Toxicological effects of As (V) in juvenile rockfish *Sebastes schlegelii* by a combined metabolomic and proteomic approach

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**A B S T R A C T**

Arsenic (As) is a metalloid element that is ubiquitous in the marine environment and its contamination has received worldwide attention due to its potential toxicity. Arsenic can induce multiple adverse effects, such as lipid metabolism disorder, immune system dysfunction, oxidative stress and carcinogenesis, in animals. Inorganic arsenic includes two chemical forms, arsenite (As (III)) and arsenate (As (V)), in natural environment. As (V) is the dominant form in natural waters. In the present study, metabolomic and proteomic alterations were investigated in juvenile rockfish *Sebastes schlegelii* exposed to environmentally relevant concentrations of As (V) for 14 d. The analysis of iTRAQ-based proteomics combined with untargeted NMR-based metabolomics indicated apparent toxicological effects induced by As (V) in juvenile rockfish. In details, the metabolites, including lactate, alanine, ATP, inosine and phosphocholine were significantly altered in As-treated groups. Proteomic responses suggested that As (V) could not only affect energy and primary metabolisms and signal transduction, but also influenced cytoskeleton structure in juvenile rockfish. This work suggested that the combined proteomic and metabolomic approach could shed light on the toxicological effects of pollutants in rockfish *S. schlegelii*.

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1. Introduction

Marine metal contamination caused by anthropogenic activities in the Bohai Sea has received great concerns from researchers (Li et al., 2018). Arsenic (As) is a metalloid element with metallic and non-metallic properties and usually classified as a metal element (Tisler and Zagorc-Koncan, 2002). Due to its non-necessity and widespread existence, As has become a typical coastal and marine contaminant in the Bohai Sea (Zhuang and Gao, 2015). Gao et al. (2014) reported that the As concentrations in seawater changed frequently and seasonally and the maximum concentration had been up to 3.4 µg/L in coastal and estuary areas along the Bohai Sea.

The toxicity of As to organisms depends on its species. Generally, the inorganic forms of As are the most toxic forms in marine ecosystems (Erickson et al., 2019). Among the inorganic forms, arsenate (As (V)) is the dominant form in natural waters.

The potential toxicity of As has received worldwide attention due to its frequent detection in the environment. As can stimulate numerous molecular events in connection with lipid metabolism disorder, immune system dysfunction, oxidative stress and carcinogenesis in organisms, which have been well documented in a variety of organisms (Chen et al., 2018a,b; Chen et al., 2019; Ramsey et al., 2013; Szymkowicz et al., 2019; Xu et al., 2013; Yu et al., 2016).

Since researchers often take advantage of traditional toxicological approaches to focus on a known class of toxicity-responsive molecules to draw out the toxic mechanisms, it is reluctant to discover new biological molecules associated with toxicological effects and presents initial and less comprehensive evaluations on toxicological reactions of pollutants in organisms (Ji et al., 2014).

Recently, omics approaches in system biology, such as transcriptomics, proteomics and metabolomics, have been proposed to...
supervise the terrestrial or marine ecosystems and these methods have been used to elucidate the potential toxicity caused by toxic substances to organisms (Chen et al., 2018a,b; García-Sevillano et al., 2013; Ji et al., 2019; Williams et al., 2011). Metabolomics focuses exactly on the alterations of low molecular metabolites (<1000 Da) within cells, tissues or biofluids in organisms (Song et al., 2018; Viant et al., 2003) and offers fresh insights into the toxicological effects of environmental stressors (Jones et al., 2008). Among modern analytical techniques, the proton nuclear magnetic resonance (1H-NMR) spectroscopy is powerful for the detection of endogenous low-molecular weight metabolites in biological samples, since all metabolites contain protons. Moreover, NMR is a rapid and nondestructive analytical technique that can provides rich structural and quantitative information of metabolites. Due to these advantages, NMR-based metabolomics has been extensively applied in ecotoxicology (Brandão et al., 2015; Cappello et al., 2016a, b). Proteomics may provide the significant discrepancies between contaminant-stressed and control conditions, which offer us a direct interpretation of toxicity mechanism after pollution exposure to organisms (Nicholson and Lindon, 2008; Salazar-Coria et al., 2019). The combination of metabolomics and proteomics can provide system-level snapshots of the metabolism of a cell or organism under the stress of toxicants (Chen et al., 2017).

Fishes are considered to be significant biomonitors in aquatic systems for the metal pollution assessment (Rashed, 2001). Rockfish Sebastes schlegelli is one of the most important economic species owing to its high demand, rapid growth and appreciated flesh. It is not only distributed in the Korean peninsula and Japan, but also distributed around the Bohai Sea, the Yellow Sea, and the East China Sea (Chen et al., 1994; Nakagawa et al., 2007). Rockfish has been widely used as experimental animal in marine ecotoxicology (Lee et al., 2018; Kim and Kang, 2017; Kim and Kang, 2015). Previous studies have illustrated that As could induce hyperglycemic effect, block energy production and normal cell signaling as well as increase the metallothionein-I and metallothionein-II mRNA levels in fish (Garg et al., 2008; Kreppel et al., 1993). However, the underlying toxic mechanisms explaining its responses to metal contaminants have been largely unexplored. The aim of this work was to unravel the toxicological responses in juvenile rockfish to environmentally relevant concentrations of As and better understand the arsenate-induced effects in rockfish S. schlegelli using a combined proteomic and metabolomic approach.

2. Materials and methods

2.1. Experimental animals and conditions

In this work, the juvenile rockfish was selected for As exposures. Seventy-two individuals of juvenile rockfish S. schlegelli (body length: 6.5–7.0 cm) were purchased from a local culturing farm in Yantai, China. After transported to the laboratory, the fish were allowed to acclimate in aerated seawater (20 °C, 33 psu) for 1 week. After acclimatization, the fish were randomly divided into three groups (control, 5 and 50 μg/L As (V) in Na₂H₂AsO₄) each containing two replicate tanks with 12 individuals. The seawater As concentration from some areas in the Bohai Sea reached 12.4 μg/L (Gao et al., 2014; Zhang, 2001). In this work, the orders of magnitude of As concentrations for exposure were environmentally relevant. During the acclimatization and exposure periods, the fish were kept under a photoperiod of 12 h light and 12 h dark, and fed with the commercial bait daily. After exposure for 14 days, fish from each tank were immediately sampled for further analyses. The whole fish samples were quickly snap-frozen in liquid nitrogen and ground into powder. Then the powder of each sample was divided into three tubes and then stored at −80 °C.

