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# First record of two potentially toxic dinoflagellates in tide pools along the Sardinian coast

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## ABSTRACT

Nowadays harmful algal blooms (HABs) represent a serious problem for the conservation of the biodiversity in the Mediterranean Sea. Nevertheless the knowledge on the presence of potentially toxic benthic microalgae in particular habitats, such as tide pools, is still scarce. In order to detect HAB-producing benthic microalgae in tide pools of the rocky intertidal zone, a pilot study was conducted in Tavolara Punta Coda Cavallo Marine Protected Area (MPA) during the late spring of 2016. Three different pools were sampled in two study sites (six pools were sampled in total) and the cell density of toxic species was estimated in each. In all the collected samples, the two potentially toxic dinoflagellates, *Prorocentrum lima* (Ehrenberg) F. Stein and *Coolia monotis* Meunier, were recorded and significant differences in their density were observed, in relation to both sites and pools.

## ARTICLE HISTORY

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## 1. Introduction

In the last few years, harmful algal blooms (HABs) have become more frequent in the Mediterranean Sea, favoured by global warming, which has led to a suite of environmental changes that benefit HAB potentials as the conversion of land-derived nutrients into harmful-algal biomass seems to be strictly related to climatic conditions (Cloern et al. 2005).

Harmful blooms are produced by a large number of microalgae, especially belonging to diatoms (*Bacillariophyceae*) and dinoflagellates rapidly expanding their distribution in the Mediterranean basin (Mangialajo et al. 2011). Some of the harmful microalgae spreading in the above-mentioned basin can produce both mucopolysaccharides and/or toxic compounds (Zingone and Oksfeldt Enevoldsen 2000). Toxic compounds, in particular, are able to enter the food chain and reach top predators and humans (Ignatiades and Gotsis-Skretas 2010; Masò and Garcés 2006). Effectively, algal toxins are responsible for die-offs of fish and shellfish and have been implicated in mortalities of marine mammals, birds and other animals (Hallegraeff 2003; Momigliano et al. 2013). Moreover, algal toxins are responsible for an array

of human illnesses associated with the consumption of contaminated seafood and the exposure to aerosolised toxins (Van Dolah 2000).

Therefore, HABs events, especially if caused by toxin producer species, are a relevant problem in marine ecosystems, causing not only environmental damage but also economic and health problems (Mangialajo et al. 2008).

Among HAB producing species, harmful benthic microalgae represent an emergent phenomenon in temperate zones (Mangialajo et al. 2011) and some of them (e.g. *Ostreopsis ovata* Fukuyo) are nowadays considered as the largest cause of HABs (Hallegraeff 2003). Nevertheless, only a few studies on the presence of potentially toxic benthic microalgae in particular habitats, such as tide pools, have been conducted till today. Consequently, despite tide pools are considered to be conspicuous components of rocky intertidal shores (Metaxas and Scheibling 1993), the information on the presence of toxic microalgae in such habitat is still very scarce.

Tide pools form a widely distributed habitat where environmental conditions are subject to wider fluctuations than in the sea (Gibson 1986) and they are considered dynamic ecosystems, changing with the flow of tides

(Jensen and Muller-Parker 1994). At high tide the pools are submerged and the system is open, as water is continually exchanged with the sea. When the tide recedes, instead, pools are isolated and they can be considered as a closed ecosystem. For this reason, deepening the knowledge on the presence of toxic microalgae in them could be particularly useful not only to manage future blooms but also to acquire useful information on the ecology of these species, specifying which environmental factors play a key role in regulating their distribution and density. Effectively, the presence of one or more toxic microalgae in tide pools could prove the ability of the observed species to survive and adapt to the remarkable diurnal and tidal changes in temperature and pH as well as in oxygen content and salinity typical of such habitats (Goss-Custard 1979).

