International Conference on *Ostreopsis* Development

Villefranche-sur-Mer / 4-8 April 2011

Final Programme

Dear Colleagues,

We are pleased to present the scientific program for the "International Conference on *Ostreopsis* Development" (ICOD).

ICOD aims to summarize existing knowledge on (1) the ecological, chemical and toxicological aspects of *Ostreopsis* species and (2) on the different approaches to management of the ecological, economic and health problems associated with blooms of *Ostreopsis*. Bringing together scientists, policy makers and managers of the Mediterranean and other temperate countries, this meeting will facilitate interactions and knowledge transfer among participants, and promote action necessary to better manage the *Ostreopsis* development and reduce the risks linked to this new phenomenon.

This event will occur in conjunction with the 2011 meeting of the French Phycological Society, which will be held on the two days prior to ICOD (4-5 April 2011).

We wish to thank all of the ICOD supporters: the "Pôle de Recherche et d'Enseignement Supérieur Euro-méditerranéen", the "Ministère du Travail, de l'Emploi et de la Santé", the "Ministère de l'Ecologie, du Développement Durable, des Transports et du Logement (LITEAU)", the "Région Provence-Alpes-Côte d'Azur", the "Conseil Général des Alpes-Maritimes" and the Villefranche-sur-mer municipality. The International Agreement RAMOGE also significantly contributed to the conference via the invitation of many scientists from the southern and eastern coasts of the Mediterranean Sea.

We also wish to thank, for their invaluable assistance before, during and quite probably after the congress, the following persons (in alphabetic order): Hazel Arceo, Valentina Asnaghi, Olivier Boebion, Stéphanie Cohu, Isabelle Courtois, Stéphane Gouy, Sophie Marro, Natacha Martini, Isabelle Palazzoli, Corinne Poutier and Pierre Thiriet.

The Organizing and Scientific Committees of the Conference warmly welcome all the participants, and hope to fulfill their expectations with interesting and fruitful discussions during ICOD.

The abstracts of the plenary sessions are presented separate, as the first part of this book. All other abstracts are presented according to the announced order of subjects of the conference.

Organizing committee

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ICOD - International Conference on *Ostreopsis* Development

Scientific programme: 6th – 8th April 2011

6th April (Wednesday)

8:30 Registration

9:00-9:30 Opening ceremony

Session 1 – Ecology, biogeography and impacts on coastal ecosystems (Chair: **Bertolotto R.**)

9:30-10:00 **Plenary lecture**: Shears N. - Ecological effects of blooms of *Ostreopsis siamensis* on rocky reef communities in New Zealand

10:00-10:30 Coffee break

10:30-10:50 Fraga S., Rodríguez F., Bravo I., Zapata M. - An approach to the study of bloom dynamics of *Ostreopsis*.

10:50-11:10 Aligizaki K., Moutopoulos D.K., Katikou P., Nikolaidis G. - Temporal, spatial and habitat variability among toxic and/or potentially toxic benthic dinoflagellates in North Aegean Sea, Greece.

11:10-11:30 Cohu S., Mangialajo L., Thibaut T., Blanfuné A., Lemée R. - *Ostreopsis* cf. *ovata* development in relation with depth, biotic substrate and environmental factors in the North Western Mediterranean Sea in 2008 and 2009 years – MediOs 2 program.

11:30-11:50 Asnaghi V., Mangialajo L., Hewitt J., Thrush S., Castellano M., Privitera D., Rossi A., Cattaneo-Vietti R., Chiantore M. - Interannual variability in *Ostreopsis ovata* bloom dynamic along Genoa coast (North-western Mediterranean): a modeling approach.

11:50-12:10 Zingone A., Scalco E., Urciuolo G., Di Cioccio D., Saggiomo V., Rossi R., Soprano V., Montresor M. - Ecological and physiological characteristics of *Ostreopsis* cf. *ovata* in the Gulf of Naples.

12:10-14:00 Lunch break

Session 1 – Ecology, biogeography and impacts on coastal ecosystems (Chair: **Fraga S.)**

14:00-14:30 **Plenary lecture**: Penna A. - Genetic diversity of the genus *Ostreopsis* Schmidt: phylogeographical considerations and molecular methodology applications for field detection.

14:30-14:50 David H., Laza-Martinez A., Miguel I., Orive E. - Phylogenetic study of the genus *Ostreopsis* along the Atlantic coast of the Iberian Peninsula.

14:50-15:10 Amany A., Halim Y. First records of *Ostreopsis* cf. *ovata* and *Ostreopsis* sp. in Alexandria coastal water – Egypt.

15:10-15:30 Cabrini M., Fornasaro D., Lipizer M. - First report of *Ostreopsis ovata* bloom in the Gulf of Trieste (Northern Adriatic Sea).

15:30-15:50 Quod J.P., Turquet J., Darius H.T., Laurent D., Chinain M. - State of the art on benthic toxic dinoflagellates in French oversea territories with special focus on *Ostreopsis*.

15:50-16:20 Coffee break

Session 1 – Ecology, biogeography and impacts on coastal ecosystems (Chair: **Quod J.P.**)

16:20-16:40 Fakhri M., Abboud Abi Saab M., Kassab M.T. - Seasonal and spatial variations of the dinoflagellate *Ostreopsis siamensis* in the Lebanese coastal waters (Eastern Mediterranean)

16:40-17:00 Totti C., Accoroni S., Colombo F., Romagnoli T. - Morphometric analysis of *Ostreopsis* cf. *ovata* cells in relationship with bloom phases.

17:00-17:20 Guidi-Guilvard L., Gasparini S., Lemée R., Blanfuné A., Cohu S., Mangialajo L., Thibaut T. - The effect of *Ostreopsis* cf. *ovata*, a toxic benthic dinoflagellate, on phytal meiofauna from the coastal NW Mediterranean.

17:20-17:40 Blanfuné A., Lemée R., Cohu S., Mangialajo L., Thibaut T. - Impact of *Ostreopsis* cf. *ovata* development on macroinvertebrates in the NW Mediterranean Sea.

17:40-19:00 **Posters session**

20:30 Official dinner (Restaurant Le Cosmo, Villefranche)

7th April (Thursday)

Session 2 – Secondary metabolites and toxicity of *Ostreopsis* (Chair: **Ciminiello P.**)

8:30-9:00 **Plenary lecture**: Yasumoto T. - Chemistry and Toxicology of Palytoxin and Related Toxins

9:00-9:20 Dell'Aversano C., Ciminiello P., Dello Iacovo E., Fattorusso E., Forino M., Grauso L., Tartaglione L. - High resolution LC-MS study of ovatoxins produced by *Ostreopsis ovata*.

9:20-9:40 Riobó P., Franco J.M., Bravo I., Fraga S., Ramilo I., Rial P., Rodriguez F., Sala M.M., Penna A., Vila M. - Determination of palytoxins in samples from *Ostreopsis* outbreaks in Llavaneres (NW Mediterranean coast)

9:40-10:00 Sechet V., Sibat M., Chomerat N., Nezan E., Grossel H., Lafontaine L., Kantin R., Ganzin N., Marco-Miralles F., Lemée R., Amzil Z. - Influence of environmental factors on development and toxicity of the epiphytic dinoflagellate *Ostreopsis* cf. *ovata* and accumulation of palytoxin analogs in seafood in French coastal areas.

10:00-10:20 Vanucci S., Guerrini F., Pezzolesi L., Dell'Aversano C., Tartaglione L., Ciminiello P., Fattorusso E., Pistocchi R. - Preliminary results on cell growth and toxin content of *Ostreopsis ovata* in the presence and in absence of the associated bacteria.

10:20-10:50 Coffee break

Session 2 – Secondary metabolites and toxicity of *Ostreopsis* (Chair: **Séchet V.**)

10:50-11:10 De Haro L., Glaizal M., Tichadou L., Drouet G., Hayek-Lanthois M. - Clinical feature of human exposure to *Ostreopsis* and/or palytoxin by seawater contact or food chain contamination.

11:10-11:30 Pelin M., Sosa S., Tubaro A., Florio C. - Toxic effects of the marine toxin palytoxin on human skin keratinocytes.

11:30-11:50 Rossini G.P., Sala G.L. - Palytoxin and other microalgal toxins belonging to different chemical classes induce cytotoxic effects involving a common set of stress response proteins.

11:50-12:10 Trotereau S., Lemée R., Biré R., Delpont C., Chabot B., Aumond Y., Oregioni D., Krys S. - Palytoxins levels in marine p roducts harvested between Villefranche-sur-mer and Nice during the summer 2009 and 2010.

12:10-14:00 Lunch break

Session 2 – Secondary metabolites and toxicity of *Ostreopsis* (Chair: **Bire R.**)

14:00-14:20 Giussani V., Faimali M., Garaventa F., Piazza V., Corrà C., Chiantore M., Gallus L., Asnaghi V., Privitera D., Isola G., Cattaneo-Vietti R., Mangialajo L. - The harmful benthic microalgae *Ostreopsis ovata* along Genoa coast: bloom dynamic and toxic effects on invertebrate and vertebrate marine organisms.

14:20-14:40 Sardo A., Rossi R., Soprano V., Ciminiello P., Fattorusso E., Cirino P., Zingone A. - The impact of *Ostreopsis ovata* on *Mytilus galloprovincialis* and *Paracentrotus lividus.*

14:40-15:00 Honsell G., De Bortoli M., Boscolo S., Dell'Aversano C., Tartaglione L., Battocchi C., Penna A., Berti F., Fontanive G., Sosa S., Poli M., Yasumoto T., Tubaro A. - *Ostreopsis* cf. *ovata* Fukuyo in the Gulf of Trieste (Northern Adriatic Sea): new details on toxin content, immunocytochemistry and ultrastructure.

15:00-15:20 Vidyarathna N.K., Granéli E. - Climate change involvement in blooms of the toxic benthic dinoflagellate *Ostreopsis ovata* worldwide: comparative studies on different strains

15:20-15:50 Coffee break

15:50-17:50 **Round table discussion** (moderator: **Rossini G.P.**)

17:50-19:00 **Posters session**

8th April (Friday)

Session 3 – Environmental, health and economic management: state of the art and perspectives (Chair: **Boissery P.**)

8:30-9:00 **Plenary lecture**: Bertolotto R. - Five years of *Ostreopsis* monitoring in Italy: lessons learned?

9:00-9:20 Lemée R., Mangialajo L., Amzil Z., Blanfuné 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., Séchet V., Soliveres O., Thibaut T. - The French research project MediOs 2 (Mediterranée *Ostreopsis*) and the relations with managers and policy makers.

9:20-9:40 Vila M., Riobó P., Bravo I., Masó M., Penna A., Reñé A., Sala M.M., Battocchi C., Fraga S., Rodriguez F., Franco J.M. - The bloom dynamics of toxic *Ostreopsis* in St. Andreu de Llavaneres (NW Mediterranean Sea).

9:40-10:00 Ennaffah B., Nafil E., Chaira K., Fraikech M., Chafik A. - The first occurrence of *Ostreopsis* cf *siamensis* in Southern Moroccan Atlantic waters.

10:00-10:20 Mangialajo L., Ganzin N., Accoroni S., Asnaghi V., Blanfuné A., Cabrini M., Cattaneo-Vietti R., Chavanon F., Chiantore M., Cohu S., Costa E., Fornasaro D., Grossel H., Marco-Miralles F., Masó M., Reñé A., Rossi A.M., Sala M.M., Thibaut T., Totti C., Vila M., Lemée R. - Trends in *Ostreopsis* cf. *ovata* proliferation along the northern Mediterranean Sea (NW Mediterranean and Adriatic Seas) coastal waters.

10:20-10:50 Coffee break

Session 3 – Environmental, health and economic management: state of the art and perspectives (Chair: **Bertolotto R**.)

10:50-11:10 Fattorusso E., Ciminiello P., Dell'Aversano C., Dello Iacovo E., Forino M., Grauso L., Tartaglione L., Zingone A., Urciuolo G., Di Cioccio D., Saggiomo V., Buia M.C., Ammendola A., Soprano V., Rossi R., Capozzo D., Bruno T., Castellano V., Arace O., Serpe L., De Maio L., Capone S., Pignalosa C., Gramegna C., Cocozziello B., Lubrano Lavadera S., De Filippo S., Montanino A., Ventimiglia S.C., Cella A. - *Ostreopsis ovata* along the Campania coasts. Results from a 4-year-long regional monitoring programme.

