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## First Morphological and Molecular Evidence of the Negative Impact of Diatom-Derived Hydroxyacids on the Sea Urchin Paracentrotus lividus

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## ABSTRACT

Oxylipins (including polyunsaturated aldehydes [PUAs], hydoxyacids, and epoxyalcohols) are the end-products of a lipoxygenase/hydroperoxide lyase metabolic pathway in diatoms. To date, very little information is available on oxylipins other than PUAs, even though they represent the most common oxylipins produced by diatoms. Here, we report, for the first time, on the effects of 2 hydroxyacids, 5- and 15-HEPE, which have never been tested before, using the sea urchin *Paracentrotus lividus* as a model organism. We show that HEPEs do induce developmental malformations but at concentrations higher when compared with PUAs. Interestingly, HEPEs also induced a marked developmental delay in sea urchin embryos, which has not hitherto been reported for PUAs. Recovery experiments revealed that embryos do not recover following treatment with HEPEs. Finally, we report the expression levels of 35 genes (involved in stress, development, differentiation, skeletogenesis, and detoxification processes) to identify the molecular targets affected by HEPEs. We show that the 2 HEPEs have very few common molecular targets, specifically affecting different classes of genes and at different times of development. In particular, 15-HEPE switched on fewer genes than 5-HEPE, upregulating mainly stress-related genes at a later pluteus stage of development. 5-HEPE was stronger than 15-HEPE, targeting 24 genes, mainly at the earliest stages of embryo development (at the blastula and swimming blastula stages). These findings highlight the differences between HEPEs and PUAs and also have important ecological implications because many diatom species do not produce PUAs, but rather these other chemicals are derived from the oxidation of fatty acids.

Key words: diatoms; hydroxyacids; sea urchin; development; genes.

Several marine diatoms are rich in polyunsaturated fatty acids (PUFAs) and have traditionally been considered as an important food source for many aquatic animals. These fatty acids are also precursors for the production of toxic short-chain polyun-saturated aldehydes (PUAs) and other oxygenated fatty acid derivatives, collectively termed "oxylipins." Production of all of these metabolites is triggered by cell damage or breakage, as during grazing or lysis of cells (Pohnert, 2005). Fatty acids

liberated from cell membranes are oxidized by lipoxygenases (LOXs) to lipid hydroperoxides (FAHs), which are then rapidly converted within seconds to PUAs and other oxylipins. Of the known oxylipins, PUAs are the far best described and most comprehensively studied. This is due to PUAs being the first group described (Miralto *et al.*, 1999) and also are commercially available, inexpensive and sufficiently stable to allow for a range of laboratory bioassays to be conducted. PUAs have important

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