Evaluation of toxic effects of petroleum hydrocarbon on *Mytilus coruscus* under different salinity based on an integrated biomarker response index

Lei Li, Cuihua Wang, Ziniu Li, Guodong Xv, Baojun Tang and Mei Jiang

East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Shanghai, China

**ABSTRACT**

An oxidative stress experiment of crude oil in Pinghu on *Mytilus coruscus* under different salinities (10, 15 and 20) for 15 days was carried out in this study. Oxidative stress of oil pollutants in digestive glands was measured by several typical biomarkers, the levels of ethoxyresouorufin-O-deethylase (EROD) activities and aryl hydrocarbon hydroxylase (AHH) activities, CYP1A1mRNA content and DNA damage degree. The integrated biomarker response index (IBR) was introduced in for the quantitative evaluation of the responses of *M. cephalus* to the toxic effects of crude oil in Pinghu under different salinities. The results showed that the different salinities of crude oil in Pinghu can induce toxic effects on the digestive glands of *M. coruscus* to different extents, and the order of the degrees of toxicity, which were higher than those of the control groups, was 20 salinity > 15 salinity > 10 salinity.

As a result of oil spill accidents, about 200 to 10 million tons of oil is discharged into the ocean every year worldwide, and this condition is extremely harmful. Oil pollution leads to a decrease in dissolved oxygen content; increases carbon dioxide and organic matter in seawater, inhibits the growth of algae, and affects the normal development of aquatic organisms and their own respiration and feeding, resulting in the death of a large number of marine organisms. In several cases, oil slicks drift to the coasts and into estuaries, and these marine oil spills can have catastrophic consequences for estuarine and offshore ecosystems [1]. Numerous bivalve mollusks are important resources in estuary coastal waters. In relation to the difficulty of avoiding oil pollution, marine mollusk bivalves (such as oysters, mussels, etc.) have greater ability to enrich petroleum hydrocarbons from the environment than fish, but their metabolism and the ability to release petroleum hydrocarbons are considerably [2]. In addition, the toxic effects of mollusk bivalves have long been a great concern among people, several hydrocarbons in the oil slow down the growth of shellfish, inhibit the phagocytic capacity of leukocytes, and damage the lysosomal membrane [3]. Given that the changes in salinity gradients in estuarine and offshore areas can affect the rheological properties of oil [4], water salinity of water may influence the solubility of toxic hydrocarbons that are present in the crude oil and the accumulation of these hydrocarbons by aquatic organisms that dwell in different salinity mediums [5]. The bivalve mollusks inhabiting estuarine and coastal areas face the dual pressures of salinity change and oil pollution. To determine the effect mechanism of different salinities, scientists focus on solving the toxic effect of oil on shellfish for the scientific assessment of biological damage of oil pollution to shellfish in the water environment with different salinity gradients.

