

PhD projet

PhD's title:	MicroCOSMA Microbial Community shifts in New Caledonia coastal ecosystems under Seasonal and Multidecadal Anthropogenic impacts
PhD supervisor (<i>accredited to supervise research</i>): Ifremer Dpt./Unit/Lab:	Dr. Raffaele Siano, HDR ODE/DYNECO/Pelagos – Brest
PhD co-supervisor: Organisation, laboratory (Ifremer Dpt./Unit/Lab):	Dr. Hugues Lemonnier RBE/LEAD – Nouvelle-Calédonie-Nouméa
Laboratory/host structure, location:	DYNECO/Pelagos – Brest

English summary – 1174 characters (max 1200)

The lagoon of New Caledonia has an exceptional marine biodiversity, which has to be preserved face to strong environmental issues due to territory development. The mining industry and coastal urbanization represent a threat to marine biodiversity by increasing terrestrial inputs of organic matter and contaminants into coastal systems through riverine run-offs during cyclonic periods. In the area, the potential impacts of terrestrial inputs on marine microorganism diversity and community structure is not clearly defined at both temporal and spatial scales. Based on the coupled approach between environmental genomics (eDNA), coastal hydrology and geochemistry, the PhD will aim at understanding human activity impacts over microbial communities under three different and complementary perspectives. (1) The evaluation of variations in microbial community structure between the dry and rainy seasons in estuarine ecosystems of ultramafic catchment areas under mining influences. (2) The analysis of shifts in microbial communities occurred over the last century in parallel to the development of the mining industry by means of the analyses of paleoarchives (*seDNA* and heavy metals) collected from sediment cores. (3) The assessment of the arrival and/or the recrudescence of blooms of potential harmful microorganisms in relation to human activity development.

Key-words:

Coastal ecology, river run-offs, anthropogenic impacts, pollutions environmental microbiology, environmental DNA, paleoecology-paleogenetics, New Caledonia, coastal management, metabarcoding

Preferred profile of the PhD student– 474 characters (max 400)

The PhD candidate must be in possession of a Master in Marine Ecology.

Specific competences:

- knowledge of the diversity, genetics and ecology of marine microorganisms;
- genetic analysis techniques;
- management of metabarcoding data;
- biostatistics (R software);
- very good knowledge of English;
- attitude to work at sea and in a team;
- availability for mobility overseas;
- interest in the management of coastal areas and biodiversity.

Detailed Research Project

1- Scientific background

In New Caledonia (NC) human and climatic pressures increase inputs from land by rivers impacting the protected coral reef and lagoon ecosystems (See annex 1 for complementary information on NC territory). These inputs are essential for biodiversity and the functioning of the Caledonian coral ecosystem (1,2) but can be potentially harmful in case of excess (3, 4, 5). In fact, the territory of NC is subject to large intense rainfall episodes, themselves influenced by strong inter-annual climatic variations due to the El Niño climatic phenomenon (6, 7, 8). The leaching of the soil following these extreme rainfall events, favored by the destruction of terrestrial environments by fires, induces in the waters of rivers marked signatures of material of terrestrial origin (9). **River run-off impacts on the oligotrophic waters of the New Caledonian lagoon represent an interesting study case for evaluating and monitoring human influences on biodiversity and functioning of tropical coral ecosystems.** River waters can be vectors and/or favor the development of microorganisms directly affecting human health (enteric bacteria, new viruses) or threatening humans via the consumption of unsafe sea products (toxic microalgae) (10, 11). In fact, the risk of Ciguatera disease increases with enrichment in nutrient salts in the environment and in the presence of cyanobacteria (12, 13). The risk of human contamination by *Vibrio vulnificus*, which has already led to the deaths of 3 people in 2008 in NC after consuming oysters (14), should increase with both global warming, rising water level and environmental enrichment due to river run offs (15, 16). In addition to microbiological risks, there are chemical risks due to the mining industry impacting water catchments. The level of metallic contamination in organisms depends on the characteristics of the receiving environments (17, 18) which can even make certain seafood products unsafe for consumption (19, 20).

