CONFERENCE NATIONALE

Phycotox Groupement de Recherche & GIS Cyano

Diversité, écologie et fonctionnement
des microalgues toxiques et cyanobactéries:
de leurs toxines et interactions avec les autres organismes aux risques écosystémiques et socio-économiques associés.

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IUEM (Institut Universitaire Européen de la Mer)

Brest, 31 Mars – 2 Avril 2015

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Mardi 31 mars

09h30 – Café de bienvenue et inscription
10h00 -10h45 – Ouverture (*H. Hégaret, R. Siano, D. Latour, P. Hess*)

SESSION 1 : Interaction micro algues toxiques et organismes marins (Chair : Philippe Soudant)

10h45 - 11h30 – Conférence plénière
Pedro R. Costa: *Transfer of biotoxins in the marine food web: case studies from the Portuguese coast* **(p. 9)**

11h30 - 11h45 – *Cochennec Laureau Nathalie, Retho M., Chauvin J., Fleury E.*
Histological study of the accumulation and depuration of king scallop, *Pecten maximus*, during toxic outbreak of *Pseudo-nitzchia* spp. in South Brittany, France **(p. 15)**

11h45 - 12h00 – *Le Gal Dominique, Terre Terrillon A., Derrien A., Gouriou J., Duval A., Le Gall C., Le Bec C.*
Pecten maximus and domoic acid: a few questions **(p. 16)**

12h00 - 12h15 – *Boullot Floriane, Benoit E., Castrec J., Hégaret H., Boudry P., Soudant P., Fabioux C.*
Characterization of the voltage-gated sodium channel and its isoforms in the Pacific oyster *Crassostrea gigas*: link with its sensitivity to paralytic shellfish toxins produced by *Alexandrium minutum* **(p. 17)**

12h15 - 12h30 – *Lassudrie Malwenn, Fabioux C., Soudant P., Lambert C., Le Goïc N., Wikfors G.H., Sunila I., Nicolas J. L., Miner P., Le Grand J., Hégaret H.*
Combined effects of toxic dinoflagellates of the genus *Alexandrium* and pathogens on bivalve physiology **(p. 18)**

Repas (pris au Restaurant Universitaire)

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14h45 - 14h30 – *Ternon Eva, Calabro K., Botana L., Thomas O.*
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15h00 - 15h15 – *Nézan E., Chomérat Nicolas, Lemonnier H., Goraguer H., Bilien G., Boulben S., Chèze K.*
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15h15 - 15h30 – *Humbert Jean François, Gugger M., Pancrace C., Calteau A., Barny M. A., Pédron J.*
Live free or attached: the dilemma of *Planktothrix* **(p. 21)**

15h30 - 15h45 – *Gerphagnon Melanie, Latour D., Sime-Ngando T.*
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15h45 - 16h00 – *Le Gland G., Sourisseau M., Chapelle A., Plus Martin*
A model of *Alexandrium minutum* blooms in inter-specific competition. A trait-based modelling Approach **(p. 23)**

16h00 - 16h15 – *Leboulanger C., Ader M., Agogué H., Bouvy M., Cadeau P., Cellamare M., Duval C., Fouilland E., Got P., Jézéquel D., Le Floc'h E., Sarazin G., Bernard Cécile*
Cyanobacteria in Lake Dziani Dzaha, a potential analog of Precambrian oceans **(p. 24)**

16h15 - 16h30 – *Siano Raffaele, Chapelle A., Antoine V., Chauvin J., Rigaut-Jalabert F., Guillou L., Hégaret H., Leynaert A., Curd A.*
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Pause café

SESSION 3 : Toxines des microalgues (Chair : Valérie Fessard)

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Presentation of the FOODCYANO project **(p. 26)**

17h15 - 17h30 – *Limon G., Cabon A., Sénant A., Cozic Valérie, Durand G.*
Risk evaluation of shellfish consumption due to toxin presence. Exposition evaluation and contribution of danger characterization **(p. 27)**

17h30 - 17h45 – *Gatti Clémence, Darius H. T., Roué M., Lonati D., Sibat-Dubois M., Savar V., Amzil Z., Chinain M.*
Novel case report of a Ciguatera Shellfish Poisoning cluster following the consumption of *Techtus niloticus* (Gastropod) in French Polynesia **(p. 28)**

17h45 - 18h00 –*Réveillon Damien, Abadie E., Savar V., Séchet V., Bardouil M., Masseret E., Brient L., Hess P., Amzil Z.*
BMAA and isomers in samples from Thau lagoon and in mollusks from other French coasts (Channel, Atlantic and Mediterranean sites) **(p. 29)**

18h00 - 18h15 – *Mondeguer Florence, Abadie E., Hervé F., Deschamps L., Bardouil M., Séchet V., Raimbault V., Berteaux T., Zendong Z., Palvaudau H., Amzil Z., Hess P.*
Pinnatoxin-G: analytical methodology, distribution in Mediterranean lagoons and accumulation in mussels (*Mytilus edulis* and *Mytilus galloprovincialis*) **(p. 30)**

18h15 - 18h30 – *Aráoz Romulo, Huguet A., Pelissier F., Hess P., Servent D., Molgo J., Fessard V.*
Pinnatoxin bioaccumulation in mussels exposed to *Vulcanodinium rugosum* **(p. 31)**

Apéritif dinatoire offert à la Bibliothèque la Pérouse

Mercredi 1 Avril

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Aurelia Tubaro : *Emerging palytoxin analogues: the opinion of the toxicologist* **(p. 11)**

09h45 - 10h00 – *Fessard Valérie, Huguet H., Hogeveen K., Lanceleur R., Sialehaamoa A., Pelissier F., Mondeguer F., Sosa S., Tubaro A., Aráoz R., Molgó J.*
Investigation on the toxic effects and intestinal crossing of pinnatoxin-A and -G and of extracts of *Vulcanodinium rugosum* cultures **(p. 32)**

10h00 - 10h15 – *Aráoz R., Ouanounou G., Iorga B. I., Goudet A., Alili D., Amar M., Benoit E., D. Servent D., Molgó Jordi*
How 13,19-didesmethyl and 13-desmethyl spirolide C phycotoxins affect the cholinergic function? Pharmacological studies on nicotinic and muscarinic acetylcholine receptors **(p. 33)**

10h15 - 10h30 – *Alarcan Jimmy, Le Hégarat L., Hessel S., Lampen A., Fessard V.*
Effect of co-exposure to marine lipophilic biotoxins on the intestinal barrier, bioactivation and molecular mode of actions **(p. 34)**

10h30 - 10h45 – *Enora Briand, Bormans M., Gugger M., Dorrestein P., Gerwick W.*
Changes in secondary metabolic profiles of *Microcystis aeruginosa* strains in response to intraspecific interaction **(p. 35)**

Pause café

SESSION 5: Toxines des microalgues (Chair : Malwenn Lassudrie)

11h15 - 11h30 – *Mazzeo Antonia, Zendong Z., Hess P., Tartaglione L., Forino M., Ciminiello P., Dell'Aversano C.*
Extraction of palytoxins from seawater and preliminary stability studies **(p. 36)**

11h30 - 11h45 – *Le Manach S., Khenfech N., Huet H., Demenou B., Qiao Q., Duval D., Arul M., Bolbach G., Clodic G., Djediat C., Bernard C., Edery E., Marie Benjamin*
Gender-specific toxicological effects of chronic exposure to pure microcystin-LR or complexe *Microcystis* extracts on the liver of adult medaka fish revealed by anatomopathology and proteomics **(p. 37)**

11h45 - 12h00 – *Borcier Elodie, Morvezen R., Miner P., Le Souchu P., Fabioux C., Le Goïc N., Cassonne A.L., Lambert C., Lassudrie M., Richard G., Guillonneau R., Charrier G., Laroche J., Boullot F., Muir F., Nunes F., Harney E., Huvet A., Soudant P., Boudry P., Hégaret H.*
Impacts of two strains of *Alexandrium minutum* on behavior and growth of king scallops *Pecten maximus* **(p. 38)**

12h00 - 12h15 – *Abi-Khalil Célina, Lopez-Joven C., Abadie E., Savar V., Amzil Z., Laabir M., Rolland J. L.*
Effect of *Alexandrium catenella* on mortality of the oyster *Crassostrea gigas* **(p. 39)**

12h15 - 12h30 – *Rolton Anne , Vignier J., Volety A., Donaghy L., Shumway S., Bricelj M. , Pierce R., Henry M., Soudant P.*
Effect of *Karenia brevis* exposure on the reproductive and related physiological processes of *Crassostrea virginica* **(p. 40)**

Repas (pris au Restaurant Universitaire)

SESSION 6 : Ecologie des microalgues (Chair : Delphine Latour)

14h00 - 14h45 – Conférence plénière
Marisa Silva : *Emergent neurotoxins in North Atlantic temperate waters and new vectors* **(p. 12**)

14h45 - 15h00 – *Legrand Benjamin, Latour D., Thouvenot A., Sabart M.*
Seasonal life cycle repartition of a Nostocale Cyanobacteria (*Anabaena macrospora*) in an eutrophic lake (Aydat, France) **(p. 41)**

15h00 - 15h15 – *Guellati F. Zohra, Saoudi A., Kadri S., Bensouila M.*
First reporting of *Planktothrix rubescens* bloom in Algeria in the Bouhamdan dam **(p. 42)**

15h15 - 15h30 – *Lemée Rodolphe, Fricke A., Jauzein C., Mangialajo L.*
Development of the toxic benthic dinoflagellate genus *Ostreopsis* in Mediterranean Sea and the associated M3-HABs European project **(p. 43)**

15h30 - 15h45 – *Jauzein Cécile, Fricke A. , Mangialajo L., Lemée R.*
Optimization of sampling and counting techniques for the monitoring of benthic toxic dinoflagellates: focus on the genus *Ostreopsis* **(p. 44)**

Pause café

SESSION 7 : Ecologie des microalgues (Chair : Laure Guillou)

16h15 - 16h30 – *Artigas Luis Felipe, Bonato S., Créach V., Didry M., Gómez F., Guiselin N., Hamad D., Hébert P. A., Houliez E., Lefebvre A., Lampert L., Lizon F., Poisson-Caillault E., Prévost E., Rijkeboer M., Thyssen M.,Veen A., Rutten T., Wacquet G.*
Monitoring of phytoplankton and Harmful Algal Blooms in coastal waters by combining innovative semi-automated tools (scanning flow cytometry & spectral fluorometry) **(p. 45)**

16h30 - 16h45 – *Lefebvre Alain, Neaud-Masson N., Maurer D., Wacquet G., Grosjean P., Colas F., Tardivel M., Artigas L.-F., Belin C.*
Optimization of the monitoring strategy for the French National Phytoplankton and Phycotoxins Network (REPHY) using semi-automated digital images analysis **(p. 46)**

16h45 - 17h00 – *Wacquet Guillaume, Grosjean P., Hamad D., Lefebvre A., Neaud-Masson N., Colas F., Maurer D., Artigas L.- F., Belin B.*
Zoo/PhytoImage: current advances in the semi-automated classification of plankton digital images **(p. 47)**

17h00 - 17h15 – *Colas Florent, Crassous M.-P., Lunven M., Compère C.*
Underwater Surface Plasmon Resonance sensor for the detection of marine biotoxin **(p. 48)**

17h15 - 17h30 – *Zendong Zita, Abadie E., Sibat-Dubois M., Herrenknecht C., Jauzein C., Lemée R., Gouriou J., Amzil Z., Hess P.*
The memory of seawater: passive sampling for the profiling of algal toxins in lagoons and open coastal seas **(p. 49)**

17h30 - 18h00 – *Assemblée Générale GDR Phycotox*

18h00 - 20h00 – *Session Poster + Apéritif offert par Novakits*

20h00 – Diner : Repas de crêpes bretonnes offert à l'IUEM

Jeudi 2 Avril

SESSION 8 : Ecologie des microalgues (Chair : Raffaele Siano)

09h00 - 9h45 – Conférence plénière
Shauna Murray : *Molecular tools in HAB ecology and seafood safety monitoring* **(p. 13)**

09h45 - 10h00 – *Dia Aliou, Guillou L., Mauger S., Bigeard E., Marie D., Valéro M., Destombe C.*
Population genetics reveal limited gene flow at regional scale of the toxic phytoplanktonic dinoflagellate *Alexandrium minutum* **(p. 50)**

10h00 - 10h15 – *Le Gac Mickaël, Destombe D., Guillou L., Siano R., Valéro M., Chapelle A.*
Intraspecific diversity and incipient speciation in *Alexandrium minutum* **(p. 51)**

10h15 - 10h30 – *Sabart Marion, Lesobre J., Legrand B., Crenn K., Sabatier P., Colombet J., Latour D.*
First evidence of anatoxin-a genes in several freshwater lakes in France: spatio-temporal diversity and phylogenetic affiliation of the sequences **(p. 52)**

10h30 - 10h45 – *Chapelle A., Alves-de-Souza C., Labry L., Sourisseau M., Guallar Morillo C., Gobet A., Morin P., Destombe C., Siano R., Lepelletier F., Dia A., Marie D., Bigeard E., Romaric V., Jeanthon C., Guillou Laure*
Evolutionary processes may increase or attenuate blooming capacity of aquatic microbes **(p. 53)**

Pause café

SESSION 9 : Ecologie des microalgues (Chair : Philipp Hess)

11h15 - 11h30 – *Audrey Mat, Massabuau J. C., Tran D.*
Alexandrium minutum disrupts cyclic activity at behavioral and gene transcription levels in the oyster *Crassostrea gigas* **(p. 54)**

