

Supplementary Material

Effects of stranded heavy fuel oil subacute exposure on the fitness-related traits of sea urchin *Strongylocentrotus intermedius*

Xuanbo Wang^A, Xishan Li^A, Deqi Xiong^{A,*}, Huishu Chen^A and Hang Ren^A

^ACollege of Environmental Science and Engineering, Dalian Maritime University, 116026, Dalian, PR China

*Correspondence to: Email: xiongdq@dlmu.edu.cn

During the exposure period, the test solution was taken every 24 h to determine the concentration of total petroleum hydrocarbons (TPH) and PAHs for 21 days. TPH concentrations of HFO was measured using ultraviolet visible spectroscopy (Biotek Epoch2) according to Standardization Administration of China GB 17378.4–2007 as described in our previous work (Duan *et al.* 2018). Briefly, take out 200 mL of solution in the upper part of the treatment tank using a measuring cylinder and were extracted with 20 mL of *n*-hexane. TPH of water samples was measured against standard curves of *n*-hexane dilutions. The absorbance measured at 225 nm with an ultraviolet visible spectrophotometer represents the TPH concentration. The recovery of TPH was measured using hydrocarbon standards, the recovery rate is above 90%. Moreover, we have analysed the 16 environmental Protection Agency (EPA) PAHs in this study as our previous work described (Duan *et al.* 2018), including naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, dibenz[*a,h*]anthracene, benzo[*ghi*]perylene. The water samples were pretreated according to the EPA 3510C liquid-liquid extraction method. The concentrate was cleaned using EPA 3630C silica gel cleaning method. Then, PAHs were analysed using gas chromatography mass spectrometry (GC/MS) (Agilent 7890B, USA). The recovery of PAHs was between 72 and 118%.

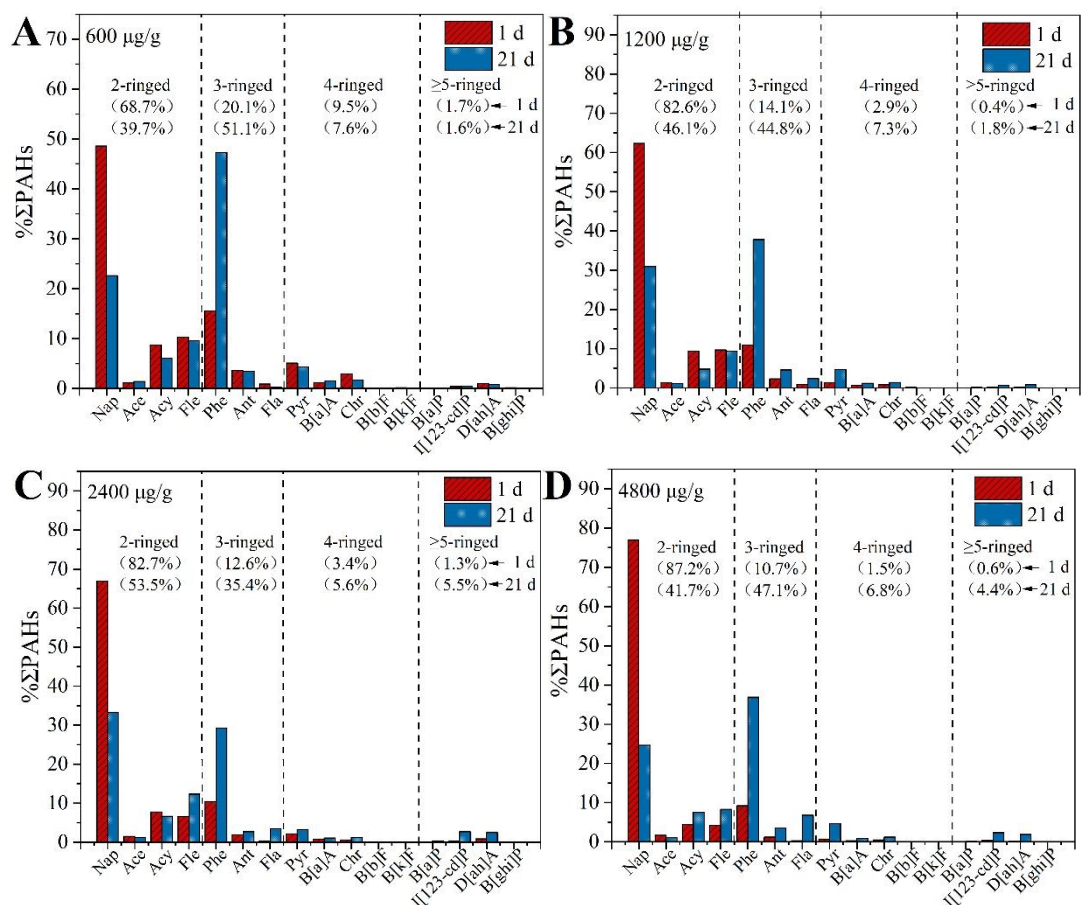


Fig. S1. The composition of PAHs in the sample water from exposure system at day 1 and day 21.

Reference

Duan, M., Xiong, D., Bai, X., Gao, Y., Xiong, Y., Gao, X., and Ding, G. (2018). 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](https://doi.org/10.1016/j.marenvres.2018.08.010)