Due to their rapid turnover and physiological plasticity, coastal unicellular planktonic microorganisms react fast to chronic or extreme environmental perturbations. Being the plankton the first essential component of marine trophic chains, variations in their community might have heavy consequences of the whole marine ecosystems through cascading effects. The most modern techniques of molecular biology based on metabarcoding and real time PCR analyses of environmental DNA (eDNA) can now provide access to a more complete analysis of microbial diversity and could improve the studies on communities or species variations carried out until now in NC (21, 22, 23, 24, 25, 26, 27, 28). Metabarcoding of contemporary eDNA has proven to be a valuable tool for ecosystem management, from freshwater quality evaluation (e.g. 29; 30) to the analysis of the ecological impacts on communities (e.g. oil spill event (31) fish farms (32) and vertebrate biomonitoring (33)). In addition, the study of ancient DNA (aDNA) from marine sediments (*seDNA*) has shown to make available valuable information about past biological communities, especially for unicellular plankton (viruses, bacteria, protists) (34; 35; 36; 37, 38, 39). In fact, the use of *seDNA* paleo-archives may provide a biodiversity baseline for the evaluation of biological variations that might have occurred during the Anthropocene epoch due to human impacts (40). The use of long-term analyses on *seDNA* and more in general of paleoecological approaches are becoming new tools for conservation science and policy actions, providing baselines to understand the evolution of nature under different stressors (41). **Both contemporary and ancient eDNA may improve the evaluation of present and past effects of anthropogenic pollutions on microbial communities and contribute to the evaluation of stability and resilience of New Caledonian estuarine ecosystems.**

2- Strategic positioning within the Department/Institute

The analysis of the land sea interface as well as the analysis of resilience and the application of conservation strategies for protected ecosystems are some of the priorities of the institute (COP: Action 1 and 2, Ifremer Research project: Topic 6, 8, 10 and challenge 1) (See annex 2 for complementary information on the strategical position of the project).

3- Scientific objectives

The main aim of this PhD project is the study of the effect of anthropogenic activities on microbial coastal communities at both seasonal and multidecadal time scales. Beyond general river-run offs impacts, we will voluntarily focus on mining industry pollution, studying the effect of metal inputs (Ni, Co, Cr, Mn), which are the most relevant sources of impact in NC, in particular in the south-west part of the territory (Dumbea, Coulée and Pirogues estuaries). Based on the coupled

approach of environmental genomics and coastal hydrology and geochemistry, the PhD will aim at understanding the effect of human activity development over microbial communities. The PhD project has been organized in 3 parts, corresponding to 3 aims. **1st aim**) Comparison of the diversity of microbial communities (eDNA metabarcoding of bacteria and protist communities) and the potential interactions among microbes (microbial networks) in estuarine ecosystems during the dry and rainy seasons (extreme rain events) in order to identify changes in community structures in relation to river run-offs. **2nd aim**) Correlation between heavy metal concentration changes and microbial community shifts using chemio-biological paleoarchives (heavy metal traces and *sed*aDNA) collected from sediment cores sampled in target ecosystems. **3rd aim**) Identification of biological terrestrial tracer of run-offs (real time PCR on eDNA of endogenous plants, soil bacteria, enteric bacteria) or potential harmful microorganisms (toxin microalgae) along the river plume (spatial approach) and the evaluation of the date of arrival and/or dynamics over the last century of these tracers using paleogenetic data (temporal approach) in order to analyze harmful recrudescence and run-off variations in relation to human activity development.

4- Methodology

The PhD project will benefit from the information and experience collected in the frame of the projects Searcé (9), PALMITO (37), POHEM (42) and PALMIRA (39) coordinated by the Phds supervisors.