2.2. NMR spectroscopy-based metabolomic analysis

Metabolite extraction of juvenile rockfish was performed using the modified extraction protocol as described previously (Ji et al., 2015). All the tissues (ca. 100 mg wet weight) were homogenized and extracted in 4 mL/g of methanol, 5.25 mL/g of water and 2 mL/g of chloroform (Lin et al., 2007; Wu et al., 2008). Only metabolite extracts in methanol/water were analyzed on a Bruker AV 500 NMR spectrometer performed at 500.18 MHz (at 25 °C) as described previously (Ji et al., 2015).

The ProMetab software in Matlab (version 7.0; The MathWorks, Natick, MA, USA) was used to process NMR spectra (Liu et al., 2011). Each spectrum was segmented into 0.005 ppm bins from 0.2 to 10.0 ppm. Then, these NMR spectra were generalized log transformed with a transformation parameter equivalent to 2.0 × 10⁻⁸ to stabilize the variance across the spectral bins (Liu et al., 2011). The obtained NMR spectral matrix data were mean-centered prior to multivariate data analysis. More details about metabolomic analysis were described in the Supporting Information.

2.3. iTRAQ-based quantitative proteomic analysis

Protein extraction was performed according to the methodology described in a previous study with some modifications (Isaacs et al., 2006). Briefly, samples (~0.1 g) from 3 individuals of juvenile rockfish were pooled as one replicate, and proteins were extracted using cold phenol extraction buffer. The concentrations of protein extracts were determined by BCA method (Smith et al., 1985). iTRAQ technique was employed to quantitative proteomic analysis. The iTRAQ labeling of peptides from fish samples were performed using iTRAQ 8-plex reagents (Applied Biosystems, Foster City, CA, USA) according to the manufacturer’s protocol. Eight samples (two biological replicates for control group and three replicates for each As-treated group) were labeled with the iTRAQ tags. Data was processed with Protein Pilot Software (version 5.0, AB SCIEX, USA) against Pericormes database using the Paragon algorithm. The experimental data from tandem mass spectrometry (MS) was used to match the theory data to identify the proteins. The protein ratios in each replicate were then quantified based on the summed intensities of the matched spectrum. These ratios from the biological replicates were evaluated by Student’s t-test combined with the Benjamini–Hochberg correction (Han et al., 2013). To calculate the relative protein levels, proteins with corrected p values less than 0.05 and average iTRAQ ratios of ≥1.3 or ≤0.77 were considered to be significantly differential. The Gene ontology (GO) databases (http://www.geneontology.org) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg/) were used to classify and group these identified proteins. Further details regarding proteomic analysis were described in the Supporting Information S1.

2.4. Determination of As in juvenile rockfish samples

Nine powered samples of juvenile rockfish in each group were dried at 80 °C to constant weights and then digested in concentrated nitric acid (HNO₃, 70%, Fisher Scientific) using a microwave digestion system (CEM, MARS). These fish samples in concentrated HNO₃ were heated in the microwave oven at 200 °C for 15 min and clear solutions were obtained. The digested samples were transferred to 10 mL polypropylene tubes and filled up to 10 mL with ultrapure water (MilliQ plus) before arsenic concentration determination by ICP-MS technique (Agilent 7500i, Agilent Technologies Co. Ltd, Santa Clara, CA, USA). The As concentrations in juvenile rockfish were expressed as means ± standard deviation. One way analysis of variance (ANOVA) was conducted on the As
concentrations from control and As treatments, respectively, using SPSS statistics. The p values less than 0.05 were considered statistically significant.

3. Results

3.1. Arsenic accumulation

Fish can effectively bioaccumulate metal contaminants from the aquatic environments (Kumari et al., 2017). The concentrations of total arsenic in juvenile rockfish from control and both As treatments were determined using ICP-MS technique. As shown in Fig. 1, the juvenile rockfish from both As treatments exhibited significant (p < 0.05) As accumulations in the whole tissues. In details, the concentrations of total As were 1.37 ± 0.17 and 1.41 ± 0.07 μg/g dry weight in the juvenile rockfish exposed to 5 and 50 μg/L waterborne As for 14 d, respectively. However, the As concentrations in both low and high As-treated groups were only 17.1% and 20.5% higher than the As concentration in the control group. In addition, the accumulations in both As treatments were not significantly (p > 0.05) different.

3.2. Metabolic responses in juvenile rockfish to As exposures

Thirty-four metabolites were identified in juvenile rockfish samples (Fig. 2). Several classes of metabolites were identified including amino acids (branched chain amino acids (BCAAs), arginine, threonine, alanine, glutamate, tyrosine, histidine, phenylalanine, glycine, etc.), organic acids (lactate and acetate), energy metabolites (phosphocholine, creatine phosphate, glucose and ATP), intermediates in the Krebs cycle (fumarate and succinate), and osmolytes (taurine, dimethylamine and trimethylamine N-oxide). Principal component analysis (PCA) was performed on the NMR spectral data from control and As-treated groups and significant separations were observed along PC1 and PC2 axes (Fig. 3), respectively. In addition, the two As exposed-groups were separated along PC2 axis, indicating the significant metabolic differences between these two As treatments. Then, O-PLS-DA was performed on the NMR spectral data from control and each As-treated group. As shown in Fig. 4A and B, the O-PLS models generated from control and As treatments indicated their robustness and reliability with Q2 values of 0.886 and 0.651, respectively.

From the loading plot (Fig. 4C), the low concentration (5 μg/L) of As increased the levels of dimethylamine, phosphocholine, glucose, and inosine, and decreased the levels of lactate, alanine, succinate and ATP. As shown in Fig. 4D, the high concentration (50 μg/L) of As induced some similar metabolic responses, including the increased phosphocholine, inosine and decreased lactate, alanine, succinate and ATP, compared with those metabolites from the low dosage group. Specifically, depleted glycine and histidine as well as increased leucine and arginine were uniquely observed in the high concentration (50 μg/L) of As-treated samples.

