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Antioxidant Response of Ridgetail White Prawn *Exopalaemon carinicauda* to Harmful Dinoflagellate *Prorocentrum minimum* Exposure and Its Histological Change

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Abstract The dinoflagellate *Prorocentrum minimum*, one of the most widespread red tide causing species, affects marine aquaculture and ecosystems worldwide. In this study, ridgetail white prawn *Exopalaemon carinicauda* were exposed to *P. minimum* cells (5×10^4 cells mL^{-1}) to investigate its harmful effects on the shrimp. Antioxidant activities and histological changes were used as indicators of health status of the shrimp. In 72 hours, the mortality of *E. carinicauda* was not affected, but its antioxidant response and histology were statistically different from those of control. Elevated superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities and depressed catalase (CAT) activity were observed in gill; while increased SOD, glutathione S-transferase (GST), CAT activities and modulated GPX activity were observed in hepatopancreas. Thus, antioxidant activities in gill and hepatopancreas seem to respond differentially to harmful alga exposure. Increased malondialdehyde (MDA) content in early a few hours indicates the damage of the antioxidant defense system. Although MDA content recovered to a low level thereafter, a series of histological abnormalities including accumulation or infiltration of hemocytes, tissue lesions and necrosis were discovered in gill and hepatopancreas. Exposure to *P. minimum* induced sublethal effects on *E. carinicauda*, including temporary oxidative damage and histological injury.

Key words *Prorocentrum minimum*; *Exopalaemon carinicauda*; antioxidant response; histological changes

1 Introduction

Shrimp culture is one of the pillars of China's marine aquaculture. In shrimp farming industry, the economically important ridgetail white prawn (*Exopalaemon carinicauda*) mainly distributed in the coasts of Yellow Sea and Bohai Sea, China (Li *et al*., 2002). Recently, the culture area of *E. carinicauda* has been extended because of its fast growth, excellent nutritional properties, good reproductive performance and wide environmental adaptability (Wang *et al*., 2005). Phytoplankton is an important constituent in shrimp ponds and plays a role in regulating the stability of community structure. However, with the addition of organic matter and nutrients, the phytoplankton growth increases and harmful algal blooms (HABs) may develop (Alonso-Rodríguez and Páez-Osuna, 2003). HAB events have been described in shrimp ponds in different regions of the world (Alonso-Rodríguez and Páez-Osuna, 2003), and in some cases, harmful algae exposure can cause morbidity and mortality and delay the growth of shrimps (Alonso-Rodríguez and Páez-Osuna, 2003; Páez-Osuna *et al*., 2003). According to Alonso-Rodríguez and Páez-Osuna (2003), heavy dinoflagellate blooms can affect shrimp by poisoning, causing anoxia and producing mucus.

Harmful algae represent a type of environmental stress, which is well known to induce oxidative stress, alter cellular redox balance (Pinho *et al*., 2005; Liang *et al*., 2014; Huang *et al*., 2015), and generate highly reactive oxygen species (ROS) (Oda *et al*., 1992, 1997; Kim *et al*., 2007). Cells have evolved antioxidant defense systems to aid organisms to eliminating ROS. The involved enzymes include glutathione S-transferase (GST), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX). If not scavenged quickly, excessive ROS can damage important biomolecules, causing cell damage and even death (El-Beltagi and Mohamed, 2013). The damages may associate with protein oxidation, DNA breaking and lipid peroxidation (LPO) (Ott *et al*., 2007). The malondialdehyde (MDA) level is an indicator of LPO induced by ROS (Winston, 1991), and reflects the degree of cell damage.

Histopathological evaluation has become an important

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tool in aquatic toxicology to provide information on the health status of organisms, and it has been implemented in many biomonitoring programs (Cuevas *et al*., 2015). The changing patterns of cells can be used as an index to determine the impact of contaminants (Moore, 1985; Boudet *et al*., 2015). In recent years, histological change has been applied in shrimp species as a tool to assess the effect of pollutants on aquatic organism (Bhavan and Geraldine, 2000; Chang *et al*., 2006; Wu *et al*., 2008; Boudet *et al*., 2015; Karthikeyan *et al*., 2015). Odendaal and Reinecke (2003) suggested that histological alteration, together with other biomarkers, could be used as tools to evaluate the toxicity of environmentally relevant chemicals in both predictive and retrospective ways.

The dinoflagellate *Prorocentrum minimum* is one of the most widespread red tide causing species which may damage marine aquaculture by affecting the survival and growth of aquatic organisms (Alonso-Rodríguez and Páez-Osuna, 2003; Azanza *et al*., 2005; Tango *et al*., 2005). The toxicity of *P. minimum* is not clear (Landsberg, 2002; Vlamis *et al*., 2015), but several death events have been associated with *P. minimum* at high concentrations ($>10^3$ cells mL^{-1}) (Landsberg 2002; Alonso-Rodríguez and Páez-Osuna, 2003; Azanza *et al*., 2005; Heil *et al*., 2005; Sierra-Beltrán *et al*., 2005). The damage may be explained by both indirect biomass effects (*e.g.*, low dissolved oxygen) and toxic effects (Azanza *et al*., 2005; Heil *et al*., 2005; Sierra-Beltrán *et al*., 2005). *P. minimum* is often found in shrimp ponds (Alonso-Rodríguez and Páez-Osuna, 2003; Xu *et al*., 2010), and *P. minimum* at high concentrations seems to cause shrimp stress, affecting its growth and making it more vulnerable to viral diseases (Alonso-Rodríguez and Páez-Osuna, 2003). However, little information is available regarding the effect of *P. minimum* on shrimp.

This study was carried out under oxygenated conditions to determine the toxic effect of *P. minimum* exposure on shrimp *E. carinicauda*, based on histological change and enzyme activities involved in antioxidant response. This study will provide new insights into the toxic mechanism of *P. minimum* on shrimp.

2 Material and Methods

2.1 Animals

Healthy adult shrimp *E. carinicauda* (mean weight = 1.62 g ± 0.16 g) were collected from a commercial farm in Ganyu, Jiangsu, China. They were acclimated in 200 L plastic containers, containing aerated seawater (salinity 30 and pH 8.2) at 25℃ ± 1.0℃ for one week prior to experiment. During acclimation, *E. carinicauda* were fed with commercial prawn pellets three times a day, and seawater was renewed every two days.

2.2 Microalga

The dinoflagellate *P. minimum* was isolated from a farm in Laoshan, Qingdao, China, where a *P. minimum* bloom occurred. In this bloom, the cell concentration reached 5×10^4 cells mL^{-1}. The alga was batch-cultured in 5 L glass flasks in filtered sea water (0.45 μm) supplemented with f/2 medium at 25℃ and under irradiance of 52 μmol photons $m^{-2}s^{-1}$ and a 14L:10D photoperiod. Alga was fixed with Lugol's solution and counted by a plankton counting chamber with a 100 μm orifice before experiment.

