

Project #138
VIROPICO-DOC

VIROPICO-DOC: Environmental control of green water events in Mediterranean lagoons under climate change

Participants :

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- Institute of Bioscience and Biotechnology of Aix-Marseille (BIAM) - Jean Alric, DR CNRS – Biophysics & Photosynthesis
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Public Abstract

Mediterranean coastal lagoons such as the Etang de Berre are among the ecosystems most vulnerable to climate change, as they experience rapid and recurrent fluctuations in temperature, salinity, light and nutrient inputs under strong anthropogenic pressure. These shallow, semi-enclosed systems act as early-warning sentinels, where microbial communities respond rapidly to environmental forcing. In several Mediterranean lagoons, episodic “green water events” caused by dense phytoplankton proliferations increasingly disrupt ecosystem functioning and threaten aquaculture activities.

Among the taxa involved, the unicellular green microalga *Picochlorum* stands out for its exceptional tolerance to environmental variability and very high growth rates, making it both ecologically successful and attractive for climate-aligned biotechnologies such as CO₂ capture and biomass production (biofuel, bioplastics, etc.). However, large-scale cultures can collapse abruptly, as observed during the VASCO2 industrial pilot, highlighting a major bottleneck for ecosystem management and biotechnology.

VIROPICO-DOC aims to develop a mechanistic and predictive understanding of how environmental variability, algal physiology and viral infection jointly shape *Picochlorum* population dynamics in the Berre Lagoon in the context of global warming. Building on the recent discovery of giant viruses infecting *Picochlorum* (Picochloroviruses), the project will assess how temperature, salinity and irradiance modulate algal physiological performance and viral infection efficiency. Controlled laboratory experiments will identify strain-specific physiological responses and environmental “windows” favouring algal proliferation, viral regulation or coexistence.

These results will be confronted with seasonal in situ time series obtained through monthly sampling in partnership with GIPREB (non-academic), combining flow cytometry, qPCR and environmental genomics. The project also includes a three-month international secondment at the National Renewable Energy Laboratory (USA), providing advanced training in genetic transformation and genome editing of *Picochlorum*. By integrating laboratory experiments with long-term field monitoring, VIROPICO-DOC delivers actionable, mechanism-based insight into bloom initiation and collapse, supporting lagoon management under climate change and the development of more resilient microalgal biotechnologies.

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1. EXCELLENCE

1.1. Pre-proposal's context, positioning and objective(s)

Context : Understanding and anticipating ecosystem transformations under **climate change** is a major scientific challenge. Mediterranean coastal lagoons, such as the Etang de Berre, are among the most exposed ecosystems, experiencing rapid fluctuations in temperature, salinity, irradiance and nutrient availability under strong anthropogenic pressure. These shallow, semi-enclosed systems act as early-warning sentinels of environmental change, where microbial communities, including photosynthetic microalgae, respond rapidly to physical and chemical perturbations. In parallel, lagoon microalgae are attracting growing interest as innovative tools to mitigate atmospheric CO₂ accumulation through biomass production, including biofuels, bioremediation and bioplastics. In this context, this project focuses on **Picochlorum**, a unicellular green microalga, and its associated viruses.

Picochlorum is particularly abundant in Mediterranean lagoons, where it forms episodic dense green water events¹ that **disrupt ecosystem functioning and threaten aquaculture** activities, as in the Thau Lagoon². These harmful green water events are expected to become increasingly frequent under ongoing climate warming. The ecological success of *Picochlorum* stems from its exceptional tolerance to environmental variability (t°, salinity, light) and from its very rapid division rate³⁻⁵. These traits explain both its dominance in highly variable ecosystems and its growing interest for **biotechnological applications**^{5,6}. However, the industrial scalability of *Picochlorum*-based bioprocesses is constrained by recurrent and unpredictable culture collapses. During the VASCO2 industrial project in Fos-sur-Mer and Palavas, sudden *Picochlorum* culture crashes were observed⁷, compromising CO₂ capture and biomass production. Our analyses identified a likely cause: a previously unknown abundant giant virus, named **Picochlorovirus**⁸.

