

The Open Access Israeli Journal of Aquaculture – Bamidgeh

As from **January 2010** The Israeli Journal of Aquaculture - Bamidgeh (IJA) will be published exclusively as an **on-line Open Access (OA)** quarterly accessible by all AquacultureHub (<http://www.aquaculturehub.org>) members and registered individuals and institutions. Please visit our website (<http://siamb.org.il>) for free registration form, further information and instructions.

This transformation from a subscription printed version to an on-line OA journal, aims at supporting the concept that scientific peer-reviewed publications should be made available to all, including those with limited resources. The OA IJA does not enforce author or subscription fees and will endeavor to obtain alternative sources of income to support this policy for as long as possible.

Editor-in-Chief

Dan Mires

Editorial Board

Rina Chakrabarti Aqua Research Lab, Dept. of Zoology, University of Delhi, India

Angelo Colorni National Center for Mariculture, IOLR, Eilat, Israel

Daniel Golani The Hebrew University of Jerusalem, Israel

Hillel Gordin Kibbutz Yotveta, Arava, Israel

Sheenan Harpaz Agricultural Research Organization, Beit Dagan, Israel

Gideon Hulata Agricultural Research Organization Beit Dagan, Israel

George Wm. Kissil National Center for Mariculture, IOLR, Eilat, Israel

Ingrid Lupatsch Swansea University, Singleton Park, Swansea, UK

Spencer Malecha Dept. of Human Nutrition, Food & Animal Sciences, CTAHR, University of Hawaii

Constantinos Mylonas Hellenic Center for Marine Research, Crete, Greece

Amos Tandler National Center for Mariculture, IOLR, Eilat, Israel

Emilio Tibaldi Udine University, Udine, Italy

Jaap van Rijn Faculty of Agriculture, The Hebrew University of Jerusalem, Israel

Zvi Yaron Dept. of Zoology, Tel Aviv University, Israel

Copy Editor Miriam Klein Sofer

Published under auspices of
The Society of Israeli Aquaculture and Marine Biotechnology (SIAMB)
&
University of Hawai'i at Mānoa
&
AquacultureHub
<http://www.aquaculturehub.org>



UNIVERSITY
of HAWAII
MĀNOA
LIBRARY



AquacultureHub
educate • learn • share • engage

ISSN 0792 - 156X

© Israeli Journal of Aquaculture - BAMIGDEH.

PUBLISHER:

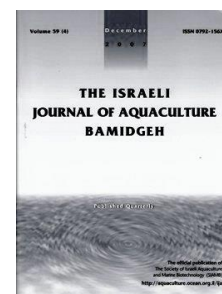
Israeli Journal of Aquaculture - BAMIGDEH -
Kibbutz Ein Hamifratz, Mobile Post 25210,
ISRAEL

Phone: + 972 52 3965809

<http://siamb.org.il>



The IJA appears exclusively as a peer-reviewed on-line open-access journal at <http://www.siamb.org.il/>. To read papers free of charge, please register online at [registration form](#).
Sale of IJA papers is strictly forbidden.



Identification of Relevant Biomarkers in Mercury Exposed Clam *Venerupis philippinarum*.

Anguo Zhang^{1,2}, Xiacong Chen¹, Ran Zhang,¹ Weiwei Zhang¹, Chunhua Jin¹ and Chenghua Li^{1*}

¹School of Marine Science, Ningbo University, Ningbo 315211, PR China

² College of Fisheries and Life Science, Dalian Ocean University, Dalian 116023, PR China

Keywords: *Venerupis philippinarum*; biomarkers; mercury; antioxidant enzymes.

Abstract

This study assessed the impact of four different concentrations (2.5, 5, 7.5, 10 µg/L) of mercury exposure on Manila clam *Venerupis philippinarum* based on the dynamic characteristics of antioxidant related enzymes including superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), acid phosphatase (ACP), alkaline phosphatase (ALP), total glutathione content, and malondialdehyde (MDA) content at 24 h and 48 h post exposure.

The results showed that hemocyte SOD activity was inhibited while GR hemocyte activity significantly increased in the 2.5 µg/L group after 24 h. Hemocyte ALP activity and (GSH) content were also elevated at the 7.5 µg/L and 10 µg/L groups when exposed for 24 h, respectively. SOD activity, GR activity, ALP activity and GSH content in the hemocytes were relatively sensitive to different concentrations of mercuric mercury (Hg²⁺), suggesting that they may act as potential biomarkers in assessing environmental mercury exposure on *Venerupis philippinarum*. At different levels of exposure, antioxidant parameters showed differing responses in gill tissue, but no biomarkers were discovered in the samples examined. We found that hemocytes are more suitable biomarkers than proteins in gill tissue. Some hemocyte biomarkers are promising candidates for monitoring mercury pollution in marine clams.

