MONITORING TOXIC *OSTREOPSIS* CF. *OVATA* IN RECREATIONAL WATERS USING A QPCR BASED ASSAY

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Abstract
Ostreopsis sp. is a toxic benthic dinoflagellate that causes high biomass blooms involving risks for human health, with negative impacts on marine biota, aquaculture activities and coastal seawater quality. This study reports the application of a rapid and sensitive qPCR method to determine *Ostreopsis* cf. *ovata* abundance in seawater and macroalgal washing seawater samples collected from different Mediterranean coastal sites. *O.* cf. *ovata* quantification is based on site-specific environmental standard curves.

Keywords: Toxic blooms, Monitoring, Dinoflagellates, Mediterranean Sea

Introduction
The dinoflagellate *Ostreopsis* sp. is an epi-benthic microalga including *Ostreopsis* cf. *ovata* known to produce potent non-protein toxins (palytoxin-like compounds and various ovatoxins). Recently, massive blooms dominated by this microalgae, have become frequent also in the Mediterranean Sea [1]. Molecular technologies based on PCR and qPCR were developed for identification and enumeration of *Ostreopsis* spp. in marine environmental samples. These methods have proved to be a fast, specific and sensitive way to confirm taxonomic identity at species level by amplifying target genes [2]. In this study, environmental samples collected at various Mediterranean beaches, were analyzed by a qPCR assay, using site-specific environmental standard curves, to quantify *O.* cf. *ovata* cells [3].

Materials and methods
A total of 18 macroalgae and 16 seawater samples were collected during the summer of 2011, 2012 and 2013 at different Mediterranean beaches: Llavaneres (Spain), Civitavecchia, Trieste, Passetto-Portonovo, Genova, Bari and Taormina (Italy). Samples were fixed, immediately lysed to obtain crude extracts containing genomic DNA or stored at +4 °C until molecular analyses. Seven qPCR site-specific LSU rDNA environmental standard curves (LSU-STD), one for each sampling site, were constructed amplifying 204 bp fragment specific for *O.* cf. *ovata*.

Results
Environmental samples of macroalgea and surface seawater were analysed by both qPCR assay and light microscopy. As LSU rDNA copy number per cell of *O.* cf. *ovata* was different in environmental samples at each of the seven sampled Mediterranean coastal site, to make *O.* cf. *ovata* cell quantification feasible, seven different site-specific LSU-STD curves were used. *O.* cf. *ovata* cells were quantified in all macroalgal samples with the exception of two samples collected at Llavaneres (Spain) in June 2011, where no cells were found. The higher concentration of *O.* cf. *ovata*, $1.18\times10^6 \pm 6.33\times10^5$ cells g^{-1}fw, was found in a sample from Genova (N Tyrrhenian Sea, Italy), while a sample from Taormina (Ioniana Sea, Italy) showed the minimum abundance (772±41 cells g^{-1}fw) by qPCR (Table 1).

Tab. 1. QPCR assay and microscopy analysis of *Ostreopsis* cf. *ovata* abundance from macroalgae samples collected in 2011, 2012 and 2013 at various Mediterranean coastal sites.

