Exposure of adult sea urchin *Strongylocentrotus intermedius* to stranded heavy fuel oil causes developmental toxicity on larval offspring

Xuanbo Wang, Xishan Li, Deqi Xiong, Hang Ren, Huishu Chen and Zhonglei Ju

College of Environmental Science and Engineering, Dalian Maritime University, Dalian, China

ABSTRACT

Heavy fuel oil (HFO) spills pose serious threat to coastlines and sensitive resources. Stranded HFO that occurs along the coastline could cause long-term and massive damage to the marine environment and indirectly affect the survival of parental marine invertebrates. However, our understanding of the complex associations within invertebrates is primarily limited, particularly in terms of the toxicity effects on the offspring when parents are exposed to stranded HFO. Here, we investigated the persistent effects on the early development stage of the offspring following stranded HFO exposure on the sea urchin *Strongylocentrotus intermedius*. After 21 d exposure, sea urchins exhibited a significant decrease in the reproductive capacity; while the reactive oxygen species level, 3-nitrotyrosine protein level, protein carbonyl level, and heat shock proteins 70 expression in the gonadal tissues and gametes significantly increased as compared to the controls, indicating that HFO exposure could cause development toxicity on offspring in most traits of larval size. These results suggested that the stranded HFO exposure could increase oxidative stress of gonadal tissues, impair reproductive functions in parental sea urchins, and subsequently impact on development of their offspring. This study provides valuable information regarding the persistent toxicity effects on the offspring following stranded HFO exposure on sea urchins.

INTRODUCTION

Around 380 million gallons of oil are released into the marine each year due to natural disasters and anthropogenic factors (oil spill from ships and oil platforms) (*Naz et al., 2021*; *Pisano et al., 2016*). Oil spills profoundly impact the coastal and marine environments, adversely affecting aquatic organisms (*Ansell et al., 2001*). Previous studies have shown that oil-derived hydrocarbons negatively impact aquatic organisms. For example, polar cod (*Boreogadus saida*) exposed to crude oil showed sublethal effects on the lipid composition of liver tissue (*Vieweg et al., 2022*). Crude oil exposure has been shown to cause growth inhibition and lipid allocation on polar cod embryonic (*Boreogadus saida*) (*Laurel et al., 2019*). Oil-derived hydrocarbons exposure of sea urchin

(*Strongylocentrotus intermedius*) significantly increased lipid peroxidation level and caused enhanced oxidative damage (*Wang et al., 2021*). In addition, nearly 40% of the more than 400 ship-sourced oil spills over the past few decades involved heavy fuel oil (HFO) (*Ansell et al., 2001*). The tidal cycle causes the seawater to contact the HFO gravel, promoting the release of sustained polycyclic aromatic hydrocarbons (PAHs) into the seawater when HFO stranded to the shore (*Carls et al., 2003*). PAHs can change in marine environments, altering the physiology of aquatic organisms (*Ma et al., 2020*; *Wang et al., 2018*). Previous studies showed that HFO stranded on gravel increased rainbow trout embryos (*Oncorhynchus mykiss*) mortality and was higher than crude oil (*Martin et al., 2014*). To our knowledge, there is limited understanding on the impacts of stranded HFO on marine benthic invertebrates. Benthic invertebrates with limited mobility generally live on the benthic substrate and are more vulnerable to threats than mobile species. Therefore, exploring the toxic effects of stranded HFO on benthic invertebrates can provide a new basis for the ecological risk assessment of HFO leakage.

Marine invertebrates have a complex life history, including short-term embryonic, larval, and long-term adult stages. The performance in one life-history stage may positively or negatively affect the subsequent life-history stages, the so-called developmental domino phenomena (*Byrne, 2012*). Marine invertebrates with short generation cycles have a strong potential for evolutionary adaptation owing to the environmental stress experienced through several generations (*Chen & Stillman, 2012*; *Ding et al., 2020*). However, whether stranded HFO causes persistent effects on the offspring in marine invertebrates remains unclear.

The slow movements are relatively impacting the adult sea urchins, as they have a though small, choice of substrate, mainly during larval settlement. In addition, sea urchin has a short generation cycle and have strong evolutionary adaptation potential. Therefore, sea urchins are a valid model for the study of oil pollution, because adults live and feed in close contact with the coastal bottom, with the ability of remodeling it by grazing. Exposure of adult sea urchins to environmental stress caused changes in their behavior and physiology (*Zhang et al., 2017*; *Chiarelli et al., 2014*). Such changes include tube foot withdrawal, decreased adsorption capacity, and spines falling off (*Axiak & Saliba, 1981*; *Bielmyer et al., 2005*). Therefore, sea urchin is a suitable experimental organism for assessing the environmental impact and ecological risk related to kind of marine stressors, such as heavy metals (*Chiarelli, Martino & Roccheri, 2019*), PAHs (*Lister, Lamare & Burritt, 2016*), nano plastics (*Manzo & Schiavo, 2022*) and other marine pollutants (*Martino et al., 2022*; *Bellas et al., 2022*). In addition, most investigations have devoted to parental fish exposed to crude oil could have adverse effects on embryos (*Bautista et al., 2021*; *Jasperse et al., 2019*; *Li et al., 2021*). However, less is known about the parental exposure of sea urchins to oil pollutants may have a negative impact on offspring. Therefore, considering the ecological importance and susceptibility to oil pollution, the persistent effects on the early development stage of the offspring of sea urchin exposure to stranded HFO pollution need to be explored.

Oil pollution may also cause the overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in marine invertebrate. Previous studies have found that

marine mussel (*Mytilus galloprovincialis*) exposed to crude oil increased ROS production, induced actin cytoskeleton disruption (*Katsumiti et al., 2019*). Crude oil exposure caused a significant increase in the ROS on sea cucumber (*Apostichopus japonicus*), further resulting in increased oxidative damage *in vivo* (*Li et al., 2020*). It has been documented that the increase of ROS and RNS within sea urchins negatively affects the DNA, proteins, and membrane lipids (*Duan et al., 2018a*; *Zhou et al., 2012*). Organisms are subjected to harmful endogenous or exogenous stresses, resulting in the overproduction of ROS and RNS in the body, leading to oxidative stress (*Di Meo et al., 2016*). The overproduction of ROS, which are highly active intermediate products formed by molecular oxygen during reduction, will affect homeostasis and induce stress response (*Dröge, 2002*). RNS are involved in the nitrosative stress in animal cells and tissues. Overproduction of RNS can induce an imbalance of 3-nitrotyrosine protein (3-NTP) in the body (*Castellano et al., 2018*). The overproduction of NTP may be associated with aging and diseases in organisms (*Johnstone et al., 2019*). Therefore, we have speculated that HFO exposure may result in ROS and RNS overproduction that would induce oxidative stress, thereby affecting the gonadal function of sea urchins. The changes in the gonadal function of sea urchins may affect gamete quality and offspring development.

This study aimed to investigate the impact of long-term stranded HFO exposure on gonadal functions, ROS, 3-NTP and protein carbonyl (PC) levels, *hsp*70 gene expression in gonads and gametes in the sea urchin *Strongylocentrotus intermedius*. We also aimed to investigate: (1) whether the long-term exposure of paternal and maternal sea urchins to stranded HFO causes toxic effects to their offspring; (2) whether the fitness of sea urchin offspring shows paternal or maternal effects following parental exposure to stranded HFO. The present study provides new and comprehensive important information related to toxicity effects of HFO on marine invertebrate's offspring.

MATERIALS AND METHODS

HFO gravel column tanks preparation

The preparation of HFO gravel column tanks followed methods as described in previous studies (*Duan et al., 2018a*; *Marty, Heintz & Hinton, 1997*), with some modifications. "380# HFO" was poured in 8 kg of clean gravel to obtain oil loading of 9,600 μg oil/g gravel. The HFO gravel was manually shaken using a polyvinyl chloride mixer for 5 min, which made the HFO evenly distributed on the gravel. The HFO gravel was kept in a dark place for 24 h to allow time for a thin oil film to completely coat the gravel. Then, the HFO gravel was transferred into an appropriate polyvinyl chloride pipe with a diameter and height of 45 and 55 cm, respectively. We used natural seawater pumped at a rate of 100 mL/min to wash the columns from bottom to top at 16 °C for 24 h and used a tank to collect the exposure fluid from the outlet of the gravel column for sea urchin exposure experiments. The control gravel column was applied with no oil.

