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Manila clam *Venerupis philippinarum* as a biomonitor to metal pollution*

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Abstract The Manila clam *Venerupis philippinarum* is a good biomonitor/bioindicator to marine metal pollution and is frequently used in aquatic toxicology. Two dominant pedigrees (white and zebra) of clam are distributed in the Bohai Sea; however, little attention has been paid to potential biological differences between these two pedigrees. In this study, we tested the sensitivity of both pedigrees to marine metal (cadmium and zinc) pollution biomonitoring and marine environmental toxicology. Results demonstrate significant biological differences in gills of white and zebra clams based on metabolic profiles and antioxidant enzyme activities. In addition, we found that hypotaurine, malonate and homarine were relatively high in white clam gills, while alanine, arginine, glutamate, succinate, 4-aminobutyrate, taurine and betaine were high in zebra clam gills. Zebra clam gills were also more sensitive to a mixture of Cd and Zn, as shown by antioxidant enzyme activities and metabolic profiles, but white clam gills could accumulate more Zn. Therefore, we suggest that the white pedigree can be used as a biomonitor to marine Zn pollution, whereas the zebra pedigree can be used for toxicology studies on Cd and Zn mixed pollution.

Keyword: Manila clam; *Venerupis philippinarum*; biomonitor; biomarker; metabolomics

1 INTRODUCTION

With rapid industrial development, a large amount of metal contaminants have been discharged into the Bohai Sea, North China (Zhang, 2001; Mao et al., 2009). Metal contaminants can be efficiently accumulated by marine organisms, especially bivalves (e.g. mussels, oysters and clams), resulting in many adverse effects to either marine organisms or ecosystems (Regoli et al., 1998; Dovzhenko et al., 2005; Sokolova et al., 2005; Felten et al., 2008; Leung et al., 2011; Thompson et al., 2011). Furthermore, the accumulated metals in organisms can be transferred into human bodies through consumption of contaminated seafood. Therefore, marine metal contaminants pose great risks to both marine ecosystems and human health. For example, cadmium (Cd) is a typical non-essential metal element that is a natural impurity of zinc mineral and other ores, and is released into the marine environment by mining, refining and plating (Choi et al., 2007). Cadmium can enter cells through calcium (Ca) channels and can inactivate many important enzymes by competing for the catalytic sites of other metals (Bouilly et al., 2006; Chang et al., 2009). Furthermore, Cd induces oxidative stresses by producing reactive oxygen species (ROS), which cause damage to biological molecules such as lipids, proteins and DNA (Chappie

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et al., 1997; Dovzhenko et al., 2005). Although zinc (Zn) is an essential element for organisms, excessive Zn can induce potential toxicities such as genotoxicity and immunotoxicity resulting in deleterious effects on fertilization, sexual maturity, and growth of the organisms (Münzinger et al., 1988; Ballatori et al., 2002; Murphy et al., 2011). In fact, improperly treated wastewater has led to severe contamination of Cd and Zn in the Bohai Sea (Zhang, 2001; Mao et al., 2009), which demonstrates the importance of monitoring these metal elements in this habitat.

In 2004, the *Mussel Watch Program* (Goldberg, 1975) was launched for biomonitoring marine contaminants in marine environments in China. Mussels and oysters have been routinely selected as biomonitors for marine heavy metal monitoring in the program in many countries (Goldberg, 1975; Regoli et al., 1993; Muñoz-Barbosa et al., 2000). However, Ji et al. (2006) reported that *V. philippinarum* was also a good biomonitor for metal pollution because of its high accumulation of various metals including Cd, Zn, Cu, Pb, Fe, Cr, Co, and Ni. Because of its wide geographic distribution and high accumulation of metals, *V. philippinarum* thus became the preferred sentinel species in the *Mussel Watch Program* in China. However, there are two dominant pedigrees (white and zebra) of the clam population in the Bohai Sea region. In the current *Mussel Watch Program* and toxicology studies, research on the biological differences between the two pedigrees is lacking, which may cause complicated biological variation in biomonitoring and marine environmental toxicology of metal contaminants. Although no report has yet been made on biological differences between the two pedigrees in accumulating metal contaminants, evidence has demonstrated that different pedigrees of clams have differential tolerances to environmental stressors (such as temperature or salinity) (Yan et al., 2005; Zhang et al., 2008). For example, Yan et al. (2005) reported that the zebra pedigree of clam had higher survival rate and tolerance to high temperature than other pedigrees. We thus propose the hypothesis that white and zebra clams differ in their biological responses to metal contaminants. To test this hypothesis, we designed an experiment to characterize toxicological indices, such as bioaccumulation of metals, biochemical indices (e.g., antioxidant enzyme activity) and molecular biomarkers (e.g., metabolites), of white and zebra clams to metal stresses. An additional aim was to identify the most suitable clam pedigree to act as a biomonitor to metal pollution in a marine environment such as the Bohai Sea.