11:10-11:30 Abbate M., Bordone A., Cerrati G., Di Festa T., Melchiorre N., Pastorelli A.M., Peirano A., Petruzzelli M.R., Ungaro N. - A new method for sampling potentially toxic benthic dinoflagellates.

11:30-11:50 Grossel H., Ganzin N. - Towards an *Ostreopsis* surveillance system along the French Mediterranean coasts.

11:50-12:10 Zingone A., Berdalet E., Bienfang P., Enevoldsen H., Evans J., Kudela R., Tester P. - The GEOHAB Core Research Project on HABs in Benthic Systems.

12:10-14:00 Lunch break

14:00-16:30 **Round table discussion** (moderator: **Boissery P**., **Grossel H**.)

16:30-18:00 Closing ceremony and appetizers in Villefranche Observatory

Posters

Ecology Session

Abbate M., Cocito S., Lombardi C., Verni F. - Who feed on *Coolia monotis* and *Ostreopsis ovata*?

Abdennadher M., Hamza A., Zouari B.A., Radai M.N. - Toxic marine dinoflagellates, *Ostreopsis siamensis* Schmidt, in Gabes Gulf (South of Tunisia, central Mediterranean).

Amorim A., Veloso V., Battocchi C., Penna A. - Occurrence of *Ostreopsis* cf. *siamensis* in the upwelling coast of Portugual (NE Atlantic).

Armi Z., Turki S., Ben Maïz N. - *Coolia monotis* and *Ostreopsis siamensis* proliferations in northern Tunisian lagoons water.

Bennouna A., El Attar J., Abouabdellah R., Chafik A., Penna A., Oliveira P.B., Palma S., Moita M.T. - Occurrence of *Ostreopsis* cf. *siamensis* blooms in Moroccan Atlantic waters from 2004 to 2010.

Bizsel N., Aligizaki K. - Detection of *Ostreopsis* cf. *ovata* in coastal waters of Turkey (East Aegean Sea).

Blasutto O., Celio M., Facchini L., Franceschini F., Gironcoli E., Moro R., Roppa D., Suraci C., Venuti M., Zanolin B., Mattassi G. - Presence of *Ostreopsis ovata* and other potentially toxic species in the Gulf of Trieste (northern Adriatic Sea) from 2006 to 2010.

Celio M., Blasutto O., Acquavita A., Stel F., Mattassi G. - Two years of *Ostreopsis ovata* monitoring in the Gulf of Trieste (northern Adriatic Sea): weather and hydrological conditions during summer 2009 and 2010.

Cohu S., Thibaut T., Mangialajo L., Labat J.P., Passafiume O., Blanfuné A., Simon N, Cottalorda J.M., Lemée R. - Impact of environmental factors on *Ostreopsis* cf. *ovata* dynamic during the summers 2007 and 2008 in Monaco (NW Mediterranean Sea).

Hamza A., Mabrouk L., Loukil A., Moufida A., Dammak L. - Variability of habitats and temporal distributions of *Ostreopsis siamensis* in southern coasts of Tunisia.

Monti M., Cecchin E. - Comparative growth of three strains of *Ostreopsis ovata* at different light intensities and allelopathic interactions on three microalgae.

Moreira A., Alonso C., Chamero D., Comas A. - Some ecological notes on *Ostreopsis* from Southern-Central Coast of Cuba.

Pannacciulli F., Fiorenzoni S., Battocchi C., Penna A. - Development of mithocondrial DNA markers for the taxonomic identification of *Ostreopsis* cf. *ovata.*

Scenati R., Marchegiani S., Mancini L. - Presence of *Ostreopsis ovata* between 2007 and 2010 along the Monte Argentario coasts, South Tuscany, in the Central Tyrrhenian Sea.

Turki S., Balti N., Aissaoui A., Armi Z. - Occurrence of planktonic and epiphytic cells of *Ostreopsis* cf. *siamensis* in Tunisian waters.

Toxicology Session

Orlandi M., Abbate M., Simonini R. - Acute effects of the toxic dinoflagellate *Ostreopsis ovata* on benthic polychaetes: first evidences from ecotoxicological tests with the dorvilleid *Dinophilus gyrociliatus.*

Selwood A., Van Ginkel R., Holland P., Boundy M., Rhodes L., McNabb P., Shears N. - A sensitive assay for palytoxins and ostreocins using LC-MS/MS analysis of cleavage fragments from micro-scale oxidation.

Taleb H., Vale P., Amanhir R., Benhadouch A., Sagou R. - Evolution of Lipophilic marins toxins in Moroccan shore.

Management Session

Armengaud A., Lasalle J.L., De Haro L., Grossel H., Lemée R., Rambaud L., Alessandrini P., Tichadou L., Glaizal M., Kantin R., Amzil Z.., Drouet G., Ricoux C., Esteve-Moussion I., Coulon O., Guillotin L., Giannetti S., Kermarec F., Malfait P. - Surveillance and prevention of the public health impact of *Ostreopsis* on the French Mediterranean coastline: summary for the period 2007 – 2010.

Dell'Aversano C., Colarusso G., Pellicanò R., della Rotonda M., Sarnelli P., Baldi L. - *Ostreopsis ovata* in Campania: a case of regional monitoring program in Italy.

Illoul H., Hernández F.R., Vila M., Adjas N., Younes A.A., Bournissa M., Koroghli A., Marouf N., Rabia S. - The genus *Ostreopsis* along the Algiers coastal waters (SW Mediterranean Sea) associated with a human respiratory intoxication episode.

Solivérès O., Hoareau L. - Economic impact of *Ostreopsis* development in the NW Mediterranean Sea.

Touahria T., Seridji R. - Monitoring of the potentially toxic genera *Ostreopsis*, *Coolia* and *Prorocentrum* (Dinophyceae) in the Bou Ismail Bay; (Algerian coast).

Abstracts

Abstracts of plenary talks and oral communications are listed in order of presentation.

1 - Plenary talks

Ecology Session

Ecological effects of blooms of *Ostreopsis siamensis* on rocky reef communities in New Zealand

Shears, N. T.

University of Auckland, New Zealand

Harmful algal blooms provide an example of an increasingly common disturbance to marine systems that is predicted to increase further with warming oceans, yet their effects on ecosystem dynamics are poorly understood and often difficult to study. Blooms of *Ostreopsis siamensis* have become a regular annual occurrence in northern New Zealand in recent years. These highly visible blooms typically occur in the summer months following periods of calm sea conditions that allow warming of surface waters. Blooms are typically restricted to shallow water and wave protected bays. The most obvious ecological effects of blooms are on sea urchins, which dramatically lose their spines during blooms. While *Ostreopsis* can result in direct mortality of sea urchins, in most cases blooms only have nonlethal effects. However, these nonlethal effects can alter ecosystem processes through increased vulnerability to physical dislodgement and predators, as well as reduced grazing rates. Given the importance of sea urchins as grazers on temperate reefs, both the lethal and nonlethal effects of *Ostreopsis* on urchins can have ecosystem-level consequences by promoting increased macroalgal abundance. The effects of *Ostreopsis* on other reef invertebrates are less well understood and the response to blooms are more subtle and highly variable; for example, the limpet *Cellana stellifera* tends to actively avoid *Ostreopsis*, whereas the large turbinid gastropod *Cookia sulcata* does not appear to be affected by blooms. Further research is needed in this system to better understand the wider ecological effects of *Ostreopsis* blooms but also the potential risks of toxins being incorporated into the foodweb.

Genetic diversity of the genus *Ostreopsis* Schmidt: phylogeographical considerations and molecular methodology applications for field detection

Penna A.[1], Fraga S.[2], Battocchi C.[1], Accoroni S.[3], Riobó P.[4], Totti C.[3], Giacobbe M.G.[5], Vila M.[6], Reñé A.[6], Aligizaki K.[7], Perini F.[1], Casabianca S.[1], Casabianca A.[1], Vernesi C.[8]

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7- School of Biology, Faculty of Sciences, Aristotle University, 54124 Thessaloniki, Greece
8- Centro Ricerca e Innovazione - Fondazione Edmund Mach, Monte Bondone, 38040 Trento, Italy

Nucleotide diversity was assessed in *Ostreopsis* species to infer phylogenetic and phylogeographical relationships especially in the Mediterranean area. The rDNA phylogeny revealed different clades corresponding to different species within the genus *Ostreopsis*. It was found that in the species *O.* cf. *ovata* different genetic lineages were correlated with macrogeographical distribution. *O.* cf. *ovata* was found to be widely dispersed throughout the coastal areas of tropical and some warm temperate seas. In the Atlantic/Mediterranean region it may constitute a panmictic population that seems distinct from Indo-Pacific populations. Species-specific identification, which is relevant for the complex of different toxins production, by traditional methods of microscopy is difficult due to the high morphological variability, and thus different species having similar morphotypes can be easily misinterpreted. Molecular primers for the species-specific identification and quantification were designed and validated using PCR based technologies. In the monitoring activities of the toxic *Ostreopsis* blooms, the PCR-based methods proved to be effective tools, complementary or alternative to microscopy for the rapid and species-specific estimation of *Ostreopsis* spp. Furthermore, new preliminary findings will be introduced with regard to population genetic of Mediterranean *O.* cf. *ovata* analysis, as well as new rapid and sensitive detection of *Ostreopsis* spp. in marine environment.

Toxicity Session

Chemistry and Toxicology of Palytoxin and Related Toxins

Yasumoto T.

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The detection of palytoxin and related toxins (PLTs) in dinoflagellates and other marine creatures are rapidly increasing. Structurally, they are difficult to define because of the largeness and existence of multiple functions and many stereocenters. PLTs are also substances difficult to isolate in sufficient quantities for toxicological studies. Structural features of the toxins produced by *Ostreopsis siamensis* and *Ostreopsis* cf. *ovata* isolated in Okinawa will be discussed together with their biological activities. The oral toxicity of PLT was studied in mice by changing the ingredients (lipids and surfactants), methods of administration (gavage, mouth membrane), and physiological conditions of mice (ulceration). PLT was most toxic when dosed through membrane. The hemolytic substance alleged to be PLT and responsible for serious and often fatal intoxication due to ingestion of the parrotfish *Scarus ovifrons* was investigated. The allegation was suggested untrue.

Management Session

Five years of Ostreopsis monitoring in Italy: lessons learned?

Bertolotto R.

ARPAL, Environmental Protection Agency of Liguria region, Via Bombrini 8, Genova 16149, Italy (rosella.bertolotto@arpal.org)

After the Genoa Ostreopsis bloom in 2005, it was clear that such phenomena were to be expected also in other parts of the Mediterranean coast: little knowledge about them was available at the time, so many scientists and experts started a new line of studies, giving to benthic microalgae the attention they never had before.
But in the meantime public health authorities had to cope with algal blooms and their effects, in terms of risk management, risk communication, consequences of bathing prohibitions: some sort of practical guide was utterly necessary. So in 2006 the italian Health Ministry appointed an expert group to devise a "Guideline on the management of the risk associated to algal blooms". These guidelines, published in 2007, offered an useful tool for local authorities in charge of managing the recreational use of the beaches and for the environmental agencies in charge of monitoring bathing waters.
Many Italian regions implemented a monitoring plan following the Health Ministry guidelines; recently, ISPRA (Italy's Central Institute for environmental protection and research) published a 3-years' national report which resumes the results of that work.
With the transposition of the Directive 2006/7/EC into the D.M.116/08, the new rules on bathing water controls become explicit about the necessity of monitoring potentially toxic microalgae when and where they are likely to bloom, and to inform people living on the coast and tourists about the potential risks associated with these blooms.
But which are these risks? Is it worth prohibiting the bathing, with all the related consequences of frightened tourists, complaining shopkeepers, empty beaches, or is it better to give plain information without too much publicity? And if so, which communication strategy is best used?

Some examples from Italian experiences will be reported.

