Conférence nationale
sur les algues toxiques Phycotox2019

Phycotox
Groupement de Recherche

Résumés des présentations et des posters

15 – 16 mai 2019

Institut Universitaire Européen de la Mer

Plouzané (Brest, France)

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Conception et mise en page du recueil de résumés : Nicolas Chomérat & Carole Demeule (IFREMER – Concarneau)

Programme de la conférence

Mercredi 15 Mai 2019

8h30 - 9h00	Café de bienvenue
9h00 - 10h00	**Session scientifique (axes 1 à 4)**
9h00 - 9h15	Introduction de la conférence *Hégaret Hélène*
9h15 - 9h30	Physiological conditions favorable to domoic acid production and accumulation in three *Pseudo-nitzschia* species *Sauvey Aurore, Fauchot Juliette, Claquin Pascal, Le Roy Bertrand*
9h30 - 9h45	Towards the warming waters? Distribution limits of the sub-tropical dinoflagellate *Ostreopsis* spp. across the Bay of Biscay *Drouet Kévin, Jauzein Cécile, Lemée Rodolphe, Laza-Martinez Aitor, Seoane Sergio, Siano Raffaele*
9h45 - 10h00	Strong constitutive expression divergence among strains but no differential expression associated with sexual reproduction in *A. minutum* *Le Gac Mickael*
10h30 - 11h00	Pause-café
11h00 - 12h30	**Session ateliers, trois ateliers en parallèle :** *1. Alexandrium* sp. et les toxines paralysantes en France Animé par *Caroline Fabioux, Mickaël Le Gac et Damien Réveillon* *2. Pseudo-nitzschia* et l'acide domoïque en France : écologie, production, devenir et conséquences sur les écosystèmes côtiers Animé par *Amandine Caruana, Juliette Fauchot et Anne Thébault* *3.* Nouvelles espèces émergentes en Europe et en France Animé par *Valérie Fessard, Philipp Hess et Nicolas Chomérat*
12h30 - 14h00	Pause-déjeuner
14h00 - 15h30	**Session de projets avec implication des professionnels**
14h00 - 14h20	Socio-economic analysis of Harmful Algal Blooms (HAB)' impact in the French Channel area and support from Sentinel-3 satellite products for detecting HABs (S-3 EUROHAB). *Raux Pascal, Chenouf Sarra, Pérez Agùndez José*
14h20 - 14h40	Impacts of Harmful Algal Blooms (HABs) in the French Channel area. Focus on the great scallop (*Pecten maximus*) fishery of the Eastern Channel *Chenouf Sarra, Pérez Agùndez José, Pascal Raux*
14h40 - 15h00	MASCOET: Maintien du Stock de Coquillages en lien avec la problématique des Efflorescences Toxiques *Blanchet-Aurigny Aline, Cugier Philippe*

15h00 - 15h20	Integrating various data products to predict risk and impacts of HAB events on the Aquaculture Sector (PRIMROSE- Project 2018-2020). Sourisseau Marc, Silke Joe, Schmidt Wiekbe, Hastie Lee, Maguire Julie, Davidson Keith, Henderson Ruth, Miller Peter, Ruiz Manuel, Matteus Marcus, Maman Luz, Ferrer Luis, Le Gac Mickael
15h20 - 15h50	Pause-café
15h50 - 17h00	**Session de projets avec implication des professionnels suivi de discussions sur la démarche prospective**
15h50 - 16h10	Culture of prey organisms of *Dinophysis* helps explain why its occurrence along the French Atlantic coast does not show trends over a 20-year period. *Gaillard Sylvain, Travers Muriel, Carpentier Liliane, Charrier Aurélie, Malo Florent, Bougaran Gaël, Irisson Jean-Olivier, Sourisseau Marc, Hégaret Hélène, Séchet Véronique, Hess Philipp*
16h10 - 16h30 :	ALERTOX-Net: detection of emerging toxins and the monitoring systems of tomorrow *Hess Philipp, Reveillon Damien, Araoz Romulo, Servent Denis*
16h30 - 17h00	**Discussions prospectives animées par Philipp Hess**
17h00 - 17h30	Session "speed talk"
17h00 - 17h05	Insights on the phytoplankton community structure in the Bay of Seine and the standing of *Pseudo-nitzschia* sp. and *Dinophysis* sp. *Lefran Angéline, Claquin Pascal, Gohin Francis, Hernandez Fariñas Tania*
17h05 - 17h10	Short-term effects of salinity and temperature stresses on DMSP and PSTs content in *Alexandrium catenella* groupe IV and *Alexandrium minutum* *Sergent Tanguy, Geffroy Solène, Herve Fabienne, Rovillon Georges-Augustin, Caruana Amandine*
17h10 - 17h15	Selectivity of the extraction solvent for maitotoxins and ciguatoxins from lyophilised biomass of *Gambierdiscus excentricus*. *Yon Thomas, Lanceleur Rachelle, Lhaute Korian, Sibat-Dubois Manoëlla , Malo Florent, Reveillon Damien, Fessard Valérie, Holland Chris, Litaker Wayne, Hess Philipp*
17h15 - 17h20	Intra- and inter-specific variability of toxin and metabolite profiles of *Dinophysis* spp. *Danthu Charline, Gaillard Sylvain, Sibat Manoella, Reveillon Damien, Séchet Véronique, Hess Philipp*
17h20 - 17h25	Towards the standardization of a new optimized post-column oxidation method for PSP toxin determination in shellfish *Bodi Dorina, Kapp Katrin, Preiss-Weigert Angelika*
17h25 - 17h30	Co-culture model Caco-2/HT29-MTX: a promising tool for toxicity investigation of phycotoxins on the intestinal barrier *Reale Océane, Huguet Antoine, Fessard Valérie*
17h30 - 20h00	**Session apéro - posters *(offert par Novakits)***

Jeudi 16 Mai 2019

9h00 - 10h30	**Session scientifique (axes 1 à 4)**
9h00 - 9h15	Neuroblastoma (neuro-2a) cell-based assay: Investigating factors affecting performance for detecting marine neurotoxins *Loeffler Christopher, Bodi Dorina, Tartaglione Luciana, Dell'avesano Carmela, Preiss-Weigert Angelika*
9h15 - 9h30	Cathepsin S and PAR-2 are new targets involved in the ciguatoxin-induced release of neuropeptides: potential applications to relieve ciguatera sensory disturbances *Pierre Ophélie, L'herondelle Killian, Fouyet Sophie, Leschiera Raphaël, Le Gall-Ianotto Christelle, Philippe Réginald, Buscaglia Paul, Mignen Olivier, Talagas Matthieu, Lewis Richard J., Misery Laurent , Le Garrec Raphaële*
9h30 - 9h45	Contribution of *sxt*A4 gene in STXs production of *A. minutum* and *A. catenella* *Geffroy Solène, Lechat Marc-Marie, Herve Fabienne, Rovillon Georges-Augustin, Guillou Laure, Amzil Zouher, Caruana Amandine*
9h45 - 10h00	Dynamics of the dinoflagellate *Lepidodinium chlorophorum* in Southern Brittany: controlling factors and consequences for exploited ecosystems *Roux Pauline, Schapira Mathilde, Siano Raffaele, Fleury Elodie, Mertens Kenneth Neil, André Coralie, Cochennec Laureau Nathalie*
10h00 - 10h15	Influence of temperature and light intensity on *Ostreopsis* cf. *ovata* growth and toxin content. *Gémin Marin-Pierre, Reveillon Damien, Herve Fabienne, Bertrand Samuel, Séchet Véronique, Lemée Rodolphe, Amzil Zouher*
10h15 - 10h30	Chemical ecology of Benthic HABs: the impact of NW Mediterranean *Ostreopsis* cf. *ovata* on copepods *Pavaux Anne-Sophie, Lemée Rodolphe, Gasparini Stéphane, Guidi-Guilvard Laurence, Marro Sophie, Rostan Julie*
10h30 - 11h00	Pause-café
11h00 - 12h30	**Session science des axes 1 à 4 et Session restitution mini-projets**
11h00 - 11h15	Salt stress response of brackish and freshwater strains of *microcystis aeruginosa* *Georges Des Aulnois Maxime, Herve Fabienne, Caruana Amandine, Briand Enora, Dittmann Elke, Bormans Myriam, Amzil Zouher*
11h15 - 11h30	Taxonomic characterization of *Ostreopsis* in the French West Indies (CARTAGO project) *Boisnoir Aurélie, Bilien Gwenael, Lemée Rodolphe, Chomérat Nicolas*

11h30 - 11h45	PARACIDE : Links between Programmed Cell Death and parasite infection pathways in microalgae *Long Marc, Szymczak Jeremy, Marie Dominique, Estelle Bigeard, Guillou Laure, Cécile Jauzein*
11h45 - 12h00	Algal Blooms from Polynesian Lagoons: New Sources of Bioactive Compounds? *Longo Sébastien, Guérineau Vincent, Girault-Sotias Pierre-Emmanuel, Servent Denis, Chinain Mireille, Araoz Romulo*
12h00-12h15	Comparison of the toxicity of palytoxin and extracts of *Ostreopsis* cf. *ovata* on a set of cell lines *Fessard Valérie, Lanceleur Rachelle, Gemin Marin-Pierre, Réveillon Damien, Zouher Amzil, Thomas Olivier*
12h15 - 12h30	Assemblée générale du GdR
12h30 - 14h00	Pause-déjeuner
14h00 - 15h00	**Restitutions des ateliers**
14h00 - 14h20	*Alexandrium* spp. et les toxines paralysantes en France *Caroline Fabioux, Mickaël Le Gac et Damien Réveillon*
14h20 - 14h40	*Pseudo-nitzschia* et l'acide domoïque en France : écologie, production, devenir et conséquences sur les écosystèmes côtiers *Amandine Caruana, Juliette Fauchot et Anne Thébault*
14h40 - 15h00	Nouvelles espèces émergentes en Europe et en France *Valérie Fessard, Philipp Hess et Nicolas Chomérat*
15h00 - 15h30	Pause-café
15h30 - 17h30	**Session science des axes 1 à 4 et clôture/remise des prix**
15h30 - 15h45	Study of the Food web transfer of pinnatoxin G and Portimine produced by the dinoflagellate *Vulcanodinium rugosum* and the effect of this HAB species on the physiology of the main copepods developing in the Mediterranean Ingril and Thau lagoon *Abadie Eric, Deschler Marie, Herve Fabienne, Derrien Amélie, Hubert Clarisse, Crottier Anaïs, Foucault Elodie, Amzil Zouher, Rolland Jean-Luc, Masseret Estelle, Antajan Elvire, Laabir Mohamed*
15h45 - 16h00	Microorganisms Chemical Mediation in a Marine Environment ? When Fungus induces Microalgal toxine production! *Bertrand Samuel, Maud Chaigne, Bagot Alizé, Gutierrez Ruth, Ruiz Nicolas, Briand Enora, Pisapia Francesco, Pouchus Yves François, Hess Philipp*
16h00 - 16h15	Role of the toxic micro-alga, *Alexandrium pacificum* (*A. catenella*) in the virulence of *Vibrio tasmaniensis* LGP32 a pathogenic bacteria for the oyster Crassostrea gigas. *Rolland Jean-Luc*

16h15 - 16h30 (Semi-)automated in vivo approaches for characterizing HAB and other phytoplankton blooms in European coastal waters: News from the Joint European Research Infrastructure for Coastal Observatories – New Expertise (JERICO-Next)
Artigas Felipe, Karlson B., Brosnahan M.L., Claquin P., Créach V., de Blok R., Debusschere E., Deneudt K., Gómez F., Grégori G., Hébert P., Kromkamp J., Lefebvre A., Lehtinen S., Lizon F., Louchart A., Möller K., Poisson-Caillault E., Rijkeboer M., Thyssen M., Seppälä J., Stemmann L., Van Dijk M., Veen A., Wacquet G.