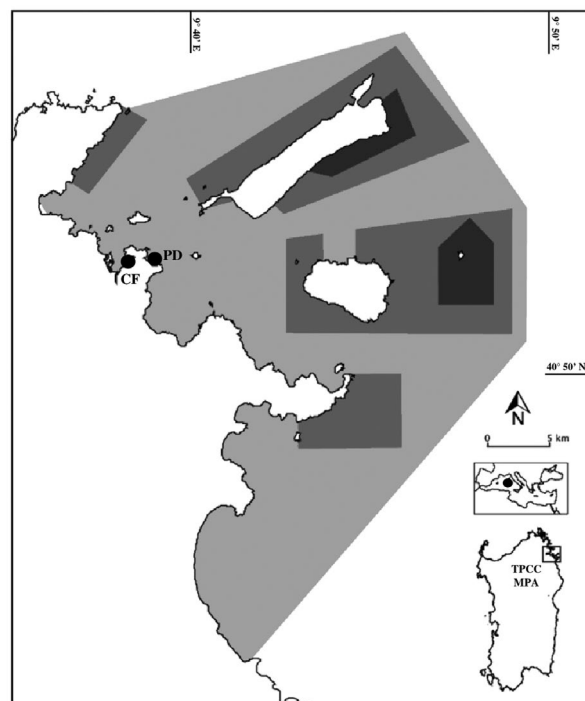
In order to detect the presence and to estimate the abundance of toxic benthic microalgae in tide pools of the rocky intertidal zone, a pilot study was conducted along the Sardinian coast in 2016 to acquire useful data to plan ad hoc mentoring programs. To verify if tide pools could represent a dissemination zone from which summer blooms of toxic microalgae originate, the research was carried out in March when cells densities of the considered species are usually very low and their presence is detectable only in the above mentioned zones.

## 2. Materials and methods

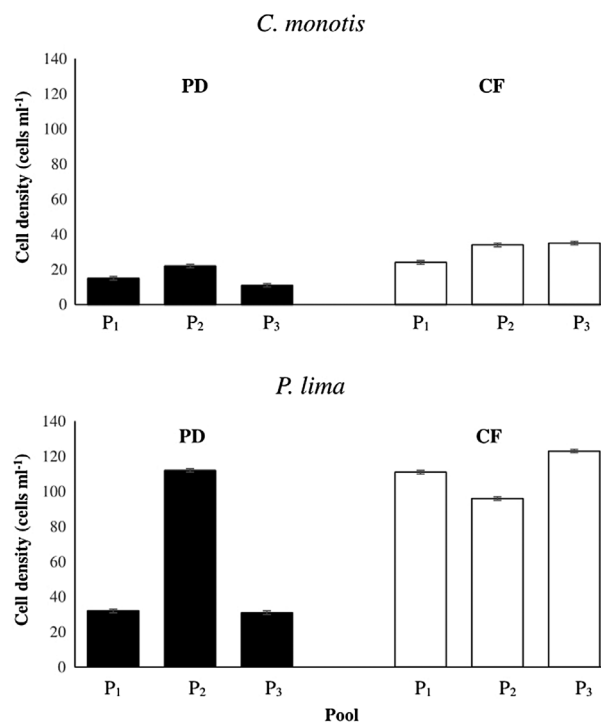
### 2.1. Study sites

The study was conducted in Tavolara Punta Coda Cavallo Marine Protected Area, along the North-eastern Sardinian coasts (Western Mediterranean Sea). Two key sites of a C area (partial protection) of the MPA where HAB producing benthic species had been recently observed (S. Caronni, personal observations) were chosen for this pilot study: Punta Don Diego bay (PD: 40°52.501' N, 9°39.306' E) and Cala Finanza bay (CF: 40°52.759' N; long: 9°38.759' E) (Figure 1). In each site, three different pools of the intertidal zone were randomly chosen, approximately 10 m apart.

The considered pools were all characterised by a maximum depth of around 30.00 cm (almost corresponding to the water depth during high tides) and by a diameter of around 60.00 cm. The mean daily water fluctuation related to tides was of about 7 cm and the mean temperature during samples was of about 17 °C. In all pools macroalgae, molluscs (limpets and gastropods), crustaceans (barnacles and little shrimps) and small fishes (blennies) as well as bare rocky surfaces were present.



**Figure 1.** Map of Tavolara Punta Coda Cavallo (TPCC) Marine Protected Area (MPA) showing the study sites. Grey tones are used to distinguish among differently protected zones of the MPA (A zone dark grey; C zones light grey).



**Figure 2.** Mean cell density (+SE) of the two species in each pool (P<sub>1</sub>, P<sub>2</sub> and P<sub>3</sub>) of the two sampling sites (PD: Punta Don Diego; CF: Cala Finanza).

## 2.2. Samples collection and laboratory procedures

In each pool three samples of water and epilithic material were collected by scraping the bare rocky surfaces of the pool at a depth of around 0.20 cm. Samples were obtained by using a brush-sampler, an efficient sampling device successfully used in other studies on benthic microalgae (Caronni et al. 2014).

