Sex-specific impacts on the accumulation of polycyclic aromatic hydrocarbons and the development of offspring of sea urchins exposed to heavy fuel oil

Xuanbo Wang¹, Xishan Li¹, Hang Ren¹, Huishu Chen¹, Zhonglei Ju¹ and Deqi Xiong¹*

¹Environmental Science and Engineering, Dalian Maritime University, Dalian, Liaoning, 116026, China
Type the corresponding author’s e-mail address: xiongdq@dlmu.edu.cn

Abstract. The sensitivity of female and male individuals of the same species to environmental stress is often different. The present study investigated sea urchins (Strongylocentrotus intermedius) of both sexes that were exposed to water-accommodated fractions of heavy fuel oil (HFO-WAF) for 7 d. Polycyclic aromatic hydrocarbons (PAHs) bioaccumulation in sea urchin gonad tissue was significantly increased following the exposure to HFO-WAF. However, PAHs bioaccumulation significantly differed between females and males, which may be related to the lipid content of gonad tissue. We found a significant increase in the percentage of anomalies in their larvae 24 h post fertilization. This indicates that parental exposure to HFO-WAF can have an adverse effect on the offspring, which may consequently affect population recovery and maintenance.

1. Introduction
After an oil spill of large quantities of heavy fuel oil (HFO) into the ocean, it forms water-accommodated fractions of HFO (HFO-WAF), which promote a prolonged toxic release[1]. PAHs were released into the water from HFO-WAF and bioaccumulation in vertebrates and invertebrates[2-3]. Previous study indicates that PAHs bioaccumulation in organisms have negative effects on their growth and offspring development[4-5]. However, most of these studies have focused on HFO-WAF exposure, and less known about the content of PAHs bioaccumulation in invertebrate organs and the development of their offspring when sea urchin has a short-time exposure to HFO-WAF. Therefore, understand the PAHs bioaccumulation of invertebrates on short-term HFO-WAFs exposure can provide more valuable information.

Sea urchins are benthic species, mainly inhabiting the seabed of shallow seas. As a source of algae consumers, sea urchin plays a key role in the composition, structure and diversity of the shallow sea biota[6]. Moreover, unlike larger swimming creatures, sea urchins lack the ability of escape from environmental stress, and are often used in toxicological experiments[7]. As a representative invertebrate, sea urchin is a great research model to explore short-term toxic effects of HFO.

Most studies focus on the effects of HFO on female sea urchins, few compared sexes differences. The present study aims at investigating the toxic effects of HFO-WAF exposure on sea urchin offspring. PAHs bioaccumulation in gonad tissue were measured in conjunction with larvae development observed, to demonstrate whether female and male sea urchins are differently affected by exposure to HFO-WAF.
2. Materials and methods

2.1. preparation of exposure solution
The exposure solution was composed of 380# HFO and filtered seawater, mixed in a volume of 1:9, and continuously stirred with a magnetic stirrer for 18 h. After standing for 6 hours, the mixed solution was diluted with seawater to concentration ratio of 0% and 5%.

2.2. Sampling of animal bioassay
Adult sea urchin (Strongylocentrotus intermedius) were purchased by Dalian Haibao Fishery Co., Ltd. Animals were transported to the laboratory by an ice boxes and kept in a recirculation system with seawater until the experiments were started. Ten female and male sea urchin individuals were randomly selected from the non-polluted culture water and distributed to the experimental solutions (0% and 5% oil loading) (N=3). The sea urchins were domesticated at 18 ± 0.5 °C with a 12 hours light and 12 hours darkness and feeding kelp Laminaria japonica for 7 days. After 7 days, female and male gonad tissues were acquired to measure PAHs bioaccumulated and component analysis by using GC (HP 6890 GC)-MS.

2.3. Embryo culture
After exposure, 1 mL KCL (0.5 mol) was injected into the female and male sea urchin coelomic to promote the gametes production. There were three independent embryo populations: maternal exposure (exposed female gametes fertilized by control male gametes), paternal exposure (exposed male gametes fertilized by control female gametes), control (control female gametes fertilized by control male gametes). The sample were cultured at 18 ± 1 °C for 24 hours post fertilization (hpf), and then part of the embryos was taken out to observe the embryo morphology using a microscope (OLYMPUS IX73).

