Effects of pyrene exposure on immune response and oxidative stress in the pearl oyster, *Pinctada martensii*

Jia Xie a, b, c, Chunfeng Zhao a, b, 1, Qian Han a, b, Hailong Zhou a, b, * Qingxiao Li d, Xiaoping Diao a, b, **

* State Key Laboratory of South China Sea Marine Resource Utilisation, Hainan University, Haikou 570228, China
* Institute of Tropical Agriculture and Forestry, Hainan University, Haikou 570228, China
* Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, 17, Chunhui Rd, Laishan District, Yantai 264003, China
* Department of Molecular Biosciences and Bioengineering, University of Hawaii at Manoa, 1955 East-West Road, Honolulu, HI 96822, USA

**Corresponding author. Institute of Tropical Agriculture and Forestry, Hainan University, 58 Renmin Road, Haikou 570228, Hainan, China.
**Corresponding author. Institute of Tropical Agriculture and Forestry, Hainan University, 58 Renmin Road, Haikou 570228, China.

E-mail addresses: Zhouhl@hainu.edu.cn (H. Zhou), diaoxip@hainu.edu.cn (X. Diao).

1 Co-first author.

Abstract

Pyrene is a polycyclic aromatic hydrocarbon (PAH) commonly observed in aquatic ecosystems, which originates primarily from the incomplete combustion of fossil fuels and the use of petroleum compounds. Pyrene can cause the immune disturbance and oxidative stress, result in immunotoxicity, DNA damage, reduce reproduction significantly, and induce behavioral changes. Marine bivalves are commonly used as bioindicators for marine pollution, and hemolymph is a metabolite transfer medium for PAH pollutant. However, the vital immune indicator responses of pearl oyster *Pinctada martensii* hemolymph exposed to pyrene is still unclear. Thus, the immunotoxic responses of pyrene on the hemolymph of the *Pinctada martensii* were investigated in this study. After exposure to pyrene for 7 days, the total number of hemocytes (THC), cell membrane stability (CMS), phagocytic activity (PA) and total glutathione (GSHT) all decreased significantly. Pyrene also caused a significant increase in lipid peroxidation (LPO). Median effective concentrations (EC50) of pyrene on THC (4.5 μg L⁻¹) and LPO (5.2 μg L⁻¹) were lower than those for CMS (13.8 μg L⁻¹), PA (12.1 μg L⁻¹) and GSHT (7.2 μg L⁻¹), which indicates that THC and LPO were more sensitive. Additionally, a clear dose-effect relationship indicated that pyrene stimulated a marked immune response, as well as oxidative stress in *P. martensii*, which demonstrates the subtle effects of pyrene exposure on marine invertebrates and the potential associated risk.

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous pollutants in the marine environment, which possess immunotoxic characteristics, carcinogenic and mutagenic [7, 56], such as which can disrupt the immune defenses of *Crassostrea gigas* [5], *Pecten maximus* [32], *Haliothys diversicolor* [27], *Mytilus edulis* [33] and *Venerupis philippinarum* [48]. PAHs occur in crude petroleum oil or as a result of incomplete combustion and have been considered priority pollutants by the U.S. Environmental Protection Agency [38]. Pyrene is a model PAH, which has been commonly found in aquatic ecosystems and has been detected in higher concentrations than other PAHs at some creosote and pyrogenic discharge sites [15, 39, 55, 80]. Aviv et al. [4] reported that a marked accumulation of pyrene in the hemolymph and gills of the mussel *Mytilus gallo-provincialis*, as well as a particular concentration in the digestive tissues. The cellular effects of pyrene included alterations of immunological responses, peroxisome proliferation, antioxidant system, and neurotoxic effects. Additionally, pyrene can cause severe oxidative stress and DNA damage [34], reduce reproduction significantly [49] and stimulate alterations in behavior [57] in marine organisms. Additionally, we have detected the concentrations of pyrene in surface water and sediment samples in the southwestern region of Hainan Province [43, 44]. Pyrene is one of the key polycyclic aromatic hydrocarbon (PAH) contaminants in estuarine and coastal areas [57] and has become ubiquitous in the marine environment, it is necessary to assess the relevant biological endpoints which will provide further information on the...
immunotoxicity of pyrene in bivalves.

