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## Hydrogen peroxide-induced oxidative damage in sea urchin (*Paracentrotus lividus*) DNA

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Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is regularly used in Scottish salmon aquaculture as an antiparasitic treatment for sea lice infestation. After application, H<sub>2</sub>O<sub>2</sub> is released into the environment and reactive oxygen species produced during the breakdown process which can induce oxidative damage in DNA of non-target marine organisms, such as sea urchins. The objective of this study was to assess oxidative DNA damage in adult sea urchins and embryos derived from gametes that were exposed to environmentally realistic H<sub>2</sub>O<sub>2</sub> concentrations. Exposure concentrations of H<sub>2</sub>O<sub>2</sub> were based on those obtained from previous models of aquaculture farm treatments. Damage to DNA was determined by the modified fast micromethod (Liu et al. 2022), which was adapted from the fast micromethod and is similar to the modified comet assay [used formamidopyrimidine-DNA glycosylase (FPG) and endonuclease-III (Endo III) for additional quantification of oxidised purine and pyrimidines]. Acute 1-h exposure of adult sea urchins to a bath treatment of 500µM H<sub>2</sub>O<sub>2</sub> resulted in significantly increased oxidative DNA damage in coelomocytes of 0.05±0.2 (mean±SEM, n=5, p<0.05, ANOVA) in strand scission factor (SSF) that returned to control levels after 3h and suggested DNA repair. Embryos cross fertilised with H<sub>2</sub>O<sub>2</sub>-exposed sperm (with confirmed DNA damage after 1mM H<sub>2</sub>O<sub>2</sub> exposure for 10min) and unexposed eggs showed elevated oxidative DNA damage of up to 0.1±0.01 SSF (mean±SEM, n=3, p<0.05, ANOVA) at 3 and 24h (blastula) post fertilisation. No evidence of DNA repair was indicated up to 24h, but average levels of SSF returned to control levels at 48h (gastrula) possibly due to DNA repair. This study used low concentrations of H<sub>2</sub>O<sub>2</sub> in short-term exposures and demonstrated DNA damage in both adult benthic sea urchins and pelagic spawned gametes with evidence of DNA repair in both cases at 3h in coelomocytes and 48h in embryos.

### Keywords:

Genotoxicity; DNA damage; oxidation; methylation; modified fast micromethod, FPG, Endo III, McrBC.