbiotic relationship, corals lose their main nutrient source, suggesting that both high- and low-temperature stresses threaten coral health and ultimately lead to bleaching.

According to the oxidative theory of coral bleaching, the impaired photosynthetic machinery of Symbiodiniaceae under temperature stress generates large amounts of reactive oxygen species (ROS) in a process that occurs prior to bleaching (Lesser et al., 1990; Nielsen et al., 2018). Similar results were obtained in the subsequent analyses of antioxidant enzyme activity. The activities and contents of CAT, SOD, POD and GSH in the symbiotic functional body tended to increase and then decrease under both high- and low-temperature stress; these results are consistent with those of previous studies (Huang et al., 2022; Meng et al., 2022). It has been concluded that under moderate temperature stress (30 °C, 19 °C), ROS levels in *P. lutea* increased, triggering antioxidant mechanisms and antioxidant enzyme activities. Whereas, at extreme temperatures (34 °C, 12 °C), ROS were over-produced, and the antioxidant mechanism then collapsed (Krueger et al., 2014; Smith et al., 2005). A decrease in GSH is a hallmark of the pro-oxidant state, as accumulated O_2^- chain-reacts with GSH, further exacerbating oxidative stress (Minhas and Thornalley, 1995; Winterbourn and Metodiewa, 1994). We observed significant declines in the density of Symbiodiniaceae,