(1st aim) The Nouméa area of NC has been selected as the target zone of this part of the project. This zone is characterized by contrasting impacts from three rivers. The first river (La Pirogue) shows a massive supply of trace metals (natural and industrial inputs). The second (La Coulée) is characterized by massive supply of trace metals but also by high inputs of nutrients (nitrate and phosphate) and dissolved organic matter. The third river (La Dumbéa) reveals high inputs of nutrients, dissolved organic and particulate matter, but lower supply of metals. This last site will be used as baseline ecosystem for the evaluation of the metallic impacts of other rivers (9). The ecological study concerns the biostatistical analyses of already acquired or in course of acquisition (cf. §5) eDNA datasets. Those datasets consist in eDNA metabarcoding data of protist and bacteria estuarine communities acquired targeting the genes encoding the V4-V5 16S rRNA (for prokaryotes, primers 43) and V4 18S rDNA (for eukaryotes, primers 44) and using approaches developed in previous studies (Tara Oceans, 45; POHEM, 42) and implemented in ongoing projects (ROME) in order to ensure the data comparison. Beyond a microbial community comparison across sampled ecosystems and seasons, the presence and the extent of terrestrial inputs will be studied using real time PCR amplification (rt-PCR) on selected species of terrestrial origins, used as bioindicators of the impacts (cf. aim 3). The identification of communities by microscopy, flow cytometry and pigment analyze (spectrofluorimetry and HPLC) will complete molecular data. Some biological processes will be measured in addition (MAP, community respiration, primary and bacterial production).

(2nd aim) Sediment cores will be collected in the zone of the Pirogues estuary affected by mining exploitation and natural metal contaminations. Sediment core sampling, and paleoarchive analyses will be carried out in collaboration (cf. §9). We will voluntarily concentrate on a time scale of about 150 years to evaluate pre- and post-industrial over the Anthropocene period. *Seda*aDNA be carefully extracted from sediment cores of muddy sediment facies following general precautions for *sed*aDNA manipulation, to avoid contamination with contemporary DNA (46) and on the base on the experience acquired in previous projects (PALMITO: 37; PALMIRA: 39). Target gene amplifications (V4 18srDNA) for metabarcoding analyses of protists paleocommunities will be performed in clean genetic laboratories available at the IRD of Nouméa. Shifts in protists communities will be evaluated by statistical analyses (MRT: Multiregression Tree Analysis) on paleogenetic data (38; 39).

(3rd aim) rt-PCR amplification on target species will be carried out on the same eDNA extracts of aim 1 and on *sed*aDNA of aim 2 used for metabarcoding analyses, in addition to new potential samples collected in the frame of the AMII CTM, in case this project will be financed (internal funds). The spatial (aim 1) and temporal (aim 2) dynamics of the terrestrial run-off bioindicators will be analyzed. eDNA of: i) enteric bacteria indicator of animal breeding impacts (*Campilobacter* spp.), ii) soil bacteria (47), iii) ectomycorrhizal fungi (48), and iv) endogenous plants of NC (49) will be amplified in samples of estuary outlets-off shore transects in order to verify the extent of terrestrial impacts, and in particular to evaluate if run-offs reach the external coral reef of the lagoon. For this part, the PhD project will benefit from the data collected in the framework of the project Bioindic on ultramafic soil (50). Harmful microalgal species will be selected for the evaluation of recrudescence of toxic species (e.g. *Gambierdiscus* spp.) (37). Other bioindicators better mirroring the effect of metal concentrations could be selected on the basis of metabarcoding analyses carried out in aims 1 and 2. The long-term dynamic of selected species will be evaluated over the Anthropocene period using *sed*aDNA. If rt-PCR will not be possible due to potential bias of *sed*aDNA quantification, we will be able at least to date the first presence of a potential bioindicator and to correlate this occurrence in the ecosystems with a potential impact. The correlation between eDNA and metal contamination will be analyzed using multivariate statistical tests.

5- Resources at disposal for the PhD student for the duration of the research project (human, technological...)