In water, the solubility of polycyclic aromatic hydrocarbons (PAHs), the main toxic substances in petroleum hydrocarbons, in water increases with the decrease in salinity [6]. Salinity changes would influence the toxic effect of petroleum hydrocarbon on marine organisms. Zanette et al. pointed out that the responses involving glutathione S-transferase (GST), catalase (CAT) and Malondialdehyde (MDA) in gill tissues of *Crassostrea gigas* to exposure to diesel changed with salinity [7]. They proved that the toxic effect of petroleum hydrocarbon on shellfish intensified with the increase of salinity and such relation may remain after purification for a period. Lysenko et al. argued that under exposure to crude oil, the GST activity in *Mytilus edulis* L. tissues was increased as a result of the reduction in salinity, however, but the responses of glutathione (GSH) and proteases to crude oil were delayed [8]. Petroleum hydrocarbons can be a typical PAHs-type inducer, which can effectively induce the content of CYP1A protein encoded by the liver CYP1A1 gene, and greatly increase the reactivity of enzymes such as ethoxyresouorufin-O-deethylase (EROD) [9]. After entering the organism, petroleum hydrocarbons can undergo biotransformation through biotransformation enzymes, and several PAHs are activated to produce carcinogenic intermediate metabolites, which combine with DNA.
damage. In addition, these biotransformations are often accompanied by the formation of a large number of free radicals. Excessive free radicals can attack nucleic acids and cause DNA chain scission directly or indirectly [10]. Aromatic hydrocarbon hydroxylase (AHH) is one of the cytochrome P450 mixed-function oxidase (MFO). After PAHs such as petroleum are combined with the body, the activity of AHH enzyme is enhanced [11]. The structure of oil pollution compounds to interfere with biochemical processes and further affects the inducing capability AHH [12]. Biomarkers have been widely used for the evaluation of biotoxic effect of pollutants on an ecosystem [13]. However, biomarkers have different sensitivities to various pollutants and such sensitivity changes with time. Therefore, the integrated biomarker (IBR) response index was introduced to reflect the responses of an organism to petroleum hydrocarbon accurately. Differences in the toxicities of pollutants can be analyzed intuitively, and the influences of the anti-oxidation effect are quantified through the quantitation of the IBR, thus enabling the prediction of toxicity differences in chemical substances accurately and effectively [14,15]. Biochemical responses in bivalve mollusks are commonly employed in environmental studies as biomarkers of aquatic contamination. In the present study, the influences of crude oil stress in Pinghu at different salinities on biomarker responses, such as oxidative stress enzyme activity, gene expression and DNA damages in the digestive gland tissues of Mytilus coruscus were discussed under experimental ecological conditions. The toxic effect of crude oil in Pinghu on M. coruscus was evaluated by an integrated the biomarker response (IBR) index. This purpose of the study is to provides basic experimental data for instruction on oil pollution based on biomarkers. This study aimed to reveal the process by which changes in salinity affect the toxic effects of bio-petroleum hydrocarbons on mussels. To provide a theoretical basis for the improvement of the biological damage assessment of oil spills, we aimed to determine pollution in waters with large salinity gradients in the coastal waters of estuaries.

1. Materials and methods

1.1 Experimental materials

In this experiment, M. coruscus was collected from an artificial breeding ground. All shellfish were cultured for one week before the experiment. The average body length and body weight of shellfish were (4.60 ± 0.21) cm and (10.45 ± 1.36) g, respectively.

Natural seawater with sands filtered (salinity = 15 and pH = 8.10 ~ 8.40) was mixed with aerated tap water and sea crystals based on a certain proportion, thus forming salinity gradients of 10 and 20. Before the experiment, M. coruscus was cultured with filtered and fully aerated natural seawater. Healthy individuals with similar specifications were selected for domestication. During the domestication, oxygen was filled in continuously. Dissolved oxygen content, water temperature and pH were kept at >5.0 mg·L⁻¹, 20.1°C ~ 22.7°C and 8.10 ~ 8.40, respectively. An 80 cm × 40 cm × 50 cm glass tank was used as the experimental container and water was changed completely every day, during which the bottom sludge was discarded. The shellfish were fed once daily with a commercial feed during the entire experiments. The domestication was implemented in seawater with 10 and 20 salinities at an interval of 5 days. The experiment began after the domestication.

Crude oil produced in the Pinghu Oil-gas field was mixed with filtered seawater, according to at the ratio of 1:10 (V:V). The mixture was oscillated continuously for 3 h in an ultrasonic oscillator and then put on static for 3 h. Later, the water phase below the surface layer was siphoned out, and it was used as the mother liquid of emulsified liquid. This mother liquid was kept in a refrigerator at 4°C and diluted before the experiments [16]. Pinghu crude oil concentration in seawater was tested by an ultraviolet spectrophotometry.

1.2 Experimental methods

The emulsified liquid of Pinghu crude oil was added to low-salinity (10) and high-salinity (20) seawater at a certain proportion. The petroleum hydrocarbon concentration was kept at 0.2 mg·L⁻¹. Meanwhile, the control groups were set with the natural clean seawater with a salinity of 15. Approximately 30 samples of M. coruscus were placed in 45 L glass tanks for both the control and three exposed groups with different salinities. Water was changed with a 100% an experimental solution at the same concentration every day, and excreta was eliminated with a siphon brush in time. Mixed feed was given to the samples twice at a fixed time and amount. The stress experiment lasted for 15 days. During the experiment, digestive gland samples of M. coruscus were collected at 5, 10 and 15 days, respectively. The samples were separated carefully, rinsed with double distilled water and then collected by small bags folded by aluminum foil. Liquid nitrogen was kept until testing.
1.3 Test indexes