16h30 - 16h45 Assessment of dispersion and transport of HAB: tracking based on satellite Chlorophyll maps
Camille Dezecache, Romaric Verney, Francis Gohin, Alain Lefebvre

16h45 - 17h30 Clôture et remise des prix

Mercredi 15 mai 2019

Communications orales

Physiological conditions favorable to domoic acid production and accumulation in three Pseudo-nitzschia species

Aurore Sauvey* [1], Juliette Fauchot † [1], Pascal Claquin [1], Bertrand Le Roy [1]

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A large diversity of diatom species of the genus *Pseudo-nitzschia* is observed on the French coast and most of these species produce the neurotoxin domoic acid (DA), resulting in ASP events and closures of great scallop fishing. We conducted batch culture experiments to estimate several physiological traits along with intracellular and extracellular dissolved DA measurements on *P. australis*, *P. pungens*, and *P. fraudulenta*, three species representative of the *Pseudo-nitzschia* community of the French coastal waters. We chose a multi-strain approach to take the intraspecific diversity inherent to *Pseudo-nitzschia* species into account, and thus better characterize interspecific differences. The objective of this study was to finely characterize the dynamics of DA production and DA accumulation as a function of growth phases, so as to identify the physiological conditions favorable to toxin production. Our ecophysiological experiments did not detect any difference in growth between *P. australis*, *P. pungens*, and *P. fraudulenta*. However, they suggest differences in nutrient requirements, uptake abilities, and light optima, consistent with their different bloom phenologies reported in different ecosystems. Regarding the dynamics of DA in *P. australis*, our results confirm that cellular DA accumulates when growth decreases or stops under silicate limitation. However, DA production was not influenced by nutrient limitation or the growth rate. Production rates were rather favored by high photosynthetic capacity. Our results therefore stress how important it is to study production rates, and not the sole cellular DA accumulation. In addition, in all three species, the dynamics of DA excretion greatly differed from the dynamics of DA production. These precise results on DA production and accumulation will be of great help to develop accurate models of DA production for *P. australis*, especially as we also took extracellular dissolved DA into account.

Mots-Clés: Pseudo nitzschia, domoic acid production, domoic acid excretion, ecophysiology, interspecific and intraspecific diversity

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†Intervenant

Towards the warming waters? Distribution limits of the sub-tropical dinoflagellate Ostreopsis spp across the Bay of Biscay

Kévin Drouet *† [1,2], Cécile Jauzein [1], Rodolphe Lemée [2], Aitor Laza-Martinez [3], Sergio Seoane [3], Raffaele Siano [1]

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Ostreopsis spp. are benthic toxic dinoflagellates responsible for numerous episodes of harmful algal blooms (HABs) throughout the world. Originally found in sub-tropical areas, reports in some temperate areas have been recently increasing. During the last two decades, toxic outbreaks were observed in the warming waters of the West Mediterranean Sea. In the colder waters of the Bay of Biscay (Atlantic Ocean), *O.* cf. *siamensis* has been detected only in few studies carried out mostly along the Spanish coast. The ongoing global warming might have affected *Ostreopsis* spp. distribution along the Bay of Biscay. In a scenario of water temperature increase of the Atlantic Ocean, it is important to identify the distribution limits of *Ostreopsis* spp. across this area and to establish sentinel monitoring areas in order to predict further expansions and prevent sanitary risks.

With this aims, we conducted a sampling campaign during summer 2018 (from August to September) along the Bay of Biscay (from the central part of the Cantabrian Sea in Spain to the Northern Brittany in France). We investigated 40 sites corresponding to habitats potentially allowing *Ostreopsis* settlement. Here we collected samples for genetic (environmental DNA) and morphological analyses using the artificial substrate method and the epiphytic microflora on local macroalgae.

Microscopy analyses allowed the identification of *Ostreopsis* spp. in 12 consecutive sheltered sites, located in the southern-east part of the Bay of Biscay (between Santander and Biarritz) at temperatures ranging from 21.1∘C to 25.9∘C and salinity ranging from 28.4 to 35. The genetic analyses based on the environmental DNA (in progress) will probably corroborate and complete these results, allowing the detection of the species even at very low concentration, finally providing the best possible picture of *Ostreopsis* distribution across the sampled area.

Mots-Clés: Ostreopsis spp., Distribution, French Atlantic coasts, Artificial substrate, Molecular tools, Climate change

*Intervenant
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Strong constitutive expression divergence among strains but no differential expression associated with sexual reproduction in A. minutum

Mickael Le Gac * [1]

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With huge genomes, permanently condensed liquid-crystalline chromosomes, and peculiar transcriptional regulation, dinoflagellates are amongst the most peculiar organisms living on the planet. In an attempt to better understand sexual reproduction and heredity in this phylum, experimental crosses were performed between clonal strains of the toxic dinoflagellate A. minutum. The major aims of this experimental approach were: 1. to characterize the various mating types, 2. to identify genes differentially expressed at two key steps of the sexual reproduction: gamete fusion and encystment, 3. to identify potential genetic markers associated with the mating types and/or mating type specific differential expression patterns, and 4. to obtain recombinant clonal offspring in order to build a genetic linkage map and to investigate genetic and phenotypic heredity within A. minutum.

*Intervenant

Socio-economic analysis of Harmful Algal Blooms (HAB)' impact in the French Channel area and support from Sentinel-3 satellite products for detecting HABs (S-3 EUROHAB).

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HAB occur naturally but deeply impact economic and non-economic activities. Face to difficulties in forecasting them, HAB events are managed on a crisis basis, leading to close contaminated areas and ban commercial products based on in situ monitoring and alert systems. There's then a need to analyse and, where possible, qualify and quantify the social and economic impacts of HAB directly on the shellfisheries, aquaculture, tourism sectors and wider local economies. A stakeholder inventory, identifying the multitude of organisations with interests in HABs, stakeholder workshops in the UK and France to discuss perceptions and impacts of HABs and definition of the existing HAB monitoring system are preliminary results of this work performed in the frame of the S-3 EUROHAB project. A regional economic analysis to identify the wider-economic impacts of HABs and the degree to which local economies are resilient to such events is under implementation. But satellite products can help in bringing a significant added value to existing monitoring systems: faster response times, spatial extension and coverage of monitoring areas. To assess to which extent it can mitigate the socio-economic impacts of HAB (monitoring and management costs, avoided economic losses, risk management by stakeholders), a cost effectiveness of the so called web-based HAB alert system is implemented. It takes into account the diversity and sometimes opposite needs from stakeholders and how different sectors respond to the information contained within it. Based on interviews and workshop, first results of stakeholder's perceptions and potential benefits as well as adverse effects of a web-based alert system are presented. This will feed a scenarios analysis related to changes in monitoring and the introduction of an HAB alarm systems.

Mots-Clés: Economic impacts, Alert system, Management, Monitoring, Stakeholder perceptions

*Intervenant

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Impacts of Harmful Algal Blooms (HABs) in the French Channel area. Focus on the great scallop (Pecten maximus) fishery of the Eastern Channel

Sarra Chenouf * [1], José Pérez Agùndez [1], Pascal Raux Pascal Raux [1]

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Over the past decades, HABs have increased in frequency, intensity and geographic distribution, making it difficult to monitor and manage them. As an important area in terms of biodiversity and economic activities, the French Channel is threatened by HABs. We implemented a systemic approach in order to address these issues in a multidimensional and integrated way. We looked at three dimensions: dynamics of HABs, the management system, and adaptation strategies of stakeholders. The aim is to identify impacts and assess them in the light of these three dimensions. Hence, the methodology developed was based on the combination of national monitoring data (REPHY-REPHYTOX) and socioeconomic data, complemented with interviews with stakeholders. Our analysis shows that management measures such as bans on collecting or catching shellfish are implemented according to the hazard level of HABs. Resource users have therefore different responses in order to minimize losses. Adaptation strategies include changing the fishing location towards non-closed areas (Eastern Channel case), changing target species (Western Channel case) and storing and postponing the sale of farmed shellfish. This study shows that the potential risk of HABs varies between areas in function of activities and adaptation strategies to HABs. We found that impacts in the Eastern Channel are different compared to the Western side. In the Eastern Channel, two types of HABs impacts have been observed. Firstly, impacts caused by shellfish bans during DSP and ASP contaminations, by which scallop fishery is the most impacted. Secondly, *Phaeocystis* blooms have negative effects on mussel farming and shrimp fisheries. In the Western Channel, the department of Finistère is the most impacted. We found that it is threatened by all of DSP, ASP and PSP toxins, contaminating many shellfish species. The system approach has allowed for a better understanding of HAB's impacts and underlined more mitigated impacts.

Mots-Clés: Harmful Algal Blooms, HAB, French Channel, impacts, system approach, scallops, toxins, management, coastal activities.

*Intervenant

MaSCoET project

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The scallop *Pecten maximus* is the third most important commercial seafood species sold in France. It is exploited by a large fleet going from the north of France to the Pertuis Charentais. Since the 2000s, all along the coast, fishermen have faced scallop fishery closures due to Pseudo-nitzschia (PSN) blooms, which can produce amnesic toxin (domoic acid: DA), responsible for ASP (Amnesic Shellfish Poisoning) in humans, rendering shellfish toxic for consumption. In this context, a number of scallop*P. maximus* fisheries have been regularly closed for several years, sometimes resulting in shifting of fishing effort toward other scallop species such as black scallops, *Mimachlamys varia*. Paradoxically, research on the subject is scarce and knowledge of these phenomena remains insufficient. In this context, the MaSCoET project aims to gather knowledge that will benefit the entire French fleet by using the Bay of Brest, highly impacted in recent years, as a study site, while associating other sites (Charente Maritime, Bay of Seine). The aims are: 1. To better understand the determinism of toxic PSN blooms; 2. To better understand why DA decontamination of scallops is very slow compared to other pectinids, including black scallop *M. varia*; 3. To improve knowledge of the ecology and population dynamics of black scallops *M. varia* in order to 4. improve the management of the fishing activity that can rely to this resource during toxic events.