2.3 Experimental Design and Sample Collection

Cells of *P. minimum* at late exponential growth phase were used. At a concentration of 5×10^4 cells mL^{-1}, *P. minimum* was used in toxic exposure treatment, and filtered seawater was used as control. The two groups were run in triplicate, and each randomly consisted of 70 healthy individuals of *E. carinicauda*. *E. carinicauda* were fed with commercial prawn pellets three times a day. The water was renewed every two days.

Eight individuals were taken randomly at 0, 3, 6, 12, 24, 48, and 72 hours each replicate. Individuals were carefully dissected. Their gill and hepatopancreas were collected, frozen in liquid nitrogen, and then stored at −80℃. Five individuals were collected each replicate at the end of experiment (72 h) for histological evaluation.

2.4 Biochemical Parameter Assays

The samples were homogenized (1:10, w/v) in cold phosphate buffer saline (pH 7.4) and centrifuged at 3000 × *g* and at 4℃ for 10 min. The supernatant was transferred and used for biochemical assays. The biochemical parameters were determined with commercial test kits (Jiancheng, Ltd., Nanjing, China) and measured on an Infinite M2000 Pro™ plate reader (Tecan, Germany).

GST was determined according to Habig *et al*. (1974) by monitoring the conjugation of reduced glutathione with 1-chloro-2, 4-dinitrobenzene (CDNB) at 340 nm. SOD activity was determined according to the xanthine/xanthine oxidase method by Marklund and Marklund (1974). CAT activity was measured by monitoring residual H_2O_2 absorbance at 405 nm (Goth, 1991). The activity of GPX was estimated with dithio-binitrobenzoic acid method (Rotruck *et al*., 1973), using H_2O_2 and GSH as substrates. The protein content was measured with the method described by Bradford (1976) using bovine serum albumin as a standard. The MDA content was measured using the thiobarbituric acid test according to Ohkawa *et al*. (1979).

2.5 Histological Observation

Shrimp were carefully dissected with their gill (wrapped up with wax paper to prevent scatter) and hepatopancreas immediately fixed in Davidson's solution for 24 h, and then transferred to 70% ethanol. After dehydrated with alcohol at a series of concentration, tissues were embedded in paraffin. Sections 5 μm in thickness were obtained with a conventional microtome and stained with hematoxylin and eosin (H.E.). The histological change was observed using a light microscope (Olympus BX60 microscope).

2.6 Statistical Analysis

The results were expressed as means ± standard deviation (SD) ($n=8$). After testing the homogeneity of variances, statistical difference between treatments was analyzed by a one-way analysis of variance (ANOVA) followed by a multiple comparison of LSD test. The level of significance was set at $P<0.05$ (*) and $P<0.01$ (**). All statistical analyses were performed using SPSS statistical package (ver. 19.0, SPSS Company, Chicago, USA).

3 Results

3.1 Effect of *P. minimum* on the Mortality of *E. carinicauda*

The mortality of *E. carinicauda* did not significantly differ between toxic (1.43% ± 1.43%) and control (0.48% ± 0.82%) treatments ($P>0.05$), suggesting that *P. minimum* exposure did not affect shrimp mortality.

3.2 Effect of *P. minimum* on Antioxidant Activity

Compared with the control, GST activity in gill only significantly increased in 6 h ($P<0.01$). Then it decreased but no significantly from 12 h to 72 h ($P>0.05$; Fig.1). In hepatopancreas, GST activity increased significantly ($P<0.01$) and peaked at 3 h (1.73 fold of control). Then it decreased but remained significantly higher than that of control ($P<0.01$; Fig.1).

SOD activity was significantly affected by *P. minimum* exposure ($P<0.01$, Fig.2). Compared with that of control, SOD activity in gill and hepatopancreas showed a similar pattern, significantly increasing first ($P<0.01$), peaking at 3 h (1.89 and 1.9 fold of control, respectively); and then decreasing and rising again until the end of experiment, always significantly different from that of control ($P<0.01$).

The activity of CAT in gill and hepatopancreas was significantly affected ($P<0.01$) by *P. minimum* exposure and showed different responding patterns (Fig.3). Compared with control, CAT activity in gill was depressed significantly at 3 h ($P<0.01$), remaining significantly lower than that of control until 12 h ($P<0.01$); then became similar to that of control from 24 to 72 h ($P>0.05$). In hepatopancreas, CAT activity of exposure group increased and remained higher than that of control in 72 h ($P<0.01$).

The activity of GPX in gill and hepatopancreas showed significant difference ($P<0.01$) between exposure and control groups (Fig.4). Compared with control, GPX activity in gill was significantly higher from 6 h to 72 h ($P<0.01$) while in hepatopancreas it increased significantly in

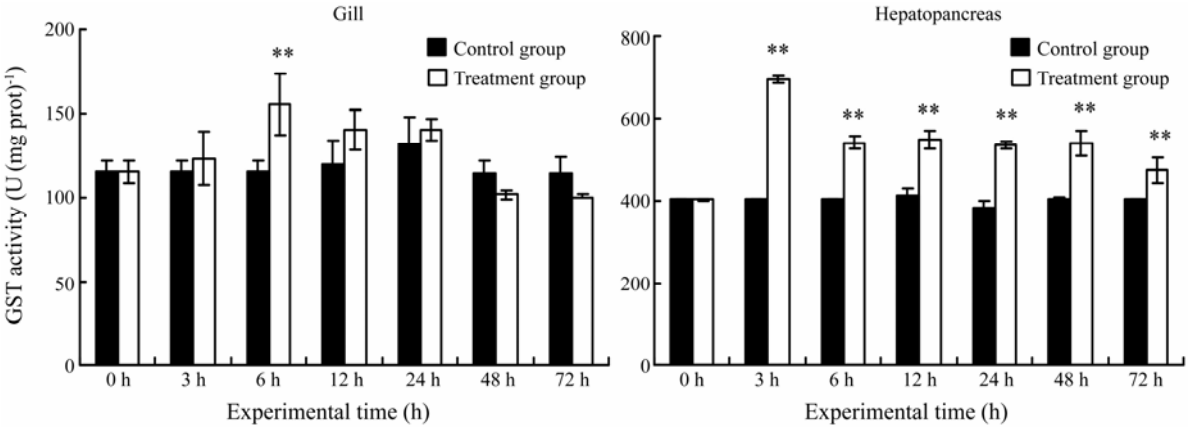

Fig.1 Effect of *P. minimum* exposure on GST activity (U (mg prot)$^{-1}$) in gill and hepatopancreas of *E. carinicauda*. Data are expressed as means ± SD (n=8). *, $P<0.05$, and **, $P<0.01$.