2 – Oral communications

Ecology Session

An approach to the study of bloom dynamics of *Ostreopsis*

Fraga S.[1], Rodríguez F.[1] , Bravo I.[1], Zapata M.[2]

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2- Instituto Investigaciones Marinas (IIM-CSIC), Eduardo Cabello 6, 36208 Vigo, Spain

Algal blooms dynamics depend on the balance between gains and losses of cells. If gains, due to replication or advection of cells are higher than losses from grazing, mortality and dispersion, a bloom will occur. While these terms have been well studied for phytoplankton, this is not the case for benthic microalgae like *Ostreopsis*. Phytoplankton communities are associated to a particular water body, but benthic dinoflagellates are associated with a substrate where water is changing continuously and where they have a limited role in their nutrient dynamics. *Ostreopsis* blooms in well illuminated shallow shores in summer and then, they need an adaptation to this high light regime. Light curves obtained by PAM fluorescence on *Ostreopsis* show they are shade-adapted organisms as it is reflected in their pigment composition showing a high peridinin:chlorophyll c_2 ratio. While phytoplankton is grazed mainly by filter feeders, it is not known which the main predators of *Ostreopsis* are. Mucilage production, in addition of affecting dispersion, it can reduce grazing pressure as well. Parasites may play an important role in the control of *Ostreopsis* populations. Although *Ostreopsis* toxins are present in the food web, the role that toxins may play in bloom dynamics is unknown. As in other benthic dinoflagellates, no *Ostreopsis* resting cysts have been identified, so they probably have not a significant role in their bloom dynamics, although they produce zygotes in laboratory experiments.

Temporal, spatial and habitat variability among toxic and/or potentially toxic benthic dinoflagellates in North Aegean Sea, Greece

Aligizaki K.[1], Moutopoulos D.K. [2], Katikou P.[3], Nikolaidis G.[1,*]

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The ecological patterns of benthic dinoflagellates, focusing on *Ostreopsis* populations which have been associated with the presence of putative palytoxin in shellfish, were studied during the period August 2003-December 2005 in 52 sampling sites along North Aegean coasts (Greece). Macrophyte, sediment and water samples were collected, temperature and salinity were measured *in situ*, while nutrient and Chl-*α* concentrations were also determined. *Ostreopsis* spp. populations displayed the clearest temporal and spatial distribution patterns. They were recurrently recorded from July to November, being inversely correlated with the photoperiod rate and positively correlated with water temperature. Significant correlations with the abiotic variables were also found for *C. monotis* and to a lesser extent for *P. lima*, *P. emarginatum*, *P. rhathymum* and *Amphidinium* spp. Furthermore, *Ostreopsis* populations were repeatedly absent, or their abundance was near the detection limit, in certain areas with the highest Chl-*α* and silicate concentrations; this resulted in a statistically significant negative correlation with the concentrations of SiO_2 and Chl-*α*. PCA analysis has indicated that *Ostreopsis* spp., as well as *C. monotis* populations, were positively affected when the substrate included the macroalgae *Padina pavonica* and *Codium fragile* and also micro-vegetation on rocks, whereas *Prorocentrum* populations were favored by the presence of *Cymodocea nodosa* and to a lesser extent of *P. pavonica*.

Ostreopsis cf. *ovata* development in relation with depth, biotic substrate and environmental factors in the North Western Mediterranean Sea in 2008 and 2009 years – MediOs 2 program

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To manage the new problematic of *Ostreopsis* development in temperate areas, a better understanding of the role of environmental factors seems necessary. In this context, a first monitoring of *Ostreopsis* abundances has been performed seven times in 2008 and early 2009 in six sites of the Gulf of Lions and Ligurian coasts, at three depths and on three different macroalgae substrates. Over the total sampling, *Ostreopsis* was observed from early March to early December, with highest concentrations measured in July. Throughout the study, two distinct groups of studied sites were observed with important epibenthic and planktonic *Ostreopsis* concentrations in the three eastern sites (up to 8.54 x 10^6 cells.g^{-1} FW and 68 x 10^3 cells.l^{-1} respectively) and very low abundances in the western sites (up to 0.04 x 10^6 cells.g^{-1} FW and 3 x 10^3 cells.l^{-1}). Concentrations were higher at 0.5 meter deep and *Dictyota* spp. seemed to be the favorite substrate. Annual variations of temperature significantly affected *Ostreopsis* development and represented probably the most impacting parameter. Phosphate and Chlorophyll a concentrations were also positively correlated with *Ostreopsis* concentrations, contrarily to Oxygen which showed a negative correlation. No significant link between *Ostreopsis* and bacteria abundances has been highlighted. In a second study, *Ostreopsis* abundance was measured with an increasing frequency in summer. An important variation in concentrations was then observed from one day to another, and also between day and night.

Interannual variability in *Ostreopsis ovata* bloom dynamic along Genoa coast (North-western Mediterranean): a modeling approach

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In the last decades the occurrence of harmful algal blooms (HABs) has increased worldwide, both in terms of frequency, magnitude and geographic distribution, probably increasing with climate change and human activities. To date, blooms of benthic dinoflagellates belonging to the tropical genus *Ostreopsis* have been reported in many temperate regions. In the Mediterranean Sea the genus *Ostreopsis* has been recorded since the '70s, but large bloom events have been reported only in recent years, as the Genoa event in 2005, that caused consequences on human health and mortality of marine organisms.
The factors involved in bloom occurrence are still debated: a relationship with sea water temperature has been highlighted in some areas, but other factors, such as wave exposure, nutrients and salinity, are also potentially involved.
In order to describe *O. ovata* bloom dynamics and provide a better understanding of factors involved in blooms, we collected a time series of data in Genoa from summer 2006 till summer 2010. Cell abundances in water column and on macrophytes were assessed throughout the year, concurrently with related physical and chemical features.
We elaborated a meaningful predictive model, realizing multiple correlations between bloom magnitude (maximum cell concentration) and length (extent of the bloom event; with N° of cells > 4000 cell/l) and water/meteorological features; this model could be used as a forecasting tool for *O. ovata* blooms prediction of particular interest in an ecological, economic and sanitary perspective and may represent a good base for managers in the attempt of forecasting toxic events.

Ecological and physiological characteristics of *Ostreopsis* cf. *ovata* in the Gulf of Naples

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From 2007 to 2010 we studied the distribution of *O.* cf. *ovata* along the Campania coasts (Tyrrhenian Sea) with the aims of gaining insights on the ecology of the species and improving the management of the risks posed by its blooms. Maximum cell abundance (<3 x 10^5 cells g^{-1} FWM) is generally recorded in mid July, but a second period of increase is detected in some years at the end of the summer. No obvious relationships were found between *O.* cf. *ovata* abundance and temperature, nutrients, macroalgal substrates or hydrodynamic conditions. In order to identify factors regulating bloom dynamics of *O.* cf. *ovata*, we also tested its growth responses over a combination of temperature, photoperiod, and irradiance conditions. The species grew at temperatures between 18 and 30°C, with the maximum growth rates (0.56-0.82 div. day^{-1}) recorded at longer day length conditions and between 18 and 26°C. Morphologically aberrant cells were abundant in the post-exponential growth phase and at the lowest and highest temperatures. Small cells were detected in several experiments. Toxin abundance and composition analysed at different temperature and photoperiod conditions showed maximum of total palytoxins in the post-exponential phase and at 18-22°C, whereas the highest percentage of ovatoxin-a was detected at 26°C. *Ostreopsis* cf. *ovata* is apparently able to grow over a broad range of temperature and day-length conditions and to perform better at longer day lengths and lower irradiances.

Phylogenetic study of the genus *Ostreopsis* along the Atlantic coast of the Iberian Peninsula

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Species of the Ostreopsidaceae are usually found living epiphytically on macroalgae and seagrasses in the tropical-subtropical areas all over the world. However, lately *Ostreopsis* spp. has been found in temperate waters, among which, the Mediterranean area has been the most commonly studied for this genus. Our study aimed to identify *Ostreopsis* along the Atlantic coast of the Iberian Peninsula, which is subjected to a marked variability in water temperature. Until recently, the identification of *Ostreopsis* species was based solely on morphological characteristics that did not allow to distinguish among morphologically similar species. To gain insight on the taxonomic composition and to investigate the geographical variability of the genus within the study area, we analysed the LSU and 5.8S-ITS molecular structures of isolated strains of *Ostreopsis*. The phylogenetic analysis was run together with other *Ostreopsis* sequences of different world locations, provided by Genbank, and revealed that the Atlantic Iberian strains belong to *Ostreopsis cf. siamensis.*

First records of *Ostreopsis* cf. *ovata* and *Ostreopsis* sp. in Alexandria coastal water – Egypt

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Ostreopsis spp. are reported for the first time from Egyptian Mediterranean waters. Macroalgal samples were collected monthly from June 2005 to December 2007 from rocks at Abu Qir from less than 1.5m depth and their associated microalgae were examined. Populations of two *Ostreopsis* species occurred in the location east from Alexandria: *O.* cf *ovata* Fukuyo and *Ostreopsis* sp..

Ostreopsis sp. was abundant and dominant during summer. It was more abundant on the brown algae *Padina sp.* and *Sargassum sp.*, less abundant on the red algae *Corallina sp., Jania* sp., *Laurencia* sp. and even less so on the green algae *Ulva spp. Ostreopsis* cf *ovata* was also found during summer months on the same algal species but at much lower abundance.

Ostreopsis sp. alternated in dominance with the benthic cyanobacteria *Oscillatoria* spp. and the diatom *Licmophora sp.* Other benthic dinoflagellates recorded at low abundance were *Amphidinium carterae, Gymnodinium* sp. and *Prorocentrum lima.*

First report of *Ostreopsis ovata* bloom in the Gulf of Trieste (Northern Adriatic Sea)

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Adriatic Italian coasts have been interested by plankton blooms since 1970, but recently events caused by benthic dinoflagellates have been reported. In the Gulf of Trieste, along the coastal waters, *Ostreopsis ovata* has been sporadically observed since 2006, while in September 2009 a high concentration of this epiphytic microalga was registered for the first time. During this bloom period, hydrological data were collected to characterize the site. A concentration of $13*10^6$cell/L *Ostreopsis ovata* was recorded, prevalently developed in shallow waters and distributed on rocks that were covered by an evident mat produced by the microalgae. To compare the growth on different substrata, from May to October 2009, macroalgae were collected in two coastal sites and the environment was characterized on the basis of physical and chemical parameters. In September, the period of the bloom, *Ostreopsis* resulted preferably associated with *Dictyota dichotoma* and *Padina pavonia*. From these preliminary observations, hard substrata, sheltered conditions and scarce hydrodynamism seem to favour the bloom of *Ostreopsis*. Moreover the bloom seemed gradually to disappear with the decrease of temperature and salinity. No toxic events were registered to human health in this area, only episodes of weak numbness and tingling on hands of operators involved in monitoring were reported.

State of the art on benthic toxic dinoflagellates in French oversea territories with special focus on *Ostreopsis*

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French oversea tropical territories are located in the Atlantic, Pacific and Indian oceans. Harmful algal blooms with health consequences have been largely documented in relationship with ciguatera fish poisoning (CFP) outbreaks, a seafood disease related to degradation of coral reefs.
During the 80 decade, studies have been performed to identify the assemblage of benthic potentially toxic dinoflagellates, and seasonally monitor them as indicators of the ciguatera risk and/or climate change.
Gambierdiscus sp is the main genus involved in the CFP syndrome, sometimes in association with *Prorocentrum spp* and *Ostreopsis spp development.*
Cyanobacteria blooms are frequently associated with abiotic substrate and may contribute to the toxic pool entering the food web. The contribution of cyanobacteria is actually studied as part of the ARISTOCYA project.
Due to increasing degradation of coral reefs health over the past decades, associated with both (i) nutrients inputs and (ii) climate change events such as coral bleaching, there is a critical trend to observe in future more potentially blooms of noxious species. For example, following the 1998 massive coral bleaching and mortality a historical bloom of *Gambierdiscus* was notified in Mayotte lagoon (Indian ocean), but without effects on human health as the strain was non toxic. More recently, an acute fish poisoning outbreak involving 2 fatal cases was reported in 2009 in Rapa (Australes archipelago, French Polynesia), involving herbivorous fishes and massive blooms of *Ostreopsis sp*, which may be a consequence of the global warming.

Seasonal and spatial variations of the dinoflagelate *Ostreopsis siamensis* in the Lebanese coastal waters (Eastern Mediterranean)

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The dinoflagellate *Ostreopsis siamensis* was noted in Lebanese coastal waters at the beginning of our regular samples in 1979. In the frame-work of a monitoring program aiming at the control of water quality along the coastline, this species like other toxic or potentially toxic microalgae, received much attention. The monthly and spatial variations of its density in water sample were carried out at 5 different rocky stations.