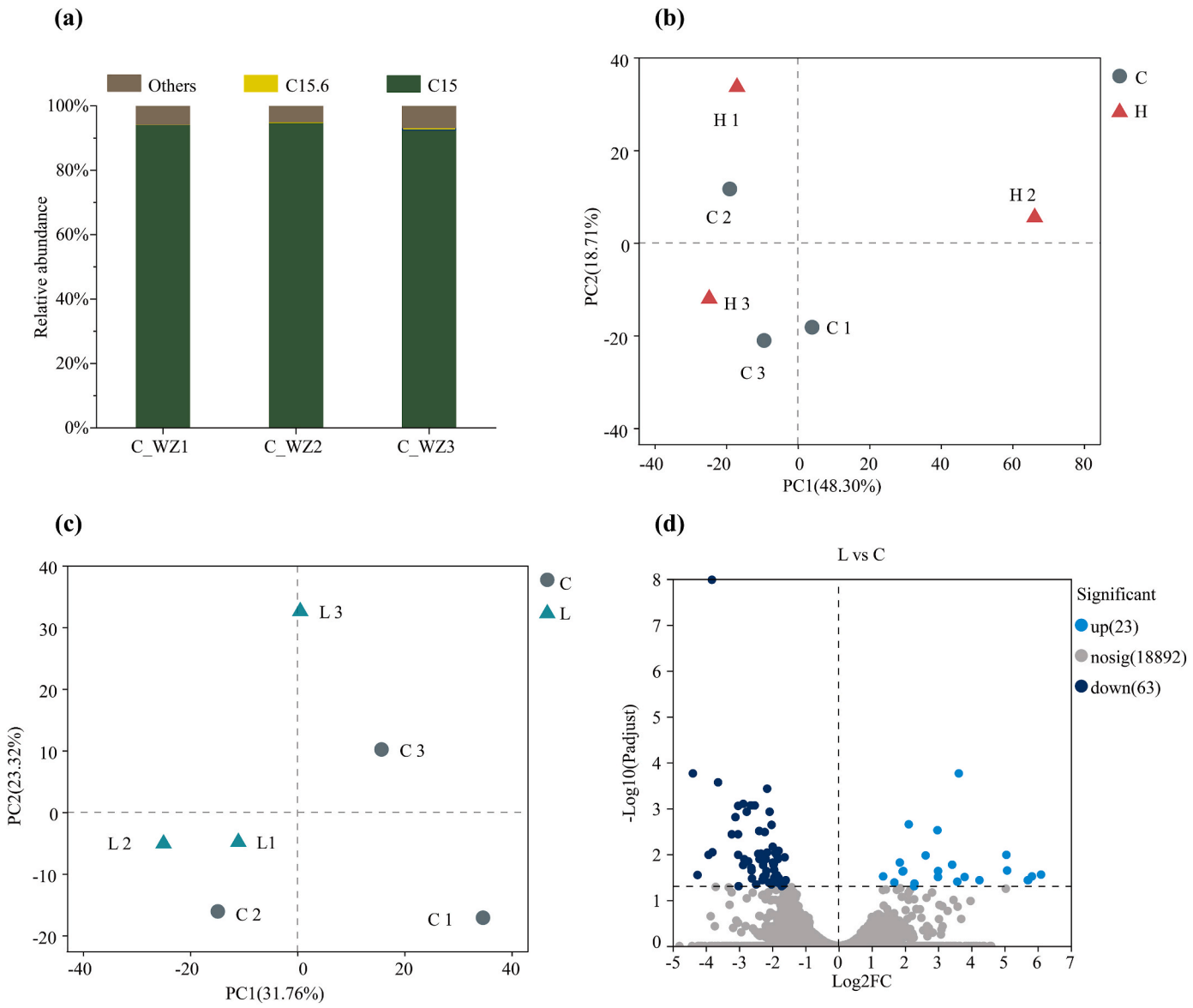

Fig. 6. Identification of lineage groups and transcriptional analysis of Symbiodiniaceae. (a) Relative abundance of different Symbiodiniaceae in coral samples. Others indicate subclades with abundance <1%. (b) PCA of the high-temperature stress group. (c) PCA of the low-temperature stress group. (d) Differentially expressed genes in Symbiodiniaceae under low-temperature stress. PCA: principal component analysis.

chlorophyll *a* concentration, and the *Fv/Fm*, which are likely indicative of the expulsion of Symbiodiniaceae. The expulsion of Symbiodiniaceae is thought to be a coral's last resort defence against oxidative stress (Downs et al., 2002), suggesting that this process of removing the main source of ROS reduces the extent of damage. Antioxidant-related GO terms were also observed in the GO enrichment analysis of DEGs downregulated at both high and low temperatures, further confirming the function of the antioxidant system. This implied that *P. lutea* experienced oxidative stress under both high- and low-temperature conditions.

4.2. High- and low-temperature transcriptional responses of P. lutea

4.2.1. Temperature stress leads to increased probability of disease in P. lutea

In the KEGG enrichment analysis at high and low temperatures, multiple upregulations in cancerous, bacterial, and viral infectious disease pathways, such as transcriptional misregulation in cancer, Hepatitis B, and Kaposi sarcoma-associated herpesvirus infection were observed. This suggests that under high- and low-temperature stress, some of the immune defences of *P. lutea* are disrupted and the probability of disease and viral infection is increased ((Fitt et al., 2001; Harvell et al., 1999). A similar observation was made for another coral species, *Porites compressa,* where there was a rapid increase in herpes virus abundance under heat stress (Thurber et al., 2008). This was further validated by the observed susceptibility of coral larvae to early molecular responses to high-temperature stress (Rodriguez-Lanetty et al., 2009).

Notably, the types of disease pathways upregulated by high- and low-temperature stress differed, with high temperatures upregulating more bacterial-viral infectious diseases in the TOP 20, whereas low temperatures upregulated more cancer-related pathways. Therefore, we hypothesized that disease susceptibility is related to the type of temperature stress experienced by corals. High temperatures can increase the number of pathogens (Harvell et al., 2002). This assumption corresponds to that of a previous study, in which models of coral white syndrome outbreaks in the Great Barrier Reef showed a strong correlation between high summer temperatures and high morbidity (Heron et al., 2010). Yu et al. (2023) observed that on Weizhou Island, there was

