

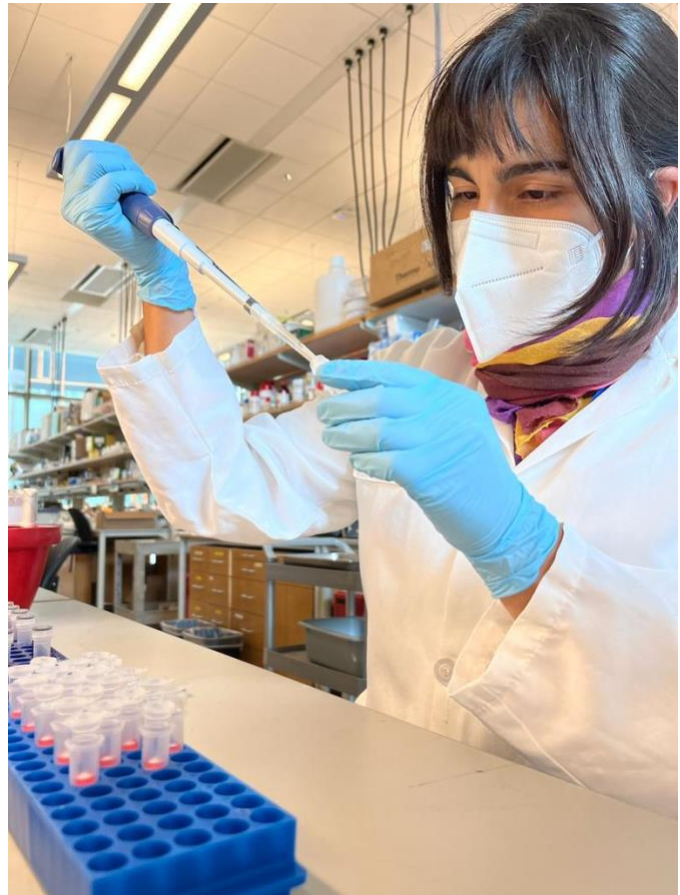


SCAR Fellowship Report



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Diversity of bloom-forming phytoplankton species in Western Antarctic Peninsula (WAP) nearshore waters



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Host Institution: Scripps Institution of Oceanography. Dr. Maria Vernet, mvernet@ucsd.edu, United States.

Dates of Activity

July 2022-October 2022

Introduction

Studies on the phytoplankton community composition and dynamics are needed to understand future changes in the western Antarctic Peninsula (WAP) ecosystem. Nearshore WAP waters, fjords, and channels congregate predators like baleen whales, seals, and penguins (Ducklow et al. 2013). Understanding the microbial diversity that sustains this food web is fundamental due to expected modifications in this region by increasing meltwater delivery from glaciers (Cook et al. 2016), in particular bloom-forming species that cycle a large proportion of the carbon in surface waters (Vernet et al. 2008).

Since 2016, the Citizen Science project FjordPhyto (www.fjordphyto.org) has been collecting water samples from the WAP coastal areas during the spring-summer season, from November to March (Cusick et al. 2020). These samples have allowed researchers to understand the phytoplankton composition of these underexplored nearshore areas (Mascioni et al. 2019). So far, we have identified that the main phytoplankton blooms are dominated by species never described for Antarctica (Scott and Marchant 2005). One of them is the first record of a dinoflagellate bloom in the WAP (Mascioni et al. 2019). Currently, analysis that combine microscopy and molecular analysis are fundamental in describing new phytoplankton species, but they are quite rare (e.g., Moro et al. 2002; de Salas et al. 2008). Recent genomic studies in WAP have found a large number of gene sequences that do not coincide with previously described organisms (e.g., Luo et al. 2016; Abele et al. 2017). In this way, it is essential to combine molecular and morphological approaches to assess phytoplankton diversity in the region. Since I already had microscopic analyses of the FjordPhyto samples, we decided to undertake a series of genetic analyses that would allow us to complement the microscopy and describe new species in the WAP.

