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ACTIVE AND RESTING HARMFUL DINOFLAGELLATES IN THE HIGHLY PRODUCTIVE MUSSEL CULTURE AREA OF THE MAR PICCOLO (NORTHERN IONIAN SEA)

FORME ATTIVE E DI RESISTENZA DI DINOFLAGELLATE DANNOSE NELL'AREA DI ALLEVAMENTO DI MITILI ALTAMENTE PRODUTTIVA DEL MAR PICCOLO (MAR IONIO SETTENTRIONALE)

Abstract – *The presence of harmful microalgae in mariculture sites represents a risk for production and human health, so the monitoring of these taxa is important for the early warning of harmful algal blooms. The aim of this work was to gain knowledge about the presence and distribution of harmful dinoflagellate taxa in the Mar Piccolo of Taranto (Northern Ionia Sea), through a long-term data series (from 1991 to 2019), using an integrated water/sediment approach. Considering both motile cells in the water and resting stages in the sediments, a total of twenty-two harmful dinoflagellates, were identified in the study area mainly during the summer and fall periods. All the taxa were potentially toxic except* Akashiwo sanguinea*,* Levanderina fissa, Prorocentrum micans *and* Scrippsiella acuminata *complex that are classified as high biomass producers. All these data could be useful to understand the dynamics of potentially toxic dinoflagellates in coastal sites devoted to aquaculture.*

Keywords: *phytoplankton, harmful algae, cysts, monitoring, public health*

Introduction - The impact of Harmful Algal Blooms (HABs) on public health and aquaculture has increased in the last decades and their detection and monitoring is urgent. The production of benthic resting stages (cysts) in the life cycle of many phytoplanktonic harmful species is part of the strategy implemented to escape unfavourable conditions in the water column. This allows to investigate the plankton not only in the water column but also through the analysis of the dormant stages in the sediments to gain a better understanding of the complex functioning of the planktonic system (Rubino & Belmonte, 2021).

The Mar Piccolo of Taranto is one of the most important shellfish farming area in Italy with an annual production of around 40,000 tons (Caroppo *et al.*, 2012). In this basin, HABs have been responsible for massive mussel mortality since 1938, and these events have been often blamed for farmers economic losses (Caroppo *et al*., 2016).

The aim of this manuscript is to demonstrate the effectiveness of integrated water/sediment monitoring through a phytoplankton long-term series of the Mar Piccolo of Taranto.

Materials and methods - From 1991 to 2019, dinoflagellates were investigated both as motile and resting forms at a total of 28 sites in the Mar Piccolo of Taranto (Fig. 1). Motile, i.e., planktonic stages, were sampled in the water column by using a Niskin bottle, while the resting cysts were isolated from surface sediments collected in triplicate by SCUBA divers in a depth range between 5 and 13 meters.

For planktonic cells, water samples, freshly collected, were fixed with Lugol's iodine solution to a final dilution of 1.0%, stored at 4 °C, and analysed within four weeks. Identification and counting were performed under an inverted microscope (Labovert FS Leitz) equipped with a digital camera AXIOCAM ICc 5 (Carl Zeiss, Oberkochen, Germany) and following the Utermöhl method (Edler & Elbrächter, 2010). The minimum

value of enumerated cells was 200 cells per sample, by assuming a confidence limit of 14% (Andersen *et al.*, 2004). Quantitative data are expressed as cells L^{-1}.
For resting forms, the sieving method described in Montresor *et al.* (2010) was adopted on aliquots of 2-3 ml of wet sediment for each replicate. Qualitative and quantitative analyses were carried out under an inverted microscope (Zeiss Axiovert 100) equipped with a Leica MC170HD (Leica Microsystems) digital camera, at ×200 (>75 µm fraction) and ×320-400 (fine-grained fraction) final magnifications. For the fine-grained fraction 200 cysts were counted at least for each replicate to obtain abundance values as homogeneous as possible and to evaluate rare species too; the >75 µm fractions were entirely examined. Quantitative data are expressed as cysts g^{-1} of dry sediment (cysts g^{-1} dw). To summarize the quantitative results, in Table 1, the classes of frequency are reported as percentages of the number of observations of each species *versus* the total of examined samples.

