



## “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

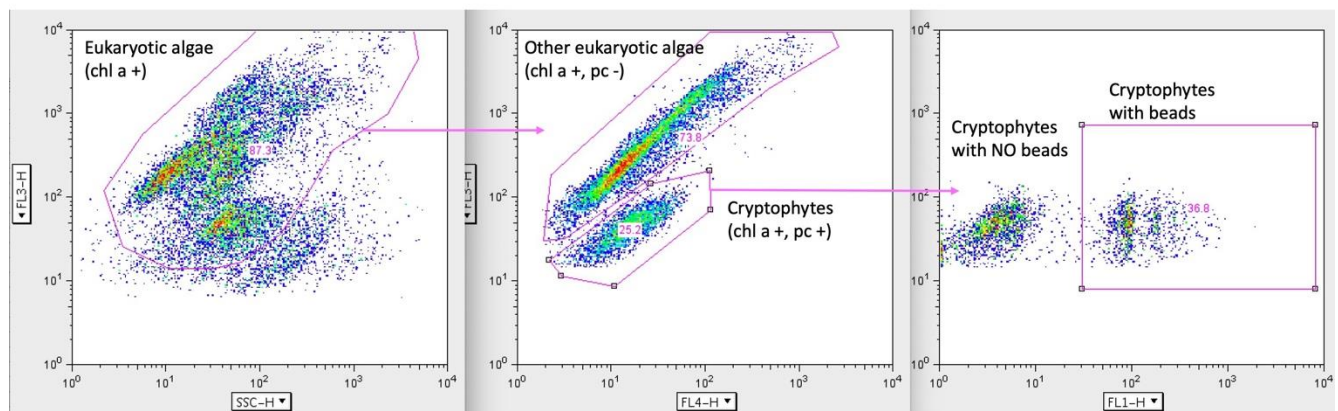
### Summary of activities

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 quantify 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 surrogate prey (1- $\mu\text{m}$  plastic fluorescent beads) for 2 to 3 h. At various times, samples were preserved for flow cytometry and cell sorting. Cells containing both chlorophyll autofluorescence (indicating photosynthetic capabilities) and surrogate prey (suggesting ingestion) were differentiated and assumed to be mixoplankton (Figure 1).

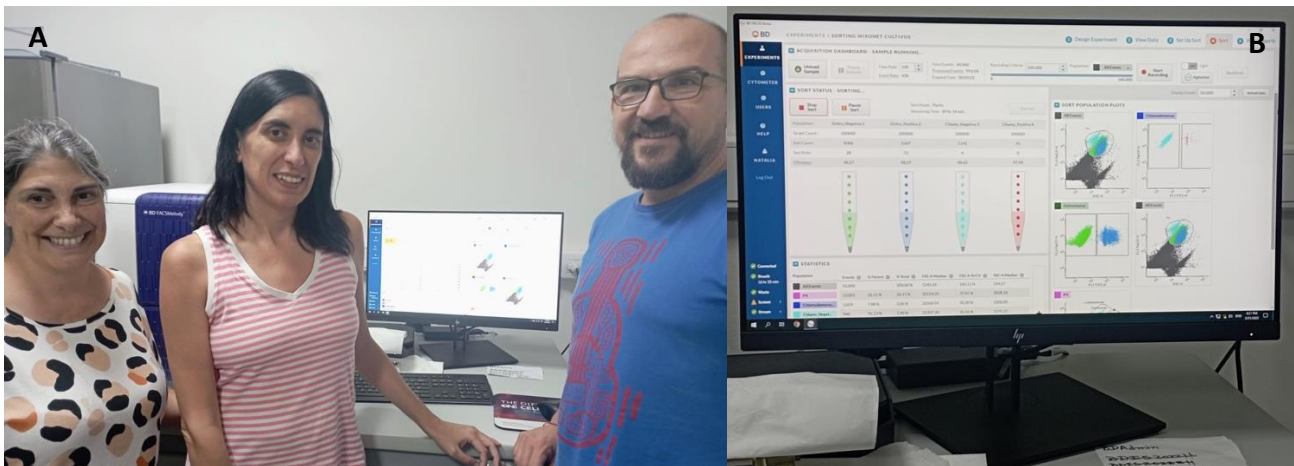
**Figure 1.** Example of data analysis by flow cytometry. After two hours of incubation with fluorescent beads, we can differentiate mixoplankton with both autofluorescence and prey.

