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The negative impact of *Ostreopsis* cf. *ovata* on phytal meiofauna from the coastal NW Mediterranean

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Abstract – The tropical benthic dinoflagellate *Ostreopsis* cf. *ovata* recently occurred in the shallow coastal NW Mediterranean where blooms have caused health problems to humans. As part of the MediOs 2 project within the French research program Liteau III, we investigated the possible effects of this toxic microalga on the meiofauna (*i.e.* metazoans ranging from 40 µm to 1 mm in size) inhabiting the very common brown macroalga *Halopteris scoparia*. The macroalga was sampled in triplicate at 0.5 m depth in six stations along the French and Italian coasts on seven occasions in 2008. *Ostreopsis* bloomed in summer in three out of the six stations with abundances ranging from 2.5 to 6.6 10^5 cells g^{-1} macroalgal wet weight. Mean metazoan meiofauna densities ranged from 1274 to 9774 individuals g^{-1} macroalgal spin-wet weight. Statistical analyses revealed that changes in the community structure were associated with high abundances of *Ostreopsis*. The most affected organisms were the nauplii suggesting a negative impact on harpacticoid copepod reproduction.

Harmful Algal Blooms (HABs) / *Ostreopsis* / toxicity / phytal metazoan meiobenthos / subtidal / Mediterranean Sea

INTRODUCTION

The worldwide occurrence of the tropical toxic dinoflagellate *Ostreopsis* cf. *ovata* Fukuyo has increased during the last 15 years, particularly in the coastal Mediterranean (Cohu *et al.*, 2011; Mangialajo *et al.*, 2011) where its summer blooms have caused respiratory affections and skin or eye irritations to people frequenting the beaches (Tichadou *et al.*, 2010). *Ostreopsis* species are preferentially epibenthic/epiphytic on hard substrates and on macrophytes. These substrates are moreover populated by an abundant and diverse faunal community, the phytal meiofauna. These organisms are small benthic metazoans in the "40 µm-1 mm" size class, intermediate between micro- and macrobenthos (Giere, 2009). Because they live in close contact with *Ostreopsis*, they may be affected by the toxic cells. As part of the MediOs 2 project within the French research program Liteau III (Lemée *et al.*, this issue), we conducted the first investigation on the possible impact of *Ostreopsis* on meiofauna associated with the very common brown macroalga *Halopteris scoparia* (L.) Sauvageau.

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MATERIALS AND METHODS

During the year 2008, six stations (CAS, RAM, SRA, RES, MAR and ITA) distributed along the French and Italian NW Mediterranean coasts (Fig. 1) were sampled seven times at different seasons, and more frequently in the summer when *Ostreopsis* is known to bloom. Specimens of *Halopteris scoparia* approximately 5 cm in length were collected in triplicate by scuba diving in large mouth plastic bottles on immersed hard bottoms at half a meter depth. Animals on the macrophytes were narcotised with Magnesium Chloride (Higgins & Thiel, 1988) before they were fixed with Borax-buffered Formaldehyde (4% final). Organisms were extracted from the macrophytes by a series of shakings/sievings under running tap water. Metazoan meiofauna taxa were identified and counted in fractions of the extracted samples under a stereomicroscope. The meiofauna-free macrophytes were centrifuged at low speed (5 minutes at ± 800 rpm) to eliminate excess water and weighed for "spin-wet weight". *Ostreopsis* abundance per macrophyte wet weight (ww) was part of the core parameters measured in the MediOs 2 project. Procedures are given in Cohu *et al*. (2011) and Blanfuné *et al*. (this issue). Changes in the meiofauna community composition were analysed using Correspondence Analysis (CA) with the help of the statistical software SPAD 3.5 (Decisia). Environmental conditions, including *Ostreopsis* abundance, were projected into CA factorial planes as supplementary variables for comparison with meiofauna attributes.

