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Parental exposure to heavy fuel oil induces developmental toxicity in offspring of the sea urchin *Strongylocentrotus intermedius*

Meina Duan[a], Deqi Xiong[a,*], Mengye Yang[b], Yijun Xiong[c], Guanghui Ding[a]

[a] *School of Environmental Science and Engineering, Dalian Maritime University, Linghai Road 1, Dalian 116026, Liaoning, China*
[b] *School of Life Science, Wuhan University, Wuhan 430072, Hubei, China*
[c] *School of Biology and Chemistry, Grinnell College, 1115 8th Ave, Grinnell, IA 50112, USA*

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ABSTRACT

The present study investigated the toxic effects of parental (maternal/paternal) exposure to heavy fuel oil (HFO) on the adult reproductive state, gamete quality and development of the offspring of the sea urchin *Strongylocentrotus intermedius*. Adult sea urchins were exposed to effluents from HFO-oiled gravel columns for 7 days to simulate an oil-contaminated gravel shore, and then gametes of adult sea urchins were used to produce embryos to determine developmental toxicity. For adult sea urchins, no significant difference in the somatic size and weight was found between the various oil loadings tested, while the gonad weight and gonad index were significantly decreased at higher oil loadings. The spawning ability of adults and fecundity of females significantly decreased. For gametes, no effect was observed on the egg size and fertilization success in any of the groups. However, a significant increase in the percentage of anomalies in the offspring was observed and then quantified by an integrative toxicity index (ITI) at 24 and 48 h post fertilization. The offspring from exposed parents showed higher ITI values with more malformed embryos. The results confirmed that parental exposure to HFO can cause adverse effects on the offspring and consequently affect the recruitment and population maintenance of sea urchins.

1. Introduction

Oil spillages of medium or heavy fuel oil (HFO), either as cargo or as bunker fuel, have caused widespread contamination and adverse impacts on coastlines and sensitive resources (Ansell et al., 2001). For example, in November 2007, the container ship Cosco Busan released 53,569 gallons of bunker fuel oil (specifically HFO 380) into San Francisco Harbor, bringing more than 3367 acres of shoreline habitat under threat of oil pollution (Cosco Busan Oil Spill Trustees, 2012). To investigate the toxicity of oil stranded onshore, oiled gravel columns have been developed (Carls et al., 1999; Martin et al., 2014; Marty et al., 1997). The system promotes partition-controlled delivery of the toxic and hydrophobic components of oil to seawater with seawater flowing through gravel substrates coated with oil. Recently, the system has been utilized to investigate the chronic toxicity of HFO to fish embryos (Martin et al., 2014). In fact, the interstitial toxic water hypothesis (Mark et al., 2003) indicates that tidal cycling could bring water into contact with oiled gravel producing an alongshore flow of contaminated interstitial seawater with polycyclic aromatic hydrocarbons (PAHs). This mechanism could increase the exposure of benthic organisms to stranded HFO or toxic interstitial water. Thus, the toxicological assessment of HFO stranded on gravel to marine benthic organisms is needed, as this can provide relevant information to the ecological risk assessment of HFO spillages.

Sea urchins, as a benthic species, often play an important role in communities inhabiting shallow gravel shores (Furman and Heck, 2009; Hernández et al., 2008; Pearse, 2006; Rose et al., 1999), which are the most difficult of all the beach types to clean and restore after an oil spill (Hayes and Michel, 2001). Sea urchins have a complex life cycle including a larval planktonic stage and a benthic adult stage. Not only are the stages of embryonic and larval development of sea urchins sensitive to oil pollution and suitable for embryo-toxicity tests, monitoring and risk assessment programs (Beiras and Saco-álvarez, 2006; Bellas et al., 2013, 2008; Lukyanova et al., 2017; Rial et al., 2013, 2014; Saco-Álvarez et al., 2008; Stefansson et al., 2016), but adult sea urchins have also been shown to be sensitive to contaminants such as metals and hydrocarbons (Bielmyer et al., 2005; Borges et al., 2010; Cunha et al., 2005; Yang and Xiong, 2015). Therefore, sea urchins represent a well-suited test organism in the evaluation of environmental effects of HFO stranded onshore and in ecological risk assessment, especially considering the ecological and economic significance of sea urchins, together with their sedentary habits and sensitivity to oil contaminants.