Experiment 2: natural water from Cava Peaje after 2 h incubation with fluorescent beads



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  $-20^{\circ}\text{C}$  for epifluorescence microscopy analysis. This will enable us to confirm that fractions sorted 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. Attempts have not been successful so far (possibly due to a low concentration of DNA) and will be continued within 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 cytometry technician, N. Menite. B, cell sorting in progress. Tubes of different colors represent collection of mixoplankton that ingested beads, putative 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 awareness on mixoplankton and SCOR-funded activities (Figure 4).

**Figure 3.** Project discussion at INTECH.

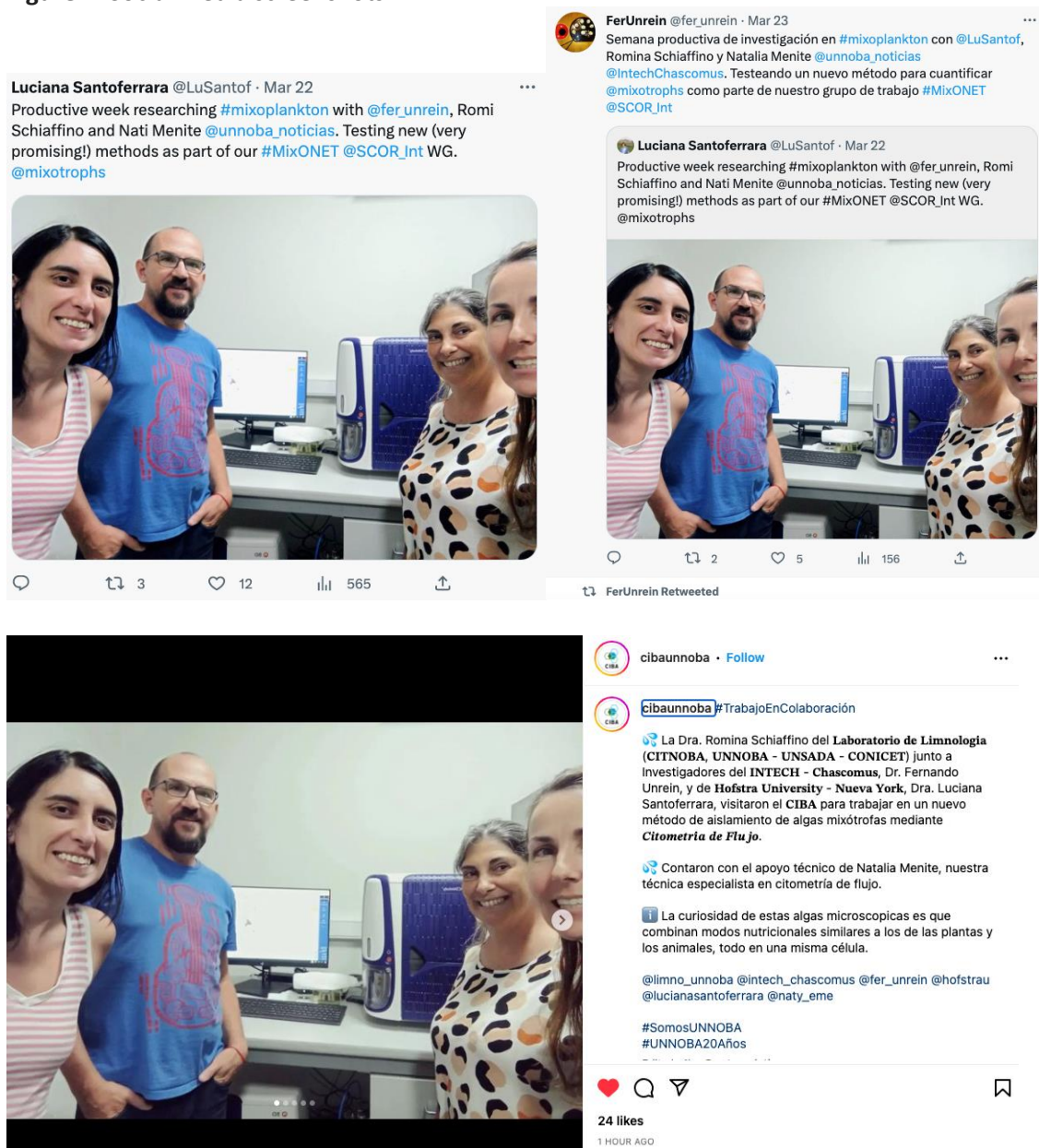


## Products and future interactions

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. Schiaffino and team were also invited to participate in the project, and they will carry on additional troubleshooting and optimization of molecular work. Possibilities of short-term visit to Dr. Santoferrara's lab by team members were also discussed, and will consider, for example, the SCOR-POGO Visiting Fellowship.

Figure 4. Social media screenshots.



The figure displays three social media posts related to a research collaboration. The top-left screenshot is a tweet from Luciana Santoferrara (@LuSantof) dated March 22, 2023. The text reads: "Productive week researching #mixoplankton with @fer\_unrein, Romi Schiaffino and Nati Menite @unnoba\_noticias. Testing new (very promising!) methods as part of our #MixONET @SCOR\_Int WG. @mixotrophs". Below the text is a photograph of four people in a laboratory setting. The top-right screenshot is a tweet from FerUnrein (@fer\_unrein) dated March 23, 2023, which retweets the tweet from Luciana Santoferrara. The text reads: "Semana productiva de