11h30 - 11h45 – *Delegrange Alice, Lefebvre A., R. Amar R., Courcot L., Vincent D.*
Seasonal variability of *Pseudo-nitzschia* sp. and domoic acid concentrations in the Southern Bight of the North Sea **(p. 55)**

11h45 - 12h00 – *Thorel M., Claquin C., Juliette Fauchot*
Life cycle in *Pseudo-nitzschia* spp.: species-specific control of the induction of sexual reproduction **(p. 56)**

12h00 - 12h15 – *Lefebvre Alain, Poisson-Caillault E., Rousseeuw K.*
Spatio-temporal dynamics of phytoplankton biomass in the English Channel: high resolution strategy and modelling using unsupervised classification and Hidden Markov Model **(p. 57)**

12h15 - 12h30 – *Présentations des ateliers (Raffaele Siano & Delphine Latour + animateurs des ateliers)*

Repas (pris au Restaurant Universitaire)

14h00 - 16h30 – Ateliers

17h00 - 18h00 – Restitution des ateliers

18h00 – Clôture de la conférence

Conférences

Plénières

Transfer of biotoxins in the marine food web: case studies from the Portuguese coast

Pedro R. Costa[1]

[1] Instituto Português do Mar e da Atmosfera (IPMA), Avenida de Brasília s/n 1449-006 Lisboa, Portugal

The Portuguese coast is impacted by three main groups of biotoxins: domoic acid (DA)-, saxitoxin (STX)-, and okadaic acid (OA)- group of toxins. The neurotoxins DA and STX have been the main focus of our research. DA is produced by *Pseudo-nitzschia* diatoms, mostly *P. australis*, and STX-group toxins are produced by the dinoflagellate *Gymnodinium catenatum*. These species and their toxins are an obvious threat for human health as they are accumulated in seafood, in particular bivalve mollusks. However, bivalves are not the only organisms exposed to harmful algal blooms. Our research has been developed to evaluate the impact of toxigenic phytoplankton blooms in pelagic and benthic marine organisms, including crustaceans, cephalopods and fish. Based on field observations and laboratory experiments we aim to assess the following: i) baseline levels of DA and STX in pelagic and benthic environments, ii) dynamics of accumulation and elimination of these groups of toxins in selected pelagic and benthic species, and iii) effects of these toxins in the selected target organisms. The main results of this research will be discussed. To finalize this communication, we will present update information on toxigenic species and/or toxins recently detected in the Portuguese coast and discuss their potential impact in the marine food web.

NOTES:

Diversity of Amphidomataceae, the dinophycean source of azaspiracid toxin

Urban Tillmann[1] *& Bernd Krock*[1]

[1]Alfred Wegener Institute, Am Handelshafen 12, D-27570 Bremerhaven, Germany

Azaspiracids (AZAs) are the most recently discovered group of lipophilic marine biotoxins of microalgal origin associated with human incidents of shellfish poisoning. It took about twelve years from the first human toxicity event until a culprit for AZA production was unambiguously identified and described as a novel species, *Azadinium spinosum*, within a newly created genus. Since then, knowledge about the diversity of *Azadinium* has increased rapidly, and today ten species of *Azadinium* are described. Moreover, *Amphidoma languida* has been described as new, and this species is the closest relative of *Azadinium* based on both molecular and morphological data. *Amphidoma* and *Azadinium* are now grouped in the family Amphidomataceae, which forms an independent lineage among other monophyletic major groups of the dinophytes. Initially, azaspiracids have been detected in *A. spinosum* only, but AZA production within the Amphidomataceae appears complex and diverse. At least two other species of *Azadinium, A. poporum and A. dexteroporum*, and also *Amphidoma languida* have been found to produce AZAs. We here will present an up-to-day overview on the current circumscription of the Amphidomataceae including various aspects of morphology, phylogeny, biogeography, and toxin production.

NOTES:

Emerging palytoxin analogues: the opinion of the toxicologist

Aurelia Tubaro[1] & *Pelin Marco*[1]

[1] University of Trieste, Department of Life Science, 34127 Trieste, Italy

Palytoxin (PLTX) is the reference compound of a group of marine toxins identified in *Palythoa* corals, *Ostreopsis* dinoflagellates and *Trichodesmium* cyanobacteria. PLTX is one of the most harmful natural compounds found in nature. Human intoxications are usually associated to ingestion of contaminated seafood, inhalation of aereosolized water during *Ostreopsis* blooms and cutaneous contact with corals and water of *Palythoa*-containing aquaria or seawater during *Ostreopsis* blooms. Recent investigations have reported an increasing number of PLTX analogues, depending on the source of origin. In the Mediterranean area, for instance, *Ostreopsis ovata* is reported to produce mainly ovatoxins (OVTXs), with a prevalence of ovatoxin-a (OVTX-a) and only traces of PLTX. Moreover, in the cyanobacteria *Trichodesmium* as well as in some *Palythoa* corals widely used as aquaria decorative elements a 42-hydroxy-palytoxin (42-OH-PLTX) was found. Very recently, a diasteroisomer with a conformational inversion on C50 was identified in *P. tuberculosa*. Given the growing cases of adverse effects attributed to PLTXs and the considerable amounts of OVTX-a detected in *Ostreopsis* and/or edible marine organisms in the Mediterranean Sea as well as the amounts of 42-OH-PLTXs detected in soft corals, it is necessary to define the toxicological profile of these new compounds. Unfortunately, from a toxicological point of view very few information are available, so far. Recently, we demonstrated that the diasteroisomer of 42-OH-PLTX detected in *P. tuberculosa* is less toxic *in vitro* than PLTX and 42-OH-PLTX. Similarly, OVTX-a seems to be less cytotoxic than PLTX on skin keratinocytes. Given the availability of higher amounts of these new toxins, these results should be confirmed by *in vivo* study. The results could have a high impact on the evaluation of the actual risk for human health associated to these toxins.

NOTES:

Emergent neurotoxins in North Atlantic temperate waters and new vectors

Marisa Silva[1,2] *& Vitor Vasconcelos*[1,2]

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[2] Faculty of Sciences, Porto University, Rua do Campo Alegre, 4169-007 Porto, Portugal

Harmful Algal Blooms (HAB) are complex to manage due to their intermittent nature and their severe impact on the economy and human health. Climate change and anthropogenic intervention contribute to the dispersion and establishment of toxin-producing invasive species that promote the settling of emergent toxins in the food-chain. Neurotoxins such as tetrodotoxin, ciguatoxin, palytoxin, cyclic imines, azaspiracids, brevetoxins and yessotoxins are commonly reported in warm waters but have also caused recently poisoning incidents in temperate zones. In order to minimize public health impacts, monitoring programs should be adjusted as well as adequate legislation. We provide evidence that monitoring for these toxins exclusively in bivalves is simplistic and underestimates the risk to public health, since new vectors have been reported for these toxins and as well for regulated toxins such as PSTs and DSTs.

NOTES:

Molecular tools in HAB ecology and seafood safety monitoring

Shauna Murray[1]

[1] Ass. Prof. Plant Functional Biology and Climate Change Cluster (C3)
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Aquaculture continues to increase in importance worldwide, as fisheries catch is in decline. In many areas of the world, HABs have become expected events for the aquaculture industry, occurring in somewhat predictable seasons and regions. Ocean temperature changes appear to be impacting the distribution and abundance of some HAB species, and may also change the physiological ecology of molluscs in response to biotoxins. In this context, the needs of monitoring authorities and the seafood industry is more focused than ever on rapid and precise HAB identification so that harvesting closures can be minimised. Information from emerging molecular genetic techniques, such as transcriptomics and environmental sequencing, have provided the first information on the genetics of marine biotoxins and the presence of previously undetected cryptic species. The harnessing of such information allows for the development of rapid tools to protect seafood safety, and to build our understanding of marine microbial ecology. I will discuss examples of tools recently developed for the detection of *Alexandrium* blooms and cryptic species of *Gambierdiscus,* both of which appear to be increasing their ranges in Australian waters, with corresponding recent spikes in ciguatera and PST incidences.

NOTES:

Abstracts

des présentations

Histological study of the accumulation and depuration of king scallop, *Pecten maximus*, during toxic outbreak of *Pseudo-nitzchia* spp. in South Brittany, France

Cochennec-Laureau N.[1], *Retho M.*[1], *Chauvin J.*[1], *Fleury E.*[1]

[1] Ifremer, Laboratoire Environnement Ressources du Morbihan Pays de Loire, Rue des résistants, 56470 La Trinité sur Mer

Toxicity of the king scallop, *Pecten maximus*, by the harmful microalgae *Pseudo-nitzchia* spp. tends to become more frequent in Quiberon Bay (South Brittany, France). This recurrence combined with the high accumulation and slow depuration capability of *P. maximus* makes these populations toxic for several years. In this way, between 2010 and 2012, fishery activities have been closed due to high domoic acid (DA) accumulation after a strong *P. australis* outbreak occurred in April 2010. This kind of toxicity have an increasingly importance both for economical and ecological point of view. From june to october 2013, we have designed an experiment i) to monitor the phytoplankton species communities and the toxin concentration and ii) to describe histological changes and lesions in *P. maximus* organs (digestive gland, muscle, gill and mantle). The aim of this study was to establish a baseline histological lesion in order to assess a semi-quantitative approach (pathological index, PI) for juveniles produced in hatchery, not previously contaminated and adults from natural bed with recurrent DA accumulation. The toxin concentration in juvenile and adult populations was respectively 31 and 35 mg/kg (of whole body). Juveniles and adult scallops depurated DA for 85 and 106 days respectively. Detailed histopathology revealed distinct patterns of lesions and alterations for both populations and periods. Severe histopathological damages were mainly observed in the digestive gland of animals contaminated by AD. The PI, representing the histological damage severities, remained high in adult before, during and after a recurrent DA accumulation. In contrast, PI decreased rapidly in juvenile scallop after the first toxic occurrence. These results suggest that histological lesions due to DA accumulation may induce severe alteration not totally reversible after several toxic outbreaks in wild adult scallops. Finally, the potential decrease of depuration activity observed in adult scallop coupling with successive intoxication processes due to seasonal toxic phytoplankton outbreaks is one of the processes responsible for the long duration of banning periods. The present study contributed to the understanding and knowledge of histopathology of an important commercial bivalve. Histopathological screening could be used as a biomarker for toxicity biomonitoring in aquatic organisms.

NOTES:

Pecten maximus and domoic acid: a few questions

Le Gal D.[1], *Terre Terrillon A.*[1], *Derrien A.*[1],*Gouriou J.*[1], *Duval A.*[1], *Le Gall C.*[1], *Le Bec C.*[1]

[1] Ifremer, Laboratoire Environnement Ressources Bretagne Occidentale, Station de Biologie Marine – Place de la Croix -BP 40537- 29185 Concarneau Cedex

The genus *Pseudo nitzschia*, known to be toxinogenic for some species, induced many fishing bans for bivalve molluscs along the French coast. For the 10 years, these prohibitions are recurrent and Domoïc acid concentrations constantly increase. For example, after the first harvest prohibition of the *Pecten maximus* (50mg AD/kg) at the end of October 2003 in the "Rade de Brest", the 2007-2008 episode revealed concentrations up to 250 mg/kg, At the beginning of March 2014 DA concentrations decreased to reach 0,5 mg/kg, In April 2014 *P.australis* bloom affected bivalves with a concentration up to 861 mgAD/kg.In this area all the bivalves were impacted and naturally decontaminated by a few weeks (around 4), except *Pecten maximus.* For this specie the decontamination kinetics was particularly slow (level 284,5 mg AD/kg on the 14/01/2015). In parallel of the REPHY health monitoring network results, several questions need to be solved.
In this presentation, four illustrations are developed:
1) Differences of kinetics and contamination levels (illustrated by a comparison between different species of Douarnenez Bay and the Glenan Islands area).
2) *Pecten maximus* matter in "Rade de Brest" .
3) Inter individual variability concerning scallops.
4) Domoïc acid migration in the different organs after freezing.

NOTES:

Characterization of the voltage-gated sodium channel and its isoforms in the Pacific oyster *Crassostrea gigas*: link with its sensitivity to paralytic shellfish toxins produced by *Alexandrium minutum*.

Boullot F.[1], *Benoit E.*[2], *Castre J.*[1], *Hégaret H.*[1], *Boudry P.*[3], *Soudan P.*[1], *C. Fabioux* [1]

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French coasts are regularly affected by blooms of the dinoflagellate *Alexandrium* spp. These micro- algae can produce paralytic shellfish toxins (PSTs),such as saxitoxin (STX) and its derivatives, which bind to voltage-gated sodium (Nav) channels and block conduction of action potential in muscle and nerve fibers. Filter feeder marine organisms, such as oysters, can accumulate high levels of PSTs, making them toxic and unsuitable for human consumption. The toxin load has been shown to vary between individual Pacific oysters, *Crassostrea gigas*, sampled from a same population and then exposed experimentally to *A. minutum*, thus suggesting a high variability in PST tolerance. The purpose of this study is (i) to characterize Nav channels and its isoforms in *C. gigas* and (ii) to determine whether Nav channel iso forms play a role in the highly variable sensitivity of *C. gigas* to PSTs. Among the three proteins annotated as "Nav" channels in the C. gigas genome, only the Nav9 has all the characteristics of a true Nav channel and was thus selected as candidate in this study. The tissular and cellular expression pattern of Nav9 was characterized by real-time polymerase chain reaction and in situ hybridization. Nav9 appeared mainly expressed in neuro secretory cells of the visceral ganglia, in nerve fibers of the skeletal muscle and in epithelial cells of the labial palps and mantle. First electrophysiological measurements performed on nerves of oysters showed a significant decrease of the global action potential when exposed to 10-7g/mL of STX. These results indicate a medium sensitivity of oysters to PSTs, which must be confirmed by further electrophysiological experiments. First sequencing analysis of Nav cDNA in muscle and visceral ganglia put in evidence three alternative splicing transcripts that could be a source of the variability of sensitivity to PSTsin *C. gigas*, as demonstrated in some insects for resistance to pyrethroids.