3.3. Proteomic responses in juvenile rockfish to As exposures

A total of 2053 proteins (homologous proteins grouped together) were identified in both As treatments (Supporting
Information S2), among which 163 proteins were significantly altered. In 5 μg/L As-treated group, 137 proteins including 83 up-regulated and 54 down-regulated proteins were found. Only 39 proteins including 22 up-regulated and 17 down-regulated proteins were found in the high concentration (50 μg/L) of As-treated group. The differential proteomic responses indicated that the juvenile rockfish adopted different strategies in response to As exposures at concentrations of 5 and 50 μg/L, respectively. Among these differentially expressed proteins (DEPs), a total of 13 proteins were commonly altered in both As treatments.

All of the DEPs were classified into diverse functional classes (Fig. 5). The DEPs in the low concentration (5 μg/L) of As-treated group covered a wide range of metabolism (46.97%), signal transduction (13.64%) and cytoskeleton (12.12%) (Fig. 5A). Meanwhile, DEPs located in the metabolism and signal transduction also constituted the majority of the altered proteins in 50 μg/L As-treated group, with proportions 32.26% and 29.03%, respectively (Fig. 5B). The full details of the DEPs were listed in Table 1. Subsequent analyses such as GO and KEGG analyses were all based on the DEPs. Under the category of biological processes, the majority of the DEPs were related to metabolism. While under the second ontology of GO enrichment analysis-cellular components, most of the DEPs were located in the cell (35%) and the organelle (28%). In addition, the vast majority of DEPs were detected for catalytic activity and binding activity (Fig. 6). A total of 11 pathways were classified in the As-treated groups that shared 4 KEGG pathways, including glycolysis/gluconeogenesis, carbon metabolism, biosynthesis of amino acids and degradation of ketone bodies. The particular pathways enriched in the high concentration (50 μg/L) of As-treated group

![Fig. 3. Mean principal component analysis (PCA) scores plot of PC1 versus PC2 for 1H NMR spectra of tissue extracts in juvenile rockfish S. schlegeli from control ( ), 5 μg/L As (■) and 50 μg/L As ( △) treatments. The classes were presented as mean ± standard deviation of PC scores for each group of samples.](image)

![Fig. 4. O-PLS-DA scores derived from 1H NMR spectra of the tissue extracts from juvenile rockfish S. schlegeli from control ( ) and As (V) treatments ( ), (A) control Vs. 5 μg/L As (V) treatment and (C) control Vs. 50 μg/L As (V) treatment and corresponding coefficient plots (B) and (D). The color map shows the significance of metabolite variations between the two classes (control and As treatment). Peaks in the positive direction indicate metabolites that are more abundant in As (V) treatment and metabolites that are more abundant in control group are presented as peaks in the negative direction. Keys: (1) Lactate, (2) Alanine, (3) Succinate, (4) Dimethylamine, (5) Phosphocholine, (6) Glucose, (7) Inosine, (8) ATP, (9) Leucine, (10) Arginine, (11) Glycine (12) Histidine. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)](image)
were butanoate metabolism, BACCs degradation and metabolic pathways.

4. Discussion

4.1. Accumulations of As in juvenile rockfish

In both As-treated rockfish samples, slight but significant (p < 0.05) accumulations of As were observed in juvenile rockfish S. schlegelii. Similarly, a slight accumulation of As was also found in the marine fish Terapon jarbua exposed to waterborne As (Zhang et al., 2012). Due to the low As bioavailability, most marine animals, such as shrimps, bivalves and fishes, have limited abilities to accumulate arsenate from seawater (Zhang et al., 2012), which was observed in juvenile rockfish S. schlegelii in this work. Although the average total As in the high concentration of As (50 µg/L)-treated group was slightly higher than that in the low concentration of As (5 µg/L)-treated group, there was no significance between these two groups. In a previous study, Kim and Kang (2015) reported that juvenile rockfish S. schlegelii could significantly accumulate As in some tissues, including liver, kidney, spleen, gill and intestine, in a dose-dependent manner. For the muscle tissue, however, there were only slight increases (p < 0.05) in As accumulations with increasing exposure concentrations from 50 to 200 µg/L (Kim and Kang, 2015). This was attributable to the reason that As moved into the actively main organs, such as liver, kidney, and spleen, resulting in the low As accumulation in muscle (Karaytug et al., 2007). In this work, the whole tissue of each fish individual with the muscle tissue occupying the largest proportion of tissue weight was used for experiments. Therefore, the similar As accumulations in muscle tissues might alleviate the difference of As concentrations in the whole tissues from both As-treated juvenile rockfish S. schlegelii. Overall, the significant accumulations of arsenic might induce toxicological effects in juvenile rockfish S. schlegelii.

4.2. Alteration of glycolysis associated with As exposures

Glycolysis can metabolize glucose into pyruvate and lactate and plays an important role in glucose metabolism (Lunt and Vander Heiden, 2011). In this work, seven proteins (glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate mutase, fructose-bisphosphate aldolase, glucose-6-phosphate isomerase, enolase 2, enolase 3 and L-lactate dehydrogenase A chain) related to glycolysis were identified to be differentially altered in As-treated groups. Among these proteins, only glycerol-3-phosphate dehydrogenase (GAPDH) and phosphoglycerate mutase were commonly down-regulated in both As-treated groups (Fig. 7). However, their decrements in the lower dose (5 µg/L) group were even greater. GAPDH is a pivotal glycolytic protein with central role in reductive force, pyruvate and energy production (Dastoor and Dreyer, 2001). Phosphoglycerate mutase is another key enzyme in glycolysis metabolism, catalyzing the conversion between 3-phosphoglycerate and 2-phosphoglycerate. These two commonly altered proteins suggested that glycolysis was inhibited by both As exposures. Furthermore, the down-regulated enolase 3, L-lactate dehydrogenase A chain, fructose-bisphosphate aldolase, glucose-6-phosphate isomerase and alpha-1,4 glucan phosphorylase in the low concentration of As-treated group also confirmed the inhibited glycolysis, as mentioned above. Interestingly, metabolomic analysis indicated that lactate (one of the products of glycolysis) was the most significantly decreased metabolite in both As-treated samples, followed by alanine (Fig. 4C and D). Amino acids are the major source of energy in carnivorous fish, because their natural diet is high in protein and low in carbohydrate (Waarde, 1983). Especially, alanine was demonstrated to be the single important source for de novo synthesis of glucose in the liver of migrating sockeye salmon (Oncorhynchus nerka) (French et al., 1983). Thus, the As-induced decrease in lactate and alanine levels confirmed the inhibited glycolysis and the maintenance of glucose stores during As exposure. Coincidentally, the increased glucose was found exactly in the 5 µg/L As-treated group, which was consistent with the proteomic responses. In As-treated clam Ruditapes philippinarum, the glucose was also increased (Wu et al., 2013). Compared with As-treated clam R. philippinarum, however, no similar DEPs related to glycolysis were found in As-treated rockfish S. schlegelii, suggesting that As influenced glycolysis via different metabolic pathways in rockfish S. schlegelii and clam R. philippinarum. Interestingly, enolase 2 and glucose-6-phosphate isomerase in the lower As-dosed group were over expressed compared to the control. This might be a compensatory reaction to the inhibited glycolysis metabolism so as to prevent the juvenile rockfish from suffering more damage under As exposure. An earlier research revealed the enhanced glycolysis and lactate fermentation in zebra fish Danio rerio caused by arsenic exposure (15 and 20 ppm) (Li et al., 2016), which was partially resembled warburg effect (Reichl et al., 1988). The contrary alteration in current study might be due to the lower exposure concentrations of As (5 and 50 µg/L). Dastoor and Dreyer (2001) reported that GAPDH could act as a nuclear repairer or a nuclear carrier of proapoptotic molecules that were associated with apoptosis and oxidative stress. In a previous work, arsenic exposure similarly down-regulated the expression levels of GAPDH in cultured lung cells (Lau et al., 2004). Therefore, the down-regulated GAPDH indicated the apoptosis or oxidative stress induced by As exposures in juvenile rockfish.

