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Winter and Summer Variations in the Physiological Parameters of Two Scleractinian Corals in Sanya Bay

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Abstract: Coral reefs in Sanya Bay have been degrading in recent decades under climate change and human activities. To identify physiological changes of scleractinian corals and corresponding influencing factors, aquatic environmental factors and physiological parameters of Pocillopora damicornis, Porites pukoensis and their symbiotic zooxanthellae were examined in four Sanya Bay coral reef areas in December 2020 (winter) and July 2021 (summer). The density and chlorophyll a+c content of the symbiotic zooxanthellae were significantly high in winter and low in summer. Superoxide dismutase and caspase3 activities of corals and zooxanthellae were high in summer and low in winter, whereas catalase activity showed the opposite pattern. The variations were consistent for both coral symbionts. Water temperature and salinity were the main factors affecting the physiological variations of corals. Compared with winter, the high temperature/low salinity aquatic environment in summer reduced the density and chlorophyll a+c content of zooxanthellae, resulting in high superoxide dismutase and caspase3 activities in the corals and zooxanthellae. In addition, turbidity was an important factor affecting the physiological characteristics of coral–zooxanthellae symbionts among the four coral reef areas. Our results have important implications for understanding the changes in coral reef communities in Sanya Bay and coral reef protection.

Keywords: Pocillopora damicornis; Porites pukoensis; zooxanthellae; water temperature; turbidity

1. Introduction

Coral reefs are marine ecosystems with high biological productivity and biodiversity, and scleractinian corals are the basis for the healthy development of coral reef ecosystems [1]. The symbiosis between scleractinian corals and zooxanthellae is the most fundamental ecological feature of coral reef ecosystems [2]. The coastal areas of Hainan Island are important coral reef habitats. However, coastal coral reefs are experiencing varying degrees of degradation under anthropogenic disturbances that include fishing, aquaculture, and waste discharge [3,4]. From the 1960s to 2009, live coral cover in Luhuitou of Sanya City has declined from 90% to 12% [5] and been maintained at around 15–20% during 2016–2020 [6]. Reef-building corals have strict requirements in terms of their growth environment, and minor environmental changes may affect coral growth and lead to coral diseases or coral bleaching. Therefore, variations in the aquatic environment have a significant impact on the growth of coral reefs. Increased seawater turbidity reduces light transmission, inhibiting the photosynthesis of symbiotic zooxanthellae [7]. An environment with high temperature [8] and low salinity [9] can induce the expression of genes involved in the oxidative stress response of adult corals, disrupt material exchange between the symbionts, and lead to the loss of symbiotic zooxanthellae, resulting in coral bleaching and thereby disturbing the balance of coral reef ecosystems [10]. At the same time, the coral–zooxanthellae symbionts can adapt to changes in the aquatic environment by regulating their physiological metabolism [11]. Wang [12] found that high concentrations of...
heavy metals can activate the peroxidase system of corals to raise superoxide dismutase (SOD) and catalase (CAT) activities to permit adaptation to physiological changes under heavy metal stress in order to maintain homeostasis. The synergistic effects among aquatic environmental factors, including high concentrations of nutrient salts, high water temperature, and strong light intensity, enhance the expression of antioxidant enzymes in the symbionts and accelerate coral bleaching [13]. In addition, different coral species exhibit different levels of sensitivity to alterations in the aquatic environment [14–17]. Therefore, studying the changes in the physiological status of coral-zooxanthellae symbionts and their corresponding relationship with variations in the aquatic environment is of great scientific importance for understanding coral reef degradation as well as coral reef protection and restoration.

