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Quantitative histopathology of the Mediterranean mussel (*Mytilus galloprovincialis* L.) exposed to the harmful dinoflagellate *Ostreopsis* cf. *ovata*

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ABSTRACT

Ostreopsis cf. *ovata* is a benthic dinoflagellate that produces palytoxin-like compounds that adversely affect both marine vertebrates and invertebrates and are reported to be responsible for human intoxication in aerosol form. In this work, a histopathological analysis accompanied by quantitative evaluation of tissue injury in mussels (*Mytilus galloprovincialis*) exposed to *O.* cf. *ovata* cells under natural and experimental conditions, provided baseline data on the health status of the mussels in terms of defensive and regressive pathological changes. We recorded a total of 15 health parameters in the digestive system, muscle, kidney and gills in mussels exposed to *O.* cf. *ovata* both in the laboratory and at sea. Animals exposed to different concentrations of *O.* cf. *ovata* cells (300, 500 and 1000 cells ml^{-1}) for 48 h showed activation of the inflammatory response, which increased with the cell concentration, mainly characterized by haemocyte aggregates actively enclosing the algae, while mussel mortality was also recorded in some cases. Moreover the use of image analysis for the evaluation of digestive tubule damage revealed a pronounced increase in the lumen in terms of its area, perimeter and circularity, with a shift in a high percentage of tubules from an adsorbing profile to an atrophic profile. Animals collected from the natural environment during a summer bloom of *O.* cf. *ovata* in the Gulf of Naples (Italy) showed comparable lesions in terms of types and severity. This is the first quantitative study assessing damage to the digestive epithelia in terms of lumen modifications in mussels exposed to *O.* cf. *ovata*.

The presented methodology provides a new technique for automating the evaluation of epithelial tubule modifications. Our results highlight the importance of monitoring the presence of *O.* cf. *ovata* in this area, taking into account the effects on the residing marine species.

1. Introduction

The mussel *Mytilus galloprovincialis* is a filter-feeding species that is used worldwide as a sentinel organism in ecotoxicological investigations and that is marketed as seafood. The presence of parasites and diseases in this species has been widely documented and may cause severe damage and contribute to decreases in both natural and cultivated populations (Carella et al., 2013a,b,c; 2011; Villalba et al., 1997). Microalgae generally play a key role in aquatic ecosystems as primary producers, but in many coastal areas, their proliferation and production of biotoxins, known as harmful algal blooms (HABs), may have important negative consequences for the trophic web, including for filter feeders (Landsberg, 2002; Mangoni et al., 2011). The pathological effects of toxic microalgae on bivalves can differ greatly, including mortality (Wikfors and Smolowitz, 1995), tissue damage (Pearce et al., 2005), cellular dysfunction (Hegaret and Wikfors, 2005), reproductive failure (Granmo et al., 1988) or reductions of the clearance rate, growth rate and condition index (Landsberg, 2002).

In the last 15 years, interest in the benthic toxic dinoflagellates *Ostreopsis* spp. has increased, related to blooms in temperate and tropical coastal waters in both the northern and southern hemispheres (Rhodes, 2011). In the Mediterranean region, blooms of *Ostreopsis* cf. *ovata* have been observed with an increasing frequency, intensity and distribution (Totti et al., 2007; Mangialajo et al., 2011). Mass mortalities of benthic organisms (Sansoni et al., 2003; see Aligizaki et al., 2011 for a review) and human health problems (see Tubaro et al., 2011 for a review; Brescianini et al., 2006) reported at times during *O.* cf. *ovata* blooms have been

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