Title
FjordPhyto: Antarctic Citizen Science Project



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# FJORD PHYTO

## ANTARCTIC CITIZEN SCIENCE PROJECT

Photo by Maria Stenzel


Allison Lee

CAPSTONE PROJECT

MASTER OF ADVANCED STUDIES

CENTER FOR MARINE BIODIVERSITY & CONSERVATION

SCRIPPS INSTITUTION OF OCEANOGRAPHY, UC SAN DIEGO

**Capstone Project Abstract**

The FjordPhyto Citizen Science project is designed to engage the International Association of Antarctic Tour Operators and their Guests in hands-on science as they journey along the fjords of the west Antarctic Peninsula. The Antarctic Peninsula is one of the fastest warming regions in the world. Melting glaciers bring an influx of freshwater and nutrients into the fjords potentially altering the biology at the phytoplankton level. Phytoplankton play a critical role in regulating the atmosphere, drawing carbon dioxide into the ocean and producing over half of the Earth's oxygen. These microscopic drifting plants make up the foundation of the food system supporting whales, seals, and penguins. FjordPhyto aims to understand how glacial meltwater impacts phytoplankton communities among various fjords throughout the austral summer. Visitors will collect phytoplankton samples and photograph images using simple-to-operate tools. Equipment and educational materials will be provided by the FjordPhyto research team as outlined in this Capstone Project. Citizen Science is a powerful tool bringing travelers and scientists together to answer critical science questions. FjordPhyto provides a fun and easy way to involve visitors in the legacy of research in Antarctica, while providing scientists with data that greatly expands the current knowledge of Antarctic fjord ecosystems.

*Capstone Advisory Committee Final Capstone Project Signature Form*

# The Invisible Forest: Using Citizen Science to Improve Understanding of Antarctic Phytoplankton

Allison M. Lee

**Spring 2017**

**MAS Marine Biodiversity and Conservation**

**Capstone Project**

Capstone Advisory Committee

Signature ______ Print Name Dr. Maria Vernet Date 6/7/17

Affiliation SIO - UCSD Email mvernet@ucsd.edu Phone ______

Signature ______ Print Name Dr. George Watters Date 6/8/17

Affiliation NOAA - SWFSC - AERD Email George.Watters@noaa.gov Phone 858-546-7198

# CAPSTONE PROJECT

## DELIVERABLES

Capstone Deliverables List: Proposed vs. Accomplished

Poster Presentation: Gordon Research Conference – Polar Marine Science

Promotional Flyers: International Association of Antarctic Tour Operator Conference

User's Guide for Fjord Phyto Antarctic Citizen Science Project

National Science Foundation Supplemental Funding Request

**CAPSTONE DELIVERABLES**
**Allison Lee**
**MAS-MBC 2016-2017**

| **Deliverables I had proposed to accomplish:** | **Deliverables I accomplished:** |
|---|---|
| *Secure Funding:*<br>- Complete a full proposal to fund the pilot phase into implementation phase. | *Secure Funding:*<br>○ Submitted a 15 page supplemental grant to NSF under the PPSR Public Participation in STEM Research |
| *Raise Awareness:*<br>- Create a poster to present at Gordon Research Polar Marine Science Conference, March 25-30, 2017.<br>- Develop an impactful 5-minute "PechaKucha 20x20" talk to deliver to non-science communities at both the Ignite Talk San Diego, May 19, and the annual IAATO conference, May 3-5.<br>- Write blog posts about the impact of Citizen Science, Fjords, and Antarctic research efforts to be published on the FjordEco website and in the IAATO Science Newsletter.<br>- Write a magazine article about the importance of ecotourism travel for conservation, and submit to Sevenseas Travel Conservation Magazine. | *Raise Awareness:*<br>○ Created a poster and presented at the Gordon Research Polar Marine Science Conference, March 25-30, 2017, Ventura, CA.<br>○ Developed a 5-minute mock pitch and delivered to Waitt Foundation for comment, March 8$^{th}$, 2017, SIO, CA<br>○ Attended the Citizen Science Association Conference to meet experts in the field and share my Capstone idea, May 21-30, 2017, St. Paul, MN.<br>○ Created promotional flyers to send to IAATO meeting, May 3-5, Edinburgh, Scotland.<br>○ Built connections with tour operators: National Geographic Lindblad Expeditions, Quixote Expeditions, Polar Latitudes, G-Adventures, Ocean Expeditions, Cheeseman Safaris.<br>○ *Still intend to write a magazine article for Sevenseas and Blogs but at a later date, not as a deliverable for this MAS.* |
| *Create Programs:*<br>- Develop a working pilot study in California to implement methods outlined above.<br>- Create simple easy-to-use identification field guides specific to Antarctic zooplankton and phytoplankton to be used by Citizen Science participants.<br>- Design webinar trainings to deliver to participating IAATO tour operators. | *Create Programs:*<br>○ *Still need to develop a working pilot citizen science project in California to implement methods.*<br>○ Created a FjordPhyto User's Guide for implementation in the field.<br>○ Created a simple to use ID guide for Antarctic zoo and phytoplankton, added to User's Guide.<br>○ Designed website with preliminary training videos, sampling procedures, data sheets and information to deliver to participating IAATO tour operators. |

# Developing an Antarctic citizen science program to understand glacial meltwater impacts on fjord phytoplankton communities

Allison Lee[1] | Dr. Maria Vernet[1] | Dr. George Watters[2] | [1]Scripps Institution of Oceanography, UCSD (SIO) | [2]Antarctic Ecosystem Research Division, U.S. National Oceanic and Atmospheric Administration (NOAA-AERD)

## Introduction

**FjordEco**, a National Science Foundation funded project, investigates the impacts of glacial meltwater on fjord ecosystem structure and function on the west Antarctic Peninsula (WAP).

**Current research programs** along the WAP (Fig.1) have contributed a great deal to our understanding of ecosystem dynamics along the continental shelf and open ocean, yet coastal fjord inlets remain to be fully characterized, particularly at the level of primary producers (Garibotti et al., 2005; Moline et al., 2008). During the 2016-2017 season, FjordEco researchers teamed up with Antarctic tour operators to collect scientific samples (Fig. 2b). As a continuation of this effort, we aim to develop a citizen science program that leverages the help of the ecotourism community to gather much needed data throughout the melt season.

**Citizen science** brings volunteers and scientists together in order to answer real-world questions using the scientific process.

**Ecotourism** is environmentally responsible travel to natural areas, distinguished by its emphasis on conservation, education, traveler responsibility and active community participation.

## Scientific Questions

1. How is the phytoplankton community composition structured within the fjords?
2. How does glacial meltwater affect the composition and phenology of phytoplankton over the course of the melting season?

## Proposed Approach

- Leverage help of the International Association of Antarctic Tour Operators (IAATO).
- Tourist vessels visit the coastal fjords steadily November through March.
- Design program around popular sites visited by zodiac landings to shore (Fig.
- Train on-board Naturalists and Citizen Science Coordinators to lead efforts in the field.
- Interested participants collect scientific data as they venture to shore which will fill the phenology and species composition gap of phytoplankton blooms during the austral summer.

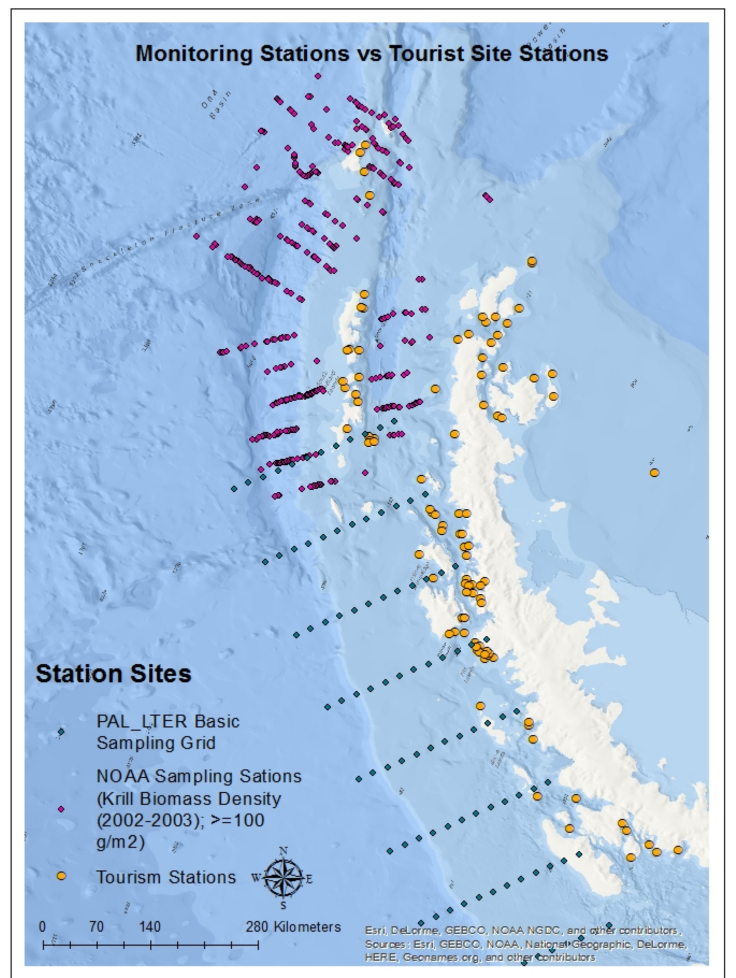


**Fig. 1**. Map showing various station sites.

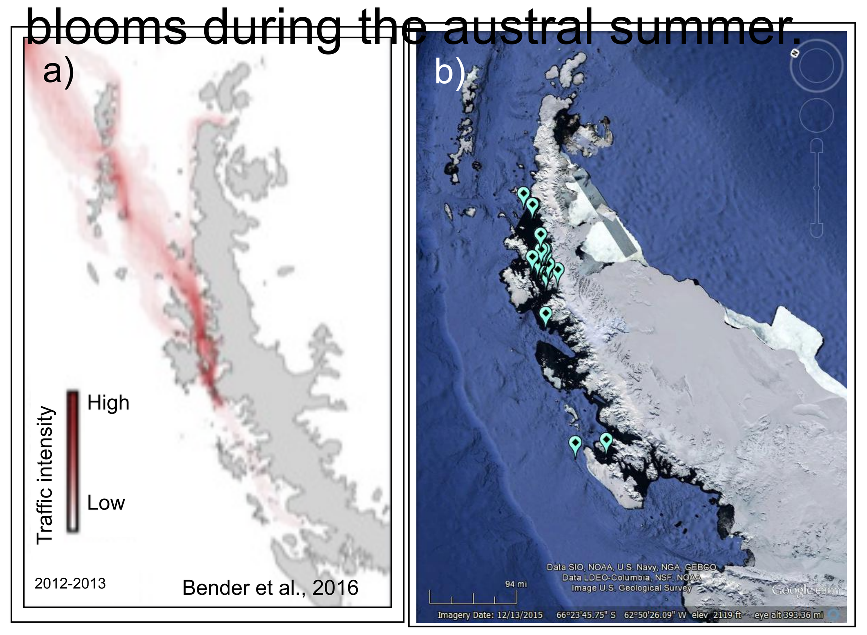


**Fig. 2.** (a) Map tourism vessel traffic. Adapted from Bender et al., 2016. (b) Sites sampled by FjordEco tourist vessel partners 2016-2017 season.

## Program Development Toolkit

Over 25 U.S. federal agencies developed The Federal Crowdsourcing and Citizen Science Toolkit (U.S. Federal Government, 2016). We will use five basic steps for planning, designing and carrying out a citizen science project with the IAATO community (adapted from Bonney et al., 2009).

- **Scope Out Your Problem**: Need more frequent sampling data throughout the glacial melt season to determine phytoplankton community composition changes and phenology of bloom within fjords of WAP.
- **Design a Project**: Using simple low-cost technology, tourists can easily contribute to scientific data. Educational material will be provided for learning opportunities.
- **Build a Community**: Leverage the Antarctic tourism community (e.g., naturalists and guests). Build greater interest in science and the understanding of scientific principles used to understand complex ecosystems.
- **Manage Your Data**: Ensure quality and usefulness of data by standardizing protocols, offering training, and data verification prior to analysis and publication. Provide collaborators with access to public data.
- **Sustain and Improve**: Provide standardized training methods and personal connection between tourists and scientists. Contribute to long-term science and increased excitement about the fjord ecosystems.

## The Community

**IAATO:** Self-organized • Founded in 1991 • 100+ companies

**Mission:** *Practice and promote safe and environmentally responsible travel to Antarctica. Support science in Antarctica through cooperation with National Antarctic Programs, including logistical support and research and to foster cooperation between private-sector travel and the international science community in the Antarctic* (IAATO, 2017).

## The Method

- Meltwater characteristics measured using a hand-held unit (Fig. 3.a).
- Plankton nets capture zooplankton and phytoplankton (Fig.3.b).
- Samples can be imaged on board using a USB microscope (Fig.3.c).
- Preserved samples will be shipped back to San Diego for further analysis in the lab.

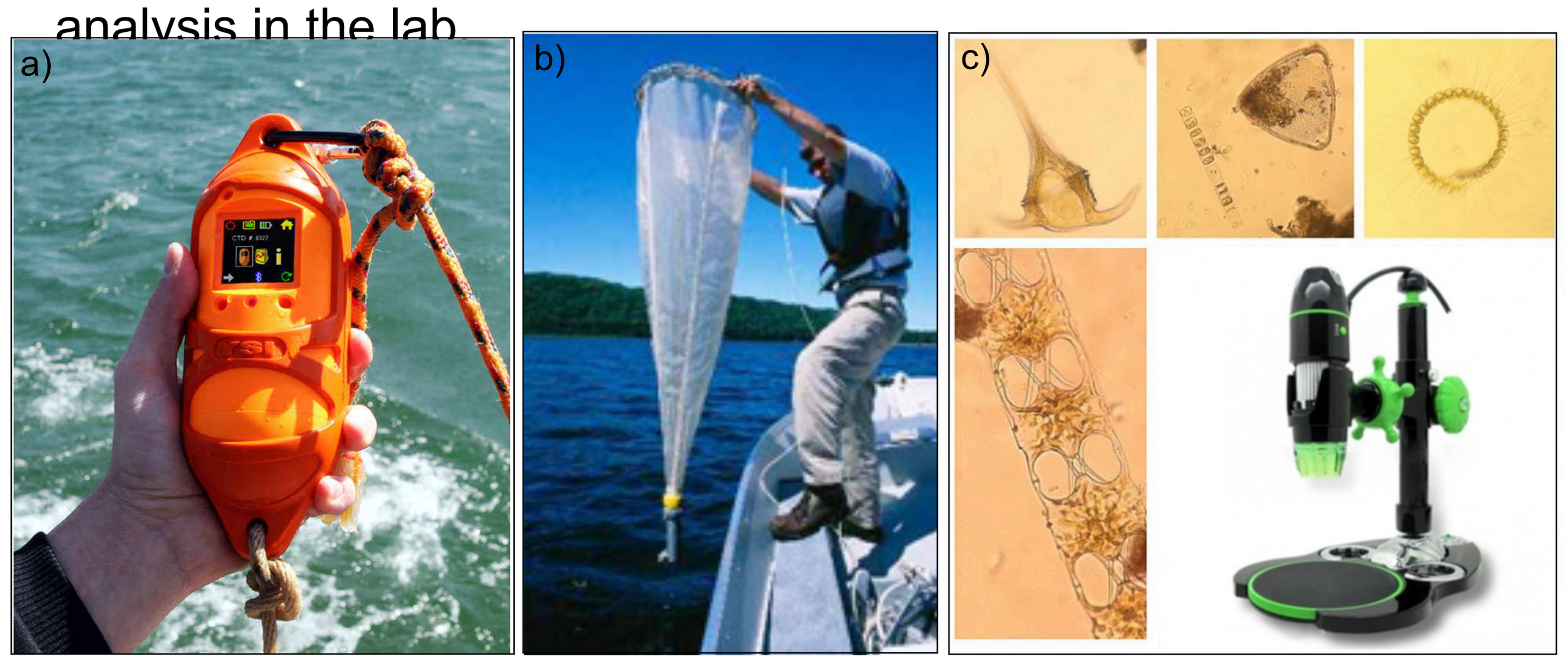


**Fig. 3.** Example resources used for FjordEco Citizen Science program. (a) Castaway CTD unit measures salinity & temperature. (b) Plankton net. (c) Images of phytoplankton taken using iPhone and microscope.

## Deliverables

- Secure funding for pilot program.
- Increase awareness through poster, presentations, blogs, and magazine articles.
- Develop training webinar videos and education material IAATO naturalists can use on board.
- Phytoplankton identification guides allow participants to identify organisms (Fig. 4.a) and upload observations to the existing iNaturalist crowdsourced platform (Fig.4.b).

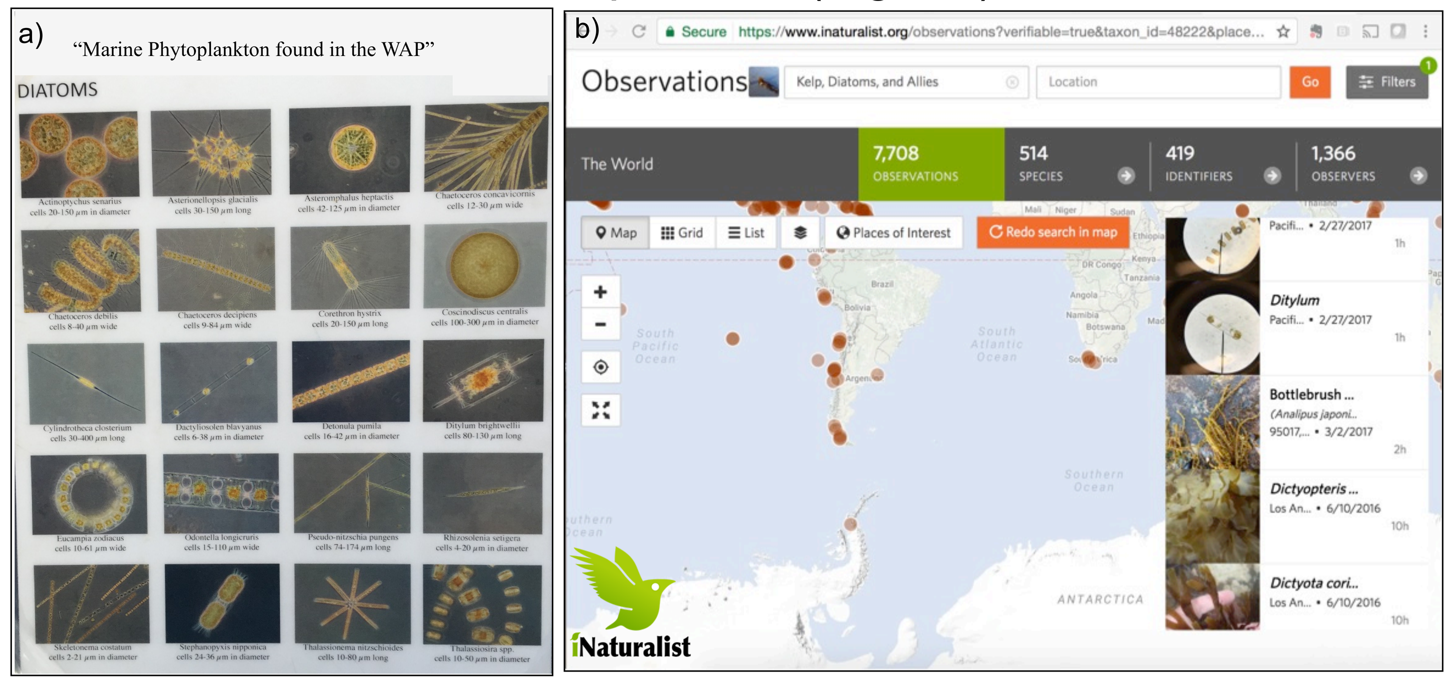


**Fig. 4**. (a) Example identification card to be developed for the WAP region. (b) Screenshot of the iNaturalist database showing current observations for "Kelp, Diatoms, and Allies". Note the lack of observations for the WAP region—the single red data point shown is an observation of brown kelp.