Mots-Clés: Pecten maximus, Mimachlamys varia, HAB, Pseudo nitzschia, domoic acid, contamination/decontamination, fisheries management, Bay of Brest

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Integrating various data products to predict risk and impacts of HAB events on the Aquaculture Sector (PRIMROSE- Project 2018-2020).

Marc Sourisseau * [1], Joe Silke [2], Wiekbe Schmidt [2], Lee Hastie [2], Julie Maguire [3], Keith Davidson [4], Ruth Henderson [5], Peter Miller [6], Manuel Ruiz [7], Marcus Matteus [8], Luz Maman [9], Luis Ferrer [10], Mickael Le Gac [1]

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Primrose planned to improve and/or to develop forecasting systems for Habs and microbial contamination in the Atlantic Area (Interreg Project) according to each country organisation and end-users feedback. The PRIMROSE project aims to develop a web portal system providing a joint front end for transnational forecasts of harmful events for the aquaculture sector. Reporting procedures will be standardised, and partly automated for an expert evaluator to have information available to make an accurate forecasts. The project involves establishing many links between national monitoring systems and opertional oceanography. Ifremer is involved in this project 1) to evaluate a new tools and sensors for monitoring coastal waters and 2) to assess the connectivity of dinophysis populations at the european scale. For the first action, an autonomous FCM system was evaluated in 2018 in the Bay of Brest and will be deployed again in 2019. For the second action some, a SNPs approach was started by sequencing strains (Spain and France) and in-situ sampling (from Portugal to Ireland).

Mots-Clés: Habs, forecast, monitoring, connectivity

*Intervenant

Culture of prey organisms of Dinophysis helps explain why its occurrence along the French Atlantic coast does not show trends over a 20-year period

Sylvain Gaillard*[1], Muriel Travers[2], Liliane Carpentier[1], Aurélie Charrier[3], Florent Malo[1], Gaël Bougaran[3], Jean-Olivier Irisson[4], Marc Sourisseau[5], Hélène Hégaret[6], Véronique Séchet[1], and Philipp Hess†[1]

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Résumé

Species of the genus *Dinophysis* are known to produce lipophilic toxins and form blooms globally. Such toxins may accumulate in bivalves and intoxicate consumers of contaminated shellfish. This study is part of the European project CoCliME which, among others, aims at assessing the potential impact of global change key factors on growth, distribution and toxicity of toxic species of the genus *Dinophysis*. It is also important to understand the ecology of prey organisms of this mixotroph since *Teleaulax amphioxeia* and *Mesodinium rubrum* have been shown to be sources of plastids for *Dinophysis*. Optimal growth and physiology of *T. amphioxeia* as a prey model were determined without acclimation using a factorial design for temperature, irradiance and pH in an experimental set-up with 15 combinations. The model predicted a higher growth rate of *T. amphioxeia* at high light intensity (400 μE), and in more acidic pH (7.6) and warmer temperature (17.6 ∘C) than current conditions in French Atlantic waters. Subsequently, *M. rubrum* was fed with *T. amphioxeia* acclimated at different conditions to investigate potential impacts of prey quality and quantity on the physiology of the predator. *M. rubrum* growth appeared to be more constrained by quantity of prey provided than by the pigment content of the prey. Monitoring over a 20 year monitoring period of *Dinophysis* and its toxins along French Atlantic coasts did

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not show a clear time trend. While no significant increase in the occurrence of *Dinophysis* was observed over this period, experimental studies indicate a potential for significant increase of *T. amphioxeia* and *M. rubrum* occurrences in future climate conditions, which may lead to increased occurrence of *Dinophysis*. The current and future impacts of HAB on the shellfish industry is currently evaluated through a survey on shellfish farmers in Southern Brittany and Pays de la Loire regions.

Mots-Clés: Dinophysis, Teleaulax amphioxeia, Mesodinium rubrum, Database, Factorial design

ALERTOX-Net: detection of emerging toxins and the monitoring systems of tomorrow

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The ALERTOX-Net project is funded by the Atlantic Interreg (2017-2020) and regroups 11 partners from the Atlantic arch to transfer knowledge from research institutes to the European fish and shellfish industry. A review of available detection methods has been carried out and will be presented.
French partners are Ifremer and CEA, which focus on the emerging toxin groups of ciguatoxins, tetrodotoxins as well as novel detection methods for Na-channel toxins and toxins active on the nACh receptor. Initial findings on method development and occurrence of such toxins in France will be presented.
An overview will be given on interactions possible with shellfish industry and developments in the other Atlantic regions.

Mots-Clés: emerging toxins, surveillance, industry sector, fish and shellfish aquaculture

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Communications orales

Neuroblastoma (neuro-2a) cell-based assay: Investigating factors affecting performance for detecting marine neurotoxins

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Marine biotoxins contaminating seafood products pose a risk to human health. These toxins are often potent in minute amounts and contained within complex matrices; requiring sensitive, reliable, and robust detection methods. The mouse neuroblastoma cytotoxicity assay (CBA-N2a assay) is a cell-based, sensitive, high-throughput, *in vitro* method useful for detecting sodium channel-specific marine biotoxins. The N2a assay can distinguish between voltage-gated sodium channel (VGSC) specific effects on cell membranes, such as toxins that activate (e.g., ciguatoxins (CTX), brevetoxins (BTX)) or block (e.g., tetrodotoxins, saxitoxins) the target VGSCs. This assays sensitivity and specificity to compounds activating VGSCsis due to the addition of the pharmaceuticals ouabain (O) and veratridine (V). However, this method has not yet been validated. Here, we investigated several parameters of the N2a assay to determine their impact on the sensitivity, variability, and ability to detect marine biotoxins; 1) cell concentration per well, 2) concentration of ouabain and veratridine administered, and 3) a modification to the cell line through a novel selection process. We defined the successful application of these factors on the N2a assay by A) a sensitivity range of 60-85% survival in the presence of the pharmaceuticals ouabain and veratridine, B) a standard deviation $< 20\%$ among replicates, and C) the successful detection of commercially available CTX3C and PbTx standards. With the successful manipulation of these factors and the defined parameters for a successful assay, we determined the impact of these conditions on detecting sodium channel activating marine biotoxins with the N2a assay. Here we present our results, with implications for future studies. LC-HRMS methods for the detection of tetrodotoxins, saxitoxins, and brevetoxins have already been developed in our laboratories, and currently CTXs are under development. This will allow the comparison of results obtained by analytical methods and the N2a assay.

Mots-Clés: Ciguatoxins, cell, based assay, brevetoxins, analytics

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Cathepsin S and PAR-2 are new targets involved in the ciguatoxin-induced release of neuropeptides: potential applications to relieve ciguatera sensory disturbances

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Ciguatera Fish Poisoning (CFP) is a widespread tropical intoxication consecutive to ciguatoxin (CTX) ingestion, which is characterized by persistent sensory disturbances, including cold allodynia and an intense pruritus (itch). Currently, there is no specific treatment. The primary target of CTXs is the voltage dependant sodium channel (Nav), which is largely expressed in sensory nerves and, to a lesser extent, in keratinocytes. By activating Nav, CTXs induce neuronal hyperexcitability but the downstream molecular mechanisms leading to sensory disorders are poorly understood. We previously showed that P-CTX-2 induces the release of the pain- and itch-associated neuropeptide substance P (SP) from a coculture of sensory neurons and keratinocytes, and demonstrated the synergistic role of keratinocytes in this release. To better understand the pathophysiology of CFP sensory disturbances, the purpose of the present study was to identify molecular actors involved in the SP release elicited by P-CTX-2. Our results demonstrate that P-CTX-2 is able to induce calcium signal in both sensory neurons and keratinocytes. Interestingly, both cells express the protease-activated receptor-2 (PAR-2), which is an emerging receptor involved in pain and itch pathophysiology. We show that antagonism of PAR-2 strongly diminished the P-CTX-2-evoked calcium signal in single cell cytosolic Ca2+ imaging in sensory neurons. Using confocal microscopy, we also show that both P-CTX-2 and brevetoxin-1 could internalize PAR-2 in keratinocytes, suggesting that PAR-2 activation by P-CTX-2 occurs through a Nav-dependent mechanism. In the coculture, the P-CTX-2-induced SP was almost completely abolished by PAR-2 or cathepsin S antagonists, and cathepsin S activity was increased after P-CTX-2 treatment. Taken together, this work reveals that PAR-2 and cathepsin S are novel actors in the P-CTX-2-induced release of neuropeptides, suggesting those are two promising pharmacological targets for specifically treating CFP sensory disorders.

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Mots-Clés: Ciguatoxin, Sensory, Substance P, Keratinocyte, Protease activated receptor 2, Cathepsin S

Contribution of sxtA4 gene in STXs production of A. minutum and A. catenella

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Microalgae of the genus *Alexandrium* are able to form blooms and may have ecosystem and health impacts in producing saxitoxin and its analogues (STXs). In the *sxt* gene cluster, *sxtA* catalyzes one of the initial steps of the STXs synthesis and is present in multiples copies in the genomes of STX-producing strains. A *sxtA* short isoform and long isoform composed respectively of three and four catalytic domains, exist in dinoflagellates. The domain *sxtA4* (aminotransferase domain) appears to be the most discriminating for PST production. We investigated the intraspecific variability in STX content and profile among twenty strains of *A. minutum* and *A. catenella*. We attempt to explain this phenotypic variability by exploring (I) the genetic variability in terms of gene copy number of the *sxtA4* gene (ii) the transcript variability of the *sxtA4* gene. Gene copy number were examined by qPCR, genome measurement by flow-cytometry and toxin content by liquid chromatography equipped with a fluorescence detector (LC/FLD). The *A. minutum sxtA4* mRNA sequences were analyzed from a transcriptomic database. Our results showed a positive correlation between the *sxtA4* CPN and the toxin content. We obtained 3-fold higher *sxtA4* CPN in *A. catenella* than in *A. minutum strains.* Two non-STX producing *A. minutum* strains contain little amounts of *sxtA4* gene isoforms (sxtA4 mRNA sequence analyses in *A. minutum* strains, *sxtA4* transcripts are presents in all strains even in the two non-toxic strains. These results suggest that SXTs synthesis depends on CPN but may be also regulated by post-transcriptional and post-translational mechanisms.