1.3.1 EROD activity
The digestive gland samples, which were weighted accurately, were added to the pre-cooled (0°C–4°C) phosphate buffer solution (Na₂HPO₄ · 12H₂O 0.125 mol/L, KH₂PO₄ 0.125 mol/L and Na₂EDTA 0.05 mol/L) at pH = 7.7 based on the volume mass ratio. The mixture was homogenized under ice-bath conditions for 3 min. The homogenate was frozen and centrifuged for 20 min at 4°C and 10000r/min. The EROD activity was tested by Pohl rapid termination fluorescence method [7]. The reaction was carried out, at 25°C, in the fluorometer cuvette containing 1 mL 0.5 mM ethoxyresorufin (in 0.1 M Tris–HCl, pH 7.4, containing 0.15 M KCl and 20% glycerol) and 100 mL of microsomal suspension. The reaction was initiated by adding 10 mL of NADPH (10 mM) and the progressive increase in fluorescence, resulting from the resorufin formation, was measured for 3 min (emission wavelengths of 530 and 590 nm). The EROD activity was reported as picomoles reesorufin per milligram protein per minute.

1.3.2 AHH activity
Digestive glands were collected in liquid nitrogen and weighted before putting in the ice bath. Next, 10 times of (w/v) 0.01 mol·L⁻¹ pre-cooled buffer solution (pH 7.6) was added to the ice bath for homogenization. The mixture was processed by freezing centrifugation at 4°C (15 000 r·min⁻¹, 20 min), and the supernate was collected to test the AHH activity immediately. A certain amount of the supernate in the reaction mixture with a total volume of 1 mL, 50 μmol Tris-HCl buffer solution, pH 7.5, 0.36 μmol NADPH and 3 μmol MgCl₂ was collected. Finally, 20 μL diphenyl (PPO) solution (10 μg PPO was dissolved in 20 μL methyl alcohol) was added as the substrate in the follow-up reaction. Later, the mixture was kept at 37°C for 30 min. Subsequently, 10 mL acetone which had been cooled in an ice bath was added to terminate the reaction and then 3.25 mL normal hexane (n-hexane) was mixed fully with the mixture. Then, 1 mL organic phase was collected and extracted by 1 mol·L⁻¹ 3 mL NaOH. The water phase was collected for testing. The excitation and emission wavelengths were set 345 nm and 510 nm, respectively. The enzyme activity unit (U) was expressed by fluorescence intensity per milligram of proteins [17].

1.3.3 DNA damage analysis
The tissue of digestive glands was cut into small pieces, placed in a 10 mL tissue grinder, and with 2 mL of pre-chilled tissue homogenate (PBS), and ground until homogenized. The ground samples were filtered through a 100-mesh cell strainer, followed by centrifugation at 1500 rpm for 5 min. The supernatant liquid was discarded, and 2 mL PBS was added to suspend the pellet to prepare a cell suspension. A self-made mini electrophoresis tank and single-layer gelling were applied to the comet electrophoresis method. Cell activity was tested by 0.5% phenolic blue staining method before gel (≥85%). Cell suspension (30 μL) and 0.7% low-melting agarose (LMA, 200 μL) were mixed and 80 μL LMA of low-melting agarose gel-cell suspension was added to a self-made mini electrophoresis tank. Each sample experiment was repeated thrice [18,19].

1.3.4 Expression of CYP1A1 mRNA
The primers of internal reference genes were designed by Primer Premier 5.0. The mRNAs of digestive glands in the different groups were extracted with a Trizol kit (Invitrogen Corporation, USA) and then treated by reverse transcription–polymerase chain reaction (PCR) kit (TakaraBelmont Corporation, Japan). The target genes were amplified by the designed real-time fluorogenic quantitative polymerase chain reaction (qPCR) in which SYBR Green fluorochrome method and 20 μL reaction system were applied. The reaction conditions were set at 30s, annealing under 95°C, 95°C for 5s and 60°C 34s, amounting to 40 cycles. The final step was the dissociation stage and the reaction process was set automatically by a tester software. Each sample experiment was repeated thrice. At the end of the reaction, a melting curve of the reaction system was drawn, and the specificity of qPCR products was analyzed. The relative quantitative results were analyzed by the relative standard curve method 2-ΔΔCT [20].

1.4 Data processing
DNA damage analysis: Comet images were analyzed by CASP12.2 software. Olive tail moment (OTM) and tail DNA content (tail DNA%) are common international evaluation indexes at present. Tail DNA%, which is tail DNA fluorescence intensity/(head DNA fluorescence intensity + tail DNA fluorescence intensity) ×100%, was computed. Tail length reflects the moving distance of DNA. The following equation was also used: Tail distance (TM) = distance from the tail center of gravity to the head center of gravity × percentage of tail DNA.