Previously, we investigated the differential metabolic responses in gill tissues of various pedigrees of clams exposed to mercury, and concluded that the white clam was the most sensitive pedigree to waterborne mercury exposure (Liu et al., 2011a). However, further study is necessary to illustrate the differential effects of various pedigrees of clams to either non-essential or essential metals based on metal bioaccumulations, antioxidant enzyme activities and metabolic responses. This can in turn provide more evidence for the selection of a suitable pedigree of clam for metal pollution monitoring and toxicology studies. In this work, two typical metals, Cd and Zn, were used as non-essential and essential metals, respectively, for clam exposures. The metabolic differences in gills of clams between the control and metal-exposed groups were determined by NMR-based metabolomics. The antioxidant enzyme activities (superoxide dismutase, glutathione peroxidase and glutathione S-transferases) and bioaccumulation of Cd and Zn were measured to test the sensitivity of the two pedigrees to marine metal (Cd and Zn) pollution biomonitoring and marine environmental toxicology.

2 MATERIAL AND METHOD

2.1 Sample collection

Two hundred adult clams *V. philippinarum* (shell length: 3.4–3.8 cm and n=100 for each pedigree) were purchased from a local culturing farm. The clams were allowed to acclimate in aerated seawater (25°C, salinity 33 part per thousand, collected from pristine environment) in the laboratory for 1 week and fed with *Chlorella vulgaris* Beij at a ration of 2% tissue dry weight daily. After acclimatization, clams were randomly divided into four tanks (one control and three heavy metal exposures). Each tank contained 25 white and 25 zebra clams, which were exposed to dissolved Cd^{2+} (20 μg/L), Zn^{2+} (50 μg/L), or a mixture of Cd^{2+} and Zn^{2+} for 48 h. Cadmium and zinc were prepared from $CdCl_2$ and $ZnCl_2$ (analytical grades) and the concentrations of stock solution of Cd^{2+} and Zn^{2+} were 200 mg/L. The experimental concentrations of Cd^{2+} and Zn^{2+} are environmentally relevant in the Bohai Sea (Zhang, 2001; Mao et al., 2009). After 48 hours of exposure, all clams were immediately dissected for gill tissues, which were flash frozen in liquid nitrogen, and then stored at -80°C before metabolite extraction (n=10), antioxidant enzyme

activity measurement (n=8) and metal determination (n=5).

2.2 Metabolite extraction

The metabolomes in clam samples were extracted using a modified extraction protocol as described previously (Lin et al., 2007; Wu et al., 2008, 2010; Liu et al., 2011a, 2011b). Briefly, the gill tissue (ca. 100 mg) was homogenized and extracted in 4 mL/g of methanol, 5.25 L/g of water and 2 mL/g of chloroform. The methanol/water layer with polar metabolites was transferred to a glass vial and dried in a centrifugal concentrator. The extracts of gill tissue were subsequently re-suspended in 600 μL phosphate buffer (100 mmol/L Na_2HPO_4 and NaH_2PO_4, including 0.5 mol/L TSP, pH 7.0) in D_2O. The mixture was vortexed and then centrifuged at 3 000 *g* for 5 min at 4°C. The supernatant substance (550 μL) was then pipetted into a 5 mm NMR tube prior to NMR analysis.

2.3 ^{1}H NMR spectroscopy, data processing and multivariate analysis

Clam samples were analyzed on a Bruker AV 500 NMR spectrometer performed at 500.18 MHz (at 298 K) as described previously (Liu et al., 2011a, 2011b). NMR spectral peaks were assigned following the tabulated chemical shifts (Fan et al., 1996; Viant et al., 2003) and using the software, Chenomx (Evaluation Version, Chenomx Inc., Canada).

All NMR spectra were processed using custom-written ProMetab software in Matlab (version 7.0; The MathWorks, Natick, MA; Purohit et al., 2004; Liu et al., 2011a). Each spectrum was segmented into 0.005×10^{-6} bins between $(0.2–10.0)\times10^{-6}$ with bins from $(4.70–5.20)\times10^{-6}$ (water) excluded from all the NMR spectra. All NMR spectra were generalized log transformed (gLog) with a transformation parameter $\lambda=1.0\times10^{-8}$ to stabilize the variance across the spectral bins (Purohit et al., 2004; Parsons et al., 2007).