2.4. Statistical analysis
For statistical analysis, the results were performed by using IBM SPSS Statistics 19. The data were compared by ANOVAs followed by Tukey’s post hoc tests, expressed as means ± standard deviation (SD). One-way ANOVA was used to analyze all data. When significant difference was found, LSD test was used to analyze pairwise multiple comparisons. The differences were considered significant at p<0.05.

3. Results

3.1. Bioaccumulated of PAHs bioassay
The results showed that the concentration of PAHs accumulated in female (384.23 ± 30.2 ng/g DW) ovaries were significantly increased than in male testes (274.76 ± 16.9 ng/g DW) (figure 1).

![Figure 1](image1.png)

Figure 1. The concentration of PAHs accumulated in gonads tissues of female and male sea urchins exposed to HFO-WAFs for 7 d. Date are presented as means ± SD. Asterisk indicated the difference between sexes (p<0.05).
3.2. Effects on offspring

At 24 hpf, delayed embryos were mainly arrested at the early gastrula and blastula stages (figure 2). We found that the percentage of abnormality embryos at 5% HFO-WAF was significantly higher in maternal (30.5% ± 1.8%) and paternal (24.73% ± 0.8%) exposure than in the control (average 13.81% ± 1.65%). Furthermore, the percentage of abnormality embryos have significantly difference between maternal exposure and paternal exposure to 5% HFO-WAF.

![Figure 2. The component of PAHs in gonads tissues of female and male sea urchins (S. intermedius) exposed to HFO-WAFs for 7 d. Date are presented as means ± SD, and asterisk indicated the difference between sexes (p<0.05).](image)

4. Discussion

In this study, we explored the toxic effects of HFO-WAFs exposure on the sea urchin for 7 d. We found that a large of PAHs were found in the sea urchin gonad tissue when sea urchin exposed to HFO. This indicated that PAHs released into the water from HFO-WAFs and was accumulated in sea urchin organ, consistent with previous studies[8]. PAHs are highly lipid soluble in seawater. However, the HFO was increasing the specific surface area in contact with seawater, resulting in the PAHs were continuously released into the seawater, which bioaccumulated in the aquatic organisms[1]. In a word, the results showed that sea urchin exposed to HFO could bioaccumulated a large number of PAHs, affecting the fitness of them. Thus, HFO-WAF presents a huge threat to the marine benthic ecological environmental.

In this study, we found that sea urchins have sex-specific differences in the bioaccumulation of PAHs. The concentration of PAHs in the ovaries of female sea urchins was higher than that in the testes of male. That may be because PAHs are fat-soluble and mainly bioaccumulation in abundant lipid tissue of organisms, and there was evidence that lipid content of ovaries was higher than in testes[9]. Therefore, the sex-specific differences of PAHs bioaccumulation may be related to the different lipid content of gonad tissue.

In this study, we found that maternal exposure to HFO-WAF cause d significant increase in the percentage of abnormality larvae. Previous study has shown that female sea urchin (Strongylocentrotus. intermedius) exposed to 0.04 - 0.3 mg/L diesel fuel hydrocarbons for 50 d caused a significant increase in 45CA2+ in the eggs and a corresponding increase in the percentage of abnormality of their offspring[10], which was consistent with the results of this study. However, Carls et al showed that female fish (Clupea. pallasi) exposed to petroleum hydrocarbons did not affect the early embryos development[11]. This difference may be related to the different sensitivity of the species to oil pollution[12]. At present, most studies focus on the impact of maternal exposure to environmental stress on their offspring development, while few studies explored paternal exposure. Our study showed that the percentage of abnormality larvae were significantly increased when paternal exposed to HFO-WAF. This may be paternal exposure to environmental stress caused changes in the gene expression of their sperm, and epigenetic mutations might stably inherit and expressed in the offspring[13]. In addition, the DNA damage of the male under environmental stress, increased the percentage of abnormality of
offspring[14]. We found that maternal exposure had significant higher in the percentage of abnormality larvae than paternal exposure. This might because that the eggs provided the early embryos with the nutrients needed for growth and development. After maternal exposure, the eggs may have been damaged, resulting in insufficient nutrients, which will lead to excessive abnormality in offspring development. In a word, this study showed that the toxic effects of HFO-WAF on sea urchin may be transgenerational across, and will seriously threaten the development of their offspring and the survival of the population.

