FINAL REPORT PROGRAM LEFE

Program LEFE CYBER	**Project Title : LINKS** Co-Limitation of light and nutrient on the mIxotrophy of nanophytoplanktonic communities in a coaStal ecosystem with strong environment gradient, the French Guiana coastal waters.	**Years 2020-2021**

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Contribution to

Other funding sources : SAD Region Bretagne

Context: Mixotrophy, the combined use of photosynthetic and heterotrophic nutrition within a single organism, constitutes an essential physiological trait in the understanding of the structural and functional biodiversity of marine plankton. Considering the expected proliferation of small phytoplankton cells in the ocean due to warming leading to ocean surface oligotrophication, the study of the nutritional modes and functional diversity of the nanophytoplanktonic communities present in a particular coastal ecosystem, the French Guiana coastal waters, would provide relevant information on the microbial loop functioning in coastal marine ecosystems co-limited by light and nutrient.

Objectives / scientific questions In the project LINKS, we proposed to investigate the effect of light and nutrient limitation on the mixotrophy of natural nanophytoplanktonic communities. The main hypothesis was that different nanophytoplankton communities prevail in the different water types presenting different levels of mixotrophy relative to the present nutrient and light conditions.

Main results The first part of the project (the part that has be funded) was a phase of preliminary tests to define the right methodologies for cell sorting and osmotrophy measurements with turbid water samples. D. Marie (research engineer from SBR and partner of this project) showed that, after a pre-filtration with 3 µm nylon filter, turbid samples of phytoplankton communities from Mackenzie River with suspended particulate matter (SPM) concentration between 5 and 100 g m^{-3} were well sorted. We proposed therefore to do this prefiltration to our samples dedicated for bacterivory measurements. For osmotrophy measurements, considering that the comments on the project was that in turbid water with suspended matter, associated bacteria would remain on the filter and contribute to the signal measured by mass spectrometry , we tested and developed a methodology to inhibit bacterial activities without modifying phytoplankton ones. For that, we tested the effect of an antibiotic cocktail (Penicilin 5000 U, Streptomycin 5 mg/mL, Neomycin 10 mg/mL; Middelburg and Nieuwenhuize 2000) and tetrasodium pyrophosphate (Ppi; Severin and Erdner 2019) on the cell abundance and the photosynthetic efficiency of five different non-axenic monospecific cultures of phytoplankton and on the cell abundance of their associated bacteria. We tested the addition of Ppi and antibiotics at three different final concentrations (0.1 mM, 0.2 mM (Severin and Erdner 2019) and 0.4 mM for Ppi and 1/2000, 1/1000 (Middelburg and Nieuwenhuize 2000) and 1/500 for antibiotics) to three replicates of 45 mL of *Isochrisis galban*a, *Dunaliella tertiolecta*, *Chaetoceros muelleri*, *Alexandrium minutum* and *Prorocentrum micans* cultures and a control (artificial seawater). At the beginning (t0), and after 2h, 5h and 24 h, we measured phytoplanktonic and bacterial abundances and maximum potential quantum efficiency of Photosystem II (F_v/F_m), index for photosynthetic efficiency. In parallel, we tested also different concentration of the antibiotics cocktail and Ppi on natural planktonic communities sampled in the Bay of Brest and measured planktonic and microbial cell abundance at t0 and after 2h, 5h and 24 h.

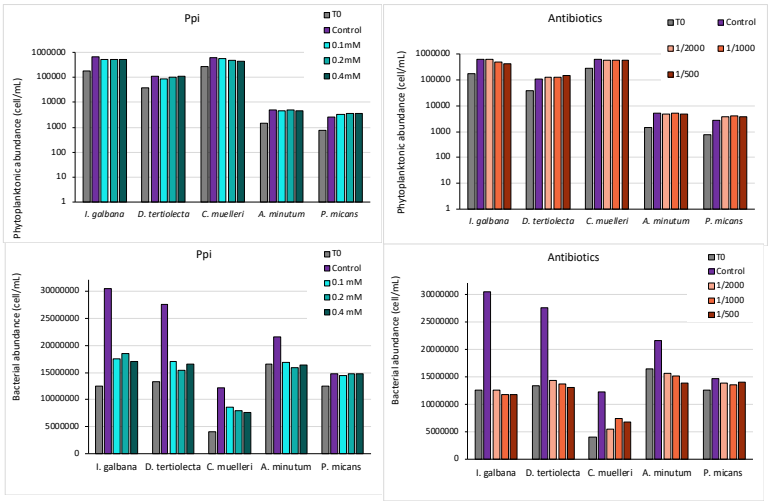

Fig. 1. Phytoplanktonic (top) and bacterial (bottom) abundance (cell/mL) after addition of Ppi (left) and antibiotics (right) at t0 and after 3h for bacteria and after 24 h for phytoplankton.

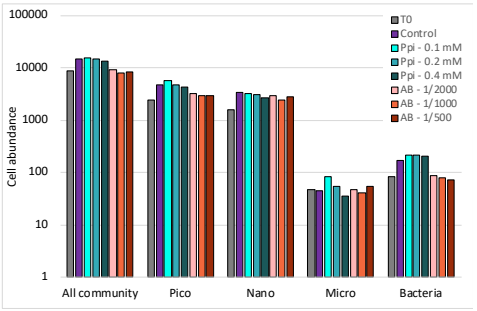

Fig. 2. Cell abundance (cell/mL for phytoplanktonic communities and 10^{-4} cell/mL for bacteria) after addition of Ppi and antibiotics at t0 and after 24 hours for natural planktonic communities.

For monospecific phytoplanktonic cultures, we observed that after 24 h, phytoplanktonic abundances were not affected by the addition of antibiotics and Ppi (Fig. 1). The associated bacteria had their abundance stabilized after the addition of the antibiotics in comparison to the untreated control (Fig. 1). However, bacterial abundance increased after the addition of Ppi (Fig. 1). We observed also that the photosynthetic efficiencies (F_v/F_m) of the different phytoplankton cultures were not reduced after the addition of Ppi and antibiotic (data not shown). For natural planktonic communities, most of the planktonic groups (nanophytoplankton, picophytoplankton and microphytoplankton) were not affected by the addition of antibiotics, showing a significant growth over 24 h- (Fig. 2) whereas bacteria were affected (Fig. 2). However, after addition of Ppi, the bacterial abundance after 24 h was significantly higher than the control (Fig. 2). From these results, we decided to use the antibiotic cocktail with a dilution of 1⁄2000 to stop bacterial activities without affection phytoplanktonic metabolism for osmotrophy measurements. We decided to apply 1⁄2000 dilution of antibiotics considering that it was the lowest concentration for which bacterial abundance was stabilized.

Future of the project After presenting some preliminary results and the cruise being accepted by the French oceanographic fleet, the LINKS project was finally accepted and funded by the LEFE 2021 call. We were able to do the cruise in October 2021 and we are currently doing laboratory analyses and collecting data.