Results showed that *O. siamensis* is almost present at all rocky shores along the Lebanese coastline from south to north between May and November, reaching 10500 cells/L in July 2001 and 5700 cells/L in September in the same year at another station. In 2008 and 2009, this species was found in some stations in February, March and April but in small numbers. Annual temperature varied between 17 ^{0}C (February –March) and 29 ^{0}C (August). Correlations between the density of this species and the environmental parameters will be presented. Until now any remarkable or undesirable event not been recorded in the Lebanese shores. Our results suggested that *O. siamensis* is a thermophilic species and constitutes a permanent component of the floristic list of Lebanese waters.

Morphometric analysis of *Ostreopsis* cf. *ovata* cells in relationship with bloom phases

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Blooms of the toxic benthic dinoflagellate *Ostreopsis* cf. *ovata* are associated with noxious effects on human health and mortality of benthic organisms, due to the production of palytoxin-like compounds. We investigated the *O.* cf. *ovata* bloom in two areas along the Conero Riviera (March-November 2009), in order to analyse morphometric characters in relation to bloom phases and environmental conditions. 1152 cells were measured in natural samples collected during summer 2009. *O.* cf. *ovata* proliferation occurred from end August to November, with the highest abundances in mid-September (1.3×10^6 cells g^{-1} fw). The morphometric analysis showed a marked variability (DV: 17.5-75 µm; W:12.5-60 µm; AP: 10-31.25 µm), and a relationship between cell dimensions and bloom phases was shown: cells in late-bloom phase had DV significantly longer (52.19 ± 9.84 µm) than both those in early-bloom (50.22±6.56 µm, p-level <0.05) and in exponential phase (49.55 ± 8.61 µm, p-level <0.001). Moreover, *O.* cf. *ovata* showed DV significantly higher in exposed sites (50.54 ± 7.16 µm) compared with sheltered ones (48.67 ± 8.06 µm, p-level <0.05), suggesting that the latter may represent more favourable sites for intense *O.* cf. *ovata* proliferation. The dimensions of *Ostreopsis* cells in the water column were significantly higher (DV: 52.74±8.24 µm) than epiphytic ones (DV: 49.70±8.10 µm, p-level <0.01), suggesting that resuspended cells in the water column derived from a mature benthic population.

The effect of *Ostreopsis* cf. *ovata*, a toxic benthic dinoflagellate, on phytal meiofauna from the coastal NW Mediterranean.

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Ostreopsis cf. *ovata* is a tropical toxic benthic dinoflagellate that recently occurred in the shallow coastal NW Mediterranean where its blooms have caused health problems on humans in contact with the cells whether epiphytic, planktonic or in sea spray. 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 *Stypocaulon scoparium*. The macroalga was sampled in triplicates at 0.5 m depth in 6 stations along the French and Italian coasts on 7 occasions in 2008. Toxic cells bloomed in summer in 3 out of the 6 stations with concentrations ranging from 2.5 to 6.6 10^5 cells g^{-1} macroalgal wet weight. Metazoan meiofauna densities ranged from 1274 to 8646 individuals g^{-1} macroalgal spin-wet weight. Statistical analyses revealed that changes in the community structure were associated with high concentrations of *Ostreopsis*. The most affected taxon was the nauplii suggesting a negative impact of *Ostreopsis* on harpacticoid copepod reproduction.

Impact of *Ostreopsis* cf. *ovata* development on macroinvertebrates in the NW Mediterranean Sea

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Harmful benthic microalgae blooms represent an emergent phenomenon in temperate zones, causing health, ecological and economic concern. The development of *Ostreopsis* cf. *ovata*, a toxic benthic dinoflagellate, is more and more observed in the North Western Mediterranean Sea. In order to understand the ecological impact on some species of macroinvertebrates, two studies were carried on edible grazers, the sea urchins *Paracentrotus lividus* and the limpets *Patella spp*. The first study performed monthly in six sites along the Liguria Sea (Genova, Nice, Villefranche-sur-mer, Saint-Raphaël, Ramatuelle, and Cassis) in 2008, showed that, whatever the depth, in surface or at 3 m deep, the density of *P. lividus* was not affected by *Ostreopsis* development. Regarding the density of *Patella spp*., only one significant decreased of the density was observed between July and August in Genova. The link between the decrease of the density of *Patella spp*. and the *Ostreopsis* bloom was not clear. A second study was carried out 2009 in the Bay of Villefranche-sur-mer in two sites, the Marinières where blooms occurred during summer and the Lido where concentrations of microalgae were much lower. Specific *Patella spp.* composition was not affected, a mortality of *Patella coerulea* and an impact on small class (< 15 mm) were observed in bloom's site. Concerning *Paracentrotus lividus*, no trace of death was noticed despite massive *Ostreopsis* bloom, in this bay during this summer.

Toxicity Session

High resolution LC-MS study of ovatoxins produced by *Ostreopsis ovata*

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In the last decades, the recurring presence of the benthic dinoflagellate *Ostreopsis ovata* has caused severe sanitary emergencies and economic losses along the whole Italian coastline. Liquid chromatography-mass spectrometry (LC-MS) technique played a key role in revealing the presence of putative palytoxin and much higher amounts of ovatoxin-a, a palytoxin-like compound, in field samples of *O. ovata*. In our ongoing research on *O. ovata* toxins we report herein on high resolution LC-MS and MS^2 investigation of *O. ovata* cultures, which disclosed the occurrence of several new ovatoxins in algal extracts. Elemental formulae very close to palytoxin's were assigned to the new compounds and information was gained about their structural features. A quantitative study of several *O. ovata* culture extracts indicated that ovatoxin-a generally represents the major component of the algal toxin profile and that the whole of the other ovatoxins constitutes up to 46% of the total toxin content of the analyzed strains; thus, their presence should be taken into account when LC-MS based monitoring programs of either plankton or contaminated seafood are carried out.

Determination of palytoxins in samples from *Ostreopsis* outbreaks in LLavaneres (NW Mediterranean coast)

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Dinoflagellates of the genus *Ostreopsis* have been related to harmful episodes in many Mediterranean coastal areas since 1998. In August 2004 one important event occurred in Llavaneres beach (Catalan coast, Spain) affecting 74 people with rhinitis and breathing problems. Since then, many Mediterranean heavy blooms of *Ostreopsis* have coincided with respiratory problems in people staying near the beach, suggesting a possible link between them.

To test this hypothesis, samples of seawater, macroalgae epiphytics, benthic marine invertebrates and aerosols were collected from this location during 2009 and 2010 in the framework of the EBITOX project for palytoxin determination.

Samples were analysed by haemolytic assay and LC–FLD and the presence of palytoxin was confirmed by LC-MS.

High levels of palytoxin were detected in macroalgae epiphytics and cultures, trace levels in seawater and no palytoxin was detected in aerosol filters neither in sea urchins. On the other hand, *Ostreopsis* cells were found in the viscera of sea urchins and PCR analysis revealed the presence of *Ostreopsis* DNA in aerosol filters. It suggests that palytoxin concentration in aerosols that might cause human intoxication is below the limit of detection of our methodologies.

Whereas there is a coincidence in time between *Ostreopsis* blooms and human intoxications by inhalation, we are not yet able to demonstrate if the causative agent is palytoxin, the entire *Ostreopsis* cells, or another agent that causes an allergic reaction.

Influence of environmental factors on development and toxicity of the epiphytic dinoflagellate *Ostreopsis* cf *ovata* and accumulation of palytoxin analogs in seafood in French coastal areas.

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Ostreopsis spp. has been first observed in 1972 along Mediterranean coasts, in the bay of Villefranche sur Mer (Taylor 1979). However, over the past ten years harmful events related to this benthic dinoflagellate have been reported in Italian, Spanish, Greek, French, Tunisian and Algerian coastal areas. In France, during a hot period in August 2006, cases of dermatitis and respiratory problems were registered in Morgiret (Marseille). At that time, a link to the proliferation of *Ostreopsis* was highlighted for the first time in that area. A specific monitoring was designed and implemented in summer 2007.

Two strains of *Ostreopsis cf ovata*, collected in 2008 from Villefranche sur Mer and Morgiret coastal waters and grown in culture, were studied to characterize their growth and toxin profile. Liquid chromatography-mass spectrometry (LC-MS/MS) indicated that both strains produced ovatoxin-a (OVTX-a) as the major component (ca. 90%), and traces of palytoxin (PLTX). Toxin content was determined at the end of exponential growth phase with highest concentration of 50 pg eq.PLTX.cell^{-1}.

Initial screening of native sea urchins, gastropods, fishes and artificially immerged shellfishes for the presence of palytoxin analogs was carried out using LC-MS/MS. The highest toxin level, 458 µg eq.PLTX/kg was found in sea urchins, but significant level of toxins, 217 µg eq.PLTX/kg, was also measured in mussels, exceeding by far the European Food Safety Agency recommended threshold value.

Preliminary results on cell growth and toxin content of *Ostreopsis ovata* in the presence and in absence of the associated bacteria

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Ostreopis ovata is a benthic dinoflagellate known to produce palytoxin-like compounds; as for different toxic microalgae a question on the role of bacteria in influencing algal growth and toxin production can be postulated. Preliminary results on effects of associated bacteria on growth and toxin content of *O. ovata*, investigated in culture experiments, are reported. Bacteria were removed using an antibiotic treatment (a cocktail of streptomycin, ciprofloxacin, gentamicin and penicillin G). The actual axenic status of antibiotic treated cultures was assessed by epifluorescence microscopy using SYBR gold dye. Toxin profile and quantification of each toxin (PLTX, OVTX-a, -b, -c, -d, -e) were performed by high resolution LC-MS on cell extracts and on media. The removal of bacteria affects slightly algal final cell yield. Bacteria-free and control cultures showed the same qualitative toxin profile. Total toxin concentrations on a cell and on volume basis were similar in bacteria-free cultures and controls during exponential phase; whereas in stationary phase, concentrations were significantly lower in bacteria-free cultures with respect to controls (on a cell basis: 34 and 48 pg $cell^{-1}$; on volume basis: 103 and 126 µg L^{-1} for bacteria-free and control cultures, respectively). Results point out that toxin production by axenic *O. ovata* cultures is not highly affected by bacteria, however the role of the latter on algal toxin dynamics needs further investigation.

Clinical feature of human exposure to *Ostreopsis* and/or palytoxin by seawater contact or food chain contamination.

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Ostreopsis species in the Mediterranean produce toxins similar to palytoxin (PTX). Experience in Europe has provided better insight into cutaneous, mucosal and respiratory exposure during *Ostreopsis* blooms. Symptoms are mainly due to direct tissues irritation. Flu-like manifestations (headache, digestive disturbances and fever) have been reported. Dyspnea due to hypersecretion and bronchospasm has been observed in the most severe cases. The exposed patient management is straightforward, but the main problem is the possibility of a mass poisoning involving several hundred persons (Liguria, 2005). The population at greatest risk is swimmers. For snorkeling or scuba diving, mouth breathing devices (snorkels or regulators) promote formation of saltwater micro-droplets deeply inhaled into the airways.

Another concern is contamination of the food chain by PTX-like toxins. Case reports have described persons who died rapidly after eating contaminated crustaceans or fish. The clinical syndrome is called palytoxicosis when it involves consumption of crustaceans and parrotfish and clupeotoxism when it involves consumption of pelagic fish (sardines, mackerels). The severity of poisoning is linked to the strong vasoconstricting properties of PTX/PTX-like toxins that can lead to life-threatening complications within a few hours including rhabdomyolysis, myocarditis, and renal failure. Until now, human poisoning due to shellfish or fish ingestion has not been reported in the Mediterranean.

Toxic effects of the marine toxin palytoxin on human skin keratinocytes

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Exposure to the marine toxin Palytoxin (PLTX), identified in *Ostreopsis* dinoflagellates as well as in *Palythoa* zoanthids, has been recently associated to dermatological lesions observed in humans during *O. ovata* blooms. To characterize PLTX effects on the skin, an *in vitro* study was carried out on human keratinocytes (HaCaT cells), a predictive model for evaluating skin toxicity. After 4h exposure, PLTX reduced plasma membrane integrity, cell mass and mitochondrial activity with increasing potency (EC_{50}s of $1.8\pm0.1x10^{-8}$, $4.7\pm0.9x10^{-10}$ and $6.1\pm1.3x10^{-11}$ M, respectively). PLTX-induced cytotoxic effects were counteracted by ouabain, an inhibitor of the main cellular target of the toxin, the Na^+/K^+-ATPase, and by removal of extracellular Na^+, but were only partially reduced by extracellular Ca^{2+} withdrawal. One hour exposure to PLTX also induced superoxide anion production that was ouabain- and Na^+-dependent but insensitive to removal of Ca^{2+} ions. Hence, among the chain of intracellular events leading to PLTX-induced irreversible cell death, the first and crucial step appears to be the increase of intracellular Na^+ levels that leads to oxidative stress and mitochondrial dysfunction. This latter event occurs at PLTX concentrations even lower than those quantifiable in seawater samples during *O. ovata* blooms along Italian coasts, corroborating the harmful potential of PLTX on skin.