Sample No.	Locality	Abundance (cells g^{-1}fw ± SD)	
		qPCR	Microscopy
1	Llavaneres (Spain)	n.d.	n.d.
2	Llavaneres (Spain)	n.d.	n.d.
3	Llavaneres (Spain)	98,677 ± 1091	169,120 ± 2039
4	Llavaneres (Spain)	342,459 ± 43,691	484,086 ± 3913
5	Civitavecchia15888 (Italy)	49,484 ± 3688	36,548 ± 2673
6	Civitavecchia15892 (Italy)	5408 ± 115	6793 ± 1708
7	Trieste Canovella 1 (Italy)	212,797 ± 66,631	241,067 ± 28,706
8	Trieste Canovella 2 (Italy)	208,733 ± 23,336	240,816 ± 6136
9	Trieste Canovella 3 (Italy)	265,722 ± 16,939	239,811 ± 34,194
10	Ancona, Portonovo (Italy)	850 ± 232	1173 ± 774
11	Ancona, Passetto 1 (Italy)	415,062 ± 29,170	417,572 ± 15,025
12	Ancona, Passetto 2 (Italy)	673,835 ± 42,287	689,281 ± 40,586
13	Genova a (Italy)	473,598 ± 30,455	455,607 ± 71,592
14	Genova c (Italy)	1,180,043 ± 63,326	1,143,652 ± 125,136
15	Genova e (Italy)	661,039 ± 10,950	656,814 ± 9676
16	Genova g (Italy)	965,409 ± 79,188	925,962 ± 97,907
17	Taormina St.1 (Italy)	2286 ± 111	1734 ± 270
18	Taormina St. 2 (Italy)	772 ± 41	713 ± 59

Generally, cell concentrations in seawater were lower than on macroalgae samples with the only exception of a sample from Bari (Ionian, Italy) ($1.90\times10^6 \pm 8.45\times10^5$ cells l^{-1}). Interestingly, one sample from Ancona (N Adriatic Sea, Italy), negative by microscopy for *O.* cf. *ovata*, showed 1106 ± 426 cells l^{-1} by qPCR (Table 2).

Tab. 2. QPCR assay and microscopy analysis of *Ostreopsis* cf. *ovata* abundance in seawater samples collected in 2011 and 2013 at various Mediterranean coastal areas.

Sample No.	Locality	Abundance (cells l^{-1} ± SD)	
		qPCR	Microscopy
19	Civitavecchia 15,887 (Italy)	1832 ± 90	1260 ± 85
20	Llavaneres (Spain)	n.d.	n.d.
21	Llavaneres (Spain)	56,320 ± 10,478	97,667 ± 12,503
22	Llavaneres (Spain)	28,405 ± 5226	66,000 ± 5657
23	Ancona, Portonovo (Italy)	1106 ± 426	n.d.
24	Ancona, Passetto (Italy)	92,600 ± 8414	98,400 ± 19,819
25	Genova b (Italy)	44,353 ± 4590	14,500 ± 2121
26	Genova d (Italy)	17,000 ± 1044	23,500 ± 707
27	Genova f (Italy)	58,000 ± 7071	29,778 ± 5441
28	Genova h (Italy)	17,500 ± 707	11,776 ± 1275
29	Bari, Trullo 1 (Italy)	115,750 ± 19,363	142,000 ± 19,305
30	Bari, Trullo 2 (Italy)	135,250 ± 27,476	154,000 ± 26,969
31	Bari, S. Spirito 1 (Italy)	1,077,265 ± 34,479	542,250 ± 148,572
32	Bari, S. Spirito 2 (Italy)	1,907,088 ± 84,540	1,198,750 ± 226,729
33	Bari, Giovinazzo 1 (Italy)	357,255 ± 24,375	131,000 ± 26,255
34	Bari, Giovinazzo 2 (Italy)	841,710 ± 39,675	256,250 ± 25,617

Significant positive correlations between *O.* cf. *ovata* cell densities on macroalgal samples and in water column (n=16, Spearman r=0.8386, p <0.0001) and between abundance determined by light microscopy and qPCR assays (n=16, Spearman r=0.9808, p <0.0001 and n=15, Spearman r=0.9263, p <0.0001 for macroalgae and surface seawater samples, respectively) were found.

Conclusions
Monitoring programs of *Ostreopsis* spp. events provide insight into the dinamics of these marine microbes and can help use to develop new strategies to mitigate their impacts on human health, economic activities and ecosystem functioning. In this study, LSU rDNA gene was used to accurately quantify *Ostreopsis* spp. abundance in natural samples by qPCR method based on the generation of environmental site-specific standard curves. This application allowed a rapid and correct quantification of *O.* cf. *ovata* in field assessing beach water quality during the monitoring activity of the study period.

References
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