Marine bivalves are commonly used as sentinel organisms for monitoring marine pollution [41] because of their longevity, sessile lifestyle, filter-feeding behavior and tolerance of harsh environmental conditions [71]. Bivalves also exhibit relatively sensitive biological responses to stress, high amounts of bioaccumulation, and low rates of metabolizing PAHs [78]. *P. martensii* is distributed throughout the Guangdong, Guangxi, Hainan, and Taiwan provinces of China and along the Japanese coast and is one of the most commercially important pearl-producing bivalves in southern China [40]. In addition, bivalves have been widely used in previous immunotoxicity response studies [1,54,75]. The host defense system of bivalve consists of both cellular and humoral components, the former includes circulating hemocytes which can kill microbes through phagocytosis and cytotoxic reactions, while the latter includes lectins, lysosomal enzymes and antimicrobial peptides [83].

The cellular defense mainly relies on the hemocytes through infiltration, aggregation, encapsulation, cytotoxic reactions, and phagocytosis of foreign particles [22]. In addition, hemolymph is a transfer medium for xenobiotic compounds, hemocytes constitute the major immune defense of mussels [14] and are susceptible to the toxic effects of xenobiotics [59]. While the gonad, gill and hepatopancreas are the target tissues for PAH toxicity, contaminants reach these organs via the hemolymph circulatory system [30]. However, no report exists currently on the immunotoxic effects of pyrene on the immune response and oxidative stress of the commercially important bivalve *P. martensii*.

Reactive oxygen species (ROS) are a challenge to aerobic life [50]. Bivalve mussels can generate reactive oxygen species (ROS) by biotransformation [68]. Under normal conditions, the ROS are kept at a low level. However, increases in the cellular ROS level can cause a shift in the balance between oxidation and antioxidation, and induce oxidative damage to membrane lipids, proteins and DNA [37]. Studies have shown that metabolites and reactive intermediates such as diol epoxides, radical cations and redox active o-quinoines of PAHs are characterized by high redox potential. This can stimulate the formation of ROS, and react with DNA to form adducts that result in mutations [64]. A variety of parameters such as granulocyte percentage, hemocyte concentration, phagocytosis, membrane stability, ROS production and oxidative burst have been used to monitor biological responses to contaminants in the marine environment [65]. Among these, hemocyte concentration and phagocytosis were most often used as biological parameters of immunotoxicity in bivalves exposed to environmental xenobiotic compounds [18,20]. Additionally, measuring malondialdehyde (MDA) is considered an indirect way to assess lipid peroxidation (LPO), which indicates the damage to cellular membrane lipids caused by ROS [77]. Detoxification activity and antioxidant defense were determined by glutathione (GSH) assay [76].

This study investigated the effects of pyrene on immune responses (including THC, CMS and PA) and oxidative stress (including GSH and LPO) in the hemolymph of *P. martensii*. It's very important to analyze the dose-response relationships of hemolymph exposed to pyrene, which can reveal a possible relationship between oxidative stress and the inhibition of hemocyte function, demonstrates potential biomarkers, and provides early warnings for marine chemical pollution.

2. Materials and methods

2.1. Pearl oyster collection and acclimation

One hundred twenty *P. martensii* (60–80 mm shell height) were hand-picked from a trawl at a local aquaculture site in Lia'nan Bay in Hainan, China in November 2012. Oysters were immediately transferred to the laboratory and housed in a 1000 L tank containing 800 L of seawater from the sampling site. There are no industries around Lia'nan Bay, so it is considered to be a clean site. The pearl oysters were cultured in a pearl farm, and the selected batch of *P. martensii* exhibited a high level of uniformity. Therefore only a few pearl oysters were dissected, and examined for spermatozoa and oocytes under the microscope. These specimens were verified to be of similar gonad maturation and at a pre-spawning stage of gametogenesis [51]. At the time of collection, ambient seawater temperature was 21 ± 1 °C. Pearl oysters were acclimated to laboratory conditions for one week in static tanks containing recirculated and filtered seawater (32±% salinity, 21 ± 1 °C). During the acclimation period, the oysters were fed twice a week with the algal concentrate (*Isochrysis* Instant Algae® (approx. 2.7 × 10^5 cells per oyster) and water was changed daily. No mortalities were observed during the whole acclimation period.