Fig. 1 - The Mar Piccolo of Taranto with the indication of the sampling stations. Green circles: water column; violet stars: sediments (QGIS 3.28.3)
Il Mar Piccolo di Taranto con l'indicazione delle stazioni di campionamento. Cerchi verdi: colonna d'acqua; stelle viola: sedimenti (QGIS 3.28.3)

Results – A total of 22 harmful dinoflagellates were identified in the study area. Among them, 20 were collected in the water column (Fig. 2), mainly during the summer and fall periods, while 2 taxa were observed only as cysts in the sediments. All these taxa are potentially toxins producers, except for *Akashiwo sanguinea*, *Levanderina fissa*, *Prorocentrum micans* and *Scrippsiella acuminata* complex (Tab. 1).
The most frequent taxa were *Scrippsiella acuminata* complex and *Akashiwo sanguinea* (Tab. 1), which reached also higher abundances respect to the other harmful dinoflagellates. *Alexandrium minutum* complex, *Dinophysis sacculus*, *Lingulaulax polyedra*, *Prorocentrum cordatum* and *P. micans* resulted less frequent and with maximum values ranging between 17.5 and 37.9 x 10^3 cells L^{-1}. All the other taxa were observed sporadically and with very low densities.
The analysis of the sediment samples revealed the presence of resting stages belonging to 6 potentially toxic species and 2 high-biomass producer taxa, i.e., *Levanderina fissa* and *S. acuminata* complex (Fig. 2). This latter taxon resulted by far the most abundant and widely distributed, being observed at all the 20 sampling sites. Two of these eight harmful species were identified only in the sediments, i.e., *Levanderina fissa* and *Protoceratium reticulatum*.

Tab. 1 – List of the harmful dinoflagellates detected in the Mar Piccolo of Taranto in water column (M, motile forms) and in sediments (C, dormant cysts). Occurrence frequency related to the harmful dinoflagellate species: "+" = <10%; "++" = 10-40%; "+++" = 40-75%; "++++" > 75%. no = not observed
Elenco delle dinoflagellate dannose rilevate nel Mar Piccolo nella colonna d'acqua (M, forme mobili) e nei sedimenti (C, cisti). Frequenza di rinvenimento delle dinoflagellate dannose: "+" = <10%; "++" = 10-40%; "+++" = 40-75%; "++++" > 75%. no = non osservato

	Life cycle stage	
	M	**C**
Akashiwo sanguinea (K.Hirasaka) Gert Hansen & Moestrup	+++	no
Alexandrium cf. *andersonii* Balech	+	no
Alexandrium minutum complex	++	++
Alexandrium cf. *pseudogonyaulax* (Biecheler) T.Horig. ex Yuki & Fukuyo	+	+
Alexandrium tamarense complex	+	+
Amphidinium carterae Hulburt	+	no
Dinophysis acuminata complex	+	no
Dinophysis caudata Kent	+	no
Dinophysis sacculus F.Stein	++	no
Gonyaulax spinifera (Clap. & J.Lachm.) Diesing	+	no
Levanderina fissa (Levander) Moestrup, Hakanen, Gert Hansen, Daugbjerg & M.Ellegaard	no	+
Lingulaulax polyedra (F.Stein) M.J.Head, K.N.Mertens & R.A.Fensome	++	+++
Margalefidinium polykrikoides (Margalef) F.Gómez, Richlen & D.M.Anderson	+	+
Ostreopsis cf. *ovata* Fukuyo	+	no
Phalacroma rotundatum (Clap. & Lachm.) Kof. & J.R.Michener	+	no
Prorocentrum cordatum (Ostenf.) J.D.Dodge	++	no
Prorocentrum lima (Ehrenb.) F.Stein	+	no
Prorocentrum micans Ehrenb.	++	no
Prorocentrum rhathymum A.R.Loebl., Sherley & R.J.Schmidt	+	no
Prorocentrum cf. *shikokuense* Hada	+	no
Protoceratium reticulatum (Clap. & Lachm.) Bütschli	no	+
Scrippsiella acuminata complex	++++	++++