Samples of seawater from the columns were collected every 24 h for chemical analysis to determine concentrations of total petroleum hydrocarbons (TPH) and PAHs in the exposure solution, as described in the previous studies (*Li et al., 2019*; *Duan et al., 2018a*). Briefly, 200 mL solution samples were extracted with 20 mL *n*-hexane using a separating

funnel. TPH concentrations were measured by ultraviolet spectrometry (Epoch 2; BioTek, Winooski, VT, USA) according to the specification for seawater analysis (GB 17378.4-2007) method with modifications. Moreover, 16 priority PAHs were also measured by gas chromatography-mass spectrometry (GC/MS) (HP6890 GC-5975 MSD; Agilent Technologies Inc., Santa Clara, CA, USA). The EPA 3510C liquid-liquid extraction method was used to pretreat the water samples. EPA 3630C silica gel cleaning method is used to clean the concentrate, and samples were measured by GC/MS. TPH concentrations were decreasing from 811.22 μg/L to 474.64 μg/L (Fig. S1). PAHs concentrations decreased from 6.04 to 1.83 μg/L (Fig. S2).

Sea urchin and HFO treatments

Adult sea urchins were provided from Dalian Haibao Fishery Co., Ltd. and transferred to the environmental toxicology laboratory. Animals were kept in tanks with circulating seawater until the exposure experiment was performed.

Sea urchins (horizontal test diameter: 52.5 ± 10.61 mm; vertical test diameters: 24 ± 5.66 mm) were randomly selected and placed in the treatment tank (20 sea urchins were raised in each tank). Two tanks (control and treatment) were controlled at 16 °C under a 12-h light: 12-h dark cycle for 21 d; three repeated experiments were arranged for each treatment. Sea urchins were fed kelp (laminaria japonica) every 3 d, and siphon was used to remove fecal waste from the bottom of each tank.

Spawning, egg production and fertilization

After exposure, the sea urchin was washed with filtered seawater, and 1 mL KCl (0.5 M) was injected into the coelom through the peristomia membrane to obtain the gametes. To determine the spawning ability, the number of spawned male and female sea urchins was divided by the total number of male and female sea urchins according to *Duan et al. (2018a)*. Each female's reproductive ability was measured by the number of eggs that the female sea urchins excreted within 30 min (*Rahman, Tsuyoshi & Rahman, 2002*).

We used fertilization success as an indicator of sperm quality (*Duan et al., 2018b*). We used a pipette to collect 100 μL dried sperm in 10-mL filtered seawater, which was mixed quickly, then, the eggs released after 30 min were added and the beaker was gently shaken to fully mix the sperm and eggs. The fertilized eggs completely sank to the bottom of the beaker, then the shaking stopped. The seawater above the fertilized eggs was removed, then clean filtered seawater was injected, the fertilized eggs were washed, the process was repeated three times. After 15 min of fertilization, three duplicate samples (1 mL) were taken, few drops of 40% formaldehyde solution were added, and then, the fertilized eggs were observed using a microscope. Bulging of the fertilization membrane indicates successful fertilization (observed in at least 100 fertilized eggs per sample).

Larval cultures

Fertilized eggs were washed thrice to remove excess sperm. And then, they were cultured in buckets (diameter = 12 cm, height = 12 cm) with clean seawater and oxygen pumps, the embryos density was 200 ind/mL at 16 ± 0.5 °C. After 30 h, adjust the hatching density
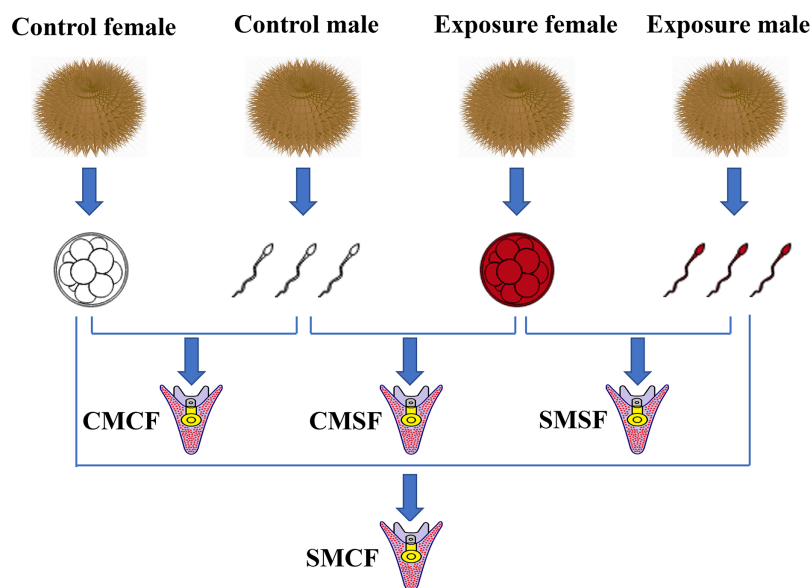
Figure 1 Schematic set-up of the experimental design. Sperm and eggs indicate that the gametes from either the control sea urchins or HFO exposure sea urchins. For the experimental design, there are four types of offspring: CMCF refers to control male crossed with control female, CMSF refers to control male crossed with exposed female, SMSF refers to exposed male crossed with exposed female, SMCF refers to exposed male crossed with control female. Full-size ⊡ DOI: 10.7717/peerj.13298/fig-1

of fertilized eggs to 0.3–0.5 ind/mL. Subsequently, the larvae were fed microalgae (*Chaetoceros gracilis*) three times a day. The seawater (salinity 34 parts per thousand) was replaced two third each day using a fine silk net (*Ding et al., 2020*; *Zhao et al., 2018*). The embryos were produced with four types of parental crosses: CMCF (control male + control female), SMCF (exposed male + control female), CMSF (control male + exposed female), SMSF (exposed male + exposed female) (Fig. 1).

Biological analysis

ROS production of sea urchin gonadal tissue was determined using a 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) method (*LeBel, Ischiropoulos & Bondy, 1992*) with the reactive oxygen species assay kit (Nanjing Jian Cheng Bioengineering Institute, Nanjing, China) according to the manufacturer's described. Briefly, fresh sample (50 mg) was washed twice with 100 nM PBS buffer and then homogenized using homogenate instrument. The sample was centrifuged at $500g$ for 20 min and collected the precipitate. Then, the precipitate was mixed with 100 μL PBS and 50 μL DCFH-DA (1 mM) and incubated at 37 °C for 60 min. The fluorescence intensity was detected with excitation at 485 nm and emission at 525 nm using a Fluorescence spectrophotometer (Cary Eclipse; Agilent, Santa Clara, CA, USA). The ROS level was calculated in arbitrary units per mg protein (AU/mg protein).

3-NTP is an indicator of nitrosative stress and RNS production (*Castellano et al., 2018*). 3-NTP content in sea urchin gonadal tissues were measured using 3-Nitrotyrosine Elisa kit (Shanghai Yun Duo Biology, Shanghai, China). Brief, sample (50 mg) homogenate supernatants were mixed with standard and detection antibody horseradish peroxidase

(HRP), and then washed thoroughly after incubation. The sample was developed with the substrate tetramethylbenzidine (TMB) and converted to yellow under the action of acid. The microplate reader ultraviolet spectrometry was used to measure the absorbance at 450 nm and calculate the sample concentration (nmol/L).

We used a protein carbonyl assay kit (Nanjing Jian Cheng Bioengineering Institute, Nanjing, China) to determine the total content of 2,4-Dinitrophenylhydrazine (DNPH) in the supernatant of sample (50 mg) homogenate (*Reznick & Packer, 1994*). The absorbance was measured at 370 nm using an ultraviolet spectrophotometer and calculated protein carbonyl content (nmol/mg protein).

Gene expression of *hsp*70

Brief, the method of gene transcription was slightly modified based on *Li et al. (2019)*. Total RNA was extracted from gonadal tissues and gametes and purified using the MiniBest Universal RNA Extraction Kit (Takara, Tokyo, Japan) according to the instructions. All samples were measured with an ultraviolet spectrophotometer to detect A_{260}/A_{280} values, and the values of all samples were between 1.85 and 2.0, with an average RNA concentration of 721.6 ± 203.7 ng/μL. Total RNA integrity was evaluated using an agarose gel. cDNA synthesis of the sample was conducted using the PrimeScript™ RT reagent Kit with gDNA Eraser (Takara, Tokyo, Japan) and using MyGene™L Series Peltier Thermal cycler (LongGene, Hangzhou, China) at 40 °C for 15 min, and 80 °C for 5 s. Then, the LightCycler® Real-Time PCR System (Roche Diagnostics, Mannheim, Germany) was used to detect the expression levels of *hsp*70 and 18S (the reference gene) genes in the gonadal tissues and gametes, and the LightCycler® 96 Ver 1.1.0 Software was used for analysis. The conditions for a qPCR were: preincubation for 10 min at 95 °C, 3-step amplification (a denaturation step for 10 s at 95 °C, a hybridization step for 60 s at 60 °C, with an annealing step for 10 s at 72 °C) followed by 40 cycles. The $2^{-\Delta\Delta Ct}$ method was used to calculate the expression of *hsp*70 (*Livak & Schmittgen, 2001*). Primer sequences used in the study were listed in Table S1 (referred to *Zhao et al., 2014*).