## Impacts

- **Science** – expanded scope of data collected; collaboration with diverse parties; research gap addressed.
- **Tourism** – increased value of the tourism experience; greater interest in science and understanding of scientific principles; deeper understanding of ecosystem; contribution to a legacy of research in Antarctica.
- **Society** – informed stewards of the ocean will share stories with friends and family; increased civic scientific literacy.
- **Management** – new information about region can be used to inform decision makers on management issues.
- **Measurement of success** – recruitment and retention of interested IAATO members.

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## Acknowledgements

Dr. Maria Vernet (SIO Committee Chair), Dr. George Watters (NOAA-AERD), Amy Van Cise (SIO).

# FJORDPHYTO

POLAR | TRAVEL | SCIENCE

## CITIZEN SCIENTISTS AS ANTARCTIC AMBASSADORS

**FjordPhyto** researchers at the world's leading **Scripps Institution of Oceanography** have developed a **Citizen Science program** where visitors and scientists work together to answer climate science questions.

### Will you join us?

**Give visitors the hands-on experience of doing real science in Antarctica!**

- Equipment and training will be provided!
- It's fun and easy!
- Educational to visitors and important for science!

**Contribute to the legacy of research in Antarctica.**

**The FjordPhyto team wants to know:**

How are melting glaciers impacting phytoplankton communities within the fjords along the peninsula?

**Phytoplankton matter because they ...**

- are the foundation of the food system, supporting whales,seals, and penguins.
- play a critical role in the climate, drawing carbon dioxide out of the atmosphere.
- contribute to over half of the Earth's oxygen -- more than the trees and plants on land, combined!

Ecosystems on the peninsula are shifting.

**Let's understand the changes together!**


Collect samples within fjords using simple-to-operate, hand-held equipment provided by the FjordPhyto research team.


View phytoplankton under the microscope and learn more about how they support life within the fjords.


Take photos with a smartphone! Researchers will analyze data and share results.

**Get involved:** Email Allison Lee at allisonlee9@gmail.com

# FJORD PHYTO

## Antarctic Citizen Science Project

***Q*uestion:** As freshwater from melting glaciers enter the fjords, how are marine phytoplankton communities adapting?


SCRIPPS INSTITUTION OF OCEANOGRAPHY
UCSD

# Travelers and Scientists Working Together

*To understand changes in marine ecosystems within the west Antarctic Peninsula Fjords*

Visitors will collect samples and images within the fjords using simple-to-operate tools. Equipment and educational materials will be provided by the research team.

Phytoplankton support whales, seals, and penguins, contribute to over half of the Earth's oxygen, and draw carbon dioxide out of the atmosphere.

## Join us and contribute to the legacy of research in Antarctica

Email Allison at allisonlee9@gmail.com




SCRIPPS INSTITUTION OF OCEANOGRAPHY
UC San Diego

USER'S GUIDE FOR

# FjordPhyto

## Antarctic Citizen Science Project

Prepared by MAS-MBC graduate Allison Lee

Center for Marine Biodiversity & Conservation

# USER'S GUIDE FOR FJORDPHYTO ANTARCTIC CITIZEN SCIENCE PROJECT

Developed by Allison Lee and Dr. Maria Vernet at
the Scripps Institution of Oceanography, University of California San Diego

**Principal Investigator:** Dr. Maria Vernet
**User's Guide Writers:** Allison Lee, Graduate Student (M.A.S.)
Dr. Maria Vernet, Research Biologist
**Guide Designer:** Allison Lee
**Website:** Allison Lee, **www.fjordphyto.wordpress.com**

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Dr. George Watters, Director, Antarctic Ecosystem Research Division (AERD, NOAA)

**FjordEco Team**

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**If you have any questions about any aspect of this user's guide, please contact Allison Lee**

allisonlee9@gmail.com

**www.fjordphyto.wordpress.com**

Scripps Institution of Oceanography is one of the oldest, largest, and foremost oceanographic institutions in the world. Founded in 1903, Scripps has provided education and public service to the world encompassing physical, chemical, biological, geological, and geophysical studies of the oceans, earth, and atmosphere.

Recommended citation: Lee, A.L., Vernet, M. 2017. *User's Guide for FjordPhyto Antarctic Citizen Science Project*. San Diego, CA: Scripps Institution of Oceanography.

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**Cover Photo**
Phytoplankton, Corethron, by Maria Stenzel

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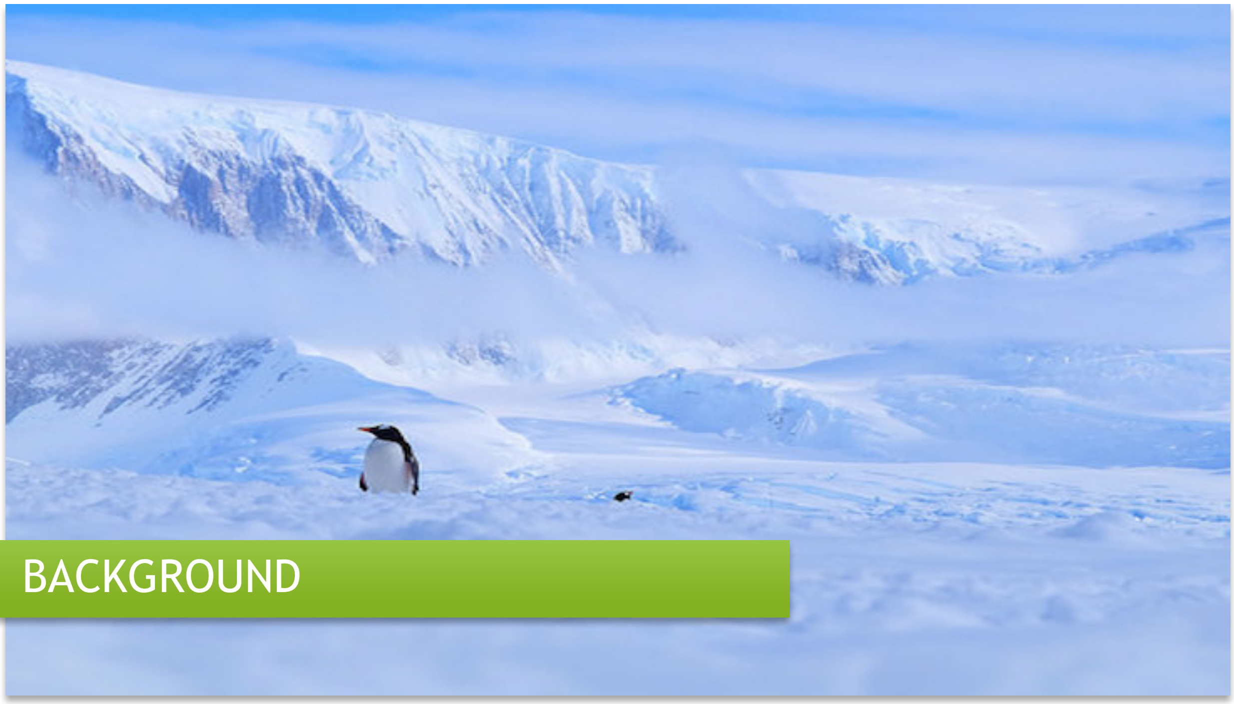


# BACKGROUND

**FjordPhyto** brings Antarctic travelers and scientists together for a Citizen Science approach to understanding fjord ecosystems along the west Antarctic Peninsula.

## Purpose

This guide is designed for Citizen Science Partners operating along the west Antarctic Peninsula who seek assistance in understanding the aims and methods of the **FjordPhyto Antarctic Citizen Science** project. Citizen science projects, also known as **Public Participation in Scientific Research (PPSR)**, engage volunteers and scientists in collaborative research to answer critical science questions. Researchers from Scripps Institution of Oceanography (University of California San Diego), have developed the FjordPhyto Antarctic Citizen Science project to leverage the help and support of the International Association of Antarctic Tour Operators (IAATO) in understanding fjord ecosystem changes over the season.

As visitors travel to the peninsula exploring various fjords and bays, they can learn more about these intact ecosystems, and contribute to science by collecting much needed samples with the help of on-board staff and science specialists.

This guide is not intended to be fully comprehensive of the project, but rather, a supplement to prior and continuing conversation with researchers in the Vernet Lab at Scripps Institution of Oceanography. Additional resources can be found on the FjordPhyto website: www.fjordphyto.wordpress.com.

The sampling methods outlined in this guide incorporate the best practices used in the lab and informal STEM (Science, Technology, Math and Engineering) settings. There is a diversity of techniques that engage the public in science-based learning, and we took an approach with the intention of engaging visitors in easy hands-on activities that contribute to overall gained knowledge and have scientific relevance. Thank you for joining us and contributing to the legacy of research in Antarctica.

# FjordEco – Scientific Research

The **FjordPhyto – Antarctic Citizen Science** project is an extension of the existing **FjordEco** project entitled "Collaborative Research: Fjord Ecosystem Structure and Function on the West Antarctica Peninsula – Hotspots of Productivity and Biodiversity? (FjordEco)" funded by the National Science Foundation (NSF, award PLR-1443705). FjordEco is a collaborative project between researchers from the Scripps Institution of Oceanography, the University of Hawaii at Manoa, and the University of Alaska at Fairbanks with a mission to understand fjord ecosystem structure and function on the west Antarctic Peninsula (WAP).

The FjordEco project is carrying out a successful field (2015-2017) and modeling (2016-2018) program to evaluate processes in physical oceanography, glacial inputs, phytoplankton dynamics, and benthic community structure and function in Andvord Bay, a glacio-marine fjord along the WAP. Andvord Bay - a potential biodiversity hotspot - is well suited for the study of fjord ecosystem drivers and the relationship between glacier - ocean – sea ice and phytoplankton composition and productivity; their findings form the basis of the supplemental study (FjordPhyto).

It is understood that each fjord along the WAP is subject to different oceanographic forces. Additionally, with changing latitude, the contribution of meltwater within the fjords is also expected to change (Cook et al. 2005). The relationship between increased meltwater and phytoplankton productivity has yet to be determined. Phytoplankton communities studied in FjordEco presented a more complex pattern than expected. a more continuous and extensive sampling along a latitudinal gradient, in combination with meltwater determinations, is needed to provide a data set to test phytoplankton composition in relation to the environmental drivers at the ocean-ice interface and an estimation of what areas are affected by these meltwater characteristics. For more information about the FjordEco project visit www.fjordeco.wordpress.com.

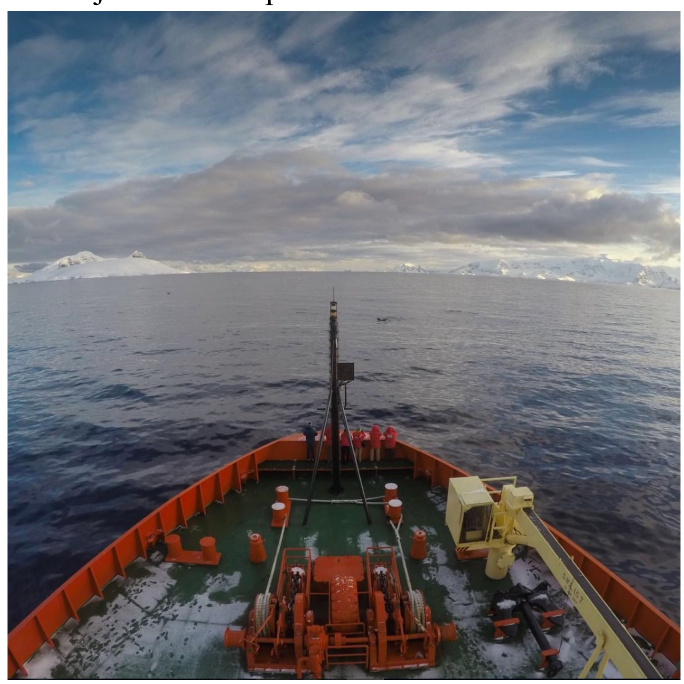


Photo courtesy of FjordEco

# FjordPhyto – Citizen Science Participation

The FjordPhyto project brings together travelers and scientists to answer critical climate science questions. Due to limitations in sampling the large, extreme environment along the west Antarctic Peninsula in a continuous way, FjordPhyto offers a community-based solution to gathering data within the fjords while engaging visitors in the region. This project leverages the help and support of the International Association of Antarctic Tour Operators (IAATO) designed around popular sites visited by zodiac landings to shore (Bender et al. 2016). As visitors travel to the peninsula exploring various fjords and bays, they can learn more about these intact ecosystems, and contribute to science by collecting much needed samples with the help of on-board staff and science specialists.

FjordPhyto aims to include sampling to: (1) characterize the spring and summer meltwater conditions at several fjords and coastal embayments along a North - South gradient. This sampling will provide an estimate of freshwater contribution from glacial meltwater to the continental shelf from these fjords; and (2) understand the timing (phenology) of phytoplankton blooms throughout the season, and their variability in relation to meltwater concentration and distribution.

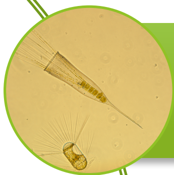
This is a fun and easy way to give visitors the hands-on experience of doing science in Antarctica. Engaging visitors in the scientific process fosters partnerships between travelers and the scientific community. Together, we can greatly expand our current knowledge of fjord ecosystems.

Other current citizen science projects occurring in Antarctica include: Penguin Watch; Happy Whale; Satellites Over Seals; and Microplastics (Adventure Scientists). FjordPhyto will be the first of these efforts to cover Antarctic microbes.

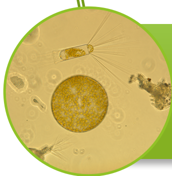
## SCIENTIFIC QUESTIONS

Fjords are known for their richness in plankton productivity (Clarke et al. 2008), benthic diversity (Smith et al. 2008), and aggregations of krill and whales (Nowacek et al. 2011). Those found along the Antarctic Peninsula are hotspots of biological activity and biodiversity.

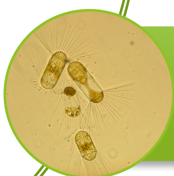
Despite the pristine nature of Antarctica, the peninsula has been one of the fastest melting regions on Earth (Turner et al. 2005). Melting glaciers bring freshwater into fjords and bays. This meltwater mixes with the saltwater marine environment creating dynamic transition zones, altering the physical and chemical properties of the water. Melting ice can bring nutrient-rich waters into the fjords, water containing enhanced concentrations of silica, nitrate, phosphate, and iron. This increase in nutrients, stimulates phytoplankton growth giving way to primary productivity in the fjords. Intense phytoplankton blooms occur throughout the summer.



**1.** Where is meltwater first observed along the peninsula, and how does it progress from within the fjord to offshore?



**2.** What is the composition of phytoplankton within various fjords and how does it change throughout the season?



**3.** Does the presence of meltwater affect the timing of phytoplankton blooms throughout the season?

Phytoplankton are microscopic, drifting plants. They use sunlight and carbon from seawater to produce energy through photosynthesis. They are primary producers. They play a critical role in drawing carbon dioxide out of the atmosphere and contribute to over half of the Earth's oxygen — more than the trees and plants on land combined!

Phytoplankton are sensitive to environmental change and the way in which they respond may drastically impact various levels of the food web. Not all phytoplankton communities are the same. The organisms that eat phytoplankton have varying preferences in their choice of food. At the base of the food system, the composition of these primary producers are pivotal to the existence of krill. Krill sit at the heart of the Southern Ocean ecosystem. They are the lynchpin which holds everything together supporting whales, seals, and penguins. Krill also happen to prefer a diet rich in a specific type of phytoplankton called diatoms.

During high primary productivity periods, diatoms are known to be abundant (Smetacek et al. 1990). Phytoplankton composition is tied with seasonal changes and diatom-rich communities can give way to flagellate micro-plankton as conditions shift throughout the growth season. The timing and reasons behind this shift have yet to be defined. As sensitive indicators of ecosystem health and change (Racault et al. 2012), phytoplankton community structure is affected by a variety of factors including, but not limited to, changes in the chemical and physical conditions such as nutrient content, salinity, and temperature (Garibotti et al. 2005), all of which are affected by meltwater.

FjordPhyto aims to capture plankton dynamics of fjord ecosystems within the austral growth period (November – March). We will target popular tourism landing sites as these biologically active locations also draw an active tourism community.

Several goals have been defined to develop a coordinated strategic partnership with the IAATO community to gather citizen science collected samples and data which aim to expand the knowledge of phytoplankton bloom phenology, composition and gene expression within fjords exposed to varying atmospheric and oceanic influences of meltwater production.

Through sampling during the tourist season, we will gain a comprehensive view of phytoplankton community structure and bloom dynamics in Antarctic fjords.

# SAMPLING KIT

The FjordPhyto kit will be assembled by the researchers at Scripps Institution of Oceanography. Within this kit you should find everything you need to implement the FjordPhyto project while in the field, traveling through Antarctica's fjords. Along with the items shown and listed in the Item Checklist, you will find detailed instructions within this guide of how to set up and use each item. This guide is not meant to be a comprehensive stand-alone guide. Some methods will be better explained through conversation and demonstration with researchers prior to use in the field. You can also find training videos online under the FjordPhyto 'Kit' tab at www.fjordphyto.wordpress.com. If there are any hesitations, contact the FjordPhyto team for best scientific practices and ideas for guest engagement.

## Item Checklist

| | | | | |
|---|---|---|---|---|
| <br>Plankton Net (20-micron/63-micron) w/ weight | <br>Line Reel | <br>Temperature, Salinity Data Device | <br>Secchi Disc, Weight, and Line | <br>Squeeze Bottle |
| <br>Large Dark Bottle | <br>Sample Bottle | <br>Sample Tube | <br>Gloves | <br>Filter (47mm, 0.45-micron) |
| <br>Reusable Filtration Setup | <br>Handheld Vacuum Pump w/ Tubing | <br>Sample Bags | <br>Microscope | <br>Data Sheet |
| <br>Tweezers | <br>Permanent Pens | <br>Eye Droppers | <br>Small Petri Dishes | <br>Funnel |

# Sampling Instructions

## Sampling Meltwater Characteristics

Water may seem like a simple fluid, but it is actually very complex. **Meltwater** comes from the melting of snow and ice from glaciers. This water is less salty than seawater with salinity averaging around 33.2 parts per thousand (ppt), and shown to be as low as 30.5 ppt (Dierssen et al. 2002). In contrast, the salinity of the world's oceans averages 35 ppt and in fresh water it is nearly 0 ppt.