Cysts abundances ranged between 17 and 2,688 cysts g^{-1} dw, with an average value of 528±481 cysts g^{-1} dw. The resting stages of *Gonyaulax spinifera* and *Scrippsiella acuminata* complex were observed with an occurrence frequency, for each species, greater than 90%. In terms of abundances of such taxa, the highest values (respectively 561 and 2,525 cyst g^{-1} dw) were observed in 2015. Concerning the other taxa, *Alexandrium minutum* group, *A. tamarense*, *L. fissa*, *Lingulaulax polyedra* and *Margalefidinum polykrikoides*, were observed for the first time in 2004; in 2004 and 2005 *A. minutum* group, *A. tamarense*, *L. fissa* and *L. polyedrum* showed the highest values (up to 80 cyst g^{-1} dw). *M. polykrikoides* was the less common species reaching approximately 10% of frequency occurrence and its highest abundance, 50 cyst g^{-1}dw, was reported in 2015. Finally, *P. reticulatum* was observed only in 2015 (24 cyst g^{-1}dw).

Conclusions – Twenty-two harmful dinoflagellate taxa were identified from the two domains of the ecosystem. This great number of harmful dinoflagellates present in the Mar Piccolo of Taranto represents a potential threat for the aquaculture industry.

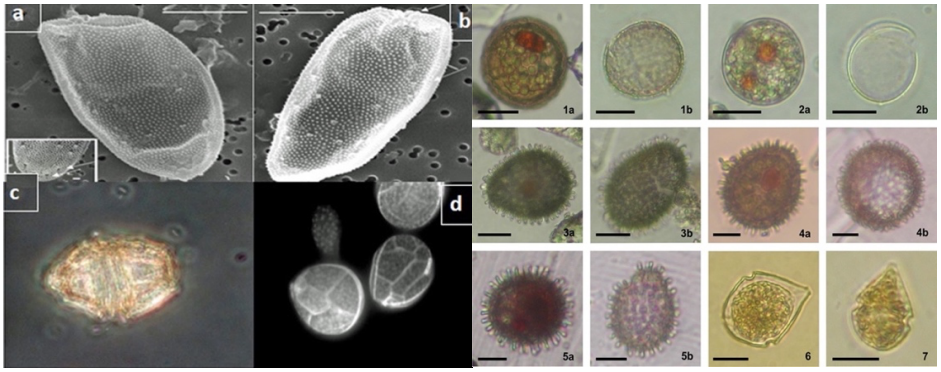

Fig. 2 – On the left: Three harmful dinoflagellates detected in the Mar Piccolo: a), b) *Prorocentrum* cf. *shikokuense* (modified from Roselli et al., 2019); c) *Lingulaulax polyedra;* d) *Ostreopsis* cf. *ovata*. On the right: Different cyst morphotypes produced by *Scrippsiella acuminata* complex, identified in the surface sediments of the Mar Piccolo; a and b refer to viable and germinated cysts respectively; scale bars = 20 µm, except Figs 4a-b and 5a-b with scale bars = 10 µm. 1a-b rough type; 2a-b uncalcified type; 3a-b large type; 4a-b medium type; 5a-b small type; 6. Vegetative stage produced by germination of 1a; 7. Vegetative stage produced by germination of 4a.
A sinistra: Tre specie di dinoflagellate dannose rilevate nel Mar Piccolo: a), b) Prorocentrum *cf.* shikokuense *(modificato da Roselli* et al*., 2019); c)* Lingulaulax polyedra*; d)* Ostreopsis *cf.* ovata*.*
A destra: Diversi morfotipi di cisti prodotti da Scrippsiella acuminata *complex identificati nei sedimenti superficiali del Mar Piccolo; a e b si riferiscono rispettivamente a cisti vitali e germinate; barre di scala = 20 µm, eccetto Fig. 4a-b e 5a-b con barre di scala = 10 µm. 1a-b tipo ruvido; 2a-b tipo non calcificato; 3a-b tipo grande; 4a-b tipo medio; 5a-b tipo piccolo; 6. Stadio vegetativo prodotto dalla germinazione di 1a; 7. Stadio vegetativo prodotto dalla germinazione di 4a.*

Among the taxa identified in the water samples, many of them are not known as resting stage producers, but this aspect needs to be investigated, because in the sediments we found many cyst morphotypes that was not possible to identify at species level. This means that a greater number of harmful/toxic species could be present in the Mar Piccolo.

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