IBR index: The normalization and assignment of biomarker testing results were performed. First, the mean of test results for the biomarker in a specific period (x) and its total mean (X) in all periods, and the standard deviation(s) were calculated first. xᵢ was normalized in each period in accordance with the following equation: Xᵢ = (xᵢ − X)/s, where Xᵢ is the value after normalization of xᵢ. If the activity of a biomarker is activated by pollution, let Z=xᵢ, otherwise, Z = -xᵢ. Let | xᵢ − min | be the...
absolute value of minimum normalized value of the biomarker in each period, and the score of this biomarker in each period is $B_i = Z + |x_{	ext{min}}|$. The value of $B_i$ of each biomarker in each period was expressed by the length of the radiation ray in the star chart, and the IBR value in a period was calculated from area of the star chart (sum of the triangular area ($A_i$) formed by the radiation rays of adjacent biomarkers). $\text{IBR} = \sum_{i=1}^{n} A_i$ and $A_i = B_i/2\sin\beta(B_i\cos\beta + B_{i+1}\sin\beta)$, where $\beta = \arctan(B_i + 1\sin a/B_{i+1} + 1\cos a$; $n$ is the number of involved biomarkers; $a$ is the included angle between two adjacent radiation rays, $a = 2\pi/n$; $B_i + 1 = B_{i+1}$. When $n = 4$, there is $a = \pi/2$ exists, and the corresponding calculation formula of $A_i$ can be simplified as follows: $A_i = (B_iB_{i+1})/2$. The higher the value of IBR, the greater the influences on the biology. The value of four biomarkers was assessed by one-way analysis of variance (ANOVA) and the least-significant difference test. A value of $p<0.05$ indicated statistical significance. Principal component analysis (PCA) was used to identify the major biomarkers of *M. coruscus*. Statistical analysis was carried out using SPSS 17.0 for Windows software and EXCEL 2010 was applied for fitting and drawing of relevant data.

2. Results and analysis

2.1 Effects of petroleum hydrocarbon on EROD activity under different salinities

Under petroleum hydrocarbon stress, EROD activity increased gradually with the decrease in salinity from 20 to 15 and then to 10. At 15 days of experiment, there is a significant difference was observed in the EROD activity between the 20-salinity experimental group and the 15-salinity control group ($p<0.05$, Figure 1). However, the 15-salinity groups and the 10-salinity groups showed no significant difference in the EROD activity with the 15-salinity control group.

2.2 Effects of petroleum hydrocarbon on AHH activity under different salinities

Figure 2 shows that the AHH activity of digestive glands in groups with different salinities exhibited an increasing trend under petroleum hydrocarbon stress. The increment in AHH activity was positively related with salinity. At 15 days, the 20-salinity group showed significant differences with the 15-salinity control group in terms of the AHH activity ($p<0.05$), whereas other experimental groups exhibited no significant difference with the control groups.

2.3 Effects of petroleum hydrocarbon on the expressions of CYP1A1 mRNA genes under different salinities

Figure 3 shows the effects of petroleum hydrocarbon on the expressions of CYP1A1 mRNA genes under different salinities. The expressions of CYP1A1 mRNA genes in all experimental groups is increased to different extents under petroleum hydrocarbon stress. The longer the experimental period, the greater the value of gene expression. No significant differences were observed between experimental groups and the 15-salinity control group in terms of the expression of CYP1A1 mRNA genes are observed.

2.4 Effects of petroleum hydrocarbon on DNA damages under different salinities

As shown in Figure 4, DNA damages in the digestive glands of different-salinity groups present varied change trends under the petroleum hydrocarbon stress. The tail DNA content increased with the increase in salinity. Compared with the 15-salinity control group, the 20-salinity group showed significant differences, in the tail DNA content at 15 days ($p<0.05$, Figure 4). However, the remaining groups showed no significant differences.

