Team: Cyanobacteria, Cyanotoxins and Environment (CCE), MNHN, CNRS

MNHN missions: Research , Collections, Teaching and Platforms (Electronic microscopy)

Foreword

Cyanobacteria are a group of prokaryotic microorganisms performing oxygenic photosynthesis and present everywhere on Earth. They have developed a large variation of acclimation mechanisms that allows them to be present in a wide variety of ecological niches in marine, freshwater and terrestrial habitats, and they are distributed in climatic zones ranging from the frozen Arctic waters to hottest deep sea hydrothermal waters. They are also capable to survive to rapidly changing environments like fluctuating light or nutrients. Cyanobacteria have a lot of beneficial properties. Cyanobacteria are important primary producers at the global scale and play essential roles in carbon and oxygen cycles. Furthermore, some strains have the ability to fix the atmospheric nitrogen and convert it into nitrates and ammonia which are needed for the growth of all living organisms. Cyanobacteria also provide food, vitamins, pigments and essential growth factors for Human or animals (Sili et al. 2012). Finally, they synthesize bioactive secondary metabolites with great potential for pharmaceutical applications and the treatment of important diseases (anticancer, antibiotic, antifungal, and antioxidant).

However, cyanobacteria also have the potential to be highly toxic for animals and humans through the synthesis of particular family of molecules, the cyanotoxins. In specific environmental circumstances, such as nutrient-rich hot summer waters, cyanobacterial populations can suddenly develop to become the most dominant photosynthetic organisms in the water, forming cyanobacterial blooms. In recent decades we've observed an increase in the occurrence of these blooms, notably including species and strains that produce toxins. In aquatic ecosystems, cyanobacterial blooms can transform clear and clean waters into a very turbid blue-green or reddish slurry. These phenomenons have been related to changes in nutrient concentrations, ratio of nutrients (*e.g.* Nitrogen/Phosphorus ratio), light intensity, temperature and stability of water column. Aquatic environments are particularly sensitive to local and global environmental changes (e.g. increase in human population, climate change). There is a direct relationship between the frequency of cyanobacterial blooms and the increase of available nutritive resources caused by human activities. Climate changes could also favor the appearance of cyanobacterial blooms suggesting that these phenomenons will be amplified in the next future (e.g. Paerl et al., 2016).

Cyanobacteria are able to survive in a large range of habitats and climates, and they have all the physiological mechanisms needed to become dominant under specific environmental conditions. Such imbalances result in a reduction in the diversity (taxonomic and functional) of communities of photosynthetic microorganisms (e.g. Sukenik et al., 2015). However, a high diversity is required in these communities to maintain or restore the characteristics of these ecosystems that make them essential for human populations (e.g. recreational activities, drinking water resources or fish farming). Consequently, we urgently need to better understand the environmental factors responsible for the maintenance of primary producer's biodiversity in aquatic ecosystems and, conversely, better understand the processes leading to an erosion of this biodiversity under the dominance of cyanobacteria (e.g. Carey et al., 2012).

The adaptive capabilities of cyanobacteria to environmental stresses (i.e. high temperature or irradiance, changes in salt concentrations, resistance to chemical pollutants) constitutes a key element toward a better understanding of the dynamics of cyanobacterial blooms and survival. Furthermore, secondary metabolites seem to have a crucial role in such processes, but it is yet poorly understood.

In the frame of the UMR 7245 MCAM, the scientific project of the CCE team for the next 5 years is closely related to the results obtained over the last five years' plan (cf Focus CCE) which opened new perspectives. In addition, this project relies on: 1. the synergy between the individual skills of each CCE's members (past and new), 2. a multidisciplinary approach focused on one model, the cyanobacteria, and which gather concepts and techniques from ecotoxicology, microbial ecology, chemical ecology, and 3. long-time national and international collaborations with complementary skills and expertise's to CCE's group.
Thus, the objectives of the CEE team for the next five years are to open up to topics yet poorly or not explored, in the international context. These main topics are listed below:

