

A new method for sampling potentially toxic benthic dinoflagellates

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Abstract – In the last decades, blooms of *Ostreopsis ovata* are increasingly frequent in several Mediterranean coastal areas, sometimes causing problems to the public health. The quantification of *O. ovata* abundances is generally performed by quantifying the number of cells per gram of macroalga, often preventing the comparison of abundances from different sites if cell quantifications are performed on different algal species. In this paper we propose a sampling method based on the use of a modified plastic syringe designed to quantify benthic cells abundances independently from the type of substratum. The method was tested in the *O. ovata* monitoring carried out in the Gulf of La Spezia (Ligurian Sea) and along the Apulian coasts (Southern Italy) since 2007. In 2009 the “syringe” method was compared with the classic methodology used in national monitoring of *O. ovata*. The syringe method seems interesting in terms of time costs and effectiveness, both for sample collection and processing, allowing quick and simple sampling of several sites along the coastline. The technique turned out to be adequate when a fast and reliable estimate of *O. ovata* “reservoir” near the bottom was required, in order to forecast toxic algal blooms in coastal areas.

Toxic dinoflagellates / *Ostreopsis ovata* / Monitoring / Sampling Methods

INTRODUCTION

Blooms of the picoplanktonic-epibenthic dinoflagellate *Ostreopsis ovata* Fukuyo 1981 have been increasingly reported during the last decades in the Mediterranean Sea. Concerning the Italian waters, the first massive bloom of *O. ovata* was reported in the Ligurian Sea (Gulf of Spezia, Marina di Massa) during August 1998 (Abbate *et al.*, 2007). In the following years, particularly during the summer seasons 2000, 2003, 2005, 2006 and 2008, huge blooms were also reported in other Italian coastal zones (Sansoni *et al.*, 2003; Di Turi *et al.*, 2003; Congestri *et al.*, 2006; Zingone *et al.*, 2006; Monti *et al.*, 2007; Totti *et al.*, 2007; Mangialajo *et al.*, 2008; Ungaro *et al.*, 2010). *O. ovata* is a potentially toxic species, causing environmental warnings during blooms, and sometimes affecting the public health (Gallitelli *et al.*, 2005; Brescianini *et al.*, 2006). This emerging

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issue was faced recently and, in recent years, national monitoring programs were planned to follow the microalgal blooms.

The need of standardized protocols, including the sampling procedure, was highlighted in order to obtain reliable and comparable information. Guidelines to sample *Ostreopsis ovata* at national level were proposed in Italy by ISPRA (2007). The method proposed is the classic method used for the qualitative and quantitative analysis of benthic microalgal communities, mostly represented by pinnate diatoms (Totti *et al.*, 2003). It consists in the collection of macroalgal samples for the quantification of microalgal cells per gram of macroalga (usually wet weight). This method does not necessarily need the use of Uthermol's chambers and inverted microscope, because the density of cells is generally high enough to allow for a direct count with a standard microscope using standard volume chambers (see Cochu *et al.*, 2011 who used 1 ml chambers). The sampling procedures suggested by ISPRA (2007) for the monitoring of *O. ovata* indicate that sampling should be done on the same substrate, but this requirement comes up against the difficulty to find the same macroalgal species in all the monitoring sites, along the whole monitoring period. In case of absence of macroalgae in the sampling sites, a standardized area of bottom has to be considered, for example scraping rocks or collecting sediments where necessary.

According to *in situ* observations, it was highlighted that *Ostreopsis ovata* only loosely adhere to the substrate with a thin filament. The dinoflagellate can be clumped between the ramifications of macroalgae (*e.g. Dictyota dichotoma*), and in calm waters may form a web of filaments covering the surface of the thallus (*e.g. Halopteris scoparia*). On bare substrate, *O. ovata* cells form often a biological film. Mechanical actions (waves, currents, swimmers, divers, etc.) might easily re-suspend cells of *O. ovata* from the substrate (Barone *et al.*, 2006; Abbate *et al.*, 2010). For these reasons we consider that a method based on the suction of seawater in the proximity of the macroalgae (or any other substrate) using a syringe may be a good solution for the quantification of cell abundances, irrespective of the substratum type.