![Figure 1](image1.png)  
**Figure 1.** EROD activity in digestive gland of *Mytilus coruscus* during petroleum exposure in different salinity stress. Data represent means ± SD. * Significantly different, ANOVA multiple comparison and Duncan’s test versus control $P<0.05$.
2.5 IBR analysis

The levels of four biomarkers under different times and salinities were integrated into the IBR in accordance with the calculation method of IBR (Figure 5). IBR is the area of the polygon in the star chart. The star chart showed that the polygon area in the 20-salinity group was significantly higher compared with those in other experimental groups. The polygon area of the 15-salinity group was the second largest, whereas that of the 15-salinity control
Figure 5. Biomarkers stellate map on digestive gland of *Mytilus coruscus* during petroleum exposure in different salinity stress.

Figure 6. IBR value difference on digestive gland of *Mytilus coruscus* during petroleum exposure in different salinity stress.
group, was the smallest. The IBR is an index that reflects environmental pressure, and the petroleum hydrocarbon stress increases with the increase in salinity. In addition, the columnar volume graph of the IBR values of all experimental groups showed different degrees of petroleum hydrocarbon stress to *M. coruscus* under different salinities. The IBR value of the 10-salinity group was considerably higher than those in the 15-salinity group and 20-salinity groups (Figure 6).

### 2.6 Statistical analysis

PCA is one of the most common evaluation methods because of its simplicity in practice and objectivity in the results. Figure 7 shows the results for the four biomarker activities. The first component (PC1), which explained 74.02\% of the variance in the model of the 10 salinity group, was characterized by the activity of three biomarkers (AHH, EROD and DNA) in the digestive gland tissues. PC2 explained 25.98\% of the variance. The score plot showed the expressions of CYP1A1 mRNA genes. Figure 7 shows the results of antioxidant properties, which were defined by three biomarkers (CYP1A1, EROD and DNA), in the 15 salinity groups, and they together explained 72.54\% of the variance in the model. EROD revealed the strongest antioxidant capacity. Two biomarkers activity (AHH and EROD) explained 62.50\% of the variance in the model of 20 salinity group, that is, PC1. Next, the indicator scores for the activities of four biomarkers of the three salinity groups were determined (Table 1). The indicators were obtained: The EROD and AHH activities with high with a value of 1.00 for both in the 20 salinity groups.

3. Discussions

Petroleum hydrocarbon possesses durable arene in water environment. Ingredients such as anthracene, phenanthrene and Benzo(a)pyrene are characteristics of lipotropic, environmental persistence and genetic toxicity. Petroleum stress may induce a series of oxidative stress reactions, including DNA breakage, lipid peroxidation and enzyme inactivation in living bodies, and even may influence the lipid oxidation of living body
significantly [21]. In this experiment, it found that the same sample made different antioxidant responses to petroleum hydrocarbon stress under various salinities in the testing period. The degrees of inductions varied between EROD and AHH activities, which were manifested by their significant growth in the high-salinity group and their low growth in the low-salinity group. Organic pollutants including petroleum hydrocarbon, can cause biotransformation in living bodies. As a result, the activities of EROD and AHH, which are typical indexes of aquatic organism may increase to a certain extent [22,23]. A study proved that similar with other abiotic stress factors, salinity stress can induce the production of excessive reactive oxygen (ROS) species and cause oxidative damages in cells [24]. Several studies discovered that salinity can influence the solubility of toxic hydrocarbons in crude oil and decrease the oil drops in chemical dispersion agents suspended in crude oils [25]. The solubility of petroleum hydrocarbon in seawater decreases with the increase of salinity [25]. Influenced by high salinity (20), the dissolution of toxic hydrocarbons (e.g. polycyclic aromatic hydrocarbon-PAH) in petroleum hydrocarbon in the experimental solution may be higher than those under low salinities (15 and 10), which led to the evident induction of petroleum hydrocarbon to enzyme activity in digestive glands throughout the exposure. Given the increase in enzyme induction, the living body can eliminate more oxygen radicals under petroleum stress. In a previous study, Zanette et al. reported that salinity influences the diesel-related biomarker responses and toxicity on oyster (Crassostrea gigas), several of those responses remained altered even after depuration, and the toxicity of petroleum hydrocarbons on the oyster increased with the increase in salinity [7]. The toxic effects of diesel on Brazilian oyster (Crassostrea rhizophoraein) increased with the increase in salinity during the diesel exposure and subsequent purification phases were performed in accordance with the work of Da Silva et al. [26]. This finding demonstrated that salinity could influence the toxicity of petroleum hydrocarbon to a certain extent.