4.3. Alteration of lipid and amino acid metabolism associated with As exposures

Fish prefer lipids and fatty acids but carbohydrates as their sources of energy (Kuiglen et al., 2010). Three altered proteins related to lipid metabolism including 3-hydroxyymethyl-3-methylglutaryl-CoA synthase (HMCL1), 17-beta-hydroxysteroid dehydrogenase type 4 (HSD17B4) and fatty acid binding protein 11a (FABP11A) were identified in As-treated juvenile rockfish. HMCL1 has important functions in lipid biosynthesis and its expression levels were the highest in both As-treated groups (Montgomery et al., 2012). HSD17B4 is indispensable in the modulation of bile acids, which is a promoter in the digestion and absorption of lipids (Mindnich et al., 2004; Chiang, 2009). This protein was uniquely up-regulated in juvenile rockfish exposed to the lower...
Table 1
The details of differentially expressed proteins (DEPs) in juvenile rockfish in response to As treatments.

| Protein accession | Protein description                                      | Fold change |
|-------------------|----------------------------------------------------------|-------------|
|                   | 5 μg/L As                                                 | 50 μg/L As  |

**Carbohydrate Metabolism**
- Q6NV33: Isocitrate dehydrogenase [NAD] subunit 1.3172
- F1QZ16: Glucose-6-phosphate isomerase 1.3161
- QPVK5: L-lactate dehydrogenase A chain (LDH-A) 0.7464
- Q8U37Q: Fructose-bisphosphate aldolase 0.7431
- F1QW7: Enolase 1, (Alpha) (Enolase 3) 0.6962
- Q5Q3C7: Alpha-1,4 glucan phosphorylase 0.6868
- Q6GQ0M: Enol2 protein (Enolase 2) 1.5375
- Q7YS54: Enolase 1-phosphate-phospho-lyase 1.4057
- Q5XJ10: Glycerinaldehyde-3-phosphate dehydrogenase 0.7164
- BIA4H6: Phosphoglycerate mutase 0.6681

**Amino Acid Metabolism**
- Q6PHK4: Serine–pyruvate aminotransferase 1.7514
- F1Q740: Valyl-tRNA synthetase 0.6904
- Q6DC37: Histamine N-methyltransferase (HMT) 0.6863

**Lipoprotein Metabolism**
- Q6G60B: Fatty acid binding protein 11a 0.7656
- Q8AYH1: 17-beta-hydroxysteroid dehydrogenase type 4 1.3922
- A2BGU5: Low density lipoprotein receptor-related protein 1.3382

**Protein Metabolism**
- Q66I80: Fatty acid binding protein 11a 0.7656
- Q8AYH1: 17-beta-hydroxysteroid dehydrogenase type 4 1.3922
- A2BGU5: Low density lipoprotein receptor-related protein 1.3382

**Mitochondrial Energy Metabolism**
- Q6PBX8: NADH dehydrogenase (ubiquinone) flavoprotein 2 1.3056
- Q6ZM23: Cytochrome c oxidase subunit V1c 1.3646
- Q37750: Cytochrome b-c1 complex subunit 6 1.4299

**Other Metabolism**
- Q6P2U5: ADP-ribosylation factor 6a 1.3459
- A9C350: Scl:ch211-189n5.11 0.7469
- Q5RCV1: Inosine-5'-monophosphate dehydrogenase 1b 1.5453

**Cytoskeleton Protein**
- F1QWU4: Myosin, light chain 6 1.6702
- P13104: Tropomyosin alpha-1 chain (Alpha-tropomyosin) 1.3689
- Q66I73: Myosin light chain, phosphorylatable, fast skeletal muscle b 1.3226
- Q6ZM60: Myosin, light chain, phosphorylatable, fast skeletal muscle 6 1.5122
- E7EYD0: Myomesin 1a (skelemin) 0.741
- B0UY61: Nebulin 0.7276
- Q7Z3V7: Protein phosphoryase methylesterase 1 1.5422

**Cytoplasmic Protein**
- Q6TNT8: Guanine nucleotide binding protein 1.7018
- Q6TNT8: Guanine nucleotide binding protein 1.7018
- Q5XJ10: Glycerinaldehyde-3-phosphate dehydrogenase 0.7164
- F1QK60: Protein tyrosine phosphatase, receptor type, U, a 1.7454