* Corresponding author: Chenghua Li; Tel.: +86-574-87608368; Fax: +86-574-87608368, Email: lichenghua@nbu.edu.cn

Introduction

Heavy metal contamination is problematic in market seafood, especially in marine and coastal environments where mercury (Hg) is considered to be one of the most hazardous, persistent, and toxic pollutants. Mercury (Hg) can be both transported and accumulated in tissues of marine organisms (Jiang et al., 2006). In aquatic systems, inorganic, and organic forms of elemental mercury are usually transformed into methyl mercury which is highly toxic (Loux 1998). Mercury (Hg) has been found in high concentrations in Bohai Sea environments (Wang et al., 2005; Wang and Wang, 2007).

Exposure to mercury can induce oxidative stress by forming reactive oxygen species (ROS) leading to increased lipid peroxidation (LPO) in marine organisms. This may affect cell viability by damaging membranes and inactivating enzymes (Nordberg et al., 2001). Lipid peroxides are unstable indicators of oxidative stress in cells that decompose to form more complex and reactive compounds such as malondialdehyde (MDA). MDA is an indicative biomarker of heavy metal pollution (Aldini et al., 2007). The level of MDA is an important indicator of lipid peroxidation and is a sensitive diagnostic index of oxidative injury in cells (Zhang et al., 2014a). Antioxidant enzymes can respond to oxidative stress to counteract the detrimental effects of ROS. These enzymes could serve as biomarkers to study the impact of mercury exposure on organisms (Verlecar et al., 2008). The major enzymatic defenses include superoxide dismutase (SOD) and catalase (CAT), inactivating the superoxide anion. Acid phosphatases act as marker enzymes for the detection of lysosomes in cell fractions and can be altered by the presence of xenobiotics (Cajaraville et al., 2000), whilst alkaline phosphatases are intrinsic plasma membrane enzymes found on the membranes of almost all animal cells. The oxidative pathways of chemical toxicity and oxidative stress biomarkers in marine organisms provide the basis for biomarker selection (Regoli and Giuliani 2014).

The Manila clam *Venerupis philippinarum*, is widely distributed along coastal and estuarine areas of China. The clam is a commercially important marine bivalve in China (Zhuang 2001). Because of their benthic and sedentary modes of life, they are easily exposed to environmental pollution, and may accumulate high concentrations of heavy metals in their tissues. Therefore, they are widely used as bioindicators of heavy metal pollution in marine environments (Zhang et al., 2011; Papo et al., 2014; Zhang et al., 2014b).

Molluscan hemocytes have several key functions, such as wound and shell repair, digestion, excretion, and internal defense (Matozzo et al., 2001). It is known that the hemocytes of bivalve mollusks may accumulate high levels of metals, mainly in lysosomes. Hemocytes of mollusks are the first line of defense against pathogenic infection, through phagocytosis and stimulation of respiratory burst, and are widely used as models in environmental toxicology, in order to determine the target of heavy metal toxic effects. Gills are the main interface between the organism and its environment hence these tissues are frequent reservoirs of environmental pollutants (Company et al., 2004). Therefore they are used in the study of biochemical biomarkers in environmental monitoring studies. Our previous study (Chen et al., 2014) showed that mercuric mercury (Hg^{2+}) affected the activity of the oxidative system. However, we have not found biomarkers that have the potential to detect mercury. There has been a rapid development of the use of enzyme biomarkers (Nicholson and Lam, 2005), and the changes of key enzymatic activities have been used as biomarkers in many bivalves (Mazorra et al., 2002; Dafre et al., 2004; Świergosz-Kowalewska et al., 2012). In this study, we investigated the role of Hg^{2+} induced oxidative stress at the protein level in four environmentally relevant exposure doses in clams *V. philippinarum*, by applying antioxidant and lipid peroxidation parameters as biomarkers.