The Sanya Coral Reef National Nature Reserve is a national marine nature reserve in China established in 1990. Coral cover was 44.43% along the coast of Sanya City in 2007 [18]. A survey on the coverage of reef-building corals in the nature reserve in 2014 revealed coral degradation within the coral reef area, with coral cover reduced to 6.33% in the Dadong Hai, 21.58% at Luhuitou, and 15% around Dongmao Island [19]. There are a large number of branching and massive corals in the Ximao Island area. Massive corals such as Galaxea fascicularis and Porites lutea replaced branching corals such as Pocillopora damicornis, becoming the dominant coral communities in recent years [20]. There has been a decreasing trend in the coverage of branching corals in the nature reserve, while massive corals are becoming the dominant populations [21]. In 2020, the coral cover in the nature reserve was 19.93% and massive corals were the dominant group [6].

Previous studies on the coral reefs in Sanya Bay have mostly been conducted on individual coral reef areas at Luhuitou, and field experiments have mostly focused on the changes in coral communities [3,20,22] or coral health assessments [19]. Little research has addressed the spatial and temporal changes in the physiological status of coral hosts and symbiotic zooxanthellae in natural environments.

In the present study, we performed field investigations and laboratory analyses to assess the aquatic environmental factors and physiological indicators of the dominant scleractinian corals Pocillopora damicornis and Porites pukoensis and their symbiotic zooxanthellae in four major coral reef areas of Sanya Bay. Following this introduction, the rest of this paper includes four sections: Section 2 describes the sample collection and data processing; Section 3 presents the variations in aquatic environmental factors along with the physiological status of the two coral species and their symbiotic zooxanthellae; Section 4 discussed the possible effects of aquatic environmental factors on the physiological status of coral polyps and symbiotic zooxanthellae; and finally Section 5 provides a brief summary and conclusion.

2. Materials and Methods

2.1. Study Area and Sample Collection

The study area was Sanya Bay, which is located south of Hainan Island in the northern South China Sea (Figure 1a). It has a tropical maritime monsoon climate. The wet season (from April to September) and dry season (from October to March) are distinct. Samples of P. damicornis and P. pukoensis were collected in four major coral reef areas: Fenghuang Island (FHI), Luhuitou (LHT), the east side of Ximao Island (XI-E), and the west side of Ximao Island (XI-W) (Figure 1b) on 28 December 2020 (winter, dry season) and 20 July 2021 (summer, wet season). The sites of XI-E, and XI-W are far away from Sanya River, while FHI and LHT are located near the river and living quarters. Five samples of P. damicornis and P. pukoensis each with an approximate size of 5–10 cm were collected from each coral reef area at a water depth of 2–5 m. Each sample was divided into small branches (P. damicornis, branching coral) (Figure 1c) or small pieces (P. pukoensis, massive coral) (Figure 1d), immediately placed in aluminum foil bags on dry ice for temporary storage, transported to the laboratory, and stored at −80 °C.
Figure 1. Location and topography of Sanya Bay (a) and the coral and seawater sampling sites (red solid circles) in the coral reef areas of Sanya Bay (b). Photos of *P. damicornis* (c) and *P. pukoensis* (d) collected at the site. The color in (a) represents the water depth (m). The bold text in (b) is the abbreviation of the survey sites. XI-W: west side of Ximao Island; XI-E: east side of Ximao Island; FHI: Fenghuang Island; LHT: Luhuitou.

While collecting coral samples, one liter of surface seawater was collected (with three replicates) and refrigerated in high density polyethylene bottles. Before starting sampling at each site, we flushed the inside and outside of the water sampler three times with local seawater to clean the water sampler. The aquatic environmental factors were measured immediately after the water samples were delivered to the laboratory. Seawater turbidity and the concentrations of nitrate (NO₃⁻), nitrate (NO₂⁻), ammonium (NH₄³⁺), reactive phosphate (PO₄³⁻), copper ions (Cu²⁺), lead ions (Pb²⁺), zinc ions (Zn²⁺) and cadmium ions (Cd²⁺) were tested according to the methods in the Specification for Marine Monitoring [23]. The sea surface water temperature at each sampling location was recorded three times on site using a mercury thermometer. When the detection rate accounted for half or less than half of the number of surface seawater samples, one-half and one-quarter of the detection limit were taken as the readings for the negative samples in the statistical calculations, respectively.

