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Management of *Ostreopsis* blooms in recreational waters along the Catalan coast (NW Mediterranean Sea): cooperation between a research project and a monitoring program

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Abstract – As shown in this report, the integration of a research project with a monitoring program improves the detection and management of *Ostreopsis* blooms in Catalonia. The research project benefits from information previously obtained from several localities by the monitoring program, which in turn profits from the specific findings and conclusions contributed by the research project.

***Ostreopsis* blooms / harmful algae / benthic or epiphytic microalgae / respiratory irritation / Mediterranean Sea**

INTRODUCTION

Reported occurrences of the genus *Ostreopsis* in temperate coastal waters have increased during the last 15 years. These events are noteworthy because the blooms produced by several of its member species cause harmful environmental effects in some areas (Shears & Ross, 2009; Mangialajo *et al*., 2011). In Catalan waters, *Ostreopsis* was detected for the first time in several

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harbors between the years 1995 and 1999 (Vila *et al*., 2001a); shortly thereafter, an intensive 1-year study of *Ostreopsis* was carried out in a shallow protected rocky habitat in Palamós (Costa Brava, NW Mediterranean) (Vila *et al*., 2001b). A spatial distribution analysis conducted in the summer of 2004 along 234 Catalan beaches identified blooms (abundances $> 10^4$ cells·L^{-1} in seawater) along a few specific beaches (2%), but only in some cases were they associated with harmful effects (Vila *et al*., 2008).

In Catalan seawaters, the first epidemiological study relating high concentrations of *Ostreopsis* with respiratory symptoms in people living close to the beach also dates to 2004 (Alvarez *et al*., 2005). The described bloom occurred in Sant Andreu de Llavaneres (hereafter Llavaneres) and the analysis of the event was the product of a collaboration between the Catalan Water Agency (ACA) and the Public Health Department. The same event occurred two years later.

In response to the growing concern regarding the consequences of Mediterranean *Ostreopsis* blooms, the Spanish Ministry for Science and Innovation funded the Project EBITOX "*Study of the biological and toxinological aspects of benthic dinoflagellates related to human health risks"* starting in 2009. The main aim of the project is to determine whether *Ostreopsis* blooms are the source of toxicity events in Llavaneres. In addition to this research project, there is an ongoing monitoring program *"Monitoring of harmful and noxious phytoplankton along the Catalan coast*," which is a collaboration contract between the ACA and the Institute of Marine Science ICM (CSIC). Here, we report on the results of the collaboration between the research project and the monitoring program and the mutual benefits obtained in terms of the management of *Ostreopsis* blooms in Catalonia.

METHODS

The program "Monitoring of harmful and noxious phytoplankton along the Catalan coast" is a collaborative contract between the ACA and ICM (CSIC) that began in the year 2000. The part of this program devoted to monitoring *Ostreopsis* blooms is based on the regular sampling of 14 beaches (Tab. 1). During the 5-year period considered in this paper, phytoplankton samples were taken monthly at each station, with an increase in the sampling frequency to twice a month at some stations during the summer months (from May to September). Thus, between 50 and 80 samples were obtained from each station. In addition, 240 beaches were visually monitored by several ACA inspectors almost daily during the summer. In case of seawater discolorations, the presence of scum, or other observations indicative of a noxious bloom, such as skin irritations in beachgoers, the ACA inspectors collected phytoplankton samples (Lugol-fixed) for microscopic observations.

The EBITOX project involves detailed study of the *Ostreopsis* population at a single site, Llavaneres (Tab. 1). The station is a fossil beach in the middle of an open and extended sandy area and is highly colonized by different species of macroalgae (including the genera *Corallina, Stypocaulon, Dictyota* and *Padina*), which are the substrate of the *Ostreopsis* population. The aims of the project are: (1) morphological and genetic characterization of different *Ostreopsis* strains; (2) studies of the temporal and spatial distribution of

Table 1. Sampling stations, listed from north to south: location and type of beach, date and level of the maximum *Ostreopsis* concentrations, and their percentage of detection in each station

