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Preliminary assessments of the impact of *Ostreopsis* cf. *ovata* (Dinophyceae) development on macroinvertebrates in the North Western Mediterranean Sea

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Abstract – The development of *Ostreopsis* cf. *ovata*, a toxic benthic dinoflagellate has increased in the North Western Mediterranean Sea, causing health, ecological and economical concerns. In order to understand the ecological impact on macroinvertebrates, a preliminary study was carried on edible grazers, the sea urchin *Paracentrotus lividus* and the limpet *Patella* spp. Their densities were followed toward the abundance of *O.* cf. *ovata*. Thus, in 2008, we performed a monthly field survey in scuba-diving in three sites of the North Western Mediterranean Sea (Genoa, Nice and Villefranche-sur-Mer) at the sea surface and at 1 and 3 m depth. Results showed that at 1 m and 3 m depth, the density of *P. lividus* was not clearly affected by a strong development of *Ostreopsis*. Decrease in *P. lividus* abundance may be due to natural variation or to fishing activities especially in France. Regarding the density of *Patella* spp., only one significant decrease was observed between July and August 2008 in Genoa. *Patella* species are living in the interdital zone, we could therefore hypothese that the limpets were intoxicated either by direct ingestion of *O.* cf *ovata* by grazing and/or by the toxins released in the surrounding water.

Macroinvertebrates / Mediterranean Sea / *Ostreopsis* / *Paracentrotus lividus* / *Patella* spp.

INTRODUCTION

Ostreopsis species produce palytoxin-like compounds (Ciminiello *et al.*, 2008), and have been responsible for human intoxication due to clupeotoxism in 1994 in Madagascar (Onuma *et al.*, 1999). Proliferation of the genus *Ostreopsis* in urbanized coastal areas can induce sanitary, socio-economical and ecological concerns.

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In the last decade, the frequency and intensity of harmful benthic microalgae blooms have considerably increased. Blooms of the dinoflagellate *Ostreopsis* spp. have long been reported in tropical areas; however, recently, those events also occurred in temperate zone (Rhodes, 2011). *Ostreopsis* blooms can now be considered like a common phenomenon along the Northern Mediterranean coasts (Mangialajo *et al.*, 2011). On the French Riviera in the South East of France, *Ostreopsis* spp. was first reported in 1972 in the bay of Villefranche-sur-Mer (Taylor, 1979). Since 2008, massive proliferation of *Ostreopsis* cf. *ovata* Fukyo occurrs every year in this bay. The same phenomenon occurrs in Italy since 1998 (Sansoni *et al.*, 2003; Simoni *et al.*, 2003; Mangialajo *et al.*, 2008; Totti *et al.*, 2010).

Human health distress was recorded, caused by direct contact with cells and/or inhalation of marine aerosols along the French Mediterranean coast (Tichadou *at al.,* 2010) and the Italian shoreline (Brescanini *et al.*, 2006; Ciminiello *et al.*, 2006; Durando *et al.*, 2007). Nevertheless, until now, no intoxication by ingestion of sea-food has been reported.

Adverse effects on marine benthic invertebrates, such as mortality or distress of sea urchins have been recorded in New-Zealand (Shears & Ross, 2009; 2010), in Brazil (Granéli *et al.*, 2002) and in Italy (Simoni *et al.*, 2003; Faimali *et al.*, 2011; Mangialajo pers. comm.). Nevertheless, the potential adverse effect of *Ostreopsis* toxin is still uncertain as others studies have shown no deleterious effects on invertebrates and vertebrates (Yasumoto *et al.*, 1986; Kodama *et al.*, 1989; Mahnir *et al.*, 1992; Lau *et al.*, 1995).

The aim of this preliminary study was to assess the potential impact of the proliferation of *Ostreopsis* cf. *ovata* on *in situ* populations of *Paracentrotus lividus* and *Patella* spp in the Mediterranean Sea.