Larval size

After 5 d post fertilization (5 dpf), we used a microscope (IX73; Olympus, Tokyo, Japan) to measure the growth-related traits of sea urchins (50 larvae for each group) (*Zhao et al., 2018*) (Fig. 2).

Statistics analysis

The results were expressed as the mean ± standard deviation (SD) of the mean. One-way analysis of variance (ANOVA) followed by Tukey's test were used for fecundity, *hsp*70 expression and larvae size. Spawning, ROS, 3-NTP and PC levels were analyzed by two-way ANOVA with sexes and treatments as fixed factors. It was considered statistically significant when $p < 0.05$. All results were analyzed using Sigma Plot 12.5 computer software (Systat Software, San Jose, CA, USA).
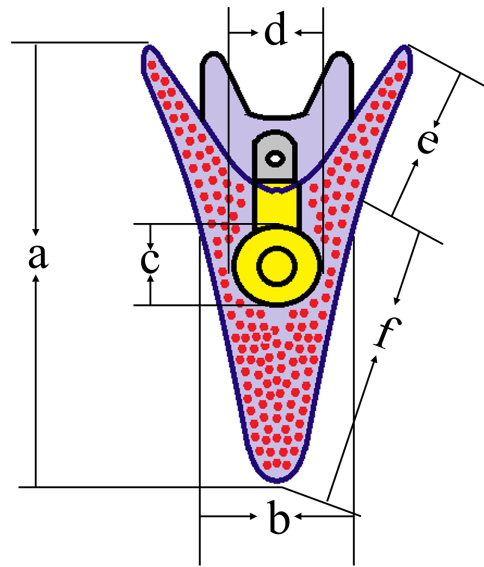
Figure 2 Conceptual diagrams showing 5 dpf larval size measurements. Lowercase letters a, b, c, d, e, f indicates larval length, larval width, stomach length, stomach width, post-oral arm length and body rod length, respectively. The size of normal larvae was measured as 446.88 ± 37.67 μm (a), 211.02 ± 34.99 μm (b), 78.36 ± 11.43 μm (c), 72.71 ± 8.45 μm (d), 94.65 ± 15.55 μm (e) and 352.23 ± 41.04 μm (f), respectively. Full-size ⬜ DOI: 10.7717/peerj.13298/fig-2
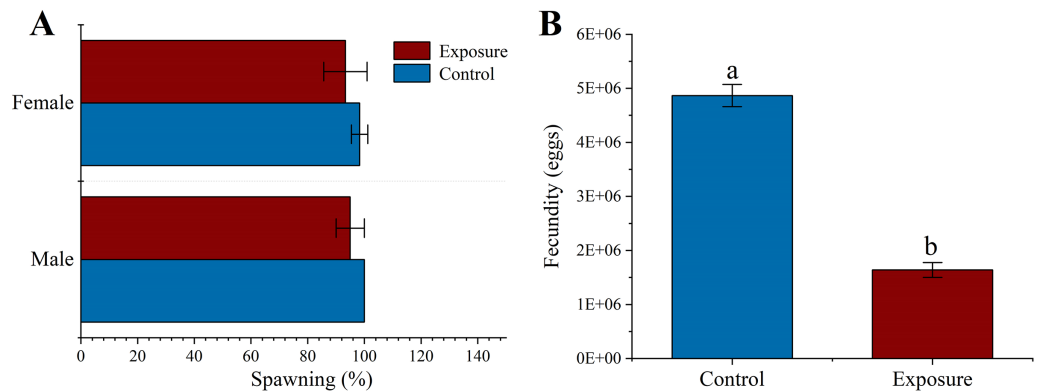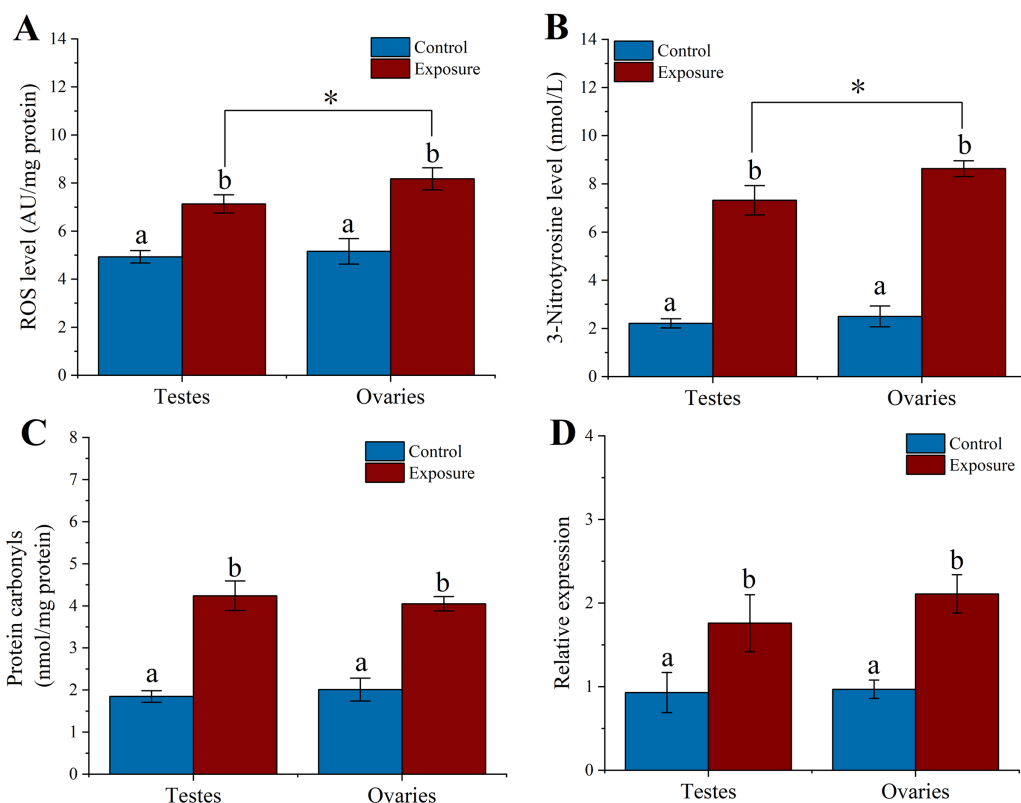

Figure 3 Effects of 21 d HFO exposure on gametes spawning (A) and fecundity (B) (mean ± SD). Different lowercase letters mean significant differences between treatments ($p < 0.05$). Full-size ⬜ DOI: 10.7717/peerj.13298/fig-3

RESULTS

Effect of HFO on spawning, egg production and fertilization

There was no significant difference in spawning at the respective HFO concentration between different exposure treatments than control ($f = 3$, $p = 0.158$ and $f = 0.125$, $p = 0.349$ for male and female, respectively) or sexual groups ($f = 0.1$, $p = 0.768$ for exposure sexes) (Fig. 3A). Female sea urchins exposed to HFO had a significant decreased egg production ($1.64 \pm 0.14 \times 10^7$ eggs) compared to control ($4.87 \pm 0.21 \times 10^7$ eggs) ($f = 507.06$, $t = 22.518$, $p < 0.001$) (Fig. 3B). We observed the fertilization rate of each

Figure 4 Effect of 21 d HFO exposure on ROS level (A), 3-Nitrotyrosine level (B), protein carbonyls level (C) and HSP70 expression (D) in sea urchin gonadal tissues (mean ± SD). Different lowercase letters mean significant differences between treatments ($p < 0.05$). An asterisk (*) indicates significant differences between testes and ovaries ($p < 0.05$). Full-size ◪ DOI: 10.7717/peerj.13298/fig-4

treatment, and the results showed that there was no significant difference between HFO treatment and control, and average fertilization rate of all treatments was 97.51 ± 1.73% ($f = 0.43$, $p = 0.548$).

Effect of HFO on parental sea urchin

To elucidate the excessive ROS production in sea urchin gonadal tissues caused by HFO, we analyzed the ROS level in sea urchin gonadal tissue after 21 d HFO exposure. Sea urchins exposed to HFO showed increases in ROS level in testes and ovaries, respectively. ROS level of testes and ovaries were significantly increased around 1.4- and 1.6-fold than control ($f = 69.551$, $t = 8.34$, $p = 0.001$ and $f = 46.306$, $t = 6.805$, $p = 0.002$, respectively) when sea urchin exposed to HFO. The ROS level of ovaries (8.18 ± 0.46 AU/mg protein) in sea urchin was significantly increased than testes (7.13 ± 0.31 AU/mg protein) after exposed to HFO ($f = 10.86$, $t = 3.295$, $p = 0.03$) (Fig. 4A).

