THEMATIC NETWORK e-Tox: *Toxic Blooms in the Anthropocene era*

BOOK OF ABSTRACTS

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ORAL COMMUNICATIONS

Student communication

Assessment of the cyanobacterial risk linked to the presence of *Pectinatella magnifica*

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Whilst the rise of heatwaves is driving more people to visit lakes, summer outbreaks of toxin-producing cyanobacterial blooms led to bathing restrictions. The Devesset (Ardèche) and Pareloup (Aveyron) lakes, are affected by these blooms and also invaded by *Pectinatella magnifica.* This invasive freshwater bryozoan forms colonies consisting in one layer of clusters-arranged zooids, attached to a bulky gelatinous matrix. The zooids produce very resistant statoblasts that remain dormant during winter and germinate in summer, generating new colonies. A rich microbiome, included harmful cyanobacteria is hosted by *P. magnifica.* Our aim is to investigate the risk for lake users, by quantifying toxins in water surrounding *Pectinatella's* spots and to assess the microbial community structure by 16S metagenomics coupled with new generation sequencing method.

During summer 2024, sampling campaigns were carried out at different invaded zones of both lakes. Microcystins and anatoxins Novakits ELISA assays were carried out in the surrounding water of colonies and microscopic observations of the bryozoan microbiome were made. *P. magnifica* biofilms and zooids content were harvested in order to constitute a DNA collection and to measure cyanotoxins by qPCR.

Except in one case, toxins were systematically detected in spots with *Pectinatella* but not in area without the bryozoan. In Pareloup lake, anatoxins exceeded the ARS quality limit, in two spots where a *Microcystis* bloom was present. *Pectinatella* microbiome seems not mirror cyanobacteria content in surrounding water because no Microcystis cells were present in *Pectinatella* but *Limnothrix sp.* and *Pseudanabaena sp.* When *Pectinatella* dies, cyanobacteria and zooids are released from the colony. Therefore, the risk could be maximal during the decaying period, in Automn. qPCR will be performed to quantify toxins and determine biofilm and zooid diversity.

Keywords: cyanobacteria, cyanotoxins, bryozoan, lake

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Harmful algal bloom research in Aotearoa New Zealand: From freshwater ecosystems to Pacific shores

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Harmful algal blooms (HABs) are increasing in frequency and duration across Aotearoa New Zealand, threatening drinking-water supplies, aquaculture, tourism, and the wellbeing of coastal and freshwater communities. This presentation will include an overview of the multidisciplinary HAB research we are currently undertaking at the Cawthron Institute -from toxic cyanobacterial blooms in lakes and rivers, and newly emerging marine benthic cyanobacterial mats, to toxin-producing dinoflagellates around the shores Aotearoa New Zealand and in the Pacific Islands, particularly the work on ciguatera poisoning. My main research focuses on the emerging HABs and marine toxins in New Zealand and their potential impacts on fish, shellfish and human health, mainly using molecular techniques such as metabarcoding, development on single species detection assays and transcriptomic analyses, which will be discussed during the presentation.

Keywords: benthic cyanobacteria, ciguatera, alexandrium pacificum, metabarcoding, transcriptomics

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*Speaker

Phytoplankton ecophysiology goes high-tech: automation to the rescue!

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The TIP is a new unique experimental prototype, located at the Ifremer site in Nantes and specifically developed for ecophysiological studies of phytoplankton. It consists of a battery of instrumented-photobioreactors, controlled for environmental forcing variables such as temperature, light, photoperiod, and pH.
This device allows for the consideration of single or multifactorial studies of various response variables for a phytoplankton strain. In the latter case, the experimental strategy is based on 23* factorial design where 3 factors are varied simultaneously to study their individual and interactive effects on the response variable. Whether it involves optimizing characteristics of the strain or describing its sensitivity to environmental forcings, the TIP offers accurately controled conditions and delivers a detailed characterization of a phytoplankton behavior.

The presentation will outline all the functionalities offered by this new experimental device. It will also address the types of studies that can be envisaged and the advantages and limitations of the TIP.

Keywords: ecophysiology, experimental device, photobioreactor

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Student communication

Adaptive Governance for sustainable fisheries: Insights from the Scallop fishery of the Eastern English Channel (France) faced with HAB and emerging challenges

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Harmful algal blooms (HAB) have been particularly intense in the Eastern English Channel over the past two decades. This phenomenon, characterized by its cyclical and unpredictable nature, represents a major driver for the dynamics in the fisheries sector, including economic, social, and governance-related changes.

As a key fisheries activity, the scallop fishing activity is strictly regulated and subject to specific exploitation conditions through binding management measures, especially in the classified deposit area of the Bay of Seine. This management framework includes a set of technical and access regulations aimed at preserving and sustainably managing this resource. Furthermore, following the emergence of HAB-related public health and socio-economic issues, a risk mitigation approach, based on continuous monitoring and commercial restrictions, has gradually been implemented through a series of regulatory reforms. Consequently, HAB have triggered significant challenges and changes in fishery governance and the operational strategies of fishermen.

The evolution of this social-ecological system requires particular attention to ensure its sustainability and resilience in the face of growing pressures, including those induced by HAB. These challenges extend beyond environmental concerns, as the sector is currently subject to a complex combination of cumulative effects within a continuously evolving context. Although scallop stocks in the Eastern English Channel remain generally in good condition and Amnesic Shellfish Poisoning (ASP) toxicities have not been reported since the 2012 crisis, fragmentation of governance continues to hinder the sector's ability to respond effectively to emerging challenges and uncertainties.

Based on an integrated spatial and temporal modelling, this research focuses on the sustainability and the effectiveness of the fishery, and explores decision-support mechanisms to foster a more adaptive, resilient, and collaborative governance approach, that meets the fishing community's needs.

Keywords: Scallop fishery, sustainability, adaptation, spatial management, governance, HAB, fishing bans, spatial modelling.

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Student communication

Seasonal dynamics of picocyanobacteria in two contrasting lakes in the Auvergne region

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Picocyanobacteria are defined as cyanobacteria with cells measuring less than 2 μm. These organisms have been observed in a variety of aquatic environments, including rivers, seas and lakes. They are known for their capacity to thrive in adverse conditions such as low light or low nutrient levels where larger cyanobacteria are unable to develop. Currently, the ecology of freshwater picocyanobacteria remains relatively unexplored. While data exist for the genera *Prochlorococcus* and *Synechococcus* which are mainly encountered in marine environments, freshwater genera such as *Aphanocapsa, Merismopedia* are poorly studied. However, we suspect them to be potential toxin producers, and they may be responsible for cases of bathing water closures that resulted in significant economic impacts. Consequently, the study of freshwater picocyanobacteria emerges as a pressing research priority. In this study, we followed the development of picocyanobacteria in two contrasting lakes in the Auvergne region. The Fades-Besserve lake is classified as a meso-eutrophic reservoir exhibiting a high diversity of cyanobacteria. Whereas Cournon d'Auvergne is a small eutrophic pond, composed predominantly by the genus *Raphidiopsis.* The development of cyanobacteria was monitored from spring to autumn by collecting an integrated sample from the 0-3 meters depth interval. Microscopic counts have yielded novel insights into the proliferation of picocyanobacteria. In the Cournon d'Auvergne pond, a proliferation of the genus *Cyanocatena* was documented for the first time. The question of its toxicity is raised, as the proliferation occurred during the bathing water period. In the Fades-Besserve lake, a divergent scenario occurred. Throughout the whole sampling period, seven genera of picocyanobacteria were identified in a consistent state. None exhibited marked predominance. Although microscopic counting remains the most widely used method, it is time-consuming, and other methods are gaining popularity. We also used flow cytometry, phycocyanin probe, and vertical Fluoroprobe profiling to give complementary information regarding picocyanobacteria proliferation.

Keywords: Cyanobacteria, Picoyanobacteria, Freshwater, Lakes, Ecological dynamics

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Deciphering *Microcystis* Phycosphere Metabolism through Multi Omics Integration and Genome Scale Modeling

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Cyanobacterial blooms pose escalating threats to ecosystems, public health, and economies. While environmental triggers of these harmful events are partially understood, the physiological and ecological mechanisms driving them remain unclear. Emerging evidence suggests that symbiotic cross-feeding interactions between cyanobacteria and their associated microbiome may enhance nutrient utilization within the phycosphere, potentially sustaining blooms. To investigate this, we employed a multi-omics approach combining metagenomics and metabolomics, enabling the reconstruction of genome-scale metabolic networks (GSMNs) to predict interactions and subsequent validation. This study focuses on 12 *Microcystis* strains and their heterotrophic bacterial consortia, sampled during a bloom in a eutrophic French lake. Metagenomic assembly identified six distinct *Microcystis* genotypes coexisting with a total 116 bacterial species. GSMN analysis revealed that despite variations in bacterial community composition across strains, core metabolic functions were conserved. Metabolism prediction from GSMNs highlighted divergent metabolite synthesis between isolated *Microcystis* strains and their native bacterial communities, suggesting metabolic complementarity. Chemical landscapes from metabolomics fitted

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well with genotype and/or GSMN predictions. Notably, certain bioactive peptides (e.g. microcystins) were strain-specific, underscoring metabolic heterogeneity. Nevertheless, only a subset of predicted metabolites from GSMNs were actually detected by metabolomics. This limited overlap between predicted and observed metabolites highlights challenges in cross-validating omics datasets, emphasizing the need for enhanced metabolite annotation pipelines. Ongoing work aims to refine these pipelines to bridge gaps between genomic predictions and metabolomic evidence. Collectively, our findings underscore the utility of integrating multi-omics and computational modeling to unravel functional synergies between cyanobacteria and their microbiome, which may drive bloom persistence. This approach advances our mechanistic understanding of microbial ecology in bloom dynamics, offering insights for mitigation strategies in aquatic ecosystems.

Keywords: microcystis, metabolism, cross, feeding interaction, metagenomics, metametabolomics, phycosphere

"MOustaCHe" : A novel blooming heterokont discovered in South Brittany in summer 2024

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"MOustaCHe" : A novel blooming heterokont discovered in South Brittany in summer 2024

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In August 2024, a significant brown-discoloured of the water was observed in Concarneau bay, caused by a bloom of an unknown species of flagellate. This event coincided with a massive mortality of conger eel, with over a thousand stranded individuals counted along the Concarneau beaches. Preliminary morphological observations under light microscopy revealed biflagellate cells of 15 μm in size, containing numerous golden green chloroplasts, and exhibiting a "mustache-like" shape. This typical heterokont displayed some resembance to species such as *Heterosigma* (Raphidophyceae) or *Pseudochattonella* (Dictyochophyceae). However, molecular analysis revealed that its LSU and SSU ribosomal DNA sequences did not match any described organism in GenBank but it was closely related to sequences of uncultured eukaryote clones obtained from environmental data. Phylogenetic analysis inferred from SSU rDNA suggested that this organism belongs to the group of Marine Ochrophyte 2 (MOCH-2), closely related to Pelagophyceae and Dichtyocophyceae, but clearly distinct. Therefore, this unknown organism represents a high-taxonomic level (new Class) previously known only from environmental sequence data.