2.2. Determination of Physiological Parameters for Coral and Zooxanthellae

2.2.1. Density and Chlorophyll Content of Symbiotic Zooxanthellae

The surface of the coral samples was rinsed using WP-462EC Teeth Flusher (Waterpik, Inc., Fort Collins, CO, USA) with pre-cooled phosphate-buffered saline (PBS) as the rinse solution. Coral and symbiont tissues (organic matter of the zooxanthellae and the coral host) were separated from coral skeletons, all rinsed slurry was collected, and the total volume of the rinse solution was measured. Then, the coral skeletons were dried in an oven at 65 °C and stored in sealed polyethylene bags for the subsequent surface area measurements. Six milliliters of well-mixed rinse solution were accurately measured to determine the density of symbiotic zooxanthellae using a hemocytometer. The counting was repeated six times for each sample to obtain the average value. The surface area of coral skeletons was measured using the paraffin-coating method [24] (linear regression R² = 0.998). The density of symbiotic zooxanthellae was expressed as the number of zooxanthellae per square centimeter of coral surface area (cells cm⁻²) [25]. Another 1 mL of coral rinse solution was collected and centrifuged at 5000 g and 4 °C for 15 min, the supernatant was discarded, the precipitate was washed twice with pre-cooled PBS, suspended in an equal volume of
100% acetone solution for 24 h in a dark place, and then centrifuged at 5000 g and 4 °C for 15 min [26,27]. The resulting supernatant was collected to measure its absorbance at 630 and 663 nm. The chlorophyll a+c content of zooxanthellae was calculated using the equation of Jeffrey and Humphrey [28], and the results were normalized to pg cell\(^{-1}\) based on the counted number of zooxanthellae cells.

### 2.2.2. Enzyme Activity of Coral Polyps and Symbiotic Zooxanthellae

The SOD, CAT, and cysteine-containing aspartate proteolytic enzyme-3 (caspase3) activities of coral polyps and symbiotic zooxanthellae were detected using the remaining rinse solution from Section 2.2.1. Specifically, 1.5 mL of well-mixed rinse solution was pipetted into a centrifuge tube and centrifuged at 5000 g and 4 °C for 15 min [27]. The supernatant containing host tissues was retained for determination of the SOD, CAT, and caspase3 activities of the coral polyps. The precipitate containing the symbiotic zooxanthellae was washed twice with pre-cooled PBS and ground after adding an appropriate amount of grinding beads. The solution obtained after grinding was centrifuged at 5000 g and 4 °C for 15 min, and the supernatant was collected to determine the SOD, CAT, and caspase3 activities of the symbiotic zooxanthellae. The protein concentrations of coral polyps and symbiotic zooxanthellae were determined using a BCA kit (PC0020; Solarbio, Beijing, China). SOD and CAT activities were examined using detection kits (A001-1 and A007-1, respectively; Nanjing Jiancheng Bioengineering Institute, Nanjing, China), with data expressed in U mg\(^{-1}\) prot\(^{-1}\). Caspase3 activity was measured using a KGA203 kit (Jiangsu KeyGEN BioTECH Co., Ltd., Nanjing, China), with data expressed as ∆OD\(_{405}\) mg\(^{-1}\) h\(^{-1}\) (OD: optical density value). The enzyme activity detection was performed according to the kit instructions.

### 2.3. Data Processing

In this paper, the aquatic environmental factors and the physiological parameters of the coral symbionts are expressed as arithmetic means. The data were statistically analyzed using SPSS 25.0 (IBM, Inc., Chicago, IL, USA), with Levene’s test and the Shapiro–Wilk test applied to assess whether the data fulfilled the normality and homogeneity of variance assumptions. One-way analysis of variance (ANOVA) was adopted if the assumptions were satisfied; otherwise, non-parametric tests were performed for comparison. Spearman’s correlation analysis and principal component analysis (PCA) were used to analyze the correlation between the physiological indicators of corals and symbiotic zooxanthellae and the environmental factors of surface seawater in the coral reef areas. A p-value < 0.05 was considered to indicate a significant difference in the statistical analyses.

