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Distributions and Interactions in Three Groups of Polar Marine Plankton

Alison Clare Cleary
University of Rhode Island, budgiegirl@att.net

DISTRIBUTIONS AND INTERACTIONS IN THREE GROUPS OF POLAR MARINE PLANKTON

ALISON CLARE CLEARY

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ALISON CLARE CLEARY

APPROVED:

Thesis Committee:

Major Professor Edward G Durbin

Jonathan A Hare

Karen F Wishner

Nasser H Zawaia

DEAN OF THE GRADUATE SCHOOL

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Abstract

Polar marine ecosystems are highly productive, with strong seasonal phytoplankton blooms, and high abundances of vertebrate predators. A key link between these phytoplankton and megafauna are the zooplankton, which package and transform phytoplankton biomass, making it available to the fish, mammals, seabirds, and other predators in the ecosystem. I investigated three groups of these important small eukaryotes. In the Bering Sea I analyzed the diet of three morphologically very similar congeners of Pseudocalanus copepods. The two copepod species with largely overlapping geographic ranges were found to have different diets, suggesting feeding differences may serve as a mechanism of niche partitioning between these two species, reducing competition and allowing them both to persist simultaneously. In the West Antarctic Peninsula region the distribution of krill, and the diversity and distribution of microeukaryotes were analyzed in winter. Krill were concentrated within the fjords along the coast, with the few krill found in more offshore stations small, young-of-the-year individuals. Microeukaryotes in the peninsula region included organisms from nearly every major eukaryotic lineage. Microeukaryote assemblages were different in surface waters, deep-waters, and sediments, with further differences by geographic location. Sequences for multiple phytoplankton groups in sediment samples suggest the importance of resting stages, and of the sediments as a seed bank for the highly seasonal phytoplankton bloom. Enhanced understanding of the ecology of these polar ecosystems may potentially allow for improvements in modeling and fisheries management in these regions, and also serves as a baseline against which future changes may be compared.

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Preface

This dissertation is in manuscript format and is composed of four manuscripts addressing related questions in the ecology of small polar marine eukaryotes. The first manuscript, "Feeding by *Pseudocalanus* copepods in the Bering Sea: trophic linkages and a potential mechanism of niche partitioning", is in press at Deep Sea Research II for inclusion in the special issue on the Bering Sea Ecosystem Study (BEST). The second manuscript, "Winter distribution and size structure of *Euphausia superba* populations inshore in the West Antarctic Peninsula", is formatted for submission to Polar Biology. The third manuscript, "Diversity and distribution of small pelagic and benthic eukaryotes in the West Antarctic Peninsula in winter", is formatted for submission to Molecular Ecology. The forth manuscript "Unexpected prevalence of parasite sequences amongst Antarctic marine protists" came out of discussions in the defense of this dissertation, and is formatted for submission to PLoS One.

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Feeding by *Pseudocalanus* copepods in the Bering Sea: trophic linkages and a potential mechanism of niche partitioning

Alison C Cleary, Edward G Durbin, Jennifer Bailey

This manuscript is in press at Deep Sea Research II and will be included in the forthcoming special issue on the Bering Sea Ecosystem Study (BEST). ED conceived of the idea for the study. JB collected the samples. JB determined species identity for each copepod individual. AC determined gut contents OTUs. AC analyzed the data. AC wrote the manuscript with contributions from ED.

Feeding by *Pseudocalanus* copepods in the Bering Sea: trophic linkages and a potential mechanism of niche partitioning

Alison C Cleary*, Edward G Durbin, Jennifer Bailey

University of Rhode Island Graduate School of Oceanography,

South Ferry Road, Narragansett, Rhode Island, 02882, USA

Running head: *Pseudocalanus* feeding in the Bering Sea

* corresponding author: acleary@my.uri.edu, (401) 874-6807

Abstract

Pseudocalanus copepods are small and abundant zooplankton in the Bering Sea ecosystem which play an important role in transferring primary production to fish and other higher trophic level predators. Four morphologically cryptic species, the primarily arctic *P. minutus* and *P. acuspes*, and the more temperate *P. newmani* and *P. mimus*, are found within the Bering Sea. *Pseudocalanus* are generally considered phytoplanktivores. However, their feeding is poorly known, despite their importance to the ecosystem. *In situ* feeding by the three most abundant *Pseudocalanus* congeners, *P. minutus*, *P. newmani*, and *P. acuspes*, was investigated by sequencing partial 18S rDNA (ribosomal Deoxyribonucleic Acid) of gut contents from 225 individuals sampled from 8 stations across the Bering Sea in May and June of 2010. The 28,456 prey 18S rDNA sequences obtained clustered into 138 distinct prey items with a 97% similarity cut-off, and included diatoms, dinoflagellates, microzooplankton, mesozooplankton, and vascular plants. *Pseudocalanus* diets reflected variations in the environment, with phytoplankton sequences relatively more abundant in copepods from stations with higher water column chlorophyll a concentrations. Feeding differences were observed between species. *P. acuspes* diet contained relatively more heterotrophic dinoflagellate sequences, and was significantly different from that of *P. minutus* and *P. newmani,* which both contained relatively more diatom sequences, and between which no significant difference was observed. Feeding differences between the two primarily arctic species may be a mechanism of niche partitioning between these spatially co-located congeners and may have implications for the effects of climate change on the success of these abundant zooplankters and their many predators in this ecosystem.

Keywords: *Pseudocalanus*, Bering Sea, trophic interactions, niche partitioning, 18S rDNA

1. Introduction

The competitive exclusion principle in ecological theory suggests that no two sympatric species can occupy precisely the same ecological niche, as one will inevitably eventually outcompete the other (Gause 1934, Hardin 1960). In the Bering Sea, four morphologically cryptic species of *Pseudocalanus* copepods have been identified (Frost 1989, Bailey et al. this issue), which at first glance appear to violate this principle. These four species are similar in both size and overall morphology and traditional morphometric and meristic traits are often unsuccessful at differentiating among them, although they are genetically distinct and can be distinguished by DNA sequencing (Frost 1989, Bailey 2012, Bailey et al. this issue). *Pseudocalanus minutus* and *P. acuspes* are considered primarily arctic, while *P. newmani* and *P. mimus* are considered primarily temperate, but all four species ranges overlap in the Bering Sea (Frost 1989, Coyle et al. 2011). In order for these species to persist in their coexistence in the Bering Sea, they must be different from each other in at least one ecologically meaningful way, such as susceptibility to disease or predators, timing of reproduction, or feeding.

As a location for investigating copepod niche partitioning, the Bering Sea is particularly interesting as it is a region with high levels of interannual variation and alternating 4 to 5 year periods of relatively warm and cool temperatures (Stabeno et al. 2012). Changes in physical forcing may have bottom up ecosystem effects, such as by

changing phytoplankton bloom dynamics (Stabeno et al. 2012, Winder & Sommer 2012). Warming has also been associated with a shift from a largely benthic ecosystem to a more pelagic dominated system, giving small zooplankton such as *Pseudocalanus* spp. an increasingly important role in the transfer of carbon and energy from primary producers to pelagic higher predators (Overland & Stabeno 2004).

Although *Pseudocalanus* spp. are small (1-2 mm in length), their abundance makes them important components of food webs and carbon cycling in the Bering Sea ecosystem (Frost 1989, Napp et al. 2002). *Pseudocalanus* are particularly important as prey for walleye pollock, *Theragra chalcogramma,* one of the most commercially important fish stocks in the US, since larval pollock diet consists of up to 60% small copepods (Coyle et al. 2011). Understanding the range of prey consumed by *Pseudocalanus in situ* and the relative importance of different prey types is essential to understanding the role this abundant consumer plays in transferring primary production to higher trophic levels.

Little is known about the specific prey types consumed by *Pseudocalanus in situ*; results published to date have lumped all *Pseudocalanus* species together (Lebour 1922, Marshall 1949, Poulet 1973). Incubation experiments in natural seawater and water from mesocosms have suggested that *Pseudocalanus* spp. copepods are able to feed effectively on particles from 4 to 102 μm diameter, with a potential preference for particles 25 to 57 μm (Poulet 1973, Harris 1982). Microscopic analysis of *Pseudocalanus* gut contents has shown that they consume diatoms, including *Coscinodiscus*, *Paralia*, *Navicula*, and *Thalassiosira* spp., and to a lesser extent, crustaceans, radiolarians, and flagellates (Lebour 1922, Marshall 1949).

The relative paucity of data on *Pseudocalanus* feeding is not surprising, since measuring feeding by small zooplankton is methodologically challenging. Previous work used incubation experiments and particle counters (Poulet 1973), but these may not be representative of feeding *in situ* and give fairly coarse resolution of prey type. Microscopic examination of gut contents provides *in situ* data, but is strongly biased towards prey with distinctive exoskeletons and typically only limited samples can be analyzed by this labor intensive approach (Lebour 1922, Marshall 1949). Recent advances in DNA analysis and sequencing have allowed for new molecular approaches to understanding zooplankton feeding. DNA barcodes from gut contents can be sequenced, and by comparing these sequences to reference databases of known organisms, the identity of every known eukaryote the predator, in this case *Pseudocalanus,* had consumed in the preceding minutes can be determined (Cleary et al. 2012, Durbin et al. 2012, O'Rorke et al. 2012, Hu et al 2014, Craig et al. 2014). Unlike microscopy-based diet analysis, 18S rDNA sequencing allows for identification of the full range of eukaryotic prey items consumed, including soft bodied and morphologically indistinct prey. Feeding differences have been inferred as mechanisms of niche partitioning in other copepods (e.g. von Vaupel Klein 1997); however, in their study feeding was not directly measured, but rather assumed to be a function of body size and morphology.

This study examined 18S rDNA in *Pseudocalanus* gut contents to elucidate feeding by *P. minutus, P. newmani,* and *P. acuspes* in the eastern Bering Sea, to address questions of niche partitioning by these cryptic congeners and improve understanding of food webs and carbon flows through this ecosystem. The gut contents of 225 *Pseudocalanus* individuals from across the shelf and shelf break region were analyzed

using Peptide Nucleic Acid (PNA) probes and a high throughput sequencing approach to determine what they had consumed, how environmental factors affected feeding, and whether there were differences in consumption between species which might serve as a mechanism of ecological niche separation.

2. Methods

2.1 Field sampling and species identification

Copepods and environmental data were collected in the eastern Bering Sea between May 19 and June 10, 2010. *Pseudocalanus* spp. copepods were collected in vertically integrated net tows from 60 m (or 1 m above the seafloor where depth <60 m) to the surface at 15-20 m min^{-1} with a 153 μm mesh 1m ring net at 8 stations (Table 1 and Fig. 1). Mixed plankton samples were immediately preserved in 95% ethanol, to minimize effects of digestion and potential net feeding, and ethanol was changed once after 12 to 24 hours to maintain concentration (Passmore et al. 2006). Temperature, salinity and fluorescence profiles were obtained with a SBE 19+ CTD at the same stations as copepods were collected, with temperature and salinity at the depth of the *in situ* fluorescence maximum used in station comparisons. Total chlorophyll and chlorophyll >5μm were measured flourometrically from extracted pigments of water collected at this same depth of maximum *in situ* fluorescence (M. Lomas, unpublished data). In the lab, individual *Pseudocalanus* spp. copepods were picked from mixed plankton samples under a dissecting microscope and rinsed thoroughly in clean 95% ethanol (Bailey et al. this issue). DNA was extracted from each whole individual using the DNeasy Blood and Tissue kit (Qiagen) (Bailey et al. this issue). Species identity of

each copepod was determined through sequencing of cytochrome oxidase gene fragments by Bailey et al. (this issue).

2.2 Gut contents 18S rDNA amplification and sequencing

Peptide Nucleic Acid Polymerase Chain Reaction (PNA-PCR) was used to amplify the partial 18S rDNA of all eukaryotes in *Pseudocalanus* gut contents from pools of DNA extracts from 5 conspecific individuals from a single net tow (Cleary et al. 2012, O'Rorke et al. 2012). Triplicate pools were analyzed of each species at each of the stations, for 15 copepods in total from each species in each net tow where sufficient individuals were collected (Table 1). One technical replicate, with all stages of analysis after DNA extraction run separately, was run on one of the pools of 5 copepods from *P. acuspes* at station 99, for an overall total of 46 samples. Each reaction contained 1x GoTaq Green master mix (Promega), 0.5 µmol L^{-1} each 960F and 1200R primers (Gast et al. 2004), 20µmol L^{-1} PNA (5'-TGCTCAATCTCGTGCGAC-3'), and approximately 0.5 ng μL^{-1} template DNA. An initial denaturation at 95° C for 30s, was followed by 30 cycles of 94° for 30s, 77° for 30s, 58° for 30s, 60° for 45s, and a final extension at 60° for 5 min. To remove any remaining genomic DNA, amplicons were electrophoresed on a 0.8% agarose gel and the entire lane between approximately 2000 and 20 base pairs was excised with a sterile scalpel based on the migration of dye fronts of Crystal 5x DNA Loading Buffer Tri-Color (Bioline).

Amplicons were gel extracted and purified using the Wizard SV kit (Promega) as per manufacturer's directions. 454 sequencing adaptors and 8 base pair sample identification tags for each of the 46 samples were attached to the amplicons in a second PCR, containing 1x GoTaq Green master mix (Promega), 0.5 µmol L^{-1} each 454-tag-

1200Rrc Forward and 454-960Frc Reverse primers (modified from Gast et al. 2004), and 30% by volume 18S amplicons from the first round of the PCR. Reverse compliments of the PNA-PCR primers were used here in the addition of 454 adaptors to maximize sequencing resolution of the more variable 3' region of the amplicon. Following an initial denaturation at 95^{o} C for 30s, 9 cycles of 94^{o} for 30s, 65^{o} for 30s, 72^{o} for 45s, were run, with a final extension at 72^{o} for 5 min. No-template controls were carried through all of the above steps along with each set of 6 samples, and visualized at the completion on a 0.8% agarose 1x TAE gel with ethidium bromide – ultra-violet light imaging to confirm the absence of contaminants.

454-tagged amplicons were purified using AmPure (Beckman-Coulter) and sample DNA concentration was quantified using the DNA 1000 kit on a 2100 Bioanalyzer (Agilent). Equimolar aliquots of amplicons from each sample were combined into one of two template pools (23 samples per pool). Each template pool was sequenced in 1/16th pico-titer plate on a GS FLX+ 454 platform (Roche) at the University of Illinois WM Keck Center following standard protocols.

2.3 Sequence data analysis

Sequence data was denoised to remove sequencing errors (Quince et al. 2009, Edgar 2010), separated by sample tag, and clustered into Operational Taxonomic Units (OTUs) with 97% identity by UClust in MacQiime (Caporaso et al. 2010). A phylogenetic tree was constructed of all prey OTUs using Fasttree in MacQiime (Price et al. 2010) and visualized in FigTree v. 1.4. Subgroups of related prey OTUs from this tree were then combined with related known organisms from GenBank using known phylogeny and BLAST (Altschul et al. 1990) to create 11 sub-trees of OTUs and related

organisms. General phylogeny of sub-trees was confirmed with literature comparisons (Baroin-Tourancheau et al. 1992, Collins 2002, Cavalier-Smith & Chao 2003, James et al. 2006).

For each OTU, the nearest taxonomic identity was determined from these sub-trees, and was typically chosen as the taxon into which both the nearest and second nearest neighbor were classified; in some instances where reference sequences were sparse and general tree topology agreed well with known phylogeny, only the nearest neighbor was used. This approach resulted in different degrees of specificity for different prey items. Literature searches were used to identify OTUs whose nearest taxonomic identity was known to be parasitic (Ho & Perkins 1985, Evans et al. 2008, Guo et al. 2012) and these OTUs were excluded from all subsequent dietary analyses. Additionally any OTUs for which the nearest taxonomic identity was *Pseudocalanus*, suggesting the sequence originated from the copepod itself, or which clustered only with mammalian pseudogenes, suggesting potential trace human DNA contamination, were excluded from prey analysis.

All data were normalized by total sequence abundance per sample prior to analysis and a variety of transformations were tested. Multivariate statistics were run on the abundance of each OTU in each 5-copepod sample in Primer6 (Clark & Gorley 2006). Results are reported for both root-transformed abundance data and presence-absence transformed data. We present both statistical results, because while presence-absence results may be less subject to potential biases arising from variations in 18S rDNA copies per cell, such an analysis will tend to give disproportionately more weight to rare items (Clarke & Warwick 2001). In the case of pyrosequencing, where even very

rare trace OTUs are detected, such a presence absence analysis may not provide the most ecologically meaningful comparisons. The relative sequence abundance analysis is similar to previous successful analysis of broadscale diet differences in larger predators (Jarman et al. 2013).

Bray-Curtis distances were calculated as a measure of the similarity of the prey assemblages recovered from each sample. Analysis of Similarity (ANOSIM) was used to determine differences between species and stations, and SIMPER analysis was used to determine which prey items drove observed differences. BioEnv was used to compare diet data with the potential explanatory variables of species, station, on-off shore, total chlorophyll and chlorophyll >5μm (M. Lomas unpublished data), mixed layer temperature, mixed layer salinity, straight line distance to nearest land, water column depth, latitude, longitude, water column stability (the difference between the density at 5m and the density at 100m depths), density at 5 m, and pycnocline depth. Sea ice was not included because none was observed at any of the stations analyzed. Linear regressions of gut contents and environmental chlorophyll were run in Excel 2007. For all analyses each sample of 5 copepods was considered a separate data point.

3. Results

3.1 Prey spectrum

A total of 28,456 prey sequences, which clustered into 138 OTUs were obtained from the 46 samples (GenBank accession #s KC952737 through KC952871). The PCR and sequencing technical replicates had a Bray-Curtis similarity of 79.75% (*P. acuspes*, station 99). Biological replicates had a lower average similarity of 48.36% between

replicate pairs. Overall average similarity across all species and all samples was 29.35%. Each sample contained on average 19.5 different OTUs (± 5.6 stdv, min 10, max 33).

OTUs found in gut contents included a wide phylogenetic range of organisms, including single- and multi-cellular autotrophs and heterotrophs (Fig. 2 and Table 2). Diatoms were among the most abundant and diverse groups of prey items. Centric diatom OTUs included four different *Thalassiosiraceae*, seven different *Chaetoceros*, and one each *Attheya*, *Amphipora*, *Porosira*, *Proboscia*, and *Rhizosolenia*. Pennate diatom OTUs included two different *Achnanthales*, and one OTU each of *Pseudo-nitzschia*, *Navicula*, *Stauroneis*, *Fragilariopsis*, and *Fragilaria*. Ten different diverse dinoflagellate OTUs were found in *Pseudocalanus* gut contents, including groups thought to be predominantly autotrophic (e.g. *Gyrodinium*) and predominantly heterotrophic (e.g. *Polykrikos*, *Amoebophrya*). Green alga in gut contents included *Chlorellaceae*, *Klebsormidiaceae*, and *Prasinococcus*. Sequences from the prymnesiophyte *Phaeocystis* were also present in gut contents. In addition to microalgae, autotroph sequences in *Pseudocalanus* gut contents included four vascular land plants: *Pinus* (pine) and *Betula* (birch) trees, a *Camellia* (tea/flower) bush and a *Poaceae* (rice/wheat) grass.

Pseudocalanus gut contents also showed evidence of predation by these small copepods, including 18S rDNA from a variety of metazoan prey. Crustacean OTUs found include a *Metridia* (copepod), three different *Euphausacea* (krill), one *Hyperiidea* (amphipod), and a *Cirripedia* (barnacle). Other metazoan OTUs included *Sagitta* and *Eukrohnia* (chaetognaths), a *Pteropoda*, an *Ophiuroid* (brittle star), *Bdelloidea* and *Plomida* (rotifers), *Ctenophora*, and *Cnidarians*. Heterotrophic protist OTUs in *Pseudocalanus* gut contents included one *Labyrinthulia*, a *Ciliophora*, a *Centrohelida*, a

slime mold, two different *Oomycetes*, two different *Saccharomyces* (yeasts), and 18 other fungi. (A full list of prey items is available in the Web Appendix)

3.2 Parasites within Pseudocalanus

Thirteen OTUs were identified as parasitic organisms and excluded from the diet analysis. These parasite OTUs included one *Digenea* trematode, six different gregarines, three *Foettingeridae* (Oligohymenophorea) ciliates, and three *Amoebophrya* dinoflagellates. Overall, parasite OTUs made up 17.7% of the total recovered sequences, with parasites in separate samples of 5 copepods ranging from 0% to 93% of the recovered sequences. *P. minutus* showed the highest percent parasitic sequences, and the highest number of different parasites per sample (mean 27% parasite sequences, 3.1 OTUs), with *P. newmani* showing intermediate parasite abundance and diversity (mean 11% parasite sequences, 2.3 OTUs), and *P. acuspes* with the lowest parasite abundance and diversity (mean 0.7% parasite sequences, 1 OTU) (ANOVA $p<0.01$ for both % parasite sequences and number of parasite OTUs by *Pseudocalanus* species).

3.3 Environmental effects on diet

Water column chlorophyll a concentration was positively correlated with the percent of gut contents sequences represented by phytoplankton (Fig. 3). This trend was most pronounced in *P. minutus* which was found over the full range of environmental chlorophyll levels (linear regression r^2=0.93), but was also present in *P. acuspes* and *P. newmani*. At the stations with the lowest environmental chlorophyll levels, relatively more microzooplankton, mesozooplankton, and gelatinous organism sequences were recovered (Fig. 5). Although the trend of increasing proportion of phytoplankton in

Pseudocalanus diets with increasing environmental chlorophyll is clear, it is not possible to determine the form of this relationship (linear, exponential, logistic, etc.) without more data from intermediate environmental chlorophyll levels.

Within the diatom OTUs, diet diversity was highest, particularly among the *Chaetoceros* spp., at intermediate chlorophyll levels, while at both high and low chlorophyll concentrations the diatom sequences in gut contents were composed mainly of a single *Thalassiosiraceae* OTU (Fig. 4). It is worth noting, however, that because many species of *Thalassiosiraceae* are identical over the 18S rDNA gene region sequenced, this OTU may contain a complex of related species. *Pseudocalanus* species and water column salinity explained 51% of the variance in BioEnv analysis, with the addition of longitude explaining an additional 1% of the variance.

3.4 Species differences

P. newmani and *P. minutus* diets were not significantly different from each other (root-transformed p=0.07, presence-absence p=0.34). *P. acuspes* diet was significantly different from *P. newmani* (root-transformed p=0.001, presence-absence p=0.01), and under the more representative root-transformed data, was also significantly different from *P. minutus* (root-transformed p=0.001, presence-absence p=0.08). *P. newmani* and *P. minutus* diet contained relatively more diatoms, while *P. acuspes* diet contained relatively more heterotrophic dinoflagellates (Fig. 5). The OTUs which explained the most of this difference (SIMPER) were a *Thalassiosiraceae*, a *Polykrikos* dinoflagellate, an *Amoebophrya*, and a *Cnidaria*.