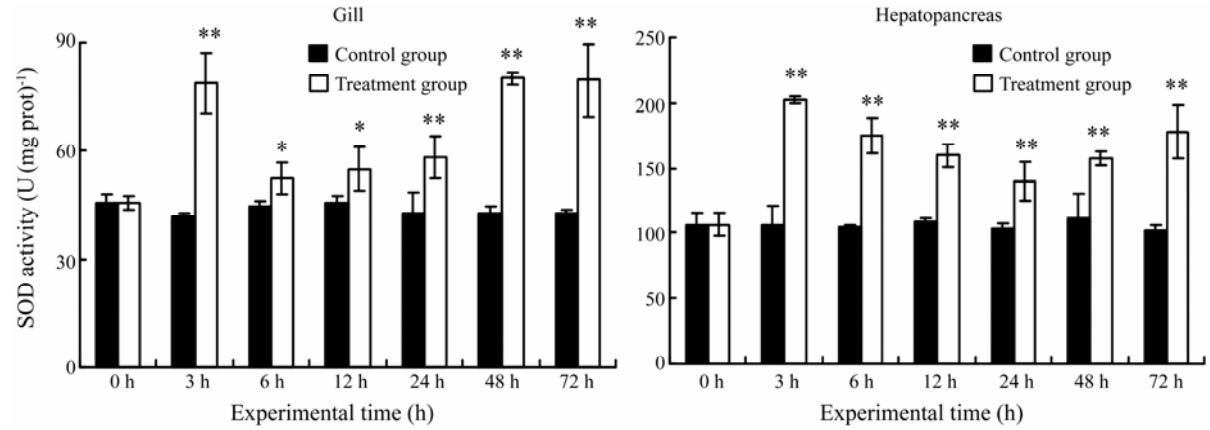

Fig.2 Effect of *P. minimum* exposure on SOD activity (U (mg prot)$^{-1}$) in gill and hepatopancreas of *E. carinicauda*. Data are expressed as means ± SD (n=8). *, $P<0.05$, and **, $P<0.01$.

3 h, maximized at 6 h (1.42 fold of control, $P<0.01$), reached that of control at 12 h ($P>0.05$) and finally decreased to the lowest at 24 and 48 h (0.47 fold and 0.51 fold of control, respectively; $P<0.01$). GPX activity in hepatopancreas increased again and reached that of control at 72 h ($P>0.05$).

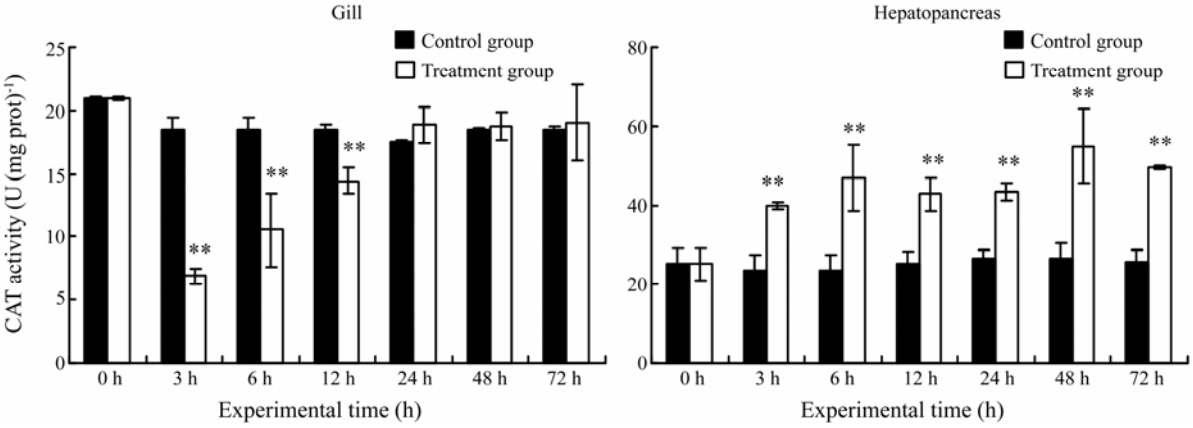

Fig.3 Effect of *P. minimum* exposure on CAT activity (U (mg prot)$^{-1}$) in gill and hepatopancreas of *E. carinicauda*. Data are expressed as means ± SD (n=8). *, $P<0.05$, and **, $P<0.01$.

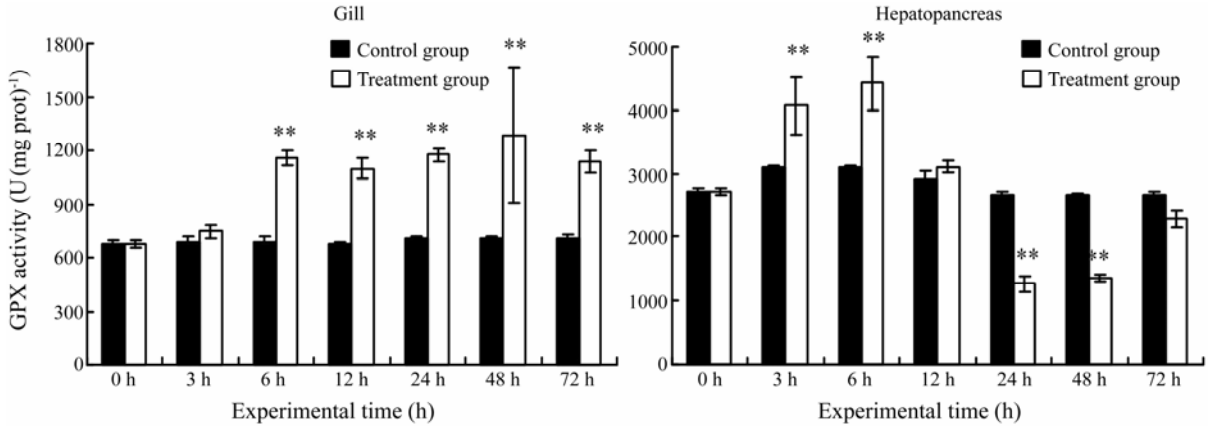

Fig.4 Effect of *P. minimum* exposure on GPX activity (U (mg prot)$^{-1}$) in gill and hepatopancreas of *E. carinicauda*. Data are expressed as means ± SD (n=8). *, $P<0.05$, and **, $P<0.01$.

3.3 Effect of *P. minimum* on Lipid Peroxidation

The content of malondialdehyde (MDA) was significantly affected by *P. minimum* exposure ($P<0.01$, Fig.5). Compared with the control, MDA content in gill and hepatopancreas was induced sharply, reaching the peak at 3 h (2.37 fold and 1.59 fold of the control, respectively; $P<0.01$). Then, it stated to decline and reached a lower level than control at the end of experiment (72 h, $P<0.01$).