All collected samples were fixed with Lugol's solution (Auinger, Pfandl, and Boenigk 2008) immediately after collection and transported to the laboratory. Cell identification and counts were carried out with an inverted microscope according to Utermöhl's sedimentation method (Utermöhl 1958). Three sub-samples for each sample were analysed after sedimentation (24 h) in specific sedimentation chambers (5 ml). The cell density of each species was expressed as the number of cells ml<sup>-1</sup>.

## 2.3. Data analysis

Statistical analysis was performed using the software GMAV5 for Windows (Underwood and Chapman 1997). Differences in the cell density of the toxic microalgae observed during the study among sites and pools were assessed through a two-way analysis of variance (ANOVAs) where site (two levels) and species (two levels) were treated as fixed factors while pool (three levels) was treated as a random factor nested in site. To test for normality and homogeneity of variances, the Shapiro–Wilk and Cochran's tests were run, respectively. A Student–Newman–Keuls test (SNK) was used for a posteriori comparisons of means for fixed factors.

## 3. Results

During the study, two potentially toxic benthic dinoflagellates were recorded in the considered tide pools: *Prorocentrum lima* (Ehrenberg) F. Stein (PL) and *Coolia monotis* Meunier (CM). The two species were observed in both study sites, although significant differences in their cell densities were recorded, as confirmed also by the statistical analysis (Table 1). In all the investigated pools, *P. lima* appeared to be more abundant than *C. monotis* (Figure 1; Table 1) with a mean cell density of 59 and 111 cells ml<sup>-1</sup>, respectively, in Punta Don Diego and Cala Finanza Bay. *C. monotis* abundance, instead, did not exceed 16 and 31 cells ml<sup>-1</sup> in the first and in the second study site respectively (PD and CF).

Finally, with regard to *P. lima*, significant differences in its cell density were recorded also among pools in the same study site. In Punta Don Diego Bay, in particular, the mean density of the microalga *per* pool varied from 32 to 112 cells ml<sup>-1</sup> (Figure 1; Table 1) and this latter value was very similar to these observed in Cala Finanza Bay (CF: 111,

**Table 1.** Results of the three-way ANOVA testing the differences in cell density among sites (two levels), pools (three levels) and species (two levels). Significant results are given in bold. SNK tests for comparisons of significant interactions were also performed.

Source	df	MS	F	P
<b>ANOVA</b>				
Site = Si	1	10,268.4444	4.76	0.0947
Pool = Po(Si)	4	33,856.0000	23.03	<b>0.0087</b>
Species = Sp	1	2159.0000	78.44	<b>0.0000</b>
SiXSp	1	3173.4444	2.16	0.2157
SpXPo(Si)	4	1470.3889	53.41	<b>0.0000</b>
Residual	24	27.5278		
Cochran's Test			C = 0.3542 ns	
<b>SNK test</b>				
SpXPo(Si)			SE = 3.0292	
PD	P <sub>1</sub>		CM < PL	
PD	P <sub>2</sub>		CM < PL	
PD	P <sub>3</sub>		CM < PL	
CF	P <sub>1</sub>		CM < PL	
CF	P <sub>2</sub>		CM < PL	
CF	P <sub>3</sub>		CM < PL	
PD	PL		P <sub>3</sub> = P <sub>1</sub> < P <sub>2</sub>	
PD	CM		P <sub>1</sub> = P <sub>2</sub> = P <sub>3</sub>	
CF	PL		P <sub>2</sub> < P <sub>1</sub> < P <sub>3</sub>	
CF	CM		P <sub>1</sub> = P <sub>2</sub> = P <sub>3</sub>	

96 and 123 cells ml<sup>-1</sup> in the three considered pools - P<sub>1</sub>, P<sub>2</sub> and P<sub>3</sub>). *C. monotis* densities, instead, appeared to be more homogeneous among pools in both the study sites, as highlighted also by the statistical analysis (Table 1).