- To revisit the microbial and chemical diversities of aquatic ecosystems with a focus on cyanobacteria and microalgae with the new OMICs and NGS approaches
- To understand the mechanisms underlying the relationships between biodiversity and functioning of aquatic ecosystems, by combining multi-trophic (e.g. microbial loop, interactions between microorganisms and macroorganisms) and multi-scale (space, time) approaches with field campaigns and experimentations (e.g. micro-, mesocosms)
- To characterize the environmental factors explaining the relationships between microbial communities of aquatic ecosystems, with modelling approaches

- To understand the responses of fishes to multicontaminations in environmental context and search for specific signatures of cyanobacterial ecotoxicological stress and toxins on aquatic organisms (multi-omics approaches)
- To increase the interface between environmental and societal sciences in order to improve the general understanding and management of the societal impacts of cyanobacterial proliferations

All these topics are closely related with each other (**Fig. 1**) and in the continuation with the results collected over the last years by our team. The arrival of a MNHN's Professor in October 2017 will reinforce our research on microbial diversity and the mechanisms implied in adaptations capacity of cyanobacteria to environmental fluctuations or stresses.

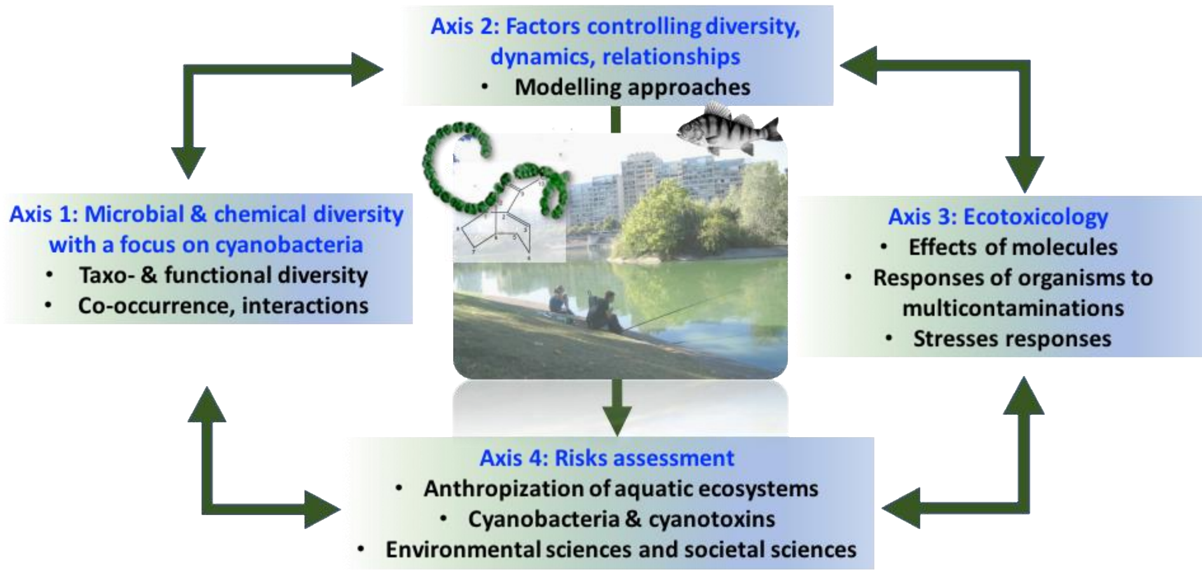

Fig. 1: Schematic representation of the major CCE research topics on cyanobacteria (Biodiversity, ecotoxicology, and transfer of knowledge to environmental and societal sciences. The picture represents the close relationship between aquatic ecosystem (i.e. urban lake) and human pressures (i.e. fishing, urbanization). The ecological status of aquatic ecosystem has consequences on ecosystems biodiversity, ecosystem functioning and good & services of these environments (© C. Bernard, MNHN).

All these topics are developed in four scientific axes, each being led by one or two researchers, depending of their skills. Most of the time, funds are not specific to only one axis and some of them are transversal to several axes.

