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**Istituto di Chimica
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**Toxigenic effects of benthic diatoms upon
grazing activity of the sea urchin**

Paracentrotus lividus

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Abstract

Diatoms are unicellular algae playing a key role as photosynthetic organisms in the world's ocean food web. The chemical ecology of planktonic diatoms is well documented, but few studies have reported on the effects of benthic diatoms on their consumers, also due to difficulties in the collection, quantification and massive culturing of benthic species. This study investigates, for the first time, the effects of diets based on four benthic diatoms, *Cylindrotheca closterium*, *Nanofrustulum shiloi*, *Cocconeis scutellum* and *Diploneis* sp. isolated from the leaves of the seagrass *Posidonia oceanica*, on the sea urchin *Paracentrotus lividus*. The results demonstrate a toxigenic effect on embryos generated by females fed for one month on these benthic diatoms by multidisciplinary approaches. (i) Morphological observations by microscope revealed a noxious effects of *C. closterium*, *N. shiloi* and *Diploneis* sp. on embryos deriving from adult sea urchin *P. lividus* fed for one month on these diets, with *N. shiloi* showing the strongest effects; on the contrary, *C. scutellum* showed no effects, producing embryos as those deriving from the control diet. Malformations of these embryos were very similar to those observed after treatment with planktonic diatom-derived oxylipins. (ii) Metabolomic analysis by *Nuclear Magnetic Resonance* ($^1\text{H-NMR}$) demonstrated that feeding on these diatoms induced variations in the levels of lipids and/or amino acids in the gonads of *P. lividus*. (iii) Molecular analysis by *de novo* transcriptome and *Real Time qPCR* showed that benthic diatoms were able to affect the expression levels of several genes, involved in different cellular processes. (iv) Chemical analyses by Gas Chromatography–Mass Spectrometry (GC-MS) and Liquid Chromatography–Mass Spectrometry have been focused on two classes of secondary metabolites isolated in

planktonic diatoms: the oxylipins and sterol sulfates. All benthic diatoms analysed in this work produced oxylipins with the only exception of *Diploneis* sp., which in turn produced some unknown compounds deriving from polyunsaturated fatty acids metabolism. Moreover, all four benthic diatoms showed the presence of StS.

This study is the first demonstration of the toxic effects of benthic epiphytic diatoms on embryos and larvae of the sea urchin *P. lividus* due to the feeding of adults during gonadal maturation. Furthermore, the present work assumes a considerable ecological relevance, opening new perspectives on the study of diatom-derived secondary metabolites influencing their grazers.