Palytoxin and other microalgal toxins belonging to different chemical classes induce cytotoxic effects involving a common set of stress response proteins

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The complexity of effects exerted by palytoxin (PlTX) in biological systems is easily appreciated by considering the variety of responses observed in animal and cellular models. Owing to this complexity, we have employed proteomic tools to study the responses induced by PlTX at a system level. The analysis of a subproteome of a human cell line has shown that PlTX causes a change in the phosphorylation state of hsp 27 and the oxidation of DJ-1, supporting the conclusion that cell stress responses, including oxidative damage, participate to the cytotoxic effect of PlTX (Sala et al., *Chem. Res. Toxicol*.; 22, 2009, 2009-2016).

A meta-analysis of proteomic data regarding the toxic responses induced by other classes of microalgal toxins, such as okadaic acid and microcystins, in biological systems reveals that several protein components participating to cell stress responses, particularly oxidative stress, are affected when the systems are exposed to effective doses of toxins. Detected changes comprise either the total levels of individual proteins, or the relative proportions of isoforms distinguishable according to their post-translational modifications.

A common set of protein effectors would emerge from our meta analysis, suggesting that cytotoxic effects exerted by microalgal toxins could share common molecular processes executing cell death responses.

Acknowledgments. Our investigations on molecular mechanisms of action of biotoxins have been supported by the Italian MIUR.

Palytoxins levels in marine products harvested between Villefranche-sur-mer and Nice during the summer 2009 and 2010

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Palytoxin (PlTX) is one of the most potent toxins known, produced by *Ostreopsis* spp. This toxin was found in different fishery products such as crabs, shellfish and various fish.
PlTX has been associated with intoxication syndromes related to skin contact or through the respiratory tract and with food poisonings in tropical areas following the consumption of contaminated crabs, sea urchins and fish.
Despite the risk related to the exposure to PlTX, there is no regulation yet. The EFSA proposed a threshold of 30 µg PlTX and ostreocin D/kg shellfish meat. The EFSA also pointed at the lack of representative occurrence data on PlTX-group toxins in shellfish in Europe.
In France, a monitoring program was put in place on the Mediterranean coast after *Ostreopsis* cf. *ovata* was identified in 2006. This surveillance is completed with research projects focusing on the ecology of *Ostreopsis* and on the contamination of fishery products.
Thus in 2009 and 2010, the General Directorate for Health funded a project coordinated by the French agency for food, environmental and occupational health safety. This project aiming at collecting data on the contamination levels of sea products in sampling sites located between Nice and Villefranche-sur-Mer, also included the Oceanographic Laboratory of Villefranche.
This project involved the sampling of 4 sites in the summer 2009 and 1 site in the summer 2010. On all sites, abundances of *Ostreopsis* cells were recorded and the toxin levels in the sampled animals were determined by hemolytic test and liquid chromatography coupled with tandem mass spectrometry.

The harmful benthic microalgae *Ostreopsis ovata* along Genoa coast: bloom dynamic and toxic effects on invertebrate and vertebrate marine organisms

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Harmful Algal Blooms (HABs) represent an emergent phenomenon causing health and economic concern worldwide, as well as in the Mediterranean Sea, where blooms of *Ostreopsis ovata* occur in summer-early autumn, with increasing regularity, sometimes leading to human intoxications and mass mortalities of marine organisms. In this work, we quantified *O. ovata* cell abundance during the summer 2010 along the Genoa coastline (Ligurian Sea, North-western Mediterranean) and investigated on the toxic effects of different environmental concentrations of *O. ovata* (laboratory cultured and field samples) using crustaceans (*Amphibalanus amphitrite*, *Artemia salina*, *Tigriopus fulvus*) and fish (*Dicentrarchus labrax*) as model organisms for the ecotoxicological screening. We performed crossed experiments testing two different temperatures across a range of cell concentrations and treatments (microalgae + growth medium; growth medium without microalgae; resuspended microalgae in sea water; control). Our results show maximum algal proliferation at the end of July (20.670 cells/ l) at 27°C sea water temperature and calm sea conditions. This finding confirms the role of sea water temperature and weather conditions in favouring bloom development in the Ligurian Sea. The LC_{50} values show the highest sensitivity in *A. salina* ($LC_{50\text{-}48h}$: < 4 cells/ml) and the lowest in *D. labrax* ($LC_{50\text{-}96h}$: > 500 cells/ml) among model organisms. The experiments have shown a much larger effect when microalgae were present, while the growth medium does not have a significant effect, at least in our experimental conditions, confirming that the toxin content retained in cells is significantly higher than that released in growth medium.

The impact of *Ostreopsis ovata* on *Mytilus galloprovincialis* and *Paracentrotus lividus*

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Blooms of *Ostreopsis* cf. *ovata* have become very common along Mediterranean coastline, where they may cause human suffering via the exposure to marine aerosol or by ingestion of contaminated seafood. Physiological damages and mortality have also been reported for benthic marine organisms. In order to study the trophic relationship and impact of these toxic dinoflagellates, we fed the sea urchin *Paracentrotus lividus* and the mussel *Mytilus galloprovincialis* with cultures and natural material of *O. ovata* collected from the Gulf of Naples (Mediterranean Sea). We also tested the effect of the prolonged exposure of these animals to cultures of *O. ovata*. Toxicity was tested with the mouse bioassay and with the LC-MS analysis. Sea urchins did not show particular damages after the ingestion of the red alga *Asparagopsis taxiformis* colonised by *O.* cf. *ovata* and became toxic in some cases, while the exposure to *O. ovata* cultures caused the partial or total loss of the spines, and – at higher concentration – the death. Mussels filtered actively *O.* cf. *ovata* cultures (10^6 cells L^{-1}) in 24 hours. In two-three days, however, they died and resulted toxic. Two-three weeks of stabulation were necessary for detoxification of naturally toxic mussels stabled in open system tanks. Our results demonstrate that both sea urchins and mussels can feed *O. ovata* and become toxic, but prolonged or intensive exposure causes permanent damages and even death in the animals.

Ostreopsis cf. _ovata_ Fukuyo in the Gulf of Trieste (Northern Adriatic Sea): new details on toxin content, immunocytochemistry and ultrastructure.

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Blooms and toxic events associated with *Ostreopsis* cf. *ovata* Fukuyo have been increasing in the Mediterranean Sea over the last few years, including the Gulf of Trieste, where this species was first detected in 2006. A bloom with more than 6,000,000 cells/L occurred in late September 2009 in shallow waters along the northern Gulf of Trieste. Cells were identified as *Ostreopsis* cf. *ovata* by morphological features and molecular analysis. High resolution LC-MS revealed for the first time in natural populations the presence of ovatoxin-a, -b, -c, -d and –e, with ovatoxin-a being the major component. Immunocytochemisty using polyclonal and monoclonal anti-PLTX antibodies localized for the first time palytoxin-like compounds in the cytoplasm of *Ostreopsis* cells, while no reaction was observed within nuclei and chloroplasts. Scanning and transmission electron microscopy revealed new details on the structure of the filamentous network attaching *Ostreopsis* to macroalgae and bottom pebbles.

Climate change involvement in blooms of the toxic benthic dinoflagellate *Ostreopsis ovata* worldwide: comparative studies on different strains

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Blooms of the benthic palytoxin-producing dinoflagellate *Ostreopsis ovata* are being recorded in tropical, sub-tropical and temperate marine waters for the last 12 years. We hypothesize that temperature increase due to climate changes is involved in the occurrence of these blooms. A Japanese (J) and a Mediterranean (M) *O. ovata* strain were exposed to temperatures between 24 – 30 ºC, and their growth and toxicity were estimated. Growth rates of strain-M were positively correlated with increasing temperatures showing the maximum at 30 ºC while strain-J had its maximum at 25 ºC. On the other hand, lower temperatures (24 - 25 ºC) induced higher cell toxicities in both strains. Increases in temperature significantly reduced the cell toxicities in strain-M but not in strain-J. Cells of strain-M were 50 times more toxic at 24 ºC than at 30 ºC while at the same temperature cells of strain-J were only 1.25 times more toxic. The present study clearly demonstrates that the effect of temperature on the growth and toxicity of *O. ovata* is strain specific, but also that increase in temperatures due to climate changes, seems involved in blooms of this species.

Management Session

The French research project MediOs 2 (Mediterranée *Ostreopsis*) and the relations with managers and policy makers

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The goal of the French project MediOs 2 (Mediteranean *Ostreopsis*) was to acquire and analyze pertinent scientific knowledge on *Ostreopsis* in areas as diverse as ecology, biology, chemistry, epidemiology or socio-economic issues in the Mediterranean Sea, in order to provide a decision-making support for administrative action.
The project was funded by the "Conseil Général des Alpes-Maritimes", the Ministry of Ecology and the "Agence de l'Eau Rhône Mediterranée Corse". The project members belong to University Pierre & Marie Curie, University of Nice-Sophia Antipolis, the CNRS, the Ifremer, the Nice Hospital and Chamber of Commerce.
This communication will summarize the main results obtained during the project, the main recommendations given in order to improve the monitoring of the toxic algae and will also focus on the relation between scientists and French managers or policy makers.

The bloom dynamics of toxic *Ostreopsis* in St. Andreu de Llavaneres (NW Mediterranean Sea)

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During the last decade, epiphytic dinoflagellates of the genus *Ostreopsis* have been related to harmful episodes in many Mediterranean coastal areas. Human intoxications characterized by respiratory difficulties, fever, and skin irritations affected people exposed to marine aerosols or bathers. Although this genus is widespread in most of the Mediterranean coasts, harmful events have been recorded only in some localities and periods. Sant Andreu de Llavaneres (Catalan coast) is one of the sites on the Mediterranean coasts where *Ostreopsis* achieves the highest concentrations. The bloom was studied in the framework of the EBITOX project. The main results are: 1) Two *Ostreopsis* species, *Ostreopsis* cf. *siamensis* and *O.* cf. *ovata,* have been identified to bloom together by a molecular PCR-based assay. 2) The epiphytic *Ostreopsis* bloom starts in late May-early July, and a few weeks later is detected in the water, where it reaches maximum concentrations in July-August; maximum epiphytic concentrations are detected from mid July to mid November. In winter, *Ostreopsis* is not detected in the water, but low epiphytic concentrations are detected sporadically. 3) Resting cysts have not been detected during the sampling of macroalgae and sediment during no-bloom season. However, short-dormancy pellicle and thin-walled cysts are observed in bloom-incubated samples. 4) The bloom is toxic; palytoxin, analyzed by haemolysis assay and HPLC-FLD is detected in epiphytic samples taken during bloom-period.

The first occurrence of *Ostreopsis* cf *siamensis* in Southern Moroccan Atlantic waters

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Within the Moroccan phytoplankton monitoring network, the epiphytic dinoflagellate called *Ostreopsis*, was first observed in Southern Moroccan Atlantic waters.
The analyse of samples of Atlantic south shores (Dakhla bay) from 2009 to 2010, revealed the presence of this dinoflagellate during summer and autumn seasons, The maximum abundance reached respectively $1.08x10^4$ and $1.04x10^4$ C / l in Boutelha (23°52,431N-15°48,022W) and PK25 (23°54,746N-15°46,060W) areas during August 2009. In 2010, the density of *Ostreopsis* was maximal at October.
This proliferation was observed with surface temperature between 23 and 28°C in 2009. The measurement and plate patterns observed suggest the presence of *Ostreopsis cf siamensis*.
This study will contribute to the biogeographically distribution of *Ostreopsis* in Moroccan waters, and its presence will be used as environmental tracer and climate indicators.