When oil pollution is combined with changes in salinity, it may have a greater influence on biological survival by increasing the uptake and accumulation of oil by aquatic organisms or by inhibiting detoxification pathways, resulting in a reduced function of biological metabolism and detoxification processes [27]. In this experiment, petroleum hydrocarbons induced antioxidant enzymes at different sampling points, whereas the tail DNA content increased with petroleum hydrocarbon exposure in all salinity. In addition, and the trend of this increment was observed throughout the exposure. This phenomenon continued to appear during the experimental period, and the higher the salinity, the more evident the degree of DNA damaged. When the environmental salinity changes, mussels can modulate their cellular permeability by changing the amount of endogenous organic matter [28]. Notably, under high salinity conditions, M. coruscus can increase the accumulation of hydrocarbons in petroleum by regulating osmotic pressure [25]. After aromatic compounds in petroleum pollution are absorbed by the organism, bioactive electrophilic compounds which are transformed through metabolism can form covalent binding with DNA, thus, the DNA conjugate is formed, thus changing the DNA structures and causing damages [29].

In vivo cells in mussels are regulated by biochemical pathways to adapt to fluctuating salinity [7]. The salinity of water will affect the solubility of hydrocarbons such as PAHs in petroleum, and then cause lipid peroxidation and damage resulting from the accumulation of these compounds in the body [30]. Lysenko et al. assessed the biological effects of crude oil contamination and desalination on the blue mussel Mytilus edulis L [8]. The biomarker data showed that the combined effects of oil compounds and desalination can be realized in both a synergistic manner. In this experiment, it was observed that CYP1A1 mRNA was induced in the digestive glands of M. coruscus under oil stress, and the expression of CYP1A1 mRNA increased with the increase in salinity, and its expression was positively correlated with salinity. Moreover, the mussel’s antioxidant defense system provides effective protection against external oxidative stress. After the PAHs in petroleum can enter organisms through respiration, feeding, and body surface infiltration, they can effectively induce the content of CYP1A1 protein encoded by CYP1A1 gene in organs and tissues [31]. Although the relationship between salinity adaptation and CYP1A1 mRNA in shellfish is unclear, the accumulation of PAH hydrocarbons reflects the adaptation of aquatic organisms to different salinity media through osmotic

| Biomarker                  | F1   | F2   |
|----------------------------|------|------|
| CYP1A1 mRNA expression     | 0.99 | 0.99 |
| AHH activity               | 0.99 | 0.99 |
| EROD activity              | 0.99 | 0.99 |
| DNA damage                 | -0.98| 0.99 |
|                            |      |      |
regulation. The differences in the expressions of CYP1A1 mRNA genes may be related to the soluble component structure of crude oil in water [32]. CYP1A1mRNA may respond to oxidative stress, depending on the intensity of oxygen partial pressure and duration of oxidative stress caused by allogenic materials.

IBR can be expressed quantitatively by the integrated biological effect of different biomarkers [33]. IBR has been widely applied in the quantitative study of bio toxicity assay. Differences in the toxic effects of different pollutants can be observed intuitively through the numerical value of IBR. In this study, all experimental groups showed different IBR values to a certain extent, proving that salinity can influence the toxic effect of petroleum pollution on aquatic organisms. The details of IBR of the different experimental groups were presented in the star chart. According to the star charts with diversified shapes of sampling points and different coverage areas, petroleum hydrocarbon imposed different degrees of toxic effects on the various experimental groups and it can cause oxidative stress to a living body [34]. Under a high salinity, a large coverage area was observed in the star chart at all sampling points, and this finding may be related to the oxidative stress of petroleum hydrocarbon to the organism. The columnar graph reflected that the cumulative IBR values for different time sequences under a high salinity was significantly higher than those under low salinities. This finding that the toxic effect of petroleum hydrocarbon on organisms intensified with the increase in salinity. IBR can be used as an effective supplement to the results of single biomarkers [33]. Based on the combination of antioxidative response of organism and analysis results, a more comprehensive theoretical foundation shall be set up for the toxic effect evaluation of pollutants. This study is conducive to interpreting the mechanism of toxicity of petroleum hydrocarbon and understanding the regulatory mechanism and effects of parameters of the anti-oxidative system.

4. Conclusions

Petroleum hydrocarbon can induce the enzyme related to the activities of EROD and AHH and expressions of CYP1A1 genes of digestive glands of M. coruscus. Moreover, they can damage the DNA in digestive gland cells with the increase in salinity. According to data changes in IBR, it showed that the toxic effect of petroleum hydrocarbon on M. coruscus under high salinity was stronger than that under low salinity.