NOTES:

Combined effects of toxic dinoflagellates of the genus *Alexandrium* and pathogens on bivalve physiology

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Oyster populations undergo regular epidemics that weaken or decimate exploited stocks and thus limit aquaculture. These diseases are caused mainly by viruses, bacteria or parasites, and occur primarily during spring and summer. This period of the year also provides favorable conditions for toxic dinoflagellate blooms in temperate areas, including species of the genus *Alexandrium*. Thus, the risk of *Alexandrium* sp. blooms and infectious diseases co-occurring in oysters is high. However, these micro-algae synthesize and excrete toxins and cytotoxic compounds responsible for physiological changes in bivalves and could lead to an immuno-compromised status. The aim of the experiences presented here was to evaluate the combined effects on bivalve physiology of exposure to the toxic dinoflagellate, *Alexandrium* sp., and infection by pathogens, through the study of different bivalve - pathogen - *Alexandrium* sp. tripartite interactions. The results of this work indicate that a unique response profile could not be identified. Thus, the exposure to *Alexandrium fundyense* increases the susceptibility of C. virginica oyster to the parasite *Perkinsus marinus*, probably via immuno-suppression, as suggested by the partial inhibition of hemocyte responses. On the contrary, the dinoflagellate *Alexandrium catenella* reduces the herpesvirus infection in oyster *Crassostrea gigas*. Additionally, the herpesvirus infection decreased Paralytic Shellfish Toxin (PST) accumulation in the oysters. These results provide a better understanding of the involvement of toxic algal blooms in the development of diseases affecting commercial bivalve species, but also of the involvement of the bivalve biotic environment in the accumulation of regulated toxins.

NOTES:

Various approaches in chemical ecology of toxic micro-algae

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Although harmful algal blooms have become major environmental and health concern due to their increased frequency and wider distribution, metabolic mechanisms involved in the production of the toxins remain scarcely described, mainly in reason to their structural complexity and the low amount of substances produced. Investigation in the field of HABs chemical ecology is currently needed to manage their impact on both human health and marine ecosystems, and this would be enabled by the recent improvements in analytical chemistry, in particular NMR and MS. Indeed, there is no way to investigate the role and the impact of toxins produced by phytoplanktonic species without acquiring a broad-spectrum knowledge of their chemical nature. In this context, we present the detailed chemical structure of one polyether isolated from a cultivated strain of *Gambierdiscus belizeanus*. We will also focus on metabolic pathways evidenced to be involved in the production of another polyether isolated from *Karenia brevis*, using radio-labeled methodology. At last, approaches in targeted and untargeted metabolomics currently carried out to assess the evolution of the toxic content of *Ostreopsis cf.ovata* under biotic stressors will be presented.

NOTES:

Ichthyotoxic micro-algae and sustainable aquaculture

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Ichthyotoxic micro-algae, cyanobacteria excluded, are generally small-sized flagellates which belong to different taxonomic groups. They are difficult to identify with light microscopy and almost impossible to recognize when samples have previously been fixed for long-time storage. Hence, this group is only partially taken into account in routine analyses for monitoring. The presence of these organisms is under-estimated although they are capable of massive proliferations, responsible for mortalities of various marine animals. In France, several research projects contributed to a better knowledge of their diversity. Species of potentially toxic Raphidophyceae, Prymnesiophyceae and Dictyochophyceae have been observed along French coasts. Since 2008, a specific effort in the study of ichthyotoxic dinoflagellates has been made in the DIALTAX action in the context of mortalities of juvenile oysters in metropolitan France or shrimps reared in farms in New Caledonia, and of the aquaculture development of scallops in Saint-Pierre-et-Miquelon. This allowed to detect the presence of i) toxic *Karenia* species such as *K. brevisulcata* in France and *K. cristata* in Miquelon waters, ii) kareniacean new taxa affiliated to known or unknown genera in France, and iii) Pfiesteriaceae species in France and New Caledonia. In addition, attempts to culture some of the species encountered in the samples have been made in order to estimate their toxic activity. Three strains of kareniaecean dinoflagellates have been obtained and haemolytic assays realized were positive for two of them.

NOTES:

Live free or attached: the dilemma of Planktothrix

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Planktothrix is known to be a toxic cyanobacterial genus proliferating in the water column of numerous eutrophic ecosystems all around the world. However, in the recent years, benthic strains belonging to this genus have been isolated in freshwater biofilms. By the sequencing of the genomes of several planktonic and benthic strains, we are studying the genomic basis of the ability of this genus to occupy varied environments and to proliferate in them.

NOTES:

Spatiotemporal variations in the fungal parasitism of cyanobacteria

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Parasitic zoosporic fungi are ecologically significant in various aquatic ecosystems through their roles in controlling host populations, and are a proven link in the trophic food web. However, studies on cyanobacteria-chytrid pairings are still scarce. In order to provide an accurate host-parasite pairing distribution and to gather informations on the design of sampling strategy used to survey cyanobacteria-chytrid pairings we investigated the spatiotemporal variations of cyanobacteria-chytrid pairings by studying vertical, horizontal and temporal distribution of the cyanobacterial host *Anabaena* macrospora and its associated chytrids belong to the genus *Rhizosiphon*, in the eutrophic Lake Aydat, France. Our results suggested that the chytrid infection was mainly forced by the host density.Therefore, in order to improve the knowledge on host-parasite interactions under natural conditions, we suggested focusing the sampling strategy on temporal rather than on spatial scales. Additionally, we reported a high chytrid infection rate on the *A. macrospora* resting spores. Through their infection, chytrids may have an important impact on cyanobaterial population from year to year,in term of both, the size of inoculum and the genetic diversity.

NOTES:

A model of *Alexandrium minutum* blooms in inter-specific competition. A trait-based modelling approach.

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Since 2010, planktonic algae *Alexandrium minutum*, cause of the PSP (Paralytic Shellfish Poisoning) syndrome, has been proliferating during early summer in the bay of Daoulas, within the roadstead of Brest. A 0D model has been designed in order to derive these blooms from physical parameters (temperature, irradiance, nutrient supply, dilution rate ...). In the model, *A. minutum* competes with 50 other plankton species with randomly attributed optimal temperature and nutrient uptake abilities. Each species competes for 3 nutrients (nitrate, phosphate and silicate) and cellular quotas are taken into account. All nutrient related parameters are calculated following an allometric function of cell volume. The only loss term is the purely hydrodynamic dilution (derived from 3D hydrodynamic model simulations) since neither mortality nor grazing is simulated. Simulations are compared with in situ data coming from the Veliger and Rephy survey programs. The model turns out to reproduce well the bloom of 2012, with a correct date of maximum concentration and a relevant order of magnitude. It reproduces the order of magnitude of 2013 concentrations, but the bloom then is too spread in time around the maximum. Further simulations on other years and estuaries will validate or invalidate this tool.

NOTES:

Cyanobacteria in Lake Dziani Dzaha, a potential analog of Precambrian oceans

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Dziani Dzaha is a small, tropical crater lake located in Mayotte Island (Comoros archipelago, Western Indian Ocean). This lake is alkaline, hypersaline with permanent anoxia below one meter depth and characterized by a high productivity with a low biomass turnover rate (ca. 15 days). Total chlorophyll a biomass vary between 500 and 650 $\mu g \cdot L^{-1}$ and is dominated by cyanobacteria (large filaments and single cells $\leq 2\mu m$). High abundance of heterotrophic prokaryotes was also reported throughout the water column. Water chemistry of the lake supports the hypothesis of the marine origin of water strongly modified by geological and microbial activities, which generated the extreme alkaline conditions observed (pH close to 11). This saline lake has recently been identified by the consortium of this project as a potential analog of Precambrian oceans. Two multidisciplinary projects (ANR DZIANI 2014-2017, PI M. Ader, IPGP, Paris & Total foundation biodiversity program DZAHA 2014-2015, PI C. Leboulanger, MARBEC, Montpellier) aim to perform a geochemical and microbiological study to fully characterize this unique ecosystem and its sedimentary record. The objectives are to characterize (i) the biogeochemical C cycle; (ii) the biogeochemical cycles of N, S and Fe and their interactions with the C cycle; (iii) the biodiversity of microorganisms (virus, cyanobacteria, eubacteria, archaea and eukaryotes) in the different compartments as a preliminary step towards addressing their role in the biogeochemical cycles and (iv) the main metabolic activities operating within the lake. This presentation will give a first insight of cyanobacteria diversity and their potential functional role in Dziani Dzaha lake.

NOTES:

Citizen participation in monitoring phytoplankton seawater discolorations

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A citizen monitoring program of water discoloration observations (*Phenomer*) caused by phytoplankton proliferations was launched throughout the coastal waters of Brittany (France) in 2013 in parallel to the ongoing phytoplankton and biotoxin monitoring network (REPHY). Beyond communication and outreach objectives, this project aims to explore the possibility to acquire scientifically valuable data on Harmful Algal Blooms (HABs) through extending the survey area of coastal waters by means of citizen alerts. A theoretically infinite number of sampling points (public observations) could contribute to identify i) HAB frequency and recurrence; ii) the distribution and extension of water discolorations; iii) the biogeography of causative taxa. During the first two years of project implementation, 14 and 32 out of respectively 40 and 75 observations corresponded to HAB events. Citizen observations contributed to evaluate the extension and duration of water discolorations phenomena. In 2013, *Noctiluca scintillans* red discolorations were observed at ca. 180 km of distance, and in 2014, *Lepidodinium chlorophorum* created impressive green discolorations, lasting over one month, being at times associated to massive fish mortalities. New harmful algal bloom risks were identified for the first time in Brittany. A bivalve mortality event coincided with a dark-brown phytoplankton bloom characterized by the dominance of the toxic raphidophytes *Heterosigma akashiwo* and the dictyochophyte *Pseudochattonella verruculosa. Phenomer* showed the value of citizen science programs in contributing towards managing and monitoring marine coastal areas at risk from HABs, whilst also providing the basis for the realization of new research projects on harmful microalgae.

NOTES:

Presentation of the FOODCYANO project

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In June 2014, EFSA (the European Food Safety Authority) launched an open call for a review of literature on cyanobacteria toxins in food. A consortium set up by ANSES (the French Agency for Food, Environmental and Occupational Health & Safety) and ISS (the Italian National Institute of Health, Istituto Superiore di Sanità) has been selected by EFSA to conduct this project. The objectives set by EFSA are as follows:

Objective 1: to conduct an extensive literature search and perform a critical review of the scientific literature on cyanobacteria toxins occurrence in food (including supplements), identifying also possible data gaps and research needs. In particular, information should be collected and analysed from studies on cyanotoxins (and congeners) found in food (fish, shellfish, food supplements, vegetables & fruits through irrigation, meat and dairy products through drinking water for livestock), sample preparation and reliable detection methods, bioaccumulation (field and laboratory), monitoring systems/programs according to existing international/national regulations/guidelines or "health alert" levels, etc.

Objective 2: to conduct an extensive literature search and perform a critical review of the scientific literature on the toxicological profiles of cyanotoxins found in food sources (including supplements), identifying also possible data gaps and research needs. In particular, information should be collected and analysed from cyanotoxin studies on the elucidation of mechanisms of toxicity both for short- and long-term exposure, considering also aggregate/cumulative exposure or concomitant exposure with other chemicals, and on environmental factors responsible for changes in toxicity profiles, etc.

Objective 3: to formulate plausible exposure scenarios to cyanotoxins from food consumption (including supplements, food sources identified in objective 1, direct and indirect uses of water, i.e. drinking water, food preparation, food industry) in the general population and identifiy susceptible groups.

This 12 months project has been contracted in December 2014. The aim of this presentation to both the GdR PHYCOTOX and GRISCYA (GIS cyanobacteria) is to make a call for data to the scientists of these two French networks to help us to provide the best state of knowledge to EFSA.

NOTES:

Risk evaluation of shellfish consumption due to toxin presence. Exposition evaluation and contribution of danger characterization

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The aim of the project is to evaluate the contamination of phycotoxins in shellfish, harvest by recreational shellfish harvesters. In this context, are realized:
-Shellfish collecting and phycotoxins analysis
-Consumption surveys to recreational shellfish harvesters, in order to evaluate their exposure.
The different survey areas were chosen in Brittany (France), according to the previous phycotoxins contamination observed by the REPHY program of IFREMER and C. Picot thesis. Four areas were selected: Brest harbor, Douarnenez Bay, Concarneau Bay and Vilaine Bay. The shellfish are collecting once a month, preferentially during the highest tides. It is during those periods that we can observe the most recreational shellfish harvesters. The study will last three years. The first shellfishes were collected in October 2013.
The shellfish species collected are different according to the site and depends on the shellfish harvest habits of the recreational shellfish harvesters in each area.
After shelled the shellfish, the phycotoxins concentration in shellfish flesh is measured in liquid chromatography with tandem mass spectrometry detection, UPLC-MS-MS. The analyzed molecules are:
• Lipophilic toxins: dinophysistoxins, okadaic acid, pectenotoxins, azaspiracids and yessotoxins and neurotoxins shellfish poisoning (NSP toxins) : spirolides, gymnodimines,
• Amnesic shellfish poisoning (ASP toxins): domoic acid and epidomoic acid,
• Paralytic shellfish poisoning (PSP toxins): saxitoxins.
Shellfish preparation (cook or not) will be tested because the toxins concentration can be influenced by this step. The results obtained in 2013 are shown. Moreover, during the collects, surveys have been done to the recreational shellfish harvesters in the different collecting areas. These surveys are about the species collected, the consumption habits, the knowing and perception of recreational shellfish harvests interdiction.

