FINAL REPORT PROGRAM LEFE

Program LEFE/ CYBER	Project Title: Biophymed Estimation of the phytoplankton biomass in the Mediterranean Sea	Years 2021 – 2022

PI: Lucile Duforêt-Gaurier, lucile.duforet@univ-littoral.fr

Laboratoire d'Océanologie et de Géosciences, UMR LOG 8187, ULCO, U. Lille, CNRS, IRD

Participating Laboratories: Mediterranean Institute of Oceanography & PRECYM Platform Aix-Marseille

Contribution to

Other funding sources : Institut des Sciences de la Mer et du Littoral, Université du Littoral Côte d'Opale

Context - Empirical relationships relating the carbon content of phytoplankton cells have been established exclusively from microcosm experiments. The Biophymed project proposes to revise these relationships for pico- and nanoeukaryotes from natural samples in the Mediterranean Sea using flow cytometry.

Objectives / scientific questions

The objectives of the Biophymed project were to:

- improve the size estimation by flow cytometry to derive the particle size distribution in natural samples from the Cytosense (CytoBuoy b.v.);
- estimate the carbon biomass in the phytoplankton groups identified in the Mediterranean Sea by CHN analysis after cell sorting;
- make some simple technical modifications to the ACCURI-C6 cytometer (BD Science) to measure the cell scattering more fully and improve size estimates by using additional information;
- better understand the impact of cell morphology on scattering.

Main results

As phytoplankton cell size is considered to be a fundamental parameter influencing several biological and ecological processes, we proposed to improve the size estimates obtained by flow cytometry. For this purpose, cell imaging is used as a reference to develop new empirical relationships between cell diameter and the optical signal measured by the flow cytometer. A new empirical method was developed using forward scatter (FWS) and fluorescence signals (FLR) from the Cytosense (Figure 1). This new method shows relative diameter errors of less than 25% (compared to cell images) instead of 50% with existing methods. In addition, the size distribution is consistent with the size distribution seen in the cell images.

At the same time, we developed an improved version of the ACCURI-C6 cytometer, consisting of adding two detectors to measure the scattering at 45° and 135° (in addition to the existing detectors at 5° and 90°). It is a proof-of-concept highlighting that measurement of four scattering angles, instead of two, allows better estimates of particle size. A such proof-of-concept will enhance the need to develop a new advanced cytometer to measure the full scatter patterns of bioparticles, that may contain much more information than what forward scatter, and perpendicular (side) scattering.

Furthermore, a protocol is now fully operational to measure the carbon content (POC) from CHN analysis on the phytoplankton populations *(synechococcus*, picoeukaryotes and nanoeukaryotes) sorted by the INFLUX MARINER cytometer (PRECYM platform, Aix-Marseille University) (ILLUSTRATION 2). New relationships will be soon established between the carbon content and biovolume.

ILLUSTRATION 1

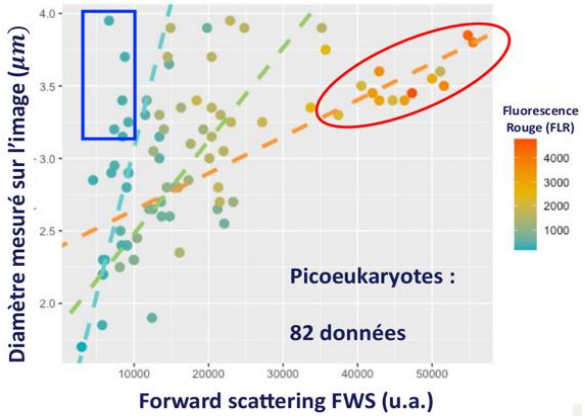

ILLUSTRATION 2

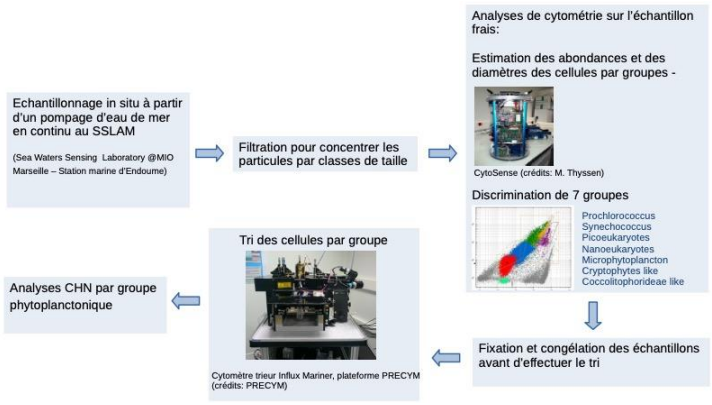

ILLUSTRATION 1: Relationship between diameter measured on phytoplankton cell images and forward scattering (FWS) as measured by the Cytosense for picoeukaryotes (the same was done for nanoeukaryotes). The red ellipse and blue square show cells of similar size but with very different intensities of red fluorescence (FLR) and very different relationships between diameter and FWS

ILLUSTRATION 2: Main steps of the protocol for the quantification of particulate organic carbon in phytoplankton groups identified by cytometry

Future of the project:

The future will consist in studying the variability of the relationship between POC and biovolume as a function of, depth, PAR, and nutrient concentration. Sampling will be also conducted in the English Channel and the variability of the empirical relationships according to the region will be addressed. The LEFE project BIOPHYMED allowed us to make a proof-of-concept, showing that the measurement of additional scattering angles is valuable to improve size estimates and open up perspectives to better characterize cell morphology. We are now part of a consortium with Purdue University and the subcontractor Miftex Corporation to develop an advanced cytometer measuring the scattering from few degrees to 160°.

Number of publications, communications and theses

A publication on the size improvements by cytometry in preparation

A communication (poster)for the Ocean Optics Conference, Las Palmas de Grand Canaria, Spain, 6-11 October 2024.

Data availability

Data will be available after the publication of the results on Seanoe (https://pangaea.de/)