3-Nitrotyrosine is considered as an indicator of apoptosis, cell damage, disease, and RNS production in aquatic organism (*Curtis et al., 2011*; *Johnstone et al., 2019*). Following 21 d of treatment, the level of 3-NTP in testes and ovaries of sea urchin was significantly increased by approximately 3.3- and 3.5-fold compared to control ($f = 192.282$,

$t = 13.867$, $p < 0.001$ and $f = 393.338$, $t = 19.833$, $p < 0.001$, respectively) (Fig. 4B). The 3-NTP level of ovaries (8.63 ± 0.33 nmol/L) were significantly increased compared to testes (7.32 ± 0.61 nmol/L) ($f = 10.655$, $t = 3.264$, $p = 0.031$).

PC level were measured in gonadal tissues from two treatments. After 21 d treatment, we found that the PC level significantly upregulated ~2.3-fold in the testes (4.24 ± 0.35 nmol/mg protein) of sea urchins exposed to HFO compared to control (1.84 ± 0.14 nmol/mg protein) ($f = 125.612$, $t = 11.208$, $p < 0.001$). Meanwhile, PC level in the ovaries (4.05 ± 0.17 nmol/mg protein) showed significantly higher than control (2.01 ± 0.27 nmol/mg protein) ($f = 126.807$, $t = 11.261$, $p < 0.001$). However, there was no significant between testes and ovaries ($p = 0.975$) (Fig. 4C).

Heat shock protein has the function of preventing protein denaturation and recovering deformed protein. When activating the HSP genes, organisms can produce a class of specific proteins that repair and protect cells by rapidly regulating the cell defense system against oxidative stress (*Wood, Brown & Youson, 1999*). Sea urchins exposed to HFO showed an increase in *hsp*70 expression in testes and ovaries in male and female sea urchin, respectively. *hsp*70 levels in testes was significantly increased about 1.89-fold compared to control ($f = 11.723$, $t = 3.424$, $p = 0.028$). *hsp*70 levels in ovaries were also significantly increased 2.18-fold when female sea urchins exposed to HFO ($f = 58.852$, $t = 7.672$, $p = 0.002$) (Fig. 4D).

Effects of HFO on gametes

We examined the level of ROS, 3-NTP, PC and *hsp*70 expression in gametes after sea urchins exposed to HFO for 21 d. We found that sperm (5.32 ± 0.23 AU/mg protein) and eggs (6.67 ± 0.57 AU/mg protein) released from exposure sea urchins showed significantly higher levels of ROS compared to control (control sperm: 4.06 ± 0.33 AU/mg protein, control egg: 4.58 ± 0.24 AU/mg protein) ($f = 29.388$, $t = 5.421$, $p = 0.006$ and $f = 34.416$, $t = 5.866$, $p = 0.004$, respectively). Eggs experienced a significant higher in levels of ROS compared to sperm following parental exposure ($f = 14.35$, $t = 3.788$, $p = 0.019$) (Fig. 5A).

Following 21 d treatment, the 3-NTP level in gametes (sperm: 5.29 ± 0.16 nmol/L, eggs: 7.88 ± 0.39 nmol/L) were significantly increased than control (control sperm: 3.84 ± 0.32 nmol/L, control egg: 4.46 ± 0.55 nmol/L) ($f = 49.692$, $t = 7.049$, $p = 0.002$ and $f = 93.487$, $t = 9.669$, $p < 0.001$, respectively). In addition, the level of 3-NTP was significantly higher in egg than in sperm following parental exposure ($f = 116.075$, $t = 10.774$, $p < 0.001$) (Fig. 5B).

PC level significantly increased 1.8- and 1.5-fold in the sperm and eggs when sea urchin exposed to HFO compared to control ($f = 39.614$, $t = 6.294$, $p = 0.003$ and $f = 45.898$, $t = 6.775$, $p = 0.002$, respectively) (Fig. 5C). In addition, we found that sea urchin exposed to HFO showed significantly increased in *hsp*70 expression in sperm (1.4-fold) and eggs (1.73-fold) compared to control, respectively ($f = 9.975$, $t = 3.158$, $p = 0.034$ and $f = 21.025$, $t = 4.585$, $p = 0.01$, respectively) (Fig. 5D).
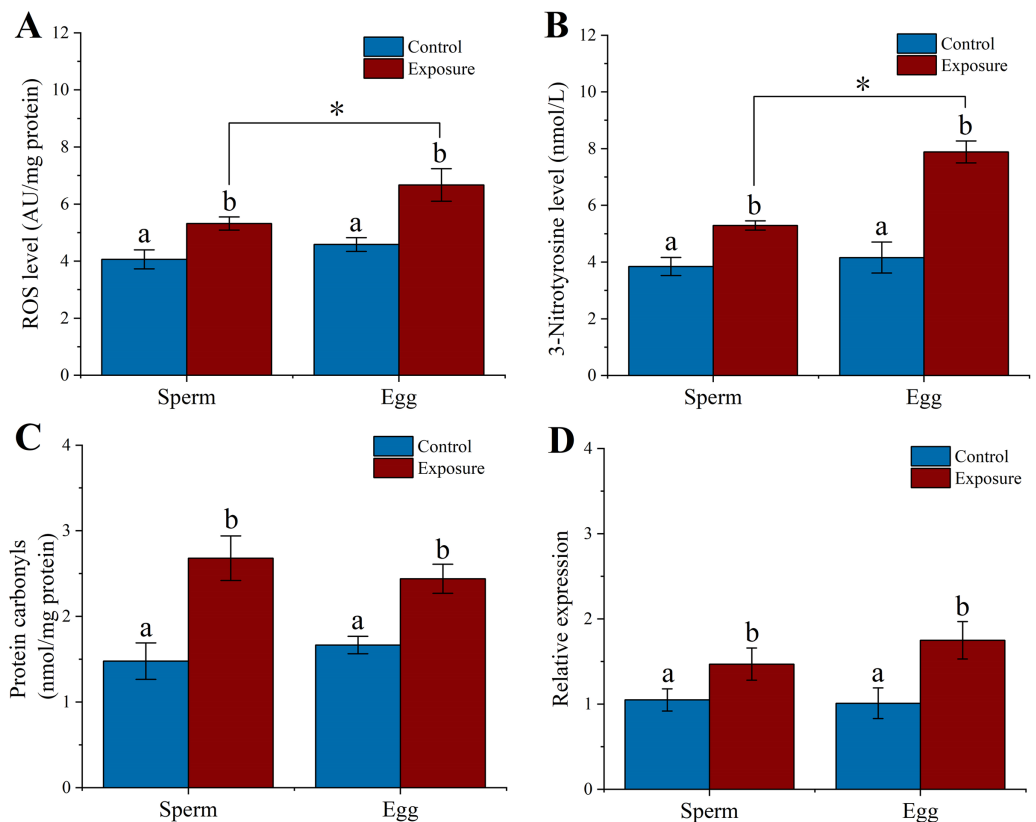
Figure 5 Effects of 21 d HFO exposure on ROS level (A), 3-Nitrotyrosine level (B), protein carbonyls level (C) and HSP70 expression (D) in sea urchin gametes (mean ± SD). Different lowercase letters mean significant differences between treatments ($p < 0.05$). An asterisk (*) indicates significant differences between sperm and eggs ($p < 0.05$). Full-size 🖼 DOI: 10.7717/peerj.13298/fig-5