## 4. Discussion and conclusions

During this pilot study, the two dinoflagellates *P. lima* and *C. monotis* were found in the intertidal tide pools of Tavolara Punta Coda Cavallo Marine Protected Area. Both species produce a variety of toxins with harmful effects on other marine organisms and human health (Laza-Martinez, Orive, and Miguel 2011; Pagliara and Caroppo 2012). *P. lima*, in particular, has been shown to produce okadaic acid and dinophysins toxins (Nascimento, Purdie, and Morris 2005; Vale, Veloso, and Amorim 2009), while *C. monotis* is able to synthesise an analogue of yessotoxin named cooliatoxin (Ben-Gharbia et al. 2016; Holmes et al. 1995). Vila, Garcés, and Masó (2001) reported that *P. lima* and *C. monotis* are frequently found associated and their assemblages seem to contribute to the polymorphism of the clinical features of ciguatera disease (Karafas, York, and Tomas 2015; Yasumoto et al. 1987), a widespread ichthyosarcotoxism which causes gastrointestinal, neurological and cardiovascular disturbances (Baumann, Bourrat, and Pauillac 2010).

Nowadays, these two species are widely distributed not only in tropical and subtropical marine areas but also in colder waters (David et al. 2014; Heil, Glibert, and Fan 2005; Rhodes et al. 2014; Vale, Veloso, and Amorim 2009).

The highest cell concentrations of these dinoflagellates have always been documented at depths superior to 0.50 m (Bomber, Norris, and Mitchell 1985; Cohu and



Lemée 2012) while their presence at around 0.20 m of depth has not been documented till today. Therefore, the results of this research, even if only preliminary, represent the first evidence of the ability of these two species to survive in very shallow waters, as already observed for other toxic microalgae, like *O. ovata* (Sbrana et al. 2017; Tawong et al. 2014).

The observation of *P. lima* and *C. monotis* in tide pools, characterised by variable and unstable environmental conditions, demonstrate their ability to adapt to such environmental conditions, proving they are characterised by a flexible behaviour, that is a complex balance between physiological optimisation and environmental stress, as asserted by Kamykowski (1981) studying other benthic dinoflagellates.

However, both the species were observed in the pools in very low abundances (<150 cells ml<sup>-1</sup>) if compared with those usually recorded on rocky substrata in coastal waters (*P. lima*: >3 × 10<sup>6</sup> cells l<sup>-1</sup>, Tango et al. 2005; *C. monotis*: 15.2 × 10<sup>3</sup> cells l<sup>-1</sup>, Ismael 2014). The reasons of such differences appear to be various. First of all, the distribution pattern of these species seems to be completely different among seasons and blooms always occur in summer or early autumn while in late spring (sampling period) the abundance of the considered species is always quite low (Ismael 2014). Even though, Ignatiades and Gotsis-Skretas (2010) found high densities of similar species in Greek coastal waters also in late spring (up to 1.2 × 10<sup>5</sup> cells l<sup>-1</sup> for a *Prorocentrum* species quite similar to *P. lima*). Therefore, as differences in cell density among tide pools of TPCC MPA and coastal waters in other geographic areas appear to be highly remarkable, it is plausible to suppose that also other factors beyond the season played a role in determining *P. lima* and *C. monotis* cell density in pools. Even if Saravia, Giorgi, and Momo (2012) asserted that the abundance of microalgae is not entirely imposed by external conditions but generated endogenously by their own growth and by their interactions with the other components of microbenthos, the above mentioned variability of the environmental conditions typical of pools and the limited depth could play a key role in limiting these species abundance. Beyond fluctuations in temperature, salinity and dissolved inorganic materials, also water motion produced by tides could significantly affect dinoflagellates abundance as their density was reported to be significantly negatively correlated with water motion (Carlson and Tindall 1985; Totti et al. 2010), especially for *P. lima* (Richlen and Lobel 2011). Even if several benthic microalgae are able to stick to rocks and cobbles using the mucous they secrete as an adhesive, it is more difficult for cells to settle and proliferate on the substratum when a high hydrodynamic stress occurs (Abelson and Denny 1997; Seuront and Spillmont, 2002).

Finally, the significant differences in *P. lima* cell density recorded among pools in the same study site indicate that a sort of patchiness in this species abundance at centimetres and metres of distance is present, as already observed for other bloom forming microalgae (Caronni et al. 2014). On this matter, Díaz et al. (2012) have recently demonstrated that multiple, often interacting ecological processes affect marine assemblages at small scales and their interaction provides the explanation for patchiness at small spatial scales.

In conclusion, even if further and more deepened studies will be necessary to better understand the abundance patterns of these two toxic dinoflagellates in tide pools of TPCC MPA, the obtained results offer some important insights into the ecology of these HAB producing microalgae and contribute to predict future blooms, planning ad hoc monitoring activities in the marine protected area. Indeed, the observed results suggest the importance of including tide pools when planning monitoring programs on *P. lima* and *C. monotis*.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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