an increase in the abundance of opportunistic bacteria on *Acropora pruinosa* and *Pavona decussate* in summer, which consisted of opportunistic pathogenic bacteria such as *Acinetobacter* and *Vibrio*. In contrast, low temperatures may inhibit bacterial and viral activity, and reduce the incidence of infectious diseases in corals. In a coral white syndrome outbreak model, winter disease outbreaks were significantly reduced (Heron et al., 2010). Previous studies have isolated *Vibrio* from *Eunicella verrucosa,* but it did not cause disease at temperatures below 15 °C (Hall-Spencer et al., 2007). However, at extremely low temperatures, *P. lutea* upregulates disease pathways associated with cancer lesions downstream of the cancer pathways typically associated with growth and proliferation. Although excessive growth and division may lead to growth anomalies (Ricci et al., 2022), the cause remains unclear. Extreme temperatures during different seasons are generally considered possible causes of growth anomalies (Peters et al., 1986; Ricci et al., 2022), and corals under extremely low temperatures are a cause of concern for their health. This strongly suggests that both high- and low-temperature stress pose serious threats to corals.

4.2.2. Pro-apoptotic response and immunomodulation of P. lutea

Oxidative stress induced by high- and low-temperature stress as well as disease susceptibility leads to cellular, DNA, and protein damage. This activates a cellular death program and multiple immune responses in corals to maintain their stability (Haines et al., 2013; Palmer, 2018). Apoptosis is a conserved process in which cells commit suicide in a controlled manner to remove potentially harmful cells from an organism (Raff, 1998). In this study, *P. lutea* upregulated several pathways related to apoptosis under both high- and low-temperature stress conditions. In many species, apoptosis is activated through the regulation of the Bcl-2 and caspase families. In contrast, the MAPK signaling cascade activates multiple transcription factors, such as p53, to regulate downstream apoptotic genes (Leu et al., 2004). In addition, the Ras/MAPK signaling pathway can promote apoptosis through specific mediators (Ouyang et al., 2012). Similar to the significant upregulation of MAPK observed in a study on *A. pruinosa* (Chui et al., 2023), this indicates that the regulatory role of MAPK in the cellular regulation of stress is a key feature. In addition, the PI3K-AKT signaling pathway is involved in the regulation of apoptosis by phosphorylating AKT (Yap et al., 2008), and the neurotrophin signaling pathway involves neurotrophic factor receptors, such as TrkA, which play a role in inducing apoptosis in neuroblastoma cells (Muragaki et al., 1997). Apoptosis is one of the mechanisms underlying coral bleaching (Weis, 2008), and the immediate exclusion of damaged symbionts under heat stress can prevent coral bleaching (Fujise et al., 2014). Simultaneously, symbiont exclusion may reduce ROS from the impaired photosynthetic machinery, as it may be the driver of commensal ROS (Cziesielski et al., 2018). These results suggest that apoptosis in *P. lutea* may improve the survival of coral hosts by removing damaged cells and dysfunctional Symbiodiniaceae under high- and low-temperature stress. The phenomenon of activated apoptotic programs in corals, which in turn reduces commensal density, has been documented in the rise of oxidative stress caused by acute hypoxia in *Pocillopora damicornis* (Zhang et al., 2023).

However, the necroptosis pathway was significantly upregulated under low-temperature stress, implying that *P. lutea* might experience an excessive apoptotic response. Necroptosis is thought to be a mode of cell death in cells that are unresponsive to tonic stressors (Haines et al., 2013), and it may lead to inflammation (Galluzzi et al., 2017). Under low-temperature stress, *P. lutea* significantly upregulated protein processing in the endoplasmic reticulum pathway, a pathway in which most genes are associated with unfolded/misfolded proteins, implying that low-temperature stress leads to endoplasmic reticulum stress in corals. Endoplasmic reticulum stress also induces apoptosis when there is excessive accumulation of misfolded proteins (Xu et al., 2005). This is similar to the process of acute cold stress in *P. lutea* reported by Wei et al. (2024). A stronger apoptotic response of *P. lutea* at low temperatures suggests that it is a warning response, although high temperatures have serious effects on corals the hazards associated with low temperature events should not be ignored.