Materials and Methods

Experimental design. Clams *Venerupis philippinarum* (wet weight 8.65 ± 1.24 g) were collected from a local aquatic farm in Ningbo, Zhejiang Province, China and acclimated for five days prior to the experiment. Temperature of the aerated artificial seawater (salinity 25 psu) was maintained at 18-20°C throughout the experiment. After acclimatization, the clams were divided into five tanks (one control group and four experimental groups) each tank containing 100 clams. The 1 g/L mercury chloride was

prepared as stock solution. For the mercury exposure experiment, the clams were exposed to four different concentrations of Hg^{2+} : 2.5, 5, 7.5 and 10 $\mu\text{g/L}$. Enzyme activity in gills and hemolymph was measured at 24 and 48 h post-exposure. At each interval, 15 clams from each group were sacrificed and gills and hemolymph was removed immediately. Hemolymph was extracted from the adductor muscle using a sterile 5 ml syringe with a 22G1/4" needle containing 1 ml of Alseve buffer (ALS buffer: 60 mM glucose, 27.2 mM sodium citrate tribasic, 9 mM EDTA, 385 mM NaCl, pH 7 and 1000 mOsm/L). Hemolymph was centrifuged at 800 rpm for 5 min to harvest the hemocytes. The gill samples were flash-frozen in liquid nitrogen and then stored at -80°C for biochemical analysis. All the determination assays were performed in triplicate. No mortalities were recorded during the 48 h experimental period.

Sample preparation and biochemical assays. 0.2 gm of gill tissue and hemocytes were resuspended in 1 mL of lysis buffer (Beyotime, China) containing PMSF and protease inhibitor cocktail. The lysate was centrifuged for 5 min at 12,000 g and the supernatant was collected for SOD, GR, GSH, MDA activity, and protein content assays. Ice-cold PBS buffer was used for ACP and ALP analysis.

Total protein content and enzymatic activities were determined by a commercial assay kit (Beyotime Biotechnology, China) and expressed as activity units or molar per microgram proteins. The absorption values were detected at 450 nm, 520nm, 340nm, 405nm, 405nm, 412nm and 535nm for SOD, CAT, GR, ALP, AKP, GSH and MDA, respectively.

Statistical analysis. Statistical analyses were carried out by one-way ANOVA using the Duncan-test to evaluate whether the means were significantly different, taking $p < 0.05$ as minimal value of significance. Statistical computations were performed with SPSS 19.0 for Windows (SPSS).

Results

SOD, GR, ALP, and GSH content in hemocytes. The results of SOD, GR, ALP activities and GSH content in hemocytes are shown in Fig. 1. SOD activity decreased significantly within 24 h in the 2.5 $\mu\text{g/L}$ group compared to the other groups ($p < 0.01$). The GR activity increased in the 2.5 $\mu\text{g/L}$ group after 24 h. The ALP activity and GSH content in hemocytes also increased significantly in the 7.5 $\mu\text{g/L}$ and 10 $\mu\text{g/L}$ group after 24 h exposure.

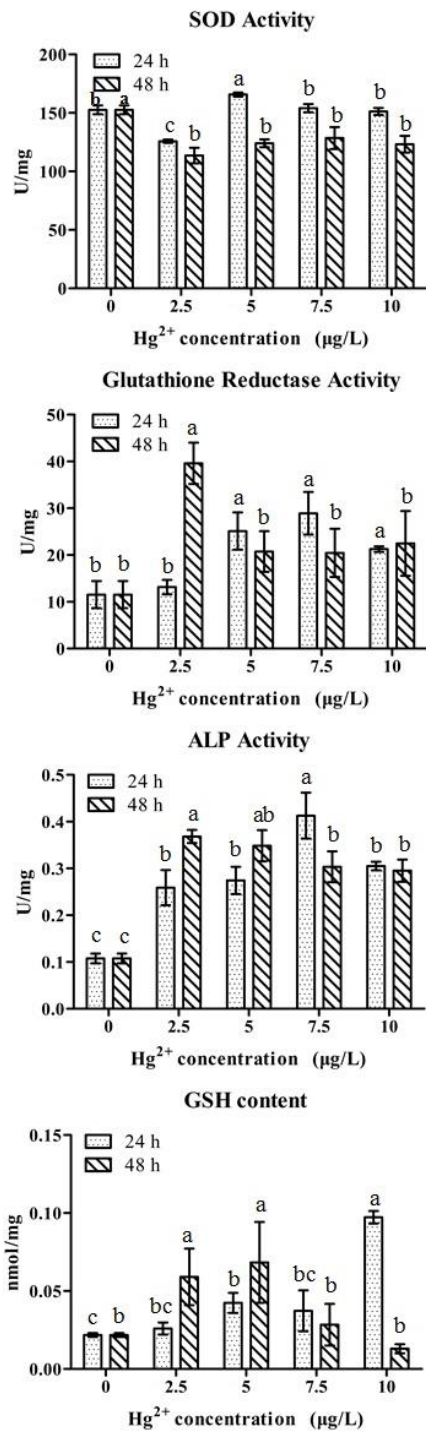


Fig. 1. The activity of SOD, GR, ALP and GSH content in hemocytes of *V. philippinarum* exposed to Hg²⁺ (0, 2.5, 5, 7.5 and 10 µg/L) for 24 h and 48 h