Acknowledgments

This work was supported by Project NO. 2019M04 supported by Special Scientific Research Fund for Central non-profit Institutes (East China Sea Fisheries Research Institute), China Agriculture Research System of MOF and MARA, National Key Research and Development (2017YFC1600705), Central Public-interest Scientific Institution Basal Research Fund, CAFS (No.2020TD14).

Disclosure statement

No potential conflict of interest was reported by the authors.

References

[1] Saco-Alvarez L, Bellas J, Nieto O, et al. Toxicity and phototoxicity of water-accommodated fraction obtained from prestige fuel oil and Marine fuel oil evaluated by marine bioassays. SciTotal Environ. 2008;394(2–3):275–282.
[2] Geyer H, Sheehan P, Kotzias D, et al. Prediction of ecotoxicological behaviour of chemicals: relationship between physico-chemical properties and bioaccumulation of organic chemicals in the mussel Mytilus edulis. Chemosphere. 1982;11(11):1121–1134.
[3] Miehelle MG, Norman AR, Moore MN. Immune inhibition in marine mussels by polycyclic aromatic hydrocarbons. Mar Environ Res. 1996;42(14): 187–190.
[4] De OCBZ, Walisson S, Santana CF, et al. Rheological Properties of water-in-Brazilian crude oil emulsions: effect of water content, salinity, and pH. Energy Fuels. 2018;32(8):8880–8890.
[5] Shukla P, Gopalan M, Wate D. Influence of salinity on PAH uptake from water soluble fraction of crude oil in Tilapia mossambica. Bull Environ Contam Toxicol. 2007;79(6):601–605.
[6] Shahunthala DR, Michael JS, Peter VH, et al. Influence of salinity and shellfish species on PAH uptake from dispersed crude oil. Mar Pollut Bull. 2006;52(10):1182–1189.
[7] Zanette J, de Almeida EA, Da Silva AZ, et al. Salinity influences glutathione S-transferase activity and lipid peroxidation responses in the Crassostrea gigas oyster exposed to diesel oil. SciTotal Environ. 2011;409(10): 1976–1983.
[8] Lysenko L, Sukhovskaya I, Borvinskaya E, et al. Detoxification and protein quality control markers in the mussel Mytilus edulis (Linnaeus) exposed to crude oil: salinity-induced modulation. Estuar Coast Shelf Sci. 2015;167(part A):220–227.
[9] Haasch ML, Lech JJ, Prince R, et al. Caged and wild fish: induction of hepatic cytochrome P-450 (CYP1A1) as an environmental biomonitor. Environ Toxicol Chem. 1993;12(5):885–895.
[10] Martinez PG, Livingstone DR. Benzo[a]pyrene-dione stimulated oxyradical production by microsomes of digestive gland of the common mussel, Mytilus edulis. Mar Environ Res. 1995;39(1–4):185–189.
[11] Paul C, Michael SS, Stekoll DR. Hepatic aryl hydrocarbon hydroxylase activities in coho salmon (Oncorhynchus kisutch) exposed to petroleum hydrocarbons. Comp Biochem Physiol Part C: Comp Pharmacol. 1984;79(2):337–341.
[12] Walton DG, Lijm F, Kiceniuk JW. Seasonal changes in aryl hydrocarbon hydroxylase activity of a marine fish Tautogo labrus adspersus (walbaum) with and without petroleum exposure. Comp Biochem Physiol Part C: Comp Pharmacol. 1983;76C(2):247–253.
[13] Lam PK, Gray JS. The use of biomarkers in environmental monitoring programmes. Mar Pollut Bull. 2003;46(2):182–186.

[14] Jiang M, Li L, Li YR, et al. Oxidative stress in shellfish *Sinonovacula constricta* Exposed to the water accommodated fraction of zero sulfur diesel oil and Pinghu crude oil. Arch Environ Contam Toxicol. 2017;73(2):294–300.

[15] Devin S, Burgeot T, Giambe ’rini L, et al. The integrated biomarker response revisited: optimization to avoid misuse. Environ Sci Pollut Res. 2014;21(4):2448–2454.

[16] Li L, Jiang M, Shen XQ. Assessment of the toxicity of crude oil in *Sinonovacula constricta* clams. Aquat Living Resour. 2015a;28:119–126.