Principal component analysis (PCA) and partial least square-discriminant analysis (PLS-DA), were used in this work to classify the sample groups, as described previously (Lindon et al., 1999; Keun et al., 2003; Rubingh et al., 2006; Wu et al., 2010; Liu et al., 2011a). SAM software was applied to find significant metabolic differences among control and heavy metal-exposed groups with appropriate false discovery rate (FDR) cutoffs. One-way ANOVA was conducted on the ratio of significantly changed bins to identify significant metabolites (at FDR<0.01; Katsiadaki et al., 2009). P=0.05 was considered significant for the ANOVA on the metabolites between control and exposed samples.

2.4 Measurement of antioxidant enzyme activities

The antioxidant enzyme activities in the gill tissues (n=8) of *V. philippinarum* were assayed using a multimode microplate reader (Infinite M200, TECAN, Switzerland) according to the manufacturer's protocols for enzyme kits (Jiancheng, Nanjing, China). In this work, the antioxidant enzymes for the activity measurement included superoxide dismutase (SOD, EC 1.15.1.1), glutathione peroxidase (GPx, EC 1.11.1.9) and glutathione S-transferases (GST, EC 2.5.1.18). Protein concentration was determined by the Coomassie brilliant blue G-250 dye-binding method with bovine serum albumin as standard (Bradford et al., 1976). The unit of each enzyme was defined as the activity of an enzyme per milligram of total protein (expressed in μmol/min/mg protein, or U/mg protein).

2.5 Cadmium and zinc concentrations in gill tissues

The gill samples (n=5) of *V. philippinarum* were dried at 80°C to constant weights. The dried tissues were digested in concentrated nitric acid (70%, Fisher Scientific) using a microwave digestion system (CEM, MAR5). The samples were heated in the microwave oven (heating to 200°C and holding at 200°C for 15 min). All completely digested samples were diluted with ultrapure water for the quantification of Cd and Zn using ICP-MS technique (Agilent 7500i, Agilent Technologies Co. Ltd, Santa Clara, CA, USA). GBW08571 Marine muscle tissue was employed as a certified reference material for metal analysis to ensure internal quality assurance/quality control (QA/QC) practices (Smith et al., 2011; Li et al., 2012). The recovery of target elements, as tested by three individual spiking experiments, was restricted within 95.5%–104.3% for Cd and 95.1%–106.2% for Zn.

2.6 Statistical analysis

Data of antioxidant enzyme activities (n=8) and metal concentrations (n=5) in the gill tissues were expressed as the mean ± standard deviation. The data were statistically analyzed using the statistical software, Statistics toolbox in Matlab (version 7.0; The MathWorks). One-way ANOVA with Tukey's

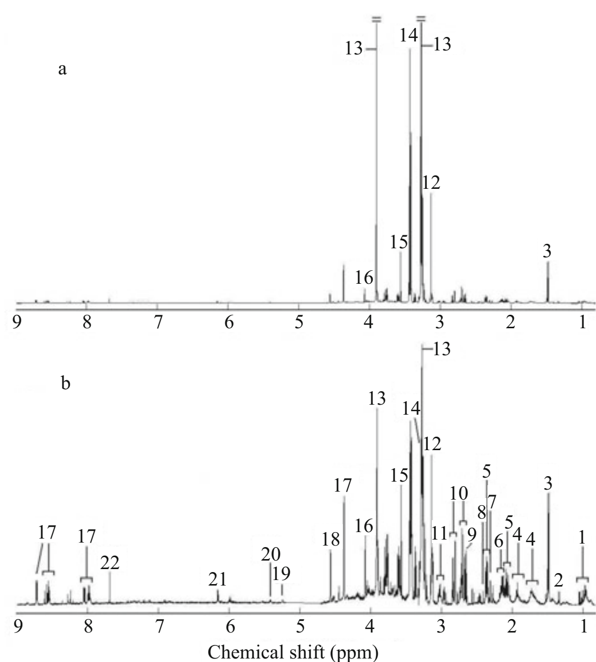

Fig.1 A representative 1-dimensional 500 MHz ^{1}H NMR spectrum of gill tissue extracts from a white clam of control group in original (a) and generalized log transformed (λ=1.0×10^{-8}) (b) forms

Keys: (1) branched chain amino acids: isoleucine, leucine and valine, (2) lactate, (3) alanine, (4) arginine, (5) glutamate, (6) glutamine, (7) acetoacetate, (8) succinate, (9) hypotaurine, (10) aspartate, (11) 4-aminobutyrate, (12) malonate, (13) betaine, (14) taurine, (15) glycine, (16) unknown 1 (4.07 ppm), (17) homarine, (18) unknown 2 (4.56 ppm), (19) α-glucose, (20) glycogen, (21) ATP, and (22) unknown 3 (7.68 ppm).