Research axes

① <u>Biodiversity and adaptive responses of cyanobacteria and microalgae:</u>

<u>In continental aquatic environments:</u>
The objectives of our team are to: 1. characterize the taxonomic and functional diversity of photosynthetic microorganisms (i.e. **Fig. 2**), 2. identify the environmental factors (anthropic, geomorphological, hydrological ...) explaining the structure of these microbial communities, 3. propose hypotheses on the mechanisms (e.g. ecophysiological characteristics, stress responses, adaptive capacities) explaining the diversity and distribution of species in a given environment. To this end, we will continue to perform field sampling campaigns in a wide variety of environments (e.g. freshwater bodies in the Ile-de-France area, French rivers, lakes in African countries including Uganda, Senegal, Ivory Coast, and in hypersaline alkaline lakes). This will allow to implement the data bases constructed by the CCE team. Experimental approaches, both in field and laboratory, will be considered as well in order to determine the environmental factors (axis 2) controlling the diversity, distribution and dynamics of photosynthetic microbial communities in regards to the adaptative capacities of the species studied (axis 3).

The characterization of photosynthetic microbial communities will be carried out both by conventional microscopy (photonics, electronics) and molecular approaches for functional diversity (PCR, qPCR or even RT-qPCR on targeted functions such as stress responses or nitrogen fixation, and NGS for meta-barcoding).

This research should enable us to acquire knowledge about: 1. the taxonomy and diversity of current organisms (individuals, populations, communities), 2. their phylogeny and evolution, 3. their adaptation and acclimatization potential, 4. their physiology, 5. the functional traits related to selected biogeochemical cycles, and 6. their interactions with all organisms (micro-, macro-) in open ecosystems. The "*in natura*" study sites of the CCE team are mainly urban and peri-urban lakes, waterbodies and rivers, which are subject to high and diverse anthropogenic pressures.

A full permanent professor position (recruited in October 2017) in microbial ecotoxicology will develop a specific and complementary research axis on the impacts of pollutants or toxins, on the diversity, structure and functions of microbial communities. The project will be developed: 1. in the different compartments of aquatic ecosystems (open water, exposed surfaces, sediment), 2. with a multi-scale approach (from environments to communities of free-living, animal- and plant-associated microbiomes), and 3. at several spatial and temporal scales (time-series samplings). The presence/absence and abundance of the different bacterial groups will be quantified with different approaches developed in the lab (e.g. microscopy, NGS and community analysis) but also using *in situ* hybridization with fluorescence-labeled probes (by FISH and CARD-FISH methods).

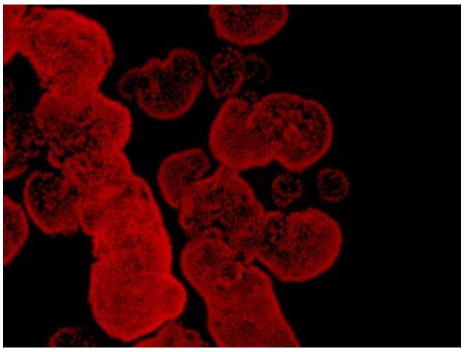

Fig 2. Auto-fluorescence d'une colonie de cyanobactérie, *Woronichinia* sp. (© C. Djediat & A. Catherine, MNHN).

Chimiodiversity of cyanobacteria:

Cyanobacteria are known to produce a very wide variety of bioactive metabolites, some of which being highly toxic for various organisms (vertebrates, zooplankton, phytoplankton, etc.) and different cellular targets (hepatotoxins, neurotoxins, dermatoxins, etc.). Biological roles and functions (in ecosystem functioning and evolution) of the numerous secondary metabolites produced by cyanobacteria remain unknown to date, but their strong biological activities suggest that they can be involved in ecological functions. These activities allow cyanobacteria to colonize specific environments or occupy new ecological niches (Dittman et al., 2015).
This research will directly benefit from the biological material of the 700 living strains of cyanobacteria available from the MNHN collection of cyanobacteria and microalgae (hosted, fed and maintained by the CCE team). Among these strains, several will be selected on their toxic potential, the new taxa description (types maintained in the collection) or on their synthesis of natural products with pharmacological potential. This research will integrate 1/ sequencing and annotation of genomes (to search for the genetic islands responsible for the specific syntheses of the different families of metabolites), as well as 2/ direct characterization of the metabolites contents of the strains studied (chemotyping of various strains of the cyanobacteria collection of the MNHN (PMC from UMR 7245) by analytical biochemical approaches developed in the UMR: mass spectrometry and NMR), 3/ according to different biotic and abiotic conditions (in order to study the determinism of the production of these different metabolites), and 4/ with regard to the diversity of the metabolites produced during natural blooms (France, Mayotte, Africa, New Zealand ...) according to the main taxa in presence.