Larval size on 5 dpf

Parental sea urchin exposed to HFO have significantly negative effect on all larval size. SMCF were significantly longer in larval length, post oral arm length and body rod length compared to CMSF ($f = 94.28$, $t = 9.71$, $p < 0.001$ for larval length, $f = 57.535$, $t = 7.585$, $p < 0.001$ for body rod length and $f = 54.443$, $t = 7.379$, $p < 0.001$ for post oral arm length, respectively), but not for larval width ($f = 1.238$, $p = 0.267$), stomach length ($f = 2.245$, $p = 0.135$) and stomach width ($f = 0.861$, $p = 0.354$). In addition, SMCF were significantly higher in larval length ($f = 318.792$, $t = 17.855$, $p < 0.001$), stomach length ($f = 24.34$, $t = 4.934$, $p < 0.001$), stomach width ($f = 49.14$, $t = 7.01$, $p < 0.001$), post-oral arm length ($f = 168.607$, $t = 12.985$, $p < 0.001$) and body rod length ($f = 214.405$, $t = 14.643$, $p < 0.001$) than SMSF, not for larval width ($f = 2.326$, $p = 0.128$). Larval length ($f = 52.482$, $t = 7.244$, $p < 0.001$), stomach length ($f = 29.09$, $t = 5.394$, $p < 0.001$), stomach width ($f = 84.836$, $t = 9.211$, $p < 0.001$) and body rod length ($f = 36.172$, $t = 7.244$, $p < 0.001$) showed smaller size in SMSF than CMSF. However, the larval width ($f = 0.306$, $p = 0.58$) and post-oral arm length ($f = 0.835$, $p = 0.125$) of SMSF were not significantly different from those of CMSF (Fig. 6).
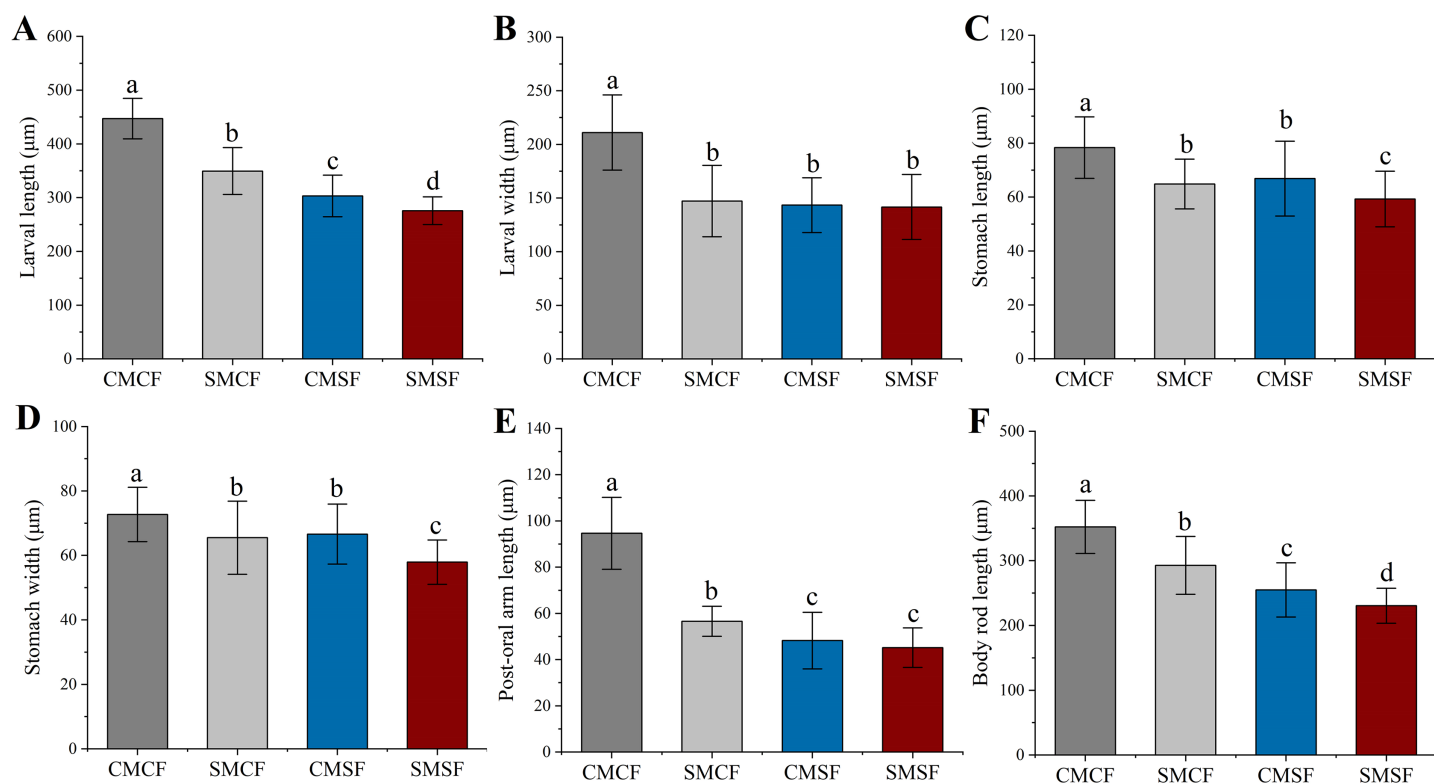
Figure 6 (A–F) Larval size of sea urchin on 5 dpf, whose parent exposed to long-term HFO or not (mean ± SD). The embryos were produced with four types of parental crosses: CMCF (control male + control female), SMCF (exposed male + control female), CMSF (control male + exposed female) and SMSF (exposed male + exposed female). Lowercase letters above the bars indicated significant difference among experimental groups ($p < 0.05$). Full-size 🖼 DOI: 10.7717/peerj.13298/fig-6

DISCUSSION

The results of this study clearly suggested that HFO exposure reduced the reproductive function of female sea urchins. In the study, the TPH concentration were decreasing from 811.22 to 474.64 µg/L, and PAHs concentrations decreased from 6.04 to 1.83 µg/L, within the concentration range after oil spill in the marine environment (*Kim et al., 2010*; *Boehm, Neff & Page, 2007*). The PAH distributions in HFO exposure solution was mainly phenanthrene and naphthalene. Egg production in female sea urchin showed a negative impact with HFO exposure, we found that the egg production in female sea urchins exposed to HFO decreased by 2.3-fold. A previous study showed that single PAHs exposure could prevented proper oogenesis and reduced fertility of sea urchin (*Schaefer & Koehler, 2009*). Organisms exposed to environmental stress exhibit increased degradation of ovarian follicles; a phenomenon referred to as follicular atresia (*Santos et al., 2008*). These data indicated that HFO exposure might inhibit egg formation in female sea urchins. In addition, we found that HFO has no effect on the spawning and fertilization of sea urchins, and the fertilization capacity after exposure could still reach more than 90%. However, a previous study found that the swimming speed of sea urchin sperm decreased after 8 weeks of exposure to oil production effluent (*Krause, 1994*). In this study, measurements limited to fertilization cannot clarify the harmful effects of HFO

on the sperm quality of male sea urchins, which requires further investigation. The gonadal function would also be negatively affected by oil-derived hydrocarbons pollution in other marine invertebrates. For instance, the HFO contamination negatively affects the gonadal function of mussels (*Bellas et al., 2008*). *Yang et al. (2021)* found that scallop (*Chlamys farreri*) exposed to PAH reduced fertility. These results support that the HFO exposure decreases the gonadal function of sea urchins and other marine invertebrates.

ROS levels increase sharply in organisms under environmental stress, leading to oxidative stress, which in turn result in severe damage to their health and survival (*Wang et al., 2019*; *Zhang et al., 2017*). In addition, RNS are nitric oxide (NO) derivatives that contain highly oxidative free radicals and nitro compounds, which may lead to an increase in oxidative stress and apoptosis, thereby severely affecting the physiological state of organisms (*Ahsan, 2013*; *Folkerts et al., 2001*). NO reactions involve nitrification and nitrosation processes as well as RNS formation (*Patel et al., 1999*). Oil-derived hydrocarbons have been observed to induce ROS and RNS overproduction and oxidative damage in various marine vertebrate and invertebrate species. For example, *Hong et al. (2016)* found that populations of live Manila clam in oil spill damaged areas displayed high levels of ROS and RNS, that caused DNA damage. *Donaghy et al. (2016)* observed that mussels exposed to oil-derived hydrocarbons in seawater for 2 years after the 2007 *Hebei Spirit* oil spill had elevated levels of oxidative stress, and an altered energetic metabolism. In this study, we found that sea urchins exposed to stranded HFO showed higher levels of ROS, 3-NTP, and PC content in the gonadal tissue and gametes. This result indicated that the sea urchin gonadal tissues and gametes accumulated hydrocarbons-derived contaminants, leading to the overproduction of ROS and RNS and the oxidative damages, which may negatively impact sea urchin health.

Marine organisms should respond to the rapid changes in the aquatic environment and resist the environmental pollutants to maintain homeostasis for survival (*Rivera Ingraham & Lignot, 2017*). Heat shock proteins (HSP) are functional proteins in organisms that respond to the changes in the environment (*Javid, MacAry & Lehner, 2007*). *hsp*70 is an important member of the HSP family (*Whitley, Goldberg & Jordan, 1999*). The high expression level of *hsp*70 can protect and repair cells by rapidly adjusting the defense system, which assists in repairing protein damage under stress conditions (*Clark & Peck, 2009*). As a molecular chaperone, *hsp*70 can impede apoptosis by blocking the release of cytochrome C, Apaf-1 oligomerization, and caspase-8 activation. In addition, *hsp*70 has been recognized as a biomarker of environmental stress in adult sea urchin (*Johnstone et al., 2019*; *Roccheri et al., 2004*). *hsp*70 also excelled in reducing apoptosis and oxidative stress (*Padmini & Tharani, 2014*). In the stress response, *hsp*70 can regulate the cell defense system to resist stress and protect cells (*Johnstone et al., 2019*; *Köhler et al., 1992*). Our study showed that male and female sea urchins exposed to HFO experienced an increase in *hsp*70 expression in gonad and gametes. *Madison, Hodson & Langlois (2015)* observed that TPH of diluted bitumen (0.95 μg/L) significantly increase *hsp*70 expression in medaka embryos. *Cruz-Rodriguez & Chu (2002)* also found that suspended clay particles spiked with PAHs (400 μg/L) disrupted the metabolism of marine bivalve oysters, resulting in a significant increase in *hsp*70 level. These results suggested that HFO

exposure increase the pressure on sea urchins, triggering more *hsp*70 expression to prevent contamination damage. Thus, *hsp*70 expression can serve as a biomarker for sea urchin exposure to different kind of pollutants, including HFO.