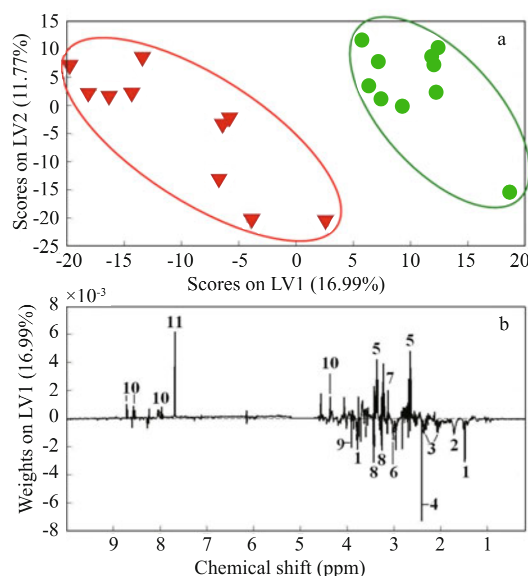

Fig.2 Partial least squares-discriminant analysis (PLS-DA) model showing (a) separations between control white (●) and control zebra (▼) clam samples, and corresponding LV1 (b) weights plot showing the metabolic differences between the different pedigrees of clam gill tissue extracts

Keys: (1) alanine, (2) arginine, (3) glutamate, (4) succinate, (5) hypotaurine, (6) 4-aminobutyrate, (7) malonate, (8) taurine, (9) betaine, (10) homarine, and (11) unknown 1 (7.68 ppm)

test was conducted on the data of antioxidant enzyme activities and metal concentrations, and significant difference was defined as P<0.05.

3 RESULT

3.1 Metabolic differences between gills of white and zebra clams

A representative ^{1}H NMR spectrum of gill tissue extracts from a white clam is shown in original (Fig.1a) and gLog transformed (Fig.1b) forms in Fig.1. Various classes of metabolites were identified, including amino acids (valine, leucine and isoleucine, alanine, aspartate, glutamate, glycine, etc.), energy related compounds (ATP/ADP, glucose and glycogen), Krebs cycle intermediates (succinate), osmolytes (betaine, taurine, hypotaurine and homarine) and a metabolite in fatty acid metabolism (acetoacetate), using the software Chenomx (Evaluation version).

PCA was applied to the ^{1}H NMR spectral data from both white control and zebra control clam samples to examine the metabolic differences in gill tissues resulting in a significant (P<0.05) separation (Data not shown). To achieve a better classification between the two control groups, PLS-DA was employed to classify white control (green cycles) and zebra control (inverted red triangles) groups, resulting in clear classification with a high Q^2 value greater than 0.4 (Fig.2a). From the first latent variable (LV1) weights plot (Fig.2b), the potentially contributive metabolites with greater weights were identified and labeled. Furthermore, the significant metabolic differences were detected after one-way ANOVA combined with a FDR at 0.01. There were several abundant metabolites including hypotaurine, malonate and homarine in the gill tissues of white clams (Fig.2b). The metabolic profile of zebra clam samples showed high levels of alanine, glutamate, succinate, 4-aminobutyrate, taurine and betaine compared with that of white clam samples.

3.2 Metabolic changes induced by metals in gills from white and zebra clams

PCA was conducted on the ^{1}H NMR spectral data from control and metal-exposed clams. However, no