Trends in *Ostreopsis* cf. *ovata* proliferation along the northern Mediterranean Sea (NW Mediterranean and Adriatic Seas) coastal waters.

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Harmful benthic microalgae blooms represent an emergent phenomenon in temperate areas, causing health and economic concern, especially in touristic areas. This is the case for the Mediterranean Sea, where *Ostreopsis* blooms occur in summer, with increasing regularity. Although a relationship with sea water temperature has been highlighted in some areas, other factors such as wave exposure, nutrients and salinity, are potentially involved; at the present state of knowledge we are not able to forecast the risk of *Ostreopsis* development.

In this study we present the occurrence and proliferation of *Ostreopsis* in 15 Spanish, French and Italian sites along the northern limit of the Mediterranean Sea. Benthic and planktonic cell abundances from 2007, 2008 and 2009 were pooled in order to search for possible general trends in *Ostreopsis* proliferation.

The results highlight that the sea water temperature does not seem to be a primary driver and that maximal abundance periods are Site-Year specific. Nevertheless general trends are observed in the two considered basins: in north-western Mediterranean Sea higher cell abundances are mostly recorded in mid-summer (end of July), while in the Adriatic Sea in early fall (end of September). Such results represent an important step in the understanding of harmful benthic microalgae blooms in temperate areas, and represent a good base for managers in the attempt of forecasting toxic phenomena.

Ostreopsis ovata along the Campania coasts. Results from a 4-year-long regional monitoring programme

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We present the preliminary results of monitoring program of *Ostreopsis ovata* and its toxins along the Campania coasts, committed and funded by the Regione Campania following the guidelines of the Italian Ministry of Health. This program has been carried out from 2007 to date.
The main aims of the project were i) to develop a surveillance programme to monitor the environmental conditions that favor microalga proliferation at levels able to harm bathers and/or seafood consumers health; ii) to study *O. ovata* bloom dynamics over a space and time scale; iii) to determine both qualitatively and quantitatively toxins produced by *O. ovata* and iv) to ascertain whether they contaminated seafood products.
A number of sites (32) in the Bay of Napoli, Salerno and Policastro as well as along Ischia, Capri and Procida coasts were sampled each year from June to late October. *O. ovata* cells density was assessed both in seawater and on seaweeds. Wild marine organisms (mussels, sea-urchins, etc.) were collected from sites where *O. ovata* was most abundant; they were tested by mouse bioassay and subsequently analyzed by liquid chromatography-mass spectrometry for the presence of palytoxin-like compounds. Geo- and hydro-morphological data as well as biological, physical and chemical parameters of the sampling sites were recorded and related to the algal bloom development. The results of this study show that *O. ovata* is widespread along the rocky shores of the whole Campania coasts. Cell abundances were often higher than 10^6cells g^{-1} FW of macroalga and of 10^4cells L^{-1} in sea-water and peaks occurred in July and September. Total palytoxins in seafood often exceeded the levels indicated as harmful for human health (up to 625 µg/kg), and their values were relatively high also several weeks after *O. ovata* peaks.

A new method for sampling potentially toxic benthic dinoflagellates.

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In the last decade, blooms of *Ostreopsis ovata* Fukuyo 1981 took place in several Mediterranean coastal areas, sometimes causing problems to public health. A new quick sampling method, aimed to investigate spatial and temporal variability of *O. ovata* abundance, was developed. *Ostreopsis* is a tycoplanktonic microalgae that does not strongly adhere to the substratum and often forms cell aggregates easily re-suspended in the water column. The sampling method involves the use of a plastic syringe, available commercially, modified in order to draw a fixed volume from a fixed surface of any type of substratum. An experimental design was employed to test the validity of the sampling method and confirmed its use. Furthermore, it highlighted the small scale large variations of *O. ovata* abundance in relation to substratum type and exposure. The technique was used in some monitoring studies carried out in 2009 in the Ligurian Sea and southern Adriatic Sea (Mediterranean) and compared with the methodology outlined in the guidelines of the Italian Ministry of the Environment. The new method proved to be less time-consuming and easier, both for the sample collection and processing, allowing rapid sampling of several sites and substrates within the same area of investigation. Such technique is strategically important when the institutional needs require a quick and reliable estimate of *O. ovata* "reservoir" near the bottom that could be re-suspended into the water column.

Towards an *Ostreopsis* surveillance system along the French Mediterranean coasts

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Within the REPHY network of Ifremer, a study on the possibility of monitoring *Ostreopsis* as part of Ifremer's institutional network will be started during the summer of 2011. At this early stage, the objective is to have an overview of the presence of macroalgal *Ostreopsis* along the coasts of the French Mediterranean. Approximately 80 stations will be selected for their *Ostreopsis* favourable geomorphological (rocky coast and shallow waters) and biological (presence of macro-algae) characteristics. Samples of macro-algae will be collected monthly throughout the summer for the detection and quantification of *Ostreopsis* in its benthic forms.
Besides this regular monitoring scheme, a complementary study is considered in order to test the potential of satellite derived Sea Surface Temperatures (SST) in an improved monitoring system. In a previous study at Ifremer, it was observed that *Ostreopsis* blooms often appear after sharp increases in water temperature. With "day to day" SST image download system, one would be able to extract and plot SST values for a number of pre-identified sites. If a sharp temperature increase is detected, samples are quickly collected and analysed to confirm the level of presence of *Ostreopsis*. The compilation of the results after an entire summer over all the selected sites will indicate if SST monitoring can be used to setup a "pre-alert" based monitoring system.

The GEOHAB Core Research Project on HABs in Benthic Systems

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The "Global Ecology and Oceanography of Harmful Algal Blooms (GEOHAB, www.geohab.info)" is an international scientific program aimed at fostering and promoting co-operative research on Harmful Algal Blooms (HABs). The overall scientific goal of GEOHAB is to "improve prediction of HABs by determining the ecological and oceanographic mechanisms underlying their population dynamics, integrating biological, chemical, and physical studies supported by enhanced observation and modeling techniques." The implementation of the science program is achieved, in part, through different comparative, interdisciplinary and international Core Research Projects (CRPs).
A new CRP, on HABs in Benthic Systems, is under development. An open science meeting (OSM) on HABs in Benthic Systems (BHABs), held in June 2010, brought together scientists working on the understanding on how particular benthic phytoplankton species (e.g. *Gambierdiscus*, *Ostreopsis*, *Prorocentrum*) proliferate and cause harm. During this OSM, research priorities and infrastructural gaps were identified regarding the taxonomy, distribution and ecology of benthic HABs.
The outcomes of the open science meeting, including the state of the art in the field of BHABs and a research implementation plan, will be part of a report currently in preparation. The OSM was followed by a training workshop on methods for collecting and processing benthic HAB samples. A new workshop focusing on sampling methodology is planned in 2011.

3 - Posters

Ecology Session

Who feed on *Coolia monotis* and *Ostreopsis ovata*?

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Coolia monotis Meunier 1919 and *Ostreopsis ovata* Fukuyo 1981 are epibenthic dinoflagellates which are able to produce toxins and responsible of toxic blooms. Conspicuous blooms of these toxic algae were observed in association with bissum detachment and mortality of molluscs and loss of echinoderm spines. However, it is still not clear if toxin production is induced by specific stress conditions (i.e. scarcity of oxygen, high density of individuals in culture) or if it related to the normal life-cycle of these species. To date, data related to toxin pathways within the trophic chain are scarce and only refer to experiments in culture whereas nothing is known from the natural environment. Given that, in order to test the effect of hydrologic and weather parameters potentially responsible of *O. ovata* and *C. monotis* blooms in the Gulf of La Spezia (western Ligurian Sea), sampling and *in situ* observations were made. At the beginning of the summer season, some benthic filter-feeders (ciliates, annelids, bryozoans) were observed to feed on cells of *O. ovata* and/or *C. monotis* and the dinoflagellates were clearly visible within the digestive system of the predators. Neither physical damage nor suffering potentially induced by the toxicity of the algae were shown by the feeders, a great preference in selecting and eating these two toxic algae were observed by some species instead.

Toxic marine dinoflagellates, *Ostreopsis siamensis* Schmidt, in Gabes Gulf (South of Tunisia, central Mediterranean)

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The toxic phytoplankton events represent the most important natural impact for aquaculture and shellfish production areas in Tunisia. Several dinoflagellates have been identified responsible for these toxic events. The study of these harmful species in laboratory cultures will help establish an efficient monitoring program and to resolve many problems in shellfish harvesting areas. In this work, two strains of *Ostreopsis siamensis* Schmidt (Ost.s0) and (Ost.s1) collected in 2008 from two areas Ras younga and Bougrara lagoon (Gabes Gulf, south of Tunisia) and grown in culture were studied to characterize their morphology, their growth and toxin profile. The two strains showed different cell seize, the Ras younga strain (Ost.s0) being bigger than the Bougrara strain (Ost.s1). Growth investigations during the exponentially phase showed that *O.siamensis* strain (Ost.s1) showed a low growth rate ($0.03d^{-1}$) than (Ost.s0) strain ($0.42\ d^{-1}$).The mouse bioassay for *O.siamensis* (Ost.s1) showed that strain was toxic to mousse and (total cell number extracted 3236 cell ml^{-1}) resulted in deaths in 12h.

Occurrence of *Ostreopsis* cf. *siamensis* in the upwelling coast of Portugal (NE Atlantic)

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The coast of Portugal is located in the warm temperate/sub-tropical transition of the North Atlantic. It is part of the major discontinuity in the eastern boundary of the NE Atlantic, being affected both by seasonal upwelling and water mass exchange with the Mediterranean basin. In October 2007, a sampling program aiming at the early detection of *Ostreopsis* species along the Portuguese coast was initiated.
So far only *Ostreopsis* cf. *siamensis* has been identified. Specimens have been recorded from two recreational marinas located on the west coast of Portugal, Cascais (38°41'36"N; 9°24'53"W) and Sines (37°57'2"N; 8°51'53"W). In the latter site *O.*cf. *siamensis* was detected in June 2008, October 2008, September 2009 and November 2010, always within a benthic dinoflagellate community which included, *C. monotis*, *P. lima*, *P. emarginatum* and *Amphidinium* spp. In Cascais it was only detected in September 2010 from plankton net samples and the only other group of benthic dinoflagellates co-occurring were species of *Amphidinium*.
Three cultures of *O.*cf. *siamensis* have been established and are kept in the culture collection of the Oceanography Centre, University of Lisbon.

Coolia monotis and *Ostreopsis siamensis* proliferations in northern Tunisian lagoons water

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A study of the harmful phytoplankton was carried out, from 2006 to 2008, in tow coastal lagoons in the north of Tunisia (Bizerte lagoon and North Tunis lagoon). The aim of this study is to follow the dynamics of potentially toxic species and to identify the risk periods relating to their efflorescence. Ostreopsidaceae species were carried out in the two sites which represent an important mollusc's production in Tunisia.
In the south part of Tunis north lagoon, recurrent *Coolia monotis* proliferation ($5x10^5$ cells.L^{-1}) took place in late spring and early summer of 2006. Proliferation development of *C. monotis* was linked to climatic conditions, water temperature and high concentrations of nitrogenous nutrients.
During the two years 2007 and 2008, maximum concentration of *C. monotis* did not exceed $2.3x10^3$ cells.L^{-1} in spring 2008, however, *Ostreopsis siamensis* reached $3.2x10^4$ cells.L^{-1} in July 2008.
In Bizerte lagoon waters, maximum concentrations for *O. siamensis* and *C. monotis* growing mainly as epiphytes or as part of the benthos were about $24.7x10^4$ and $17.5x10^3$ cells.L^{-1} in July 2006 and May 2008, respectively. High concentrations of *O. siamensis* were also found in July 2007 ($3.75x10^4$ cells.L^{-1}) as well as during August and November 2008.

Occurrence of *Ostreopsis* cf. *siamensis* blooms in Moroccan Atlantic waters from 2004 to 2010.

