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TOXIC ALGAL BLOOMS ALONG THE URBAN COAST: A CITIZENS' POINT OF VIEW

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ALGAE
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SOUTHERN ADRIATIC SEA
CITIZEN SCIENCE

ABSTRACT. – In this study, a Citizens' Observatory, the Osservatorio del Mare a Molfetta (OMM), has monitored the proliferation of the toxic microalgae *Ostreopsis ovata* along the coast of Molfetta, an Italian city on the Southern Adriatic Sea, where blooms have been reported since summer 2009. To establish an alternative low cost and effective monitoring protocol, planktonic cells were counted for four years in seawater sampled at two stations along the urban coast. We show that: (i) *O. ovata* blooms occur along the coast of the city of Molfetta; (ii) the presence of *O. ovata* was detected from May up until January of the following year; (iii) blooms of *O. ovata* are concomitant with those of non-toxic diatoms of the genus *Coscinodiscus*, which were predominant in the phytoplankton community; (iv) during the time frame of this study, *Ostreopsis* and *Coscinodiscus* proliferation start at the station closest to the urban centre. The traditional *O. ovata*-monitoring protocols, based on single sampling station/city every 15 days between June and September, need improvement. The weekly sampling of seawater along the urban coast from June to January is an effective and low-cost method to predict toxic algal blooms in our city. As a local citizen's observatory, we intend to support traditional monitoring programs by providing our data set to improve the surveillance.

INTRODUCTION

Benthic Harmful Algal Blooms (BHABs) are detrimental to the environment, marine organisms and humans (Faimali *et al*. 2012). The microalgae of the genus *Ostreopsis* live attached to benthic substrates such as brown seaweeds, rocks, shells of invertebrates and proliferate at high rates in shallow coastal areas close to the shoreline down to a few meters of depth. In the Mediterranean basin, *Ostreopsis ovata* Fukoyo, 1981, is one of the most common microalgae of tropical origin that produces palytoxin-like toxins (ova-toxins) (Fukuyo 1981, Penna *et al*. 2012). Ova-toxins represent a threat for human health via entry into the human food chain, inhalation or direct contact when cells are resuspended in surface seawater (Totti *et al*. 2010, Vila *et al*. 2012, Pelin *et al*. 2016). *Ostreopsis ovata* blooms in the Mediterranean Sea have become more frequent gaining the attention of scientific communities, managing institutions and public governance. Since the earliest 1990s, high concentrations of *O. ovata* have been recorded all around the Mediterranean Sea (Gallitelli *et al*. 2005, Kermarec *et al*. 2008, Blanfuné *et al*. 2015), along the Italian coasts, in the Ligurian, Sardinian, Tyrrenian, Ionian, and the northern and southern Adriatic Sea (Totti *et al*. 2010, Mangialajo *et al*. 2011). A notable bloom took place in 2005 in northern Italy along the coastline at Genoa where more than 200 cases of infection were recorded with clinical symptoms such as skin erythema, dispnoea, colds, fever and conjunctivitis (Ciminiello *et al*. 2006, Mangialajo *et al*. 2008, Tichadou *et al*. 2010). Most of the cases of infection were associated with inhalation rather than direct contact (Durando *et al*. 2007). Since summer 2009 in Molfetta (the city were the present study took place), regular monitoring of *O. ovata* blooms at coastal and recreational seawaters has been carried out by the regional agency for environmental protection every two weeks from June to September at a single sampling station (at Prima Cala in Molfetta), enabling the detection of blooms mostly in August and September (Ungaro *et al*. 2010, http://www.arpa.puglia.it).

Herein, trained volunteers from the Osservatorio del Mare a Molfetta (OMM) report the occurrence of *Ostreopsis* blooms at two public beaches on the waterfront of Molfetta. From July 2016 to December 2019, the concentration peaks of *O. ovata* were closely monitored on the basis of weekly sampling as well as its occurrence well beyond summer. Our data show that further efforts are required to understand the ecology of *O. ovata* and to improve the surveillance and alert systems in our city.

MATERIALS AND METHODS

Community-based environmental monitoring: OMM is a community of volunteers dedicated to promoting through the participative methodology of citizen science: i) the visual census and protection of coastal and marine ecosystems at urban level; ii) the public's right of access to environmental informa-

tion in order to empower citizens to make key decisions relating to environmental issues. In addition, OMM provides incentives to scientists to engage with citizens in order to establish the absence or presence of a cause-and-effect association between marine biodiversity loss and habitual pollution/consumption practices.