CAT, ACP and MDA content in hemocytes. CAT activity, ACP activity and MDA content are shown in Fig. 2. Hemocyte CAT activity and ACP activity in all groups were significantly enhanced compared to the control group. Dose-dependent expression profiles of hemocyte CAT were detected at 24 h in the first three exposure doses. No correlation was found for ACP expression and exposure doses at the time intervals tested. Clam groups exposed to mercury had higher MDA levels at 24 h, when compared to control group however there was no significant change in MDA levels at 48 h in mercury exposed groups.

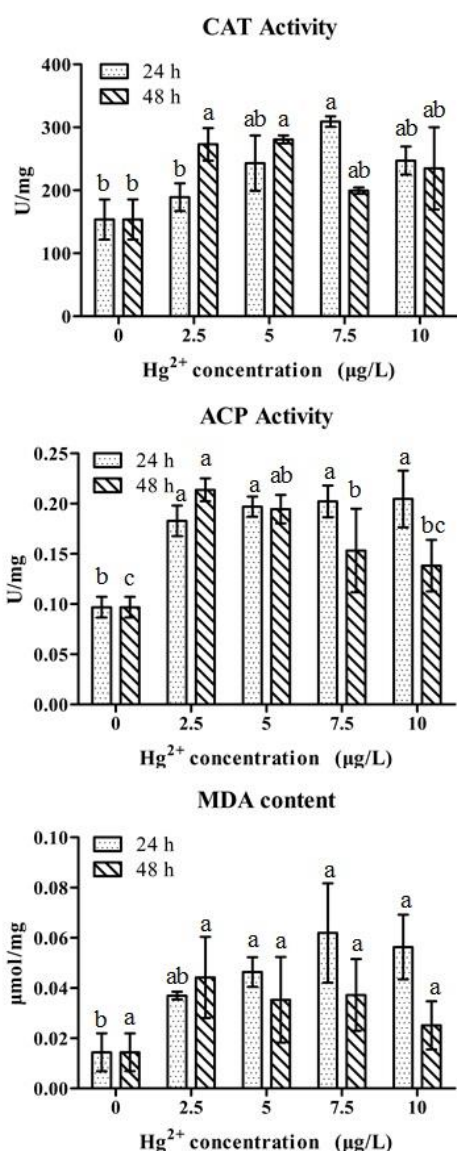


Fig. 2. The activity of CAT, ACP and MDA content in hemocytes of *V. philippinarum* exposed to Hg²⁺ (0, 2.5, 5, 7.5 and 10 µg/L) for 24 h and 48 h

Antioxidant parameters in gills. Antioxidant levels varied in examined gill tissues examined (Fig. 3). SOD activity in gill tissue was high. It was lowest in 5 µg/L group compared to the other groups at 24 h. CAT activity was time and dose-dependent. CAT activity was highest in 10 µg/L group at 48 h ($p < 0.01$). GR response in gill samples was higher than in the control. Decreased GSH content in the gills was observed in all experimental groups. It gradually increased as concentration increased at 24 h exposure, but remained lower than in control group. GSH content in the gills in the 48h exposure group followed an inverted parabolic pattern and peaked in the 5 µg/L group. ACP activity was similar to ALP activity in the gills (see Fig. 3). Highest ACP and ALP activity was found in the 7.5 µg/L mercury group at 48 h ($p < 0.05$). Lowest ACP and ALP activity was detected in 5 µg/L group at 48 h. Results obtained in this study show that lower levels of lipid peroxidation (MDA levels) were observed in the gills of clams exposed to 5 µg/L and 7.5 µg/L mercury at 24 h and 48 h, respectively ($p < 0.05$).