[17] Li L, Jiang M, Shen XQ. Variation of antioxidant/detoxification enzyme activities in response to benzo[a]pyrene in the gazami crab *Portunus trituberculatus*. Aquat Living Resour. 2015b;28:45–51.

[18] Pohl RJ, Fouts JR. A rapid method for assaying the metabolism of 7-ethoxycresorufin by microsomal subcellular fractions. Anal Biochem. 1980;107(1):150–155.

[19] Ümit A, Burak EI, Fahriye ZN, et al. Alterations in blood parameters, DNA damage, oxidative stress and antioxidant enzymes and immune-related genes expression in Nile tilapia (*Oreochromis niloticus*) exposed to glyphosate-based herbicide. Comp Biochem Physiol Part C. 2021;249:109147.

[20] Wong CK, Yeung HY, Woo PS, et al. Specific expression of cytochrome P450IA1 gene in gill, intestine and liver of tilapia exposed to coastal sediment. Aquatic Toxicol. 2001;54(1–2):69–80.

[21] Tang D, Shi S, Li D, et al. Physiological and biochemical responses of *Scytonema javanicum* (cyanobacterium) to salt stress. J Arid Environ. 2007;71(3):312–319.

[22] Lyons MC, Wong DKH, Mulder I, et al. The influence of water temperature on induced liver EROD activity in Atlantic cod (*Gadus morhua*) exposed to crude oil and oil dispersants. Ecotoxicol Environ Saf. 2011;74(4):904–910.

[23] Janina B, Aleksandras R, Galina G, et al. Environmental genotoxicity and cytotoxicity studies in mussels before and after an oil spill at the marine oil terminal in the Baltic Sea. Environ Monit Assess. 2012;184(4):2067–2078.

[24] Choo KS, Snoeijis P, Pedersen M. Oxidative stress tolerance in the filamentous green algae *Cladophora glomerata* and *Enteromorpha ahlneriana.* J Exp Mar Biol Ecol. 2004;298(1):111–123.

[25] Blondina GJ, Singer MM, Lee I. Influence of salinity on petroleum accumulation by dispersants. Spill Sci Technol Bull. 1999;5(2):127–134.

[26] Da Silva AZ, Zanette J, Ferreira JF, et al. Effects of salinity on biomarker responses in *Crassostrea rhizophorae* (Mollusca, Bivalvia) exposed to diesel oil. Ecotoxicol Environ Saf. 2005;62(3):376–382.

[27] Ramachandran SD, Sweezey MJ, Hodson PV, et al. Influence of salinity and fish species on PAH uptake from dispersed crude oil. Mar Pollut Bull. 2006;52(10):1182–1189.

[28] Deaton LE. Hyperosmotic volume regulation in the gills of the ribbed mussel, *Geukensia demissa*: rapid accumulation of betaine and alanine. J Exp Mar Biol Ecol. 2001;260(2):185–197.

[29] Bellas J, Saco-Alvarez L, Nieto O. Ecotoxicological evaluation of polycyclic aromatic hydrocarbons using marine invertebrate embryo-larval bioassays. Mar Pollut Bull. 2008;57(6–12):493–502.

[30] Sol EM, Porte C, Albaig ESJ. Hydrocarbons, PCBs and DDT in the NW Mediterranean deep-sea fish *Mora moro*. Deep Sea Res Part I: Oceanogr Res Pap. 2001;48(2):495–513.

[31] Beyer J, Sandvik M, Hylland K, et al. Contaminant accumulation and biomarker responses in flounder (*Platichthys flesus*L.) and Atlantic cod (*Gadus morhua* L.) exposed by caging to polluted sediments in Sørjorden, Norway. Aquatic Toxicol. 1996;36(1):75–98.

[32] Tor O, Palma-Fleming HAN, Navarro JM. Organic pollutant burden of the giant mussels *Choromytilus* chorus from the south-central Chilean coast. Chemosphere. 2004;55(2):267–275.

[33] Lee L, Jiang M, Shen XQ. Variability in antioxidant detoxification enzymes of *Sinonovacula constricta* exposed to benzo[a]pyrene and phenanthrene. Mar Pollut Bull. 2016;109(1):507–511.

[34] Nwaogu L. Effect of Chronic Exposure to petroleum hydrocarbon pollution on oxidative stress parameters and histology of liver tissues of native fowl (*Gallus domesticus*). Int J Biochem Res Rev. 2014;4:233–242.