MATERIALS AND METHODS

From February to December 2008, two sites along the French coast (Nice and Villefranche-sur-Mer) and one in Italy (Genoa) were sampled (Fig. 1) in February, April, June, July, early August, late August and December.

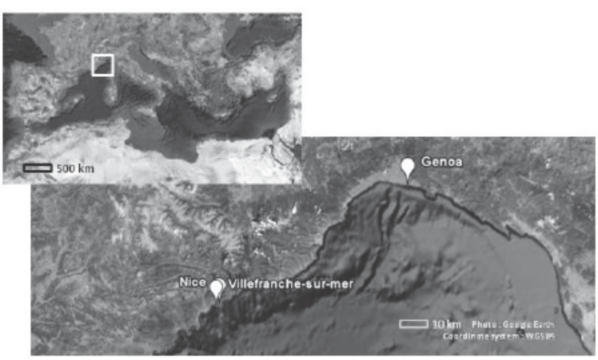

Fig. 1. Maps of the sampling sites along the North Western Mediterranean coast.

To assess the impact of *O.* cf. *ovata* on *P. lividus* population, sea urchin density was measured in ten 1 m^2 quadrat replicates for each date and site at 1 and 3 m depth. No data were available from 3 m depth in Villefranche-sur-Mer where sea urchins were absent. For each sampling date, the abundances of *O.* cf. *ovata* were counted on the Phaeophyceae *Halopteris scoparia* (Linnaeus) Sauvageau at 1 and 3 m depth (n = 3). Macroalgal samples were collected with surrounding water in plastic flasks and fixed with a 2% acid Lugol solution. The cells were separated from the substrate by a vigoruous shake and the water was filtered on a 500 µm mesh grid. Dinoflagellate cells were counted with an optical microscope using calibrated square chambers of 1 ml (Sedgwick Rafter). Results were expressed as numbers of cells per gram of fresh weight of macroalgae ($Cell.g^{-1}FW$) (see methods in Cohu *et al.,* 2011).

To assess the impact of *O.* cf. *ovata* on the *Patella* spp. population, the density of individuals was measured for each date and site on six 1 m long replicate transects. Limpets graze microalgae, cyanobacteria and recruits of macroalgae on the rocks, in the intertidal zone, where *Ostreopsis* from the surrounding water can deposit. We therefore also collected the surrounding water with a plastic flask and fixed it with a 2% acid Lugol solution. Dinoflagellate cells were counted with an inverted microscope using the Utermöhl method on 50 ml of seawater. Results are expressed as number of cells per liter ($Cell.l^{-1}$) (see methods in Cohu *et al.,* 2011).

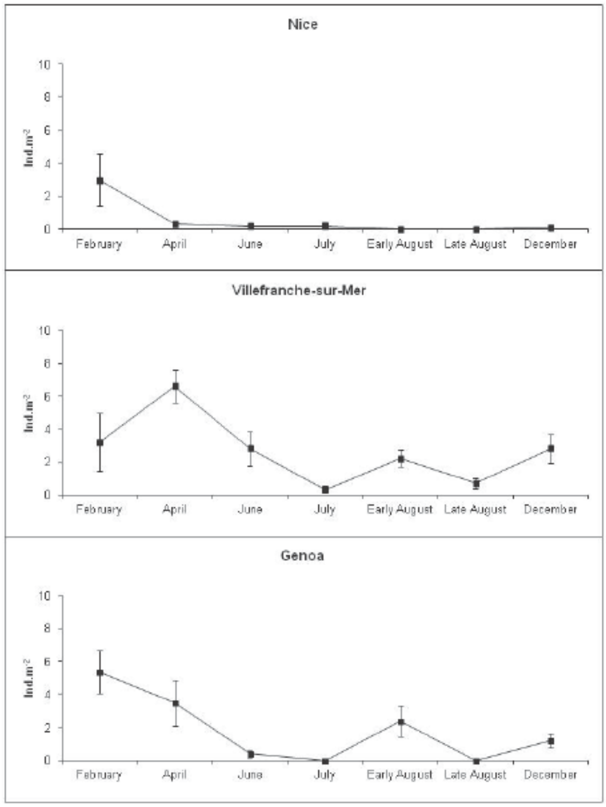

Fig. 2. Evolution of the mean density (± SE) of *Paracentrotus lividus* at 1 m depth, between February and December 2008, in the studied sites.

