UNIVERSITY OF CALIFORNIA SAN DIEGO

PHYTOPLANKTON COMMUNITY SUCCESSION IN RELATION TO SALINITY AND TEMPERATURE ALONG THE WESTERN ANTARCTIC PENINSULA: A FOUR-YEAR STUDY LEVERAGING PARTICIPATORY SCIENCE

A Dissertation submitted in partial satisfaction of the requirements
for the degree Doctor of Philosophy

in

Oceanography

by

Allison Michelle Cusick

Committee in charge:

Maria Vernet, Co-Chair
Peter Franks, Co-Chair
Omar Akbari
Andrew Allen
Jack Gilbert
Fiammetta Straneo

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The Dissertation of Allison Michelle Cusick is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

University of California San Diego

2025

DEDICATION

I dedicate my doctoral thesis work to all the curious minds who seek to leave the world a better place, who care for the environment and all the biodiversity of life on Earth. I dedicate my thesis to all those who are still discovering their passions - who don't yet know "what they want to be when they grow up". May you find your own path by following your passions, holding fast with grit and determination – persevere, build your resilience, see problems as opportunities to grow, and be conscientious of your actions and how it impacts others around you. If doors don't yet exist to open, build them. If you open a door, hold it open so others may follow. Build bridges between worlds. It will not be easy, but you can achieve extraordinary things! Of course I dedicate this work to my parents, Cindy and Jim, for creating me and to all my extended family for influencing who I have become. I dedicate this work to all the amazing individuals in the polar expedition cruise sector who made FjordPhyto come to life through their own support and enthusiasm for getting people involved in science while on vacation! Thank you for supporting this work, thank you for supporting me. Without the collective efforts of thousands of people, this thesis and many of the 'deliverables' of FjordPhyto participatory science program would not have been possible. Thank you to everyone who has contributed!

EPIGRAPH

"Don't ask what the world needs. Ask what makes you come alive and go do it. Because what the world needs is people who have come alive." - *Howard Thurman*

"Exploration is in our nature. We began as wanderers, and we are wanderers still. We have lingered long enough on the shores of the cosmic ocean. We are ready at last to set sail for the stars." – Carl Sagan

"People ask: Why should I care about the ocean? Because the ocean is the cornerstone of earth's life support system, it shapes climate and weather. It holds most of life on earth. 97% of earth's water is there. It's the blue heart of the planet — we should take care of our heart. It's what makes life possible for us. We still have a really good chance to make things better than they are. They won't get better unless we take the action and inspire others to do the same thing. No one is without power. Everybody has the capacity to do something." – Her Deepness Sylvia A. Earle

"The best scientists and explorers have the attributes of kids! They ask question and have a sense of wonder. They have curiosity. 'Who, what, where, why, when, and how!' They never stop asking questions, and I never stop asking questions, just like a five year old." – Her Deepness Sylvia A. Earle

"Participating in the FjordPhyto science boat was the first time in a long time where I've felt that child-like sense of curiosity." – Participant in the FjordPhyto science boat activity 2017

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LIST OF ABBREVIATIONS

CHEMTAX	Chemical Taxonomy
CSESP	Citizen Science for Earth Systems Program
DsN1	Days since Nov. 1
HPLC	High Performance Liquid Chromatography
IAATO	International Association of Antarctica Tour Operators
MAST	Marine Stramenopiles
NASA	National Aeronautics and Space Administration
NSF	National Science Foundation
OPP PPSR	Office of Polar Programs Public Participation in STEM Research
SO	Southern Ocean
SSS	Sea Surface Salinity
SST	Sea Surface Temperature
STEM	Science Technology Engineering and Math
NAP	Northern Antarctic Peninsula
CAP	Central Antarctic Peninsula
WAP	Western Antarctic Peninsula

ACKNOWLEDGEMENTS

I have thousands of people to thank who have been formative in my career as a scientist and the research I have been able to partake in and conduct dating back to my first internship in science in 2006. At the culmination of this doctoral degree, I have reached the age of 41 years and have actively pursued STEM disciplines since 2002, growing in different ways as a scientist over the last 23 years. This STEM journey over two decades, with the last eight years focused on my work in graduate school, has taught me an incredible amount about science and being a scientist, of which I will forever be both grateful and traumatized. To say the least, I could use a sabbatical now!

First and foremost, the success of the FjordPhyto program in which the data for this thesis arises would not be possible without the >8,000+ people who have contributed to supporting the program including the support from cruise expedition operators, polar expedition guides facilitating the program, the curious enthusiastic travelers who participated, the science research team and collaborating students, collaborators, and the financial support from NSF, NASA, donations, seed grants, and fellowships. Without these people also believing in the program, believing in the team, believing in me, nothing about this dream could have become a reality. A special huge thank you to my colleague, close collaborator (lifelong!) and friend Dr. Martina Mascioni who ran the program with me for years and was instrumental in supporting our growth logistically, scientifically, and for me personally on an emotional and morale level. I cannot express enough how much all of this support has meant to me. Thank you to all the students who asked me to be their mentor or sit on their committee, thank you for contributing your passions for the project and for trusting me to share my perspective on the work you brought to the table.

Thank you to Dr. Alaina Smith and Nik Yanek-Chrones for all the coding help and morale uplift, meeting for long hours every week at cafes around San Diego to work together data wrangling a large dataset the last year of My PhD. Without these two individuals in the trenches with me, I would also not have gotten through my final year of my PhD. Thank you to the Flower Pot Café (previously known as La Jolla Pannikin) for providing great coffee and vibes.

I would like to thank my co-advisor Dr. Peter Franks for being the first faculty we interacted with at Prospective PhD Open House in 2017. On that day he told everyone in that room that we belonged in science and his statement of inclusivity resonated deeply with me. I thank Peter for adopting me into the Frank's lab in 2019 and taking me under his wing especially in the final years to push my thesis to the finish line. I would not have been able to get there without his encouragement and close supervision. Dr. Franks created a space in his lab for us to discuss anything we wanted and provided unwavering mentorship to me. He taught me about writing a thesis proposal, publishing a paper with a team of co-authors, about Biological Oceanography, about eating good food, about being kind and investing in supporting others career growth and mental health. He taught me to honor the human side of being a scientist. He taught me so much more than I'll ever be able to articulate, and I am forever grateful for his guidance and mentorship! In the words of Peter, "Go be smart, and be nice to people!"

I would like to thank my co-advisor, Dr. Maria Vernet. I am grateful for her initial meeting with me over lunch in 2016 when I was in the Scripps Institution of Oceanography, Marine Biodiversity and Conservation program looking for ideas that would allow me to continue my passion for phytoplankton research, genetics work, systems thinking, ecology goals, ecotourism, and working in Antarctica. Thank you for introducing me to Robert Gilmore, the

FjordEco team, our colleagues in Argentina Dr. Martina Mascioni and Dr. Clara Iachetti and for working with me to write two full proposals first in 2017 to the National Science Foundation Office of Polar Programs (NSF OPP) and then in 2021 and 2024 to NASA's Citizen Science for Earth Systems Program (NASA CSESP). I gained valuable insights into idea generation, grant writing, and budgeting to fund life into the FjordPhyto program. This program has become bigger and more impactful than any of us had ever imagined it would.

I would like to thank Dr. Andy Allen for serving as my first-year advisor, for providing access to his lab and wonderful team at the J. Craig Venter Institute, for being positive and supportive of my work. Andy brought an enthusiasm for the molecular life of diatoms, nerding out over phytoplankton and encouraging a fun lab culture going to Padres games and socializing with each other at happy hours. Thank you to Dr. Allen for collaborating with me over the years, and for collaborating together to start a new initiative - the Genomics At Sea Program (GASP) - with Viking expedition ships to further innovative ways of doing phytoplankton research and supporting the next generation of scientists.

Thank you specifically to the Allen Lab members Hong Zheng for her patience and support in helping me process hundreds of DNA and RNA samples from FjordPhyto, and to doing the library prep for the RNA samples. Thank you to Dr. Ariel Rabines, Dr. Rob Lampe, and Dr. Zoltan Füssy, Dr. Chase James for help with the automated pipelines and bioinformatics which allowed me to analyze the data for my thesis. Thank you to Dr. Füssy for annotating the RNA data from FjordPhyto, Sermilik fjord in Greenland (Dr. Fiamma Straneos work), and Hearts in the Ice Svalbard Norway (Sunniva Sorby and Hilde Fålun Strøm's initiative). While this data did not end up making it into my PhD thesis, I have confidence it will emerge in other scientific work and continue to provide novel contributions to the polar science field.

Thank you to all my curricular group cohorts from the MAS MBC program ’17 and from the PhD 2017 cohort who have been a source of support and comfort during my graduate school experience. Thank you to my office mates Dr. Tammy Russell and Dr. Jack Pan for being great colleagues and ears to listen and commiserate on the woes of grad life. I have met so many wonderful people and forged strong friendships.

Thank you to my past housemates and closest friends who have been through thick and thin with me, through the highest highs and the lowest lows. I’ll have a lot of traumas to unpack from grad school and neglected and broken friendships to repair! Thanks for sticking through it with me - I will never be able to express how grateful I am to you all, even to those of you who saw me as a monster at my lowest of times. Thank you to my German Shepherd Misha who lived 14.5 years and was by my side for the most formative adults years of my life. She was an amazing adventure companion and a part of my soul. RIP Misha. Thank you to my mom and dad and my family who have helped form who I am today.

Thank you to my committee members for supporting my PhD endeavors and for all the professors who taught me a wealth of information in their classes.

I worked in STEM for 10 years prior to graduate school in the fields of neuroscience, immunology, field ecology, ornithology, and rainforest biology. I even worked as a Swedish baker in Seattle’s Pike Place Market and as a bartender at a friend’s salsa dance studio. I never wanted to go to graduate school. To be honest, I didn’t think I was smart enough to go and the only stories I had heard were about how toxic it was for students. I was the first in my family to go beyond high school and getting a bachelor’s degree felt like a very great accomplishment (which it is)! I was inspired to pursue a STEM degree in college because I wanted to travel the furthest any human could travel and I saw that many NASA astronauts had STEM degrees; I

loved being outdoors, understanding the natural world, traveling and I hoped someday I would reach the stars.

Thank you to all my past supervisors, mentors, and lab mates who never made me feel like I couldn't be a scientist just because I didn't have a graduate degree. I want to especially thank Dr. Monica Orellana for hiring me as her technician in 2010 at the Institute for Systems Biology (ISB) and introducing me to the world of bioreactors, systems thinking, phytoplankton and oceanography. I thank Dr. Orellana for including me as a co-author in my first scientific publication which was truly the first time, I thought to myself that maybe I could be a "real scientist" too, even if I felt like I was "just a technician". Technicians and people who do not have higher education credentials to back their expertise are not to be undervalued or underestimated, ever. Thank you to everyone at ISB for the professional development and sense of belonging in science no matter what my academic credentials. I also want to thank Monica for sending me to Antarctica in 2013, trusting me to go in her place as Co-Principal Investigator. I want to thank the entire TRACERS team who I worked with on the *RV Nathanial B Palmer* in the Ross Sea. You all embraced me and treated me like one of the team. Because of that opportunity, my life and career path changed.

I want to thank Dr. Cassandra Brooks who I met during that voyage. She was working on her PhD in larval toothfish ecology and policy advocating for the Ross Sea Marine Protected Area. Because of her, I saw that scientists could have more impact in the world than simply publishing papers. She revealed to me that scientists could literally change the world with their work. She encouraged me to pursue an interdisciplinary master's degree, which I started in 2016 through Scripps Institution of Oceanography's Master of Advanced Studies in Marine Biodiversity and Conservation at the University of California San Diego. The ironic thing is that

prior to my Antarctic experience I had vowed I would never work with the ocean as I thought it was too wet, too cold, too windy – and I get seasick. I recognize the irony now that I have pursued three graduate degrees (MAS in Marine Biodiversity and Conservation, MSc in Marine Biology, and PhD in Oceanography) all focused on the coldest, windiest, most hostile and wonderful ocean in the world surrounding Antarctica. Through my time in graduate school, I felt like a pioneer and have somewhat felt in a way I scratched the itch to becoming an astronaut, exploring the furthest reaches of the mind and the most incredible places on Earth.

Thank you to all the UAW union strikers and 48,000+ students and postdocs who fought for increase wages for students and to the faculty and administration who supported the increase for student living. I took a financial risk going back to school in my mid-thirties and as the living wage in La Jolla skyrocketed over my eight years there, financial ruin was staring me in the face. My saving grace was an offer to move into subsidized graduate housing studio the last five months of my PhD in 2024 which gave me a safe affordable haven leading up to my defense. With that security I could focus and ground myself to write the thesis and get to the finish line.

Thank you to the friends and colleagues who housed me couch surfing when I could no longer afford to rent a room near school for six months of 2024. I will never be able to express just how much it meant to me in a time of desperate need for mental, emotional, and physical stability in the last year of my PhD.

Thank you to all the staff and administration at SIO and UCSD who have been helpful at enriching my graduate school experience. Thank you to the IOD office team from 2016 to 2025. Thank you to the SIO Communications team who have been encouraging of scientists putting their work out on public channels and for providing me the opportunity to attend the UNFCCC COP27 in Egypt to speak about my work in a policy forum. Thank you to the SIO Dive Locker

and Small Boating team and to Shayna Brody for the craziest wild card filming with Jeff Goldblum for the World According to Jeff Goldblum.

Thank you to the startBlue 2024 team for offering me a position in cohort 3 which transformed the way I think about doing science through the lens of innovation and entrepreneurship. Thank you specifically to Vanessa Scott and Damon Stanwell-Smith for nominating me for the Chancellors Innovation Award and the Committee who selected me to be the UCSD Inaugural Chancellor's Student/Alumni Award recipient in 2024. Thank you to everyone in the Office of Design and Innovation.

Thank you to the many organizations and individuals that have supported my research through funding: NSF OPP, NASA CSESP and SCoPE, UCSD/SIO Fellowships and Department funds, the Hurtigruten Foundation and the generous donors who made my work possible.

Thank you to the IAATO Secretariat 2016 to 2025 – particularly Amanda Lynnes and Damon Stanwell-Smith, for giving me the opportunity to pitch the idea of FjordPhyto at the 2017 Field Staff Conference in Iceland. Thank you to the founding members of the Polar Collective for early years of support understanding how field operations work. Thank you to the Operator managers who brought me on board as an Expedition Team member each year giving me my start in the expedition guiding world while representing institutional collaborations.

I would like to put a placeholder here to thank anyone I have missed!

And last, I would like to thank myself for the endurance and fortitude, for grit, for not giving up during the difficult times when I wanted to. I hope the path I've carved leaves a wide-open road for others to follow and inspiration to carve their own.

Chapter 1, in part, contains unpublished material currently being prepared for submission for publication of the material. Cusick, A. M., Tsukada, A., Hammar, F., Mascioni, M. M. A

review of spatio-temporal sampling investigating phytoplankton community structure and succession along the coastal Western Antarctic Peninsula The dissertation author was the primary author of this chapter.

Chapter 2, in full, is a reprint of the material as it appears in Oceanography. Cusick, A.M., R. Gilmore, A. Bombosch, M. Mascioni, G.O. Almandoz, and M. Vernet. 2020. Polar tourism as an effective research tool: Citizen science in the Western Antarctic Peninsula. *Oceanography* 33(1):50–61, https://doi.org/10.5670/oceanog.2020.101. The dissertation author was the primary investigator and author of this paper.

Chapter 3, in part, is currently being prepared for submission for publication of the material. Cusick, A., Smith, A., Mascioni, M., Johnson, C., James, C. C., Pinpokintr, A., Zheng, H., Allen, A. E., Reynolds, R. A. Revealing Phytoplankton Succession and Community Dynamics: A Multi-Year Spatio-Temporal Analysis Along the Nearshore Antarctic Peninsula (61°S-68°S). The dissertation author was the primary researcher and author of this material.

Chapter 4, in part, is currently being prepared for submission for publication of the material. Cusick, A., Smith, A., Yanek-Chrones, N., Mascioni, M., Johnson, C., Zheng, H., Allen, A. E., Reynolds, R.A., Franks, P. J. S. A metabarcoding-based analysis of seasonal and interannual patterns of phytoplankton in relation to temperature and salinity in coastal surface waters of the Western Antarctic Peninsula. The dissertation author was the primary researcher and author of this material.

VITA

2006 Bachelor of Science in Biology (minor Earth & Space Sciences), University of Washington

2017 Master of Advanced Studies in Marine Biodiversity & Conservation, Scripps Institution of Oceanography, University of California San Diego. *Capstone Project: Developing a Citizen Science Program to Understand Glacial Meltwater Impacts on Fjord Phytoplankton Communities in Antarctica.*

2021 Master of Science in Marine Biology, Scripps Institution of Oceanography, University of California San Diego

2025 Doctor of Philosophy in Oceanography, University of California San Diego

PROFESSIONAL APPOINTMENTS

2005 – 2008 University of Washington, Department of Immunology, *Research Scientist*

2006 Allen Institute for Brain Science, *Undergraduate Intern*

2008 – 2009 University of Washington, College of Forest Resources, *Field Biologist*

2009 Instituto Tecnológico y de Estudios Superiores de Monterrey, *Field Biologist*

2010 - 2016 Institute for Systems Biology, *Research Associate III*

2016 – 2017 Scripps Inst. of Oceanography, UC San Diego, *Graduate Student (MAS)*

2017 Institute for Systems Biology, Dynamic DNA, *Summer Instructor*

2017 – 2020 Viridos (Synthetic Genomics, Inc. Target CW), *Senior Research Associate*

2017 – 2025 Scripps Inst. of Oceanography, UC San Diego, *Graduate Student (MSc, PhD)*

2017 – Present Antarctic Tour Expedition Ship Operators, *Polar Expedition Guide*

AWARDS

2013 Antarctic Service Medal - U.S. Government, National Science Foundation

2016 Merit Scholarship, CMBC, Scripps Institution of Oceanography UCSD, $2k

2017 Graduate Student Excellence Travel, Scripps Institution of Oceanography, UCSD, $3k

2017 National Science Foundation Award PLR-1443705 (PI: Dr. Vernet), $100k, *Research Team Member*

2018 Ragen FHL Endowed Scholarship, Friday Harbor Labs at University of Washington, $500

2018 Michael M. Mullin Fellowship, Scripps Institution of Oceanography, UCSD, $7k

2019 Hurtigruten Foundation, Molecular Learning Lab, *M/V Roald Amundsen*, $4k

2021 NASA Award, #20-CSESP2020-0039, #22-CSESP2022-0005, (PIs: Dr. Vernet, Dr. Reynolds), ~$1.19M, *Research Team Member*

2022 NASA SCoPE Seed Grant, PRO-OCEANOS OCEANOS (PI: Allison Cusick), $20k

2023 NASA SCoPE Seed Grant, PRO-OCEANOS II OCEANOS (PI: Allison Cusick), $20k

2024 NASA SCoPE Seed Grant, PRO-OCEANOS III OCEANOS (PI: Allison Cusick), $20k

2024 NASA SCoPE Seed Grant, POLARIS Infiniscope (PI: Allison Cusick), $20k

2024 Viking Genomics at Sea Program (PIs: Dr. Allen, Dr. Vernet), $600k, *Research Team Member*

2024 UCSD Inaugural Chancellor's Student/Alumni Innovation Award (FjordPhyto), $25k

2024 NASA Award, #24-CSESP24-0023 (PIs: Dr. Vernet, Dr. Reynolds), ~$814k, *Research Team Member*

SEA GOING EXPEDITIONS

2013 Ross Sea, Antarctica**,** *R/V Nathaniel B Palmer,* 53-days

2015 Puget Sound, Washington, *R/V Clifford A. Barnes,* two 7-day voyages

2016 Station ALOHA, Hawaii, *R/V Ka'imikai-o-Kanaloa,* 5-days

2016 San Diego, California, *R/V Gordon Sproul,* two 2-day voyages

2017 Antarctic Peninsula, *M/S Hebridean Sky,* 29-days

2019 Antarctic Peninsula, *M/S Ocean Nova; M/S Akademik Ioffe,* 43-days

2019 Antarctic Peninsula, *M/S Roald Amundsen; M/S Magellan; M/S Ocean Nova,* 65-days

2021 San Diego, California, *R/V Gordon Sproul,* Chief Scientist, two 1-day voyages

2022 Antarctic Peninsula, *M/S Magellan*, *M/S Viking Octantis,* 74-days

2023 Antarctic Peninsula, *M/S Roald Amundsen,* 28-days

2025 Antarctica Peninsula, *M/S Viking Octantis,* 41-days

PROFESSIONAL ORGANIZATIONS

2006—2016	Association for Women in Science - *Member and Volunteer*
2015—2016	Women in Bio - *Program and Events Committee Member*
2016—2021	Association of Polar Early Career Scientists (APECS)—*Member*
2016—Present	Association for Advancing Participatory Science – *Member*
2017—2020	The Polar Citizen Science Collective - *Scientific Advisor*
2018	American Association for the Advancement of Science (AAAS) - *Member*
2019—2022	Polar Tourism Guiding Association (PTGA) – *Member*
2020—Present	Science in the Wild – *Board of Advisors*
2020—2021	Association of Polar Early Career Scientists (APECS)—*Council Member*
2020—Present	Oceans360 - *Board of Advisors*
2021—2023	Greenland Ice Sheet Ocean Science Network (GRISO) -*Board of Advisors*

PUBLICATIONS

Cusick, A.M., Mascioni, M., Dixon, B., Cajiao, D., Gilmore, R., Cheeseman, T. (2024 *in revision).* Can a citizen science project enrich travelers' experience in Antarctica? Case study of a preliminary evaluation of the FjordPhyto project. *The Polar Journal Public Engagement with the Polar Regions*.

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ABSTRACT OF THE DISSERTATION

PHYTOPLANKTON COMMUNITY SUCCESSION IN RELATION TO SALINITY AND TEMPERATURE ALONG THE WESTERN ANTARCTIC PENINSULA: A FOUR-YEAR STUDY LEVERAGING PARTICIPATORY SCIENCE

by

Allison Michelle Cusick

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Maria Vernet, Co-Chair
Peter Franks, Co-Chair

In this dissertation I examined the dynamic seasonal and interannual patterns of phytoplankton communities in the coastal waters of the Western Antarctic Peninsula (WAP). The rapid warming of the WAP has led to significant changes in environmental conditions, yet the impact on phytoplankton communities, particularly in relation to environmental drivers,

remains poorly understood. In examining a synthesis of 245 published studies, including 42 key works, I recognized discrepancies in the conclusions regarding observed coastal phytoplankton succession responses to environmental conditions such as changing temperature and salinity that may likely arisen from overly sparse sampling. This highlights the need for coordinated high-frequency sampling to improve understanding of phytoplankton dynamics in response to climate change. I demonstrated the transformative role polar tourism could play in expanding our knowledge (Cusick et al. 2020), showing how data collected aboard expedition vessels can significantly enhance the spatial and temporal resolution of ecological studies in remote regions, while also addressing the critical need for community engagement in advancing polar research. My data were acquired through four 5-month sampling seasons along the WAP (61°S–68°S) through a participatory "citizen" science program, "FjordPhyto". Using metabarcoding sequencing of samples, I identified 65 phytoplankton taxa across seven major groups revealing high interannual variability in phytoplankton community structure. I investigated how environmental variables, particularly sea surface temperature and salinity, shape phytoplankton succession and community structure. Diatoms dominated early-season cold, salty waters, while mixotrophic species such as dinoflagellates, marine stramenopiles (MAST), and haptophytes increased in richness later in the season with warm and fresh conditions. Diatom species showed high interannual variability with significant correlation to timing, temperature, and salinity, and each season featured a consistent low-salinity assemblage of 4-6 diatom species and 1-2 dinoflagellate species, though the exact combination changed each year. A revised conceptual mandala based on these data shows two possible succession sequences: a main sequence in early season with diatoms or an alternate sequence with cryptophytes and dinoflagellates. This mandala offers insights into community responses to environmental drivers. By integrating

traditional and innovative methodologies within a participatory science framework, my dissertation advances understanding of protist biodiversity in the WAP.

Chapter 1 A review of spatio-temporal sampling investigating phytoplankton community structure and succession along the coastal Western Antarctic Peninsula

Abstract

The Antarctic marine ecosystem is characterized by strong seasonality, with phytoplankton communities playing a key role in biogeochemical cycles. Understanding the drivers of the composition, population dynamics and succession of Antarctic phytoplankton communities is constrained by logistical challenges caused by the region's extreme weather, and the wide variety of sampling approaches employed in response to logistical limitations.

Here, I present a synthesis of the literature focused on quantifying phytoplankton succession in the nearshore region of the Antarctic Peninsula. I examined 245 studies published between 1942 and 2024, with an emphasis on the 42 studies that addressed phytoplankton community structure and succession patterns using primarily microscopy and molecular tools as a method for organism identification. The studies I review describe substantial spatial and temporal variability in phytoplankton communities. Different assemblages were recorded depending on their location and time of year.

Spatial scope – I reviewed sampling efforts including two large-scale gridded sampling station programs: the Pal-LTER program (USA), and the GOAL program (Brazil). Studies investigating succession were published from three locations: the Rothera Time Series (UK) at Adelaide Island, 68°S, the Pal-LTER station (USA) at Anvers Island, 65°S, and Carlini Station (Argentina) at King George Island, 62°S.

Temporal scope - The reviewed studies described distinct and highly variable seasonal patterns. However, most of the studies collected samples infrequently, and during a narrow window of time relative to the growth season (October to April) – typically single days or

focused on the anticipated peak-bloom month of January. Only nine studies sampled repeatedly throughout October through April. The reviewed sampling efforts were mostly snapshots in time, though broad generalizations were made regarding phytoplankton community responses, even though there was a paucity of empirical data.

Taxonomic scope – A wide range of taxonomic diversity was represented in the studies; however, the number of possible taxa recorded depended on when and where the samples were collected. In general, diatoms were recorded as dominating the early season in the southern region influenced by sea ice; in contrast, flagellates and haptophytes (specifically *Phaeocystis* sp.) were recorded in northern regions with their presence attributed to water column stratification and glacial meltwater input.

Method comparison - Some reviewed studies highlighted that not all species within a group responded in the same way to the environmental drivers. Therefore, generalizing a group's response should be done with caution. Broad taxonomic groups of phytoplankton, including phylum, division, and class, were challenging to compare in the present review because studies employed disparate methods – from molecular techniques and microscopy to pigment analysis, chlorophyll-*a* analysis, and estimated biomass – that cannot be directly compared.

In conclusion - With climate change rapidly affecting the Antarctic Peninsula, there is an imperative to understand phytoplankton community dynamics and species' responses to a changing environment. I propose that a strategic and coordinated sampling effort of increased temporal frequency and broader spatial scale, with repeatedly visited stations and comparable methods used for species identification, would vastly improve our understanding of the spatio-temporal variability and environmental drivers of phytoplankton community structure and succession. This effort could be achieved through collaboration with the International

Association of Antarctic Tour Operators in which private expedition cruise industry operators maintain a fleet of more than 60 vessels visiting 300+ possible sites across five months every austral season.

Introduction

Introduction to the coastal Antarctic Peninsula Ecosystem

The Antarctic Peninsula (AP) is an ecologically significant region of the world, where phytoplankton blooms provide organic carbon that sustains a wide range of organisms locally (Arrigo et al. 1998), and species that migrate globally to feed during the austral summer months such as Arctic terns and humpback whales (Egevang et al. 2010, Modest et al. 2021). The nearshore coastal regions of the western Antarctic Peninsula (WAP) are particularly productive (Garibotti et al. 2003), where fjords and embayments provide habitat and refuge for diverse pelagic and benthic organisms. These biodiversity hotspots (Grange & Smith, 2014) support taxa including krill, fish, penguins, seabirds, seals, and whales (Nowacek et al. 2011; Espinasse et al. 2012; Bernard & Steinberg, 2013; Lydersen et al. 2014). The most diverse group of phytoplankton in the WAP is diatoms, which thrive seasonally in cold, nutrient-rich waters (Armbrust 2009, Tréguer et al. 2018). In polar oceans, diatoms dominate the overall biomass compared to other phytoplankton groups (Gilbertson et al. 2022). At large, diatoms in the Southern Ocean (SO) contribute more than 85% to the total primary production (Rousseaux and Gregg 2014). The dominant phytoplankton grazer, krill (*Euphausia spp.*), selectively feed on large lipid-rich, centric and chain-forming diatoms (Haberman et al. 2003) in the pelagic and under sea-ice habitats (Thomas and Dieckmann 2002, Kooistra et al. 2007, Trefault et al. 2021).

Climate change impacts on the Peninsula

The SO is connected to all oceans of the world, acting as a central hub ventilating and regulating climate patterns through oceanic, atmospheric and biogeochemical processes (Turner

et al. 2009, Meredith et al. 2019). Through primary production and carbon sequestration, the SO absorbs 40% of total global oceanic carbon dioxide from anthropogenic and natural sources (Cavicchioli et al. 2015).

As a result of climate change, the SO and Antarctic continent are warming disproportionately rapidly (Meredith et al. 2019) compared to the global average and have experienced accelerated ice mass loss (Shepherd et al. 2018). The fastest rates of surface air temperature warming have been recorded on the WAP, with an increase of annual mean surface air temperature of 3°C since 1950 (Turner et al. 2005) and warming during winter of ~6 °C from 1950–2011, with a recent cooling (Turner et al. 2013, 2016). Temperature rises in the air and ocean have driven substantial environmental changes, including a decrease in sea ice extent and glacial mass (Stammerjohn et al. 2008). Along the WAP, 87% of the glaciers are in retreat (Cook et al. 2005), contributing to increased glacial melt and reduced salinity of WAP coastal waters (Meredith et al. 2013, 2019).

The nearshore WAP is characterized by tidewater glaciers creating an ice-ocean transition that is influenced by air and ocean temperature fluctuations (Cook et al. 2016), oceanic currents (Cape et al. 2019), sea ice dynamics (Stammerjohn et al. 2008), high speed katabatic wind events (Lundesgaard et al. 2020) and nutrient inputs (Forsch et al. 2021). The combination of reduced sea ice increased glacial meltwater input, and regional stratification is altering the physical and chemical properties of the marine environment, affecting the growth, distribution, and timing of primary producers (Moore et al. 2018, De Lima et al. 2019).

Changes in phytoplankton community size structure and species composition has resulted in shifts in primary production and food availability for phytoplankton grazers (Montes-Hugo et al. 2008, Schloss et al. 2014, Ferreira et al. 2020). The reduction in mean phytoplankton size

influences higher trophic levels, e.g., a decrease in sea ice has resulted in reduced diatom size and abundance, which has subsequently led to declines in Antarctic krill populations (Atkinson et al. 2004). In turn, these size shifts impact predator populations, including population declines of the penguins *Pygoscelis adeliae* and *Pygoscelis antarctica* (Trivelpiece et al. 2011; Barbosa et al. 2012). The impacts of climate change on phytoplankton are anticipated to affect not only life in the SO but also ecosystems across the globe, as numerous highly migratory animals rely on these feeding grounds during the austral summer (Chown et al. 2015).

Identifying phytoplankton communities to species level with the advent of molecular tools

Many studies have investigated phytoplankton by measuring chlorophyll-*a* in situ or from satellite remote sensing, via cell counts and carbon biomass estimates, and by classifying size fractions or broad taxonomic groupings using pigment analysis. Identifying taxa to species level using morphology through microscopy and flow cytometry has its limitations when discriminating among similar species (Arrigo et al. 2017, Trefault et al. 2021). Recent studies have cited the benefit of consistent and accurate species-level identification, as species-specific responses are increasingly being investigated, and within-group species shifts often determine community dynamics and total primary productivity (Schofield et al. 2017).

The introduction of genomic tools has expanded our ability to identify phytoplankton species not documented through more traditional methods (Gast et al. 2006). Improvements in sequencing techniques have facilitated identification of species making up communities in samples, conventionally referred to as DNA metabarcoding (amplicon sequencing). Typically, conserved gene regions (e.g., 16S and 18S rRNA) are targeted to distinguish between prokaryote and eukaryote microbial species (Egas et al. 2017). The Tara Oceans project investigated the global distribution of marine microbes, uncovering unexpected species in polar oceans (Malviya

et al. 2016, Carradec et al. 2018). The first metabarcoding studies in Antarctica focused on bacteria in terrestrial ecosystems (Cavicchioli 2015), with more recent studies investigating protist eukaryote communities in marine coastal environments (e.g., Luo et al. 2016, Brown et al. 2021, Hamilton et al. 2021). To date, over 350 species of phytoplankton have been identified in the SO (Gilbertson et al. 2022); however, these taxa remain underrepresented in online molecular databases (Abele et al. 2017, Egas et al. 2017, Krinos et al. 2024) due to the paucity of environmental sequencing initiatives focused specifically on polar microbes (Sunagawa et al. 2019, Gilbertson et al. 2022). The present synthesis concludes that frequent sampling at multiple stations, using sequencing approaches following ecologically significant events such as the first day of sea ice retreat or fast ice break up, could resolve gaps in our knowledge and provide higher resolution of species occurrence, patchiness of habitat/ niche specific processes and community structures (Abele et al. 2017).

A fragmented understanding of phytoplankton in the coastal WAP

Several publications noted that the WAP has been infrequently studied and is poorly understood compared to more accessible marine ecosystems in the world (Smith et al. 1995, Henley et al. 2019, Gutt et al. 2020). In their review of over 450 publications from 2010 to 2020, Gutt et al. (2020) reported that while the WAP is recognized as a climate-change hotspot, statements regarding increasing blooms and the threats to fjord biodiversity posed by glacial melting were limited in both quantity and quality of evidence, resulting in contradictory findings.

We lack comprehensive knowledge about the base of the food web and the dynamic response of primary producers (Egas et al. 2017, Ferreira et al. 2020). Spatial and temporal responses are challenging to compare and may mask divergent regional responses driven by the highly variable physical environment (Vanzan et al. 2015, Brown et al. 2019). Antarctica is one

of the most data-limited regions of the world, and, given the difficulties conducting research in its extreme conditions, any rigorously collected data will contribute to improved understanding and prediction of phytoplankton dynamics (Smith et al. 1999).

As the WAP is one of the fastest warming regions globally, there is an urgency to understand how the influx of freshwater from melting glaciers will influence phytoplankton communities. Before we can answer this question, we must first ask: What is our current understanding of phytoplankton community structure and succession? How many studies to date have investigated phytoplankton succession in the WAP? What was the temporal resolution of their sampling and what was the spatial coverage? What methods did they use to identify phytoplankton and to what taxonomic levels did they discriminate identifications?

Here, I present a synthesis of the published literature that reported on phytoplankton communities and environmental drivers in the WAP. My aim is to understand whether phytoplankton sampling intensity has been sufficient in WAP to address the questions posed above, or if over-interpretation from limited data has resulted in inaccurate conclusions based on low sampling efforts (Ferreira et al. 2020, Gutt et al. 2020).

Literature review methodology

I conducted a literature review that focused on molecular approaches to studying eukaryotic photosynthetic protists (phytoplankton) with an emphasis on the dynamics of phytoplankton succession in the Antarctic Peninsula. I aimed to trace the origins of key statements found in highly cited publications that present divergent results and investigate why contradictory findings have been regularly reported.

I started the present review by dissecting the seminal syntheses from the International Panel on Climate Change Chapter 3: Polar Regions report by Meredith et al. (2019), Henley et

al. (2019), Gutt et al. (2020) and Ferreira et al. (2020). All these syntheses highlighted the differing conclusions regarding phytoplankton bloom characteristics and size shifts of phytoplankton communities in the WAP. I have compared the declared scope of each study as presented in their title and introduction with their reported results and considered the efficacy of conclusions drawn. The following keywords were used in select combinations for the searches: *amplicon sequencing, DNA, monitoring, molecular phytoplankton, protist, spatio-temporal, succession, time-series, 18S.*

The review process involved the following steps:

1 **Database Searches:** I searched Google Scholar for keywords, identifying studies that employed molecular tools and / or investigated eukaryote protists and phytoplankton. For references not found on Google Scholar, reports archived in the public domain were identified using Google searches and were accessed through the libraries of UC San Diego and the Scott Polar Research Institute, University of Cambridge, UK.

2 **Focus on Succession Studies:** I narrowed the scope of my review to studies addressing phytoplankton succession within the WAP region, avoiding broader studies related to the entire SO or other regions around Antarctica. For the present review, I have defined a "season" as the austral summer, the period of peak insolation between October and April, and I classified studies as being a succession or time series when they sampled the same sample station, either (i) more than once in successive months; (ii) for two or more months in each season; or (iii) by intense sampling over a short time window (e.g., daily sampling over 14 days).

3 **Evaluation of Methodologies:** I assessed each publication for its methodological approach, with care to distinguish between studies that utilized microscopy or molecular techniques. The studies that relied solely on chlorophyll-*a* or biomass measurements were not

included in the summary evaluation as the data were considered too coarse. Five select pigment (no molecular nor microscopy) studies were noted for context (Moline and Prézelin, 1996, Schofield et al. 2017, Pan et al. 2020, Feng et al. 2022, Mendes et al. 2023). I tabulated selected metadata based on location of study, month and dates of study, method of identifying taxa, key questions posed, and key findings. Studies that discussed significant phytoplankton community observations, seasonal investigation, time-series succession, or used molecular methodologies were included in the analyses.

4 **Analysis of Taxonomic Levels:** I documented the taxonomic resolution reported in each study, focusing on how results were classified at various taxonomic levels.

Results

The reviewed literature spans the publication years 1942 to 2024 and comprises 245 individual publications or reviews. Selected studies of the Antarctic Peninsula that specifically focused on succession, time-series data, community structure with spatio-temporal resolution, or used molecular and microscopy methods, totaled 42 studies (Table 1.1).

Table 1.1: Summary of studies conducted on WAP – Includes studies through National Programs by vessel or at station (e.g., Argentina - Carlini Station, USA - Palmer Station, Poland - Arctowski Station, UK - Rothera Station). Grey shaded region indicates samples collected within that month (specific timing or date noted when specified within publication).