5. Conclusion
This research highlights detrimental effects of HFO-WAF. In this study, we explored the sex-specific difference in the toxic effects of HFO-WAF on sea urchin for 7 d. Our results showed that sea urchin exposure to HFO for 7 d caused PAHs accumulation in gonads of parental. Female ovaries were significantly more sensitive to HFO-WAF exposure than male tastes. Female and male exposure to HFO-WAF had negative affected on the larval development, which may be related to PAHs, and further investigation is needed. This study increases our understanding of sex-specific in the toxic effects of HFO-WAF on sea urchin and provides valuable information into marine environmental safety.

Acknowledgments
This work was supported by the National Natural Science Foundation of China (40976064).

References
[1] Martin, J.D., Adams, J., Hollebone, B., King, T., Brown, R.S., Hodson, P.V., (2014) Chronic toxicity of heavy fuel oils to fish embryos using multiple exposure scenarios. Environ. Toxicol. Chem., 33: 677–687.
[2] Li, X.S., Xiong, D.Q., Ding, G.H., Fan, Y.M., Ma, X.R., Wang, C.Y., Xiong, Y.J., Jiang, X., (2019) Exposure to water-accommodated fractions of two different crude oils alters morphology, cardiac function and swim bladder development in early-life stages of zebrafish. Chemosphere., 235: 423–433.
[3] Duan, M.N., Xiong, D.Q., Bai, X., Gao, Y.L., Xiong, Y.J., Gao, X., (2018) Transgenerational effects of heavy fuel oil on the sea urchin Strongylocentrotus intermedius considering oxidative stress biomarkers. Mar. Environ. Res., 141: 138–147.
[4] Lister, K.N., Lamare, M.D., Burritt, D.J., (2016) Dietary pollutants induce oxidative stress, altering maternal antioxidant provisioning and reproductive output in the temperate sea urchin Evechinus chloroticus. Aquat. Toxicol., 177: 106–115.
[5] Lister, K.N., Lamare, M.D., Burritt, D.J., (2017) Maternal antioxidant provisioning mitigates pollutant-induced oxidative damage in embryos of the temperate sea urchin Evechinus chloroticus. Sci. Rep-UK., 7: 1954.
[6] Hernández, J.C., Clemente, S., Sangil, C., Brito, A., (2008) The key role of the sea urchin diadema aff. antillarum in controlling macroalgae assemblages throughout the Canary Islands (eastern subtropical Atlantic): An spatio-temporal approach. Mar. Environ. Res., 66: 259–270.
[7] Saco Alvarez, L., Bellas, J., Nieto, O., Bayona, J.M., Albaiges, J., Beiras, R., (2008) Toxicity and phototoxicity of water-accommodated fraction obtained from Prestige fuel oil and marine fuel oil evaluated by marine bioassays. Sci. Total. Environ., 394: 275–282.
[8] Yang, B., Xiong, D.Q., (2015) Bioaccumulation and subacute toxicity of mechanically and chemically dispersed heavy fuel oil in sea urchin (Glyptocidaris crenulata). Sci. Mar., 79: 497–504.
[9] Fernandez, C., Pergent, G., (1998) Effect of different formulated diets and rearing conditions on growth parameters in the sea urchin Paracentrotus lividus. J. Shellfish. Res., 17: 1571–1581.
[10] Zhadan, P.M., Vaschenko, M.A., (1993) Effect of diesel fuel hydrocarbons on embryogenesis and $^{45}$Ca$^{2+}$ uptake by unfertilized eggs of sea urchin, Strongylocentrotus intermedius. Comp. Biochem. Phys. C., 105: 543–548.
[11] Carls, M.G., Hose, J.E., Thomas, R.E., Rice, S.D., (2000) Exposure of Pacific herring to weathered crude oil: Assessing effects on ova. Environ. Toxicol. Chem., 19: 1649–1659.
[12] Buric, P., Jaksic, Z., Stajner, L., Sikiric, M.D., Jurasin, D., Cascio, C., Calzolai, L., Lyons, D.M., (2015) Effect of silver nanoparticles on Mediterranean sea urchin embryonal development is species specific and depends on moment of first exposure. Mar. Environ. Res., 111: 50–59.
[13] Curley, J.P., Mashoodh, R., Champagne, F.A., (2011) Epigenetics and the origins of paternal effects. Horm. Behav., 59: 306–314.
[14] Lewis, C., Galloway, T., (2009) Reproductive consequences of paternal genotoxin exposure in marine invertebrates. Environ. Sci. Technol., 43: 928–933.