In the present work the "syringe" method, adopted for the first time in the Ligurian Sea during the 2006 *Ostreopsis ovata* blooms (Abbate, 2007), is applied and compared with the "macroalgal sampling method" (ISPRA, 2007). The comparison is done using data collected in Ligurian and Apulian coastal areas during *Ostreopsis* blooms occurred in the year 2009.

MATERIALS AND METHODS

Ostreopsis ovata was sampled using a modified plastic syringe PIC indolor[®] of 100 cc (Abbate *et al.*, 2010). The syringe was cut at about one cm from its tip in correspondence of the Luer-Lock connection, obtaining a suction hole with a suction surface of about 20 mm². To limit the suction of a sample of the desired volume (we expect un-comparable results if different water volumes are sampled) the syringe was modified by inserting a lock flange on the cylinder and installing a stop on the piston rod (Fig. 1). The sampling of the microalgae was carried out holding the syringe at a slight (~30°) angle with respect to the macroalgae surface, so as not to occlude the tip completely. Sampling was performed in each sampling station sucking 20 ml of water (sub-sample) above the

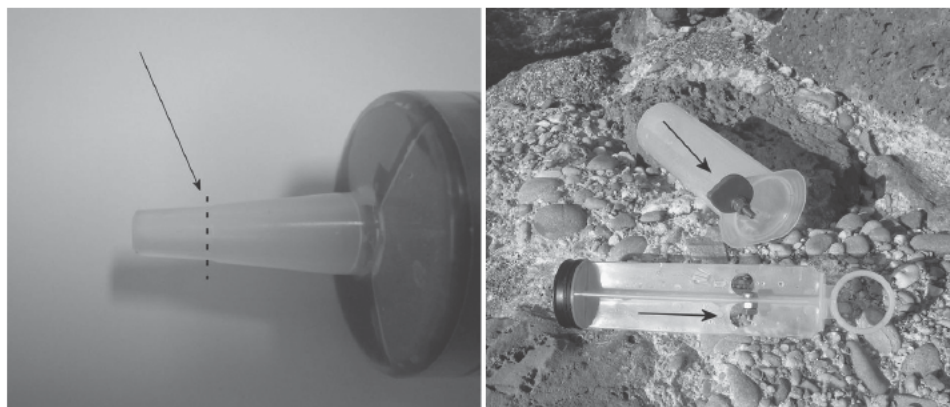


Fig. 1. The modified syringe. On the left: the arrow indicates the cut section on the syringe tip. On the right: lock flange on the syringe cylinder and the stop on the piston rod (see the respective arrows).

surface of each macroalga in three random points in a circle of 10 cm in diameter. The above reported procedures were strictly standardized (e.g. the same angle on the macroalga surface) in order to obtain reliable and replicable samples. The three sub-samples were then mixed to obtain a 60 ml sample. Three replicates of 60 ml were collected in each site and for each macroalga. Samples were fixed with lugol solution or formalin. In laboratory cell counts were carried out according to the Utermöhl method (Utermöhl, 1958). Density was expressed as cells ml^{-1} .

In order to test the sensitivity of the syringe method to *Ostreopsis ovata* presence and abundance and to compare it with the macroalgal sampling method, two experiments were performed in two Italian marine-coastal areas. The first one was conducted from July to September 2009 in “Baia Blu” (La Spezia Gulf-Ligurian Sea; $44^{\circ} 03.700' \text{ N}$; $09^{\circ} 55.478' \text{ E}$), where the syringe method and the macroalgal sampling methods were used to sample *O. ovata* on a shore colonized by *Halopteris scoparia*. The second one was performed in the framework of the monitoring program of the Apulian region. Samples were collected twice in a month in three different sites from North to South of the Adriatic Apulian coast (Tremiti, Bari and Brindisi), both with the syringe method and with the macroalgal sampling method. Three different macroalgal substrata were considered: *Laurencia* spp. in Tremiti islands; *Corallina elongata* in Bari coastal area; *Dictyopteris polypodioides* in Brindisi coastal area.