Citation	Seasons (years) sampled	Oct	Nov	Dec	Jan	Feb	Mar	Apr	Station
Long Term Successive Season Sampling									
Lange et al. 2018	1980–2013	33 year review all months							Carlini
Moline and Prezelin 1996	1991-1994	Launching first three years of Palmer LTER							Palmer
Kim et al. 2016	1993–2013								Palmer
Kopczyńska 1996	1994-1995	every 7 - 10 days							Arctowski
Kopczyńska 2008	1996 - 1998, 2003 - 2005	once to four times a month							Arctowski
Rozema et al. 2017	1997-2012								Rothera
Clarke et al. 2008	1998 - 2005	every 5 days in summer, and every 7 days in winter							Rothera
Abele et al. 2017	2007-2009	biweekly in spring and summer and once per month during the rest of winter							Carlini
Leeuwe et al. 2020	2013-2017	less than twice a week	twice a week					less than twice a week	Rothera
Nardelli et al. 2023	2017–2018, 2018–2019		16 November–26 March						Palmer
FjordPhyto (this study)	2017-2024		once a month to weekly						WAP
Short Term Successive Season Sampling									
Schofield et al. 2017	1991-2013								Palmer
Garibotti et al. 2005	1996, 1997 and 1999								Palmer
Mendes et al. 2023	2008-2018								
Ferreira et al. 2020	2008-2020								
Lima et al. 2019	2009–2015 (not 2011-2012, 2012-2013)		"early"			"late"			Comandante Ferraz, Botany Point, Machu Picchu, Thomas Point, and Arctowski
Antoni et al. 2024	2010-2020 (no 2015)								Carlini
Lin et al. 2021	2012-2016								Palmer
Brown et al. 2021	2012–2016								Palmer
Costa et al. 2023	2013-2015								
One Season Sampling									
Piquet et al. 2011	1998				17, 19, 20, 26, 28, 31	1, 5, 12, 13, 19, 26, 27			Rothera
Annett et al. 2010	2006-2007								Rothera
Luria et al. 2014	2008								Palmer
Vanzan et al. 2015	2010-2011			14, 23		21	01		Brazilian Antarctic Station Comandante Ferraz, Botany Point, the Peruvian Antarctic Station Machu Picchu, Thomas Point, and the Polish Antarctic Station Arctowski
TARA OCEANS	2011				03, 06, 09, 17, 22				
Egas et al. 2017	2012					4–15			
Luo et al. 2016	2013				17, 23				
Fuentes et al. 2019	2013-2014				January 28 to February 14, 2013; February 9 to March 3, 2014.				Arturo Prat
Lin et al. 2017	2014								Palmer
Alcaman Arias et al. 2018	2014					11, 14	3, 4		Arturo Prat
Arrigo et al. 2017	2014		1-21						Palmer
Trefault et al. 2021	2014-2015	30, 31	01		10-21		10, 11, 13, 14		
Feng et al. 2022	2015-2016			Dec 19 to Jan 14					
FjordEco	2015-2016		27-31	01-20				04-26	
Costa et al. 2020	2016					14-24			
Liu et al. 2022	2018					02			
Grattepanche et al. 2022	2019		06-31	01-09					
Liu et al. 2023	2020						8, 9, 10, 11, 12		

I extracted the stated questions from each introduction (total of 63 questions) and summarized the main theme each study investigated. The questions fell within five general themes:

i) What is the Phytoplankton community structure?

ii) Are there specific dominant taxa of interest?

iii) What is the temporal community variability intraseasonally and interannually?

iv) What is the spatial variability of community structure?

v) What is the influence of environmental drivers on phytoplankton communities?

To visualize the questions posed in each study, I compiled the question text and generated a word cloud (Figure 1.1); overall the study questions are posed to generate understanding of *phytoplankton community structure and seasonal environmental change of the Antarctic Peninsula*.

Figure 1.1: A word cloud illustrating the most frequently used terms from 62 questions across 42 key publications in the literature, focusing on the understanding of phytoplankton community and/or succession dynamics. The larger the word, the more often it appears in the questions drawn from each study. Each word shown occurred a minimum of four times. Word cloud generated using WordClouds https://www.wordclouds.com/.

Historical phytoplankton sampling efforts

The first scientific effort to understand the diversity of life in the SO came from the James Clark Ross Antarctic expedition of the ships HMS Erebus and HMS Terror in the years 1839–1843. In the publication, *Botany of the Antarctic Voyage* (Hooker 1847), based on the South Polar Ocean leg of the voyage, 'microscopic vegetables belonging to the order *Diatomaceae'* were noted on icebergs, pack ice, and brash ice 'making a pale ochreous colour'. There were further expeditions after Ross; notably the HMS Challenger Expedition (1872-1876) which documented diatoms, through dredged bottom samples, and famously initiated the field of Oceanography (Castracane 1886, El-Sayed 2005).

Observations of seasonal variations in Antarctic phytoplankton were not undertaken until the 1930s, beginning with the Discovery Expeditions (Hart 1934, 1942). John Hart collected samples over many years from extensive areas of the SO and demonstrated a correlation of seasonal progression of maximum phytoplankton production with increasing latitude. Subsequent studies, such as those by Kopczyńska (1981) and a comprehensive 33-year review of phytoplankton around King George Island by Lange et al. (2018), have expanded on Hart's findings. Lui et al. (2022) noted that research prior to genomics availability primarily focused on species identifiable through optical and electron microscopy, resulting in good characterization of diatoms and dinoflagellates; however, most small, flagellated taxa remained unidentified due to the limitations of optical identification of morphology.

To date relatively few studies investigating seasonal cycles have been conducted around the Antarctic Peninsula (Boyce et al. 2010). Single-year studies have provided insights into the seasonal cycle at select coastal locations, including Palmer Station near Anvers Island (Krebs 1983, Smith et al. 1995), and King George Island in the South Shetland Islands (Kopczyńska

1981). However, the longest series of year-round measurements comes from Signy Island in the South Orkney Islands (Clarke et al. 1988, Clarke and Leakey 1996). These studies revealed previously unrecognized dynamics, notably that while the summer phytoplankton bloom was dominated by diatoms, the nanoflagellate bloom was also ecologically significant due to its longer duration; they also noted persistence of the phytoplankton during winter months, including chlorophyll levels of nanoflagellates that surpassed those of diatoms.

National research station sampling programs by region

Northern Region

Phytoplankton of the Northern Antarctic Peninsula (NAP, defined here as north of 64°S) have been more extensively researched than other AP regions, beginning with studies conducted at the Polish Arctowski Station in Admiralty Bay (Figure 1.2). These early investigations, carried out between December 1977 and March 1978, provided quantitative and qualitative insights into the seasonal distribution of major algal groups and species (Kopczyńska 1980, 1981, 1996).

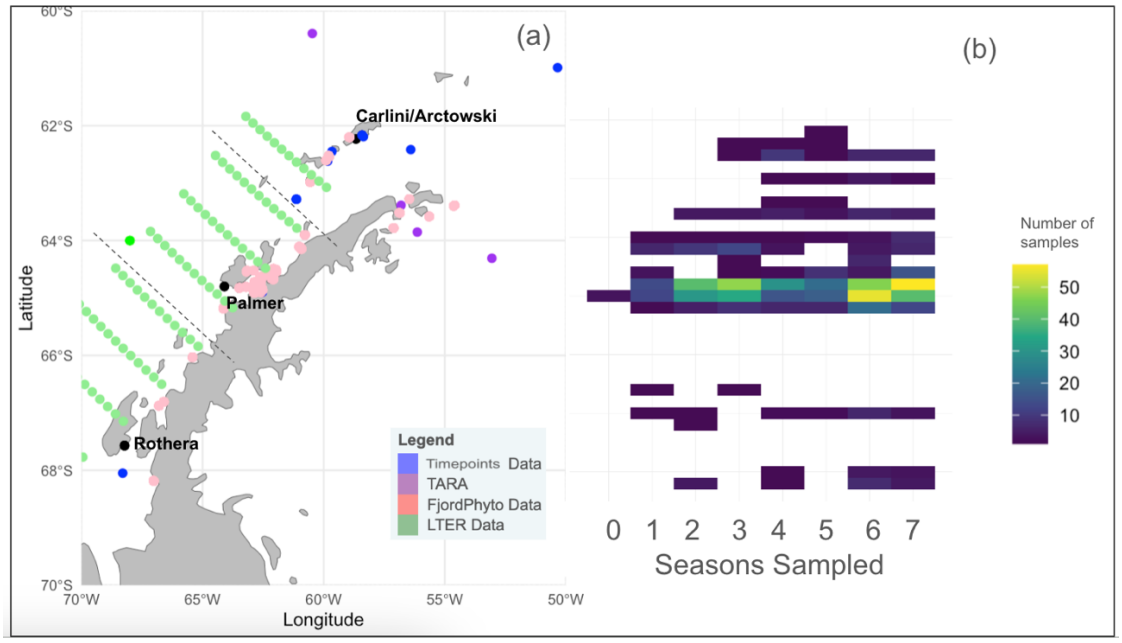

Figure 1.2: Map showing sampling locations along the WAP (a). Grey dotted lines divide the WAP into three sections (NAP, CAP, and SAP). Points show the location of cardinal sampling

stations within the large-scale Pal-LTER peninsula grid (green dots) which covers an area of 900 km (roughly parallel to the peninsula) by 200 km (on to offshore). Black dots represent national program stations doing (published) time series: Carlini Station (Argentina) is in Maxwell Bay (Potter Cove) of King George Island. Palmer Station (USA) is located at the southern end of Anvers Island. Rothera Station (UK) is located at the southern end of Marguerite Island in Ryder Bay. Blue dots represent studies that were single sampling events (Table 1.1). Purple dots represent locations sampled by the Tara Oceans expeditions. Red dots represent locations sampled by the FjordPhyto program. Heatmap depicts Number of samplings over each of the 7 consecutive seasons by latitude (b).

A long-term ecosystem research program was initiated in 1992 as a collaborative effort between the Instituto Antártico Argentino (IAA) and the Alfred Wegener Institute Helmholtz Center for Polar and Marine Science in Germany (AWI) at Carlini Station on King George Island (Abele et al. 2017). Initial studies relied on microscopic observations of phytoplankton communities and pigment fingerprinting techniques (HPLC-CHEMTAX).

Molecular approaches were used in Potter Cove during the programs ClicOPEN (IPY), IMCOAST, and IMCONET, which included insights on effects of climate change on the microbial eukaryotic diversity (Abele et al. 2017). Apart from Admiralty Bay, sampling efforts in the NAP have been limited to opportunistic summer oceanographic cruises, resulting in a series of snapshots of local phytoplankton communities (e.g., Rodriguez et al. 2002, Hernando et al. 2015, Costa et al. 2020).

The Brazilian High Latitude Oceanography Group (GOAL) have undertaken annual summer cruises in the NAP spanning 12 years (2008 - 2020). These were limited to February sampling (Mata et al. 2018). One of the main aims of GOAL's work was to better understand spatial variability present within the NAP (Mendes et al. 2012, Gonçalves-Araújo et al. 2015, Costa et al. 2020).

Interestingly, the Tara Oceans project has been highlighted as pioneer study for microbial oceanography; however, their contribution to Antarctic sampling was limited to only two stations

near the AP, taken opportunistically in January 2011 (Ibarbalz et al. 2023), providing much less molecular insight into Antarctic phytoplankton than its reputation asserts.

Central Region

The central region of the Antarctic Peninsula (CAP, defined here in between 64°S and 66°S) includes Anvers Island where the Palmer Long-Term Ecological Research (LTER) program was established in 1991 (Figure 1.2). One of the key aims of the LTER long-term study is to investigate the natural variability of the marine ecosystem that cannot be fully evaluated or separated from potential anthropogenic impacts without sustained observation. While the Palmer LTER has established stations that cover a large area, from 64°S to 70°S, sampling is temporally limited to January and early February, during the peak summer phytoplankton bloom (Smith et al. 1995).

Southern Region

The southern region of the Antarctic Peninsula (SAP, defined here as south of 66°S) includes the Rothera Time Series (RaTS) in Ryder Bay (68°S) conducted by the British Antarctic Survey (BAS) from Rothera Research Station (Figure 1.2). This time series represents one of the most comprehensive and sustained measurement programs in the Southern Antarctic Peninsula (SAP) and is a continuation of long-term year-round sampling from Signy Island Station (60°S).

Spatial Variability

Studies in the present review occurred between 60°S and 68°S along the AP (Figure 1.3). One of the earliest large spatial studies was undertaken by the BIOMASS-FIBEX cruise in February-March 1981, when 151 stations were sampled in the Bransfield straight, NAP, South Shetland Islands, and Drake Passage area (Kopczyńska and Ligowski 1982). A total of twelve studies (28%) focused on covering a large spatial area, notably the Pal-LTER grid (Garibotti et

al. 2005, Luria et al. 2014, Schofield et al. 2017, Lin et al. 2017, 2021, Brown et al. 2021, Arrigo et al. 2017), the GOAL sampling grid in the Bransfield Strait (Costa et al. 2020, 2023), and the Chinese Antarctic Expeditions (Lui et al. 2022 and 2023) covering the NAP and SO (Figure 1.3). When I compiled the sampling locations of the studies other than those listed above, collectively the sampling covers the length of the AP, but tends to be focused locally and not homogeneously distributed on a larger spatial area. Moving from south to north along the peninsula, in the SAP, seven studies (16%) were published by the RaTS in Ryder Bay. In the CAP, 15 studies (37%) were published around Anvers Island. In the NAP, five studies (12%) were published, as well as 15 studies (37%) from the South Shetland Islands region (these mainly came out of the National Research Stations (RaTS, Palmer, Carlini) (Figure map yellow stars). In addition, two studies (5%) were published from SO locations near the Peninsula (Figure 1.3).

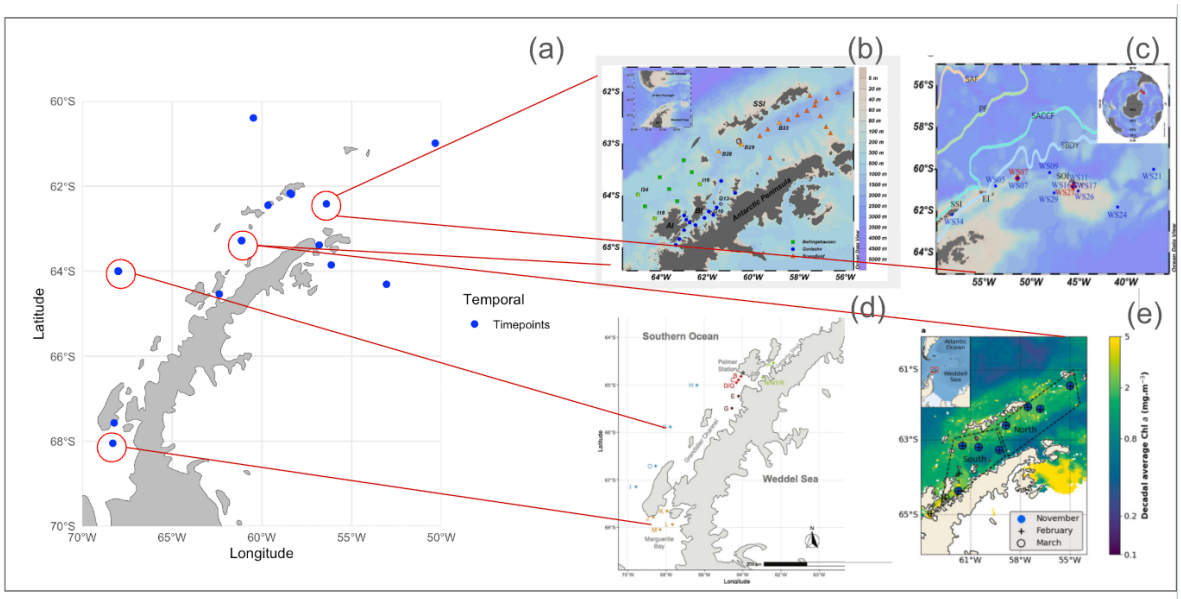

Figure 1.3: Map showing station sampled for spatial scale (a). Note that some of the dots represent a bigger sampling grid (shown on the right following the red line, b-e). For example, the blue dot at 64°S, 68°W represents the Palmer LTER grid (not shown, see Figure 2) and the blue dot in the Bransfield Strait (~between 64°S and 61°S represent sampling grids similar to those shown on right (b, e). Maps (b-e) are modified from Costa et al. 2020 (b), Liu et al. 2022 (c), Grattepanche et al. 2022 (d), Costa et al. 2023 (e).

Much of the data recorded to date on the spatial dynamics of AP phytoplankton comes from focused efforts at a few national research stations, all of which have reported substantial differences in the phytoplankton groups present, biomass values, and productivity from different regions of the coastal WAP. These differences have been ascribed to the latitudinal dependence of local hydrography of the region (Lange et al. 2018, Schofield et al. 2018). Two studies reported inshore-to-offshore gradients of community composition with inshore communities hosting abundant pennate diatoms and offshore comprising centric diatoms, with higher biomass inshore (Garibotti et al. 2003), and one study suggested that seasonal blooms initiate near the shore and propagate offshore (Kopczyńska 2008).

Temporal Variability

Of the papers reviewed, 21 studies (50%) specifically reported on time series to investigate phytoplankton seasonality (Figure 1.4). Notably, the first studies of seasonal succession on the AP were conducted at the Polish station Arctowski in 1978 (Kopczyńska, 1980, 1982). The approaches have primarily focused on the summer bloom period around January each season (Garibotti et al. 2003, Annett et al. 2010, Lima et al. 2019).

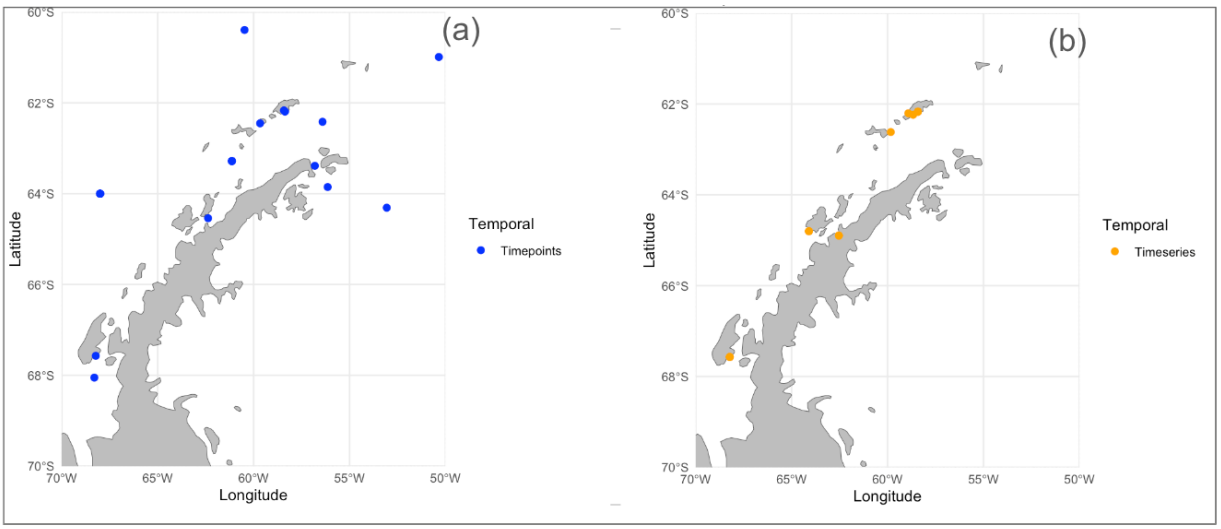

Figure 1.4: Maps showing stations sampled at timepoints (a) and stations that sampled timeseries (including more than 3 months within a given season (b). Note that some dots represent bigger sampling grids not shown (see Figure 1.3).

Eighteen studies (43%) sampled only a single austral summer season (with sampling occurring in any given month from November to April); nine studies (21%) reported sampling from two to five or more seasons to assess interannual variation, and 11 studies (26%) reported sampling for more than three successive months in the season contributing to time-series/succession results (Table 1.1).

Taxonomic Variability

Many studies identified distinct communities of phytoplankton taxa; however, studies were highly variable in their reports of the present or dominant taxa, depending on the location and date of sampling (Lange et al. 2018, Ferreira et al. 2023). A compiled list of phytoplankton taxa reported in the South Shetland Islands was collated from 75 studies in Lange et al. (2018) which included the major phytoplankton groups: diatoms, dinoflagellates, cryptophytes, haptophytes, chlorophytes, and prasinophytes. A few studies distinguished phytoplankton only by size classes (Kopczyńska 2008, Grattepanche et al. 2022). Some studies reported the

dominance of nanoplankton (Vanzan et al. 2015) and a year-round dominance of nanoflagellates and picoplankton (cryptophytes, prymnesiophytes, prasinophytes) representing 71-99% of the total recorded phytoplankton cell counts (Kopczyńska 2008). In one study, diatoms were dominant, comprising 63% of recorded phytoplankton when they were sampled in mid-January (Feng et al. 2022); another study that also sampled mid-January found dinoflagellates to surpass diatoms, contributing 28% of the total sample counts (Luo et al. 2016). A third mid-January study found that cryptophytes dominated the samples with 23% of the total counts (Lin et al. 2021).

Some notable genera of diatoms included *Thalassiosira* and *Fragilariopsis* that dominated the early season in most studies, with the occasional presence of *Corethron* and *Odontella* (Moline and Prézelin 1996, Costa 2023, Antoni 2024). Throughout the season, studies reported that haptophytes (*Phaeocystis sp.*), cryptophytes, and dinoflagellates also contributed a substantial component of abundance or carbon biomass. Large diatoms and cryptomonads typically accounted for most of the phytoplankton biomass, while unidentified photosynthetic flagellates were numerically dominant (Garibotti et al. 2003, Annett et al. 2010, Mascioni et al. 2019). One study using microscopy reported that prymnesiophytes were often numerically dominant, however surface waters consistently yielded biomass dominance by diatoms in highly variable interannual assemblages (Annett et al. 2010). One study reported a dinoflagellate as a potentially Harmful Algal Bloom (HAB) species (Lin et al. 2021), and another presented a new lineage of dinoflagellate not previously documented in Antarctic waters (Mascioni et al. *submitted*).

Recent studies report on the detection of bloom genera that were previously undocumented, such as the diatoms *Odontella* (Garibotti et al. 2003, Costa et al. 2023) and

Shionodiscus (Antoni et al. 2024), and various flagellates (Mascioni et al. 2019). The studies attributed the presence of novel bloom taxa to changing environmental conditions. Each study concluded that community structure was variable, and that more attention needed to be given to novel or bloom-forming species not previously detected.

While preparing the present review, I observed that each study assessed was reporting on different phytoplankton groups, driven by the particular focus of the study. For instance, studies variously reported on the most abundant or dominant species, the species with the largest contribution to carbon biomass, or novel bloom species. Studies recorded to differing taxonomic levels for each major phytoplankton group, and many studies did not report to species level. Of the reviewed studies that sampled multiple seasons, high interannual variability was always reported. Because of the limited temporal scope of many studies, the ability to deduce a successional community change throughout the season was limited.

Identification methods determine taxonomic variability

Of the 42 reviewed studies, many methods were utilized, with 21 using molecular tools to identify taxa present to species level, 22 studies using microscopy, and 14 studies using pigment analysis (Table 1.1). Prior to the use of molecular tools, most studies relied on optical microscopy and pigment analysis (Biggs et al. 2019, Leeuwe et al. 2020). This was underscored by Lui et al. (2022), who noted that previous research on eukaryotic microbial diversity in the SO has predominantly focused on pigment compositions and microscopic observations (Lange et al. 2007, Luria et al. 2014).

Taxonomic identification with metabarcoding has increasingly been used by studies in the WAP to investigate intraseasonal variation (e.g., Hamilton et al. 2021, Trefault et al. 2021). One study examined intraseasonal variation and interannual phytoplankton community structure

over two consecutive seasons (Abele et al. 2017). Depending on the method of identification used, different numbers of species were reported. For example, one study using molecular techniques detected 156 species of photosynthetic eukaryote (Trefault et al. 2021). Liu et al. (2023) used molecular techniques to identify 67 species of diatoms; Lange et al. (2018) through a compilation of 75 publications over a 33-year period in the NAP, reported a total of 371 species of diatoms identified mainly by microscopy methods.

The differing capacities for taxonomic discrimination of the different identification methods have made comparisons challenging. There is a risk of taxonomic bias towards the more morphologically distinct and better-preserved species, depending on the identification method used. For example, some naked dinoflagellates possess fragile membranes that are easily damaged in preserved samples; in addition, the similarity of pigment composition of some dinoflagellates and haptophytes makes distinguishing them difficult (García-Muñoz et al. 2013, Wasilowska et al. 2015).

History of molecular tool usage globally and in Antarctica

The advent of DNA metabarcoding to identify phytoplankton taxa to species level has been effective in other ocean regions (Vaulot et al. 2022), but remains nascent in use in the AP. To understand the delay in the deployment of molecular identification technologies in Antarctica, it is useful to understand the history of molecular use in global oceans.

The J. Craig Venter Institute then initiated marine whole-metagenome studies, starting with a pilot sampling project in the Sargasso Sea (Venter et al. 2004), which was followed by the JCVI Global Ocean Sampling (GOS) expedition (2004). Falkowski & De Vargas (2004) were the first to apply shotgun DNA sequencing to microbial communities in the open ocean. Early studies used Sanger chromatographic sequencing and second-generation sequencing platforms

(Metzker et al. 2010) and as the molecular technologies evolved and progressed, Illumina bridge amplification-based sequencing emerged, which allowed for high throughput capabilities and lower error rates on longer read lengths (Ambardar et al. 2016, Wilms 2021). The continued technological advancements in sequencing technologies, improved power of bioinformatics and computational software, and decreasing sequencing costs have made it possible to conduct population genomic studies on a global scale (Muir et al. 2016).

The Tara Oceans project further expanded the understanding of marine microbial biodiversity globally by integrating genetic, morphological, and functional data with environmental data on a global ocean scale from 2009 – 2013 from a single sailing yacht platform, with a brief foray into Antarctic waters in January 2011 (De Vargas et al. 2015, Sunagawa et al. 2023). Building on these efforts, the EU-funded Micro B3 project explored global marine microbial biodiversity through a coordinated initiative branded as Ocean Sampling Day (OSD), in which multiple locations around the world were all sampled on the same day, June 21, 2015 – including Antarctica, although this day was in austral midwinter (Kopf et al. 2015).

Molecular tools were introduced to Antarctic planktonic research in 1998 by López-García et al. (2001) to study planktonic protist and microbial assemblages in marine habitats. They constructed 18S rRNA environmental gene libraries comparing surface stations to samples taken from up to 3,000 m depth at the Polar Frontal Zone in the Drake Passage. There followed a rapid increase in molecular studies with an emphasis on microbial prokaryotes (Diez et al. 2001, Piquet et al. 2008, Wolf et al. 2014), while research on phytoplankton eukaryotes remained relatively underrepresented (De La Iglesia & Trefault, 2012). Reviewed studies included the sampling of a range of SO water masses (Liu et al. 2019, Swalethorp et al. 2019), glacial-

influenced coastal ecosystems (Luo et al. 2009, Moreno-Pino et al. 2016, Piquet et al. 2010), and sea ice and melt ponds (Kilias et al. 2014) around Antarctica.

As molecular studies of Antarctic marine waters increased, numerous novel and previously unobserved groups and lineages were revealed, specific to the WAP region. In the NAP, Luo et al. (2016) and Moreno-Pino et al. (2016) reported the presence of Stramenopile lineages from MAST-1, MAST-2, MAST-7 and MAST-8, a group of phytoplankton recently revealed through molecular analysis. An interesting observation from these two studies is that, despite the locations being proximal, there was high variability in the communities observed at the sampling sites – which may also have been due to sampling on different days.

In the CAP, Luria et al. (2014) compared Archaea, Bacteria, and Eukarya. They found differences among microbial eukaryote assemblages between northern and southern stations within the Palmer-LTER sampling grid during January. In the SAP, Piquet et al. (2011) and Rozema et al. (2016) used various molecular tools including DGGE, clone libraries, and Illumina MiSeQ sequencing. These and similar studies have revealed unexpectedly high diversity among smaller-sized eukaryotes (≤ 5 mm) in samples collected from various oceanic regions, including the SO (Lopez-García et al. 2001, Moreira & Lopez-García 2002).

Species identification importance and limitations

Advances in DNA molecular sequencing technology have driven our ability to quantify the diversity of microscopic eukaryotes found in SO (Dasilva et al. 2014, Liu et al. 2021). Despite such technological advances, limitations persist, particularly in naming conventions of online taxonomic databases. Many species are identified through comparison to known organisms or cultures; the lack of sequences from identified polar microbes results in misidentification of species (Hamilton et al. 2021). This is common in phytoplankton taxonomy.

Many species names have changed over time (e.g., *Gonyaulax tamarensis, Alexandrium tamarense, Alexandrium fundyense,* and *Alexandrium catenella* all refer to the same species; *Gonyaulax polyedra* is also known as *Lingulodinium polyedra* and *polyedrum*) (Lange et al. 2018). These taxonomic anomalies underscore the need for standardized sampling practices, DNA extraction and metabarcoding methods, and naming conventions, to enhance clarity in the study of marine eukaryotic diversity and improve our understanding of species-specific responses in Antarctic ecosystems.

Discussion

In the present review, I have summarized current understanding of the temporal and spatial sampling efforts aimed at identifying phytoplankton communities and succession in the WAP. This synthesis highlights the contributions of molecular tools to our understanding of phytoplankton taxa and identifies knowledge gaps.

Overall, three major points that stand out from this literature synthesis:

1. Study questions are still being posed at a foundational level, often focused on the community structure and environmental drivers giving rise to patterns observed.

2. Temporal and spatial sampling in coastal areas is often very patchy (Montes-Hugo et al. 2009), resulting in snapshots that provide only limited insight into seasonal succession of community composition or changes linked to environmental drivers.

3. Different identification methods (e.g., molecular, microscopy, pigment) make comparing results of the distinct taxa identified difficult; more attention should be given to identifying phytoplankton to species level in order for a better understanding of the community.

From my assessment of the review literature, it is my assertion that because many studies capture only sample limited local phytoplankton communities over narrow windows of time at

point locations, their data are insufficient to address questions regarding seasonal phytoplankton community structure and corresponding environmental drivers. Without sufficient data, it is dangerous to impute causation from correlations. While the comprehensive, multidecadal programs at national research stations can inform our understanding of phytoplankton succession, their focus on restricted time windows (e.g., peak phytoplankton growing seasons or maximum biomass, Pal-LTER sampling only January to early February) (Ferreira et al. 2020), or limited spatial coverage (e.g., Carlini Station, Palmer, and Rothera studies are long-term sampling however only at one location), reduces our ability to truly understand the drivers of succession in other places of the WAP.

The WAP is a highly dynamic region, with high interannual variability and local-scale environmental factors that play an essential role in shaping phytoplankton dynamics (Kim et al. 2018). The interactions among physical and biological processes span a wide range of temporal scales, from 0.1 seconds to 10,000 years, and spatial scales from < 1 mm to 10,000 km, encompassing everything from molecular processes to global climate change (Cronin et al., 2012). For example, Moline and Prézelin (1996) propose that temperature data supports the idea that water masses in coastal areas can be replaced within time scales ranging from less than a day to a few days. Such rapid changes are linked to abrupt fluctuations in local primary production, which may subsequently result in sudden shifts in the structure of local phytoplankton communities. The rapidity of changes is particularly relevant when considering the AP, which is affected by both local-scale and large-scale dynamics.

The present synthesis highlights three sampling improvements that will enable improved understanding of phytoplankton dynamics in the WAP:

1) High-frequency, long-duration temporal sampling is needed for elucidating succession.

2) Organisms need to be identified to the species level to enable understanding of species-specific responses to environmental drivers and the dynamics of the emergent communities: not all species within groups (e.g., diatoms) respond the same way to environmental perturbations.

3) Collaborative efforts among key stakeholders conducting research in Antarctica will improve temporal and spatial sampling efforts more than any one national research station or ship-based campaign alone. The significance of high-resolution, long-duration temporal sampling to understand succession cannot be overstated (Smith et al. 1995).

Collaboration as an effective solution

Collaborative efforts among researchers and institutions have often been successful demonstrations of how to enhance our understanding of phytoplankton dynamics. By integrating a breadth of expertise and resources, collaborations can more effectively tackle complex ecological questions and deepen our collective knowledge. Over the past decade, there has been a concerted initiative to combine physical, chemical, and biological data to elucidate the mechanisms driving phytoplankton bloom dynamics in the SO (Newman et al. 2019).

The National Academies of Sciences, Engineering, and Medicine (NASEM) recognized the significance of such collaborative initiatives in their 2015 Strategic Vision for Antarctic and Southern Ocean Research. The 2015 vision proposed an Antarctic genomics initiative aimed at catalyzing the research needed to decode the genomic and functional bases of organismal adaptation in response to changing environments across the spectrum of Antarctic life.

The divergence in results reported within the literature can plausibly be attributed to the variability of temporal sampling among the studies. Each study captured a unique snapshot of taxa/species dominance at different sampling times and highlighted the rapid community

changes following significant physical/meteorological perturbations. As a result, only those studies that specifically focused on temporal succession are relevant for understanding the dynamics of phytoplankton community structure over the season. Previous research has highlighted that temporal patterns and succession are more reliable predictors of community composition than traditional measures of biomass and chlorophyll-*a* (Moline and Prézelin, 1996). The reviewed studies collectively emphasize the importance of considering temporal dynamics when assessing community structure in nearshore ecosystems. Publishing positive results with limited data, and associating correlation with causation can lead to over-generalization, extrapolation, and unsupported speculation; these issues are particularly prevalent when extreme conditions limit sampling efforts, and where it is logistically difficult to conduct research. The research community is also susceptible to working in an 'echo chamber' of confirmation bias (e.g., "Cryptophytes are replacing diatom-dominated communities (Mendes et al. 2023)") which in turn could misinform future research priorities.

My observation - *what we do know about Antarctic phytoplankton that is independent of when and where they were sampled?*

Reflecting on the 82 years of phytoplankton sampling around the AP indicates that traditional sampling strategies provide too few data points or time series, at too few stations; due to high interannual variability of environment, conventional sampling cannot provide a complete picture of the phytoplankton community structure or how it responds to environmental drivers throughout the season. These limitations support the approach of mobilizing a fleet of Antarctic-based vessels that sample simultaneously throughout the season (Figure 1.5).

Sampling efforts on the AP should encompass complete seasonal cycles to enable separation of long-term ecosystem changes from pronounced seasonal and interannual variability

(Gilbertson et al. 2022), which are particularly significant in remote, data-limited, high latitudes with intense environmental seasonality. Synoptic sampling – involving simultaneous collection of samples across broad geographic areas – provides valuable snapshots of spatial variability. This synoptic spatial sampling must be combined with repeated fine-scale temporal sampling to inform our understanding of how the distribution, abundance, and composition of phytoplankton communities respond to varying environmental conditions.

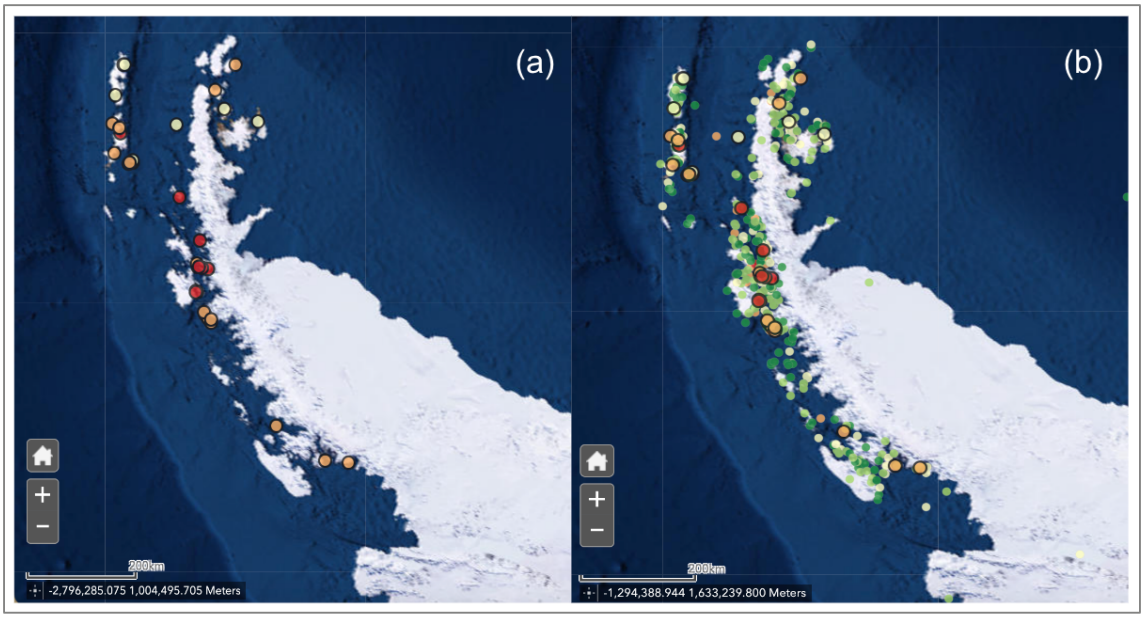

Figure 1.5: Map showing Visited sites in Antarctica by vessel-based operations with the top 25 most visited locations (warm colors, a) and all potential sites visited (green and warm colors, b). Retrieved and modified from https://antarctictreaty.maps.arcgis.com/apps/webappviewer.

One way to achieve such synoptic sampling is through partnership with the expedition cruise industry through the framework of citizen science as the ships visit numerous locations along the WAP during the austral insolation months spanning late October through early April (Figure 1.5, Cusick et al. 2020, *Chapter 2*). I propose that a strategic and coordinated sampling effort with increased temporal frequency and broader spatial scale, with repeatedly visited

stations and comparable methods used for species identification, would vastly improve our understanding of the spatio-temporal variability and environmental drivers of phytoplankton community structure and succession.

The citizen science sampling program, FjordPhyto, was conceptualized in 2016 (inspired by a pilot effort with 2 expedition vessels during the NSF funded FjordEco program) and initiated in 2017 under funding from the NSF Office of Polar Programs Public Participation in STEM Research to collaborate with 5-7 expedition vessels (Lee Cusick 2017). FjordPhyto provides all the scientific gear and sampling tools needed to partnering operators and provides rigorous online and in-person training for designated polar expedition guides. Trained guides then facilitate engagement of travelers in collecting biological and environmental data as they frequently and repeatedly visit numerous locations each voyage (Figures 1.5 and 1.6). Each science boat sampling event takes 1-1.5 hours to complete and includes collection of *in situ* salinity, temperature, chlorophyll-*a* data using a Conductivity, Temperature, Depth instrument (CTD), oxygen isotope metrics, Secchi depth measurements, and preserved microscopy and eDNA samples, all of which contribute as a '*validation safari'* ground-truthing satellite remote sensing data (Pan et al. 2024). The program's subsequent funding came from NASA's Citizen Science Earth Systems Program allowing it to grow from 7 to 11 partner operators and continue to the present (Table 1.2). This program, which has now received endorsement as a Southern Ocean Observing System (SOOS) effort establishes a time series monitoring program with over 750 unique science boat sampling events which has potential legacy for great impacts on participant experience and science.

Table 1.2: Summary of sampling results from the FjordPhyto seasons (2016–present).