Our study provides a valuable opportunity to characterize morphological, physiological and chemical features of this unknown organism.
Strains of MOCH-2, isolated during the bloom, enabled detailed morphological characterization using both scanning and transmission electronic microscopy. Additional analyses were undertaken to investigate the potential toxicity of this novel algal blooming species in order to assess its environmental risk.

Keywords: bloom, marine ochrophyte, taxonomy, environmental DNA, microscopy, conger eel mortality

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*Speaker

Student communication

Uncovering the cytotoxicity of cyanopeptides from *Microcystis* extracts on two fish cell lines

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Cyanobacteria produce toxins harmful to animals and humans, causing both acute and chronic health effects. Microcystins (MCs), a class of cyanopeptides mainly produced by *Microcystis*, are involved in poisonings of humans and wildlife. Consequently, many countries have established regulatory limits for MCs in drinking and recreational waters. Beyond MCs, other cyanopeptides (*e.g.* aeruginosins, microginins, microcyclamides) produced by many cyanobacterial genera have demonstrated enzyme inhibition, cytotoxicity, sublethal and lethal effects. However, the toxicity of these non-MC cyanopeptides remains incompletely determined, and no regulations currently address them.

This study characterizes the toxicity of complex metabolic extracts from 18 *Microcystis* strains producing various cyanopeptide families, with (MC+) or without (MC-) MCs. Acute cytotoxicity bioassays were conducted using two fish cell lines - carp leukocyte culture (CLC) and rainbow trout liver Waterloo 1 (RTL-W1). Cell mortality and concentration were assessed via flow cytometry after 24 h and 48 h. A Weight-of-Evidence (WoE) approach was applied to identify the most toxic extracts, first calculating the percentage variation relative to methanol control (reference), then determining a toxicity index normalized between reference and 3,4-dichloroaniline, a highly toxic industrial compound recommended by the OECD as a positive control.

Results showed mild to moderate cytotoxicity, with mortality ranging from 5% to 46% compared to 89% mortality in the positive control. Effects generally intensified at 48h for both MC+ and MC- extracts. Some extracts induced direct cell mortality, while others primarily affected cell concentration without causing cell death, suggesting cytostatic effects. The WoE approach identified several extracts causing significant effects on both cell lines at 48 hours, including PMC 823.12 (MC-), PMC 831.12 (MC+), and PMC 815.12 (MC+).

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Particularly noteworthy is PMC 823.12, a MC- strain producing microcyclamides and microginins that demonstrated substantial toxicity. This highlights the need for further investigation into the toxicity mechanisms of these understudied compound families.

Keywords: cellular bioassays, cytotoxicity, cyanotoxin, Microcystis, weight of evidence

Stratégie internationale et besoin de concertation française pour une évaluation, gestion et réglementation des microalgues et toxines marines en soutien des secteurs aquacole, de la pêche et du tourisme

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Les microalgues marines et leurs toxines ont des effets sur les organismes marins et l'Homme, soit par exposition directe soit par les empoisonnements subits suite à la consommation de poissons et fruits de mer contaminés par les toxines algales. Pour remédier à ces risques, la plupart des pays côtiers ont mis en place une surveillance et gestion des zones côtières. A l'échelle internationale, un *Groupe Intergouvernemental sur les Microalgues Toxiques et Nuisibles* a été créé en 1992 au sein de la Commission Océanographique Intergouvernementale (UNESCO-IOC-IPHAB). Depuis 2024, ce groupe est également formellement soutenu par la *Food & Agriculture Organisation* (FAO).

Cette présentation expliquera le fonctionnement de l'IPHAB, le progrès des sous-groupes thématiques (taxinomie, toxines, systèmes d'information et communication, systèmes d'alerte rapide, microalgues benthiques/ciguatéra et ichthyotoxiques, dessalement de l'eau de mer) et régionales (Pacifique de l'Ouest, Caraïbes, Amérique du Sud et Afrique) ainsi que les dernières décisions mises en avant lors de la réunion du 18-20 mars 2025, avec un focus sur celles pertinentes pour la communauté scientifique et les acteurs socio-économiques français.

Les ambitions du nouveau programme décennal GlobalHAB en tant que coopération entre la COI et le Comité Scientifique de la Recherche Océanique (SCOR) seront explicitées autant que les défis pour l'action de la décennie HAB-Solutions, récemment labélisé par les Nations Unies.

Keywords: Coconstruction de services écosystémiques et climatiques, menaces et opportunités liées aux HABs, coopérations internationale et française

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Characterization of marine microalgae toxicity to different organisms using in vitro bioassays

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Toxic microalgal blooms can impact marine organisms, but their effects and underlying toxic mechanisms remain poorly understood. On the French coasts, several species detected through REPHY monitoring network or citizen-science Phenomer program may threaten aquaculture and fishing activities. Ifremer LER-BO maintains _˜100 dinoflagellate strains isolated from the French coast. Among them, some genera/species are suspected to alter marine life, either through allelochemicals excreted into the surrounding environment, or intracellular toxins released after cell lysis.

In recent studies, we focused on characterizing French strain toxicity to marine organisms, using a range of *in vitro* bioassays targeting fish, shellfish and phytoplankton models.

Toxicity to fish was assessed using a fish gill cell line (RTgill-W1) as target, since gills are usually the most affected organ during ichthyotoxic blooms, leading to death by asphyxia.

Reprotoxicity to bivalves was assessed through exposure of microalgae or their compounds to oyster gametes, using viability and fertilization rates as endpoints.

Hemolytic potential of microalgal compounds was determined using finfish or mammalian erythrocytes, and bivalve hemocytes, as target cells.

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*Speaker

Allelopathic effects, defined here as toxic effects to other phytoplankton species, were assessed using a strain of the ubiquitous diatom *Chaetoceros* sp. as a target.

Using those bioassays, toxic compounds production was evidenced in several strains of dinoflagellates from the French coast, including *Alexandrium*, *Karlodinium*, *Karenia* and *Ostreopsis* species. While toxicity differed among species, high intra-specific variability was also highlighted.

Toxicity to fish and shellfish models indicated that presence of these dinoflagellate species along the French coasts may threaten aquaculture activity, while their allelopathic activity possibly disrupt phytoplankton communities. This work points towards future work focusing on characterizing the toxins responsible for these effects as well as deciphering their mode of action. Finally, the role of this toxicity in the often mixotrophic behavior of dinoflagellates remains to be further studied.

Keywords: Ichthyotoxicity, Allelopathy, Fish, Shellfish, Aquaculture

Student communication

Physiological adaptations of Dziani Dzaha's *Limnospira*

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It is well known that cyanobacteria can colonize different ecosystems all over the world, such as oceans, lakes, rock surfaces, soils, ... and one hypothesis for their high ability to colonize and adapt in different ecosystems and niches could be the microdiversity within a population (Leboulanger and al., 2017).

To study the cyanobacterial microdiversity, we used Lake Dziani Dzaha in Mayotte, a thalassohaline lake, where only microorganisms have been reported and where *Limnospira platensis*, a filamentous cyanobacterium, is the dominant primary producer (Cadeau and al., 2017). This genus is described as a cosmopolite, occurring in several habitats around the world (Cellamare and al., 2018) and in Dziani Dzaha, *Limnospira* colonize all ecological niches (i.e. photic or aphotic, oxic or anoxic zones of the water column, biofilms, stromatolites). Since 2010, the lake has been stable, with several seasonal clines (Bernard and al., 2019). However, an earthquake in 2018 has profoundly altered the overall dynamics of the lake, the entire water column is now mixed and water intrusion affects pre-existing habitats. In Dziani Dzaha, the scientific consortium has already described several *Limnospira* genotypes with unknown phenotypic capabilities (Roussel and al., 2024). In this context, the Master 2 project aims to learn more about the links between the origin of *Limnospira* isolates (water column or stromatolites) and their ability to adapt to laboratory conditions: light quality and quantity, salinity.

The scientific project is organized as follow :

- Creation of a database on MNHN Dziani Dzaha's Limnospira strains (n = 120);

- Characterisation of strain phenotypes, from different niches and physico-chemical conditions : growth rate, total biomass, photosynthetic pigment concentrations and overall metabolome analysis;

Comparison for each laboratory condition will be done between strains.

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[*] Speaker

With this project we hope to deepen the scientific knowledge on adaptativity of the main primary producer of Dziani Dzaha: *Limnospira*.

Keywords: Physiology, Adaptation, Ecological niches, Limnospira platensis, Cyanobacteria, Salinity, Light

Influence des proliférations de cyanobactéries et des métabolites bioactifs associés sur le microbiote intestinal des poissons

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Cyanobacterial blooms are becoming increasingly frequent and intense in freshwater ecosystems due to climate change and nutrient enrichment. These blooms produce a wide range of secondary metabolites, including toxins, with known direct effects on aquatic organisms. However, the potential for these compounds to indirectly affect the organism physiology via perturbing its intestinal microbiota remains poorly understood. The intestinal microbiota plays a fundamental role in fish health, contributing to digestion, immune defence, and regulation of the stress response processes. This PhD project aims to investigate how exposure to cyanobacterial metabolites alters the gut microbiota of freshwater fish, and how these taxonomic and/or functional microbial changes may, in reversely, impact the ability of the host to respond to environmental stress. Using both *in vitro* and *in vivo* approaches, including both gnotobiotic and xenic zebrafish (*Danio rerio*) and xenic medaka (*Oryzias latipes*), the project will explore the sensitivity of gut-associated bacteria to several cyanobacterial strains and whether these different cyanobacterial exposures disrupt the protective role of the microbiota against potential pathogenic infection. It will also explore how cyanobacterial morphology and secondary metabolite composition may influence feeding behavior, toxin transfer and microbial community structure within the host gut. By integrating microbiology, ecotoxicology, and host–microbiota interactions, this research aims at better understanding the complex effects of cyanobacterial blooms on fish holobionts and highlights the intestinal microbiota as a potential participant of host vulnerability.