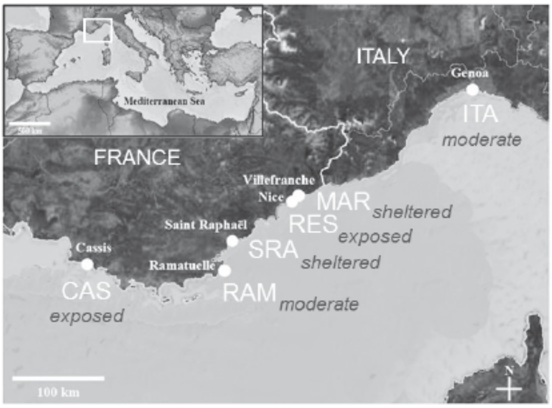

Fig. 1. Location of the 6 sampling sites distributed along the French and Italian NW Mediterranean coasts: CAS (43° 12.770' N - 5° 32.113' E), RAM (43° 11.265' N - 6° 38.730' E), SRA (43° 25.189' N - 6° 51.520' E), RES (43° 41.474' N - 7° 17.546' E), MAR (43° 42.161' N - 7° 19.191' E) and ITA (44° 23.286' N - 8° 59.623' E). Exposure to wave action is indicated for each site.

RESULTS

At all stations, when *Ostreopsis* was absent or in very low numbers, harpacticoid copepods and nauplii (larval copepods) were the most abundant meiofaunal groups on the macrophytes (see example in Tab. 1). They were followed by the nematodes. Ostracods were present, as well as "temporary" crustaceans belonging to the meiofaunal size category only as newly settled larvae that later grow to become macrofauna. They included isopods, tanaids and amphipods. Polychaetes and molluscs (gastropods and bivalves) also belong to the "temporary" meiofauna. Turbellarians and halacarid mites were present. The less represented taxa (regrouped as "others") included insects, pycnogonids, kinorhynchs and tardigrades.

Table 1. Typical taxonomic composition and contribution to total meiofauna on the macrophytes *Halopteris scoparia* in the absence of *Ostreopsis*: example of station RAM in spring where a mean of 4000 individuals of meiofauna occurred in the triplicate macroalgal samples. Nauplii are larval copepods. Amphipods, Isopods and Tanaids are temporary meiofauna (see text); the less represented taxa, *i.e.* Insects, Pycnogonids, Tardigrades and Kinorhynchs are elsewhere regrouped as "others". Contribution of these groups is in last column

Taxon	*Abbreviation*	%	%
Copepods	Cop	18.55	
Nematodes	Nem	16.09	
Polychaetes	Poly	2.38	
Ostracods	Ost	3.66	
Nauplii	Nau	49.72	
Amphipods	Amphi	2.72	2.93
Isopods	Iso	0.06	
Tanaids	Tana	0.14	
Gastropods	Gastro	3.14	
Bivalves	Biv	0.74	
Turbellarians	Turb	1.47	
Halacarids	Hala	0.91	
Insects	Ins	0.11	0.41
Pycnogonids	Pycno	0.08	
Tardigrades	Tardi	0.09	
Kinorhynchs	Kin	0.14	
Total		100	

At all stations mean total meiofauna abundances on the macrophytes exhibited significant temporal fluctuations (Fig. 2). They followed a clear seasonal pattern, reaching maximum numbers during the summer when water temperature was warm. Differences between stations occurred however in the maximum mean abundances, which were higher in stations CAS (9774 individuals.g^{-1} of macrophyte) and MAR (7993 individuals.g^{-1}) than in the other stations. The lowest summer maximum occurred in station RAM (4840 individuals.g^{-1}). Moreover, in some instances and particularly in station ITA, mean abundances dropped amidst the summer as if the expected increase had been interrupted. In Fig. 2 the stations are set in a geographical order. From West to East the maximum water temperature increased from around 24°C in stations CAS and RAM to around 28°C in stations MAR and ITA. *Ostreopsis* cell abundances on the macrophytes closely followed the temperature increase both within the stations, and among the stations. The maximum summer abundance was relatively low in the Western stations, *i.e.* below 10,000 cells.g^{-1} of macrophyte at stations CAS and RAM. It gradually increased towards the East, reaching a value 100 times higher at station ITA. Furthermore, at station ITA, the toxic bloom lasted at least 20 days (time interval between sampling periods 4 and 5), whereas it was shorter in all the other stations (Fig. 2). So the summer decrease in total meiofauna mean abundances at station ITA could be the result of an outstanding development of *Ostreopsis*.