Project Objectives

1. Characterize bloom-forming phytoplankton species in previously underexplored nearshore waters along the WAP by combining detailed microscopy and molecular techniques in the frame of a citizen science project.
2. Obtain training to become proficient in using molecular tools to elucidate phytoplankton biodiversity.
3. Create outreach materials on Antarctic phytoplankton to increase Antarctic literacy with the public.

Methods, Execution and Results

During my visit at Scripps Institution of Oceanography, Dr. Vernet's collaborator, Dr. Andrew Allen, kindly allowed me to use his laboratory at the J. Craig Venter Institute (JCVI) to perform the molecular analyses that my internship included. JCVI is a leading institute in genomic analysis and particularly in Dr. Allen's lab they already work with microalgae and phytoplankton, so they had the necessary instruments to carry out my analysis.

In the laboratory I worked on the extraction, purification, and quantification of DNA from different samples from Antarctica. In a first training phase, I practiced with environmental samples from the FjordPhyto project sampling during the last summer (2021-2022). Then I performed the same task with culture samples that I had brought from my laboratory in Argentina. In order to use my lugol-fixed samples I followed Auinger et al. (2008) protocol. I also applied the same techniques with fixed samples

that I had previously analyzed with microscopy in La Plata, i.e., samples with blooms of unidentified organisms. We performed metagenomic analysis on the bloom samples to obtain genetic information. In addition, since the organisms we were specifically interested in were dinoflagellates, we searched the literature for dinoflagellate-specific primers. We ordered custom primers and performed the extraction with them following the literature protocols (Sunesen et al. 2020, Ott et al. 2022). Finally, the DNA extracted from the various analyses performed in the laboratory was sent for sequencing via Sanger or Illumina platforms, depending on the case.

In a second training phase, I isolated single cells under the microscope for DNA extraction, cells with different fixatives and from live cultures to evaluate the effectiveness of the methods. From these analyses and a literature search, we performed a series of different protocols in order to make the extraction and subsequent amplification of DNA. Although we tested many protocols and with a large number of cells (n=48) from different samples and with different fixatives, we did not obtain positive results in most cases. In the literature review, we found that the chances of obtaining DNA from a single cell dropped considerably when performed on samples that were fixed and stored for long periods of time (Hamilton et al. 2015, Hernández-Rosas et al. 2018). Although the isolated cell DNA analysis protocols did not come to fruition, we have some clues as to what might work and what other things could be tested in the future, as time was not enough.

Project Outcomes

The main results of this training are the numerous genetic sequences obtained that will allow us to identify the organisms that form blooms, as well as those organisms that could be new species for the WAP. These sequences are extremely valuable, because they will also have a "curation" at the morphological level, meaning that these sequences will be contextualized with microscopic observations, improving optical descriptions and identifications. These sequences will be available in online gene libraries (i.e., Genbank). Ultimately, the data from this internship will be made available on the SCAR Antarctic Biodiversity Portal which currently has very low phytoplankton taxa.

In terms of personal development, this internship allowed me to professionally acquire new methodological tools, I had never performed molecular analysis prior to this experience and after this intensive training I feel capable of performing them anywhere. In addition, by working with various instruments and protocols gave me the tools to interpret a great variety of results.

Publications, Presentations and Products

During my stay in San Diego, I worked on a manuscript that is scheduled to be submitted to a scientific journal in February 2023. This work describes phytoplankton biomass, cell abundance, composition, and succession throughout the spring-summer months (November to March) in six WAP coastal areas connected to the Gerlache Strait. The sampling locations are between 64° and 65° S and were visited for three consecutive years. Significant differences in phytoplankton community composition and succession patterns were found. These differences were mainly associated with the presence of a surface thermal front in the Gerlache Strait, which separates warmer waters to the north and colder waters to the south, which was confirmed by the analysis of 10 years of satellite images. Results corresponding to

this manuscript were presented virtually during the SCAR 2022 conference. I participated in this event while in San Diego.

As for the results of this internship, data analyses of the sequenced WAP blooms are underway. While in San Diego I concentrated on lab analyses as those are more challenging. I will perform data analysis from Argentina. We plan to publish the metagenomic results, and the new protocols developed, in collaboration with my hosts and collaborators at Scripps and JCVI.