2.2. Experimental design

Pyrene was purchased from Sigma (St. Louis, Missouri, USA), and dissolved in acetone to a concentration of 1 mg mL^-1_. The total volume of stock solution was 30 mL, and it was stored in the dark at −20 °C. These working solutions were diluted to reach the final tested concentrations of 4, 8, 16, 32, and 64 μg L⁻¹ and the final concentrations of acetone were lower than 0.01% of the tank volume. The experiment was performed in a 20 L glass tank with 15 oysters placed in each tank. The collected pearl oysters were divided into seven total groups, which included one acetone solvent control (0.01%) and one seawater control group with three replicates.

The pearl oysters were quickly transferred to the tanks and then exposed to a treatment for 7 days. To maintain water quality and constant pyrene concentrations, seawater (32±% salinity, 21 ± 1 °C) was pumped from Lia'nan Bay and filtered through a sand filter with 300 μm mesh before use. The seawater was also changed with new pyrene-fortified seawater every 24 h. The solvent blank and seawater blank were also included in the experiment and water was completely changed every 24 h. Oysters were not feed and were aerated during the exposure period, all other conditions were kept in the same conditions as those used for acclimation.

No mortalities were observed in any of the tanks after 7 days. To eliminate differences between individual oysters, hemolymph was collected from 9 pearl oysters in each tank. For each oyster, approximately 0.1 mL or less of hemolymph was drawn from the posterior adductor muscle via a 1 mL syringe and then transferred to an Eppendorf tube with the same volume of physiological saline (0.02 M HEPES, 0.4 M NaCl, 0.1 M MgSO4, 0.01 M KCl, 0.01 M CaCl2; pH 7.4) [32]. Then, the nine samples from each treatment were stored on ice to minimize cell aggregation, while the hemolymph used for GSH and LPO were stored at −80 °C until tested.

2.3. Pyrene analysis

To confirm variations in pyrene concentration over 24 h, 1 L of water was collected from the tanks immediately after the initial pyrene fortification and after 24 h of exposure, but prior to changing out the seawater. Pyrene concentration was determined according to the procedure described by Li et al. [43,44]. 500 mL of seawater was collected using 1 L amber Duran bottles. Pyrene was extracted by passing 500 mL of sample through a C18 solid phase cartridge at a flow rate of 5 mL min⁻¹ and eluting with 10 mL of methylene chloride/n-hexane (v/v = 1:1). The eluents were dried...
with anhydrous Na₂SO₄, concentrated under a gentle stream of nitrogen to near dryness, dissolved in 1 mL of methanol, and then analyzed on an Acquity Ultra Performance Liquid Chromatography (UPLC) system (Waters Massachusetts, USA). Separation was performed on an Acquity UPLC C18 column (1.7 µm, 2.1 mm × 50 mm) with an injection volume of 5 µL. The flow rate remained constant at 0.4 mL min⁻¹, and the column temperature was maintained at 30 °C. The correlation coefficient of each calibration curve was higher than 0.999. The recoveries ranged from 72.5% to 109.7%, while the relative standard deviations ranged from 6% to 22%. The limits of detection (LOD) were 0.12−1.07 ng L⁻¹.

2.4. Bioassays

2.4.1. Total hemocyte count (THC)

THCs (cells/mL) were counted using an improved Neubauer hemocytometer [32]. Briefly, a 20 µL hemolymph sample (blended from 9 oysters) was diluted at 1:4 ratio with Baker’s formol calcium, and then placed in the hemocytometer and counted under an inverted phase contrast microscope (Olympus IX50) with 40× magnification. Counts were made in triplicate for each treatment.