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Ostreopsis siamensis Schmidt bloom was detected for the first time in 2004, on the central upwelling coast of Morocco by the HAB monitoring program providing an opportunity to identify the species involved and to investigate some oceanographic features of bloom dynamics. In particular, it allowed the study of the bloom relationship with prevalent winds, sea surface temperature, surface circulation patterns and nutrients rates. In parallel, the effect of *Ostreopsis siamensis* blooms on shellfish quality was evaluated. The species identification was confirmed by genetic studies. *O. siamensis* bloom occurred under surface temperatures ranging from 20 to 24°C. The blooms in seawater reached $3.7x10^3$ cells.L^{-1} in autumn 2004. The blooms became frequent, longer and increased in concentration with $12x10^3$cells.l^{-1} in 2008 to 10^5 cells.l^{-1} observed in summer - autumn 2009 and 2010. The detection of toxins in mussels collected from the same area, by mouse bioassay, evidenced the presence of lipophilic toxins. At "Cape Ghir", which is an important upwelling center, the cell maxima occurred in rocky areas, well exposed to winds and waves, and not in wind sheltered areas " Douira- Dar sfint", localized in south of the Cape. These results revealed the importance of hydrodynamism on the re-suspension of this epiphytic species in the water column. Further research is needed to examine the species ecology and toxicity through the isolation and maintenance of the species in cultures.

Detection of *Ostreopsis* cf. *ovata* in coastal waters of Turkey (East Aegean Sea)

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The records of *Ostreopsis* populations in the Mediterranean Sea are increasing lately. However, the occurrence of this genus in Turkish coasts is currently not well documented. In this study, the first record of *Ostreopsis* cf. *ovata* in Cesme Bay (Eastern Aegean coast) is reported. Water samples were collected in the frame of the national monitoring project (monthly samplings in ten stations) supported by national scientific and technical research council (TUBITAK Project no.107Y225). *Ostreopsis* populations were detected in samples taken from 0.2 m below the surface near the mouth of small tributaries in October 2010 at shallow water (about 0.4 m depth). Cells of *Ostreopsis* were about 50.0–70.0 μm in dorsoventral diameter (DV) and 35.0–47.5 μm in transdiameter (W), while the length of the apical pore plate (Po) was about 10 μm. The cell dimensions and the general cell shape fit those of *O. ovata*; thus, the specimens detected in turkish coasts are currently identified as *O.* cf. *ovata.* Densities in surface samples at the sampling site ranged between 26 x 10^3 and 65 x 10^3 cells L^{-1}. This adundance exceeds the alert threshold value (30 x 10^3 cells L^{-1} of seawater) defined by the Villefranche Oceanography Laboratory (IFREMER). The detection of *Ostreopsis* populations in such high abundance levels, comparable to those determined in Greek and other western Mediterranean coasts, indicates the necessity of further study of benthic dinoflagellates in Turkish coasts.

Presence of *Ostreopsis ovata* and other potentially toxic species in the Gulf of Trieste (northern Adriatic Sea) from 2006 to 2010

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In the Adriatic Sea *Ostreopsis ovata* was first detected in the southern coastal areas and only later in the northern coastal areas. Monti et al. (2007) report the first record of *O.* cf. *ovata* in the Gulf of Trieste in October 2006. From 2006 to 2008 the Regional Environmental Protection Agency of Friuli Venezia Giulia - ARPA-FVG revealed the presence of this dinoflagellate in the water column in some areas offshore of Trieste cliff. From June to August 2009 the Italian government (Ministero dell'Ambiente e della Tutela del Territorio e del Mare) funded a national "Monitoring Project" to check the presence of *O. ovata* along Italian coasts. According to this project ARPA-FVG investigated four different sampling areas but no blooms were detected during the monitoring period. At the end of September 2009 a bloom of *O. ovata* ($3x10^6$ cell L^{-1}) was revealed in a different area not included within the "Monitoring Project" areas. During the bathing season 2010 ARPA-FVG increased the monitoring activities and 10 bathing sampling stations were checked to detect the presence of *O. ovata*. Only a few cells of this dinoflagellate were found in two coastal sites and in the water column of the Marano lagoon. No blooms of *O. ovata* were detected in 2010. During the monitoring activities other potentially toxic dinoflagellates like *Prorocentrum lima, Coolia monotis, Amphidinium carterae, Dinophisis caudata, D. fortii* and *D. tripos* were found.

Two years of *Ostreopsis ovata* monitoring in the Gulf of Trieste (northern Adriatic Sea): weather and hydrological conditions during summer 2009 and 2010.

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During summer 2009 and in particular since August the Gulf of Trieste presented air and sea water temperature higher than those observed during 1999-2008 reference period. In general, in the Gulf of Trieste, an increase of 0.12-0.23 °C/year in the surface sea water has been demonstrated from 1991 to 2003 (Malačič et al., 2006), to date this positive trend is still evident. In general, many authors report that high abundance of *Ostreopsis ovata* are observed in sea water characterized by relative high values of temperature, salinity and dissolved oxygen. In the Gulf of Trieste a bloom of this dinoflagellate ($3x10^6$ cell/L) occurred at the end of September 2009. In this month, daily observations of sea surface temperature showed an increase of about 1-2°C respect to that observed during the reference period. During summer 2010 in the Gulf of Trieste no bloom of *O. ovata* was detected in the sea water and only 46,17 cell/gr wet weight of macroalgae were found. From June to September 2010 relative low sea surface temperatures were recorded, in particular the average value was 20.7°C while in the same months of the reference period (1999-2008) the average temperature was 22.9°C. The comparison between the average sea surface temperatures observed during the summer period of 2009 and 2010 presents a decrease of about 2.8°C. Although many other environmental factors affect the development of *O. ovata*, sea water temperature is probably one of the most important factors that trigger *O. ovata* blooms.

Impact of environmental factors on *Ostreopsis* cf. *ovata* dynamic during the summers 2007 and 2008 in Monaco (NW Mediterranean Sea).

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The development of the toxic dinoflagellate *Ostreopsis* cf. *ovata* along temperate coasts has become a growing problem over the last decades, especially in the North Western Mediterranean Sea. To prevent ecological and public health risks in the very touristic beach of Larvotto (Monaco), a weekly monitoring of epibenthic and planktonic *Ostreopsis* abundances has been conducted during the summers 2007 and 2008, in five sites on a small spatial scale (few hundred meters). In addition to the *Ostreopsis* cells quantification, the main environmental factors have been measured, in order to explain the spatial and temporal variations of the observed blooms. During the two studied years, epibenthic and planktonic blooms occurred most often simultaneously and a high variation of concentrations at small spatial scale was observed. We found distinct patterns in epibenthic *Ostreopsis* occurrence between the two studied years, with an early and very marked bloom in 2007 (maximal mean concentration of 1.6×10^6 cells.g^{-1} FW in late June), compared to a later and less abundant bloom development in 2008 (0.4×10^6 cells.g^{-1} FW in mid-July). Differences in bloom timing corresponded with very different hydroclimatic scenarios in 2007 (hot spring and relatively cold summer) and 2008 (standard year). Although annual temperature variations seemed to influence *Ostreopsis* development, no direct impact of summer seawater temperature was evident. Wind may favour the dispersal of benthic and planktonic toxic cells. No significant pattern was observed between *Ostreopsis* concentrations and rainfall or nutrient concentrations. However, the present study suggests that further investigations are needed, for example the potential role of ammonium and the mixotrophic nutritional mode.

Variability of habitats and temporal distributions of *Ostreopsis siamensis* in southern coasts of Tunisia

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Dinoflagellate ecology is based on multiple adaptive strategies and species having diverse habitat preferences and seasonal distribution. In the southern coasts in Tunisia, ten years of study and monitoring of phytoplankton diversity and distribution showed some particularities for dinoflagellate *Ostreopsis siamensis* in this region. This species is recorded in various habitats, at different levels from intertidal shores to the deep waters through alternative epiphytic or pelagic phases. In fact, these habitats include water column, patches of sediment, macroalgea or phanerogam leaves.
This study describes data concerning spatiotemporal distribution of *Ostreopsis siamensis* in the coasts of gulf of Gabes that reported in ten years of phytoplankton monitoring program and the variability of the habitat where this species is sampled. The distribution of this species seems to be very patchy, varying in abundance within among habitats and with a specificity of station where it is collected. There is temporal variation of the distribution and the greatest development is recorded from spring to the end of summer. Furthermore in some habitat specificity of abiotic parameters impose other conditions for fluctuation for this species. However, we found significant correlations between the abundance of epiphytic *Ostreopsis siamensis* and its abundance in the water column (surface and bottom).

The genus *Ostreopsis* along the Algiers coastal waters (SW Mediterranean Sea) associated with a human respiratory intoxication episode.

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During June and July 2009, a hundred cases of human respiratory intoxications were registered in Algerian beaches (Algiers Coast, SW Mediterranean), 40 people of which had to be hospitalized. The Agency for the Protection and Promotion of Algiers Wilaya coasts (APPL) contacted ENSSMAL few days after this outbreak. The affected beaches were monitored since July to the end of the bloom. The most plausible explanation is intoxication due to dinoflagellates of the genus *Ostreopsis*. Although the monitoring started after the intoxication, relatively high *Ostreopsis* concentrations coincided with this harmful episode in five rocky beaches located in the western Algiers area. The highest concentration (5 920 cells·l^{-1}) was detected in Martin in 19/7/2009. Since July 2009 a monitoring program is conducted in 13 beaches of Algiers region by APPL. We present in this work the spatial distribution of *Ostreopsis* spp in Algiers beaches during the toxic episode and temporal distribution of this genus during an annual cycle.

Comparative growth of three strains of *Ostreopsis ovata* at different light intensities and allelopathic interactions on three microalgae.

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Three strains of *Ostreopsis ovata* were isolated from water and algae samples collected either along the Tyrrhenian (Campania region: D483 strain) or the Adriatic (Marche region: CBA-T strain and Friuli Venezia Giulia region: OS2T strain). The cells were cultured at 25°C under 15:9 h L:D cycle (ca. 50 μmol m^{-2} s^{-1} cool white lamp), at salinity 36 and K/2 medium. Evaluation of growth profile of the three *O. ovata* strains in batch cultures were analyzed at four light intensities (10, 100, 400, 650 μmol m^{-2} s^{-1}). The results indicated in 10 μmol m^{-2} s^{-1} the lowest value for the growth. The three strains showed similar growth rates with higher values at the intensity of 100 μmol m^{-2} s^{-1} (0.56 day^{-1}).
The allelopathic effect of the filtrate of *Ostreopsis ovata* cultures on growth of *Coscinodiscus granii*, *Prorocentrum minimum* and *Coolia monotis* was studied. Filtrate from culture in exponential and senescent growth phase was considered. The results revealed a weak allelopathic effect on the growth of the three microalgae with an inhibition on the growth of *Coolia monotis*.

Some ecological notes on *Ostreopsis* from Southern-Central Coast of Cuba

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Potentially toxic microalgae of *Ostreopsis* genus from the southern-central region of Cuba were surveyed between September 2010 and January 2011, mainly in the estuarine complex Cienfuegos Bay and its adjacent coast. The highest abundance of the genus was recorded during the dry season (January) in a shallow bay of the eastern coast. *Ostreopsis siamensis* (2. 16 x 10^{4} cells g^{-1}) and *Ostreopsis ovata* (3. 05 x 10^{3} cells g^{-1}) were the dominant benthic species. Other potentially toxic dinoflagellates were observed in moderate or low abundance such as *Prorocentrum belizeanum*, *Gambierdiscus* sp., *Amphidinium operculatum*, *Prorocentrum rathymum* and *Prorocentrum lima*. During the rainy season, the cyanophyte *Lyngbya* sp. was the dominant benthic species and *Ostreopsis siamensis* (7.20 x 10^{3} cells g^{-1}) was the second species in abundance. Populations of *Ostreopsis* were very scarce in the estuarine complex Cienfuegos Bay. In this ecosystem, *Ostreopsis ovata* was reported during the dry season in moderate concentration. The presence of these populations of *Ostreopsis* should be taken into account for future toxicological studies due to the occurrence of dermatotoxic and ciguatera episodes in the region.

Development of mithocondrial DNA markers for the taxonomic identification of *Ostreopsis* cf. *ovata*

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Morphological features not always allow exact species identification of toxic dinoflagellates, while the use of molecular markers can help in this task. So far, a few markers were developed for this purpose in dinoflagellates but not for amplification of the mitochondrial genome that, in some species, can be a suitable region for taxonomic studies. No attempts were made to amplify mithocondrial genes in *Ostreopsis* spp. and the literature states that the only genes certainly present in the mtDNA of dinoflagellates are Cytochrome Oxidase I and III (COI and COIII) and Cytochrome b (Cytb).
We used universal primers, developed for other dinoflagellates, to target COI and Cytb in *Ostreopsis* cf. *ovata*. Several attempts were made to PCR amplify these regions and we finally succeeded in producing fragments of 350 bp for COI and 550 bp for Cytb. These products were sequenced and species-specific primers for *O.* cf. *ovata* designed, respectively amplifying fragments of 300 bp for COI and 463 bp for Cytb. So far, these sets of primers were tested on few isolates of *O.* cf. *ovata* but, when applied to other Mediterranean and worldwide isolates, not always worked. New sets of primers are now under construction to allow amplification of mtDNA in *O.* cf. *ovata* isolates coming from various areas of the species distribution and able to amplify larger portions of the target genes. Such mtDNA markers could be useful not only for taxonomic studies but also in population genetic analyses.