* Corresponding author.
E-mail address: xiongdq@dlmu.edu.cn (D. Xiong).

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Reproductive success is crucial for species survival. Reproductive impairments of adult sea urchins may affect fertilization success, embryo development, and consequently, population survival and recruitment. It has been reported that oil-related contaminants induced a decrease in the fertilization success of sea urchins (*Strongylocentrotus purpuratus* Stimpson) caged in polluted sites (Krause, 1994) and a higher percentage of abnormalities in the offspring of indigenous sea urchins (*Sterechinus neumayeri*) (Lister et al., 2015). Similar results were also obtained in the offspring of sea urchins collected in the coastal zone of Amur Bay (Vaschenko and Zhadan, 2003) and in the Bay of Brest (Guillou et al., 2000) (*Strongylocentrotus intermedius* and *Sphaerechinus granularis*, respectively), which suffered from heavy metal pollution. These results obtained in field studies would constitute a basis for predicting possible population responses under oil-related pollutants. External (e.g., photoperiod and temperature) (Shpigel et al., 2004) and internal (e.g., reproductive cycle) (Schäfer et al., 2011; Schäfer and Köhler, 2009) factors can modify the effects of contaminants on biological responses. For this reason, these data from complex marine ecosystems are sometimes difficult to interpret. Therefore, laboratory experiments conducted under controlled conditions without confounding factors are essential to assess the subsequent effects of parental exposure to oil on the early developmental stages for providing the scientific basis and correct evaluation in marine pollution monitoring.

There are very few laboratory studies available about the impacts of parental exposure to oil on offspring in sea urchins. For example, diesel fuel oil (Vashchenko, 1980) and dietary PAHs exposure (Lister et al., 2016, 2017) to adult sea urchins have been shown to cause negative effects on development of offspring (*Strongylocentrotus nudus* and *Evechinus chloroticus*, respectively). However, the subsequent effects on early life stages after parental sea urchins were exposed to stranded HFO are unknown. It has been well documented that both the chemical (e.g., methylphenanthrenes) (Le Bihanic et al., 2014) and physical characteristics (e.g., dispersability) (Wu et al., 2012) of the oil in the environment can affect the availability of toxic components to organisms. HFO is characterized by lower concentrations of low-molecular-weight (MW) volatile compounds such as BTEX (benzene, toluene, ethylbenzene and xylene), higher concentrations of high-MW alkanes, and alkyl-PAHs compared to diesel fuel oil (Fingas, 2014). HFO has physical properties (i.e. it has high density, viscosity and pour point) that would inhibit the rate of oil-water partitioning of the toxic constituents (Ansell et al., 2001); however, when HFO is stranded on gravel, the increasing surface area of HFO exposed to water could promote the continuous release of the toxic constituents from HFO to water (Martin et al., 2014). Given these characteristics of stranded HFO, it is uncertain whether parental exposure to stranded HFO could harm the development of offspring.

Furthermore, most field and laboratory toxicity studies related to sea urchins focus on maternal exposure (Lister et al., 2016; Migliaccio et al., 2015; Roepke et al., 2006; Schweitzer et al., 2000; Zhadan and Vaschenko, 1993), but the significance of paternal exposure to HFO to offspring is virtually unknown. Although the impacts of sperm exposure to environmental stress on the sperm quality and subsequent fate of offspring in sea urchins have been well documented (Au et al., 2002; Caplat et al., 2010; Gambardella et al., 2013, 2015a, b; Lu and Wu, 2005; Nahon et al., 2009; Pagano et al., 1985), the effects on offspring following exposure of adult male sea urchins remain to be determined. Few studies reported that paternal exposure to oil-related contaminants at a single concentration induced the abnormalities of offspring in sea urchins (Lister et al., 2017; Vashchenko, 1980); however in those studies, developmental analyses were conducted without distinguishing different adverse effects on embryo morphology and development. Thus, in the present study, a more sensitive integrative toxicity index (ITI) was adopted to discriminate the various phenotypes in offspring derived from exposed sea urchins, which assigned different scores to various embryonic malformations depending on their severity and the stage at which they appeared (Morroni et al., 2016).