NOTES:

Novel case report of a Ciguatera Shellfish Poisoning cluster following the consumption of *Techtus niloticus* (Gastropod) in French Polynesia.

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Ciguatera Shellfish Poisoning (CSP) is a form of seafood poisoning related to the consumption of various marine invertebrates highly popular in Pacific Islands Countries and Territories (PICTs). Until recently, only CSP incidents associated with the consumption of giant-clams (*Tridacna maxima*) and sea urchins (*Tripneutes gratilla*) have been sporadically reported in French Polynesia. Here, we provide the first description of an outstanding poisoning episode, which occurred in Nuku-Hiva Island (Marquesas archipelago, French Polynesia) following the consumption of trochus (gastropod) belonging to the species *Tectus niloticus*. In June 2014, nine tourists (two French, two Dutch and five Italian) developed signs of a "Collective Foodborne ToxiInfection" which occurred following the consumption of trochus collected in Anaho Bay, located in the northern part of Nuku Hiva island. Seven of them quickly received medical care at the island's hospital in order to treat typical major gastrointestinal and neurological disorders which clearly evoked a Ciguatera-like syndrome (cold allodynia, itching, dysesthesia and paresthesia of the extremities). However, the rapid onset of symptoms ($\leq$ 2 hours) and the unusual oropharyngeal burning sensation experienced by a majority of patients underpin the possible presence of other toxic compounds distinct from ciguatoxins. Two of the five Italian eventually returned to Italy a week after the poisoning event, and continued receiving medical attention from the Poison Control Centre and National Toxicology Information Centre of Pavia. In addition, all 9 tourists were contacted 5 months later, and were asked to complete a medical questionnaire documenting the acute and chronic phases of their poisoning. This questionnaire has pointed out that a majority of them were still experiencing symptoms even several months after the poisoning incident, mainly characterized by gastrointestinal and/or neurological signs of variable intensity and quality. In parallel, the preliminary results of the toxicological analysis conducted on *T. niloticus* specimens collected one month after the toxic episode in the area reputed toxic in Anaho Bay, strongly suggest the presence of both ciguatoxic compounds and Azaspiracid-2 analogs. These latter are known to be responsible for a diarrhetic syndrome and are commonly produced by the dinoflagellate *Azadinium* sp. which has been previously reported in West Pacific waters. These preliminary investigations highlight the role of T. niloticus as a possible vector of CSP in French Polynesia. The suspected co-occurrence of ciguatoxins and Azaspiracids-2 compounds coincides with clinical manifestations observed in patients (high prevalence of gastrointestinal signs, in acute and chronic phase). Further investigations are underway to confirm the toxic organisms and toxins involved characterize the chronic evolution of symptoms and hopefully identify therapeutic solutions. Finally, such findings should be of great interest to local health authorities of most PICTs where T. niloticus constitute a significant subsistence and economic resource, especially in the purpose of improving seafood poisoning risk management programs that are on-going in the Pacific region.

BMAA and isomers in samples from Thau lagoon and in mollusks from other French coasts (Channel, Atlantic and Mediterranean sites)

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BMAA and isomers (DAB, AEG andBAMA) are non proteinogenic amino acids that have been found in higher trophic levels in many ecosystems, e.g. in the Baltic Sea. BMAA has been associated with neurodegenerative diseases, especially amyotrophic lateral sclerosis (ALS). As a polar molecule, its bioaccumulation in the brain of patients is uncommon and but possibly justified by incorporation of BMAA in proteins during protein synthesis. Many different analytical methods were employed to detect BMAA that led to reports of controversial concentrations of BMAA in the environment. To overcome this difficulty, an LC-MS/MS method has been optimizedto unambiguously identify BMAA and isomers in biological samples.In France, these BMAA and isomers have recently been found in mollusks from Thau lagoon, where a cluster of ALS had previously been reported. With the aim of improving knowledge on the distribution of BMAA in the Thau lagoon ecosystem, mussels, periphyton (biofilms attached to mussel) and plankton were collected using nets between July 2013 and October 2014. The screening of these samples showed an annual presence of BMAA, DAB and AEG in mussels of the lagoon, and also in periphyton and seston samples. The concentrations found could suggest bioaccumulation of BMAA and DAB in the mussels. Indeed, concentrations of 0.58 and 0.84, 2.6 and 3.3, 4 and 7.2 µg/g DW of BMAA and DAB were quantified in plankton collected with nets, periphyton and mussels, respectively. Unlike other toxins (i.e. both cyanotoxins and phycotoxins), BMAA and DAB did not seem to accumulate only in digestive glands but were also found in significant amounts in remaining flesh of the mussels, maybe as a function of protein metabolism and synthesis. The continuous detection of BMAA in mussels from Thau Lagoon led us to screen this toxin in other ecosystems. As a consequence, mollusks of nine locations chosen for geographical spread and to reflect important shellfish production areas were screened. BMAA, DAB and, to a lesser extent, AEG were systematically found in concentrations similar to those in Thau lagoon (except for the oysters from Ronce and Leucate where significantly lower concentrations were observed). All these results raised questions about the origin of BMAA and DAB detected in mollusks collected from several sites along the French coast, as well as about the link between mollusk consumption and the ALS cluster observed near the Thau lagoon.

NOTES:

Pinnatoxin-G: analytical methodology, distribution in Mediterranean lagoons and accumulation in mussels (*Mytilus edulis* and *Mytilus galloprovincialis*)

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In a previous study, Pinnatoxin G (PnTX-G) had been identified as a toxin accumulating in mussels and clams in Ingril Lagoon, a small lagoon on the French Mediterranean coast. The levels found in shellfish from this lagoon were sufficient to explain positive mouse bioassays (MBA) in the absence of other regulated toxins. The present study has been commissioned by the French Directorate General for Food and the Directorate General for Health in order to: (i) gain knowledge on the distribution of PnTX- G in other Mediterranean lagoons, (ii) better characterise the analytical methodology used (liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), and (iii) better understand the accumulation in shellfish of PnTX-G and potentially other bioactive or toxic compounds produced by *Vulcanodinium rugosum*, the dinoflagellate producing PnTX-G. A one-year study (2013 – 2014) in five Mediterranean lagoons (Prévost, Vic, Ingril, Thau and Leucate) revealed that mussels at Ingril were by far the most contaminated, with mussels in the other four lagoons typically accumulating PnTX-G to levels less than those that would cause positive MBAs. Limits of quantification (LoQ) and matrix effects in the LC-MS/MS analysis of PnTX-G have been characterised on two models of tandem mass spectrometers (API-4000 and API-5500 Q-Traps). The LoQs were sufficiently low on both instruments to detect and reliably quantify concentrations below those that would cause positive MBAs (50 µg PnTX-G / kg whole flesh). Matrix effects did not significantly differ between instrument models; however, matrix effects differed between clams, mussel whole flesh and mussel hepatopancreas, with the latter matrix yielding the least signal for PnTX-G in crude extract (50% signal suppression) and clams showing the least signal suppression. Live mussels (*M. edulis* and *M. galloprovincialis*) have been exposed to live cells of *V. rugosum* in three separate accumulation experiments. Depending on the experiment, mussels accumulated PnTX-G to concentrations of approximately 20 to 65 µg kg-1 whole flesh. These levels are lower than those observed in mussels from Ingril lagoon, probably due to (i) lower toxin cell quota in the cultured *V. rugosum* than those in situ and (ii) shorter exposure times in the laboratory (24 – 72h). However, the levels are comparable to or higher than those observed in mussels from the other four lagoons. Portimine, another recently identified, major metabolite of *V. rugosum*, also accumulated in mussels in the laboratory. Concentrations of portimine in mussels were estimated to exceed those of PnTX-G five-fold; however, the ratio of portimine over PnTX-G in *V. rugosum* is much higher (ca. 20), and hence, portimine is either less accumulated or metabolised or excreted faster in mussels.

NOTES:

Pinnatoxin bioaccumulation in mussels exposed to *Vulcanodinium rugosum*.

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Vulcanodinium rugosum is a newly described marine dinoflagellate found in the Méditerranéen lagoon of Ingril (France) and in the coastal zones of New Zealand, Australia and Japan. While the Ingril *V. rugosum* strain produces mainly pinnatoxin-G, the strains isolated from South Pacific coasts produce pinnatoxin-E, F and G. Pinnatoxins are potent antagonists of nicotinic acetylcholine receptors belonging to the cyclic imine toxin family. The distinguishing feature of these compounds is the presence of a cyclic imine moiety that is common to gymnodimines, spirolides, pteriatoxins, prorocentrolides and spiro-prorocentrimines. Pinnatoxins exhibit fast acting neurotoxicity provoking mouse death by respiratory arrest within minutes following intraperitoneal administration, gavage or after voluntary intake. As cyclic imine toxins could be bioaccumulated by shellfish, their potential impact on human health is of concern. In the frame of a study financed by the French governmental institutions (DGAL and DGS), we have studied the bio-accumulation of pinnatoxin-G on mussels exposed for 4 days to a culture of *V. rugosum*. The analysis by Microplate-Receptor Binding Assay showed that both, mussel control specimens and mussels exposed to the toxic dinoflagellate were positive for the presence of toxins targeting nicotinic acetylcholine receptors. The samples were analyzed by ultra-performance liquid chromatography-tandem mass spectrometry in order to determine the nature of the nicotinic toxins. Actually, it was found that all the mussels were contaminated with 13-desmethyl-spirolide-C (~20 nM) explaining why control mussels were positive by the bioassay. However, only mussels exposed to *V. rugosum* cultures contained pinnatoxin-G (~2 nM). Altogether, these analyzes demonstrated the bioaccumulation of pinnatoxin-G *in-vitro* by mussels exposed to *V. rugosum*. The study of mussels extracts collected from the Ingril lagoon showed that these specimens were naturally contaminated with gymnodimine-A, 13-desmethyl-spirolide-C, pinnatoxine-A, pinnatoxin-G and the 28-*O*-faty acyl pinnatoxin-G, an storage sub-product resulting from the acylation of pinnatoxin-G by the mussels. More studies are needed to show whether the Ingril lagoon constitutes a natural reservoir for dinoflagellate species producing cyclic imine toxins, mostly when Ingril lagoon is located in a Region where conchylicultural activities are important. Finally, the development of new detection methods will be discussed.

NOTES:

Investigation on the toxic effects and intestinal crossing of pinnatoxin-A and -G and of extracts of *Vulcanodinium rugosum* cultures

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During a previous study devoted to investigate the effects of pinnatoxin-G (PnTX-G) and *Vulcanodinium rugosum* extracts, we showed that, although PnTX-G induced no cytotoxic effect on different cell lines, the crude extract and some fractions of *V. rugosum* were cytotoxic indicating that other toxic compounds are produced by this dinoflagellate. Moreover, a receptor binding assay (RBA) for detecting pinnatoxins in shellfish extracts was tested and showed promising results. In this new study, we further investigated: i) if pinnatoxin-G but also –A as well as the main 2 toxic fractions of *V. rugosum* can induced some specific in vitro effects, ii) if PnTX-A and –G can cross a human intestinal cell barrier model, iii) which in vivo acute effects PnTX- G and *V. rugosum* extracts induce after gavage to mice, and iv) if other toxic compounds produced by *V. rugosum* may accumulate in shellfish. PnTX-G and -A did not affect cell viability of human intestinal Caco2 and neuroblastoma Neuro2a cells up to 30 nM after 24h. Similarly, no induction of inflammation measured by IL8 release or nuclear translocation of NFkB was observed as well as apoptosis (activation of caspase 3) and DNA damage (phosphorylation of H2Ax). On the contrary, the two main toxic fractions from *V. rugosum* induced cytotoxicity, apoptosis, DNA damage and inflammation, even though some specific morphological modifications were observed for each fraction. These results confirm that toxic compounds, other than PnTX-G, are produced by the dinoflagellate. Whether with pure toxin, crude extract or PnTX-G-enriched fraction, we showed that PnTX G can moderately cross the in vitro intestinal barrier (up to 20% of the loaded amount after 24h). However, PnTX-A highlighted a different behavior. A LD50 = 200 µg/kg was established for PnTX-G by gavage with mouse death after only 15-20 min, symptomatic of some effect on the nervous system. Slight alterations of the small intestine (moderate mucosa degeneration and atrophy of the villi) were observed from histological examination, only for PnTX-G or extract doses inducing lethality. Extracts from mussels fed with *V. rugosum* showed a higher toxicity level on Neuro2a cells compared to control mussel extracts, suggesting accumulation of toxic compounds other than PnTXs. Identification of these toxic compounds and their cell targets should be performed.