The saltier the water is, the more dense and heavy it becomes. Because meltwater is less salty that seawater, and therefore less dense, it sits as a lens on top of the seawater. Liquids of different densities do not mix well, including water. Without any strong winds to stir things up, a stable surface layer can be created. This stable environment can significantly influence the marine ecosystem by providing favorable conditions for phytoplankton to bloom.

The amount and extent of meltwater entering the fjords seasonally is not well characterized (Vernet et al. 2001). Additionally, the timing of these meltwater intrusions varies from season to season. The presence of meltwater is more common in the late summer (January) to early fall (March) and influences the nearshore and offshore ecosystems. The depth of meltwater layers can vary from a few meters down to 40 meters.

As part of the FjordPhyto project, you will measure certain meltwater characteristics, such as salinity, turbidity, temperature, and depth, at various points within the fjords. As guests venture to shore in a Zodiac, Field Guides will deploy a small, rugged, easy to use device called a CastAway-CTD overboard. Devices like this are used by Oceanographers, Hydrographers, Hydrologists, Aquatic Fisheries Biologists, Coastal Engineers, even Fishermen. This device has been tested to operate effectively down to 100 meters as well as in the frigid waters of Antarctica. However, during your trip to the fjord, you will only need to deploy this unit to depths of 30 meters.

Prior to using this device in the field, familiarize yourself with the manufacturer's Quick Start Guide before operation. For additional instructions on use, refer to the training videos posted under FjordPhyto 'Kit' tab on the FjordPhyto website (www.fjordphyto.wordpress.com).

While in Antarctica on expedition we are interested in measuring meltwater characteristics at the mouth (entrance) of each fjord, as well as at the landing site nearer to shore (glacial terminus). As an example of points of interest, refer to the starred regions on the map of Neko Harbor (FIGURE 1). At your chosen sampling location deploy the CastAway-CTD unit off the side of the Zodiac. The instrument is designed to free fall at a rate of 1 m/s. If you wish for it to go faster, you can add a small weight (provided in the kit).

This unit features an internal GPS that records latitude and longitude both before and after each profile is taken. With the built-in LCD display, you can immediately view plots of conductivity, salinity, temperature, and sound speed plotted against depth while in the field. Raw data can be easily downloaded via Bluetooth to a Windows computer for detailed analysis and /or export at any time. Be sure to export and save all data to send to the Vernet Lab at Scripps Institution of Oceanography for further analysis and plotting.

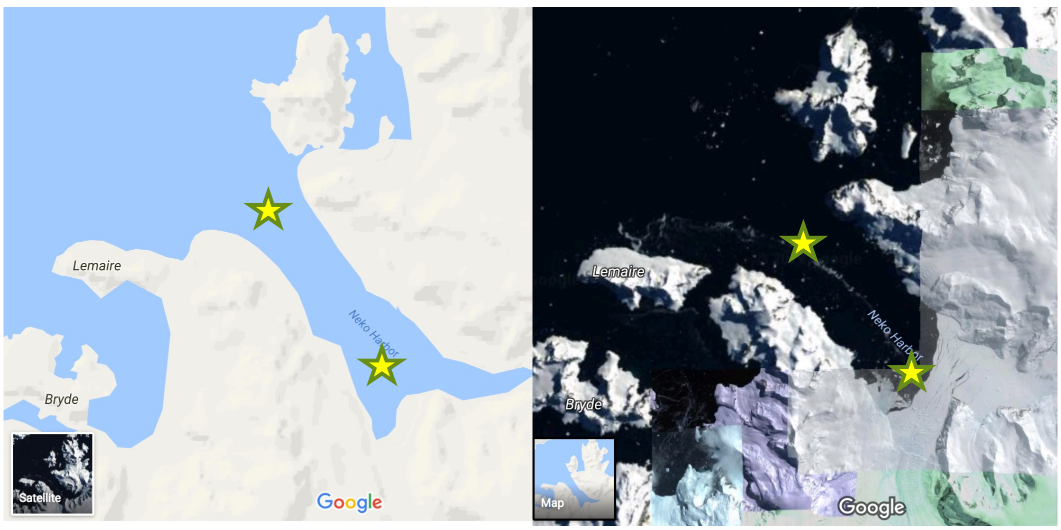
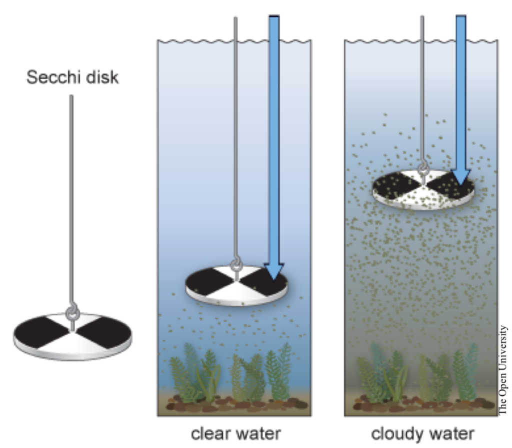
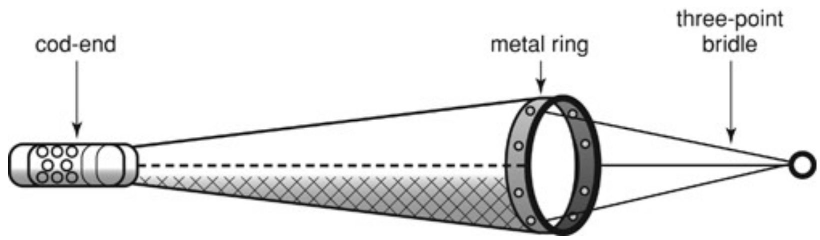



***Figure 1****. Land and satellite view of Neko Harbor, a popular fjord found along the west Antarctic Peninsula. Yellow stars represent regions of interest for CastAway-CTD deployment. Measurements should be taken at the mouth, or entrance to each fjord, as well as at the landing site, or glacial terminus point closest to the glacier.*

Another characteristic of meltwater can be defined from turbidity. Turbidity is a measure of the fine sediment and organic matter suspended in the water. This suspended material influences backscattering of light from the sun. Traditionally this is measured using a simple device called a Secchi disc. This flat circular plate, 20-30cm in diameter, with alternating black and white quadrants, is lowered into the water on the shadow side of the vessel until it cannot be seen by the eye. The depth at which it disappears is recorded as the Secchi depth. Although simple, if taken carefully the Secchi depth can provide excellent information on the light attenuation in the water column (Lee et al. 2015).

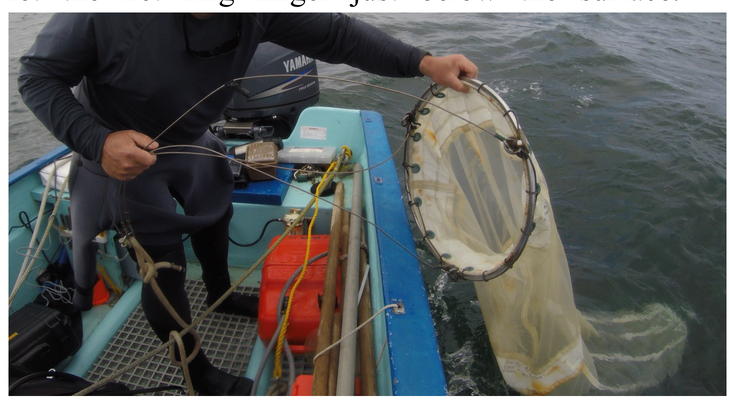


# Using the Plankton Net

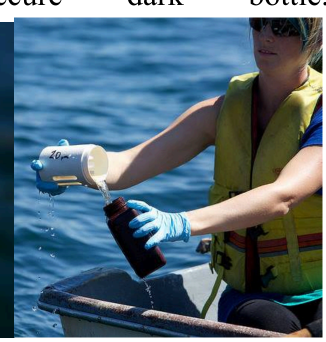



*Standard plankton net*

In your kit we have provided a plankton net of mesh size 20-micron (µm). The mesh size on plankton nets is specifically designed to allow some organism to pass through, while others remain trapped in the cod-end of the net. Much the same as a fishing net, but for microscopic things! Smaller phytoplankton will be retained in the 20 µm net. Prior to deployment you will attach line to the ring at the three-point bridle end. At the other end of the net you will find the cod-end, which is the detachable portion allowing you to concentrate a sample of plankton into one easy-to-detach container. This is a durable plastic cylinder with a ring holding it to the net. Prior to deployment of the net, make sure the cod-end is secured closed. Lower the net over the zodiac. Have a helper record as much information as possible (e.g., date, time, GPS location, weather state, winds, length of tow time). As you first drop the net overboard, you will get an air bubble between the cod-end and the base of the net mesh, but with gentle plunging motion this should quickly fill with water and begin to sink. Let out enough line to begin moving the zodiac. As you slowly move forward let out more line so the line angle is ~45° (at this point you should have released ~40 m of line) then tie off securely to the back of the zodiac, taking care to tie-off away from the path of the motor propeller. Continue to tow the net at a slow speed (~1.5 kts) for approximately ten minutes. If you happen to be in a dense bloom causing the net to clog easily, do a tow for less time (~ five minutes). At a speed of ~1.5 – 2.0 kts more strongly swimming organisms (zooplankton) will be captured. After the tow, begin to bring the net in and completely on board. Do not let the net ring linger just below the surface.



Before pulling the net and cod-end up fully, dunk it up and down in the water a couple of times to rinse excess plankton off the sides. Once the entire net is on-board, carefully undo the cod-end and preserve sample in a secure dark bottle.



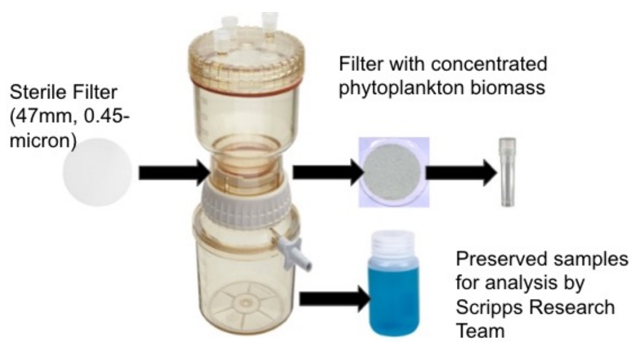
Did you see any salps or other organisms on your journey? Record those observations on the data sheet as well! After collection, rinse all equipment with fresh water and let dry.

For more instruction on how to use the plankton net, see the training videos posted on the FjordPhyto website.

**What are we hoping to catch?** Phytoplankton (plant-drifters) and zooplankton (animal-drifters). We will not be able to catch everything that is in the water, but plankton tows will give us a pretty good representation of what lives in that community. Phytoplankton are very diverse and range in size from 2 µm to 500 µm! As reference, a phytoplankton around 300 µm can be seen with the naked eye, resembling a grain of sand. To read more about these wondrous organisms, see the Educational Discussion section in this guide.

As soon as possible, once you're back at the "lab" area, swirl the contents of the dark bottle to mix the sample. Pour a portion of this sample into a smaller bottle (provided, labeled with an 'M' for microscope). This will be reserved for the microscope activity. The bulk of the remaining sample will be used during the filtration step, so that the samples can be preserved on filters for researchers to analyze back at Scripps. At Scripps, researchers will look at the samples under a high-powered microscope, as well as identify organisms to a species level by extracting DNA and using genetic tools for analysis. Those results will take time, but will be published on the FjordPhyto website as soon as possible, so you can reference the work to which you contributed.

## Setting Up the Filtration Unit



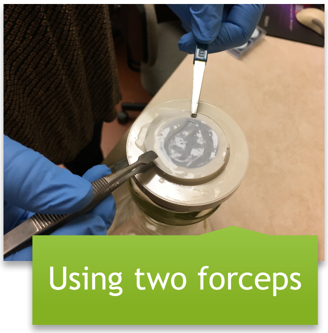
Sterile Hand-held Filtration Setup

The following steps are more easily understood by watching the demonstration video posted on the FjordPhyto website under the Sampling Kit tab. Please refer to the training video on setting up the filtration unit prior to performing this activity. Before you start, put on sterile gloves. Don't worry, you will not be handling any dangerous materials or liquids, the gloves will help keep human microbes out of our seawater sample.

Using tweezers, make sure you add the filter in between the top and the bottom before you pour your sample in! Once you have the unit set up properly, pour your sample in the top. As you hand-pump the sample through the filter you're left with clear water in the bottom and coloration (green to brown) on the filter. We want to save both! The filter should have coloration and will be carefully folded using sterile tweezers. Refer to the images below for instructions on folding.

Then put the folded filter into a screw-top tube that already has the proper preservation solution in it (pre-assembled by researchers). This solution is not harmful to humans, but if some should get on you it is a good idea to wash your hands with soap and warm water. Add the appropriate label to the tube and record information on provided data sheet. The filtered water remaining in the bottom container will be poured into a small tube and frozen for researchers to analyze nutrient concentrations back in the lab.

# Folding the Filter


Using two forceps

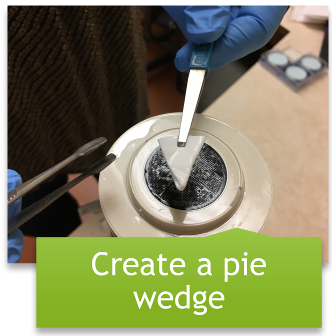

fold filter in half


Flatten the fold


Fold in half again


Flatten the fold


Fold in half again


Create a pie wedge


Place in proper tube

**Step 1:** Put on gloves.

**Step 2:** First separate the filtration unit by removing the top half from the bottom half.

**Step 3:** Open the individually wrapped sterile filter.

**Step 4:** Using tweezers, add filter (white circle) to the unit.

**Step 5:** Screw the top of the filtration set up to the bottom making sure not to catch the filter. Twist the white ring around until it is secure.

**Step 6:** Attach hose and hand pump. Make sure the set up is stable and won't topple over. Also make sure you've plugged the other port opposite of where you attached the pump.

**Step 7:** Pour contents into the top half of the unit, to the 500mL mark.

**Step 8:** Hand filter. This will take a couple of minutes but is a good point to engage other participants and share the activity. Work those forearms!

**Step 9:** Once all the liquid is filtered through, the filter should have green to brown appearance. Make sure the filter looks "dry" and not like a slushy. Remove carefully with tweezers.

**Step 10:** Using two forceps, fold filter in half taco style, then half again like a crepe. It's like doing

origami with chopsticks! Iinsert the folded filter into the proper labeled screw-top tube. Record information on the data sheet.

**Step 11:** Repeat this three times total making sure you've added a filter to each tube.

**Step 12:** Put the sample tubes in the proper sample collection bag, with corresponding data sheet. These samples should be stored in the freezer (~20°C).

# Taking Images with the Microscope

It is not until you look at a concentrated drop of seawater under the microscope that the Invisible Forest comes alive. Within that one drop there are countless organisms living in a world all their own. Most people have never had the chance to look at phytoplankton or zooplankton so closely before. Through this microscope activity, we hope you will gain an appreciation and amazement for the little things that make a big impact on our planet.

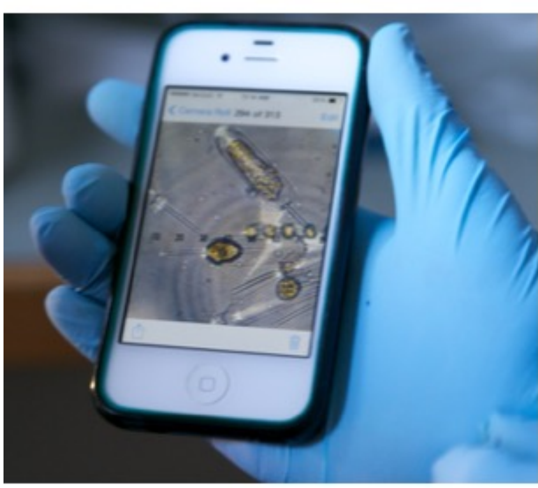
"*In the end we will conserve only what we love, we will love only what we understand, and we will understand only what we are taught*." – Baba Dioum, Forest Engineer.

To read more about these organisms, see the Educational Discussion section as well as the Plankton Identification Guide in the Appendices section.

Once the microscope is set up, use the eye droppers and petri dishes to look at the concentrated seawater under the microscope. At first you will need to start at a lower power to survey the sample and see if there are any interesting organisms. As you find different size organisms you can work your way up to higher power magnification to bring them in focus. Play around!

Share what you know about plankton and their role in the ecosystem. Read the Educational Discussion section on Antarctic Southern Ocean Food Webs as well as the sections on Phytoplankton. Additionally, we have provided a beautiful coffee table book by Christian Sardet, called *Plankton: Wonders of the Drifting World,* with the hopes these up close images will continue to inspire you to learn about the Invisible Forest.

Before taking photos with your smartphone or camera, make sure the geotag settings and date and time are properly set. This is very important! We want to host these images on a map of the Antarctic Peninsula, but we will need to know the accurate GPS location where these organisms were collected. We are looking for crisp, non-blurry images. We've included examples below:



Maria Stenzel

# Uploading Images to iNaturalist

Not familiar with iNaturalist? iNaturalist is a website and smartphone app providing a public database for recording observations of species biodiversity taken by anyone around the world. The site was acquired by California Academy of Science in 2014 and serves as a tool for crowdsourced species identification and as a record of organism occurrence around the world.

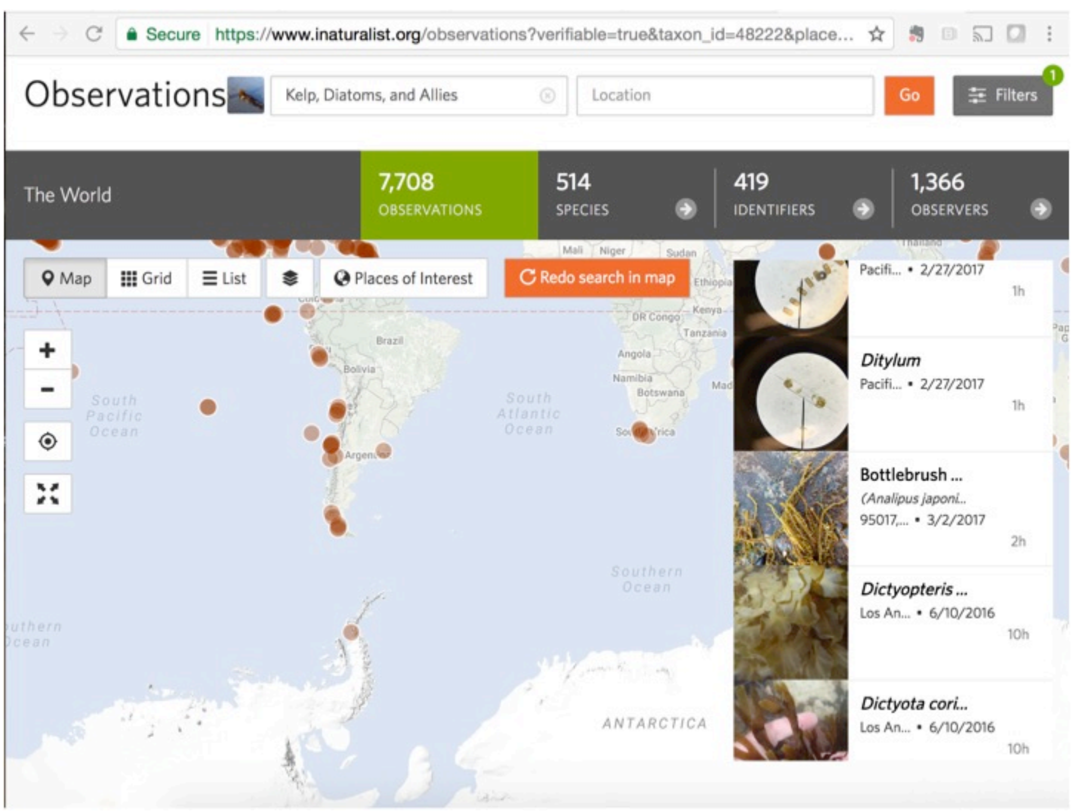
Anyone, anywhere can snap a photo of an organism in the field and upload it to the iNaturalist database. Yes, you can even use this tool for phytoplankton observations made in Antarctica!