P. lutea also responds to temperature stress via other immune modulation mechanisms. Under high-temperature stress, coral hosts upregulate innate immune pathways, such as phagosome and cytokine–cytokine receptor interactions, which are instrumental in pathogen clearance and inflammation elimination (Flannagan et al., 2012). In contrast, the upregulation of pathways such as proteasomes and ribosomes under low-temperature stress may enhance the ability to clear unfolded/misfolded proteins and synthesise proteins, which in turn maintains cellular protein homeostasis. *P. lutea* downregulates cellular and genetic information-processing-related pathways in response to DNA damage at low temperatures. For example, p53 responds to DNA damage by participating in the p53-p21-RB signaling pathway, resulting in the downregulation of a large numbers of cell cycle genes (Engeland, 2022). The downregulation of the DNA replication pathway has also been observed in *Acropora solitaryensis* studies (Yuyama et al., 2022). However, downregulation of these pathways may lead to the accumulation of damaged DNA in corals, exacerbating the harmful effects of temperature stress, which may be a helpless means of coral survival.

4.2.3. Reduction of energy consumption through metabolic inhibition and other means

To ensure its survival under temperature stress, *P. lutea* redistributes energy in various ways, including through metabolic inhibition. Among the DEGs downregulated by low-temperature stress in *P. lutea* hosts, several metabolism-related pathways, including arginine and proline metabolism, drug metabolism and other enzymes, were enriched. This is similar to the idea derived from previous studies, which may be the autonomous regulation of energy supply by corals in the event of a symbiotic relationship breakdown and the loss of most of its energy sources (Barott et al., 2021; Jiang et al., 2022). Reduced energy expenditure is achieved by mass Symbiodiniaceae excretion as Symbiodiniaceae becomes parasitic (Huang et al., 2022). In addition to responding to DNA damage, downregulated pathways (such as the cell cycle and DNA replication) result in the division of DNA-damaged cells, which inhibits cell proliferation during stress. Energy is conserved, which allows corals to withstand adverse environments (DeSalvo et al., 2010). Most of the pathways downregulated in our KEGG enrichment analysis of DEGs at high temperatures were also related to metabolism. It is worth mentioning that the significance of pathway enrichment was lower under high-temperature stress than under low-temperature stress. This may be related to the temperature history of *P. lutea*, where frequent high SST situations have made it more tolerant to high temperatures. Nevertheless, its main strategy is to reduce metabolic processes in response to stress. In addition, the fact that most of the downregulated GO terms were related to ion transport suggests that corals choose to regulate energy under temperature stress by reducing unnecessary activities under special circumstances to reduce energy consumption.

4.3. P. lutea hosts may be critical for responding to temperature stress compared to Symbiodiniaceae

The symbiotic relationship between corals and Symbiodiniaceae may be related to environmental conditions and their long-term co-evolution. The composition of Symbiodininceae is different in most corals in the South China Sea at different latitudes (Chen et al., 2019). However, in this and other studies, C15 was highly represented in the Symbiodiniaceae composition of *P. lutea* in different regions (Chen et al., 2019; Huang et al., 2022), possibly due to the screening effect of *P. lutea* on the type of Symbiodiniaceae (Gong et al., 2018), which forms a specialised symbiosis. In contrast, C15 is considered a more tolerant symbiotic algal type (Al-Hammady et al., 2022). The high PP lutein de-epoxidation and strong light acclimation signals of C15 enable it to adapt to different

depths of light intensities (Ziegler et al., 2015), and it can be seen that this relatively specific symbiotic relationship between *P. lutea* and C15 is more conducive to its growth and reproduction. Therefore, this is also considered to be one of the factors contributing to *P. lutea* becoming a dominant species at relatively high latitudes (Gong et al., 2018).

Because of the specialised symbiosis of *P. lutea* with C15, we compared it with the reference genome of C15 and analysed changes in Symbiodiniaceae under high- and low-temperature stress. The transcriptional responses of C15 to high- and low-temperature stress were not significant. In particular, the difference from the control in PCA under high-temperature stress was not significant and no DEGs were detected, whereas under low-temperature stress, the extent of the C15 response was modest and no statistically significant data were derived from the enrichment analysis of DEGs. This is similar to the results of a previous study in which Symbiodininceae of *P. lutea* in other regions of the South China Sea did not actively respond to temperature changes under low-temperature stress (Huang et al., 2022). Bhagooli et al. (2003) also determined that coral hosts play an important role in determining the susceptibility to coral bleaching. This may be caused by changes in the energy supply relationship of the coral under temperature stress, forcing it to excrete large quantities of Symbiodiniaceae or Symbiodiniaceae for direct degradation *in situ*, which remains a coral-host-dominated sacrificial process (Al-Hammady et al., 2022). Drury et al. (2020) suggested that the host played a key role in the overall response of corals to temperature stress. Thus, *P. lutea* hosts are the key to an integrated response to temperature stress.

