“Exceptional” Call for SCOR Project and Working Groups Scholars

Activity Report

SCOR Working Group
SCOR Working Group 165. Mixotrophy in the Oceans – Novel Experimental designs and Tools for a new trophic paradigm (MixONET)

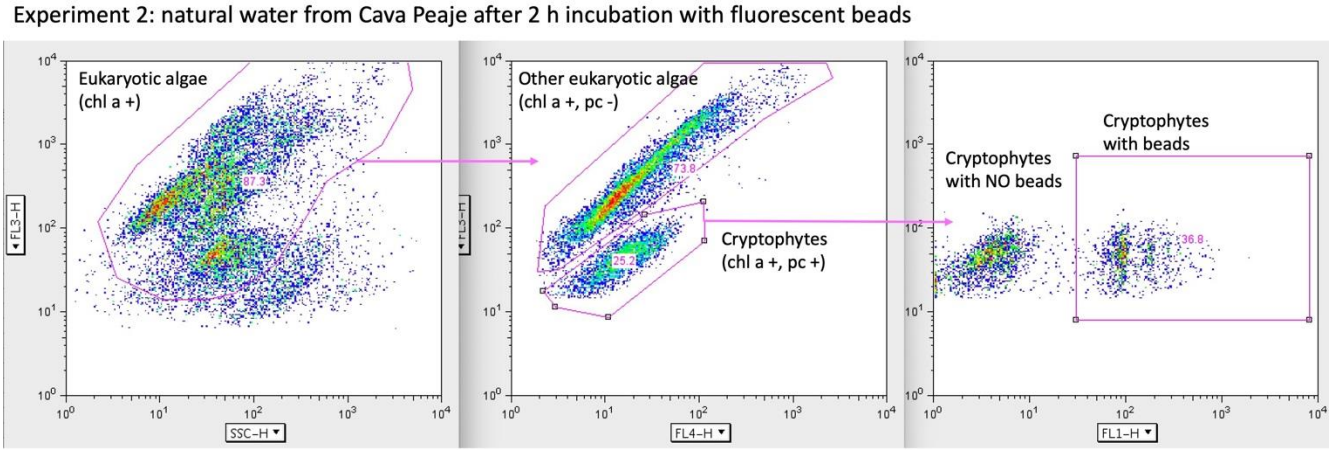
Researchers
Luciana Santoferrara, Hofstra University, New York, USA (visitor)
Fernando Unrein, INTECH (UNSAM-CONICET), Buenos Aires, Argentina (Host)

Dates and locations of the visit
March 16-19, 2023: INTECH (UNSAM-CONICET), Chascomus, Buenos Aires, Argentina
March 20-24, 2023: UNNOBA, Junín, Buenos Aires, Argentina

Summary of activities
This project aligns with MixONET’s ToR 2 (Biological oceanography research methods under the mixoplankton paradigm) and ToR 3 (Development of new biological oceanography methods for the study of mixoplankton). The goal of the project is to establish a novel method to quanfy and identify mixoplankton.