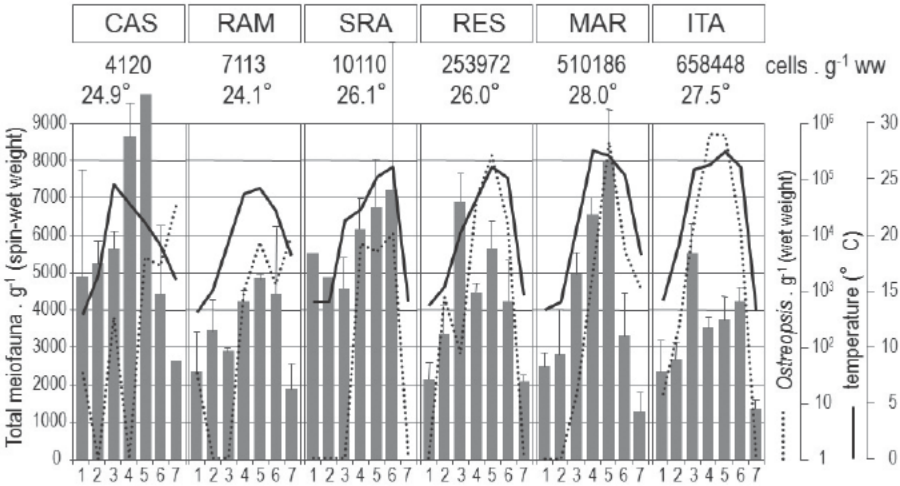

Fig. 2. Temporal fluctuations of mean (and standard deviation) total meiofauna abundances on the macrophytes collected at the 6 stations CAS, RAM, SRA, RES, MAR and ITA on 7 occasions (1: February-March; 2: April-May; 3: June; 4: early July; 5: late July; 6: August; 7: fall-winter) during the year 2008 (units on the left). Black curve is water temperature, dotted line mean *Ostreopsis* abundance on the macrophytes (units on the right). Maximum temperature and *Ostreopsis* summer abundance at each station are above the graphs.

A first CA, applied to all the samples collected, showed that most of the variability in the meiofauna assemblage (31.6% for axis 1) was seasonal and essentially driven by water temperature. Because *Ostreopsis* abundance was strongly correlated to water temperature, this analysis did not discriminate between the two environmental variables.

In an attempt to minimise the influence of water temperature, we considered the situations when it was almost constant, at least above some threshold (here 24°C), and performed another CA, based on the summer samples only (Fig. 3). Axis 1 represented more than 38% of the variability. It clearly opposed nematode richness to nauplii richness as well as station SRA to station RAM. But this axis had little to do with *Ostreopsis* abundances. So the nematode-nauplii opposition would not be due to an *Ostreopsis* effect but rather to differences in the sedimentary conditions at these two stations. Observation of the extracted samples showed that macrophytes from the sheltered station SRA were much richer in sediment than those from the moderately exposed station RAM. The presence of sediment increases the contribution of nematodes to the phytal meiofauna assemblage whereas harpacticoid copepods and offspring (nauplii) are favoured in exposed phytal zones (Giere, 2009).

The *Ostreopsis* axis was clearly Axis 2 (19.2% of the variance). It opposed richness in halacarids (and gastropods to a lesser extent) to richness in nauplii. The samples and the stations were distributed along this axis from the richest in *Ostreopsis* (ITA) to the poorest (RAM and CAS). This suggests that strong *Ostreopsis* blooms, like the one that occurred at station ITA, would favour the development of halacarids, and have a negative effect on nauplii. This conclusion is further illustrated in Fig. 4 that compares the two extreme stations with respect to the extent of *Ostreopsis* development, *i.e.* RAM with low