Mots-Clés: Alexandrium catenella, Alexandrium minutum, saxitoxin (STX), copy number variation, sxtA4

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Dynamics of the dinoflagellate Lepidodinium chlorophorum in Southern Brittany: controlling factors and consequences for exploited ecosystems

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During the last decades, the coastal zone of Southern Brittany (France) influenced by the Loire and Vilaine Rivers has been heavily impacted by more frequent harmful algal blooms. More particularly, green seawater discolorations caused by massive development of the dinoflagellate *Lepidodinium chlorophorum* are recurrently reported along and offshore the coastline of the Bay of Vilaine. While this dinoflagellate does not produce toxins, massive blooms of *L. chlorophorum* have been supposed to be harmful for bivalves. In particular, exopolymers excreted by this dinoflagellate could accentuate the episodes of hypoxia in the ecosystem, altering the rheological properties of seawater, and eventually causing problems to cultured bivalves. Despite the potential harmful effect of *L. chlorophorum,* few studies have specifically targeted this dinoflagellate. In this presentation, the main objectives developed in an ongoing Phd project on this species will be presented. The project will aim at: 1) identifying environmental factors controlling the space and time dynamics of *L. chlorophorum* in Southern Brittany*,* including distribution of the seed banks, on the basis of both long term data analysis and new data collected from the sediment and the water column, 2) estimating concentrations and molecular composition of the exopolymers produced by this species *in vitro*, verifying their presence during different phases of a bloom, and investigating their consequences on seawater viscosity and hypoxia, 3) assessing potential consequences of green seawater discolorations on oyster behavior and growth.

Mots-Clés: Eutrophication, Harmful Algal Bloom, Lepidodinium chlorophorum, Exopolymers, Viscosity, Hypoxia, Oyster growth

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Influence of temperature and light intensity on Ostreopsis cf. ovata growth and toxin content.

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Ostreopsis cf. *ovata* is a benthic toxic dinoflagellate forming bloom in summer period in the Mediterranean Sea. For the last fifteen years, frequency and intensity of blooms reported have increased in this temperate area. Since 2006, it is being monitored every year because blooms have been correlated with mortality of marine organisms and human illness (e.g. skin irritation, cough, fever, sore throat, dyspnea). This toxic dinoflagellate was also identified as a producer of palytoxin and palytoxin-like molecules named ovatoxins (OVTX-a to -l identified so far). As global warming has been suggested as a major actor involved in the increase of *Ostreopsis* blooms, we studied the combined effects of temperature and light intensity on growth and toxin content of one strain of *Ostreopsis* cf. *ovata* isolated in Villefranche-sur-Mer in summer 2014. After acclimation, three temperatures (23, 27 and 30 ∘C) and light intensities (200, 400 and 600 μmol photons m-2 s-1) were tested in batch cultures and samples taken at 6, 10, 14, 18 and 21 days to monitor the growth and quantify intracellular OVTXs. In all culture conditions, stationary phase appeared when nutrients were consumed, except at 30 ∘C at 200 μmol photons m-2 s-1 where the growth stopped while half of nutrients was still available. OVTX contents were higher during the stationary phase, as previously reported. Interestingly, the highest OVTX quota was observed at 23 ∘C per cell and per biovolume unit despite the increase of biovolume at this temperature. While temperature seemed to have a more pronounced effect on growth and OVTXs quota than light intensity, OVTXs profile (relative ratios of OVTX -a to -e) were constant whatever the growing phase and conditions.

Mots-Clés: Ostreopsis cf. ovata, Ovatoxins, Dinoflagellate, Depth, Macroalgae, Bloom monitoring

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Chemical ecology of Benthic HABs: the impact of NW Mediterranean Ostreopsis cf. ovata on copepods

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Blooms of toxic benthic dinoflagellates genus *Ostreopsis* have increased in frequency and intensity, notably in the Mediterranean Sea. Adverse effects on Human health have been recorded (skin/eye irritation, fever, headaches and breath difficulties) by direct contact with the microalgae or their bio aerosols. The toxicity of these dinoflagellates was attributed to the presence of palytoxin and analogs. Deleterious effects of *Ostreopsis* cf. *ovata* blooms on marine organisms have previously been reported, without knowing however if they were due to anoxic/hypoxic conditions or to the real toxicity of this dinoflagellate. In this context, the aim of the study was to assess the effect of *Ostreopsis* cf. *ovata* on the harpacticoid copepod *Sarsamphiascus* cf. *propinquus,* a phytal meiobenthic species that lives in direct contact with the toxic dinoflagellate. This model copepod was exposed, *in vitro*, to ecologically realistic concentrations of *O.* cf. *ovata* and to *Licmophora paradoxa*, a non-toxic competitive benthic microalga. The possible toxic effect of *O.* cf. *ovata* was tested on survival rates, fecal pellets production (as a proxy of feeding) and reproduction (using fertility and fecundity ratios). Regarding acute toxicity evaluation, *S.* cf. *propinquus* proved to be the most tolerant organism to *O.* cf. *ovata*, reported to date (LC50 (48h) > 20 000 cells.mL-1), even if hatching success and egg production were affected by the presence of the toxic dinoflagellate, suggesting a reprotoxic effect. These deleterious effects on reproduction are liable to modify the composition of benthic communities and suggest chemical defense of *O.* cf. *ovata* against grazers by affecting the future generations.

Mots-Clés: Ostreopsis cf. ovata, meiobenthic copepods, reprotoxicity, benthic HABs, chemical

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SALT STRESS RESPONSE OF BRACKISH AND FRESHWATER STRAINS OF MICROCYSTIS AERUGINOSA

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Blooms of *Microcystis*are most often observed in freshwaters but increasing numbers of bloom events have been recorded in coastal areas. Hydrologic events like storm and drouhgts are promoting the transfer of *Microcystis* and microcystins (MCs) across the freshwater-to-marine continuum. Salinity represents one of the main abiotic factor controlling the presence and toxicity of this genus. Therefore, understanding the impact of salinity on *Microcystis*physiology and toxin production and release is crucial to assess the potential environmental risk. Physiological responses of *Microcystis aeruginosa*including toxin production were investigated during five days using two toxic strains (PCC 7806 and PCC 7820) respectively from brackish and freshwater origins. After a sudden salt shock from salinity 3.4 to 14.4, PCC 7806 and PCC 7820 presented distinct limits of salinity tolerance. Their growth were inhibited above a salinity of 8.4 and 6.7 respectively. PCC 7806 produced 2 variants of MCs (MC-LR > dmMC-LR), and PCC 7820 produced 6 variants of MCs (MC-LR > MC-LW > dmMC-LR > MC-LY > MC-LF). For both strains, the sudden increase in salinity led to a decrease of MCs concentration per cell with no modification in their toxin profiles. Further experiments are being conducted to compare the short term physiological response to salinity shock in both strains with the relative expression of several genes using qPCR. Genes involved in MCs synthesis (*mcyA*), in oxidative responses (*sod*, *gshB*, *groel*), photosystem activity (*psbC*and *psbA*) and sucrose synthase (*sppA*and *spsA*) were evaluated. Also, osmolyte contents are studied using targeted analyses.

Mots-Clés: Salinity, Microcystis, Brackish, Transfer

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Taxonomic characterization of Ostreopsis in the French West Indies (CARTAGO mini-project)

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Among 11 *Ostreopsis* species, only the 2 last discovered species have been described with the morphogenetic approach. Several *Ostreopsis* species have been found for the first time in the Caribbean Sea and described only with morphological criteria. Genetic sequences for holotype coming for the Caribbean Sea are missing and *i*) prevents comparisons with new DNA sequences, *ii*) limits phylogeographical studies, and *iii*) leads to uncertain identifications.
Epiphytic dinoflagellates were collected at Rivière Sens, Gosier, Bois Jolan and Chapelle Beach (Guadeloupe Island). The samples were collected between 2017 and 2018 to start the morphogenetic assessment of *Ostreopsis* species in the Caribbean Sea. Six groups were found after comparisons to accessible sequences in GenBank.

- 1 group close to *O. lenticularis*,

- 3 groups close to *O.* cf. *siamensis* with 3 different percentages of correspondence,

- 1 group that corresponds with the morphological description of *O.* cf. *heptagona*

- 1 group close to *O.* cf. *ovata*

The genetic group close to *O.* cf *lenticularis* were found at all the sampled stations. The 3 groups close to *O.* cf. *siamensis* were found at distinct stations. The group that corresponds to the morphological description of *O. heptagona* was found only at Chapelle Beach and the group related to *O.* cf. *ovata* was found only at Rivière Sens.

Mots-Clés: diversity, taxonomy, molecular phylogeny, Ostreopsis

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PARACIDE : Links between Programmed Cell Death and parasite infection pathways in microalgae

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In coastal marine environments, mechanisms leading to decline and termination of phytoplankton blooms are still poorly understood. Loss factors in bloom dynamics can be controlled by physico-chemical stressors and/or complex biological interactions. This last includes grazers, pathogens, sexual reproduction, internal signaling systems call and programmed cell death (PCD or cell suicide). PCD has only been rarely reported in the field due to the diversity of cell deaths and due to the lack of knowledge on the factors inducing it. While parasitism and PCD were reported, they were only studied separately. Viral infection can induce PCD, therefore a similar causal link could exist between parasitism and PCD. This project aims to study potential links between PCD and parasitism. This work is based on the hypothesis that PCD could be induced by phytoplankton during parasitic infection to mitigate the parasite propagation. Preliminary experiments were performed to study the different responses of the host in different host/parasite couples, searching for potential induction of PCD by microalgal cells in response to parasite exposure. Fourteen dinoflagellates strains were tested as hosts, including 4 toxic species (*Alexandrium minutum, Ostreopsis cf. ovata*, *Ostreopsis siamensis, Prorocentrum lima*) and 4 non-toxic ones (*Scrippsiella trochoidea*, *Scrippsiella donghaienis*, *Heterocapsa triquetra, Prorocentrum micans*). They were exposed to two different types of parasites, the Syndiniales *Amoebophrya* sp. and the chytrid *Dinomyces arenysensis*. The kinetic of infections was studied over five days and the physiologic responses of hosts (growth, encystment, membrane permeability, lysis, hallmarks of apoptosis) were monitored by flow-cytometry and microscopic observations. The first results highlighted a high variability in the host/parasite compatibility related to different phenotypes. This suggests that resistant strains have a wide range of defensive mechanisms to prevent the parasitic infection.

Mots-Clés: Parasite, Programmed cell death, apoptosis, dinoflagellate, syndiniales, chytrid

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Algal Blooms from Polynesian Lagoons: New Sources of Bioactive Compounds?