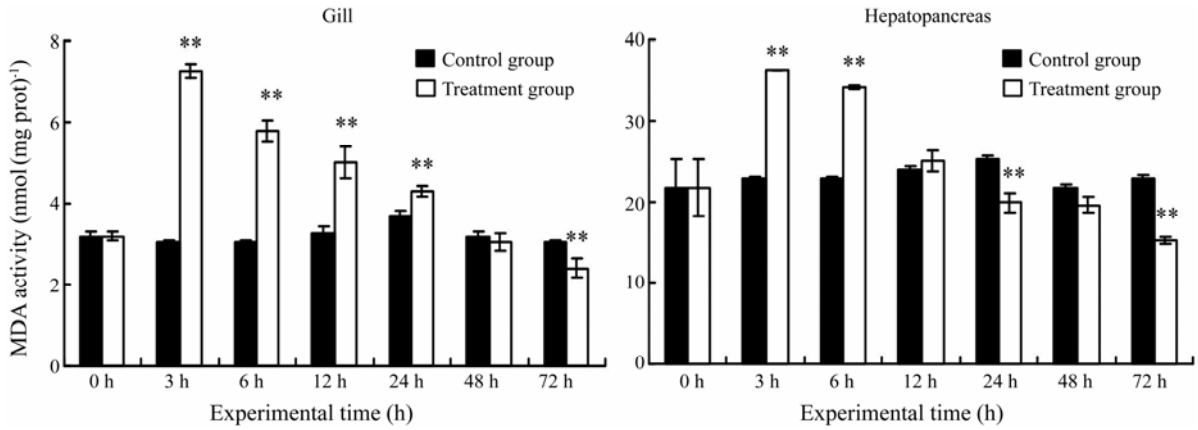

Fig.5 Effect of *P. minimum* exposure on the content of MDA (nmol (mg prot)$^{-1}$) in gill and hepatopancreas of *E. carinicauda*. Data are expressed as means ± SD (n=8). *, $P<0.05$, and **, $P<0.01$.

3.4 Effect of *P. minimum* on Histological Change

After 72 hours of exposure to *P. minimum*, structural change was shown in gill of *E. carinicauda* (Fig.6). In the control, the gill showed healthy connective tissues (CT) in the septa of filaments and in the axes (Fig.6a). In addition, the gill lamellae (L), with uniform interlamellar space (ILS), were covered by a monolayer epithelium as a thin cuticle that consisted of pillar cells (PC) and hemocytes (HC) (Fig.6b). The gill of exposure group showed significant pathological change. The hemocytes (HC) increased in number, and accumulated in the hemocoelic space (Fig.6c). After exposed to *P. minimum*, some distal filaments were completely disordered, resulting in abnormal gill tips (AG) (Fig.6c). The gill lamellae showed lifting of lamellar epithelium (LLE), swelling (SL), and even fusion (FL) of gill lamellae. Histopathologies were followed by hemocytic infiltration in the hemocoelic space (Fig.6d). In addition, abnormal connective tissues (CT) with sloughing epithelium were shown in the axes of the gill (Figs.6e). Necrotic cells (NC), with nuclei becoming pyknotic or karorrhexis, were seen in gill lamellae and axes (Fig.6d, f). Exposure to *P. minimum* altered the well-organized structure of gill.

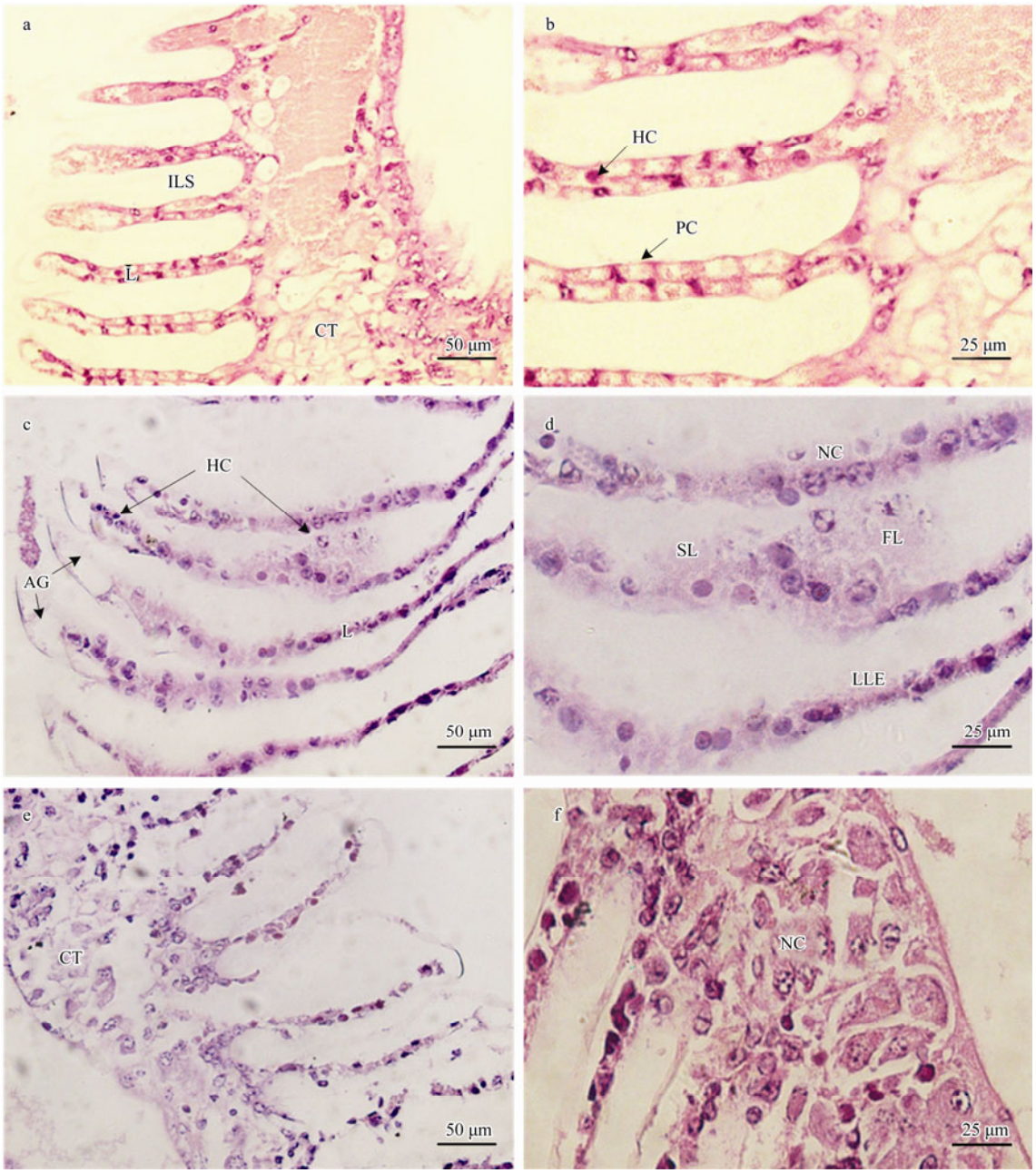

Fig.6 Effect of *P. minimum* exposure on the gill of *E. carinicauda*. (a–b) Typical structure of the gill (control): healthy connective tissues (CT), gill lamellae (L) with uniform interlamellar space (ILS), optimum number of hemocytes (HC) in hemocoelic space, pillar cells (PC) specialized epithelial cells. (c–f) Histopathologies (exposure group): accumulation or infiltration of hemocytes (HC) in hemocoelic space, non-uniform interlamellar space of gill lamellae (L), lifting of lamellar epithelium (LLE), swelling (SL) and even fusion (FL) of gill lamellae, abnormal gill tips (AG) and necrotic cells (NC) in gill lamellae, abnormal connective tissues (CT) with sloughing epithelium and necrotic cells (NC) in gill axes.