This discovery opens a largely unexplored research field. Picochloroviruses constitute both a potential constraint for *Picochlorum*-based biotechnological processes and a regulator of lagoon microalgal populations, likely involved in green water termination. Understanding these host-virus interactions is essential for sustainable lagoon management and climate-aligned biotechnology.

Preliminary results : An ongoing collaboration between BIAM and MIO investigates the adaptive capacities of *Picochlorum* and its viruses under environmental variability, aiming to establish this host-virus pair as a model system for Mediterranean lagoons⁹. A strain collection of several *Picochlorum* species isolated from the Berre, Thau and Prevost lagoons has been established, with physiological and genomic characterisation in progress. Within the CNRS-funded VIROPICO project, multiple *Picochlorovirus* strains were isolated from the Berre Lagoon and cultured with the model host *Picochlorum oklahomense* PicoA. Host and viral genomes were sequenced and annotated, revealing viral genes involved in host photosynthetic performance⁹. These results provide a strong foundation for the proposed doctoral project.

Scientific objectives and hypotheses: The central objective of this doctoral project is to establish a mechanistic and predictive framework linking environmental variability, algal physiology and viral infection to *Picochlorum* population dynamics in the Berre Lagoon. This objective is addressed through three testable hypotheses:

H1 - Environmental modulation of host physiology. *Picochlorum* displays predictable physiological responses to temperature, salinity and irradiance, defining environmental "windows" for optimal growth, stress and limitation. Different *Picochlorum* strains are further expected to exhibit strain-specific adaptations of their photosynthetic apparatus, enabling them to thrive within distinct optimal conditions. We expect unimodal response curves allowing estimation of strain-specific optima and tolerance breadth.

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H2 - Environmental control of viral infection efficiency. The efficiency of *Picochlorovirus* infection (adsorption, replication kinetics, latent period, burst size and infectivity) depends on host physiological state and is modulated by the same environmental drivers. We hypothesise that environmental conditions favouring maximal algal growth do not necessarily maximise viral replication, leading to distinct environmental “windows” promoting host proliferation, viral regulation or coexistence.

H3 - Mechanistic understanding informs in situ dynamics. Bloom initiation and collapse of *Picochlorum* populations in the Berre Lagoon occur when in situ environmental conditions enter or exit the physiological and infection-favourable ranges defined experimentally. We expect that lab-defined physiological optima, stress thresholds and infection windows will explain and predict seasonal host-virus dynamics in the field.

Positioning relative to the state of the art: Although phytoplankton-virus interactions are known to influence marine and freshwater ecosystems, they remain poorly characterised in highly dynamic coastal and lagoon systems, particularly under the combined effects of temperature, salinity and irradiance changes associated with climate warming. For the genus *Picochlorum*, knowledge is still fragmentary: its genome, resilience mechanisms and photosynthetic plasticity have only recently been described in a few strains, and its viral partners have been identified even more recently.

The *Picochlorum*-*Picochlorovirus* system thus represents an emerging, largely unexplored model to study how environmental forcing shapes microbial population dynamics in Mediterranean lagoons and how viruses act as natural regulators of algal blooms. Despite the biotechnological potential of *Picochlorum*, no predictive framework currently exists to anticipate lagoon bloom risks or to prevent viral crashes in industrial cultures, and in situ monitoring of *Picochlorovirus* is virtually absent from management programmes. The proposed project addresses these scientific and operational gaps by coupling laboratory experimentation with field data generated in collaboration with GIPREB.

Methodology: The doctoral project combines controlled laboratory experiments, viral infection assays, and in situ ecological monitoring into an integrated research framework to understand how environmental variability shapes *Picochlorum* population dynamics and their regulation by viruses in Mediterranean lagoons. It is structured in three complementary, multidisciplinary work packages that address the hypotheses H1→H3.

WP1: Laboratory characterisation of *Picochlorum* physiological responses to environmental conditions (BIAM). Twelve diversified *Picochlorum* strains from Mediterranean lagoons (Berre, Thau, Palavas) will be exposed to gradients of temperature, salinity and irradiance reflecting seasonal variability. Environmental responses will be initially assessed through growth rates and high-throughput chlorophyll fluorescence measurements of key photosynthetic parameters. Based on strain-specific differences, a deeper characterisation of the photosynthetic apparatus will be conducted under selected conditions using pigment profiling and advanced non-invasive biophysical analyses.