Sampling Years	2016 - 2017	2017 - 2018	2018 - 2019	2019 - 2020	2020-2021	2021 - 2022	2022 - 2023	2023 - 2024	2024-2025
Funding Source	MAS MBC/Capstone Mullin Fellowship	NSF OPP PPSR	Donation/auction/Mullin Fellowship	NA	NA	NASA CSESP (pilot)	NASA CSESP (Implementation)	NASA CSESP (Implementation)	NASA CSESP (Implementation)
Season Start and End Dates	Nov 16, 2016 - Mar 1, 2017	Nov 13, 2017 - Mar 18, 2018	Nov 2, 2018 - Mar 19, 2019	Nov 6, 2019 - Mar 2020	COVID-19	Nov 28, 2021 - Mar 26, 2022	Nov 11, 2022 - Mar 20, 2023	Nov 11, 2023 - April 20, 2024	October 31, 2024 - TbD
Sites Visited	12	15	12	27	COVID-19	29	39	43	TBD
Highest Frequency Sampling at One Site	12	13	15	10	COVID-19	8	25	23	TBD
Total Samples Collected (including replicates collected by lead researchers)	38 (microscopy only)	122	82	67	COVID-19	68	141 (846-1000 unique samples)	194 (910 unique samples)	TBD
Participants	380	1220	820	670	COVID-19	680	846-1410	1552 - 2300	TBD
Number of Tour Operators Participating	2	6	7	5	COVID-19	6	8	10	11
Number of Vessels	2	5	8	5	COVID-19	6	11	15	15
Operator Name, Vessel Name	G-Adventures M/V Expedition Polar Latitudes M/V Hebridean Sky	Antarctica21 M/V Hebridean Sky G-Adventures M/V Expedition Lindblad Expeditions M/V Orion Polar Latitudes M/V Hebridean Sky Private Charter M/V Hans Explorer Quixote Expeditions M/V Ocean Tramp	Antarctica21 M/V Ocean Nova Cheesemans' Ecological Safaris M/V Ioffe G-Adventures M/V Expedition Hurtigruten M/V Midnatsol Polar Latitudes M/V Hebridean Sky M/V Island Sky Ocean Expeditions M/V Australis Quixote Expeditions M/V Hans Hansson	Antarctica21 M/V Ocean Nova G-Adventures M/V Expedition Hurtigruten M/V Roald Amundsen Polar Latitudes M/V Island Sky Quixote Expeditions M/V Hans Hansson	COVID-19	Antarctica21 M/V Magellan Aurora Expeditions M/V Greg Mortimer Hurtigruten M/V Roald Amundsen Polar Latitudes M/V Seaventure Quixote Expeditions M/V Hans Hansson Viking Expeditions M/V Viking Octantis	Antarctica21 M/V Magellan M/V Ocean Nova Aurora Expeditions M/V Greg Mortimer Albatross Expeditions (AUIP) M/V Ocean Victory Hurtigruten M/V Roald Amundsen M/V Nansen Intrepid M/V Ocean Endeavor Polar Latitudes M/V Seaventure Quixote Expeditions M/V Hans Hansson Viking Expeditions M/V Viking Octantis M/V Viking Polaris	Antarctica21 M/V Magellan M/V Ocean Nova Albatross Expeditions (AUIP) M/V Ocean Victory Aurora Expeditions M/V Sylvia Earle M/V Greg Mortimer Bark Europa M/V Bark Europa Hurtigruten M/V Roald Amundsen M/V Fram M/V Fridtjof Nansen Intrepid M/V Ocean Endeavor Polar Latitudes M/V Seaventure Oceanwide (Blue Green Expeditions) M/V Ortelius Quixote Expeditions M/V Ocean Tramp	Antarctica21 M/V Magellan M/V Ocean Nova Albatross Expeditions (AUIP) M/V Ocean Victory Aurora Expeditions M/V Sylvia Earle M/V Greg Mortimer Bark Europa M/V Bark Europa Hurtigruten M/V Roald Amundsen M/V Fram M/V Fridtjof Nansen Intrepid M/V Ocean Endeavor Polar Latitudes M/V Seaventure Oceanwide (Blue Green Expeditions) M/V Ortelius Quixote Expeditions
Number of Feedback Surveys Recieved	0	43	38	TBD	0	TBD	TBD	TBD	TBD

Conclusions

The present synthesis provides a summary of the spatio-temporal sampling efforts of studies investigating phytoplankton community dynamics in Antarctic waters from 1942. It highlights significant gaps in our understanding of phytoplankton communities, particularly concerning their seasonal succession and the cadence of key taxa that arise under observed environmental conditions. Spatially, the use of fixed sampling grids, single fixed stations, or regional vessel-based efforts restrict our ability to comprehensively assess phytoplankton distributions across the AP. Temporal sampling gaps from a lack of repeated sampling throughout the entire austral growth season, and a bias toward studies that focus on peak bloom periods in mid or late season, have led to incomplete and possibly misleading conclusions about community dynamics. Taxonomically, the increasing multitude of methodologies to study organisms at various taxonomic levels, combined with insufficient data on polar species, complicate our understanding of species-level niche preferences, dynamics, and ecological interactions. Using molecular tools can complement traditional methods, enhancing our knowledge of seasonal community dynamics, and capturing the overall biodiversity of blooms that build up during the summer months. Molecular analyses have not yet linked DNA sequence information to the corresponding morphological features of species. Hence, the identities of many sequence entries remain unknown. In future work, molecular information can be coupled to phytoplankton culture information, to better link morphological and genetic identification.

My synthesis highlights the necessity of year-round sampling to comprehensively describe phytoplankton communities in Antarctic coastal waters. To understand the impacts of climate change on these communities, we must establish a solid understanding of the dynamics underlying species assemblages; this requires sustained, high temporal-resolution sampling.

Fostering better coordination among key stakeholders and researchers from various disciplines and countries will enhance the detection of issues that need to be addressed in future Antarctic scientific expeditions. Such collaborations not only support the establishment of research priorities relevant to different Antarctic values, but also strengthen the science-policy interface. They could also contribute to Biodiversity observatory networks, such as the Group on Earth Observations Biodiversity Observation Network (GEO BON), the Genomic Observatories Meta-Database (GEOME), and the Ocean Biomolecular Observing Network (OBON). The Scientific Committee on Antarctic Research (SCAR) has supported collaborative efforts that collect and share eDNA samples (e.g., the Antarctic Genetic Archive) and data (e.g., the SCAR Antarctic Biodiversity Portal or GenBank); and the Southern Ocean Observing System (SOOS) and Antarctica InSync aim to foster synchronized coordination efforts in Antarctica which is very timely for the upcoming 5th International Polar Year currently in planning stages.

Acknowledgements

Chapter 1, in part, contains unpublished material currently being prepared for submission for publication of the material. Cusick, A. M., Tsukada, A., Hammar, F., Mascioni, M. M. A review of spatio-temporal sampling investigating phytoplankton community structure and succession along the coastal Western Antarctic Peninsula The dissertation author was the primary author of this chapter.

Chapter 2 Polar Tourism as an Effective Research Tool: Citizen Science in the Western Antarctic Peninsula

ABSTRACT. The Antarctic Peninsula is one of the fastest warming regions in the world, with over 87% of its glaciers in retreat. The resulting influx of glacial meltwater to the coastal ecosystems may influence the succession patterns of primary producers. It is critical to document these dynamics, gathering time-series data throughout the seasonal growth period, because any shifts in primary production can affect higher trophic level organisms in the nearshore food web. The Antarctic tourism industry maintains a fleet of vessels that visit the peninsula's nearshore waters throughout the austral summer, November to March. We developed a citizen science (CS) program—FjordPhyto—to leverage these vessels as platforms for gathering data about the region. Trained staff and travelers collect environmental data and biological samples that are sent to researchers. Preliminary results include phytoplankton species identification, cell abundance determination, carbon biomass estimates, and euphotic depth measurement at multiple sites. We show that CS is a valid tool that can enhance research in Antarctica, while also providing an enriching experience to travelers interested in learning more about science in polar environments.

INTRODUCTION

The poles are the fastest warming regions on Earth, garnering global attention from scientists, policymakers, and the public (Larsen et al., 2014). Yet, not enough is known about their ecosystem structures and functions to predict how they will change in future climate scenarios. In the Southern Hemisphere, the Western Antarctic Peninsula (WAP) has warmed rapidly in the second half of the twentieth century, resulting in retreat of more than 87% of its marine glacier fronts (Cook et al., 2016; Turner et al., 2016). It is critical to understand how glacial meltwater patterns impact phytoplankton communities in coastal fjords because any shifts in primary production can affect higher trophic level organisms in the nearshore food web.

The high-latitude oceans are notoriously difficult to monitor because of their harsh environments. Research is logistically challenging; surface moorings are ineffective due to the presence of sea ice and frequent collisions with icebergs; ocean color satellites using visible light are hampered by persistent cloud cover; and research vessels can provide in-depth sampling only during short time periods. Although several nations have research programs that collect long-term data, most are restricted to short time periods (e.g., one month) or at static locations (Henley et al., 2019). As a result, we have relatively sparse spatial and temporal year-round coverage of the WAP's 1,300 km nearshore waters compared to more accessible regions in the world. Knowledge of seasonal patterns is patchy, and studies provide varying evidence of how these ecosystems will respond at regional scales (Moline et al., 2004; Constable et al., 2014).

The WAP coastline has hundreds of islands, straits, fjords, and embayments where glaciers deliver ice and meltwater. These nearshore locations appear to be hotspots of biodiversity with high biological productivity (Vernet et al., 2008). They provide refuge and are aggregation zones for baleen whales (Nowacek et al., 2011), penguins, and seals (e.g., Santora and Reiss, 2011; Bernard and Steinberg, 2013) whose foraging efforts coincide with krill distribution (Espinasse et al., 2012). Additionally, the fjords' seafloors harbor an abundance of benthic animals such as amphipods, polychaetes, and echinoderms (Grange and Smith, 2013). These pelagic and benthic animal aggregations within the nearshore may be fueled by intense phytoplankton blooms that occur throughout the summer and fall (Smith et al., 2008). This "invisible forest" (Falkowski, 2002) of microscopic organisms plays a large role in providing oxygen to the environment and cycling carbon. Seasonal shifts in phytoplankton taxa are significant in the WAP, following changes in day length (e.g., Rozema et al., 2017; Schofield et al., 2017). Using improved molecular tools, understanding the shifts in phytoplankton taxonomic groups down to the species level can shed light on their functional roles (e.g., Lin et al., 2017). Furthermore, integrating molecular techniques with taxonomical analysis will reveal species diversity shifts in this invisible forest (Figure 1) with future climate scenarios. Understanding such dynamics requires gathering data repeatedly, over a full season, for many years or even decades (Magnuson, 1990). How could this type of coverage be achieved over the long term?

One platform that has not yet been leveraged is the fleet of tour expedition vessels run by members of the International Association of Antarctica Tour Operators (IAATO) for visits to the WAP during five months of each year. These ships cover hundreds of nautical miles, making daily landings repeatedly over a season (Bender et al., 2016). Although Antarctica is devoid of permanent residents and indigenous peoples, between researchers, fishers, and travelers, there is a considerable human presence. The ship-based tourism community is growing, with an estimated 59,367 visitors making landings in 2019/2020, outnumbering scientists more than 10 to one (IAATO, 2019a). Partnership with these vessels would allow sampling over a larger temporal and spatial extent, and could prove an effective method for characterizing phytoplankton community succession in response to glacial meltwater within nearshore fjord ecosystems.

These vessels also allow valuable opportunities for citizen science (CS), or Public Participation in STEM Research (PPSR), with travelers helping to collect reliable data that can be published in peer-reviewed scientific literature (McKinley et al., 2017). There are more than 3,000 active CS projects around the globe (see https://scistarter.org/), and the field is currently developing best practices (Shirk et al., 2012). CS provides a way to fill gaps in research while increasing enthusiasm for science among the public (Kennicutt et al., 2014; Miller, 2016).

CITIZEN SCIENCE PROJECT DEVELOPMENT

Following the US government's Federal Crowdsourcing and Citizen Science Toolkit (US Federal Government, 2019), we developed the FjordPhyto project so that staff and travelers on Antarctic tour vessels could contribute to research questions investigating a succession of phytoplankton communities within coastal fjords. The toolkit includes five basic steps for planning, designing, and carrying out a CS project (adapted from Bonney et al., 2009): (1) scope your problem, (2) design a project, (3) build a community, (4) manage your data, and (5) sustain and improve. Additional resources from the Biological Sciences Curriculum Study (BSCS) within the National Science Foundation (Edelson, Kirn, et al., 2018), along with a workflow Logic Model sheet from the CitSci2017 workshop (Phillips et al., 2014), were used to further guide the initial development of the project.

The following describes the five basic steps considered during the design and development of the FjordPhyto project.

STEP 1: SCOPE YOUR PROBLEM

Scientific Rationale

As glaciers melt along the coast, they deliver ice and freshwater to the marine environment with a maximum signal late in the summer (Dierssen et al., 2002). It is unknown how the freshwater influx from these coastal tidewater glaciers will influence the ecosystem at the level of primary producers (i.e., phytoplankton; Hernando et al., 2015). It has been hypothesized that freshwater provides a stratified environment beneficial for phytoplankton blooms (Dierssen et al., 2002), and studies show

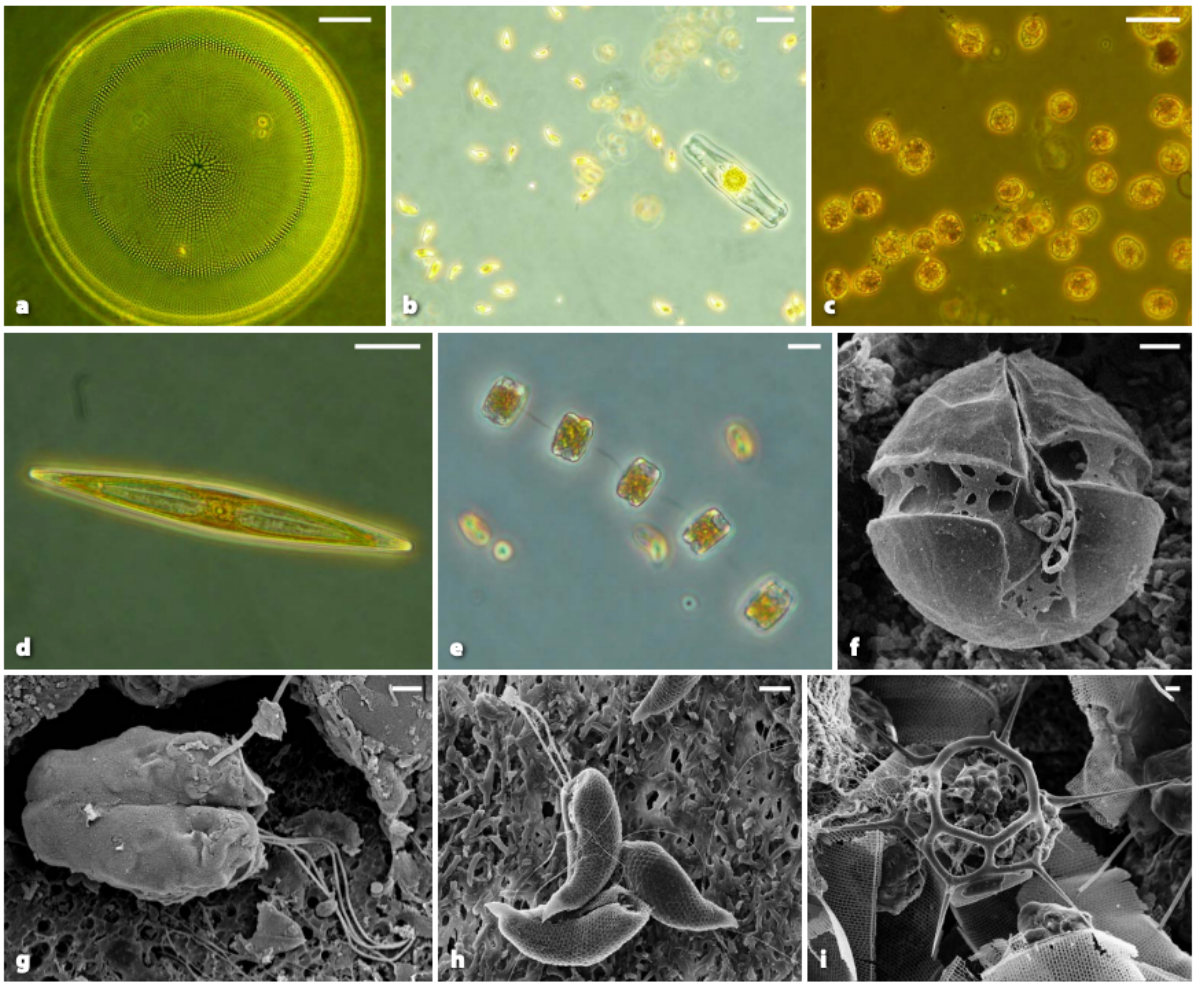

FIGURE 1. Microscope images of phytoplankton taxa from samples collected in the nearshore regions of the Western Antarctic Peninsula display different shapes and sizes. (a) Diatom *Coscinodiscus bouvet*, (b) cryptophytes with diatom (note size differences), (c) small dinoflagellates, (d) Naviculoid diatom, (e) chain-forming diatom genus *Thalassiosira*, (f) dinoflagellate genus *Peridiniella*, (g) prasinophyte genus *Pyramimonas*, (h) cryptophytes, and (i) silicoflagellate *Dictyocha speculum*. (a–e) Light microscope scale bar is 10 µm. (f – i) Scanning electron microscope scale bar is 2 µm.

FIGURE 2. Travelers assist trained tour ship staff in collecting phytoplankton samples from the field. *Photo credit: Robert Gilmore*

there can be cryptophyte abundance in mixed layers enriched in meltwater (Moline et al., 2004; Mendes et al., 2013), although other environmental factors can be equally important (e.g., Brandini and Rebello, 1994; Lange et al., 2015; De Lima et al., 2019). Present-day observations indicate that WAP coastal waters have an early spring diatom bloom from October through November, presumably related to sea ice retreat (Ross et al., 2000; Kim et al., 2018), and a later summer diatom bloom occurring in January. Before and after diatom blooms, smaller flagellates (e.g., cryptophytes) dominate (Kozlowski et al., 1995; Rozema et al., 2017).

Different taxa contribute varying amounts of carbon biomass to the ecosystem (Garibotti et al., 2005). Regardless of the phytoplankton succession in any given season, it remains unclear how shifts will affect higher trophic level organisms in the nearshore food web (Corbisier et al., 2004; Saba et al., 2014). This knowledge is crucial to understanding recruitment of larval *Euphausia superba*—Antarctic krill—a key zooplankton species of the Southern Ocean (Ross et al., 1996) that feeds higher trophic levels. Krill select for larger diatom and chain-forming phytoplankton (Haberman et al., 2003) for reproductive success; thus, changes in phytoplankton communities may affect krill populations (Nicol et al., 2010) as well as nearshore food webs containing fish and amphipods (Sailley et al., 2013). Sampling of both phytoplankton and meltwater along the WAP may help predict future changes.

Objectives

FjordPhyto has both scientific and educational objectives:

1. Scientific aims: (a) Determine the seasonal and interannual changes in meltwater intrusion at fjords and coastal embayments at multiple sites, and (b) characterize the phytoplankton community diversity and species succession during the austral growth period (November to March).
2. Education and broader impact aims: (a) Create a sampling program under which travelers can participate in the scientific process with the guidance of trained staff; (b) gather data at popular landing sites near glaciers, creating a time-series data set; and (c) increase ocean literacy of travelers through education and participation in CS projects.

STEP 2: DESIGNING A CITIZEN SCIENCE PROJECT

The FjordPhyto project is designed to be an engaging bonus activity that complements the cruise experience without competing for time allotted to other activities offered. During each voyage, travelers learn about FjordPhyto as a CS project that will be carried out during their trip. Informative talks are offered by staff, information is posted around the ship, and announcements are made. At each site of interest, staff leading the project organize interested travelers to board a rubber inflatable boat typically seating five to 18 people (Figure 2). Sampling can be carried out during either landing or cruising excursions to or from shore. Once the group reaches the GPS location, ship's

staff distribute tasks among participants. Staff explain the protocols and the importance of research on Antarctic fjord ecosystems and assist travelers in sampling (Cusick, 2018a; Sear, 2018; respectively).

Training

A high priority was designing a sampling program using easy-to-operate scientific equipment and sampling protocols. Protocols and videos were developed, field tested, and refined with staff input (Lee, 2017). Before each field season, researchers meet with ship staff in person or via teleconference to identify sampling locations and to discuss the transfer of necessary gear to the ship as well as end-of-season logistics, including sending data and samples to Ushuaia, Argentina, and California, United States. Continuous communication and feedback via email throughout the seasons further guide development of this program.

Choosing Sample Locations

Samples are taken from November to March at several locations along the WAP between King George Island (62°S, 58°W) and Marguerite Bay (near 68°S, 68°W; Figure 3). IAATO uses a ship scheduling tool that allows management of landing site visits in accordance with the Antarctic Treaty System (ATS) and IAATO requirements for minimal or transitory impact on the environment. This coordination is based on the legally binding agreement signed as Measure 15 (2009) during the Antarctic Treaty Consultative Meeting (ATCM) XXXII (IAATO, 2009) and provides a reliable method for determining sites that might be visited during each season.

The specific sampling locations for FjordPhyto are chosen based on popular landing sites located near tidewater glaciers (Figure 3). Other considerations include logistical ease for cruise operators to conduct the activity based on time restrictions at that site and the frequency of IAATO-member ships visiting those sites. Landing sites are regulated (via ATS and IAATO requirements) to restrict the number of vessels that can visit any site at a given time. Typically, ships follow a predictable yet flexible itinerary traveling similar routes. This coordination permits many sites to be visited repeatedly over the season.

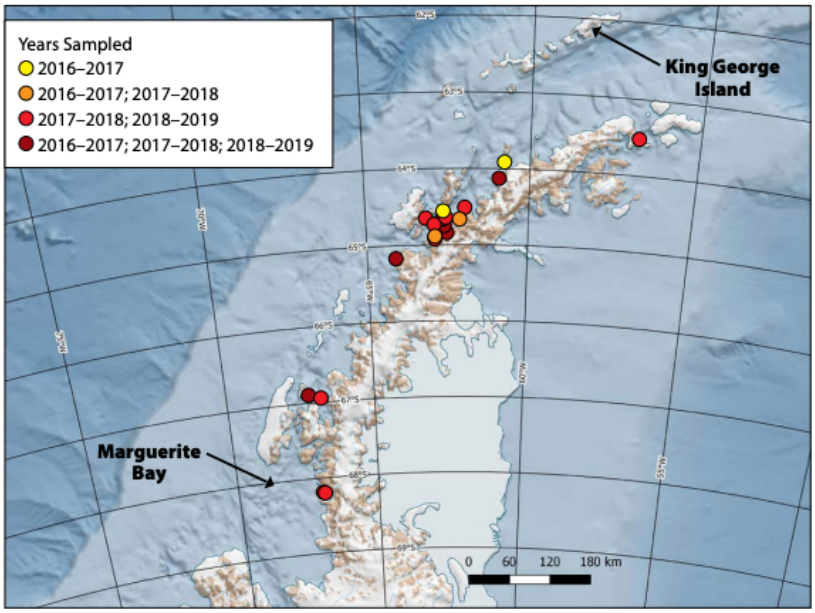

FIGURE 3. A map of the Western Antarctic Peninsula shows the locations of samples collected from 2016 to 2019. The map was created using QGIS Desktop 2.18.24.

Sampling Meltwater

CTD Measurements. Using an annually calibrated SonTek CastAway® CTD, participants collect water column profiles of temperature and salinity at each sampling location (Figure 3). The CTDs are deployed manually and can withstand pressures to 100 m (Figure 4). The range and accuracy of the device is based on company-calibrated specifications with an accuracy of 0.25% of measured value and a drift that does not exceed 0.075°C per year for temperature and 0.380% per year for salinity. Data that are later exported to researchers for defining the presence of meltwater and calculating mixed layer depths include pressure, depth, temperature, conductivity, specific conductance, salinity, sound velocity, and density (Thomson and Fine, 2003; Pan et al., 2019).

Secchi Depth. With supervision, CS participants measure water turbidity by submerging a Secchi disk to the depth where it disappears (the oldest turbidostat known to oceanography; Wernand, 2010; Figure 4). The Secchi disk-determined depth can provide excellent information on light attenuation and can be used to calculate euphotic depth (Z_{eu}) where photosynthetic available radiation is 1% of its surface value in the water column (Figure 5; Aas et al., 2014). In this project, commercially available disks were used for the estuarine coastal environment known to have higher turbidity and varying optical properties due to meltwater and tidal variations compared to those of the open ocean (Hou et al., 2007).

Sampling Phytoplankton Communities

CS participants collect surface phytoplankton samples (Figure 4). These samples are later analyzed in the laboratory by microscopy for cell abundance and carbon biomass, and by high-throughput sequencing for species diversity and function. Under the constraints of this project, only surface phytoplankton can be evaluated, and replicates are taken when

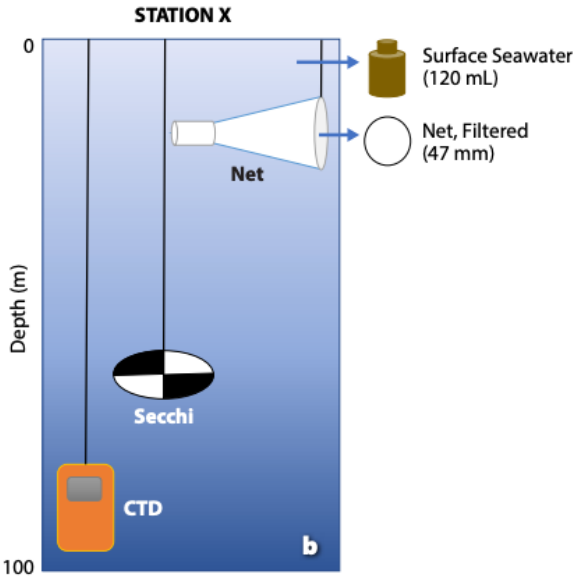

FIGURE 4. Sampling methods. (a) Citizen scientists assist staff with a phytoplankton net tow. *Photo credit: Mathew Farrell* (b) Schematic of sampling methods. Two measurements (CTD and Secchi depth) and two samples are collected at each station. Surface seawater is collected in bottles for examination by microscopy to determine cell abundance and carbon biomass, and high-throughput sequencing of net-collected phytoplankton provides data on species diversity and function.

possible. Permits are not needed to sample phytoplankton from seawater as per the United States Antarctic Program following the Antarctic Conservation Act (ACA of 1978, NSF 01-151).

Microscopy. Surface seawater (0–5 m depth) is collected and preserved by filling pre-rinsed amber HDPE bottles (120 mL) and adding a Lugol's solution (4% final concentration; Edler and Elbrächter, 2010), which is a less toxic preservation option that stains more fragile organisms. Samples are kept in a cool, dark place until the end of the season, when they are offloaded and sent to Universidad Nacional de La Plata, Argentina, for researchers to analyze (as described in detail in Mascioni et al., 2019). The measurement error is <10% with 400 cells counted per sample.

Molecular Genetics. A SEA-GEAR Corporation plankton net (20 µm mesh) is towed at idle speed for 10 minutes to concentrate surface phytoplankton (0–5 m, Figure 4a). The sample is then filtered (0.2 µm) using a hand-operated vacuum pump. Filters are inserted into pre-filled tubes with Invitrogen RNA*later* Stabilization Solution and frozen on board the ship until the end of the season. In the absence of deep-freezing capabilities, this nontoxic reagent rapidly permeates cells to stabilize and protect genetic material. According to manufacturer specifications, genetic material can be protected from potential degradation if left at room temperature for up to one week. This benefit allows samples to be unfrozen during air transit to San Diego, California, where researchers then freeze tubes until they can be processed.

Educational Outreach

Aboard a tour vessel, every day provides multiple opportunities for education. Formal and informal lectures are typically offered by staff and guest scientists on topics that include exploration, natural history, and science. FjordPhyto provides lecture slides detailing aspects of oceanography and the importance of phytoplankton in the polar and global ecosystems. Vessels that do not have microscopes on board are given a Celestron TetraView LCD Digital Compound Microscope and the book *Plankton* by Christian Sardet (2015) for further engagement with the phytoplankton world. A portion of the plankton net tow can be reserved for viewing on board, offering additional opportunities for teaching about the invisible forest and primary producers.

STEP 3: BUILDING A COMMUNITY

Establishing Capacity

Before launching any CS project, it is important to establish the capacity of interested partners. IAATO members work together within the ATS framework to deliver safe, environmentally responsible operations in Antarctica. One of IAATO's missions is to support science in

various ways. They report their activities annually under the ATCM, making this information, including annual reports, tourism statistics, and guidelines for travelers and tour operators, available publicly on their website (IAATO, 2019b). In addition, FjordPhyto researchers presented at various conferences before and during the project's launch to gain perspective from multiple stakeholders, including private-sector tour operators and staff, polar researchers, grant program managers, and CS practitioners (Cusick, 2017a,b).

Pilot and Launch

FjordPhyto was tested during the 2016–2017 season with two IAATO-member operators (see section below on Phytoplankton Diversity in Fjords; Mascioni et al., 2019). With funding for subsequent seasons, sampling efforts were expanded (detailed herein), and partnerships with tour operators increased (Table 1). An estimated 2,500 travelers have directly participated in sample collection since the launch of the program, with additional participants attending the onboard lectures. FjordPhyto is open to all interested IAATO ships; however, we have not been able to fully accommodate increasing interest and integrate new partners due to program funding constraints (see Discussion).

STEP 4: DATA MANAGEMENT

One of the most common questions and recognized concerns about CS is data quality. Many CS studies have shown that these challenges can be successfully managed (Wiggins et al., 2011; Miller-Rushing et al., 2012) to provide valid results publishable in peer-reviewed scientific journals (Crall et al., 2011). To reduce the possibility of poorly collected data, researchers chose user-friendly equipment and developed clear protocols to ensure sampling consistency among all participants. Researchers train the ship staff annually in person at meetings and on ships in the field, with reinforcement via teleconference. The same gear is distributed to all ships, and instrumentation is calibrated annually by the manufacturer. Researchers participate in cruises to take additional replicates. All data files are transferred to researchers who identify missing or erroneous information. Additionally, quality control procedures exist for downstream sample processing to ensure the integrity of microscopy and genetic samples.

To date, samples are being processed and form the basis of two PhD theses. Once published in scientific journals (e.g., Mascioni et al., 2019), the environmental data will be sent to a long-lived repository (e.g., BCO-DMO), and metadata will be deposited in the Antarctic Master Directory. Genetic sequencing data will be stored in online databases (e.g., GenBank BioProject).

STEP 5: SUSTAIN AND IMPROVE

To sustain and improve a CS project, there must be communication among the scientists, operators, staff, and travelers. In addition to coordinating logistics and analyzing samples for publication, researchers must be available to solve issues that occur during the season, gauge strengths and weaknesses of the program, share results in a timely manner, and secure funding to ensure the project's longevity.

The impact of the FjordPhyto program is gauged in two ways. First, are samples scientifically relevant and do they advance knowledge of this region? Peer review will help determine whether the data are of sufficient quality to provide scientific merit (e.g., Mascioni et al., 2019). Second, is there a positive impact on travelers' participation and learning? The latter question is partially evaluated by distributing questionnaires to staff and travelers, asking for feedback and suggestions for improvements. The responses received (Table 1) help guide further project development (see Discussion).

The personal contact information of participants is not shared with the researchers for privacy reasons. Therefore,

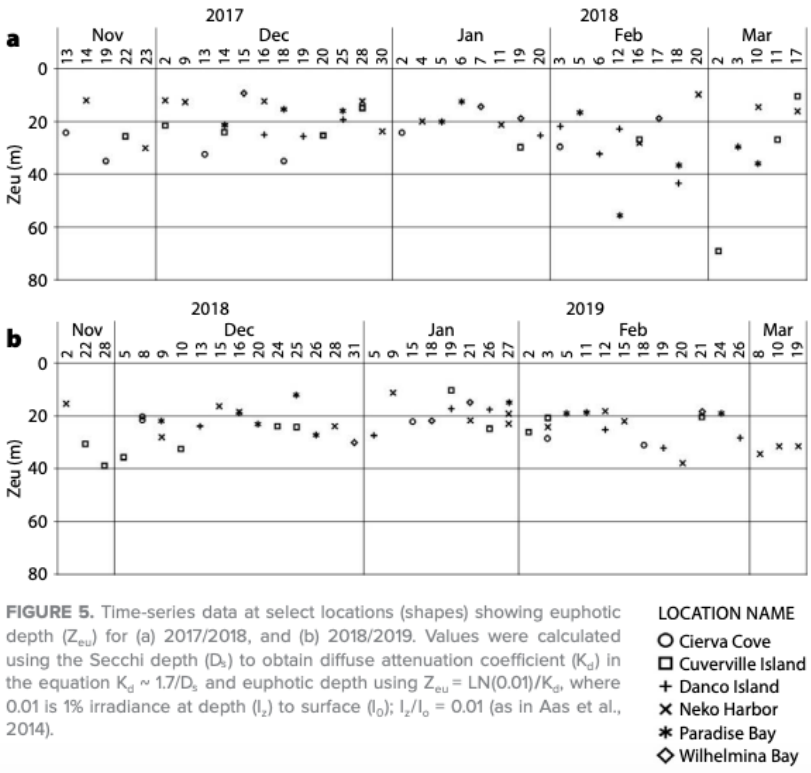

FIGURE 5. Time-series data at select locations (shapes) showing euphotic depth (Z_{eu}) for (a) 2017/2018, and (b) 2018/2019. Values were calculated using the Secchi depth (D_s) to obtain diffuse attenuation coefficient (K_d) in the equation $K_d \sim 1.7/D_s$ and euphotic depth using $Z_{eu} = LN(0.01)/K_d$, where 0.01 is 1% irradiance at depth (I_z) to surface (I_o); $I_z/I_o = 0.01$ (as in Aas et al., 2014).

in an effort to maintain engagement and disseminate results, we developed a website (https://www.fjordphyto.org) and social media accounts (@FjordPhyto on Facebook, Twitter, and Instagram) where we post updates. We also present our project to the scientific and CS communities by attending and speaking at conferences (Cusick, 2018b,c, 2019; Mascioni, 2019).

PHYTOPLANKTON DIVERSITY IN FJORDS

The identity, abundance, and biomass of the phytoplankton sampled in FjordPhyto's first year reveal the seasonal development of phytoplankton communities at varying sites, north to south (Figures 3, 5, and 6). For example, Figure 6 shows data from Cierva Cove (64°09'18"S, 60°55'12"W), Wilhelmina Bay (64°37'13.44"S, 62°12'07.14"W), and Neko Harbor (64°50'34.44"S, 62°32'13.13"W). At these three sites, small flagellates were present during the entire sampling period and usually dominated the phytoplankton community at the beginning of the season. Most of the phytoplankton carbon biomass (as estimated using cell-volume conversion described in Mascioni et al., 2019) came from large cells, diatoms, and dinoflagellates, although prasinophyceans were also important in Neko Harbor during January (Figure 6). In Wilhelmina Bay, in mid-December, there was a cryptophyte bloom of organisms morphologically related to the genus *Plagioselmis*, presumably a new species for Antarctica (Mascioni et al., 2019). During this first season of sampling, we also identified two other flagellate blooms, *Pyramimonas* sp. in Neko Harbor, and an unidentified unarmored dinoflagellate at Danco Island (data not shown).

DISCUSSION

After three years of operation, we consider FjordPhyto a successful CS project, proven to be feasible in the field. It provided scientifically relevant samples, and it was well received by IAATO operators and travelers (Table 1). We attribute its success to the efforts of dedicated staff and scientists who provided travelers with a meaningful experience.

When scoping the problem, we started by setting realistic goals regarding the project's capacity, scale, participant interests, and scientific priorities. Due to the difficulty in collecting data from this region, we consider CS a beneficial approach to addressing questions on phytoplankton community succession, and further, relating that to meltwater over the growth season. Although samples are limited to ship-visited locations, involving more ships can extend the spa-

TABLE 1. Sampling results of three seasons (2016–2019).

	2016–2017	2017–2018	2018–2019
Season Start–End Dates	Nov 16, 2016–Mar 1, 2017	Nov 13, 2017–Mar 18, 2018	Nov 2, 2018–Mar 19, 2019
Sites Visited	12	15	12
Highest Frequency Sampling at One Site	12	13	15
Total Samples Collected (including replicates collected in tandem by lead researcher)	38	122	82
Number of Tour Operators Participating	2	6	7
Number of Vessels	2	5	8
Operator Name, Vessel Name	G-Adventures, M/V *Expedition* Polar Latitudes, M/V *Hebridean Sky*	Antarctica21, M/V *Hebridean Sky* G-Adventures, M/V *Expedition* Lindblad Expeditions, M/V *Orion* Polar Latitudes, M/V *Hebridean Sky* Private Charter, M/V *Hans Explorer* Quixote Expeditions, M/V *Ocean Tramp*	Antarctica21, M/V *Ocean Nova* Cheesemans' Ecology Safaris, M/V *Ioffe* G-Adventures, M/V *Expedition* Hurtigruten, M/V *Midnatsol* Polar Latitudes, M/V *Hebridean Sky* and M/V *Island Sky* Ocean Expeditions, M/V *Australis* Quixote Expeditions, M/V *Hans Hansson*
Number of Feedback Surveys Received	0	43	38

tial and temporal sampling.

Samples collected from November to March allow us for the first time to obtain seasonal data from these previously underexplored nearshore sites along the WAP in a cost-effective way. The results to date (Figures 5 and 6; Mascioni et al., 2019) are more encouraging than expected, with unknown species being discovered. Although the seasonal progression follows the expected response to day-length changes, there is large variability in the timing of abundances of different taxa. We expect this variability is in part due to physical properties, particularly those related to meltwater input from the glaciers (Dierssen et al., 2002). We will be able to test this hypothesis with further analysis of data collected during subsequent seasons.

Sampling methods chosen for FjordPhyto avoid the need for ultra-freezer space (e.g., liquid nitrogen) to store and transport easily-degradable biological material. Therefore, compromises were made as to what types of data could be collected to best address our scientific aims. We consider surface sampling to be representative of the mixed layer phytoplankton (Garibotti et al., 2005; Pan et al., 2019), albeit some error is expected and will be addressed as time and instrumentation permits. This project would additionally benefit from analysis of inorganic nutrients and chlorophyll concentrations; however, samples must remain frozen until analysis. To best address the objective of determining seasonal and interannual intrusion of meltwater through salinity data, a first-order effort will be based on salinity and optics, as developed by Pan et al. (2019). Further efforts to quantify the fraction of meltwater from sea ice versus glacial melt requires $\delta^{18}O$ oxygen isotope analysis (Meredith et al., 2013) and is considered in planning for future sampling efforts.

Building an Antarctic CS community relies on support from tour operators, staff, and travelers. Within the travel industry, CS projects must be integrated into an already full schedule of activities. FjordPhyto was designed to fit within a one-hour time window that would complement normal operations. If CS programs are to be successful on board, staff need to be given time to make projects a priority.

A unique problem in this collaboration is the frequent turnover and rotation of staff mid-season. Because these remote regions lack a reliable Internet connection, researchers must speak with all CS-identified staff at the beginning of the season to discuss potential issues that may arise such as broken or lost equipment and to avoid the "telephone effect" and the degradation of instructions. To ensure that instructions are clear, we also provide in-person training and detailed protocols for reference once the season is underway.