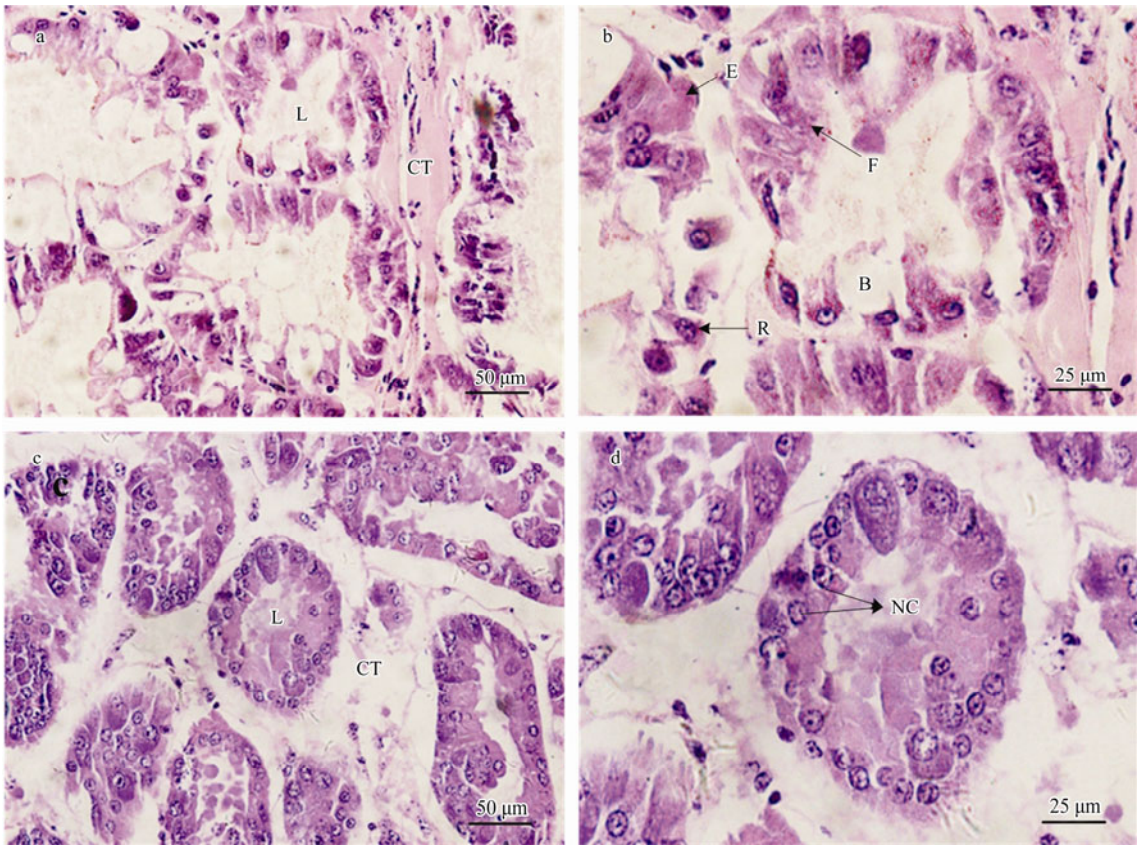

Fig.7 Effect of *P. minimum* exposure on the hepatopancreas of *E. carinicauda*. (a) Typical structure of the hepatopancreas (control) with star-shaped lumen (L) and healthy connective tissues (CT). (b) Typical structure of hepatopancreas tubules: contain undifferentiated embryonic cells (embryonalzellen, E cells), secretory cells (blasenzellen, B cells), fibrous cells (fibrillenzellen, F cells), and absorptive and storage cells (restzellen, R cells). (c–d) Histopathologies (exposure group): abnormal structure of the hepatopancreas with irregular lumen (L) and unhealthy connective tissues (CT), increased number of R-cells and necrotic cells (NC).

The histological change in hepatopancreas was showed in Fig.7. In control, the hepatopancreas of healthy shrimp consisted of a well-organized structure with healthy connective tissues (CT) and star-shaped lumen (L) with a single layer of epithelial cells (Fig.7a). Cells of healthy organisms show a typical differentiation into embryonic cells (E cells) at the distal blind end; absorptive and storage cells (R cells), and fibrous cells (F cells) at middle and proximal zones; and secretory cells (B cells) at the proximal zone (Fig.7b). Exposure to *P. minimum* had induced some histological changes in the hepatopancreas of shrimp (Fig.7c). The connective tissues with sloughing epithelium scattered in the space of tubular, increase in the space of adjacent tubules, conspicuous atrophy and necrosis of tubules, irregular lumens lost of the star shape seeming like compressed, reduction in the abundance of B cells, and increase in the number of R and necrotic cells. In addition, some morphological changes were seen in tubular epithelial cells: a decrease of the epithelial cell height, cell rupture and cells detached from the basal lamina (Fig.7d).

4 Discussion

The mortality of shrimp *E. carinicauda* (ridgetail white prawn) seems not to be affected by the exposure to toxic dinoflagellate *P. minimum* (5×10^4 cells mL^{-1}) for 72 hours. However, antioxidant response and histological change were significantly affected by exposure.

The glutathione S-transferase (GST) plays a vital role in detoxification and protection of tissues from oxidative damage in aquatic organisms (Goto *et al*., 2009). The phycotoxin microcystins (MCs) increased the GST activity, after injection with a sub-lethal dose of an extract of toxic *Microcystis aeruginosa*, in shrimp *Litopenaeus vannamei* (Gonçalves-Soares *et al*., 2012). In the present study, GST activity in gill of shrimp was only induced at 6 h of exposure to *P. minimum*. Whereas GST activity in hepatopancreas was increased throughout the experiment, suggested that GST played an important role in shrimp hepatopancreas during the exposure period, and possibly after exposure.

