J. Ocean Univ. China (Oceanic and Coastal Sea Research)
https://doi.org/10.1007/s11802-019-3846-1
ISSN 1672-5182, 2019 18 (3): 727-734
http://www.ouc.edu.cn/xbywb/
E-mail:xbywb@ouc.edu.cn

Exposure to *Prorocentrum minimum* Induces Oxidative Stress and Apoptosis in the Ridgetail White Prawn, *Exopalaemon carinicauda*

MU Cuimin[1), 2)], GE Qianqian[1), 2)], and LI Jian[1), 2), *]

1) *Key Laboratory for Sustainable Utilization of Marine Fisheries Resources of Ministry of Agriculture*, *Yellow Sea Fisheries Research Institute*, *Chinese Academy of Fishery Sciences*, *Qingdao* 266071, *China*
2) *Function Laboratory for Marine Fisheries Science and Food Production Processes*, *Qingdao National Laboratory for Marine Science and Technology*, *Qingdao* 266071, *China*

(Received March 10, 2018; revised May 15, 2018; accepted December 16, 2018)

Abstract *Prorocentrum minimum* is a bloom-forming, planktonic mixotrophic dinoflagellate, and can cause stress in shrimp ponds. In this study, healthy *Exopalaemon carinicauda* were exposed to 5×10^4 cells mL^{-1} *P. minimum* for 72 hours to investigate the adverse effect of *P. minimum* on shrimps. Elevated superoxide dismutase (SOD) activity and malondialdehyde (MDA) content, reduced total antioxidant capacity (T-AOC) and catalase (CAT) activity, and regulatory glutathione peroxidase (GPX) activity were found in the hemolymph of *E. carinicauda* after exposure to *P. minimum*. In this study, *P. minimum* exposure induced oxidative stress and caused significant oxidative damage to *E. carinicauda*. *P. minimum* exposure increased the expression of *HSP70* gene in the hemocyte, gills and hepatopancreas. Compared with the enhanced level of *caspase-3* gene mRNA in the hemocyte and gills, the up-regulation of *caspase-3* gene in the hepatopancreas was only observed from 3 to 6 h, and then the mRNA level of glutathione-S-transferase (GST) gene increased. These results indicated that *GST* might be involved in the shrimp hepatopancreas' defense against *P. minimum* exposure. The present study demonstrates that exposure to *P. minimum* could induce oxidative stress and apoptosis in *E. carinicauda*. The SOD activity, HSP70 and GST (in the hepatopancreas) were evoked to protect cells from oxidative stress and apoptosis. This study will provide new insights into the toxic mechanism of *P. minimum* on shrimps.

Key words *Prorocentrum minimum*; *Exopalaemon carinicauda*; oxidative stress; apoptosis; HSP70

1 Introduction

The ridgetail white prawn, *Exopalaemon carinicauda*, an economically important species in the shrimp farming industry, is mainly distributed in the Yellow Sea and Bohai Seacoasts in China (Li *et al*., 2002). The culture area of *E. carinicauda* has expanded in recent years because of its fast growth, excellent nutritional properties, good reproductive performance, and wide environmental adaptability (Wang *et al*., 2005). However, shrimp pond waters are often enriched with organic matter and nutrients, which provide a good environment for the expansion of harmful algae. According to Alonso-Rodrıguez and Páez-Osuna (2003), large blooms of harmful algae could result in economic losses by increased shrimp mortality or reduced growth.

Harmful algae represent a type of environmental stress, which induces oxidative stress and alterations in the cellular redox balance (Pinho *et al*., 2005; Liang *et al*., 2014; Huang *et al*., 2015). Environmental stress can also accelerate the generation of highly reactive oxygen species (ROS) (Oda *et al*., 1992, 1997; Kim *et al*., 2007). Antioxidant defense systems are evoked to help organisms eliminate ROS. If ROS are not eliminated timely, they can damage important biomolecules inducing cell damage or even cell death via apoptosis (Winston, 1991; El-Beltagi and Mohamed, 2013). As a useful marker for detecting stress-induced apoptosis, caspase is crucial in regulating the progression of apoptosis (Franco *et al*., 2009; Cheng *et al*., 2015). Glutathione-S-transferases (GSTs) are dimeric multifunctional enzymes that are involved in detoxification, protection of tissues from oxidative damage, targeting for transmembrane transport, ligandin binding, and the nonenzymatic binding for intracellular transport (Sheehan *et al*., 2001; Blanchette *et al*., 2007; Goto *et al*., 2009). The mechanism of detoxification and protection from oxidative stress has been well documented in marine organisms (Blanchette *et al*., 2007; Goto *et al*., 2009). Heat shock proteins (HSPs) represent defense systems that protect the host from different kinds of stresses and maintain biological homeostasis (Sørensen *et al*., 2003; Jiang *et al*., 2012). HSP70, one of the most conserved HSPs, is a key regulator of signal transduction pathways controlling cell

* Corresponding author. Tel: 0086-532-85830183
E-mail: lijian@ysfri.ac.cn

homeostasis and cell death (Mayer and Bukau, 2005). HSP70 protects cells against oxidative stress and is involved in anti-apoptotic responses (Mayer and Bukau 2005; Jiang *et al*., 2012; Bermejo-Nogales *et al*., 2014).