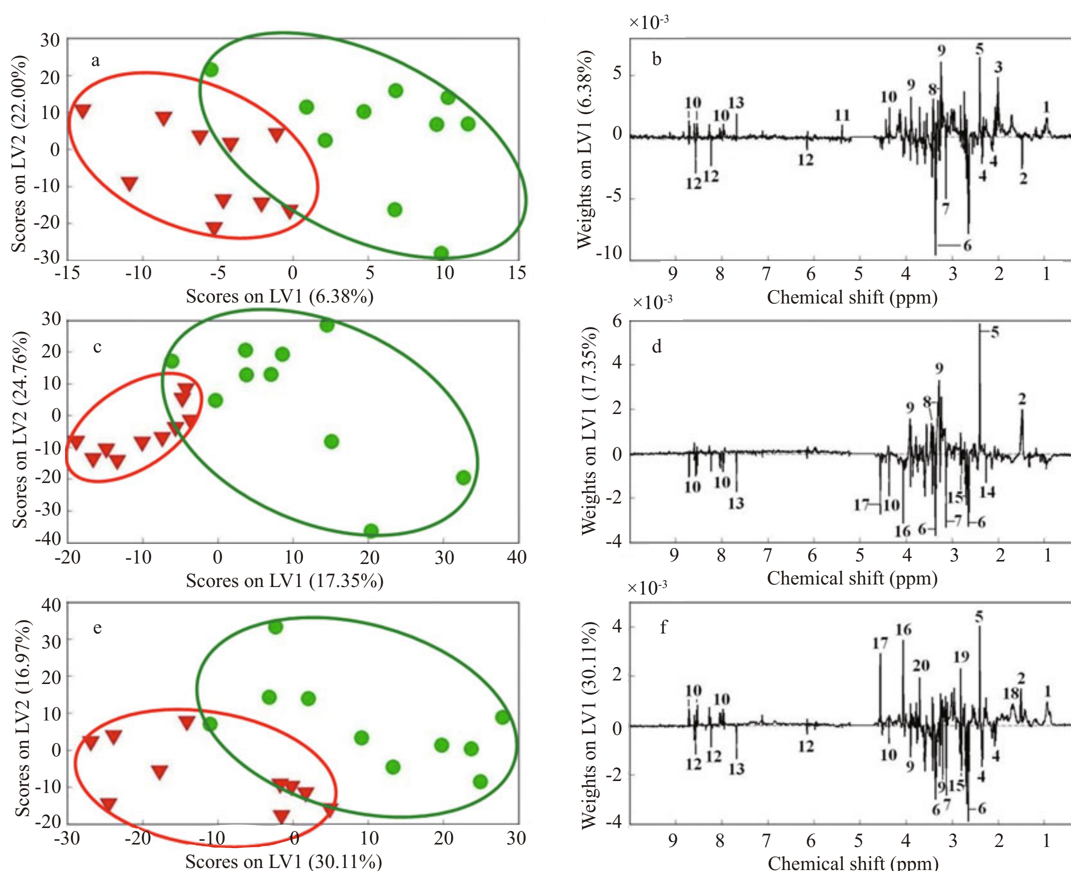

Fig.3 Partial least squares-discriminant analysis model showing separations between control (▼) and Cd (a), Zn (c) and mixed Cd/Zn (e) exposed (●) white clams, and corresponding LV1 weights plots, (b), (d) and (f) showing the metabolic differences between the control and metal-exposed clam samples after exposure for 48 h

Keys: (1) branched chain amino acids: valine, leucine and isoleucine, (2) alanine, (3) proline, (5) succinate, (6) hypotaurine, (7) malonate, (8) taurine, (9) betaine, (10) homarine, (11) glycogen, (12) ATP, (13) unknown 1 (7.68 ppm), (14) acetoacetate, (15) aspartate, (16) unknown 2 (4.07 ppm), (17) unknown 3 (4.56 ppm), (18) arginine, (19) unknown 4 (2.82 ppm), and (20) unknown 5 (3.71 ppm).

statistical significances from either white or zebra pedigrees were found between control and metal-exposed groups (data not shown). Therefore, the supervised pattern recognition method, PLS-DA, was applied for the separations between control and metal-treated groups. As shown in Figs.3 and 4, the control (triangles) and each metal-treated groups (cycles) were clearly separated along LV1 axis with Q^2 values larger than 0.4. The potentially contributive metabolites for the classification between control and metal-exposed groups were identified and labeled in the corresponding LV1 weights plots (Figs.3b, 3d, 3f, 4b, 4d, and 4f). For the white clam samples, the significant metabolic biomarkers induced by Cd in white clam gills included increased branched chain amino acids, proline, succinate, taurine, betaine, homarine and glycogen, together with decreased alanine, glutamate, hypotaurine, malonate and ATP. In Zn-treated white clams, significantly elevated alanine, succinate, betaine and taurine were found as well as depleted acetoacetate, aspartate, malonate, hypotaurine and homarine in gill tissues. For the exposure of white clams to mixed Cd/Zn, the inducible metabolic biomarkers comprised the up-regulated branched chain amino acids, alanine, arginine, succinate and homarine, and down-regulated glutamate, aspartate, hypotaurine, malonate, betaine and ATP in gill tissues.