Using their own devices such as light microscopes, computers, smartphones and cell counting chambers, OMM volunteers have carried out bibliographical research involving background reading of publicly available environmental data, sea water sampling, cell counts and data analysis.

Study sites and sampling: In the city of Molfetta, two public beaches which are crowded in summer 5 km apart from each other were chosen as sampling sites: Prima Cala, which is closer to the urban center (41°11'5N; 16°36'54"E), and Gavetone (41°11'37N; 16°38'11"E) (Fig. 1).

Benthos was collected weekly at 8:00 am from July 2nd 2016 to December 27th 2019. At each site, 40 ml of seawater samples were taken close to the shore from the bottom of four shallow tide pools (0.2-0.4 m depth) using a 10 ml syringe with the tip cut off and collected in a single tube.

Cell counting and identification of planktonic species: Within 2 hours after sampling, collection tubes were gently inverted 10 times. 1 mL of seawater was taken, dispensed into the Sedgewick-Rafter counting chamber, and planktonic cells were counted in duplicate under the light microscope. Photographs were taken using personal mobile phones by laying the camera on the eyepiece lens of the microscope. Microalgae identification was performed by the use of publicly available guides (Avancini *et al*. 2006; https://www.algaebase.org/)

Statistical analysis: Statistical analysis was performed with the GraphPad Prism software using an ANOVA model followed by Tukey's post-test.

RESULTS

Ostreopsis ovata *abundance*

Fig. 1 shows the location of the two sampling stations, Prima Cala and Gavetone, located 5 km apart.

From July 2016 to December 2019, weekly sampling of seawater showed that maximum blooming of *O. ovata* occurred in summer 2016, 2017, 2018 and 2019 (Table I). The maximum peaks of proliferation were detected in August at Prima Cala and September at Gavetone with an average of 14.50 ± 13.27 days advance at Prima Cala relative to Gavetone. At the latter sampling station, one exception occurred in summer 2016 with a major peak in August. In general, *O. ovata* was more abundant at Prima Cala than at Gavetone with highest concentration peaks that were 2.77-, 6.18-, 1.6- and 1.2-fold higher during summer 2016, 2017, 2018 and 2019, respectively. The highest peak was measured during the first week of August 2018 at Prima Cala with a value of 13,242 ± 315.36 cells/ml (Table I).