Prorocentrum minimum is a planktonic, mixotrophic bloom-forming dinoflagellate, and is one of the most widespread red tide species. The majority of *P. minimum* clones in culture cannot provide clear evidence of *P. minimum* toxicity; whereas the clones isolated from Japan contained hepatotoxic components, and the clones isolated from France contained neurotoxic components (Heil *et al*., 2005). Vlamis *et al*. (2015) were the first to indicate a possible link between the presence of *P. minimum* in seawater and tetrodotoxin (TTX, an extremely potent neurotoxin) in Greek bivalves. Although the potential toxicity of *P. minimum* is not clear (Landsberg 2002; Vlamis *et al*., 2015), a number of events causing damages are associated with high concentrations of *P. minimum* ($>10^3$ cells mL^{-1}) (Alonso-Rodrıguez and Páez-Osuna, 2003; Azanza *et al*., 2005; Heil *et al*., 2005; Tango *et al*., 2005). According to the previous studies, high concentrations of *P. minimum* can have detrimental effects on the survival or growth of aquatic organisms, such as fish (Azanza *et al*., 2005; Heil *et al*., 2005; Sierra-Beltrán *et al*., 2005; Tango *et al*., 2005), shellfish (Luckenbach *et al*., 1993; Hégaret and Wikfors 2005; Heil *et al*., 2005) and shrimp (Alonso-Rodrıguez and Páez-Osuna, 2003; Páez-Osuna *et al*., 2003; Sierra-Beltrán *et al*., 2005).

P. minimum is often found in shrimp ponds (Alonso-Rodrıguez and Páez-Osuna, 2003; Páez-Osuna *et al*., 2003; Sierra-Beltrán *et al*., 2005). Among the thirteen recorded *P. minimum* blooms in Mexico from 1942 to 2005, six occurred in shrimp ponds (Sierra-Beltrán *et al*., 2005). In these events, high concentrations ($>1.2\times10^3$ cells mL^{-1}) of *P. minimum* could cause shrimp stress by affecting their survival and growth, and making the organisms more vulnerable to viral diseases. However, few studies are available on the mechanisms of *P. minimum*'s effects on shrimp. The aim of this study was to determine the mechanisms of *P. minimum* on *E. carinicauda* by evaluating the effects of *P. minimum* exposure on the defense systems, such as antioxidant activities, HSP70, GST, and the major executioner of cell apoptosis caspase-3.

2 Materials and Methods

2.1 Animals

Healthy adult *E. carinicauda* (mean weight = 1.62 ± 0.16 g) used in the experiment 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 the experiment. During the acclimation, *E. carinicauda* were fed with commercial prawn pellets for three times each 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 red tide of *P. minimum* occurred. In this bloom event, the concentration was 5×10^4 cells mL^{-1}. Alga was batch-cultured in 5 L glass flasks with 0.45 μm membrane-filtered sea water supplemented with f/2 medium at 25℃, with irradiance at 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 the experiment.

2.3 Experimental Design and Sample Collection

Cells of *P. minimum* in the late exponential growth phase were used in the experiment. 5×10^4 cells mL^{-1} of *P. minimum* was used in treatment group, and filtered seawater was used as control. These two groups were run in triplicate, and each one was randomly distributed with 70 healthy *E. carinicauda*. *E. carinicauda* were fed with commercial prawn pellets three times each day. The water was renewed every two days to ensure the concentration of *P. minimum*.

Eight samples were taken randomly at 0, 3, 6, 12, 24, 48 and 72 h from each replicate. Hemolymph was extracted from the hearts of eight samples with a 1 mL syringe which contained an equal volume of cold anti-coagulaion buffer (30 mmol L^{-1} trisodium citrate, 0.34 mol L^{-1} sodium chloride, and 10 mmol L^{-1} $EDTANa_2$, at a pH of 7.55 with osmolality adjusted to 780 mOsm kg^{-1} using 0.115 mol L^{-1} glucose) (Liu and Chen, 2004), and then transferred to 1.5 mL sterile centrifugal tubes. After centrifuging the mixture at 3000 × *g* for 10 min under 4℃, the supernatant was collected and stored at −80℃ for antioxidant parameters and biomolecule damage determination. Hemocytes were collected and placed in 0.5 mL TRIzol reagent (Takara, Dalian, China), and stored at −80℃ for RNA extraction. Gills and hepatopancreas were also carefully collected and were ground to fine powder in liquid nitrogen. 0.1 g powder was homogenized with 1.0 mL TRIzol reagent (Takara, Dalian, China) and stored at −80℃ for RNA extraction.