The main consensus from many CS practitioners and participants emphasizes the importance of feedback from the scientists themselves. FjordPhyto results are shared through putting post-trip materials online and distributing annual reports to the tour operators, who may distribute these materials directly to travelers. It is important that operators and CS participants understand the timeline of the scientific process as well. It may take months to years to fully process, analyze, and publish peer-reviewed work. Regardless, scientists can still provide simple updates each season so that staff can show travelers the impacts of authentic scientific endeavors.

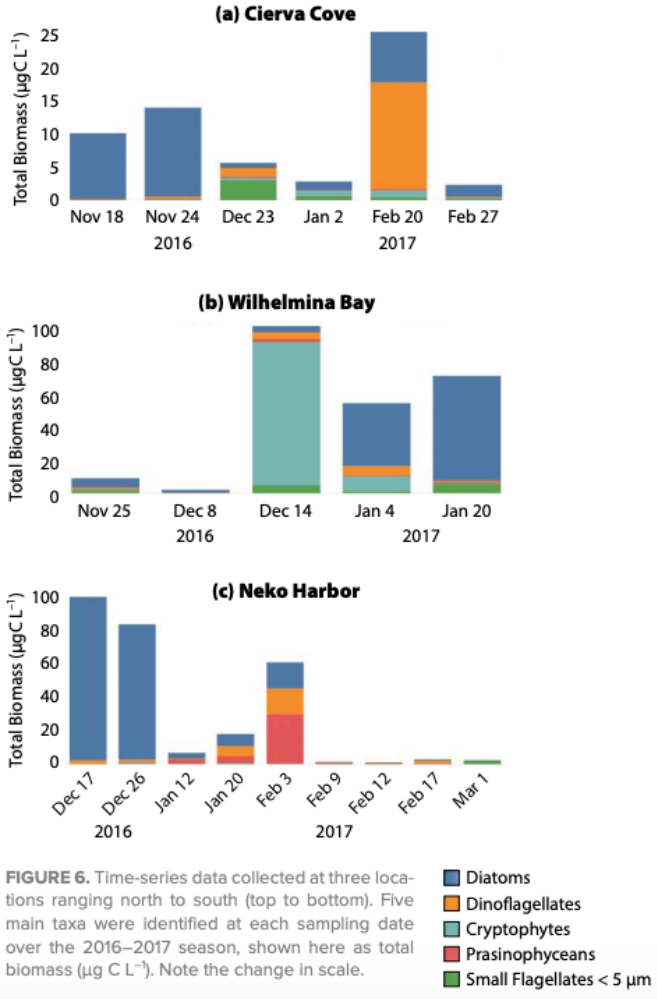

FIGURE 6. Time-series data collected at three locations ranging north to south (top to bottom). Five main taxa were identified at each sampling date over the 2016–2017 season, shown here as total biomass (µg C L^{-1}). Note the change in scale.

To ensure the growth and sustainability of FjordPhyto partnerships, we rely on frequent feedback from staff. Between the 2017/2018 and 2018/2019 seasons, we received 81 feedback survey responses. Travelers who participated expressed greater appreciation for the role of science: "Participating in [CS] allowed me to be more than just a tourist. It also reinforced the Antarctic as a crucial part of climate change." Because CS projects are a recent addition within the polar tourism industry, we have not yet quantitatively assessed attitudes toward science and the environment. This is something being considered by other researchers, and collaboration with social scientists may provide a possible metric for future growth. In addition to travelers' personal testimonies, staff enthusiasm is equally important. As one staff person indicated, "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 [CS] Program was an absolute success!"

Within the research and tour industry there is a paradigm shift to embrace CS as a valid research tool. Federal agencies are investing in CS projects, and polar tour operators are marketing CS projects as an attraction for travelers to remote regions (Walsh, 2019). CS projects are non-revenue-producing and need to rely on diverse funding sources to sustain them long term. By comparison to the costs of established national research programs, the real savings of a CS project is in both ship time and personnel time that ranges in hundreds of thousands of dollars over a five-month period. All other costs pertaining to sample analysis, access to the ship, salary, and graduate student tuition costs remain the same. Overall, savings could range between 60% and 80% when doing research from IAATO ships. To date, some of the FjordPhyto costs have been offset by federal grants; however, FjordPhyto relies on donations and fellowships from individuals and foundations to carry out the research.

FjordPhyto can serve as a powerful low-cost tool for furthering research on phytoplankton communities. Results from the first year of sampling have already brought invaluable insights on phytoplankton composition and phenology in nearshore waters. Chlorophyll values in an unarmored dinoflagellate bloom at Danco Island are estimated at ~27.5 µg Chl-*a* L^{-1} (Mascioni et al., 2019), a concentration comparable to blooms near Anvers Island and Bransfield and Gerlache Straits (Rodriguez et al., 2002). However, the previous blooms have been related to other taxa, such as diatoms, prymnesiophytes, prasinophyceans, or cryptophytes, but never to dinoflagellates. Although dinoflagellates are known to dominate the phytoplankton community in Admiralty Bay during February (Lange et al., 2015), the concentration found (around 7×10^3 cells L^{-1}) is two orders of magnitude lower than those found in this study (9×10^6 cells L^{-1}). Seasonally, the 2016 springtime phytoplankton biomass was dominated by diatoms (Figure 6), although numerically the most abundant group was the small flagellates (<5 µm; Mascioni et al., 2019), as seen in WAP continental shelf waters (Garibotti et al., 2005). Abundant phytoplankton in nearshore waters are supported by relatively shallow mixed layers, usually <40 m (Figure 5; Mitchell and Holm-Hansen, 1991). Our results suggest that numerically, the diatom spring bloom may not always develop in these nearshore waters (see Schloss et al., 2012). The biomass at WAP fjords seems to be dominated by planktonic diatoms, such as *Odontella weisflogii* that is well known in WAP productive environments (Varela et al., 2002; Garibotti et al., 2005), and it is somewhat different from that in King George Island coastal waters, where benthic diatoms constitute the highest biomass (Lange et al., 2015). However, with only one year of sampling, it is not possible to generalize. Low sea ice cover in the fjords and passages in 2016 may have caused a delay in water-column stratification and prevented the formation of the classic diatom bloom, as observed in Marguerite Bay (Rozema et al., 2017).

CONCLUSIONS

With rapid rates of environmental change and significant growth in tourism to the WAP, we saw an opportunity for a joint effort among many stakeholders to answer critical science questions and obtain observations over time that would not be possible or affordable using traditional scientific approaches. While CS should not be considered a replacement for studies by scientists, FjordPhyto can enhance the scientific process and satisfy travelers wanting an enriching experience. Additionally, Antarctic policy and conservation are influenced by more than scientific data. Sharing in the scientific process increases the public understanding of science, allowing travelers to gain new perspective on ocean life. Participants may return home with a deeper understanding of the polar environment and could go on to influence the future protection of the icy continent.

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Chapter 3 Revealing Phytoplankton Succession and Community Dynamics: A Multi-Year Spatio-Temporal Analysis Along the Nearshore Antarctic Peninsula (61ºS-68ºS)

Abstract

The prevailing paradigm is that in Antarctica phytoplankton growth starts in early austral spring with a mixed diatom community, followed by a summer bloom, and terminating with a mixed flagellate community. However, the long-term monitoring required to quantify phytoplankton diversity and its changes in space and time is challenging in the Antarctic due to harsh climate and difficult logistics. The paucity of appropriately resolved data brings this successional paradigm into question. Here, we conducted sampling between 2017 and 2022 along the nearshore region of Antarctic Peninsula (61°S-68°S) to test the Southern Ocean succession paradigm. Our approach included analyzing molecular samples (18S V9) collected through the citizen science initiative "FjordPhyto", quantifying the micro- and nano-eukaryote community structure across 32 sites within four distinct geographical regions, between the months of November and March. We analyzed the diversity, community composition, and succession patterns of species within seven photosynthetic groups: Diatoms, Dinoflagellates, Cryptophytes, Haptophytes, Rhodophytes, and Green algae. Through hierarchal cluster analysis, three distinct assemblages were identified, predominantly characterized by high relative read abundances of either *Porosira_sp.*, *Geminigera_cryophila*, or diverse mixed-diatom taxa. Overall, we found diversity in Antarctic photosynthetic protists to be higher than previously reported for high latitude regions. While there was evidence that seasonal succession followed previously reported patterns, we found that this succession pattern changed across regions and over time. Across four years, these recurrent communities exhibited the characteristic succession pattern of the Southern Ocean in only two years, with large variability at the community, group

and species level depending on location and month sampled. The findings reveal distinct community compositions that consistently characterize this region. Changes within the community across regions and over time suggest that variability in the underlying environment and changes in ecological pressure could drive shifts in community composition with climate change. This more complete seasonal overview of microeukaryote dynamics contributes to our understanding of succession and the role of primary producers in coastal ecosystems, paving the way for further exploration of the environmental drivers and ecological integration, and advancing knowledge of biodiversity and biogeography in polar regions.

Introduction

Photosynthetic protists – single-celled eukaryotic organisms we refer to as phytoplankton – are responsible for fundamental ecological and biogeochemical processes, including inorganic carbon fixation into organic molecules (primary production), and oxygen production. Phytoplankton also serve as a mediator of atmospheric carbon sequestration through the biological carbon pump – the sinking and preservation of organic carbon in the ocean's sediments (Cavicchioli et al. 2015, Deppeler and Davidson 2017). The strength of the biological carbon pump varies in time and space depending largely on patchiness of phytoplankton production.

The nearshore region of the Western Antarctic Peninsula (WAP) is known to be particularly productive, with abundant phytoplankton blooms, and with higher phytoplankton biomass compared to shelf and offshore regions (Vernet et al. 2008, Rozema et al. 2017, Costa et al. 2020, Mascioni et al. 2019). Because of their high productivity, these ecosystems have been identified as biodiversity hotspots and are home to whales, penguins, seabirds, seals, krill, fish, and rich benthic fauna (Nowacek et al. 2011, Espinasse et al. 2012, Bernard & Steinberg 2013,

Pettit et al. 2015, Ziegler et al. 2020). High phytoplankton species diversity contributes to an ecosystem's productivity; identifying variability in taxa throughout the season can inform how an ecosystem may respond to environmental changes (Lin et al. 2017). Characterizing phytoplankton species diversity and succession is key to understanding the significance of changes experienced in coastal ecology (Litchman et al. 2006).

The most significant efforts to characterize species diversity in the WAP have been through national research stations, with vessel-based expeditions ultimately providing only snapshots in time, characterizing the stages of growth representative of only the period sampled (Chapter 1: Table 1.1). Early studies focused on the importance of diatoms (Kopczyńska 2008), while later studies included analyses of flagellates, such as cryptophytes, dinoflagellates, haptophytes and prasinophytes. Such studies provided evidence that, even during diatom blooms, there was a persistent dominant assemblage of nanoflagellates and picoplankton (Garibotti et al. 2005, Clarke et al. 2008, Annett et al. 2010, Rozema et al. 2017, Schofield et al. 2017, Mascioni et al. 2023).

Succession is a fundamental ecological process of gradual and predictable changes in community composition over time, often following a disturbance (Margalef 1958, Pequin et al. 2022, Staude et al. 2023, Poorter et al. 2023). In polar regions the winter sea ice formation and polar night act as annual disturbances to phytoplankton growth through low irradiance and isolation of surface waters from the atmosphere (Petrou et al. 2016).

In the Antarctic, phytoplankton growth and succession occur in the relatively short austral summer season: five months of continuous high solar irradiance that follow the darkness of polar night (Vernet et al. 2008). In early spring, following sea ice retreat, a mixed community of diatoms flourish, sometimes followed by a few chain-forming diatom species growing to large

concentrations (blooms), succeeded by abundant flagellates (e.g., cryptophytes and dinoflagellates) in the late summer (Petrou et al. 2016, Van Leeuwe et al. 2020). In lower sea-ice years, the succession changes, with early spring communities reduced in diatoms and instead dominated by flagellates, giving way to bloom-forming diatoms, and ending in late summer with abundant flagellates (Rozema et al. 2017, Pan et al. 2021, Mascioni et al. 2023). These seasonal dynamics provide an excellent system to deepen our understanding of the temporal and spatial dynamics of phytoplankton patches. However, our insights are constrained, as year-round information on phytoplankton abundances, especially during "shoulder seasons" (September – November, March—May) is limited; many studies have focused only on discrete spring or summer periods (e.g., December - January), and are unable to resolve annual phytoplankton community successions (Chapter 1).

Long-term monitoring studies in the Antarctic are challenging due to harsh climate, challenging logistics, and the difficulty of securing continuous research funding (Medlin et al. 2000, Clarke & Leaky 1996). This has left a fundamental gap in our understanding of how phytoplankton communities are distributed along the WAP, and how/whether communities recur during seasonal succession. This has hindered our ability to quantify long-term trends or predict shifts in community composition and abundance in response to environmental change. Thus, the extent to which phytoplankton communities exhibit seasonal and interannual variability in this highly dynamic environment remains extrapolative; more frequent and continuous sampling will help to fill this significant data gap.

Here, we achieved increased spatial and temporal phytoplankton sampling coverage and resolution along the coastal areas of the Antarctic Peninsula (AP) by utilizing the novel “FjordPhyto” citizen science partnership (Cusick et al. 2020) with tour expedition vessels within

the International Association of Antarctica Tour Operators (IAATO). Through metagenomic analyses we examined trends in phytoplankton diversity, community composition, and seasonal succession at multiple taxonomic levels to deepen our understanding of phytoplankton dynamics in this region. The main taxonomical groups reported in the literature include a predominance of diatoms, bloom-forming diatoms, and flagellates – especially dinoflagellates and cryptophytes (e.g. Abele et al. 2017). As previously reported for coastal stations, we expected phytoplankton diversity (e.g., species richness, evenness, Shannon Index) to decline in the summer during blooms, and increase again in the fall, independent of location (Staude et al. 2023, Hamilton et al. 2021). Individual species are expected to form characteristic communities, following the classic model that early season begins with mixed diatoms, then gives way to bloom-forming diatoms, and concludes with communities predominantly composed of flagellates (Petrou et al. 2016). Furthermore, within these communities, there would be individual species responding independently at different times in the summer period (Emery 2010, Chang et al. 2018).

Materials and Methods

Study location

Through collaboration with IAATO-member vessels participating in the FjordPhyto citizen science program, 32 different location sites were visited along the AP between the months from November to late-March/April during four consecutive austral summer seasons (2017-2018, 2018-2019, 2019-2020, 2021-2022), excluding the COVID-19 pandemic season (2020-2021) when no ships sampled (Figure 3.1). Locations were visited based on scheduled ship activity within the South Shetland Islands (region 61°00'-63°37'S, 53° 83'–62°83'W) and the AP's northern, western, and southern nearshore coasts (between 63°24′S and 68°11′S). For ease of description, locations were numbered 1 – 32 beginning in the south with Stonington Island

extending north to the South Shetland Islands (Supplementary Table 3.1), and grouped by geographic proximity categorized into four broad regions: South Shetlands Islands (referred to herein as the "Shetlands"), "Northern", "Middle", and "Southern" (Figure 3.1A, Supplementary Table 3.1).

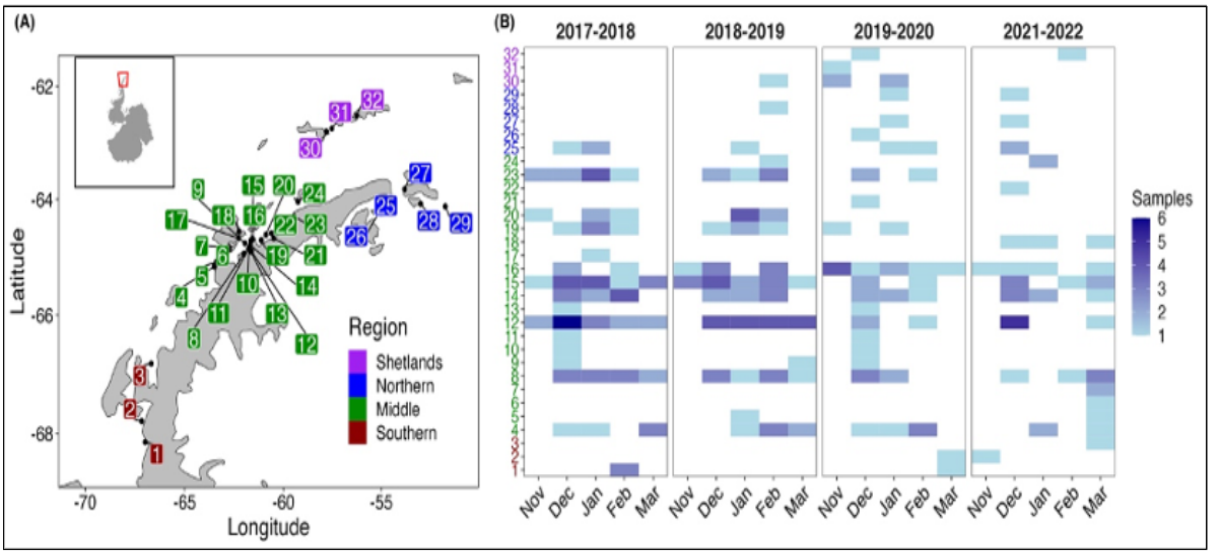


Figure 3.1: Location of sites visited along the AP where samples were collected using FjordPhyto protocols, in collaboration with IAATO operators (A). Stations are labeled from 1 – 32 and labels are colored according to which region they are associated with (red = Southern region, green = Middle region, blue = Northern region, and purple = Shetlands region. Total number of samples collected at each site (Y-axis) over all months (November – March; X-axis) across all sampling seasons (B).

Sample collection

To investigate questions of phytoplankton community seasonality and diversity over time, molecular and biological samples were collected from numerous sites repeatedly visited over the seasons (Figure 3.1B). Samples in this publication were collected as described in Cusick et al. (2020), under constraints of IAATO expedition vessel operations. At each site sampled (Supplementary Table 3.1), a 20 µm phytoplankton net (bridle mouth diameter 30 cm, SeaGear Corp) was towed just under the surface (0-1 m) at idle speed from a rubber inflatable boat out of

direct line of the engine path. This mesh size targets microeukaryote protists larger than 20-µm. After 10 minutes, the net was brought on board and the 1-L cod end jar concentrate was poured through a filtration column (Nalgene 1000 mL Reservoir) as soon as possible. This passed through a pre-sterile 0.2 µm - 47 mm filter (PALL Supor Hydrophilic polyethersulfone). Samples preserved in RNAlater stabilization solution (Invitrogen) within a cryovial tube (3-mL) then frozen onboard the ship at -20 °C. At the end of each austral season, samples were transported to Scripps Institution of Oceanography, UC San Diego California, USA, at the end of each austral season, where they were kept frozen at -80 °C until analysis.

DNA extraction, PCR, sequencing

All 271 samples from 2017-2022 were processed and prepared for metabarcoding sequencing at the J. Craig Venter Institute (JCVI), San Diego, California, USA. The RNAlater was first reduced from the cryovial tube and the biomass was retrieved from the filter through pelleting in a centrifuge. Following the protocol from NucleoMag Plant Kit for DNA Purification (Macherey-Nagel, Düren, Germany), genomic deoxyribonucleic acid (gDNA) was extracted from samples using the epMotion 5075TMX robot (Eppendorf, Hamburg, Germany).

A Nanodrop spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) was used to assess the extracted DNA for quality and quantity. DNA was amplified via a one-step polymerase chain reaction (PCR) using the TruFi DNA Polymerase PCR kit (Azura, Raynham, MA, USA) targeting the18S hyper variable V9 region of the nuclear rRNA gene. For 18S V9, the 1389 F (TTGTACACACCGCCC) and 1510 R (CCTTCYGCAGGTTCACCTAC) primer set was used (Amaral-Zettler et al. 2009). Each reaction was performed in a final volume of 25 µL with an initial denaturing step at 95 °C for 1 min followed by 30 cycles of 95 °C for 15 sec, 56

°C for 15 sec, and 72 °C for 30 sec. DNA quality was assessed by putting 2.5 µL of each PCR reaction on a 1.0% agarose gel to confirm amplification.

PCR products were purified using the Zymo Research DNA cleanup and Concentrator Kit and OneStep PCR inhibitor Removal Kit. PCR quantification was performed using Invitrogen Quant-iT PicoGreen dsDNA Assay kit (Invitrogen, USA) following manufacturer's instructions. Samples were then pooled in equal proportions (20-ng) into an Eppendorf tube (1.7 mL) for the 18Sv9 data followed by another 0.8x AMPure XP bead purification. The pools were evaluated on an Agilent 2200 TapeStation and quantified with Qubit HS dsDNA.

The extracted DNA samples were sequenced at the Institute for Genomics Medicine (IGM) at the Genomics Center of the University of California, San Diego using next-generation sequencing single Illumina MiSeq lanes (PE150, 2×150bp, for 18S) with a 15% PhiX spike-in. Custom mock community spike-ins (Lin et al. 2019) were not included in this study's sequencing runs because the volume of water filtered during the net tow in Antarctica was undetermined.

Bioinformatics analysis

Raw paired-end sequence data were analyzed in the QIIME2 (v2022.11) environment with DADA2 in which data were demultiplexed, trimmed, denoised, and annotated using the protocol from Allen Lab GitHub (https://github.com/allenlab/QIIME2_18Sv9_ASV_protocol#protocol-for-processing-18sv9-sequences-in-qiime2). Trimmed reads were then quality-filtered, denoised, merged, and chimera-removed with DADA2 (version 1.16) and amplicon sequence variants (ASVs) were identified using the naïve Bayesian classifier method (Wang et al. 2007, Callahan et al. 2016). Taxonomic annotation of ASVs was performed against the Protist Ribosomal Reference database (PR2,

v4.14.0) (Guillou et al. 2013) for 18S V9 amplicons with 97.0% cut off. Each Feature ID (unique ASV) was annotated to one of eight levels: Kingdom, Supergroup, Division, Class, Order, Family, Genus, Species (https://pr2-database.org/documentation/pr2-structure/ last updated in April 2023) following the PR2 naming convention.

The total 18S V9 rRNA amplicon dataset resulted in 1295 unique ASVs and 11,491,065 total reads observed across 271 total samples. Due to differences in library sizes across samples, we chose to rarefy the data set down to a library size of 7,000 which removed 38 samples that had smaller library sizes (Supplementary Figure 3.1, McMurdie and Holmes 2014, Gloor et al. 2016, Cameron et al. 2020). The remaining 228 final samples had a depth of sequencing saturated for all samples (Supplementary Figure 3.1). Because different taxa and cells are known to produce varying levels of DNA (Needham and Fuhrman 2016, Liu et al. 2022, DeVargas et al. 2015) and due to the lack of spike-in community for comparison, ASV reads are reported as relative abundances and do not reflect the direct cell count numbers (Lin et al. 2017, 2019).

Classification of ASVs into Phytoplankton groups

To focus on emerging patterns in space and time, we first removed the single sample collected in April and we removed ASVs that had fewer than 5 total reads across all samples. This left 513 ASVs and a total of 1,629,929 high-quality reads. For the purposes of focusing on microeukaryote protist diversity, we categorized 228 ASVs into seven distinct phytoplankton groups that contained photosynthetic taxa based on most common reported in literature (Hamilton et al. 2021, Pan et al. 2020, Mascioni et al. 2021, Mendes et al. 2018, Abele 2017, Trefault et al. 2021), herein referred to as: Diatoms (Class Bacillariophyta), Dinoflagellates (Division Dinoflagellata), Cryptophytes (Division Cryptophyta), Marine Stramenopiles (Class MAST), Haptophytes (Division Haptophyta), Rhodophytes (Division Rhodophyta), and Green

algae (Division Chlorophyta and Prasinodermophyta) (Table 3.1). Part of the plankton composition includes species of seaweeds in the Rhodophyte and Green algae groups; these were included as they constitute pieces of blades detached from whole organisms and are available for carbon absorption and food for benthic organisms. These were added to the eukaryote photosynthetic >20 µm plankton (Mincks et al. 2008).

Table 3.1: Summary of phytoplankton groups showing the total number of ASVs, total number of reads, and total number of genera and species

	# ASVs	# reads	# Genera	# Species
Diatoms	87	1235488	21	27
Dinoflagellates	73	76133	24	24
Cryptophytes	8	58308	1	1
MAST	27	48084	15	15
Haptophytes	11	17797	4	5
Rhodophytes	5	9297	5	5
Green algae	17	3287	12	12
Other	285	181613	123	125

We removed 285 ASVs (totaling 181,836 reads) that were not classified within one of the phytoplankton groups and labeled as “Other” (which made up 11.1% of all the reads in the dataset, Table 3.1) additionally including the removal of Metazoa, Telonemia and Opisthokonta fungi and small eukaryotes with low read abundances including the Ochrophyte mixed flagellates: (Bolidophyceae, Pelagophyceae, Dictyochophyte), and Chlorophyta (Mamiellophycea).

Quantifying Diversity and Community composition

Community diversity within each sample was examined using three different metrics: richness, evenness, and Shannon diversity index. Species richness was calculated as the total number of species or ASVs observed in a sample. Pielou's species evenness measure was calculated as the Shannon-Weiner diversity index value divided by the natural log of the richness in each sample. Shannon-Weiner diversity index was calculated using the *vegan* package in R (v2.6-4, Dixon 2003, Oksanen 2015) and considers species abundance and community evenness.

To define community composition, we aggregated the reads for all ASVs within a species and calculated the Spearman correlation coefficient between each pair of phytoplankton species. We then converted the correlation matrix to a matrix of Euclidean distances between the inverse of the correlation matrix, which creates a measure of dissimilarity. Hierarchical clustering was conducted on the dissimilarity matrix using an updated Ward's minimum variance method where the dissimilarity matrix was squared before clustering (Murtagh and Legendre 2014). We determined the optimal number of clusters (k) to be 3 based on various algorithms such as Gap Statistics, Within Sum Square (Elbow), and Silhouette. The hierarchical clustering split each species into 3 community clusters based on Spearman correlations. Here, each sample can contain all three clusters, but the relative dominance of clusters might change over space and time. All analyses were performed in RStudio Version 2023.06.1+524 (RCore Team 2015) and data visualizations were created using *ggplot2.*

Seasonal succession for each species of phytoplankton during each sampling season was defined using Locally Weighted Scatter Plot Smoothing (LOESS) regression fitting. We chose a LOESS fit as opposed to a Generalized Additive or Linear Model to reduce the bias of assuming functional forms in the data. For each sample, the relative abundance of each species was calculated by adding up all the reads from each ASV within a species and dividing by the total

reads across all phytoplankton. Additionally, the co-located time of sample collection was converted to "Days since start of season" by calculating the number of consecutive days that had elapsed since November 1st for each season. For example, if a sample was collected on December 2, 2017, or February 4, 2019 then there would have been 30 and 161 days since the start of the season. For each species in each season, we plotted the relative abundance versus the days since start of season. We then fit the LOESS regression to predict the relative abundance at each day in the season (Supplementary Figure 3.2). Using the fitted curve, we calculated the day of the season when the relative abundance was at its maximum, and then calculated the weighted standard deviation around the maximum. These values were used to determine whether a species was an "Early", "Mid", or "Late" season dominant, and whether this dominance was more localized (i.e., the standard deviation was small), or was more generally distributed (i.e., the standard deviation was large). Due to the patchiness of the sampling effort, we chose to use the regression fit as opposed to the raw data to avoid sampling bias and focus on the overall trends. We found that the LOESS fit captured the seasonal pattern well (Supplementary Figure 3.2).

Results

Section I –Phytoplankton taxonomic composition and spatial and temporal distribution

Diatom species

We identified 87 diatom ASVs representing 27 unique species across 21 genera (Table 3.1). The top 10 diatom genera across all samples were *Porosira_sp.,* and unknown *Bacillariophyta*_sp*., Stellarima_microtrias, Polar-centric-Mediophyceae_sp., Odontella_sinensis, Eucampia_sp.*, *Chaetoceros_sp., Shionodiscus_ritscheri., Chaetoceros_socialis* and *Proboscia_alata* (Figures 3.2 and 3.3).

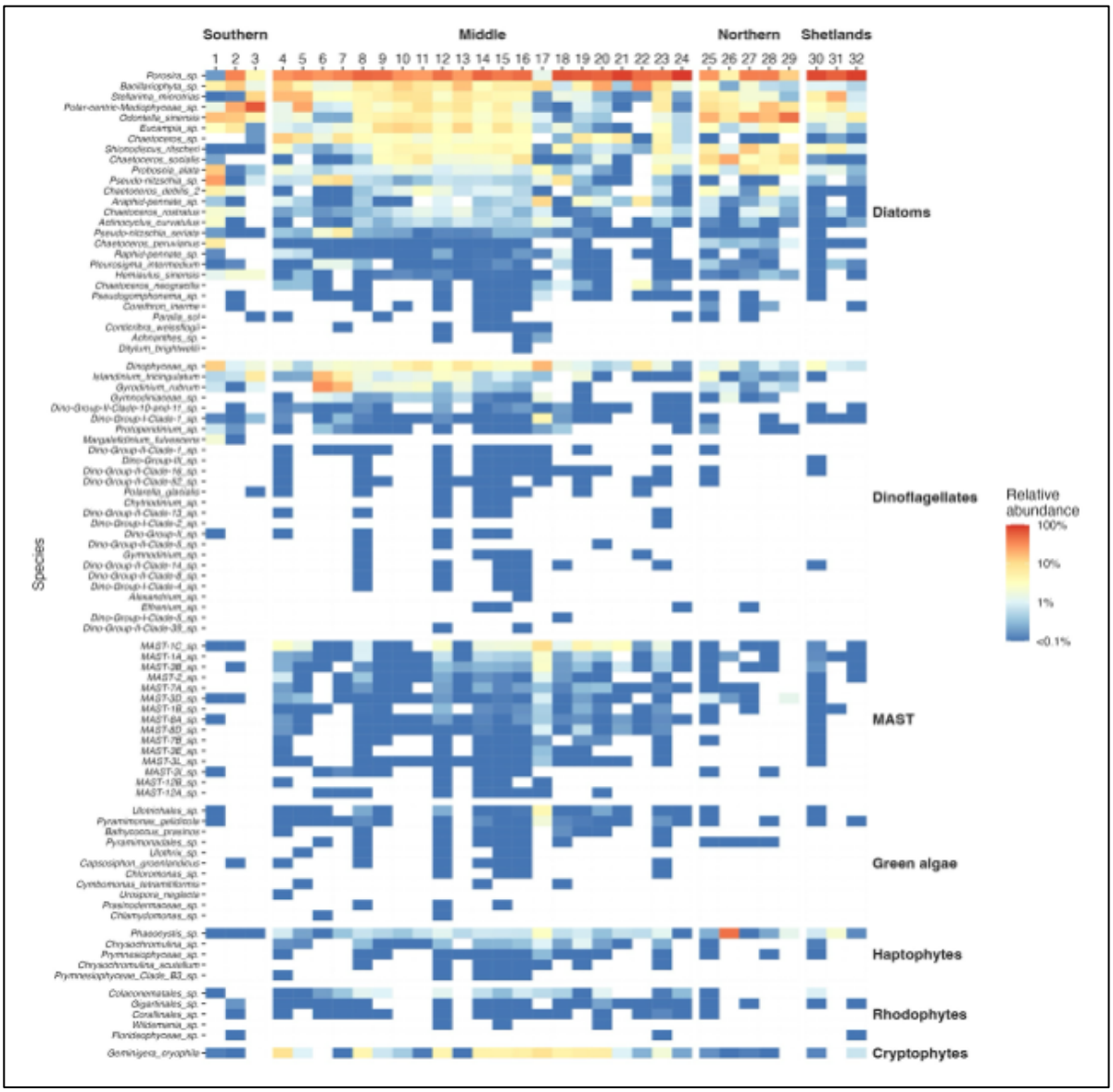


Figure 3.2: Heatmap showing the relative abundance in percentages for each species at each station sampled (1-32). Colorbar is log scaled to show differences more easily between abundant and rare species. Species names on Y-axis are arranged according to phytoplankton group. Within each phytoplankton group, species are ordered on the Y-axis by relative abundance across all samples with the top species in each group first. Relative abundances are presented as percentages and calculated as the number of reads of a given ASV divided by the total reads of all ASVs for a given sample multiplied by 100. White box denotes absence of reads for a particular species in samples at each site.

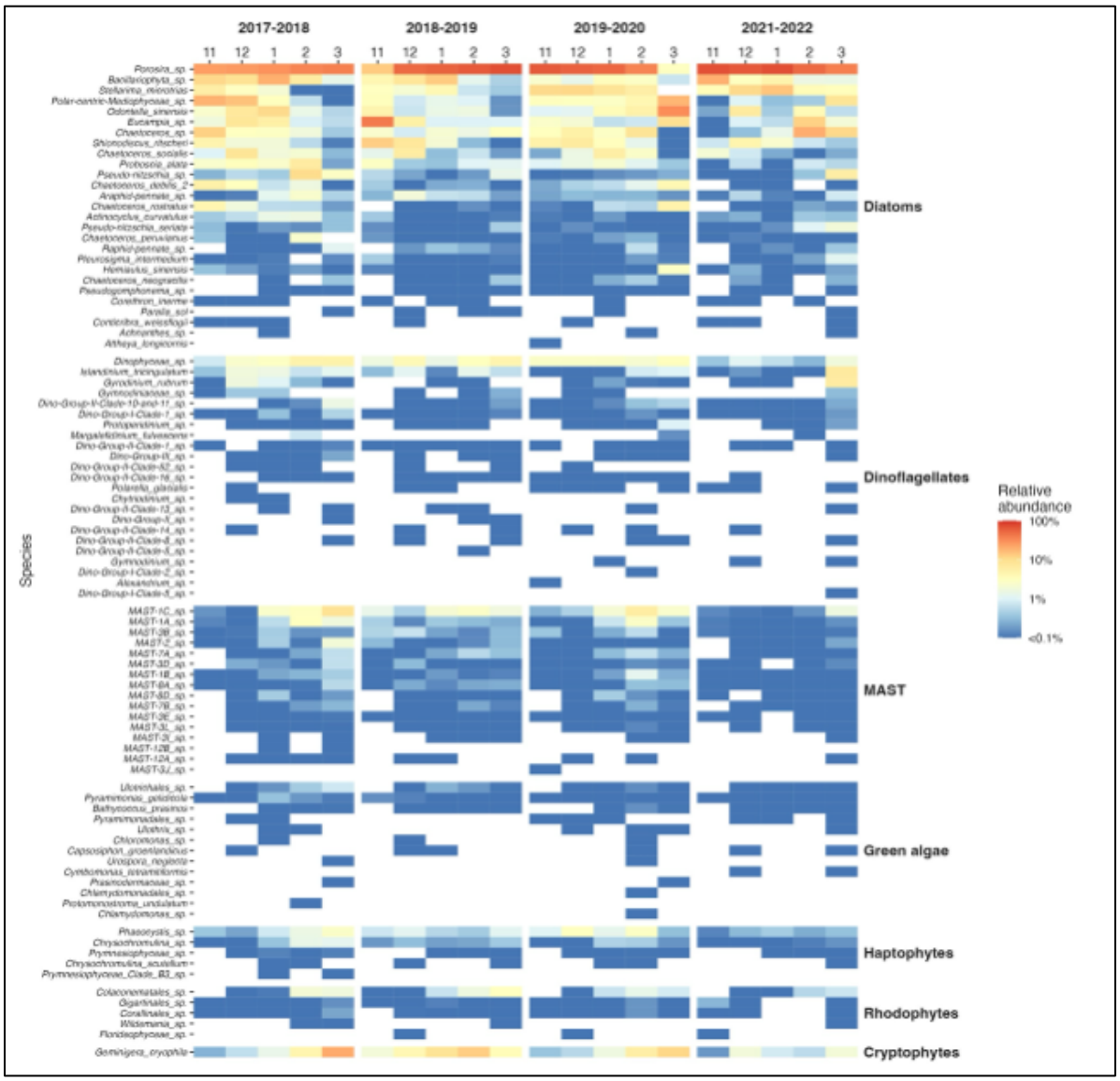


Figure 3.3: Heatmap showing the relative abundance in percentages for each species in each month across 4 sampling seasons. Colorbar is log scaled to show differences more easily between abundant and rare species. Species names on Y-axis are arranged according to phytoplankton group. Within each phytoplankton group, species are ordered on the Y-axis by relative abundance across all samples with the top species in each group first. Relative abundances are presented as percentages and calculated as the number of reads of a given ASV divided by the total reads of all ASVs for a given sample multiplied by 100. White box denotes absence of reads for a particular species in samples at each site.

Large spatial variability was observed in diatom distributions, with some genera found in all locations and some in only certain locations (Figure 3.2). Most of the species found only at

specific locations were: *Chaetoceros_debilis_2, Chaetoceros_peruvianus, Chaetoceros_neogracilis, Pseudogomphonema_sp., Corethron_inerme (*most likely misidentified as the well-known WAP *Corethron pennatum*), *Hemiaulus_sinensis*, *Paralia_sol, Pseudo-nitzchia_seriata Conticribra_weissflogii, and Achnanthes_sp.* Sites in the Southern and Northern regions – like Detaille Island (site 3), Charlotte Bay (site 21), and Portal Point (site 22) – had fewer diatom species than other sites. However, sites in the Middle region – like Neko Harbour (site 12), Danco Island (site 14), and Cuverville Island (site 15) – had almost all the diatom species present.

Most diatom genera encountered in the microplankton were present at all months and all seasons except for *Achnanthes_sp., Attheya_longicornis, Conticribra_weisflogii., Paralia_sol., Corethron_inerme, Pseudogomphonema_sp.* (Figure 3.3). Most diatoms appeared in the beginning of the season, but some, such as *Polar-centric-Mediophyceae_sp., Odontella_sinensis, Eucampia_sp., and Chaetoceros_sp.*, appeared at higher relative abundances of reads in the late season (Figure 3.3).

Dinoflagellate species

We identified 73 dinoflagellate ASVs representing 24 unique species across 24 genera (Supplementary Table 3.2). Within the dinoflagellate group the most abundant genera were *Dinophyceae_sp., Islandinium_tricingulatum,* and *Gyrodinium_rubrum.* Dinoflagellate distributions were spatially restricted (Figure 3.2). Only a few sites had a high diversity of dinoflagellates: Petermann Island (site 4), Paradise Harbour (site 8), Neko Harbour (site 12), Danco Island, Cuverville Island, Orne Harbour, Dallman Bay (sites 14 – 17, respectively). Most sites in the Northern and Shetlands region had low dinoflagellate diversity. Dinoflagellate diversity varied strongly throughout the season, except for *Dinophyceae_sp.* and

Islandinium_tricingulatum that were present in every month and season (Figure 3.3). Only three out of 24 dinoflagellate genera were always present at the beginning of the season (starting in November). By December of each austral season, at least half of the dinoflagellate genera were present through to March (Figure 3). Commonly occurring genera throughout different months included *Dinophyceae_sp., Islandinium_tricingulatum,* and *Gyrodinium_rubrum, Gymnodiniaceae_sp.,* and *Dino-Group-I-Clade-1_sp.* Less frequently observed genera included *Margalefidinium_fulvescens*, *Chytriodinium_sp., Dino-Group II-Clade-2, 5, 8, 14_sp.* and *Alexandrium_sp., and Dino-Group-I-Clade-5_sp.*

Cryptophyte species

We identified eight cryptophyte ASVs representing one unique species across one genus, *Geminigera_cryophila* (Supplementary Table 3.2). *G. cryophila* was present at all sites except (site 3) in the Southern Region, (site 6) and Heroina Island (site 29) in the Northern, and Yankee Harbour (site 32) from the Shetlands sites (Figure 3.2). *G. cryophila* was most abundant at Petermann Island (site 4), Paradise Harbour (site 8), Neko Harbour (site 12), Danco Island, Cuverville Island, Orne Harbour, Dallman Bay, Fournier Bay, Wilhelmina Bay, and Enterprise Island (sites 14 -20, respectively). *G. cryophila* was present in all months and seasons but had a higher relative abundance as the season progressed with highest reads in February and March (Figure 3.3).