The superoxide dismutase (SOD) plays an important role in transforming the superoxide radical (O^{2-}) into H_2O_2 and O_2, thereby protecting organisms from oxidative stress (El-Beltagi and Mohamed, 2013; Winston, 1991). In the present study, SOD activity was significantly stimulated by *P. minimum* exposure, and remained a higher level until the end of the experiment (72 h). Significantly increased SOD activity at 3 h indicated that large amounts of O^{2-} were induced by exposure to *P. minimum*, and the antioxidant activity was evoked to pro-

tect the organism. The temporary decline in SOD activity may associate with temporary oxidative damage.

Both catalase (CAT) and glutathione peroxidase (GPX) can convert H_2O_2 into H_2O and O_2 (El-Beltagi and Mohamed, 2013), protecting tissues and cells from LPO and H_2O_2 (Ran *et al*., 2007). Thus, CAT and GPX could reduce tissue injury by removing H_2O_2. In our study, the CAT activity was depressed in gill but increased in hepatopancreas. Meanwhile, the GPX activity showed almost an opposite pattern. In hepatopancreas, GPX activity increased to the peak in 6 h suggesting its effective protection at earlier time. The modulated GPX activity after that may be associated with the injury of hepatopancreas cells. These results suggested that CAT and GPX function in hepatopancreas and gill, respectively. Similarly, after exposure of fish *Corydoras paleatus* to different concentrations of microcystin-RR, the activity of CAT was enhanced in liver at all concentrations, but it was not detected in gill (Cazenave *et al*., 2006). These preliminary results raise intriguing questions about the differential antioxidant response in diverse organs (Cazenave *et al*., 2006; Jos *et al*., 2005). Thus, more studies are needed to elucidate the differential antioxidant response in gill and hepatopancreas.

The gill and hepatopancreas are the main organs for organism protection from oxidative damage. In the present study, elevated SOD activity, depressed CAT activity and increased GPX activity were observed in gill, while increased SOD, GST, CAT activities and modulated GPX activity were observed in hepatopancreas. These results indicated that different antioxidant responses activated in gill and hepatopancreas after exposed to toxic *P. minimum*. This finding is consistent with those obtained from fish *C. paleatus* exposed to different concentrations of microcystin-RR (Cazenave *et al*., 2006).

Reactive oxygen species (ROS) play important roles in host defense. However, overproduction and residuals can lead to cellular damage and lipid peroxidation (LPO) (Ryter *et al*., 2007; El-Beltagi and Mohamed, 2013). As an important product of LPO, malondialdehyde (MDA) is often used to reflect the degree of cell damage (Jia *et al*., 2014; Li *et al*., 2015; Ren *et al*., 2015; Wang *et al*., 2015; Wei and Yang, 2015). High levels of MDA were observed in gill and hepatopancreas of shrimp *E. carinicauda* at the beginning of experiment (3–12 h). Then MDA declined to lower levels than that of control. Oscillations in MDA level suggested a temporary oxidative damage in the defense system of shrimp from exposure group.

Antioxidant capability of an organism under certain conditions can reflect its health status (Xu and Pan, 2013). Histopathological evaluations were performed to determine whether it is applicable to the species *E. carinicauda* after exposure to toxic algae. In aquatic organisms, gill is a crucial organ that plays an important role in the transport of respiratory gases and regulates the osmotic and ionic balance of organisms (Ghate and Mulherkar, 1979). The gill of shrimp is in direct contact with the environment and represents the first line of defense, being frequently affected by extreme environments, such as heavy metals and various toxic compounds (Ghate and Mulherkar, 1979; Mallatt, 1985; Bhavan and Geraldine, 2000). In the present study, exposure to toxic dinoflagellate *P. minimum* induced serious effects on the gill of ridgetail white prawn. The histopathologies observed in the shrimp gill were similar to the gill change reported in prawns exposed to organic or inorganic pollutants (Ghate and Mulherkar, 1979; Victor *et al*., 1990; Bhavan and Geraldine, 2000; Li *et al*., 2007). Mallatt (1985) stated that the swelling and lifting of the lamellar epithelium and the hyperplasic changes in gill lamellae might simply reflect a physiological adaptation to stress. However, the adaptation might result in reduced oxygen consumption and disruption of the osmoregulatory function of aquatic organisms (Ghate and Mulherkar, 1979).

The hepatopancreas in crustaceans is analogous to the liver in vertebrates, and it is a sensitive organ liable to be damaged by waterborne pollutants. Thus, the occurrence of pathologies in hepatopancreas is often used to monitor the effects of different toxic compounds (Vogt, 1987; Bhavan and Geraldine, 2000; Wu *et al*., 2008; Boudet *et al*., 2015). In the control of this study, the hepatopancreas comprised a mass of blind tubules with little intertubular space occupied by hemolymph. The well-organized structure in the shrimps from control was similar to those from other shrimp species (Bhavan and Geraldine, 2000; Li *et al*., 2007; Wu *et al*., 2008). Exposure to toxic *P. minimum* altered the well-organized structure of hepatopancreas. Similarly, structural change was observed after the exposure of white shrimp *Palaemonetes argentinus* and *L. vannamei* to heavy metals, comprising infiltration of hemocytes, separation of necrotic cells from the basal lamina, and formation of necrotic tubules (Wu *et al*., 2008; Boudet *et al*., 2015). Histopathologies are related to environmental quality, demonstrating the high sensitivity of hepatopancreas to the change in environmental quality (Boudet *et al*., 2015). In the present study, most of the histological changes in hepatopancreas of *E. carinicauda* exposed to *P. minimum* were in the tubules, and most of R cells were observed in these tubules. The R cells are the most readily and severely affected among different cell types in the tubular epithelium of hepatopancreas (Wu *et al*., 2008; Boudet *et al*., 2015). R cells do not only absorb and store nutrients, but also they are involved in detoxification (Vogt, 1987; Bhavan and Geraldine, 2000; Sousa and Petriella, 2000; Sousa *et al*., 2005). The increased GST activity and R cells indicate that the detoxification process might be involved in the shrimp hepatopancreas defense against harmful algal exposure.

The gill and hepatopancreas of shrimp *E. carinicauda* are sensitive to toxic dinoflagellate *P. minimum*, showing temporary oxidative damage and histological lesions. The antioxidant defense systems were activated in different ways in these two organs, higher GPX activity in gill and higher GST and CAT activities in hepatopancreas. In conclusion, the antioxidant response combined with histological evaluation could be highly sensitive to evaluate the negative effect of harmful algae on shrimp, including ridgetail white prawn *E. carinicauda*. The exposure to

toxic dinoflagellate *P. minimum* caused damage in gill and hepatopancreas. The typical physiological activity and health of *E. carinicauda* are likely to be seriously affected. However, further studies are needed to clarify the detailed mechanism of toxic effect of *P. minimum* on shrimp.