② Factors controlling diversity and dynamics of photosynthetic microorganisms:

Following the description of biodiversity in microbial coomunities, the objective of this research is to answer the following questions: 1/ What are the mechanisms responsible for diversity-functioning relationships at key trophic levels (microbial loop and microbio-metazoan interfaces) (NGS approaches, microcosms)?, 2/ How do environmental factors affect the structure and functioning of communities? Indeed, the structure of microbial communities in ecosystems results from complex biotic and abiotic interactions.

The data obtained for the period 2014-2018 on the diversity of photosynthetic microorganisms (taxonomic, chemical and functional diversity) in the framework of several programs (ANR CYANOTOX, PULSE for the Ile-de-France region, Monaco Foundation for the region Mediterranean, ANR DZIANI for Mayotte) associated with environmental data, allow us to address these issues using statistical analysis and modeling approaches. According to the sites studied, the observation scales will be local (e.g. Lac Dziani, Mayotte) or regional (e.g. Ile de France or rivers watersheds).
The use of co-occurrence networks should make it possible to understand the potential relationships between micro-organisms and also between micro-organisms and the environment (Jeanbille et al., 2016).

All this work allows to:
=> Answer questions and to test concepts on microbial interactions with biotic and abiotic factors
=> Propose scenarios for the evolution of aquatic ecosystems
=> Reinforce modeling approaches, *in natura*

③ Ecotoxicology of cyanobacteria:

The aim of this work is to study the toxicological effects of chronic exposure to cyanotoxins, cyanobacteria or bloom extracts, in particular in key organisms in aquatic ecosystems such as fish (indigenous, *in situ* or model organisms, *in vivo*). We will take special care to integrate different experimental scales combining *in situ* exposures in natural conditions and also in the laboratory under controlled conditions. The toxicological response of organisms to toxin mixtures will be studied at various scales, ranging from life traits of organisms to the most fundamental molecular levels. This allows to obtain a synthetic view of toxicological responses specific to the effects of different cyanotoxins, of the various toxin extracts, or of the different strains or blooms of cyanobacteria. This work involves microcosm and mesocosms experiments, pathological analyzes by histology, high-throughput proteomic, transcriptomic, genomic and metabolomic analyzes, as well as analyzes of toxin profiling. How these exposure events influence the diversity and composition of microbiotes associated to the animals (skin, gills, gut…) will also be analyzed to understand the potential role of microorganisms occurring at the interface between the animal and its environment.

The main projects we wish to develop concerns:1. the description of emerging cyanotoxins, in particular those with potential endocrine-disrupting or goitrogenic-disrupting (thyroid regulation system) effects, 2. the characterization of ecotoxicological stress signatures of cyanobacteria and their toxins on aquatic organisms or model species, *via* multi-omic integrative approaches. This research need to integrate different levels of experimental or sampling scales.

=> They require strengthening the genomic, proteomic, metabolomic and transcriptomic approaches for the search for specific molecular signatures of toxic substances and their effects (cf Unit focus 3)

④ Risks related to cyanobacteria and decisional support

Cyanobacteria, by their ability to proliferate and produce toxins, pose a risk to the functioning of aquatic ecosystems but also threaten the uses that can be made of these ecosystems by human populations (production of drinking water, irrigation, bathing, fishing ...). Thus, scientists working in aquatic environments but also managers and users of these aquatic ecosystems share common concerns. The work carried out up to then in the team have progressively led us to the interface between environmental sciences and societal sciences.

