Assessing Oxidative Stress in the Sea-ice Diatom *Fragilariopsis cylindrus*

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Environmental factors have been shown to increase reactive oxygen species (ROS) in phytoplankton, including light, salinity, metal and viral exposure. Excessive buildup of ROS can cause widespread oxidative damage to a cell, thus organisms have developed various antioxidant mechanisms including the sulfur compound glutathione. Certain marine phytoplankton species produce another sulfur compound, dimethylsulphonioproprionate (DMSP), which plays important climate and biogeochemical roles. Cellular concentrations of DMSP are responsive to various environmental variables but its physiological role is still poorly understood. Recently it has been hypothesized to serve as a cellular antioxidant due to its ability to readily react with hydroxyl radicals. In order to better understand associations between environmental stressors, DMSP accumulation, and oxidative stress, the purpose of the current work was to optimize an *in vivo* method to quantify cellular ROS levels. The membrane permeable compound 2',7'-dichloro-dihydrofluorescein (DCFH-DA) was selected because following esterase conversion and oxidation by ROS within the cell, DCFH-DA produces the green-fluorescent compound 2',7'-dichlorofluorescein (DCF) which is retained within the cell and its emitting fluorescence has minimal overlap with chloroplast autofluorescence. Hydrogen peroxide (HO) and methyl viologen (MV) treatments, both known to increase intracellular ROS, were used to optimize assay parameters. Intracellular green fluorescence was measured at 520nm by flow cytometry. Sixty minutes was found to be a suitable stain incubation time. At the highest stain concentration tested the mean intracellular green fluorescence was elevated in non-treated controls similarly to HO and MV treatment groups, suggesting at high concentrations the stain generates ROS. However at lower stain concentrations HO and MV treatments both showed increased green fluorescence relative to untreated controls. These results demonstrate the utility of this method to assess ROS in a sea-ice diatom despite certain assay limitations. Future experiments will look at salinity and light stress effects on DMSP and ROS accumulation, as well as global proteome changes.

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