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References

Alonso-Rodríguez, R., and Páez-Osuna, F., 2003. Nutrients, phytoplankton and harmful algal blooms in shrimp ponds: A review with special reference to the situation in the Gulf of California. *Aquaculture*, **219** (1-4): 317-336.

Azanza, R. V., Fukuyo, Y., Yap, L. G., and Takayama, H., 2005. *Prorocentrum minimum* bloom and its possible link to a massive fish kill in Bolinao, Pangasinan, Northern Philippines. *Harmful Algae*, **4** (3): 519-524.

Bhavan, P. S., and Geraldine, P., 2000. Histopathology of the hepatopancreas and gills of the prawn *Macrobrachium malcolmsonii* exposed to endosulfan. *Aquatic Toxicology*, **50** (4): 331-339.

Boudet, L. N. C., Polizzi, P., Romero, M. B., Robles, A., Marcovecchio, J. E., and Gerpe, M. S., 2015. Histopathological and biochemical evidence of hepatopancreatic toxicity caused by cadmium in white shrimp, Palaemonetes argentinus. *Ecotoxicology and Environmental Safety*, **113**: 231-240.

Bradford, M. M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, **72** (1-2): 248-254.

Cazenave, J., Bistoni, M. D. L. A., Pesce, S. F., and Wunderlin, D. A., 2006. Differential detoxification and antioxidant response in diverse organs of *Corydoras paleatus* experimentally exposed to microcystin-RR. *Aquatic Toxicology*, **76** (1): 1-12.

Chang, C. C., Lee, P. P., Hsu, J. P., Yeh, S. P., and Cheng, W., 2006. Survival, and biochemical, physiological, and histopathological responses of the giant freshwater prawn, *Macrobrachium rosenbergii*, to short-term trichlorfon exposure. *Aquaculture*, **253** (1–4): 653-666.

Cuevas, N., Zorita, I., Costa, P. M., Franco, J., and Larreta, J., 2015. Development of histopathological indices in the digestive gland and gonad of mussels: Integration with contamination levels and effects of confounding factors. *Aquatic Toxicology*, **162**: 152-164.

El-Beltagi, H. S., and Mohamed, H. I., 2013. Reactive oxygen species, lipid peroxidation and antioxidative defense mechanism. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, **41** (1): 44-57.

Ghate, H., and Mulherkar, L., 1979. Histological changes in the gills of two freshwater prawn species exposed to copper sulphate [India]. Short communication. *Indian Journal of Experimental Biology*, **17** (8): 838-840.

Gonçalves-Soares, D., Zanette, J., Yunes, J. S., Yepiz-Plascencia, G. M., and Bainy, A. C. D., 2012. Expression and activity of glutathione S-transferases and catalase in the shrimp *Litopenaeus vannamei* inoculated with a toxic *Microcystis aeruginosa* strain. *Marine Environmental Research*, **75**: 54-61.

Goth, L., 1991. A simple method for determination of serum catalase activity and revision of reference range. *Clinica Chimica Acta*, **196** (2-3): 143-151.

Goto, S., Kawakatsu, M., Izumi, S., Urata, Y., Kageyama, K., Ihara, Y., Koji, T., and Kondo, T., 2009. Glutathione S-transferase π localizes in mitochondria and protects against oxidative stress. *Free Radical Biology and Medicine*, **46** (10): 1392-1403.

Habig, W. H., Pabst, M. J., and Jakoby, W. B., 1974. Glutathione S-transferases the first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry*, **249** (22): 7130-7139.

Heil, C. A., Glibert, P. M., and Fan, C., 2005. *Prorocentrum minimum* (Pavillard) Schiller: A review of a harmful algal bloom species of growing worldwide importance. *Harmful Algae*, **4** (3): 449-470.

Huang, X., Chen, L., Liu, W. J., Qiao, Q., Wu, K., Wen, J., Huang, C. H., Tang, R., and Zhang, X. Z., 2015. Involvement of oxidative stress and cytoskeletal disruption in microcystin-induced apoptosis in CIK cells. *Aquatic Toxicology*, **165**: 41-50.

Jia, R., Cao, L. P., Du, J. L., Wang, J. H., Liu, Y. J., Jeney, G., Xu, P., and Yin, G. J., 2014. Effects of carbon tetrachloride on oxidative stress, inflammatory response and hepatocyte apoptosis in common carp (*Cyprinus carpio*). *Aquatic Toxicology*, **152**: 11-19.

Jos, A., Pichardo, S., Prieto, A. I., Repetto, G., Vázquez, C. M., Moreno, I., and Cameán, A. M., 2005. Toxic cyanobacterial cells containing microcystins induce oxidative stress in exposed tilapia fish (*Oreochromis* sp.) under laboratory conditions. *Aquatic Toxicology*, **72** (3): 261-271.

Karthikeyan, V., Selvakumar, P., and Gopalakrishnan, A., 2015. A novel report of fungal pathogen *Aspergillus awamori* causing black gill infection on *Litopenaeus vannamei* (pacific white shrimp). *Aquaculture*, **444**: 36-40.

Kim, D., Nakashima, T., Matsuyama, Y., Niwano, Y., Yamaguchi, K., and Oda, T., 2007. Presence of the distinct systems responsible for superoxide anion and hydrogen peroxide generation in red tide phytoplankton *Chattonella marina* and *Chattonella ovata*. *Journal of Plankton Research*, **29** (3): 241-247.

Landsberg, J. H., 2002. The effects of harmful algal blooms on aquatic organisms. *Reviews in Fisheries Science*, **10** (2): 113-390.

Li, N., Hou, Y. H., Ma, D. D., Jing, W. X., Dahms, H. U., and Wang, L., 2015. Lead accumulation, oxidative damage and histopathological alteration in testes and accessory glands of freshwater crab, *Sinopotamon henanense*, induced by acute lead exposure. *Ecotoxicology and Environmental Safety*, **117**: 20-27.

Li, N., Zhao, Y. L., and Yang, J., 2007. Impact of waterborne copper on the structure of gills and hepatopancreas and its impact on the content of metallothionein in juvenile giant freshwater prawn *Macrobrachium rosenbergii* (Crustacea: Decapoda). *Archives of Environmental Contamination and Toxicology*, **52**: 73-79.

Li, X. Z., Liu, R.Y., and Liang, X. Q., 2002. The zoogeography

of Chinese Palaemonoidea fauna. *Chinese Biodiversity*, **11** (5): 393-406.

Liang, Z. X., Li, J., Li, J. T., Tan, Z. J., Ren, H., and Zhao, F. Z., 2014. Toxic dinoflagellate *Alexandrium tamarense* induces oxidative stress and apoptosis in hepatopancreas of shrimp (*Fenneropenaeus chinensis*). *Journal of Ocean University of China*, **13** (6): 1005-1011.