2.4.2. Plasma protein concentrations

Plasma protein concentrations were determined in triplicate according to the method of Bradford [10], using bovine serum albumin (BSA) as a standard. Briefly, diluted hemolymph samples were transferred in 5 µL aliquots to a microplate. 5 µL of physiological saline control and 5 µL of protein standard were added. An aliquot of 200 µL of diluted BioRad reagent (1:5 distilled water) was added to each well and the color was read at 595 nm after 20 min of incubation at 20 °C.

2.4.3. Cell membrane stability (CMS)

CMS was assessed in triplicate by determining the retention of neutral red (NR) dye [8] according to the procedure described by Hannam et al. [32]. Briefly, hemolymph samples (50 µL) were pipetted onto a 96-well microplate. After incubating the samples for 45 min at 4 °C, non-adhered cells were removed by a physiological saline rinse and 200 µL aliquots of 0.004% NR solution were added to each well. After incubating for 3 h at 20 °C, excess NR dye was washed with physiological saline, and 200 µL acidified ethanol was added to break down cellular membranes and to resolubilize the dye. The optical density (OD) of the retained neutral red was measured using a spectrophotometer at 550 nm, and the result was expressed as a function of protein content.

2.4.4. Phagocytic activity (PA)

The PA of hemocytes was measured using the uptake of neutral red-stained zymosan particles isolated from Saccharomyces cerevisiae, according to a previously established method [62]. Briefly, hemolymph samples were incubated at 4 °C for 1 h. Then, non-adhered cells were removed by rinsing with physiological saline (100 µL × 2). Next, 50 µL of dyed zymosan suspension was added. The microplate was incubated for 30 min at 20 °C, and then the phagocytic uptake was halted through the addition of 100 µL BFC. Excess zymosan suspension was removed with a physiological saline rinse, and 100 µL of acidified ethanol was added to solubilize the dye before recording the absorbance at 550 nm. Phagocytic uptake of zymosan particles by hemocytes was calculated against a standard curve and expressed as a function of protein content in a 50 µL sample.

2.4.5. Total glutathione (GSH₂)

Total glutathione in the hemolymph lysate, including the reduced form (GSH), the oxidized form (GS), and glutathione disulfide (GSSG), was determined following the method of Owens and Belcher [58]. Hemolymph samples were centrifuged at 200 g for 5 min (4 °C). The supernatant was removed, and cells were suspended in physiological saline. The hemocytes were lysed through sonication in an ice bath and stored at −80 °C until analysis. Hemolymph lysate samples (80 µL) were thawed on ice prior to adding 80 µL of 5.5'-dithiobis- (2-nitrobenzoic acid) (DTNB) solution. Aliquots of 40 µL DTNB-treated samples were transferred to a microplate, and 210 µL of glutathione reductase solution was added. After maintaining equilibrium for 1 min, 60 µL of 1 mM NAPDH was added to start the reaction, and the change in absorbance was measured kinetically at 405 nm for 10 min. Concentrations of total glutathione were calculated against a 40 µM GSH standard, and results were expressed as nmol GSH mg⁻¹ protein.

2.4.6. Lipid peroxidation (LPO)

Oxidative damage was described by hemocyte LPO, which was evaluated according to a modified version of the method of thiobarbituric acid reacting substances, (TBARS) used by Camejo et al. [11]. Hemolymph samples were thawed on ice and transferred in 40 µL aliquots to a microplate containing 10 µL BHT to prevent additional LPO. One hundred µL of extraction buffer was added to each well, followed by the addition 50 µL trichloroacetic acid (TCA) solution and 75 µL TBA solution. After incubating for 60 min at 60 °C, the plate was cooled on ice and the absorbance at 530 nm was recorded. The results were measured as malondialdehyde equivalents (MDAₑ) calculated against a standard curve using 1,1,3,3-tetraethoxypropane (0−24 µM); results were expressed as nmol MDA mg⁻¹ protein.