I plan to present preliminary results during upcoming international conferences, e.g., the XIII SCAR Biology Symposium to be held from July 31st to Aug 4th 2023 in Christchurch, New Zealand.

Capacity Building, Education and Outreach Activities

From the citizen science project FjordPhyto we carry out important scientific communication activities in social media. As an active participant in the project, I have made numerous videos and posts that can be seen on the project's social media channels @fjordphyto, Instagram, Facebook, Twitter, and YouTube. In addition, thinking about reaching a wider audience, we have recently started to make all Instagram and Facebook posts bilingual, in Spanish and English, and my main contribution here is the translation into Spanish. Furthermore, all the results, publications, interviews, reports, and more produced by the project are available on our web page www.fjordphyto.org.

As part of this fellowship, I was invited last summer (2021-2022) to embark on two IAATO member cruises. On board these cruises I had the opportunity to be in close contact with polar guides and travelers conducting FjordPhyto sampling in the field. These trips allowed me to collect new phytoplankton samples that were analyzed during my stay in San Diego, as well as to work together with tourism, sharing my knowledge in lectures, sampling experiences, and in the laboratory on board, among other things.

During my stay in San Diego, I had the opportunity to participate with Allison Cusick in the NightSchool program organized by the California Academy of Sciences, that comprises virtual chats with experts on different topics open to the public.

I also had the opportunity to participate in the Scripps Student Symposium in person. Although I did not have the opportunity to present, I was able to listen to the talks of other doctoral students, network, and learn about the different research topics carried out at the Scripps Institution of Oceanography.

While in San Diego, I met two polar guides from IAATO ships, Dr. Daniel Moore and Ms. Emily Cunningham, who were trained in FjordPhyto methods to continue in the upcoming 2022-2023 season, and also discuss different opportunities to do science on polar ships.

Future Plans and Follow-ups

During my stay in San Diego, the Vernet Lab received new funding from NASA for the next three years. In addition to collecting phytoplankton and oceanographic measurements, the FjordPhyto project will correlate ground measurements to satellite observations in the WAP. After I finish my PhD in 2023, I plan to keep

participating actively in the FjordPhyto project and therefore collaborating with Dr. Maria Vernet, Dr. Rick Reynolds, and graduate student Allison Cusick.

Most of the results of this internship are still in process, i.e., sequencing Illumina takes about two months. Once I have these results, I will start the stage of computational analysis. During this period, I will keep in touch with my internship tutors and collaborators for analysis and subsequent manuscript writing. I anticipate these data will become part of my post-doctoral fellowship proposal.

Personal Impact

This internship is definitely one of the highlights within my PhD career. Although this internship was delayed for 2 years during the COVID-19 pandemic and occurred later than expected, at the end of my PhD, it allowed me to create links and connections with other PhD students, postdocs, and researchers from other institutions.

This visit was an excellent way to come back to lab work after two years of being remote and virtual, it allowed me to get closer to a lot of colleagues with whom I once exchanged e-mails or a zoom meeting, being able to interact in person was highly rewarding and very valuable. It also allowed me to expand my professional circle and learn how people work in other parts of the world.

Financial Statement

The funds from the SCAR Fellowship covered flights and travel costs from Buenos Aires (Argentina) to San Diego, California (USA), accommodation, subsistence, health insurance, and local transportation for three months of living in La Jolla, California. The SCAR Fellowship was supplemented by an IAATO Fellowship, as the funds were insufficient for a 3-month stay in California. In addition, the SCAR funds covered the costs for supplies (isolation kits, primers, working solutions, etc.) and sample sequencing with Illumina and Sanger techniques.

Acknowledgements and References:

I would like to thank the support of the SCAR secretariat during the pandemic, I could not have done this internship without their understanding. I would also like to thank Dr. Maria Vernet and Allison Cusick, for hosting me at Scripps Institution of Oceanography, for their patience and help in making this visit possible, and for introducing me to Dr. Allen, who welcomed me to his laboratory. Finally, a special thanks to Dr. Allen and his entire team who opened the doors of his laboratory at the J. Craig Venter Institute to allow me to carry out all the laboratory experiments.

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