These measurements will provide mechanistic insight into regulatory and photoprotective strategies underlying strain-specific adaptation and will quantify the impact of photosynthetic plasticity on cellular energy metabolism and stress resilience through CO₂ assimilation efficiency and reactive oxygen species production. Resulting strain-specific response curves will define optimal, suboptimal and limiting conditions for growth, enabling comparative analyses of environmental tolerance, phenotypic plasticity and susceptibility to environmental stress and viral regulation. This WP will be conducted at BIAM under the supervision of S. Viola and J. Alric.

WP2: Environmental modulation of *Picochlorovirus* infection dynamics (MIO+BIAM). WP2 investigates how environmental drivers modulate *Picochlorovirus* infection dynamics through their effects on host physiological state. Infection assays will be

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conducted using a reduced and representative subset of environmental conditions identified in WP1, corresponding to optimal, suboptimal and stress regimes for *Picochlorum* growth across gradients of temperature, salinity and irradiance.

Using the *Picochlorum* PicoA-*Picochlorovirus* model system, key viral life-cycle parameters will be quantified, including adsorption efficiency, latent period, replication kinetics, infectivity and burst size. Host and viral abundances will be measured using flow cytometry and qPCR, ensuring methodological continuity with WP3. Photophysiological tools developed in WP1 will be applied to infected cultures under contrasting conditions favouring either algal growth or viral replication. Comparison of infected and non-infected cultures will allow discrimination between abiotic stress and virus-induced effects, including altered photosynthetic efficiency, population heterogeneity and stress signatures.

Integration of WP1 and WP2 will define environmental “windows” promoting host proliferation, viral regulation or coexistence, providing a mechanistic basis to link environmental variability with bloom development and collapse.

WP3: Linking laboratory-derived mechanisms to in situ population dynamics (MIO+GIPREB). The PhD student will participate monthly in GIPREB’s ecological monitoring of the Berre Lagoon, collecting project-specific samples in parallel with routine observations. GIPREB is a public organisation (Syndicat mixte) responsible for coordinating the lagoon’s restoration strategy, producing long-term environmental datasets, and interfacing between scientific research and management authorities. Samples will be processed at GIPREB and MIO and complemented by long-term monitoring datasets to generate time series of *Picochlorum* abundance, *Picochlorovirus* load and key environmental parameters. Host and viral abundances will be quantified by flow cytometry and qPCR, ensuring methodological consistency with WP2. In parallel, environmental genomics approaches will be used to explore population dynamics and microdiversity, including shotgun metagenomics of viral fractions and targeted barcoding of algal communities using pan-*Picochlorum* PCR primers previously developed and validated at MIO. These analyses will be conducted with the support of Elsa Mendes, bioinformatics engineer at MIO.

Field observations will be interpreted in light of the physiological optima, stress thresholds, and infection-favourable windows defined experimentally in WP1 and WP2. By bridging controlled laboratory experiments with long-term in situ observations, WP3 will assess the ecological relevance and predictive power of host-virus interaction mechanisms and will provide a framework to anticipate how climate-driven environmental variability may reshape *Picochlorum* bloom dynamics in Mediterranean lagoons. Beyond the scope of the doctoral project, the datasets generated during the PhD will provide empirical inputs to an existing mechanistic model developed at MIO, which integrates physiological responses, viral infection parameters, and in situ environmental forcing to explore *Picochlorum* bloom dynamics. While the development of this model will remain independent of the doctoral work, it contributes to the broader objective of predicting and managing algal blooms.

An international secondment of 3 months will be carried out in the laboratory of Anagha Krishnan at the National Renewable Energy Laboratory (NREL, Colorado, USA), which provides recognized expertise in genetic transformation and genome editing approaches for *Picochlorum*¹⁰⁻¹² not available in the host laboratories (MIO and BIAM). The main objective of this secondment is to train the PhD student in state-of-the-art functional genomics tools for microalgae. Although these approaches will not be directly implemented within the core PhD work packages, they will strengthen the student’s conceptual and technical background for deciphering gene function in microalgae and their viruses.

