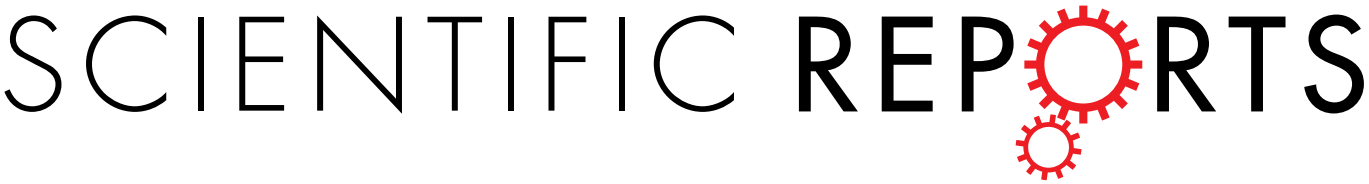

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Subtle reproductive impairment through nitric oxide-mediated mechanisms in sea urchins from an area affected by harmful algal blooms

Oriana Migliaccio[1], Immacolata Castellano[1], Davide Di Cioccio[1], Gabriella Tedeschi[2], Armando Negri[2], Paola Cirino[1], Giovanna Romano[1], Adriana Zingone[1] & Anna Palumbo[1]

The health of the sea urchin *Paracentrotus lividus*, a key species in the Mediterranean Sea, is menaced by several pressures in coastal environments. Here, we aimed at assessing the reproductive ability of apparently healthy *P. lividus* population in a marine protected area affected by toxic blooms of *Ostreospsis* cf. *ovata*. Wide-ranging analyses were performed in animals collected prior to and during the bloom, as well as at several times thereafter, during the reproductive season. Adults showed a low fertilization rate, along with high nitric oxide (NO) levels in the gonads and the nitration of the major yolk protein toposome, which is an important player in sea urchin development. Serious developmental anomalies were observed in the progeny, which persist several months after the bloom. NO levels were high in the different developmental stages, which also showed variations in the transcription of several genes that were found to be directly or indirectly modulated by NO. These results highlight subtle but important reproductive flaws transmitted from the female gonads to the offspring with the NO involvement. Despite a recovery along time after the bloom, insidious damages can be envisaged in the local sea urchin population, with possible reverberation on the whole benthic system.

In the Mediterranean Sea, the sea urchin *Paracentrotus lividus* represents a key species in the benthic communities, where it controls the dynamic, structure and composition of shallow macroalgal assemblages through its grazing activity[1]. In addition, *P. lividus* represents a food source for fishes and other animals, including humans that consider its gonads a culinary delicacy. The health of *P. lividus* and its safety as food are therefore of great interest at multiple levels. Nevertheless sea urchins continuously face the impact of a variety of increasing pressures, acting single or simultaneously in the coastal environments, including eutrophication, ocean acidification, warming, hypoxia, chemical contamination and harmful algal blooms (HABs). These impacts are more deleterious for benthic organisms because of their limited or null motility, and in case of abundant or structuring species may have detrimental consequences on the biodiversity and functionality of the whole benthic system[2].

Among HABs are blooms of species of the benthic dinoflagellate genus *Ostreopsis*, which produces ovatoxins[3–5], i.e. palytoxin- like molecules that are deemed to be responsible for a number of health problems in humans[6] (for a review). *Ostreopsis* cf. *ovata*, first reported in subtropical waters[7], has been increasingly recorded also in temperate seas and in the Mediterranean Sea[8], with intense blooms in July/August in the western and in September-October in the Adriatic Sea, (e.g.[9]) and in the eastern basin[10]. Occasionally, mortality of benthic organisms, including sea urchin, or damages to the exoskeleton have been reported during *O.* cf. *ovata* blooms[11,12], whereas fertilization impairment and embryo/larvae mortality were observed upon exposure to *Ostreopsis* cultures[13,14].

In the Mediterranean Sea, the reproductive cycle of *P. lividus* generally starts in autumn and has a main peak in spring[15], which limits the possibility of a direct impact of *Ostreopsis* blooms on the reproduction. However,

[1]Stazione Zoologica Anton Dohrn, Naples, Italy. [2]D.I.P.A.V. – Section of Biochemistry, University of Milan, Milan, Italy. Correspondence and requests for materials should be addressed to A.Z. (email: adriana.zingone@szn.it) or A.P. (email: anna.palumbo@szn.it)