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Toxigenic effects of two benthic diatoms upon grazing activity of the sea urchin: morphological, metabolomic and *de novo* transcriptomic analysis

Nadia Ruocco^{1,2,3}, Susan Costantini⁴, Valerio Zupo⁵, Chiara Lauritano⁶, Davide Caramiello⁷, Adrianna Ianora⁶, Alfredo Budillon⁴, Giovanna Romano⁶, Genoveffa Nuzzo³, Giuliana D'Ippolito³, Angelo Fontana³ & Maria Costantini¹

Diatoms are unicellular algae playing a key role as photosynthetic organisms in the world's ocean food webs. 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. Here for the first time we investigate the effects of feeding on two abundantly occurring benthic diatoms, *Nanofrustulum shiloi* and *Cylindrotheca closterium*, isolated from the leaves of the seagrass *Posidonia oceanica*, on the sea urchin *Paracentrotus lividus*. Adult *P. lividus* were fed for one month on diets of either one of the two diatoms and on the green alga *Ulva rigida*, used as a feeding control. By combining morphological, metabolomic and *de novo* transcriptomic approaches, we demonstrate toxigenic effect on embryos generated by females fed with these benthic diatoms. Furthermore, chemical analysis reveal the presence of polyunsaturated aldehydes only for *N. shiloi*, and a high production of other oxylipins (cytotoxic compounds on their grazers and on cancer cell lines) for both diatoms, including some additional peaks not correlated to the canonic oxylipins commonly observed in planktonic diatoms. These findings open new perspectives in the study of diatom secondary metabolites influencing their grazers.

Diatoms are unicellular eukaryotes, representing one of the largest and ecologically groups and exclusively depositing biogenic silica. The siliceous wall is transparent, allowing the entrance of the light, and perforated, making possible the diffusion and excretion of materials¹. They contribute about 20% of global photosynthetic fixation of carbon (about 20 Pg carbon fixed per year), which is more than all the world's tropical rainforests, also playing important roles on earth and in oceans as oxygen synthesizers and biomass sources². Functionally, diatoms are single cells but they can appear as filaments, chains, or colonies, and they are abundant in nearly all aquatic habitats, living either in the water column (planktonic species) or attached to any single substratum (benthic species). Moreover, diatom morphology can be considered as an additional environment assessment tool to the biological indices to evaluate for example the anthropogenic eutrophication effects³.

Diatoms have been regarded as beneficial to the growth and survival of primary consumers such as planktonic and benthic filter feeders. However, many planktonic diatoms have been discovered to produce a wide range

¹Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121, Napoli, Italy. ²Department of Biology, University of Naples Federico II, Complesso Universitario di Monte Sant'Angelo, Via Cinthia, 80126, Napoli, Italy. ³Bio-Organic Chemistry Unit, Institute of Biomolecular Chemistry-CNR, Via Campi Flegrei 34, Pozzuoli, Naples, 80078, Italy. ⁴Unità di Farmacologia Sperimentale, Istituto Nazionale Tumori "Fondazione G. Pascale", IRCCS, Napoli, Italy. ⁵Center of Villa Dohrn Ischia-Benthic Ecology, Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, P.ta S. Pietro, Ischia, Naples, Italy. ⁶Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121, Napoli, Italy. ⁷Unit Marine Resources for Research, Stazione Zoologica Anton Dohrn, Naples, Italy. Correspondence and requests for materials should be addressed to M.C. (email: maria.costantini@szn.it)