Presence of *Ostreopsis ovata* between 2007 and 2010 along the Monte Argentario coasts, South Tuscany, in the Central Tyrrhenian Sea

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Here we report the occurrence of the potentially toxic dinoflagellate *Ostreopsis ovata* in four areas of Monte Argentario, a promontory on Tyrrhenian Sea, monitored during the summertime from 2007 to 2010. During this period, we collected samples, made identification and count of the *Ostreopsis* cells. Areas studied are used for bathing, are mainly rocky, relatively low, and rich in macroalgae. The macroalgae samples were collected at a depth of 50 cm, submerging a plastic bag, so to have the macroalgae and the pore water into. Samples were then shacked manually and transferred to 1 liter bottles. In lab, samples, fixed in Lugol, were observed by inverted microscopy ,and counted by Utermöhl method.In fixed samples was present other toxic and potentially toxic species , *Prorocentrum lima* mainly, and order *Coolia monotis* and *Amphidinium carterae. Ostreopsis ovata* was always the dominant species, and during four seasons examined the maximum concentrations always corresponded with the second half July, when the water temperature reached the highest levels. However, since Ostreopsis occurs in the area, no case of human health problems has been reported. Regarding my part we had not other options in the laboratory to extend the research. But it would need studies and specific means to better observe the presence of Ostreopsis, to learn the ecology, such as environmental parameters affect its life cycle, relationship with other species, allelopathic phenomena, a number of insights that would certainly help.

Occurrence of planktonic and epiphytic cells of *Ostreopsis* cf. *siamensis* in Tunisian waters

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The aim of this communication was to synthesize the data collected about the occurrence of *Ostreopsis cf siamensis* in Tunisian waters since this species has been recently associated with many socio-economic consequences in Mediterranean recreational waters. We summarize ecology features knowledge on *Ostreopsis* occurrence in diverse areas distributed along Southern and Northern coastal Tunisian waters. The goal of this national Tunisian program was to acquire and analyze pertinent scientific knowledge on *Ostreopsis* occurrence in coastal areas since it was distributed largely in the Mediterranean Sea, as in pelagic and benthic zones, and could represent an invasive species for the bordering countries.

Monitoring of the potentially toxic genera *Ostreopsis*, *Coolia* and *Prorocentrum* (Dinophyceae) in the Bou Ismail Bay; (Algerian coast)

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A potentially toxic epiphytic dinoflagellate assemblage on macro algae was studied for 1 year in a shallow protected rocky habitat in Bou Ismail Bay (West of Algiers bay, Algerian basin). The assemblage was monitored on Rhodophyte macroalgae: *Corallina* sp. The dominant dinoflagellates were *Ostreopsis* cf. *ovata*, and the accompanying species were *Coolia monotis* and *Prorocentrum lima*. The diatom *Coscinodiscus* sp. and *Lichmophora* sp. were abundant components of the assemblage. The dinoflagellate assemblage follows a clear seasonal pattern, achieving maximum cell concentration during spring (3507 cell/ml; 87 675 cell/gr) and summer with significant relative changes in the species composition. Morphometric measurements were used for epiphytic dinoflagellates: in field specimens, cells of *Ostreopsis ovata* had a length between 50-70 µm and width comprised between 25-45 µm.

Toxicology Session

Acute effects of the toxic dinoflagellate *Ostreopsis ovata* on benthic polychaetes: first evidences from ecotoxicological tests with the dorvilleid *Dinophilus gyrociliatus*

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In order to improve the current knowledge on the *Ostreopsis ovata* effects likely to occur on marine invertebrates, 96-hour acute ecotoxicological tests with the laboratory-cultured polychaete *Dinophilus gyrociliatus* (Dorvilleidae) were performed. During summer 2010, seawater samples containing *O. ovata* were collected from costal hard bottoms near La Spezia (Ligurian Sea) on July (12th, *O. ovata* density about 3500 cells ml^{-1}; 26th, 1500 cells ml^{-1}), August (23rd, 300 cells ml^{-1}) and September (6th, 160 cells ml^{-1}). At each date, specimens of *D. gyrociliatus* were exposed to four experimental treatments: artificial seawater (control), unfiltered seawater (original sample), and filtered seawater; the fourth treatment provided the dilution/concentration of the original sample, in order to obtain about 200 *O. ovata* cell ml^{-1} (reference sample). Mortalities were low (0%-14% after 96h) in controls and filtered seawater treatments at all dates, and in the groups exposed to original and reference samples collected in late August and early September. On the other hand, the experiments performed in July evidenced that almost all worms (98-100%) died within 2-8 hours when exposed to original samples, while the mortality exceeded 75% after 48h of exposure to reference samples in both dates. These preliminary results suggest that *O. ovata* toxicity would depend both on microalgae density and growth phase, and that it could harm polychaetes living in temperate marine coastal areas.

A sensitive assay for palytoxins and ostreocins using LC-MS/MS analysis of cleavage fragments from micro-scale oxidation

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Direct analysis of palytoxin compounds by LC-MS with electrospray ionisation is difficult due to the complex mass spectra. They are large molecules (FW 2500 – 2800 Da) containing three nitrogens which favours formation of multivalent parent ions with the charge spread across multiple different cationised species, all with large ^{13}C isotope contributions. This leads to ambiguities in assigning the true MW and low and variable sensitivity and specificity for trace detection using SIR or the preferred MRM transition $[M.H_2]^{2+}$ > m/z 321, especially in biological extracts. To simplify analysis and improve sensitivity for trace analysis, a method has been developed that uses LC-MS/MS analysis of the substructures generated by oxidative cleavage of the macro-structure at the gem-diols. Periodate oxidation of palytoxins or ostreocins generates two nitrogen-containing fragments. One fragment is common for all palytoxins and ostreocins and is analysed to quantify the sum of all toxins present. The other fragment varies depending on the parent toxin and is analysed for confirmation of the particular palytoxin or ostreocin present. The conditions for micro-scale oxidation of palytoxin were optimised and a rapid analysis for the fragments was established using LC-MS/MS (Acuity uPLC coupled to a Quattro Premier TSQ with Z-spray ion source, Waters-Micromass). Linear calibration ranges (MRM) for the quantitation and the confirmation fragments from oxidation of palytoxin and ostreocin-D were established. LOQs with confirmation were ca 1 ng/mL. The method is applicable to both algal cultures and shellfish. Shellfish flesh extracts were free of interferences with an LOQ for palytoxin of 5 µg/kg. The method is suitable for regulatory testing with secure quantitation of all palytoxin analogues using the one palytoxin calibration.

Evolution of Lipophilic marins toxins in Moroccan shore

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Liquid-chromatography coupled to mass spectrometry (LC-MS) analysis were carried out in shellfish from the Moroccan Atlantic coast harvested between 2004 and 2007 for the confirmation of lipophilic toxins, namely diarrhetic shellfish poisoning (DSP) and Azaspiracids (AZAs) in samples of blue mussel previously found positive by mouse bioassay. The toxin profile was dominated by okadaic acid (OA) alone, or simultaneous presence of OA and dinophysistoxin-2 (DTX2). After alkaline hydrolysis, both of these toxins were found esterified. The presence of several acyl esters of OA was demonstrated in the most toxic sample, with dominance of C16:0, C14:0 and C16:1 fatty acid esters. The dominance of OA contamination started in spring, while DTX2 usually was only detected in mid or late summer. After the first spring toxin peak other DSP peaks might follow, that might be separated in time as much as four and half months. Azaspiracids were dominated by azaspiracid-2 (AZA2) followed by AZA1 (13-26%). AZA3 was rarely detected, and maximal concentrations found were between 3 and 8% of total AZA1/3. Time series showed maximal concentration of AZAs appeared in July.
The simultaneous screening of both toxin families demonstrated that the maximal concentrations of AZAs usually followed the decline in DSP toxin's concentration or occurred during periods of low DSP levels.

Management Session

Surveillance and prevention of the public health impact of *Ostreopsis* on the French Mediterranean coastline: summary for the period 2007 – 2010

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Ostreopsis is a marine micro-alga that recently developed in the Mediterranean Sea. In 2005, 240 people have been affected by febrile respiratory syndromes and skin disorders in an outbreak that occurred in Genoa, Italy, leading to 20 hospital admissions. In September 2006, in Marseilles, France, a few people also presented various symptoms related to the presence of *Ostreopsis*, after swimming around the Frioul islands, a short distance offshore Marseilles.
In France, since 2007, the Ministry of Health has set up a system combining epidemiological and environmental surveillance, together with the prevention of health problems related to the presence of *Ostreopsis* in sea waters. That system covers the whole French Mediterranean coastline and involves several partner-institutions such as Ifremer, the Centre of toxicology expertise in Marseilles, the Regional health authorities and the Inter-regional epidemiological Units for the southern regions of France. Upon alert, a specific Task force, comprising of scientists and experts in risk evaluation and management, provides ad hoc recommendations to the concerned local decision-makers (e.g. Mayors, State representatives). Due to a limited knowledge of a number of parameters — e.g., causes of blooming *Ostreopsis*, toxicity threshold of *Ostreopsis* concentrations in sea waters — the surveillance system has been adapted on pragmatic bases, between 2007 and 2010. A research program is currently associated with surveillances activities, in order to gradually improve the system on scientific evidences (e.g., oral toxicity levels of Palytoxine accumulating in seafood products).

Ostreopsis ovata in Campania: a case of regional monitoring program in Italy

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Following two years of preliminary targeted investigations, a monitoring plan on the distribution and the impacts of *O. ovata* was established in the Campania Region by Regional Committee Decree n. 2106 of 31/12/2008.
The plan spans from June through October and includes three activity phases: routine, alert and alarm. Routine activity takes place in periods and at places where *O. ovata* concentrations and the deriving risks are low. It includes an analytical component, with observation and sampling of coastal seawater, benthic macroalgae and seafood, along with an educational component and a syndromic surveillance and risk communication plan. The next phases take place when a risk due to possible toxic aerosol and seawater or to toxin accumulation in specific seafood is highlighted by the routine activity. Measures taken in this case differ depending on the kind of anticipated risk. The alert corresponds to phases of possible risks due to increasing *O. ovata* concentrations, leading to bloom development. It implies more intense controls and sampling on the different matrices. The alarm is triggered by high *O. ovata* concentrations and/or high levels of toxins in the seafood. Immediate measures are taken in this case to prevent risks due to exposure of the population to toxins.
The Campania Monitoring Plan has allowed to identify risk areas and to enact restrictive measures which have been so far effective in protecting public health.

Economic impact of *Ostreopsis* development in the NW MEditerranean Sea

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The economy of the PACA region (Provence-Alpes-Cote d'Azur), the area selected for study, is strongly linked to the sea: fishing, aquaculture, ports and marinas, but especially seaside tourism: Tourists spend 11 billion Euros in the region each year, largely during the summer.

This preliminary study focuses on the potential impact of *Ostreopsis* on the tourism economy of the PACA region, although this phenomenon, as well as other forms of marine pollution, can in fact be observed throughout the Mediterranean basin.

Surveys were conducted among beach users and businesses located nearby. Research was also carried out on the Internet to locate news articles dealing with *Ostreopsis*.

It appears from this work that, under current conditions (scenario 1), the general public remains largely unaware of *Ostreopsis*, which is often masked or confused by other types of pollution. Its economic impact remains zero at the regional level, despite difficulties for some businesses. If amplification of the phenomenon leads to the precautionary closure of a large beach area (scenario 2), the impact becomes negative at the regional level and the losses amount to several hundred thousand Euros. If the phenomena become severe and recurrent, leading to a significant drop in tourism in the region (scenario 3), with widespread alarming national and international press coverage, the losses would amount to millions or tens of millions of Euros.

Notes

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