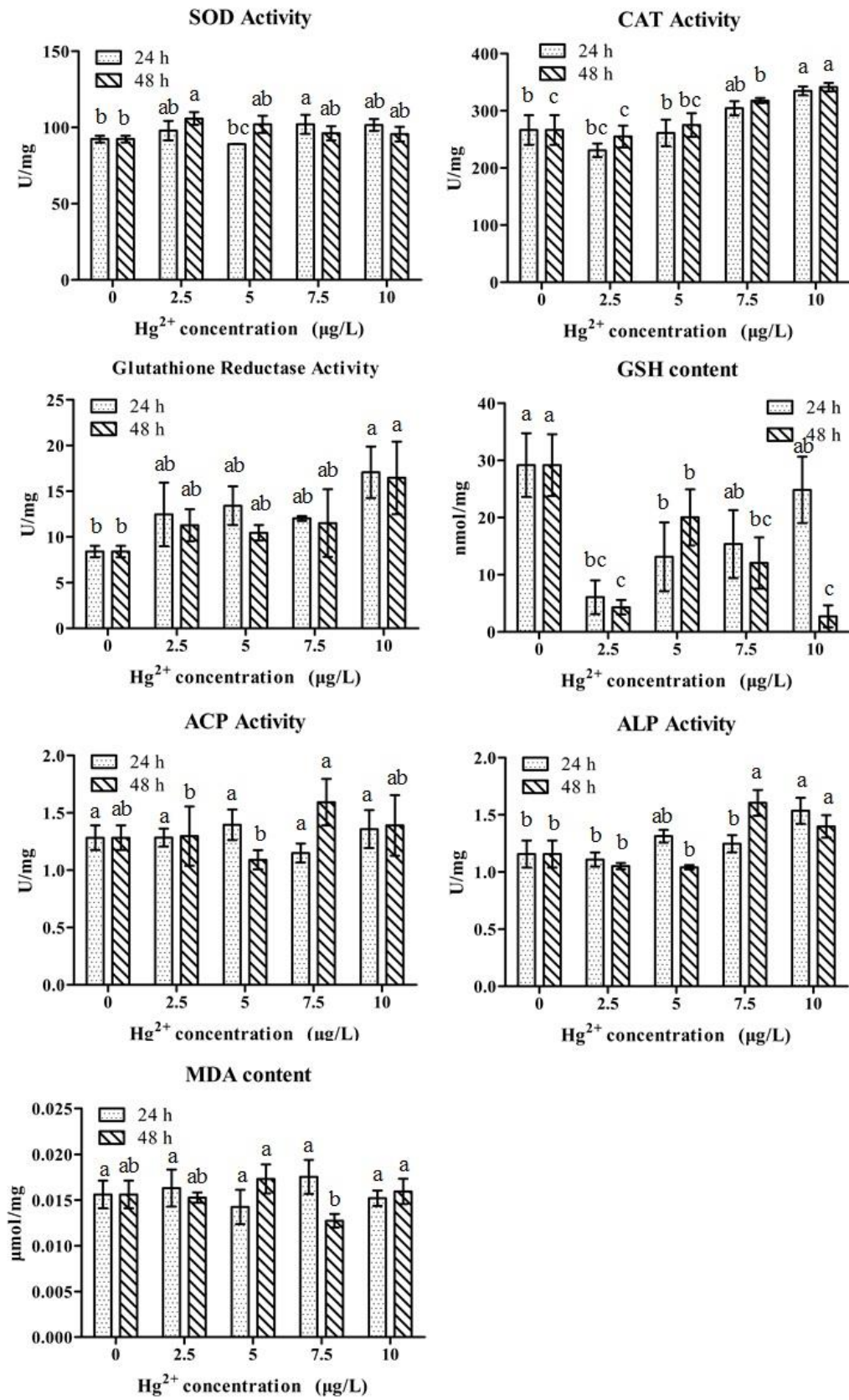


Fig. 3. The response of antioxidant parameters in gills of *V. philippinarum* exposed to Hg²⁺ (0, 2.5, 5, 7.5 and 10 µg/L) for 24 h and 48 h.

Discussion

Antioxidant activity. Several studies have indicated that antioxidant enzyme activity may increase when bivalves are exposed to low heavy metal concentrations, or during short-term exposure, but they may decrease or may even be inhibited at higher dosages, or after prolonged exposure. The results of our study support these previous findings (Géret et al., 2002a; Siraj Basha and Usha Rani, 2003; Wang et al., 2011)

Many studies have indicated the role of SOD and CAT in relation to oxidant stress as well as in protecting cells against environmental pollutions (Monteiro et al., 2010; Umasuthan et al., 2012). In the current study, the hemocytes and gills showed different patterns of change in SOD activity. SOD activity in hemocytes of the clam *V. philippinarum* decreased, while in the gill it increased at all Hg dosages. Differences in the antioxidant response between the hemocytes and gills may be related to their different physiological functions. Molluscan hemocytes are the cellular mediator of internal defense, mainly through phagocytosis and encapsulation, with subsequent enzymatic and oxidative destruction of invading foreign particles (Yang et al., 2015). In contrast, the gills are located in the ventilated mantle cavity and interact directly with the marine environment. As the gill epithelium is very thin, gills are frequent receptors of environmental pollutants (Rajalakshmi and Mohandas, 2005). Antioxidant enzyme responses observed in our study suggest that hemocytes are primarily responsible for detoxification in *V. philippinarum*. Decreased SOD activity in hemocytes of the clam *V. philippinarum* may indicate that SOD activity was inhibited during the initial hours but with the increase in metal concentrations and time, SOD was gradually inhibited (Jing et al., 2007). Decreased SOD activity detected in our results was compatible with previous studies (Verlecar et al., 2007).