Station name	*Latitude and Longitude*		*Type*	*Maximum concentration (cells·L^{-1})*	*Date*	*Detection percentage (%)*
Montjoi	42º 15'02''N	3º 13'41''E	Cove (rocky area)	24566	02/09/08	34
Canyelles Petites	42º 14'45''N	3º 11'55''E	Cove (rocky area)	20689	02/09/08	50
Desemb. Muga	42º12'10''N	3º06'44''E	Open (sandy beach)	80	03/08/06	1
Estartit	42º02'29''N	3º11'48''E	Open (sandy beach)	160	13/08/08	5
Fosca	41º51'26''N	3º08'38''E	Cove (sandy and rocky)	2800	29/08/08	29
Canyet	41º45'34''N	2º58'51''E	Cove (rocky area)	14878	09/08/06	33
Ses Illetes	41º 43'21''N	2º 56'22''E	Open (rocky area)	34445	25/07/06	29
Cavaió	41º 34'46''N	2º 33'57''E	Open (sandy beach)	1760	28/07/08	8
Llavaneres	41º 33'07''N	2º 29'31''E	Open beach, rocky platform in a sandy area	205632	28/07/08	40
Castelldefels	41º15'44''N	1º56'25''E	Open (sandy beach)	1680	27/08/10	5
Llarga	41º07'45''N	1º18'52''E	Open (sandy beach)	n.d.		0
Arenal	40º59'09''N	0º54'49''E	Open (sandy beach)	200	13/09/06	8
Alguer	40º53'04''N	0º48'20''E	Open (sandy and rocky)	2400	22/07/09	30
Platjola	40º33'27''N	0º39'47''E	Open (sandy beach)	320	25/08/08	2

n.d.: not detected (< 40 cells·L^{-1})

Ostreopsis; (3) the description of the *Ostreopsis* life cycle; (4) determination of the *Ostreopsis* toxin profile in isolated and cultivated strains; (5) determination of *Ostreopsis* cell abundances and toxin levels in marine aerosols; and (6) measurements of toxins in samples of biota (mollusks) taken from areas of bloom occurrence.

Phytoplankton and phytobenthos samples (alive, Lugol-fixed, formalin-fixed, and filtered) were sampled between two and five times a month in Llavaneres during the potential bloom season and monthly during the rest of the year. Living samples were used for *Ostreopsis* isolation, culture establishment, and life cycle experiments. Lugol-fixed samples were used to determine cell concentrations, for microscopy-based identification, and for molecular analyses (Battocchi *et al*., 2010). Formalin-fixed samples were used for observation of the nuclei and thecal plates (Bravo *et al*., 2012), and filtered samples for palytoxin analyses (Riobó *et al*., 2006; Riobó *et al*., 2008). During a bloom, marine aerosols were periodically sampled with high-volume air samplers fitted with 15-cm diameter quartz fiber filters. The aerosols were then analyzed for the presence of palytoxin and *Ostreopsis* cells (by qualitative real-time PCR and SEM observation using gold-coated samples). Finally, benthic fauna were also sampled for palytoxin content.

RESULTS AND DISCUSSION

Ostreopsis in recreational Catalan waters

Regular phytoplankton monitoring of several stations over a period of several years was able to provide accurate information on the presence of *Ostreopsis*, the frequency of its blooms, and their temporal distribution along the Catalan coast (Fig. 1). Data from the monitoring program were crucial in the choice of Llavaneres as a pilot station for the EBITOX project. At present, the monitoring program also provides sampling facilities and data obtained from other stations along the Catalan coast.

Ostreopsis was detected in the plankton of 13 of the 14 Catalan beaches inspected (Tab. 1). Among these, the highest *Ostreopsis* concentrations were found in Llavaneres, where a maximum was detected in summer 2008 ($> 2 \cdot 10^5$ cells·L^{-1} at the end of July). While at four other beaches (Montjoi, Canyelles Petites, Canyet and Ses Illetes) high concentrations were also detected, they were at least one order of magnitude lower ($> 10^4$ cells·L^{-1}) than at Llavaneres. Overall, *Ostreopsis* accounted for 29-50% of the samplings at the five beaches. These areas of high *Ostreopsis* abundances were characterized by rocky bottoms or coves with rocky side walls; by contrast, at sandy beaches the *Ostreopsis*