NOTES:

How 13,19-didesmethyl and 13-desmethyl spirolide C phycotoxins affect the cholinergic function? Pharmacological studies on nicotinic and muscarinic acetylcholine receptors

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The dinoflagellates *Alexandrium ostenfeldii* / *Alexandrium peruvianum* are known to produce the potent lipophilic marine toxins known as spirolides, which have been detected in contaminated shellfish. The spirolides are referred to act as fast acting phycotoxins, since they induce rapid onset of neurotoxic symptoms when administered by intraperitoneal injection or orally, followed by rapid death in laboratory mice. The aim of the present work was to further characterize the mode of action of 13,19-didesmethyl and 13-desmethyl spirolide C phycotoxins on various nicotinic acetylcholine receptors (nAChRs) subtypes and to determine whether they act on muscarinic receptors (mAChRs). For this, functional electrophysiological studies using isolated nerve-muscle preparation, as well as patch-clamp recordings on skeletal embryonic myocytes, and voltage-clamp recordings on *Xenopus* oocytes expressing various nAChR subtypes in their membranes have been used. The interaction of the two toxins with their putative receptors was also studied in competition binding experiments with specific radiolabelled ligands of various mAChR and nAChR subtypes. The results obtained indicate that 13-desmethyl spirolide C interacts efficiently with sub-nanomolar affinities and low selectivity on muscle- and neuronal-nAChRs, the 13,19-didesmethyl spirolide C is more selective of the muscular and homopentameric α7 receptors and recognizes only weakly the neuronal heteropentameric receptors, especially the α4β2 subtype. On the other hand, both spirolides interact with low micromolar affinity on the five mAChRs. Thus, the presence of an additional methyl group on the spiroimine moiety modified significantly the pharmacological profiles of these phycotoxins by decreasing their affinity on neuronal nAChRs. In conclusion, our results indicate that the toxicity of the spirolide C analogs here studied is mainly due to their high inhibition potency on various peripheral and central nAChRs and not to their low ability to interact with mAChR subtypes.

NOTES:

Effect of co-exposure to marine lipophilic biotoxins on the intestinal barrier, bioactivation and molecular mode of actions

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Marine lipophilic biotoxins are secondary metabolites produced by a restricted variety of phytoplankton, a type of microalguae which constitutes the first nexus of marine food chain. Biotoxins include different groups of compounds which occur with an increased frequency in combination in shellfish. Contamination of shellfish by phycotoxins is a worlwide phenomenon and consumption of such shellfish may result in food intoxication with a large panel of symptoms on human health. Therefore, this three-year thesis, in collaboration with BfR from Berlin, is aimed at addressing the effects of a panel of marine phycotoxins (alone or in combination) on the intestinal barrier. Particularly, three main questions will be investigated: the integrity of the epithelium after exposure to phycotoxins, their transport across the intestinal barrier and finally their toxic effects. A strong review on the metabolism of phycotoxins will also be undertaken in order to determine the detoxification of the compounds, the formed metabolites, the pathways involved and also the ability of phycotoxins to be bioactivated. Okadaïc acid (OA) is one known example of such marine biotoxins which can provoke food intoxication. Little is known about its detoxification, especially concerning the role of phase III transporters. Therefore, the influence of three xenobiotic-metabolizing enzymes inhibitors was assessed on *in vitro* hepatic cell line model HepaRG. Voriconazole, a specific CYP3A4 inhibitor, ketoconazole, a known CYP3A4 and weak P-gP inhibitor and verapamil a potent P-gP and weak CYP3A4 inhibitor were incubated one hour before one day co-treatment with OA. Several cytotoxicity parameters, including apoptosis (caspase-3 activation), double strand break DNA (phosphorylation of H2AX) and cell viability (DAPI staining), were followed by means of high content analysis. The results of this work bring additional evidence for confirming the role of CYP3A4 in the detoxification of OA and also show that P-gP may play a key role in its excretion.

NOTES:

Changes in secondary metabolic profiles of *Microcystis aeruginosa* strains in response to intraspecific interactions

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The freshwater cyanobacteria *Microcystis* proliferate in eutrophic freshwater ecosystems and produce bioactive compounds including the well-known harmful toxins, the microcystins (MC). There is growing evidence that these secondary metabolites play an important role in shaping community composition through biotic interactions; however, for the most part, their biological role and mode of regulation of the production are poorly understood. As natural cyanobacterial populations consist of a mixture of producing and non-producing strains for each class of bioactive peptides, we tested if the production of a range of peptides by co-existing cells could be regulated through intraspecific interactions. With an innovative purpose-built co-culturing chamber together with a MS/MS-based molecular networking approach, we monitored the growth and compared the metabolic profiles of a MC-producing as well as two non-MC-producing *Microcystis* strains under mono- and co-culture conditions. These strains in monoculture grew comparably, however, the non-MC-producing mutant produced higher concentrations of cyanopeptolins, aerucyclamides and aeruginosins than the wild-type. Moreover, physiological responses to co-culturing were reflected in a quantitative change in which major peptides were produced. Using recently developed advanced mass spectrometry techniques we have identified new analogues in several known classes of peptides as well as a few new compounds of unknown structure. This work provides fresh insights into the factors that regulate the production of MC and other secondary metabolites in cyanobacteria, and suggests that these compounds may have interchangeable or complementary functions allowing bloom-forming cyanobacteria to efficiently colonize and dominate in highly variable aquatic environments.

NOTES:

Extraction of palytoxins from seawater and preliminary stability studies

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Palytoxin is one of the most potent marine toxins and may be produced by several different species of the soft coral Palythoa (order: Zoanthidae). Ovatoxins are analogues of Palytoxin produced by the microalga Ostreopsis cf. ovata, which typically blooms in tropical and subtropical areas. However, more recently, it has also been detected across the Mediterranean and Southern-Atlantic coasts of Europe. The release of high concentrations of palytoxin analogues (putative palytoxin and ovatoxins) in seawater during a massive bloom causes severe toxic outbreaks due to inhalation and/or dermal exposure. In addition, several cases of poisonings have been reported in aquarium hobbyists from incidental contact with palytoxin-producing Palythoa sp. In order to prevent human illness following palytoxin or ovatoxin exposure, a reliable method for an early monitoring of toxins in seawater is needed. Different types of stationary phases (HLB, STRATA-X, SP-850, SP-207, HP-20, RP-18, Carbograph) and elution conditions were evaluated in terms of recovery yield and reproducibility. Best recoveries were obtained by using HLB, HP-20 and RP-18. Furthermore, it emerged that Carbograph is a stationary phase able to completely retain palytoxin. For this reason it could be used in the detoxification procedures of the home aquaria. Preliminary studies assessed the stability and recovery of palytoxin during different evaporation procedures (nitrogen stream, freeze drying, vacuum concentration). Freeze-drying did not significantly improve recovery in comparison to a gentle stream of nitrogen. Strong acid negatively affected recovery of palytoxin during evaporation procedures, while acetic acid seemed to slightly facilitate recovery. A strong influence was also observed for the materials of the containers used in evaporation. Silanised glass and Teflon surfaces yielded significantly higher recoveries than unsilanised glass or polypropylene tubes. Palytoxin recovery from evaporation through centrifugal vacuum concentration appeared not to be strongly influenced by different materials of the containers; however, the best recovery was obtained using Teflon containers. Overall, HLB, HP-20 and RP-18 stationary phases were most appropriate for recovery of palytoxins from seawater. Finally, optimised evaporation thus appears to be a critical step to minimise losses of palytoxin and analogues in preparative isolation procedures.

NOTES:

Gender-specific toxicological effects of chronic exposure to pure microcystin-LR or complexe *Microcystis* extracts on the liver of adult medaka fish revealed by anatomopathology and proteomics

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Cyanobacterial toxic blooms often occur in freshwater lakes and constitute a potential health risk to human populations, as well as to fish and other aquatic organisms. The most commonly detected cyanotoxins in the freshwater environment, microcystins, are potent hepatotoxins, deregulating the kinase pathway by inhibiting phosphatases 1 and 2A. Although toxicological effects have been clearly linked to the in vitro exposure of vertebrates such as fish to purified microcystins, cyanotoxins are produced by the cyanobacteria together with numerous other potentially toxic molecules, and their overall and specific implications for the health of aquatic organisms chronically and environmentally exposed to microcystin-producing cyanobacteria have still not been clearly established and remain difficult to assess.
The medaka fish (Oryziaslatipes) was chosen as an experimental aquatic model for studying the cellular and molecular toxicological effects on liver - being in the same time the main tissue target of hepatotoxin and the xenobiotic detoxifying organ - of chronic exposure to pure microcystin-LR, complex extracts of biomasses of cyanobacteria producing- or non-producing microcystins, and of a Microcystis natural bloom. Young mature fishes were maintained during 28 days in aquaria containing 5 µL.L-1 of microcystins (or equivalent cyanobacteria biomass), corresponding to environmental concentration frequently observed during summer blooms. Fish were then sacrificed, and livers and gonads collected for analyses. Interestingly our results show a higher susceptibility of females to the different treatments in comparison to males at both cellular and molecular levels. Indeed, although hepatocyte lyses increase with microcystin-containing treatments, they appear always more intensive in females, when glycolysis cellular reserves appear also more decreased. Proteomic investigations reveal even more contrasted views with clear divergent proteinaceous compositions between males and females with deep differences in their de-regulation induced by the treatments too, especially for proteins involved in metabolic and gene expression processes. Furthermore, the liver effects induced by cyanotoxin chronic exposures were also correlated to ovarian impact with reduction of mature ovocytes. Overall, our observations underline the functional involvements of the female liver in the oogenesis (choriogenesis and vitellogenesis) and their potential ecotoxicological consequences for fish populations during hepatotoxin-producing cyanobacteria blooms.

NOTES:

Impacts of two strains of *Alexandrium minutum* on behavior and growth of kingscallops *Pecten maximus*

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The king scallop *Pecten maximus*, is an economically important bivalve in Europe, especially in France. Due to their repartition, they can be impacted by harmful algal blooms (HAB). Massive blooms of *Alexandrium minutum* have appeared for the first time recently in the Bay of Brest, causing contamination of many commercial bivalve species, including scallops, rendering them toxic for consumption and causing public health issues. It is thus important to understand their impacts on the biology and physiology of the king scallop. This study aimed to evaluate under controlled conditions the effects of two strains of *A. minutum* on king scallops *P. maximus*. The first strain produced paralytic shellfish toxins (PST), while the other strain was not producing PST but secreted other extracellular compounds that may have hemolytic, cytotoxic or allelopathic effects. Juvenile king scallops were experimentally exposed to three different conditions (food algae, food algae and the PSP-producing *A. minutum* strain or food algae and a non-PSP producing *A. minutum*) to assess their escape response to predators, daily growth, histological effects and their sensitivity to the accumulation of PSP toxins. A 7-day exposure of king scallops to non-PST producing strain, inhibited growth for three days, caused an increase in the time of reaction to the presence of a predator, and seemed to cause tissue damages in the digestive tubules. This suggests a cytotoxic effect of extracellular compounds causing damage when in direct contact with tissue. The king scallops exposed to PST-producing *A. minutum*, however, did not stop growing but appeared to have a less efficient escape response, potentially related to impaired muscle, as observed histologically. This suggests that additional effect of toxins and extracellular compounds led to weakness of the adductor muscle and tissue alterations.

NOTES:

Effect of *Alexandrium catenella* on mortality of the oyster Crassostrea *gigas*

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Since several years an alarming worldwide expansion of Harmful Algae Bloom (HAB) was observed in coastal and / or confined waters. *Alexandrium catenella,* a paralytic shellfish poisoning (PSP) producer, is now regularly found in Thau lagoon (south of France) at concentrations up to 15x106 cellules. l-1. In the same periods, events of oyster's mortality are also observed causing huge economic losses. The purpose of this study is to investigate the relationship between these toxic events and mortality phenomenon. For that, we conducted a field work to evaluate the presence of the toxic *A. catenella* in Thau lagoon during the years 2013 and 2014 in which high mortality of oysters spats were observed in spring. At the same time, we experimentally infected spats with the pathogenic bacterium *V. tasmaniensis* LGP32 and kept them unfed or previously fed either with the toxic alga *A. catenella* or with nontoxic algae, *Alexandrium tamarense* or *Tisochrysis lutea*. Results showed that the toxic *alga* was present in the lagoon when spats' mortalities occurred suggesting *A. catenella* could be involved in these events. Moreover, our laboratory experiment showed that the exposure to *A. catenella* increases the susceptibility of spats to one of its pathogens, *V. tasmaniensis* LGP32. Those results both together suggest for the first time that, in the environment, toxic algae could be implicated in oysters' mortality. Further studies should be conducted to determine the main factors of this alga implicated in weakening oysters and their immune system against their pathogen which lead to these large scale mortalities.

NOTES:

Effects of *Karenia brevis* exposure on the reproductive and related physiological processes of *Crassostrea virginica*

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Blooms of the brevetoxin producing dinoflagellate species, *Karenia brevis*, are a re-current and sometimes devastating phenomenon in the Gulf of Mexico. The eastern oyster, *Crassostrea virginica*, is regularly exposed during blooms and for long periods yet, little is known about the effect of *K. brevis* upon this species. Following separate exposures of adult *C. virginica* to *K. brevis* in the field and, after a relatively short- term (10 days) exposure to high bloom concentrations (1000 and 5000 cells mL-1) of *K. brevis*, inflammatory responses and a corresponding response of a significant increase in total circulating hemocytes were recorded in tissues in direct contact with *K. brevis* cells and/ or associated toxins. Brevetoxin was present in both sperm and oocytes obtained from oysters exposed to a field bloom of *K. brevis*. Maternal transfer of PbTx to the offspring via the oocytes, may have resulted in the negative effects recorded on larval development, but only up to the end of the lecithotrophic phase. The direct exposure of early life stages of *C. virginica* to different cell preparations of *K. brevis* revealed dose-dependent negative effects. Results suggest other toxic compounds in addition to PbTx may be involved in toxicity and that the majority of negative effects occur during embryonic divisions. Below a threshold, larvae showed the ability to recover from negative effects. Eastern oysters are more susceptible to *K. brevis* exposure than previously realized. The resulting multiple stressor effects on adult and early life stages combined with near- annual exposure to blooms of *K. brevis* could cause significant bottle necks on recruitment of this species.