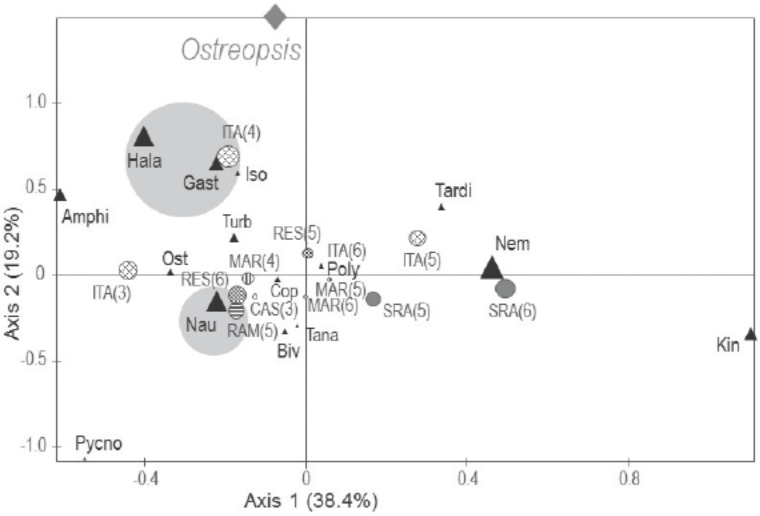

Fig. 3. First two factorial axes of the Correspondence Analysis including the samples collected at the 6 stations CAS, RAM, SRA, RES, MAR and ITA during the summer 2008 (3: June; 4: early July; 5: late July; 6: August) when the water temperature was above 24°C. Active variables are the taxa contributions to the meiofauna community on the macrophytes (triangles). Supplementary variables are environmental data (diamond). Circles represent samples and stations. Shaded areas highlight the main result of the analysis. See Tab. 1 for abbreviations.

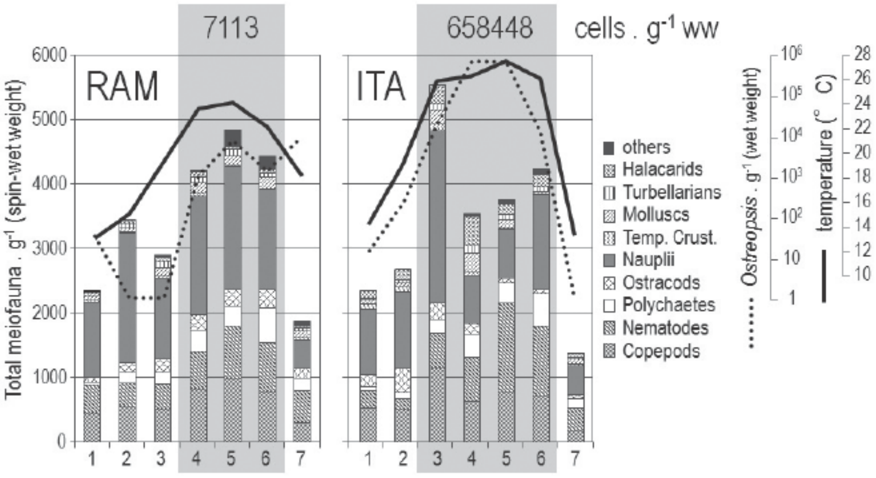

Fig. 4. Temporal fluctuations of meiofauna taxa mean abundances on the macrophytes collected at the 2 stations RAM and ITA on 7 occasions (1: February-March; 2: April-May; 3: June; 4: early July; 5: late July; 6: August; 7: fall-winter) during the year 2008 (units on the left). Black curve is water temperature, dotted line mean *Ostreopsis* abundance on the macrophytes (units on the right). Maximum *Ostreopsis* summer abundance at each station is above the graphs. Shaded area is the summer period. See text for definitions of "Temporary Crustaceans" (Temp. Crust.) and "others".