4. Discussion

4.1 Prey item diversity

The range of prey items found in *Pseudocalanus* spp. gut contents in this study was very large. 138 different prey item OTUs were identified, and an average of 19 of these prey OTUs were present in each sample of five conspecific copepod individuals. Prey items were taxonomically diverse, and included soft-bodied organisms which could not have been detected with traditional microscopy-based techniques, and unusual prey items which would not have been detected by more targeted molecular approaches.

Diatoms in *Pseudocalanus* spp. gut contents included species known to be common in the Bering Sea, and which have been previously observed in *Pseudocalanus* spp. gut contents. *Thalassiosiracea* spp. were the most frequent and abundant sequences in gut contents, and are typically one of the major constituents of spring blooms in this region (Aizawa et al. 2005). All of the groups of diatoms that early studies observed microscopically in *Pseudocalanus* gut contents (Lebour 1922, Marshall 1949) were also detected with DNA in this study, with the exception of *Paralia*, a tachypelagic species typically associated with resuspended sediments, and hence more commonly found in shallower waters.

Pine and birch tree DNA in *Pseudocalanus* gut contents likely originated from pollen blowing out to sea and being consumed by these copepods. Birch pollen abundance in southern Alaska peaks in late May (Municipality of Anchorage, 2013), during the time these *Pseudocalanus* were collected. Terrestrial organic matter represents a large input of carbon to the ocean through both riverine and atmospheric transport, but its fate and potential incorporation into marine food webs is poorly understood (Hedges et al. 1997). Birch tree DNA was found in just under half of the samples and all but one

of the stations analyzed, and pine tree DNA was found in nearly a third of the samples and at every station, although overall sequence abundance of both these tree OTUs was much lower than that of the most common diatom prey items. Pine pollen is typically 30 to 50μm in size, while birch pollen is slightly smaller, around 15 to 30μm (Davis & Goodlett 1960). Both of these fall into the 25 to 57μm range previously suggested as optimal for *Pseudocalanus* consumption (Poulet 1973). Further understanding of the quantitative importance of terrigenous material ingested by zooplankton may be useful in modeling carbon fluxes in this ecosystem, particularly the fluxes of carbon from the land to the marine environment, and in predicting Bering Sea ecosystem responses to continuing climate change, with potential changes in the timing and magnitude of inputs, such as pollen, from the terrestrial realm.

4.2 Method assessment

While this data was rigorously quality controlled, it is possible that some of the sequences recovered did not originate from direct ingestion of the sequenced organism by a *Pseudocalanus*. It is possible that trace amounts of prey were adhered to the exoskeleton of the copepods. Copepods used in this analysis were rinsed thoroughly in fresh ethanol prior to DNA extraction and there were no visible organisms attached to the exoskeleton. Although this approach is less rigorous than some which have been suggested (Greenstone et al. 2012, O'Rorke et al. 2013) rinsing with ethanol has been considered to be effective in removing contaminants in some cases (Greenstone et al. 2012) and given the small size of *Pseudocalanus* and the relative permeability of marine crustacean exoskeletons, this approach reduced surface contaminants without risk of damaging gut contents DNA. It is still possible that some of the "prey" DNA, was in fact

adhered to the exterior of the copepod, particularly that of gelatinous organisms, such as ctenophores and chaetognaths (Durbin & Casas 2014, O'Rorke et al. 2013). However, this external contamination is likely to be relatively low in abundance compared with true gut contents. Additionally, external contaminants would be likely to affect all *Pseudocalanus* species equally within a sample; something not supported by our observations of substantial differences between species.

In order to eliminate parasites, we took a conservative approach of excluding from the dietary analysis sequences of any organism known to be parasitic. This may have excluded some parasitic organisms which had been consumed as prey items. Alternately, true parasites not yet reported in the literature as such would have been included as prey. We found that known parasites tended to show relatively low frequency amongst samples but high sequence abundance, since unlike prey, parasites are alive and are not in the process of digestion. On this basis we excluded one additional OTU with very poorly resolved phylogeny which was present in a single sample at high abundance. While starved copepod controls would have been ideal to determine which sequences might originate from symbionts of *Pseudocalanus*, given our conservative approach with the analysis of the available samples, we do not think that any inadvertent inclusion of parasite OTUs as prey significantly affected our diet results.

Although DNA sequences can be used to identify the organisms consumed, they do not provide information on the life stage or body part of the organisms which were consumed. Some of the sequences recovered may thus have originated from feeding on eggs, larvae, fragments of carcasses, exuvea, or fecal pellets of the sequenced organism either singly, or in aggregates or marine snow. Copepods, including species

morphologically similar to *Pseudocalanus* have been observed to feed on marine snow (Green & Dagg 1997), and such feeding may explain some of the unusual prey items observed here. Feeding on fecal pellets might potentially explain euphausiid sequences found in *Pseudocalanus* gut contents. Euphausiids produce thin string-like fecal pellets which are bound by a membrane, and this membrane contains euphausiid DNA. *Pseudocalanus* feeding on euphausiid fecal pellets would consume this membrane, and therefore contain euphausiid 18S rDNA. Prey DNA has been found in feces of copepods (Nejstgaard et al. 2003, Vestheim et al. 2005, Durbin et al. 2012). Although it is possible that the contents of the fecal pellet might also be detected in the *Pseudocalanus* gut contents, this DNA would have already been subject to digestion and degradation in the euphausiid gut, so this doubly digested prey is unlikely to show a strong DNA signature in our analysis. Prey-of-prey has been observed as a potential challenge in studies using species or group specific primers (Sheppard et al. 2005). However, in the universal primer approach applied here, such doubly digested prey-of-prey, derived from fecal pellets or gut contents of consumed prey, is likely to be strongly outcompeted by the more abundant and less degraded prey consumed directly. Thus, though prey-of-prey may be detected, they are likely to occur at very low frequency and abundance and unlikely to be a significant source of error in the diet analysis.

Technical variation from PCR and sequencing was low, with replicates providing very similar gut contents information, lending confidence to the reproducibility of pyrosequencing gut contents. Biological variability between groups of copepods of the same species from the same net tow was higher, with Bray-Curtis similarities on average less than 50%, suggesting difference between individuals in their feeding over the time

immediately prior to capture. As DNA is rapidly digested, this variability is not surprising since individual copepods will vary in their recent feeding history. This variability between biological replicates was still considerably lower than the variability observed between different species and stations sampled, and did not obscure overall patterns in diet.

Direct comparisons of sequence data with ingested biomass suggest that, at least for larger predators, relative sequence abundance offers a broadscale indication of the relative importance of different prey items (Deagle et al. 2010, Murray et al. 2011). In our study the strong correlation between relative abundance of phytoplankton OTU sequences and environmental water column chlorophyll a concentrations suggests these abundances may offer at least a semi-quantitative view of the abundance of each prey item; these OTU abundances may potentially reflect the total prey biomass consumed rather than the number of prey individuals consumed. Additionally, although potential biases due to variations in copy number per cell, digestion, extraction and PCR amplification efficiency are likely to vary between different prey items, they are likely to be consistent across different *Pseudocalanus* individuals. Because these biases are likely to be relatively uniform across all the samples analyzed, relative OTU abundance is used to provide a comparison between species which is less influenced by the rare sequences, and thus may be more representative of actual *in situ* feeding.

4.3 *Effects of environmental variation*

Pseudocalanus gut contents included a greater proportion of sequences from phytoplankton where environmental chlorophyll a levels were higher. This suggests that when phytoplankton were abundant, *Pseudocalanus* consumed them, whereas when

phytoplankton were relatively rare, *Pseudocalanus* fed on alternative prey items such as meso- and microzooplankton. This ability to feed on alternative prey items may be important to *Pseudocalanus* during periods of low phytoplankton abundance, such as winter and late summer. Switching between herbivory and carnivory has been observed in other copepod species as a function of the relative abundances of different prey types (Landry 1981, Kiørboe et al. 1996). Prey switching may not only buffer the copepods themselves from changing environmental conditions, but also allow for a refuge from predation for low abundance prey items, thus helping to maintain diversity in the plankton (Landry 1981).

Amongst the environmental variables analyzed, salinity was the best predictor of diet. Salinity changes have been previously found to explain temporal variations in copepod assemblages, and it has been hypothesized that this is due to bottom up effects (Pershing et al. 2005, Mountain & Kane 2010, Ji et al. 2012). Salinity variations may drive changes in water column stability with effects on the relative success of diatoms and dinoflagellates; potentially leading to different prey assemblages available for *Pseudocalanus* to feed on (Mountain & Kane 2010, Hinder et al. 2012). However, water column stability, pycnocline depth, and density at 5m were not found to be good predictors of diet here. Thus, in this case, salinity may be acting rather as a tracer for different water masses with varying prey assemblages.

4.4 Niche separation

P. minutus and *P. newmani* showed very similar diets, while *P. acuspes* diet was significantly different from that of *P. minutus* and *P. newmani*. Diet differences were maintained across the range of chlorophyll a concentrations encountered. These diet

differences were driven largely by broad differences in the types of prey consumed, with *P. acuspes* consuming relatively more heterotrophic dinoflagellates and to a lesser extent macrozooplankton, while *P. minutus* and *P. newmani* consumed relatively more diatoms. Of the diatoms consumed, no differences in selection for specific types were observed between any of the three species. Feeding differences between species may result from or be a reflection of small-scale variations in copepod species distribution. Species preferences for locations in the water column are poorly known, and may impact feeding, by affecting the available prey field of different species. Differences in broad prey types consumed suggest feeding differences may also potentially be driven by differences in feeding behavior. Copepods typically catch diatoms and other non-motile prey by maintaining a relatively stationary position and generating a feeding current, whereas motile prey such as dinoflagellates are typically captured by passively sinking copepods detecting hydromechanical signals from the prey items and then capturing them in a form of ambush feeding (Kiørboe et al. 1996). *P. minutus* have been observed to filter water through their mouthparts almost constantly, consistent with our observation of a diet relatively rich in non-motile diatoms (Tiselius & Jonsson 1990). Differences observed between *P. minutus* and *P. newmani* versus *P. acuspes* diets suggest potentially different allocations of effort to these two feeding strategies.

P. minutus and *P. acuspes*, which are both considered primarily arctic (Coyle et al. 2011), and hence occupy largely overlapping geographic ranges, show diet differences, suggesting potential ecological niche partitioning through feeding differences. By contrast *P. minutus* and *P. newmani* which have largely similar diets are more geographically separated, as *P. newmani* is considered to be a primarily temperate

species (Coyle et al. 2011), suggesting they may fill similar ecological niches in these different geographic areas. Feeding differences have previously been suggested as a mechanism of niche partitioning amongst co-located copepods (Maly & Maly 1974).

4.5 Potential implications for the future

The Bering Sea is experiencing rapid warming and changes in the patterns of interannual variation in response to anthropogenic climate change; and the effects of these changes have already been observed in both physical and biological components of the Bering Sea (Overland & Stabeno 2004, Stabeno et al. 2012). Overall *Pseudocalanus* abundance has been found to be either negatively correlated with or independent of temperature in the Bering Sea (Coyle et al. 2011, Stabeno et al. 2012). Changes in sea ice and storminess may affect water column stability, with implications for the relative success of diatoms and dinoflagellates (Edwards & Richardson 2004, Edwards et al. 2006, Hinder et al. 2012), and any such changes could have opposite implications for *P. minutus/P. newmani* and *P. acuspes.*

Diet has been shown to affect zooplankton fecal pellet sinking speeds, leading to variations in the efficiency of the biological pump and the sequestration of carbon in the deep sea (Atkinson et al. 2012). Diets rich in diatoms are associated with denser faster sinking fecal pellets and more efficient carbon export (Atkinson et al. 2012). *P. minutus* and *P. newmani* feeding may thus be a relatively efficient mechanism for the export of carbon from the mixed layer, while feeding by *P. acuspes* may instead enhance microbial recycling within the upper water column. Interspecific differences in susceptibility to changing environmental variables, and roles in the biological pump suggest that

ecosystem monitoring efforts might benefit from differentiating these morphologically cryptic congeners.

Given that *P. minutus* and *P. newmani* have similar diets it is possible that the more temperate *P. newmani* may take over some of the southern part of the range of *P. minutus*, under continued warming. Such a geographic shift in the transition zone between *P. minutus* and *P. newmani* would likely have minimal top down effects since their diet is so similar. However, *P. minutus* stores lipids, while *P. newmani* does not (Coyle et al. 2011), which may change their nutritional value as prey items to higher trophic levels, with potentially negative implications for commercially important predator species such as walleye pollock, ecologically important groups such as seabirds, and culturally important organisms even higher up the trophic web, such as marine mammals. The high diversity of prey items found in *Pseudocalanus* gut contents suggests these organisms are able to consume a wide range of different prey types, and may potentially be able to feed opportunistically. Opportunistic feeding may allow *Pseudocalanus* populations to help buffer higher trophic levels in this productive ecosystem from bottom-up effects induced by changing climate.

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Table 1: Characteristics of sampling locations and species analyzed at each station. Station (Stn) numbers correspond to Bailey (2012), species numbers indicate how many individuals of each species were analyzed, latitude and longitude are in decimal degrees, all dates are in 2010, total bottom depth is in meters and chlorophyll a (Chla) represents the >5 µm size fraction in µg L^{-1}at the depth of maximum chlorophyll a fluorescence.

Stn	*P. acuspes*	*P. minutus*	*P. newmani*	*Latitude*	*Longitude*	*Date Sampled*	*De pth*	*Chla*
49	0	30	30	59.8998	-178.8960	May 19	486	20.1
55	0	15	0	58.2043	-174.2357	May 21	381	0.4
87	0	15	15	55.4315	-168.0608	May 29	205	6.2
99	15	0	15	56.8536	-164.5056	May 31	73	3.8
156	0	15	15	62.1890	-175.1521	June 5	79	3.8
163	0	15	0	59.8934	-178.8983	June 7	657	4.1
175	15	0	0	59.9003	-172.2170	June 9	73	0.8
179	15	15	0	58.8301	-168.1589	June 10	46	0.8

Table 2: Most abundant Operational Taxonomic Units (OTUs) from *Pseudocalanus* gut contents, with nearest taxonomic identity, total number of sequences, presence/absence count out of 45 total samples, and cumulative % of total sequences. These top 29 OTUs account for 27,044 sequence reads, which is 95% of the total prey sequence reads found. The complete list of gut contents OTUs can be found in the Web Appendix.

Rank	*GenBank Ass. #*	*Prey Organism*	*Total*	*Count*	*Cumulative %*
1	*KC952766*	*Thalassiosiraceae* diatom	9451	45	33.2%
2	*KC952848*	*Polykrikos* dinoflagellate	4239	29	48.1%
3	KC952832	*Cnidaria*	2189	30	55.8%
4	KC952803	*Ameobophrya* dinoflagellate	1974	40	62.7%
5	KC952751	*Mertensia* cnidarian	1340	31	67.5%
6	KC952857	*Fragilaria* diatom	1003	19	71.0%
7	KC952818	*Fragilariopsis* diatom	867	27	74.0%
8	KC952820	*Ophiuroid* brittle star	857	2	77.0%
9	KC952779	*Euphausiid* krill	723	37	79.6%
10	KC952790	*Polykrikos* dinoflagellate	508	15	81.4%
11	KC952860	*Sagitta/Krohnitta* chaetognath	495	37	83.1%
12	KC952772	*Phaeocystis* prymnesiophyte	454	21	84.7%
13	KC952833	*Chaetoceros* sp. diatom	452	18	86.3%
14	KC952738	*Chaetoceros* sp. diatom	379	12	87.6%
15	KC952837	*Chaetoceros* sp. diatom	349	23	88.8%
16	KC952807	*Chaetoceros* sp. diatom	255	15	89.7%
17	KC952745	*Pezizomycete* fungus	230	26	90.5%
18	KC952802	Grain (rice/wheat/corn)	212	15	91.2%
19	KC952765	*Betula* sp. Birch tree	205	21	92.0%
20	KC952752	*Metridia* copepod	124	10	92.4%
21	KC952748	*Pinus* sp. Pine tree	115	14	92.9%
22	KC952749	*Rhizochaete* fungus	94	16	93.2%
23	KC952767	*Agaromycete* fungus	93	17	93.5%
24	KC952867	*Navicula* diatom	91	10	93.8%
25	KC952824	*Porosira* diatom	77	4	94.1%
26	KC952756	*Semaeostomae* cnidarian	76	4	94.4%
27	KC952777	Dinoflagellate	70	8	94.6%
28	KC952791	*Cryothecomonas* flagellate	63	1	94.8%
29	KC952823	*Oligohymenophorea* ciliate	59	4	95.0%

Figure legends

Figure 1: Map of sampling locations in the Bering Sea. The 25, 50, 75, 100, 200 and 1000m isobaths are shown. Stations 49 and 163 were in the same location on different dates.

Figure 2: Phylogenetic tree of all prey 18S rDNA sequences recovered from *Pseudocalanus* spp. Circle size indicates in how many of the 45 samples each Operational Taxonomic Unit (OTU) was identified, with no circle indicating less than 10. Branch and circle colors indicate prey identity and correspond to colored text. Gelatinous organisms fall into two distinct clades as this group is defined by morphology, and includes the phylogenetically diverse ctenophores, cnidarian, and chaetognaths.

Figure 3: Environmental chlorophyll a concentrations and phytoplankton as a percent of total prey sequences in *Pseudocalanus* gut contents. Circles and solid line show *P. minutus*, triangles and dotted line show *P. acuspes*, squares and dashed line show *P. newmani*. All trend lines show linear regressions.

Figure 4: Diatom diversity in gut contents of each sample arranged as a function of chlorophyll a concentration. Colored bars indicate the proportion of the total diatoms in gut contents contributed by each diatom OTU (left-hand axis). White circles show water column chlorophyll a concentration (right-hand axis). Vertical white lines separate each station, while brackets below the x axis indicate the copepod species, with "a" *P. acuspes*, "m" *P. minutus*, and "n" *P. newmani*.

Figure 5: Differences in diet among species and with chlorophyll a concentration. Pie charts show the relative abundance of sequences belonging to each type of prey in *Pseudocalanus* gut contents.

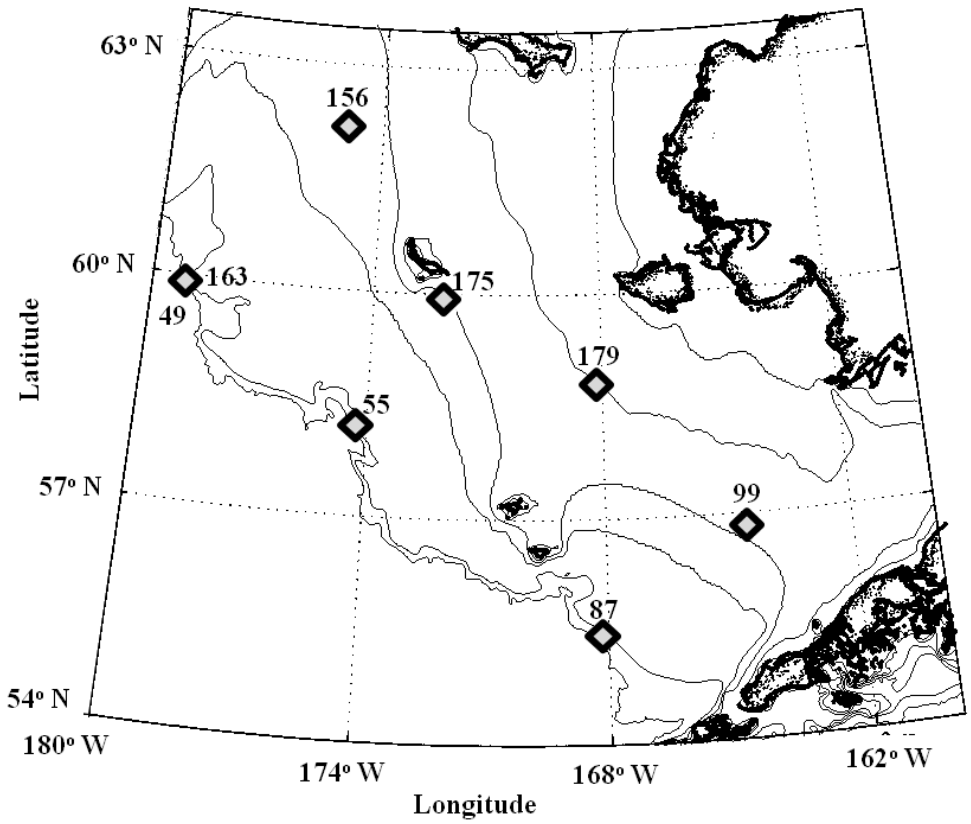
63° N
60° N
57° N
54° N
180° W
174° W
168° W
162° W
Latitude
Longitude
156
163
49
175
179
55
99
87