NOTES:

Seasonal life cycle repartition of a Nostocale Cyanobacteria (*Anabaena macrospora*) in an eutrophic lake (Aydat, France)

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Autumnal blooms of the nostocalean cyanobacteria *Anabaena macrospora* occur annually over vast areas of Aydat lake (France). Nevertheless, we are still unable to predict the intensity of these blooms and the damages caused to the use of this lake. Akinetes, which are a specialized type of cell for resting stage, play a key role in the annual cycle of this cyanobacterium and could be used as a predictor tool for the management of the lake. Monitoring approaches during the bloom event coupled with core sediment sampling allow us to investigate the spatio-temporal akinete distribution in different parts of Aydat lake. During the pelagic proliferation phase, maximum akinetes' abundance were reached at the end of the vegetative cell peak. In sediment, the akinetes' abundance was generally more important in sediment from deep zones than from littoral zones, with values rocketing up to 700 000 akinetes.gDW^{-1} of sediment. However, less than 10% had not undergone a lysis and were potentially able to germinate during the next spring. Despite these weak proportions from the pelagic and the littoral sediments, viable akinetes still present at the surface of the lake sediment constituted a sufficient pool to be a starting point for future proliferations. The next step will be to compare this abundance of viable akinetes in the sediment with the next summer proliferation to know if a predictive tool can be developed.

NOTES:

First reporting of *Planktothrix rubescens* bloom in Algeria in the Bouhamdan dam

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Since the last decade, the occurence of cyanobacterial blooms in different water body in Algeria is increasing; in addition to this and for the first time the water took a reddish color in Bouhamdene dam (Guelma).Since the reporting of this phenomenon on may 2012, we performed multiple measures of physical and chemical parameters *in situ* (pH, T°, dissolved oxygen, conductivity and phycocyanin), followed by measuring nutrient concentration (N-NH_4, N-NO_3, N-NO_2, P-PO_4) and microcystins concentration using Microcystins-ADDA ELISA, microtiter plate. On the basis of morphologic and anatomic characters using microscopic observation, we identified the species *Planktothrix rubescens* (De Candolle ex Gomont) Anagnostidis et Komarek 1988; this cyanobacteria is known to be rich of phycoerythrin witch is responsible of the brown-redish color of water. Microcystin was detected only in the period where *Planktothrix rubescens* densities were in their high level 3 625 600 cell/mL, with a concentration of 0,47µg/L in raw water. We observed a similar evolution of *P.rubescens* cells counting and phycocyanin concentration. Also, for nutrient concentration which didn't exceed the guidelines values due to their consumption by P.rubescens for their proliferation. The high densities and the low toxicity of *Planktothrix rubescens* bloom need further investigations using molecular analysis along with an extension of study period and the study of the influence of ongoing climatic parameters.

NOTES:

Development of the toxic benthic dinoflagellate genus *Ostreopsis* in Mediterranean Sea and the associated M3-HABs European project.

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In recent decades, the Mediterranean Sea has been affected by the development of toxic benthic dinoflagellate genus *Ostreopsis*. Blooms have been so far reported in Spain, France, Italy, Croatia and Greece, and *Ostreopsis* occurrence has been recently reported also in other Mediterranean Countries, such as Tunisia, Algeria, Egypt and Lebanon. *Ostreopsis* bloom events may have environmental and health consequences. In tropical areas, *Ostreopsis* species produce palytoxin and its derivatives which are thought to be responsible for tropical poisonings *via* the consumption of contaminated seafood. Around the Mediterranean Sea, blooms of *Ostreopsis* occasionally cause cases of skin and eye irritations and less frequently respiratory distress. *Ostreopsis* proliferation can also cause significant ecological damage, with mass mortalities of invertebrates occurring during blooms. The pan-Mediterranean project "Risk Monitoring, Modelling and Mitigation of Benthic Algal Blooms along Mediterranean coasts" will be presented. The M3-HABs project aims to provide a common pan-Mediterranean strategy for monitoring benthic toxic microalgae, with particular reference to *Ostreopsis* spp. One of the main goals is to develop common procedures and protocols, making monitoring process more cost and time effective. This project will also allow to increase the knowledge on environmental drivers affecting harmful algal blooms and translate this into a forecasting tool, improving the general awareness of the risks related to *Ostreopsis*.

NOTES:

Optimization of sampling and counting techniques for the monitoring of benthic toxic dinoflagellates: focus on the genus *Ostreopsis*

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Harmful events associated with benthic toxic microalgae have been reported more frequently over the last decade. Outbreaks of *Ostreopsis* cf. *ovata* are exhibiting a similar trend along the Mediterranean coast. However, our knowledge of benthic HAB species is nascent: they have received considerably less attention than their planktonic counterparts. As a consequence, a large diversity of sampling and counting techniques are currently used for the monitoring of benthic toxic species. An optimization and standardization of these techniques will directly benefit to our understanding of the ecological control of benthic HABs, helping for comparison between studies. One of the main goal of the European project M3-HABs is to optimize sampling, preserving and counting techniques for the monitoring of *Ostreopsis* cf. *ovata* blooms along Mediterranean coasts. Results of experiments and survey that were conducted during summer 2014 in Villefranche Bay (South East of France) will be presented. In particular, different sampling strategies were tested, allowing for a comparison between "classical methods" (planktonic sampling and benthic sampling on macroalgal substrates) and non-destructive methods such as sampling with syringes and artificial substrates. In particular, different types of artificial substrates were deployed and tested. An optimal range of porosity (from 1 to 3 mm) was defined for the mesh size of artificial substrates and the study resulted in an efficient and easy to handle monitoring device. Other tests on the optimization of the separation step between epiphytic microalgal cells and macroalgal substrates were also run and will be discussed.

NOTES:

Monitoring of phytoplankton and Harmful Algal Blooms in coastal waters by combining innovative semi-automated tools (scanning flow cytometry & spectral fluorometry)

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In order to address phytoplankton dynamics in aquatic systems and to detect short term changes in phytoplankton composition, there is a need for innovative and reproducible monitoring procedures that could be applied at high frequency. Indeed, current methodologies and monitoring practices are not equipped to deal with fast changes in phytoplankton composition, which can reflect changes in the environmental status of aquatic systems and/or the occurrence of harmful events (Harmful Algal Blooms-HAB). Amongst recent research, the DYMAPHY project (Development of a DYnamic observation system for the assessment of MArine water quality, based on PHYtoplankton analysis, 2010-2014, www.dymaphy.eu), co-funded by the European Regional Development Funds (ERDF), aimed at contributing to a better assessment of the quality of marine waters in the Eastern English Channel and Southern North Sea (INTERREG IV A "2 Seas") through the study of phytoplankton and related environmental parameters, at high spatial and temporal resolution. By combining innovative techniques as pulse-shape recording scanning flow cytometry (CytoSense, Cytobuoy©) and spectral fluorescence (Fluoroprobe and AOA bbe©), compared to reference techniques, the DYMAPHY project proposed within a cross-border (France, England and Netherlands) effective work, better-standardized procedures and greater automation in data analysis for monitoring phytoplankton. This approach was tested in common inter comparison/calibration exercises, as well as in common international cruises in different coastal ecosystems of the "2 Seas" area. A discussion is carried out on the limits of integration of these methodologies in routine monitoring systems.

NOTES:

Optimization of the monitoring strategy for the French National Phytoplankton and Phycotoxins Network (REPHY) using semi-automated digital images analysis.

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The REPHY Observation network provides hundreds of thousands of samples of marine phytoplankton of crucial importance (i) to increase knowledge on phytoplankton ecology and impacts of global change on ecosystems, (ii) to protect human health from phycotoxins bio-accumulation in marine products. These samples are analysed using conventional inverted microscopy for phytoplankton counts. This needs to be improved to move towards a higher resolution in space and/or in time using alternative approaches associated with semi-automatized methodologies. This is of importance to be in phase with the processes involved in phytoplankton blooms and dynamics, but also to improve the homogeneity of the results by decreasing the observer bias, store additional parameters, and for the long term archiving of the data. To our knowledge, the use of semi-automatic analysis of phytoplankton digital images in the framework of a national monitoring programme is without equivalent today. We will introduce the strategy and on-going works on devices and software. We will also discuss the performances of the phytoplankton training set derived from English Channel and Atlantic samples. We will conclude about its implementation in a real operational mode. Use of such a tool combined with spectral fluorimetry, flow cytometry, HPLC, should bring the big picture about phytoplankton determinism and dynamics, and about harmful algal blooms. Use of such a kind of high resolution survey with modern tools should play a crucial role in implementing a new optimized REPHY observation network in the context of the Water Framework Directive (WFD), the Marine Strategy Framework Directive (MSFD), or Regional Sea Convention (*e.g.* OSPAR) to take on board new challenges to assess environmental status.

NOTES:

Zoo/PhytoImage: current advances in the semi-automated classification of plankton digital images.

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Advances in the field of image analysis and classification algorithms based on *machine learning* principle, led to the development of methods that can automatically analyze plankton samples. These automated approaches make up the basis of the Zoo/PhytoImage workflow (http://www.sciviews.org/zooimage) which provides a quick overview of sample composition, helping to detect harmful algae blooms. However, due to the difficulty in discriminating plankton images, the classification error can be significant, especially for rare groups for which it easily can exceed 30-40%. In this context, a manual validation of all predicted items recently became a standard practice in automatic recognition of plankton. This semi-automated identification remaining time-consuming, we present here two additional statistical techniques to reduce the number of particles to validate/correct: (i) a method where only the most wrong predictions, automatically identified, are manually validated, followed by (ii) an active learning step where the validation information is fed back to adjust the recognition tool and thus to automatically correct a part of remaining errors induced by the classification algorithm. These techniques are applied to several phytoplankton samples from English Channel and North Sea. The results show that it is possible to catch 90 to 95% of the error into the suspects, and to reduce over 50% the number of particles to correct manually.

NOTES:

Underwater Surface Plasmon Resonance sensor for the detection of marine biotoxin

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For the last decades, biosensors have demonstrated their ability to detect several kinds of molecule at very low concentration. In particular, Yu et al developed an assay for measuring the concentration of DA in shellfish extracts at concentration as low as 0.1ppb. On the other hand, Trainer et al reported that DA can be present in the range of 0.06 ng.mL-1 – 3 ng.mL-1 for cell concentrations of *Pseudo-nitzschia* spp. ranging from 3,000 to 500 000 cells.L-1. Such a DA concentration range is consistent with SPR biosensing capabilities.An underwater SPR biosensor assay was then developed for underwater detection of domoic acid. At first the chip is functionnalized with DA through modified OEG chemistry. The sample is mixed with the antibodies and the mixture is then injected in the sensor after 30 min of incubation time. Antibodies that did not react with the DA are then quantified. Such an assay enabled us to detect DA at concentration of the order of 0.1ppb during laboratory and shipboard experiments. These first results will be exposed and discussed.

NOTES :

The memory of seawater: passive sampling for the profiling of algal toxins in lagoons and open coastal seas.

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Marine biotoxins constitute a major problem to human health as they may accumulate in fish and shellfish and render seafood improper for human consumption. In this context of public health, monitoring programmes use, among others, chemical analysis (LC-MS/MS) for the detection and quantification of lipophilic toxins in seafood. Previous studies have shown the benefit of using passive sampling, e.g. Solid Phase Adsorption Toxin Tracking(SPATT), in combination with LC-MS/MS analysis, for the detection of dissolved toxins produced by either pelagic or benthic microalgae. SPATT samplers with different weights of HP-20 resin (300 mg, 3 g and10 g)have been deployed at several sites on French Mediterranean and Atlantic coasts to evaluate their capacity to capture microalgal toxins. The different sites can be categorised into open sea areas (the Bay of Villefranche-sur-mer and the Bay of Concarneau (VivierandScoréstations)) or semi-enclosed lagoons (Ingril Lagoon). Subsequently, samples were analysed using liquid chromatography coupled to low and/or high resolution mass spectrometry. Our results confirm indeed that toxins from pelagic, epiphytic and benthic microalgae may be detected using SPATTs. Okadaic acid, dinophysistoxin-1 (DTX-1), 13-desmethyl spirolide C and pectenotoxin-2 have been found in both lagoons and open sea areas. The capacity of SPATT samplers to detect ovatoxins remains as yet to be demonstrated. However, pinnatoxin G, produced by *Vulcanodinium rugosum*, a microalga with mostly epiphytic life stages, as well as DTX-1, produced by *Prorocentrum lima*, have been detected in SPATTs from Ingril Lagoon. Hence, the capability of SPATT samplers to detect, in principle, toxins from otherwise cryptic micro-algae (benthic or epiphytic, small and low-abundance species) is stressed by our results.

NOTES:

Population genetics reveal limited gene flow at regional scale of the toxic phytoplanktonic dinoflagellate *Alexandrium minutum*

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The genetic structure of marine phytoplankton species is assumed to depend mainly on dispersal by currents. This study investigates the genetic structure of the toxic dinoflagellate *Alexandrium minutum* along the Brittany coast. This microalga is one of the major causes of PSP (Paralytic Shellfish Poisoning) all over the world, and durably installed in several estuaries along the French Brittany coast (particularly in the Penzé and the Rance estuaries). Recently (during summer 2012 and 2013), this species was suddenly blooming in previously preserved areas along the French Brittany coast (the Aber Wrac'h and the Daoulas estuaries). We therefore compared samples from these two sites to other Brittany estuaries in which blooms were recurrent using population genetics approaches with seven microsatellites markers. Our results revealed that populations are spatially structured and show high genetic diversity. Interestingly the new blooms observed in Aber Wrac'h and Daoulas are genetically different from each other and also different from other blooms. These results suggest that migration is relatively limited between estuaries. In addition, the genetic differentiation between estuaries could also be closely linked to the life history traits. Indeed, the rapid development of blooms in a new site, followed by the formation of a large number of cysts accumulated in estuaries could be a particular system characterized by high genetic buffer and limiting arrival of immigrants by competition (monopolization hypothesis).