2.4 Effect of *P. minimum* on Oxidative Damage in Hemolymph

The total antioxidant capacity (T-AOC), catalase (CAT), glutathione peroxidase (GPX) and superoxide dismutase (SOD) activities, and protein and malondialdehyde (MDA) contents were determined with commercial test kits (Jiancheng, Ltd., Nanjing, China) and measured on an Infinite M2000 Pro™ plate reader (Tecan, Germany).

One unit of T-AOC was defined as 0.01 increase of absorbance value caused by 1 mg protein per minute at 37℃. CAT activity was measured by monitoring residual hydrogen peroxide (H_2O_2) absorbance at 405 nm (Goth, 1991). The activity of GPX was estimated based on the dithiobinitrobenzoic acid method (Rotruck *et al*., 1973) using H_2O_2 and glutathione (GSH) as substrate. The SOD activity was determined according to the xanthine/xanthine oxidase method by Marklund and Marklund (1974). The protein contents were measured by the method of Bradford (1976) at 595 nm, using bovine serum albumin as a stan-

dard. The MDA content, an indicator of lipid peroxidation (LPO), was measured using the thiobarbituric acid test according to Ohkawa *et al*. (1979).

2.5 Effect of *P. minimum* on Related Gene Transcription

Samples were thawed on ice, total RNA was extracted with TRIzol reagent (Takara, Dalian, China), and remaining genomic DNA was purified by RNase-free DNase (Takara, Dalian, China) according to the manufacture's protocol. Purity and concentration of nucleic acids were determined by spectrophotometry (A260/A280) and electrophoresis on 1.5% agarose gel. Finally, 2.0 μg verified RNA sample was used for cDNA synthesis with PrimeScript™ Real-time PCR Kit (Takara, Dalian, China), and the product was stored at −20℃.

Table 1 Primers used for real-time PCR

Gene	GenBank number	Forward Prime Sequence (5' to 3')	Reverse Prime Sequence (5' to 3')
GST†	KF430648.1	GATGGCTCAATGGCTTCCTA	AGGCATATTGGCTTTGCATC
HSP70†	HQ185257.1	GGACCTGTTGCTGTTGGACG	TTGGTGGGGATGGTGGTGTT
Caspase-3†	KJ408215.1	GTAAACGCAAGGGAATCCAA	GGACTGCTACTTCCCTGCTG
18S††	HQ172894.1	TATACGCTAGTGGAGCTGGAA	GGGGAGGTAGTGACGAAAAAT

Notes: † Target gene; †† Internal control gene.

Different changes in gene expression were determined by real-time RCR (RT-PCR), which was performed on the ABI 7500 System (Applied Biosystems, USA). The target genes and the primers were shown in Table 1. The primers were designed using Primer 5 software.

The RT-PCR was carried out in the buffer with a total volume of 10 μL, containing 5 μL of Eva Green 2× qPCR Master Mix (ABM, Canada), 1 μL of the diluted cDNA, 0.3 μL of each primer and 3.4 μL of deionized water. The PCR program included one cycle of 95℃ for 30 s, then 40 cycles of 95℃ for 5 s and 60℃ for 34 s, followed by 1 cycle of 95℃ for 15 s, 60℃ for 1 min and 95℃ for 15 s. After the program, the threshold cycle (CT) values were obtained from the samples. The $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001) was used to analyze the expression levels of the target genes.

2.6 Statistical Analysis

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

3 Results

3.1 Effects of *P. minimum* on Antioxidant Activities in the Hemolymph

The antioxidant activities of T-AOC, CAT, GPX and SOD in the hemolymph were shown in Fig.1. Compared with the control group, the T-AOC activity was depressed significantly at 3-48 h, then recovered to the control level at 72 h ($P<0.01$, Fig.1A). The CAT activity significantly declined after exposure to *P. minimum* ($P<0.01$), from less than half of the control at 3 h to less than one third at 72 h (Fig.1B). The GPX activity was significantly affected by *P. minimum* ($P<0.05$). It increased at 3 h then decreased and rose again at 72 h (Fig.1C). Compared with the control group, the SOD activity was evoked at once and last for 72 h ($P<0.01$, Fig.1D).