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In the frame of the collaborative mini-project EPSOM " Efflorescences algales des lagons Polynésiens : nouvelles SOurces de Molécules bioactives ? " supported by the GDR PHYCOTOX, we studied > 30 extracts from the Polynesian Marine Phytoplankton Culture Collection from Louis Malardé Institute. This work describes a methodology developed to discover novel ligands directed against nicotinic acetylcholine receptors from complex phytoplankton extracts. The methodology uses purified electrocyte membranes from *Torpedo marmorata in-solution* or immobilized on the bottom of 96-wells microplates. In these preparations, the nicotinic acetylcholine receptors are in their native form and surrounded by their natural lipids and clustering proteins. Thereby, *Torpedo*-nAChRs are stable and functional for years. We address the question of the chemical nature of the toxin or ligand detected. This is a novel concept. Most bioassays enable the detection of any given analyte, but the chemical nature of the binder remains unknown. The receptor-binding assay allows the direct capture of ligands from phytoplankton extracts by the nicotinic receptor target. Following a washing step under stringent conditions, the captured toxins are eluted. Mass spectrometry was used to determine the chemical nature of the captured ligand. Further, the putative eluted nicotinic ligands were analyzed by two-electrode voltage-clamp electrophysiology using *Xenopues laevis* oocytes expressing human nicotinic acetylcholine receptors confirming the presence of antagonists of this family of receptors in so far unforeseen marine phytoplankton species. Taken together, the proposed technology (WO 2012/101378 A1)* is suitable for the discovery of unknown and unanticipated neurotoxins from marine and continental aquatic environments.
*WO2012101378A1 "Method for manufacturing an analysis substrate, and use thereof for detecting toxins". R. ARAOZ et al.
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Mots-Clés: Nicotinic acetylcholine receptor (nAChR), Torpedo electrocyte membranes, Microplate receptor binding assay, Cyclic imine toxins, Harmful Algal Blooms (HABs), MALDI, TOF

*Intervenant

Comparison of the toxicity of palytoxin and extracts of Ostreopsis cf ovata on a set of cell lines

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Ostreopsis cf *ovata* has been recently involved in human health problem along the Mediterranean coast in Italy, France and Spain. This species has shown to produce ovatoxins which are analogs of palytoxin (PlTX). If several analogs (from a to h) of ovatoxins have been identified, only few data on their toxicity have been published due to the absence of purified compounds. Crude extract from *O. ovata* cultures as well as fractions containing or not ovatoxins were tested using the cytotoxicity MTT assay after 24 hours of treatment on a range of cell types: nervous system (enteric glial cells and neuroblastoma Neuro2A cells), intestinal (Caco2 cells), pulmonary (A549 cells) and hepatic (HepaRG cells). The results were compared with the ones obtained with the commercially available palytoxin.

Our data with PlTX showed an inhibitory concentration 50% (IC50) around 0.07 nM (187 pg/ml) for EGCs and around 5 nM (13.4 ng/ml) for differentiated Caco2 cells. The IC50 of the crude extract ranged from 3 to 60 ng/ml depending on the cell line. For a pooled fraction enriched in ovatoxins, the IC50 ranged from 3 to 15 ng/ml depending on the cell line. On the contrary, the IC50 was generally above 200 ng/ml with a pooled fraction without ovatoxins.

Mots-Clés: palytoxine, ovatoxines, Ostreopsis, toxicité in vitro

*Intervenant

Study of the Food web transfer of pinnatoxin G and Portimine produced by the dinoflagellate Vulcanodinium rugosum and the effect of this HAB species on the physiology of the main copepods developing in the Mediterranean Ingril and Thau lagoon

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Vulcanodinium rugosum a HAB dinoflagellate species has been recently described in Ingril lagoon (French Mediterranean) by Nézan & Chomérat (2011) and it's dangerously spreading to other lagoons. It produces pinnatoxins and portimine mainly accumulated by mussels. In France, Thau lagoon is fourth aquaculture zone in terms of shellfish production with 10% of national production. It is connected to the Ingril lagoon. *V. rugosum* could threat the shellfish activity. High pinnatoxin concentrations were detected several months per year in Ingril lagoon while less amounts of theses toxins were observed in Thau. Toxin transfer through food web compartments is poorly studied, especially though copepods which may represent up to 80% of mesozooplankton fraction. Nothing is known on the transfer of the *V. rugsoum* toxins transfer. pinnatoxin G and portimine concentrations have been investigated twice a month in three planktonic fractions (20-60μm ; 60-200 μm ; $>$ 200 μm) in Ingril and Thau lagoons. First data showed that portimine concentrations were higher than pinnatoxin G concentrations in the three fractions in Thau and Ingril. Both toxins were present in the microphytoplankton fraction (20-60μm) containing *V. rugosum* in Ingril and to a lesser extent in Thau. These toxins were detected in the upper fractions, especially in the $>$ 200 μm fraction which mainly contained copepods. Using laboratory controlled experiments, the main copepod species were incubated with *V. rugosum* cells to investigate the potential effect of this algae and the produced toxins on the feeding behavior and the fecundity of the selected copepods. Preliminary results showed that the biology of the copepods originated from Ingril was less impacted by *V. rugosum* in comparison the the copepods sampled in Thau lagoon. These experiments are in progress.

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Mots-Clés: Vulcanodinium rugosum, pinnatoxin, portimine, copepods, transfer, Mediterranean

Microorganisms Chemical Mediation in a Marine Environment – When Fungus induces Microalgal toxine production!

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During identification studies of marine microalgae by molecular biology, different DNA fungal sequences were observed (unpublished data). Among those contaminating sequences, more than 40% were attributed to fungi, mostly Ascomycota, evidencing their presence in the microalgal samples. This observation raised the question of interactions existing between fungi and microalgae.
In fact, in the marine environment, many microorganisms are present; and much information is reported on bacterial and microalgal symbiotic interactions [1]. However, similar interactions between fungi and microalgae [2, 3], which exists in terrestrial environments forming lichens, are less documented in the marine environment.

To study interaction between microalgae and fungi, fungal isolation was achieved using traditional isolation strategy [4] from cultures of different benthic dinoflagellates (*Gambierdiscus*, *Ostreopsis* and *Prorocentrum* species), yielding fungi belonging to various genera, e.g. *Aspergillus* or *Wallemia*.

To analyze in-depth such interactions in an environment similar to coastal waters, a specific technical setup was devised. Chemical analysis using a metabolomic approach [5, 6], based on liquid chromatography coupled to high-resolution mass spectrometry, indicates that such chemical mediation exists.

This study shows the tight connection between microbial interactions and microalgal toxin production.

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Mots-Clés: microbial co, culture, fungus, Microalgal, Metabolomics, Prorocentrum lima

Role of the toxic micro-alga, Alexandrium pacificum (A. catenella) in the virulence of Vibrio tasmaniensis LGP32 a pathogenic bacteria for the oyster Crassostrea gigas.

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Bacteria, like all bacterioplankton, are subject to numerous biotic and abiotic constraints in the environment, such as predation and the availability of nutrients essential for their growth. If the association of these vibrios with phyto and zooplankton has been shown, the ecological consequences of there interactions are unknown. In particular, the effects of toxic microalgae on the virulence of vibrios have, to our knowledge, never been explored before.Here, our objective was to characterize the unknown role of microalgae / vibrio interaction in the expression of vibrio virulence.

Taking care to place our experiments in conditions close to those encountered in the environment, we found that cell extracts of *Alexandrium pacificum* both stimulated the growth and increased the virulence of *Vibrio tasmaniensis* LGP32. Moreover, we showed that a series of LGP32 proteins, already known to be involved in known mechanisms of virulence in bacteria were differentially expressed upon contact with the microalgae.

In order to validate the role of the identified differentially expressed proteins in the virulence of LGP32, we will study the ability of the depleted LGP32 mutants of these proteins to interact with Alexandrium, as well as the virulence of these mutants in experimental infections of juvenile oysters.

Mots-Clés: virulence, oyster, interaction, vibrio, alga

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(Semi-)automated *in vivo* approaches for characterizing HAB and other phytoplankton blooms in European coastal waters: News from the Joint European Research Infrastructure for Coastal Observatories – New Expertise (JERICO-Next)

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(Semi-)automated *in vivo* approaches are being deployed since the last decade, in order to better understand phytoplankton temporal and spatial distribution in coastal systems, by improving the detection of phytoplankton community changes affecting growth rates, size structure, taxonomic and/or pigmentary composition. These changes can occur at different time and spatial scales, evidencing rapid as well as long-term changes in environmental conditions. Therefore, when implemented in automated environmental monitoring platforms, as fixed stations, moorings, research vessels and/or ships of opportunity, these (semi-)automated techniques can represent valuable early-warning systems of community changes, as the occurrence of blooms and, in particular, of harmful algal blooms (HAB), which can lead to disruption of marine food webs and mass mortalities of marine organisms and which are of special interest in areas of fishing, aquaculture and tourism. The discrimination and operability of automated techniques addressing phytoplankton diversity (at nearly taxonomical and/or functional levels) and productivity were improved within the the Joint European Research Infrastructure network for Coastal Observatories – Novel Expertise for coastal observatories (JERICO-Next – H2020, 2015-2019), in order to provide early warning platforms for the occurrence, duration and spatial extent of phytoplankton blooms and, by extent, of noxious episodes. Scientists inter compared, worked on technical and analytical improvements and applied a combination of phytoplankton automated observation approaches, based on single cell/particle or

bulk optical characteristics, in several European coastal systems ranging from oligotrophic (West Mediterranean) to mesotrophic (southern Bay of Biscay, Celtic seas) and eutrophic systems (eastern Channel, southern North Sea, Skagerrak/Kattegat, Baltic Sea), characterised by different types of potential HABs developments (dinoflagellates, diatoms, haptophytes, cyanobactetria). Three main groups of techniques, image in-flow or *in situ* acquisition and analysis, pulse shape-recording automated flow cytometry, as well as multispectral and variable fluorometry and spectrophotometry, are being critically explored and implemented in different sites and platforms. A summary of the main results gathered by a combination of these techniques is presented and recommendations on future applications of these techniques and remaining challenges are discussed in the frame of their implementation in operational marine observing systems.

Assessment of dispersion and transport of HAB: tracking based on satellite Chlorophyll maps

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Harmful Algal Blooms might be observed combining *in situ* sampling and satellite data to optimize the spatio-temporal coverage of the existing phytoplankton observatories. Except when using highly complex hydrodynamical/biogeochemical modelling, an intuitive, easy to implement and decision-helping method/tool is lacking to automatically follow their initiation, extent and movement, which could form the basis of an alert system.
The aim is to present the methodology and the first steps of an ongoing study about the spatial and temporal characterization of blooms dynamics, based on interpolated Chlorophyll satellite products. After identifying individual bloom patches from Chlorophyll maps based on a threshold, neighboring patches are merged and their centroids are identified. The major remaining challenges, once all patches/centroids are individually identified and associated with their dates, are to build a consistent genealogy of the patches in space and time. This is made difficult by possible merging or separation of parents or children patches. Two solutions to this issue are to separate areas characterized by one major bloom or to use as input available risk maps of presence of HAB. In addition, pertinent metrics of patch size and direction of displacement have to be determined.
Such a tool might be combined with published methods such as spectral classification and Markov modelling. By integrating other environmental variables, we would be able to build a powerful dataset capable of observing and explaining the spatio-temporal dynamics of phytoplankton blooms, including HAB. The overall objective might be to forecast such HAB events. All these geoprocessing operations are made using free and open source software R and GRASS GIS.