2.5. Statistical analyses

Biological endpoints were measured in three replicates for a total of nine individuals per treatment. The experimental data were expressed as the mean ± standard deviation (SD). Raw data were analyzed for normality using the Shapiro-Wilk and Levene’s tests and for variance using Levene’s test. The median effective concentration (EC₅₀) represents the concentration of pyrene where 50% of its effect is observed. The calculation of EC₅₀ was using GraphPad Prism 5 (GraphPad software, Inc., USA). One-way analysis of variance (ANOVA) and Tukey’s test were applied to determine significant differences in various biological parameters. A p < 0.05 was considered to be significant, and different letters (a, b, c, etc) indicated significant differences between each treatment. All analysis was performed using SPSS 16.0 software.

3. Results

3.1. Pyrene concentration variations

Seawater samples were taken 0 h and 24 h after exposure. The concentrations of pyrene at the initial time (0 h) ranged from 88% to 93% of the nominal exposure concentrations (Table 1). 0 h after exposure, the pearl oysters were not in the tank, so the loss of pyrene may be due to pyrene binding to the tank walls. Pyrene levels in the exposure tanks decreased after 24 h of exposure with a 34–62% reduction in initial pyrene concentration in all treatment groups (Table 1). The loss of pyrene may be due to the binding of the drug to the tank walls and oysters shells, absorption by the pearl oysters, or photolysis. No pyrene was detected in the control group.

3.2. Total hemocyte count

Upon exposure to 4–64 µg L⁻¹ of pyrene, in general, the total
number of hemocytes decreased \( (p < 0.05) \) following a dose-dependent relationship (Fig. 1a). Compared with control group, THC significantly decreased at 8 \( \mu \text{g L}^{-1} \) of pyrene, however there has no significance among 8, 16, 32 and 64 \( \mu \text{g L}^{-1} \) of pyrene. The lowest THC (5.68 \( \times 10^6 \) mL\(^{-1}\)) was observed in the 64 \( \mu \text{g L}^{-1} \) treatment group, in which the number of hemocytes decreased by 52\% in comparison to the control group \( (p < 0.01) \). The effective concentration of pyrene causing a 50\% (EC\(_{50}\)) decrease in THC was approximately 4.5 \( \mu \text{g L}^{-1} \) (Table 2). The no observable effect concentration (NOEC) occurred at 0 and 4.0 \( \mu \text{g L}^{-1} \) of pyrene (Table 2). In addition, there was no significant difference between the blank seawater and solvent control groups \( (p > 0.05) \).

### 3.3. Cell membrane stability

Pyrene exposure significantly affected CMS at concentrations of 16, 32 and 64 \( \mu \text{g L}^{-1} \) \( (p < 0.01) \) (Fig. 1b), similar to the THC, there has no significance among 16, 32 and 64 \( \mu \text{g L}^{-1} \) of pyrene. The greatest decrease in cell membrane stability was observed with the highest pyrene concentration (64 \( \mu \text{g L}^{-1} \)), which was 74\% lower than the control group after 7 d \( (p < 0.01) \). The EC\(_{50}\) for CMS was 13.8 \( \mu \text{g L}^{-1} \) of pyrene, while the LOEC and NOEC were 8.0 and 4.0 \( \mu \text{g L}^{-1} \), respectively (Table 2).

### 3.4. Phagocytic activity

PA in \( P. \) martensii was significantly altered by pyrene at concentrations of 8, 16, 32 and 64 \( \mu \text{g L}^{-1} \) \( (p < 0.01) \), there has no significance among 32 and 64 \( \mu \text{g L}^{-1} \) of pyrene. With the number of phagocytosed particles decreasing from 24.88 \( \times 10^5 \) mg\(^{-1}\) proteins in the control group to 11.05 \( \times 10^5 \) mg\(^{-1}\) proteins in the 64 \( \mu \text{g L}^{-1} \) treatment group \( (p < 0.01) \) (Fig. 1c). The EC\(_{50}\) for PA was 12.1 \( \mu \text{g L}^{-1} \) of pyrene, while the LOEC and NOEC were 8.0 and 4.0 \( \mu \text{g L}^{-1} \), respectively (Table 2).