Keywords: Ecotoxicology, Cyanobacteria, Cyanotoxins, Gut microbiota, Fish

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[*] Speaker

Student communication

Microdiversity, the driving force behind *Limnospira* (cyanobacterium) adaptation in Dziani Dzaha (lake, Mayotte)

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Lake Dziani Dzaha in Mayotte is characterized as an extreme environment by its steep physico-chemical gradients. The biomass of the lake is exclusively microbial, dominated by the cyanobacteria *Limnospira platensis* (commonly known as spirulina). Several niches can be found in this lake: stromatolites, biofilms, a water column with light penetration in the first meter (photic zone) and then aphotic below, except for a few wavelengths in the dark red. All these niches are colonized by *Limnospira platensis* and previous study (Roussel et al., 2023) showed that they are genetically similar (99.9% similarity for the gene encoding 16S rRNA). However, the sequenced genomes reveal significant variation in gene contents between strains, known as microdiversity. Microdiversity has mainly been studied at a large scale, in models of small marine cyanobacteria such as *Prochlorococcus* or *Synechococcus* (Biller et al., 2014, Bhaya et al., 2007) with research focusing on the role of microdiversity in adaptation to ecological niches at different latitudes or at very different depths in the marine water column. We do not yet know whether this concept applies to a filamentous cyanobacterium at the very small scale of a lake.

The project therefore aims to determine whether microdiversity is a driving force behind the adaptation of *Limnospira* from Dziani Dzaha to different ecological niches. Three axes will be investigated: comparative genomics - attempting to associate genotypes and ecological niches, phenotypic analysis - cultural approach to attempt to associate phenotypes and ecological niches, and finally transcriptomics - attempting to link genotypes to phenotypes and thus microdiversity to niche partitioning.

Keywords: Microdiversity, Genotype, Phenotype, Adaptation, Ecological niches

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Evaluation de l'impact des nouvelles préconisations de gestion des proliférations de cyanobactéries sur les usages des plans d'eau de la région Grand-Est

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Les proliférations de cyanobactéries toxinogènes dans les plans d'eau constituent un enjeu sanitaire, environnemental et sociétal. En effet, en raison des capacités de nombreux genres de cyanobactéries à produire des toxines, les populations humaines et animales fréquentant ces écosystèmes peuvent être exposées à ces toxines. Pour réduire ce risque d'exposition, la DGS a proposé en 2003 des premières recommandations pour la surveillance des cyanobactéries et pour la gestion des risques associés pour les humains. Suite aux travaux de l'Anses publiés en 2020, la DGS a révisé ses recommandations en 2021, en prenant en compte, dans les protocoles de surveillance sanitaires, de nouvelles cyanotoxines et en modifiant les critères et les seuils définissant des niveaux d'alerte.

L'étude présentée s'inscrit dans le cadre du projet NoCyano financé par l'Agence de l'Eau Rhin-Meuse. Nous avons analysé les données de contrôles sanitaires des sites de baignade naturelle acquises dans sept départements, pendant 14 années (2010-2023), par l'ARS Grand Est. Les objectifs étaient à la fois de rechercher combien de ces sites étaient touchés par des proliférations de cyanobactéries, de déterminer quels étaient les genres présents et leur potentiel toxinogène, et d'évaluer l'impact des modifications des préconisations sanitaires introduites en 2021 par la DGS sur la baignade dans les plans d'eau.

Nos analyses ont révélé que près de la moitié des sites de baignade de la région Grand Est ont été confrontés à des proliférations de cyanobactéries toxinogènes au moins une fois entre 2010 et 2023. Les principaux genres dominants sont *Aphanizomenon*, *Dolichospermum* et *Microcystis* et les principales toxines détectées les microcystines et/ou les anatoxines. La nouvelle instruction de 2021 de la DGS conduit à une augmentation du nombre de recommandations de fermeture de baignades, notamment en raison de la présence d'anatoxine-a, toxine qui n'était généralement pas recherchée avant les nouvelles directives de la DGS.

Keywords: cyanobactéries, cyanotoxines, évaluation des risques, région Grand Est

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*Speaker

Toxicity of different Preparations of *Alexandrium minutum* culture (intracellular, extracellular and membrane debris) on in vitro aquatic organism models (bivalve, diatoms, and fish)

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Alexandrium minutum can have detrimental effects on marine biota by producing Paralytic Shellfish Toxins (PSTs) and Bioactive Extracellular Compounds (BECs). These toxins can affect a wide range of taxa, including microalgae, crustaceans, and fish, and have been reported to impact bivalves at different life stages. However, the toxic mechanisms of *A. minutum* remain unclear and may depend on the strain, the toxins produced, and the target organism. This study assesses the in vitro toxicity of different culture fractions from *A. minutum* against various biological models.
Three strains were selected based on their previously reported toxicity: Da1257 (producing PSTs but no BECs), AM89BM (producing both PSTs and BECs), and CCMI1002 (secreting BECs only). Cultures were divided into three fractions: supernatant (extracellular toxins), intracellular content from lysed cells, and cell membrane debris. Bioassays were conducted to evaluate the toxicity of each fraction on a diatom (*Chaetoceros* sp.), a fish gill cell line (RTgill-W1, from rainbow trout), and gametes of the Pacific oyster (*Crassostrea gigas*).

All strains showed toxicity to the tested models, especially the "PST + BEC" and "BEC only" strains, even at environmentally relevant bloom concentrations. For these strains, toxicity followed the pattern: intracellular $>$ extracellular $>>$ membrane debris. Specifically, the ex-

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*Speaker

tracellular fraction of the ”PST only” strain was not toxic; *Chaetoceros* sp. appeared sensitive to both PSTs and BECs; oyster fertilization was highly affected by extracellular fractions; and fish gill cells were moderately impacted by all strains. BECs may be produced intracellularly, contributing to overall toxicity.

These findings suggest that BECs may exert stronger adverse effects than PSTs during bloom events. *A. minutum* toxins appear to act through multiple mechanisms depending on the target, potentially affecting co-occurring organisms in complex ways.

Keywords: Allelopathy, BECs, PSTs, Reprotoxicity, Viability

Cyanobacterial bloom dynamics in lake Léon: a multi-seasonal modeling approach

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Cyanobacterial blooms are known to cause significant ecological and public health concerns by altering the physico-chemical conditions of aquatic environments and releasing harmful toxins. This issue has recently been observed in lake Léon (Landes, France), where the proliferation of cyanobacteria has become a major issue. Various chemical (e.g., nutrient concentrations), physical (e.g., water level), and meteorological factors are known to favor the development of cyanobacterial blooms. The aim of this study is to test the relative importance of these factors on the development of cyanobacteria biovolume in the lake Léon. To this end, the relevant variables have been compiled from 2008 onwards and incorporated into a series of linear models. These models were constructed both across the entire study period and at a seasonal scale to identify the specific conditions that contribute to the biovolume variability over time. Physico-chemical factors-particularly nutrient levels such as phosphorus-show strong correlations with cyanobacterial biovolumes. Meteorological conditions also play a key role, although the timing and intensity of their effects vary by parameter and season. Among these, temperature emerges as the most influential variable. Additionally, water level is significantly associated with biovolume during the summer months. Seasonal persistence was also observed, with biovolumes depending on those of the preceding seasons. Consequently, the occurrence of blooms appears to result from the interplay of these multiple factors. These findings align with existing literature and offer new insights into cyanobacterial proliferation in the specific context of lake Léon. Nevertheless, expanding the dataset-both in terms of duration and resolution-would improve model reliability and support the development of predictive tools for managing future public health risks.

Keywords: cyanobacteria, Léon, lake, phosphorus, bloom, toxins, nutrients, temperature, biovolume, water level, cyanobacterial bloom, ecology, linear model, meteorology, model, season

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[*] Speaker

Photo-acclimation and photo-adaptation in *Microcystis*

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The evolution of populations within aquatic ecosystems is closely linked to the interactions between these organisms and the biotic and abiotic conditions of the environment. Factors such as nutrient concentration and the quantity and quality of available light vary spatially, generating micro-niches where organisms modify their physiology to optimise their growth. Among the factors influencing the structure of phytoplankton communities, light plays a fundamental role. In photoautotrophs such as cyanobacteria, light triggers diverse ecophysiological responses, enabling them to maintain stable metabolic performance in a fluctuating light environment. As a result, cyanobacteria have developed various adaptation and acclimation mechanisms to different light conditions. Among these, the quantity and regulation of their pigments, such as phycocyanin, play a key role in optimising the use of incident light. However, the role of photobiological acclimation processes in the structuring of communities and the proliferation of phytoplankton populations in fresh water remains poorly described in the literature. Among these cyanobacteria, the genus *Microcystis*, known for its toxicity, is extremely involved in massive proliferation events (blooms), significantly disrupting the functioning of aquatic ecosystems. A detailed study of the impact of light on this genus, based on complementary physiological approaches, opens up new prospects for identifying ecotypes based on the characteristics of their photoresponses.

Keywords: Cyanobactéries, lumière, adaptation, acclimatation

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[*] Speaker

Student communication

Gene expression analysis of a synthetic community associated with the ichtyotoxic *Prymnesium parvum* in response to different nutrient limitations

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Prymnesium parvum is a haptophyte microalgae capable of synthesizing ichthyotoxins under nutrient limitations. This toxin production can significantly impact biodiversity and thus, the coastal economies reliant on fishing, aquaculture and tourism. Interactions between *P. parvum* and the associated bacterial community play a key role in shaping the algal growth and physiology. To explore the underlying mechanisms of these mutualistic interactions, co-cultures of *P. parvum* and a synthetic bacterial community were performed under various nutrient-limited conditions in photobioreactors.

This study focuses on the gene expression analysis of the synthetic community associated with *Prymnesium parvum.* To investigate bacterial responses under different conditions, RNA-seq analysis was performed using RNA extracted on day 14 of co-cultures. Differential gene expression analysis revealed significant differences in bacterial activity across conditions and among bacterial species, highlighting their distinct roles and contributions.

These findings provide novel insights for future experiments to better understand the expression of the metabolic pathways involved. Understanding the interactions between P. parvum, bacteria and the environment contributes to a more comprehensive view of the ecological impacts of P. parvum blooms.