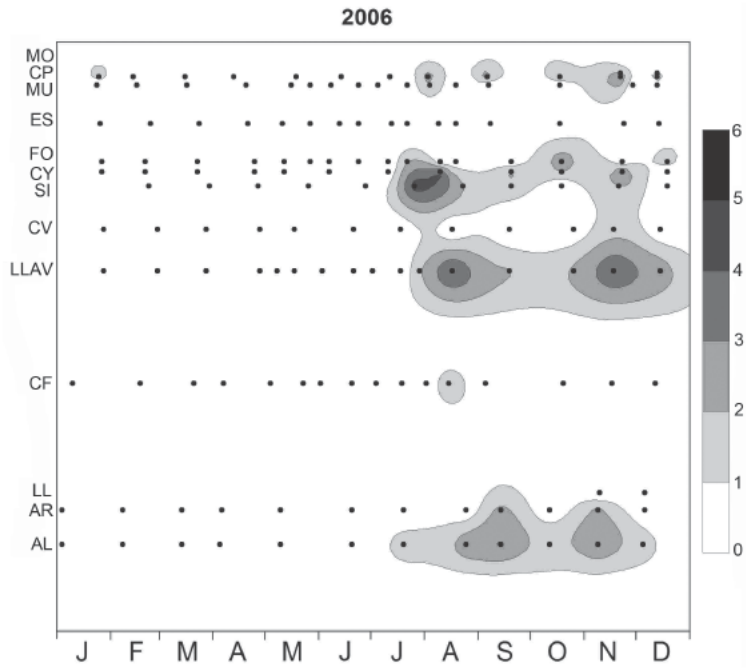

Fig. 1. Spatio-temporal distribution of *Ostreopsis* along the Catalan coast during 2006. Station codes: MO (Montjoi), CP (Canyelles Petites), MU (Desemb. Muga), ES (Estartit), FO (Fosca), CY (Canyet), SI (Ses Illetes), CV (Cavaió), LLAV (Llavaneres), CF (Castelldefels), LL (Llarga), AR (Arenal), AL (Alguer), PJ (Platjola). Gray scale indicates the *Ostreopsis* concentration in the water column. 0: *Ostreopsis* not detected; 1: 10^1 cells·L^{-1}; 2: 10^2 cells·L^{-1}; 3: 10^3 cells·L^{-1}; 4: 10^4 cells·L^{-1}; 5: 10^5 cells·L^{-1}; 6: 10^6 cells·L^{-1}.

concentration never exceeded 2000 cells·L^{-1}. Furthermore, the rocky substrates were in all cases colonized by macroalgae, indicating that *Ostreopsis* blooms in Catalonia are related to the presence of macroalgae. The development of blooms directly upon hard substrata (rocks and mollusk shells), as observed in some areas of Italy (Totti *et al.*, 2010), has never been documented in our study area.

The genus *Ostreopsis* was simultaneously detected in several stations during the second half of the each study year (*i.e.*, beginning in June or July) (Figs 1-5). Among the 13 different beaches where *Ostreopsis* was detected, maximum abundances were reached at seven in 2008, at four in 2006, at one in 2009, and at one in 2010 (Tab. 1). At each of these sites, the highest plankton concentrations were detected between the end of July and the beginning of September, consistent with the findings reported for other NW Mediterranean sites (Mangialajo *et al.*, 2011). In addition, as was the case at those sites, a second bloom occurred in Llavaneres, from October to November, or in November (Figs 1-4). In 2010, the *Ostreopsis* bloom started at the beginning of August and lasted until mid-November.

The highest concentrations of *Ostreopsis* in the plankton were detected during sampling carried out within the framework of the EBITOX project, most likely due to the high sampling frequency during the bloom season. Indeed, these measurements revealed concentrations of 10^5-10^6 cells·L^{-1}, detected on a few occasions during the blooms, whereas concentrations of 10^3-10^4 cells·L^{-1} were usually recorded in July and August. The highest epiphytic concentrations determined were of the order of several millions of cells·g^{-1} fresh weight macroalgae. *Ostreopsis* cells were hardly found during the winter, the exception