Ostreopsis abundance and ITA with the strongest *Ostreopsis* bloom. During the summer, the mean contribution of halacarids to the meiofauna assemblage was lower at station RAM than at station ITA (1.4% against 6.8%, respectively). Moreover, the nauplii contribution remained high during the entire summer at station RAM (around 40%), but decreased substantially (from 49% to 20%) at station ITA when *Ostreopsis* reached its maximum abundance. As a consequence, the summer drop in total meiofauna mean abundances (from 5524 to 3528 individuals.g^{-1} between periods 3 and 4) at station ITA mainly resulted from the decrease in mean nauplii numbers (from 2680 to 740 individuals.g^{-1}). Note that copepod numbers also declined (from 1128 to 620 individuals.g^{-1}), along with those of the other crustaceans (ostracods and the temporary fauna).

DISCUSSION

Meiofauna includes many different taxa, which contribute numerically in different proportions to the total assemblage depending on the habitat (Giere, 2009). In the present study, when *Ostreopsis* was absent or in low numbers on *Halopteris scoparia*, the meiofauna community composition was typical of phytal assemblages (Hicks & Coull, 1983) where copepods and nauplii largely dominate over all the other taxonomic groups.

The marked temporal variations in the meiofaunal population abundances found in the present study are similar to those usually reported in the literature. Since reproductive and development rates (and ultimately abundance) are known to be positively correlated with temperature, it is not surprising that most taxa have their maximum abundance in warmer months (Giere, 2009). Other factors such as food supply may also control or modify abundance of a local population. Potential food is rarely limiting in phytal habitats where the trophic resources are the components of the algal “Aufwuchs” community, *i.e.* dense populations of heterotrophic bacteria, cyanobacteria, diatoms, phytoflagellates, fungi and ciliates, associated with mucilage on algal surfaces (Hicks & Coull, 1983). Nevertheless, because this epigrowth is also subject to seasonal fluctuations, food availability can be a significant feature superimposed on any temperature influence as shown by the results of our first Correspondence Analysis. In this analysis we could not discriminate between the influence of temperature and that of *Ostreopsis* (*i.e.* one of the components of the epigrowth of *Halopteris scoparia*) on the meiofauna community. In our second Correspondence Analysis we found that less than 20% of the variability in the meiofauna community of *Halopteris scoparia* was due to *Ostreopsis*, and only in summer. This somewhat low impact is probably because the *Ostreopsis* blooms were relatively small in most of the sites of our study. Only in station ITA was the *Ostreopsis* bloom intense enough (both in magnitude and duration) to induce a clear negative effect on nauplii numbers. Because nauplii were essentially larval copepods, this result suggests that *Ostreopsis* had a negative effect on copepod reproduction. Studies by Ianora *et al.* (e.g. 2003, 2004) have demonstrated that toxins of some planktonic diatoms and dinoflagellates had a negative effect on planktonic copepod reproduction, e.g. maternal “toxic” diets affected not only egg hatching success, but also the development of nauplii that did hatch. In the phytal habitats, benthic copepods feed on the different components of the algal epigrowth, and such a diverse diet leads to high reproductive performances (Guidi, 1984). In periods of dense blooms, *Ostreopsis* cell numbers may become so high on the macrophytes that the toxic cells prevail over all the other components of the epigrowth. In such a situation, the phytal fauna is probably "forced" to feed on *Ostreopsis*. Although harpacticoids are capable of selective feeding, food discrimination and rejection (Hicks & Coull, 1983), they may not necessarily detect the toxicity of *Ostreopsis*. Meiofauna has been widely used in pollution studies to assess ecosystem health and it is now well established that harpacticoid copepods are one of the most sensitive meiobenthic taxon to all kinds of pollutants (Giere, 2009). However no previous work has ever been carried out on the impact on meiofauna of benthic Harmful Algal Blooms (HABs) or *Ostreopsis* blooms. Only sparse laboratory observations have been made on "attacks" of benthic copepods and nematodes by *Ostreopsis* (Barone, 2007) or on ingestion of *Ostreopsis* cells by Turbellarians and Polycheates (Abbate, personal communication). Several field studies of megabenthic organisms, have moreover reported mass mortalities of sea urchins and shellfishes during *Ostreopsis* blooms in Italy and New Zealand (see Cohu *et al*., 2011, and references therein).