Two sets of environmental samples and metabarcoding data (18s rDNA for protists and 16s rDNA for bacteria) will be available before the beginning of the PhD. These datasets correspond to the sample surveys carried out in 3-7-day cruises along estuary outlets-off shore in the frame of the project Searsé in September 2019 (dry period) and February 2020 (heavy rain) using concomitantly eulerian and lagrangian sampling strategies. Another sampling cruise has been carried out in December 2020 during a different dry season. More than 40 environmental parameters have been processed and all sequencing of eDNA samples will be completed in April 2020, before the PhD start. These data sets will be used to address the 1st aim of the project. In order to finance aim 2 and 3 of the PhD, additional funding is currently being requested in the framework of (1) the new “Au fil de l’eau” project call of the Cresica Research Consortium (2021-2023) and (2) the future research grant from NC Northern Province, Southern Province and Ifremer (2022 - 2027). These new projects will assemble all collaborators of the PhD project. The PhD project will be carried out both in Brest and Nouméa (50% of the time at DYNECO and LEAD, respectively). It will start in Brest waiting for the stabilization of the COVID-19 crisis, then a period in Nouméa is planned, before finishing the PhD in Brest. Phd’s tutors will plan reciprocal visit in Nouméa and Brest to follow student’s work, sampling and analyses. **Thanks to this double site planning of the PhD, the student will benefit of the facilities of both the Ifremer centers of Brest and Nouméa** (See annex 3 for complementary information on PhD resources availability).

6- Expected results and valorization (publications and public dissemination)

This Phd project will provide an integrated and multidisciplinary analyses of the impact of river-run-offs and metal pollutions on the New-Caledonian lagoon. A long-term reconstruction of the industrial pollutions and its effect over the microbial population will be provided for the first time, using the eDNA approach never applied so far in this territory for the microbial compartment. We will generate new data and analyses that could be useful for the protection of the biodiversity of the lagoon ecosystems and the analysis of the coral reef resilience. We plan 3 scientific articles, corresponding to the 3 parts of the work, to be published in high impact factor journals (ISME journal, Environ. Microbiology). The results of this Phd will be discussed in the frame of local scientific partnerships (Cresica, ENTROPIE) but also with local stakeholders and administrations in order to improve the monitoring and management of the lagoon. General public presentations will be also programmed in the frame of local manifestations but also in continental France to better teach and transmit the environmental issues of oversea territories.

7- Originality and innovation

The New Caledonian estuarine territory, its climate, and the water characteristics of the lagoon offer the best opportunity to study the impact of terrestrial run-offs due to heavy rains over oligotrophic water of tropical ecosystems. The extreme contrasting conditions that the lagoon experience from the dry season (“pristine” waters) to the rainy season (run-off impacts) will certainly enhance the possibility to study drastic changes in microbial composition of this marine ecosystems, likely allowing the identification of bioindicators of terrestrial inputs. In addition, the development of the mining industry over the coastal ecosystem of the NC territory will help in understanding the impact of metal pollution in a pristine, oligotrophic ecosystem. Often in coastal ecosystems metal pollutions are superposed to other pollutions (agricultural, breeding), complicating the analyses of the metal impacts. The NC territory offer the great opportunity to disentangle this pollution and to study *in situ* the specific effect of metal inputs over the microbial compartments.

8- Does the project come under the ABS Nagoya Protocol and/or does it involve the use of genetic resources?

Yes, for the use of genetic (eDNA) material from New Caledonia (See annex 4 for complementary information)

9- Potential partnerships

The PhD student will benefit from internal (Ifremer) and external collaborators of the Cresica and UMR ENTROPIE partnerships. The speciality and the contribution of each collaborator of the project are specified in Annex 5.

10- Provisional schedule

		20	2022					2023				2024		
		21	1	2	3	4	5	6	7	8	9	10	11	12
	Trimester													
	PhD period in Brest													
	PhD period in Nouméa (NC)													
Aim 1	Analyses on already available metabarcoding data													
	Redaction of paper 1, Congress.													
Aim 2	Sediment core sampling cruise preparation													
	Sediment core sampling													
	SedaDNA extraction and amplifications													
	Sequencing of <i>sedaDNA</i> (metabarcoding)													
Aim 3	real time qPCR analyses on target species													
Aim 2/3	Analyses and correlation with other environmental data													
Aim 2	Redaction of paper 2													
Aim 3	Redaction of paper 3, Congress													
	Phd manuscript redaction and defense													