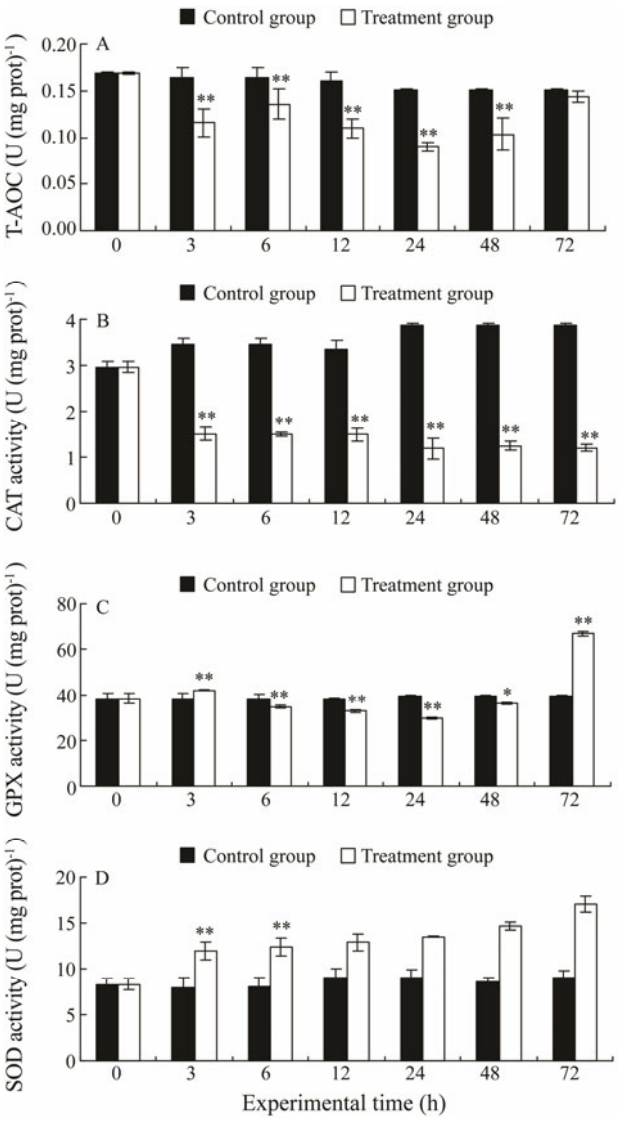

Fig.1 Effects of *P. minimum* exposure on antioxidant activities in the hemolymph of *E. carinicauda*. Data in all cases are expressed as means ± standard deviation (SD) ($n=8$). * means $P<0.05$ compared with the control, and ** means $P<0.01$ compared with the control.

3.2 Effects of *P. minimum* on Lipid Peroxidation in the Hemolymph

The contents of MDA were significantly affected by *P. minimum* exposure ($P<0.01$, Fig.2). Compared with the control group, the MDA content in the hemolymph increased significantly at 12 h and last for 72 h ($P<0.01$).

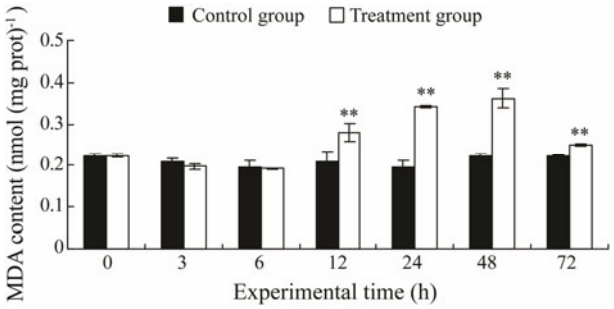

Fig.2 Effects of *P. minimum* exposure on the contents of malondialdehyde in the hemolymph of *E. carinicauda*. Data are expressed as means ± standard deviation (SD) ($n=8$). ** means $P<0.01$ compared with the control.

3.3 Effect of *P. minimum* on *GST* Gene Expression

The effects of *P. minimum* exposure on the relative expression levels of *GST* gene in the hemocyte, gills and hepatopancreas of *E. carinicauda* were shown in Fig.3. The expression level of *GST* gene in the gill was not significantly affected by *P. minimum* exposure ($P>0.05$, Fig.3B). The expression level of *GST* gene in the hemocyte was only increased at 12 h and 72 h ($P<0.05$, Fig.3A); while in the hepatopancreas, the *GST* mRNA expression increased significantly after 12 h and reached to the peak level at 48 h (2.78-fold of control, $P<0.01$, Fig.3C).