Despite the important gender confounding factors, few studies distinguished oil-related pollution sexual effects on sea urchins, and we found sex-specific differences in ROS and 3-NTP. We previously found higher polyunsaturated fatty acid contents in ovaries than in testes of sea urchins (*Duan et al., 2018a*). *Kozhina, Terekhova & Svetashev (1978)* found that eggs have a significantly higher level of polyunsaturated fatty acids contents than sperm. Our findings showed that ovaries and eggs had greater ROS and 3-NTP levels than testes and sperm after HFO exposure, respectively. Probably, in the presence of HFO derivatives, the higher energetic request for egg production in ovaries produces a high level of ROS and RNS with a higher oxidative potential for the oxidation targets such as polyunsaturated fatty acids, with possible cascade effect due to their modified structure.

Polyunsaturated fatty acids are substrates for ROS, RNS attack and production of lipid peroxidation (*Lushchak, 2021*). These results indicated that HFO exposure increased ROS and 3-NTP content in gonads and accelerated oxidative stress production. These processes resulted in gonadal oxidative damage and the transfer of hazardous sub-stances from parental individuals to their gametes, which may harm the growth and development of sea urchin offspring.

Phenotype of offspring may reflect that the petroleum hydrocarbon pollution influences the phenotypic plasticity and evolutionary adaptation of marine invertebrates. When parental sea urchins were exposed to stranded HFO for 21 d, the larval length of offspring whose parents were exposed to HFO were significantly shorter, and paternal exposure larval length were significantly higher than maternal and parental exposure. This may be due to paternal sea urchin exposed to PAHs had less oxidative damage on their early pre-feeding stages embryos (*Lister, Lamare & Burritt, 2017*). Our study revealed that the exposure of both parents to HFO resulted in a shorter larval stomach length and width. These results indicated a negative effect of HFO exposure on the stomach size of sea urchins. Larval post-oral arm and body rod lengths are related to the feeding ability and health of the sea urchins (*Adams et al., 2011*). After HFO exposure, the motor behavior of sea urchin larvae may be reduced, resulting in a weakening of the larval feeding ability. The energy obtained through feeding is distributed to cellular repair, reducing the energy supply for larval growth and development, thereby exhibiting degradation or delayed development of the post-oral arm and body rod (*Zhao et al., 2016*). Here, we found that the maternal exposure of sea urchins to HFO results in more harmful effects on the development of offspring.

CONCLUSIONS

Oil spill is a great threat to marine invertebrates in shallow waters. Sea urchins live near the coast and are susceptible to contact with oil pollution due to their slow movements. In this study, we found that sea urchin exposed to HFO had a significant negative impact on the gonadal functions and gamete health, providing evidence for the parent-to-gametes transfer of stranded HFO toxic substances. The HFO exposure transmission effects are

complex, and sea urchins exposed to HFO affect larvae size generationally. Paternal and maternal effects were highly trait dependent. We found that maternal individuals may play essential roles in the negatively effects of HFO exposure on larvae size. The results confirmed that female and male exposure to HFO can cause negative effects on their offspring which in turn affects sea urchin population maintenance and recruitment. This study could enrich our understanding of the persistent effects on the offspring of HFO exposure on sea urchins. The results of this study can provide relevant information for marine ecological risk assessment after oil spill accidents.

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ADDITIONAL INFORMATION AND DECLARATIONS

Competing Interests
The authors declare that they have no competing interests.

Author Contributions
- Xuanbo Wang conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Xishan Li conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Deqi Xiong conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Hang Ren performed the experiments, prepared figures and/or tables, and approved the final draft.
- Huishu Chen performed the experiments, prepared figures and/or tables, and approved the final draft.
- Zhonglei Ju performed the experiments, prepared figures and/or tables, and approved the final draft.

Data Availability

The following information was supplied regarding data availability:

The raw data are available in the Supplemental Files.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.13298#supplemental-information.

REFERENCES

Adams DK, Sewell MA, Angerer RC, Angerer LM. 2011. Rapid adaptation to food availability by a dopamine mediated morphogenetic response. *Nature Communications* **2(1)**:592 DOI 10.1038/ncomms1603.

Ahsan H. 2013. 3-Nitrotyrosine: a biomarker of nitrogen free radical species modified proteins in systemic autoimmunogenic conditions. *Human Immunology* **74(10)**:1392–1399 DOI 10.1016/j.humimm.2013.06.009.

Ansell DV, Dicks B, Guenette CC, Moller TH, Santner RS, White IC. 2001. A review of the problems posed by spills of heavy fuel oils. *International Oil Spill Conference Proceedings* **2001(1)**:591–596 DOI 10.7901/2169-3358-2001-1-591.

Axiak V, Saliba LJ. 1981. Effects of surface and sunken crude oil on the behaviour of a sea urchin. *Marine Pollution Bulletin* **12(1)**:14–19 DOI 10.1016/0025-326X(81)90134-X.

Bautista NM, Crespel A, Crossley J, Padilla P, Burggren W. 2021. Parental transgenerational epigenetic inheritance related to dietary crude oil exposure in danio rerio. *The Journal of Experimental Biology* **18**:97 DOI 10.1242/jeb.222224.

Bellas J, Saco-Alvarez L, Nieto O, Beiras R. 2008. Ecotoxicological evaluation of polycyclic aromatic hydrocarbons using marine invertebrate embryo-larval bioassays. *Marine Pollution Bulletin* **57(6–12)**:493–502 DOI 10.1016/j.marpolbul.2008.02.039.

Bellas J, Rial D, Valdes J, Vidal-Linan L, Bertucci JI, Muniategui S, Leon VM, Campillo JA. 2022. Linking biochemical and individual-level effects of chlorpyrifos, triphenyl phosphate, and bisphenol A on sea urchin (*Paracentrotus lividus*) larvae. *Environment Science and Pollution Research* **269(4)**:126 DOI 10.1007/s11356-022-19099-w.

Bielmyer GK, Brix KV, Capo TR, Grosell M. 2005. The effects of metals on embryo-larval and adult life stages of the sea urchin, *Diadema antillarum*. *Aquatic Toxicology* **74(3)**:254–263 DOI 10.1016/j.aquatox.2005.05.016.

Boehm PD, Neff JM, Page DS. 2007. Assessment of polycyclic aromatic hydrocarbon exposure in the waters of Prince William sound after the Exxon Valdez oil spill: 1989–2005. *Marine Pollution Bulletin* **54(3)**:339–356 DOI 10.1016/j.marpolbul.2006.11.025.

Byrne M. 2012. Global change ecotoxicology: identification of early life history bottlenecks in marine invertebrates, variable species responses and variable experimental approaches. *Marine Environ Research* **76**:3–15 DOI 10.1016/j.marenvres.2011.10.004.

Carls MG, Thomas RE, Lilly MR, Rice SD. 2003. Mechanism for transport of oil-contaminated groundwater into pink salmon redds. *Marine Ecology Progress Series* **248**:245–255 DOI 10.3354/meps248245.

Castellano I, Migliaccio O, Ferraro G, Maffioli E, Marasco D, Merlino A, Zingone A, Tedeschi G, Palumbo A. 2018. Biotic and environmental stress induces nitration and changes in structure and function of the sea urchin major yolk protein toposome. *Scientific Reports* **8(1)**:1–11 DOI 10.1038/s41598-018-22861-1.

Chen X, Stillman JH. 2012. Multigenerational analysis of temperature and salinity variability affects on metabolic rate, generation time, and acute thermal and salinity tolerance in daphnia pulex. *Journal of Thermal Biology* **37(3)**:185–194 DOI 10.1016/j.jtherbio.2011.12.010.

Chiarelli R, Agnello M, Bosco L, Roccheri MC. 2014. Sea urchin embryos exposed to cadmium as an experimental model for studying the relationship between autophagy and apoptosis. *Marine Environmental Research* **93**:47–55 DOI 10.1016/j.marenvres.2013.06.001.

Chiarelli R, Martino C, Roccheri MC. 2019. Cadmium stress effects indicating marine pollution in different species of sea urchin employed as environmental bioindicators. *Cell Stress and Chaperones* **24(4)**:675–687 DOI 10.1007/s12192-019-01010-1.