Mallatt, J., 1985. Fish gill structural changes induced by toxicants and other irritants: A statistical review. *Canadian Journal of Fisheries and Aquatic Sciences*, **42** (4): 630-648.

Marklund, S., and Marklund, G., 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry*, **47** (3): 469-474.

Moore, M. N., 1985. Cellular responses to pollutants. *Marine Pollution Bulletin*, **16** (4): 134-139.

Oda, T., Akaike, T., Sato, K., Ishimatsu, A., Takeshita, S., Muramatsu, T., and Maeda, H., 1992. Hydroxyl radical generation by red tide algae. *Archives of Biochemistry and Biophysics*, **294** (1): 38-43.

Oda, T., Nakashima, T., Shikayama, M., Kawano, L., Ishimatsu, A., and Muramatsu, T., 1997. Generation of reactive oxygen species by raphidophycean phytoplankton. *Bioscience, Biotechnology, and Biochemistry*, **61** (10): 1658-1662.

Odendaal, J., and Reinecke, A., 2003. Quantifying histopathological alterations in the hepatopancreas of the woodlouse *Porcellio laevis* (Isopoda) as a biomarker of cadmium exposure. *Ecotoxicology and Environmental Safety*, **56** (2): 319-325.

Ohkawa, H., Ohishi, N., and Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, **95** (2): 351-358.

Ott, M., Gogvadze, V., Orrenius, S., and Zhivotovsky, B., 2007. Mitochondria, oxidative stress and cell death. *Apoptosis*, **12** (5): 913-922.

Páez-Osuna, F., Gracia, A., Flores-Verdugo, F., Lyle-Fritch, L. P., Alonso-Rodrí, R., Roque, A., and Ruiz-Fernández, A. C., 2003. Shrimp aquaculture development and the environment in the Gulf of California ecoregion. *Marine Pollution Bulletin*, **46** (7): 806-815.

Pinho, G. L. L., Moura da Rosa, C., Maciel, F. E., Bianchini, A., Yunes, J. S., Proença, L. A. O., and Monserrat, J. M., 2005. Antioxidant responses and oxidative stress after microcystin exposure in the hepatopancreas of an estuarine crab species. *Ecotoxicology and Environmental Safety*, **61** (3): 353-360.

Ran, Q., Liang, H., Ikeno, Y., Qi, W., Prolla, T. A., Roberts, L. J., Wolf, N., VanRemmen, H., and Richardson, A., 2007. Reduction in glutathione peroxidase 4 increases life span through increased sensitivity to apoptosis. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, **62** (9): 932-942.

Ren, X., Pan, L., and Wang, L., 2015. Toxic effects upon exposure to benzo[a]pyrene in juvenile white shrimp *Litopenaeus vannamei*. *Environmental Toxicology and Pharmacology*, **39** (1): 194-207.

Rotruck, J., Pope, A., Ganther, H., Swanson, A., Hafeman, D. G., and Hoekstra, W., 1973. Selenium: Biochemical role as a component of glutathione peroxidase. *Science*, **179** (4073): 588-590.

Ryter, S. W., Kim, H. P., Hoetzel, A., Park, J. W., Nakahira, K., Wang, X., and Choi, A. M., 2007. Mechanisms of cell death in oxidative stress. *Antioxidants and Redox Signaling*, **9** (1): 49-89.

Sierra-Beltrán, A. P., Cortés-Altamirano, R., and Cortés-Lara, M. C., 2005. Occurrences of *Prorocentrum minimum* (Pavillard) in México. *Harmful Algae*, **4** (3): 507-517.

Sousa, L. G., and Petriella, A. M., 2000. Histology of the hepatopancreas of the freshwater prawn *Palaemonetes argentinus* (Crustacea, Caridea). *Biocell*, **24** (3): 189-195.

Sousa, L. G., Cuartas, E. I., and Petriella, A. M., 2005. Fine structural analysis of the epithelial cells in the hepatopancreas of *Palaemonetes argentinus* (Crustacea, Decapoda, Caridea) in intermoult. *Biocell*, **29** (1): 25-31.

Tango, P. J., Magnien, R., Butler, W., Luckett, C., Luckenbach, M., Lacouture, R., and Poukish, C., 2005. Impacts and potential effects due to *Prorocentrum minimum* blooms in Chesapeake Bay. *Harmful Algae*, **4** (3): 525-531.

Victor, B., Narayanan, M., and Nelson, D. J., 1990. Gill pathology and hemocyte response in mercury exposed *Macrobrachium idea* (Heller). *Journal of Environmental Biology*, **11** (1): 61-65.

Vlamis, A., Katikou, P., Rodriguez, I., Rey, V., Alfonso, A., Papazachariou, A., Zacharaki, T., Botana, A. M., and Botana, L. M., 2015. First detection of tetrodotoxin in Greek shellfish by UPLC-MS/MS potentially linked to the presence of the dinoflagellate *Prorocentrum minimum*. *Toxins*, **7** (5): 1779-1807.

Vogt, G., 1987. Monitoring of environmental pollutants such as pesticides in prawn aquaculture by histological diagnosis. *Aquaculture*, **67** (1-2): 157-164.

Wang, X. H., Qu, R. J., Huang, Q. G., Wei, Z. B., and Wang, Z. Y., 2015. Hepatic oxidative stress and catalyst metals accumulation in goldfish exposed to carbon nanotubes under different pH levels. *Aquatic Toxicology*, **160**: 142-150.

Wang, X. Q., Yan, B. L., Ma, S., and Dong, S. L., 2005. Study on the biology and cultural ecology of *Exopalaemon carinicauda*. *Shandong Fisheries*, **22** (8): 21-24.

Wei, K. Q., and Yang, J. X., 2015. Oxidative damage of hepatopancreas induced by pollution depresses humoral immunity response in the freshwater crayfish *Procambarus clarkii*. *Fish and Shellfish Immunology*, **43** (2): 510-519.

Winston, G. W., 1991. Oxidants and antioxidants in aquatic animals. *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology*, **100** (1-2): 173-176.

Wu, J. P., Chen, H. C., and Huang, D. J., 2008. Histopathological and biochemical evidence of hepatopancreatic toxicity caused by cadmium and zinc in the white shrimp, *Litopenaeus vannamei*. *Chemosphere*, **73** (7): 1019-1026.

Xu, H. L., Min, G. S., Choi, J. K., and Zhu, M. Z., 2010. Temporal population dynamics of the dinoflagellate *Prorocentrum minimum* in a semi-enclosed mariculture pond and its relationship to environmental factors and protozoan grazers. *Chinese Journal of Oceanology and Limnology*, **28** (1): 75-81.

Xu, W. J., and Pan, L. Q., 2013. Enhancement of immune response and antioxidant status of *Litopenaeus vannamei* juvenile in biofloc-based culture tanks manipulating high C/N ratio of feed input. *Aquaculture*, **412-413**: 117-124.

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