### 3. Results

#### 3.1. Aquatic Environmental Factors in the Coral Reef Areas

Temperature (Figure 2a) and salinity (Figure 2b) of surface seawater showed obvious differences between winter and summer. The average water temperature in summer was 29.4 °C, higher than the average water temperature in winter of 25.2 °C. The average salinity in summer of 33.6 was slightly lower than the average salinity of 34.1 in winter. In summer, the salinities at Fenghuang Island and Luhuitou near the Sanya River were slightly lower than those on the two sides of Ximao Island, while the salinity difference in winter was not significant. Seawater turbidity was significantly influenced by the river in winter and summer. The highest turbidity (2.9 nephelometric turbidity units, NTU) was evident at Fenghuang Island near the Sanya River estuary, followed by the values obtained at Luhuitou and the east side of Ximao Island. The lowest turbidity (0.7 NTU) was evident on the west side of Ximao Island far from the Sanya River estuary (Figure 2c). The distribution of nutrient salts and heavy metal ions differed between winter and summer as well. In winter, the highest dissolved inorganic nitrogen (DIN) concentration (7.74 µmol L\(^{-1}\)) was observed at Luhuitou. The value was approximately three times the value in the other coral reef areas (Figure 2d). The highest PO\(_4^{3-}\) concentration was evident in summer on
the east side of Ximao Island (0.22 μmol L⁻¹). This value was approximately three times the concentrations in the other coral reef areas (Figure 2e). Lead (Pb²⁺) and zinc (Zn²⁺) concentrations exhibited seasonal variations, with high values in winter and low values in summer at most of the sampling sites except for the west side of Ximao Island (Figure 2g,h). In comparison, the concentrations of copper (Cu²⁺) and cadmium (Cd²⁺) did not show apparent differences between winter and summer (Figure 2f,c). The concentrations of DIN, PO₄³⁻, and heavy metals (Cu²⁺, Pb²⁺, Zn²⁺, Cd²⁺) in the four coral reef areas of Sanya Bay (Figure 2d–i) were all lower than Class I of the Sea Water Quality Standard of China (GB 3097–1997) (DIN ≤ 14 μmol L⁻¹, PO₄³⁻ ≤ 0.48 μmol L⁻¹, Cu²⁺ ≤ 5 μg L⁻¹, Pb²⁺ ≤ 1 μg L⁻¹, Zn²⁺ ≤ 20 μg L⁻¹, Cd²⁺ ≤ 1 μg L⁻¹) [29].

Figure 2. Aquatic environmental factors of surface seawater in four coral reef areas of Sanya Bay in winter and summer. The site locations (x-axis) are shown in Figure 1b. (a): water temperature; (b): salinity; (c): turbidity; (d): DIN; (e): PO₄³⁻; (f): Cu²⁺; (g): Pb²⁺; (h): Zn²⁺; (i): Cd²⁺.

3.2. Winter and Summer Variations in the Density and Chlorophyll a+c Content of Symbiotic Zooxanthellae

The density of zooxanthellae symbiotic with *P. damicornis* ranged from 9.63 to 15.98 × 10⁷ cells cm⁻² in winter and 5.31 to 14.76 × 10⁷ cells cm⁻² in summer (Figure 3a). The density of zooxanthellae symbiotic with *P. pukoensis* ranged from 10.43 to 15.89 × 10⁷ cells cm⁻² in winter and 5.38 to 16.07 × 10⁷ cells cm⁻² in summer (Figure 3c). All the coral reef areas except Luhuitou exhibited a pattern of high density in winter and low density in summer. Significance analysis revealed apparent differences between the density of zooxanthellae symbiotic with *P. damicornis* in winter and summer on the west side of Ximao Island. The density of zooxanthellae symbiotic with *P. pukoensis* displayed significant differences between winter and summer at Fenghuang Island, the two sides of Ximao Island.