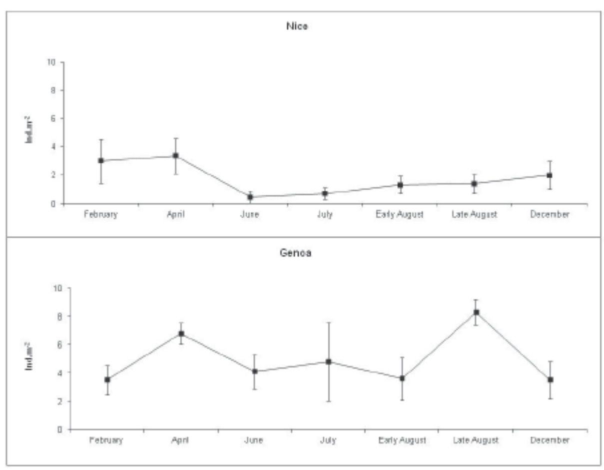

Fig. 3. Evolution of the mean density (± SE) of *Paracentrotus lividus* at 3 m depth, between February and December 2008, in the studied sites.

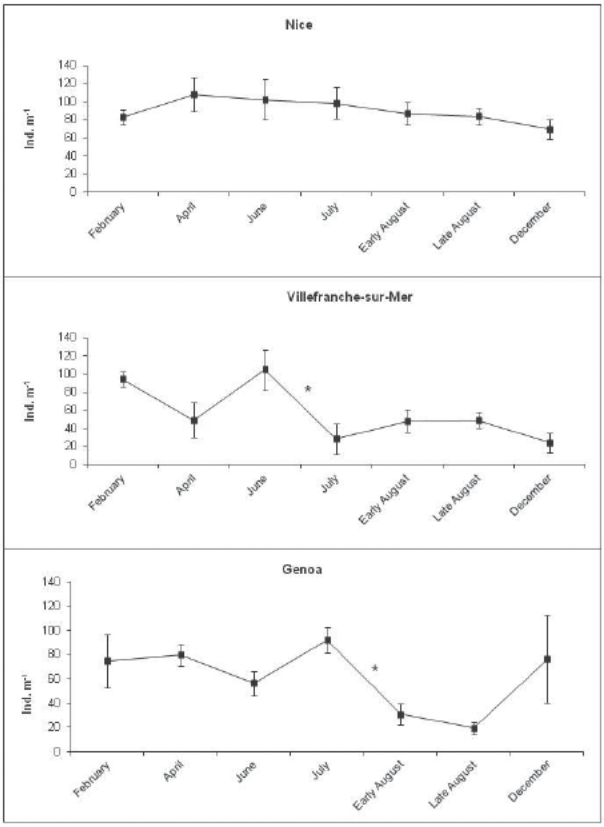

Fig. 4. Evolution of the mean density (± SE) of *Patella* spp. between February and December 2008, in 3 sites along the North Western Mediterranean coast.

Statisticals analysis

Data did not follow the assumptions of parametric tests, we therefore used non parametric Kruskall & Wallis (KW) tests to highlight month by month differences in the densities of sea urchins and limpets. When *O.* cf *ovata* was dectected, densities of *P. lividus* and *Patella sp.* were compared with non parametric SNK procedures.