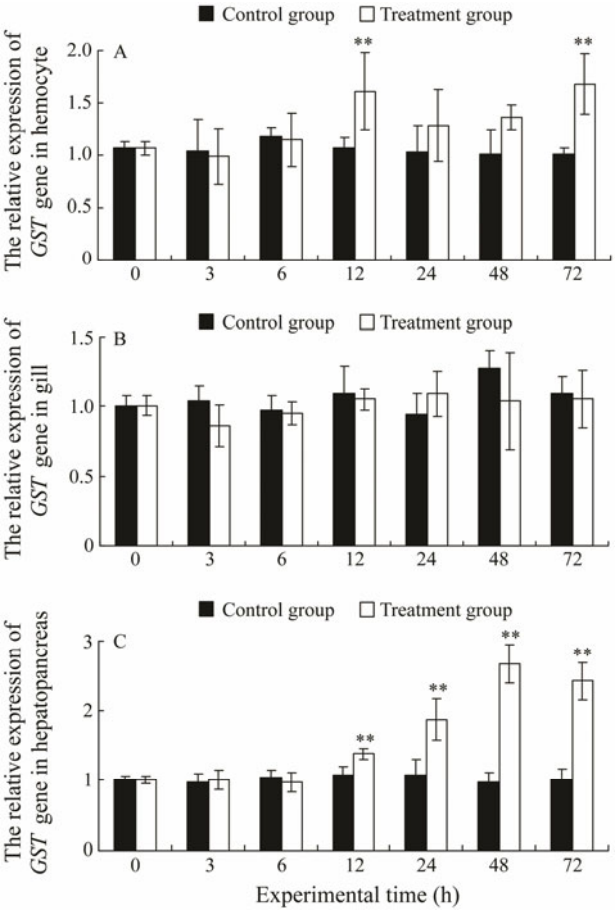

Fig.3 Effects of *P. minimum* exposure on the relative expression levels of *GST* gene in the hemocyte, gill and hepatopancreas of *E. carinicauda*. Data are expressed as means ± standard deviation (SD) ($n=8$). ** means $P<0.01$ compared with the control.

3.4 Effect of *P. minimum* on *HSP70* Gene Expression

The effects of *P. minimum* exposure on the relative expression levels of *HSP70* gene were shown in Fig.4. The mRNA level of *HSP70* in the hemocyte increased significantly after exposure to *P. minimum* for 6 h and reached to the peak level at 48 h (2.32-fold of control, $P<0.01$, Fig.4A). The mRNA levels of *HSP70* in the gill and hepatopancreas were evoked immediately by *P. minimum* and lasted for 72 h ($P<0.01$, Fig.4B, C).

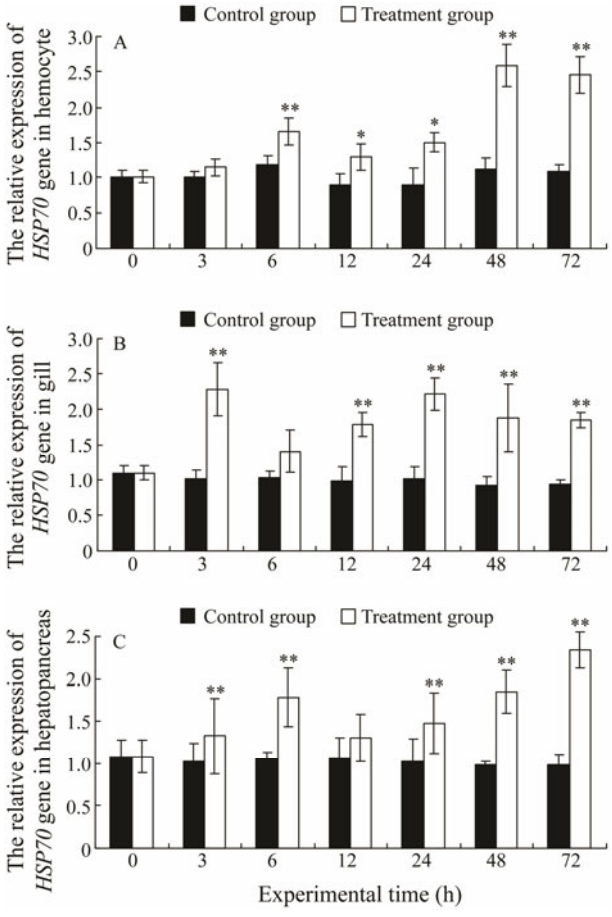

Fig.4 Effects of *P. minimum* exposure on the relative expression levels of *HSP70* gene in the hemocyte, gill and hepatopancreas of *E. carinicauda*. Data in all cases are expressed as means ± standard deviation (SD) ($n=8$). * means $P<0.05$ compared with the control, and ** means $P<0.01$ compared with the control.

3.5 Effect of *P. minimum* on *Caspase-3* Gene Expression

The expression patterns of *caspase-3* gene were shown in Fig.5. Compared with the control group, the mRNA levels of *caspase-3* in the hemocyte and gills were up-

regulated at 6 h and reached to the highest levels at 24 h (3.45-fold of control) and 72 h (2.38-fold of control), respectively ($P < 0.01$, Figs.5A, B). The mRNA level of *caspase-3* in the hepatopancreas reached the peak level at 3 h (1.46-fold of control, $P < 0.01$), then decreased and recovered to the control level at 12 h (Fig.5C).