11. Annex 1: Complementary information on New Caledonia territory

For more than 20 years, New Caledonia (NC) has been recognized as one of the “hot spots” of global biodiversity, home of four primordial ecoregions, and one of the highest endemic richness (51, 52). This territory has an exclusive economic zone (EEZ) of almost 1,500,000 km². Its maritime space includes reefs with varied structures, lagoon areas, mangroves and very large oceanic areas. NC is also the seat of rapid demographic, economic and societal changes. While the mining industry has significantly shaped land and coastal landscapes over the past century, there is a strong desire to diversify the country's economy through tourism, aquaculture and agriculture. This results in strong urbanization and growing, diversified anthropogenic pressures on coastal areas to which are added the consequences of climate change, which are still poorly understood (53; 54; 55, 55, 56, 57).

12. Annex 2: Complementary information on the strategic PhD project positioning at Ifremer

The Action 1 of the COP (*Contrat d'Objectifs et de Performance*) establishes the scientific objectives of Ifremer for 2019-2023. The project MicroCOSMA contributes to three of Ifremer objectives: the evolution of coastal ecosystems in a global change perspective (topic 6 of Ifremer Research project), the cumulative impact of stressor (topic 8) and the evaluation of socio-ecosystems and biodiversity face to global changes (topic 10). To contribute to these issues, MicroCOSMA will develop and apply a multidisciplinary, integrated *in situ* observation strategy (biology and chemistry) using new approaches (eDNA), following challenge 1 of the Ifremer Research project (COP Action 2).

The utilisation of the eDNA approaches to evaluate microbiological community changes and risks are the aims of ongoing key research project of Ifremer (project ROME). The eDNA approach in the marine coastal ecosystems in NC is a new, promising tool for the evaluation of the biodiversity, the analyses of anthropogenic impacts and the elaboration of conservations strategies. This tool has never been used so far in NC for the analyses of microorganism diversity and ecology. This PhD project will allow the affirmation of Ifremer and LEAD/DYNECO as the only team integrating this tool in microbial coastal ecology analyses within the context of local (Cresica) and national (UMR ENTROPIE) partnership strategies.

By providing an exhaustive evaluation of microbial shifts, including pathogens and toxic species, with a new tool (eDNA) in an oligotrophic, protected environment (NC lagoon), MicroCOSMA will contribute: i) to future research perspectives on the evaluation of co-occurring stressors on coastal ecosystem (*exposome*) and ii) the development of multidisciplinary approaches fostering the protection and conservation of marine ecosystems in a holistic perspective (Eco-One-Health). The research and technical advancements achieved during MicroCOSMA could contribute to build up a long-term integrated observatory (coastal and off-shore) for ecosystem protection in NC. This project could help its consortium to eventually contribute and potentially lead this microbial eDNA long-term marine observatory in NC.

MicroCOSMA contributes to development of new research initiatives in the French over-sea territories. The interdepartmental collaboration between the research units ODE/DYNECO and RBE/LEAD, elaborated in 2018 (project politique inter-centre POLCA) and concretely started in 2019 (project Searsé), will further evolve in the frame of this project. The Searsé project is part of a larger program entitled "*au fil de l'eau*" supported by the Cresica Research Consortium of which ifremer is an integral partner (<https://www.cresica.nc/>). The purpose of Cresica is to coordinate various multi-disciplinary research actions along the land-sea continuum in NC. This PhD project will also contribute to the objectives of axes 2 (understanding marine organisms), 3 (characterization of community dynamics) and 4 (protecting marine biodiversity) of the UMR ENTROPIE joined by Ifremer-LEAD in January 2020 (<http://umr-entropie.ird.nc/index.php/home>).