You can take images in the field, and once you are back in regular Wi-Fi service, observations will be added to the iNaturalist database. As long as the geotag, date and time settings are properly set, you can wait to do this after your trip.

iNaturalist has many ‘How-to’ tutorials in written and video format with detailed explanations on how to use the program. If you have already downloaded iNaturalist to your smartphone, open the app and start taking pictures. These will automatically be uploaded to the database once you’re in an area reliable service. Otherwise you can take pictures as normal and upload them to iNaturalist at a later time.

We’ve created a specific project for this activity called FjordPhyto on iNaturalist, so when you’re selecting a folder to upload your observations to, be sure to add them to that project!

The more observations we take, the more we can document the changing phytoplankton community along the Antarctic Peninsula throughout the season!




Screengrab of iNaturalist observations for 'Kelp, Diatoms, and Allies'. Note the lack of observations along the Antarctic Peninsula. With your photos and the FjordPhyto project we can start populating this region of the world!

iNaturalist

# EDUCATIONAL DISCUSSIONS

## History

### Polar Exploration & Science Expeditions

Human history in Antarctica is less than 200 years old. Early sealers and whalers from the late 1700's pushed further and further south to hunt and this exploration eventually led the discovery of Antarctica. The first sightings of the continent were recorded and charted in 1820. An American, Nathaniel Palmer, and Russian, Thaddeus Gottlieb von Bellingshausen made the first sighting of the peninsula and that same year Edward Bransfield charted the region. Commercial fishing did not begin until much later, by the Soviet Union in 1967 with exploitation of the finfish resources.

The Wilkes Expedition in 1839 – 1843 is remembered for the development of Antarctic scientists, as noted in the publication by James D. Dana describing the abundant krill, *Euphausia superba.* In 1839 – 1843 J.D. Hooker, a botanist/surgeon, noted a summer diatom bloom on the first scientific voyage, the James Clark Ross, aboard *Erebus* and *Terror* Expeditions. The first International Polar Year was set from 1882-1883 for scientific study in the continent. The first studies focused on geological observations but shortly after included the mission to document the biology and ecology of the Southern Ocean. *Belgica* scientific expedition was the first ship to perform year-round scientific observation including studies of phytoplankton by Mangin, and morphology of krill (Euphausia superba) by Zimmer. More scientific expeditions followed with a flurry of expeditions between 1900 – 1926. By this time, features of the western coast of the Antarctic Peninsula were fairly well located, and a reasonably accurate reconnaissance map could be made for the Islands of the Scotia Arc and the coast of the Antarctic Peninsula as far south as Marguerite Bay.

In 1931, H. H. Gran hypothesizes that phytoplankton development was slowed by vertical mixing within the water column. Only after stabilization of the water column layers could a bloom develop and deplete the nutrients. This key finding stays with biological oceanographers today as we continue to try and understand how the influx of glacial melt water stabilizes the marine environment allowing the phytoplankton to consume nutrients and to bloom.

In 1931 the International Council of Scientific Unions (ICSU) was founded to promote international scientific activity in the different branches of science and its application for the benefit of humanity. The ICSU is one of the oldest non-governmental organizations in the world.

During the 19th and 20th centuries, England, Chile, and Argentina began to lay territory claims for whaling and seal hunting land bases. More countries followed suit and in the 1940's countries like New Zealand, Norway, Australia, and France claimed other parts of the continent. In the 1950's a fear that Antarctica would become a launch pad for nuclear war concerned a group of scientists. In 1952 the ICSU started to plan for a collaborative year of scientific study through what is called the International Geophysical Year and was to take place between 1957 and 1958. The ICSU created the Scientific Committee on Antarctic Research (SCAR) bringing together scientists from various countries active in research to promote the development of Antarctic science. This was also the first time a female scientist worked in Antarctica. Russian women had been traveling with whaling ships decades prior, but the marine geologist Professor Maria Klenova was the first female scientist to head down aboard the *Ob*, one of the research vessels used to establish a Russian base in preparation for the IGY. In 1959 twelve countries came together to protect the Antarctic continent as a place for peace and science and the Antarctic Treaty came into force 1961. The Antarctic Treaty protected the land, but not the oceans and governance was organized as consensus-based, where all countries had to agree on the outcomes before final decisions would be made. Until this time, the oceans remained open access for fishing until 1980 when the Convention on Conservation of Antarctic Marine Living Resources (CCAMLR) Treaty came about. It declared that fishing was permissible as long as environmental impacts were taken into consideration. The Ross Sea fishery was very rich in resources and took off in the 1990s. With concern over the depleting Toothfish stock, a group of scientists urged CCAMLR to establish a Marine Protected Area (MPA) in 2002. The official Ross Sea proposal was presented in 2012 but rejected as consensus was not achieved. Every year following, it continued to be rejected until October 2016. All countries with the authority to make decisions came to a consensus that the Ross Sea MPA would be adopted, protecting the region for 35 years. This 1.55 million square kilometer region is now the world's largest MPA and signifies a huge diplomatic achievement.

For a more complete history on scientific expeditions to Antarctica, please read El-Sayed, S. Z. (1996). Historical perspective of research in the Antarctic Peninsula region. *Foundations for Ecological Research West of the Antarctic Peninsula*, 1-13. You can also find great information on Antarctica's history according to the National Science Foundation's report, NSF (1997). *Antarctica Past and Present.* Retrieved from https://www.nsf.gov/pubs/1997/antpanel/4past.htm.

# Food Web

## Southern Ocean Food Web

The Southern Ocean is one of the most productive oceans in the world. Because of the cold waters and rich nutrient sources, these provide foundation for the base of the food web, the primary producers. Southern Ocean food webs are relatively simple and intact compared to those found around the world. A food web is simply the natural interconnected web of what-eats-what in an ecological community and intact food webs have top apex predators consuming smaller species and grazers below them. As an example, baleen whales, seals, and seabirds eat krill. Krill are the backbone of the Southern Ocean ecosystem and krill graze on the microscopic plants, or phytoplankton. This is a very short food chain. It is important to note that krill have specific tastes in phytoplankton. During a portion of their life history, the stomachs of krill have been filled with diatoms. Diatoms are an abundant type of phytoplankton covered in siliceous glass and dominate Southern Ocean environments. A really cool example of this short food chain involves whale poop. As Antarctic krill (*Euphasia superba*) eat phytoplankton, they concentrate the iron in their tissues. The baleen whales then eat massive amounts of Antarctic krill and once they've digested their meal, they poop the iron rich feces back into the ocean. This iron provides nutrients for the phytoplankton stimulating a bloom, and the cycle is repeated! Because blooms can become enormous enough to see from space, they provide a rich food source and promote some of the largest aggregations of whales ever reported to be seen at densities 1000+ whales/$m^3$, known as super aggregations (Novaweck 2011).

The long-term studies from the United States Palmer Long Term Ecological Research (Pal-LTER) program, scientists reveal that the northern region of the WAP is changing from the classic short polar food chain, dominated by larger phytoplankton that are eaten by krill, transitioning to a microbial food web dominated by smaller phytoplankton and bacteria which are favorable to organisms like salps (Garzio & Steinberg 2013). Understanding how climate variability affects multiple levels in food web is essential for understanding and predicting ecosystem responses to climate change. Much of the food web dynamics are still poorly studied in the Southern Ocean as it remains a harsh remote region in which to operate. What is clear, is that many of the changes that the region is experiencing will impact the food web, restructuring some of the relationships that are more well known (Bernard et al. 2012).

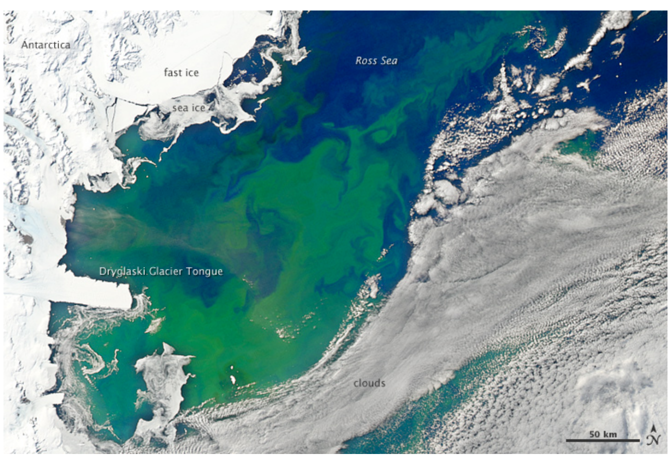
# Phytoplankton

Phytoplankton are microscopic plant-like organisms that drift through the marine environment. The term 'phyto' means plant, and the term 'plankton' was coined in 1887 by Victor Hensen to mean free floating and suspended bodies living passively in water (Gopinathan et al., 2007). Phytoplankton use sunlight, water, carbon dioxide and nutrients to make food through a process called photosynthesis. Because of their need for light, they are found within the first 100-200 meters of the ocean. As they photosynthesize, they draw carbon out of the ocean, ultimately drawing carbon dioxide out of the atmosphere, playing a critical role in regulating the Earth's carbon cycle. As a primary producer, they make up the foundation of the food web, supporting whales, seals, and penguins. A byproduct of photosynthesis is oxygen and phytoplankton produce over half of the Earth's oxygen — more than the trees and plants on land combined! That is one mighty invisible forest. Phytoplankton belong to the class of Algae and are made up of multiple Classes known as Diatoms (made of glass), Dinoflagellates (whirling whips), Blue-green algae, Phytoflagellates (plant whips) and many others including Silicoflagellates (containing silica) and Coccolithophores (containing calcium discs). They can live as single-cell individuals or form chains, rafts, and colonies.

### Role of Plankton:

**1) Base of the food web.**
**2) Produce over half of Earth's oxygen.**
**3) convert inorganic carbon to organic carbon, regulating planet's climate.**

Phytoplankton are monitored through the use of satellites (e.g., Sea-viewing Wide Field-of-View Sensor, or SeaWiFS), robotic Autonomous Underwater Vehicles, and direct sampling of the seawater.



*This image shows a phytoplankton bloom in the Ross Sea, Antarctica as seen on January 22, 2011 by the Moderate Resolution Imaging Spectroradiometer (MODIS) on NASA's Aqua satellite. NASA image courtesy Norman Kuring at NASA Goddard Space Flight Center.*

When primary producers are removed or reduced in an ecosystem, this change can trigger changes in the populations that prey upon them. In the Antarctic ecosystem changes in phytoplankton can disrupt the predator krill which is a keystone species holding the food web together. You can read more about food webs in the previous section. If phytoplankton decrease they will also take up (sequester) less carbon, causing $CO_2$ levels in the atmosphere to rise. Without phytoplankton in the oceans, our Earth's atmosphere would have a lot more $CO_2$ than present day levels (currently ~ 400ppm).

Phytoplankton are like sentinels, prompting scientists to pay attention to other changes occurring in a marine ecosystem (Racault et al. 2012) and their community structure is affected by a variety of factors including, but not limited to, changes in the chemical and physical conditions such as nutrient content, salinity, and temperature (Garibotti et al. 2005), all of which are affected by meltwater coming in to the fjords. Species composition (who is there and how many) are used as indicators to monitor environmental stresses on watersheds. Just like wildflowers blooming in a field, not all fields have the same flowers. It is the same with phytoplankton. Not all blooms will have the same composition of phytoplankton types and the presence of different types can tell scientists something about the ecosystem.

The Southern Ocean is mostly dominated by diatoms. Diatoms live in marine and fresh water environments and can be found floating in the sea (planktonic), living on the bottom (benthic), or living on or within other animals such as whales, Crustacea, foraminifera, and in seaweed. They are covered in a silica glass shell and come in beautiful architectural shapes and sizes. They have captivated the attention of scientists and artists alike, and have been studied for nearly 300 years. Their sizes range from 2 µm to 500 µm and there is thought to be upwards of 100,000 different species. Most of the common species you may see in the Antarctic Peninsula have been grouped by shape (morphology) for the purposes of this guide but scientists identify diatoms based on other features like shape, color, and function.

## What are scientists seeing with the FjordEco Research?

The distribution of phytoplankton in the ocean is dependent on light, time of day, temperature, nutrients, salinity and seasonal cycles. Favorable conditions for phytoplankton, especially the diatoms include winter ice and reduced winds which allow for the water column to stabilize and stratify (or separate into layers). Diatoms are known to be abundant during periods of high primary production (Smetacek et al. 1990) and factors favoring diatom growth are considered to maximize production in WAP inner waters (Garibotti et al. 2003, 2005; Kozlowski 2008). In the WAP shelf waters, periods of high production are dominated by diatoms larger than 20 µm (e.g. summers of 1996 and 2005, Kozlowski et al. 2011) while smaller plankton such as flagellates become more abundant later in the growth season due in part to increased grazing (Ross et al. 2000; Walsh et al. 2001)

Phytoplankton communities studied through the NSF funded FjordEco project presented a more complex pattern than expected. Present-day models indicate that coastal waters along the west Antarctic Peninsula have an early diatom bloom occurring October-November. This is presumably related to sea ice retreat (Ross et al. 2000), and a summer diatom bloom occurring in January; Flagellates such as cryptophytes, dominate in between and after these diatom dominated blooms (Kozlowski et al. 1995). Krill grazing on phytoplankton and meltwater coming in to the fjords have been proposed as supporting the growth of these cryptomonad blooms (Walsh et al. 2001, Moline et al. 2004), however this hypothesis has not been confirmed (Garibotti et al. 2005). In 2015, a large and productive cryptomonad bloom was observed in December in Andvord Bay while diatoms dominated later in the season during April 2016, at a time of maximum meltwater concentration entering the fjord. Conditions in WAP sub-polar fjords are ideal to maintain diatom growth throughout the growth season: stratification is provided by a continuous source of meltwater from glaciers under conditions of low wind mixing (Fryxell, 1989). Iron and nitrate input either from glacier melt and/or deep mixing (Buck et al. 2010), and turbulence over sills generated by tides (Rodriguez et al. 2001, Tozzi et al. 2004) maintain these systems nutrient rich.

Thus, it is necessary to have a more continuous and extensive sampling program along the latitudinal (North – South) gradient of the peninsula. Phytoplankton sampling, in combination with meltwater determinations, is needed to provide a data set which will allow researchers to investigate the phytoplankton composition in relation to the environmental drivers at the ocean-ice interface and an estimation of what areas are affected by these meltwater characteristics. In this way, we will be able to detect timing of bloom, number of blooms in a season and their relationship to the main oceanographic factors, and provide data to test the alternative hypothesis that cryptomonads are favored during melting events (Moline & Prezelin, 1996).

For more information on the beauty and diversity of phytoplankton visit the FjordPhyto website for artists such as Ernst Haeckel's famous drawings, Klaus Kemp's tedious mosaics, and short video episodes from the Tara Oceans Plankton Chronicles.

# Fjords

Fjords are deep-sided valleys carved by glacial ice. They are the transition zones between the land and sea. Fjords along the peninsula have tidewater glaciers, influenced by the tides, and are known as glacio-marine fjords. They are widespread at temperate to polar latitudes and form important boundary zones (e.g., Powell & Domack 1995). At high latitudes such as the Arctic and Antarctic, fjord ecosystems are major pathways carrying glacial ice to the sea and are thus highly sensitive to frozen water and ocean interactions induced by a warming climate (e.g., Syvitski et al. 1989, Pritchard & Vaughan 2007, Kedra et al. 2010, Weslawski et al. 2011).

## Why study fjords?

Glacio-marine fjords along the WAP appear to be intense, potentially climate sensitive, hotspots of biological production and biodiversity. However, fjords in the Antarctic are understudied systems. The structure and dynamics of these fjord ecosystems are very poorly understood. In comparison, fjords in the Arctic are well understood. Because the Arctic experiences more warming and freezing cycles, the ecosystem overall experiences more frequent mixing. Despite the increased influx of nutrients, these rapidly changing systems are not ideal for phytoplankton blooms. Thus, we do not see Arctic fjords as being the same hotspots for biodiversity that we are finding in Antarctica. There are four lines of evidence to suggest that fjords along the WAP are intense hotspots of productivity and biodiversity, in contrast to sub-polar, glacio-marine fjords in the Arctic. **(1)** Feeding baleen whales are frequently concentrated in and near WAP fjords along the Danco/ Graham Coast in summer-autumn months, apparently in response to dense aggregations of krill (e.g., Nowacek et al. 2011). **(2)** Geological studies indicate that meltwater processes are weak in WAP fjords, resulting in low turbidity and land based sedimentation rates providing nutrients and stability for phytoplankton blooms to occur. **(3)** Seafloor photo-surveys in WAP fjords reveal extraordinary benthic megafaunal abundance and distinct community structure compared to similar depths (450-600 m) on the open Antarctic continental shelf (Grange & Smith 2013). **(4)** Pilot phytoplankton studies indicate that intense blooms may occur in WAP fjords throughout the summer-autumn, potentially fueling krill/whale aggregations contributing to the flux of high organic carbon to the fjord floor (benthos) (G. Ferreyra, unpubl. data).

## The question is, how much of this productivity relates to meltwater?

What scientists are finding in the WAPs shelf-slope system is that it has different characteristics in the water compared to the open areas of the ocean. In fact, nearshore quantities of phytoplankton pigments (chlorophyll) from the images are revealing roughly four times (4x) higher pigment than off shore areas. Some of these changes are from seasonal summer warming when glacial meltwater runs off into the region. This meltwater lowers surface water salinity causing an increase in the frequency and abundance of certain phytoplankton groups.