4.4. Key processes in P. lutea temperature stress and further considerations

We observed a relatively low number of upregulated and downregulated genes under high-temperature stress. This limited gene expression suggests that the stress response to high temperatures is concentrated within specific stress pathways. These pathways are likely critical for the temperature response of *P. lutea*, as similar processes have been observed under low-temperature stress, and the effects include antioxidant activity, immune regulation, and metabolic inhibition. Related signaling pathways, such as the PI3K-Akt and MAPK signaling pathways, are consistent under both high- and low-temperature stress, and the temperature response of *P. lutea* appears to be similar (Huang et al., 2022, 2024; Wei et al., 2024). This seems to explain why *P. lutea* does not have a trade-off between high- and low-temperature tolerance (Nielsen et al., 2020), and why high- and low-temperature stresses are the same type of stimulus for corals and will use the same set of coping programs. *P. lutea* survives at relatively high latitudes and repeatedly activates this set of regulatory mechanisms in response to the fluctuating seasonal temperatures, making it more active and exhibiting greater plasticity. This was also verified in a previous study in which *P. lutea* from the subtropical Daya Bay was more active in response to high and low temperatures than populations from the Xisha Islands. The Daya Bay populations experienced less albinism under the same stress conditions (Huang et al., 2022) (including unpublished data). Natural selection plays an important role in species evolution and local adaptation limits the adaptive potential of some corals (Baumann et al., 2021), thereby emphasising the importance of relatively high-latitude corals under future climate change.

However, the short-term acclimation of corals to different seasons can alter their tolerance to temperature stress. For instance, *Acropora muricata* samples collected in summer and winter exhibited distinct transcriptional responses to identical high- and low-temperature conditions. This variation may stem from acclimatization to the corresponding seasonal temperatures, as suggested by Lee et al. (2018) and Shiu et al. (2017). Similarly, *Astrangia poculata* collected in autumn may have acclimatized to summer's high temperatures, leading to a heightened transcriptional response to cold stress, as reported by Wuitchik et al. (2021).

Our sampling at the end of spring was strategic to avoid short-term acclimation to the cold of winter or heat of summer. Additionally, the coral samples were subjected to a one-month indoor recovery period between collection and the start of the experiment. This period likely mitigated seasonal differences and reduced the impact of the short-term thermal history. Despite our efforts to counteract the effects of short-term thermal histories, such influences are inevitable. Therefore, to gain a comprehensive understanding of the mechanisms underlying the coral temperature responses, it is essential to collect coral samples across different seasons.

CRediT authorship contribution statement

Wen Huang: Writing – review & editing, Validation, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Zhihua Huang:** Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Enguang Yang:** Resources, Methodology, Investigation, Data curation, Conceptualization. **Linqing Meng:** Methodology, Data curation, Writing – review & editing. **Jinlian Chen:** Validation, Methodology. **Ronghua Tan:** Validation, Methodology. **Zunyong Xiao:** Software, Formal analysis. **Yupeng Zhou:** Software, Formal analysis. **Mingpei Xu:** Software, Formal analysis. **Kefu Yu:** Resources, Project administration, Funding acquisition, Conceptualization.

Data statement

Raw ITS2 sequencing datasets have been deposited in the NCBI Sequence Read Archive under accession number PRJNA1026307. Raw RNA-seq data are available from the NCBI SRA (BioProject: PRJNA1025981). All data generated from this study have been deposited in public databases as described above.

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Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2024.106858.

Data availability

Raw ITS2 sequencing datasets have been deposited in the NCBI Sequence Read Ar-chive under accession number PRJNA1026307. Raw RNA-seq data are available from the NCBI SRA (BioProject: PRJNA1025981).

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