Another experiment was performed to test if the syringe method was able to detect *Ostreopsis ovata* variability on different substrates and sites. It was performed in July 2008 in the Fiascherino Bay (La Spezia Gulf-Ligurian Sea; $44^{\circ} 03.700' \text{ N}$; $09^{\circ} 55.478' \text{ E}$) on a shore colonized by algal mat (Sp1), *Halimeda tuna* (Sp2), *Dictyota dichotoma* (Sp3). Samples were collected 5 meters apart with the syringe method at 30 cm depth at three stations, characterized by different expositions to wave actions (St.1 low hydrodynamism, St.2 moderate hydrodynamism, St.3 high hydrodynamism). Differences in densities among sites and species were compared through two-ways ANOVA; prior to analysis, Levene’s test was employed to assess homogeneity of variances; as variances were heterogeneous, data were log-transformed. Post hoc comparisons were done using Student-Newman-Keuls (SNK) tests.

RESULTS

In the field, the application of the syringe method is very simple and quick: the collection of the three subsamples per macroalgal species takes no more than 10 minutes per site.

Ostreopsis abundances measured in “Baia Blu” are reported in Fig. 2. The highest values were recorded with the two methods in the second half of July. The correlation of the abundances calculated with the two methods is reported in Fig. 3

Similarly, the monitoring carried out in the Apulian region highlighted a high correlation between the estimates from the two methods ($n = 24$; $r = 0.87$, $P < 0.001$). Higher abundances were recorded in September in Tremiti and Bari and in August in Brindisi (Fig. 4).

The study of *Ostreopsis ovata* variability according to the sites and to the substrates (macroalgal species) using the syringe method in Fiascherino bay showed a significant interaction between the two tested factors (Tab. 1). *Ostreopsis* abundances are consistently different on all sites (algal mats > *Halimeda tuna* > *Dictyota dichotoma*).

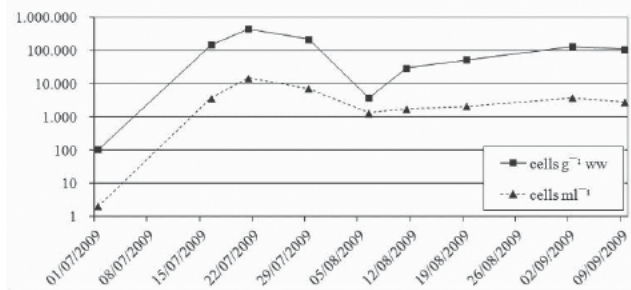


Fig. 2. Abundances measured with the classic macroalgal method (cells g⁻¹ ww) and with the syringe method (cells l⁻¹) in the Ligurian Sea (Baia Blu).

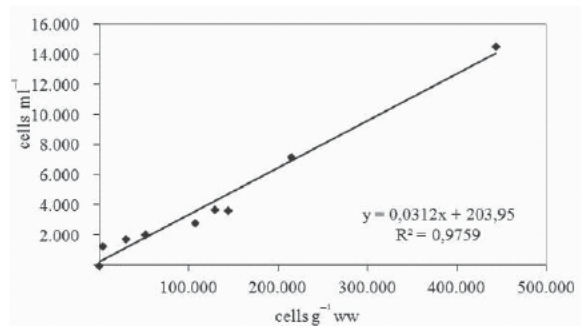


Fig. 3. Correlation between the abundances calculated with the classic macroalgal method (cells g⁻¹ ww) and with the syringe method (cells l⁻¹) in the Ligurian Sea (Baia Blu).