13. Annex 3: Complementary information on PhD resource availability

In Brest the student will be supervised for the molecular ecology and paleogenetic part (eDNA) of the thesis and will benefit from the collaboration and help of the SeBiMer bioinformatic facility. During the stay in NC, sediment core samples and potentially new eDNA samples will be collected taking advantage of the experience and facilities of the LEAD team for environmental samplings. The PhD will be formed to genetic analyses by technician and researchers of the LEAD team in Nouméa and Saint-Vincent, with the collaboration of the DYNECO team. The LEAD laboratory disposes of all instruments and expertise allowing genetic analyses in loco (PCR, qPCR, fridges, etc.), other facilities will be available at the IRD and at the University of NC (controlled atmosphere laboratory for *seadaDNA*). In Nouméa, the student will be supervised for the hydrological part and will benefit from the collaborations with members of the Cresica consortium and UMR ENTROPIE (see annex 6) who will ensure analyses on nutrients, metals and organic matter compounds (cf. § 9, annex 5). Microbial metagenomics datasets from neocaledonian ultramafic soil is already available to identify terrestrial eDNA in the samples (54, 55, 56). This dataset will help in the identification of terrestrial bioindicators and will be carried out in collaboration with members of the IAC (Institut Agronomique Néo-Caledonien).

14. Annex 4: Complementary information on ABS Nagoya Protocol.

All genetic analyses (DNA extractions, PCR analyses) will be performed in Nouméa. Illumina Mi-Seq sequencing for metabarcoding analyses need specific facilities that are not available in NC. Hence, amplification products of both water and sedimentary DNA must be exported to France, and sequenced in Toulouse (www.genotoul.fr platform). A list of all samples will be created following ABS protocols and Ifremer internal rules elaborated in the project MORSE following special procedures for New Caledonia territory.

15. Annex 5: Complementary information on the future collaborators and their roles

The PhD student will be in charge of biostatistical analyses of communities (metabarcoding data, aim 1), sampling and treatment of sediment cores (aim 2), and of all genetic analyses (*seadaDNA* extraction, aim 2 and rt-PCR on tracer species, aim 3). The aims and the valorization of the project will be attained with the benefit of multiple collaborations here listed:

Microbial Ecology

- **Nolwenn Callac** (Ifremer/LEAD – microbial ecology, bioinformatics): support for analyzing the bacteria and Archaea diversity.
- **Thierry Jauffrais** (Ifremer/LEAD – microalgae physiology): support for analyzing the physiology of phytoplankton compartment and toxic microalgae in the context of our sampling.
- **Michèle Gourmelon** (Ifremer/DYNECO/Pelagos – microbial ecology, enteric bacteria): identification of bacterial contaminations.
- **Martine Rodier** (MIO-IRD Marseille – phytoplankton ecology in South Pacific): support and data expertise for the microphytoplankton analyses.

eDNA and Tropical Ecology

- **Laurent Vigliola** (IRD Nouméa - eDNA analyses on fish populations): support for eDNA sampling facilities and expertise.
- **Fabian Carriconde** (IAC – Nouméa): Support for analysing and identifying terrestrial DNA.

Bioinformatics

- **Alexandre Cormier and Patrick Durand** (Ifremer/SeBiMer): metabarcoding analysis support and PhD formation.

Metal chemistry

- **Farid Juillot** (IRD Nouméa – Geochemistry and mineralogy): support for the heavy metal analyses in sediments and for the identification of metal sources from terrestrial inputs.

Organic matter chemistry

- **Cécile Dupouy** (IRD Fidji - Biogeochemistry): support for organic matter analyses.
- **Stéphane Mounier** (IRD Toulon- biogeochemistry): support for the identification of the dissolved organic matter sources.

Sedimentology

- **Sabine Schmidt** (CNRS/EPOC- sedimentology): sediment dating analyses

- **Axel Ehrhold** (Ifremer/GM- coastal paleo-sedimentology): sediment granulometry analyses
- **Stephan Jorry** (Ifremer/GM): support for analyses on paleoreconstructions.

Data analysis and run-off modelling

- **Romain Le Gendre** (Ifremer/LEAD: modelling): river run-off simulations
- **Simon Van Wynsberge** (Ifremer/LEAD: population ecology): support for analysing of *in situ* ecological data
- **Nazha Selmaoui-Folcher** (Univ. New Caledonia, informatics, artificial intelligence): support fo data mining and statistical analyses. §

16. List of references

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