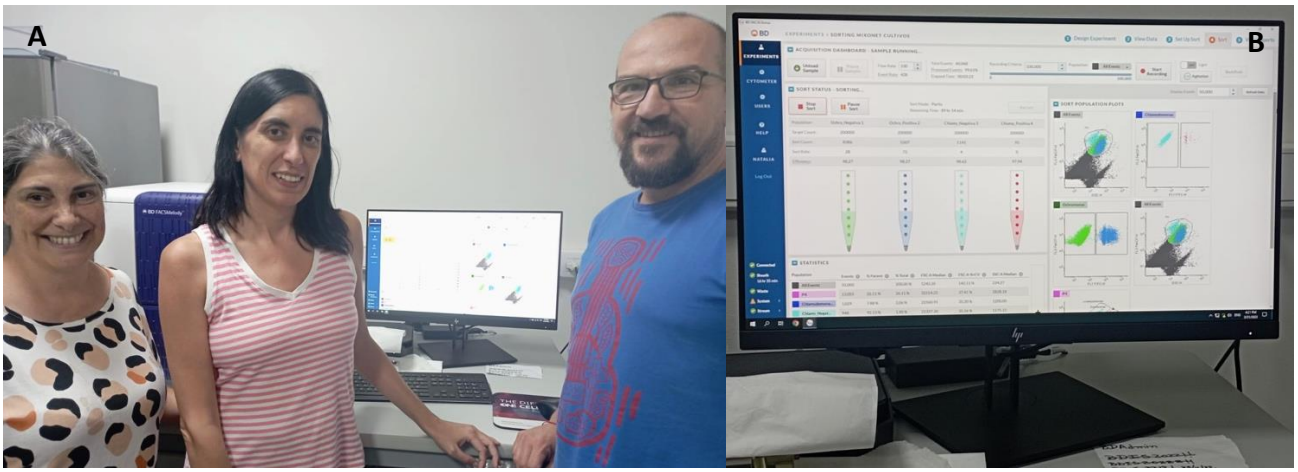
At INTECH, three experiments were conducted using either natural water samples or mixed microalgal cultures including mixoplankton species. Samples were incubated with subrogate prey (1-µm plastic fluorescent beads) for 2 to 3 h. At various times, samples were preserved for flow citymetry and cell sorting. Cells containing both chlorophyll autofluorcence (indicating photosyntetic capabilieites) and subrogate prey (suggesting ingestion) were differentiated and assumed to be mixoplankton (Figure 1).

Figure 1. Example of data analysis by flow cytometry. After two hours of incubation with fluorencent beads, we can differentiate mixoplankton with both autofluorecence and prey.



At UNNOBA, fractions with and without mixoplankton were sorted for two goals (Figure 2). On one hand, sorted fractions were concentrated on filters, stained with DAPI and stored at -20ºC for epifluorence microscopy analysis. This will enable us to confirm that fractions sorded as mixoplankton indeed contain subrogate prey in their cytoplasm. On the other hand, fractions were collected in lysis buffer, immediately subjected to freezing-defrizing for cell lysis, and used for polymerase chain reaction of the 18S rRNA gene. Eight rounds of PCR reactions were done to troubleshoot two sets of primers, master mix and lysis conditions. Attemps have not been succesful so far (possibly due to a low concentration of DNA) and will be continued whithin the next month, including steps for DNA extraction. Success in this task will enable DNA sequencing and detailed taxonomic identification of mixoplankton.

Figure 2. The BD FACS Melody cell sorter available at UNNOBA enabled us to sort mixoplankton and non-mixoplankton fractions. A, from right to left: F. Unrein, L. Santoferrara and UNNOBA's flow citometry technician, N. Menite. B, cell sorting in progress. Tubes of different collors represent collection of mixoplankton that ingested beads, putitative mixoplankton that did not ingest beads after the incubation, and non-mixoplankton.



In addition to Drs. Unrein and Santoferrara, the activity involved Dr. Romina Schiafinno and technician Natalia Menite at UNNOBA. During the visit at UNNOBA, Drs. Unrein and Santoferrara had multiple chances to discuss the project and other research experiences with Dr. Schiafinno's team of three female members, including PhD and MS students. At INTECH, Drs. Unrein and Santoferrara gave a 1.5 h talk and discussion about the project with attendance of about 10 researchers and PhD students (Figure 3). The project was also announced on social media to raise awarness on mixoplankton and SCOR-funded activities (Figure 4).

Figure 3. Project discussion at INTECH.

Products and future interactions

The ingestion experiments, flow cytometry and cell sorting were successful, and we trust the molecular work will yield products in new attempts within the next month. Given the innovative nature of our approach, preliminary results will be presented at an international meeting, possibly the ISME-LAT meeting happening in Buenos Aires, Argentina later this year. The method will also be tested in other aquatic environments and a publication in an international, peer-reviewed journal will be produced.

This activity has opened the door for collaboration among Drs. Unrein and Santoferrara. Funding opportunities are being explored to continue this work. Dr. Schiafinno and team were also invited to participate in the project, and they will carry on additional troubleshooting and optimization of molecular work. Posibilities of short-term visit to Dr. Santoferrara's lab by team members were also discussed, and will consider, for example, the SCOR-POGO Visiting Felowship.

Figure 4. Social media screenshots.