During the secondment, the student will establish a genetic transformation strategy using a ClonNat resistance marker to select stable transformants. In parallel, CRISPR/Cas9-based genome editing will be tested for targeted inactivation of nuclear genes in *Picochlorum*. As

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a proof of concept, a key gene involved in arginine metabolism (arginine succinyltransferase) will be disrupted to generate a selectable auxotrophic phenotype. This training will enhance the scientific ambition and originality of the project by providing a gene-centered perspective complementary to the physiological, ecological, and environmental analyses of the thesis. Upon return, the acquired expertise will be transferred to MIO and BIAM through protocol adaptation and internal training, enabling future studies on *Picochlorum* adaptation and virus-alga interactions using advanced genetic approaches and strengthening the long-term competitiveness of the host teams.

Originality and innovative aspects of the planned research. This project is original in both its conceptual framework and its methodological integration. By linking algal physiology, viral infection dynamics and environmental variability, it moves beyond traditional descriptive ecology to deliver a predictive, mechanism-based understanding of green water dynamics. A key innovation is the construction of quantitative physiological and infection response landscapes, describing how *Picochlorum* growth and *Picochlorovirus* infection parameters vary across realistic environmental gradients. These experimentally derived responses are then projected onto long-term in situ observations, allowing direct testing of their ecological relevance. The project is among the first to place virus-mediated regulation at the centre of bloom dynamics in Mediterranean lagoons, a component largely absent from current monitoring and management frameworks despite its potential importance. The project links laboratory experiments and GIPREB long-term field data to connect microbial ecophysiology, viral ecology and ecosystem-scale dynamics.

1.2. Interdisciplinary dimension of the project

This project is interdisciplinary and intersectoral by nature, combining algal physiology, marine virology, microbial ecology, environmental genomics, functional genomics, and long-term ecosystem monitoring to address research questions that cannot be resolved within a single disciplinary or academic framework.

The academic partners provide complementary expertise for the successful completion of the project and supervision of the fellow. BIAM contributes internationally recognised expertise in algal physiology, photosynthesis and photophysiological measurements, which is required to characterise *Picochlorum* growth, stress responses and physiological limits under environmental variability (WP1). MIO provides expertise in marine virology, virus-host interactions, flow cytometry, qPCR and environmental genomics, enabling the quantification of *Picochlorovirus* infection dynamics and viral diversity (WP2-WP3).

The interdisciplinary scope of the project is reinforced by the integration of functional genomics as a complementary dimension, through the international secondment, providing a link between physiological traits, viral susceptibility and underlying genetic determinants.

Methodologically, the project relies on integration across biological scales, from cellular physiology and viral infection kinetics to population-level dynamics observed in situ. Rather than juxtaposing disciplinary approaches, laboratory-derived physiology and infection response are coupled with long-term field observations, enabling causal interpretation of bloom initiation, persistence and collapse.

The fellow will be primarily hosted at MIO, with regular stays at BIAM for physiological experiments and monthly field activities conducted with the non-academic partner GIPREB within its routine monitoring of the Berre Lagoon. This organisation ensures sustained interaction between academic teams, the international partner and the environmental management authority, reinforcing the project's interdisciplinary and intersectoral dimensions.

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2. IMPACT

2.1. Expected impact of the project on the candidate's career

This PhD project will provide the student with a highly competitive scientific profile at the interface of algal physiology, marine virology, microbial ecology and environmental monitoring. By combining controlled laboratory experiments with long-term field datasets generated in collaboration with GIPREB, the student will be trained to work at the science-management interface. Through an integrated training programme combining laboratory experimentation, long-term field observations and interdisciplinary supervision, the student will acquire advanced analytical skills applicable across biological scales, from molecular mechanisms to ecosystem-level dynamics. The close interaction with lagoon managers will further expose the student to real-world constraints of ecosystem monitoring, data scarcity and decision-making.