Table 1. Two-ways ANOVA. Site: Site 1 less exposed, Site 2 moderate exposition, Site 3 exposed. Substrate: Sp.1 (algal mat), Sp.2 (*Halimeda tuna*), Sp.3 (*Dictyota dichotoma*)

Source of Variation	df	MS	F	p-level
Site	2	1.1323	44.42	0.0000
Substrate	2	5.6953	82.31	0.0006
Site x Substrate	4	0.0692	2.71	0.0364
Error	72	0.0255		
Total	80			

* SNK: In all sites Algal mats > *Halimeda tuna* > *Dictyota dichotoma* (all differences at $p < 0.01$).

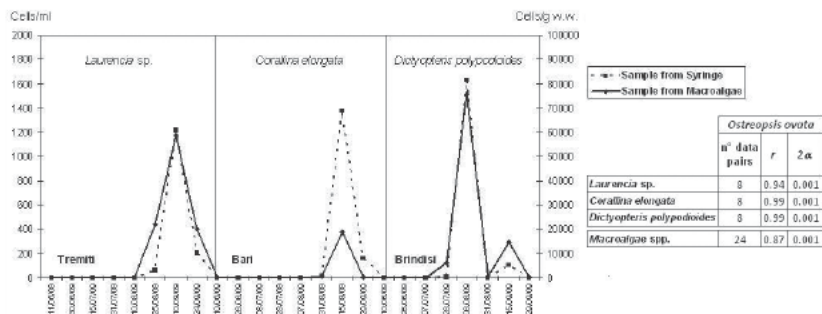


Fig. 4. Comparison among the results the abundances calculated with the classic macroalgal method (cells g⁻¹ ww) and with the syringe method (cells l⁻¹) in the Southern Adriatic Sea.

DISCUSSION AND CONCLUSIONS

The first application of the Italian protocols for monitoring *Ostreopsis ovata* abundances (ISPRA, 2007) pointed out some issues to be discussed and improved, among which the sampling procedure. In particular, the procedure indicated by ISPRA (2007) national guide-lines seems to be inappropriate for some substrate typologies such as the bare rocks and gravels. In fact, the accurate collection of rocks or gravel from the bottom is complicated by the nature and fragility of *O. ovata* cells “anchoring” system, with high risk of losing cells and making the sampling unrepresentative. The syringe method proposed in this paper allows a simple, non destructive and quick collection of samples from stones, rocks or any biological substrata (e.g. mussel beds, Abbate *et al.*, 2010). The syringe method proposed is very easy, quick and may also be performed by personnel with a moderate training.

In our study we showed that the syringe method is appropriate for comparing *Ostreopsis ovata* abundances during blooms, as reported for the Ligurian Sea (Baia Blu) and the Apulian region. The study on natural variability of *Ostreopsis* abundances on three different substrates performed in Fiascherino Bay allowed highlighting consistent trends in the three sites concerning the different substrates considered. In the Apulian waters, the estimates of *O. ovata* density by syringe method were used to obtain a clear and synthetic view of the *O. ovata* occurrence, and the potential relationships with anthropogenic pressures, through the use of a frequency-abundance synthetic index (Ungaro *et al.*, 2010). Additionally, the trends in *Ostreopsis* proliferations observed both in the Ligurian Sea and in the Adriatic Sea (highest values respectively in July and September) are in agreement with what observed in the same two basins by other authors (Mangialajo *et al.*, 2011).

The comparison between the two methods provided promising results, showing high correlations both in the Ligurian Sea and in the Adriatic Sea. The new proposed method does not reduce the amount of information in relation to the routine monitoring targets (localization and quantification of *O. ovata* blooms warnings), and remains a low-cost method in terms of unitary cost and time (although in general the classic macroalgal samples do not need to sediment in Utermöhl's chamber, that is a time consuming process).

Nevertheless, a more detailed comparison of the two methods is needed in order to improve the statistics, potentially find conversion factors with the more common macroalgal species and therefore provide comparable values with other studies.

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