Keywords: Prymnesium parvum, haptophyte microalgae, ichthyotoxins, nutrient limitation, harmful algal blooms, algae, bacteria interactions, synthetic bacterial community, mutualistic interactions, gene expression analysis, RNA, seq, bacterial response

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*Speaker

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Phosphorus starvation activates allelopathic activity in the dinoflagellate *Karlodinium*

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Toxic microalgae can release toxins that damage or kill other cells, a chemical interaction known as allelopathy. This can give a competitive edge to toxic phytoplankton over their competitors, more specifically in a context of limited resources. However, toxin production seems to bear a significant energetic cost, and is favoured only when most beneficial to the producer cell.

We designed a series of experiments to investigate the allelopathic activity of 3 strains of the ichthyotoxic dinoflagellate *Karlodinium.* We cultured them with a target organism (the cryptophyte *Teleaulax amphioxeia*) that served as competitor and prey. We used flow cytometry to observe the effect of *Karlodinium* on the cryptophyte algae, in nutrient-replete conditions (i.e., exponential growth) and under phosphorus starvation.

For 2 *Karlodinium* strains, while cryptophytes displayed no symptoms of toxicity when cultivated with dinoflagellates grown in nutrient-replete conditions, the contact with phosphorus-starved *Karlodinium* cells or their supernatant caused a sudden and dramatic increase in the fluorescence of photosynthetic pigments, followed by a gradual decrease and eventually death of *T. amphioxeia* cells. For the third Karlodinium strain , no toxic effects on the cryptophytes were observed in either condition.

Our results show that a potent allelopathic activity in toxic *Karlodinium* strains can be activated by phosphorus starvation. From these results and on the basis of further analysis of transcriptomics and toxin data, we propose to discuss the molecular mechanisms at play, as well as the ecological drivers that favour the selection of allelopathy in planktonic dinoflagellates.

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*Speaker

Keywords: Allelopathy, Karlodinium, Dinoflagellates, Phosphorus

Student communication

Understanding the causes of Cyanobacteria proliferations and their impacts on local activities and aquatic wildlife on Lake Bairon, France

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Cyanobacterial blooms increasingly occur due to soils and water bodies anthropisation and in addition to climate change, especially with intensification of heatwave and extreme hydrological events. Several French regions are impacted by these blooms, including regions with continental climate, such as Champagne-Ardenne region in North-East France. This region was affected by the post-World War II agricultural raise and by land consolidation, including fertilizers and pesticides high uses. The Ardennes is known for its variety of landscapes and keeps count of two lakes where summer recreates activities are organised. The Bairon lake is one of the two Ardennes lakes frequented by about 200,000 people by year for bathing, hiking, fishing, or sail activities, with about 40,000 swimmers. The Bairon lake is commonly touched by cyanobacterial blooms during summer periods, which caused swimming closure. Many studies have been conducted to understand Bairon lake's eutrophication, in demand to local authorities, including stream and lake water bodies analysis and cyanobacteria monitoring.

The project aims to investigate the causes of cyanobacteria proliferations, and to assess these blooms consequences on aquatic communities and human activities. The first step is to characterize nitrogen and phosphorus entrances by water quality analyses in all of drainage basin of Bairon lake through preliminary highlight of the main run-off spots thank to geographic information system (GIS). Secondly, a collaborative work will be begun on summer to evaluate potential fishing contribution of baiting with fishermen supply. Phytoplanktonic communities, cyanobacterial biovolume and cyanotoxins will be monitored from April to August. Bioaccumulation in the biota will be assessed, principally macro invertebrates and fish consumed by humans, to identify potential bioaccumulator or bioindicator species.

An initial analysis of historical cyanobacterial monitoring data from Lake Bairon revealed a dominance of the diazotroph genus *Aphanizomenom* and *Dolichospermum*.

Keywords: Eutrophication, Toxigenic cyanobacteria, Cyanotoxins bioaccumulation, aquatic ecosystem

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[*] Speaker

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The development and challenges of developing a qPCR assay for the detection of anatoxin and guanitoxin producing cyanobacteria in environmental samples

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Timely and accurate identification and reporting of Cyanobacteria is critical for the management of toxin producing events. The current Phytoxigene Quantitative PCR (qPCR) assay has been widely used to screen for the potential risk of the toxins Microcystin/Nodularin, Cylindrospermopsin and Saxitoxin, plus total cyanobacteria by the 16SrRNA gene since 2016. Studies have previously demonstrated that there is a very high correlation between toxin gene detection and toxin production. The results from these studies indicate that this method can predict HAB risk and simplify the process of toxin analysis. Anatoxin- is a secondary, bicyclic amine alkaloid and cyanotoxin with acute neurotoxicity and can cause death in all mammals, with dog deaths attracting the most attention. The toxin is produced by multiple cyano-bacteria genera throughout the world, most commonly by benthic organisms including *Microcoleus*, *Dolichospermum*, *Oscillatoria* and *Aphanizomenon*. Guanitoxin, formerly known as Anatoxin-a(s), is a neurotoxin most commonly produced by the benthic cyanobacteria. A lesser understood toxin as it is more labile, genetic screening for a gene responsible for its's production will provide a better understanding of its scope and subsequent risk. Phytoxigene has developed a multiplex qPCR assay that will detect the presence of an Anatoxin and Guanitoxin gene required for Anatoxin and Guanitoxin synthesis. The presentation will highlight the development process and challenges related to the design of this assay

Keywords: qPCR Monitoring anatoxin guanitoxin

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[*] Speaker

Student communication

Etat des lieux des cyanobactéries benthiques dans les plans d'eau de France métropolitaine

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Although less studied than their planktonic counterparts, benthic cyanobacteria in water bodies may represent a major health and ecological issue due to their ability to produce a broad spectrum of cyanotoxins. Apart from a few descriptive studies, often triggered by cases of animal poisoning, their spatial and temporal dynamics, as well as their taxonomic diversity, remain largely unknown in French freshwater ecosystems.

In recent years, their presence has been mentioned with increasing frequency in surveys carried out to monitor the ecological status of water bodies, raising a central question: are we really witnessing an increase in their occurrence - under the effect of global change and anthropogenic pressures - or is this an old phenomenon that has simply been neglected until now?

Against this backdrop, this study offers an initial overview of benthic cyanobacteria in lakes in mainland France. It is based on the analysis of data collected between 2006 and 2024, as part of the macrophyte sampling protocols used to assess the ecological status of lakes, as required by the Water Framework Directive. As colonies of benthic cyanobacteria are visible to the naked eye, they are counted during these campaigns and then identified in the laboratory.

The aim of this work is to (1) characterize the geographical and temporal distribution of these organisms, (2) identify taxa which may produce cyanotoxin, (3) locate areas of high abundance, and (4) initiate the identification of environmental factors influencing the abundance of benthic cyanobacteria.

The initial results, presented here in the form of maps and graphical analyses, have already enabled us to identify trends and gain a better understanding of the environments concerned. These initial findings show the presence of benthic cyanobacteria able to emit toxins in approximately 2/3rd of studied lakes.

Keywords: Dynamiques spatiales et temporelles, cartographie, plan d'eau, cyanobactérie benthique

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[*] Speaker

POSTERS

Première observation d'une efflorescence de *Pyrodinium bahamense* dans le golfe d'Aden, Djibouti : caractérisation morpho-moléculaire et abondance spatio-temporelle

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À la fin de l'été 2023, en septembre, une intense décoloration de l'eau (marée rouge) accompagnée d'une mortalité de poissons a été observée dans le golfe de Tadjourah. Cette décoloration du milieu marin était causée par un dinoflagellé potentiellement toxique, *Pyrodinium bahamense*. La prolifération, localisée près des côtes entre les îles Moucha/Maskali et la ville de Tadjourah, était d'une ampleur suffisante pour être détectée par imagerie satellitaire. *P. bahamense* a formé une efflorescence presque monospécifique (90 %), avec une densité de 1,8 × 10^6 cellules l-^{1} (22 septembre 2023). Pour la première fois, nous avons identifié moléculairement le ribotype toxique de l'Indo-Pacifique dans la région du golfe d'Aden. Cette découverte met en évidence un risque émergent associé à ce ribotype toxique dans le bassin oriental de la Méditerranée. Cette région, déjà confrontée à divers défis environnementaux, pourrait voir sa vulnérabilité accrue avec l'apparition de nouveaux risques. DOI : https://doi.org/10.1080/09670262.2024.2447871.

Keywords: Djibouti, Gulf of Tadjoura, Harmful Algal Blooms (HABs), microalgae, phytoplankton, red tide

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*Speaker

Identifying cholinergic ligands from the marine *Oscillatoria cf. bonnemaisonii*

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Anthropogenic impact on the reef ecosystems in Raivavae Island (French Polynesia) triggered the proliferation of benthic marine *Oscillatoria* that was shown to be associated with human health problems through consumption of "bénitiers" giant clams of the genus *Tridacna* from affected areas. Methanolic extracts of *Oscillatoria* cf. *bonnemaisonii*, which were collected from the reef areas of Raivavae Island, blocked nerve-evoked muscle twitch and synaptic transmission in neuromuscular preparations. The blockade of the amplitude of miniature-endplate potentials suggested the muscle nicotinic acetylcholine receptor (nAChR) as a potential target. Microplate receptor-binding assay, using Torpedo marmorata electrocyte membranes rich in nAChR of muscle type and biotinylated α-bungarotoxin as toxin tracer, was used for the bio-guided purification of the marine cyanobacterial nicotinic ligands by preparative HPLC. High resolution mass spectrometry (HRMS) analyses identified the analytes in the active fraction composed of structure-related lipophilic compounds with overlapping elution times. Two-electrodes voltage clamp electrophysiology recordings on Xenopus laevis oocytes expressing nAChR of mussel type confirmed the blocking activity of these molecules. Marine cyanobacteria are a valuable source of novel bioactive molecules

Keywords: Marine cyanobacteria Oscillatoria, nicotinic acetylcholine receptor (nAChR), bioactivity based molecular networking, toxin discovery, two, electrodes voltage clamp

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*Speaker

Combined In Vitro Toxicity of Co-occurring Harmful Algal Bloom Species *Alexandrium minutum* and Dinophysis spp. on Pacific Oyster Gametes

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Harmful algal blooms (HABs) frequently overlap with the spawning season of bivalves such as the Pacific oyster *Crassostrea gigas*, an important aquaculture species, potentially affecting their reproductive success. The co-occurrence of multiple HAB species, as reported globally, may further amplify the negative effects of toxic blooms on bivalve reproduction, posing risks to population sustainability. Although the individual impacts of certain toxic microalgae on oyster physiology are well documented, little is known about the combined effects of co-occurring HAB species. To address this gap, this study investigated the *in vitro* effects of co-occurring HAB species on oyster gamete toxicity, focusing on whether interactions are additive, synergistic, or antagonistic. Co-occurring HAB species were identified using data from the French phytoplankton monitoring network (REPHY). A flow cytometry-based fluorescent bioassay assessed gamete toxicity and reactive oxygen species (ROS) production following exposure to individual and binary mixtures of HAB species at sub-lethal concentrations.