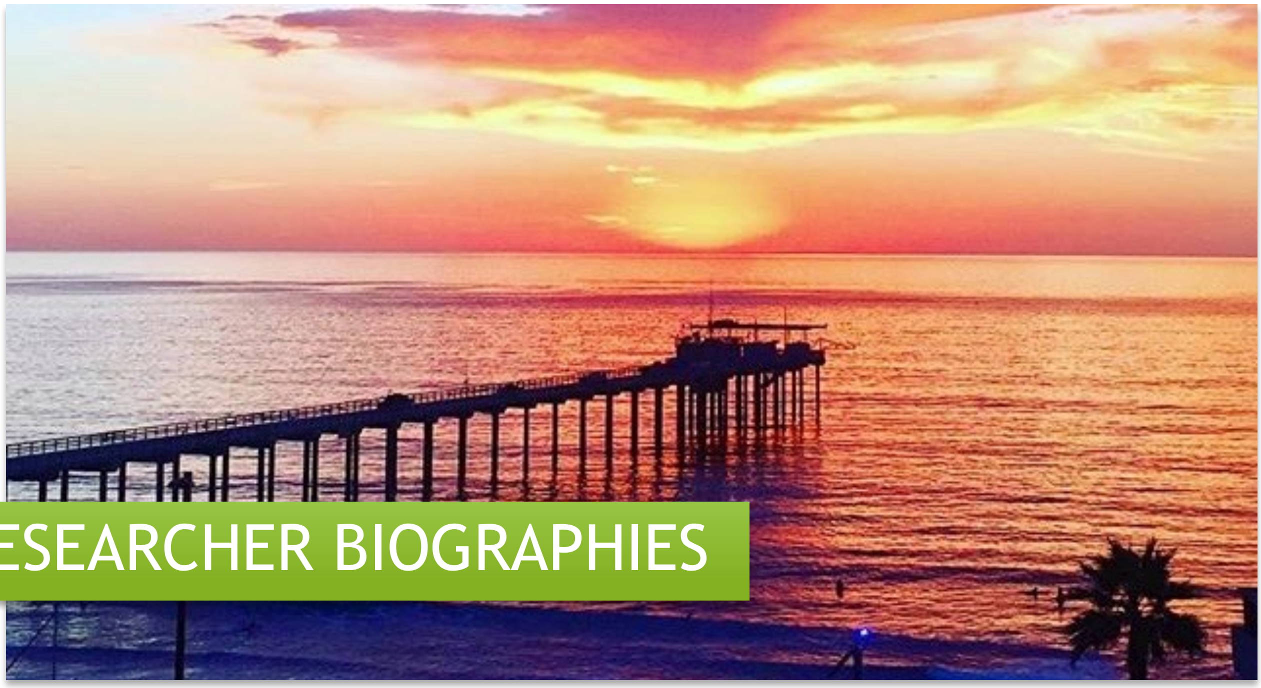
Additionally, scientists are seeing regional differences between the fjords in the north and south of the peninsula. As global warming reduces surface water salinity around the peninsula as a result of glacial meltwater runoff, this increases the frequency and abundance of certain phytoplankton groups that prefer these conditions. Further analysis of these findings reveals a change in phytoplankton composition from large-celled diatoms (a preferred food of Antarctic krill) to now much smaller cryptophytes (or single celled photosynthetic algae) (Montes-Hugo et al. 2008).

# West Antarctic Peninsula

The length of the Antarctic Peninsula (AP) extends along a North to South gradient exposing it to extreme weather and complex ocean circulation patterns. According to NASA's GRACE Satellite observations, the peninsula has lost a significant amount of ice mass making it one of the most rapid warming regions in the world (Ducklow et al. 2013). Since the beginning of the U.S. Palmer Long Term Ecological Research program (1990), the greatest impacts in this region are climate change, duration of sea ice presence, and seasonal inputs from glacial melt water. Over the past 50 years, the peninsula has warmed by ~ 7°C decreasing the duration of sea ice by almost 100 days since 1978 (Ducklow et al. 2013). The AP lost 20 Gt $yr^{-1}$ of ice during the period 1992 to 2011 (Shepherd et al. 2012). Nearly all of this ice melt from the glaciers, flows into the oceans contributing to global sea level rise. The net contribution by the AP to sea level rise has been 0.16 $\pm$ 0.06 mm $yr^{-1}$ during the period 1992 to 2007 (Pritchard and Vaughn 2007). The extent of the retreating glaciers is rapidly migrating southward, at a rate faster than the atmospheric warming trends, suggesting that the atmosphere might not be the sole driver of glacier retreat in the region. A key driver of ice loss is summer melting (Abram et al. 2013). However, the relationship between temperatures and melt is not linear. Alternatively, Pritchard and Vaughn (2007) attribute the 12% increase in the ice delivered to the ocean by the tidewater glaciers of the Antarctic Peninsula from 1992 to 2005 to a dynamic response of thinning of the glacier front, not to enhanced lubrication by meltwater or enhanced snowfall. This lack of agreement on the processes causing glacier retreat in WAP is further complicated by our lack of understanding of the role of oceanic influence, a process critical to ice shelf retreat (Cook et al. 2016).

The Upper Circumpolar Deep Water in the Antarctic Circumpolar Current (UCDW-ACC) flows westerly and floods the WAP continental shelf (Martinson et al. 2008) with water >1.7 $^{o}$C. In this way, relatively warm water is accessible to glacier fronts within WAP fjords. The sensitivity of glaciers to oceanic melting makes them vulnerable to the increased UCDW circulation. Model studies predict that any excess heat brought by increased UCDW on the WAP shelf in response to stronger southern winds is lost on the shelf due to increased mixing and it does not reach the ice front (Dinniman et al. 2012). Despite these controversial results on heat loss from the UCDW on the WAP, meltwater is being delivered to the ocean and phytoplankton respond to its presence.

The melting of ice can bring up nutrient-rich water with enhanced levels of macronutrients such as silicic acid, nitrate, phosphate and micronutrients, such as iron, all key for phytoplankton growth. These nutrients are high in concentration within the first 5 km from the glacial front in Barilari Bay. Thus, fjords are similar to other natural fertilization features in the Southern Ocean known to increase productivity through enhanced nutrient availability (FjordEco data). In this way fjords have the potential to enrich the WAP coastal waters and could be partially responsible for the high productivity observed not only in the fjords but in WAP coastal waters (Vernet et al. 2008). Fjords are known for their richness in plankton productivity (Clarke et al. 2008), benthic diversity (Smith et al. 2008), and presence of krill and whales (Nowacek et al. 2011). It is these same locations that draw the tourism community to this region.



# RESEARCHER BIOGRAPHIES

## Dr. Maria Vernet
## Principal Investigator

Dr. Maria Vernet is a Senior Research Biologist at Scripps Institution of Oceanography. Dr. Vernet has conducted research in international settings since 1987 when she first traveled to the Arctic and in 1988 to Antarctica. She participated in one of the first research teams to study the effect of ultraviolet radiation on marine phytoplankton after the discovery of the Antarctic ozone hole in 1985. She has also participated in research studying free-floating icebergs and the ecosystems of the Larsen B Ice Shelf. Her field expeditions have taken her into the Atlantic, Pacific, Arctic and Southern Oceans with a variety of internationally assembled research teams. Maria received her Ph.D. (1983) and M.S. (1981) in Biological Oceanography from the University of Washington and her B.S. (1975) in Biological Sciences from the University of Buenos Aires, Argentina. (Photo by Maria Stenzel). **Fun Fact:** Maria sometimes goes by the nickname Vernizzle.

## Allison Lee
## Graduate Student

Allison Lee is a graduate student at Scripps Institution of Oceanography receiving a Master's degree in Marine Biodiversity and Conservation. She has extensive experience conducting experiments on phytoplankton in the laboratory to investigate their genetic response to projected 21st century environmental scenarios such as ocean acidification and climate change. Her sea-going field expeditions have taken her throughout Washington's Puget Sound, Hawaii's Pacific Ocean, and Antarctica's Ross Sea. Her first expedition to Antarctica occurred in 2013 where she lived aboard the U.S. Antarctic Programs' icebreaker *Nathaniel B. Palmer* for 53-days tracing the fate of algal carbon export in the Ross Sea. Allison received her B.S. (2006) in Biology with a minor in Earth and Space Sciences from the University of Washington. (Photo by Meredith Jennings).

**Fun Fact:** Allison ran a marathon on the Ross Ice Shelf, Antarctica, dressed as a banana, the same day she boarded the *Palmer* icebreaker for a two-month expedition at sea.

# APPENDICES

## APPENDIX A: Data Sheet 1 & 2

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Images: Maria Stenzel

# FJORD PHYTO

## Data Sheet

version 2

## 1) IDENTIFICATION

Date (MM/DD/YYYY): ____

Time: ____ UTC/Local (circle one)

Tour Company: ____

Vessel Name: ____

Zodiac Operator Name: ____

(Optional) Citizen Scientist Name(s):

Fjord/Site Name: ____

Mouth / Glacial Terminus (circle one)

## 2) METEOROLOGICAL CONDITIONS

Air Temp (Celcius): ____

Water Temp (Celcius): ____

Wind Speed (kts): ____

Wind Direction: ____

Weather (circle all that apply):

| | | | |
|---|---|---|---|
| sunny | partly cloudy | overcast | fog |
| rainy | windy | breezy | calm |

## 3) PHYTOPLANKTON SAMPLING

| Net Size: | Depth: | Completed (Y/N) |
|---|---|---|
| 20µm | 0-30m | |
| 63µm | 0-30m | |

| | | |
|---|---|---|
| GPS Coordinates START: | Lat: | Long: |
| GPS Coordinates END: | Lat: | Long: |

Boat Speed: ____ kts

| | | |
|---|---|---|
| Secchi Depth Reading | m | m |
| | disc disappeared | isc reappeared |

HydroColor Measurement (Y/N): ____

Castaway CTD deployed (Y/N): ____

## 4) FILTRATION

| | Multiple Filters Preserved (3 minimum) | | | Solution |
|---|---|---|---|---|
| RNA | | | | RNALater |
| DNA | | | | Ethanol |

Place a check in each box for # filters preserved

## 5) MICROSCOPE

Images taken Y / N
Uploaded to iNaturalist? Y / N
Magnification:
Other: salp? (Y/N)

## 6) COMMENTS

Images: Maria Stenzel

# FJORD PHYTO
## Data Sheet

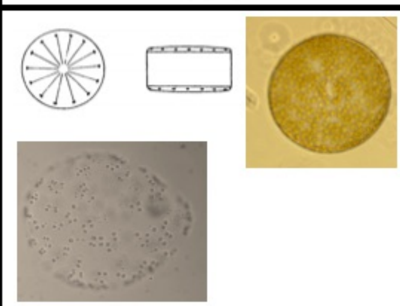
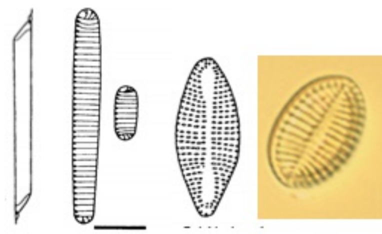
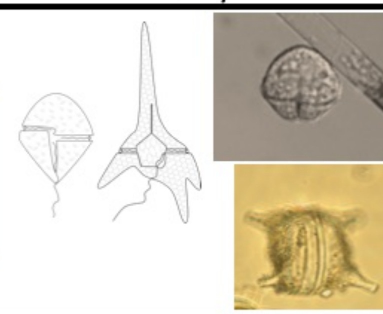
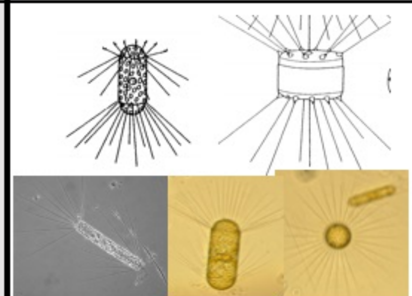
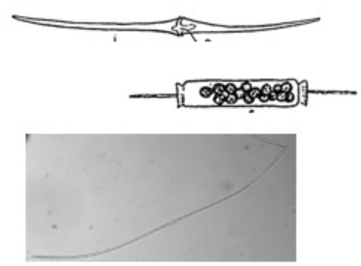
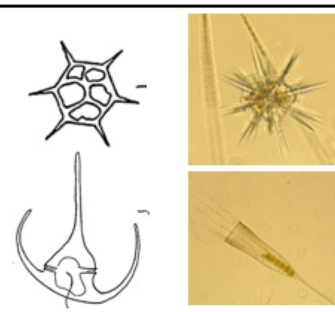
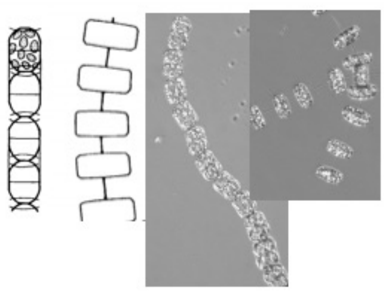
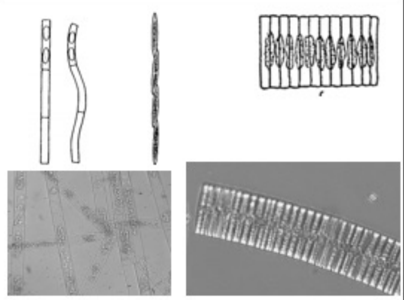
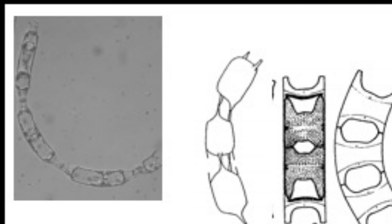
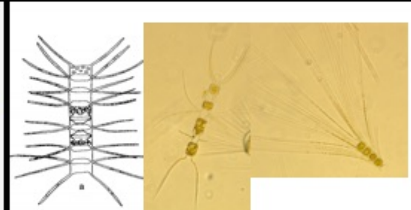
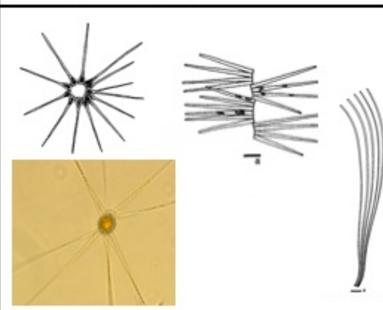
version 1

| Sample # | Date | Time | Location | Latitude | Longitude | Wind Speed (kts) | Beaufort | Wind Direction | Water Temp (ºC) | Air Temp (ºC) | Swell (m) | Conductivity (µS/cm) | Salinity (PPT) | Secchi Depth (m) | Comment about water clarity (if possible) | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Example: | | | | | | | | | | | | | | | | |
| 1 | 26 Dec 2016 | 1400 | Neko Harbor | 64°40.002S | 062°38.60W | 8 | 2.5 | NE | 1.3 | -4 | 3 | 30.21 | 35 | 16 | Very Green Water | 2 castaway deployments |

# APPENDIX B

## Plankton ID Guide

This Phytoplankton Identification Guide provides a basic view of the diversity of phytoplankton that live within the marine environment. For the purposes of this guide, phytoplankton categories are artificially grouped into shapes (not evolutionary lineages) to help provide a visual key for orienting people who are new to phytoplankton. We have provided images of some of the more commonly seen species found in the Southern Ocean but please note, identifying phytoplankton is much more complicated and it takes an expert taxonomist to achieve the more detailed and accurate trees. Images courtesy of Hasle et al. 1997, Sverdrup et al. 1942, Dr. Amy Leventer, Maria Stenzel, and the Vernet Lab.

| | Round | Elongated | Funky |
|---|---|---|---|
| SINGLE no spines |  |  |  |
| SINGLE spines |  |  |  |
| CHAINS no spines |  |  |  |
| CHAINS spines |  |  | |

# APPENDIX C

## Consent to Participate in Citizen Science

"Citizen Science" is scientific research conducted at least partly by volunteers. To support laws protecting professional scientists, we, the members of FjordPhyto Citizen Science, distinguish our work via the following agreements:

**1.Compensation:** Participation in FjordPhyto Citizen Science is completely voluntary. None of us is to be compensated for our contributions, and any of us is free to stop contributing at any point without penalty.

**2. Open Access:** We agree to publish the work of our group where it can be accessed for free through the World Wide Web. To protect confidentiality, personally identifying information will be removed from research data specific to individuals.

**3. Intellectual Property:** Each participant in FjordPhyto Citizen Science retains ownership of his/her own intellectual work. However, we give all other members of FjordPhyto Citizen Science permission to use our intellectual contributions as they see fit, including publishing them and contributing them to other research efforts. Each of us is individually responsible not to contribute stolen work.

**4. Regulation:** Conflicts could arise if things do not go as planned. We agree not to endure, impose or seek any penalties not mandated by the laws governing the State of California. We count all citizens as responsible to advance any new legislation justified by new discoveries, and we prioritize our duty to support such civil process above the scientific activities and economic interest of FjordPhyto Citizen Science.

**5. Attribution:** Our contributions may be credited to us using our names as printed on this consent form or may instead be credited to "Members of FjordPhyto Citizen Science" (as the list of contributors may become impractically long). The group and its members can be contacted Scripps Institution of Oceanography, currently located at 8622 Kennel Way, La Jolla, CA 92037.

I enter into all five of these agreements by signing below:

Name (printed):__________________________________________________
Signature:_______________________________________ Date:__________

# APPENDIX D

## Glossary of Terms

**Abundance:** The quantity or number of a particular type of organism within a specific community.

**Benthic:** Occurring at the bottom of a body of water.

**Biodiversity:** The diversity of living organisms in a particular area or region.

**Biomass:** The total mass of an organism in a given area, volume, or habitat.

**Bottom Up Forcing:** when primary producers or primary consumers are removed or diminished in an ecosystem triggering a change in population size of those who prey on them.

**Chlorophyll:** A pigment in all plants which allows them to perform photosynthesis. Chlorophyll a is a naturally green photosynthetic pigment that can be measured directly and is used as an indicator for algal biomass.

**Diatom:** Single-celled algae encased in a silica shell that live in freshwater and marine environments. This group has been estimated to produce nearly 20 % of Earth's oxygen.

**Dinoflagellate:** Single-celled algae which are photosynthetic. Most are found in marine environments. Some species of dinoflagellates are toxic and are responsible for harmful algal blooms in other regions of the world. From Greek *dinos* "whirling" and *flagellum* "whip".

**DNA:** A molecule that carries genetic instructions used in the growth, development, functioning, and reproduction of all living organisms. It is part of the genetic material (genes) found within every cell also known as Deoxyribonucleic acid (see Gene).

**Fjord:** Deep-sided valley cut by glaciers.

**Food Web:** Natural interconnected web of what-eats-what in an ecological community.

**Gene:** hereditary information that is transferred from parent to offspring and determines some characteristic of the offspring (see DNA).

**Genetic Material:** In modern molecular biology and genetics, genetic material of an organism consists of DNA or RNA. It includes both the coding region (genes) and the non-coding region. Genetic material can be found in the mitochondria, chloroplasts, and nucleus of a cell. A central tenet of molecular biology states that DNA makes RNA makes proteins.

**Grazing:** a method of feeding in which herbivores such as krill feed on plants such as phytoplankton.

**Larval Stage:** The juvenile form of an animal that often looks very different from the adult. In marine animals, larva are typically free-floating as plankton and change form as they mature.

**Megafauna:** Refers to large animals.

**Metagenomics:** The study of genetic material directly extracted from an environmental sample. It is used as a way to individually identify organisms to the species level and tells us which phytoplankton and microbes are present in a sample. Think of it as Meta- GENE- Omics.