Fig. 1

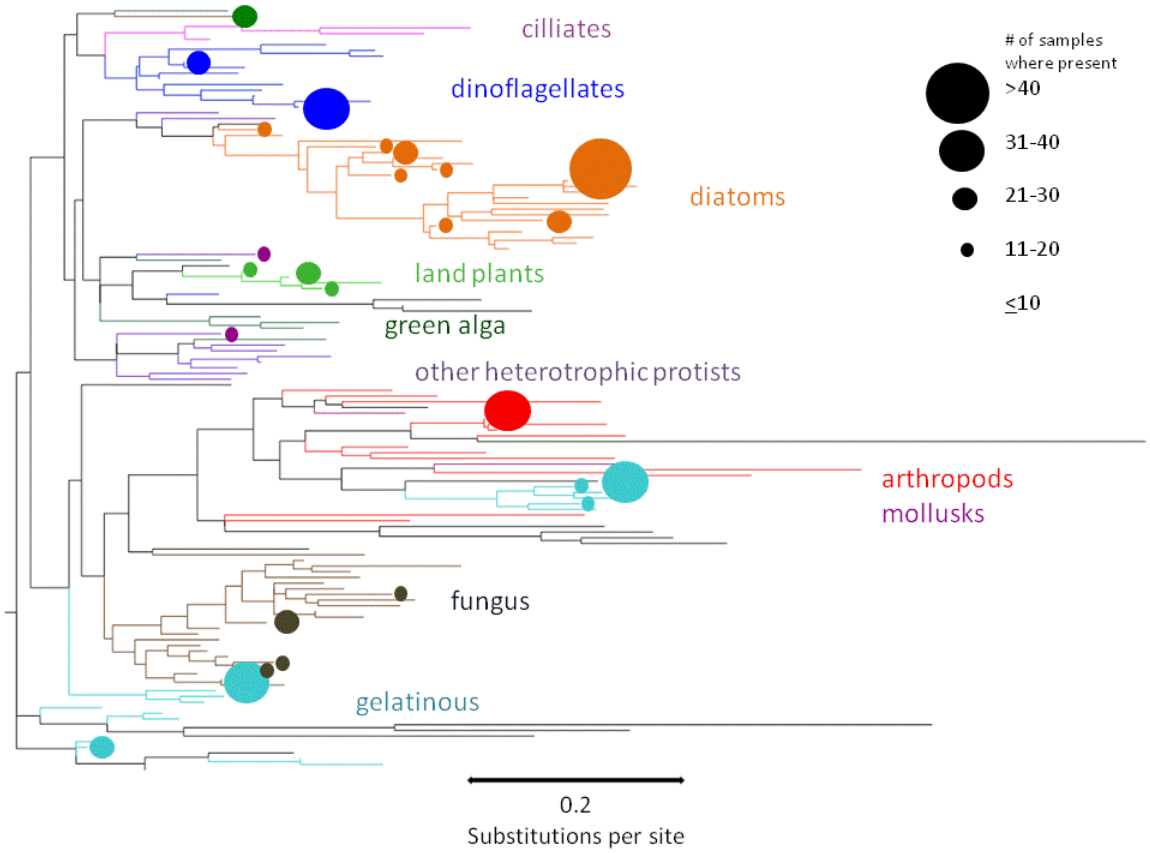
cilliates
dinoflagellates
diatoms
land plants
green alga
other heterotrophic protists
arthropods
mollusks
fungus
gelatinous
# of samples where present
>40
31-40
21-30
11-20
≤10
0.2
Substitutions per site

Fig. 2

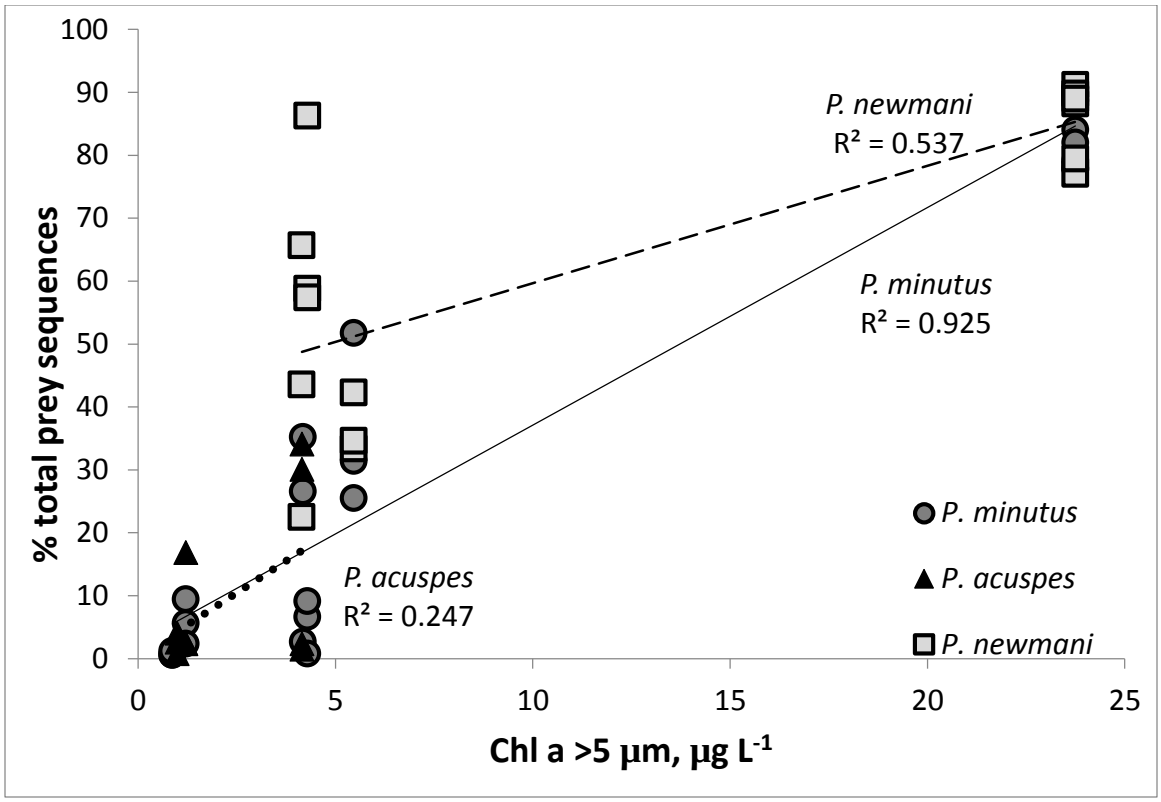
100
90
80
70
60
50
40
30
20
10
0
% total prey sequences
0
5
10
15
20
25
Chl a >5 µm, µg L-1
P. newmani
R² = 0.537
P. minutus
R² = 0.925
P. acuspes
R² = 0.247
P. minutus
P. acuspes
P. newmani

Fig. 3

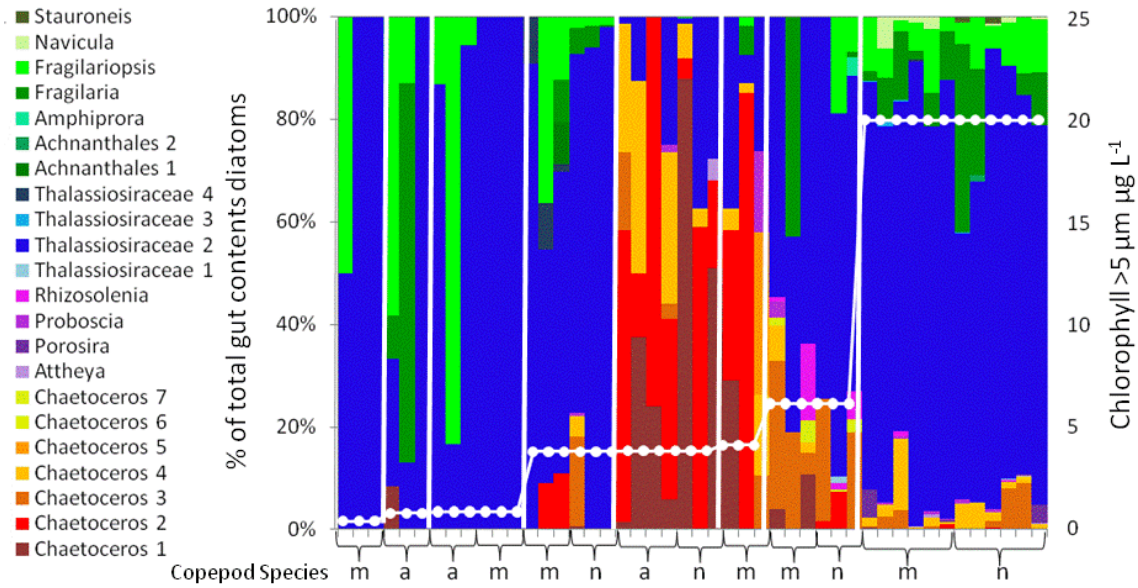
Stauroneis
Navicula
Fragilariopsis
Fragilaria
Amphiprora
Achnanthales 2
Achnanthales 1
Thalassiosiraceae 4
Thalassiosiraceae 3
Thalassiosiraceae 2
Thalassiosiraceae 1
Rhizosolenia
Proboscia
Porosira
Attheya
Chaetoceros 7
Chaetoceros 6
Chaetoceros 5
Chaetoceros 4
Chaetoceros 3
Chaetoceros 2
Chaetoceros 1
% of total gut contents diatoms
100%
80%
60%
40%
20%
0%
25
20
15
10
5
0
Chlorophyll >5 µm µg L-1
Copepod Species
m a a m m n a n m m n m n

Fig. 4

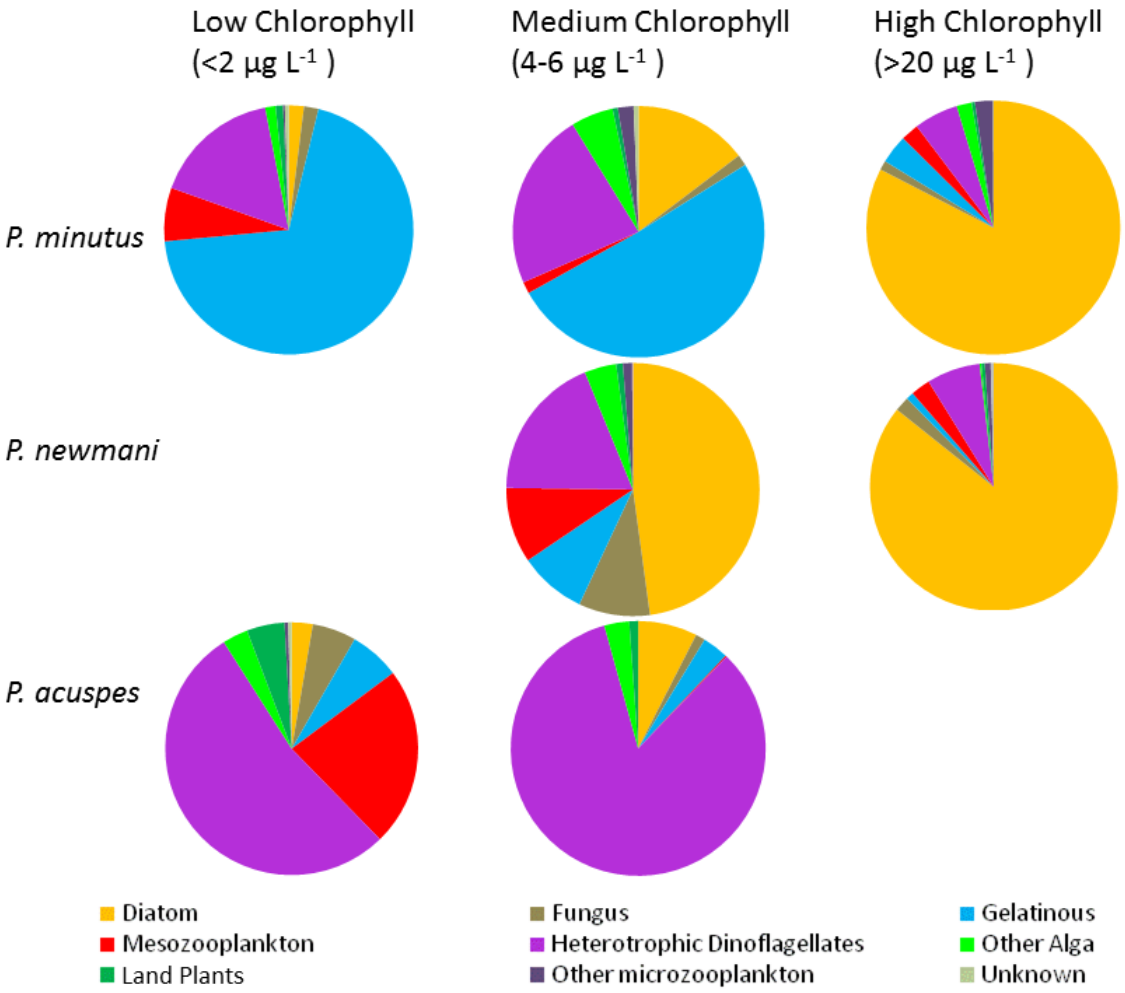
Low Chlorophyll
(<2 µg L-1)
Medium Chlorophyll
(4-6 µg L-1)
High Chlorophyll
(>20 µg L-1)
P. minutus
P. newmani
P. acuspes
Diatom
Mesozooplankton
Land Plants
Fungus
Heterotrophic Dinoflagellates
Other microzooplankton
Gelatinous
Other Alga
Unknown

Fig. 5

Winter distribution and size structure of *Euphausia superba* populations inshore in the West Antarctic Peninsula

Alison Cleary, Maria Casas, Edward Durbin, Meng Zhou

This manuscript is in preparation for submission to Polar Biology. ED conceived of the idea for the study. MC collected length weight data and MZ collected acoustic data. AC analyzed the data. AC wrote the manuscript with contributions from ED.

Winter distribution and size structure of *Euphausia superba* populations inshore in the West Antarctic Peninsula

Alison Cleary*[1], Maria Casas[1], Edward Durbin[1], Meng Zhou[2]

1 – Graduate School of Oceanography, University of Rhode Island, Narragansett, Rhode Island, USA

2 – Department of Environment, Earth and Ocean Sciences, University of Massachusetts Boston, Boston, Massachusetts, USA

* Corresponding author: acleary@my.uri.edu, (415)300-6556

Abstract

Antarctic krill, *Euphausia superba*, are a key component of food webs in the maritime West Antarctic Peninsula, and their life history is tied to the seasonal cycles in sea ice and primary production in the region. Previous work has shown a general in-shore migration of krill in winter, however the very near shore has not often been sampled as part of these surveys. We investigated distribution, abundance, and size structure of krill in two bays along the peninsula, and in the adjacent Gerlache Strait using vertically stratified MOCNESS net tows and acoustic biomass estimates. Krill abundance was high within bays, with net estimated concentrations exceeding 60 krill m^{-3}, while acoustic estimates were an order of magnitude higher. Krill within bays were larger than krill in the Gerlache Strait, though they had slightly lower condition indices. Within bays, krill aggregations were observed near the seafloor during the day, and exhibited diel vertical migration higher into the water column at night, potentially balancing optimizing feeding, predation risk, and metabolic costs. This abundance of nearshore krill helps to refine an increasingly complex picture of krill ecology and adaptability. Including these nearshore krill may increase populations in stock assessments and understanding the ecological role of these nearshore krill aggregations may have implications for managing the krill fishery this region.

Key_words: *Euphausia superba*, winter, distribution, DVM, Andvord Bay, Gerlache Straight

Introduction

Antarctic krill, *Euphausia superba*, (hereafter "krill") are key members of Southern Ocean ecosystems. Krill serve as important prey to many megafauna; in the West Antarctic Peninsula (WAP) region krill make up over 90% of the diet of numerous species of baleen whales and brush-tailed penguins, and over a third of the diet of additional species of seals, birds, and fish (Quetin & Ross 1991). These small pelagic crustaceans have a huge global biomass, estimated at over 300 million tons, with 70% of the stock found in the narrow Atlantic sector from 0^o to 90^o W, encompassing the WAP (Atkinson et al. 2008).

This huge biomass of krill in the Southern Ocean is not distributed evenly on any spatial scale, with strong patchiness on scales from thousands of kilometers around the continent, to meters within and between aggregations (Atkinson et al. 2008, Hamner & Hamner 2000). Krill are at the "awkward boundary between plankton and nekton" (Atkinson et al. 2008); their distribution can be strongly influenced by current flows, but they are also strong swimmers, capable of moving up to 15 cm s^{-1} horizontally or vertically (Lascara et al. 1999).

Much of the research on krill distributions in Antarctica has been focused on the productive and more accessible summer season (Atkinson et al. 2008). A few studies have investigated patterns in the seasonal distributions of krill, both in general and along the WAP. The general paradigm has been that krill spend the summer feeding in aggregations along the shelf break and in the waters beyond, with females laying eggs into the deep waters beyond the shelf; while in the fall and winter krill migrate to more inshore areas (Siegel 1988, Nicol 2006, Lascara et al. 1999, Atkinson et al. 2008). The

smallest individuals have generally been found closest in-shore, where they may rely on sea ice for refuge from predation or food resources (Atkinson et al. 2008, Lascara et al. 1999, Siegel 1988, Lawson et al. 2004). The ecological reasons for this migration remain unclear, although it has been suggested it may improve feeding or reduce advection out of the favorable WAP region (Siegel 1988).

Many of the studies that have addressed seasonal variations in krill distribution have sampled much lower levels of krill in winter as compared to other seasons. Lascara et al. (1999) found krill in winter at just one of their 25 acoustic stations, with total biomass estimates an order of magnitude lower than those in the same region in summer. Seasonal sampling in Marguerite Bay also observed much lower biomass in winter than in fall (Lawson et al. 2004). Earlier surveys showed similar changes, with over an order of magnitude more krill estimated to be present in summer than in winter, when only 0.086 individuals m^{-3} were found, leading to the idea of a winter krill "vacuum" (Siegel 1988).

Unlike for smaller zooplankton, seasonal changes in observed krill abundance cannot be a result of population growth or contraction. Krill are long lived, taking two to three years to reach sexual maturity, with lifespans estimated at five to seven years (Lascara et al. 1999). Additionally, the fall/winter reduction in krill abundance, and corresponding spring increase, is evident in krill from a wide range of sizes (Atkinson et al. 2008, Siegel 1988) Given both the multiyear life cycle of krill, and the parallel abundance patterns amongst different age classes, the seasonal decline and increase in observed krill abundance is more likely to be due to krill entering and exiting the sampled

waters. Limited nearshore sampling has suggested this may be where large krill are found in winter (Lawson et al. 2004).

Although krill are thought to move inshore in winter, the very most inshore regions of the WAP have been poorly sampled, and are missed by many standardized sampling programs. The coast of the WAP is complex and convoluted, with a series of deep bays and fjords. Vessel safety considerations mean that standardized or randomized transects of the type most often used in broad scale surveys are typically not possible within these areas (Johnston et al. 2012). Sampling within Wilhelmina Bay has shown the presence of krill "super-aggregations" suggesting this very nearshore region may be important winter habitat for krill (Nowacek et al. 2011).

In this project we used adaptive sampling with nets and acoustics to investigate the distribution, abundance, and size structure of krill within Andvord and Flandres Bays and in adjacent comparison areas of the Gerlache Strait in winter. By sampling krill in this poorly known very nearshore region in winter we aimed to refine our understanding of krill seasonal distributions, and the implications of these distributions for the ecology and life history of *E. superba* in the WAP.

Materials and Methods

Setting

Krill and acoustic data were collected on the Research Vessel Ice Breaker Nathaniel B. Palmer between May 16 and June 15 2013 (figure 1 and table 1). Where possible paired day-night tows were analyzed. Tows 7 and 8 occurred in Andvord Bay, tows 14 and 15 in Flandres bay, tows 18 and 19 in the Gerlache Strait, and a single tow,

tow 20 was analyzed from Palmer Deep, the most offshore area sampled. Mixed layer chlorophyll was uniformly low throughout the study, with all values below 0.4 µg L^{-1} as determined from fluorometric measurements of extracted pigments. Mixed layer temperatures ranged from -1.7°C to +0.5°C, with salinities from 33.4 to 34.4 psu, both as measured by 911plus CTD (SeaBird) (See appendix C). Sampling locations were based on bathymetry as observed with multibeam, and maintaining safe distances from coastlines and large icebergs.

Net Sampling

Vertically stratified samples of krill were collected with a 1 m^2 Multiple Opening Closing Net Environmental Sensing System (MOCNESS) (Wiebe et al. 1976) equipped with nine 333 µm mesh black nets. The maximum dimension of the mesh in a 333 µm net is 0.47 mm along the diagonal; since krill have a typical width to length ratio of 1:8 (Zhou & Dorland 2004), the largest krill which would be able to go through the mesh would be 4 mm in length. The MOCNESS is therefore quantitatively sampling krill individuals greater than 4 mm in length. Two LED strobe lights (Brightwaters Instruments) were attached to the frame above the net mouth, and flashed continuously throughout all tows at approximately 2 flashes per second with a nominal light output of 3 watts, in order to reduce net avoidance behavior by krill (Sameoto et al. 1993, Wiebe et al. 2004). The net was towed obliquely at a 45° angle at 1.5 to 2 knots from 50 m above the seafloor to the surface. Net opening and closing depths varied between tows and were determined based on real-time acoustic data in order to maximize resolution of aggregation structure. Each net filtered between 53 and 900 m^3 of water.

Net catches were processed immediately. Catches were split on board the ship using a bucket splitter, (a 20L cylinder with a 5 cm diameter tube extending from the bottom to a T-junction with identical 5cm outflow tubes pouring into 2 separate 20L cylinders) as many times as required for a sample of roughly 150 to 200 ml biovolume. Split samples were preserved in a 4% final concentration solution of sodium borate buffered formalin in seawater. In the laboratory, catches were further split if necessary for a final target sample size of 100 individuals. The final counted sample ranged from the full net catch to a 128^{th} split. Krill were removed from the few non-krill zooplankton, and all krill individuals were measured for length and dry weight. Only juvenile and adult krill were analyzed, larvae were not included, setting a de-facto lower size limit close to the 1.5 cm length at which krill typically molt from larvae to juveniles (Siegel 1987). Both Standard 1 (base of eyestalk to posterior end of uropods) and Discovery (front of eye to tip of telson) lengths were used, as these have both been widely reported in the literature (Everson 2000). Measuring both of these metrics allows the results of this study to be broadly comparable, and also provides a conversion for use in comparing other studies. Individual krill were dried at 60^{o}C for 24 or more hours and weighed on a BP310S microbalance (Sartorius).

Data processing was conducted in Excel and MatLab to calculate numbers and biomass per m^3 of water filtered and per m^2 of water column in each 0.25 cm length increment size fraction for each net. Size bins are designated in the figures by their upper size cut-off. Condition index was calculated as: $condition = \frac{1000 * dry\,weight}{standard1\,length^3}$ with weight as dry weight in grams and length as Standard1 in cm (Ricker 1975). Length-weight analysis and all regressions were conducted using MatLab's curve-fitting toolbox.

Based on length weight analysis extreme outliers were removed from the data set as they were unlikely to be true *E. superba* (n=4). Statistical differences between tows were investigated using ANOVA and Tukey tests (multcompare) as implemented in MatLab. Cluster analysis was conducted to look at the assemblage of different sized krill in each net using UPGMA with the number of krill in each 25mm size bin, normalized by the total number of krill in the net, as input and calculations done with the Cluster and Linkage functions in MatLab.