Keywords: Harmful algal blooms (HABs), Crassostrea gigas, Gamete toxicity, Co occurring HAB species, Combined effects

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Insights into the in vitro ichthyotoxicity on fish gill cells and toxin production of *Karlodinium veneficum* (Dinophyceae) strains from French waters

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Strains of the dinoflagellate *Karlodinium veneficum* are frequently detected in French coastal waters, but their ichthyotoxicity and potential to produce karlotoxins (KmTxs) remain unclear. To investigate this, four French strains (DIA2C2, IFR-CC-20-44, MAR1F7, CBC7) and one American strain (CCMP 2936, previously characterized and used here as a reference) were evaluated. Both intra- and extracellular methanolic extracts (INT, EXT) were assessed for their biological activity using a resaruzin-based assay, lactate dehydrogenase (LDH) release, mitochondrial membrane potential (TMRE dye), and liquid chromatography coupled to mass spectrometry to detect KmTxs. Cytotoxic activity differed by strain and fraction, with IFR-CC-20-44 EXT and CCMP 2936 INT showing the highest toxicity, while CBC7 showed no toxicity. The French isolates generally showed distinct cytotoxic mechanisms compared to the American strain. The KmTx profiles of the toxic strains displayed an unexpectedly high chemodiversity with 93 KmTx-like molecules detected. Three of the French strains produced similar KmTx profiles dominated by KmTx-5 and and a KmTx-5 analogue. In contrast, the American strain CCMP 2936 produced a completely unique KmTx composition, with no overlapping KmTxs found in the French strains. Notably, CBC7 did not produce any KmTxs and was non-toxic. However, among the other *K. veneficum* strains, no clear link was found between the cytotoxicity of their extracts and total KmTx content, suggesting that individual KmTx analogue may vary in potency, as well as pointing to a possible contribution from other uncharacterized bioactive compounds.

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Keywords: Cytotoxicity, K. veneficum strains, karlotoxins, RTgill W1 cells, France

Detection and quantification of cyanotoxins in the mucilage of natural *Microcystis* colonies

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During blooms of colonial cyanobacteria such as *Microcystis*, microcystins (MCs) can be intracellular, extracellular (dissolved in the water or adsorbed to environmental particles) or trapped within the *Microcystis* mucilage, an extracellular polymeric substance (EPS) made of polysaccharides, nucleic acids, phospholipids and proteins. This study investigated the detection and quantification of MCs within the mucilage of a Microcystis-dominated bloom, employing methods to separate and quantify attached EPS from cyanobacterial cells. The lowest MCs concentration was measured on the filter by classical filtration (112.44 ± 4.94 μg L-1) and methanol extraction, representing free MCs (intracellular and associated with EPS of the mucilage). Centrifugation followed by lyophilization of the pellets significantly elevated particulate MCs concentrations (153.98 ± 15.65 μg L-1), likely due to the inclusion of extracellular MCs adsorbed onto particles. However, fixation and extended solubilization (10 to 60 min) by heating further increased particulate MCs concentrations, yielding values 1.6 to 1.9 times higher than those obtained via classical filtration, depending on the solubilization duration. It also demonstrated a significant contribution (up to 72%) of MCs associated with attached EPS, indicating that alternative methods such as centrifugation, fixation, lyophilization and heating allow for the quantification of MCs forms not captured by simple filtration. Additionally, a shift in MCs variants between intracellular and attached EPS was observed. We discuss the implications of these findings, particularly the role of heating in detecting mucilage-associated MCs, its impact on MCs quotas andbioavailability, and the degradation of MCs associated with EPS by heterotrophic bacteria.

Keywords: microcystins, Microcystis, extracellular polymeric substances

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Exploitation of amphidinols for biopesticide production: environmental triggers, excretion patterns, and the distribution of active and less active forms in *Amphidinium carterae* PBR cultures

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In a world where access to sustainable and uncontaminated food is a growing challenge, transitioning agriculture toward biopesticide use is a crucial step forward. *Amphidinium carterae*, a marine microalga, is a promising non-model organism for biopesticide biomanufacturing. The species is known for the production of amphidinols (AMs), macromolecular toxins with potent antifungal properties. However, to achieve scale-up production of AMs from *A. carterae*, the industry lacks better understanding of the environmental triggers that maximize AM yields, increase the proportion of biologically active AM forms, and optimise the ratio of these molecules inside and outside the cells. Our study focused on optimizing photobioreactor culture conditions to enhance AM yields and increase the proportion of biologically active AMs. Using 18 one-liter photobioreactors, we tested the effects of light irradiance and stationary-phase duration on AMs production. Intra- and extracellular AMs were sampled across multiple points of the culture's stationary phase and quantified via mass spectrometry. Results showed the presence of extracellular AMs ($<$ 2% of the total) only in traces, emphasizing the potential importance of focusing on intracellular AMs for large-scale production. Increased irradiance and prolonged cultivation boosted AM yields, with most accumulation occurring by the 5th day of the stationary phase. Moreover, early stationary-phase harvesting resulted in the best ratio of biologically active to non-active AM forms, while additionally supporting the benefits of exploiting AMs at the beginning of the stationary phase. In conclusion, this study highlighted the importance of light irradiance and stationary-phase duration in AM production while advancing *A. carterae* as a scalable, system for the biomanufacturing of biopesticides.

Keywords: biopesticide, amphidinol, Amphidinium, irradiance, growth phase

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‡Speaker

Optimization of the Extraction Procedure and Strains Screening for the analysis of *Prymnesium parvum* toxins by LC-HRMS

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Prymnesium parvum is a microalgae known to produce potent ichthyotoxins called prymnesins, which have been implicated in harmful algal blooms and massive fish kills worldwide. Prymnesins are large polyether compounds classified into three types A, B, and C based on their carbon backbone structures, with several analogues differing in glycosylation, chlorination and the degrees of unsaturation. One of the major challenges in prymnesins analysis is the lack of commercially available standards, which complicates both the detection and quantification of these toxins. This highlights the need to develop and optimize extraction protocols that enable efficient recovery of both intracellular and extracellular prymnesins for analysis by liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS). In this study, we compared different sample preparation strategies to improve toxin extraction. Among the tested conditions, 100% methanol provided the best signal response, indicating higher extraction efficiency. In addition, we evaluated the impact of filtration versus centrifugation for cell harvesting, to ensure that prymnesins detected in the supernatant did not originate from poorly pelleted residual cells. Following this optimization, we screened several strains of *P. parvum* to assess their prymnesins production profiles. LC-HRMS analysis revealed features consistent with prymnesins types A, B and C, suggesting a toxin production profile specific to each strain. However, further studies are required to confirm the identity of the analogues detected and to explore the structural diversity within each prymnesins class. These findings provide insights into prymnesins profiling and represent a first step toward establishing a robust and reproducible analytical workflow.

Keywords: Microalgae, Prymnesium parvum, Strains Screening, LC, HRMS, Prymnesins Toxins

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A multi-omic approach to characterize the interaction between *Alexandrium minutum* and its microbiome

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Phytoplankton taxonomy, physiology and metabolism may influence the diversity and structure of associated bacterial communities. To test this hypothesis, we investigated the bacterial community composition (comparing free-living and phytoplankton-attached fractions), physiology, and metabolome of *Alexandrium minutum* and its natural microbiome over 48 days of co-culture. The most abundant bacterial families associated with *A. minutum* were *Flavobacteriaceae*, *Rhodobacteraceae*, *Marinobacteraceae*, *Alteromonadaceae* and *Methylophagaceae.* Both fractions (i.e., free-living vs. attached) and the phytoplankton growth phases were identified as structuring factors of the bacterial community. *Flavobacteriaceae* tended to be attached to the *Alexandrium* cells, reaching up to 80% of the attached fraction at the end of the exponential growth phase, whereas *Marinobacteraceae* and members of *Alphaproteobacteria* (*Rhodobacteraceae* and *Hyphomonodaceae*) were enriched the free-living fraction during the exponential growth phase and the stationary phase, respectively. Interestingly, time was a significant factor influencing the evolution of the metabolome in both fractions. In light of these results, we are aiming to link metabarcoding and metabolomics datasets to determine if variations in algal cells metabolites could shape the microbiota of *A. minutum.*

Keywords: toxic dinoflagellate, bacteria, metabarcoding, metabolomic

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ChronosAlgae, Algae explore the future climate change

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Le projet ChronosAlage proposé à la région des Pays de la Loire vise à étudier les impacts du changement climatique sur des espèces clès phytoplanctoniques. En effet, il devient de plus en plus probable que nous dirigeons vers une augmentation des températures très importante en 2100 (+4○C) selon le scénario RSP6. Ce changement climatique aura des répercussions majeures sur l'écosystème régional et notamment sur la biodiversité. Les secteurs économiques liés au tourisme, la pêche, l'aquaculture et l'agriculture subiront des transformations profondes. L' objectif est d'anticiper l'évolution du phytoplancton régional à l'horizon 2100, selon le scénario RSP6. Pour aborder ce sujet, une expérience d'évolution dirigée sera réalisée en laboratoire en simulant les conditions climatiques futures. L'analyse des réponses adaptatives des espèces étudiées permettront d'établir un modèle prédictif afin d'anticiper leur évolution dans ce contexte de changement climatique. L'adaptation de certaines espèces phytoplanctoniques régionales influencera probablement l'ensemble de l'écosystème pouvant avoir des conséquences directes pour la vie économique et sociale des habitants de la région. Les résultats obtenus viseront à sensibiliser les acteurs sociétaux et politiques aux possibles conséquences de ce changement climatique et à surtout contribuer à la mise en place de stratégies pertinentes pour la résilience de la biodiversité et des activités économiques attenantes.