Marine Stramenopiles (MAST) species

We identified 27 Marine Stramenopiles (MAST) ASVs representing a total of 15 species across 15 genera (Supplementary Table 3.2). Within the MAST group nearly all the genera were present in many Middle region sites (Figure 3.2). Most sites, such as Petermann Island (sites 4), Paradise Harbour (site 8), Neko Harbour (site 12), Danco Island, Cuverville Island, Orne

Harbour, and Dallman Bay (sites 14-17, respectively) (Figure 3.2), exhibited the presence of MASTs. Dallman Bay (site 17) showed the highest relative abundance, with a relative dominance of *MAST-1C_sp.* The Southern sites showed few to no MASTs (Petermann Island, site 3; Heroina Island site 29). Peltier Channel (site 6), Mikkelsen Harbour (site 24), Vega Island (site 26), Eden Rocks (site 28), and Yankee Harbour (site 32) had the fewest reads. Notably, Heroina Island (site 29) only had MAST-3D *sp.* and no others. Overall, MAST taxa were consistently observed across all months (Figure 3.3), but fewer were present in November. Notably, *MAST-3J_sp.* was exclusively observed in 2019-2020.

Green Algae species

We identified 17 Green algae ASVs representing a total of 12 planktonic and benthonic species across 12 genera (Supplementary Table 3.2). Within the Green algae group the taxa present in all samples were *Ulotrichale_ sp.* (a family of benthic associated species), *Pyramimonas_gelidicola* (nanoplanktonic alga, ~10 um), and *Bathycoccus_prasinos (*picoplanktonic alga). All other species were rare and had sporadic appearances. The site with highest abundances of green algae, in particular *Ulotrichales_sp.* and *Pyramimonas_gelidicola,* was Dallman Bay (Site 17). The sites with higher numbers of most Green algae species present were Neko Harbour (site 12) and Orne Harbour (site 16) (Figure 3.2). The Southern, Northern, and Shetlands regions were mostly devoid of green algae, except for the three most abundant species (Figure 3.2). *Pyramimonas_gelidicola* occured in every month in all seasons (Figure 3). *Ulotrichales_sp.* and *Bathycoccus_prasinos* appeared in most months, but *Ulotrichales_sp.* seemed to occur earlier in the year compared to *Bathycoccus_prasinos* (Figure 3.3).

Haptophyte species

We identified 11 haptophyte ASVs representing a total of 5 species across 4 genera (Supplementary Table 3.2). Within the haptophyte group, *Phaeocystis_antarctica* and *Chrysochromulina_sp.* were the predominant genera in all samples (Figures 3.2 and 3.3). Conversely, *Prymnesiophyceae_Clade_B3_sp.* was less frequently encountered, with minimal occurrences and reads relative to the other haptophyte species. *Phaeocystis_antarctica* was detected in 217 samples across all sites (Figure 3.2). Haptophytes were present in all regions but made up less than 0.1% of the reads in the Southern region. The highest reads were recorded at Vega Island (site 26). Both *Phaeocystis_antarctica* and *Chrysochromulina_sp.* were consistently detected throughout all months, whereas *Prymnesiophyceae_Clade_B3_sp.* was only observed in January or March 2017-2018, or exclusively in March 2019-2020 (Figure 3.3).

Rhodophytes species

We identified 5 Rhodophyte ASVs representing a total of 5 species across 5 genera (Supplementary Table 3.2). The most abundant rhodophyte species was *Conaconemetales_sp.* with a total of 7,913 reads; it was mostly present in the Middle region (Figure 3.2). *Wildemania_sp.* was observed in all seasons except for 2019-2020, predominantly appearing in the latter half of the summer during February and March (Figure 3.3). On the other hand, *Florideophyceae_sp.* typically appeared slightly earlier in the season, notably in December and occasionally in November (Figure 3.3).

Section II – Phytoplankton Communities and their Spatial, and Temporal variability and Diversity

The phytoplankton communities within the WAP fjords and inland channels exhibited a broad range in spatial diversity patterns, differing in total and average species richness, evenness, and Shannon index (Figure 3.4). The middle regions showed the highest total species richness

(Figure 3.4A), with up to 91 ASVs found in samples from sites such as Neko Harbour (site 12). The sites with the lowest species richness were Yankee Harbor (site 32) in the Shetlands, Detaille Bay (site 3) in the Southern region, and Heroina Island (site 29) in the Northern region, with total numbers of species of 27, 28, and 31, respectively (Figure 3.4A). Dallman Bay (site 17) in the Middle region had the highest mean species richness (alpha diversity) with an average of 74 species, the highest evenness with a value of 0.798, and the highest Shannon diversity index with a value of 3.44 (Figure 3.4B-D). In contrast, Mikkelsen Harbour (site 24) in the Northern region had the lowest average species richness with 25.7 species, the lowest evenness with a value of 0.135, and the lowest Shannon diversity index with a value of 0.436 (Figure 3.4B-D).

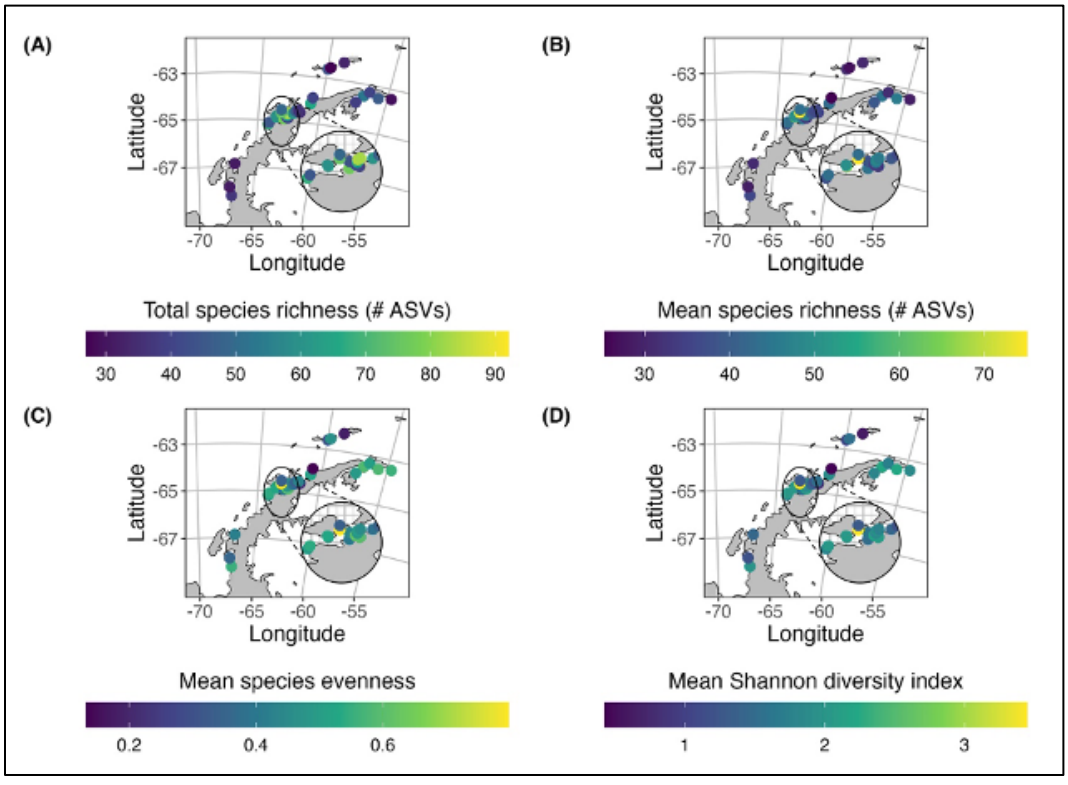


Figure 3.4: Diversity metrics from all sites displayed spatially over the AP. (A) Total species richness measured as the total number of ASVs that could be found at each of the 32 sites, (B) Mean species richness measured as the average number of ASVs that was found in each sample at a given site, (C) The mean evenness of species (J) within each site, and (D) the mean Shannon Diversity Index (H') at each site. The inset plot is zoomed in on sites in the Middle region that

are close together for easier visualization. Each color bar is scaled according to the measured metrics, but darker points represent lower values, and brighter points represent higher values.

Within a given sampling season, species richness increased from November to March whereas species evenness and Shannon diversity were higher in the beginning of the season and at the end of the season (Figure 3.5). The 2017-2018 season deviated from this pattern slightly, with higher reported values for richness, evenness, and Shannon diversity in the middle of the sampling season compared to the subsequent sampling seasons (Figure 3.5). The temporal variability in species richness, evenness, and Shannon diversity varied among the seasons sampled, with an overall increase in species richness over the months sampled for any given season (Figure 3.5A). The evenness of species early each season started highest for the first three years sampled (0.45 – 0.70) then dropped as the season progressed and increased again in the late season (February and March). The 2017-2018 year had the steadiest evenness throughout the season (Figure 3.5B). The Shannon diversity index for each season declined for 2018-2019 over the year but increased for the 2019-2020 and 2021-2022 seasons. The 2017-2018 year held a consistent Shannon diversity index through the year (~2) with a maximum of 3.44 (Figure 3.5C).

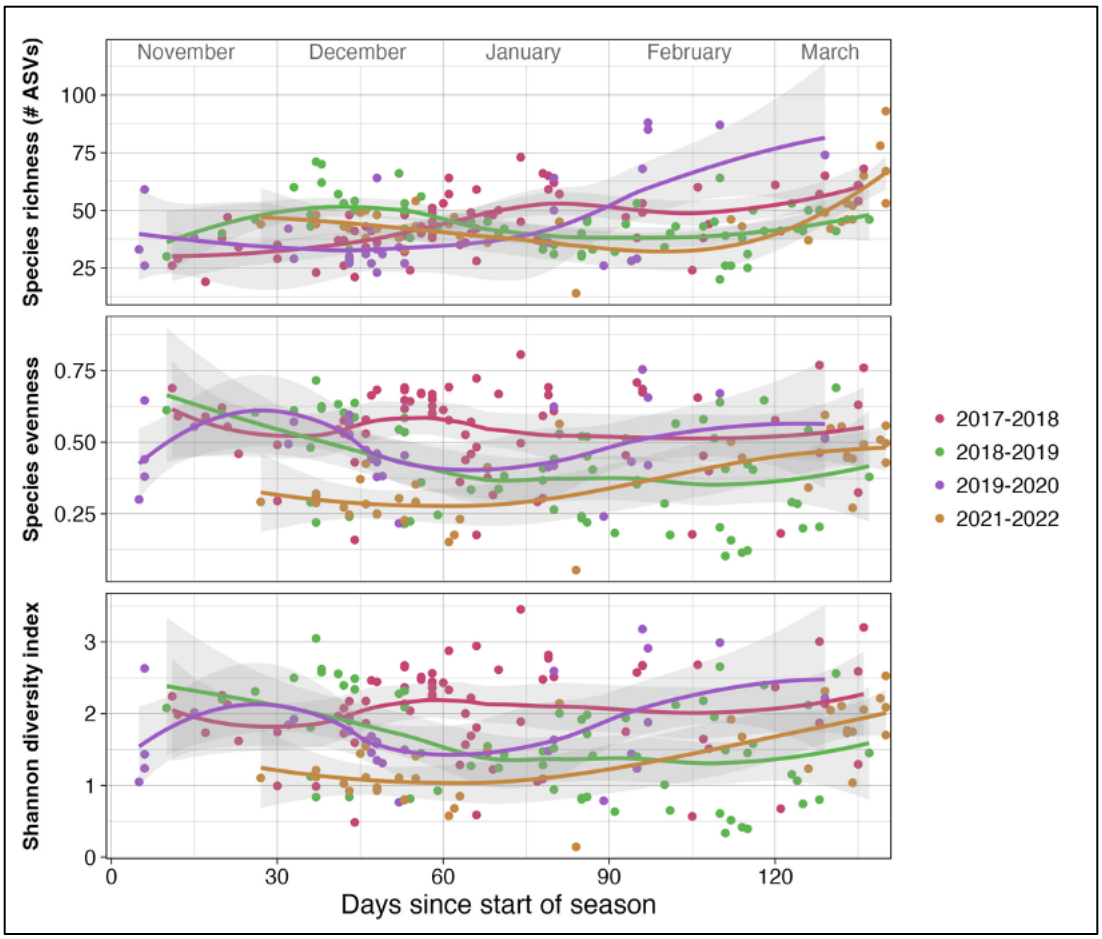


Figure 3.5: Variability in (A) richness, (B) evenness, and (C) Shannon diversity across all locations sampled (points) beginning November 1st (Day 0) from all sampling seasons (represented by distinct colors). Lines are LOESS fits for each diversity metric over each season (colors) with shaded gray areas representing the 95% confidence interval.

Section III – Co-Occurring taxa and Community Composition

Our analyses revealed groups of phytoplankton taxa with different correlation strengths to other taxa (Figure 3.6), giving rise to three clusters (Figure 3.7): Cluster 1 (blue), Cluster 2 (grey), Cluster 3 (red). Cluster 1 is comprised of 15 species that are, in general, more highly correlated with each other. The strongest positive correlations were between *Proboscia_alata*, *Chaetoceros_debilis_2, Polar-centric-mediophyceae_sp., Odontella_sinensis, Hemiaulus_sinensis, Actinocyclus_curvatulus,* and *Eucampia_sp.* Conversely, *Raphid-pennate_*

sp. was negatively correlated with *Polar-Centric-Mediophyceae_sp., O. sinensis, and H. sinensis*, in addition to *Porosira_sp.*

Figure 3.6: Species co-occurrence Matrix (Spearman's correlation plot) divided into three clusters (colored bars) using the hierarchical clustering Ward's method ("ward.D2"). Each species identified in the dataset is displayed with warmer colors represent stronger positive correlations and cooler colors represent stronger negative correlations.

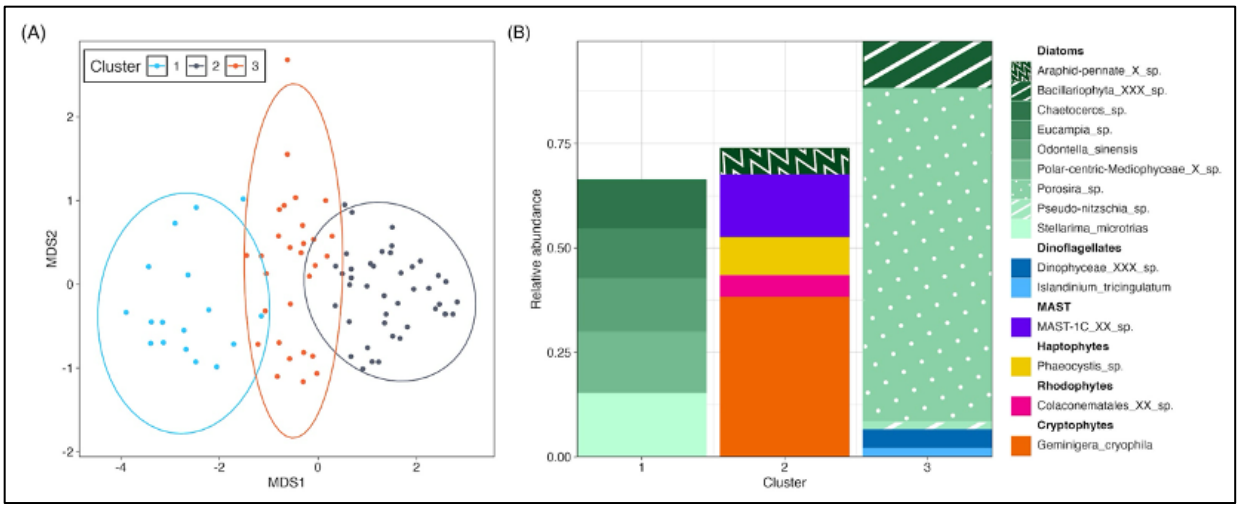


Figure 3.7: Cluster analysis and associated assemblages of taxa. A) Nonmetric multidimensional scaling (NMDS) of species (points) grouped into three distinct clusters (blue = 1, grey = 2, and red = 3) based on Spearman's correlation (see Figure 3.4). The ellipses show the 95% confidence interval from a multivariate t-distribution. B) The top 5 most abundant by reads phytoplankton species within each cluster. Cluster 1 represents a mixed diatom community; Cluster 2 is dominated by the cryptophyte *Geminigera_cryophila*. Cluster 3 is dominated by the diatom *Porosira_sp.* The diatom species *Araphid-pennate_X_sp.* (waves), *Bacilliariophyta_XXX_sp.* (white diagonal lines), *Prorosira_sp.* (white dots), and *Psuedo-nitzchia_sp.* (white diagonal lines) are given distinct patterns to better identify species across clusters.

Cluster 2 (grey) is made up of 34 highly positively correlated species with ten species that strongly correlated with each other more than with any other species (*MAST-1A, -1B, -1C, -8A, -3L, -7A, -7B, Colaconematales_sp., Chrysochromulina_sp., Geminigera_cryophila*). The ten most highly positively correlated species in Cluster 2 were strongly negatively correlated with all species in Cluster 1 (Figure 3.6). *Colaconematales_sp., MAST-7B,* and *MAST-7A* were closely associated with *Dino-Group-I-Clade-10_sp.* and *-11_sp.*, as well as *Bathycoccus_prasinos.*

Cluster 3 is made up of 39 species that correlated positively with each other, but were not strongly correlated with other species or clusters. The diatom *Porosira_sp.* was found in Cluster 3 and was negatively correlated with nearly all other species, and specifically very strongly negatively correlated with the Cluster 1 species *O. sinensis, H. sinensis, Eucampia_sp., P. alata,*

Chaetoceros_debilis_2, Gyrodinium_rubrum, Chateocero_ rostratus, Bacillariophyta_sp., the Cluster 2 species *Prymesiophyceae_sp., MAST-8D, MAST-2, Dino-Group-I-Clade-1, MAST-3D,* and the Cluster 3 species *Pseudo-nitzschia_seriata, Dinophyceae_sp., and Islandinium_tricingulatum, Protoperidinium_sp.*

The top five most dominant species in Cluster 1 were *Chaetoceros_sp., Eucampia_sp., Odeontella_sinensis, Polar-centric-Mediophyceae_X_sp.,* and *Stellarima-microtrias* (Figure 3.7). The top five most dominant species in Cluster 2 were *Geminigera_cryophila, Colaconematales_XX_sp., Phaecsystis_sp., MAST-1C_XX_sp*., and *Araphid-pennate_X_sp.* (Figure 3.7). The top five most dominant species in Cluster 3 were *Bacillariophyta_XXX_sp., Porosira_sp., Dinophyceae_XXX_sp.,* and *Islandinium_tricingulatum* (Figure 3.7).

Section IV – Taxonomic Succession

Succession at phylum and species level

The analyses of seven main phytoplankton groups revealed temporal variability in species composition, with each species exhibiting a unique timing of peak relative proportion of ASV reads at a specific day during the spring-summer-early fall period (Figure 3.8). Diatoms exhibited a mixed occurrence, with some species consistently peaking early in the season, while others emerged in the middle or later months (Figure 3.8). Notably, diatoms like *Shionodiscus_ritscheri, Hemialus_sinensis, Chaetoceros_sp., C._rostratus, C._peruvianus, Pseudo-nitzchia_seriata., Corethron_inerme, Porosira_sp., Polar_centric_mediophyceae_X_sp., and Proboscio_alata* were consistently early-season species (Figure 3.8). Conversely, pennate species such as *Raphid-pennate*_sp., *Pseudo-nitzschia_sp.*, *Pleurosigma_intermedium*, chain-forming *Chaetoceros_neogracilis*, and *Paralia_sol* consistently appeared late in each sampled year (Figure 3.8). However, there was a marked shift observed across different sampling seasons,

with early-occurring diatoms transitioning slightly later in the early season, and mid-summer diatoms occurring either earlier or later depending on year (Figure 3.8). In 2021-2022, more than half of the diatom species appeared late in the season (Figure 3.8). Additionally, certain dinoflagellates, such as *Islandinium_tricingulatum* and *Gymnodiniaceae_X_sp.,* initiated early in the season, while others emerged in the middle or late season (Figure 3.8). Haptophytes, notably *Pyramimonas_sp.*, displayed variable timing, with some occurring early or late, but others consistently appearing mid to late season (Figure 3.8). Similarly, green algae tended to peak in mid to late season, while MAST species typically peaked late, although exceptions like *MAST 8D, 3B, and 7B* occasionally occurred early in certain seasons (Figure 3.8). Rhodophytes exhibited a mid to late occurrence pattern, with *Corallinale_XX_sp.* being earliest in the first two years of observation (Figure 3.8). In 2021-2022, nearly every species from every phytoplankton group appeared in the late season, except for some early-season species such as *MAST-3B_XX_sp., MAST -8A_XX_sp., Polarella_glacialis,* and mixed diatoms (Figure 3.8).

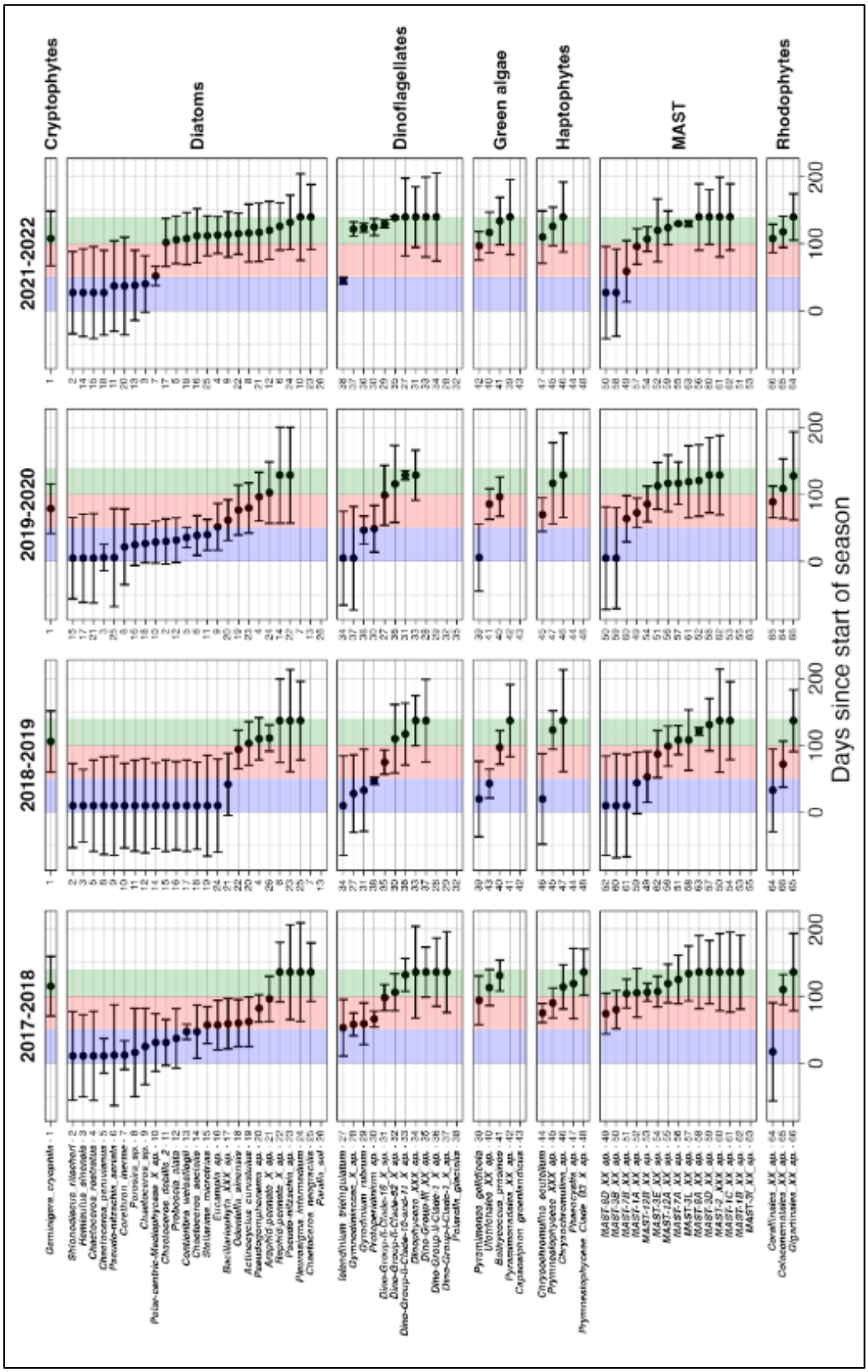

2017-2018
2018-2019
2019-2020
2021-2022
Cryptophytes
Diatoms
Dinoflagellates
Green algae
Haptophytes
MAST
Rhodophytes
Days since start of season
0
100
200

Figure 3.8: Variability in temporal occurrence (Day 0 = November 1st) of species level (primary Y-axis) and group level (secondary Y-axis) succession across four sampling seasons for only the stations located in the Middle region. Each point represents the day of the season when the relative proportion of reads was maximized for each species with error bars representing the standard deviation around the maximum (see Supplemental Figure 3.2). Species are given numbers (left Y-axis) relative to the order in the 2017-2018 season. Species along the Y-axis for each panel are ordered by the earliest to latest maximum and the order of species changes between subsequent sampling years (see numbers at he left of each panel). Shaded colors in the background represent "Early" (blue: 0-50 days), "Mid" (red: 50-100 days), and "Late" (green: 100-140 days) season ranges.

Succession at community level

There was marked spatial and temporal variability in cluster distributions (Figure 3.9, Supplemental Figure 3.3). To compared over a season, each sample was characterized based on the dominant cluster for that event, although patterns at individual stations did not always agree with the annual trends. Different clusters appeared and disappeared depending on month and year sampled. Additionally, some clusters seemed to dominate at certain locations and regions over other clusters.

During the first sampling season (2017-2018), Cluster 1 dominated across most sites and transitioned to Cluster 2, representing a shift from a mixed diatom community to a *Geminigera_cryophila* dominated community in the late season. In the next sampling season (2018-2019), we observed a similar trend: Cluster 1 was more prevalent across all sites at the beginning of the season, then the community shifted towards a mix of Cluster 3 and some of Cluster 2 in the late season, representing a dramatic shift from a mixed diatom community to dominance by a bloom-forming *Porosira_sp.* or flagellate *Geminigera_cryophila*. In the 2019-2020 sampling season, Cluster 3 was observed early before the community transitioned towards a dominance of Cluster 2. The COVID-19 Pandemic year had no samples (2020-2021), but in the following sampling season (2021-2022), the *Porosira_sp.*-dominated Cluster 3 again was

observed at the start of the season and shifted to the *Geminigera_cryophila*-dominated Cluster 3 by the end of the season, with some occurrence of flagellate-dominated Cluster 2, but in low enough occurrences to not characterize the sample as predominant (Supplemental Figure 3.3).

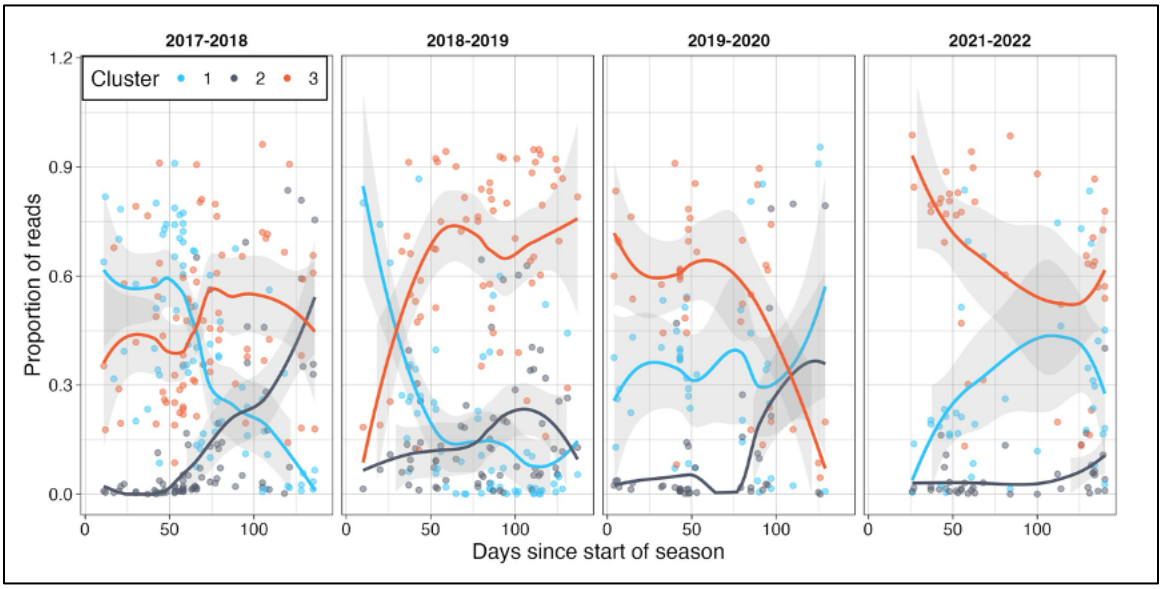


Figure 3.9: Temporal occurrence of clusters for all sites sampled. Each color point represents the dominant cluster found in that sample (see Figure 3.7). Points represent a sampling site. Cluster 1 (blue) represents a mixed diatom community, Cluster 2 (grey) is dominated by the cryptophyte *Geminigera_cryophila*. Cluster 3 (red) is dominated by the diatom *Porosira_sp.* Line is the LOESS fit and shaded gray areas representing the 95% confidence interval.

Discussion

By leveraging collaborative citizen science efforts aboard expedition vessels and focusing on nearshore regions, this study presents the first comprehensive attempt to establish seasonally resolved time series (November to March) along the WAP, sampling across four seasons, spanning the region from 61°S - 68°S, with the most frequently visited sites in the middle region (Figure 3.1). This research contributes a substantial molecular dataset (18S V9) to Antarctic phytoplankton ecology and microeukaryote genetics, demonstrating that cost-effective, user-friendly sample collection methods from routinely visited locations can support long-term

seasonal and interannual monitoring. Sampling efforts are influenced by opportunistic movements of expedition vessels, facilitated by improvements in ship strength, expansion of ice-free areas, and exploration of new sites (Bender et al., 2016). Utilizing ships of opportunity, we expanded the spatio-temporal sampling coverage of the nearshore WAP (Figure 3.1) to A) determine baseline information about which phytoplankton taxa (i.e., phyla, genus and species) were found throughout the 5-month austral summer over four seasons (Section I, Figures 3.2 and 3.3), B) examine the community diversity (Section II and III, Figures 3.4 and 3.5) and structure (Figures 3.6 and 3.7) at different scales, and C) detect patterns in succession at species, phyla, and community levels (Section IV, Figure 3.8 and 3.9, Supplemental Figure 3.3). Our findings using molecular approaches to species identification provide a more complete description of the nearshore phytoplankton taxa than previously possible. The data enabled novel insights into community structure and succession; however, these data are also limited by particular challenges and considerations discussed below.

Identifying Phytoplankton taxa and challenges in comparing studies

This study provided representatiosn of the surface phytoplankton taxa through next-generation sequencing analysis, with 228 unique phytoplankton ASVs identified. However, we found some key discrepancies between the observed versus the expected taxa that may result from differences in methods, and/or from the limited taxonomic database for polar protists. In our dataset, we assigned 285 ASVs to "other" eukaryotes that could potentially contain eukaryote protists. While previous studies have highlighted the importance of smaller-celled or rare phytoplankton taxa in nearshore environments (Clarke et al. 2008, Mascioni et al. 2021), we focused on micro-planktonic organisms that are able to be captured in a 20-μm net. Although the reads of nanoplanktonic (cells 2-20 μm) species could be underestimated compared to those of

micro-planktonic and chain-forming species, the presence and sometimes abundance of cells <20 µm such as *Geminigera_cryophila, Pyramimonas_sp*., MAST, and *Prymnesiophyte_sp*. indicates that net sampling can contribute to biodiversity studies in this region. The taxa that were highlighted in other studies as being the dominant species but not in this study could result from differences in sample processing, gene markers used, and sorting or size-fraction methods (Trefault et al., 2021). DeVargas et al. (2015) noted that about one-third of eukaryotic ribosomal diversity in their data set did not match any reference barcode in the extensive V9_PR2 database, and were attributed to the "unassigned" category (2.6% of their dataset) that included mostly rare and minute taxa. Polar microbes are particularly underrepresented in molecular databases, and taxonomic assignments given by the PR2 database can be incorrect; this could lead to species being misidentified entirely (Hamilton et al., 2021). Comparing differences in molecular taxonomic assignment to microscopic identification from the same FjordPhyto samples have also produced differing species lists (Mascioni et al. 2019, 2021, 2023). Three detailed examples are highlighted below, showing discrepancies in diatoms, cryptophytes, and dinoflagellates.

In this study, the diatom *Odontella_sinensis* (assigned by PR2) is most likely *Odontella weissflogii* as identified consistently by microscopy (Mascioni et al. 2019, 2023). Additionally, using microscopy, Mascioni found *Corethron_pennatum* to be one of the most abundant species in terms of cell counts (cells/mL). However, in this molecular dataset the unique ASV within the genus *Corethron* showed very low relative abundances, despite occurring in many sites along the peninsula (Figure 3.2). As *Corethron pennatum* is a large cell (20-40 um), it is easily retained by a 20-um mesh net, and sampling bias cannot account for its few appearances (Figures 3.2 and 3.3). Additionally, the *Corethron* ASVs were assigned by PR2 to the species *Corethron_inerme*, but it is well known that this is a misidentification: it should attributed to the well-known WAP

species *Corethron pennatum*. As another example, we observed widespread distribution and high ASV read counts dominated by *Porosira_sp.,* a diatom. The genus *Porosira* is extensively cited in studies of Antarctic coastal waters as *Porosira glacialis* (Al-Handal et al. 2022, Trefault et al. 2021). However, studies utilizing microscopy methods did not identify any *Porosira* species in these samples; they may have been grouped and classified as small centric diatoms (Mascioni et al., 2021, 2023).

The eight Cryptophyte ASVs found in this study were identified as *Geminigera_cryophila* according to PR2 assignment (Vaulot et al., 2022). However, the PR2 database has only one cultured cryptophyte, and previous studies have found evidence of novel WAP cryptophytes in the TPG clade VII (Hamilton et al., 2021), meaning that the microdiversity within the cryptophyte group is likely underrepresented.

Dinoflagellates are not as well studied in Antarctica (Liu et al., 2022). However, we were expecting to find some key dinoflagellate genera such as *Oxytoxum, Peridiniella, Prorocentrum,* and *Protoperidinium* that were previously identified in samples from this region (Mascioni et al., 2019, 2023; Hamilton et al., 2021); these genera were absent from our analyses. Most likely, the discrepancies can be attributed to the fact that databases are limited in 18S gene sequences from smaller, undescribed dinoflagellates in Antarctica (López-García et al., 2001, Gast et al., 2006). Liu et al. (2022), who targeted dinoflagellate communities and used dinoflagellate specific primers for taxonomic assignment to DINOREF database, found 742 ASVs categorized to the genus or species level, with dominant taxa (75% of dinoflagellate reads) associating to *Dinophyceae, Gyrodinium, Gymnodinium, Polarella*, and *Islandinium,* as found in this study.

In summary, despite advances in ‘omics technologies and growing use of eDNA for species-level detection (Deagle et al. 2017), the lack of consensus on universal primer standards

and misidentifications from database assignments for protist eukaryote taxa underscores the necessity of integrating molecular methods with complementary approaches like microscopy, pigment analysis, and flow cytometry (among others) for a more holistic understanding of phytoplankton communities.

Phytoplankton community structure and diversity patterns along the WAP

Diatoms are the dominant group of phytoplankton both throughout the region (Figure 3.2), and especially in the early months of the season (Figure 3.3). However, toward the later months of a given season, diatoms become less abundant and are replaced by other phytoplankton groups such as dinoflagellates, MASTs, and cryptophytes (Figure 3.3). The high presence of diatoms in the early season was expected, as diatoms are known to dominate phytoplankton biomass in the form of blooms (Egas et al. 2017). They are the major taxon fixing carbon in the WAP, independent of their abundance (Mascioni et al. 2021), and are a major food source for the dominant zooplankton (Clearly et al. 2018). They are also a major source of organic carbon sedimentation in this region (Lin et al. 2017).

Multiple studies have highlighted the role of cryptophyte blooms on the WAP continental shelf (e.g., Garibotti et al. 2005, Kozlowski 1995, Brown et al. 2021), and particularly in coastal environments (Schofield et al., 2017), including nearshore waters (Mendes et al. 2018) and fjords (Hamilton et al. 2021). The results of this study show that the cryptophytes occur from the South Shetland Islands to Marguerite Bay (61°00'S - 68°00'S), and occur in late season (Figures 3.8 and 3.9, Supplemental Figure 3.3). We found a strong presence of *Geminigera_cryophila* in the WAP, consistent with other findings (Brown et al. 2021, Trefault et al. 2021), and we detected *Geminigera* dominance late in the season, as reported by Mendes et al. (2019, 2021). This study shows higher relative read abundances of cryptophytes in the Middle and Southern regions,

consistent with Mascioni et al. (2019) who observed them in 93% of the samples from the central region. However, cryptophytes in that study generally had high abundances in early summer and contributed greatly to total phytoplankton abundance, particularly between the end of November and the first days of January. This pattern was seen in this study at Neko Harbour only in 2017-2018 (Figure 3.3). *Geminigera_cryophila* is strongly positively correlated with multiple species of MAST, haptophytes and rhodophytes that all showed high abundances in the later months of the season (Figure 3.3 and Figure 3.6). This period coincides with times of high glacial meltwater concentration in nearshore waters (Moline et al. 2004; Pan et al. 2019). Several studies have suggested that cryptophytes are replacing diatoms as the dominant taxon in the WAP (Costa et al. 2023, Ferreira et al. 2022). However, other phytoplankton groups such as dinoflagellates have also been found to dominate in late season when high meltwater was also reported to be prevalent (Lima et al. 2019).