Similar to the variations in the density of symbiotic zooxanthellae, the overall chlorophyll a+c content of the zooxanthellae symbiotic with two coral species was high in winter and low in summer. The chlorophyll a+c content of the zooxanthellae symbiotic with *P. damicornis* was 2.15 to 2.97 pg cell⁻¹ in winter and 0.52 to 0.89 pg cell⁻¹ in summer, with significant differences between winter and summer at all four sampling sites (Figure 3b).
The chlorophyll a+c2 content of the zooxanthellae symbiotic with *P. pukoensis* was 0.56 to 1.53 pg cell\(^{-1}\) in winter and 0.79 to 1.06 pg cell\(^{-1}\) in summer (Figure 3d), with a characteristic pattern of high content in winter and low content in summer on the east side of Ximao Island.

![Figure 3](image_url)

**Figure 3.** Density (a,c) and chlorophyll a+c2 content (b,d) of zooxanthellae symbiotic with *P. damicornis* and *P. pukoensis* in winter and summer. The site locations (x−axis) are shown in Figure 1b. Asterisk means that there are significant differences between them.

### 3.3. Variations in Enzyme Activity of *P. damicornis* and Its Symbiotic Zooxanthellae

SOD, CAT, and caspase3 activities of *P. damicornis* and its symbiotic zooxanthellae both varied significantly between winter and summer (Figure 4). SOD and caspase3 activities were higher in summer than in winter, while CAT activity was lower in summer than in winter. Significance analysis revealed that the SOD activity of *P. damicornis* was significantly higher in summer than in winter in the Fenghuang Island and Luhuitou coral reef areas (Figure 4a). Caspase3 activity was apparently higher in summer than in winter in all four coral reef areas (Figure 4c), with a summer level approximately 1.7 to 6.25 times the winter level. CAT activity of *P. damicornis* (Figure 4b) was higher in winter than in summer in the four coral reef areas, with significant differences observed on the east side of Ximao Island. SOD activity of symbiotic zooxanthellae was apparently higher in summer than in winter in all four coral reef areas (Figure 4d). Caspase3 activity of symbiotic zooxanthellae was generally low in winter and high in summer, though it only exhibited significant winter and summer differences in the Fenghuang Island area (Figure 4f).

### 3.4. Variations in Enzyme Activity of *P. pukoensis* and Its Symbiotic Zooxanthellae

Variations in SOD, CAT, and caspase3 activities of *P. pukoensis* and its symbiotic zooxanthellae were consistent between winter and summer across the four coral reef areas. All areas displayed higher SOD and caspase3 activities and lower CAT activities in summer than in winter (Figure 5). Significance analysis of SOD activity of *P. pukoensis* revealed significant seasonal differences in the Fenghuang Island and Luhuitou coral reef areas.
(Figure 5a). The seasonal differences in SOD activity of the symbiotic zooxanthellae were significant in all four coral reef areas, with a summer level of approximately three times the winter level (Figure 5d). The CAT activity of the coral and symbiotic zooxanthellae was significantly higher in winter than in summer, while SOD activity displayed the opposite pattern (Figure 5b,e). Caspase3 activity of the coral varied in the range of 1.03 to 1.76 ΔOD405 nm mg⁻¹ h⁻¹ in summer, with a prominent pattern of high level in summer and low level in winter in the Luhuitou coral reef area (Figure 5c). Caspase3 activity of the symbiotic zooxanthellae ranged from 1.92 to 2.98 ΔOD405 nm mg⁻¹ h⁻¹, which was approximately 1.4 to 5 times the winter value; the summer level was significantly higher than the winter level at both Luhuitou and the east side of Ximao Island (Figure 5f).

Figure 4. SOD, CAT, and caspase3 activities of *P. damicornis* (a–c) and its symbiotic zooxanthellae (d–f) in winter and summer. The site locations (x–axis) are shown in Figure 1b. Asterisk means that there are significant differences between them.