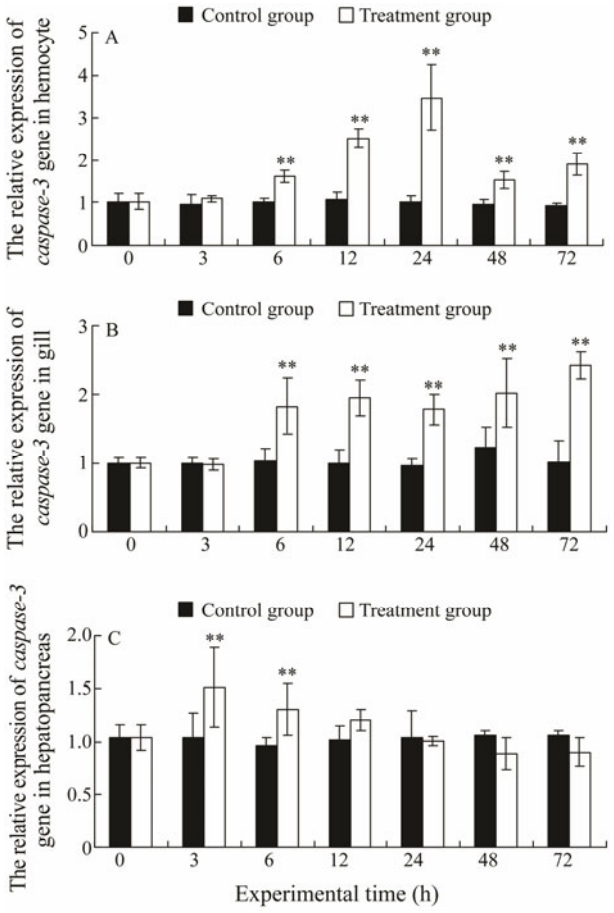

Fig.5 Effects of *P. minimum* exposure on the relative expression levels of *caspase-3* gene in the hemocyte, gill and hepatopancreas of *E. carinicauda*. Data in all cases are expressed as means ± standard deviation (SD) ($n = 8$). ** means $P < 0.01$ compared with the control.

4 Discussion

Oxidative stress is defined as an imbalance between oxidants and antioxidants in favor of the oxidants, leading to potential damages (Sies, 1997). It was suggested to be a central element in the regulation of apoptotic pathways (Ott *et al*., 2007; Ryter *et al*., 2007; Franco *et al*., 2009). Therefore, in this study healthy prawns were exposed to *P. minimum* at a concentration of 5×10^4 cells mL^{-1} for 72 hours to investigate the adverse effect of *P. minimum* on oxidative stress and apoptosis in *E. carinicauda*.

More attention has been focused on antioxidant research of aquatic organisms because of their peculiarly susceptible to oxidative stress as a result of environmental perturbations (Liu and Chen, 2004; Castex *et al*., 2010; Ren *et al*., 2014). Under oxidative stress, shrimp can evoke an integrated antioxidant system, including enzymatic and non-enzymatic antioxidants, to maintain normal oxidant status. The antioxidant defense systems benefit organisms to eliminate ROS, which was produced by oxidative stress. If they are not eliminated quickly, the overproduction of ROS can damage important biomolecules, leading to cell damage or death (El-Beltagi and Mohamed, 2013).

Like other crustaceans, shrimp completely depend on an innate immune system, which includes humoral and cellular responses to defend against the potentially harmful and pathogenic organisms (Bachère *et al*., 2004; Vazquez *et al*., 2009). As an essential element in the vascular system, hemolymph plays important roles in both humoral and cellular immunity (Bachère *et al*., 2004). Therefore, in this work oxidative damage in the hemolymph was investigated to evaluate the oxidative stress caused by *P. minimum* exposure.

The T-AOC can reflect the health status of an organism under certain conditions (Xu and Pan, 2013). The repressed T-AOC activity suggests that the physiological status of *E. carinicauda* was affected after exposure to *P. minimum*. SOD plays an important role in transforming the superoxide radical (O^{2-}) into H_2O_2 and O_2, and is the first enzyme to respond to O^{2-} and protect organisms from oxidative stress (Winston, 1991; El-Beltagi and Mohamed, 2013). In the present study, the SOD activity was significantly stimulated by *P. minimum*, and remained at a higher level than the control until the end of the experiment. The increased SOD activity indicates that a large amount of O^{2-} was converted into H_2O_2 and the antioxidant activities were evoked by exposure to *P. minimum*. Both CAT and GPX can convert H_2O_2 into H_2O and O_2 (El-Beltagi and Mohamed, 2013), protecting tissues and cells against LPO and H_2O_2 (Ran *et al*., 2007). Reduced CAT activity and fluctuating GPX activity were observed in this study, which suggests that the antioxidant activities might be insufficient to reduce the ROS level and the induced oxidative damage. MDA is an important product of LPO caused by ROS (Winston, 1991), and is often used to reflect the degree of cell damage (Jia *et al*., 2014; Li *et al*., 2015; Ren *et al*., 2015a, b; Wang *et al*., 2015; Wei and Yang, 2015). The increased MDA content after 12 h in the treatment group indicated that the host defense system could not eliminate ROS quickly, and was damaged by the overproduction and residuals of ROS (Ryter *et al*., 2007; El-Beltagi and Mohamed, 2013).