### 3.5. Total glutathione

Pyrene caused a significant depletion of glutathione (GSH + GSSG) in \( P. \) martensii (Fig. 2a). GSH\(_T\) levels were the lowest (8.22 nmol mg\(^{-1}\) protein) in the specimens placed in the highest pyrene concentration group (64 \( \mu \text{g L}^{-1} \)), and there has no significance among 8, 16, 32 and 64 \( \mu \text{g L}^{-1} \) of pyrene. The GSH\(_T\) levels were 55\% less than the concentration recorded in the control group. The EC\(_{50}\) for glutathione depletion was 7.2 \( \mu \text{g L}^{-1} \) of pyrene, while the LOEC and NOEC were 4.0 and 0 \( \mu \text{g L}^{-1} \), respectively (Table 2).

### 3.6. Lipid peroxidation

LPO was significantly affected by pyrene exposure \( (p < 0.01) \) (Fig. 2b). LPO levels increased significantly begin at the concentrations of 8 \( \mu \text{g L}^{-1} \), which increased with elevating exposure to pyrene \( (8–64 \mu \text{g L}^{-1}) \), there has no significance among 8, 16,
Table 2
Summary of LOEC, NOEC and EC50 of pyrene on varying physiological and biochemical parameters of Pinctada martensii.

| Parameters | LOEC (µg L⁻¹) | NOEC (µg L⁻¹) | EC50 (µg L⁻¹) |
|------------|---------------|---------------|---------------|
| THC        | 4             | 0             | 4.5           |
| CMS        | 8             | 4             | 13.8          |
| PA         | 8             | 4             | 12.1          |
| GSH₄        | 4             | 0             | 7.2           |
| LPO        | 8             | 4             | 5.2           |

LOEC: lowest observable effect concentration.
NOEC: no observable effect concentration.
EC50: median effective concentration.

32 µg L⁻¹ of pyrene. In the 64 µg L⁻¹ pyrene treatment, a maximum LPO of 9.35 nmol MDA mg⁻¹ protein was observed, which was 72% higher than that of the control group. The EC50 for LPO was 5.2 µg L⁻¹, and the LOEC was 8.0 µg L⁻¹ (Table 2).

4. Discussion

4.1. Pyrene concentration

A reduction in pyrene concentrations over 24 h may indicate bioavailability and uptake, as well as attachment to the walls of the tank and oyster shells. The log Kow value of pyrene is 4.57 [73]. To maintain water quality and constant pyrene concentrations, it is necessary to regularly exchange the water in the tank every 24 h, replacing it with fresh seawater fortified with the appropriate concentration of pyrene.