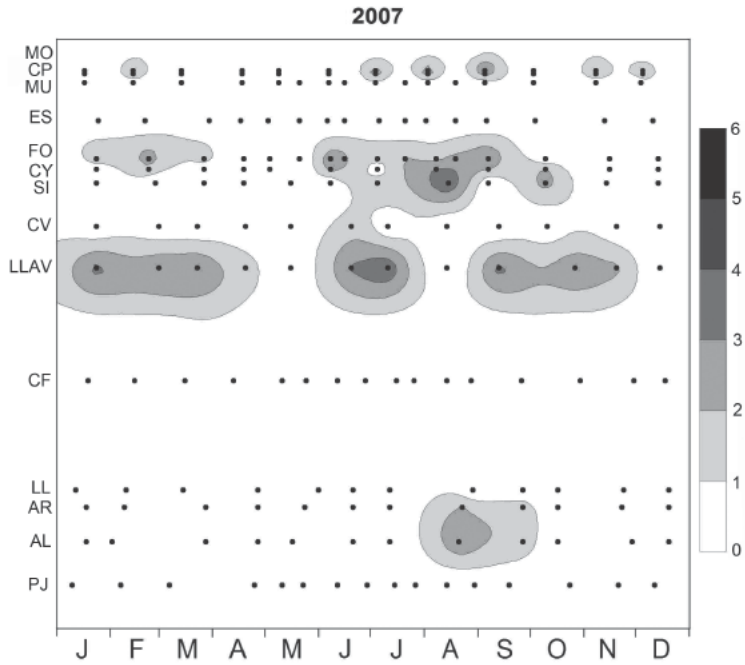

Fig. 2. Spatio-temporal distribution of *Ostreopsis* along the Catalan coast during 2007. Station codes and *Ostreopsis* concentrations as in Fig. 1.

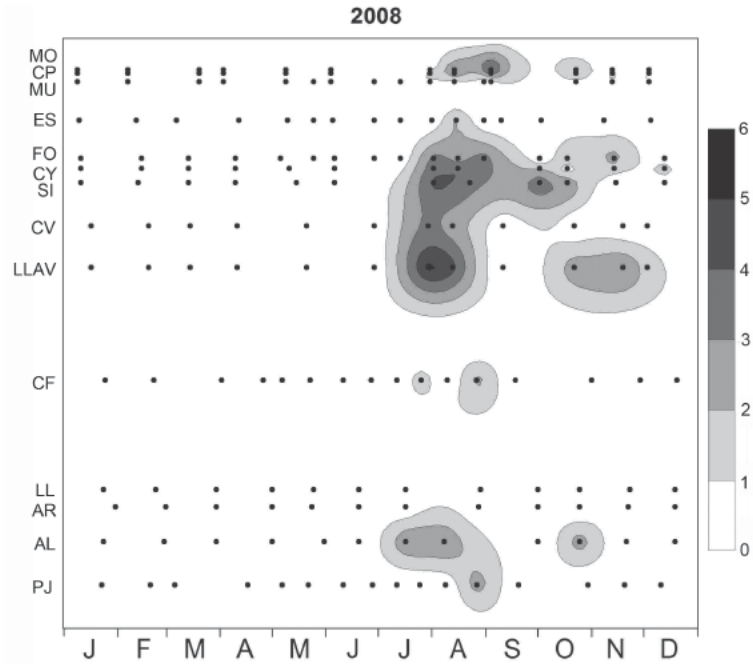

Fig. 3. Spatio-temporal distribution of *Ostreopsis* along the Catalan coast during 2008. Station codes and *Ostreopsis* concentrations as in Fig. 1.

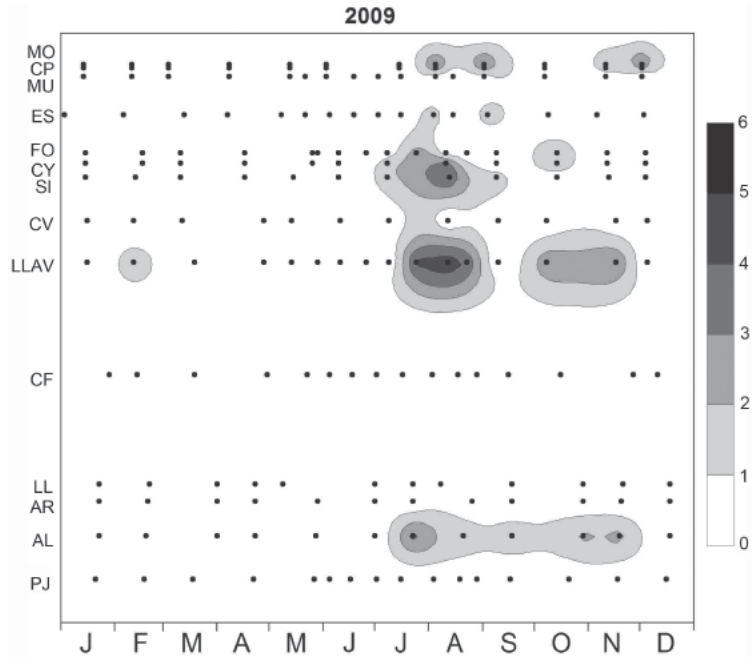

Fig. 4. Spatio-temporal distribution of *Ostreopsis* along the Catalan coast during 2009. Station codes and *Ostreopsis* concentrations as in Fig. 1.