Mots-Clés: modélisation spatialisée, HAB, satellite, suivi spatio, temporel

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Speed talks

Insights on the phytoplankton community structure in the Bay of Seine and the standing of Pseudo-nitzschia sp. and Dinophysis sp.

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Nowadays, researchers have a better understanding of the influence of abiotic parameters on toxic species involved in Harmful Algal Blooms (HABs). Studies show that nutrient inputs, temperature and salinity with the stratification that they entail are major drivers of HABs. For the next two years, the PhD study at the origin of this work will aim to better understand the impact of environmental changes on phytoplankton in the Bay of Seine. What ecological niche – combining abiotic and biotic influence – characterizes toxic blooms? How functional traits structures the phytoplankton community? What relations can be made to explain the toxicity at the Seine plume interface? To answer those, we first need to have insights on the communities' structure throughout the Bay. The database related to the REPHY monitoring program for the Bay of Seine gathers the abundance of 284 taxons starting as early as 1987. A complete and regular database for the period 2000 - 2018 is explored to understand the resilience of the phytoplankton community at different coastal stations. Besides, the phenology and timing of the toxic genera *Pseudo-nitzschia sp.* and *Dinophysis sp.* is also investigated to describe the spatial variability of the blooms within the bay. First observations show a heterogeneous pattern along the coast. Indeed *Pseudo-nitzschia sp.* blooms are affecting the areas of the Bay of Veys and Ouistreham while *Dinophysis'* maximum abundances were found around the mouth of the Seine river. Moreover, an increase of lipophilic toxins (produced by *Dinophysis sp.* and causing Diarrheic Shellfish Poisoning (DSP)) has led to recurrent farms closures in the past few years with a long period in 2014. Whereas Domoic Acid concentrations (causing Amnesic Shellfish Poisoning (ASP)) remain below the EU-sanitary level since 2013 even though annual blooms are still monitored, suggesting specific local conditions that would fit further studies.

Mots-Clés: Phytoplankton community structure, Bay of Seine, Pseudo, nitzschia, Dinophysis, REPHY

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Short-term effects of salinity and temperature stresses on DMSP and PSTs content in Alexandrium catenella groupe IV and Alexandrium minutum

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Harmful algal blooms intensity and frequency are reported to increase in association with climate change. These bloom events may have negative impacts on coastal activity and human health. Some species of marine dinoflagellates which produce Paralytic Shellfish Toxins (PSTs) may be involved in these toxic blooms. Among them, *Alexandrium minutum* and *Alexandrium catenella* group IV are known as major producers of saxitoxin and analogues (STXs). These neurotoxin compounds interfere with sodium channels and disrupt nerve impulses of contaminated shellfish consumers. Marine dinoflagellates are also a group able to produce considerable amounts of dimethylsulfoniopropionate (DMSP), a precursor of DMS involved in climate regulation. DMS molecules have the property to form condensation nuclei in the atmosphere and are responsible for cooling the earth. Interestingly, it was suggested that there might be a link between STXs and DMSP production, with the common precursor methionine, involved in the two biosynthesis pathways. In our experiments, four strains of *Alexandrium minutum* and *Alexandrium catenella* group IV were investigated: AM1231, AM1232, ACA-15 and H8-4. The salinity conditions tested were 20, 35, 43 and 48 psu for *A. minutum* and 23, 38, 46 and 51 for *A. catenella*. In addition, two temperatures of 18∘C and 25∘C were coupled to these salt stresses. During this study, three sampling times T6h T24h and T48h were performed to evaluate the effects of a short-term stress. The DMSP and STXs concentrations were analyzed by liquid chromatography coupled to a mass spectrometer (LC-MS). In this study, the objectives are to assess the effects of salinity and temperature changes on the production of DMSP and STXs. Secondly, it aims to clarify the cellular role of both metabolites for marine dinoflagellates and whether there is a competition between the metabolic pathways of these two molecules under stress conditions.

Mots-Clés: Alexandrium sp., Dimethylsulfoniopropionate (DMSP), Paralytic Shellfish Toxins (PSTs), Salinity stress, Temperature stress

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Selectivity of the extraction solvent for maitotoxins and ciguatoxins from lyophilised biomass of Gambierdiscus excentricus.

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Ciguatera is a food poisoning caused by the ingestion of fish or shellfish accumulating toxins produced by the genus *Gambierdiscus*. Ciguatera is endemic in the Caribbean area, which is the second-most impacted zone after the South Pacific Ocean.
The initial isolation of ciguatoxins was carried out from fish livers due to the higher content of toxins in this organ. So far, only few methods have been reported the isolation of toxins from dinoflagellates (due to slow growth rate and large biomass required). All procedures using methanol or acetone for extraction of highly lipophilic ciguatoxins (CTXs) and methanol or aqueous ethanol for the more hydrophilic maitotoxins (MTXs).

This study aims at clarifying the selectivity of the extraction solvents for isolation of toxins from *G. excentricus,* previously shown to be an MTX- and CTX-producer. *G. excentricus* biomass was obtained during 6 months of semi-continuous culture and preserved through lyophilisation. Evaluation of the extraction solvent was carried out monitoring MTX4 (the only known toxin in *G. excentricus*) by mass spectrometry, while the extraction efficiency of CTXs was evaluated by the cell based-assay (Neuro2a) and by fluorescence-Receptor Binding Aassay (fRBA). As MTXs interfere in the detection of CTXs by the Neuro2a assay, toxin-selective extraction would be advantageous. Acetone yielded ten times more ciguatoxin-like activity than the extraction with 100% methanol followed by liquid-liquid partitioning between aqueous methanol and dichloromethane. Acetone also extracted less than 3% MTX4 resulting in a lower interference of this compound in the Neuro2a-assay. Finally, three times more MTX4 was extracted from lyophilised cell pellets when using aqueous methanol (75%) compared to 100% methanol.
This study demonstrated the importance of the extraction solvent for both optimal recovery of target analytes and selectivity of this initial step in the isolation procedure for the further characterization of toxins produced by *G. excentricus*.

Mots-Clés: Benthic dinoflagellate, Caribbean ciguatoxins, Sample preparation, Bioassay

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Intra- and inter-specific variability of toxin and metabolite profiles of Dinophysis spp.

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Toxic species of the genus *Dinophysis* produce two types of lipophilic toxins: okadaic acid (OA) and its derivatives the dinophysistoxins (DTXs) and pectenotoxins (PTXs). DTXs are responsible for the diarrheic shellfish poisoning (DSP) in humans by consumption of contaminated bivalves. Globally, the accumulation of DTXs in shellfish leads to many closures of shellfish farms, resulting in a significant economic impact.
Due to a lacking knowledge on the life cycle of *Dinophysis*, it is currently not clear whether *Dinophysis* spp. form distinct local populations in different regions of the Bay of Biscay or whether the populations are connected, i.e. of a single origin and simply transported along the coast with currents from South to North. Therefore, we studied the metabolite profiles of several strains and species isolated from the French Atlantic and Mediterranean coasts of *Dinophysis* in culture. If profiles of strains of the same species from different locations differ significantly despite identical culture conditions, it is likely that the populations should be considered distinct.
To compare intra- and inter-specific variability of *Dinophysis*, five strains of *D. sacculus* (from Thau lagoon (Mediterranean Sea), Arcachon Bay and Vilaine Estuary (Atlantic area)) and two strains of *D. acuta* and *D. acuminata* from Arcachon Bay were selected. All strains were fed with three *Mesodinium rubrum* cells per cell of *Dinophysis*, except *D. acuta* which received nine cells of *M. rubrum* per cell. Cultures were sampled during exponential growth phase to quantify intra- and extracellular toxins and other metabolites by liquid chromatography coupled to tandem mass spectrometry. Analysis is still ongoing.

Mots-Clés: Dinophysis spp., toxins, metabolites, geographic origin, transport, connectivity

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Towards the standardization of a new optimized post-column oxidation method for PSP toxin determination in shellfish

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Paralytic shellfish poisoning (PSP) toxins can cause acute gastrointestinal and neurologic symptoms after ingestion for example from shellfish. Therefore, PSP monitoring in shellfish is implemented in the European food safety system. Therefore, robust, precise, sensitive, specific, and rapid analytical methods are mandatory to determine the presence of PSP toxins in shellfish.
Since 2019 a pre-column oxidation HPLC method with fluorescence detection, known as the Lawrence method (AOAC 2005.06) is determined as the regulatory reference method for PSP monitoring in shellfish in the EU. Although the Lawrence method is a powerful method, it is complex, time consuming and requires great expertise. Other internationally recognized methods can be used for monitoring as long as no arbitral analysis is claimed.

The aim of our work is to standardize an alternative method for PSP monitoring purposes which is less elaborate while providing a high level of precision and specificity. The post column oxidation method (PCOX), published as AOAC 2011.02, is simpler and faster than the Lawrence method with some drawbacks e.g. regarding separation of certain PSP toxins. We based our optimization on this method and carried out the following steps:

- Adjustment of the oxidation reagents and their flow rates, as well as the HPLC gradient for increased sensitivity
- Change of extraction solvent from chloric acid to acetic acid for toxin profile preservation
- Implementation of a hydrolysis step to enable quantification of GTX6, C3 and C4 as well as separation of GTX4 and GTX6

During in-house verification we proved comparability of results of our optimized PCOX to those achieved from AOAC 2005.06. Our next step is the conduction of a collaborative study to enable the method's standardization by the European standardization committee (CEN). For this purpose we are aiming at the acquisition of contaminated sample material and participating laboratories among the experts meeting at Phycotox2019.

Mots-Clés: Parayltic shellfish poisoning, postcolumn oxidation, HPLC, FLD, validation

*Intervenant

Co-culture model Caco-2/HT29-MTX: a promising tool for toxicity investigation of phycotoxins on the intestinal barrier

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Lipophilic phycotoxins produced by marine microalgae can accumulate in edible shellfish. Some of them are documented to affect the gastrointestinal tract provoking acute intoxications in humans. However, for some toxins, the absence of proven human intoxications makes it difficult by public health authorities to estimate the risk for humans following acute exposure. Investigation of toxins toxicity through both *in vitro* and *in vivo* studies can provide key information. In fact, several phycotoxins have been shown *in vivo* to induce toxic effects on the intestinal epithelium such as cell detachment, fluid accumulation and villous erosion. Nevertheless, most of the toxicity data have been obtained *in vitro* on intestinal epithelial cell monolayers with a single cell type. Recently, co-culture models have been developed to mimic more closely the human intestinal barrier and are expected to improve evaluation of the toxicity of ingested compounds. Using such relevant co-culture model with enterocytic Caco-2 cells and HT29-MTX goblet cells, we investigated the effects of four phycotoxins (okadaic acid (OA), yessotoxin (YTX), pectenotoxin-2 (PTX2) and azaspiracid-1 (AZA1)). Cell viability, permeability, production of mucus and inflammation were evaluated using various approaches such as TEER, ELISA, histology and High Content Analysis. Our results showed that OA and PTX2 affected the monolayer permeability and that YTX and AZA1 increased the mucus layer through histological analysis. Only OA seems to induce inflammation through IL8 cytokine release. Additional results using RT-PCRq will highlight the pathways and genes affected by these toxins on the investigated processes. This co-culture model appears to be a promising tool to evaluate and compare the effects of phycotoxins on the human intestinal barrier.