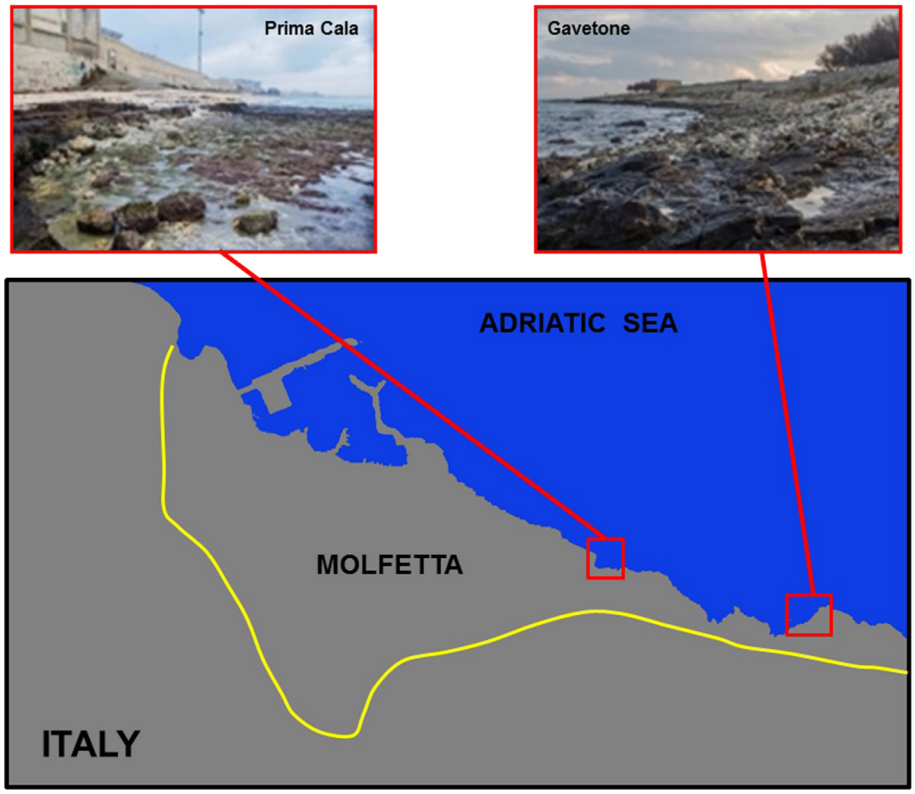

Fig. 1. – Study sites and sampling. In the city of Molfetta, two public beaches, crowded in summer, were chosen as sampling sites that are 5 km apart from each other. Prima Cala is closer to the urban centre (41°11'5N; 16°36'54"E) than Gavetone (41°11'37N; 16°38'11"E).

Table I. – Abundance of *Ostreopsis ovata* and *Coscinodiscus* sp. measured at the two sampling stations: Prima Cala and Gavetone. $P < 0.05$ is considered significant.

Species	Sampling station	First detection	Last detection	Maximal peak occurrence	Cell number (cells/ml ± SD)	Significance	Difference between the two sampling stations
Ostreopsis ovata	Prima Cala	Jul. 2016	Jan. 2017	Aug. 2016	6040 ± 14.85	P < 0.0001	
		Jun. 2017	Jan. 2018	Aug. 2017	6572 ± 197.98	P < 0.0001	
		May 2018	Jan. 2019	Aug. 2018	13242 ± 315.36	P < 0.0001	
		Jun. 2019	Jan. 2020	Aug. 2019	4791 ± 173.95	P < 0.0001	
							P > 0.05
Ostreopsis ovata	Gavetone	Jul. 2016	Jan. 2017	Aug. 2016	2173 ± 65.77	P < 0.0001	
		Jun. 2017	Jan. 2018	Sep. 2017	1063 ± 12.72	P < 0.0001	
		Jun. 2018	Jan. 2019	Sep. 2018	8064 ± 814.59	P < 0.0001	
		Aug. 2019	Jan. 2020	Sep. 2019	4140 ± 25.45	P < 0.0001	
Coscinodiscus sp.	Prima Cala	Jul. 2016	Feb. 2017	Aug. 2016	34553 ± 219.58	P < 0.0001	
		May 2017	Jan. 2018	Sep. 2017	27460 ± 2582.32	P < 0.0001	
		Apr. 2018	Feb. 2018	Sep. 2018	7983 ± 250.31	P < 0.0001	
		Mar. 2019	Jan. 2020	Aug. 2019	11232 ± 25.46	P < 0.0001	
							P < 0.01
Coscinodiscus sp.	Gavetone	Jul. 2016	Jan. 2017	Aug. 2016	3110 ± 57.98	P < 0.0001	
		Apr. 2017	Dec. 2017	Sep. 2017	7890 ± 33.94	P < 0.0001	
		Apr. 2018	Jan. 2019	Sep. 2018	4572 ± 280.01	P < 0.0001	
		Apr. 2019	Jan. 2020	Oct. 2019	9194 ± 463.15	P < 0.0001	

Ostreopsis ovata firstly appeared in July at Prima Cala and Gavetone in 2016 and in June at both sampling stations in 2017. In 2018, first detection occurred in May at Prima Cala and in June at Gavetone. Finally, in 2019, *O. ovata* was first detected in June at Prima Cala and only in August at Gavetone. Interestingly, the final detections of the dinoflagellata occurred in January of the following year. However, Tukey's post-test indicated that there is no significant difference ($p > 0.05$) in the distribution of *O. ovata* between the two sampling stations (Table I).