In zebra clam gills, the significant metabolic responses to Cd exposure included increased alanine, hypotaurine, glutamine, succinate, betaine and homarine as well as decreased arginine, acetoacetate, aspartate, malonate, taurine and ATP. Zn exposure induced obvious increases in alanine, hypotaurine, malonate, betaine and homarine and decreases in arginine, acetoacetate, aspartate and taurine. For exposure of zebra clams to mixed Cd/Zn, elevated alanine, acetoacetate, hypotaurine, glycine and

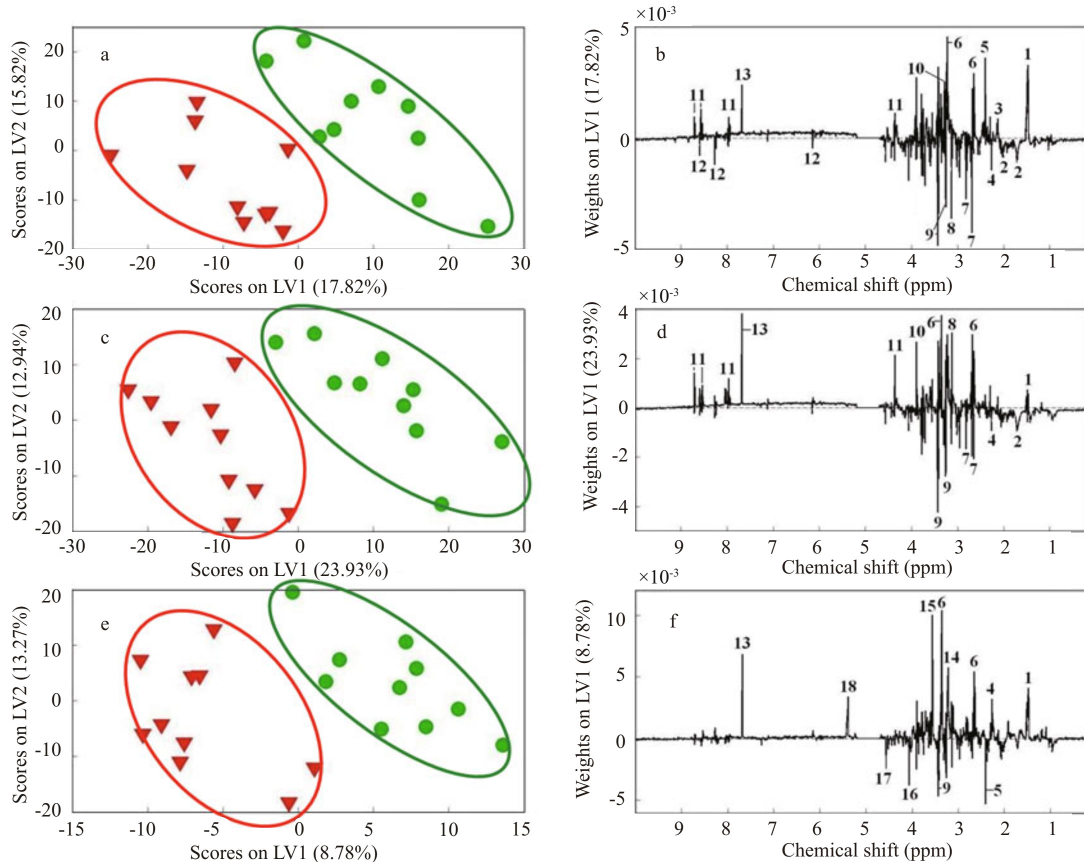

Fig.4 Partial least squares-discriminant analysis model showing separations between control (▼) and Cd (a), Zn (c) and mixed Cd/Zn (e) exposed (●) zebra clams, and corresponding LV1 weights plots, (b), (d) and (f) showing the metabolic differences between the control and metal-exposed clam samples after exposure for 48 h

Keys: (1) alanine, (2) arginine, (3) glutamine, (4) acetoacetate, (5) succinate, (6) hypotaurine, (7) aspartate, (8) malonate, (9) taurine, (10) betaine, (11) homarine, (12) ATP, (13) unknown 1 (7.68 ppm), (14) phosphocholine, (15) glycine, (16) unknown 2 (4.07 ppm), (17) unknown 3 (4.56 ppm), and (18) glycogen.

glycogen, and depleted succinate and taurine were apparent in samples.

3.3 Antioxidant enzyme activities in gills from white and zebra clams exposed to metals

After exposure to heavy metals for 48 h, no significant alterations were found in SOD, GPx and GST activities in all metal-treated white clam gills (Table 1). The activities of GST were significantly ($P<0.05$) decreased in mixed Cd/Zn-exposed zebra clam gills.