RESULTS

Impact on *Paracentrotus lividus*

Although we detected important blooms of *O.* cf. *ovata* in Nice, Villefranche-sur-Mer and Genoa, with concentrations higher than 200.000 $Cell.g^{-1}FW$ in July and at the beginning of August (Tab. 1), we did not observe any decrease of *P. lividus* densities related to high abundances of *O.* cf. *ovata.*

At 1 m depth, in Nice, the decrease of *P. lividus* densities ($H_{(6,67)}$ = 21,76 ***) observed between February and April was not linked to *O.* cf. *ovata* (Fig. 2, Tab. 1). In this site densities remained very low from April to December (SNK, p > 0,05). In Villefranche-sur-Mer, densities were considerably variable ($H_{(6,65)}$ = 30,70***) and the decrease observed from April to July could not be linked with high abundances of *O.* cf. *ovata*. During the summer period, densities of sea urchins did not show any significant difference (SNK test p > 0,05) (Fig. 2, Tab. 1). In Genoa, densities of *P. lividus* were also highly variable ($H_{(6,70)}$ = 21,76***). The decrease of the densities observed from February to June were not significant month to month and could not be linked to high abundance of *O.* cf. *ovata* (Fig. 2, Tab. 1).

At 3 m depth, in Nice the densities of *P. lividus* did not vary significatively during the study period ($H_{(6,65)}$ = 9,84 ns) (Fig. 3, Tab. 1). In Genoa, the densities of *P. lividus* were highly variables ($H_{(6,70)}$ = 17,86***) and no significant decrease of *P. lividus* density could be linked with high abundance of *O.* cf. *ovata* (Fig. 3, Tab. 1).

Table 1. Mean *Ostreopsis* cf *ovata* abundances (± SE) on *Halopteris scoparia* (Cell.g^{-1}FW) at 1 m (a) and 3 m depth (b), and in the surrounding water (Cell.l^{-1}) at 0.5 m depth (c) in the three sites studied between February and December 2008

a)

Site	*February*	*April*	*June*	*July*	*Early August*	*Late August*	*December*
Nice	0	0	13 ± 22	311.552 ± 422.125	204.768 ± 89.303	8.085 ± 2.469	0
Villefranche-sur-Mer	0	0	37 ± 64	3.104 ± 3.571	236.495 ± 67.465	3.617 ± 2.024	1.701 ± 1.331
Genoa	0	0	13.449 ± 8.850	281.218 ± 116.901	183.002 ± 124.998	23.171 ± 8.778	0

b)

Site	*February*	*April*	*June*	*July*	*Early August*	*Late August*	*December*
Nice	0	0	0	28.587 ± 11.824	124.352 ± 17.236	3.260 ± 1.013	0
Villefranche-sur-Mer	0	51 ± 47	37 ± 63	764 ± 991	18.203 ± 14.415	4.986 ± 2.788	861 ± 380
Genoa	0	0	4.273 ± 3.753	200.581 ± 57.459	219.953 ± 71.550	45.864 ± 44.064	0

c)

Site	*February*	*April*	*June*	*July*	*Early August*	*Late August*	*December*
Nice	0	0	0	5.873 ± 5.319	6.280 ± 2.346	140 ± 60	0
Villefranche-sur-Mer	0	0	0	127 ± 30	4.447 ± 1.804	507 ± 514	7 ± 11
Genoa	0	27 ± 30	307 ± 197	6.213 ± 1.796	33.880 ± 29.204	107 ± 58	0

Whatever the site and the date of sampling, no signs of suffering of *P. lividus* (*i.e.* loss of spine) had been observed.

Impact on *Patella* spp.

At 0.5 m depth, we recorded high abundance of *O. cf. ovata* in the 3 sites in July and early August (from 4.447 to 33.880 Cell.l^{-1}) with a maximum abundance recorded in Genoa (33.880 Cell.l^{-1} early August, Tab. 1). Although the densities of *Patella* spp. did not significatively differ through time in Nice ($H_{(6,42)}$ = 2,88 ns), they were highly variable in Villefranche-sur-Mer ($H_{(6,42)}$ = 2.88***) and in Genoa ($H_{(6,42)}$ = 17,40**). In Villefrance-sur-Mer, the significant decrease of the density of *Patella* observed from June to July (SNK, $p < 0{,}05$) could not be linked to the abundance of *O.* cf. *ovata*. The following months densities remained similar (SNK, $p > 0{,}05$) (Fig. 4, Tab. 1). In Genoa, the densities of *Patella* were highly variable during the study period ($H_{(6,42)}$ = 17,40**). The significant decrease (SNK, $p < 0{,}05$) observed from July to early August could be linked to the high abundances of *O.* cf. *ovata* (Fig. 4, Tab. 1). This event occurred during the maximum abundance of *O.* cf. *ovata* recorded in this site.