Acoustics

Acoustic Doppler Current Profiler (ADCP) data were continuously collected and processed from a hull-mounted unit (Teledyne) with all instruments, settings and preliminary data processing as per Zhou & Dorland (2004). ADCP data were observed in real-time at sea, and were further examined in the laboratory by looking at profiles of 2 to 4 hour blocks of time throughout the cruise in MatLab. Binned and processed ADCP data (8 m depth bins from 32m depth to 400m depth and 6 minute time bins) was used to compare acoustic and net estimates of biomass. ADCP backscatter was analyzed to investigate patterns in the depth of krill aggregations. For each time interval the depth of maximum biomass was found in the ADCP record. These calculations excluded the 10m immediately above the seafloor, as determined by Knudsen echosounder, due to potential noise from side-lobes of the ADCP beams, and excluded any time interval in which the maximum biomass did not exceed 100 grams m^{-3}, as such time intervals may indicate areas without krill or bad data due to bow-thruster noise. In order to compare MOCNESS and ADCP estimates of krill biomass, for each MOCNESS net the corresponding acoustic backscatter estimate was calculated by averaging all ADCP bins within the time

interval of the complete tow and the depth interval of the net. The complete tow time rather than the net time was used because the ADCP is sampling the water directly under the vessel, and the net is behind the vessel, so the full tow time should give a more representative sample. MOCNESS counts were multiplied by mean weight in each net to give a biomass concentration, and a wet weight to dry weight conversion factor of 0.2 was applied (Schmidt et al. 2011). Latitude and Longitude from MOCNESS logs and ADCP files were compared to ensure the correct time range of bins were being averaged, and the sum of all measured krill was calculated to ensure all krill were accounted for in the calculations, which were initially conducted with size fractionated counts.

Results

Net Sampling

In total, 3051 krill were counted and measured from 39 discrete depth interval nets in 7 tows encompassing two bays and the more offshore Gerlache Strait and Palmer Deep regions. Krill ranged in length from 0.9 to 5.1 cm and in dry weight from 0.001 g to 0.217 g. The two length measurements, Standard 1 and Discovery, were highly correlated with Discovery length =1.002* Standard1 lnegth +0.09. The slope was not significantly different from 1 and the line fit had an r^2 of 0.999. For the remainder of the results Standard 1 length is used. Krill-length weight fit the expected power model, with weight=0.0014*length$^{2.98}$ and r^2=0.934(figure 2). This model fit the data from all samples well, although differences were seen, and are discussed in the condition index. The exponent was not significantly different from the theoretical 3 (Ricker 1975), indicating that for the post-larval krill sampled here growth was isometric.

The distribution of krill lengths showed a strong peak at sizes of 2.75 to 3.25 cm, with some evidence of smaller secondary peaks at 1.25 to 1.5 cm and 4.25 to 4.5 cm (figures 3 & 4). The larger size classes showed a fairly consistent pattern of contributions from the different MOCNESS tows, but the smallest four size classes showed disproportionately high contributions from the Gerlache Strait stations, with tows 19 and 20 making up over 50% of the krill 2 cm and under, despite making up only 2% of the overall total (figures 3 & 4). The peak in abundance between 2.75 and 3.25 was observed in all sampling locations, but with higher abundances in the fjords, while the small size class peak at 1.25 to 1.5 was most noticeable in the more offshore Gerlache Strait and Palmer Deep samples (figure 4).

Both the abundance and average size of krill were higher in the inshore stations than in the Gerlache stations (figure 5 and table 2). Tow 14 (Flandres Bay at night) had by far the highest average abundances with 34.15 krill m^{-3}, followed by 8 and 15 (Andvord & Flandres Bays, respectively in the day). Tows19 and 20 (Gerlache Strait & Palmer Deep, respectively, at night) had the lowest average abundance, with 0.71 and 0.16 krill m^{-3} respectively. Length distributions of krill were significantly different between all tows except for tows 8, 14, and 15, (Andvord daytime, Flandres nighttime, and Flandres daytime, respectively) which are not significantly different from each other and had the highest mean values of 3.1, 2.9, and 2.8 cm respectively. Tows 7 and 18 (Andvord nighttime & Gerlache daytime, respectively) had intermediate mean lengths of 2.6 and 2.5 cm respectively, while tows 20 and 19 (Gerlache Strait & Palmer Deep nighttimes, respectively) had the lowest mean lengths at 1.6 and 1.4 cm respectively. Not only did these more offshore tows 19 and 20 have the highest relative abundances of krill

in the smallest size fractions, they also had the highest total abundances of krill less than 2 cm in length (figures 3 & 4).

The condition index showed similar groupings, but with different trends from length. Tows 8, 14, and 15 (Andvord daytime, Flandres nighttime, and Flandres daytime, respectively) were again not significantly different from each other, but these tows had the lowest condition krill. Tow 7 (Andvord nighttime) was different from all other tows, with an intermediate condition. Tows 18, 19, and 20 (Gerlache Strait daytime & nighttime & Palmer Deep) were not significantly different from each other and showed the highest condition. Condition was found to be most variable in the smallest individuals, particularly those under 2 cm in length. Considering only these smallest individuals greatly reduces the size of the data set, and the power to discern trends, but the overall pattern remains similar; with tow 14 intermediate and not different from any other tows, tow 18 having the highest condition and different from all except 7 and 14, with 20 next highest and again different from all except 14, while tows 8, 15, and 19 were not significantly different from each other and exhibited the lowest condition indexes.

Vertical patterns of krill abundance showed both diel and spatial differences (figure 6). Within Andvord and Flandres Bays a diel pattern was observed with krill more concentrated and deeper during the day, and more dispersed and shallower at night (figure 6 & table 2). In the Gerlache Strait the major day-night difference observed was not in the vertical distribution but in the overall size class distribution with larger krill collected in the day than at night. No data is presented for Palmer Deep 100- 150 or 500-700 meters as the cod-ends were lost at sea.

Cluster analysis showed one large grouping, and one very distinct smaller grouping (figure 7). This smaller grouping consisted of all of the nets in tows 19 and 20 (Gerlache & Palmer deep nighttimes), and the surface net in tow 18 (Gerlache daytime). These offshore nets tended to have smaller krill relative to the net samples collected in bays. Four of the six nets in tow 7 (Andvord nighttime) made up a sub-cluster within the large grouping; tow 7 tended to have a more even distribution of size classes, with both more small krill and more large krill, but relatively fewer medium krill, as compared to the other tows within bays. The large grouping included nets from tows 8, 14, 15, and below surface 18, which clustered with no clear patterns. These nets all tended to have relatively large fractions of krill in the 2.75 to 3.5 size classes, with relatively few of the largest and smallest size fractions.

Acoustic sampling

Net estimates and acoustic estimates of krill biomass for each net do not correlate particularly well (figure 8). A linear fit has a slope of over 15 and $r^2 = 0.20$, and even the best fit with a power curve has an $r^2=0.23$. Looking more closely at the points which fall far from the trend line, the two points near the top of the y axis correspond to two nets in tow seven, in which the ADCP showed narrow, strong bands of scatterers, but the net catches were low. In the opposite direction, the two points furthest along the x axis which appear too low in terms of ADCP estimates were from a school with a particularly high spectrum broadening, an indication of relatively high swimming behavior (Zhou & Dorland 2004).

General observations from the acoustic data were similar to patterns observed in MOCNESS tow catches, despite the poor point-by-point comparison. Krill abundance

was higher within bays than in the Gerlache Strait and offshore regions, with particularly high abundances near the coast (Figure 9). Within the bays sampled krill biomass was generally higher than outside the bays. During the day, krill were typically concentrated near the seafloor, in dense layers typically 50 to 100 m thick. Where the seafloor was shallower than 300 m, these aggregations were right on the sediment interface; where the seafloor was deeper, the aggregations tended to separate from the sediment but stay deep in the water column, typically between 200 and 300 m. At night the krill tended to come up in the water column and form less dense aggregations. These night time aggregations were typically concentrated between 100 and 200 m depth. Peak krill abundance typically followed these patterns, with peak abundance either close to the seafloor or between 100 and 200 m depth (Figure 10). Outside of the bays in the Gerlache Strait area acoustic observations were generally very low, with the few observations of higher biomass generally close to the coasts.

Discussion

High krill abundances found in the fjords in winter were above densities typically observed in the WAP. Within fjord abundances ranged from 235 – 8061 krill m^{-2} and 54 - 1733 grams m^{-2}, well in excess of previously observed average values. Across the WAP as a whole including the offshore areas during the summer, averages of 3.4 krill (2.1 g WW) m^{-2} have been observed with nets (Siegel et al. 2013). In the Elephant Island region long term abundances have averaged 45 krill m^{-2}, or 0.23 krill m^{-3} (Siegel et al. 2013). Further afield to the north, krill biomass has been estimated for South Shetlands (1-60 g m^{-2}), and South Georgia (1.87-40.57 g m^{-2}), and to the south vertically integrated krill biomass in Marguerite Bay (1.3-77.7 g m^{-2}) (Lawson et al. 2008 and references therein).

Abundances observed here in the more offshore Palmer Deep and Gerlache Strait stations are in-line with previous observations in the area, with 156-284 krill and 8-43 grams m^{-2}. The similarity of the observed abundances in the shelf region to previous surveys suggests the observed high abundances within fjords are not artifacts of our method, but rather indicate true, though typically under-sampled, regions of particularly high winter krill abundance.

The observed abundances suggest that fjords may be important habitat for krill in the WAP region, at least in early winter as sampled here. Previous suggestions of the importance of nearshore habitat for *E. superba* include Lawson et al.'s (2008) observation of highest krill abundances in fall and one of two winters close to shore in Marguerite Bay, and Zhou et al.'s (1994) observations of high krill abundance in the Gerlache Strait region.

Distributions of size fractions

The overall size distribution considering all tows together observed here is similar to previous observations. Zhou et al. (1994) observed a peak in krill length frequency of 22 mm for krill collected in the Gerlache Strait. This peak size is between that observed in the present fjord samples and the present offshore samples. His sampling was in an area geographically between the fjords and offshore areas analyzed in this study, at the same time of year. Brinton et al. (1987) observed size frequency distributions in March of 1984 which showed a pattern similar to what was observed here, with a main peak around 30 mm and a smaller peak around 6 mm, with MOCNESS tows near Elephant Island. Hernandez-Leon (2001) observed a smaller size distribution, with a peak around 17mm length outside of Wilhelmina Bay, very close to our study region in summer; while Siegel

(1987) encountered more larger individuals than were encountered in this study, with many krill over 5 cm in length. The differences in the overall size caught in this study and that of Siegel may be due to methods, where Siegel's RMT trawl with larger mesh and higher tow speeds may be more difficult to escape than the MOCNESS used here. Krill collected within the fjords in winter mainly fell into the size of age 1+ krill, individuals which would be roughly 18 months old at the time of sampling (figures 3 & 4). Although the peaks in the length frequency observed in this study do not correspond particularly well with published length-at-age estimates for this region and season (figure 3), the largest peak observed here is between previous estimates for age 1+ krill and age 2+ krill, while the small secondary peak is between previous estimates for young-of-the-year and age 1+ krill from the same time of year as our sampling (Siegel 1987, Quetin et al. 1996). It seems most likely that the krill in this study are slightly larger than the krill used in the previous estimates, suggesting the small peak observed contains mainly young-of-the-year, individuals which had been born the previous summer, while the main peak observed was composed of age 1+ krill, with some larger and older krill in the higher end tail of the observed distribution.

Broad scale surveys have typically observed smaller krill inshore of larger krill (Lascara et al. 1999, Siegel 1988, Atkinson et al. 2008, Quetin & Ross 2003). This is different from the pattern observed here, over a smaller scale, with the smallest young-of-the-year krill collected offshore of the larger age 1+ krill, with the 2+ and older krill not observed and hypothesized therefore to be further offshore. Siegel (2005) suggested a seasonal pattern in which the age 1+ and older krill move further onshore in the winter, with the 1+ krill moving the closest onshore, and the young-of-the-year krill staying out

over the shelf break. This conceptual model is not fully in agreement with the observations here, where young-of-the-year were on the shelf and age 2+ and older krill were largely absent from the shelf area. These broadscale surveys have typically not sampled the very most inshore regions, and the few instances of such very near shore sampling have observed high acoustic biomass with either large krill individuals (Lawson et al. 2004) or juveniles (Lawson et al. 2008). It has been suggested that small krill may utilize sea ice in inshore areas for food resources and habitat (Schmidt et al. 2011, Quetin et al. 1996). It is possible small krill within the bays could have been missed in this study if they were strongly associated with the under-ice habitat, as this part of the water column is not effectively sampled by the MOCNESS. However, previous work with nets more suited to sampling the ice-water interface and SCUBA observations have observed very few individuals in this habitat in winter (Meyer 2012, Quetin & Ross 1996). During the period of sampling for this study, the water was largely open and sea ice was mainly small pancakes. During the late fall and early winter when this sampling was conducted the ice algal community is not yet established, and the underside of the such small ice pancakes are unlikely to be a particularly favorable habitat for small krill, which prefer the more complex structure of established ice and pressure ridges. Thus our under-sampling of this area is unlikely to explain the offshore bias to our distribution of the smallest krill.

There are several possible explanations for the observed pattern of small young-of-the-year krill existing on the shelf, larger age 1+ krill being present mainly within the fjords (figure 4), and age 2+ and older krill somewhere beyond the sampling area, potentially further off shore over the shelf break or beyond. Potential explanations

include differences in swimming speed, advection, metabolic requirements, top-down effects of cannibalism, and some combination of these factors. The roles of each factor are unclear, and some combination may help explain the distributions observed here. Krill swimming speed is a function of individual length, with larger individuals typically exhibiting faster sustained swimming speeds (Kils 1981). Different age classes of krill are of distinctly different lengths, with concomitant differences in sustained swimming speeds. The interactions of these differences in swimming speeds with the advective WAP environment may play a role in the distribution of size classes. Young of the year krill, which were found mainly in the offshore stations of Palmer Deep and Gerlache Strait, may be too small to effectively swim against currents. In the winter these krill may still be passively drifting in the more offshore gyre circulation. The circulation along this part of the WAP is complex with northeastward flow in the Antarctic Circumpolar Current near the shelf break, and south-westward flow in the Antarctic Coastal Current near shore, with a series of smaller gyres connecting these two systems and acting as retention areas for krill (Amos 1984, Quetin & Ross 2003, Nicol 2006). Young of the year krill sampled in this region may still be in the process of drifting inshore following developmental ascent, the growth period in which krill mature from eggs through furcilia while slowly ascending from deep waters (Hempel & Hempel 1986). It has also been suggested that the smallest size classes of krill in the Bransfield Strait may originate in areas of the Weddell or Bellinghausen Seas (Brinton 1991). Thus, the small individuals observed here may have originated in the Gerlache region, or in either of these upstream regions.

Age 1+ krill were highly abundant in the fjords. These fjords may serve as a refuge from advection out of the favorable WAP region. Potentially, age 1+ krill may migrate into these fjords to save energy they might otherwise need to expend in swimming against currents and avoid being swept out of the highly productive WAP.

Larger krill were not observed in the fjords, nor in the offshore samples in winter. Such 2+ and older krill were collected in summer with identical sampling gear (Durbin, unpublished data), suggesting these larger krill are not simply missed due to increased net avoidance skills at greater age and size. It is possible these largest and strongest swimming krill may spend the winters even further from land, where water from the Antarctic Circumpolar Current (ACC) may advect in potential zooplankton prey (Loeb et al. 2009). These largest krill would be better able to swim long onshore offshore seasonal migrations, and better able to maintain position against a current, potentially allowing them to take advantage of these higher food resources not available to smaller krill individuals with lower swimming speeds. There is some evidence of larger krill individuals beyond the shelf break in previous broadscale surveys (Lascara et al. 1999, Siegel et al. 2013). Further winter sampling is needed to better understand the migration and habitat use by age 2+ and older krill in winter.

Krill are known to behave cannibalistically; this has been observed in laboratories ((Cleary, unpublished data) and through gut contents analysis (Ligowski 2000). Avoiding cannibalism has been suggested to be one of the factors driving the life history patterns of krill (Nicol 2006). Since krill are frequently the biomass dominant marine organism in this ecosystem, predation by large krill may be an important mortality factor for small krill. This conspecific predation may be one of the factors keeping young-of-the-year

krill in more offshore waters away from predation by age 1+krill, and/or one of the factors driving age 1+ krill into the fjords and away from the age 2+ and larger individuals. In relatively enclosed bays where a range of sizes of krill were observed together, and encounter rates between large and small krill are potentially high, it may be that by the time of sampling, age 1+ krill had effectively removed many of the small krill from the population. Rather than having been cleared, these different habitat usage strategies may be evolved responses to minimize losses due to cannibalism.

Different ages of krill may also have different metabolic requirements. Young-of-the-year krill have had only a few months to grow, and are not thought to be able to survive prolonged starvation (Meyer et al. 2009). Age 2+ and larger krill may gain a reproductive advantage by winter feeding to fuel early spring reproduction. Early spring reproduction is thought to lead to higher success rates of the offspring by giving them a longer summer season to mature (Ross & Quetin 1986). Age 1+ krill may be in a unique position of having enough resources accumulated to be able to survive prolonged starvation, while also not needing to fuel reproduction in the coming summer. Pelagic food resources in the nearshore areas are low, with vanishingly low concentrations of chlorophyll and very few mesozooplankton (Durbin, unpublished data). The ACC is thought to carry mesozooplankton and potentially other prey items into the offshore areas of the WAP region (Loeb et al. 2009). The ACC however can also potentially advect krill out of the favorable WAP region, and into less productive areas to the north. Thus the more offshore shelf break areas may offer a risky, but potentially rewarding habitat for krill, with potential for food, but also risk of advection. The age-specific food requirements of krill may determine the balance of this risk-reward.

Another unexpected observation was that offshore krill tended to have higher condition indices than krill sampled in bays, even when including only the smallest size classes for which condition was most variable. At first glance it would seem that krill should be concentrated in the areas with the most favorable environmental conditions, which would lead to krill in areas of higher abundance also being in better condition, opposite to the observed pattern. However, krill in the bays may have higher competition for food resources, because of the high densities found there. Offshore krill may also have been advected into the region from areas with more favorable conditions.

No clear patterns in size distribution with depth were observed, with different tows showing different patterns. Cluster analysis also showed that station was a better predictor of the size distribution of a tow than depth, as nets within a tow clustered together more than nets of a particular depth. There has been a previous suggestion that different sizes of krill may utilize different parts of the water column in a form of niche partitioning (Schmidt et al. 2011, Nicol 2006), but no consistent evidence of this was observed in this study.

Near bottom aggregations

Acoustic and net tow observations found krill aggregations in close proximity to the seafloor within bays during the daylight hours. This pattern was more evident in the acoustic data as net tows were restricted to 50 m above the seafloor due to concerns of vessel safety around icebergs. Our near bottom aggregation observations add to the growing evidence that the sediment interface can be an important krill habitat. Schmidt et al. (2011) reviewed studies to date on epibenthic krill aggregations, showing over 14 different studies observing epibenthic *E. superba*. This behavior is widespread, with

aggregations observed in the WAP, as well as in Terre Adélie in East Antarctica (Schmidt et al. 2011). Widespread near bottom aggregations have been previously observed acoustically in winter to the south of our study region (Lawson et al. 2004). Epibenthic krill aggregations may be utilizing sediment food resources, consuming epibenthic and sublittoral diatoms which grow year round in some locations (Ligowski 2000), or feeding on detritus settled out from the water column, which is preserved by the cold temperatures as a kind of "food bank" (Schmidt et al. 2011, Smith et al. 2006). If benthic food resources are indeed important to krill, particularly at times when water column food resources are scarce (Schmidt et al. 2011), energetic balance and access to these food resources may help explain why near-bottom krill aggregations were observed mainly in the very inshore. Relatively shallow inshore bays may reduce the energetic costs of swimming to and from the sea-floor, as compared to similar journeys in the deeper offshore areas; potentially an important savings during the winter period when krill energy budgets may be tight. Thus, sediment feeding may provide a greater net energy gain in shallow inshore areas as compared to deeper areas in the Gerlache Strait and offshore, potentially explaining the higher abundances of near bottom krill observed in the nearshore region.

Diel patterns

Krill were observed to spend the daylight hours at depth and in fairly high concentration aggregations, while during the night krill were more dispersed throughout the upper water column. Similar patterns have been observed in this area previously, with Guzman (1983) and Zhou & Dorland (2004) all noting a pattern of descent at dawn and ascent after dusk. Water column phytoplankton was not available to krill during the

winter period of this sampling, but such diel cycling may be driven in part by endogenous rhythms not sensitive to temporal variations in the available prey field (Teschke et al. 2011).

Minimizing predation risk can be one of the main drivers of Diel Vertical Migration (DVM) behavior. The most prominent predators of krill during our sampling in the bays were humpback whales. Humpback whale abundance within these bays is high in late fall and winter, with estimates of 0.68 whales km^{-2} in Andvord bay, and 1.75 to 5.1 whales km^{-2} in the adjacent Wilhelmina Bay (Johnston et al. 2012, Nowacek et al. 2011). These whales were frequently observed during sampling. Outside of the bays, whale abundance is much lower, at less than 0.1 whale km^{-2}, and they were rarely noted during sampling or transit through these areas. Unlike toothed whales, humpback whale feeding relies on a "significantly visually based prey locating component" (Friedlaender et al. 2009). Humpback whales in the WAP have been observed to show strong diel patterns in feeding, with all or nearly all observed feeding occurring at night (Friedlaender et al. 2013), and similar patterns in whale diel behavior were anecdotally observed during krill collections here. Thus krill may reduce predation risk by avoiding the surface waters, where they are likely to be most accessible to whales, during daylight hours when visibility is greatest for the whales. The distribution of humpback whales in this region also tracks our observed pattern of DVM; in bays where whales were abundant, strong DVM was observed, whereas in the more offshore areas in the Gerlache Strait where whales are relatively scarce, no clear signal of DVM was observed.

With benthic food resources available at the seafloor and very little food in the water column, and a refuge from whale predation at the seafloor as well, why would krill

come to the upper part of the water column at night? Krill may be balancing multiple predation risks, or potentially minimizing metabolic costs. Although prominent, whales are not the only krill predators in the sampled bays. Mackerel icefish (*Champsocephalus gunnari*) were also anecdotally observed in underwater video on the seafloor around the time of sampling, and these predators may pose a predation risk to epibenthic krill. DVM has been suggested to help reduce metabolic costs in zooplankton, by allowing them to spend the day in deeper cooler waters where their metabolic rates are reduced (McLaren 1963). In winter in the bays along the WAP this situation was somewhat reversed, as surface cooling led to a water column in which the coldest waters were consistently those above about 120 meters, with warmer waters below (data not shown). Thus krill moving from daytime near the seafloor up into the water column at night may be experiencing reduced temperatures, allowing for reduced metabolism and energy savings. For example, a krill individual moving from the daytime biomass peak in Andvord Bay up to the nighttime peak would go from 0.09 ^{o}C to -0.14^{o}C for a reduction of 0.25^{o}C, and an individual which chose to transit further would experience an even greater change. Krill metabolism is strongly affected by temperature; for larval krill a change from 0^{o}C to -1^{o}C leads to almost halving of its energy requirement (Quetin & Ross 1989). Krill are efficient swimmers, and transiting from their daytime depths to nighttime depths would only cost a couple of Joules of energy each way (Swadling et al. 2005). It may be that the energy savings from spending the day at slightly colder temperatures outweigh the costs of swimming to and from the seafloor, but more data are needed to understand this trade-off.