3.4 Cadmium and zinc concentrations in gills from white and zebra clams exposed to metals

Table 2 illustrates the metal accumulations in gills from white and zebra clams. After exposure to metals for 48 h, both white and zebra clams showed no obvious accumulation of Cd and Zn in gill tissues from all metal-exposed groups. The average amounts of Zn in both Zn- and mixed Cd/Zn-exposed white clam gills were higher than that of control groups, although these differences were not statistically significant. However, white clam gill samples exhibited significantly ($P<0.05$) higher amounts of Zn in both Zn- and mixed Cd/Zn-exposed groups compared with the corresponding zebra clam gill samples (Table 2).

4 DISCUSSION

4.1 Biological differences between white and zebra clams

In the *Mussel Watch Program*, the Manila clam *V. philippinarum* is the preferred biomonitor species because of its wide geographic distribution and high accumulation of metal contaminants compared with mussels (e.g., *Perna viridis*, *Mytilus galloprovincialis*), oysters (e.g., *Crassostrea gigas*, *Crassostrea*

Table 1 The activities (U/mg protein) of SOD, GPx and GST in gills from white and zebra pedigrees of Manila clams (*Venerupis philippinarum*) after exposure to Cd, Zn and Cd/Zn mixture for 48 h

Treatment	White pedigree			Zebra pedigree		
	SOD	GPx	GST	SOD	GPx	GST
Control	12.06 ± 1.94	1 368.76 ± 758.20	400.86 ± 282.97	9.52 ± 4.06	767.94 ± 473.57	371.83 ± 111.36
Cd	10.76 ± 3.14	1 147.08 ± 1 281.57	395.14 ± 254.91	6.64 ± 1.27	711.58 ± 298.75	288.86 ± 82.92
Zn	12.24 ± 3.33	1 233.15 ± 1 700.63	500.82 ± 317.67	7.73 ± 0.88	965.39 ± 665.40	304.17 ± 134.38
Cd+Zn	15.08 ± 7.27	1 664.76 ± 780.29	204.65 ± 142.98	8.47 ± 1.67	798.63 ± 255.35	260.77 ± 78.93*

Values are presented as the mean ± standard deviation. Significant difference (*$P<0.05$) between control and treated-groups was tested by one-way analysis of variance.

Table 2 The accumulated concentrations (μg/g) of cadmium (Cd) and zinc (Zn) in gills from white and zebra pedigrees of Manila clams *Venerupis philippinarum* after exposure to Cd, Zn and Cd/Zn mixture for 48 hours

Treatment	Cd concentration (mean ± SD)		Zn concentration (mean ± SD)	
	White pedigree	Zebra pedigree	White pedigree	Zebra pedigree
Control	1.19 ± 0.54	1.78 ± 1.01	135.61 ± 40.89	114.47 ± 5.44
Cd	1.92 ± 1.53	1.77 ± 0.95	138.60 ± 12.02[a]	110.47 ± 5.91[a]
Zn	1.28 ± 0.48	1.37 ± 0.78	134.64 ± 18.32	118.58 ± 82.71
Cd+Zn	2.56 ± 1.55	1.20 ± 0.38	149.98 ± 16.94[b]	124.00 ± 15.17[b]

Values are presented as the mean±standard deviation. [a] meant that the statistical differences in Zn contents between white and zebra groups exposed to Zn were less than 0.01. [b] meant that the statistical differences in Zn contents between white and groups exposed to mixed Cd and Zn were less than 0.05.

rivularis) and other clams (e.g., *Meretrix meretrix*, *Mactra quadrangularis*). In regular biomonitoring of metal contaminants, however, the potential biological differences between different pedigrees of clams have been ignored. The biological differences could produce varying biological responses (e.g., metal accumulation) in different pedigrees of Manila clams exposed to metals, which can undoubtedly introduce significant variations for pollution biomonitoring. In addition, the biological differences can also distort biological interpretation because of differential tolerances and sensitivities of clams to marine contaminants in aquatic toxicology studies.

Based on the results of pattern recognition and statistical analysis, we found clear metabolic differences between white and zebra clam gills. These include high levels of hypotaurine, malonate and homarine in white clam gills as well as high levels of alanine, arginine, glutamate, succinate, 4-aminobutyrate, taurine and betaine in zebra clam gills (Fig.2). Since both white and zebra clams have a similar genotypic milieu, differential phenotypic fingerprints (e.g., metabolic differences) may be caused by differential gene regulation and corresponding enzymes related to various metabolisms. Interestingly, the GPx activities between control white and control zebra clam samples were obviously different with a statistical significance approaching 0.05 (P=0.078). Therefore, toxicological interpretation could be distorted when testing oxidative stresses induced by contaminants (e.g., metals) in mixed pedigrees of clams. Thus, it is necessary to select a sensitive pedigree of clam as biomonitor for metal pollution biomonitoring and toxicology study.