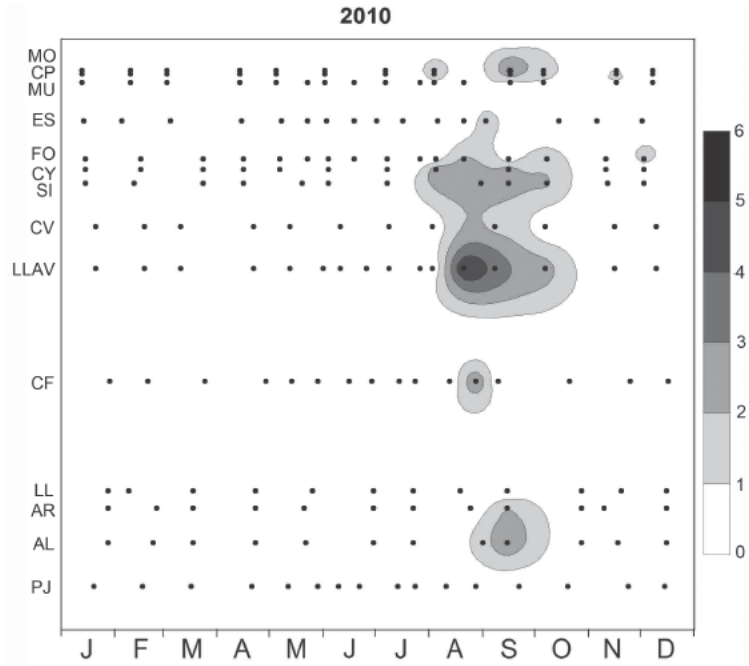

Fig. 5. Spatio-temporal distribution of *Ostreopsis* along the Catalan coast during 2010. Station codes and *Ostreopsis* concentration as in Fig. 1.

being in 2007 when their presence was detected at five stations at concentrations of < 2100 cells·L^{-1} (from January to April in Llavaneres), up to 400 cells·L^{-1} (from January to March in Fosca), and up to 100 cells·L^{-1} (during February in Montjoi, Canyelles Petites, and Canyet).

Information flux between institutions involved in Ostreopsis management

As part of the cooperation between the EBITOX research project and the monitoring program, ICM (CSIC) scientists involved in the project contact the ACA whenever the *Ostreopsis* concentration reaches ~10^5 cells·g^{-1} fw macroalgae or ~10^4 cells·L^{-1} in seawater at Llavaneres. This threshold is based on our observations that at those concentrations health problems in humans can occur if the wind is blowing landwards. The ACA regularly provides warnings about such events to the Public Health Department and the local authorities as well as to Red Cross and lifeguards in the affected area (Fig. 6). The ACA warns that at particular risk are children, the elderly, people with a history of respiratory problems, and individuals with a high degree of exposure to aerosols, such as employees at nearby restaurants, residents at beach-side apartments, lifeguards, and regular beachgoers that are in contact with aerosols for hours. Information is also provided regarding the possible symptoms (Alvarez *et al.*, 2005), which include rhinitis, throat irritation, cough, expectoration, migraine, conjunctivitis, and even fever (38ºC) of several hours duration.

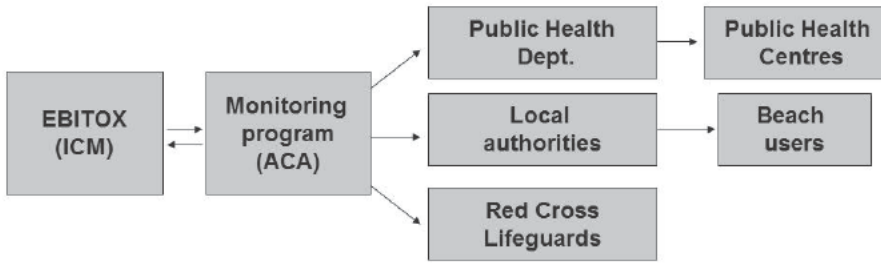

Fig. 6. Information flux between institutions involved in the management of *Ostreopsis* blooms in Catalonia.