4.2. Immunity responses of P. martensii to pyrene

The relationship immunity-environment is complex, immune system is strongly influenced by environmental conditions, including the environmental pollution, such as pyrene. Pyrene is potent immunosuppressors, even at a low concentration [24]. Bivalves rely exclusively on an innate, nonlymphoid system of immune reactions. Hemocytes play a crucial role in the immune system of bivalve mollusks when they carry out immune surveillance [74]. Thus, the number of hemocytes can be a useful reflection of the host’s health [2]. In this study, hemocyte counts decreased during exposure to pyrene, with concentrations ranging from 4 to 64 µg L⁻¹ of pyrene. These results are consistent with those found on Arctic scallops Chlamys islandica exposed to oil [30] and abalones exposed to benzo(a)pyrene (BaP) [26]. However, some studies reported that THCs were significantly increased in bivalves after oil spill exposure [3,16] and phenanthrene stress [32]. This may be because of differences in the pathways that are involved in responding to various chemicals in different species. PAHs can cause cytolysis in lysosome-rich cells like hemocytes [53], and the significant decrease in THCs observed in this study was likely due to PAH-induced cell death. Other research has shown that decreases in THCs persisted for up to 20 days in bivalves after PAH exposure [35,61]. Decreases in THC and cell membrane stability may result from the lipophilic binding of pyrene to membrane lipids, which endangers basic cellular functions by altering fluidity and ionic pumps [12]. Because PAHs can penetrate bivalve membrane systems [45], this type of decrease in cell membrane stability has also been studied in bivalves exposed to phenanthrene and water from North Sea oil-wells [31,32], dispersed oil [6], and low molecular weight PAHs, such as anthracene and phenanthrene [23]. The observed reduction in phagocytic activity is consistent with significantly damaged cell membrane stability, which suggests that decreases in membrane size may have contributed to lower phagocytic activity. Phagocytic processes depend on the membrane properties of hemocytes. However, PAHs can alter the fluidity of cell membranes [46] and restrict membrane deformation, which is essential to phagocytic endocytosis [28]. This result is supported by observations on the effects of a low-dose, short-term stimulation of phagocytosis on the bivalve response to contaminants such as diesel, metals [63,70], water from industrial sources [31], and pesticides [66].

4.3. Antioxidant responses of P. martensii to pyrene

Marine bivalves can take in contaminants from the water column, suspended particulate material, food sources, and bottom sediments [42]. They have been widely used as indicator species for pollutants in marine environments [69]. The toxic effects of PAHs depend not only on the availability of the contaminants but also on such factors such as species, photoinduced potential, metabolism, temperature, salinity, and pH. For example, one previous study Fernandez-Tajes et al. [19] showed a higher sensitivity of clams and cockles to pollution load levels. Additionally, results from Pelletier et al. [60] indicated that the glochidial larvae of Utterbackia imbecillus are sensitive to photoactivated pyrene at environmentally

Fig. 2. Antioxidant responses: variation trends of GSH₄ (a) and LPO (b) of P. martensii after exposure to pyrene for 7 d. Data are expressed as Means ± SD. Significant differences are indicated by different lower case letters (a, b, c) (Tukey’s test, p < 0.05).
relevant concentrations, and demonstrated that the LC50 for pyrene at 24 h was 2.63 (2.38–2.90) µg L−1 in the glochidia of Utterbackia imbecillis. Additionally, phototoxicity can affect marine species [82]. For these reasons, biomonitoring has become an alternative and a complement to chemical measurement for PAH assessment. In particular, the parameters of antioxidant systems can be useful biological indicators for determining not only exposure to contaminants but also toxicity measures such as GSH and LPO. GSH plays a key role in the antioxidant defense system, and can remove hydrogen peroxide (H2O2) [29]. Increases in total GSH concentrations reflect an up-regulation of antioxidant defenses [36]. However, in this study a significant decrease in total GSH concentrations was observed. This likely causes the antioxidant capacity to be overwhelmed, and could result in a mass oxidation of GSH and lead to the excretion of oxidized molecules (GSSG) [47].

5. Conclusions

The homoeostasis in bivalves are key indicators of their health and stress, and the status of an invertebrate’s health may be predicted by measuring its homoeostatic function. This study revealed that exposure to pyrene significantly inhibited immune-related activity in oysters, as exhibited by significant decreases in THC, CMS and PA. Furthermore, a significant correlation between oxidative stress and changes in immune cell counts indicated that the PAH-induced stimulation of ROS production was likely to be a primary contributor to reductions in an organism’s immunocompetence. LPO is the most sensitive oxidative damage endpoint. Significant oxidative damage and reduction in THC after PAH exposure demonstrated that P. martensii was susceptible to the immunotoxic effects of pyrene.

Acknowledgements

This work was funded by the National Natural Science Foundation of China (31160126), the Specialized Research Fund for the Midwest Program of Hainan University (ZXB11H-XK002) and the Specialized Research Fund for the Doctoral Programme of Higher Education of China (20114601120001). The authors would like to thank the editor and the anonymous reviewers for their valuable comments and suggestions regarding this paper.

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