Figure 5. SOD, CAT, and caspase3 activities of *P. pukoensis* (a–c) and its symbiotic zooxanthellae (d–f) in winter and summer. The site locations (x–axis) are shown in Figure 1b. Asterisk means that there are significant differences between them.
4. Discussion

We observed that SOD and caspase3 activities were relatively high in summer, while CAT activity was relatively low for both the P. damicornis symbionts and P. pukoensis symbionts, with the opposite enzyme activity patterns, in winter (see Figures 4 and 5). The consistent variations in the enzyme activity of the two coral species indicate that the P. damicornis and P. pukoensis symbionts in the same habitat have consistent physiological responses to environmental changes. In summer, the high SOD activity suggests a high level of reactive oxygen species (ROS) produced in the symbiont cells [30] and activation of the ROS scavenging mechanism. Excessive accumulation of ROS in cells can damage unsaturated fatty acids in the biological membranes of symbiotic zooxanthellae and coral polyps, leading to peroxidation of membrane lipids and increased biological membrane permeability, culminating in programmed cell death [31]. Caspase3 is the core enzyme involved in the execution of programmed cell death. The activation of caspase3 marks the irreversible stage of apoptosis [30]. The high SOD activity of the corals and zooxanthellae in summer indicates that the ROS scavenging mechanism was activated and that the excessive ROS had caused damage to the cells and induced the expression of caspase3.

To explore the relationship between the changes in the physiological parameters of the coral symbionts and the aquatic environmental factors, we performed Spearman’s correlation analysis (Figure 6) and PCA (Figure 7). The density and chlorophyll a+c2 content of the zooxanthellae symbiotic with P. damicornis were obviously high in winter and low in summer in the four coral reef areas. Correlation analysis showed that the chlorophyll a+c2 content of the zooxanthellae symbiotic with P. damicornis was significantly negatively correlated with surface seawater temperature and significantly positively correlated with salinity (Figure 6a). Increased temperature [14,32] and decreased salinity [15] are critical factors promoting the release of zooxanthellae from corals and reduction of chlorophyll content. In the present study, the SOD activities of the two species of coral polyps and their symbiotic zooxanthellae were positively correlated with water temperature and negatively correlated with salinity, while the correlation trends of CAT activity were the opposite. PC1 revealed strong positive correlations between water temperature and the SOD activity of two species of coral polyps, a strong negative correlation with salinity, and a strong negative and positive correlation of CAT activity with water temperature and salinity, respectively (Figure 7). These results suggest that the increase in seawater temperature and decrease in salinity in summer are responsible for the low chlorophyll a+c2 content of the zooxanthellae symbiotic with P. damicornis, and are the main aquatic environmental factors regulating the SOD and CAT activities in coral–zooxanthellae symbionts. The relatively high correlation coefficients (Figure 6) indicate that the effect of high temperature on the activation of SOD expression in the zooxanthellae symbiotic with two coral species is more significant than that of low salinity.

The chlorophyll a+c2 content of the zooxanthellae symbiotic with P. pukoensis was higher in winter than in summer, except at Fenghuang Island (Figure 3). However, the winter and summer variations were significantly milder than those in the density and chlorophyll a+c2 content of the zooxanthellae symbiotic with P. damicornis. Zhang et al. [33] discovered similar seasonal variations in the chlorophyll content of the zooxanthellae symbiotic with different scleractinian coral species in the northern South China Sea; all zooxanthellae displayed a high content of chlorophyll in autumn and winter and low content in spring and summer. Correlation analysis did not reveal any significant correlations of chlorophyll a+c2 content of the zooxanthellae symbiotic with P. pukoensis with the changes in aquatic environmental factors, while the chlorophyll a+c2 content of the zooxanthellae symbiotic with P. damicornis was prominently influenced by temperature and salinity (Figure 6). These results indicate that compared with the zooxanthellae symbiotic with P. pukoensis, the chlorophyll a+c2 content of the zooxanthellae symbiotic with P. damicornis is more susceptible to changes in aquatic environmental factors, especially water temperature and salinity.
These findings indicate that in addition to temperature and salinity, heavy metal ions (Cd\(^{2+}\) and Cu\(^{2+}\)) and reactive phosphate have a specific impact on the expression of caspase3 in coral–zooxanthellae symbionts (P. damicornis) and P. pukoensis.) Figure 6 shows the correlation coefficients between aquatic environmental factors in winter and summer and the physiological parameters of P. damicornis symbionts (a) and P. pukoensis symbionts (b). Asterisk means that the significance level (p-value) is less than 0.05.