Clark M, Peck L. 2009. HSP70 heat shock proteins and environmental stress in Antarctic marine organisms. *Marine Genomics* **2(1)**:11–18 DOI 10.1016/j.margen.2009.03.003.

Cruz-Rodriguez LA, Chu FLE. 2002. Heat-shock protein (HSP70) response in the eastern oyster, crassostrea virginica, exposed to PAHs sorbed to suspended artificial clay particles and to suspended field contaminated sediments. *Aquatic Toxicology* **1060(3–4)**:157–168 DOI 10.1016/S0166-445X(02)00008-5.

Curtis L, Garzon C, Arkoosh M, Collier T, Myers M, Buzitis J, Hahn M. 2011. Reduced cytochrome P4501A activity and recovery from oxidative stress during subchronic benzo[a]pyrene and benzo[e]pyrene treatment of rainbow trout. *Toxicology and Applied Pharmacology* **254(1)**:1–7 DOI 10.1016/j.taap.2011.04.015.

Di Meo S, Reed T, Venditti P, Victor V. 2016. Role of ROS and RNS sources in physiological and pathological conditions. *Oxidative Medicine and Cellular Longevity* **2016**:1245049 DOI 10.1155/2016/1245049.

Ding J, Zheng D, Sun J, Hu F, Yu Y, Zhao C, Chang Y. 2020. Effects of water temperature on survival, behaviors and growth of the sea urchin *Mesocentrotus nudus*: new insights into the stock enhancement. *Aquaculture* **519(16)**:734873 DOI 10.1016/j.aquaculture.2019.734873.

Donaghy L, Hong HK, Kim M, Park HS, Choi KS. 2016. Assessment of the fitness of the mussel *Mytilus galloprovincialis* two years after the Hebei spirit oil spill. *Marine Pollution Bulletin* **113(1–2)**:324–331 DOI 10.1016/j.marpolbul.2016.10.007.

Dröge W. 2002. Free radicals in the physiological control of cell function. *Physiological Reviews* **82(1)**:47–95 DOI 10.1152/physrev.00018.2001.

Duan M, Xiong D, Bai X, Gao Y, Xiong Y, Gao X, Ding G. 2018a. Transgenerational effects of heavy fuel oil on the sea urchin *Strongylocentrotus intermedius* considering oxidative stress biomarkers. *Marine Environmental Research* **141**:138–147 DOI 10.1016/j.marenvres.2018.08.010.

Duan M, Xiong D, Yang M, Xiong Y, Ding G. 2018b. Parental exposure to heavy fuel oil induces developmental toxicity in offspring of the sea urchin *Strongylocentrotus intermedius*. *Ecotoxicology and Environmental Safety* **159**:109–119 DOI 10.1016/j.ecoenv.2018.04.067.

Folkerts G, Kloek J, Muijsers R, Nijkamp P. 2001. Reactive nitrogen and oxygen species in airway inflammation. *European Journal of Pharmacology* **429(1–3)**:251–262 DOI 10.1016/S0014-2999(01)01324-3.

Hong HK, Donaghy L, Kang CK, Kang HS, Lee HJ, Park HS, Choi KS. 2016. Substantial changes in hemocyte parameters of manila clam *Ruditapes philippinarum* two years after the *Hebei Spirit* oil spill off the west coast of Korea. *Marine Pollution Bulletin* **108(1–2)**:171–179 DOI 10.1016/j.marpolbul.2016.04.033.

Jasperse L, Levin M, Rogers K, Perkins C, Bosker T, Griffitt RJ, Sepulveda MS, De Guise S. 2019. Parental exposure to deepwater horizon oil in different environmental scenarios alters

development of sheepshead minnow (*Cyprinodon variegatus*) offspring. *Marine Environmental Research* **150**:104762 DOI 10.1016/j.marenvres.2019.104762.

Javid B, MacAry PA, Lehner PJ. 2007. Structure and function: heat shock proteins and adaptive immunity. *Journal of Immunology* **179(4)**:2035–2040 DOI 10.4049/jimmunol.179.4.2035.

Johnstone J, Nash S, Hernandez E, Rahman MS. 2019. Effects of elevated temperature on gonadal functions, cellular apoptosis, and oxidative stress in Atlantic sea urchin *Arbacia punculata*. *Marine Environmental Research* **149(2007)**:40–49 DOI 10.1016/j.marenvres.2019.05.017.

Katsumiti A, Nicolussi G, Bilbao D, Prieto A, Etxebarria N, Cajaraville MP. 2019. In vitro toxicity testing in hemocytes of the marine mussel *Mytilus galloprovincialis* (L) to uncover mechanisms of action of the water accommodated fraction (WAF) of a naphthenic North Sea crude oil without and with dispersant. *Science of The Total Environment* **670**:1084–1094 DOI 10.1016/j.scitotenv.2019.03.187.

Kim M, Yim UH, Hong SH, Jung JH, Choi HW, An J, Won J, Shim WJ. 2010. Hebei spirit oil spill monitored on site by fluorometric detection of residual oil in coastal waters off Taean, Korea. *Marine Pollution Bulletin* **60(3)**:383–389 DOI 10.1016/j.marpolbul.2009.10.015.

Köhler HR, Triebskorn R, Stöcker W, Kloetzel P-M, Alberti G. 1992. The 70 kD heat shock protein (hsp 70) in soil invertebrates: a possible tool for monitoring environmental toxicants. *Archives of Environmental Contamination and Toxicology* **22(3)**:334–338 DOI 10.1007/BF00212095.

Kozhina VP, Terekhova TA, Svetashev VI. 1978. Lipid composition of gametes and embryos of the sea urchin *Strongylocentrotus intermedius* at early stages of development. *Developmental Biology* **62(2)**:512–517 DOI 10.1016/0012-1606(78)90232-4.

Krause PR. 1994. Effects of an oil production effluent on gametogenesis and gamete performance in the purple sea urchin (*Strongyloncentrotus purpuratus stimpson*). *Environmental Toxicology and Chemistry* **13(7)**:1153–1161 DOI 10.1002/etc.5620130717.

Laurel BJ, Copeman LA, Iseri P, Spencer ML, Hutchinson G, Nordtug T, Donald CE, Meier S, Allan SE, Boyd DT, Ylitalo GM, Cameron JR, French BL, Linbo TL, Scholz NL, Incardona JP. 2019. Embryonic crude oil exposure impairs growth and lipid allocation in a *Keystone Arctic Forage* fish. *IScience* **19**:1101–1113 DOI 10.1016/j.isci.201908.051.

LeBel CP, Ischiropoulos H, Bondy SC. 1992. Evaluation of the probe 2′,7′-dichlorofluorescin as an indicator of reactive oxygen species formation and oxidative stress. *Chemical Research in Toxicology* **5(2)**:227–231 DOI 10.1021/tx00026a012.

Li XS, Liao GX, Ju ZL, Wang CY, Li N, Xiong DQ, Zhang YL. 2020. Antioxidant response and oxidative stress in the respiratory tree of sea cucumber (*Apostichopus japonicus*) following exposure to crude oil and chemical dispersant. *Journal of Marine Science and Engineering* **8(8)**:547 DOI 10.3390/jmse8080547.

Li XS, Xiong Q, Ding GH, Fan YM, Ma XR, Wang CY, Xiong YJ, 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 DOI 10.1016/j.chemosphere.2019.06.199.

Li XS, Xiong DQ, Ju ZL, Xiong YJ, Ding GH, Liao GX. 2021. Phenotypic and transcriptomic consequences in zebrafish early-life stages following exposure to crude oil and chemical dispersant at sublethal concentrations. *Science of The Total Environment* **763**:143053 DOI 10.1016/j.scitotenv.2020.143053.

Lister KN, Lamare MD, Burritt DJ. 2017. Maternal antioxidant provisioning mitigates pollutant-induced oxidative damage in embryos of the temperate sea urchin *Evechinus chloroticus*. *Scientific Reports* **7(1)**:1954 DOI 10.1038/s41598-017-02077-5.

Lister KN, Lamare MD, Burritt DJ. 2016. Dietary pollutants induce oxidative stress, altering maternal antioxidant provisioning and reproductive output in the temperate sea urchin *Evechinus chloroticus*. *Aquatic Toxicology* **177**:106–115 DOI 10.1016/j.aquatox.2016.05.013.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR. *Methods* **25(4)**:402–408 DOI 10.1006/meth.2001.1262.

Lushchak VI. 2021. Interplay between bioenergetics and oxidative stress at normal brain aging: aging as a result of increasing disbalance in the system oxidative stress-energy provision. *Pflügers Archiv-European Journal of Physiology* **473(5)**:713–722 DOI 10.1007/s00424-021-02531-4.