**Signal Transduction**
- Q7ZUR5: Signal sequence receptor, gamma 1.5329
- Q5XJ6: Paxillin a (Fragment) 1.3608

**Other Protein**
- Q6P2U5: ADP-ribosylation factor 6a 1.3459
- A9C350: Scl:ch211-189n5.11 0.7469
- Q5RCV1: Inosine-5'-monophosphate dehydrogenase 1b 1.5453
- Q503G2: Prolyl endopeptidase (Zgc:110670) 1.4001
- F1QJC9: Collagen, type I, alpha 1a 1.4004
- Q7Z3V7: Protein phosphoryase methylesterase 1 1.5422
- Q6P2U5: ADP-ribosylation factor 6a 1.3459
- A9C350: Scl:ch211-189n5.11 0.7469
- Q5RCV1: Inosine-5'-monophosphate dehydrogenase 1b 1.5453
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- Q503G2: Prolyl endopeptidase (Zgc:110670) 1.4001
- F1QJC9: Collagen, type I, alpha 1a 1.4004
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- Q503G2: Prolyl endopeptidase (Zgc:110670) 1.4001
- F1QJC9: Collagen, type I, alpha 1a 1.4004
- Q7Z3V7: Protein phosphoryase methylesterase 1 1.5422
concentration of As. These DEPs indicated that juvenile rockfish facilitated their lipid regulation in response to As exposure so as to ensure the energy supply. Strangely, the FABP11A was down-regulated slightly (0.76-fold) in the juvenile rockfish exposed to the low concentration of As. As a member of FABP family, FABP11A primarily functions in regulation of fatty acid uptake and intracellular transport. Previous studies demonstrated that the members of the FABP family functioned as modulation of cell growth and proliferation (Chmuryńska, 2006). Therefore, the down-regulation of FABP11A suggested that As might disturb cell growth and proliferation in juvenile rockfish.

Perturbed amino acid metabolism has been reported in fish exposed to As (Kumari et al., 2017; Li et al., 2016; Palaniappan and Vijayasundaram, 2009). In As-treated zebrafish, the levels of some amino acids (threonine, glycine, etc.) were reduced, which was related to dysfunctional energy metabolism (Li et al., 2016). However, Palaniappan and Vijayasundaram (2009) found that As exposure might reduce protein synthesis and increase amino acids in freshwater fingerlings Labeo rohita. In this work, the amino acids, arginine and leucine, were significantly increased, while alanine, glycine and histidine were decreased in either low or high concentration of As-treated juvenile rockfish, indicated by metabolomic analysis (Figs. 4C and D). The amino acids are essential for energy production via TCA cycle (Sears et al., 2009), suggesting the dysfunctional energy metabolism induced by As in juvenile rockfish, together with the altered metabolites (succinate and ATP) involved in energy metabolism. The enzyme, asparagine synthetase (ASNS), catalyzes the biosynthesis of asparagine through an ATP-dependent reaction using glutamine as a source of nitrogen (Zalkin, 1993). The ASNS in the low concentration (5 μg/L) of As-treated group was down-regulated. In addition, amino acid metabolism is involved in protein synthesis, as mentioned above. Ribosomal proteins perform the crucial function of protein biosynthesis (Ruszczyk et al., 2008). The altered levels of ribosomal proteins (RPL24, RPL3 and RPL27) were accordingly observed in the As-treated samples. Since aminoacyl-tRNA plays a critical role in ribosomal protein synthesis by transporting amino acid, the down-regulated valyl-tRNA synthetase (VARS), combined with the down-regulated ASNS and altered composition of ribosomal proteins, indicated the disturbances in protein synthesis/degradation.

| Protein accession | Protein description                                      | Fold change |
|-------------------|---------------------------------------------------------|-------------|
| Q7ZVY0            | Cytotoxic granule-associated RNA binding protein 1      | 0.7579      |
| Q9XER7            | Macrophage migration inhibitory factor                 | 0.7382      |
| Q9DDU5            | Glutathione S-transferase pi                           | 0.7505      |
| Q8IMW7            | Parvalbumin 4 (Pvalb4 protein)                         | 0.7457      |
| F1QYE2            | Tenasin C                                              | 0.6738      |
| F1R968            | Complement component 1, q subcomponent-binding protein |             |
| BBJ6              | Tripartite motif-containing 63b                        |             |
| A6NA21            | Polymerase 1 and transcript release factor             |             |
| F1R2T2            | Fras1-related extracellular matrix 1b                  |             |
| Q7SZY8            | Glyoxylate reductase                                    | 0.7513      |
| Q24W2             | Lysozyme (EC 3.2.1.17)                                 | 0.7342      |
| B0R1T4            | Retinol-binding protein 1b, cellular                    | 0.7287      |
| Q7ZUP0            | Acidic leucine-rich nuclear phosphoprotein 32 family member A | 0.7044 |
| Q6NSP1            | Adaptor-related protein complex 1                      | 0.6885      |
| Q08C7K            | Insulin-like growth factor 2 mRNA-binding protein 1    | 1.3361      |
| FQRUB6            | Chloride intracellular channel protein (Fragment)      | 0.6653      |
| Q6NYD2            | Sideroflexin (Fragment)                                | 2.2156      |

Table 1 (continued)