=> One of the current objectives of the CCE team is to reinforce our activities at the interface environmental and societal sciences

This requires the development and validation of monitoring tools for cyanobacteria in particular and aquatic environments in general, but also decision-support tools for the managers. This effort will not be limited to management and uses of aquatic environments in a French context, but will be extended to tropical and subtropical countries where quality of aquatic environments constitutes an even greater scientific and societal concern.
The approaches that we will be developed are diverse, depending on whether they are concerned with toxins, cyanobacteria or, more generally, with the diversity and dynamics of phytoplankton communities in aquatic environments related to anthropogenic pressures.

Thus, in the frame of this axis we aim at:
=> Develop standardized tests for detection, assay, and identification (discrimination) of MCs based on the use of antibodies;
=> Validate an aerial sensor to monitor the horizontal distribution of cyanobacteria;
=> Adapt the French phytoplankton index (IPLAC), developed in response to the European Water Framework Directive, to tropical and subtropical countries;
=> Develop scenarios for predicting the evolution of phytoplankton diversity in relation to eutrophication and anthropogenic pressures;
=> Define indicators and scoreboards for monitoring the quality of aquatic environments with the objective to set up scientific observatories for these environments

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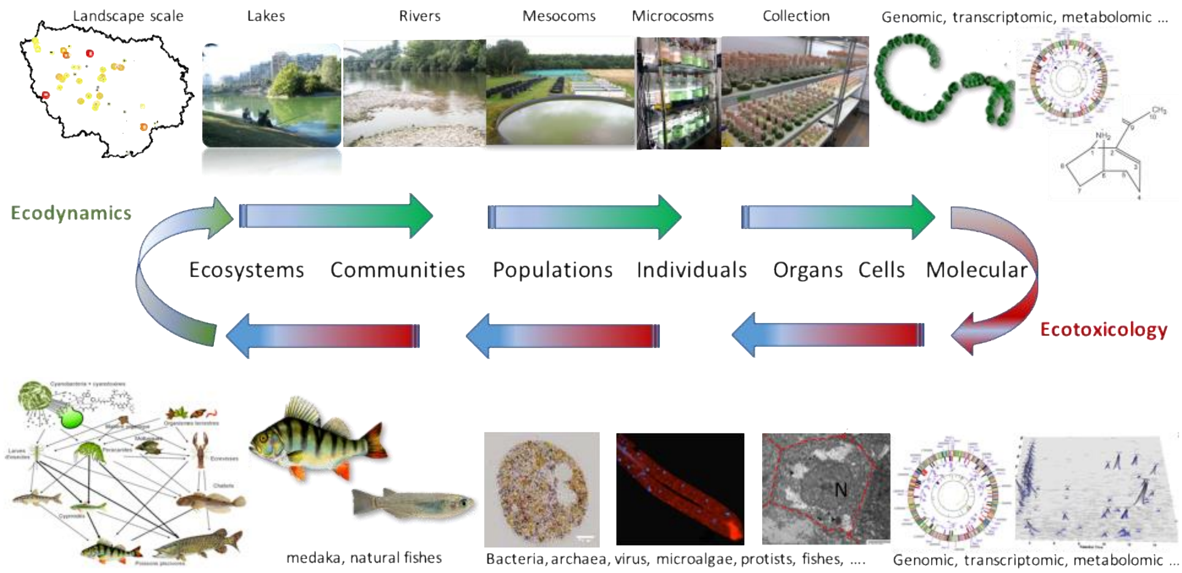
Models and techniques:



Team organization (2018)

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Collaborations

iEES,
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ANSES,
ENVA,
ENS,
UPMC,
ESPCI,
IPGP,
LIENS,
Ecologie Microbienne Lyon, …

Networks:

GIS Cyano,
Ecotoxicomics,
GDR Aquatic toxicology,
RFMF,
Metabohub

Funds

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CNRS EC2CO
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Conseil Général de Seine Saint Denis