In our research, increased CAT activity was detected in the gills and hemocytes of *V. philippinarum*. Significant inhibition of CAT activity was found in the gills of the clam *Ruditapes decussates* during the first two days of cadmium (Cd) exposure (Géret et al., 2002a). The hemocytes and gills of *V. philippinarum* showed different patterns of change in GSH activity. GSH activity increased in the hemocytes, whereas GSH activity decreased in the gills. These results indicate that SOD, CAT, and GSH activity of bivalves respond differently to mercury exposure in different species. Mercury concentration and exposure duration as well as environmental factors may also result in different responses of these antioxidants to oxidative stress.

Most studies have focused on the effects of exposure to heavy metals on antioxidant enzyme activities in bivalves (Isani et al., 2003; Almeida et al., 2004; Company et al., 2006; Verlecar et al., 2008). However, few studies have been conducted to determine ACP activity and ALP activity of bivalve exposure to heavy metal pollutants (Jing et al., 2006; Li et al., 2008). In the present study, results indicated that ACP activity in hemocytes of *V. philippinarum* exposed to Hg was activated, whereas, it was not activated in the gills. Hyperactivity of gill acid phosphatase in mussel *Lamellidens corrianus* (Lea) occurred at the time of mercury stress however when it dissipated, levels of enzyme activity returned to normal (Rajalakshmi and Mohandas, 2008). ACP activity in the gills of *Mactra vereformis* exposed to Cd was not activated until Day 3 and Day 5 (Wang et al., 2011). ALP is sensitive to metals, and the effects of metals on ALP have been reported in the clam *Scrobicularia plana* (Mazorra et al., 2002) and *Mactra vereformis* (Wang et al., 2011). In the current study, Hg exposure affected ALP activity in the hemocytes of *M. Vereformis*, however, a significant impact on gill ALP activity of *M. Vereformis* was seen only in the 10 µg/L Hg treatment group. Differences in enzyme activity in *V. philippinarum* may be due to the fact that metals bind differently in the hemocytes and in the gills of clams, and metal detoxification in the gills may be less effective than in the former.

MDA content can be used as a biomarker in evaluating LPO levels. In this study, MDA gradually accumulated in hemocytes of *V. philippinarum* exposed to mercury, and MDA content increased compared to the control. Similar results were found in the gills of the bivalve *M. vereformis* (Wang et al., 2011) and *R. decussatus* (Géret et al., 2002b). ROS in cells was not eliminated, causing oxidative stress, LPO, and eventually, MDA

accumulation. This agrees with conclusions of previous studies (Huang et al., 2010). Minor decreases were observed in gill MDA levels after both 24 h and 48 h of mercury exposure was tested. An inverse trend between activity of gill ACP and degree of LPO was observed, the former increasing and the latter decreasing. We conjecture that reductions in LPO were probably due to protective action of ACP against oxidative damage in clams.

Biomarker investigation. Biochemical biomarkers are increasingly used in heavy metal risk assessment of the marine environment (Eason and O'Halloran, 2002) because of their potential as rapid early warning signals against potentially damaging effects caused by stressors. ACP can be used as a reliable tool for biological assessment of metal pollution (Rajalakshmi and Mohandas, 2005). Few studies have evaluated the ACP activity of bivalves as a useful marker for monitoring heavy metal pollutants. In the current study, results indicated that SOD and GR activity in hemocytes of the clam *V. philippinarum* may be potential biomarkers to monitor concentrations of 2.5 µg/L Hg²⁺. ALP activity and GSH content could be good biomarkers indicating Hg²⁺ pollution of different concentrations. No biomarkers were discovered in gill tissues, therefore hemocytes of the clam *V. philippinarum* are more suitable as biomarkers. Although some studies have demonstrated how heavy metal pollution activates antioxidants of bivalves, few studies have addressed the way these act as biomarkers for a given concentration of mercury in the marine environment. Further study is needed to determine whether biomarkers selected in our study have early warning characteristics able to detect mercury pollution in aquaculture environments.