Fig. 6. Gene ontology classification of proteins that were expressed in both treated samples. Green and red bars indicated 5 and 50 μg/L As-treated groups, respectively. The significant first 20 entries of three ontologies were generally displayed and the rest items are not displayed in the graph. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
Mitochondria are involved in the regulation of energy metabolism and 95% of the energy required for cell survival is provided by the mitochondrial respiratory chain which is the main site of oxidative phosphorylation and ATP synthesis. The respiratory chain or electron transport chain (ETC) locates within the inner membrane of mitochondria, and consists of NADH ubiquinone oxidoreductase (Complex I), succinate ubiquinone oxidoreductase (SQR) (Complex II), ubiquinone-cytochrome c reductase (QCR) (Complex III), cytochrome oxidase (COX) (Complex IV) and the F1F0-ATP synthase. In this study, one of the most remarkable changes associated with As stress was the up-regulation of proteins related to oxidative phosphorylation and ATP synthesis. Expressions of cytochrome c oxidase subunit 4 (COX4) were up-regulated in juvenile rockfish exposed to both As treatments (1.62- and 1.34-fold, respectively). NADH dehydrogenase (ubiquinone) flavoprotein 2 (NDUFV2), cytochrome c oxidase subunit Vc (COX6C), cytochrome b-c1 complex subunit 6 (UQCRH) and ATP synthase F0 complex were also up-regulated compared with those in control group. Rezin et al. (2008) reported the dysregulation of mitochondrial respiratory chain complex proteins in rat models exposed to chronic stress such as food deprivation, isolation and flashing light. These up-regulated proteins suggested a disturbance of the mitochondrial respiratory chain in juvenile rockfish. One previous study reported that increased oxidative phosphorylation might be an adaptive and protective mechanism in coping with external pressures (Bisgaard et al., 2007). For this reason, the disturbance of the mitochondrial respiratory chain in juvenile rockfish revealed an energetic readjustment process in fighting against As stress. Moreover, adenine phosphoribosyltransferase (APRT) was found to be over expressed dramatically in both As-treated groups. APRT has the function of promoting the biosynthesis of ATP through a salvage reaction leading to the formation of AMP (Shao et al., 2015). In addition, isocitrate dehydrogenase is an indispensable enzyme of the Krebs cycle. Thus the up-regulated isocitrate dehydrogenase in 5 µg/L As-treated group combined with the altered APRT in both As-treated groups indicated that juvenile rockfish underwent disturbance in energy metabolism. Furthermore, the elevated ADP/ATP concentration ratios could stimulate the activity of isocitrate dehydrogenase (Denton and McCormack, 1980). Therefore, the up-regulation of isocitrate dehydrogenase meant the possible depletion of ATP, which was clearly detected in the metabolic profiles from As-exposed juvenile rockfish samples (Fig. 4C and D). The coherence between metabolomic and proteomic biomarkers suggested the complementarity between proteomics and metabolomics in toxicological biomarker discovery. In As-exposed clam R. philippinarum, the contrary metabolic alterations compared with these in As-exposed rockfish S. schlegelii, including depleted succinate and elevated ATP, were observed (Wu et al., 2013). These differences revealed species-specific strategies in energy metabolism in rockfish and clam to As exposure. In addition, NDUFV2 is an important subunit of the NADH dehydrogenase, which is involved in production of reactive oxygen species (ROS) in mitochondria (Turrens and Boveris, 1980). The increased expression of NDUFV2 implied that juvenile rockfish suffered from clear oxidative stress under As (5 µg/L) treatment. Apparently, isocitrate dehydrogenase can also take part in redox reaction by producing NADH. The up-regulation of isocitrate dehydrogenase was in conformity with the over expressed NDUFV2, suggesting the oxidative stress induced by As (5 µg/L) treatment in juvenile rockfish.
collagens were up-regulated with statistical significances. Collagens are not only responsible for the resilience of multicellular organism and mechanical resistance, but also participate in signaling pathways of defining cellular shape and behavior (Daley et al., 2008). The altered collagens suggested the disruption in signaling pathways via the extracellular matrix in juvenile rockfish induced by As. Ca\textsuperscript{2+} is a highly versatile intracellular signal that can regulate many different cellular functions (Carafoli et al., 2001). In this work, calcium uniporter protein was significantly up-regulated and calcium-transporting ATPase was significantly down-regulated in 5 \( \mu \)g/L As-treated group (Table 1). In addition, as one subtype of voltage-gated calcium channels in cytomembrane, voltage-dependent L-type calcium channel subunit alpha was significantly up-regulated in 50 \( \mu \)g/L As-treated group. These altered proteins implied the disturbed calcium homeostasis in juvenile rockfish exposed to As. G proteins are not only involved in signaling pathways but also critical for stress responses (Clapham and Neer, 1993). The significant alteration of G protein implied the cellular injury induced by As exposure. CK1 protein kinases belong to a large family of Ser/Thr kinases in eukaryotes that can be used to regulate diverse cellular processes and coordinate in an orderly manner through different signal transduction pathways (Gross and Anderson, 1998; Knippschild et al., 2005). Serine/threonine-protein phosphatases and tyrosine protein phosphatase belong to protein phosphatases family with the functions of regulating various cellular processes through different signaling pathways (Tian and Wang, 2002). Increased abundances of CK1 protein kinases and serine/threonine-protein phosphatases were detected in low (5 \( \mu \)g/L) and high (50 \( \mu \)g/L) concentrations of As-treated groups, respectively. Moreover, tyrosine protein phosphatase significantly increased its protein abundance in both As-treated groups. These altered proteins suggested the disturbance of signaling pathways in juvenile rockfish exposed to As.

4.6. Cytoskeleton changes induced by As

A total of eight proteins related to cytoskeleton were altered in the As-treated samples. Keratin 4 was commonly expressed in both As-exposed groups. The other seven proteins (myosin light chain 6, tropomyosin alpha-1 chain, myosin light chain, myomesin 1a, myosin heavy chain a, septin 15, mapping protein (actin filament) muscle Z-line) were uniquely detected in the low dose of As-exposed group. The cytoskeleton proteins have the central role in cell structure and intracellular organization and perturbations in the architecture of any of the three main cytoskeletal networks can result in marked pathologies (Fletcher and Mullins, 2010). Metals or metalloids can induce cytoskeletal injuries in marine bivalves, which has been confirmed in both laboratory and field experiments by proteomics (Wu et al., 2013; Xu et al., 2016). The DEPs related to cytoskeletal injuries usually include actins, tubulins and myosin light chains in marine bivalves (clam R. philippinarum and oyster Crassostrea hongkongensis), which were also observed in As-treated rockfish in this work. Therefore, the alterations of these eight cytoskeleton-associated proteins suggested the cytoskeleton injuries induced by As exposures in juvenile rockfish.

5. Conclusions

In this study, the toxicological effects were characterized in juvenile rockfish S. schlegelii exposed to As with environmentally relevant concentrations (5 and 50 \( \mu \)g/L) of As (V) for 14 days, at metabolite and protein levels. A schematic illustration of pathways in juvenile rockfish exposed to As (V) was presented in Fig. 7. Combined proteomic and metabolomic analyses showed that As significantly influenced diverse biological processes in juvenile rockfish, such as glycolysis, mitochondrial energy metabolism, lipid and amino acid metabolisms and the regulation of Ca\textsuperscript{2+} signaling pathways in juvenile rockfish. Although these two dosages of As induced some similar effects in juvenile rockfish S. schlegelii, the distinction between these two As treatments were also clearly observed from the metabolomic and proteomic profiles. Especially, more DEPs were altered in the low concentration (5 \( \mu \)g/L) of As-treated group, which suggested that the low dose of As could activate physiological changes in juvenile rockfish to counteract the adverse situation, while the high dose of As (50 \( \mu \)g/L) induced an evasion from the stressful situation. This study demonstrated that the combined proteomic and metabolomic analysis could provide a perceptive view into the influences of environmental metal pollution in the marine fish at molecular levels.

Ethical statement

All procedures were strictly performed according to the Guidelines of the Chinese Council on Laboratory Animal Care (2011), which was approved by the Animal Research Ethics Board of Chinese Academy of Sciences.