In this dataset, absolute bloom intensities and species abundances could not be quantified by ASV read counts (as there were no mock community spiked into samples, as discussed in Lin et al. 2017). However, the ASV reads provide relative abundances, and we can assume that the species with higher relative abundances showing maxima over the season may be indicative of 'blooming' taxa – the same genera that found in the clusters (Figure 3.7). Taking the example of *Porosira_sp.,* a widespread genus that appears to thrive in the Shetlands, Northern and Middle sites (Figure 3.2), it is interesting to note that when this taxon's reads are high, it negatively correlates with species that are prevalent in the other clusters. If we assume that a rare species (i.e., not always present) has decreased opportunities to grow to high abundance in the environment, the widespread presence of *Porosira_sp.* (Cluster 3) would support the formation of Cluster 3 where/when this genus dominates. Based on the k-means correlation (Figure 3.6), if

Porosira_sp. were strongly negatively correlated with other taxa, it might signify that *Porosira_sp.* blooms more rapidly than other microplankton size-fraction species (>20 μm), ot somehow suppresses the growth of other species.

The structures of the clusters, as defined by the k-means analysis, indicated that the negative correlations between taxa were based on times of the season; the high read abundances of certain taxa were positively correlated with some taxa but uncorrelated with others, suggesting a consistency to community formation (Figure 3.6). The dominant taxa in these clusters broadly mirror the main communities reported in the Southern Ocean (Petrou et al. 2016), as well as findings reported by Garibotti et al. (2003) for nearshore and continental shelf WAP regions. Three unique assemblages identified by Mascioni et al. (2021) and Pan et al. (2021) in the central WAP region and were dominant in the fjords, differed somewhat from the dominant taxa found in the three clusters in this study (Figure 3.7). The drivers of these assemblages, or clusters, could be environmental (e.g., increased presence of glacial meltwater), or though biological. Lima et al. (2019) reported distinct sets of functional groups: 1) trophic traits (mixotrophy and heterotrophy), usually associated with flagellates, such as found in cluster 2 (Figure 3.7); 2) coloniality/chain formation, the presence of raphe and high surface/volume ratio, as expected in a mixed diatom community such as found in cluster 1; and 3) morphological traits including cells >35 μm in size, autotrophy and the presence of silica, as in the diatom species that dominate cluster 3 (the *Porosira sp.* cluster). To understand whether these clusters predictably arise due to environmental/biological drivers, will require considerably more detailed investigation than is possible with these data.

Phytoplankton diversity

One of our key expectations was that phytoplankton diversity (e.g., species richness, evenness, Shannon Index) would decline during the summer due to the dominance of the community by blooms, and increase again in the fall, independent of location (Staude et al. 2023; Hamilton et al. 2021). Our analyses largely support this expectation, showing a decline in diversity during bloom events in the summer, followed by an increase in diversity later in the season, particularly in the fall. This trend reflects the dynamic nature of the WAP ecosystems, where species richness fluctuates with seasonal productivity. This study captured a higher diversity in phytoplankton communities than previously reported from global studies (Figure 3.4, Figure 3.5). Ibarbalz et al. (2019) reported that the Shannon diversity index (S') reached ~2.0 at the poles for photosynthetic protist groups (e.g., diatom, dinoflagellates, haptophytes). In this study, we have captured overall higher diversities in mid-summer, peaking at 2.4-3.44. This is similar to findings by Hamilton et al. (2021), who showed the highest diversity of ASVs to be an S' of 3.49 in spring 2015 communities in Andvord Bay.

The diversity of the phytoplankton community is strongly correlated with the diversity of diatoms (Supplementary Figure 3.4). Diatoms dominate the diversity indices ($R^2 = 0.72$, $p < 2.2x10^{-16}$), while other groups, except for the cryptophytes, have significant but considerably lower significance (R^2 from 0.0015 to 0.13, $p < 2.2x10^{-16}$), with dinoflagellates the second-most influential group ($R^2 = 0.15$, $p < 2.2x10^{-16}$) (Supplementary Figure 3.4).

Geographically, we found the highest diversity to occur in the middle region where all three clusters were found (Figure 3.4). This could indicate a boundary or edge zone that supports higher diversity of species by combining species from two different domains (e.g., a northern geographic region and a southern one) (Mascioni et al. 2023). The middle region is where warm deep Antarctic Circumpolar Current (ACC) water from the south meets cold Weddell Sea deep

water from the north (Cook et al. 2016) in nearshore WAP waters. Previous studies found that a surface front in the Gerlache Strait east of Anvers Island had an influence on separating phytoplankton communities north and south of this mesoscale feature (Parra et al. 2017, Mascioni et al. 2023). These results underscore the important roles that oceanographic factors may play in driving phytoplankton diversity in the region.

Multiple levels of complexity within succession

Our results align with the classic Antarctic succession model: diatoms dominate early in the season, decrease in abundance toward the end, and are replaced by increasing populations of flagellates (Petrou et al. 2016). This study revealed a more detailed progression, beginning with a well-mixed diatom community, followed by mid-season blooms of diatoms like *Porosira sp.,* and ending with a shift to cryptophytes, dinoflagellates, MASTs, and pennate diatoms (Figure 3.8). This general pattern, observed from the South Shetland Islands to Marguerite Bay, has been reported in previous studies (Lin et al. 2017, Pan et al. 2021, Mascioni et al. 2023). However, many of these studies relied on short-term sampling or single-site observations, which may limit the robustness of their conclusions regarding the full-season structure of community succession.

Successional changes occur at both the species and community levels (Poorter et al. 2023). Groups and clusters in our study showed similar succession, for example, while diatoms occurred throughout the season, other groups like dinoflagellates and haptophytes appeared at different times, typically in mid- to late summer. However, within each group, the individual species that occurred at distinct times exhibited high interannual variability, not occurring within the same timing as the subsequent or prior season. This variability suggests that succession is more complex and influenced by multiple factors.

To understand these results, we could consider Clements' Super-Organism concept of succession which proposes that all species in an ecosystem work together to form a stable climax community, where stable environmental conditions allow species composition to remain unchanging over time (Clements 1936). While the high frequency of sampling in this study increases the likelihood of observing a stable climax community, the high interannual variability in species composition shows that detecting a consistent climax community of phytoplankton on the WAP may not be possible (Figure 3.9). In contrast, Gleason's Individualistic Species concept suggests that communities are a random assembly of species regulated by environmental factors, which leads to more unpredictable changes (Gleason 1926). This concept helps explain the temporal variability we observed in individual species (Figure 3.8), supporting the hypothesis that succession is shaped by complex interactions between environmental conditions and species responses.

Despite the variability observed, our dataset shows evidence of a continuity in community formation from one season to the next. For example, the 2018-2019 fall community dominated by *Porosira sp.* (Cluster 3) reappeared in the spring of 2019-2020, suggesting that the end-of-season community may have been "frozen" in the winter sea ice, seeding the spring community (McMinn et al. 2010). Additionally, a significant marine heatwave in 2020 (Latorre et al. 2023) likely contributed to the drastic shifts in species and cluster succession patterns observed between the 2019-2020 and 2021-2022 seasons (Figures 3.8 and 3.9, Supplemental Figure 3.3).

The observed variability in our data highlights the importance of considering both spatial and temporal scales when interpreting succession patterns. In the WAP, seasonal succession varies by location, including latitude and proximity to shore (Garibotti et al. 2003, Luria et al.

2014). Studies of succession that report high variability in species richness often reflect a spatial-scale dependence (Staude et al. 2023). While richness may decline at smaller scales due to the dominance of bloom-forming species, it tends to increase at larger scales as both early- and late-successional species coexist. As noted by Staude et al. (2023), space-for-time studies like Petrou et al. (2016) show decreased richness at the sea ice edge, while temporal data indicates increased richness at local scales.

Ultimately, community structure emerges from the balance between environmental changes, nutrient consumption, mortality, and species biotic interactions (Behrenfield et al. 2021). Given the patchy and highly variable nature of phytoplankton in the WAP, to understand the drivers of species succession patterns it will be essential to quantify these interacting factors and a range of spatio-temporal scales.

Conclusions

Based on the comprehensive seasonal sampling achieved through the FjordPhyto citizen science framework, this study provides insights into phytoplankton community structure and succession along the WAP. Our analyses of the large metagenomic data set identified key taxa and recurrent communities. The known misidentification of some taxa emphasizes the importance of integrating molecular techniques with traditional methods like microscopy and pigment analysis to overcome discrepancies in taxonomic identification. These efforts are essential for accurately characterizing phytoplankton diversity and succession at the species and community levels – particularly in polar regions, where data gaps are common.

The study supports the classic Antarctic succession model, in which diatoms dominate early in the season and are replaced by flagellates such as dinoflagellates, MASTs, and cryptophytes later on. However, we observed significant interannual variability in the species

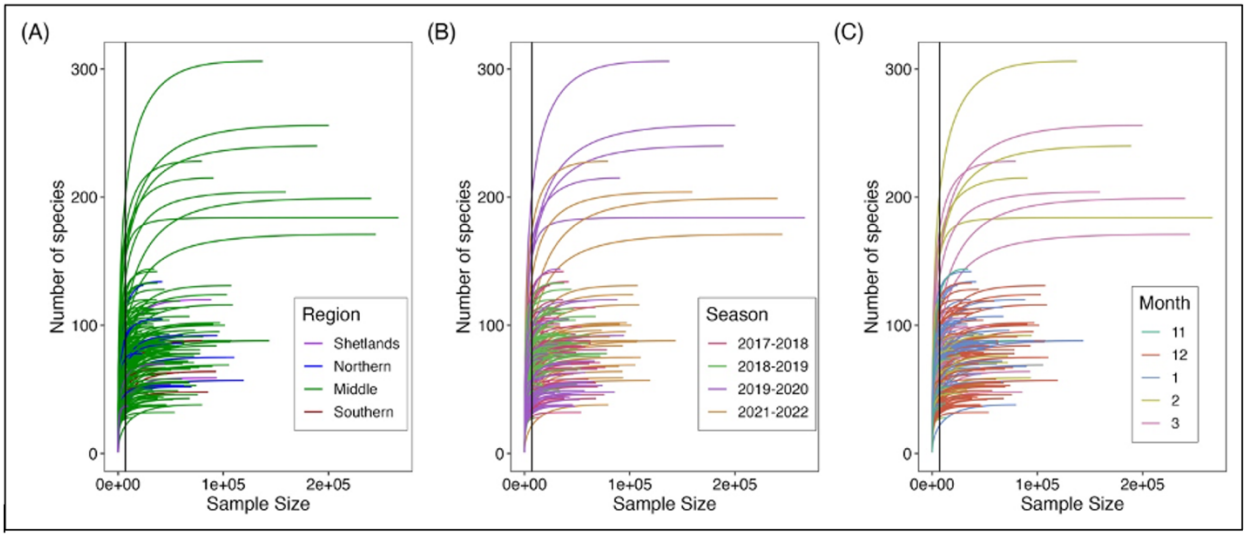
that occurred within the phytoplankton groups, suggesting that succession is influenced by a combination of environmental factors and species-specific traits. Our results underscore the complexity of succession in this region, pointing to the need for better understanding of environmental and trait-based drivers shaping community dynamics.

Our analyses revealed higher phytoplankton diversity in the WAP than previously reported, with peak diversity indices reaching 2.4–3.44 in mid-summer, primarily driven by diatom species. The highest diversity was found in the middle region of the WAP, where a thermal front exists at the intersection of cold Bransfield Strait waters from the Weddell Sea, and the southern intersection of warm Antarctic Circumpolar Current waters.

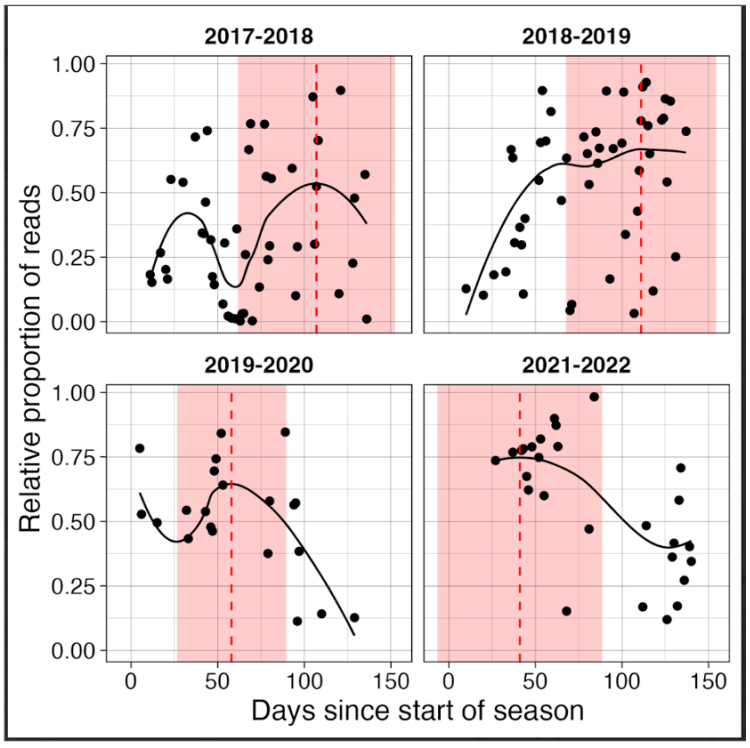
Our findings underscore the need for a more nuanced understanding of succession and co-occurring species, emphasizing high interannual variability and the influence of large-scale spatial and temporal factors. This highlights the complex and unpredictable nature of phytoplankton communities in the WAP and calls for continued monitoring and improved molecular tools to enhance our understanding of these ecosystems.

Acknowledgements

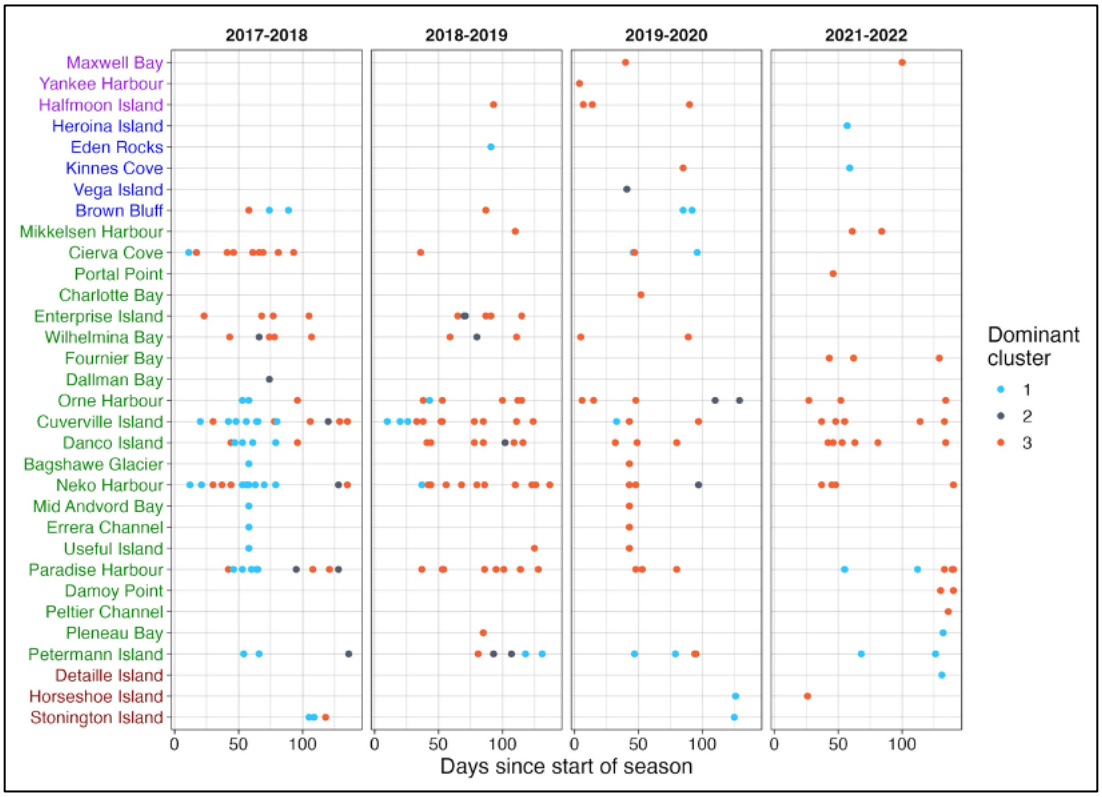
Chapter 3, in part is currently being prepared for submission for publication of the material. Cusick, A., Smith, A., Mascioni, M., Johnson, C., James, C. C., Pinpokintr, A., Zheng, H., Allen, A. E., Reynolds, R. A. Revealing Phytoplankton Succession and Community Dynamics: A Multi-Year Spatio-Temporal Analysis Along the Nearshore Antarctic Peninsula (61°S-68°S). The dissertation author was the primary researcher and author of this material.



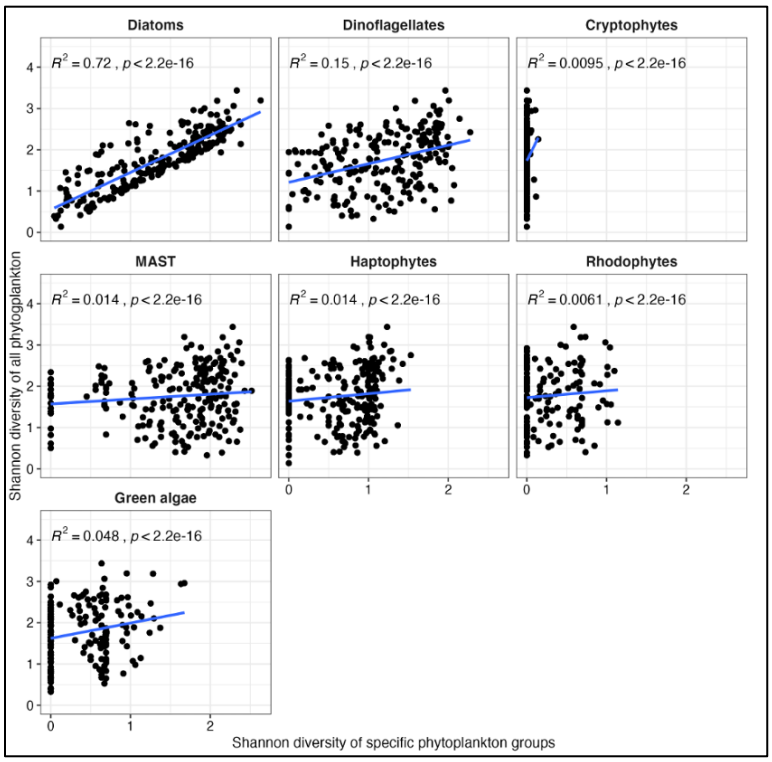
Supplementary Figure 3.1: Rarefaction curves for each sample colored by region (A), season (B), and month (C). Solid vertical line represents the library size cut off to which all samples were rarefied down to (7000 total reads).



Supplementary Figure 3.2: Relative proportion reads, each sample what the total reads was and show relative proportion per each genus. The black line is the loess regression fit; red vertical dotted line is max reads on a day in the season. Shaded red is standard deviation.



Supplementary Figure 3.3: Spatio-temporal occurrence of clusters for all sites sampled. Each color point represents the dominant cluster found in that sample (see Figure 3.7). Sampling stations (Y-axis) are colored by the associated region (red = Southern, green = Middle, blue = Northern, purple = Shetlands, see Figure 3.1). Cluster 1 (blue) represents a mixed diatom community, Cluster 2 (grey) is dominated by the cryptophyte *Geminigera_cryophila*. Cluster 3 (red) is dominated by the diatom *Porosira_sp.*



Supplementary Figure 3.4: Shannon diversity index of each phytoplankton group: Diatoms, Dinoflagellates, Cryptophytes, MAST, Haptophytes, Rhodophytes, and Green algae within each samples (X-axis) compared to the Shannon diversity index of all phytoplankton within each sample (Y-axis). Blue lines represent a linear fit with R^2 and *p* values reported. A Shannon diversity index value of zero means there was only one species of each phytoplankton group present in that sample.

Supplemental Table 3.1: Coordinate location of all sampled sites and their associated site number.

Site Number	Site Name	Latitude	Longitude
1	Stonington Island	-68.186	-66.997
2	Horseshoe Island	-67.836	-67.189
3	Detaille Island	-66.870	-66.790
4	Petermann Island	-65.173	-64.116
5	Pleneau Bay	-65.089	-64.046
6	Peltier Channel	-64.854	-63.495
7	Damoy Point	-64.820	-63.520
8	Paradise Harbour	-64.912	-62.862
9	Useful Island	-64.725	-62.858
10	Errera Channel	-64.810	-62.670
11	Mid Andvord Bay	-64.808	-62.672
12	Neko Harbour	-64.851	-62.542
13	Bagshawe Glacier	-64.860	-62.556
14	Danco Island	-64.746	-62.622
15	Cuverville Island	-64.671	-62.611
16	Orne Harbour	-64.638	-62.534
17	Dallman Bay	-64.650	-63.150
18	Fournier Bay	-64.533	-63.150
19	Wilhelmina Bay	-64.642	-62.160
20	Enterprise Island	-64.539	-61.980
21	Charlotte Bay	-64.583	-61.633
22	Portal Point	-64.502	-61.756
23	Cierva Cove	-64.156	-60.873
24	Mikkelsen Harbou	-63.896	-60.776
25	Brown Bluff	-63.520	-56.873
26	Vega Island	-63.833	-57.366
27	Kinnes Cove	-63.316	-56.466
28	Eden Rocks	-63.495	-55.702
29	Heroina Island	-63.423	-54.692
30	Halfmoon Island	-62.595	-59.974
31	Yankee Harbour	-62.524	-59.778
32	Maxwell Bay	-62.223	-58.859

Chapter 4 A metabarcoding-based analysis of seasonal and interannual patterns of phytoplankton in relation to temperature and salinity in coastal surface waters of the Western Antarctic Peninsula

Abstract

Despite decades of studies on phytoplankton community structure, gaps remain in our understanding of how environmental variability influences these organisms within the Antarctic Peninsula (62°- 68°S) – the third-fastest-warming region on Earth. Current studies predict that increasing sea surface temperatures and decreasing salinity from glacial meltwater input may drive shifts from diatom-dominated communities to communities of smaller flagellates and cryptophytes, impacting ecosystem dynamics such as trophic structure and biogeochemical cycles. Although many hypotheses have been proposed, it remains uncertain whether the observed trends are consistent across the peninsula, intraseasonal and interannually. This study employed a high-resolution temporal sampling approach through collaboration with the tour expedition industry via the FjordPhyto citizen science program. Sampling was conducted nearshore over five months (November through March) during the austral summers of 2017-2022. We utilized a molecular dataset (18S V9 rRNA) from 260 surface net (20-μm) tows to identify phytoplankton and analyzed relative read abundance in relation to sea surface temperature and salinity from 331 CTD profile casts. Overall, salinity decreased over the season while temperature peaked at mid-season. The highest ranges for both temperature and salinity occurred during the 2019-2020 season, whereas the most restricted conditions were observed in the 2021-2022 season. Phytoplankton samples revealed 65 taxa across seven major groups: Cryptophytes, Diatoms, Dinoflagellates, Green algae, Haptophytes, Marine Stramenopiles (MAST), and Rhodophytes. The community richness increased over the season and showed no significant relationship to salinity or temperature. Species richness within groups varied with the

time of season, temperature, and salinity conditions. Diatoms dominated the early season in colder, saltier waters. In contrast, a greater abundance of mixotrophic species, such as dinoflagellates, MAST, and haptophytes, were noted later in the season when waters became warmer and fresher. Species within the groups of dinoflagellates, green algae, haptophytes, MAST, and rhodophytes showed similar responses to timing (an increased richness in late season), temperature (an increased richness with high-temperature conditions) or salinity (an increased richness in low salinity), with high interannual variability. Diatom species showed highly variable responses to timing, temperature, and salinity. Notably, each season featured a persistent low-salinity assortment of species consisting of 4-6 diatom species and 1-2 dinoflagellate species, although the exact combination varied from year to year. The patterns of phytoplankton community composition and richness were generalized into a simple conceptual mandala relating species and groups to time of season, temperature, and salinity. These conceptual schematics can help visualize the environmental context of overall and interannual patterns. This first-of-its-kind high-resolution molecular dataset provides critical insights into fluctuations of phytoplankton groups and species with environmental drivers such as surface salinity and temperature, offering valuable information for predicting community structure response to future environmental changes.

Introduction

The western Antarctic Peninsula (WAP) is the third-fastest-warming region on Earth, and recent anthropogenic-induced changes in oceanographic conditions have led to dramatic shifts in coastal phytoplankton communities (Clarke et al. 2007, Montes-Hugo et al. 2009, Moreau et al. 2015). These changes have altered the phytoplankton species and community size structure (Barrera-Alba et al. 2012, 2015, Lange et al. 2014, Vanzan et al. 2015). The current literature

suggests that increased warming will promote reduced sea ice earlier than average in the season, resulting in more open-ocean-like, well-mixed conditions (Ferreira et al. 2020). However, increased glacial melt and freshening of the coastal region (Meredith et al. 2013, Cook et al. 2016) are expected to lead to increased surface stratification and consequent decreased nutrient fluxes, driving a continued decline in diatom diversity and increased growth of smaller-sized bloom-forming species (e.g., small diatoms, *Phaeocystis*, cryptophytes; Mascioni et al. 2019, Antoni et al. 2020, Ferreira et al. 2023, Moline et al. 2004, Mendes et al. 2013). Researchers have reported a reduction in diatoms (Mendes et al. 2023) and declines in biomass in the southern region of the WAP (Kim et al. 2018) and decline in community diversity over the last half of the previous century (Lin et al. 2021), but the northern region showed increase in biomass in the South Shetland Islands. A recent study contradicts these findings, indicating the last 25 years have seen an increase in biomass and duration of blooms over the peninsula (Ferreira et al. 2024).

Decades of sampling efforts in the WAP have demonstrated that fjord environments provide nutrient-rich conditions beneficial for phytoplankton blooms (Kopczynska 1981, Kim et al. 2018), resulting in greater nearshore primary production than in offshore areas (Holm-Hansen and Mitchell 1991, Vernet et al. 2008). The timing of phytoplankton succession patterns and the structures of associated species drive important ecosystem dynamics, including trophic cascades, biogeochemical cycles, and carbon sequestration (Petrou et al. 2016, Turner et al. 2024). All phytoplankton have unique optimal growth conditions driven by preferences for environmental variables, such as sea surface temperature, salinity, light, and nutrient concentrations (Petrou et al. 2016). These preferences influence competition among species and set the conditions controlling species spatial and temporal distributions. At the start of the austral growth summer

season (October-November), the return of sunlight increases sea surface temperature and drives sea ice retreat, activating dormant diatom communities (Petrou et al. 2016). Along with the diatoms, an assemblage of mixed species of phytoplankton persists throughout the austral growing season and into austral winter (Pan et al. 2021, 2023, Mascioni et al. 2023). Within the WAP, periods dominated by nanophytoplankton—chlorophytes, small dinoflagellates, and prasinophytes—were observed to be associated with glacial meltwater stratification during summer, with decreased nutrients were decreased (Buma et al. 1992, Moline et al. 2004, Kozlowski et al. 2011, Mendes et al. 2012). In contrast, other studies measure iron-rich glacial meltwater which is found to directly enhance the abundances of diatoms and cryptophytes, both over the shelf and within fjords (Moline and Prézelin, 1996, Annett et al. 2015, Dierssen et al. 2002a; Pan et al. 2019, Buck et al. 2010, Mendes et al. 2013; Rozema et al. 2017). Further contradictions within the literature state well-mixed colder waters are dominated by diatoms and the haptophyte *Phaeocystis antarctica* (Arrigo et al. 1999, Abele et al. 2017), while other studies found diatoms associated with stratified, warmer, saltier waters (Mendes et al. 2013; Höfer et al. 2019, Leeuwe et al. 2020), suggesting that these preferences can be genus, species, or even population specific.

Phytoplankton community responses to environmental change are highly variable, and phytoplankton taxonomic groups are not affected uniformly (Carmeno and Falkowski 2009, Pan et al. 2021, Ferreira et al. 2020). Unfortunately, the extreme conditions of the Antarctic region make it difficult to set up continuous or consistent sampling programs; the consequent lack of appropriate data contributes to the varied and contrasting results found in the literature (Chapter 1). Through the FjordPhyto citizen science sampling framework, formed as a collaboration with the tour expedition industry to cover a large spatio-temporal region of the WAP (Cusick et al.

2020, Chapter 2), this study aims to broaden our understanding of the seasonal succession patterns and the environmental drivers that influence phytoplankton community dynamics. Here we analyze the phytoplankton community structure over time and quantify species- and group-level relationships to sea-surface temperature and sea-surface salinity conditions. The following hypotheses (H) were derived from the current literature, fit into two overarching themes, and are used to guide these analyses:

Theme 1: How does species richness at both the community and group level change over time and as salinity decreases and temperature increases?

a) Previous literature has suggested that community richness decreases over time due to decreasing diatom richness.
    i. H1- Phytoplankton community richness decreases over time and with increasing temperature (Lin et al. 2021) and decreasing salinity.
    ii. H2 – Diatom richness decreases over time with increasing temperature, and reduced salinity (Montes-Hugo et al. 2009, Mendes et al. 2013, 2018).
    iii. H3 - Flagellate richness increases as salinity decreases (Montes-Hugo et al. 2009, Mendes et al. 2013, 2018).
    iv. H4 - Cryptophytes occur in greater proportion later in the season (Moline and Prézelin 1996, Kim et al. 2016).

Theme 2: Do individual groups and the species within groups show consistent relationships to salinity and temperature over time?

b) Previous literature has suggested that responses to salinity and temperature is group- (Garibotti et al. 2005, Kim et al. 2016, Pan et al. 2021, Mascioni et al. 2021, 2023) and species-specific (Hernando et al. 2015, Costa et al 2020).

v. H5 - Diatoms (and species within), peak in abundance in low temperatures and high salinity (Petrou et al. 2011, Sackett et al. 2013, Mascioni et al. 2023).

vi. H6 - Cryptophyte abundance increases in high temperature (Lin et al. 2021, Moline et al. 2004, Mendes et al. 2018) and low salinity (Moline and Prézelin, 1996, Moline et al. 2004, Schofield et al. 2017, Costa et al. 2023).

Methods

Along the western Antarctic Peninsula 33 sites were sampled during expedition cruises that ranged from the South Shetland Islands (61°00'-63°37'S, 53°83'–62°83'W) and the northern, western, and southern nearshore coasts of the Antarctic Peninsula (63°24′S to 68°11′S) (Figure 4.1). In collaboration with expedition vessels and travelers through the FjordPhyto citizen science program (Cusick et al. 2020), phytoplankton samples and CTD profiles were collected between November and March over four seasons (2017-2022), excluding the 2020-2021 COVID-19 season. Samples were collected under constraints of IAATO expedition vessel operations and typically at a sea state level 2 or below. Due to complications of operations prior to and after the global pandemic, only limited data were collected during the late season 2019-2020 and early season 2021-2022. Below, we outline the methods used for collecting, processing, and analyzing phytoplankton communities and the environmental measurements, as well as the methods for quantifying species maximum abundance for sea surface temperature and salinity. All analyses were performed in RStudio (RCore Team, 2015) and data visualizations were created using ggplot2. To ensure transparency and reproducibility, relevant data and code for analysis and visualization are available on GitHub (to be indicated at time of publication).

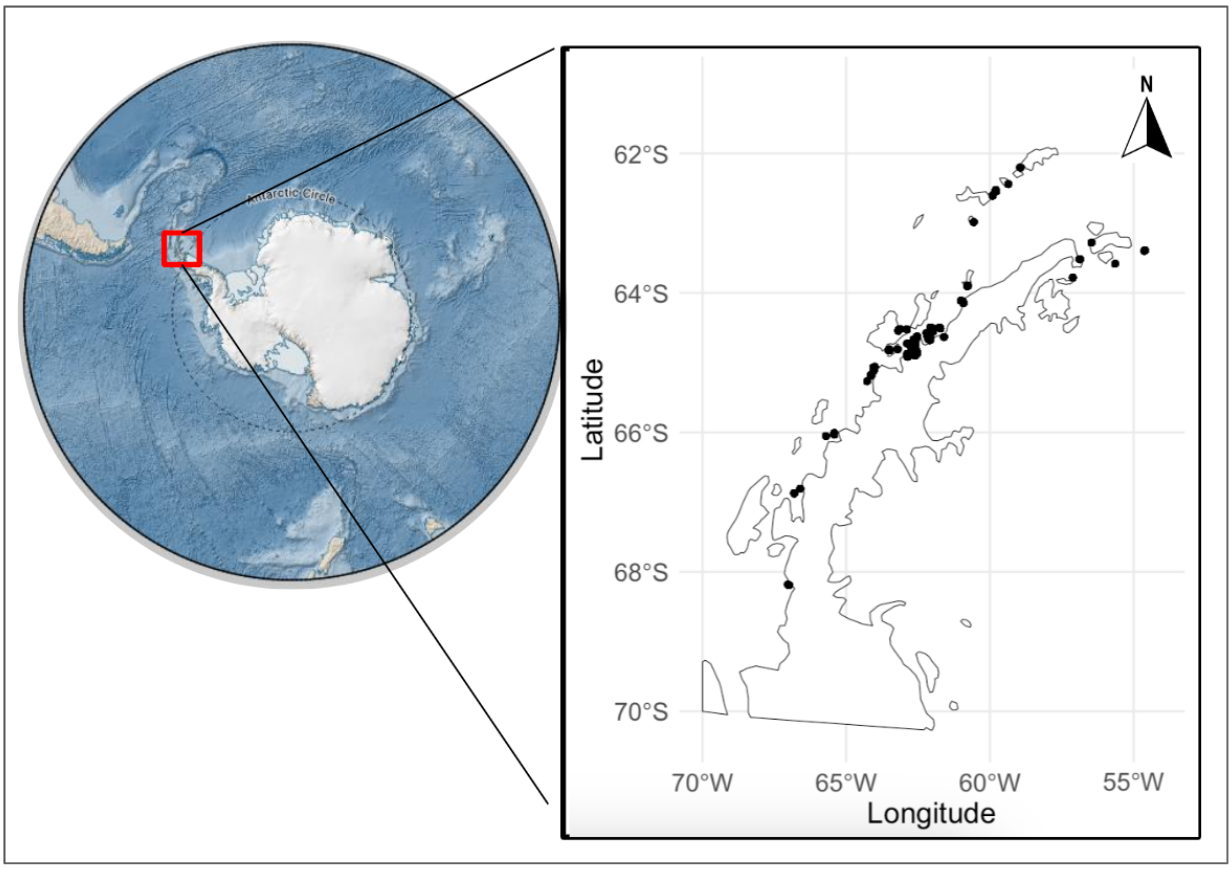


Figure 4.1: Location of sites visited along the WAP where samples were collected using FjordPhyto protocols, in collaboration with IAATO vessels (black points).

Phytoplankton sample collection and data processing

At each site, phytoplankton were collected from the surface by towing a 20-µm phytoplankton net (bridle mouth diameter 30 cm, SeaGear Corps) for 10 minutes. The sample was then filtered through a pre-sterile 0.2 µm - 47 mm filter (PALL Supor Hydrophilic polyethersulfone), preserved in RNAlater (Invitrogen), and frozen at -20°C onboard the ship until transport to Scripps Institution of Oceanography at the University of California San Diego in La Jolla, California, USA, where the samples were then frozen at -80°C until processing.

DNA extraction, PCR amplification, sequencing

All 260 samples from 2017-2022 were processed at the J. Craig Venter Institute (JCVI), San Diego, California, USA and prepared for metabarcoding sequencing. The RNAlater was removed before extracting DNA with the NucleoMag Plant Kit (Macherey-Nagel, Düren, Germany) using the epMotion 5075TMX robot (Eppendorf, Hamburg, Germany). DNA quantification was determined using a Nanodrop spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) and amplified using the TruFi DNA Polymerase PCR kit (Azura, Raynham, MA, USA) targeting the 18S hyper variable V9 region of the nuclear 18S rDNA gene (hereafter 18S V9). For 18S V9, a 25µL volume reaction was performed using the 1389 F (TTGTACACACCGCCC) and 1510 R (CCTTCYGCAGGTTCACCTAC) primer set (Amaral-Zettler et al., 2009). PCR was run with an initial denaturing step at 95°C for 1 min followed by 30 cycles of 95°C for 15 sec, 56°C for 15 sec, and 72°C for 30 sec. DNA quality was assessed on agarose gel to confirm amplification. The Zymo Research DNA cleanup and Concentrator Kit and OneStep PCR inhibitor Removal Kit were used to purify PCR product, quantified using the Invitrogen Quant-iT PicoGreen dsDNA Assay kit (Invitrogen, USA). Samples were pooled (20-ng each), purified with the 0.8x AMPure XP bead purification, evaluated on an Agilent 2200 TapeStation, and quantified with Qubit HS dsDNA. Samples were sequenced at the Institute for Genomics Medicine (IGM) at the Genomics Center of the University of California, San Diego using Illumina MiSeq lanes (PE150, 2×150bp, for 18S) with a 15% PhiX spike-in. Because the volume of water filtered during the net tow in Antarctica was undetermined, custom mock community spike-ins (Lin et al. 2019) were not included in this study.

Bioinformatics analysis

Sequence data was processed using QIIME2 (v2022.11) and DADA2 following protocols from Allen Lab GitHub (https://github.com/allenlab/QIIME2_18Sv9_ASV_protocol#protocol-

for-processing-18sv9-sequences-in-qiime2). Amplicon sequence variants (ASVs) were identified using the naïve Bayesian classifier method (Wang et al. 2007, Callahan et al. 2016) and taxonomic assignment was given using the Protist Ribosomal Reference database (PR2, v4.14.0) (Guillou et al. 2013, Vaulot et al. 2022) for 18S V9 amplicons with 97.0% confidence interval cut off. Samples were rarefied using the vegan package in R to ensure a minimum library size of 7,000 reads (McMurdie and Holmes 2014, Gloor et al. 2016, Cameron et al. 2020). ASV reads are reported as relative abundances and do not reflect the direct cell count numbers (Lin et al. 2017, 2019).

Determination of ASVs into Phytoplankton groups

To focus on the 'phytoplankton' community, we categorized 105 ASVs into seven distinct phytoplankton groups that contain photosynthetic taxa based on most common reported in literature (Hamilton et al. 2021, Pan et al. 2020, Mascioni et al. 2021, Mendes et al. 2018, Abele 2017, Trefault et al. 2021), herein referred to as: cryptophytes (Division Cryptophyta), diatoms (Class Bacillariophyta), dinoflagellates (Division Dinoflagellata), green algae (Division Chlorophyta and Prasinodermophyta), marine stramenopiles (Class MAST), haptophytes (Division Haptophyta), and rhodophytes (Division Rhodophyta). The observed phytoplankton taxa (n = 65) cover organismal sizes ranging from <3 mm (e.g., MAST) to ~200 mm (e.g., diatom) despite using a 20-µm net tow. Diversity in the context of this study refers to number of species (i.e., richness). Many of the samples had the PR2 database-identified diatom *Porosira_glacialis*, whose read abundance was highest in the samples, swamping the relative read abundances of all other taxa. For this reason, in some visualizations *Porosira* was removed to better reveal the patterns of the relatively less-abundant species.