The current experiment was undertaken to simulate an offshore HFO spill event. The main objectives of the current study were (1) to determine whether HFO stranded on a shoreline could affect offspring fitness in the sea urchin *S. intermedius*, an ecologically important species inhabiting intertidal and subtidal rocky bottoms in northern regions of Asian Pacific coastal waters (Agatsuma, 2013), and (2) to identify whether the offspring derived from different parental crosses differed from each other in the types of developmental abnormalities. Therefore, in the present study, adult sea urchins (*S. intermedius*) were exposed to effluents from oiled gravel columns for 7 days to explore reproductive toxicity to adult sea urchins and carry-over effects on their offspring. Furthermore, a more effective ITI method was utilized to discriminate the various phenotypes in offspring following parental (maternal, paternal, or both) exposure. This information could improve the understanding of the impacts of HFO spillages on aquatic organisms at a population level and be complementary to field studies.

2. Materials and methods

2.1. Chemicals

HFO 380, a bunker fuel oil with a viscosity of 729.8 mm^2/s at 50 °C and density of 0.9821 g/cm^3, was provided by Dalian Marine Fuel Co., Ltd.

2.2. Oiled gravel column

Stranded HFO 380 was prepared in accordance with the methods of previous studies (Carls et al., 1999; Martin et al., 2014; Marty et al., 1997). Oil was poured on 1.8 kg of clean gravel (rounded pebbles, 10–50 mm in diameter) at 25 °C, and then the gravel was shaken intensely for 2 min. The nominal concentrations were 400, 800, 1600, 3200 and 6400 μg oil/g gravel. Polluted gravel was kept for 24 h in the dark and transferred into columns structured by chlorinated polyvinyl chloride pipe (diameter = 10.8 cm, height = 35 cm, Fig. S1). The columns were rinsed with seawater at 20 mL/min for 24 h at 18 ± 1 °C before the bioassays. Then, the oiled gravel columns were flushed with clean seawater at 20 mL/min to obtain exposure solutions.

The lowest oil loading of oiled gravel used in the present study was based on the results of Zhadan and Vaschenko (1993), which demonstrated that the long-term (50 days) exposure of female sea urchins (*S. intermedius*) to water soluble hydrocarbons of diesel fuel oil (the concentrations of total petroleum hydrocarbons (TPH) up to 300 μg/L) caused disturbances in embryogenesis and early larval development. Based on our preliminary experiments, 400 μg/g was chosen because it would provide TPH concentrations near those measured by Zhadan and Vaschenko (1993). As expected, measured TPH concentrations in the 400 μg/g group decreased from 618.5 to 308.6 μg/L during the exposure period.

The highest oil loading was based on our preliminary trials with female sea urchins. Oil loadings of 4000, 8000 and 16,000 μg/g were tested (TPH concentrations at day 1 were 1159, 1790 and 3198 μg/L, respectively), and egg production was recorded. The 16,000 μg/g oiled gravel resulted in spontaneous spawning after 2 days of exposure and the female sea urchins exposed for 7 days were less likely to spawn, failing to produce eggs. No spontaneous spawning was observed in the 4000 and 8000 μg/g groups during the exposure period. Females in the 4000 μg/g group produced adequate eggs to be used in a subsequent experiment, whereas eggs collected in the 8000 μg/g group were insufficient. To ensure sufficient eggs in our formal experiment, the highest oil loading selected should be lower than 8000 μg/g.

Therefore, oil loadings of 400, 800, 1600, 3200 and 6400 μg/g were chosen to ensure the induction of significant biological effects on offspring and sufficient eggs produced by exposed adult sea urchins. Additionally, the TPH concentrations at day 1 in the five test solutions