Declaration of competing interest

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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Appendix A. Supplementary data

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References

Bisgaard, C.F., Jayatissa, M.N., Enghild, J.J., Sánchez, C., Artemychyn, R., 2007. Pro- teomic investigation of the ventral rap hippocampus links DRP-2 to escatalam- trim treatment resistance and SNAP to stress resilience in the chronic mild stress model of depression. J. Mol. Neurosci. 32, 132–144.
Brandão, F., Cappello, T., Raimundo, J., Santos, M.A., Maisano, M., Mauceri, A., Pacheco, M., Pereira, P., 2015. Unravelling the mechanisms of mercury hepato- toxicity in wild fish (Liza aurata) through a triad approach: bioaccumulation, metabolomic profiles and oxidative stress. Metallomics 7, 1352–1363.
Cappello, T., Brandão, F., Guilherme, S., Santos, M.A., Maisano, M., Mauceri, A., Canário, J., Pacheco, M., Pereira, P., 2016a. Insights into the mechanisms un- derlying mercury-induced oxidative stress in gills of wild fish (Liza aurata) combining \(^{1}H\) NMR metabolomics and conventional biochemical assays. Sci. Total Environ. 548–549, 13–24.
Cappello, T., Pereira, P., Maisano, M., Mauceri, A., Pacheco, M., Fasulo, S., 2016b. Advances in understanding the mechanisms of mercury toxicity in wild golden grey mullet (Liza aurata) by \(^{1}H\) NMR-based metabolomics. Environ. Pollut. 130–148.
Carafoli, E., Santella, L., Branca, D., Brini, M., Carafoli, E., Santella, L., Branca, D., Brini, M., 2001. Generation, control, and processing of cellular calcium signals. Crit. Rev. Biochem. Mol. Biol. 36, 107–260.
Chen, D., Ye, Z., Duan, Y., Feng, D., 1994. Preliminary studies on the biology of reproduction groups and the fry breeding in black rockfish Sebastes schlegelii. Acta Oceanol. Sin. 16, 94–101 (in Chinese).
Chen, H., Diao, X., Wang, H., Zhou, H., 2018a. An integrated metabolomic and pro- teomic study of toxic effects of benzaldehyde on gills of the pearl oyster Pinctada martensi. Ecotoxicol. Environ. Saf. 156, 330–336.
Chen, L., Song, D., Zhang, W., Zhang, C., Zhang, L., 2019. The dynamic changes of arsenic bioaccumulation and antioxidant responses in the marine medaka
Oryzias melastigma during chronic exposure. Aquat. Toxicol. 212, 110–119.

Chen, L., Zhang, W., Guo, Z., Zhang, L., 2018b. Effects of acclimation on arsenic bioaccumulation and biotransformation in freshwater medaka Oryzias melastigma after chronic arsenic exposure. Environ. Pollut. 238, 17–15.

Chen, L., Hu, Y., He, J., Chen, J., Giesy, J.P., Xie, P., 2017. Responses of the proteome and metabolome in livers of zebra fish exposed chronically to environmentally relevant concentrations of microcystin-LR. Mar. Pollut. Bull. 120, 134–138.

Chiang, Y.J., 2009. Bile acids: regulation of synthesis. J. Lipid Res. 50, 1955–1966.

Chumyrzina, A., 2006. The multigene family of fatty acid-binding proteins (FABPs): function, structure and polymorphism. J. Appl. Genet. 47, 39–48.

Clapham, D.E., Neer, R.J., 2003. New roles for G-protein βγ dimers in transmembrane signaling. Nature 365, 403–406.

Daley, W.P., Peters, S.B., Larsen, M., 2008. Extracellular matrix dynamics in development and regenerative medicine. J. Cell Sci. 121, 255–264.

Daston, T., Z. Doniger, J.L., Borys, W., 2015. Nuclear translocation of cAMP-regulated calcium-activated chloride channel in C. elegans. Cell. Signal. 25, 996–1007.

Li, C., Li, P., Tan, Y.M., Lam, S.H., Chan, E.C.Y., Gong, Z., 2016. Metabolomic characterization of liver injury caused by acute arsenic toxicity in zebrafish. PLoS One 11, 1–18.

Li, H., Gao, X., Gu, Y., Wang, R., Xie, P., Liang, M., Ming, H., 2018. Comprehensive large-scale investigation and assessment of trace metal in the coastal sediments of the West Yellow Sea. Mar. Pollut. Bull. 130, 138–145.

Lin, C.Y., Wu, H., Tjeerdema, R.S., Vian, M.R., 2007. Evaluation of metabolite extraction strategies from tissue samples using NMR metabolomics. Metabolomics 3, 55–67.

Liu, X., Zhang, L., You, L., Yu, J., Zhao, J., Li, L., Wang, Q., Li, L., Li, C., Liu, D., Wu, H., 2011. Differential toxicological effects induced by mercury in gills from three pedigrees of Manila clam Ruditapes philippinarum by NMR-based metabolomics. Ecotoxicology 20, 177–186.

Lunt, J.E., van der Heiden, M.G., 2011. Aerobic glycolysis: meeting the metabolic requirements of cell proliferation. Annu. Rev. Cell Dev. Biol. 27, 441–464.

Mindrich, N., Röller, G., Adamski, J., 2004. The role of 17 beta-oxydihydroxysteroids. Mol. Cell. Endocrinol. 218, 7–20.

Montgomery, C., Piet, Z., Watkins, P.A., Miezirkzio, H.M., 2012. Identification and characterization of an extramitochondrial human 3-hydroxy-3-methylglutaryl-CoA lyase. J. Biol. Chem. 287, 32327–32336.

Nakagawa, M., Okouchi, H., Adachi, J., Hattori, K., Yamashita, Y., 2007. Effectiveness of stock enhancement of hatchery released black rockfish Sebastes schlegeli in Yamada Bay—evaluation by a fish market survey. Aquaculture 263, 295–302.

Nicholson, J.K., Lindon, J.C., 2008. Metabolomics. Nature 455, 1054–1056.

Palaniappan, P.L.K., Vijayasundaram, V., 2009. The effect of arsenic exposure and the deficiency of DMSA on the antioxidant levels and the lipids of the gills of Lewis rats. Lebensm. Wiss. Technol. Food Chem. 47, 1752–1759.

Ramsey, K.A., Bosco, A., Mckenna, K.L., Carter, K.W., Elliott, J.G., Berry, L.J., Sly, P.D., Lattimer, J.K., Zosky, G.J., 2017. In utero exposure to arsenic alters lung development and genes related to immune and mucociliary function in mice. Environ. Health Perspect. 121, 244–250.