Mots-Clés: Co, culture, phycotoxin, toxicology, permeability, mucus

*Intervenant

Posters

Molecular networks development for the microalgae genus Dinophysis from data of high resolution mass-spectrometry (HRMS)

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Some dinoflagellates of the genus *Dinophysis* are toxin-producers and represent the main threat to shellfish farming in France. Indeed, they are responsible for gastrointestinal illness (Diarrhetic Shellfish Poisoning, DSP) caused by the consumption of shellfish contaminated by two families of toxins: okadaic acid (OA) and their analogues dinophysistoxins (DTXs) and pectenotoxins (PTXs).
Targeted chemical analysis by low resolution liquid chromatography-mass spectrometry (LC-MS/MS) are routinely used for the monitoring survey of regulated known toxins (OA, DTX1, DTX2 and PTX2) in shellfish and to determine toxin profiles in microalgae. To identify unknown toxin analogues, a novel approach – Molecular Networking – was used based the untargeted analysis of mass spectrometry fragmentation data obtained by liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS).

Molecular networks are based on MS fragmentation comparison of to highlight spectral similarities related to compound structural similarities. Before obtaining informative network creation, it is mandatory to optimize data-dependent LC-HRMS acquisition conditions. Therefore twenty-one different condition were compared (1) in a targeted manner for improved toxin clustering and (2) in an untargeted manner to provide a global overview of *Dinophysis* spp. metabolic diversity. The presence of isobaric compounds made important the pre-process of LC-HRMS data by Mzmine 2 software which provides deconvoluted peak lists. Finally, the influence of various parameters on the quality of molecular networks was investigated using the Global Natural Product Social Molecular Networking (GNPS) platform.
Molecular networking, as a bioinformatics approach, provides a more complete and structured overview of chemodiversity from harmful algae, as-well-as highlighting toxinic diversity of *Dinophysis* spp. Thus, it is a valuable and innovative tool to study in-depth *Dinophysis,* toxin-producers.

Mots-Clés: molecular networking, HRMS, bioinformatic, toxin, microalgae

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Tools development for metabolic characterization of diatom cells by flow cytometry

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In microalgae, interferences of cellular metabolism, energy and stress status are important for the regulation of growth, in relation with environmental abiotic or biotic factors that regulate their ecophysiology. The aim of the present work was the development of flow cytometry tools to characterize the cell status of diatoms in term of vitality and metabolic activity. For this purpose, the DiBac4, a probe used in viability test, was validated in several diatom species: *Nitzschia alexandrina*, *Entomoneis paludosa, Phaeodactylum tricornutum, Navivula sp, Skeletonema costatum, Cylindrotheca fusiformis*, *Amphora sp* and *Pseudo Nitzschia multistriata.* For oxidative stress (ROS production), the two probes CM-H2DCFDA and Bes-H2O2-AC were validated in *E. paludosa* and *C. fusiformis.* We then use the viability tool DiBac4 to study diatoms/bacteria relationships. For this purpose, *E. paludosa* and *C. fusiformis* were grown with/or without antibiotics, and the diatom's viability was followed in relation with the associated bacteria during the microalgal growth. In a second experiment, oxidative status of these two diatoms was studied during osmotic stress. *Entomoneis paludosa* and *C. fusiformis* were grown under a range of salinity in natural sea water enriched with 50, 80, 100 or 200 g.L-1 NaCl, and cytometric measurements using CM-H2DCFDA and Bes-H2O2-AC were used to determine the ROS production of the cells. In conclusion, these preliminary results show that the cellular probes DiBac4 and CM-H2DCFDA & Bes-H2O2-AC can be used in diatoms for the determination of cell viability or oxidative status (ROS) respectively. These tools are good indicators of microalgal metabolic status during growth in response to biotic or abiotic regulations.

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Mots-Clés: diatoms, flow cytometry, viability, oxydative stress

In vivo and in vitro pinnatoxins A and G reversibly block transmission at the skeletal neuromuscular junction.

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The dinoflagellate *Vulcanodinium rugosum,* first isolated from Ingril, a French Mediterranean lagoon, is known to produce the pinnatoxins (PnTXs) and the portimines. PnTXs (A-H) constitute an emerging family of phycotoxins belonging to the cyclic imine group. Interest has been focused on these fast-acting highly-potent toxins because they are widely found in contaminated shellfish. Despite their highly complex molecular structure, PnTXs have been chemically synthetized by the Zakarian's group, and demonstrated to act on various nicotinic acetylcholine receptors (nAChRs). To the best of our knowledge, neither PnTX-A nor PnTX-G and analogs, obtained by chemical synthesis with high degree of purity ($>$ 98%), have been studied *in vivo* or *in vitro* on adult mouse and isolated nerve-muscle preparations expressing the mature muscle-type (a1)2b1de nAChR. Our results show that PnTX-A and PnTX-G acted on the neuromuscular system of anesthetized mice and blocked the compound muscle action potential (CMAP) in a dose- and time-dependent manner with similar ID50 values (dose required to block 50% of the CMAP), as determined using an *in vivo* minimally invasive electrophysiological method. The decrease of CMAP induced by both toxins *in vivo* was reversible within 6-8 h. PnTX-A and PnTX-G, applied to isolated *extensor digitorum longus* (EDL) nerve-muscle preparations, blocked reversibly isometric twitches evoked by nerve stimulation. Both toxins exerted no direct action on the contractile machinery of muscle fibers, as revealed by direct muscle stimulation. In addition, PnTX-A and PnTX-G blocked synaptic transmission at mouse neuromuscular junctions. PnTX-A aminoketone analog (containing an open form of the imine ring) had no effect on neuromuscular transmission. These results indicate the importance of the cyclic imine for interacting with adult mammalian muscle-type nAChR.
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Mots-Clés: pinnatoxin A, pinnatoxin G, neuromuscular system, in vivo, muscle contraction, synaptic transmission, nicotinic acetylcholine receptors

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Uncharacterized bioactive extracellular compounds of Alexandrium minutum are highly deleterious for oyster gametes

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Blooms of *Alexandrium minutum* are recurrent along French coasts and estuaries, which are oyster production areas. Toxic strains of *A. minutum* produce Paralytic Shellfish Toxins (PST) responsible for shellfish poisoning in consumers who ate contaminated shellfish. *A. minutum* strains also produce uncharacterized Bioactive Extracellular Compounds (BEC) with allelopathic and cytotoxic activities. *A. minutum* blooms generally occur during the spawning period of marine bivalves and may have an impact on gamete quality, fertilization success, embryogenesis, and larval development and recruitment. The aim of the present study was to investigate the effects of three strains of *A. minutum* producing only PST, only BEC, or PST and BEC on gametes of the ecologically and economically important Pacific oyster *Crassostrea gigas*. Gamete cellular characteristics (viability, reactive oxygen species, and mitochondrial membrane potential) and quality, spermatozoa motility and methylation, and oocyte morphology were assessed after 2 h of *in-vitro* exposure to a range of concentrations of *A. minutum*. Our results reveal that the *A. minutum* strain producing only BEC is more deleterious for oyster gametes than *A. minutum* strains producing PST or PST + BEC.

*Intervenant

Alexandrum minutum exposure impacts protein composition of oyster mucus

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Mucus is a viscous secretion at the interface of the animal and its environment. It is the outermost line of protection against environmental stressors, including pathogens, ultrafine particles, and toxins. In filter feeding species such as bivalves, mucus of the gills is the portal of entry of food, trapping and transporting food particles, toward the interior of the organism. Some microalgae species produce toxins that affect bivalve physiology. In particular, toxic strains of *Alexandrium minutum* regularly bloom along French coasts and estuaries in production areas of oysters. Toxic strains of *A. minutum* produce Paralytic Shellfish Toxins (PST) responsible for shellfish poisoning in consumers who ate contaminated shellfish. *A. minutum* also produces uncharacterized Bioactive Extracellular Compounds (BEC) with allelopathic and cytotoxic activities on phytoplankton cells and oyster gametes, embryos, and external tissues of adult. The aim of the present study was to investigate the effects of toxic *A. minutum* on protein composition of mucus in the oyster *Crassostrea gigas*. Oysters were exposed to *A. minutum* strain (AM89BM) producing PST and BEC at 2.60 × 107 of algal cells/oyster/24h for 1 week, in the range of concentrations of *A. minutum* measured during natural blooms. Mucus of oysters was sampled before and after the exposure period. Label-free quantitative proteomic study (LC/MS-MS) of mucus revealed differentially accumulated proteins among feeding regimes.

*Intervenant

Bacterial communities associated with Alexandrium: study of their structure and composition in culture and in situ by a metabarcoding and network analyses approach.

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Toxic microalgae blooms have negative impacts on ecosystem function and public health. Abiotic factors, such as nutrients and temperature, are well known to contribute to these proliferations, however the role of biotic interactions between microalgae and bacteria are still poorly understood. Nowadays, high-throughput sequencing techniques coupled with metabarcoding analyses enable to study the microalgae/bacteria association both in culture and in the natural environment. For example it remains unclear, if the associated microbiota is strain/population specific and if the bacteria/microalgae consortium found in culture are also occurring in nature. The aim of the present work was to apply bioinformatic and biostatistics approaches on metabarcoding dataset obtained from culture and environmental samples to identify microalgae/bacteria association at the species/strain and the population level. First, the bacterial communities (BCs) associated with different *Alexandrium* species and strains, maintained under controlled conditions were studied. Second, the dynamic of the BC within a bloom of *A. minutum* during three consecutive sampled years in the Brest bay were analyzed. For both projects, a pipeline of metabarcoding data analyses was created. The scripts were implemented in python and R language, utilizing dada2 and phyloseq packages and the Snakemake workflow manager.
Preliminary results on cultivated strains showed that BCs associated with strains cultivated in English Channel were distinct to the strains cultivated in Mediterranean Sea, suggesting the potential influence of culture media (the salinity) on associated BCs. Concerning the environmental study, a high heterogeneity in the intensity of the bloom were observed within a seasonal bloom and between sampled years. Are the structure of bacterial community associated with *A. minutum* may contribute to the observed variability? A co-occurrence network analysis was implemented to visualize ASVs distribution among *A. minutum* bloom. Its analyze is still in progress and would improve our understanding of microalgae/bacteria interactions.