The international secondment will further strengthen the fellow's career profile by providing high-level training in functional genomics approaches developed for *Picochlorum*. Although not directly implemented within the PhD research, this training will equip the student with a rare mechanistic understanding of gene function in microalgae and their viruses, significantly increasing competitiveness for postdoctoral positions and future research.

Beyond disciplinary expertise, the project will foster key transferable skills, including project management, experimental design, data integration, science communication and interdisciplinary collaboration. The student will also gain experience in presenting results to both scientific audiences and environmental stakeholders, notably through exchanges organised by GIPREB among scientists working on the Étang de Berre.

Altogether, this training will open diverse career pathways, positioning the young researcher for postdoctoral research in microbial ecology, marine biology or environmental virology, as well as for careers in environmental agencies, ecosystem monitoring institutions and applied research or R&D sectors related to algal biotechnology and environmental management.

2.2. Expected impact for the thematic axis

The project directly addresses the thematic axis "Climate change and environmental challenges" by focusing on the mechanisms through which environmental variability shapes microbial population dynamics in climate-sensitive coastal ecosystems. Mediterranean lagoons are among the ecosystems most vulnerable to climate warming, and understanding their biological responses is essential for anticipating future ecological trajectories.

Scientifically, the project will advance the state of the art by providing a mechanistic framework that links environmental drivers (temperature, salinity, irradiance), algal physiology, and viral infection processes. By integrating viruses into the study of algal bloom dynamics, the project fills a major knowledge gap in lagoon ecology, where viral regulation remains largely overlooked despite its potential importance. The results will improve our understanding of how climate driven changes alter host/virus interactions, bloom development, and bloom termination in Mediterranean lagoons and comparable coastal systems.

Beyond fundamental advances, the project has applied relevance for ecosystem management. By identifying environmental thresholds and favourable "windows" for algal proliferation and viral regulation, the project will provide actionable knowledge for interpreting changes in water quality, water transparency and biological states of the lagoon. These processes directly affect lagoon uses such as clam fishing, bathing activities and overall ecosystem services.

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In close collaboration with GIPREB, the project will contribute to improving the interpretation of long-term environmental datasets and to strengthening the capacity of managers to anticipate algal proliferation events with potential socio-economic impacts. The identification of practical indicators and molecular tools to monitor algal and viral populations will directly support adaptive lagoon management under climate change.

More broadly, the project also informs climate-aligned biotechnological strategies by identifying environmental and biological constraints affecting the stability of microalgal cultures. By clarifying the role of viruses in culture collapse, the project contributes to the development of more resilient microalgal production systems for CO₂ mitigation and biomass generation, thereby supporting societal efforts towards sustainable and climate-compatible technologies.

2.3. Dissemination, exploitation and communication activities planned

Scientific dissemination will include the publication of results in peer-reviewed international journals in the fields of microbial ecology, marine biology and environmental sciences. The PhD student will present the project outcomes at international conferences and scientific workshops. In line with open science principles, relevant datasets (environmental measurements, sequencing data) will be deposited in appropriate public repositories.

Exploitation and stakeholder engagement will be ensured through close interaction with GIPREB, the lagoon management authority of the Étang de Berre. Project results will be shared through technical meetings, written reports and joint data interpretation sessions with lagoon managers and scientists involved in the long-term monitoring of the lagoon. These exchanges will strengthen dialogue between research and management and enhance the operational value of scientific results.

Communication and public engagement activities will benefit from GIPREB's strong outreach actions. The PhD student will contribute to exchanges between scientists working on the Étang de Berre and will participate in awareness-raising activities targeting schools, bathing safety staff and the general public. These actions will help disseminate scientific knowledge on algal blooms, water quality and climate-driven ecosystem change.

Additional outreach will include contributions to institutional communication channels (MIO, BIAM and GIPREB websites and social media), participation in national events such as the Fête de la Science, and local dissemination events organised by GIPREB. Through these activities, the project will promote public understanding of microbial and viral processes in lagoon ecosystems and their relevance for ecosystem uses, environmental management and climate adaptation. In addition, the PhD student will give a public talk at the Bar des Sciences de Marseille focusing on the ecological importance and societal relevance of viruses in marine ecosystems.