Rashed, M.N., 2001. Monitoring of environmental heavy metals in fish from nasser lake. Environ. Int. 27, 37–42.

Reichl, F., Sznitzin, L., Kreppel, H., Forth, W., 1988. Effect of arsenic on carbohydrate metabolism after single or repeated injection in Guinea pigs. Arch. Toxicol. 62, 473–475.

Roin, C.T., Cardoso, M.R., Gonçalves, C.N., Giannini, F., De Santis, A.C., Biondi, G., Rinaldi, R., Erié, G., Comin, C.M., 2008. Inhibition of mitochondrial respiratory chain in brain of rats subjected to an experimental model of depression. Neurochem. Int. 53, 395–400.

Ruszczyk, A., Joerink, M., Geldenaa, C., Hermens, T., Savelkoul, H.F., Wiegtjes, G.F., 2008. cDNA expression library screening and identification of two novel anti-ubiquitin: ubiquitin and receptor for activated C kinase (RACK) homologue, of the fish parasite Tripanosoma caudatum. Fish Shellfish Immunol. 25, 84–90.

Salaizar-Corza, L., Rocha-Gómez, M.A., Matadamas-Martínez, F., Yépez-Méjula, L., Vega-López, M., 2019. Proteomic analysis of oxidized proteins in the brain and liver of the Nile tilapia (Oreochromis niloticus) exposed to a water-accommodated fraction of Maya crude oil. Environ. Toxicol. Saf. 171, 609–620.

Sears, D.D., Hisagi, H., Hisao, A., Yu, J.G., Courtney, C.H., Orfrecio, J.M., Chapman, J., Subramanian, S., 2009. Mechanisms of human insulin resistance and thiazolidinedione-mediated insulin sensitization. Proc. Natl. Acad. Sci. U.S.A. 106, 18911–18916.

Shao, W., Chen, J., Fan, S., Lei, Y., Xu, H., Zhou, J., Cheng, P., Yang, R., Rao, C., Wu, B., Liu, H., Xie, P., 2015. Combined metabolomics and proteomics analysis of major depression in an animal model: perturbed energy metabolism in the chronic restraint stress rat cerebellum. OMICS 19, 383–392.

Smith, P.K., Krohn, R.I., Hermansson, G.T., Malta, K., Gartner, F.H., Provenzano, M.D., 1985. Measurement of protein using bichromonic acid. Anal. Biochem. 150, 76–85.

Song, Y., Cai, T., Yin, Z., Zhang, X., Zhang, W., Qian, Y., Qiu, J., 2018. Stereoselective effects of ibuprofen in adult zebrafish (Danio rerio) using UPLC-TOF/MS-based metabolomics. Environ. Pollut. 241, 730–739.

Sproviero, D.B., Sims, K.C., Schwindinger, L.K., Tattanlli, C.M., Powell, R.R., Bruce, T.F., Bridges, B.A., Bain, L.J., 2019. Exposure to arsenic during embryogenesis impairs olfactory sensory neuron development and differentiation into adulthood. Toxicology 420, 73–84.

Tian, Q., Wang, J., 2002. Role of serine/threonine protein phosphatase in Alzheimer’s disease. Neurosignals 11, 262–269.

Titter, T., Zagor-Choncak, J., 2002. Acute and chronic toxicity of arsenic to some aquatic organisms. Bull. Environ. Contam. Toxicol. 69, 421–429.

Turrens, J.F., Boveris, A., 1980. Generation of superoxide anion by the NADH dehydrogenase of the mitochondrial inner membrane. Biochem. J. 202, 799–802.

Vance, J.P., 1999. Phospholipids of cellular membranes. Annu. Rev. Biochem. 68, 489–520.

Wang, H., Mackenzie, C., Bartie, K.L., 2007. Identification and characterization of an extramitochondrial human 3-hydroxy-3-methylglutaryl-CoA lyase. J. Biol. Chem. 282, 32327–32336.

Williams, T.D., Turan, N., Diab, A.M., Wu, H., Mackenzie, C., Barrie, K.L., Hussaino, O., Lyons, L., Forrest, G.D., Herbertt, J.M., Abraham, J.K., Katsiadaki, I., C. L., Taggart, J.B., George, S.G., Vian, M.R., Chipman, K.J., Falciani, F., 2011. Towards a system level understanding of non-model organisms sampled from the environment: a network biology approach. PLoS Comput. Biol. 7, e1002126.
Wu, H., Liu, X., Zhang, X., Ji, C., Zhao, J., Yu, J., 2013. Proteomic and metabolomic responses of clam *Ruditapes philippinarum* to arsenic exposure under different salinities. *Aquat. Toxicol.* 136–137, 91–100.

Wu, H., Southam, A.D., Hines, A., Viant, M.R., 2008. High-throughput tissue extraction protocol for NMR- and MS-based metabolomics. *Anal. Biochem.* 372, 204–212.

Xu, H., Lam, S.H., Shen, Y., Gong, Z., 2013. Genome-wide identification of molecular pathways and biomarkers in response to arsenic exposure in zebrafish liver. *PLoS One* 8, e68737.

Xu, L., Ji, C., Wu, H., Tan, Q., Wang, W.-X., 2016. A comparative proteomic study on the effects of metal pollution in oysters *Crassostrea hongkongensis*. *Mar. Pollut. Bull.* 112, 436–442.

Yu, D., Ji, C., Zhao, J., Wu, H., 2016. Proteomic and metabolomic analysis on the toxicological effects of As (III) and As (V) in juvenile mussel *Mytilus galloprovincialis*. *Chemosphere* 150, 194–201.

Zalkin, H., 1993. *Adv. Enzymol. Relat. Area Mol. Biol.* 66, 203–309.

Zhang, W., Huang, L., Wang, W.-X., 2012. Biotransformation and detoxification of inorganic arsenic in a marine juvenile fish *Terapon jarbua* after waterborne and dietborne exposure. *J. Hazard Mater.* 221–222, 162–169.

Zhang, X., 2001. Investigation of pollution of Pb, Cd, Hg, as in sea water and deposit of Bohai Sea area. *Heilongjiang Environ. J.* 25, 87–90 (in Chinese).

Zhuang, W., Gao, X., 2015. Distributions, sources and ecological risk assessment of arsenic and mercury in the surface sediments of the southwestern coastal Laizhou Bay, Bohai Sea. *Mar. Pollut. Bull.* 99, 320–327.