Summary

Krill were observed to be abundant in the near shore region of the WAP in winter, with high concentrations observed in Andvord and Flandres Bays. Age 1+ individuals dominated krill assemblages in bays, while the more offshore Palmer Deep and Gerlache Strait were characterized by low overall abundances and primarily young-of-the-year krill individuals. Offshore krill had higher condition indices, suggesting either reduced competition for food, or advection into the area from a different source region. Near bottom krill aggregations were observed in all of the sampled bays, and may have been utilizing sediment food sources. Diel vertical migration from these near bottom daytime aggregations up into the water column at night was observed within bays, but no clear migration pattern was observed in the Gerlache straight. Observed DVM may be an adaptation to trade-offs between availability of sediment food resources, whale predation, and temperature influences on metabolism. These coastal fjords are currently under sampled and may harbor significant parts of the total population, with implications for overall stock assessment, and managing the fishery for krill in the WAP region, Including the observed size distributions in the near shore may help refine recruitment estimates for krill, which are to date largely based on more offshore surveys, and may be biased by missing this fraction of the population (Quetin & Ross 2003). Overall these results illustrate some of the complexities of understanding krill distributions, and indicate the importance of considering the role of very nearshore habitat for *Euphausia superba*.

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Tables

MOC tow	Location	Latitude	Longitude	Date	Time (local)
7	Andvord Bay	-64 48.23	-62 41.56	May 23	22:13
8	Andvord Bay	-64 50.89	-62 35.82	May 24	09:41
14	Flandres Bay	-65 03.88	-63 19.11	May 29	21:47
15	Flandres Bay	-65 00.92	-63 15.28	May 30	09:16
18	Gerlache Strait	-64 51.93	-63 46.30	May 31	11:28
19	Gerlache Strait	-64 51.94	-63 46.12	May 31	20:35
20	Palmer Deep	-64 54.62	-64 13.78	June 1	05:46

Table 1: MOCNESS station information, dates are in 2013, time is local 24 hour time.

MOC tow	7	8	14	15	18	19	20
# krill analyzed	265	286	443	462	578	644	373
Mean length	2.64 (1.01)	3.08 (0.71)	2.93 (0.43)	2.84 (0.72)	2.47 (1.00)	1.45 (0.30)	1.65 (0.64)
Mean weight	0.036 (0.04)	0.045 (0.03)	0.038 (0.02)	0.035 (0.02)	0.034 (0.03)	0.006 (0.01)	0.010 (0.01)
Mean condition	1.558 (0.75)	1.358 (0.32)	1.427 (0.23)	1.361 (0.43)	1.719 (0.56)	1.708 (0.42)	1.765 (0.51)
Abundance	0.785	6.569	34.156	6.794	0.987	0.711	0.162
BWMD	99.52	234.67	159.41	190.35	117.02	149.37	128.10
Krill m^{-2}	235	1807	8061	2038	251	284	156
Krill g WW m^{-2}	53.96	430.01	1733.18	413.90	42.64	7.51	13.27

Table 2: Krill length, weight, and condition for each tow with mean (standard deviation) as well as the number of krill analyzed and and overall abundance for each tow. Length is in cm, weight is dry weight in grams, $condition = \frac{1000 * dry\ weight}{standard1\ length^3}$, abundance is in krill m^{-3}, BWMD is biomass weighted mean depth and is calculated as $\sum_{i=1}^{x} \frac{n_i}{N} * Z_i$ where x is the number of nets sampled, n_i is the biomass in net I (m^{-2}), N is the total biomass in the tow, and Z_i is the midpoint of the net depth interval. Krill m^{-2} gives water column total abundances, and Krill g WW m^{-2} gives water column total wet weight biomass of krill assuming a 1:5 ratio of dry weight to wet weight (Tyler 1973).

Figures

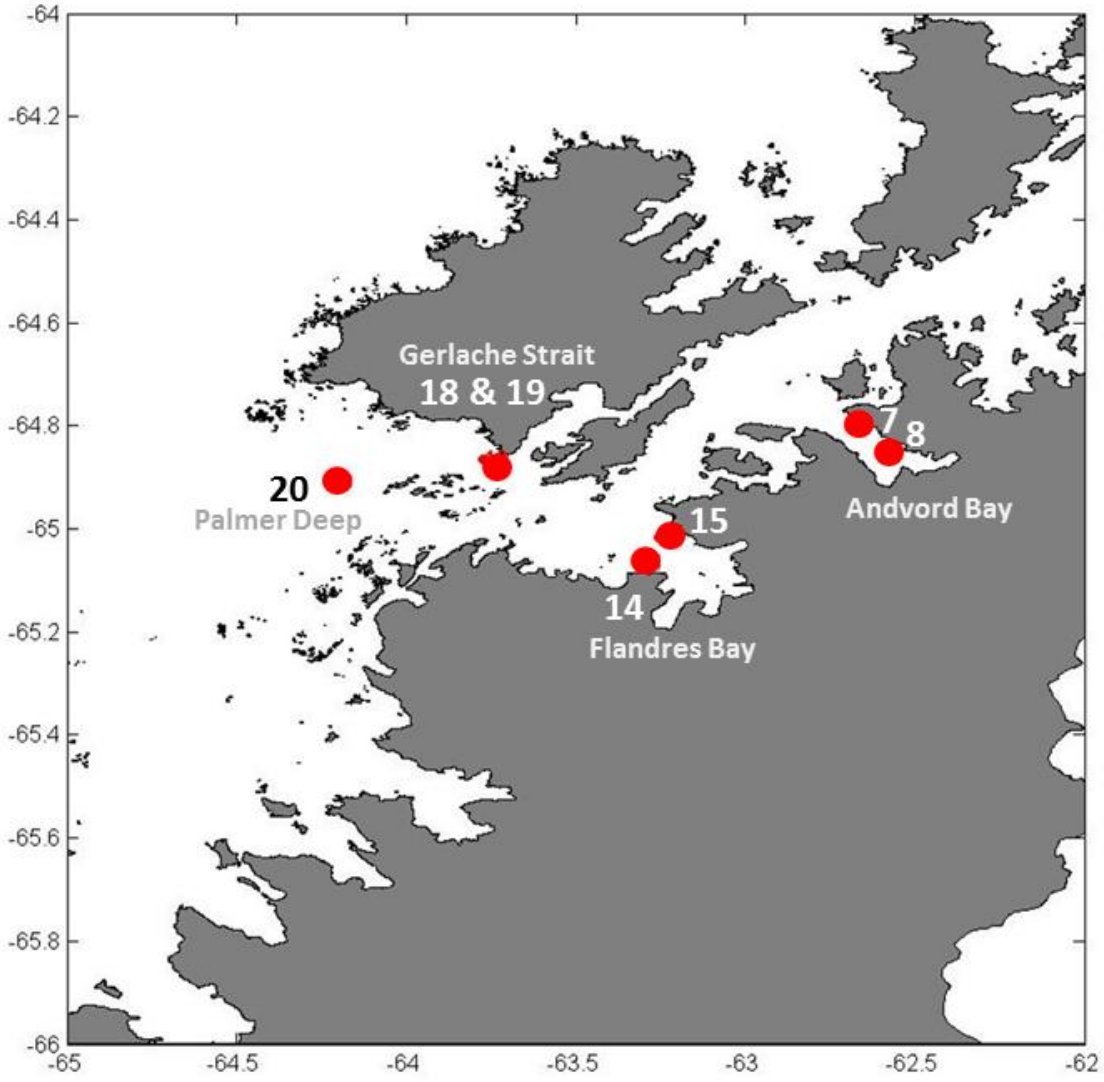

Figure 1: MOCNESS tow locations. Tows 18 and 19 occurred at the same location at different times.

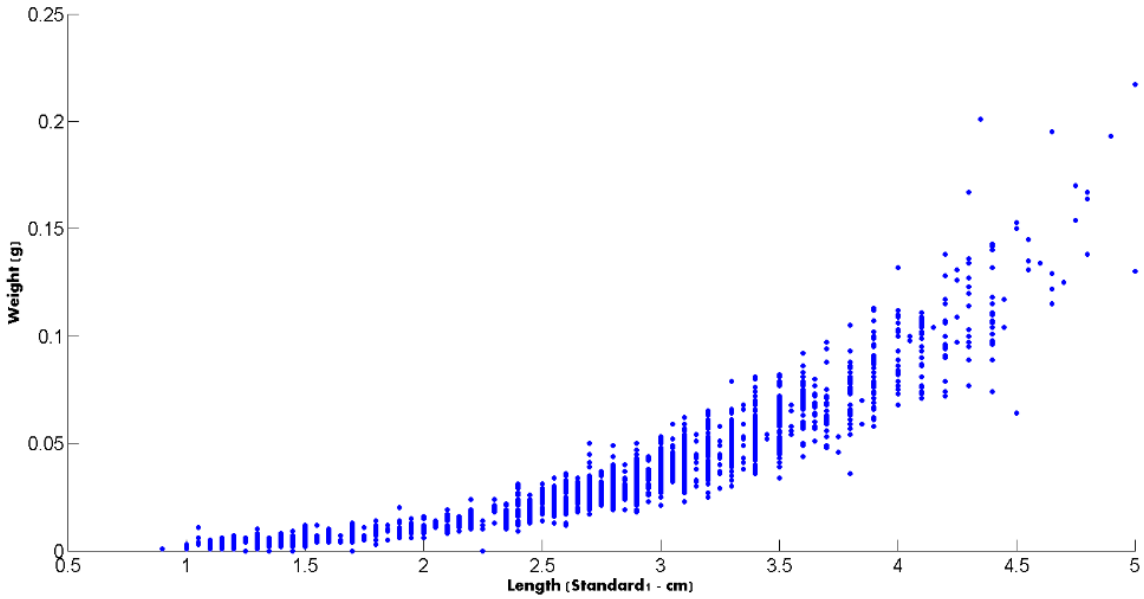

Figure 2: Length-weight relationship for all measured krill, with weight as dry weight in grams, length as Standard1, and weight = 0.014* length$^{2.98}$

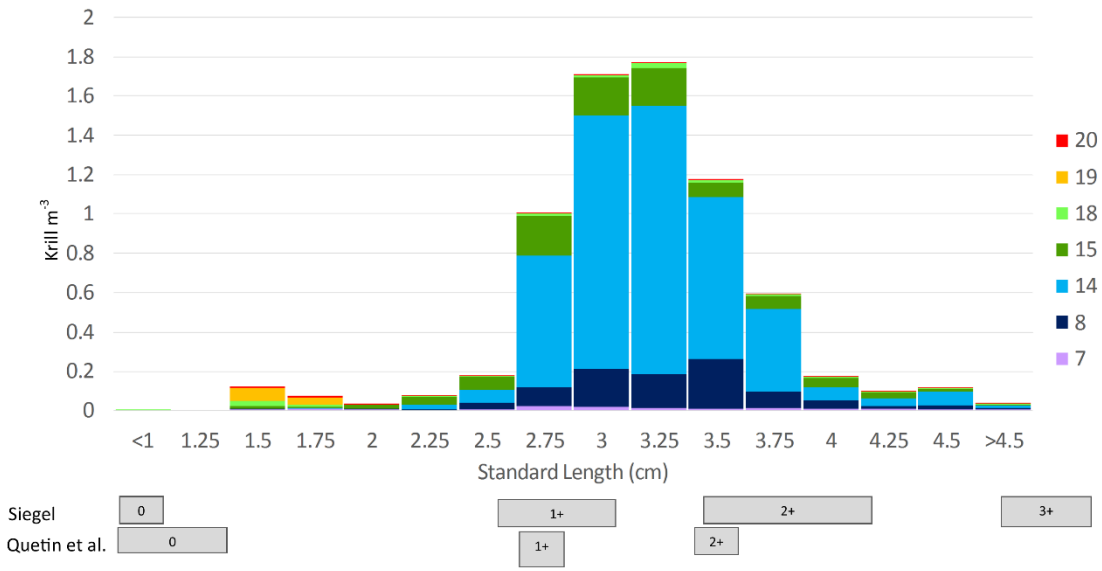

Figure 3: Histogram of lengths of krill sampled. Height of bars indicates mean numbers per m^3 throughout the study region. Length bins are 0.25 cm intervals and are labeled by the upper size limit of each bin. Color indicates in which MOCNESS tow the individuals were collected. Boxes below the plot show the size-at-age from available winter literature values, where Siegel is 1987, and Quetin et al. is 1996. The preponderance of age 1+ krill is clear in the main peak between 2.75 and 3.5 cm.

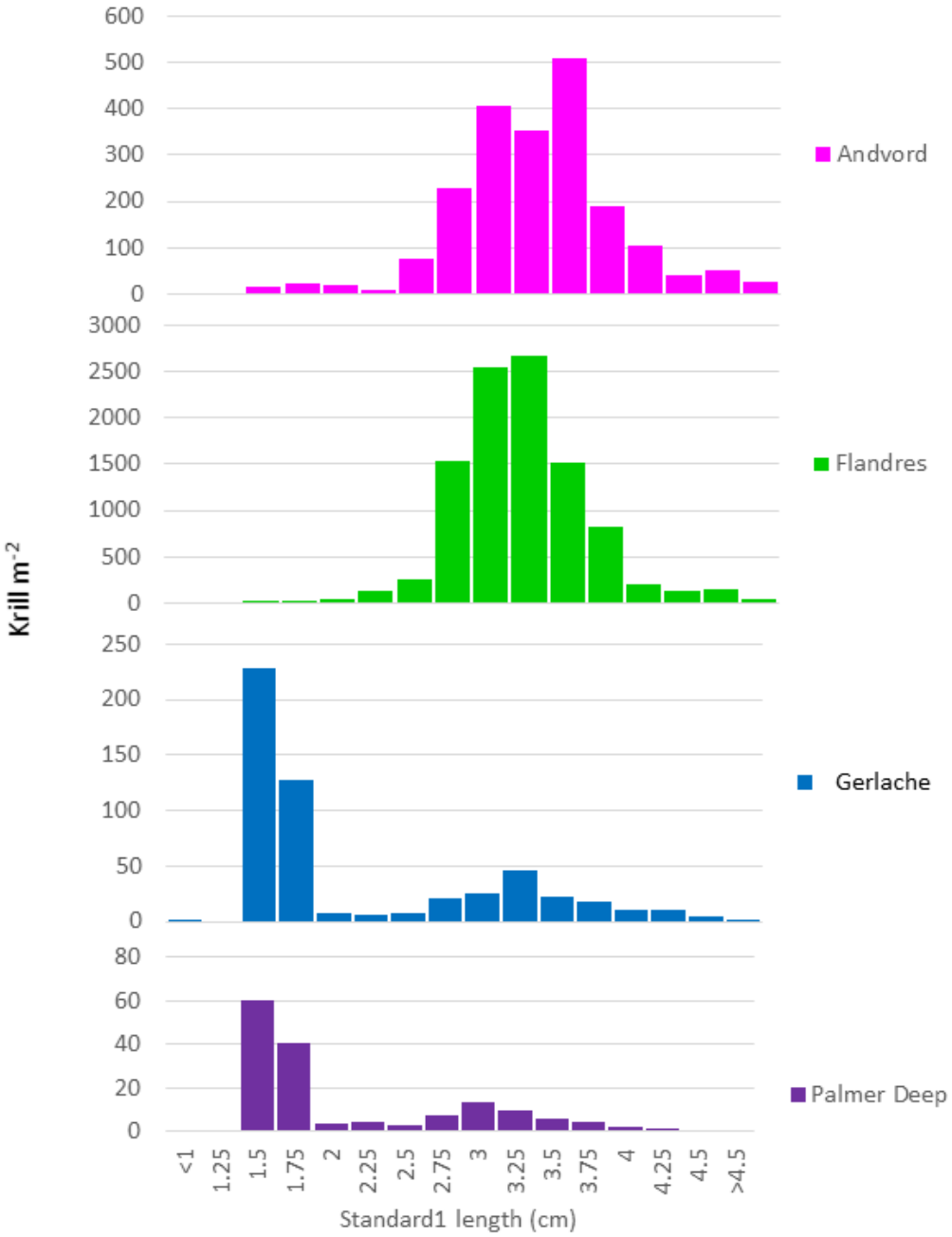

Figure 4: Length frequency histograms by location. These histograms show the similar distribution between Andvord and Flandres Bay's and the high contribution of young-of-the-year in the more offshore Gerlache Strait and Palmer Deep samplings.

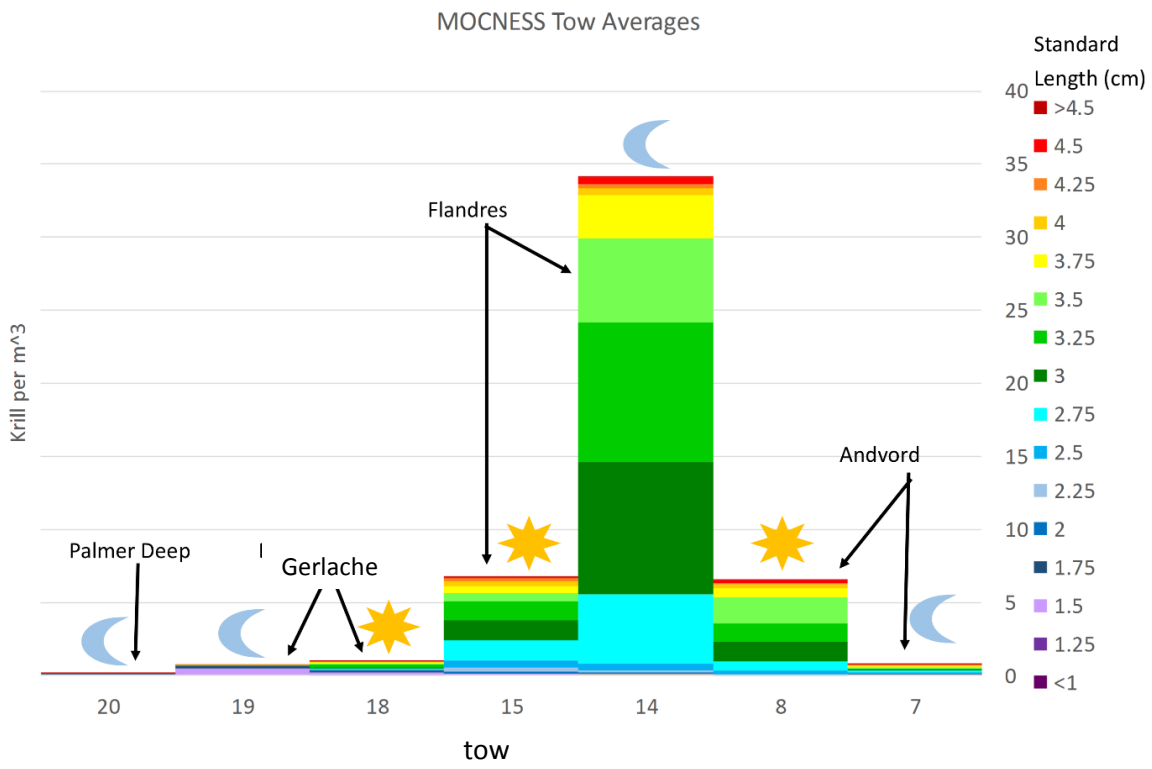

Figure 5: Average krill m^{-3} for each tow. Colors indicate the number of each krill in each size fraction. Blue moons show night time tows, yellow suns show daytime tows.

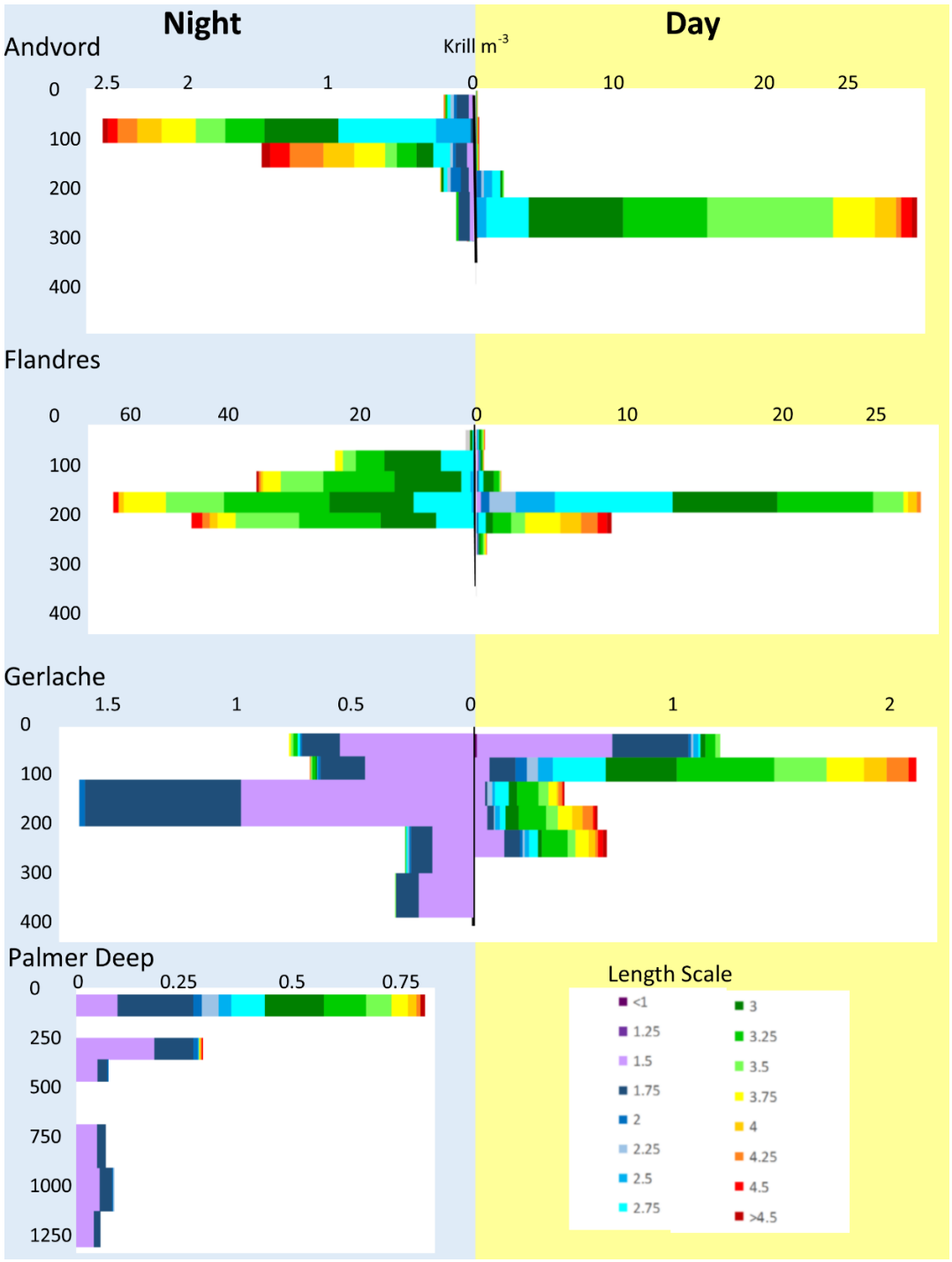

Figure 6: Vertical profiles of size fractions of krill. All plots show the upper 400 m of the water column with depth in meters on the y axis, and bar widths filling the depth interval they sampled. Please note that the abundance scale is different in each plot to show details.