Keywords: phytoplankton, evolution of organisms, evolution experiment, predictive model, evolutionary genetics, climate change, adaptation of organisms

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[*] Speaker

From Blooms to Bioactives: Investigating the chemical diversity and the toxic potential of *Microcystis* (Cyanobacteria)

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Rising global temperatures and shifts in climate patterns, including more frequent droughts and intense rainfall, are expected to intensify toxic cyanobacterial blooms. These blooms pose significant risks to human health, water resources, fisheries, and ecosystems. Among cyanobacteria, *Microcystis* is particularly concerning due to its production of various harmful bioactive compounds, many of which remain poorly appreciated in terms of their toxicological impacts. Indeed, the current understanding of the cyanobacterial metabolome remains incomplete, with many compounds unidentified due to gaps in database annotations. Evidence suggests that toxic effects extend beyond microcystins (MCs), highlighting the need for more comprehensive chemical characterization and toxicity assessment of other potentially toxic compounds. This study focuses on six highly toxigenic strains of *Microcystis*, selected among the most toxic of the Paris Muséum Collection (PMC) and Pasteur Cultures of Cyanobacteria (PCC) collection, according to preliminary tests on whole methanolic extracts. Initially, to investigate these strains, three advanced methods were used: genome mining, molecular networking, and (eco)toxicological risk assessment. The strains are notable for their embryo toxicity and teratogenic effects on Medaka fish and diverse fish cell lines, suggesting that novel metabolites may contribute to their toxicity, linked to specific geno-/chemotypes of the strains. Then, the metabolite characterization was first optimized on two strains presenting the highest toxicity, PMC 830.12 and PMC 727.11, using a dichloromethane/methanol mixture at room temperature. C_{18} Solid-phase extraction yielded five fractions of decreasing polarity. Each fraction was analyzed by ultra-high-performance liquid chromatography – tandem high-resolution mass spectrometry (UHPLC-HRMS/MS) and the resulted data were pre-processed using MZmine3 and finally, feature-based molecular networks were generated on the online platform GNPS, allowing to identify various chemical families, including the expected non-ribosomal peptides. The most promising hydro-methanolic fractions from PMC 830.12 and PCC 727.11 strains were further fractionated by reversed-phase HPLC, thus permitting to isolate metabolites for toxicity evaluation and extensive structure characterization by NMR spectroscopy and mass spectrometry.

Keywords: Microcystis, chemical screening, toxicological evaluation, molecular networking, genome mining, structural analysis

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*Speaker

New insights into the influence of temperature and nutrients on the growth of *Microcystis aeruginosa* and the metabolism of its phycosphere thanks to metabolomics

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Cyanobacterial blooms are a phenomenon of growing international concern, due to their potential ecological, human and economic impacts. While certain abiotic factors are known to contribute to these phenomena, the underlying ecological and physiological mechanisms remain only partially understood. Recently, some studies have highlighted the potential role of biotic interactions between heterotrophic bacteria and cyanobacteria in the phycosphere (i.e. a few tens of microns microenvironment surrounding cyanobacteria) in promoting cyanobacterial blooms. In this context, the COMIC project aims to better understand the occurrence of cyanobacterial blooms by studying cross-feeding metabolic interactions between Microcystis aeruginosa and heterotrophic bacteria through the coupling of metagenomic, meta-metabolomic and modeling approaches. More specifically, COMIC aims (i) to compare cross-feeding metabolic interactions in the phycosphere between cyanobacteria and heterotrophic bacteria in 12 strains of Microcystis and (ii) to characterize the influence of environmental factors (temperature and nutrients) on cross-feeding interactions in one strain. This internship is part of the project's second objective. To this end, an 8-weeks experiment (i.e. 2 growth cycle) under controlled conditions is being carried out to characterize the successive influence of temperature (22 vs 30◦) and nutrient conditions (oligotrophic vs eutrophic) on cyanobacterial growth, as well as metabolism and interactions within its phycosphere. Every week, measurements of optical density, biomass, cell concentration and chlorophyll A are performed to characterize cyanobacterial growth. At the same time, the physiological state of the cyanobacteria is assessed using photosynthetic yield measurements. In addition, weekly biomass samples are taken for metabolomic analysis to study changes in microcystins production and the overall metabolism of the consortium.

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[*] Speaker

Biomass samples will further undergo metagenomic analysis to assess bacterial taxonomy and model metabolism and phycosphere interactions, with weekly nutrient monitoring. Overall, these investigations will contribute to gain understanding of the role of Microcystis phycosphere metabolism and metabolic interactions in cyanobacterial blooms.

Keywords: Metabolomics, Cyanobacteria, Cross feeding, Phycosphere, Microcystis aeruginosa, Bacterial communities, bloom

Student poster

Effect of Hydrodynamics on the distribution and accumulation phytoplankton communities in a shallow lake

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Cyanobacterial blooms are increasingly proliferating on a global scale and pose a significant threat to ecosystem services. In the southwest of France, lake Leon (Landes) has experienced such cyanobacterial blooms, leading to health and economic consequences for the local citizens. Wind and waves can play a crucial role by shifting phytoplankton groups (PG) and by suspending sediments, which can promote algal growth in remobilizing nutrients. In this ongoing study, we aim to analyse the role of hydrodynamics in the spatial distribution of phytoplankton communities, with a particular focus on the spatial and temporal effects of wind and waves on PG. To characterize wind and waves we will use meteorological data such as the wind speed, direction and occurrence. We plan to use the Fetch distance (distance over which wind blows without encountering an obstacle in a given direction) to quantify the wind exposure and the Effective Displacement Index (EDI) for quantifying the effects of cumulative wind over several days. PG are monthly surveyed at 37 sampling points, evenly distributed across the lake. A Principal Component Analysis (PCA) will be applied to the PG concentrations to visualize the spatio-temporal patterns of their variation. We will use a time series model (ARIMAX) to test the effect of a potential wind lag (EDI) on the total concentration of PG. Finally, a Generalized Additive Model (GAM) will be used to show how the Keddy Index and the EDI spatially influence the distribution of GP. We will also incorporate results from the sediment resuspension model into the GAM as an additional spatial explanatory variable. Ultimately, this study aims to contribute to a better understanding of the spatial and temporal distribution of phytoplankton communities in lake Léon. The findings may provide valuable insights for lake managers in addressing future cyanobacterial blooms.

Keywords: Hydrodynamic, Phytoplankton, Spatial modeling, Distribution, Concentration, Wind, Wave, Keddy

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More than just disorder - metabolite diversity of *Microcystis* strains shows tight correspondence to genotype and may contribute to ecotype specificities

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Microcystis is one of the most common bloom-forming cyanobacteria in freshwater ecosystems worldwide. This species remarkably produces numerous bio-active accessory metabolites, which are believed to be potentially involved with different ecological and/or physiological processes. Their genuine contribution to the evolutive success of *Microcystis* blooms remains undetermined. To better depict the potential relation between the local genetic diversity of blooming *Microcystis* populations and the respective associated chemical diversity, we conducted a joined genomic and metabolomic analysis of 65 *Microcystis* strains collected from various lakes from France and European countries. Interestingly, both core- and noncore-gene phylogenetic analysis place 59 of these strains in 12 distinct genetic clades of at least 2 genomes, being widely distributed along the whole *Microcystis* phylogeny and presenting specific signatures of accessory metabolite biosynthesis. The chemical analysis of metabolite diversity produced by these strains, cultured under lab conditions, reveals the production of stable metabolite corteges, beyond little variations along replication, growth phases and culture conditions. Indeed, these strains belonging to 12 different genotypes correspond to 13 distinct metabotypes according to an accurate one-metabotype-for-one-genotype rule. This observation reveals that *Microcystis* collected from certain environments present a large set of genetic and subsequent corresponding metabotype diversity, whereas all strains originating from certain other lakes present a net genetic uniformity. Overall, our investigations reveal that the production of accessory metabolites constitute well conserved chemical traits across the different *Microcystis* genetic clades, suggesting these molecules may be involved in key adaptative and selective processes, that still remains unspecified.

Keywords: cyanobacteria, accessory metabolites, diversity, ecology, cultures

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*Speaker

LEON-BLOOM: origin, spatial and temporal dynamics of cyanobacteria blooms in lake Léon, France.

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Lake Léon (Landes) has recently experienced important cyanobacteria blooms, leading to severe restrictions on recreational activities of this popular tourist waterbody of the Atlantic coast in France. To investigate the origins of these algal blooms, the dynamics of biological patterns, and to provide management strategies for mitigation, the "Léon-Bloom" research project established a collaboration between environmental watershed managers and research scientists. The project aims to identify the potential sources of algal growth and gain a better understanding of their spatial and temporal dynamics. The project is structured in several workpackages, each investigating a potential mechanism. (1) Firstly, nutrient fluxes from the watershed are analyzed, including the use of passive phosphorus samplers. We also set up an experimental design to investigate the role temperature, light and phosphorus on the development of phytoplankton biomass. (2) Secondly, a chemical analysis of the lake's sediments is carried out resorting to sediment coring and experimentation to measure the potential of phosphorus release under anoxic conditions. (3) Thirdly, the role of temperature and oxygen on phytoplankton composition will be assessed using a statistical modeling approach. We measured temperature and oxygen in several stations of the lake to calibrate these models using autonomous high-frequency sensors. (4) The phytoplankton community is studied both spatially and temporally, at the taxonomic and algal group level. Cyanotoxins are also regularly monitored. Finally, two modeling-based workpackages will focus on (5) analyzing the role of wind in the spatial distribution of the plankton community and physical parameters and (6) developing remote sensing methods for monitoring algal concentrations in this lake. Ultimately, we aim to decipher the relative contribution of

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wind, nutrients (fluxes and internal release), and environmental variables, to understand the conditions of cyanobacterial blooms emergence.

Keywords: phytoplankton, phosphorus, temperature, oxygen, wind, teledetection, sediment

Renaturation of a small urbanized oxbow lake: evolution of water quality before/after COVID period

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The Cournon d'Auvergne pond is a small eutrophic oxbow lake totally disconnected from the Allier River. Toxic cyanobacterium *Raphidiopsis raciborskii* blooms was recurrent since 2015. These used to lead to a loss of site attractivity with numerous swimming areas closing during summer. In this context, the town council took several actions focusing on ecosystem renaturation. Before said actions (2017-2019), the pond showed a hypereutrophic system with phosphorus concentrations up to 100 μg/l and a poor ecological diversity. Fish were dominated by burrowing (carps and breams) and invasive species (catfishes, pumpkinseeds). Anthropic pressure was also consistent with an annual frequentation of around 300,000 people and a high baiting-based fishing pressure. In 2018-2019, the first renaturation started with bank vegetablization and floating ecosystem drafts implantation. During COVID period (2020-2021), cyanobacterial blooms weren't observed and phosphorus concentrations decreased to values around 20-25 μg/l. The confinement periods may have considerably reduced anthropic pressure (fishing was forbidden, dog-walking was restricted...). Then, fish biomanipulations were also performed, significantly reducing carp biomass, and introducing black bass, thus improving diversity. Reed debs and waterlilies were also implanted. Despite these actions, an intermediate situation has been clearly observed since 2022. Phosphorus concentrations evolved between 18 and 75 μg/l with a mean of 38 μg/l leading to cyanobacterial blooms. These were less important than during the 2017-2019 period. Furthermore, other genera were able to proliferate such as the picocyanobacterium *Cyanocatena*. All these data allow to conclude that since 2019 water quality has significantly improved. But the 2022-2024 period findings moderate this improvement with a new increase of phosphorus concentration, independent from meteorological conditions, probably due to unexpected parameters (bank erosion due to fishing, cycling and walking activities, dog dejections). Further investigations are needed to better characterize these factors and in order to keep a good water quality on the site.