The above-mentioned parameters were sensitive to Cd²⁺ exposure, but further investigation is needed to see whether they can be used as biomarkers to evaluate the aquatic environment Cd²⁺ pollution.

Conclusions

Considering the contaminants in aquatic ecosystems, antioxidant parameters represent biomarkers of interest due to the specific nature of their response. Shell abnormalities should be considered as bioindicators of unfavorable environmental conditions although their potential as bioindicators of metal pollution is unclear from data available in the literature. Results presented and discussed here with bivalve *V. philippinarum*, indicate that SOD, GR, and ALP activity, and GSH content in hemocytes are potential biomarkers of mercury pollution in aquatic environments. The abilities of the biomarkers selected in our study are the focus of our further research.

Acknowledgments

This work was financially supported by the Project of International Cooperation in the Zhejiang province (2012C24022), the Key Laboratory of Marine Ecology and Environment & Disaster Prevention and Mitigation of Shandong Province (No. 201408), and the K.C. Wong Magna Fund at Ningbo University.

References

- Aldini G., Dalle-Donne I., Facino R.M., Milzan I.A. and M. Carini,** 2007. Intervention strategies to inhibit protein carbonylation by lipoxidation-derived reactive carbonyls. *Med. Res. Rev.*, 27: 817-868.
- Cajarville M.P., Bebianno M.J., Blasco J., Porte C., Sarasquete C. and A. Viarengo,** 2000. The use of biomarkers to assess the impact of pollution in coastal environments of the Iberian Peninsula: a practical approach. *Sci. Total Environ.*, 247: 295-311.
- Chen X., Zhang R., Li C. and Y. Bao,** 2014. Mercury exposure modulates antioxidant enzymes in gill tissue and hemocytes of *Venerupis philippinarum*. *ISJ-Invert. Surviv. J.* 11: 298-308.
- Company R., Serafim A., Bebianno M.J., Cosson R., Shillito B. and A. Fiala-Medioni,** 2004. Effect of cadmium, copper and mercury on antioxidant enzyme activities and lipid peroxidation in the gills of the hydrothermal vent mussel *Bathymodiolus azoricus*. *Mar. Environ. Res.*, 58: 377-381.