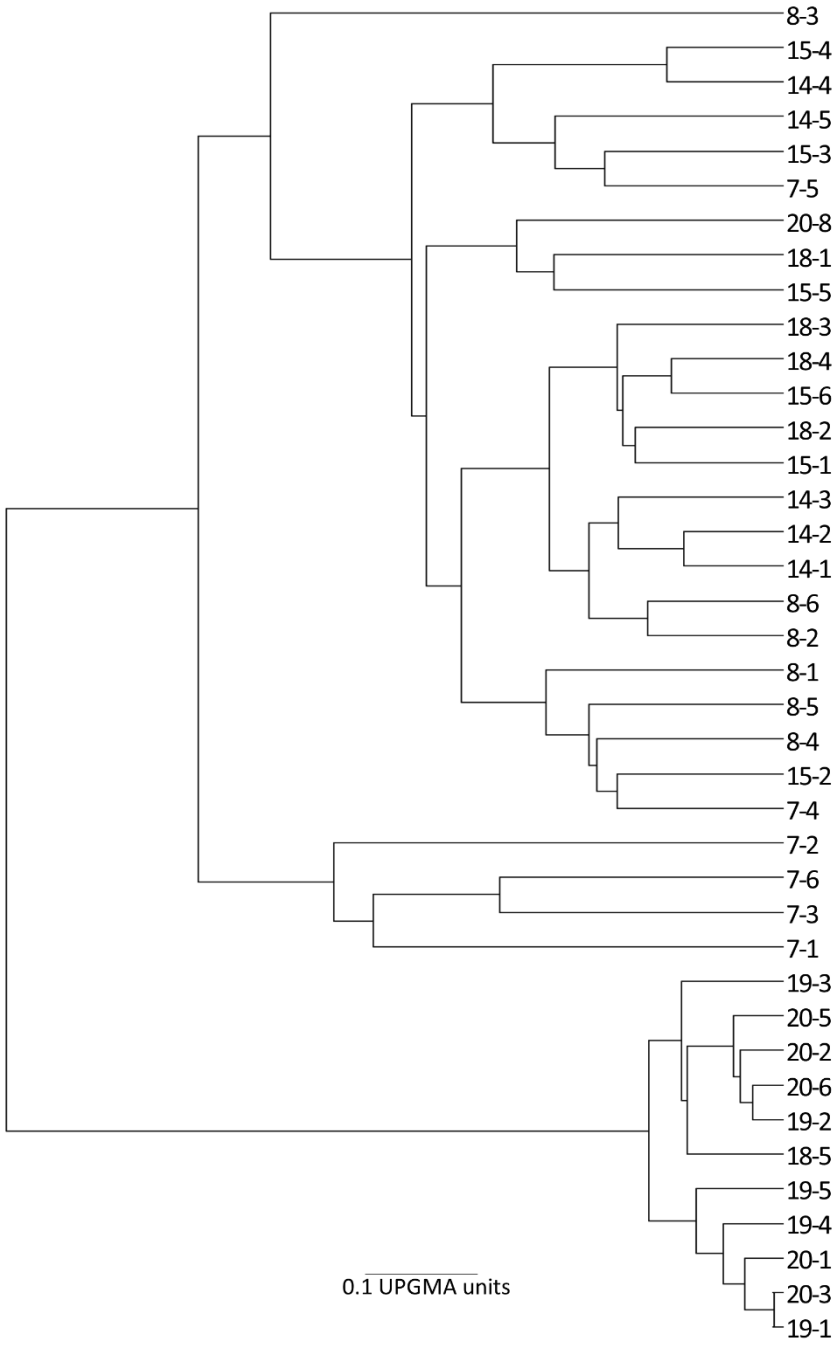

Figure 7: UPGMA cluster analysis of normalized size distributions for all nets. Nets are labeled as Tow-net, where net 1 is the deepest net and higher numbered nets are progressively shallower.

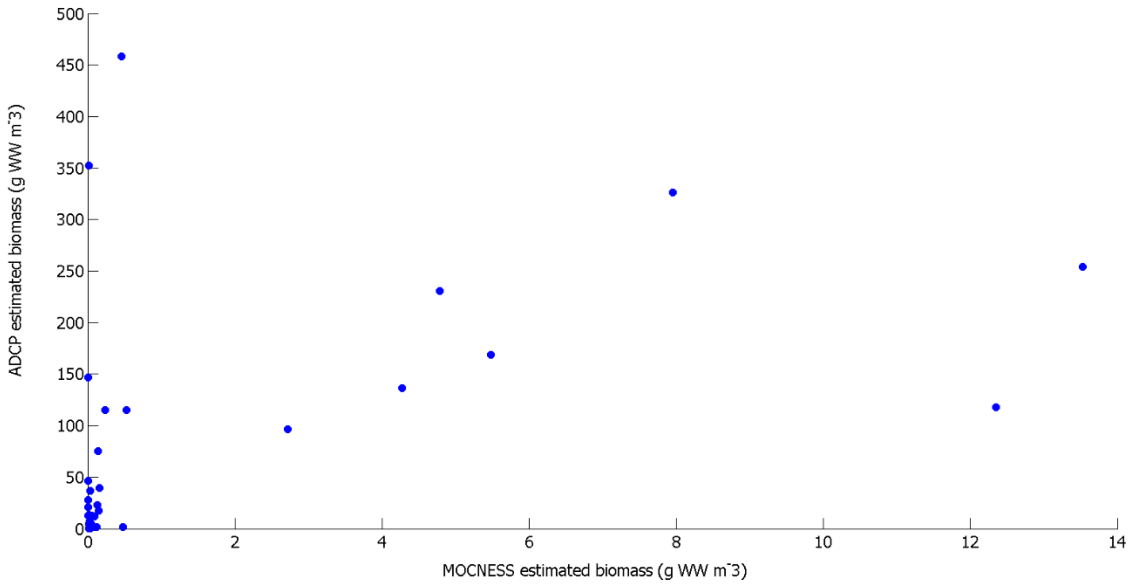

Figure 8: Comparison of krill biomass as estimated with MOCNESS net catches and ADCP acoustics. Each point represents one MOCNESS net. Note the different x and y scales.

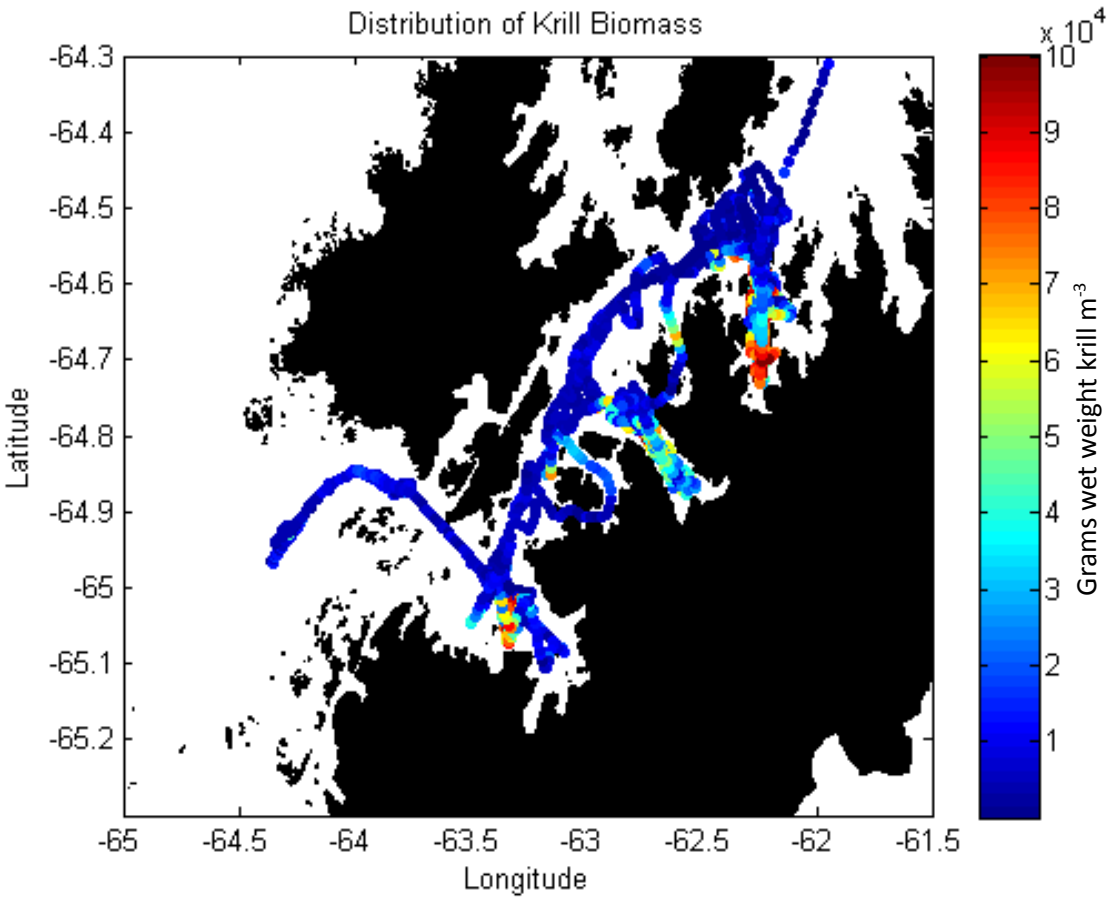

Figure 9: Spatial distribution of krill in the West Antarctic Peninsula in winter (May-June) from acoustic estimates. Each point indicates the vertically integrated biomass from the surface to 400m or 10m above the seafloor in g wet weight m^{-3} averaged over a 6 minute time interval.

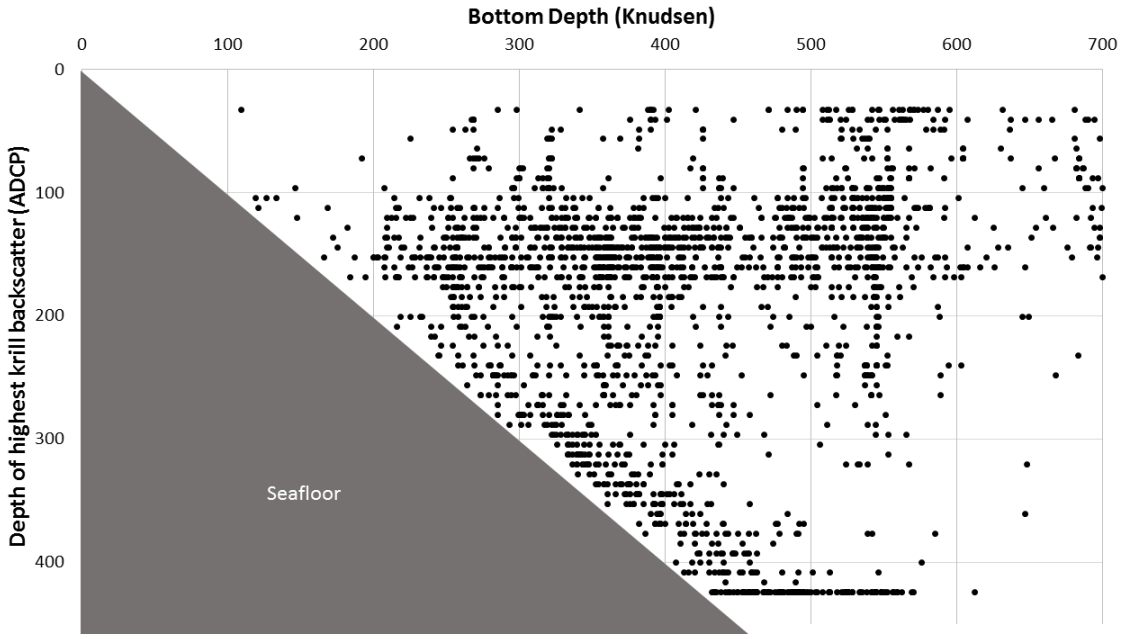

Figure 10: Krill peak abundance depth over the range of bottom depths. Krill tended to either occur close to the seafloor (points along the triangle hypotenuse) or in a layer 100-200 meters depth (points in horizontal layer).

Diversity and distribution of small pelagic and benthic eukaryotes in the West Antarctic Peninsula in winter

Alison Cleary, Edward Durbin

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Diversity and distribution of small pelagic and benthic eukaryotes in the West Antarctic Peninsula in winter

Alison Cleary*, Edward Durbin

Graduate School of Oceanography, University of Rhode Island, Narragansett, Rhode Island, USA

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* Corresponding author: acleary@my.uri.edu, (415) 300-6556

Abstract

The West Antarctic Peninsula is a highly productive and highly seasonal ecosystem. Although the krill and larger organisms in this region are historically well studied, the diversity, distribution, and ecological roles of the microeukaryotes are poorly known. In summer, diatoms and *Phaeocystis* dominate the water column, but the distribution of microeukaryotes in winter, particularly heterotrophic microeukaryotes, is largely unknown. We investigated these organisms in winter using 18S rDNA sequencing of the 0.2 µm to 5,000µm size fraction from surface waters, deep waters, and sediments in 4 locations along the Peninsula. Just under 3 million quality-controlled sequencing reads revealed over 11,000 operational taxonomic units (OTUs), including sequences representative of almost every major eukaryotic lineage. Different assemblages of OTUs were found in surface waters, deep waters, and sediments, with further differences within sample types by location. Water column samples included alveolates, diatoms, other stramenopiles, and a range of other groups, while sediment samples contained mainly cercozoa, diatoms, and metazoans. A *Chaetoceros socialis* –like OTU was observed at high abundance in all sediment samples with additional sequences in sediments from chrysophycea, cryothecomonas, and pedinellales, all phytoplankton known to form resting stages. These sediment phytoplankton OTUs suggest the sediments in this region may serve as a "seed bank" for phytoplankton diversity during the dark winter period. Improved information on the microeukaryote communities and their spatial partitioning provides a baseline against which future communities can be compared in a time of rapid

anthropogenic climate change, and suggests poorly known groups which may be ecologically important.

Introduction

The West Antarctic Peninsula (WAP) is a highly productive ecosystem. This region supports large populations of megafauna, with over 3 million penguins breeding in the area, and whale densities in excess of 1 whale per km^2 (Ducklow et al. 2007, Johnston et al. 2012). Anthropogenic pressure from multiple sources is also high in the WAP. The largest fishery in the Southern Ocean, that for the krill *Euphausia superba*, hereafter krill, is concentrated in this area, with catches in excess of 200,000 tons annually (Nicol et al. 2011). The WAP is also experiencing some of the most rapid climate change on earth; winter temperatures have increased by 5 – 6^o C over just the last 50 years (Ducklow et al. 2007).

The WAP is a historically well-studied ecosystem. Long-term surveys through the Antarctic Marine Living Resources (AMLR) and Palmer Long Term Ecological Research (palLTER) programs have documented the community of net phytoplankton (>50 µm), meso- and macro-zooplankton, penguins, whales, and seals, and the interannual variations in their abundances (Ducklow et al. 2007, Walsh 2014). However, the smallest eukaryotes, those not typically sampled by plankton nets, have often been left out from these surveys, particularly the non-photosynthetic single-celled protists (Heger et al. 2013). The microeukaryotes are operationally defined as that fraction of the eukaryotic organisms which are too small to be visualized by the naked eye, thus encompassing organisms ranging from a single micron in length through roughly 1000 µm, a range of 3 orders of magnitude. This relative lack of data on the smallest eukaryotes is not particular

to the WAP. Throughout marine ecology the smallest eukaryotes have often been excluded from analysis, largely due to methodological limitations, and protists are the least explored component of the biosphere (Caron et al. 2009, Heger et al. 2013). The diversity of microeukaryotes is incredible – molecular data have revealed a plethora of kingdom-level groups, expanding our view from an earlier, though still recent, understanding of 7 supergroups to include 13 additional deep branching lineages (Pawlowski 2013). Many of these new high level taxonomic groups have only a few described species, with little known of their distribution or ecology (Pawlowski 2013, Dawson & Pace 2002). In recent years, advances in DNA sequencing and the increasing availability of massively-parallel pyrosequencing have made broad-scale and in-depth surveys of microscopic organisms possible, and even more recent advances in computational algorithms and reference databases have made such analyses practical for eukaryotes (Caron et al. 2009, Quast et al. 2013).

Microeukaryotes can be important in the WAP marine ecosystem, and their importance is probably not yet fully realized (Knox 2007, Ducklow et al. 2005). At times, large proportions of the primary production in the region can be the result of cells to small to be identified by typical light microscopy; in some cases over 80%, of the chlorophyll and primary production occurs in the nano, pico, and ultra phytoplankton (Knox 2007). The classical paradigm of the Antarctic as a simple food web of diatoms-krill-whales is increasingly recognized as an over simplification. The microbial loop of bacteria and protists rapidly cycling through organic carbon is now thought to be playing important roles, particularly outside of the spring diatom bloom (Azam et al. 1983, Ducklow et al. 2007). For example the small protistan heterotrophic nanoplankton have

been suggested to exert top down control on bacterial populations in the region (Ducklow et al. 2007). Increased understanding of the roles of microorganisms, both prokaryotic and eukaryotic may improve our understandings of how carbon flows through this ecosystem, and what prey are available to krill, and hence available to be transferred to the whales, penguins, and other charismatic vertebrates. Thus, uncovering the poorly known microeukaryotic community may potentially improve mechanistic understanding of WAP marine ecosystems, and predictive power of models for the region.

The WAP experiences extreme seasonality, with the classical spring bloom period of melting sea ice, long days, and high primary production, contrasting with a winter of extensive sea ice, short days, and extremely low primary production (Ducklow et al. 2007). Primary production over the annual cycle in the WAP is similar to that observed in temperate regions, but compressed into less than half the time (Ducklow et al. 2007). There has also been seasonality to research in the region, with many studies focusing on the active spring and summer seasons. Though less studied, winter is a particularly interesting period in the WAP because many organisms must find ways to persist through months of low light availability and low abundances of typical pelagic food sources. Benthic-pelagic coupling has been suggested to play an important role in these seasonal cycles, with sediment potentially serving as both a "seed bank" of resting phytoplankton spores and a "food bank" of phytodetritus available to heterotrophs (Smayda 2011, Mincks et al. 2005).

In this research, we investigated the diversity and distribution of all small (0.2 µm to 5,000µm) eukaryotes in the waters and sediments of the WAP in winter through 18S rDNA sequencing. The distribution of these molecular sequences was used to investigate

the range of organisms present in the WAP, the assemblages of these organisms, their utilization of different habitat types, and their overwintering strategies.

Materials and Methods

Field Collections - Samples were collected between May 18 and June 3, 2013, on the RVIB Nathaniel B Palmer during cruise NBP1304 (Table 1). Water samples were collected in Niskin bottles on a CTD rosette. Surface water was collected from the surface mixed layer at 20 m depth. Deep-water was collected at 10m above the seafloor within bays, and at 600m in Palmer Deep (bottom depth 1345m) (Table 1, Figure 1). For each sample, 2L of whole seawater was filtered by peristaltic pump onto a 0.2 µm membrane filter, thus collecting all organisms or pieces of organisms between 0.2 µm and approximately 5 mm diameter. Surface and deep-water samples were collected from Flandres Bay, Gerlache Strait, and Palmer Deep; Surface water only was collected in Andvord Bay (Figure 1, Table 1). Filters were placed in individual cryovials and immediately frozen at -80^{o}C. Temperature and salinity were recorded simultaneous with sample collection with a SBE 911plus CTD (SeaBird). Photosynthically active radiation (400-700 nm λ) was recorded from a Biospherical Licor instrument.

Sediment samples were collected with a megacorer (Ocean Scientific International Limited (OSIL)) from sampling sites identified as topographically smooth by multibeam bathymetry, and free from excessive ice rafted rock debris by underwater camera observation. Cores were recovered and processed immediately indoors, with sampling completed within 30 minutes of collection. To access sediments, overlaying water was gently removed by peristaltic pump and cores were extruded from the core tubes to just below the level of the sediment surface. The surface most layer of sediment

was sampled using autoclaved popsicle sticks, placed in cryovials, and immediately frozen at -80^{o} C. Sediment samples collected all organisms less than approximately 5 mm. Sediment was sampled from two locations in Wilhelmina Bay, one with high krill abundance, and one with low krill abundance, from two locations in Andvord Bay, one shallower and one deeper, and from one location in Flandres Bay and one location in Palmer Deep (Figure 1, Table 1). Samples were collected from 3 separate cores from one of the Andvord Bay corings, and from a single core in each of the other corings. All samples were shipped on dry ice from the dock in Chile to the lab at the University of Rhode Island and stored at -80^{o} until analysis. Bottom depth was recorded by shipboard Chirp 3260 echo sounder (Knudsen).

Laboratory Processing – Total DNA was extracted from water filters with the DNeasy blood and tissue kit (Qiagen). Volumes of the initial lysis buffers were all doubled to ensure the filter was submerged and all material was lysed. Total DNA was extracted from 0.25 grams of each sediment sample using the PowerSoil kit (MoBio) with the bead-vortex lysis option, as per manufacturers instructions. Only samples of the same type (water or sediment) were extracted on the same day. All extractions were conducted in a laminar flow hood with project-dedicated pipettes, tips, and chemicals, to minimize possible contamination.