Keywords: eutrophication, urbanization, cyanobacteria, COVID, phosphorus

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[*] Speaker

Toxicity of *Karenia* species on Greenshell mussel reproduction: insights into the potential bloom impact on aquaculture in New Zealand

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Karenia is a genus of dinoflagellates that includes species capable of inducing harmful algal blooms (HABs). Blooms of *Karenia* species occur globally, particularly *Karenia mikimotoi*, which can reach concentrations up to 10^8 cells·L^{-1}. These blooms can cause mass mortalities of marine fauna, including in aquaculture, resulting in significant economic losses. Exposure to *K. mikimotoi* has been shown to induce hemolytic effects, fish gill damage, and harmful impacts on bivalves.
In New Zealand, *Karenia* spp. can bloom during summer, overlapping with the reproductive period of the Greenshell™ mussel (*Perna canaliculus*). In 1998, a *K. brevisculcata* bloom caused mass mortality of benthic organisms in Wellington Harbour. Species such as *K. mikimotoi*, *K. brevisculcata*, *K. papilionacea*, *K. selliformis*, and *K. longicanalis* are now monitored by the Cawthron Institute due to their bloom-forming potential. However, their effects on early life stages of Greenshell™ mussels, a species of major economic importance in New Zealand, remain poorly characterized.

This study aimed to assess how New Zealand *Karenia* species affect *P. canaliculus* reproduction. Embryo-larval development tests, sperm viability assays, and ROS (reactive oxygen species) measurements were conducted to evaluate the toxicity of the five species at bloom-level concentrations. Additionally, three strains of *K. mikimotoi* were tested to assess intraspecific variability.

Results showed that toxicity is species- and strain-dependent. After two hours of exposure, sperm viability was unaffected, but variations in ROS production indicated sublethal effects. One *K. mikimotoi* strain significantly impaired embryo-larval development, possibly due to

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gymnodimine-C. *K. selliformis* and *K. papilionacea* also had significant effects, though the toxins involved remain unidentified.
These findings suggest that, at environmental bloom levels (10^6–10^7 cells·L^{-1}), *K. mikimotoi*, *K. selliformis*, and *K. papilionacea* may impair early development of Greenshell™ mussels, posing a risk to aquaculture.

Keywords: Reprotoxicity, Bivalve, Karenia, Environmental Dose, Aquaculture

Chemical characterization and developmental toxicity of cyanobacterial metabolites in the embryonic development of Medaka fish (*Oryzias latipes*)

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The eutrophication of aquatic environments, amplified by anthropogenic activity, promotes the proliferation of cyanobacteria. These proliferations induce major ecological disturbances: hypoxic stress, modification of turbidity, and the release of secondary metabolites, some of which are toxic. While certain cyanotoxins, such as microcystins, anatoxins-a, saxitoxins are now quite well known, other families of bioactive metabolites (*e.g.* aeruginosins, anabaenopeptins, or microginins, which are frequently observed in freshwater environments), remain poorly characterized, both toxicologically and ecologically. These compounds, originating from various cyanobacterial strains (*Microcoleus, Nostoc, Aphanizomenon, Microcystis*), exhibit variable polarity profiles that may influence their bioavailability, their passage through biological barriers (*e.g.* fish embryonic chorion), and their physiological targets. This chemical diversity observed between cyanobacterial strains, particularly in terms of range of bioactive compound polarity, raises the question of their respective toxic potential for aquatic organisms. In this context, we explore the toxicity of various metabolic fractions of different cyanobacterial strains on distinct developmental stages of embryos of the Medaka fish (*Oryzias latipes*) presenting protective chorion barrier or not. On one side, the early embryos (0 *dpf*) allow the evaluation of the early impact on a primal key developmental stage of compounds that can pass through the chorionic barrier. Whereas, on the other side, the latter chorion-free larvae (9 *dpf*) exhibits more direct and diverse exposure pathways (branchial, cutaneous), and molecular targets (well-developed organs expressing divers phenotypic cell lines) providing complementary toxicological information. This work aims to better understand and establish correlations between the chemical profiles of cyanobacteria bioactive metabolites (*i.e.* polar to apolar contents of different strains), their observed toxicological effects on fish developments and their potential ecotoxicological consequences for aquatic environment biota.

Keywords: Chemical characterization, toxicity, cyanobacteria, metabolites, Medaka fish

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Culture optimisation of *Vulcanodinium rugosum* for biomass production and purification of portimine

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Climate and global change increase the impact of harmful algal blooms and promote the redistribution of species, thus increasing risks to human health. *Vulcanodinium rugosum* is a bentho-pelagic dinoflagellate that produces toxic cyclic imines, i.e. pinnatoxins and portimines, the latter have been linked to acute dermatitis (60 swimmers in Cuba, 2015; > 1,000 Senegalese fishermen, 2020 and 2021).

To obtain purified toxins in the frame of the Inflamatox-project, it was necessary to establish cultures of *Vulcanodinium rugosum* and analyze the toxins produced. To this end, four strains available in the laboratory were screened for their toxin profile. All four strains produced portimine, and only two additionally produced pinnatoxin G. However, for simplicity of purification and growth performance, a French strain producing only portimine analogues was selected.

Accurately counting cells was a major challenge, due to mucus production and transitions between pelagic and benthic phases. To address this, we adapted the counting methods by combining microscopy and automated tools, thus clarifying the reliability of cell density measurements. Finally, biomass production was optimized for harvesting 8.3g every three weeks, resulting in 90 grams after 6 months.

In parallel, the efficiency of previously published purification processes was independently assessed on small amounts of *V. rugosum* extract. These included liquid-liquid partitioning (LLP, _˜100% recovery), size-exclusion chromatography (SEC, _˜80% recovery), normal-phase silica SPE (_˜90% recovery), and semi-preparative C18 chromatography. The next step will be to apply these orthogonal techniques sequentially on a single extract to purify portimines from the selected strain. The success of the purification strongly depends on the available biomass.

The purified toxins will help the partners further understanding of portimines action mechanisms and increase their understanding of the resulting pathology. The optimized cultivation and pu-

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rification processes could also ultimately be used to develop certified standards and reference materials for improved coastal zone management.

Keywords: Vulcanodinium rugosum, Portimine, Biomass production, Purification

Effets de l'ingestion de cyanobactéries productrices de cyanopeptides émergents sur le métabolome du bivalve *Dreissena polymorpha*

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Les proliférations de cyanobactéries deviennent de plus en plus fréquentes et intenses dans les écosystèmes aquatiques eutrophes à travers le monde. Ces proliférations massives de cyanobactéries représentent un risque environnemental et sanitaire en raison de leur capacité à produire des métabolites secondaires dont certains sont réglementés et appelés cyanotoxines. Les cyanobactéries produisent environ 600 oligopeptides différents, regroupés en plusieurs familles structurales. Néanmoins, l'attention des études écotoxicologiques s'est majoritairement portée sur les cyanotoxines réglementées, telles que les microcystines (MCs). Ce travail a pour objectif d'évaluer la toxicité potentielle de cyanobactéries produisant des cyanopeptides émergents (aéruginosines, microginines et microcyclamides) ainsi que des MCs, en mettant l'accent sur leur impact sur le métabolome d'un bivalve d'eau douce. À cette fin, *Dreissena polymorpha* a été exposée pendant 7 jours à trois souches de *Microcystis* produisant différents cyanopeptides, à quatre concentrations distinctes (10, 100, 200 et 500 μg/L de chlorophylle a). Une analyse métabolomique non ciblée des extraits de glandes digestives a ensuite été réalisée par chromatographie liquide couplée à la spectrométrie de masse. L'exposition aux trois souches de cyanobactéries engendre des modifications significatives du métabolome, notamment à 500 μg/L de Chla. À cette dose, la souche PMC 826-12, productrice de microcyclamides, entraîne une perturbation globale du métabolome plus marquée que les autres souches, y compris celle produisant des MCs, impactant potentiellement les voies du stress oxydatif et du métabolisme énergétique. Un effet apoptogène potentiel a également été observé, soulignant les conséquences cellulaires induites par cette souche. Ces résultats permettront de mieux comprendre l'activité biologique de cyanopeptides encore peu étudiés et de comparer leur toxicité à celle des cyanotoxines actuellement réglementées.

Keywords: Cyanobactéries, cyanopeptides émergents, Dreissena polymorpha, métabolome

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Implementation of CRISPR/Cas9 ribonucleoprotein -mediated genome editing in the ichthyotoxic haptophyte *Prymnesium parvum*

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Gene editing technologies involves the modification of a specific target gene to understand its function and/or obtain new phenotype. Recent advances using Clustered Regulatory Interspaced Short Palindromic Repeats (CRISPR)-Cas-mediated approach have provided major breakthroughs in biology. This approach has significant potential for a range of applications, from functional genomics studies, to strain improvement for biotechnological applications. Unfortunately, this approach is still poorly developed in non-model organisms and not at all in toxic microalgae. CRISPR molecules may be delivered into cells as DNA, RNA, or pre-formed ribonucleoproteins (RNPs). Among these, RNPs stand out due to their rapid genomic targeting and prompt degradation. Here we describe the establishment of CRISPR-RNP technique in the ichthyotoxic haptophyte species *Prymnesium parvum.* To assess the feasibility of the CRISPR-RNP approach, the single-copy nuclear APT gene, encoding adenine phosphoribosyl transferase (APT), was targeted in *P. parvum.* Inducing indels at this locus results in cellular resistance to the toxic compound 2-fluoroadenine (2-FA). Following the selection and design of functional single-guide RNAs (sgRNAs), successful CRISPR ribonucleoprotein (RNP) delivery must be ensured. However, low cell penetration efficiency poses a challenge for RNP delivery in certain cell types like microalgae. Different approaches for CRISPR-RNP delivery will be evaluated in this project, and preliminary findings using electroporation will be presented. By establishing CRISPR-RNP technology in *P. parvum*, previously unexplored avenues are opened for research and development. In particular, our work will help in elucidating the physiological mechanisms and trophic strategies of toxic and harmful microalgae, exploring chemical ecology, and assessing current and emerging risks associated with algal toxins.