The present study revealed that intense *Ostreopsis* blooms had a negative impact on phytal meiofauna. The community was not affected as a whole, but the number of nauplii was considerably reduced. This suggests that *Ostreopsis* affected harpacticoid copepod reproduction. Further studies are needed, particularly experimental work including toxicity bioassays, to understand the mechanisms involved.

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REFERENCES

BARONE R., 2007 — Behavioural trait of *Ostreopsis ovata* (Dinophyceae) in Mediterranean rock pools: the spider's strategy. *Harmful algae news* 33: 1-3.

BLANFUNE A., COHU S., MANGIALAJO L., LEMÉE R. & THIBAUT T., 2012 — Preliminary assessments of the impact of *Ostreopsis* cf. *ovata* (Dinophyceae) development on macroinvertebrates in the North Western Mediterranean Sea. *Cryptogamie, Algologie* 33(2): 129-136.

COHU S., THIBAUT T., MANGIALAJO L., LABAT J.P., PASSAFIUME O., BLANFUNE A., SIMON N., COTTALORDA J.M. & LEMÉE R., 2011 — Occurrence of the toxic dinoflagellate *Ostreopsis* cf. *ovata* in relation with environmental factors in Monaco (NW Mediterranean). *Marine pollution bulletin* 62: 2681-2691.

GIERE O., 2009 — *Meiobenthology. The microscopic fauna in aquatic sediments*, 2nd Edition. Berlin, Springer-Verlag, 527 p.

GUIDI L.D., 1984 — The effect of food composition on ingestion, development, and survival of a harpacticoid copepod, *Tisbe cucumariae* Humes. *Journal of experimental marine biology and ecology* 84: 101-110.

HICKS G.R.F. & COULL B.C., 1983 — The ecology of marine meiobenthic harpacticoid copepods. *Oceanography and marine biology annual review* 21: 67-175.

HIGGINS R.P. & THIEL H., 1988 — *Introduction to the study of meiofauna*. Washington D.C., London, The Smithsonian Institution Press, 488 p.

IANORA A., POULET S.A. & MIRALTO A., 2003 — The effect of diatoms on copepod reproduction: a review. *Phycologia* 42: 351-363.

IANORA A., TURNER J.T., ESPOSITO F., CAROTENUTO Y., D'IPPOLITO G., ROMANO G., FONTANA A., GUISANDE C. & MIRALTO A., 2004 — Copepod egg production and hatching success is reduced by maternal diets of a non-neurotoxic strain of the dinoflagellate *Alexandrium tamarense*. *Marine ecology progress series* 280: 199-210.

LEMÉE R., MANGIALAJO L., AMZIL Z., BLANFUNE A., CHOMERAT N., COHU S., GANZIN N., GASPARINI S., GROSSEL H., GUIDI-GUILVARD L., HOAREAU L., LE DUFF F., MARRO S., SIMON N., NEZAN E., PEDROTTI M.-L., SECHET V., SOLIVERES O. & THIBAUT T., 2012 — Interactions between scientists, managers and policy makers in the framework of the French project "Mediterranean *Ostreopsis*" (2008-2010). *Cryptogamie, Algologie* 33(2): 137-142.

MANGIALAJO L., GANZIN N., ACCORONI S., ASNAGHI V., BLANFUNE A., CABRINI M., CATTANEO-VIETTI R., CHAVANON F., CHIANTORE M., COHU S., COSTA E., FORNASARO D., GROSSEL H., MARCO-MIRALLES F., MASO M., RENE A., ROSSI A.M., SALA M.M., THIBAUT T., TOTTI C., VILA M., LEMÉE R., 2011 — Trends in *Ostreopsis* proliferation along the Northern Mediterranean coasts. *Toxicon* 57: 408-420.

TICHADOU L., GLAIZAL M., ARMENGAUD A., GROSSEL H., LEMÉE R., KANTIN R., LASALLE J.L., DROUET G., RAMBAUD L., MALFAIT P. & HARO L.D., 2010 — Health impact of unicellular algae of the *Ostreopsis* genus blooms in the Mediterranean Sea: experience of the French Mediterranean coast surveillance network from 2006 to 2009. *Clinical toxicology* 48: 839-844.