**Metatranscriptomics:** The study of the function of a group of organisms. It is used as a way to understand the genetic activity of phytoplankton and microbes present in an environmental sample. As a segment of DNA (gene) is copied into RNA, this is called transcription. Think of it as Meta-TRANSCRIPT-Omics

**Oceanographic Forces:** Events which contribute to changing dynamics in a region. These can include tidal forces, current forces, warming forces, biological forces, etc.

**Omics:** Refers to the collective technologies used to explore the roles, relationships, and actions of various types of molecules that make up the cells of an organism.

**Organism:** An individual plant, animal, or single-celled life form.

**Phenology:** The study of seasonal cycles in relation to climate and plant and animal life (e.g., timing of phytoplankton blooms over the austral summer season).

**Phytoplankton:** Microscopic algae that live in the water and produce their own food through photosynthesis. Phytoplankton make up the foundation of the marine food web.

**Photosynthesis:** Process in which green plants and algae convert sunlight, carbon dioxide, and water into chemical energy in the form of sugars. Generally involves the green pigment, chlorophyll ($6CO_2 + 6H_2O$ + light energy -> $C_6H_{12}O_6 + 6O_2$).

**Primary Productivity:** The amount of material (biomass) produced by plants and algae (primary producers) through the process of photosynthesis.

**RNA:** A molecule present in all living cells that acts as a messenger carrying instructions from DNA to control the creation of proteins. This molecule is essential in various biological roles coding decoding and regulating the expression of genes. It is part of the genetic material found within every cell also known as Ribonucleic acid.

**Sill:** A shelf of bedrock that is higher than the surrounding bedrock. Typically found at the mouth of a fjord.

**Stratification:** The formation of distinct layers within a column of water.

**Watershed:** An area of land that drains streams, rainfall, and other precipitation into a common outlet such as the mouth of a bay or fjord.

**Zooplankton:** Drifting marine animals, like invertebrates or very small crustaceans such as krill, or larval fishes that inhabit the oceans of the world and graze on phytoplankton.

# APPENDIX E

BEAUFORT SCALE OF WIND VELOCITY

DEVELOPED IN 1805 BY SIR FRANCIS BEAUFORT, U.K. ROYAL NAVY

(NOAA SPC, 2017)

| Beaufort Number | Wind Speed (mph) | Seaman's Term | Appearance of Water |
|---|---|---|---|
| 0 | Under 1 | Calm | Smooth and mirror-like |
| 1 | 1-3 | Light Air | Scaly ripples, no foam crests |
| 2 | 4-7 | Light Breeze | Small wavelets, crests glassy, no breaking |
| 3 | 8-12 | Gentle Breeze | Large wavelets, crests begin to break, scattered whitecaps |
| 4 | 13-18 | Moderate Breeze | Small waves 1-4 ft. becoming longer, numerous whitecaps |
| 5 | 19-24 | Fresh Breeze | Moderate waves 4-8 ft taking longer form, many whitecaps, some spray |
| 6 | 25-31 | Strong Breeze | Larger waves 8-13ft, whitecaps common, more spray |

# APPENDIX F

## What Will Happen with the Samples?

The samples collected during each voyage to the fjords in Antarctica will be sent back to the research team at Scripps Institution of Oceanography. It is important that these samples are preserved correctly during their entire journey back to the U.S.A. These samples will be analyzed in many ways:

1. **Microscopy.** Using high powered microscopes, researchers are able to identify individual phytoplankton species as well as look at the structure and shape of each individual cell. The phytoplankton community is so diverse that sometimes it is not possible to identify individual species based on their shapes alone. Other methods, such as using the genetic material in metagenomics studies, can help us solve those mysteries.
2. **Metagenomics.** This is a technology used as a way to individually identify organisms to the species level by looking at the genetic information (or genes) present in a sample. The phytoplankton biomass that is collected on the filter and preserved contains this important information. Each organism has a unique set of genes and this method can tell us which phytoplankton and microbes are present in a sample taken in the environment. This can be an easier way to observe which species exist as some are too small to see clearly under a microscope.
3. **Metatranscriptomics.** This is a technology used as a way to observe what the organisms in a population might be doing in response to their environment. The phytoplankton biomass that is collected on the filter and preserved contains this important information. Various fjords in the west Antarctic Peninsula may be experiencing different amounts of fresh meltwater input from the glaciers. This meltwater may bring different amounts of nutrients and we can observe if the phytoplankton are responding to their changing environment by using metatranscriptomics to look at a genetic level. Metatranscriptomics tools provide insight into how organisms respond to environmental cues (Caron et al. 2016) and can provide indication of ecosystem shifts in health and be predictive of how populations may transition or collapse (Alexander et al. 2015).
4. **Nutrient Analysis.** Every fjord has a different amount of fresh water input from the glacier and surrounding rock. This freshwater brings minerals and nutrients from the land to the marine environment. The phytoplankton eat these nutrients in their basic chemical form. In phytoplankton studies, researchers are interested in measuring specifically the concentration of phosphate, nitrate, ammonium, silicate, and iron. Although special steps are needed to measure iron in the environment, phosphate, nitrate, ammonium, and silicate are more straightforward. Silicate is used to build the hard shell of the diatom. Phosphate, nitrate, and ammonium are used in algal growth and metabolism.

There are only a few investigations comparing phytoplankton communities between Southern Ocean environments at the genomic level but a growing number of studies are exploring changes associated with seasonal timing of blooms (Medlin et al. 2000, Pearson et al. 2015, Caron et al. 2016). As molecular tools continue to advance, a greater understanding can be gained from samples collected in this region over the season.

# APPENDIX G

## Previous Season's Citizen Science Data

Our current partners, Polar Latitudes and G-Adventures, graciously collected seawater samples for the Scripps Research Team last season 2016-2017. Mapped below are the GPS locations (latitude and longitude) showing where each sample or measurement was taken. This is a glimpse into the results from their efforts. Samples are currently being analyzed under high powered microscopes at the Universidad Nacional de La Plata in Argentina. The research team at Scripps looks forward to receiving more samples in the future as the full FjordPhyto project is implemented on board during each voyage. If enough samples are collected throughout the season, we can start to build a complete picture of how phytoplankton are responding to their changing environment. The more samples we have from various fjords along the peninsula, the more we may be able to understand the influence of meltwater on these vital organisms.

Citizen Science Samples Collected 2016-2017 Season

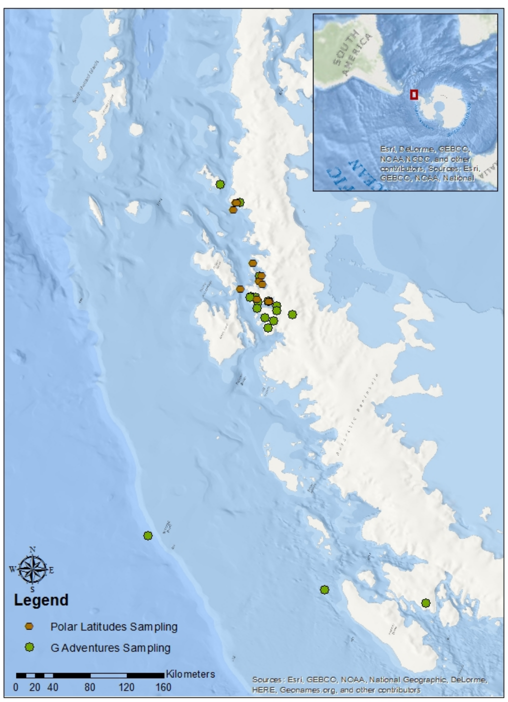



Figure 1. *Phytoplankton samples collected by Polar Latitudes (orange) and G-Adventures (green) at various GPS coordinates throughout the 2016-2017 season.*

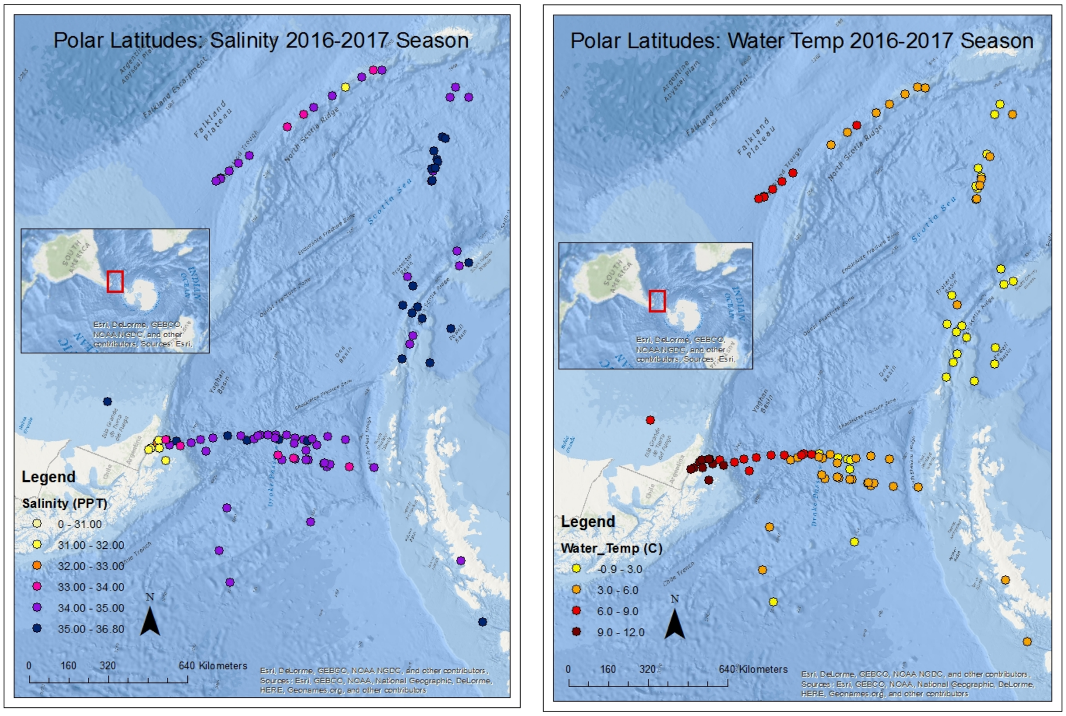



Figure 2. *Calibrated measurements taken by Polar Latitudes during their voyage across the Drake Passage. (left) Salinity measured in parts-per-thousand (ppt). Note that Antarctic waters are much saltier than water found near South America. (right) Temperature measured in Celsius. Note the distinction in temperature difference between Antarctic cold water and South American warmer waters loosely marking the Polar Front.*

Note: Maps were created using ESRI ArcGIS with projections in Geographic Coordinate System: GCS_WGS_1984 and WGS_1984_Antarctic_Polar_Stereographic Projections.

# APPENDIX H

## Acknowledgements & Photo Credits

We would like to additionally acknowledge the following partners who provided invaluable support, mentoring, and feedback in the design of this project: Amy Van Cise - Ph.D. Candidate, Scripps Institution of Oceanography/NOAA Southwest Fisheries Science Center; Bridget Altman, M.A.S., Director of Community Outreach, Ocean Sanctuaries; Claudia Ludwig, M.Ed., NBCT, Director Systems Education Experiences, Institute for Systems Biology; Bob Gilmore, Citizen Science Coordinator, Polar Latitudes; Annette Bombosch, Citizen Science Coordinator, Polar Latitudes; Susan Adie, Expedition Operations Manager, G-Adventures; Ted Cheeseman, Cheeseman Safaris/Happy Whale; Elissa Marton, Senior Marketing Manager, and Mike Greenfelder at Lindblad Expeditions; Lindsey Kern, M.Sc. Candidate, Jack Pan, PhD. Candidate, Phil Zerofski, Marine Collector, Dr. Eric Allen, Kelly Sweich, M.A.S., Nina Rosen, M.A.S. at Scripps Institution of Oceanography. We would also like to acknowledge the input and personal conversations from various researchers and citizen science experts at the Gordon Research Conference on Polar Marine Science 2017 and at the Citizen Science Association Conference 2017. Photo credits for each section: Background Section, Fjord, Andvord Bay by B. Jack Pan; Scientific Questions Section, Phytoplankton in Cod-end by Hsiao-Ching Chou; Scientific question bubbles by Maria Stenzel; Sampling Kit Section by Allison Lee; Education Discussions Section by FjordEco Team. All other images by Allison Lee unless otherwise noted.

# APPENDIX I

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## SUMMARY OF PROPOSED WORK

***Supplement Funding Request for:* "Collaborative Research: Fjord Ecosystem Structure and Function on the West Antarctica Peninsula – Hotspots of Productivity and Biodiversity? (FjordEco)"**

PI: Dr. Maria Vernet, Scripps Institution of Oceanography, University of California San Diego

### I. PROJECT BACKGROUND

As an extension to the existing "Collaborative Research: Fjord Ecosystem Structure and Function on the West Antarctica Peninsula – Hotspots of Productivity and Biodiversity? (FjordEco)" award PLR-1443705 that investigates the impacts of glacial meltwater on Andvord Bay phytoplankton composition, abundance and productivity in the west Antarctic Peninsula (WAP), we request supplemental funds to expand the scope of the project. The expanded scope includes developing a FjordEco citizen science project that seeks to investigate the phytoplankton growth season and phenology of blooms throughout several fjords along the WAP, or **FjordPhyto.**

We aim to: (1) include sampling to characterize the spring/summer conditions at several fjords and coastal embayments along a latitudinal gradient, between King George Island at 62$^{o}$S to Peterman Cove at ~65$^{o}$S to provide an estimate of meltwater distribution in inland waters and freshwater contribution to the continental shelf from these fjords; and (2) understand the phenology of phytoplankton blooms and their variability in relation to meltwater concentration and distribution.

The FjordEco project is carrying out a successful integrated field (2015-2017) and modeling (2016-2018) program to evaluate *processes* in physical oceanography, glacial inputs, phytoplankton dynamics, and benthic community structure and function in Andvord Bay, a glacio-marine fjord along the sub-polar WAP. Andvord Bay, a potential production/biodiversity hotspot, is well suited for the study of fjord ecosystem drivers and the relationship between glacier-ocean-sea ice and phytoplankton composition and productivity; their findings form the basis of the present study. However, the fjords in the WAP are subject to differential forcing and contribution to meltwater is expected to change with latitude, due to both gradients in atmospheric temperature (Cook et al. 2005) as well as oceanic influence (Cook et al. 2016).

Due to limitations in sampling this large, extreme environment in a continuous way, we propose the FjordPhyto project. This complementary project will allow us to leverage support of the International Association of Antarctic Tour Operators (IAATO) by designing a citizen science program around popular sites visited by zodiac landings to shore (Bender et al. 2016). We propose to carry out this research under the guidelines of NSF document 17-047, Public Participation in STEM Research (Science, Technology, Engineering and Mathematics) or PPSR. Preliminary collaboration with IAATO tour operators in 2015-2016 and 2016-2017 indicates this project is feasible.

### II. INTRODUCTION

Fjords are deep estuaries carved by glacial ice, typically containing one or more sills and sediment-floored basins (Syvitsky et al. 1987, Howe et al. 2010). Fjords with tidewater glaciers (glacio-marine fjords) are widespread at temperate to polar latitudes and form important boundary zones between the cryosphere and the ocean (e.g., Powell & Domack 1995). At high latitudes, fjord ecosystems are major conduits carrying glacial ice to the sea and are thus highly sensitive to cryosphere-ocean interactions and climate warming (e.g., Syvitski et al. 1989, Pritchard & Vaughan 2007, Kedra et al. 2010, Weslawski et al. 2011). Why study fjords? Glacio-marine fjords along the WAP appear to be intense, potentially climate sensitive, hotspots of biological production and biodiversity, yet the structure and dynamics of these fjord ecosystems are very poorly understood. Four lines of evidence suggest that sub-polar fjords along the WAP, including the Danco and Graham Coasts, are intense hotspots of productivity and biodiversity, in contrast to sub-polar, glacio-marine fjords in the Arctic. **(1)** Feeding baleen whales are frequently concentrated in and near WAP fjords along the Danco/ Graham Coast in summer-autumn months, apparently in response to dense aggregations of krill (e.g., Nowacek et al. 2011). **(2)** Geological studies indicate that meltwater processes are weak in WAP fjords, resulting in low turbidity and terrigenous sedimentation rates (0.1–0.3 cm $y^{-1}$), allowing high phytodetrital flux and accumulation of siliceous muds only 5-10 km from glacial termini (e.g., Griffith & Anderson, 1989, Domack & Ishman 1993, Domack & McClennen 1996, Boldt et al. 2013). **(3)** Seafloor photo-surveys in WAP fjords reveal extraordinary benthic megafaunal abundance and distinct community structure compared to similar depths (450-600 m) on the open Antarctic continental shelf (Grange & Smith 2013). **(4)** Pilot phytoplankton studies indicate that intense blooms may occur in WAP fjords throughout the summer-autumn, potentially fueling krill/whale aggregations and yielding high phytodetrital and fecal fluxes to fjord benthos (G. Ferreyra, unpubl. data). The question is, how much of this productivity relates to meltwater?

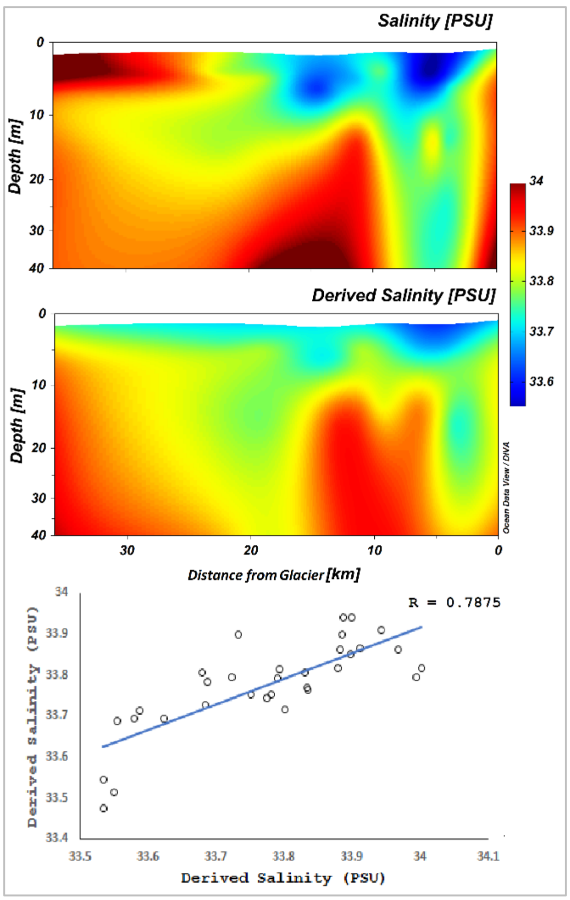


***Figure 1**. Observed and predicted Salinity in Andvord Bay, April 2016. Low salinity is observed in surface waters (0-30 m) within 5 km of the glacier, extending seaward in the top 5 m depth. This Salinity is modeled from Temperature and optical variables (particle backscatter and inorganic absorption) (Pan et al. in prep.).*

Results from FjordEco indicate meltwater is characterized by physical and optical properties, substantiated by Delta 18-O levels and transmissometer profiles, that can be combined in a predictive linear model (Fig. 1). Phytoplankton communities studied in FjordEco presented a more complex pattern than expected. Present-day paradigms indicate that WAP coastal waters have an early diatom bloom (October-November), presumably related to sea ice retreat (Ross et al. 2000), and a summer diatom bloom in January; flagellates (e.g., cryptophytes) dominate in between and after diatom blooms (Kozlowski et al. 1995). Krill grazing and meltwater have been proposed as supporting cryptomonad blooms (Walsh et al. 2001, Moline et al. 2004), however this hypothesis has not been confirmed (Garibotti et al. 2005).