- Dafre A.L., Medeiros I.D., Müller I.C., Ventura E.C. and A.C.D. Bairy,** 2004. Antioxidant enzymes and thiol/disulfide status in the digestive gland of the brown mussel *Perna perna* exposed to lead and paraquat. *Chem-biol. Interact.*, 149: 97-105.
- Eason, C. and K. O'Halloran,** 2002. Biomarkers in toxicology versus ecological risk assessment. *Toxicology*, 181: 517-521.
- Géret F., Serafim A., Barreira L. and M.J. Bebianno,** 2002a. Response of antioxidant systems to copper in the gills of the clam *Ruditapes decussates*. *Mar. Environ. Res.*, 54: 413-417.
- Géret F., Serafim A., Barreira L. and M.J. Bebianno,** 2002b. Effect of cadmium on antioxidant enzyme activities and lipid peroxidation in the gills of the clam *Ruditapes decussatus*. *Biomarkers*, 7: 242-256.
- Huang W., Cao L., Liu J., Lin L. and S. Dou,** 2010. Short-term mercury exposure affecting the development and antioxidant biomarkers of Japanese flounder embryos and larvae. *Ecotox. Environ. Safe.*, 73: 1875-1883.
- Jiang G., Shi J. and X. Feng,** 2006. Mercury pollution in China. *Environ. Sci. Technol.*, 40: 3672-3678.
- Jing G., Li Y., Xie L. and R. Zhang,** 2007. Different effects of Pb²⁺ and Cu²⁺ on immune and antioxidant enzyme activities in the mantle of *Pinctada fucata*. *Environ. Toxicol. Phar.*, 24: 122-128.
- Loux, N.T.,** 1998. An assessment of mercury-species-dependent binding with natural organic carbon. *Chem. Spec. Bioavailab.*, 10: 127-136.
- Matozzo V., Ballarin L., Pampanin D.M. and M.G. Marin,** 2001. Effects of copper and cadmium exposure on functional responses of hemocytes in the clam, *Tapes philippinarum*. *Arch. Environ. Con. Tox.*, 41: 163-170.
- Mazorra M.T., Rubio J.A. and J. Blasco,** 2002. Acid and alkaline phosphatase activities in the clam *Scrobicularia plana*: kinetic characteristics and effects of heavy metals. *Comp. Biochem. Phys. B.*, 131: 241-249.
- Monteiro D.A., Rantin F.T. and A.L. Kalinin,** 2010. Inorganic mercury exposure: toxicological effects, oxidative stress biomarkers and bioaccumulation in the tropical freshwater fish matrinxã, *Brycon amazonicus* (Spix and Agassiz, 1829). *Ecotoxicology*, 19: 105-123.
- Nicholson, S. and P.K.S. Lam,** 2005. Pollution monitoring in Southeast Asia using biomarker in the mytilid mussel *Perna viridis* (Mytilidae: Bivalvia). *Environ. Int.*, 31: 121-132.
- Nordberg, J. and E.S.J. Arnér,** 2001. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. *Free Radical Biol. Med.*, 31: 1287-1312.
- Papo M.B., Bertotto D., Quaglio F., Vascellari M., Pascoli F., Negrato E., Binato G. and G. Radaelli,** 2014. Histopathology and stress biomarkers in the clam *Venerupis philippinarum* from the Venice Lagoon (Italy). *Fish. Shellfish. Immunol.* 39: 42-50.
- Rajalakshmi S. and A. Mohandas,** 2008. Impact of mercury on the activity pattern of a marker enzyme in a freshwater bivalve. *Environmentalist.* 28:249-252.
- Regoli F. and M.E. Giuliani,** 2014. Oxidative pathways of chemical toxicity and oxidative stress biomarkers in marine organisms. *Mar. Environ. Res.*, 93: 106-117.
- Świergosz-Kowalewska R., Bednarska A. and A. Kafel,** 2006. Glutathione levels and enzyme activity in the tissues of bank vole *Clethrionomys glareolus* chronically exposed to a mixture of metal contaminants. *Chemosphere*, 65: 963-974.
- Umasuthan N., Bathige S.D.N.K., Revathy K.S., Lee Y., Whang I., Choi C.Y., Park H.C. and J. Lee,** 2012. A manganese superoxide dismutase (MnSOD) from *Ruditapes philippinarum*: Comparative structural-and expressional-analysis with copper/zinc superoxide dismutase (Cu/ZnSOD) and biochemical analysis of its antioxidant activities. *Fish. Shellfish. Immunol.*, 33: 753-765.
- Verlecar X.N., Jena K.B. and G.B.N. Chainy,** 2008. Modulation of antioxidant defences in digestive gland of *Perna viridis* (L.), on mercury exposures. *Chemosphere*, 71:1977-1985.

- Wang X.Y., Yang H.S., Liu G.B. and Q. Wang,** 2011. Enzyme responses and lipid peroxidation in gills and hepatopancreas of clam *Macra vereformis*, following cadmium exposure. *Chin. J. Oceanol. Limnol.*, 29: 981-989.
- Yang H.S., Hong H.K., Donaghy L., Noh C.H., Park H.S., Kim D.S. and K.S. Choi,** 2015. Morphology and immune-related activities of hemocytes of the mussel *Mytilus coruscus* (Gould, 1861) from east sea of Korea. *Ocean. Sci. J.* 50(1):77-85.
- Zhang H., Pan L. and Y. Tao,** 2014a. Toxicity assessment of environmental pollutant phenanthrene in clam *Venerupis philippinarum* using oxidative stress biomarkers. *Environ. Toxicol. Phar.* 37: 697-704.
- Zhang H., Pan L. and Y. Tao,** 2014b. Antioxidant responses in clam *Venerupis philippinarum* exposed to environmental pollutant hexabromocyclododecane. *Environ. Sci. Pollut.*, 21: 8206-8215.
- Zhang L., Liu X., Chen L., You L., Pei D., Cong M. and H. Wu,** 2011. Transcriptional regulation of selenium-dependent glutathione peroxidase from *Venerupis philippinarum* in response to pathogen and contaminants challenge. *Fish. Shellfish. Immunol.*, 31: 831-837.
- Zhuang Q.Q., 2001.** Fauna sinica phylum mollusca class bivalvia family veneridae. Beijing: Science Press (in Chinese).
- Wang Y.W., Liang L.N., Shi J.B. and G.B. Jiang,** 2005. Study on the contamination of heavy metals and their correlations in mollusks collected from coastal sites along the Chinese Bohai Sea. *Environ. Int.*, 31: 1103-1113.
- Wang C.Y. and X.L. Wang,** 2007. Spatial distribution of dissolved Pb, Hg, Cd, Cu and As in the Bohai Sea. *J. Environ. Sci-China*, 19: 1061-1066.