Abundance of associated diatoms

Other planktonic species have been observed together with *O. ovata*, in particular a variety of diatoms of the Bacillariophyceae class and the following genera: *Coscinodiscus*, *Licmophora*, *Gyrosigma*, *Cocconeis*, *Amphora*, *Campylodiscus*, *Achanantes*, *Bacteriastrum*, *Dactyliosolen*, *Tabellaria*, *Cyclotella*, *Navicula* and *Phaeodactylum*. *Coscinodiscus* and *Ostreopsis* were recorded as the predominant genera in summer. *Coscinodiscus* was more abundant than *Ostreopsis*, (with only one exception in summer 2018) and bloomed almost simultaneously in summer with *O. ovata* (Table I). *Coscinodiscus* reached peaks of proliferation higher than those of the dinoflagellate with concentrations that were 5.72- and 1.25- in 2016, 4.17- and 7.4- in 2017, and in 2019, 2.34- and 2.22-fold higher at Prima Cala and Gavetone, respectively. During summer 2018, *O. ovata* bloom started almost 4 weeks before than that of *Coscinodiscus spp*. at both sampling stations, and the dinoflagellate peaks were 1.62- and 1.01-fold higher than those of the diatom at Prima Cala and Gavetone, respectively. Tukey's post-test revealed that there is a significant difference ($p < 0.01$) in the distribution of *Coscinodiscus* between the two sampling stations (Table I).

DISCUSSION

In this study, volunteers at a citizens' observatory using their own devices have detected: i) the existence of *O. ovata* blooms along the coast at the city of Molfetta; ii) the presence *O. ovata* along the city waterfront from late spring up to winter; iii) the concomitance of *O. ovata* blooms with those of *Coscinodiscus*; iv) peaks of abundance of *Coscinodiscus* higher than those of *Ostreopsis;* v) both *Ostreopsis* and *Coscinodiscus* proliferations start at the station closest to the urban centre.

Recurrent health problems, *i.e.*, a respiratory syndrome caused by *O. ovata* blooming in the Mediterranean area, have necessitated the establishment of a surveillance and alert system in several countries including France, Italy, Monaco and Spain (Cohu *et al*. 2011a, b, 2013, Lemée *et al*. 2012, Vila *et al*. 2019). At Molfetta, at Prima Cala, regular public monitoring of the toxic microalga started in 2009 (http://www.arpa.puglia.it). The levels of *O. ovata* are measured during the summer season only. We have monitored the dinoflagellata over the whole year from July 2016 to December 2019 on a weekly basis at two sampling stations along the waterfront at Molfetta. *Ostreopsis* cells adhere to benthic substrates through filaments and mucilaginous substances forming mucilage and mats that can be easily detached from the benthos

into the water column by high hydrodynamic conditions (*e.g.*, Vila *et al*. 2012, Mangialajo *et al*. 2008, Cohu *et al*. 2013). This method enabled us to demonstrate the presence of *O. ovata* over a long period of time that extends well beyond the summer season. *O. ovata* appears in spring and is present up to winter. *Ostreopsis* blooms have in fact been detected in October in the northern Adriatic Sea (Mangialajo *et al*. 2011). Moreover, our data show that peaks of proliferation occur at different time at distinct places. Pfannkuchen *et al*. (2012) demonstrated that *Ostreopsis* blooms might remain undetected with a high potential to affect human health at the coast. This may be particularly true with the present public surveillance system which triggers alert status when the cell density of *O. ovata* in the water column is above 30.000 cell/L, with a prolonged period of 1 to 7 days under conditions of low hydrodynamics and high temperatures (Funari *et al*. 2014). This idea is reinforced by our findings that *O. ovata* does not appear at the same time and is not present in seawater at the same concentrations at sites that are only 5 km apart.

Herein, we also report the co-occurrence of the proliferation of *Ostreopsis* and *Coscinodiscus*, which was observed during the four years of this study. One possible explanation is the formation of epiphytic dinoflagellate assemblage on macroalgae that have been previously described in the literature. Abundant components of these assemblages include *Ostreopsis* and the diatom of the genus *Coscinodiscus* (Vila *et al*. 2001). At the same time, a correlation between benthic diatoms species diversity and seawater quality has been recently described (Ryabushko *et al*. 2019).

In summary, this study shows that citizens using their own devices can provide useful data to monitor *O. ovata* blooms. In addition, since recurrent respiratory syndromes caused by inhalation of *O. ovata* have been recorded in the Mediterranean area, we recommend to policy makers and managers i) monitoring of the abundance of the toxic microalgae in the bioareosol, and ii) launching a territory-wide investigation on the algal respiratory syndrome in order to establish the appropriate threshold concentration to activate an effective alert system.

Further studies to elucidate the presence/absence of a relationship between *Ostreopsis*/*Coscinodiscus* proliferation and city-caused pollution such as agricultural and urban runoff are also necessary.

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