Mots-Clés: Alexandrium, bloom, bacteria, metabarcoding, network

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Accumulation and metabolization of paralytic shellfish toxins in Sydney rock oysters selected for growth

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The aquaculture industry can be strongly impacted by harmful algal blooms through toxin accumulation in shellfish, which become unsafe for human consumption, resulting in harvesting closures. Therefore, identifying the factors influencing toxin levels in shellfish is an important public-health and socio-economic challenge. Selection for growth is widely used worldwide to increase shellfish industry profitability, however, the effect of growth rate on algal toxin accumulation remains unclear.
In New South Wales, Australia, 30 years of selective breeding has led to numerous Sydney rock oysters (*Saccostrea glomerata*) families with different growth and disease resistance characteristics, providing an ideal opportunity to study the effect of selection for growth on toxin accumulation.

Experimental exposures to paralytic shellfish toxin (PST)-producer *Alexandrium* spp. at field-realistic concentrations were conducted to compare different *S. glomerata* phenotypes.

In the first experiment, oyster families selected for both fast growth and disease resistance (WMr-Winter Mortality resistance; B2-dual resistance to Winter Mortality and QX) were compared with a non-selected wild oyster. While toxicity of all oyster types was similar, a difference in PST analogue composition indicated different abilities to metabolize the toxins.

In a second experiment, two oyster families with contrasted growth (slow *vs* fast growth) were analysed for PST content, and ecophysiological rates (respiration and ingestion) were assessed during the experiment. Toxicity was two-fold higher in the fast growth family and was associated with a higher ingestion rate. Additionally, a difference in metabolization of PST analogues was identified between the two oyster families.
Altogether, these results suggest that higher growth rate in oysters is associated with higher feeding rates, resulting in higher toxin uptake. This study also highlights that selection can modify toxin metabolization ability in oyster tissues, illustrating the metabolic differences between oyster types. Considering these results, oysters selected for fast growth may be at higher risk for phycotoxin accumulation.

*Intervenant

Mots-Clés: paralytic shellfish toxins (PSTs), oyster, growth rate, biotransformation

Looking for candidate metabolites of Alexandrium minutum allelopathy

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Besides from the well-known intracellular "paralytic shellfish toxins (PST)", *A. minutum* produces uncharacterized exudates which have been implicated in ichtyotoxic, hemolytic and allelochemical activities, but the nature of these extracellular toxins remain unknown. The aim of the Phycotox GDR project ALAsKA was to verify that candidate *A. minutum* allelochemicals are biosynthesized by the toxic species.
In previous experiments potential allelochemical candidates were identified from exudates of the highly toxigenic strain CCMI1002 of *A. minutum*. In parallel, the metabolomes of 6 strains of *A. minutum* of the bay of Brest germinated from sediments of different ages (1998 and 2006) were analyzed in phosphorous-limited conditions and across different growing phases. Allelochemicals and intracellular metabolites were both analyzed by high resolution mass spectrometry, respectively by LTQ XL Orbitrap and LC-QTOF. In this work we compared the allelochemical candidate's mass spectrum with *A. minutum* metabolomic fingerprinting to look for corresponding molecules
One single candidate with a mass of 408.308 ± 0.001 (m/z), was found both in the allelopathic extract and in the lipophilic fraction of the metabolomes. Systematic identification and structural characterization of this candidate via public databases result to an unknown compound. It was higher abundant in the metabolomes of 2006-strains than in 1998-ones. This suggests a highly intraspecific variability of the biosynthesis of the allelochemicals. No significant differential production of this metabolite was observed between exponential and stationary growing phases. In addition, the identification of the same molecular candidate in the metabolome databases of other non-toxic dinoflagellates (*Scripppsiella donghaienis*) suggests that the allelochemical compound might be common to toxin and non-toxic species. To verify this hypothesis, the biological activities of both the *A. minutum* and *S. donghaienis* strains will be quantified and correlated to the presence and abundance of the potential allelochemicals compound.

Mots-Clés: Alexandrium minutum, allelopathy, metabolite

*Intervenant

Diversity of benthic microphytoplankton associated to Ostreopsis cf. ovata bloom in NW Mediterranean Sea.

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Ostreopsis cf.ovata is a toxic benthic dinoflagellate forming Harmful Algal Blooms (HABs) which can cause ecological, sanitary and economic issues. Common in tropical areas, this microalgae has spread to temperate region such as the Mediterranean Sea. Since several years, this specie blooms every summer in Rochambeau, located near our laboratory, in Villefranche Bay (South of France, NW Mediterranean Sea). Moreover monitoring the abundance of this toxic algae, our program also include measured of abiotic parameters such as temperature, salinity and nutrients.
Ostreopsis cf. *ovata* was associated to others species (Accoroni et al. 2016) and chemical interactions between those microalgae has been hypothesized (Ternon et al. 2018). In order to better understand the successions and the interactions between benthic species of microalgae, a monitoring of all the benthic microalgae started during summer 2018 at 3 sites (A,B,C) about 10 meters apart at 0.5 meters depth in the Rochambeau area. Microalgae were sampled on macroalgae substrates, each week from 27th June to 25th July (before, during and after *Ostreopsis* cf. *ovata* bloom).
During this period, 3 main conclusions came up: (a) high quantity of diatoms were found, even during *O.* cf. *ovata* bloom, (b) some diatoms growth with *O.* cf. *ovata*, while others are inhibited, and (c) the 3 sites (A,B and C), even if 10 meters apart, show important variation in the composition and the phenology of benthic microalgae.

Mots-Clés: Ostreopsis cf.ovata, diatoms, diversity, monitoring

*Intervenant

Innovative workflows towards more transparent and integrative metabolomics analysis

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During their life cycle, Alexandrium minutum and Scrippsiella donghaienis can produce resistant and revivable cysts that can accumulate in marine sediments for hundreds of years. Our working hypothesis assumes that these two genera have "recorded" their adaptation to ecosystem changes in these cells preserved in the form of cysts. From these revived cysts, toxic and non-toxic dinoflagellate cultures (Alexandrium minutum and Scrippsiella donghaienis) were analyzed by a metabolomic approach to investigate the possible adaptive responses of phytoplankton to these changes in coastal ecosystems. The strains were obtained from isotopically dated sediments, 1986 and 1996 for "ancient" and 2006 for "modern". In total, 84 samples were obtained. Each experimental condition (species combinations, age, and growth phase) was grown in triplicate. Extracts from different growth phases of the ancient and modern strains of Alexandrium minutum and Scrippsiella donghaienis were analyzed by LC-HRMS and compared via two workflows: a chemometrics platform, Agilent Mass Profiler Professional (MPP) and the collaborative portal "Workflow4Metabolomics". It is interesting to note that regardless of the workflows used (MPP or Galaxy), the metabolomic profiles of cultures reactivated at different ages were more different than those of different growth phases. While A. Minutum did not generally show the specific metabolites of the different stages but only common compounds, S. donghaienis contained metabolites specific for P deficiency. Molecular network analysis (MZmine2_GNPS_ Cytoscape_MetGem) provided a better understanding of these metabolites produced in phosphorus deficiency condition

Mots-Clés: Metabolomics workflows, Bioinfo Pipeline, Molecular Network, Dinoflagellates cyst, Harmful Algal Blooms (HAB), Paleoecology

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A fast and early-warning lateral flow test for the detection of cyclic imine toxins

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Cyclic imine toxins produced by marine dinoflagellates are antagonists of nicotinic acetylcholine receptors (nAChR). Shellfish accumulate these neurotoxins following filter feeding on toxic dinoflagellates vectoring them to humans. Herein is presented a lateral flow test for detecting agonists and antagonists of nAChRs. The test is based on the high affinity of neurotoxins for their receptor target and on the immobilization of *Torpedo*-electrocyte membranes on high porosity filter membranes as support for the lateral flow test " NeuroTorp ". We used biotin-α-Bungarotoxin as tracer and gold-neutravidin as toxin conjugate for colorimetric detection. Biotin-latex-antibodies were immobilized in the control band of the lateral flow test. In the absence of cyclic imine toxins, the complex [biotin-α-Bungarotoxin - gold-neutravidin] binds to the receptor; consequently, the test band and the control band show a colored precipitate. When a toxic sample is applied, the nicotinic toxin will displace the biotinylated toxin tracer, consequently, the test band will not be colored or its intensity will be lower than in the control run. Dose-dependent inhibition of biotin-α-Bungarotoxin binding to *Torpedo*-nAChRs by α-Bungarotoxin was performed to determine the affinity of the toxin. Quantification was performed using a CDD-based imaging reader (NG Biotech, France). The performance of the lateral flow test NeuroTorp was tested at different interval times after fabrication. NeuroTorp is stable for $>$ 30 days whether it is stored at 4∘C or at -20∘C. Finally NeuroTorp was used to detect cyclic imine toxins on shellfish extracts. The cyclic imine toxins present in these natural samples was quantified by UPLC-MS/MS. NeuroTorp is a ready-to-use low-cost point-of-care warning device for rapid and early detection of nicotinic neurotoxins in freshwater and marine environments by end-users. Acknowledgements. The authors acknowledge the funding support of the LABEX LERMIT to the prematuration project DETECNEUROTOX, and of Interreg/ Atlantic Area for ALERTOX-NET EAPA_317/2016 project.

Mots-Clés: Lateral Flow Test, Nicotinic acetylcholine receptors, cyclic imine toxins, anatoxin, a, HABs

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Looking for tetrodotoxins in France: analytical development and screening of mollusks (oysters and clams) and marine bacteria within the ALERTOX-Net project

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The ALERTOX-Net project (Interreg Atlantic Area 2017-2020) gathers 11 partners from 5 countries to develop an innovative toxicity alert system for safer seafood products (e.g. detection and alert methods for industries) focusing on emerging and non-regulated marine toxins in shellfish. The reports of tetrodotoxin (TTX) in mollusks from several European countries (e.g. Greece, England, the Netherlands, Italy) prompted us to develop a hydrophilic interaction liquid chromatography-tandem mass spectrometry (HILIC-MS/MS) method for the screening of bivalves and marine bacteria as, unlike other phycotoxins, TTX had been suggested to be of bacterial origin in marine organisms.
Among several HILIC columns (e.g. amide, zwitterionic phases) ZIC-HILIC gave the best sensitivity with suitable resolution while ultrafiltration with 3 kDa cut-off filters was used as an alternative to the classical SPE clean-up (using graphitized porous carbon cartridges). This HILIC-MS/MS method is highly sensitive and selective as it is capable of detecting 6 and quantifying 4 analogues of TTX (LOQ
Using data from the REPHY network and reported criteria for the presence of TTXs in England (*i.e.* temperature > 15 ∘C, inter-tidal or shallow water in estuarine areas), we sampled 4 sites during summer 2018 and detected TTX at three sites in North Brittany and Normandy (2-10

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μg/kg in clams and oysters) suggesting that a more comprehensive screening of TTX should be performed in France. For screening of bacteria, we selected strains previously isolated from the water column, from shellfish associated with mortality episodes and from healthy oysters; analysis of these strains is in progress.

Mots-Clés: Tetrodotoxin, shellfish, bacteria, HILIC, MS/MS

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