Keywords: Genome editing, CRISPR/Cas9 ribonucleoprotein, Haptophyte, Prymnesium parvum

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*Speaker

Targeted screening of toxins in Florida marine cyanobacteria species using LC-MS/MS analysis

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Marine cyanobacteria are widespread in a variety of environments, from estuarine areas to open oceans, and are known for their ability to produce a wide range of secondary metabolites, including cyanotoxins. Cyanobacteria blooms are becoming increasingly common worldwide, and cyanotoxins can have an impact on aquatic ecosystems, as well as posing risks to animals and humans. To date, more than 2000 secondary metabolites have been identified from benthic marine cyanobacteria, particularly from the genera *Moorena*, *Lyngbya*, and *Okeania*, but many of these compounds remain poorly studied and largely uncharacterized.
In this study, we set out to identify several families of toxins potentially present in marine cyanobacteria strains mainly collected from the Florida region. Twenty-seven strains, representing ten identified species, were isolated and cultured for morpho-molecular characterization. Toxin profiles were determined by Liquid Chromatography coupled to Mass Spectrometry (LC-MS/MS). We targeted approximately forty characterized cyanotoxins, including paralytic shellfish toxins (PSTs), anatoxins, cylindrospermopsins, microcystins, and lyngbyatoxins.

Following this screening, a dmMC-RR analogue was detected in two *Moorena* species. No paralytic shellfish toxins, anatoxins, or cylindrospermopsins were found in the analyzed samples. Regarding lyngbyatoxins, a specific analytical method was developed in-house based on a contaminated sample originating from New Zealand, which enabled their identification and quantification. These compounds are currently being screened in samples using the new optimized method.
This targeted analytical approach provides valuable insights into the diversity of toxins among marine cyanobacterial strains. To further explore their chemical diversity and potentially discover new or less well-characterized metabolites, a complementary non-targeted high-resolution mass spectrometry (HRMS) approach will be developed.

Keywords: marine cyanobacteria, lyngbyatoxins, cyanotoxins, LC MS/MS analysis

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[*] Speaker

Risk Assessment of Marine Biotoxins in New Caledonia: species diversity, toxin profile and trophic transfer

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Gambierdiscus and *Fukuyoa* are known to produce neurotoxins associated with ciguatera poisoning (CP). In New Caledonia (NC), located in the southwestern Pacific Ocean, a significant knowledge gap remains regarding CP and the microalgae linked to this foodborne illness. Other toxic benthic dinoflagellates often co-occur with *Gambierdiscus*, such as the genus *Ostreopsis*, which produces palytoxin analogues (neurotoxin), or the genus *Prorocentrum*, which produces diarrhetic toxins (okadaic acid and derivatives). These toxins accumulate through the food web, pose serious public health risks, impact coastal fisheries, and may contribute to the toxic cocktail associated with ciguatera, potentially explaining the variability of observed symptoms.

Over the past few years, we have conducted numerous studies to document the diversity of toxic benthic dinoflagellates and the occurrence of marine biotoxins in NC. On the one hand, live samples were collected from multiple sites across the territory. After isolation and culturing, strains of dinoflagellates were identified by morpho-molecular approach and toxin profile were characterized by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). A major outcome was the description of a new strain of *Gambierdiscus polynesiensis* with an atypical ciguatoxin profile. On the other hand, a seasonal single-site sampling approach, combining passive samplers (SPATT) and shellfish tissue analysis was conducted to explore the diversity of toxins in coastal waters of the bays surrounding Nouméa. Eight microalgal toxin groups were detected in shellfish, of which three are internationally regulated. In addition, following CP outbreaks in Lifou, targeted LC-MS/MS analyses of fish tissues confirmed the presence of ciguatoxins, providing direct evidence of trophic transfer and human exposure.

Altogether, these findings underscore the complexity of marine toxin risks in tropical ecosystems and highlight the need to develop risk management and public health strategies in NC.

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Keywords: Ciguatera, ciguatoxins, LC, MS/MS, benthic dinoflagellates, marine biotoxins, SPATT, Gambierdiscus

Toxicity of *Ostreopsis* spp. cultures from the Basque coast: bioassays on a diatom, oyster gametes and fish gill cells

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Since 2020, the Basque coast is subjected to *Ostreopsis cf. siamensis* and *O. cf. ovata* seasonal blooms. During proliferations, mucus produced forms a benthic biofilm that detach in the water column. Symptoms in humans and in situ mortality of benthic fauna have been observed. *O. cf. ovata* produces ovatoxins, whereas no toxin is known from *O. cf. siamensis*. *O. cf. ovata* toxicity on some marine organisms has been reported, but *O. cf. siamensis* toxicity is still poorly understood, despite a recent study indicating toxicity to crustaceans. The present study aims at investigating toxicity of *Ostreopsis* spp. Basque strains on marine organisms, via bioassays targeting a diatom, fish gill cells and oyster gametes. In addition, role of mucus in toxicity was explored.

Three strains of *O. cf. ovata* and of *O. cf. siamensis* were used. Oyster gametes were exposed for 2h to different concentrations of *Ostreopsis* spp. cultures, and fertilization rate was estimated. The RTgill-W1 fish gill cell line was exposed to *Ostreopsis* spp. methanolic extracts for 24h and viability was assessed.
A strain of *Chaetoceros* sp. was exposed to *Ostreopsis* spp. cultures and intracellular methanolic extracts for 24h, then viable cells were counted. In addition, toxicity after successive filtrations (from 80µm to 0.2µm) was also tested against *Chaetoceros*.

Overall, *O. cf. ovata* was more toxic than *O. cf. siamensis*. Oyster and fish models were more sensitive than *Chaetoceros*. At in situ bloom concentrations, *O. cf. ovata* was toxic to all models, whereas *O. cf. siamensis* had deleterious effects only in the fish and oyster assays. Toxicity in fractions appeared to be decreasing with filter pore size, suggesting mucus toxicity, possibly due to toxin accumulation in the exopolymer matrix.
This study shows that *Ostreopsis* spp. from the Basque coast can alter marine life, although precise toxic mechanisms remain to be elucidated.

Keywords: Microalgae ; Dinoflagellate ; benthic ; Ostreopsis ; toxins

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*Speaker

Insights in gene expression regulation during phagotrophy in marine microalgae

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Microorganisms capable of growing by autotrophy (photosynthesis) and heterotrophy (feeding on pre-existing organic matter) are called mixotrophs. In the aquatic ecosystems, mixotrophy is much more widespread than initially thought. Mixotrophy could also be an important physiological trait for microalgae forming massive harmful algal blooms (HABs). *Prymnesium parvum* is an ichthyotoxic species that causes heavy losses in aquaculture and in coastal environments. The toxins produced by this species (called prymnesins) have a potential role in mixotrophy making possible to immobilize preys. Here, the cryptophyte *Teleaulax amphioxeia,* containing phycoerythrin, was used as prey in order to be able to differentiate prey from predators using their fluorescence by flow cytometry. To better characterize gene expression during phagotrophy, cells were sorted by flow cytometry to select *P. parvum* cells at different stages of the phagocytose/digestion process. RNAseq analyses were performed in sorted cells in order to target separately genes involved in the processes of phagocytosis and digestion.

Keywords: mixotrophy, prymnesium parvum, gene expression

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†Speaker

Payment for Ecosystem Services: An Efficient Approach to Reduce Eutrophication?

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The CPES (Channel Payments for Ecosystem Services) project developed PES schemes remunerating farmers for their activities in improving water quality by reducing the emissions of nutrients (both nitrogen and phosphorus) or erosion from agricultural activities. Catchment-wide approaches were tested in six case studies, and ecology, hydrogeology, agroeconomy, law, and farming expertise was combined. Collaborations were established with farmers, their associations, chambers of agriculture, and local and regional stakeholders.

One case study concerned Lac au Duc (Brittany), a reservoir suffering from recurrent cyanobacterial blooms. Curative actions to control cyanobacteria had limited success. The main sources of excess P entering the lake were of farming origin but varied between the sub-catchments according to hydrogeological characteristics and agricultural practices. Long-term prevention possibilities to ameliorate agricultural practices including their costs were developed with the farmers.

During the project, PESs were successfully applied for ground water protection by the water supply companies as drinking water protection has a strong business case to preserve this ecosystem service. For recreational or multi-purpose lakes and reservoirs, more effort is needed to find financing for the monetary requirements to address farmer's transition to ameliorate land management.

Keywords:

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*Speaker

CYANOACTION Effective Lake management: reducing cyanobacteria by actions in the catchment

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The increasing occurrence of toxic cyanobacterial blooms as a consequence of anthropogenic eutrophication in freshwater ecosystems threatens water security and quality worldwide for humans and animals and severely disrupts ecosystem balance. Besides, significant economic losses to water companies, fisheries, recreational activities, and other businesses caused by cyanobacterial blooms require greater attention from policymakers. Therefore, the implementation of thorough monitoring strategies for cyanobacteria, along with evaluation of the risks of bloom incidents and a plan for prevention and control measures, are now required in water safety plans by water suppliers. A suite of in-lake control methods exists, but little guidance exists on their efficiency. Decreasing nutrient input from the catchment is crucial for long-term prevention/ reduction of blooms and would be possible by the transition towards more sustainable agricultural practices, but it requires implementation strategies.

The COST action, CYANOACTION, started in the autumn of 2024, aims to increase and broaden capabilities throughout Europe for effective and sustainable management of water resources using a catchment-wide approach. To achieve this, CYANOACTION seeks to establish a unique network of experts, end users, and stakeholders. Gathering and distributing information on early warning detection methods and experience in the effectiveness of both long and short-term control methods will underpin Decision Support Tools for water managers and authorities. These tools will ensure the selection of appropriate methods tailored to specific water bodies. The overarching goal is to increase the mutual exchange between scientific knowledge and management practices in order to decrease cyanobacterial blooms while considering the diverse characteristics of water bodies, their catchment, and the regulatory frameworks across Europe.

Keywords: CYANOACTION, cyanobacteria, catchment, management

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