DISCUSSION

These results are the first *in situ* attempt, over a year on a large spatial scale, to assess the potential influence of *Ostreopsis* cf. *ovata* in the Mediterranean Sea. This field work assessed the correlation of the abundance of *O.* cf. *ovata* and the density of *Paracentrotus lividus* and *Patella* spp.

Mortalities of marine organisms concomitant to the presence of *Ostreopsis* have been reported by several authors (Granéli *et al.*, 2002; Simoni *et al.*, 2003; Shears & Ross, 2009; 2010; Faimali *et al.,* 2011). Some mortalities of *P. lividus* with a possible link to high abundance of *O. cf. ovata* have been reported in Marseilles (Ifremer pers. com.) and in Genoa (Mangialajo pers. observation). In our study, when we detected a drecrease of *P. lividus* density, we could not relate it to a high abundance of *O.* cf. *ovata.* Decrease in *P. lividus* abundance may be due to natural variation or to fishing activities especially in France. In 2008 maximum abundance of *O.* cf. *ovata* was 11 times lower than the one recorded in Genoa in 2006 (Mangialajo *et al.,* 2008). We could hypothese that (i) the lethal abundance of dinoflagellates was not reach to impact *P. lividus*, (ii) in Genoa, some individuals of *P. lividus* have adapted to *O.* cf. *ovata* after the two massive blooms in 2005, 2006 (*P. lividus* can live up to 13 years) and (iii) other environmental factors (low shore, high temperature, anoxia, anthropogenic pollution) may also be involved in mortalities as cumulative factors. Indeed, in Genoa the sampling sites is characterised by a degraded environmental quality (vicinity of a harbor, highly urbanized coast) and in Marseilles affected sea urchins were collected under fish farms.

We recorded a significant decrease in *Patella* spp. densities linked to high abundance of *O.* cf. *ovata* in Genoa. Individuals were living in the intertidal zone, grazing on microalgae, cyanobacteria, recruits of macroalgae and encrusting algae. These species forage on reduce surfaces (few cm^2). *Ostreopsis* cf. *ovata* was also present at the sea surface and therefore could have deposited on the rocks or on the algae (authors personal observations). We could hypothese that the limpets were intoxicated by direct ingestion while grazing and/or by the toxins released in the surrounding water. The decrease of the density of *Patella* in Genoa is unlikely due to human collection, since this activity is not common in the region. Until now, no human intoxication by ingestions of seafood has been reported (Aligizaki, 2008).

We are aware that this study does not lead to clear conclusions on cause/effects relationships. Nevertheless given the lack of evidence on mortalities of invertebrates due to *Ostreopsis* bloom, it is worth to show results of observational correlative studies. *Ostreopsis* cf. *ovata* is developping in all the Mediterranean Sea, we recorded the species in almost all the sites sampled along the French Mediterranean coast (personnal observation of the authors). Potentially blooms could occur everywhere. These preliminary results suggest that the impact of *O*. cf. *ovata* on macroinvertebrates is not clear and require further *in situ* studies. To improve the sampling design, we recommend that future *in situ* studies should be carried out in no-take Marine Protected Areas to avoid the artefact due to the recreational gathering of *P. lividus* and *Patella* spp. Moreover, we suggest to intentionally place *P. lividus* individuals in zone infested by a bloom of *O.* cf. *ovata*, to monitor the impact of blooms on the survival of *P. lividus.*

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