GSTs not only play an important role in detoxification reactions by catalyzing the conjugation of activated xenobiotics to endogenous water-soluble substrates, but also provide the cell with protection against a range of harmful electrophiles produced by oxidative damage to membranes (Sheehan *et al*., 2001; Blanchette *et al*., 2005; Hayes *et al*., 2005; Goto *et al*., 2009). Up-regulation of *GST* gene and increased GST activity were observed in the hepatopancreas of *Litopenaeus vannamei* after injected with a sublethal dose extract of toxic *Microcystis aeruginosa* (Gonçalves-Soares *et al*., 2012). Liang *et al*. (2014) also reported enhanced GST activity in the hepatopancreas of *Fenneropenaeus chinensis* after exposure to 1000 cells mL^{-1} of *Alexandrium tamarense* from 12 to 72 h. In the

present study, no change of *GST* gene expression in the gill and up-regulation of *GST* gene in the hepatopancreas were observed, suggesting that *GST* might play an important role in the shrimp hepatopancreas after exposure to *P. minimum*. Compared with the induced level of *caspase-3* gene expression in the gill, the up-regulation of *caspase-3* gene in the hepatopancreas was only observed from 3 to 6 h, after which time the mRNA level of *GST* increased. The results indicate that GST might be involved in the shrimp hepatopancreas' defense against harmful algal exposure.

HSPs play an important role in stress resistance, and the expression levels of HSPs are suggested to be a balance between the benefits and costs (Sørensen *et al*., 2003). Up-regulation of *HSP70* gene was found in the present study, suggesting that the levels of non-native proteins increased to assist the cellular homeostasis. HSP70 is a key regulator of signal transduction pathways controlling homeostasis, proliferation, differentiation and death of cells (Mayer and Bukau, 2005). HSP70 also protects cells against oxidative stress and is involved in anti-apoptotic responses (Mayer and Bukau, 2005; Jiang *et al*., 2012; Bermejo-Nogales *et al*., 2014). The induced *HSP70* gene in this work might be triggered by ROS production and the denatured proteins, and might reflect a protective response against exposure to *P. minimum*.

Apoptosis can be activated by two known pathways, the extrinsic death receptor pathway and the intrinsic mitochondrial pathway (Ott *et al*., 2007; Ryter *et al*., 2007). In each pathway, caspase-3 is the major executioner of active cell apoptosis (Kumar, 2007). The up-regulation of *caspase-3* gene in this study suggested that *P. minimum* could induce apoptosis in the hemocytes and gill of *E. carinicauda*. Environmental stressors are well known to induce apoptotic cell death via the intrinsic pathway (Ryter *et al*., 2007), and oxidative stress is suggested to be the central element in the regulation of the apoptotic pathways (Franco *et al*., 2009). The results of this study suggest that exposure to *P. minimum* could cause oxidative stress, evoke the SOD activity, increase *HSP* expression, and induce apoptosis in *E. carinicauda*. Similar results were reported by Huang *et al*. (2015), which suggested that oxidative stress and cytoskeletal disruption might interact with each other and jointly lead to apoptosis and renal toxicity induced by microcystins (MCs).

5 Conclusions

Since few study on the mechanisms of *P. minimum*'s effects on shrimp is available, this study was carried out under oxygenated conditions to determine the mechanisms of the toxic effect of *P. minimum* on *E. carinicauda*. We demonstrated that exposure to *P. minimum* affected the physiological health of *E. carinicauda* by inducing oxidative damage and apoptosis. The SOD activity, *HSP70* and *GST* (in the hepatopancreas) were evoked to protect cells from oxidative stress and apoptosis. Further study is needed to determine the mechanism in more details. Our results will help us understand the mechanisms of *P. minimum* on shrimp.

Acknowledgements

This work was supported by the Program of Shandong Leading Talent (No. LNJY2015002), the Scientific and Technological Innovation Project Financially Supported by Qingdao National Laboratory for Marine Science and Technology (No. 2015ASKJ02), and the Qingdao Planned Projects for Postdoctoral Research Funds (No. ZQ 5120 1617013).

References

Alonso-Rodrıguez, 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.

Bachère, E., Gueguen, Y., Gonzalez, M., De Lorgeril, J., Garnier, J., and Romest, B., 2004. Insights into the anti-microbial defense of marine invertebrates: The penaeid shrimps and the oyster *Crassostrea gigas*. *Immunological Reviews*, **198** (1): 149-168.