CAT activity of the zooxanthellae symbiotic with P. damicornis was significantly positively correlated with Pb\(^{2+}\) concentration (Figure 6a). High concentrations of Pb\(^{2+}\) can activate CAT in the zooxanthellae symbiotic with P. damicornis to scavenge the free radicals produced by the organism under heavy metal stress in order to adapt to the changes in the external environment and maintain the homeostasis of the organism [12], while a low concentration inhibits CAT expression. In addition, the caspase3 activity of the zooxanthellae symbiotic with P. damicornis was significantly positively correlated with reactive phosphate and Cd\(^{2+}\) concentrations (Figure 6a), and the caspase3 activity of P. pukoensis was significantly negatively correlated with salinity and Cu\(^{2+}\) concentration (Figure 6b). These findings indicate that in addition to temperature and salinity, heavy metal ions (Cd\(^{2+}\) and Cu\(^{2+}\)) and reactive phosphate have a specific impact on the expression of caspase3 in coral–zooxanthellae symbionts.

Figure 7. Principal component analysis for aquatic environmental factors in winter and summer and physiological parameters of P. damicornis symbionts (a) and P. pukoensis symbionts (b). SOD: superoxide dismutase of coral polyps; CAT: catalase of coral polyps; caspase3: cysteine-containing aspartate proteolytic enzyme-3 of coral polyps; SOD–Z: superoxide dismutase of zooxanthellae; CAT–Z: catalase of zooxanthellae; caspase3–Z: cysteine-containing aspartate proteolytic enzyme-3 of zooxanthellae; DIN: dissolved inorganic nitrogen.

CAT activity of the zooxanthellae symbiotic with P. damicornis was significantly positively correlated with Pb\(^{2+}\) concentration (Figure 6a). High concentrations of Pb\(^{2+}\) can activate CAT in the zooxanthellae symbiotic with P. damicornis to scavenge the free radicals produced by the organism under heavy metal stress in order to adapt to the changes in the external environment and maintain the homeostasis of the organism [12], while a low concentration inhibits CAT expression. In addition, the caspase3 activity of the zooxanthellae symbiotic with P. damicornis was significantly positively correlated with reactive phosphate and Cd\(^{2+}\) concentrations (Figure 6a), and the caspase3 activity of P. pukoensis was significantly negatively correlated with salinity and Cu\(^{2+}\) concentration (Figure 6b). These findings indicate that in addition to temperature and salinity, heavy metal ions (Cd\(^{2+}\) and Cu\(^{2+}\)) and reactive phosphate have a specific impact on the expression of caspase3 in coral–zooxanthellae symbionts.
In addition to seasonal variations, it is noteworthy that the chlorophyll a+c content of the zooxanthellae symbiotic with the two coral species showed consistent differences in four monitoring sites, being basically low in the coral reef areas near the Sanya estuary (Fenghuang Island and Luhuitou) and high in the coral reef areas far from the estuary (the east and west sides of Ximao Island) (Figure 3). We performed Spearman’s rank correlation analysis on the seawater turbidity in winter and summer with the corresponding physiological indicators of coral–zooxanthellae symbionts (figure not shown). The results revealed a significant negative correlation between the chlorophyll a+c content of the zooxanthellae symbiotic with *P. damicornis* and the turbidity in summer ($r = -1, p < 0.05$), and no correlation in winter. Chlorophyll, as the main photosynthetic pigment of zooxanthellae, plays a key role in the coral–zooxanthellae symbiotic relationship [34]. Elevated concentrations of suspended solids can decrease the photosynthetic rate of zooxanthellae [35]. The chlorophyll a+c content of the zooxanthellae symbiotic with *P. damicornis* in summer shows an increasing trend with the increase in the distance to Sanya River (FHI→LHT→XI-E→XI-W) (Figure 3b). The chlorophyll a+c content at the XI-W site was about two times that at the FHI site in summer. These results correspond to the variation of turbidity among the four coral reef sites (Figure 2c).