In 2015, a large and productive cryptomonad bloom was observed in December in Andvord Bay (>7 mg chlorophyll a $m^{-3}$) while diatoms dominated in April 2016, a time of maximum meltwater concentration, at the end of the austral summer. Thus, a more continuous and extensive sampling along a latitudinal gradient, in combination with meltwater determinations, is needed to provide a data set to test phytoplankton composition in relation to the environmental drivers at the ocean-ice interface and an estimation of what areas are affected by these meltwater characteristics.

## III. CITIZEN SCIENCE

The FjordPhyto program could serve as a powerful tool in bringing together travelers and scientists to answer critical climate science questions. IAATO steadily brings visitors throughout the year occupying the coastal fjords during times when scientists may be unable to sample. The citizen science model is a proven program that enlists the help of non-scientists to collect simple data that fulfills scientific research studies (Shirk et al. 2012, Gommerman et al. 2012). It is a cost-effective way to fill gaps in research and to spread enthusiasm for science through the civil community. There is a need to increase awareness and education of scientific principles among the public (Miller 2016, Kennicutt et al. 2014) and by engaging and instructing Antarctic guests on tourist ships, this program will address this need. In addition, scientists can gather vital data without incurring a significant cost – by leveraging the help of the already-existing tourism community in Antarctica. Tourism is expected to grow, and the rise of Antarctic tourism has closely mirrored trends in Gross Domestic Product (GDP) (Bender et al. 2016, IAATO, 2016a). Expected visitors making landings for the most recent 2015-2016 season was estimated to be 35,205 (IAATO, 2016c). Current citizen science projects occurring in Antarctica include: Penguin Watch: https://www.penguinwatch.org/; Happy Whale: https://happywhale.com/; Satellites Over Seals: http://phys.org/news/2016-07-antarctica-comfort-couch.html; and Microplastics (Adventure Scientists): http://www.quixote-expeditions.com/science/. However, these efforts are only based on photographs, and none of them cover Antarctic microbes.

The Federal Crowdsourcing and Citizen Science Toolkit (U.S. Federal Government, 2016) includes five basic steps for planning, designing and carrying out a citizen science project (adapted from Bonney et al. 2009). This program identifies five basic steps: i) Scope of the Problem; ii) Design a Project; iii) Build a Community; iv) Analyze and Manage Your Data; v) Sustain and Improve Your Project. As outlined in Section V (Proposed Research), we intend to follow these steps in implementing the FjordPhyto project.

## IV. HYPOTHESES

Three main hypotheses will be tested in this study:

**H1a:** *The presence of meltwater will be observed first in the northern fjords, extending towards the south as the season progresses*.

**H1b:** *The area influenced by meltwater will progress offshore as the season progresses, such that at the beginning of the season (November) meltwater will be mostly constrained inside fjords whereas meltwater will be measured in inland channels (e.g. Gerlache Strait) and further offshore in later summer.*

**Justification H1:** Antarctic and sub-Antarctic tidewater glaciers cover an area of 130,200 km$^2$, and are the largest component of marine-influenced glaciers on Earth (Gardner et al. 2013). For the period 1992 to 2011, the Antarctic Peninsula (AP) lost 20 Gt yr$^{-1}$ of ice (Shepherd et al. 2012). Nearly all of these glaciers flow and terminate in the ocean. For the period 1992 to 2007, the net contribution by the AP to sea level rise has been 0.16 ± 0.06 mm $yr^{-1}$ (Pritchard and Vaughn 2007). On average, AP the glaciers have been retreating and losing shelf area, broadly following the pattern of atmospheric warming (Cook et al. 2005). The extent of the retreating glaciers is rapidly migrating southward, at a rate faster than the atmospheric trends, suggesting that the atmosphere might not be the sole driver of glacier retreat in the region.

A key driver of ice loss is summer melting (Abram et al. 2013). However, the relationship between temperatures and melt is not linear: summer melt happens after a threshold of 0 $^{o}$C, and is related to the sum of the >0 $^{o}$C days (positive degree days), not their mean temperature. Alternatively, Pritchard and Vaughn (2007) attribute the 12% increase in the ice delivered to the ocean by the tidewater glaciers of the Antarctic Peninsula from 1992 to 2005 to a dynamic response to frontal thinning of the glacier, not to enhanced lubrication by meltwater or enhanced snowfall. This lack of agreement on the processes causing glacier retreat in WAP is further complicated by our lack of understanding of the role of oceanic influence, a process critical to ice shelf retreat (Cook et al. 2016).

The Upper Circumpolar Deep Water in the Antarctic Circumpolar Current (UCDW-ACC) impinges the Antarctic Peninsula in its westerly flow and floods the WAP continental shelf (Martinson et al. 2008), with physical characteristics of >1.7 $^{o}$C . In this way, relatively warm water is accessible to glacier fronts within WAP fjords (e.g. Barilari Bay has a deep-water temperature of +0.9 $^{o}$C, Cape et al. in prep.). Northern AP fjords, such as Andvord Bay have a colder deep temperature due to the presence of Weddell Deep Water. In Andvord Bay the FjordEco project measured -1.2 $^{o}$C and -0.6 $^{o}$C deep temperature in December 2015 and April 2016, respectively. The sensitivity of glaciers to oceanic melting makes them vulnerable to the increased UCDW circulation trend. Poleward shift of westerlies and acceleration of the ACC bringing UCDW, is believed to be instrumental in ice shelf melting (McKay et al. 2016). However, model studies predict that any excess heat brought by increased UCDW on the WAP shelf in response to stronger southern winds is lost on the shelf due to increased mixing and it does not reach the ice front (Dinniman et al. 2012). Independent of these controversial results on heat loss from the UCDW on the WAP, meltwater is being delivered to the ocean and phytoplankton responds to its presence.

The melting of ice by the modified UCDW can bring up nutrient-rich water, both with enhanced macronutrients (silicic acid, nitrate, phosphate) and micronutrients, such as iron. These nutrients, needed for phytoplankton growth, are in high concentration in the first 5 km away from the glaciers in Barilari Bay (Cape et al. in prep.). Thus, fjords are similar to fronts, shelf-break upwelling, eddies, icebergs, and other natural fertilization features in the Southern Ocean known to increase productivity through enhanced nutrient availability (Forsch et al, in prep, FjordEco data). In this way fjords can be net exporters of nutrients, in addition to meltwater and ice (Meredith et al. 2013) with the potential to enrich the WAP coastal waters and could be partially responsible for the high productivity observed not only in the fjords but in WAP coastal waters (Vernet et al. 2008). Fjords are known for their richness in plankton productivity (Clarke et al. 2008), benthic diversity (Smith et al. 2008), krill and whales (Novacek et al. 2011). It is these same locations that draw the tourism community to this region (see Fig. 2 and Fig. 3).

**H2:** *Phytoplankton composition will be dominated by picoplankton, cryptophytes, diatoms and single-cell prymnesiophytes, as observed on the shelf (Kozlowski et al. 2011). The dominance of any one group will be function of the season progression and presence/absence of meltwater. (Sea ice retreats early in the northern WAP and waters with sea ice cover will not be sampled from IAATO ships, so this factor is not considered).*

**Justification H2:** Diatoms are known to be abundant during periods of high primary production (Smetacek et al. 1990) and factors favoring diatom growth are considered to maximize production in WAP inner waters (Garibotti et al. 2003a, 2005; Kozlowski 2008). In this way phytoplankton composition is tied with productivity and has the potential to modulate carbon flux to depth. In the WAP shelf waters, periods of anomalously high production are dominated by >20 μm diatoms (e.g. summers of 1996 and 2005, Kozlowski et al. 2011) while flagellates become more abundant later in the growth season due in part to increased grazing (Ross et al. 2000; Walsh et al. 2001). Conditions in WAP sub-polar fjords are ideal to maintain diatom growth throughout the growth season: stratification is provided by a continuous source of meltwater from glaciers under conditions of low wind mixing (Fryxell, 1989). Fe and nitrate input either from glacier melt (5-20 nM of Fe, K. Kim and R. Bundy, pers. comm.) and/or deep mixing (Buck et al. 2010), and turbulence over sills generated by tides (Rodriguez et al. 2001, Tozzi et al. 2004) maintain these systems nutrient rich. Indeed, these conditions were observed in December 2015 and April 2016, with nitrate concentration >18 μm and >75 μm silicic acid (Ekern, unpubl. data). Dissolved Fe was measured at 4-7 nM within the fjord (Forsch, unpubl. data). We expect cryptomonads and prymnesiophytes to be the dominant flagellate taxa (Garibotti et al. 2005) although prasinophytes and other pico-eukaryotes could also be abundant (Kozlowski et al. 2011). The combination of water column sampling of physical and biological variables will provide data to estimate meltwater from Salinity and Temperature in combination with phytoplankton composition measured by microscopy and DNA. In this way, we will be able to detect timing of bloom, number of blooms in a season and their relationship to the main oceanographic factors, and provide data to test the alternative hypothesis that cryptomonads are differentially selected during melting events (Moline & Prezelin, 1996).

**H3:** *Phytoplankton communities respond to the differing physical oceanographic properties among various fjords at the genetic level. Therefore, gene activity will reflect expression patterns related to environmental conditions of the fjords (i.e., genes responsible for environmental sensing, anaerobic metabolism, nitrate reduction, iron uptake, carbon concentration and assimilation, salinity regulation, etc.).*

**Justification H3:** Phytoplankton are sensitive indicators of ecosystem health and change (Racault et al. 2012) and their community structure is affected by a variety of factors including, but not limited to, changes in the chemical and physical conditions such as nutrient content, salinity, and temperature (Garibotti et al. 2005), all of which are affected by meltwater. Gene expression and comparative metatranscriptomics tools provide insight into how microbial organisms respond to environmental cues (Caron et al. 2017). Metatranscriptomics can provide indication of ecosystem shifts and health at the cell level and be predictive of how populations may transition or collapse (Alexander et al. 2015).

There are only a few investigations comparing phytoplankton communities between Southern Ocean environments at the genomic level but a growing number of marine microbial studies are exploring changes in gene expression patterns associated with phenology of blooms (Medlin et al. 2000, Pearson et al. 2015, Caron et al. 2017). In the past decade, there has been an increase of available sequence information; the Marine Microbial Eukaryotic Transcriptome Sequencing Project (MMETSP) database, to be used in this study, provides valuable information on eukaryotic sequences from the marine environment (Keeling et al. 2014).

## V. PROPOSED RESEARCH

FjordPhyto (the FjordEco citizen science project) aims to capture plankton dynamics within the austral growth period (November – March). Through sampling during the tourist season, we will gain a comprehensive view of phytoplankton community structure and bloom dynamics in Antarctic fjords exposed to varying environmental forcing. Several goals have been defined to develop a coordinated strategic partnership with the IAATO community to:

a. Create a sampling program in the AP fjords and Antarctic coastal waters where guests can participate under the guidance of IAATO field experts (Citizen Science Coordinators);
b. Gather citizen science collected samples and data which aim to expand the knowledge of phytoplankton bloom phenology, composition and gene expression within fjords exposed to varying atmospheric and oceanic influences of meltwater production; and
c. Increase Ocean Literacy of visitors to the WAP by involving and educating them on the science process and principles known of WAP fjord ecosystems.

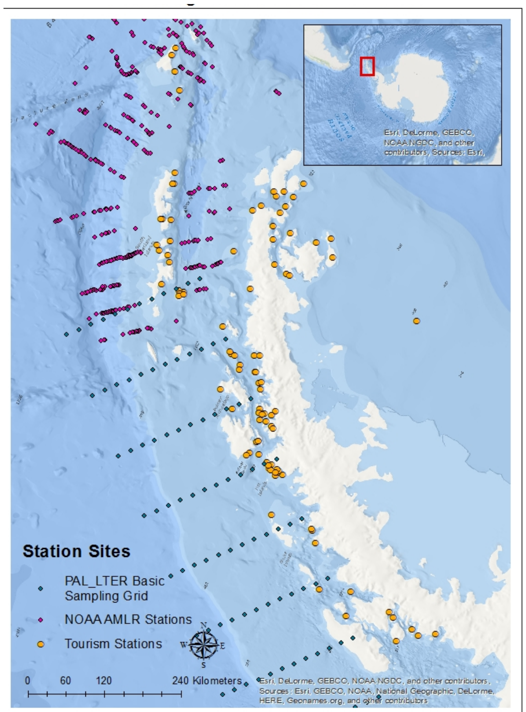


*Figure 2. Locations visited by tourist ships in 2016-2017 season (yellow circles) in comparison with monitoring from Antarctic Marine Living Resources NOAA project (AMLR) (purple dots) and the Long-Term Ecological Research project (Palmer LTER) (blue dots). (Disclaimer: Note some coastal sampling stations are on land. The GIS mapping at high-latitudes is not exact yet, we are working with the GIS Lab Director at UCSD, Ms. Heidi Batchelor, to improve accuracy of basemap and projection. However, the map gives an approximate idea of locations sampled by the different field programs.*

## i. SCOPE OF THE PROBLEM

This first step in the Federal Crowdsourcing and Citizen Science Toolkit has been developed in the Introduction and Hypotheses sections (I-V).

## ii. DESIGN A PROJECT

**Mentorship.** The project will be developed and evaluated with the mentorship of two experienced directors on outreach efforts, Ms. Claudia Ludwig and Ms. Bridget Altman (see letters of support). The PI and graduate student will perform e-meetings when establishing the project and protocols, and will meet at the end of the field season to evaluate guests' data and Citizen Science Coordinators evaluation of each cruise (see below section V. Sustain and Improve Your Project).

**Geography.** The program will be designed around popular sites visited by zodiac landings to shore (IAATO, 2016b, Bender et al. 2016). Throughout a given season there are typically 25 top fjord destinations predetermined annually for each tour operator's itinerary and shared among the IAATO community for coordinated efforts (see Fig. 2). This coordination comes about through the legally binding agreement of Measure 15 (2009) as a result of the Antarctic Treaty Consultative Meeting (ATCM XXXII, Baltimore) (IAATO, 2017a) and provides a reliable method to pre-determine sites with each upcoming season.

The top 25 most popular landing sites (yellow circles in Fig. 2), are ranked as: 1) Neko Harbour (in Andvord Bay), 2) Cuverville Island, 3) Goudier Island, 4) Half Moon Island, 5) Whalers Bay, Deception Island, 6) Petermann Island, 7) Brown Station, 8) Jougla Point,

9) Danco Island, 10) Brown Bluff, 11) Vernadsky Station, 12) Telefon Bay, 13) Barrientos Island, Aitcho Islands, 14) Orne Harbour, 15) Yankee Harbour, 16) Mikkelsen Harbour, 17) Damoy Point/Dorian Bay, 18) Paradise Bay, 19) Pléneau Island, 20) Hannah Point, 21) Port Charcot, 22) Great Wall Station, 23) Yalour Islands, 24) Waterboat Point/Gonzalez Videla Station, 25) Bellingshausen Station. These sites are visited repeatedly by the IAATO ships bringing more than 35,000 tourists per season (Bender et al. 2016). Trips follow itineraries spanning 10-12 days, from the end of October to mid-March, allowing for at least 8 trips per year. Preliminary data from 2016-2017 season provided sampling 40 phytoplankton samples with physical variables from surface waters, eight of them from Neko Harbor, generating a time series in Andvord Bay.

**Sampling.** We will follow approaches developed by Plankton Planet (E.B. 2017). Specific details and protocols will be developed before the first sampling season. Before the field season we will meet with IAATO ships participating in this project (e.g., Polar Latitudes, see letter from Mr. Bob Gilmore, and G-Adventures, see letter from Ms. Susan Adie) in order to coordinate sampling locations, train the Citizen Science Coordinators in each vessel, develop a protocol to upload citizen science photos and blogs to the internet, and shipping of data and samples to the US.

All techniques and methods will come with easy-to-follow print-out instruction materials and training videos accessible through the FjordPhyto website (www.fjordphyto.wordpress.com, in development). In addition, in-person training at the IAATO Field Operators Conference (September, 2017) will explain the use of each piece of equipment in the kit to Citizen Science Coordinators in the field. The PI and a graduate student will participate in at least one cruise each in order to train the Citizen Science Coordinators on the IAATO vessels. The participation is contingent to space availability, it will be done early in the project in order to provide training and best sampling practices (see letter from G-Adventures and Polar Latitudes).

Preliminary testing locally in California will help determine usability and effectiveness of methods prior to training Citizen Science Coordinators. Pilot participants (i.e., non-scientists) will beta test instructional videos, data recording methods, and provide feedback on how easy the program is to use. Continuous user feedback will guide further development of this program.

In the field, all necessary materials and equipment will be provided in a kit. The FjordPhyto sampling kit will contain plankton nets of two mesh sizes (20 µm and 63 µm for micro-phytoplankton, and micro-zooplankton and small copepods), a hand-held CTD (conductivity-temperature-depth) instrument, sample bottles and preservation solution, filtration set ups with hand pumps, filters, gloves, tweezers, data sheets, and a portable projection microscope. Educational material will also be provided and include phytoplankton taxonomy identification cards and microphotograph books (e.g., Plankton by Christian Sardet) for the IAATO Citizen Science Coordinators to share with the participants.

Phytoplankton and water measurement sampling (detailed below in Techniques) will be carried out from the Zodiacs during landing excursions on the return to the main ship (to insure sample freshness). After collection of phytoplankton with nets, participants will filter samples with a hand pump onto provided filters. Samples will be handled as detailed in the provided easy-to-follow instructions within each kit. Participants will record data electronically and on waterproof print-out data sheets and send back to researchers at SIO along with collected samples. We will provide templates with the information to be filled by the guests. Electronic and hand-recorded data will be extracted and excel spreadsheet files will be sent via email to researchers at SIO along with samples for further analyses.

**Sampling methods:**

**1. Meltwater characteristics:** Using low cost calibrated equipment, participants will deploy sensor at specified points along Zodiac landing routes. Using a calibrated SonTek CastAway® CTD, information on water temperature and salinity can be collected. Additionally, water turbidity will be measured with a Secchi disk. Once on board, supernatant resulting from phytoplankton filtration (see below) will be collected and stored at -20 °C for nutrient analysis.