18S rDNA was amplified using universal eukaryotic primers (Gast et al. 2004) modified by the addition of a variable position in the reverse primer to improve priming of ciliates (Cleary et al. in press), and to include adaptors for Illumina sequencing, and a variable number (0-3) of ambiguous bases to offset the amplicons and increase the variability at each read position for improved base calling. Each reaction contained a final

concentration of 1x Pfu Ultra II clear buffer (Agilent), 1x Bovine Serum Albumin (New England Biolabs), 0.25 mM equimolar mixture of all four deoxynucleotide triphosphates (dNTPs) (Promega), 0.1 µM each primer (forward and reverse), 1x Pfu Ultra II polymerase (Agilent), and 20% by volume DNA template at extracted concentrations. Pfu is a high fidelity polymerase with proof-reading activity, and has twenty times lower rate of PCR errors than the more commonly used taq polymerases, leading to increased sequence accuracy (Agilent User Manual). Thermocycling consisted of 95^{o} for 30s, followed by cycles of 94^{o} for 30s, 58^{o} for 45s, and 72^{o} for 30s, with a final extension of 72^{o} for 5 min. Samples were amplified for the minimum number of cycles necessary to obtain sufficient DNA for sequencing in order to reduce amplification biases and over-representation of abundant targets. Water samples were all amplified for 35 cycles, as were sediment samples 28, 29, 30, 35, and 37, while the remaining sediment samples were amplified for 30 cycles. Amplicon presence and size was confirmed with gel electrophoresis. Amplification of no-template blanks included in each PCR showed no signs of contaminating DNA.

Amplicon purification and sequencing was done at the URI Genomics and Sequencing Center. Amplicons were AmPure cleaned, re-amplified to add sample identification tags, and quantified on a BioAnalyzer (Agilent). Amplicons were pooled into one half Illumina Miseq run, and sequenced for 500 cycles, allowing for almost complete overlap of the amplicon.

Data Analysis – Paired ends of reads were joined if the entire overlap region was identical in both read directions; if not the reads were discarded. Amplicons were then assigned sample-specific names and pooled for further analysis. Primers, and any

sequence data beyond the end of the amplicons, were trimmed, and any sequences in which the exact primer sequence was not found were again discarded. This fairly stringent approach to QC likely eliminated most sequencing errors, as they are unlikely to occur identically in the two directions of sequencing. Amplicons were clustered into 97% Operational Taxonomic Units (OTUs). 97% sequence identity has been commonly used as a proxy for species, and while not the most biologically meaningful approach, is computationally feasible for large sequence data sets (Sogin et al. 2006). Any OTU with only a single sequence in it was discarded because such singletons may be erroneous and add significantly to computation time, without adding much to the overall interpretations of the data; this discarding of singletons is commonly applied to environmental data sets (Logares et al. 2014). All of the above analyses were conducted in Qiime – virtual box (Caporaso et al. 2010)

Taxonomic identity was assigned to each OTU through automated comparison in Qiime with the Silva database (Quast et al. 2013). BLAST searching was used to confirm and in some cases refine taxonomic assignments (Altschul et al 1990). For certain groups, sequence data was further investigated using oligotyping (Eren et al. 2013), which clusters sequences based only on the base positions with the greatest Shannon entropy, allowing for more biologically meaningful groupings with less noise. Oligotyping is currently only computationally feasible on closely related groups of sequences, and was used to investigate the *Chaetoceros* and *Telonema* sequences. A dissimilarity matrix of all samples was constructed using the Bray-Curtis metric. Principal coordinates were calculated in Qiime, and visualized in MatLab. In order to

determine the significance of observed groupings, ANOSIM and ADONIS were calculated in Qiime (Legendre & Anderson 1999).

Results

A diverse range of eukaryotic sequences was recovered from the samples. After quality control, 2,817,417 sequences were used in further analysis. These sequences clustered into 11,621 OTUs, 6,972 of which could be assigned some level of taxonomic identity. 1,715 OTUs were observed in both sediment and water samples, while 3,105 OTUs were found exclusively in water samples, and 6,803 OTUs were found exclusively in sediment samples. 2,107 OTUs were found in only a single sample. Shannon diversity metrics (Shannon & Weaver 1949) for the water column and sediment were very similar, with 4.575 for the water column, and 4.578 for the sediment. OTUs from 17 of the 20 major eukaryotic lineages were observed, including ophistokonts, amoebozoa, excavata, picobiliphytes, centrohelids, rhizaria, haptophytes, telonemia, alveolates, stramenopiles, and chlorophytes (table 2). The only high-level lineages not observed, or not identified, were rappemonae, collodictyonidae, and rigidifilida (Figure 2).

Sequence assemblages contained a few highly abundant OTUs, and a large proportion of rare OTUs. Only the 11 most abundant OTUs individually contained more than 1% of the total number of sequences. The top 20 OTUs (shown in Figure 3 and Table 2) combined made up slightly less than 52% of the total sequences. The most abundant 425 OTUs combined include 90% of the sequence reads, while the top 4,223 OTUs combined include 99% of all the sequencing reads. The least abundant 7,398 OTUs, which composed 64% of the total OTU diversity, combined to make up only 1% of the total sequence reads.

Sediment samples had a slightly higher proportion of rare OTUs than water samples. In the water samples, 91.6% of the OTUs had an individual sequence abundance of less than 0.01% of the total water sequences, while in sediment this percentage of rare OTUs was 92.6%. Similarly, water samples had more abundant OTUs, with 0.41% of the OTUs individually making up more than 1% of the total water sequence abundance, and only 0.11% of the OTUs reaching this threshold in sediment samples. Most of the top 20 OTUs were present mainly in one sample type, that is, surface water, deep-water, or sediment, though some were distributed throughout the source types (Figure 3, Table 2). Unknown groups made up a larger fraction of sediments sequences than of water column sequences. In surface waters, organisms unclassifiable to levels lower than "eukaryote" made up less than 8% of the total sequences, whereas in sediments this most poorly known category encompassed 31% of the total sequences.

The most abundant OTU overall was a type of *Chaetoceros* diatom, which is identical to reference sequences for *C. socialis*, *C. debilis*, and *C. setoense* over the sequenced gene region. This *Chaetoceros* OTU was found almost exclusively in sediment samples (Figure 3, Table 2). The next most abundant OTU was a *Scrippsiella*-like dinoflagellate, which was found mainly in surface waters, but was present in all sample types. The third most abundant OTU overall was another diatom, this one found predominantly in the surface waters. This third-most abundant OTU diatom's sequence is 100% identical over the sequenced region to representative sequences from *Thalassiosira*, *Minidiscus*, and *Cyclotella* references (as such it is categorized only as Mediophycea in the diatom analysis).

Multivariate analysis shows separation of the three source types (surface water, deep-water, and sediment), and additional separation by sampling site within the source types. Principal coordinate analysis shows these three distinct groupings (Figure 4). Surface water samples cluster most closely together. Deep-water samples cluster closer to the surface water than to the sediment, with the shallower deep-water samples from Flandres Bay clustering nearest the surface water samples, and the deepest deep-water samples from Palmer Deep clustering furthest away from the surface water samples. Sediment samples clustered separately from both surface and deep-water samples. Samples from Palmer Deep sediment clustered notably closer to the water column samples than did other sediment samples.

ANOSIM analysis showed that overall sample type is a significant factor in determining the community microeukaryote assemblages, with $p=0.001$. Within each sample type, location (Flandres, Andvord, Gerlache, Wilhelmina, Palmer Deep) was also a significant explanatory variable in these assemblages; for surface waters and sediment $p=0.001$, while for deep-water $p=0.004$. ADONIS analysis confirmed the significance of these clusterings, and showed they explain a large part of the observed variance. Overall, ADONIS showed sample type was significant at $p<0.001$ and explained 59% of the total observed variance. Within each sample type location was significant, with $p<0.001$ for all three types. Within surface waters, location explained 66% of the total variance, within deep-waters location explained 69% of the total variance, and within sediment samples location explained 53% of the total variance.

Considering the overall distribution of all OTUs across all samples, there are clear differences in the relative abundances of different phylogenetic groups in the different

sample types (Figure 5, Table 3). Surface waters show higher relative abundances of non-diatom stramenopiles, picozoa, haptophytes, and telonema. Deep water showed higher relative abundances of alveolates, metazoa, and radiolaria. Sediment showed higher relative abundances of cercozoa, apicomplexa, and unknown organisms. Both surface waters and the sediment had high relative abundances of diatoms, as compared to the deep water.

Results for specific groups follow, clustered by major lineage as per Keeling et al. (2005) and Quast et al. (2013), and arranged in alphabetical order for simplicity.

Alveolates

Apicomplexa, a phylum best known for its member *Plasmodium* which causes malaria in humans (Lee 2008), were observed in 38 of the 40 samples, but were at very low relative abundance in water samples (Figure 5). Within water samples the apicomplexa were mainly *Lankasteria*, with a few sequences of other apicomplexa including *Eugregarinia* (Figure 6). In deep water samples apicomplexa sequences included cryptosporidium OTUs. Sediments, where apicomplexan sequences were at their highest relative abundances, showed fairly even distributions of types of apicomplexa, with OTUs representative of *Eimeriorina*, eugregarines, fipodium and selidium gregarines, *Lecudina*, sarcosystis and rhytidocystis all observed.

Ciliate sequences were present in all samples, with highest relative abundances in surface samples, and lowest relative abundances in the sediments (Figure 5). In the surface waters ciliates were mainly choreotrichia and oligotrichia, with small relative abundances of *Salpingella* and *Strombilidium* (Figure 7). Although choreotrichia

sequences were not classifiable to lower levels, this group includes the tintinnids. *Strombilidium* sequences were more abundant in Andvord surface waters than in the other sampling locations. Choreotrichia and oligotrichia were also in high relative abundances within deep waters, but they were joined by oligohymenophorea sequences. The ciliate assemblage within the sediments appeared more diverse, with no single dominant type and a mixture of *Euphlota*, haptoria, hypotrichia, spirotrichia, and spirotrachelostyla. Ciliate sequence relative abundance was correlated to that of dinoflagellates, with a linear $r^2 = 0.61$.

Dinoflagellates were not a large percentage of the sequences in any sample, but were present in all samples, and showed higher relative abundances in water samples as compared to in sediments (Figure 5). In the surface waters dinoflagellate sequences belonged mainly to the gymnodiniphycidae, *Gyrodinium*, and haplozoa (Figure 8). Deep water assemblages were similar to those observed in surface waters, with the addition of smaller relative abundances of dinophycea, peridiniphycidae, and suessiasea. The sediments were quite variable in their composition of dinoflagellates, which is not surprising given the low abundance of dinoflagellate sequences in them (0.18% of sediment sequences). Palmer Deep sediments notably had a high relative abundance of kareniacea, and in Wilhelmina Bay the coring in a high krill abundance area showed notably high relative abundances of *Protoperidinium*.

Other alveolates were present in all samples, with the highest relative abundances found in deep water, where they made up 27% to 62% of the overall total sequences (Figure 5, Table 3). Many alveolate sequences were not classifiable to lower taxonomic groupings, but amongst those that were, syndiniales dominated (Figure 9). In

particular syndiniales groups I and II had the highest relative abundance, with lower relative abundances for groups III and IV. Syndiniales group I had the highest relative abundance within the sediment samples, while in deep water samples group II made up the bulk of the alveolates, and in surface waters a fairly even mixture of the two types was observed. amoebophrya were also observed, and had higher relative abundances in water than in sediment. In sediment samples, *Perkinsidae* made a consistent presence, making up over 10% of the alveolates, and low relative abundances were observed of protalveolata and duboscquella.

Cryptophytes

Cryptophytes were found in all samples, but had their highest relative abundances in the surface waters (Figure 5). Within the sediments, cryptophytes were notably higher in relative abundance at Palmer Deep. In surface waters, and deep waters except those at Palmer Deep, cryptophyte sequences were composed mainly of reads within a single OTU of cryptomonadales. Small relative abundances of one OTU of teleaulax were also present in the water samples, and less frequently in the sediment. In the sediment samples, and in the deep water at Palmer Deep, cryptophytes were composed mainly of a single OTU of Rhodomonas, with smaller contributions from 4 OTUs of goniomonas.

Excavates

Excavates were observed mainly in deep waters, with a few present in surface waters, and few in sediments, and were consistently present but at low relative abundances (Figure 5). In sediments excavates were present in highest relative

abundances in Palmer Deep sediments, with lower but still above average relative abundances in Flandres Bay sediments. Euglenozoa made up 99.5% of the excavate sequences. In the water column, excavates were mainly diplonemia, with some contribution from neobodina, particularly in surface waters (Figure 10). In the sediment a more mixed community of excavates was observed. Petalomonas made up the largest relative abundance, with additional high relative abundances of diplonemia, and smaller relative abundances of bodo, carpediomonas, and rhynchopus, with a few sequences of tetramitia.

Haptophytes

Haptophytes, single celled division of algae best known as the group containing the coccolithophores, were found mainly in surface waters (Figure 5). Across all samples haptophytes were a mixture of *Chrysochromulina* and *Phaeocystis*, with an overall contribution of 64% *Chrysochromulina* and 32% *Phaeocystis*. Small relative abundances were observed from other prymnesiophycea, and other prymnesiales. In the deep waters of Flandres Bay pavlophycea OTUs were also observed in low relative abundance. Haptophytes were at very low abundances in the sediment, with *Phaeocystis* making up less than 0.007% of the total sediment sequences.

Ophistokonts

Metazoan (multicellular animal) sequences had their highest relative abundances in deep waters, with similarly high relative abundances in sediments (Figure 5). Surface water metazoans included higher relative abundances of non-copepod arthropods, and Porifera (sponges) (Figure 11). Porifera sequences classified into 30 OTUs, but sequence

abundance was dominated by 2 OTUs, both belonging to the desmospongiae. One of these desmospongiae OTUs dominated the porifera sequences in Flandres Bay (71%), while in the other surface waters the 2 OTUs were both present in a fairly even mixture with 31% to 59% of the aforementioned OTU. Sediment samples showed high relative abundances of nematode sequences (round worms), as well as playthelminthes (flat worms), and kinorhyncha (mud dragons). Annelid (segmented worms) sequences showed high relative abundances in many samples, particularly in the deep water of Flandres bay. Copepod sequences were also found across all sample types. Within the water column copepods were a mixture of an OTU with a sequence identical to representative sequences of *Oithona* sp., an OTU with a *Pseudocalanus/Microcalanus* like sequence, an OTU more similar to reference sequences for *Euchirella/Scaphocalanus*, and an OTU most closely related to *Tisbe/Nemesis* type copepods. *Oithona* copepods were also one of the dominant zooplankters identified taxonomically in co-occurring net tows (data not shown). In the sediment, copepod sequences were mainly of the harpacticoid *Diathrodes* (Boxshall & Halsey 2004).

Fungus sequences were present in all samples, but always at low abundances, with less than 0.1% of the total sequences in the surface and deep waters, and 0.51% of the sediment sequences (Table 3). Fungus sequences were composed largely of chytridiomycetes and glomeromycotina, with smaller contributions from agarimycetes, taphrinomycotina, yeasts, and others.

Picozoa

Picozoa sequences were observed in all samples, with highest relative abundances in surface waters (Figure 5, Table 2). No further taxonomic information was available for these poorly known organisms.

Plantae

Chlorophyte (green alga) OTUs had highest relative abundances found in the surface samples (Figure 5). In these surface samples Chlorophyte OTUs were mainly *Bathycoccus* and *Micromonas* (Figure 12). In deep waters these two groups were also important, but mamiellophycea and organisms from the clade VII group were also present at high relative abundances. Within sediments the few chlorophyte OTU sequences observed were mainly associated with clade VII, although there were also sequences of mamiellophycea, *Micromonas*, prasinophytes, nephroselmidophycea and ulvophycea.

Rhizaria

Cercozoa OTUs were, like the apicomplexa, mainly observed in sediment samples (Figure 5). The few cercozoa present in surface waters were mainly cryothecomonas (Figure 13). While the deep water also had a relatively low overall abundance of cercozoan OTU sequences, those present were more diverse, with *Phaeodaria* and *Paradinium* present, and at higher relative abundances in Palmer Deep. Deep water in Flandres Bay had a larger relative contribution of protapsidae. Both deep water and sediment samples included OTUs of chlorarachniophyta. In sediment, where cercozoa had their largest relative abundance, the cercozoan assemblage was variable by station, but consistently composed largely of cryothecomonas and silicofilosea. In

Andvord Bay these two types were present in roughly equal relative abundances, while in Flandres Bay there is a lower relative abundance of silicofilosea sequences. At Palmer Deep a higher proportion of cercozoa sequences were unclassifiable to lower taxonomic groupings. Within Wilhelmina Bay, the coreing in the high krill concentration area had lower relative abundances of *Phaeodaria*, as compared to the coreing outside of the area of high krill abundance.

Foraminifera were present in 39 of the 40 samples, including surface waters. The greatest relative abundance of foraminifera was found in the Palmer Deep sediments, with lesser but still above average relative abundances in the deep waters at Palmer Deep and Gerlache Strait (Figure 5). The foraminifera sequences were 97% categorized as *Epistominella*, closely related to *E. exigua*. Other foraminifera present at low relative abundances included *Reophaxis* and *Bulmina marginata*.

Radiolarians were observed mainly in deep water samples (Figure 5). Most of the radiolarian sequences observed (78%) were categorized as radiolarian type B – the *Sticholonche* and related radiolarians. BLAST searching these sequences confirmed their taxonomic affiliation with *Sticholonche*.

Stramenopiles

Diatoms had high relative abundances in both surface waters and the sediment, and lower but still present abundances in deep waters (Figure 5). The water column diatoms were composed mainly of sequences from the single OTU with the third highest overall abundance (Figure 14). This OTU is classified as Mediophycea, as it 100% identical over the sequenced region to representatives from *Thalassiosira*, *Minidiscus*,

and *Cyclotella*. In addition to the dominant Mediophycea OTU, surface water samples contained *Corethron* sequences, and a low relative abundance of OTUs classifiable as *Thalasiossira*, while deep water included additional *Porosira* sequences. A small 2-5 µm cylindrical diatom, likely our abundant Mediophycea OTU, and *Corethron* were observed by microscopy in surface water samples during the cruise (K Whitaker pers. comm.). Sediment diatom sequences were composed almost exclusively of a single OTU of *Chaetoceros*. This sediment *Chaetoceros* OTU is identical over the sequenced region to reference sequences of *C. socialis, C. debilis,* and *C. setoense*.This *Chaetoceros* OTU was the most abundant sequence overall across all of the eukaryotic sequences(Figure 3). Oligotyping analysis showed this OTU is composed 98% of a single unique sequence – this one unique *Chaetoceros* sequence makes up 28% of all of the sediment sequences observed across all taxa. Within sediment samples the relative abundance of diatoms was correlated with depth, with higher relative abundances at shallower depths, and lowest diatom abundances in the deepest sediment from Palmer Deep. A linear fit to this comparison of diatom relative abundance with depth gave an r^2 of 0.76, while an exponential fit to this data gave an r^2 of 0.89.

Non-diatom stramenopiles were present in all samples, with highest relative abundances found in surface waters (Figure 5). The largest fractions of the non-diatom stramenopile sequences came from the numbered MArine STramenopile (MAST) clades, rather than from taxonomically described groups (Figure 15). MAST-1 sequences were present in all samples, with higher relative abundances in water samples. MAST groups 3, 7, and 8 were also at high relative abundances in water samples, while group 12 was present mainly in sediment samples. All samples had a low relative abundance signal

from bolidomonas. Surface waters also had a high relative abundance of pelagophyseae, and the Andvord and Gerlache samples also had a smaller relative abundance of phaeophycea. Deep water assemblages were intermediate between those of the surface and those of the sediment, with Palmer Deep deep water most resembling the sediment community. Both deep water and sediments had high relative abundances of chrysophyseae and labyrinthulomycetes. For Palmer Deep, deep water and sediment also contained bicoseocida sequences. Sediment stramenopile assemblages included a large relative abundance of pedinellales, with smaller contributions from peronosporomycetes, *Pirsonia*, and paraphysomonas.

Telonema

Telonema sequences were found in all samples, and had highest relative abundances in the surface waters (Figure 5). All telonema sequences were classified as unknown/uncultured, so oligotyping was used to investigate whether there were biologically separate groupings with differences in distributions. Each oligotyped OTU was assigned a letter name in order of overall abundance. Clear differences in the telonema community assemblage were observed between different sample types (Figure 16). In surface waters telonema sequences mainly belong to OTUs A & B. Deep water samples were mainly B, with others, including O & P which were not found in surface waters or the sediments. Sediments were mainly OTUs F & I. Telonema assemblages also showed more geographic variation than was seen in most groups investigated, with clear differences between stations. The northern samples from Andvord Bay and Gerlache Strait showed clear differences in telonema assemblages from the southern

samples from Flandres Bay and Palmer Deep within surface water. Within the sediment, Andvord had higher relative abundances of F as compared to the other locations.

Discussion

Range of Organisms and the Rare Biosphere

The 11,621 OTUs found in the WAP region samples covered much of the known range of diversity of eukaryotes with OTUs representative of almost all of the kingdom level eukaryotic lineages (Pawlowski 2013). OTUs which were not classifiable by comparison with Silva were simply classified as "unknown", although selected BLAST-searching suggests some of these organisms represent poorly known groups, including heliozoa, which may be underrepresented in the database, thus making our observed diversity of types of organisms potentially an underestimate. Although none of the sequences which were individually investigated showed any signs of errors, it is also possible some fraction of these unkown OTUs may represent PCR or sequencing errors which evaded our quality filtering. Roughly 10% of the estimated total diversity of protists has been described, and this fraction is highly variable between phylogenetic groups (Heger et al. 2013). Thus is it perhaps not surprising that we observed a noticeable fraction of reads which are completely unidentifiable (Table 3), and additional organisms which were identifiable only to very broad kingdom level groups (Figures 6 through 16). The unknown fraction was highest amongst sediment samples, and lowest in surface waters, suggesting the taxonomic diversity of sediment organisms in the WAP may be less well characterized than that of the water column community, and may be an interesting area for future investigations.

Many of the OTUs observed were present in very low abundances, composing the 'rare biosphere' which has often been observed in deep sequencing studies of mainly bacterial and archeal assemblages, but also microeukaryote assemblages (Sogin et al. 2006, Logares et al. 2014). In comparison with microeukaryote communities observed in the coastal surface water of Europe, our water samples contained relatively few abundant OTUs with only 0.41% of the total OTUs representing over 1% of the total sequence reads, while Logares et al. (2014) found typically higher values, with 0.9 to 2.7% of the OTUs having this level of abundance. Similarly we observed more rare OTUs with 91.6% of the OTUs observed having less than 0.01% of the total reads, as compared to the 66%-77% observed by Logares et al. Differences in target gene fragments, sequencing depth, and data analysis methods (97% OTUs here vs 95% OTUs in Logares et al. 2014) may artificially inflate or deflate the number of rare OTUs, so these results should be regarded cautiously until there are more studies of marine microeukaryotes with which to compare the results. Nevertheless, this higher fraction of rare organisms in WAP is interesting; the rare biosphere has been suggested to represent a range of organisms waiting for the environmental conditions to be right, and for them to increase in abundance (Logares et al. 2014). The higher proportion of rare OTUs in the WAP winter sampling, as compared to European coastal waters, may be a reflection of the extreme seasonality in the WAP. Many organisms in the rare biosphere may be spending the winter period in a dormant state, and may not become active members of the ecological community until the spring increase in solar irradiance and water column stratification, or the concomitant increase in primary production..