NOTES:

Intraspecific diversity and incipient speciation in *Alexandrium minutum*

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The emergence of high-throughput sequencing technologies enables the analysis of genetic diversity at the genomic level, even in non - model species. The transcripts of 18 *A. minutum* strains isolated from different estuaries/years were sequenced, assembled de novo, annotated and analyzed to investigate intraspecific diversity. Several 100000 variant sites were identified (~1.9 % of the sites of the reference transcriptome), revealing a highly diverse species. Of special interest was the identification of an incipient speciation event between two groups of strains. The transcriptome -wide pattern of genetic divergence will be discussed with special emphasis on both the evolutionary processes and the functional implications associated with the divergence.

NOTES:

First evidence of anatoxin-a genes in several freshwater lakes in france: spatio-temporal diversity and phylogenetic affiliation of the sequences

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Anatoxin-a is a neurotoxin potentially produced by many cyanobacterial genera. Despite the occurrence of potentially producers in lakes, very few ecological studies on this toxin have been performed to date. In this context, we wanted to determine if anatoxin-a production was widespread among lakes where potential anatoxin-a producing genera were present. For this purpose, we studied several freshwater lakes in the French Massif Central by targeting two anatoxin-a genes: *anaC* and *anaF*. Knowing the importance of the sediment in the annual life cycle of planktonic cyanobacteria, both planktonic and benthic compartments of the lakes were studied. Our results revealed a significant presence of *anaC* and *anaF* genes among the lakes studied. Most of the sequences retrieved in our study were different from those available in the databases and they were phylogenetically related to Nostocales and Oscillatoriales. They were variable within and among lakes which underlie a diversity of potentially producing strains. Our results also highlight that lacustrine sediment should be further taken into account in the way of evaluating the toxic potential of cyanobacteria in lakes.

NOTES :

Evolutionary processes may increase or attenuate blooming capacity of aquatic microbes

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Beside that microorganisms are essential components of all aquatic systems, they can also be toxic and/or proliferate becoming important threats. Microbial invasiveness is classically explained by stochasticity of elements under favorable environmental parameters (niche-based model). Although poorly known, evolutionary processes may additionally increase or attenuate their blooming capacity. Here, we tested this hypothesis using a 25-year time series recording bloom occurrences of the toxic dinoflagellate *Alexandrium minutum* along the French coasts (31 sites). In the 5 sites where this species durably installed, maximal annual abundances had a boom-and-bust dynamic, typical of an increasing number of invasive species. In the Penzé estuary, the boom period (11 consecutive years) corresponded to the highest growth rates observed during the whole monitoring survey, linked to frequent toxic blooms with more than 2 Million cells L^{-1}. During that period, bloom occurrences were pretty well predicted by a binomial logistic regression model based on few abiotic parameters only (water temperature, tide coefficient and run off). This was not the case anymore before and after the boom period. Trade-off between resources allocated into growth and capacity to resist to newly adapted/emerging pathogens may explain lower *in situ* growing capacity and absence of toxic blooms during the bust period (9 consecutive years). As for invasive species, fitness and ability of *A. minutum* to produce toxic blooms change over time, demonstrating that populations differentially adapted to local conditions. Taking into account evolutionary processes may help to better predict blooming capacity of microbes.

NOTES:

Alexandrium minutum disrupts cyclic activity at behavioral and gene transcription levels in the oyster _Crassostrea.gigas_.

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Marine organisms living in coastal areas inhabit a very complex biotope, submitted to the solar cycle but also to tides, semilunar and lunar cycles. Their behavior and physiology reflect these environmental cycles: tidal, daily and lunar rhythms have been described in intertidal organisms. Biological rhythms are a fundamental property of life, tightly linked to metabolism and allowing organisms to anticipate their changing environment. Generated by an endogenous molecular clock, they are synchronized by environmental factors. Resonance between the endogenous clock and the oscillating environment is considered as adaptive. In permanently immersed oysters *Crassostrea gigas*, valve activity is mainly driven by tidal cycles, although no tidal clock has been detected. Under entrainment regime (simulated tidal cycles of water current + continuous darkness) in the laboratory, oysters fed on the non-toxic dinoflagellate *Heterocapsa triquetra* (210 ± 11 cells/ml) expressed a tidal valve-activity cycle. Additionally, the gene *cryptochrome*, well-known in terrestrial species for its role in the circadian clock, oscillated at tidal frequency in the striated adductor muscle. Under the same entrainment regime, we then exposed oysters to *Alexandrium minutum* (211 ± 12 cells/ml then 853 ± 160 cells/ml). *A. minutum* disturbed both the cyclic behavior of oysters and the striated muscle expression of *Cgcry*, following a dose-response relationship. We also investigated behavioral, physiological and genotoxic impacts of *A. minutum* (1600 cells/ml) on oysters entrained by a 12:12 light:dark cycle. At behavioral level, oysters remained open but exhibited reduced valve-opening amplitude, correlated to the amount of toxin accumulated in the digestive gland. They also showed increased micro-closures. In the gills, mRNA expression levels of genes involved in oxidative and mitochondrial metabolism, endogenous clock immunity and detoxification processes were reduced. DNA impacts, both quantitative and qualitative, were observed as well. These works showed new impacts of the toxic alga *A. minutum* in the oyster *C. gigas*, both at behavioral, physiological and DNA levels. The disruption of the cyclic activity of oysters exposed to environmentally relevant concentration of *A. minutum* may also induce a loss of fitness, underlining new questions in aquatic ecotoxicology and ecology.

NOTES:

Seasonal variability of *Pseudo-nitzschia* sp. and domoic acid concentrations in the Southern Bight of the North Sea

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Phytoplankton community dynamics was assessed during a seasonal survey (2012-2013) in the Southern Bight of the North Sea and demonstrated the importance of *Pseudo-nitzschia* sp. during the phytoplankton spring bloom. In fact, three *Pseudo-nitzschia* species (*P. delicatissima, P. pungens, P. fraudulenta*) were SEM identified in 2012, and two (*P. delicatissima, P. pungens*) in 2013 and were related to high domoic acid concentration (DA) in the field. During spring 2012, DA concentrations reached 229 pg mL^{-1} and coincided with the presence of the three species making it difficult to clearly identify the potentially toxic species. By contrast, *P. pungens* was the only species present in the field in association to 102 pg DA mL^{-1} in autumn confirming its toxicity. Since REPHY alert threshold (i.e. 3×10^5 cell.L^{-1} for *P. delicatissima*) was crossed once during this survey, the Southern Bight of the North Sea should be considered as a risky zone with regard to DA seafood contamination (Amnesic Shellfish Poisoning). Finally, DA concentrations were not related to *Pseudo-nitzschia* sp. Abundance leading to reconsider abundance thresholds as ASP alert indicator. In this context, it seems necessary to assess the relationship between DA concentration in seawater and in molluscs flesh, but also to develop early warning tools for toxic phytoplankton species in the field (e.g. flowcam, cytometry, real-time PCR or SEM screening).

NOTES:

Life cycle in *Pseudo-nitzschia* spp.: species-specific control of the induction of sexual reproduction

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In toxic diatoms such as *Pseudo-nitzschia,* life cycle may influence population dynamics and toxin production. Better understanding of *Pseudo-nitzschia* sexual reproduction is thus crucial. The life cycle of four *Pseudo-nitzschia* species from the English Channel (*P. pungens*, *P. fraudulenta*, *P. delicatissima*, *P. australis*) was studied in cultures. Similar sexual stage succesions were observed for the four species. Mating experiments were carried out under three light intensities (30, 100 and 300 µmol photons m² s-1) and a temperature gradient ranging from 8 to 26°C. The influence of light and temperature on the induction and timing of sexual reproduction was different among *Pseudo-nitzschia* species. Sexual stages were observed between 8 and 20°C in *P. fraudulenta* and *P. pungens*, but only below 16°C in *P. delicatissima*. The onset of the sexual phase occurred earlier under higher temperatures and higher irradiances. Furthermore, the existence of intraspecific mating barriers amongst populations of *Pseudo-nitzschia* species of different geographic origin was tested. Sexual compatibility between *P. pungens* strains (clade I) from the English Channel and the Atlantic Ocean was observed. These results bring new understanding on the factors controlling the occurrence of sexual reproduction in *Pseudo-nitzschia* spp.

NOTES:

Spatio-temporal dynamics of phytoplankton biomass in the English Channel: high resolution strategy and modelling using unsupervised classification and Hidden Markov Model

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In order to further knowledge on phytoplankton bloom dynamics in an anthropised ecosystem frequently dominated by *Phaeocystis globosa* blooms along the French coast, a high space/time resolution strategy was implemented in the English Channel to complement conventional low resolution monitoring programmes. In the area covered by conventional programmes such as the French national Phytoplankton and Phycotoxins observation network (REPHY) and its regional derived Nutrient monitoring network (SRN), the MAREL Carnot instrumented station measures a series of physico-chemical, meteorological and biological parameters every twenty minutes since 2004. More recently, a Pocket Ferry Box (sampling frequency: 1 min., spatial resolution: 200 m) connected to a spectral fluorometer was also implemented in the English Channel on board the RV "« Côtes de la Manche ». Three scientific campaigns were planned during different stages of the *Phaeocystis globosa* bloom in 2012. A time series segmentation system is proposed allowing detection and modelling of recurrent states but also rare or pulse, extreme events. This system named uHMM is based on a Hidden Markov Model (HMM) built from an unsupervised training (u) : measured parameters are the unique knowledge required by the tool. Applied to the problems of forecasting all stages of phytoplankton development, this tool permits to segment automatically multi-sensor time series in several environmental states. uHMM system is also able to label a new time measurements according to the trained states or to reject this event as an unknown event or state. Phytoplankton biomass (based on MAREL Carnot data) or spectral group biomass (based on Pocket Ferry Box data) forecastings, and environmental status dynamics highlighted by the unsupervised Hidden Markov Model are discussed. Then results are compared to the ones obtained using low resolution conventional programmes. The authors proposed some insights (i) to help the definition of an optimized phytoplankton monitoring programme using both low and high resolution strategies, (ii) to improve the proposed modeling and classification tool, to better assess the phytoplankton dynamics, drivers of change (towards eutrophication, for example), direct and indirect effects of blooms (including HAB) and the water quality at large.

NOTES:

Posters

Effect of nitrate, ammonium and urea on toxin content and growth of *Vulcanodinium rugosum* a harmful dinoflagellate developing in Mediterranean waters

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Vulcanodinium rugosum, a recently described dinoflagellate species producing a potent neurotoxin (pinnatoxineG) developed in French Mediterranean lagoons and was responsible of shellfish poisoning. Until now, the biology and physiology of *V. rugosum* have never been investigated. We studied the growth characteristics and toxicity of a *V. rugosum* strain isolated in Ingril lagoon (North-Western French Mediterranean Sea) and cultivated in Enriched Natural Sea Water (ENSW) with organic (urea) and inorganic (ammonium andnitrate)nitrogen, a temperature of 25°C and irradiance of 100 µmole.m-2.s-1. Results showed that ammonium was assimilated by the cells more rapidly than nitrate and urea. *V. rugosum* is thus an osmotrophic species using urea, such feature may give this dinoflagellate a competitive advantage in the environment when inorganic nutrients are scarce. The growth rate of *V. rugosum* is relatively greater with ammonium (0.19 d-1) and urea (0.20 d-1) than that with nitrate (0.12 d-1). However, the production of pinnatoxin G and chlorophyll a was significantly lower with urea as a nitrogen source. The relatively low growth rate and the capacity of this species to produce continuously temporary cysts could explain why in situ cell densities of this species are not high.

NOTES:

Adaptative proteomic response to metal stress by a mediterranean strain of the invasive toxic dinoflagellate *Alexandrium catenella*

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Non axenic cultures of the invasive toxic dinoflagellate *Alexandrium catenella* (ACT03 strain isolated from the Thau Lagoon) were carried out in the presence of lead (Pb 12 µM) and without metal contamination (control). Soluble proteins were extracted from cultivated cells (end of exponential phase-early stationary phase), then separated and characterized by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). Proteins of interest, called stress proteins, for which amounts significantly varied in response to metal contamination in comparison to control, were identified by the LC-MS/MS technique. Contamination by Pb 12 µM induced perturbations of the soluble proteome from *A. catenella* ACT03, resulting in: (*i*) very significant over-expression ($p < 0.01$) of five proteins among which some were involved in the response to oxidative stress (proteasome α subunit) and in the energy metabolism (ATP-synthase β subunit) (*ii*) significant over-expression ($0.01 < p < 0.05$) of a protein associated with photorespiration and phosphorus metabolism (phosphoglycolate phosphatase) (*iii*) disappearance of many proteins implicated in photosynthesis activity (ribulose 1,5-bisphosphate carboxylase; ferredoxine-NADP reductase), in carbohydrate metabolism (malate dehydrogenase; ribose 5-phosphate isomerase), in chaperone activity (binding immunoglobulin protein; heat shock protein 90) and in bioluminescence of dinoflagellates (luciferin binding protein). These results show the harmful impact of a trace metal on the soluble proteome from *A. catenella* ACT03 and also suggest an adaptative proteomic response, *via* over-expression of some proteins. They could allow this invasive dinoflagellate to resist to oxidative stress induced by metal contamination, to stimulate its energy metabolism or even to detoxify its cells, so as to survive and develop in ecosystems impacted by pollution.