In addition, correlation analysis revealed that turbidity is significantly positively correlated with the caspase3 activity of *P. damicornis* in winter ($r = 1, p < 0.05$) and that of *P. pukoensis* in summer ($r = 1, p < 0.05$) (figure not shown). Wu et al. [21] pointed out that the elevated concentration of suspended solids in the water body is an important reason for decreased coral cover in other regions of the Sanya Coral Reef National Nature Reserve. The results of this study revealed the lowest seawater turbidity on the west side of Ximao Island and the highest at Fenghuang Island, consistent with our observation that the apparent status of coral growth on the west side of Ximao Island is better than that at Fenghuang Island (e.g., the corals collected on the west side of Ximao Island are darker in color), indicating that turbidity is one of the key factors affecting the physiological characteristics of coral–zooxanthellae symbionts in different coral reef areas of Sanya Bay and that the relatively low turbidity on the west side of Ximao Island is likely to be more favorable for coral growth. Sanya River is a main source of fresh water and suspended solids, which affects the spatial distribution of salinity and turbidity in the bay to a great extent [36]. Therefore, it is necessary to enhance the monitoring of aquatic environmental factors in Sanya River, especially for the protection of coral reef areas near river estuary such as Luhuitou and Fenghuang Island. Furthermore, there is a transformation of the dominant coral community in Sanya Bay from branching corals to massive corals, which is associated with the changes in aquatic environmental factors. This has important implications for other coral reef areas near river estuaries, namely, that attention should be paid to changes in riverine materials, as these may have effects on coral communities.

5. Conclusions

Based on the investigation and measurement of the aquatic environmental factors of surface seawater and the physiological parameters of two scleractinian coral–zooxanthellae symbionts (*P. damicornis* and *P. pukoensis*) in four coral reef areas of Sanya Bay in winter and summer, this study analyzed the winter and summer variations in physiological parameters of coral–zooxanthellae symbionts and explored the corresponding influencing factors. The physiological parameters of both *P. damicornis* and *P. pukoensis* symbionts show significant winter and summer variations, with consistent variation trends. In winter, the density and chlorophyll a+c content of the zooxanthellae symbiotic with corals are relatively high, the SOD and caspase3 activities of coral polyps and symbiotic zooxanthellae are low, and the CAT activity is high. The patterns in summer are the opposite. The winter and summer variations in the physiological parameters of these two coral species are closely related to water temperature and salinity. An aquatic environment with high temperature and low salinity in summer could reduce the density and chlorophyll a+c content of the zooxanthellae symbiotic with these corals and activate the expression of SOD
in coral symbionts, resulting in higher caspase3 activity. Compared with the zooxanthellae symbiotic with \textit{P. pukoensis}, the chlorophyll a+c2 content of the zooxanthellae symbiotic with \textit{P. damicornis} are more prominently regulated by the aquatic environmental changes between winter and summer. The difference in turbidity is significant among different coral reef areas, with the highest turbidity observed at Fenghuang Island near the Sanya River estuary and the lowest on the west side of Ximao Island far from the estuary. Turbidity is the key factor determining the distribution pattern of chlorophyll a+c2 content of the zooxanthellae symbiotic with the corals, which is low in the coral reef area near the estuary and high in the region far from the estuary. Under frequent extreme heat events in the most recent decade, high temperatures in summer are likely to exacerbate the negative effects of other environmental factors, especially turbidity, on the coral symbionts, and to adversely affect the coral reefs in Sanya Bay.

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References


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