4.2 Differential responses of white and zebra clams to metal exposures

The bioaccumulation of heavy metals in soft tissues of bivalves is often measured as a key index in metal pollution biomonitoring. Since gills of bivalves are one of the main target organs for metal accumulation (Panfoli et al., 2000), it was used in this study to determine heavy metal accumulation by clams. However, both white and zebra clams did not accumulate significant amounts of Cd in gills because of the short exposure time (48 h). Since Zn is an essential element for organisms, the baseline levels of Zn were approximately 100 times higher than Cd in clam gill tissues (Bebianno et al., 1995; Romeo et al., 1995). Therefore, the accumulation of environmentally relevant Zn (50 μg/L) was also not apparent in both white and zebra clams with a short-term exposure. However, white clam gill samples exhibited significantly ($P<0.05$) higher amounts of Zn in both Zn- and mixed Cd/Zn-exposed groups compared with corresponding zebra clam gill samples (Table 2). These findings indicate that white clams are more preferable than zebra clams for marine Zn pollution biomonitoring.

Antioxidant enzyme activities are routinely used for testing oxidative stresses induced by toxicants (Regoli et al., 1995; Regoli et al., 2011). In white clam samples, no obvious alterations in tested antioxidant enzyme activities (SOD, GPx and GST) were found in metal exposed samples. However, GST activities in mixed Cd/Zn-exposed zebra clam samples were significantly ($P<0.05$) decreased. These findings suggest that, compared with white clams, the gill tissue of zebra clams is more sensitive to mixed metal exposure.

Metabolomics is a well-developed system in biology and has been successfully employed in toxicology based on the metabolite fingerprinting for environmental pollutants (Bundy et al., 2004; Viant et al., 2006; Wu et al., 2010; Zhang et al., 2011). In this study, metal treatments (Cd, Zn, and Cd+Zn) induced significant metabolic responses in both white and zebra clam gills. However, the altered metabolic profiles differed between white and zebra clam samples exposed to a single metal or a mix of metals. Besides some similar metabolic responses, including increased succinate, betaine and homarine, and decreased malonate and ATP, some contrarily altered metabolites, such as alanine, hypotaurine and taurine, were found in either white or zebra clam samples exposed to Cd. Uniquely, increased branched chain amino acids, proline and glycogen and decreased glutamate were discovered in Cd-treated white clam samples as well as increased glutamine and decreased arginine, acetoacetate and aspartate in Cd-treated zebra clam samples. These distinctive metabolic responses between white and zebra clam samples meant differential toxicological effects in white and zebra clam gills exposed to Cd. For example, depleted phosphocholine indicated Cd-induced disturbance in energy metabolism in white clam samples, which was not found in zebra clam samples (Viant et al., 2006). For Zn exposure, the metabolic profiles between white and zebra clam samples showed several contrarily altered metabolites, including taurine, hypotaurine and homarine, which were known osmolytes in clams. This meant that Zn exposure affected osmoregulation in both white and zebra clam gills through different mechanisms. Although the mechanisms were unclear, the differential responsive mechanisms suggested that one pure clam pedigree should be used in toxicology study. After exposure with mixed Cd/Zn, the metabolic responses were dissimilar to those of Cd- or Zn-treated samples from either white or zebra clams, which could indicate that a mix of Cd and Zn induces synergetic effects in clam gills. Based on the metabolic responses, both Cd and Zn induced more metabolic biomarkers in zebra clam gills, which suggests that zebra clam gills may be more suitable for marine environmental toxicology.

5 CONCLUSION

A pedigree of the Manila clam *V. philippinarum* can be selected for biomonitoring marine metal pollution and for studying marine environmental toxicology. The metabolic profiles showed higher levels of hypotaurine, malonate and homarine in white clam gills and higher levels of alanine, arginine, glutamate, succinate, taurine, 4-aminobutyrate and betaine in zebra clam gills. The toxicological responses (e.g., metabolic changes, antioxidant enzyme activities, and bioaccumulation of metals) were different between white and zebra clams. Based on the relatively high accumulation of Zn in gills, it can be concluded that the white clam is a preferable biomonitor used for marine Zn pollution biomonitoring. However, the gills of zebra clams were more sensitive to Cd and Zn exposures because of the sensitive responses in antioxidant enzyme activities and metabolic profiles, which suggests that zebra clam gills are more suitable for toxicology studies on mixed Cd and Zn. Our results suggest that both the species and strain or pedigree should be considered in biomonitoring programs in certain geographical scales, because of the biological differences and responses to environmental pollutants.

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