Physical water column data collection and processing

Data from 327 distinct casts of a profiler measuring conductivity, temperature, and depth (CTD) were taken using the manufacturer-calibrated SonTek CastAway®-CTD or RBR Concerto with Turner Chl-*a* Fluorometer (added in 2021). The instruments were deployed covering depth profiles from 0 to 100 meters of the water column whenever possible. Processing and cleaning steps included merging the individual files containing 65956 individual datapoints. Only the downcasts and rows with non-negative values of depth, pressure, and salinity were used leaving a remaining 289 casts. As such, and since phytoplankton samples were only collected at the surface, we used only the average sea surface temperature (SST) and sea surface salinity (SSS) values in the top 2 meters from each cast to characterize the hydrographic properties of surface layer for our subsequent analysis.

Determining environmental conditions at the phytoplankton taxon's maximum

Sequences from 160 surface net-collected samples had corresponding matches to valid CTD casts were analyzed further for this study. In general, high relative read abundances from each identified organism imply that those phytoplankton species are in greater absolute abundance in that sample (Shelton et al. 2022). Therefore, we made the critical assumption that the observed SST and SSS values associated with the sample in which a species exhibited its maximum proportion of reads indicated that the species preferred those conditions (Supplementary Figure 4.1).

Given the above assumptions, we quantified each species by first adding up the rarefied read counts for all ASVs of each species and divided the sums by 7,000 (i.e. the maximum library size or total number of reads within each sample). This transformed raw reads into a normalized proportion and allowed us to compare across samples. We then found the samples in which each species exhibited its maximum proportion of reads. The observed SST and SSS

values associated with those samples were assumed to be a favorable environmental condition. To allow comparison within seasons and interannually, we converted the date that each sample was collected to a linear measure of days since November 1 (DsN1) (i.e., the start of the austral summer season sampling).

To identify potential communities emerging in each SST and SSS condition - indicated by species at peak read abundance - each variable was divided into three bins:

SST: Low (<0°C), Medium (0-1°C), High (>1°C)

SSS: Low (<32.5 PSU), Medium (32.5-33.5 PSU), High (>33.5 PSU)

DsN1: Early (0-50 days), Mid (50-100 days), Late (>100 days).

For each species, we used the above assumptions to identify which bin encapsulated that taxon's environmental condition favorable for peak relative read abundance both across all samples and within each of the four sampling seasons (2017-2018, 2018-2019, 2020-2021, and 2021-2022).

Results

Oceanographic Surface Characteristics

Overall, SSS freshened over the season, while SST warmed, peaking at mid-season, then cooled again by the late season (Figure 4.2 in black). The SSS ranged between 28.78 PSU and 34.42 PSU with a mean of 33.5 ± 0.59 PSU (Figure 4.2 in red). The CTD casts that matched with 18S data (n = 160) included 12 samples in low SSS, 53 in mid and 95 in high SSS. The highest SSS was recorded in the 2017-2018 sampling season and the lowest during the 2019-2020 year.

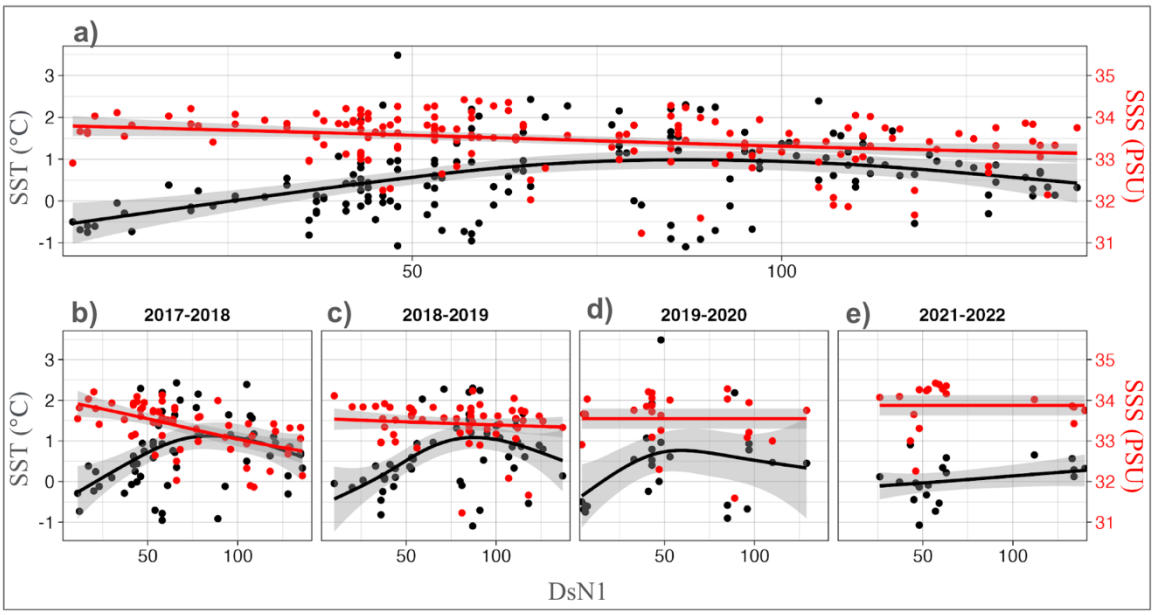


Figure 4.2: Environmental conditions showing SST (left Y-axis) and SSS (right Y-axis, red), over the austral season (DsN1) for overall dataset (a) and interannual for all seasons sampled (b - e). Generalized additive model trendlines were added for visualization purposes where shaded grey depicts the standard deviation.

The SST ranged from -1.33 °C to 3.83 °C with a mean of 0.744 °C (Figure 4.2). The CTD casts that matched with 18S data included 42 in low SST, 72 in mid, and 46 in high. The highest SST was recorded in 2019-2020 sampling season and the lowest during the 2018-2019 year.

The CTD casts that matched with 18S data over the season included 55 in early, 68 in mid, and 37 in late season.

SSS and SST showed strong interannual variability among the seasons, however there was a consistent annual seasonal cycle of warming and freshening, while on average over all the years there was an increase in SSS and decrease in SST. In the 2017-2018 season, the SSS was on average 33.3 ± 0.58 PSU and SST was on average 0.74 ± 0.87 °C. During the 2018 – 2019 season, the SSS was on average 33.4 ± 0.54 PSU with SST on average at 0.71 °C ± 0.83 °C. During the 2019-2022 season, the SSS on average was at 33.5 PSU ± 0.63 with the SST on

average at 0.43 °C ±1.05 °C. During the 2021-2022 season, SSS on average was 33.9 ± 0.56 PSU and SST on average was 0.04 ± 0.50 °C (Figure 4.2).

Community Structure and Relationship to Environmental Conditions

To test the hypothesis that time (i.e., DsN1), SST, and SSS influence community richness and the richness of each phytoplankton group, we ran 24 individual linear models testing the significance of each relationship (Figure 4.3, Supplementary Figure 4.2, and Table 4.1). At the community level, phytoplankton species richness increased significantly over time ($R2 = 0.12$, $p < 0.01$) but there was no significant effect of changing SSS or SST on community richness (Figure 4.3a-c and Supplementary Figure 4.1a-c). We found that the richness of all phytoplankton groups was significantly correlated with time (DsN1), however diatoms were significantly negatively correlated ($R2 = 0.16$, $p < 0.01$), with decreasing richness over time (Figure 4.3 and Supplementary Figure 4.2). As SSS increased, the richness of diatom species increased significantly ($R2 = 0.1$, $p < 0.01$), and the richness of haptophytes ($R2 = 0.05$, $p < 0.01$) and MAST ($R2 = 0.06$, $p < 0.01$) decreased significantly. There was no significant effect of increasing SSS on the richness of cryptophytes, dinoflagellates, green algae, or rhodophytes (Figure 4.3 and Supplementary Figure 4.2). As SST increased, diatom richness decreased significantly ($R2 = 0.12$, $p < 0.01$), and haptophyte ($R2 = 0.12$, $p < 0.01$) and MAST ($R2 = 0.12$, $p < 0.01$) richness increased significantly. However, there was no effect of SSS on the remaining phytoplankton groups (Figure 4.3 and Supplementary Figure 4.1). $R2 = 0.12$, $p < 0.01$).

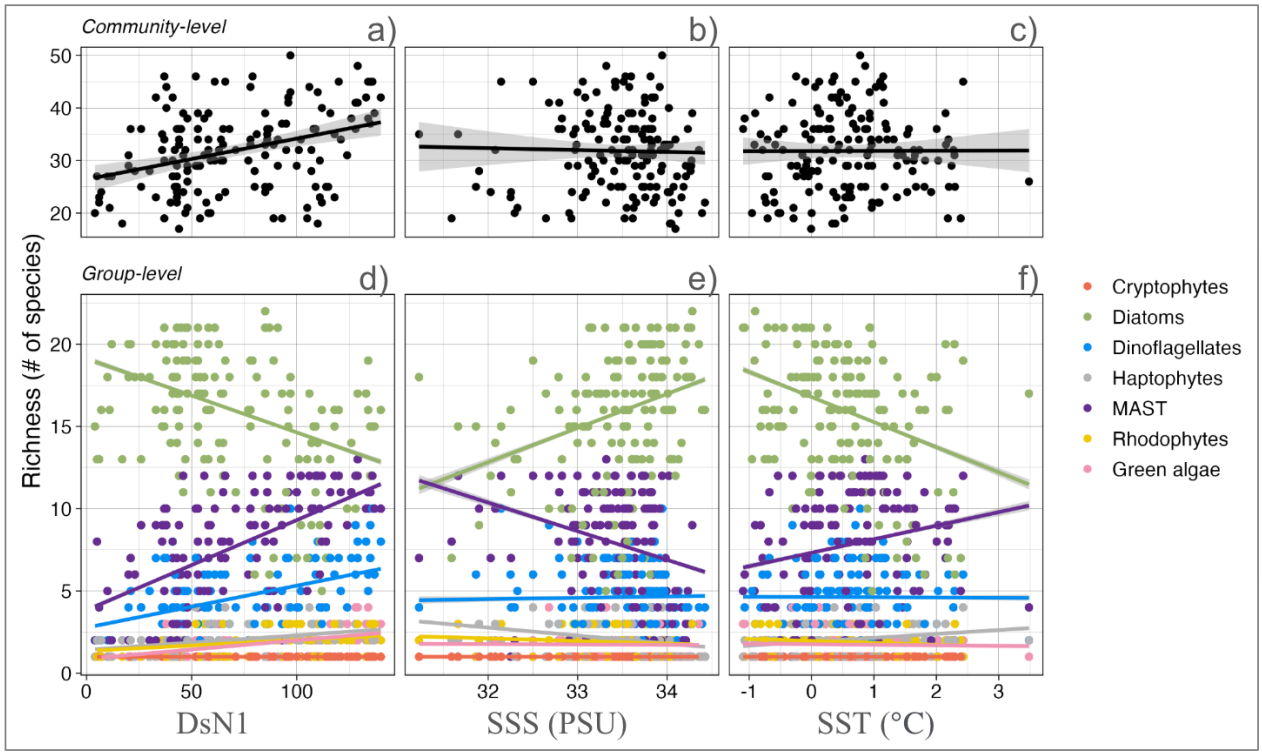


Figure 4.3: Species richness (number (#) of species) for the entire phytoplankton community (top row) across all years as a function of DsN1 (a), SSS (PSU; b), and SST (°C; c). Black lines are a regression line built using a linear model with 95% confidence interval shown in grey shading. Species richness (number of species) for each phytoplankton group (bottom row) as a function of DsN1 (d), SSS (PSU; e,) and SST (°C; f). The general linear model is used for the regression trend line and shows 95% confidence interval in grey shading. The R-squared determines the goodness of fit and the p-value determines statistical significance (shown in Table 4.1). Most all trends are statistically significant with very strong to strong evidence against the null hypothesis for groups and DsN1, SSS and SST (Table 4.1).

Table 4.1: Summary of results from linear models describing the effect of time (DsN1), SSS (PSU), and SST (°C) on species richness (# species) for each phytoplankton group. Linear models were run independently with reported R-squared (the proportion of variation in richness explained by the independent variable) and the p-value (measure of significance). Significant results reject the null hypothesis that the independent variable (DsN1, SST, SSS) has no effect on the richness of phytoplankton communities, and the orange shading highlights the phytoplankton groups that were not significant for the given variable.

Group	r.squared	adj.r.squared	p-value	rejects the null hypothesis
Days since Nov. 1				
Cryptophytes	0.497	0.490	5.810E-13	very strong
Diatoms	0.159	0.153	6.100E-07	very strong
Dinoflagellates	0.167	0.161	8.450E-07	very strong
Haptophytes	0.150	0.142	2.750E-05	very strong
MAST	0.259	0.253	2.170E-10	very strong
Rhodophytes	0.082	0.071	8.218E-03	strong
Green algae	0.187	0.179	7.060E-06	very strong
Sea Surface Salinity (PSU)				
Cryptophytes	0.499	0.496	1.520E-23	very strong
Diatoms	0.099	0.094	4.850E-05	very strong
Dinoflagellates	0.000	-0.006	8.355E-01	does not reject
Haptophytes	0.11	0.104	2.760E-05	very strong
MAST	0.075	0.069	5.831E-04	very strong
Rhodophytes	0.013	0.003	2.643E-01	none
Green algae	0.000	-0.008	9.462E-01	none
Sea Surface Temperature (°C)				
Cryptophytes	0.502	0.499	1.050E-23	very strong
Diatoms	0.127	0.122	3.680E-06	very strong
Dinoflagellates	0.000	-0.006	9.690E-01	does not reject
Haptophytes	0.066	0.06	1.361E-03	very strong
MAST	0.052	0.045	4.580E-03	strong
Rhodophytes	0.006	-0.004	4.445E-01	does not reject
Green algae	0.001	-0.007	7.733E-01	does not reject

To explore species responses to DsN1, SSS, and SST, we calculated the Pearson correlation coefficient from the relationship between reads and the environmental variables for each species in each phytoplankton group (Figure 4.4). We found that most species within each group either had no significant relationship or followed the relationship we saw at the group level (i.e., Figure 4.3). However, there were five species of diatom that showed significant positive relationships to increasing DsN1 (prevalence in the Late season): *Chaetoceros_neogracilis, Pleurosigma_intermedium, Pseudo-nitzschia_sp., Pseudo-nitzschia_seriata, Raphid-pennate_X_sp.* (Figure 4.4). This was contrary to the group-level trend of a significant negative relationship to increasing DsN1 (Figure 4.3).

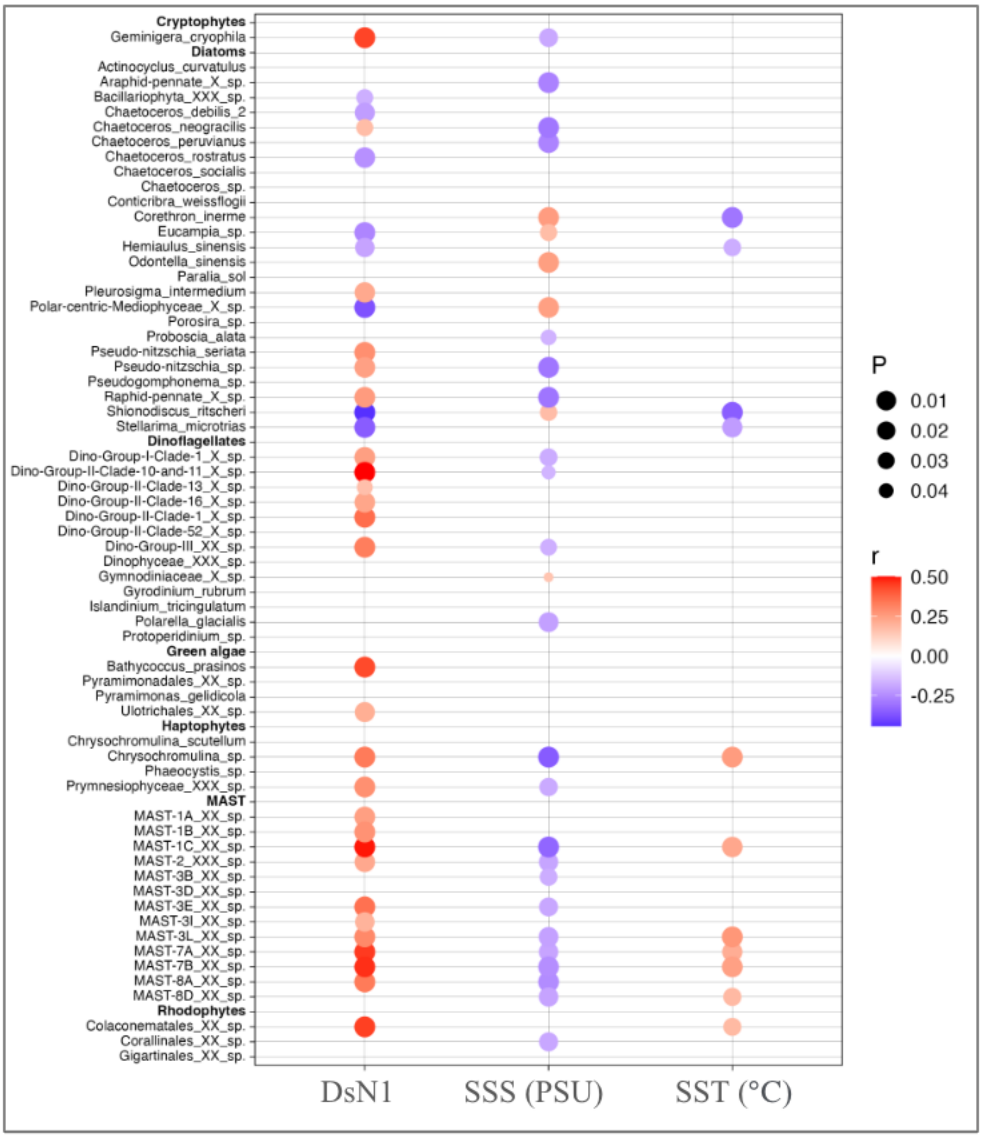


Figure 4.4: The Pearson correlation of species identified to various conditions. The color scale shows strong negative correlations (blue) and strong positive correlations (red) for DsN1, SSS (PSU) and SST (°C). The darker the red represents late, high SSS, high SST, the darker the blue represents early, low SSS, low SST. P-values are depicted by size of point. All points shown have statistically significant values ($p < 0.05$), but the size of the points provide more information. Species without a colored point indicate no statistically significant correlation to the three variables.

In summary, community and group richness increased significantly with DsN1 but the responses at the species level were highly variable and were sometimes the opposite. Although SST and SSS do not significantly affect the community richness (Figure 4.3), some

phytoplankton groups like diatoms, haptophytes, and MAST, and their species, showed significant relationships.

Variability in Succession of Phytoplankton within Surface Waters

Overall, the surface phytoplankton community showed a prevalence of diatoms in the early months. In 2017-2018, in the early DsN1, we found that diatoms made up an average of 93.4% of the reads across all samples, and all other groups were <5% of the total reads. Samples collected in the mid-DsN1 comprised diatoms (81.4%), dinoflagellates (7.3%) and MAST (7.0%); all others were less than <5%. Finally, in late DsN1, samples showed the highest relative read abundances for diatoms (39.9%), cryptophytes (22.8%), MAST (16.8%), dinoflagellates (11.6%), haptophytes (7.0%); all the other groups were <5% (Figure 4.5).

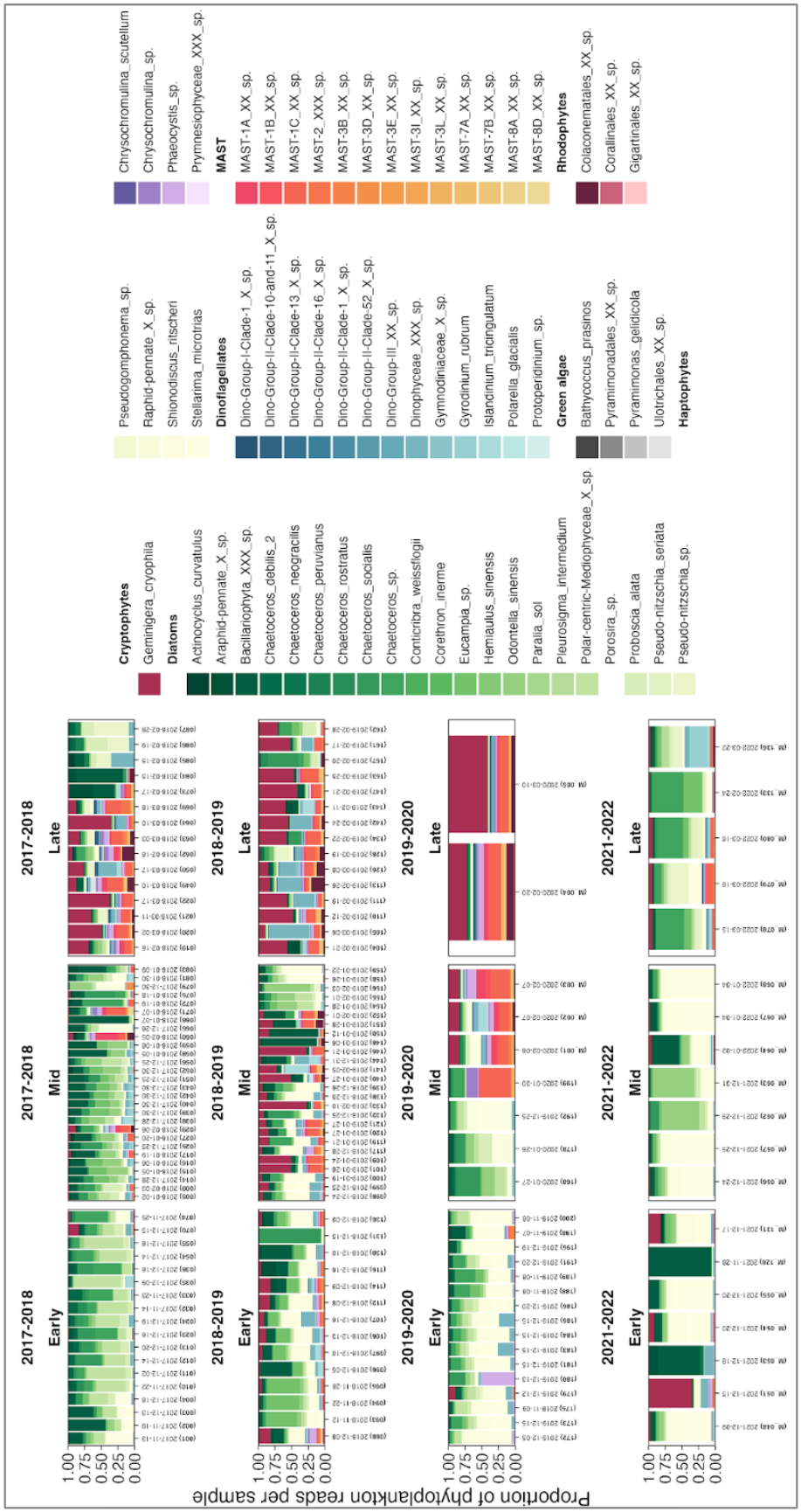

Cryptophytes
Geminigera_cryophila
Diatoms
Actinocyclus_curvatulus
Araphid-pennate_X_sp.
Bacillariophyta_XXX_sp.
Chaetoceros_debilis_2
Chaetoceros_neogracilis
Chaetoceros_peruvianus
Chaetoceros_rostratus
Chaetoceros_socialis
Chaetoceros_sp.
Conticribra_weissflogii
Corethron_inerme
Eucampia_sp.
Hemiaulus_sinensis
Odontella_sinensis
Paralia_sol
Pleurosigma_intermedium
Polar-centric-Mediophyceae_X_sp.
Porosira_sp.
Proboscia_alata
Pseudo-nitzschia_seriata
Pseudo-nitzschia_sp.
Pseudogomphonema_sp.
Raphid-pennate_X_sp.
Shionodiscus_ritscheri
Stellarima_microtrias
Dinoflagellates
Dino-Group-I-Clade-1_X_sp.
Dino-Group-II-Clade-10-and-11_X_sp.
Dino-Group-II-Clade-13_X_sp.
Dino-Group-II-Clade-16_X_sp.
Dino-Group-II-Clade-1_X_sp.
Dino-Group-II-Clade-52_X_sp.
Dino-Group-III_XX_sp.
Dinophyceae_XXX_sp.
Gymnodiniaceae_X_sp.
Gyrodinium_rubrum
Islandinium_tricingulatum
Polarella_glacialis
Protoperidinium_sp.
Green algae
Bathycoccus_prasinos
Pyramimonadales_XX_sp.
Pyramimonas_gelidicola
Ulotrichales_XX_sp.
Haptophytes
Chrysochromulina_scutellum
Chrysochromulina_sp.
Phaeocystis_sp.
Prymnesiophyceae_XXX_sp.
MAST
MAST-1A_XX_sp.
MAST-1B_XX_sp.
MAST-1C_XX_sp.
MAST-2_XXX_sp.
MAST-3B_XX_sp.
MAST-3D_XX_sp.
MAST-3E_XX_sp.
MAST-3I_XX_sp.
MAST-3L_XX_sp.
MAST-7A_XX_sp.
MAST-7B_XX_sp.
MAST-8A_XX_sp.
MAST-8D_XX_sp.
Rhodophytes
Colaconematales_XX_sp.
Corallinales_XX_sp.
Gigartinales_XX_sp.
2017-2018
2018-2019
2019-2020
2021-2022
Early
Mid
Late
Proportion of phytoplankton reads per sample
1.00
0.75
0.50
0.25
0.00

Figure 4.5: Samples from net-collected DNA (18S V9) communities over the season. Each season is divided into early, mid, and late time frames and taxa are represented as proportion of relative reads within phytoplankton identified per sample. Note that although the diatom *Porosira_glacialis* was detected with the PR2 database, due to overwhelmingly high read abundance, it was removed for visualizations. Phytoplankton species are all colored various shades of one representative color per group (e.g., all diatom species are shades of green).

2018-2019: The early DsN1 comprised diatoms (76.2%), dinoflagellates (7.5%), cryptophytes (7.3%), and all other groups were <5%. In the mid DsN1, diatoms (58.1%), cryptophytes (18.4%), MAST (10.7%), dinoflagellates (8.1%) dominated, and all other groups were <5%. Late DsN1 showed cryptophytes (33.5%), diatoms (23.9%), dinoflagellates (18.1%), MAST (16.6%) and rhodophytes (5.3%) to dominate, and all other groups were <5% (Figure 4.5).

2019-2020: The early DsN1 comprised diatoms (86.4%) and dinoflagellates (6.6%), and all other groups were <5%. In the mid DsN1, diatoms (52.8%), MAST (24.91%) dominated with dinoflagellates (7.5%), cryptophytes (7.25%), haptophytes (7.1%), and all other groups were <5%. In the late DsN1, cryptophytes (42.6%), MAST (27.6%), rhodophytes (9.64%), diatoms (6.8%), dinoflagellates (6.3%) and haptophytes (6.3%) dominated with Green algae <5% (Figure 4.5).

2021-2022: In the early DsN1, diatoms dominated (77.1%), followed by cryptophytes (13.7%), dinoflagellates (5.74%) and all other groups <5%. In mid DsN1, diatoms dominated (95.9%) and all other groups were less than 5%. In the late DsN1 diatoms (75.9%) and dinoflagellates dominated (11.0%), followed by MAST (5.6%) and all other groups <5% (Figure 4.5).

While the relationship of DsN1, SST, and SSS varied among species, overall, 22% of species occurred in early DsN1, 29% of species occurred in mid DsN1, and 49% of species occurred in late DsN1 (see Figure 4.3a). The high SSS bin had 37% of species, while 38% of

species had their maxima in mid SSS, and 25% in low SSS. Overall, 28% of species occurred in high SST, 46% in mid SST, and 26% species in low SST – with the most species (14%) having maxima in late DsN1, at low SSS, and mid SST conditions (Figure 4.6).

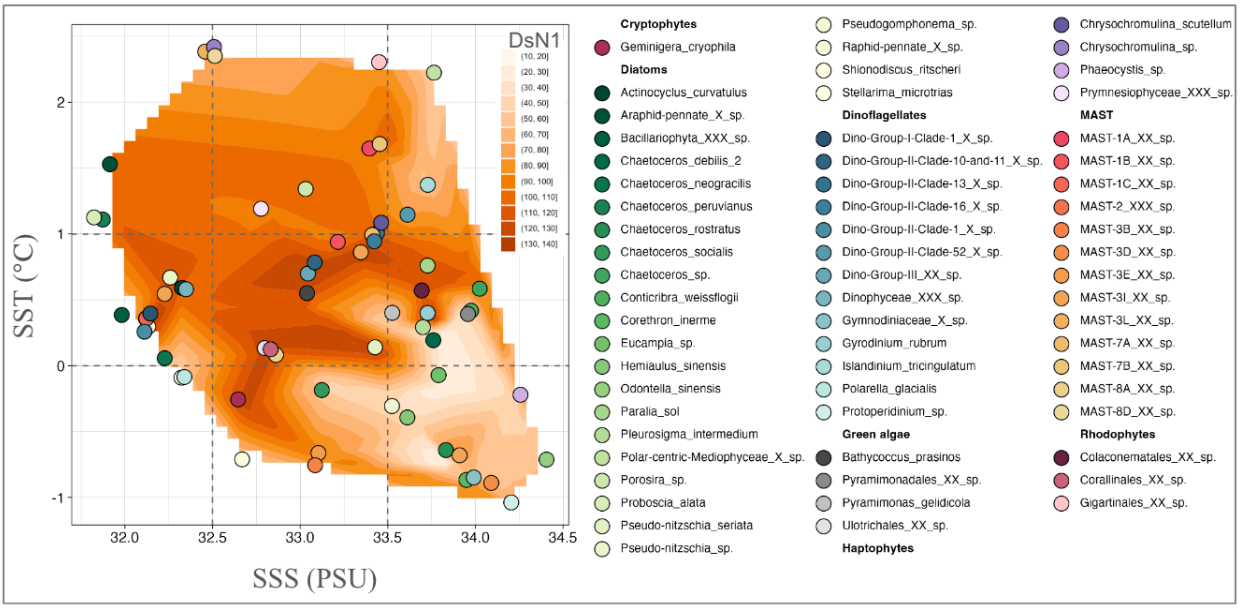


Figure 4.6: The maximum read count for each phytoplankton species corresponding to SST and SSS location according to DsN1 (depicted as a shaded orange contour with light orange depicting early season (or Early DsN1). Phytoplankton groups are colored one representative color per group (e.g., all diatom species are shades of green). Dashed grey lines represent the low, mid, and high range cut off. The dots overlapped in some cases, so they've been jittered for better viewing. Exact values can be seen in Supplementary Figure 4.1. Phytoplankton species are all colored various shades of one representative color per group (e.g., all diatom species are shades of green).

We can also look at species dynamics within each broad phytoplankton group: of the diatom species, 40% had maxima in early DsN1, 16% in mid DsN1, and 44% in late DsN1. The maximum read abundances for 36% of the diatom species were observed in low SST, 44% in mid SST and 20% in high SST environments. Regarding SSS, 48% of diatom species had maxima in high SSS conditions, and 36% in low SSS.

In contrast, 31% of dinoflagellate species had maxima in high SST waters, 15% of species in low SST waters, and 54% in mid SST waters. Dinoflagellates showed their highest percentage of species in late DsN1 (62%), 31% in mid DsN1, and only one species in early DsN1 (*Polarella_glacialis*). Dinoflagellate species maxima were found over a range of SSS: 31% of species were in low, 31% mid, and 38% high.

Of the MAST species, none had maxima in early DsN1, 53% in mid DsN1, and 47% in late DsN1. Regarding SSS, 60% of the MAST species had maxima at mid SSS, with only 20% in low or high SSS conditions (Figure 4.6). Similarly, a consistent community of mixed MAST was found with maxima varying between low (27%), mid (40%), and high (33%) SST.

Maxima of green algae were equally split between early or late DsN1, high or mid SSS, while 100% were found in mid SST. The haptophytes displayed a broader distribution over DsN1, observed at all times of DsN1, with 75% of species in mid SSS and high SST settings (Figure 6). Rhodophyte maxima were found mid to late DsN1, in mid to high SSS, and in mid to high SST conditions (Figure 6); cryptophytes exhibited maxima in late DsN1, mid SSS and low SST (Figure 4.6).

The interannual variability of occurrence of species maxima with respect to SST or SSS was high (Figure 4.7). Out of all 65 species, only one (the diatom *Hemiaulus_sinensis*) had a maximum only during the early season, low SST, and high SSS environment in each year.

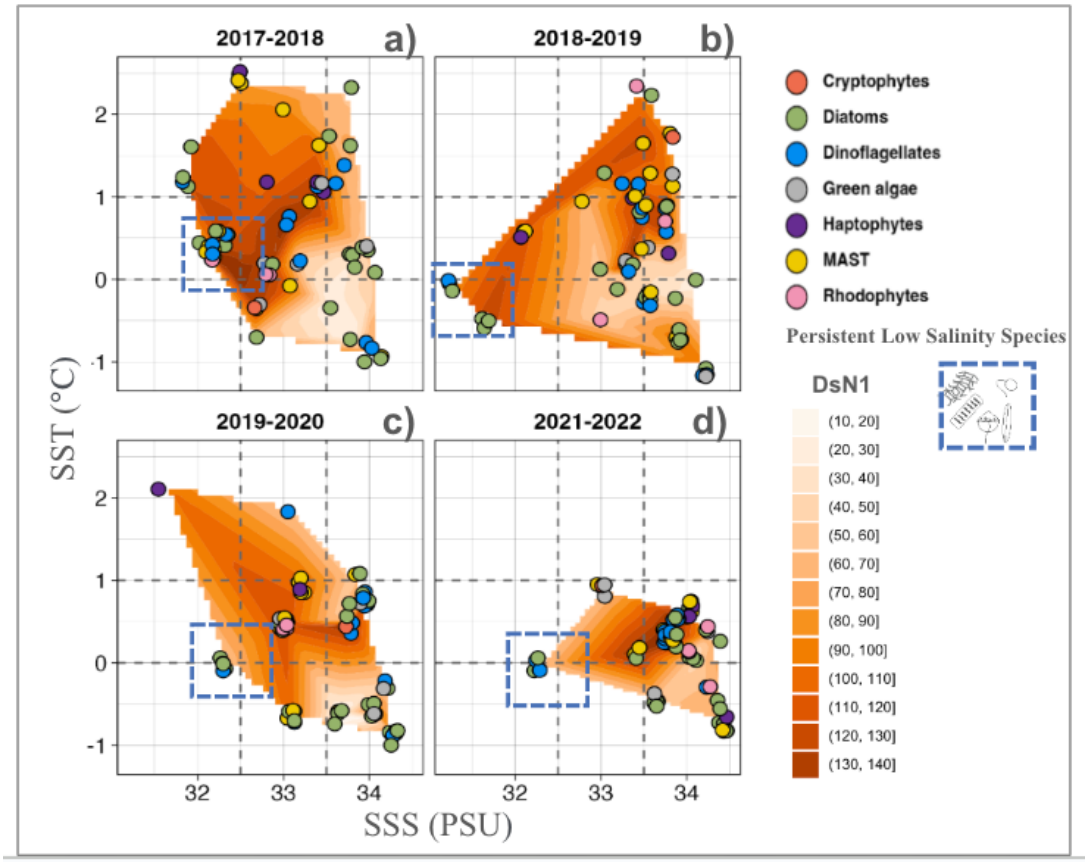


Figure 4.7: Interannual the determined maximum read abundance position for each phytoplankton group (a-d). The maximum read count for each group is corresponding SST and SSS location according to DsN1 (depicted as a shaded orange contour). Phytoplankton groups are colored one representative color per group (e.g., all diatom species are shades of green). The dots overlapped in some cases, so they've been jittered for better viewing but do not represent the exact x-y coordinate. Dashed grey lines represent the low, mid, and high range cut off. Dashed blue box represents the Persistent Low Salinity Species.

For each sampling season (Figure 4.7a-d), phytoplankton groups are reported in the same order, to facilitate comparison of group dynamics within each season, and interannually. The timing (DsN1) depicted within each season reflects the range of the environmental variables (i.e., SST, SSS) associated with the maximum read abundances for each species within a group (Figure 4.7). The full extent of the SST and SSS range in each season (Figure 4.2) represents the possible conditions phytoplankton species are found in during the season (Figure 4.5).

For the 2017-2018 season (Figure 4.7a), the Cryptophyte *Geminigera* had maxima under mid SSS and low SST conditions. 54% of Diatom species had maxima in high SSS conditions, though 38% of diatom species were found in low SSS conditions (see Persistent Species in Low Salinity). Dinoflagellates and green algae had maxima equally split between species in low, mid and high SSS and mid to high SST. Maxima of haptophytes were in mid SSS and high SST conditions. MASTs were prevalent in mid to high SST and low to mid SSS. Rhodophytes had maxima in mid SST and mid SSS.

During the 2018-2019 season (Figure 4.7b), the cryptophyte *Geminigera* had a maximum in high SSS and high SST. Diatom maxima were found under low, mid, and high SSS and low, mid, and high SST, but there was an assemblage of low SSS species similar to the prior season (see Persistent Species in Low Salinity). Dinoflagellates and green algae occurred in mid to high SSS and mid to high SST. Haptophytes occurred in low and high SSS but mid SST conditions. MASTs were prevalent in low to high SSS and mid to high SST. Rhodophytes occurred in mid to high SSS and low, mid, and high SST.

In the 2019-2020 season (Figure 4.7c), the cryptophyte *Geminigera* had a maximum in high SSS, and mid SST. Most diatom species maxima were found in high SSS and low SST conditions, with a persistent community observed in low SSS and low SST conditions (see Persistent Species in Low Salinity). The dinoflagellates showed a maximum in high SSS and low and mid SST. Green algae occurred at a maximum in high SSS and low SST. Haptophyte maxima occurred in low to mid SSS and mid to high SST conditions. MASTs were prevalent in mid SSS and low to mid SST. Rhodophytes had maxima in mid SSS, mid SST conditions. In this season, there was an assemblage of low SSS species similar to the prior seasons (see Persistent Species in Low Salinity).

During the 2021-2022 season (Figure 4.7d) the cryptophyte *Geminigera* had a maximum in mid SSS and mid SST. The diatoms had maxima in high SSS and mid to low SST. Dinoflagellates showed a more restricted range of environmental conditions than during other seasons to the high SSS and mid SST. The low SSS and low SST assemblage of diatoms and dinoflagellates was also present as in all prior seasons (see Persistent Species in Low Salinity).