investigación en #mixoplankton con @LuSantof, Romina Schiaffino y Natalia Menite @unnoba\_noticias @IntechChascomus. Testeando un nuevo método para cuantificar @mixotrophs como parte de nuestro grupo de trabajo #MixONET @SCOR\_Int". The bottom screenshot is a post from Cibaunnoba, dated one hour ago, with 24 likes. The text describes a collaboration between the CIBA laboratory and researchers from UNNOBA, UNSADA, CONICET, INTECH-Chascomus, Hofstra University, and Luciana Santoferrara. The post mentions the use of flow cytometry for the isolation of mixotrophic algae and lists the participating researchers and their affiliations.

**Luciana Santoferrara** @LuSantof · Mar 22  
Productive week researching #mixoplankton with @fer\_unrein, Romi Schiaffino and Nati Menite @unnoba\_noticias. Testing new (very promising!) methods as part of our #MixONET @SCOR\_Int WG. @mixotrophs

**FerUnrein** @fer\_unrein · Mar 23  
Semana productiva de investigación en #mixoplankton con @LuSantof, Romina Schiaffino y Natalia Menite @unnoba\_noticias @IntechChascomus. Testeando un nuevo método para cuantificar @mixotrophs como parte de nuestro grupo de trabajo #MixONET @SCOR\_Int

**cibaunnoba** · Follow  
#TrabajoEnColaboración  
La Dra. Romina Schiaffino del Laboratorio de Limnología (CITNOBA, UNNOBA - UNSADA - CONICET) junto a Investigadores del INTECH - Chascomus, Dr. Fernando Unrein, y de Hofstra University - Nueva York, Dra. Luciana Santoferrara, visitaron el CIBA para trabajar en un nuevo método de aislamiento de algas mixótrofas mediante **Citometría de Flujo**.  
Contaron con el apoyo técnico de Natalia Menite, nuestra técnica especialista en citometría de flujo.  
La curiosidad de estas algas microscópicas es que combinan modos nutricionales similares a los de las plantas y los animales, todo en una misma célula.  
@limno\_unnoba @intech\_chascomus @fer\_unrein @hofstrau @lucianasantoferrara @naty\_eme  
#SomosUNNOBA  
#UNNOBA20Años