NOTES:

Degradation of cyanobacterial secondary metabolites by a natural bacterial community associated to *Microcystis*

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Microcystis commonly forms large mucilaginous colonies with numerous bacteria embedded in the mucilage. Positive and negative interactions between *Microcystis* species and its associated bacteria have been reported in relation to different processes, such as (i) competition or exchange of nutrients, (ii) inhibition or (iii) enhancement of the cyanobacterial growth, (iv) degradation of cyanobacterial toxins and (v) formation of aggregates. But none of these studies have investigated the potential role of bacteria in the production of cyanobacterial secondary metabolites. Our main questions are: (1) Is there difference in the secondary metabolic profile of *Microcystis* strains under axenic and non-axenic conditions? (2) Which heterotrophic bacteria are associated with *Microcystis aeruginosa*? (3) Are there specific interaction between the cyanobacterium and the associated bacteria? In the present study, the secondary metabolite profiles of MC- and non-MC-producing *Microcystis* strains in co-culture with or without a natural bacterial community were compared. Mass spectrometric analysis of cell and media extracts showed that most of the peptides measured were produced by the cyanobacterial strains independently of the presence of bacteria, they were still detected in the axenic media but totally absent in the non-axenic media. This work shows that the bacterial community was able to utilize cyanobacterial peptides as carbon source and in turn can contribute to sustain cyanobacterial growth by nutrient recycling. This mutualistic interaction may explain the ecological success of *Microcystis*. The analysis of the bacterial diversity by high throughput sequencing is in progress.

NOTES:

Comparative study of the behavioral and physiological responses of the oyster *Crassostrea gigas* exposed to three strains of *Alexandrium minutum* producing different toxin types (PSTs, extracellular compounds or both)

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In France, blooms of the dinoflagellate *Alexandrium* spp, producer of Paralytic Shellfish Toxins (PSTs), are regularly detected on the coastline. PSTs accumulate into oysters and can become deadly to consumers at high doses. However PSTs are not the only toxic molecules produced by *Alexandrium* species. They also produce extracellular substances, some of which have allelopathic or ichthyotoxic properties and are excreted in the environment thereby impacting phytoplankton, zooplankton but also marine invertebrates and fishes, without implicating any PSTs. Indeed, research showed a hemolytic action of these molecules on mammal cells and harmful effects on bivalve hemocytes. The aim of this work was to test the effects of three strains of *Alexandrium minutum* producing either only PSTs, only none-PST extracellular compounds, or both PSTs and none-PST extracellular compounds, on the Pacific oyster *Crassostrea gigas*. During the exposure to these three strains, oyster behavioral response was monitored by valvometry, recording opening and closing of the valves, in order to discriminate between effects due to PSTs and effects induced by extracellular bioactive compounds. Additional physiological responses such as tissue damages and hemocyte cellular response were assessed respectively by histology and flow cytometry. Other tissue samples were also preserved for further analyses, such as gene expression and activity of enzymes involved in antioxidant defense like Glutathione S-Transferase.

NOTES:

Influence of chemical multicontamination on the structure of ultraphytoplankton community in Toulon Bay (NW Mediterranean, France)

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Toulon Bay is an ecotone between a very urbanized terrestrial environment and the Mediterranean Sea. This coastal marine ecosystem is submitted to an important anthropogenic pressure, leading to multiple inputs of inorganic and organic pollutants. A biological and physico-chemical mapping, realized by sampling 42 sites of the bay, highlighted important spatial gradients of ultraphytoplankton community structure, as well as of inorganic contamination by NO3-, Cd, Cu, Pb and Zn. Analysis by flow cytometry showed that photosynthetic picoeukaryotes may be particularly resistant to the multicontamination of the bay. Indeed, their abundance and relative proportion (up to 80 %) were maximal in the most contaminated areas. Even though they represented a low proportion (< 10 %) of total ultraphytoplankton abundance, *Prochlorococcus*-like picocyanobacteria presented a similar distribution pattern showing their resistance to this potential chemical stress. Conversely, *Synechococcus*-like picocyanobacteria appeared to be particularly sensitive to multicontamination gradients. Their abundance and their relative proportion (up to 86 %) were maximal in the least contaminated areas, whereas they dropped in the most contaminated ones. These biological spatial shifts were strongly correlated to the inorganic contamination gradient of the bay. The metallic pollution seemed to be the main abiotic structuring factor, explaining statistically 55 % of the observed biological variability, whereas the contribution of nutritional resources (NO3-, PO43- and DOC) did not exceed 9 %. Among the four measured trace metals, Pb was the most structuring for the ultraphytoplankton community, followed by Cd, Zn and Cu. In keeping with this in situ study, experiments on controlled conditions will precise the differences of sensitivity and the resilience capacity of the different ultraphytoplanktonic groups in Toulon Bay. Physiological and genetic markers will be used to that purpose. Experimental setups are currently developed to compare the respective influence of nutrients and trace metals enrichment and bioavailability.

NOTES:

The ANR HAPAR project (2014-2019)

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A new ANR project, focused on parasites of microalgae, is starting this year (défi 1, climatic changes, Coord. L. Guillou, associating the Station Biologic of Roscoff, the Genoscope, and the Ghent University). As for invasive and proliferating plants and animals, the recent increase of the frequency of massive inshore microalgal blooms may well originate from geographical and temporary disruptions between microalgae and their natural enemies. Because of their virulence and abundant offspring, such parasites have the potential to control microalgal populations, and therefore toxic microalgal blooms. In the frame of the ANR HAPAR, we will try to better understand mechanisms underlying the specificity of these natural biological controls and their capacity to infect a novel host by the means of several approaches, from experiments in the laboratory to the acquisition of complete genomes, transcriptomes and proteomes of parasitic strains having different degrees of host specialization. This project is also linked to the development of a web-based interface dedicated to parasites living in aquatic systems.

NOTES:

Impact of ploidy and gametogenesis patterns on toxin accumulation in Japanese oyster *Crassostrea gigas* exposed to a natural bloom of *Alexandrium minutum*

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This study investigated the accumulation of Paralytic Shellfish Toxin (PST) of both diploid and triploid oysters (*Crassostrea gigas*), focusing on sex differences, but also on differences between triploid α and β exposed to a natural bloom of *Alexandrium minutum*. Oysters were sampled during the summer 2014 in the Bay of Daoulas (Bay of Brest), where a bloom of *A. minutum* occurred. PST levels were measured using an ELISA assay and histological sections allowed also sex determination and gametogenesis stage. In order to assess if gametogenesis patterns could influence the accumulation of PST in triploid oysters, gametogenesis patterns α (which produce numerous gametes) and β (which produce few gametes) were identified according to Jouaux *et al* (2010). Ploidy analyses using flow-cytometry confirmed that all oysters were either diploid or triploid as presumably identified. PST accumulation showed significant differences between diploid and triploid oysters (T-test, p-value<0.05): diploid oysters accumulated more PSTs than triploid oysters. Male represented 23%, female 41% and hermaphrodite 36% of the triploid oysters and all triploid oysters reached stage III (maturation). The assessment of gametogenesis pattern showed that 49% of the triploid oysters were α, but no significant difference in PST accumulation between α and β triploid oysters or between sexes could be observed. This study showing higher PST accumulation in diploid oysters, as opposed to what has been previously described in the literature (Haberkorn *et al*., 2010; Gueguen *et al*., 2012) reveals that the origin of the oysters may play a role in oyster PST accumulation. This study also highlights that a large number of triploid oysters are able to produce gametes, but that neither sex, nor α or β patterns seem to influence PST accumulation in oysters.

NOTES:

Ionic mechanisms implicated in ciguatoxin-induced membrane hyperexcitability and cell swelling

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Ciguatoxins (CTXs) are a family of lipid-soluble, highly oxygenated, heat stable, cyclic polyether compounds responsible for ciguatera, a human seafood intoxication linked mainly to benthic dinoflagellates (*Gambierdiscus sp.*) and acquired by eating contaminated fish. The aim of the present study was to determine the ionic mechanisms implicated in Pacific ciguatoxin-1B (P-CTX-1B)-induced membrane hyperexcitability and cell swelling of NG108-15 neuroblastoma cells and frog myelinated axons, using electrophysiology and confocal microscopy. No marked variation in the three-dimensional projected area of NG108-15 neuroblastoma cells stained with FM1-43, measured as an index value of cell volume, was detected during the action of P-CTX-1B, although the toxin induced a significant and tetrodotoxin (TTX)-sensitive increase in the relative fluorescence intensity of Sodium Green-loaded cells. Taking into account that P-CTX-1B produced transient repetitive action potentials only after cell stimulation but not spontaneously, these results strongly suggest that Na^+ ions flowing through toxin-modified voltage-gated sodium channels are not sufficient to produce cell swelling, and that those flowing through unmodified sodium channels activated during spontaneous and repetitive action potentials are necessary. In frog myelinated axons, substituting external NaCl by NaMeSO4 did not affect P-CTX-1B-induced spontaneous and repetitive action potentials and axonal swelling. This indicates that the toxin action was not dependent on external Cl^- ions. In contrast, substituting external NaCl by LiCl suppressed the spontaneous action potentials and prevented the axonal swelling induced by the toxin. This strongly suggests that the selectivity of toxin-modified voltage-gated sodium channels is less for Li^+ than for Na^+ ions, and further supports that Na^+ entry through channels opened during spontaneous action potentials is required to produce cell swelling. Finally, blocking voltage-gated potassium channels with tetraethylammonium (TEA) or 3,4-diaminopyridine did not prevent P-CTX-1B-induced spontaneous and repetitive action potentials but markedly reduced axonal swelling. Interestingly, blocking potassium channels with TEA and allowing K^+ efflux with valinomycin restored a marked toxin-induced axonal swelling that was reversed by hyperosmolar D-mannitol and prevented by TTX. In conclusion, water movements responsible for cell swelling are dependent on both Na^+ influx and K^+ efflux during the action of P-CTX-1B. This may help to understand the human neurological symptoms induced by ciguatera fish poisoning, especially a decreased nerve conduction, and paves the way for further studies regarding treatment of this poisoning.

NOTES:

Culture of *Gambierdiscus* species for evaluating extraction efficiency and the inter- and intraspecific variability of growth as function of nutrition

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Ciguatera fish poisoning (CFP) is a food-borne illness caused by consumption of fishes contaminated with polyether toxins known as ciguatoxins (CTXs). Originally known as a tropical disease, CFP is being increasingly reported from areas previously not considered endemic. The benthic dinoflagellate Gambierdiscus is considered to be the primary producer of CTXs. This genus has recently been shown to have an increasing number of species, also discovered in areas where it had not been observed before.This genus also produces other types of potent toxins, such as maitotoxins and others. CTXs are bio-accumulated and transformed in herbivorous and carnivorous fish along the marine trophic chain. The study presented here is part of a PhD aiming at the elucidation of hitherto unidentified Ciguatoxins in recently discovered species from the Caribbean and North East Atlantic seas. Cultures have been obtained from culture collections and from international collaborators for evaluation of growth characteristics and toxin production. To date, a total of 19 strains are currently maintained in our laboratory, belonging to eleven different species from the Pacific, Caribbean and North East Atlantic areas. Extraction techniques were evaluated using an ultrasonic probe and a bead-mill. As the toxins of most of the strains are not yet known, extraction efficiency was evaluated using algal metabolites of similar nominal mass and chromatographic retention times as CTXs. These initial trials suggest that both techniques have very similar extraction efficiency. These trials also suggest that duplicate extraction of pellets is sufficient for retrieval of more than 95% of the metabolites in the molecular size range of CTXs. Different culture media (L1, L1+ soil extract, K2, K2 + soil extract, Kmodified and IMK2) were evaluated for their influence on growth of one species (CCMP 1653, a Hawaiian species). For this particular species, no significant major differences were detected in cell volumes; however, some differences were observed in cell densities. Further studies will focus on bioguided fractionation of toxic strains in combination with high resolution mass spectrometry to pinpoint previously undescribed compounds.

NOTES:

Integrative model of bioaccumulation and detoxification of paralytic shellfish poisonning (PST) by oyster (*Crassostrea gigas*) based on the dynamic energy budget theory

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France being the largest consumer of oysters in Europe, oyster farming is deeply rooted in French heritage. The Japanese oyster (*Crassostrea gigas*), is the oyster most exploited in France, but also in the world. By their feeding mode type filter-feeder, these bivalves are sensitive to toxic algal blooms. If these ones don't systematically cause a lethal effect on oysters, they can weaken them and make them unfit for human consumption. There are different types of phytoplankton toxins that can be grouped under designation : amnesic, neurotoxic, diarrhetic and paralytic. For the latter group, saxitoxin are synthesized by the microalgae of *Alexandrium* genus and accumulate in tissues of bivalves. In recent years, much work has been done on the interaction between *C. gigas* and saxitoxin. To better understand these interactions, mathematical models have been developed without describe accurately the kinetics of accumulation and detoxification in paralyzing toxins (PSTs). Those based on DEB theory (Dynamic Energy Budget) (Kooijman, 2000) have been widely applied to the study of bivalves energetic. This type of model has already allowed to quantify the growth and reproduction of *C. gigas* under different environmental forcing. It has also been applied to the study of host-pathogen interactions (Flye-Sainte-Marie *et al.*, 2009) and the kinetics of accumulation and detoxification of contaminants (Bodiguel *et al.*, 2009; Echinger *et al.*, 2010). The aim of my thesis work is to develop a model based on DEB theory, to describe interactions between PSTs and oysters. Indeed, physiological impacts on oysters after contamination with PSTs have been demonstrated. For example, paralytic toxins (PSTs) alter the immune response (overproduction and phagocytosis of hemocytes), the behavior (modification of valvar rhythms, production of pseudo-faeces) or the organs integrity (myoatrophy, inflamed gills). The objective of this thesis, integrated into the ACCUTOX project, is being based on the DEB model, to describe the one hand, accumulation and detoxification kinetics of PSTs in C. gigas but in the other hand the effect of these toxins on the physiology of the oyster.

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