Persistent Species in Low Salinity

In each season, there were phytoplankton species persistently found in low SSS but at various SST and DsN1, containing a mix of three to six diatoms with one or two dinoflagellate species (Figure 4.7). These low-salinity taxa were typically found in the late DsN1 (Figures 4.3 and 4.5), with slight changes in species composition depending on the sampling season. No low-salinity species was present in every season, and most were present in only one or two seasons. In alphabetical order from observed diatoms to dinoflagellates: *Araphid-pennate_X_sp.* was present in three seasons: 2017-2018, 2019-2020, and 2021-2022; *Bacillariophyta_XXX* was present in two seasons 2019-2020, 2021-2022; *Chaetoceros_rostratus* was present in one season 2018-2019; *Chaetoceros_peruvianus* was present in two seasons 2017-2018, 2018-2019; *Chaetoceros_neogracilis* was present in two seasons 2018-2019, and 2021-2022; *Proboscia_alata* was present in one season 2017-2018; *Pseudo-nitzschia_sp.* was present in two seasons 2017-2018, 2018-2019; *Pseudo-nitzschia_seriata* was present in one season 2018-2019; *Pseudogomphonema_sp.* was present in one season 2017-2018; *Raphid-pennate_X* was present in one season 2018-2019; *Stellarima_microtrias* was present in two seasons 2018-2019, 2019-2020; *Dinophyceae_XXX_sp.* in one season 2021-2022; *Polarella_glacialis* in two seasons 2018-2019 and 2021-2022; and *Protoperidinium_sp.* was present in two seasons 2017-2018, 2019-2020.

These species can also be arranged into the years they grouped together: In 2017-2018 the community contained five diatom species (*Araphid-pennate_X_sp., Chaetoceros_peruvianus, Proboscia_alata., Pseudo-nitzschia_sp., Pseudogomphonema_sp.*), and one dinoflagellate (*Protoperidinium_sp.).* In 2018-2019 the community consisted of six diatom species (*Raphid-pennate_X, Chaetoceros_rostratus, Chaetoceros_peruvianus, Chaetoceros_neogracilis, Pseudo-nitzschia_seriata, Stellarima_microtrias*) and one dinoflagellate (*Polarella_glacialis*). In 2019-2020 the community was made up of four diatom species (*Araphid-pennate*, *Bacillariophyta_XXX, stellarima_microtrias*) and the same dinoflagellate, *Polarella_glacialis*. By 2021-2022 the community consisted of three diatom species (*Araphid-pennate_X_sp., Bacillariophyta_XXX, Chaetoceros_neogracilis*) and two dinoflagellate species (*Polarella_glacialis*, and *Dinophyceae_XXX_sp.).*

Discussion

This study presents an analysis of phytoplankton from net-collected metabarcoding samples aimed to a) examine phytoplankton community structure using molecular methods from the high temporal-resolution sampling program FjordPhyto, and b) determine how succession and diversity varied with time (DsN1), temperature (SST) and salinity (SSS), and test specific hypotheses commonly reported in the literature. The hypotheses presented in the introduction fell under two main themes: 1) How does species richness at both the community and group level change over time and as salinity decreases and temperature increases? and 2) Do individual groups and the species within show consistent relationships to salinity and temperature over time? The discussion will address each hypothesis in turn.

H1 - Phytoplankton community richness decreases over time and with increasing temperature (Lin et al. 2021) and decreasing salinity.

Our data rejected H1, showing that phytoplankton community richness increased over the season (Figure 4.3). We could not detect a significant relationship of community richness with salinity or temperature (Supplementary Figure 4.2). The significant correlation to salinity and temperature occurred at the group level (Figure 4.3, Supplementary Figure 4.2, Table 1); even within a phytoplankton group not all species behaved the same (i.e., species had divergent correlations (or no correlation) to salinity or temperature) (Figure 4.4). This contrasts with Lin et al. (2021) who reported that eukaryote protist community richness and evenness declined with an increase in temperature that occurs later in the season.

H2 - Diatom richness decreases over time with increasing temperature and reduced salinity (Montes-Hugo et al. 2009, Mendes et al. 2013, 2018).

Our observations showed diatom richness was higher in early season in low-temperature, high-salinity waters, and richness decreased significantly over time with low salinity, and high temperatures (Figure 4.3), which does not reject this hypothesis. The significance of the diatom correlations with salinity were species dependent, and diatom species were less likely to be correlated with temperature (Figure 4.4, see Persistent Species in Low Salinity, and discussion on the group of persistent low-salinity species).

In the literature, some authors have reported a late-season increase in the richness of diatoms and other groups (e.g., Moline and Prézelin, 1996; Garibotti et al. 2005, Pan et al. 2020). Our data tend to reject these observations, showing a decrease in the richness of diatoms with time. Brown et al. (2021) showed diatoms had a greater richness than cryptophytes, which was also seen here. Our data confirm other studies in showing that diatoms often have maxima at the beginning of the season, presumably capitalizing on increasing sunlight and replete inorganic

nutrients in the coastal WAP waters (Kim et al. 2016, Leeuwe et al. 2020, Carvalho et al. 2020, Nardelli et al. 2023).

H3 - Flagellate richness increases as salinity decreases (Montes-Hugo et al. 2009, Mendes et al. 2013, 2018).

The identification of flagellates in the literature has typically been done via microscopy; therefore 'flagellates' are a common reported finding. In this study I interpret flagellates to include the cryptophytes, dinoflagellates, green algae, haptophytes, and MASTs. The data in this study showed significant trends with salinity only for haptophytes and MASTs, with increasing richness as salinity decreased ($P < 0.01$). There were no significant correlations for cryptophytes, dinoflagellates, or green algae, supporting the rejection of this hypothesis. The species within the flagellate groups that did show significant correlation to SSS were negatively correlated (high abundance in low salinity), except for one dinoflagellate species *Gymnodiniaceae_X_sp.* which had a positive correlation with salinity.

H4 - Cryptophytes occur in greater proportion later in the season (Moline and Prézelin, 1996, Kim et al. 2016).

Cryptophytes had a significant positive correlation to DsN1 during the season, with a maximum abundance later in the season (Figures 4.4, Table 4.1, $P < 0.05$) (except in 2021-2022 when diatoms dominated throughout the season) (Figure 4.5). In general, cryptophytes were the dominant group late in the season, with the highest proportion reaching 42.56% in the late season of 2019-2020. (Figure 4.5). Cryptophytes are thought to take advantage of diverse nutrient niches later in the season (Hamilton et al. 2021, Vanzan et al. 2015), consistent with our data showing three late-season maxima out of the four seasons. However, it is worth noting that cryptophytes were not the only group that had greater proportions of reads than diatoms late in the season. The

phytoplankton groups that flourished in the late season include groups with species that are mixotrophs (i.e., cryptophytes, dinoflagellates, haptophytes) and bacterivorous or parasitic groups (MAST) (Lin et al. 2012).

The next two hypotheses (H5, H6) group under the second theme and address previous findings that suggested that responses to salinity and temperature are group- (Garibotti et al. 2005, Kim et al. 2016, Pan et al. 2021, Mascioni et al., 2021, 2023) and species-specific (Hernando et al. 2015, Costa et al 2020), specifically focusing on two groups of phytoplankton: diatoms and cryptophytes.

H5 - Diatoms (and species within), peak in abundance in low temperatures and high salinity (Petrou et al. 2011, Sackett et al. 2013, Mascioni et al. 2023).

This study showed that the diatoms had a significant relationship with SST and SSS, but distinct species displayed environmental maxima at varying SSS and SST and had high interannual variability. This hypothesis was not rejected at the group level for diatoms, but was often rejected at the species level (Figure 4.4), revealing variable species-specific correlations that were the opposite of the group-level trends. Only two diatoms correlated significantly positively with SST: *Corethron_inerme* and *Hemiaulus_sinensis*. The diatom species that were significantly positively correlated with SSS were *Corethron_inerme, Eucampia_sp., Odontella_sinensis, Polar_centric_mediophyceae_X_sp.*, and *Shionodiscus_ritscheri* (Figures 4.4 and 4.6). Surprisingly, there were some diatoms with significant negative correlations with SSS, with maxima at low SSS (see Persistent Species in Low Salinity).

A group of persistent low-salinity species

One unexpected finding from this study was a group of species that showed maxima in low SSS conditions. These species included 4-6 species of chain-forming *Chaetoceros* diatoms

and pennate diatoms, and 1-2 species of dinoflagellate from either *Dinophyceae_XXX_sp., Polarella_glacialis*, *or Protoperidinium_sp*. The exact species making up this low-SSS group changed from season to season (Figure 4.7). Hernando et al. (2015) found that phytoplankton responses (test-forming diatoms, prasinophytes, cryptophytes, and phytoflagellates) to low salinity were species-specific. DeLima et al. (2019) noted that diatoms and dinoflagellates (unspecified to a further level) were codominant when meltwater dominated their study site in the South Shetland Islands. Moline et al. (2004) and Mendes et al. (2018) had noted certain species of diatom and dinoflagellate that seemed to be associated with low salinity; however, the range of salinity defined as "low" in these studies often fell in the high SSS range of the present study. Various studies have shown that both warm- and cold-water conditions had bloom-forming diatoms (Moline et al. 2004, Schofield et al. 2017, Rozema et al. 2017, Ferreira et al. 2020, Petrou et al. 2016, Mascioni et al. 2023). Previous studies have observed large diatom blooms (e.g., *Corethron pennatum, Fragiliara spp., Licmophora sp., Pseudo-nitzschia sp., Thalassiosira spp., Chaetoceros spp., Odontella weissflogii*) associated with low salinities derived from glacial meltwater plumes (Mendes et al. 2012, Hofer et al. 2019, Costa et al. 2021). In this study the diatom genera *Fragilaria, Licmophora*, and *Thalassiosira* were not detected by 18S amplicon sequencing. *Corethron* and *Odontella*, however, were identified and were significantly correlated with higher salinities, but not low salinity (Figure 4.4). Because the literature often suggests that cryptophytes emerge in low-salinity waters (Mendes et al. 2023), this finding of persistent non-cryptophyte, low-salinity species was unexpected but exciting, and broadens our understanding of the species and groups that might thrive in low-salinity waters.

H6 - Cryptophyte abundance increases in high temperature (Lin et al. 2021, Moline et al. 2004, Mendes et al. 2018) and low salinity (Moline and Prézelin 1996, Schofield et al. 2017, Costa et al. 2023).

Our analyses did not detect any correlation of cryptophyte abundance (relative ASV reads) with temperature, but did detect a significant negative correlation with salinity; we therefore reject H5 regarding temperature but not H5 regarding low salinity (Figure 4.4). In this study, although only one species was identified by the PR2 database and described here, there were eight unique ASVs that could in fact be incorrectly ascribed to only one cryptophyte species, as found in Hamilton et al. (2021). Although, as mentioned before, general statements about trends for cryptophytes must be taken with caution, as there was high interannual variability observed in this study: in some years cryptophytes were relatively abundant in early season (Figure 4.5) when conditions were cold and salty (in 2018 – 2019 and 2021- 2022, Figures 4.2 and 4.3), and mid- and late-season when temperature and salinity conditions were high (2018 – 2019, 2019 – 2020).

This finding is consistent with observations of cryptophyte prevalence in low-salinity waters in the WAP (salinity reported ≤ ~33.6 PSU) (Moline et al. 2004, Schofield et al. 2017, Mendes et al. 2018, Ferreira et al. 2020). In the southern region near Marguerite Bay, Kim et al. (2016) and Leeuwe et al. (2020) showed late-season dominance by cryptophytes in warmer, fresher waters in summer, as glacial ice melting promoted water column stability (Moline and Prézelin, 1996, Moline et al., 2001; Garibotti et al., 2005; Dierssen et al., 2002; Meredith et al., 2013). However, Leeuwe et al. (2020) also observed apparently random occurrences of the mixotrophic cryptophytes. Predictive models used by Pan et al. (2021) showed no relationship of cryptophyte abundance in fjords based on decreasing salinity from meltwater discharge.

Cryptophytes may not be restricted to the late season, as they are known to do well in the high-light conditions found in mid-season at maximum insolation (Mendes et al. 2023). In contrast, cryptophytes were also associated with an increase in cloudy days and changing light levels (Vernet et al. 2012). Studies report numerous potential environmental drivers of cryptophyte variability, including shortened sea ice cover, glacier retreat, input of meltwater (Montes-Hugo et al. 2009; Mendes et al. 2013, 2018), and stronger winds and deeper summer mixed layer depths (Vernet et al. 2008, Petrou et al. 2016, Lange et al. 2016, Montes-Hugo et al. 2009, Schofield et al. 2017, Rozema et al. 2017). However, as there is high regional variability in the WAP, these environmental correlates all depend on where and when samples were collected from the peninsula.

The discrepancies between this study and others could arise from the fact that previous sampling efforts were limited, and only recently have publications reported cryptophytes specifically as an emerging group of importance (Schofield et al. 2017, Mendes et al. 2018, Pan et al. 2020, Brown et al. 2021, Hamilton et al. 2021, Mendes et al. 2023), though this had been noted 10 to 20 years ago (Garibotti et al. 2003, Kozlowski et al. 2011).

More broadly, our data showed no indication that cryptophytes, more than any other observed group, are replacing diatom-dominated communities as suggested by Mendes et al. (2023).

Overall relationship of phytoplankton succession to salinity and temperature

In general, these hypotheses tests indirectly bring up the dynamics of succession. There is an overall seasonal succession pattern which begins with diatoms early in the season, and transitions to other species-rich groups (e.g., dinoflagellates, MASTs) throughout the season (Figure 4.5). This was consistently observed in each season. However, the interannual variability

of species timing or relationship to SSS and SST was large (Figures 4.5 – 4.7). Moline and Prézelin (1996) showed a consistent and repeated annual pattern in phytoplankton succession over three seasons, suggesting that the constant timing of the spring diatom bloom occurred despite highly variable environmental conditions, and that succession itself is a predictor.

In the metacommunity ecology framework (Chase et al. 2020), environmental filtering and biotic interactions act at a local level on species and communities, and community fluctuations at a regional level determine patterns of species abundance, occurrence, composition. The observation that species in this study peaked at specific times and environmental (SST, SSS) conditions suggests that the community composition should vary with the local conditions.

If particular species of phytoplankton reach peak abundance under certain environmental conditions of temperature or salinity, an assumption could be made that those same taxa would occur in the same set of conditions every year and contribute to the community richness in a predictable way. Instead, we see high interannual variability in species associations with the environment (Figure 4.7), making predictability of group response or community diversity challenging (Pan et al. 2020, Moline and Prézelin 1996). Our observations of individual species' maxima across a broad range of environmental conditions of salinity and temperature may reflect the ability of these species to exploit a range of conditions that optimize their growth and survival (i.e., their resilience).

Some studies concluded that a basic assemblage of phytoplankton was always present throughout the season, but that the dynamic temperature or salinity conditions trigger blooms of any of the possible species present (Antoni et al. 2023, Mascioni et al. 2021, Costa et al. 2020). Pan et al. (2020) showed that a range of possible drivers could explain the increases in

abundance of each group but concluded that more field observations with higher-resolution temporal sampling need to be conducted in order to confirm the parameters that best predict the outcome. The species in this study that correlate strongly to salinity or temperature could be able to bloom if those conditions arise in the environment. As more novel observations of bloom-forming species are being made (Mascioni et al. 2019, Costa et al. 2020, Mendes et al. 2023, Antoni et al. 2024), there is a need to obtain a species-level understanding of the drivers of these fluctuations.

This study supports the need to address more variables than considered here to understand community structure; the focus should be at the species level. This could be achieved with mesocosm or lab experiments, as well as performing metatranscriptomic analyses and investigating how gene transcript levels from phytoplankton correlate with environmental variables (Sunagawa et al. 2019). Performing experimental lab tests requires using cultured species; this proves difficult for polar species as many such species are not cultured or culturable. Some studies have performed mesocosm experiments in the field, examining the effects of salinity on phytoplankton physiology and assemblage composition (Hernando et al. 2015). Such studies show that results depend on the phytoplankton community present at the start of the experiment (Antoni et al. 2024).

Polar mandala: A community succession model

A simple schematic can aid in depicting our current knowledge of phytoplankton seasonal succession in relation to environmental conditions. A geometric diagram known as a mandala shows the relationship of phytoplankton species to a time-space continuum defined by environmental parameters. A classic mandala used in phytoplankton ecology was developed by Margalef (1979) and improved upon by Glibert et al. (2016) to show phytoplankton succession

in relation to red tide blooms, with turbulence and nutrient concentrations as the axes. The main sequence shows succession of groups of species, all of which have different traits or characteristics that allow them to flourish in specific conditions (Glibert 2016, Wohlrab et al. 2018).

Along the WAP, several studies highlight the influence of salinity and temperature (and their correlates) as the main environmental drivers of various phytoplankton groups (Olsen et al. 2013, Moreno-Pino et al. 2016). Moreno-Pino et al. (2016) showed a significant influence of environmental filtering on the composition of phytoplankton communities in the surface waters, and proposed that different species co-occur based on their shared tolerances or requirements for a particular environment. De Lima et al. (2018) analyzed seven organismal functional groupings in Antarctica which reflected different adaptive strategies responsible for the structuring of the microphytoplankton community in scenarios dominated by meltwater. To provide insight into the phytoplankton patterns from the dataset presented here, a Polar Mandala was created (Figure 4.8) that synthesizes the observed phytoplankton taxa into a succession sequence related to DsN1, SST, and SSS at the WAP coast. This mandala builds upon the tradition of using mandalas in ecology to visually synthesize complex relationships between environmental drivers and biological communities in simplified ways.

The axes of the mandala reflect two critical environmental drivers: temperature and salinity, with a time continuum (early to late season) overlaid to capture seasonal transitions. The mandala highlights the main phytoplankton succession pathway of large centric diatoms dominating under high-salinity and low-temperatures, typical of early-season conditions with limited freshwater input (Sackett et al. 2013). As the season progresses and salinity decreases

coinciding with increased meltwater input (Meredith et al. 2018), temperatures rise and smaller pennate diatoms, dinoflagellates, haptophytes, and green algae begin to flourish.

The mandala also identifies an alternate succession sequence in which mixotrophic phytoplankton, such as cryptophytes and dinoflagellates, dominate under conditions of lower salinity and higher temperatures, often associated with low-sea-ice years (Turner et al. 2018). The integration of species and phytoplankton groups into this simple framework offers a to predict how phytoplankton communities might respond to shifting environmental conditions driven by to climate change. With these shifts, we expect mixotrophic protist plankton to become more dominant (Flynn et al. 2013). By visually mapping the various taxa to the most foundational level of environmental drivers (i.e., salinity and temperature), the mandala provides a view of phytoplankton community structure and implications for understanding ecosystem functions such as carbon cycling, food web dynamics, and ecosystem resilience in the rapidly changing polar environment.

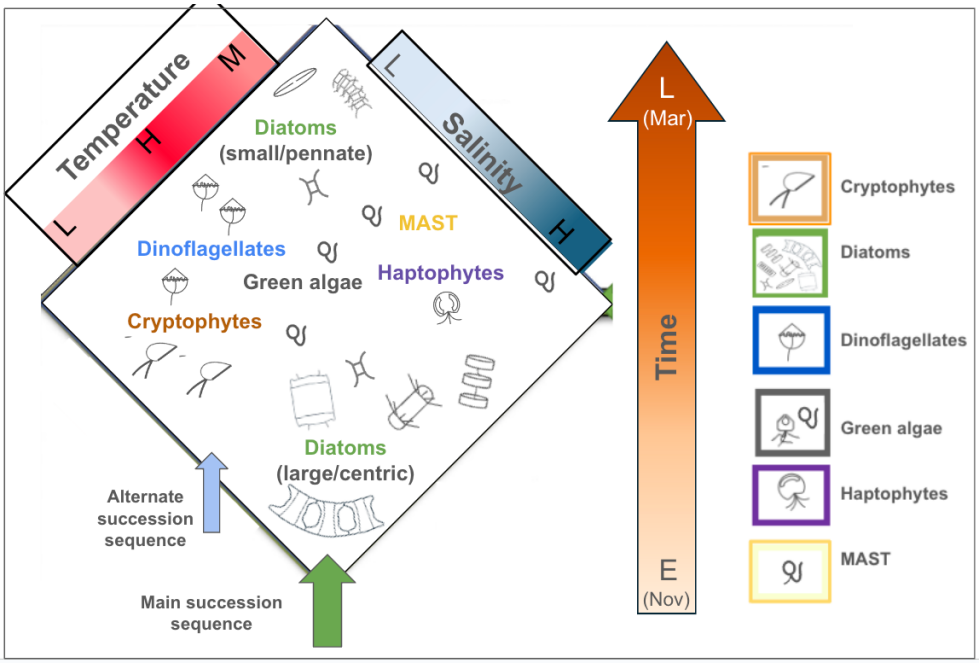


Figure 4.8: Polar phytoplankton mandala developed from the conceptual understanding of generalized spring-summer bloom sequence discussed by Glibert et al. 2022. The axes include:

(1) “Time” or Days since the start of Nov 1 (DsN1, in orange) where E is early season (November) and L is late season (March); (2) “Salinity” or sea surface salinity (PSU, in blue) which is linear to Time; and (3) “Temperature” or sea surface temperature (°C, in red) which is non-linear to Time. Days since the start of Nov 1 is used in this study to linearize time as the austral growth season progresses and the sequence of succession can begin at either the Main succession sequence or the Alternate succession sequence. The mandala highlights the main phytoplankton succession pathway, where large centric diatoms dominate under high salinity and low temperatures, typical of early-season conditions with limited freshwater input (Sackett et al. 2013). As the season progresses and salinity decreases coinciding with increased meltwater input (Meredith et al. 2018), temperatures rise and smaller pennate diatoms, dinoflagellates, haptophytes, and green algae begin to flourish, marking a shift in community composition. The mandala also identifies an alternate succession sequence, where phytoplankton such as cryptophytes and dinoflagellates dominate under conditions of lower salinity and higher temperatures, often associated with low sea ice years (Turner et al. 2018). The integration of species and phytoplankton groups into this framework offers a hypothesis-testing tool to predict how phytoplankton communities might respond to shifting environmental conditions due to climate change. Note that all scales are relative, and placement of phytoplankton groups and icons are representative and do not imply that all species within a given group will respond similarly (as previous figures show).

Limitations in this study and previous studies

One main finding from this study's opportunistic sampling strategy, which involved partnering with expedition vessels through a participatory science framework, is that sampling efforts can be made with a greater temporal resolution than scientific cruises allow, including repeatedly visiting multiple sites for five months each season.

We know that phytoplankton are patchy, and that the choice of sampling location will influence the observations (Montes-Hugo et al. 2009). Previous studies that sampled over short stretches of time may not have accurately captured the full seasonal succession patterns, potentially leading to misleading generalizations (Chapter 1).

Despite numerous efforts to understand the drivers of the phytoplankton community composition, reviews from Henley (2019), Gutt et al. (2020) and Ferreira et al. (2020) concluded that there remains a high level of uncertainty in how climate change will impact these primary producers. In Antarctica, traditional methods of identifying phytoplankton – including

chlorophyll-*a*, biomass, pigment or microscopy – do not always allow consideration of the genus or species level variability. Attention has not previously been given to rare or smaller species like MAST, haptophytes, green algae, dinoflagellates. Metabarcoding approaches allow taxonomic identification to the species level. Given the incredible diversity of protists (Burki et al. 2021), a species focus is imperative and the practice of reporting phytoplankton at group level may be too broad to identify the mechanisms that drive change entire communities.

Some important limitations to this study include the fact that samples were taken from the surface when the sea state was less than Beaufort scale two. Thus, the observations may reflect communities found in more calm or stratified waters (e.g., wind less than 20 knots, less surface chop), leading to the potential for unsupported generalizations.

The samples acquired here using a 20-μm net are biased toward microeukaryotes in the surface community, though 18S V9 amplicon sequencing also revealed smaller micro- and nano-eukaryotes, such as MASTs. Misidentifications can also arise from metabarcoding due to the limited polar protist species in the databases, leading to mis-represented and mis-identified taxa (Krinos et al. 2024, Vaulot et al. 2022). In this study the PR2 database identified the diatoms *Hemiaulus_sinensis* and *Corethron_inerme* as present, but these are not polar species and are not observed by microscopy. For example, under microscopy the diatom *Corethron pennatum* is often seen, but never *C. inerme* (Mascioni pers. comms.). Similarly, novel species can be observed as in Hamilton et al. (2021), where a new cryptophyte was identified. In Brown et al. (2019) 94 cryptophyte ASVs were found, but only two ASVs contributed to the community. An additional caveat to mention is that several species that we identified under the microscope in high abundances (Mascioni et al. 2019, 2021, 2023) were not detected as high relative read abundances in the metabarcoding data set (e.g., *Corethron_inerme* identified here makes up a

very small fraction of reads in each sample). Additionally, with 18S data, species like *Porosira* swamp the signal of read abundance. Yet under microscopy, this taxon is not the most abundant. There is an urgent need to improve molecular database taxonomic associations with other traditional methods of taxa identification (Pierella Karlusich et al. 2020) to ensure that comparison among studies can become more comprehensive and complementary.

Additionally, discrepancies in comparing this study with others could come from differing definitions of the environmental conditions. If sampling efforts among studies are restricted in time, duration, or location, the range of environmental conditions covered in each study will be different. It is important to point out that the definitions of low/high temperatures or low/high salinities vary in the literature; there is a need to critically evaluate the definitions, as these variations can significantly impact the detection and characterization of phytoplankton communities or preferences. For example, Moline et al. (2004) recorded salinity ranges of 33.3 PSU to 33.8 PSU – which fall in the 'high' range of this study, and their temperatures ranged from -1 °C to 1 °C – which is the low to mid-range for this study. Mendes et al. (2018) found surface salinity to range from 33.4 PSU to 34 PSU (the high salinity bin of this study), and their temperature range varied between 0 °C to 2 °C – which encompasses the entire temperature range of this study. The bins defined in this study reflect the total range of salinity and temperature measured from the sampling effort. Future attempts to review the entire range of possible conditions and come up with a peninsula-wide standard definition for 'low' 'mid' and 'high' salinity and temperature would help with understanding how taxa respond to environmental conditions as they shift with climate change.

Conclusions

Our dataset, spanning five months across each of four seasons, reveals significant seasonal and interannual variability in phytoplankton groups within Antarctic coastal waters, strongly correlating certain species with sea surface salinity and temperature.

This study demonstrated that phytoplankton groups appear in a predictable succession pattern each year, with a dominance of diatoms at the beginning of the season and community richness increasing to late season due to increasing abundances of groups like cryptophytes, dinoflagellates, MASTs, haptophytes, and rhodophytes. The phytoplankton group occurrence is shaped by complex interactions with the environment over the season, with statistically significant correlations with time, SST or SSS, but with high interannually variability. Within each group, the responses indicate that species are more plastic than expected, showing correlations with SSS and SST that were sometimes the opposite of the group correlations. Shifts in phytoplankton community composition and succession patterns in response to changing SST and SSS can propagate to higher trophic levels. These organisms may rely on certain species being present at certain times. Changes in the phytoplankton community may generate trophic mismatches during critical periods of feeding for higher trophic organisms such as krill, salps, or forage fish and could have impacts even further (Deppeler and Davidson 2017, Youngflesh et al. 2017).

This dataset represents the largest temporally resolved molecular data set gathered from the Antarctic Peninsula, encompassing five months during each of four austral summer sampling years through the FjordPhyto program. These samples help to establish a baseline for monitoring phytoplankton communities to species level in relation to the environment, enabling differentiation of general succession trends from high interannual variability. The FjordPhyto citizen science framework enabled more frequent and repeated sampling of sites over the WAP

during the austral summer than previously (Chapter 1). There is a need for enhanced temporal-resolution sampling to better capture short-term changes in phytoplankton communities. Metabarcoding will improve our understanding of phytoplankton dynamics at the species level and will complement traditional quantification from pigment or microscopy analyses, with database identification further enhanced by cultured organisms. Efforts from this study can improve marine monitoring and collaboration, supporting initiatives like the Ocean Biomolecular Observing Network (OBON) and the Southern Ocean Observing System (SOOS) data goals.

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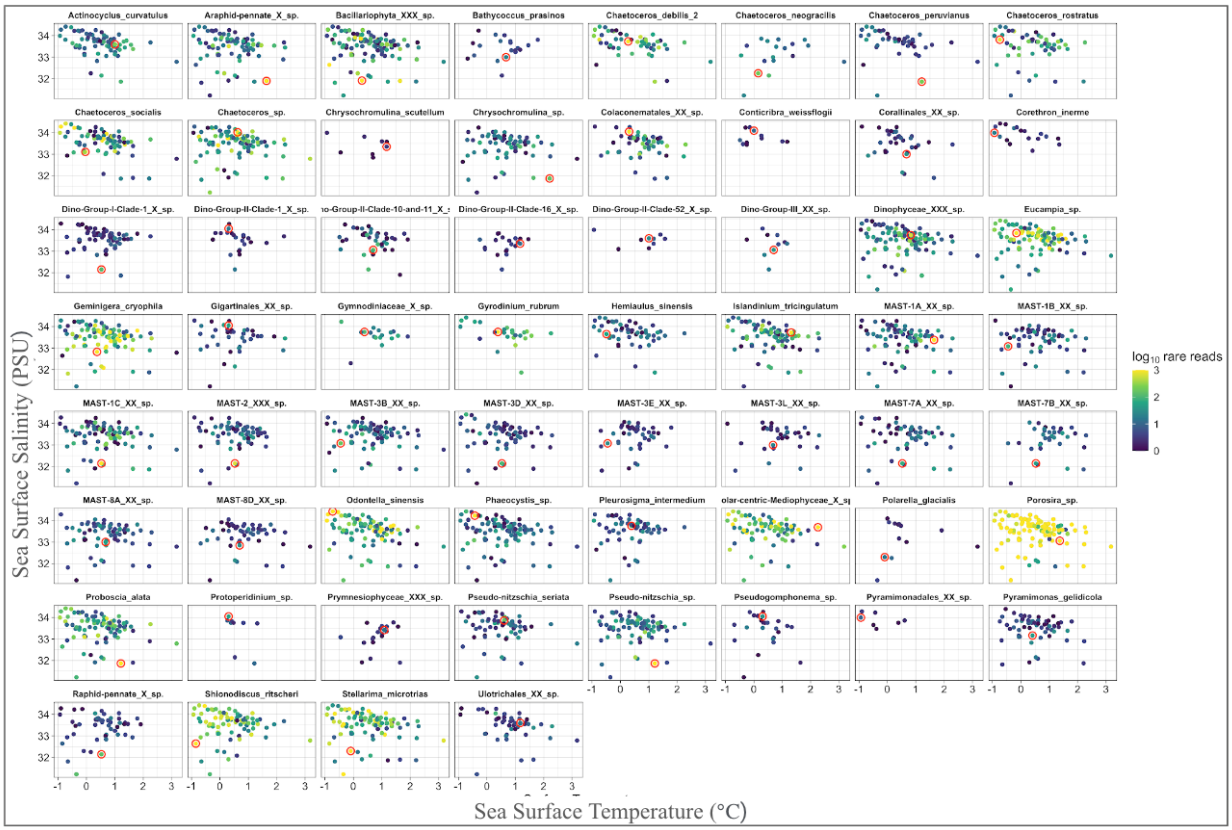
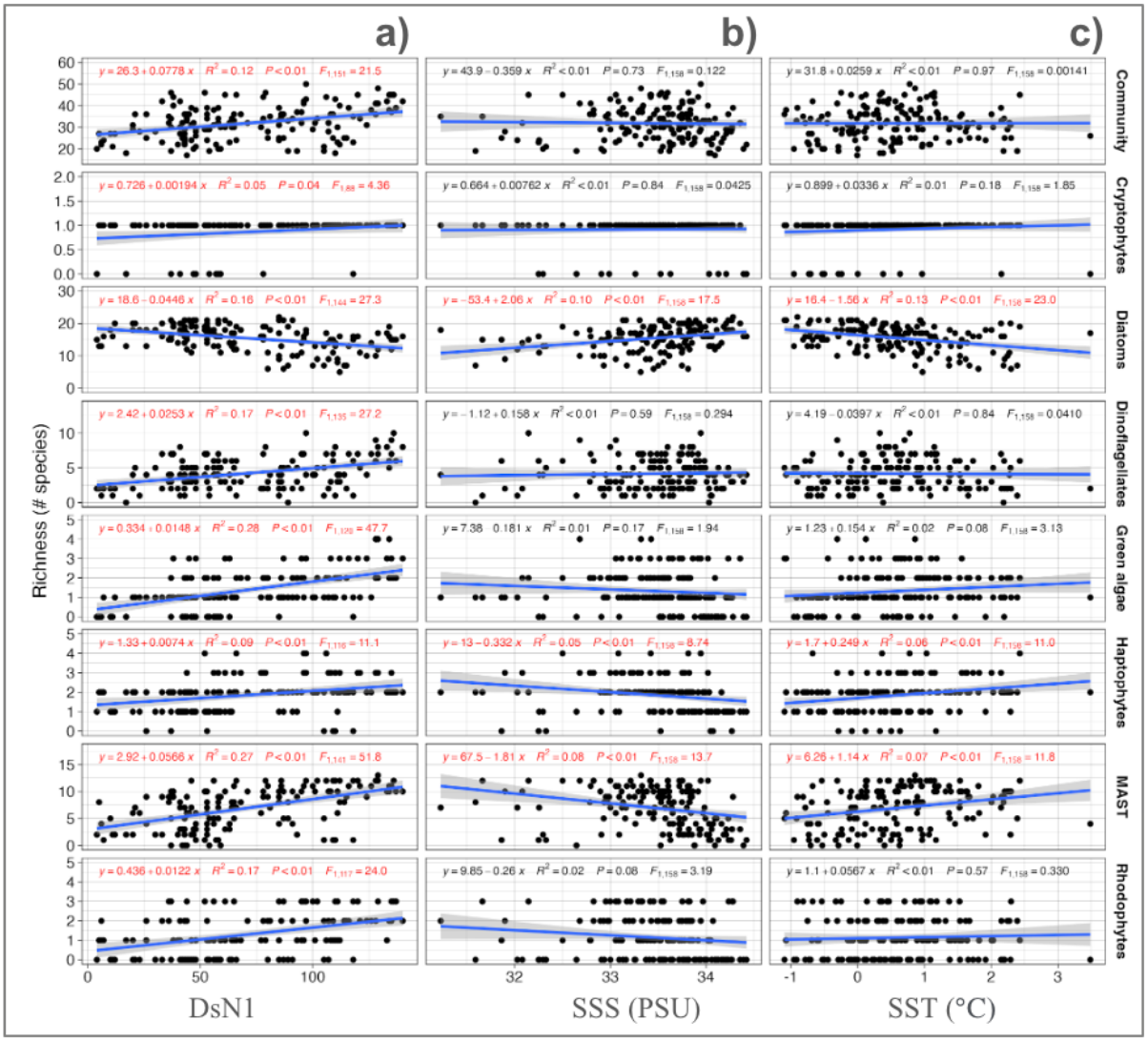
Chapter 4, in part is currently being prepared for submission for publication of the material. Cusick, A., Smith, A., Yanek-Chrones, N., Mascioni, M., Johnson, C., Zheng, H., Allen, A. E., Reynolds, R., Franks, P. J. S. A metabarcoding-based analysis of seasonal and interannual patterns of phytoplankton in relation to temperature and salinity in coastal surface waters of the Western Antarctic Peninsula. The dissertation author was the primary researcher and author of this material.



Supplementary Figure 4. 1: Overall maximum reads of each species in its corresponding SSS and SST location.



Supplementary Figure 4.2: Species richness (number of species) for the entire phytoplankton community and corresponding phytoplankton groups (secondary y-axis) across all seasons as a function of DsN1 (a), SSS (PSU); b), and SST (°C; c). Black lines are a regression line built using a linear model with 95% confidence interval shown in grey shading. The R-squared determines the goodness of fit and the p-value determines statistical significance (shown in Table 4.1). Most all trends are statistically significant with very strong to strong evidence against the null hypothesis for groups and DsN1, SSS and SST (Table 4.1), except the relationship between SST and SSS for the Cryptophytes, Dinoflagellates, Green algae, and Rhodophytes (Table 4.1).

Chapter 5 Conclusion

Dissertation synopsis

Chapter 1 synthesizes the spatio-temporal sampling efforts of phytoplankton community dynamics studies in Antarctic waters since 1942, revealing significant gaps in understanding, particularly regarding seasonal succession and the environmental conditions that influence key taxa. Spatially, fixed sampling grids, single stations, and vessel-based regional efforts limit our ability to fully assess phytoplankton distributions across the Antarctic Peninsula. Temporal gaps, stemming from a lack of repeated sampling throughout the entire austral growth season and a focus on peak bloom periods, have led to incomplete conclusions about community dynamics. Additionally, the increasing variety of taxonomic methodologies, combined with limited data on polar species, complicates our understanding of species-level ecological interactions. Molecular tools offer a promising complement to traditional methods, enhancing our understanding of seasonal dynamics and the biodiversity of summer blooms. However, the connection between phytoplankton taxa and corresponding environmental conditions remains incomplete. This synthesis emphasizes the necessity of year-round sampling to understand phytoplankton community dynamics and their responses to climate change. Coordinated, high-temporal-resolution sampling and collaboration among international researchers will strengthen future scientific efforts, foster science-policy interfaces, and contribute to global biodiversity observatories like GEO BON, GEOME, OBON, and SCAR-supported initiatives. These efforts are particularly timely in the context of the upcoming 5th International Polar Year.

Chapter 2 discusses that with rapid rates of environmental change and significant growth in tourism to the WAP, we saw an opportunity for a joint effort among many stakeholders to answer critical science questions and obtain observations over time that would not be possible or

affordable using traditional scientific approaches. While CS should not be considered a replacement for studies by scientists, FjordPhyto can enhance the scientific process and satisfy travelers wanting an enriching experience. Additionally, Antarctic policy and conservation are influenced by more than scientific data. Sharing in the scientific process increases the public understanding of science, allowing travelers to gain new perspective on ocean life. Participants may return home with a deeper understanding of the polar environment and could go on to influence the future protection of the icy continent

Chapter 3 This study highlights the importance of increasing seasonal sample collection from underexplored regions to gain a comprehensive understanding of phytoplankton dynamics. Through metagenomic (18S V9) analysis within the Fjord Phyto citizen science framework, we identified key taxa and recurrent communities, advancing our knowledge of regional diversity and seasonal succession in the Antarctic Peninsula's coastal fjords. Our findings support the hypothesis of a shift from diatoms to cryptophytes in response to increasing glacial meltwater, providing deeper insights into community structure and biodiversity through molecular methods. Overall, this study enhances our understanding of phytoplankton ecology in the Western Antarctic Peninsula and underscores the need for continued long-term monitoring and improved molecular identification tools to fully grasp the role of primary producers in marine biodiversity and productivity.

Chapter 4 of this thesis highlights significant seasonal and interannual variability in phytoplankton communities within Antarctic coastal waters, showing predictable patterns of succession over the austral summer. Diatoms dominate at the start of the season, with increased community richness toward the end due to the proliferation of cryptophytes, dinoflagellates, MASTs, haptophytes, and rhodophytes. Phytoplankton group occurrence is influenced by

complex environmental interactions, particularly sea surface salinity (SSS) and temperature (SST), with species exhibiting more plasticity than expected. These shifts in community composition can lead to trophic mismatches, impacting higher trophic levels such as krill and fish. This study, representing the largest temporally resolved molecular dataset for the Antarctic Peninsula, establishes a baseline for future monitoring and emphasizes the need for enhanced sampling resolution to capture short-term community changes. The FjordPhyto citizen science framework has proven essential for more frequent sampling and, alongside metabarcoding, offers valuable insights into phytoplankton dynamics to support global marine monitoring efforts.

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