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3. IMPLEMENTATION (2 pages max)

3.1. Work plan and interaction between tasks. The research is organised into three interconnected work packages (WPs), ensuring a progression from controlled laboratory experiments to ecosystem-scale interpretation. This structure allows the fellow to progressively acquire experimental, analytical and conceptual skills while achieving all scientific objectives within the 36-month duration.

WP1. The first year will be dedicated to WP1 at BIAM. This WP aims to quantify the physiological response of lagoon *Picochlorum* strains across gradients of temperature, salinity and irradiance. Growth and photophysiological parameters will be used to define optimal, suboptimal and stress conditions. This initial phase provides the mechanistic basis of the project and equips the student with skills in algal ecophysiology, experimental design and data analysis. The outcomes of WP1 will guide WP2, as they inform the selection of environmentally relevant conditions under which viral infection experiments will be studied.

WP2 will be conducted during the second year of the PhD at MIO, with occasional analyses performed at BIAM for targeted photophysiological measurements on infected algae. Viral infection assays will allow testing of how host physiological state modulates viral infection efficiency, replication dynamics and host mortality. MIO provides infrastructure and expertise in marine virology, enabling the fellow to acquire advanced skills in flow cytometry, viral quantification and molecular approaches. By linking environmental drivers, host physiology and infection outcomes, WP2 aims to elucidate the mechanisms through which viruses contribute to the regulation of *Picochlorum* blooms and their collapse. The results generated in WP2 feed into WP3 by defining environmental “windows” favourable to algal proliferation or viral control.

WP3 runs throughout the duration of the PhD and integrates laboratory findings with field observations from the Berre Lagoon. Monthly field sampling (one day per month) will be conducted by participating to the ongoing survey campaign piloted by GIPREB. Samples will be stored at MIO and mainly analysed during Years 2 and 3. Sequencing and physico-chemical analyses will be delegated to dedicated core facilities at MIO, while primary sequence processing will be performed by an experienced MIO bioinformatician. WP3 evaluates whether experimentally defined physiological optima (WP1) and infection-favourable conditions (WP2) can explain and predict seasonal patterns of *Picochlorum* proliferation and decline in situ. By confronting laboratory and field data, WP3 provides the ecosystem-level validation required to achieve the project’s predictive ambition.

International secondment will take place after completion of WP1 (Months 13–15) at NREL. Positioned at this stage, it does not interfere with experimental work and provides training in functional genomics applied to *Picochlorum*. Continuity of WP3 field sampling during the secondment will be ensured by the MIO team, guaranteeing uninterrupted data collection.

Expected scientific outputs and deliverables:

- Strain physiological response curves of *Picochlorum* across environmental gradients
- Quantification of environmental windows favouring *Picochlorovirus* infection, host proliferation or coexistence.
- A validated flow cytometry/qPCR workflow for joint host/virus monitoring
- Long-term time series of *Picochlorum* and *Picochlorovirus* abundances coupled to environmental data from the Berre Lagoon.
- A mechanistic framework to interpret and anticipate bloom initiation and collapse

Doctoral training is distributed across the 3 years of the PhD and complements research activities without overloading the fellow. The fellow will benefit from training programmes of the doctoral schools in Environmental and Biological Sciences. As BIAM and MIO are members of the IM2B institute, interdisciplinary training will be accessed through the Plinius



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4. ETHICS SELF-ASSESSMENT

All experimental work will be conducted on microalgal cultures and viruses under controlled laboratory conditions, or through environmental sampling of water from the Étang de Berre, without any intervention on protected species or habitats.

Field sampling will be carried out within the framework of existing monitoring programmes coordinated by GIPREB and in compliance with national and local regulations governing environmental monitoring. No invasive procedures, environmental risks or health and safety issues beyond standard laboratory and field practices are expected.

Data generated within the project (physiological measurements, molecular data, sequencing data) do not include personal or sensitive information and will be managed in accordance with open science and data management best practices.

Therefore, no ethical issues are anticipated, and no specific mitigation measures are required beyond standard good scientific practice and institutional safety procedures.

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