Ma Y, Sun Y, Li Y, Zheng H, Mi W. 2020. Polycyclic aromatic hydrocarbons in benthos of the northern Bering sea shelf and Chukchi sea shelf. *Journal of Environmental Sciences* **97**:194–199 DOI 10.1016/j.jes.2020.04.021.

Madison B, Hodson PV, Langlois VS. 2015. Diluted bitumen causes deformities and molecular responses indicative of oxidative stress in Japanese medaka embryos. *Aquatic Toxicology* **165**:222–230 DOI 10.1016/j.aquatox.2015.06.006.

Manzo S, Schiavo S. 2022. Physical and chemical threats posed by micro(nano)plastic to sea urchins. *Science of The Total Environment* **808(4)**:152105 DOI 10.1016/j.scitotenv.2021.152105.

Martin JD, Adams J, Hollebone B, King T, Brown RS, Hodson PV. 2014. Chronic toxicity of heavy fuel oils to fish embryos using multiple exposure scenarios. *Environmental Toxicology and Chemistry* **33(3)**:677–687 DOI 10.1002/etc.2486.

Martino C, Chianese T, Chiarelli R, Roccheri MC, Scudiero R. 2022. Toxicological impact of rare earth elements (REEs) on the reproduction and development of aquatic organisms using sea urchins as biological model. *International Journal of Molecular Sciences* **23(5)**:2876 DOI 10.3390/ijms23052876.

Marty GD, Heintz RA, Hinton DE. 1997. Histology and teratology of pink salmon larvae near the time of emergence from gravel substrate in the laboratory. *Canadian Journal of Zoology-Revue* **75(6)**:978–988 DOI 10.1139/z97-119.

Naz S, lqbal MF, Mahmood I, Allam M. 2021. Marine oil spill detection using synthetic aperture radar over Indian ocean. *Marine Pollution Bulletin* **162**:111921 DOI 10.1016/j.marpolbul.2020.111921.

Padmini E, Tharani J. 2014. Heat-shock protein 70 modulates apoptosis signal-regulating kinase 1 in stressed hepatocytes of Mugil cephalus. *Fish Physiology and Biochemistry* **40(5)**:1573–1585 DOI 10.1007/s10695-014-9949-0.

Patel R, McAndrew J, Sellak H, White CR, Jo H, Freeman B, Darley-Usmar V. 1999. Biological aspects of reactive nitrogen species. *Biochimica Et Biophysica Acta* **1411(2–3)**:385–400 DOI 10.1016/S0005-2728(99)00028-6.

Pisano A, De Dominicis M, Biamino W, Bignami F, Gherardi S, Colao F, Coppini G, Marullo S, Sprovieri M, Trivero R, Zambianchi E, Santoleri R. 2016. An oceanographic survey for oil spill monitoring and model forecasting validation using remote sensing and in situ data in the Mediterranean Sea. *Deep-Sea Research Part II-Topical Studies In Oceanography* **133**:132–145 DOI 10.1016/j.dsr2.2016.02.013.

Rahman M, Tsuyoshi U, Rahman S. 2002. Effects of egg size on fertilization, fecundity and offspring performance: a comparative study between two sibling species of tropical sea urchins (*Genus Echinometra*). *Pakistan Journal of Biological Sciences* **5(1)**:114–121 DOI 10.3923/pjbs.2002.114.121.

Reznick AZ, Packer L. 1994. Oxidative damage to proteins: spectrophotometric method for carbonyl assay. *Methods in Enzymology* **233**:357–363 DOI 10.1016/s0076-6879(94)33041-7.

Rivera Ingraham G, Lignot J-H. 2017. Osmoregulation, bioenergetics and oxidative stress in coastal marine invertebrates: raising the questions for future research. *Journal of Experimental Biology* **220(10)**:1749–1760 DOI 10.1242/jeb.135624.

Roccheri M, Agnello M, Bonaventura R, Matranga V. 2004. Cadmium induces the expression of specific stress proteins in sea urchin embryos. *Biochemical and Biophysical Research Communications* **321(1)**:80–87 DOI 10.1016/j.bbrc.2004.06.108.

Santos HB, Thomé RG, Arantes FP, Sato Y, Bazzoli N, Rizzo E. 2008. Ovarian follicular atresia is mediated by heterophagy, autophagy, and apoptosis in *Prochilodus argenteus* and *Leporinus taeniatus* (Teleostei: Characiformes). *Theriogenology* **70(9)**:1449–1460 DOI 10.1016/j.theriogenology.2008.06.091.

Schaefer S, Koehler A. 2009. Gonadal lesions of female sea urchin (*Psammechinus miliaris*) after exposure to the polycyclic aromatic hydrocarbon phenanthrene. *Marine Environmental Research* **68(3)**:128–136 DOI 10.1016/j.marenvres.2009.05.001.

Vieweg I, Bender ML, Semenchuk PR, Hop H, Nahrgang J. 2022. Effects of chronic crude oil exposure on the fitness of polar cod (*Boreogadus saida*) through changes in growth, energy reserves and survival. *Marine Environmental Research* **174**:105545 DOI 10.1016/j.marenvres.2021.105545.

Wang HT, Li YY, Xia XH, Xiong XY. 2018. Relationship between metabolic enzyme activities and bioaccumulation kinetics of PAHs in zebrafish (Danio rerio). *Journal of Environmental Sciences* **65**:43–52 DOI 10.1016/j.jes.2017.03.037.

Wang JH, Xue XH, Liu Q, Zhang SZ, Peng ML, Zhou J, Chen LJ, Fang FG. 2019. Effects of duration of thermal stress on growth performance, serum oxidative stress indices, the expression and localization of ABCG2 and mitochondria ROS production of skeletal muscle, small intestine and immune organs in broilers. *Journal of Thermal Biology* **85**:102420 DOI 10.1016/j.jtherbio.2019.102420.

Wang XB, Ren H, Li XS, Chen HS, Ju ZL, Xiong DQ. 2021. Sex-specific differences in the toxic effects of heavy fuel oil on sea urchin (*Strongylocentrotus intermedius*). *International Journal of Environmental Research and Public Health* **18(2)**:499 DOI 10.3390/ijerph18020499.

Whitley D, Goldberg S, Jordan W. 1999. Heat shock proteins: a review of the molecular chaperones. *Journal of Vascular Surgery* **29(6)**:748–751 DOI 10.1016/0955-0674(91)90125-I.

Wood LA, Brown IR, Youson JH. 1999. Tissue and developmental variations in the heat shock response of sea lampreys (*Petromyzon marinus*): effects of an increase in acclimation temperature. *Comparative Biochemistry and Physiology A-Molecular and Integrative Physiology* **123(1)**:35–42 DOI 10.1016/S1095-6433(99)00035-5.

Yang YY, Pan LQ, Zhou YY, Xu RY, Miao JJ, Gao ZY, Li DY. 2021. Damages to biological macromolecules in gonadal subcellular fractions of scallop *Chlamys farreri* following benzo[a]pyrene exposure: contribution to inhibiting gonadal development and reducing fertility. *Environment Pollution* **283**:117084 DOI 10.1016/j.envpol.2021.117084.

Zhang LJ, Zhang LL, Shi DT, Wei J, Chang YQ, Zhao C. 2017. Effects of long-term elevated temperature on covering, sheltering and righting behaviors of the sea urchin Strongylocentrotus intermedius. *PeerJ* **5(6)**:e3122 DOI 10.7717/peerj.3122.

Zhao C, Ji N, Zhang BL, Sun P, Feng WP, Wei J, Chang YQ. 2014. Effects of covering behavior and exposure to a predatory crab charybdis japonica on survival and HSP70 expression of juvenile sea urchins *Strongylocentrotus intermedius*. *PLOS ONE* **9(5)**:e97840 DOI 10.1371/journal.pone.0097840.

Zhao C, Sun P, Wei J, Zhang LS, Zhang WJ, Song J, Chang YQ. 2016. Larval size and metamorphosis are significantly reduced in second generation of inbred sea urchins

Strongylocentrotus intermedius. Aquaculture **452(2)**:402–406
DOI 10.1016/j.aquaculture.2015.11.024.

Zhao C, Zhang LS, Shi DT, Ding JY, Yin DH, Sun JN, Zhang BJ, Zhang LL, Chang YQ. 2018.
Transgenerational effects of ocean warming on the sea urchin *Strongylocentrotus intermedius.*
Ecotoxicology and Environmental Safety **151(9)**:212–219 DOI 10.1016/j.ecoenv.2018.01.014.

Zhou DY, Qin L, Zhu BW, Li DM, Yang JF, Dong XP, Murata Y. 2012. Optimisation of
hydrolysis of purple sea urchin (*Strongylocentrotus nudus*) gonad by response surface
methodology and evaluation of in vitro antioxidant activity of the hydrolysate. *Journal of the
Science of Food and Agriculture* **92(8)**:1694–1701 DOI 10.1002/jsfa.5534.