**2. Biodiversity of zooplankton and phytoplankton:** By using plankton nets, participants will collect and preserve phytoplankton samples (micro- e.g., >20 μm) and zooplankton (micro- e.g., >63 μm) within fjords. Sampling location is preferential at 0-5 km from glacier terminus, within 0-30 m of the water column, where glacial meltwater presence is observed and predicted (see Fig.1). Nets will be deployed to 30 meters and towed slowly behind the Zodiac for a specified amount of time. Contents of net collection will be poured into dark amber bottles and kept at ambient temperatures for processing back on the ship. Once on board, a portion of the sample will be reserved to share with participants on the cruise ship for educational viewing purposes under the microscope, while the bulk of the sample will be filtered by citizen science participants using filtration set up and protocols provided in the kit. Concentrated phytoplankton biomass will be collected onto 0.2-micron polycarbonate filters. Filters will be inserted into tubes pre-filled with appropriate preservation solution and frozen at -20°C. In the event that freezers are not available, alternate preservation methods will be used including submersion in RNA Later (for RNA extraction) and in 96% v/v ethanol which acts as a preservative solution providing high-quality yields (for DNA extraction) (Bressan et al. 2014, Black et al. 2003, and Stein et al. 2013). Microscopy samples will be fixed with Lugol's solution (or 2% formaldehyde), and kept in a dark and cool place. In the USA, further analysis using microscopy and genetic extraction techniques will be carried out, as described in section iv. Analyze and Manage Your Data. Permits are not needed to sample and preserve phyto- and micro-zooplankton as per United States Antarctic Program following the Antarctic Conservation Act.

**3. Taxonomic Identification using images and iNaturalist:** In the field, Citizen Science Coordinators will help guests on board the IAATO ships to make an approximate identification of the phytoplankton and microzooplankton observed in real time. They will have access to custom taxonomy identification guides (created by the PI and graduate student) and supplemental material such as the book *Plankton* by Christian Sardet. For this purpose, kits will include USB Microscopes (e.g., the Aven 26700-300 zipScope) which will have sufficient magnification power to view the large phytoplankton species collected by the nets.

Participants will take images of phytoplankton under the microscope or on a projection screen using their mobile devices. Photos, which are time, date, and location stamped, will provide preliminary qualitative taxonomy data on phytoplankton community composition within the fjords. Images will be uploaded to the growing, free and publically available crowd sourced database iNaturalist, available for download as a smart phone app. iNaturalist app can be used with no service - in most places with no cell service, the phone can still connect to GPS and get the correct location.

iNaturalist is a *crowdsourced species identification system* and an *organism occurrence recording tool*. The site was acquired by California Academy of Sciences in 2014 and serves as a public record of observations taken by anyone around the world. This tool provides an excellent existing platform for Antarctic citizen science participants to view preliminary results while further analysis is conducted on field collected samples by researchers back in San Diego.

Once images are uploaded to the database, organisms are identified through participation of crowd-sourced community, scientific community, and volunteer site curators using the 'Identify' tool created by iNaturalist developers. Taxonomic data utilizes the Catalogue of Life and uBio data sources and observations can move from the "Needs ID" status to the "Research Grade" level when the community agrees (more than 2/3 of identifiers) on a taxon to species-level ID or lower (Shepard, 2017). Data will also be uploaded to the FjordPhyto website where further materials, species identification, blogs, reports, and data will be hosted and shared with IAATO partners.

iii. BUILD A COMMUNITY

IAATO members come together annually to ensure best practices and environmentally responsible operations in Antarctica and provide annual reports, tourism statistics, and Visitor and Tour Operator Guidelines on their publically accessible website. One mission of IAATO is to "*Support science in Antarctica through cooperation with National Antarctic Programs, including logistical support and research and to foster cooperation between private-sector travel and the international science community in the Antarctic"* (IAATO, 2017b).

This FjordEco citizen science program – FjordPhyto - will be one of a kind, truly engaging visitors with the active process of hands-on sampling required for scientific work. By strategically partnering with interested tour operators and volunteer guests, we can build the citizen science program that naturally fits into pre-existing tour itineraries and cooperatively build an excursion program for guests on tourist ships.

A preliminary trial of FjordPhyto was tested during the 2016-2017 season with two IAATO tourist companies: Polar Latitudes and G-Adventures who collected the preliminary data in 2016-2017. Anywhere from 1-3 seawater samples were collected on any given voyage (12-24+ samples) with a range of 5- 15 passengers participating between 12 voyages (150-180 guests in the season) (see Fig. 3). The data collected from this pilot run will contribute to a portion of graduate student's degree, Allison Lee, Master of Advanced Studies (MAS) at Scripps Institution of Oceanography.

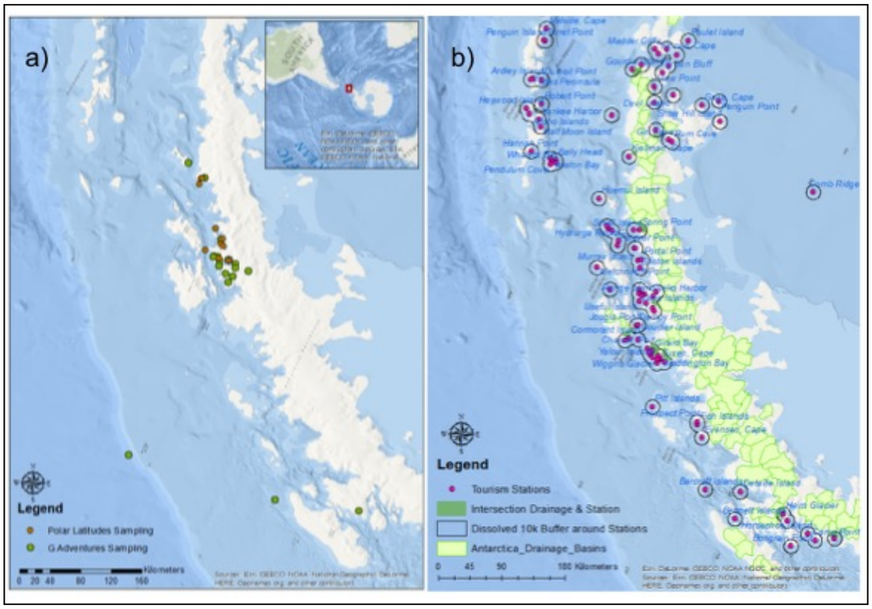


*Figure 3. Locations of tourism landing sites in any given season. Left panel: Sites where water and phytoplankton samples were collected during the 2016-2017 season by two IAATO companies G-Adventures (green circles) and Polar Latitudes (orange circles). Right panel: All sites where tour ships visit during any given season (magenta circles). Drainage basins (data retrieved from ArcGIS Online) are mapped and show intersection points (dark green) where drainage basins come within a 10km range of tourism landing sites. Higher number of tourism sites correlate with areas where drainage enters the ocean, potential production/biodiversity hotspots (see I. Project Background).*

Citizen Science Coordinators on board the IAATO ships will be responsible for coordinating shipment all biological samples, water samples, electronic Excel spreadsheet data and photo data to the Vernet Lab at SIO, San Diego. Data and samples will be processed and analyzed by a graduate student as part of her/his thesis and shared online through two public websites (see section iv. Analyze and Manage Your Data).

iv. ANALYZE AND MANAGE YOUR DATA

Samples shipped back to researchers at SIO will have been properly stored and transported upon arrival to port (e.g. Ushuaia, Argentina). All data and photos collected in the field will be loaded onto a provided hard-drive which will be sent to Scripps researchers at the end of the season. Example of data collected during the 2016-2017 season by G-Adventures and Polar Latitudes included: Place, Date, Time, Latitude, Longitude, Water Temperature ($^{o}$C), Air Temperature ($^{o}$C), Precipitation, Wind, Swell, Conductivity, PPT, and Secchi depth (measured in meters). Microscopy data will also be uploaded to the FjordPhyto website.

**Online Sharing Platforms:**

1. **iNaturalist:** Images of phytoplankton taken by guests under the microscope will be uploaded by guests (if bandwidth is available on board the IAATO ships) or researchers to the online datamap iNaturalist, and will contribute to populating the sparsely documented observations for “Kelp, Diatoms, and Other Allies” of the Antarctic Peninsula region.

2. **FjordPhyto:** FjordPhyto (https://fjordphyto.wordpress.com/) (in preparation) will be the sister website to FjordEco (https://fjordeco.wordpress.com/). Results from analyzed data and samples will be shared online with the IAATO community through non-technical trip reports and blog posts to retain interest and engagement. IAATO Citizen Science

Coordinators will have access to the results in time to share with guests during the following annual season.

3. **Visualization**: Maps for spatial visualization of fjords sampled, and any patterns emerging from taxonomy community composition data will be shared. Data will be synthesized after each austral season and presented through iNaturalist, FjordPhyto website, and scientific conferences. Data from alternate sources, such as remotely sensed data or weather information, can help cross check volunteer-collected data and will be used to do so. Data management will follow best practices, rigorously documenting any processing methods, including details on merging values, correcting data, statistical analysis, and omitting data.

**Sample analysis:** The samples collected for scientific purposes will be analyzed in the labs, either at Scripps Institution of Oceanography (physics, transcriptomics, turbidity) or at the University of La Plata, Argentina (see letter from Dr. Almandoz in support of microscopy analysis).

1. **Phytoplankton composition and abundance**: Taxonomic analysis can provide abundance and diversity estimates through microscopy and targeted metagenomics approach. Species identified from this work will corroborate and add to basic qualitative taxonomic efforts performed by citizen scientists with a microscope in the field. To identify organisms at the lowest possible taxonomical level, microalgae preserved in 2% formaldehyde and diatoms cleaned of organic matter (Hasle and Fryxell 1970; Prygiel and Coste, 2000) and mounted on permanent glass slides with Naphrax medium (Ferrario et al. 1995) will be examined using a phase contrast and a differential interference contrast Leica DM 2500 light microscope (LM) equipped with a Leica DFC420 digital camera. For more in-depth studies, the material, mounted on stubs and coated with gold-palladium according to Ferrario et al. (1995), will be examined using a Jeol JSM-6360 LV scanning electron microscope (SEM). In order to describe certain taxonomic and ecological characteristics of the organisms, such as, colony formation, etc., many of the observations will be done on whole cells, both at LM as SEM. Permanent slides will be deposited at the Herbarium of the División Científica Ficología (LPC), Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, La Plata, Argentina.

   To estimate cell abundance, all phytoplankton groups from samples fixed in Lugol's, will be examined at 400X magnification with an Iroscope SI-PH inverted microscope (Utermöhl 1958). Previously, 100 ml of each sample will be settled for 48 h in sedimentation chambers. At least 300 individuals will be counted in random fields, and the results will be expressed as number of cells per liter of seawater.

   To estimate the relative abundance of the diatom species, at least 500 valves present in the permanent slides prepared from the net samples will be counted at 1000X magnification using LM and oil immersion, and the results will be expressed as percentages.

2. **RNA analysis for gene transcription (Metatranscriptomics):** We aim to explore gene expression activity within phytoplankton populations among various fjords. Total RNA will be extracted from filters, treated for DNA contamination and enriched for eukaryotic mRNA through a poly-A pull-down onto oligo-dT beads (e.g., Malviya et al. 2016). The enriched RNA sample will then go through Illumina library preparation and sequenced

(paired-end reads) at the IGM Genomics Center at the University of California, San Diego along with targeted metagenomic samples. The IGM Genomics Center uses FastQC to visualize and determine quality of raw sequence data, which will then be cleaned and trimmed. All sequence reads from phytoplankton assemblage will be mapped to available genomes available at the Marine Microbial Eukaryotic Transcriptome Sequencing Project (MMETSP) database on iMicrobe and NCMBI BioProject websites.

3. **DNA Analysis for targeted genomics (metagenomics):** Using a targeted metagenomics approach we will explore genus-level diversity and community composition of eukaryotic phytoplankton. Total DNA will be extracted from filtered sea water collected by citizen scientists. Following methods outlined for the Tara Oceans project in Gimmler et al. 2016, total DNA will be extracted from samples. For a targeted approach, the hypervariable V9 region of the nuclear gene encoding 18*S* rRNA will be amplified through polymerase chain reaction (PCR). Libraries will be prepared for paired-end Illumina sequencing and sent to the IGM Genomics Center at the University of California, San Diego. Reads will be aligned with sequences of the non-redundant sequence database of the National Center for Biotechnology Information (NCBI) and MMETSP. Alignments will be clustered into meaningful operational taxonomic units (OTUs) and assigned taxonomy by comparison to reference sequences in the above-mentioned database.

4. **Nutrient analyses:** Seawater samples will be collected (5mL) and frozen for analysis in San Diego. At time of analysis, samples will slowly be thawed at room temperature. Using methods outlined in Valenzuela et al. (2012), phosphate, nitrate and nitrite can be measured using colorimetric analysis and a spectrophotometer. Phosphate will be measured using Malachite Green Phosphate Assay Kit (Sigma-Aldrich). Nitrate and nitrite will be measured using the Szechrome NAS colorimetric analysis kit from Polysciences, Inc. All protocols will follow manufacturer instruction. Silicate will be carefully measured according to Coradin et al. (2004) protocol using a colorimetric ammonium molybdate- blue silicomolybdic assay. Nutrient analysis methods have been adapted for small volume analysis as demonstrated in Valenzuela et al. (2012).

**Data Analysis**: The physical (CTD), optical (Secchi disk depth), phytoplankton composition and abundance (microscopy and DNA) and phytoplankton gene expression to environmental forcing (metatranscriptomics) will be analyzed in order to test the 3 hypotheses in this project:

**1. Meltwater: definition, distribution and concentration**: Meltwater will be defined from Salinity and Turbidity. Fine sediment suspension and low salinity characterized the meltwater in Andvord Bay in 2016 (Pan et al. in prep.). These properties were indicative of meltwater: salinity correlated with Delta 18-O (Fig. 4) and higher particle backscatter measured with a Hydroscat (Hobbie Labs). In this project, we will estimate turbidity with a Secchi disk, the first turbidostat. Although simple, if taken carefully the Secchi depth can provide excellent information on the light attenuation in the water column (Lee et al. 2015). If nutrient concentrations are available they will provide further information on meltwater (Lee et al. 2015). Results of meltwater distribution and concentration will be plotted spatially using ArcGIS (GIS) and Ocean Data View(ODV) software.

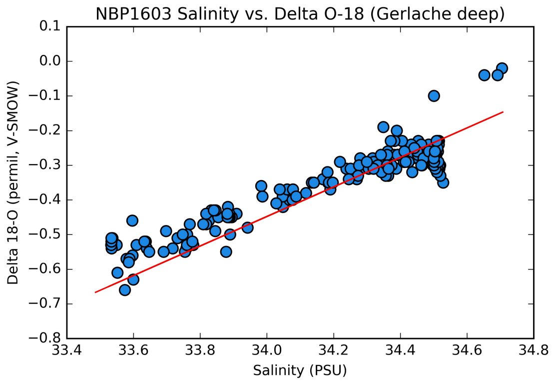


***Figure 4.*** *Correlation between Delta 18-O and salinity in Andvord Bay during April 2016. The red line depicts mixing between two sources, UCDW (a Delta 18-O of zero) and glacier ice (delta 18-O of -13.6). Based on the mixing line, an estimate of %meltwater can be obtained, usually the range is 0%-5% meltwater.*

**2. Phytoplankton diversity and function:** By probing abundance data of rDNA and microscopy with Community Similarity Analyses, we aim to evaluate eukaryotic biodiversity and taxonomy and variance between ecological patterns and phenology of blooms across the fjords sampled in the WAP region. Results will compare with current literature and provide further insights to diversity within Southern Ocean phytoplankton communities. Cluster analysis groups of organisms based on their phenotypic or genotypic similarities in order to infer the phytoplankton communities. This method minimizes within-group variation and maximizes between-group variation in order to reveal well-defined categories of samples, and therefore reduce the dimensionality of the data set to a few groups.

Metagenomic analysis will be performed using the program MEGAN5 for taxonomic, functional, and comparative analyses. All sequences will be archived to the MMETSP database. Statistical analysis will include but not limited to a Spearman's rank correlation to compare gene activity of samples between fjords and one-way analysis of variance (ANOVA) will be used to determine statistically significant differences between species diversity of samples taken from various fjords over a time-series. Metatranscriptomic profiles will be compared to the species diversity associated with the targeted 18s diversity profiles. Analysis will indicate how expression patterns compare between various fjord properties in relation to species diversity. This rich dataset will be made publically available so researchers can use these findings to further curate their ocean and climate models for phytoplankton in the Southern Ocean.

**3. Response of phytoplankton to environmental variables**: Multivariate analysis will be used to provide understanding on the what biological variables correlate with physics, nutrients and meltwater. After plotting and mapping the environmental and phytoplankton data with ArcGIS and ODV in order to obtain a first view of distribution, we will use Correspondence Analysis (CA) to answer the question whether a certain species (or group of species) occurs at specific sites, as a measure of their ecological preferences (e.g., do they prefer fjords with less meltwater?). CA has been used successfully in microbial ecology to determine whether patterns in microbial OTU distribution could reflect differentiation in community composition as a function of seasons, geographic origin, or habitat structure (Ramette 2007).

**4. Data Storage:** Data collected in the field will be stored on an external hard drive and metadata entered in paper or electronically in an excel-type file. In the US, data will be organized at Scripps Institution of Oceanography, with quality controls to establish accuracy, instruments calibration; data will be plotted to ensure quality and detection of outliers (Helly et al. 2011). Once clean the data will be organized in text files, suitable for plotting (ODV, GIS, etc.), analysis (R, Matlab) and visualization. These files will be subsequently submitted to appropriate depositories: oceanographic/ environmental data will be sent to a long-lived repository (e.g. BCO-DMO) and metadata will be deposited in the Antarctic Master Directory. iMicrobe and NCMBI BioProject websites will be used to store DNA and RNA sequencing data. Photos will be stored in iNaturalist and Wordpress web site. Phytoplankton diatom slides prepared permanently in Naphrax will be stored at the Frenguelli Diatom Collection at the University of La Plata, Argentina.

v. SUSTAIN AND IMPROVE YOUR PROJECT

Success of the program is defined in two ways:

**1.Scientific relevance:** Direct feedback from scientists on whether data is of high quality, has meaning, and provides scientific merit, and

**2. Guest participation and learning:** Are the participants having a good time with the activity? Are they learning something new? Do they understand the scientific relevance, why the data and samples are collected? Do they come away after the experience with a better understanding of the ecosystem? Are they motivated to tell their friends and family about their experience in Antarctica?

Measures of these successes can be partially evaluated using feedback surveys provided in the kit. All surveys will only be intended for improvements to development of the program, excluding IRB review. The Citizen Science Coordinator from Polar Latitudes, Mr. Bob Gilmore, indicates, “These [measures] are greatly influenced on how well we, as expedition staff educate them (guests) throughout the voyage... and this is something that we take extremely seriously! If the passengers go home educated and have a new perspective on this planet, how they fit into it and are motivated to tell their family and friends all about their experience in Antarctica and are also motivated to change possible former environmentally destructive behavior, then our Citizen Science Program was an absolute success!!”

By building a strong relationship with IAATO Tour Operators, Citizen Science Coordinators, Guests and Researchers, continual feedback can be obtained which will lead to improvements in the program’s growth.

The long-term vision includes a well-functioning network of strategically coordinated operators that can provide samples from multiple fjords throughout the five-month austral summer. This supplemental request will initiate such a program. Further work will be carried out through future requests to NSF and other agencies involved in funding Citizen Science programs.

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