Bermejo-Nogales, A., Nederlof, M., Benedito-Palos, L., Ballester-Lozano, G. F., Folkedal, O., Olsen, R. E., Sitjà-Bobadilla A., and Pérez-Sánchez, J., 2014. Metabolic and transcriptional responses of gilthead sea bream (*Sparus aurata* L.) to environmental stress: New insights in fish mitochondrial phenotyping. *General and Comparative Endocrinology*, **205**: 305-315.

Blanchette, B., Feng, X., and Singh, B. R., 2007. Marine glutathione S-transferases. *Marine biotechnology*, **9** (5): 513-542.

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**: 248-254.

Castex, M., Lemaire, P., Wabete, N., and Chim, L., 2010. Effect of probiotic *Pediococcus acidilactici* on antioxidant defences and oxidative stress of *Litopenaeus stylirostris* under *Vibrionigripulchritudo challenge*. *Fish and Shellfish Immunology*, **28** (4): 622-631.

Cheng, C. H., Yang, F. F., Ling, R. Z., Liao, S. A., Miao, Y. T., Ye, C. X., and Wang, A. L., 2015. Effects of ammonia exposure on apoptosis, oxidative stress and immune response in pufferfish (*Takifugu obscurus*). *Aquatic Toxicology*, **164**: 61-71.

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.

Franco, R., Sánchez-Olea, R., Reyes-Reyes, E. M., and Panayiotidis, M. I., 2009. Environmental toxicity, oxidative stress and apoptosis: Menage a trois. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, **674** (1): 3-22.

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 Chi-*

mica 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.

Hayes, J. D., Flanagan, J. U., and Jowsey, I. R., 2005. Glutathione transferases. *Annual Review of Pharmacology and Toxicology*, **45**: 51-88.

Hégaret, H., and Wikfors, G. H., 2005. Effects of natural and field-simulated blooms of the dinoflagellate *Prorocentrum minimum* upon hemocytes of eastern oysters, *Crassostrea virginica*, from two different populations. *Harmful Algae*, **4** (2): 201-209.

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.

Jiang, J., Shi, Y., Shan, Z., Yang, L., Wang, X., and Shi, L., 2012. Bioaccumulation, oxidative stress and HSP70 expression in *Cyprinus carpio* L. exposed to microcystin-LR under laboratory conditions. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, **155** (3): 483-490.

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.

Kumar, S., 2007. Caspase function in programmed cell death. *Cell Death and Differentiation*, **14** (1): 32-43.

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, 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.

Liu, C. H., and Chen, J. C., 2004. Effect of ammonia on the immune response of white shrimp*Litopenaeus vannamei* and its susceptibility to *Vibrio alginolyticus*. *Fish and Shellfish Immunology*, **16** (3): 321-334.

Livak, K. J., and Schmittgen, T. D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods*, **25** (4): 402-408.

Luckenbach, M., Sellner, K., Shumway, S., and Greene, K., 1993. Effects of two bloom-forming dinoflagellates, *Prorocentrum minimum* and *Gyrodinium uncatenum*, on the growth and survival of the eastern oyster, *Crassostrea virginica* (Gmelin 1791). *Journal of Shellfish Research*, **12** (2): 411-415.

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.

Mayer, M., and Bukau, B., 2005. Hsp70 chaperones: Cellular functions and molecular mechanism. *Cellular and Molecular Life Sciences*, **62** (6): 670-684.

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.

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., 2014. Effect of florfenicol on selected parameters of immune and antioxidant systems, and damage indexes of juvenile *Litopenaeus vannamei* following oral administration. *Aquaculture*, **432**: 106-113.

Ren, X., Pan, L., and Wang, L., 2015a. The detoxification process, bioaccumulation and damage effect in juvenile white shrimp *Litopenaeus vannamei* exposed to chrysene. *Ecotoxicology and Environmental Safety*, **114**: 44-51.

Ren, X., Pan, L., and Wang, L., 2015b. 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.

Sheehan, D., Meade, G., and Foley, V. M., 2001. Structure, function and evolution of glutathione transferases: Implications for

classification of non-mammalian members of an ancient enzyme superfamily. *Biochemical Journal*, **360** (1): 1-16.

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.

Sies, H., 1997. Oxidative stress: Oxidants and antioxidants. *Experimental Physiology*, **82** (2): 291-295.

Sørensen, J. G., Kristensen, T. N., and Loeschcke, V., 2003. The evolutionary and ecological role of heat shock proteins. *Ecology Letters*, **6** (11): 1025-1037.

Tango, P., 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.

Vazquez, L., Alpuche, J., Maldonado, G., Agundis, C., Pereyra-Morales, A., and Zenteno, E., 2009. Review: Immunity mechanisms in crustaceans. *Innate Immunity*, **15** (3): 179-188.

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.

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**: 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.

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**: 117-124.

(Edited by Qiu Yantao)