For example, in the summer of 2011, local authorities at Llavaneres warned the public about the bloom by placing information panels close to the affected beaches. Although it was explained that bloom-related symptoms and illness are usually mild, in persistent cases these individuals were advised to seek medical attention. Luckily, the year 2011 was not particularly bad in terms of sanitary cases. Nevertheless, after an intensive sampling day at the beach, members of our research team experienced symptoms such as muscle pain and fever, while the diver who collected the samples over a period of one hour suffered conjunctivitis.

At Llavaneres, the area affected by *Ostreopsis* blooms is not heavily frequented by beachgoers, because it is a very narrow beach. However, during the intense bloom of August 2010, two snorkelers swimming in the area for approximately one hour reported serious irritation of the lips, sore throat, and moderately itchy eyes and commented on the metallic taste of the water. In accordance with the policies of other Mediterranean countries affected by *Ostreopsis* blooms, the local authorities at Llavaneres have decided not to close the beach during bloom events. In 2011, the media (press and local radio) became aware of an ongoing bloom but the reported information did not cause public alarm.

EBITOX conclusions useful for bloom management

As most of the main findings of the EBITOX project have yet to be published, only those most important for bloom management are discussed herein.

1) Two *Ostreopsis* species, *Ostreopsis* cf. *siamensis* and *O.* cf. *ovata*, both of which are toxic, were identified during several blooms at Llavaneres by a molecular PCR-based assay (Penna *et al.*, 2005; Battocchi *et al.*, 2010; Riobó *et al.*, submitted); however, despite the use of the same methodology, only *O.* cf. *ovata* was detected at the same beach during the 2010 bloom (Bravo *et al.*, 2012).

2) The appearance of *Ostreopsis* cells in epiphytic samples began during a period extending from late May until early July; a few weeks later, the cells were detected in the water. The highest concentrations were reached in July-August, with the highest epiphytic concentrations detected from mid-July to mid-November (Vila *et al.*, submitted; Mangialajo *et al.*, 2011). Although, in general, *Ostreopsis* was not present in winter, low epiphytic concentrations were detected sporadically.

3) The concentration of *Ostreopsis* cells in water can vary highly over a small temporal scale (hours). Thus, for a representative picture of *Ostreopsis* biomass, in addition to planktonic samples benthic samples should be collected.

4) Benthic samplings during the non-bloom season revealed the absence of resting cysts among the macroalgae and sediments. However, short-dormancy pellicle cysts were observed in bloom-incubated samples; these cysts were able to survive for a period of 6 months (Bravo *et al*., 2012).

5) The toxicity of the bloom was assessed in a hemolysis assay (Riobó *et al.*, 2008) and by HPLC-FLD (Riobó *et al*., 2006), which detected palytoxin in epiphytic samples obtained during the bloom period. Toxin content varied between 0.3 and 5 pg palytoxin equivalents·cell^{-1} depending on the culture conditions (Riobó *et al*., submitted).

6) Palytoxin was not detected in aerosols; however, a few *Ostreopsis* cells were found by means of a molecular assay of rt-PCR (unpublished data), suggesting that the respiratory and irritation effects associated with *Ostreopsis* blooms might be due to an allergic reaction rather than to intoxication.

7) Finally, benthic fauna was very scarce in areas of bloom occurrence; however, the few sea urchin specimens collected tested positive for palytoxin (Riobó *et al*., submitted).

Final remarks

The presence of *Ostreopsis* in the study area has gained attention due to the temporal coincidence of algal blooms and respiratory irritations in humans, but of equal importance is the fact that this genus can cause shellfish contamination (Aligizaki *et al*., 2008), and even the mortality of benthic invertebrates (Sansoni *et al*., 2003). Decreases in sea urchin populations paralleling increases in *Ostreopsis* have been observed in some areas (Shears and Ross, 2009). Although prior to 2007 regular samples were not collected at Llavaneres beach, local residents anecdotally related that mussels, now absent, were previously abundant in the area and that sea urchins, now very scarce, were previously very abundant. In August 1998, a mass mortality of invertebrates occurred in Llavaneres (Vila *et al*., 2008). In phytoplankton samples analyzed during that event, *Ostreopsis* abundances were as high as $2{\cdot}10^5$ cells·L^{-1}. Since then, consistent with the residents' reports, the invertebrate community of Llavaneres beach has not recovered. However, despite the scarcity of sea urchins, those analyzed during the EBITOX project contained only low amounts of palytoxin.

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