Spatial variations in assemblages

Microeukaryote assemblages were variable between sample types, with clear differences between surface waters, deep waters, and sediment samples. These clusterings were seen in PCA and statistically confirmed with ANOSIM and ADONIS analyses ($p<0.001$). Sediment is, in many ways, a different habitat from the water column, with surfaces and refuges, different nutrient and chemical substrate availabilities, and without the concern for organisms of sinking out of the habitat. Causative factors driving differences between surface and deep waters are less clear, though solar irradiance is likely to be an important driver. Temperatures throughout the water column were within 1.5 degrees of 0^{o}C, and salinities were within 1 psu (table 1). Solar irradiance is likely the largest difference between the surface and deep water environments, and has previously been considered the main limiting factor for phytoplankton assemblages over much of the year in the Southern Ocean (Fryxell 1989). In winter there is relatively little incoming solar irradiance, with our highest values of photosynthetically active radiation (PAR), observed in the 20m depth samples taken around noon, at only 5 µmol photons m^{-2} s^{-1}, below the level which has been used to induce light limitation in various phytoplankton (Harrison et al. 1990). All deep samples and night time surface samples had undetectable light levels. Surface waters contained higher relative abundances of some photosynthetic groups, including diatoms, haptophytes, other stramenopiles, and chlorophytes (Figure 5 & Table 3). Thus, despite the relatively low levels of light, and few hours of light daily, photosynthetic organisms appear to be concentrated in the part of the water column where they could best utilize this energy. Surface waters also contained higher abundances of certain non-photosynthetic groups which have been associated with sea ice, including picozoa,

telonema and ciliatas, suggesting these groups may spend time in the surface waters before the ice and associated community develop. Deep waters contained higher relative abundances of metazoans, euglenas, and radiolarians than were found in surface waters.

Differences between locations were also significant within sample types. Within the sediment samples, Palmer Deep samples clustered closer to the water column samples (Figure 4). Palmer Deep assemblages included more of some more typically water associated groups of organisms, such as cryptomonads, paraphysomonas, and kareniacea. Palmer Deep is much deeper than the other sediment samples, and is an area with much lower krill abundances than within the bays (Cleary et al. in prep). These sediments may thus be experiencing less re-processing and grazing and may reflect a signal from organisms sinking out of the water column after the spring bloom. Within Wilhelmina Bay, the sediment samples from an area with low krill populations, showed higher relative abundances of *Phaeodaria* (cercozoa) than a nearby coreing in a high krill population area. It is possible these soft bodied sediment organisms may be being grazed down by krill feeding at the seafloor (Schmidt et al. 2011). Krill aggregations were observed at the sediment interface during the sampling period, suggesting krill may be actively grazing on these benthic organisms (Cleary et al. in prep)

Ciliates and Dinoflagellates

Ciliates have previously been found to be at higher abundances in water influenced by the Bellinghausen Sea than in more coastal waters with stronger Gerlache water influence (Alder & Boltovskoy 1991). This is in agreement with the finding of highest relative abundance of ciliate sequences in the surface waters of Palmer Deep, the most offshore of our stations. It has been suggested that ciliate abundance in the Southern

Ocean may be driven by patterns in the abundance of their preferred prey, dinoflagellates (Alder & Boltovskoy 1991). We see evidence of this in the sequence data, with relative abundances of ciliates and dinoflagellates correlated. However, *Gymnodinium* dinoflagellates, which were the largest fraction of the observed dinoflagellate OTUs, have been shown to feed on ciliates, with preferences for small Oligotrichs, which were one of the most abundant ciliate sequence groups (Bocktahler & Coats 1993). Dinoflagellates have shown a linear increase in feeding rate with ciliate prey concentration, with maximum ingestion rates of 1.5 ciliates per day (Bocktahler & Coasts 1993). *Gymnodinium* dinoflagellates are major herbivores in the Southern Ocean (Sherr & Sherr 1994). Thus, while the correlation between ciliates and dinoflagellates may suggest a trophic interaction between these groups as one of the factors determining their distributions, it is unclear whether ciliates or dinoflagellates are the predators. It is also possible that a third factor, such as the availability of bacterial food, or the distribution of predators, drives the abundances of both ciliates and dinoflagellates in a similar way. *Euphausia superba* were abundant in the region at the time of sampling, and these krill are known to consume microzooplankton such as ciliates and dinoflagellates, so krill predation pressure may be an important factor in the distribution of these groups (Knox 2007, Cleary et al. in prep).

Small flagellates

Small flagellates are an ecologically important group in marine ecosystems, due to their role in bactivory, and the resulting release of nutirents to phytoplankton (Logares et al. 2012). The uncultured groups of Marine Stramenopiles – designated here following the common nomenclature as MAST – followed by the group number, are thought to

consist of such small flagellates, with sizes typically in the 1-5 um fraction (Logares et al. 2012). MAST group sequences were fairly common in our samples, particularly in the surface waters. Most MAST groups are thought to be largely planktonic, corresponding to our observed distribution, but MAST group 12 is thought to be active mainly in sediment environments (Logares et al. 2012), which is where we found this group's highest relative contribution to the stramenopiles.

Picozoa, which were found in all samples but had highest relative abundances in the surface waters, are very small heterotrophic flagellates, with maximum sizes only around 3 μm (Seenivasan et al. 2013). These poorly known organisms are globally distributed (Seenivasan et al. 2013). These picozoans feed on particles <0.15 μm, likely marine colloids, such as exudates from phytoplankton, and potentially viruses, using a unique pattern of locomotion described as "jump, drag, and skedaddle" (Seenivasan et al. 2013). The high relative abundance of these organisms in the surface waters suggests they may be utilizing colloid-sized exudates remaining from the spring phytoplankton bloom. It is possible these picozoa play an important role near the base of the WAP food web in winter; they utilize a food source so small as to be inaccessible to most heterotrophs, and may be important prey in this winter period with low photosynthetic biomass. Microscopic analyses of surface waters from the WAP have previously found that "small unidentified flagellates (usually less than 5 μm) are always numerically dominant" (Ducklow et al. 2007). Our sequencing suggests that at least some of these small flagellates are likely to be members of the picozoa.

Telonema are another poorly known group which may contribute to the abundance of small unidentified flagellates in the WAP. Small flagellates such as these

telonema and picozoa groups may play an important role in marine ecosystems by increasing nutrient availability to phytoplankton through bacterial grazing and nutrient excretion (Azam et al. 1983). Although only 2 species of telonema have been described, earlier molecular work suggests at least 20 phylogenetic groupings amongst these organisms, and our analysis identified 24 phylogenetically distinct groups within the WAP (Klaveness et al. 2005, Bråte et al. 2010, Pawlowski 2013). The described species of telonema are small, pear-shaped heterotrophic flagellates, ranging from 6 to 20 µm in length, and are thought to feed on bacteria, small flagellates, and pico/nano phytoplankton (Klaveness et al. 2005, Bråte et al. 2010). Telonema are broadly distributed, and are thought to be one of the most widely reported heterotrophic flagellates (Klaveness et al. 2005). Telonema are frequently encountered in sea ice, and their abundances in water have been correlated with distance to the ice (Bråte et al. 2010). At the time of sampling in early winter, sea ice was still forming, and the ice algal community was not yet developed; the distribution of telonema sequences mainly in surface waters may suggest staying in the surface waters as an adaptation to take advantage of this sea ice habitat when it becomes available.

Although these telonema are poorly known, they are of interest due to their high relative sequence abundance particularly within the surface waters, where they made up over 10% of the sequence reads in some samples, and their presence in all sample types suggests they may play an important, and as yet largely unknown, role in the WAP ecosystem. In Arctic waters, telonema are on occasion the numerically dominant flagellates (Klaveness et al. 2005). The very clear differences in the telonema assemblages between surface waters, deep waters, and particularly sediments, suggests

that within this poorly known group different members employ different ecological strategies. Types A, D, and L had much higher relative abundances within the surface water, suggesting they may prey on small phytoplankton present there in winter or be awaiting sea ice community development, while types O & P were found predominantly in deep waters, suggesting perhaps different feeding strategies. Types F & I dominated the sediment telonema sequences; telonema to date have been mainly identified in water samples, and their flagellate morphology would not appear to be adapted to a benthic lifestyle, so the presence of telonema types F & I across all sediment samples may suggest the ability of these groups to potentially form resting spores, and possible presence of a type of cysts has previously been suggested (Bråte et al. 2010). Alternatively these sediment telonema may be residing in the nephloid layer of the water column. Our sediment sampling captured the sediment-water interface, and it is possible that some of the relatively low abundance groups in the sediments, such as the telonema may have come from the very near bottom waters, and types F & I may be adapted to a deeper lifestyle.

Foraminifera

The foraminifera *Epistominella exigua*, which was found mainly in sediment samples but also in the water column, is a globally distributed species, which is thought to be genetically homogenous over its full geographic range (Lecroq et al. 2009). This species is 100-200 µm in size, and is one of the most common deep sea foraminifera (Lecroq et al. 2009). *E. exigua* relies on phytodetrital food resources, and is therefore typically found in highly seasonal environments, such as in the WAP (Lecroq et al. 2009). This phytodetritivorous life style likely explains why the highest relative

abundance of foraminifera was observed at Palmer Deep, since Palmer Deep sediments appear to be most influenced by sedimentation of phytodetritus.

Porifera

Porifera are important components of benthic communities in the WAP (McClintock et al. 2005, Knox 2007). However, their high relative abundances in the surface waters were surprising, particularly as most sponges have larvae which are lecithotrophic and only briefly free swimming (McClintock et al. 2005). It is unclear how sponge DNA became distributed in the pattern we observed, with porifera making up a large fraction of the metazoan sequences in the surface waters, but almost absent from deep waters and sediments. It is possible some of these desmospongiea have longer larval periods, perhaps to allow for greater dispersal, or in order to match settling time of the larvae with the seasonal phytodetritus flux. Iceberg scour or anchor ice may also dislodge pieces of sponges and carry them to the surface waters.

Sediments as a Seed Bank

Cryothecomonas, our highest relative abundance cercozoan, has often been reported in polar waters and sea ice (Thaler & Lovejoy 2012, Durbin & Casas 2013). We found higher relative abundances of cryothecomonas in surface waters as compared to deep waters, which is in agreement with earlier studies finding highest abundances near the surface, and particularly in association with sea ice (Thaler & Lovejoy 2012). Cryothecomonas are generalist predators, with different species grazing on diatoms, protists, or bacteria, and their abundance has been correlated with total chlorophyll (Thaler & Lovejoy 2012). Described species of cryothecomonas are 9 to 32 μm in length,

but molecular probes suggest there are species or life stages less than 5µm in length (Thaler & Lovejoy 2012). In addition to their presence in surface waters, cryptheco monas made up over a third of the cercozoa sequences in sediment samples, the sample type in which cercozoa showed highest relative abundances overall. To date, living cells of cryothecomonas have not been reported from sediment samples (Thaler & Lovejoy 2012). However, there are reports of the related and morphologically similar *Protapsis* in sediments, which may potentially be misidentifications of cryothecomonas (Thaler & Lovejoy 2012). Cyst like cells have also been observed in cultures and water column samples (Thaler & Lovejoy 2012), so such cysts may act as a resting stage for cryothecomonas, allowing them to settle out to the sediment over winter, and re-enter the water column at the time of the ice edge bloom, when environmental conditions would be expected to be optimal for their lifestyle.

Diatoms in sediments were also suggestive of resting spores, which could seed the spring bloom (Durbin 1978). The dominant sediment diatom OTU, which made up just under a third of all sediment sequences, was a *Chaetoceros sp*., whose sequence is identical over the target region to reference sequences for *C. socialis, C. debilis,* and *C. setoense. C. socialis* is known to form resting spores, and such spores have been observed in morphology-based analyses of Wilhelmina Bay near bottom waters (Ferrario et al. 1998). *Chaetoceros* resting spores can be highly abundant in WAP sediments, with estimates of 300 to 900 million spores per gm dry weight of sediment (Crosta et al. 1997). The correlation of the relative sequence abundance of this *Chaetoceros* OTU in sediments with the depth of the overlaying water column may suggest predation on these spores during sinking, or may simply reflect geographic variations in the intensity of the

spring bloom. This gradient in *Chaetoceros* spore concentration with depth has been observed previously, and has been interpreted as an indication of the neritic environment serving as the main habitat for these spore-forming diatoms (Crosta et al. 1997). *Fragilariopsis*, a group associated with sea ice and ice edge blooms, form resting spores which have also been observed in this region (Ferrario et al. 1998); we only found this diatom type in sediment samples. *Porosira* is another diatom group known to form resting spores (Fryxell 1989) and we saw this group mainly in sediments with lower relative abundances in deep waters.

Other stramenopile OTUs show a similar pattern to that of the diatoms, with a few types up in the water column, and sequences of known spore-forming types found in the sediment. The pelageophyceae which made up 30-60% of the non-diatom stramenopiles in the surface waters are small (3-5μm), round, and non-descript members of the photosynthetic ultraplankton (Lee 2008). Many species from this group of tiny cells have just a single chloroplast and single mitochondrion (Lee 2008). The pelageophyceae are known to thrive at low temperatures, making their high relative abundance in WAP's coldest waters, near the surface, not surprising (Lee 2008). Chrysophyceae, our most abundant sediment stramenopile, is a golden-brown alga which is known to survive unfavorable periods as a resting statospore (Lee 2008). Similarly, Pedinellales, which were present at a moderately high relative abundance, but only in sediments, form a type of resting cyst under certain conditions (Thomsen 1988).

Marine sediments have been suggested to serve as a "seed bank" for the spring bloom, particularly in areas of high seasonality such as the WAP (Smayda 2011). Our results support this seed bank idea in the WAP – a large fraction of the sequences found

in the sediment samples are derived from organisms with largely pelagic life-style, but which are known to form resting spores. Thus we see DNA likely originating in resting spores from *Chaetoceros* of the hyalochaete (spore forming) section (Fryxell 1989), *Porosira*, and *Fragilariopsis* diatoms, from statospores of Chrysophycea, and from cysts of *Cryothecomonas* and Pedinellales. Resting spores in these sediments may help to initialize the spring bloom and contribute to phytoplankton diversity in the region.

Sediments as a food bank?

In addition to their role as a 'seed bank' for the spring bloom, it has also been suggested that WAP sediments may also act as a 'food bank' for heterotrophs, by preserving the phytodetritus which falls out for the spring bloom and providing a more steady food source for herbivores and detritivores during the winter period of low pelagic food availability (Minks et al. 2005). Minks et al. (2005) argued that elevated levels of photosynthetically-derived pigments in WAP sediments support this hypothesis of bulk phytoplankton biomass preservation into the winter, and its potential role as a food resource (Minks et al. 2005). DNA is degraded more rapidly than bulk biomass and pigments, and it is perhaps not surprising we did not see DNA evidence of the sediments acting as a food bank. The high abundance of bulk phytoplankton biomass in the sediments is suggested to be due to temperature limitations on extracellular enzymatic breakdown leading to the necessity of high substrate concentrations of these large molecules (Mincks et al. 2005). Since DNA is a smaller molecule composed largely of sugars, it may be small enough to be brought within the predator cell, and thus not subject to the same temperature limitation on substrate concentration as bulk biomass.

We found negligible abundances of OTUs from types of non-spore forming phytoplankton in the sediment. Spring blooms in the Southern Ocean generally contain several species, often a mixture of *Thalassiosira*, *Eucampia, Odontella, Rhizosolenia, Navicula*, *Proboscia*, and/or *Corethron*, in addition to a variety of *Chaetoceros* (Fryxell 1989, Knox 2007) – yet we only observe DNA sequences for the resting-spore forming groups of *Chaetoceros* and *Fragilariopsis.* Even within the *Chaetoceros*, the dominant species within blooms are typically the more heavily silicide forms of the *Phaeoceros* section which do not form spores (Fryxell 1989), but might be expected to be more resistant to degredation, and DNA sequences from these heavily silicified types were not important components of the sediment OTU assemblage, again suggesting the sediments are not preserving DNA from the bulk phytodetritus. Considering the non-diatom phytoplankton, *Phaeocystis* can be an important component of WAP assemblages. Summer blooms of *Phaeocystis* can reach high abundances and biomass, particularly in the inshore and Gerlache Strait regions (Ducklow et al. 2007). When these blooms terminate *Phaeocystis* settling rates out of the water column and down to the sediment can exceed 4 g C m^{-2} day^{-1} (DiTullio et al. 2000). If the sediments were acting as a food bank, and preserving the cells sinking out from the spring bloom, one might expect that *Phaeocystis* would be present, or even common, in sediment 18S assemblages. Instead what was observed was exceedingly low abundances of Phaeocystis OTUs, with these OTUs making up less than 0.007% of the total sediment sequences. This suggests that at least for Phaeocystis DNA the sediment was not acting as a food bank in the early winter in the WAP. If the sediments were acting as a food bank preserving the phytoplankton falling out of the spring bloom, we would expect to see a sediment assemblage of

phytoplankton OTUs similar to what would be expected in a spring bloom. However, this is not the pattern of OTUs we observe, suggesting that at least for small, bioavailable molecules like DNA, settling phytodetritus is rapidly consumed by herbivores or bacteria or degraded. This is not surprising given the abundance of organisms grazing on any available sediment phytodetritus. Within our sequence data we find benthic grazers including foraminiferans, worms (annelids, platyhelminthes, and nematodes), and kinhoryncha, all of which can feed on benthic phytodetritus and other sedimenting organic matter. Outside of the size range sampled in our sequencing efforts, Antarctic krill, *Euphausia superba*, are also likely to be an important grazer on benthic materials, as near bottom aggregations and feeding behavior have been observed previously, and were frequent during the sampling for this project (Cleary et al. in prep, Schmidt et al. 2011). It is possible that the shelf food bank may be seen more strongly in deeper areas; our deepest sediment samples, from Palmer Deep, showed notably more types of phytoplankton, and in greater relative abundances, suggesting they may be conforming more to the food bank model and preserving the phytoplankton and their DNA from the spring bloom. Palmer Deep is also a region with much lower abundances of krill, and hence lower grazing pressure on benthic food resources (Cleary et al. in prep).

Conclusions

In summary, during the winter in the WAP a highly diverse assemblage of microeukeryotes was observed, with different assemblages in surface waters, deep waters and sediments, and further variation by geographic location within all sample types. Microeukaryote ssemblages included OTUs from nearly all described kingdoms of eukaryotes. Groups with high relative sequence abundance included both well-known

organisms, such as diatoms and metazoans, and as yet largely undescribed groups such as Picozoa and Telonema.

The WAP shows high seasonality, with strong spring blooms and long summer days followed by dark winter with very low photosynthetic production and biomass (Ducklow et al. 2007). The extreme seasonality appeared to influence the observedour distributions of OTUs. This seasonality is clearest in sediment samples, which appear to be serving as a seed bank. These benthic assemblages are dominated by sequences of pelagic organisms, spending the cold winter months as resting cysts or spores on the seafloor. These OTUs of diatoms and other groups are potentially available to seed a bloom when the light and stratification of spring arrive in the WAP.

Though the WAP has sometimes been considered a simple ecosystem, with a food web dominated by diatoms-krill-whales, molecular analysis showed an incredible diversity of organisms, likely filling a diverse range of complex ecological roles, and interacting in ways yet to be understood. Including these small and less familiar organisms in our understanding and modeling of the WAP may help to improve mechanistic understanding and future modeling of this important and fascinating region.

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Tables

Samples	Type	Date	Time (local)	Depth	Latitude	Longitude	Location	Map Symbol	Temp C	Salinity (psu)
1,2,3	Water	24-May	23:05	20	-64.847	-62.611	Andvord Bay	1	-0.892	33.655
4,5,6	Water	29-May	11:15	250	-65.047	-63.301	Flanders Bay	2	0.718	34.474
7,8,9,79	Water	29-May	11:15	20	-65.047	-63.301	Flanders Bay	2	-0.767	33.636
10,11,12	Water	1-Jun	10:40	600	-64.967	-64.355	Palmer Deep	3	1.427	34.661
13,14,15	Water	1-Jun	10:40	20	-64.967	-64.355	Palmer Deep	3	-0.864	33.646
16,17,18	Water	3-Jun	00:50	300	-64.795	-63.121	Gerlache Strait	4	-0.236	34.507
19,20,21	Water	3-Jun	00:50	20	-64.795	-63.121	Gerlache Strait	4	-0.581	33.838
22,23,24	Sediment	18-May	18:25	520	-64.686	-62.235	Wilhemina Bay (in krill)	5		
25,26,27	Sediment	22-May	18:00	628	-64.535	-62.235	Wilhemina (out of krill)	6		
28,29,30	Sediment	26-May	20:20	545	-64.812	-62.733	Andvord Bay	7		
31,32,33	Sediment	27-May	16:00	356	-64.811	-62.718	Andvord trawl line	8		
34,35,36	Sediment	30-May	16:30	725	-65.003	-63.311	Flanders Bay	9		
37,38,39	Sediment	1-Jun	13:20	1345	-64.967	-64.355	Palmer Deep	3		

Table 1: Sampling locations and metadata. Sample numbers correspond to independent water filters or sediment scrapings, except in the case of 79 which is a technical replicate for 9. All dates are in 2013, time is in Chilean local 24 hr time, depth is in meters, map symbol corresponds to figure 1. Salinity and temperature data are not available for sediment samples.

OTU #	Taxonomic Identity	Surface Water	Deep Water	Sediment
12711	Chaetoceros socialis/debilis diatom	0.01	0.10	29.32
7747	Scrippsiella-like dinoflagellate	13.18	10.17	0.32
7743	Thalasiossira/Stephanodiscus/Cyclotella diatom	14.62	1.71	0.37
7744	Heliospora	3.88	4.38	3.28
2567	Heliospora	0.03	0.05	7.64
8258	Aureococcus stramenopile	7.43	0.20	0.00
8259	Heliospora	2.29	2.00	2.27
3669	Cryothecomonas	0.43	0.04	3.78
11332	Polycheate metazoan	0.14	6.46	0.79
14627	Stramenopile	4.00	0.76	0.00
16996	Choreotrichia cilliate	2.82	1.15	0.00
16265	Silicofilosea rhizarian	0.00	0.03	2.07
5278	Syndiniales alveotate	0.16	4.76	0.00
7516	Karlodinium alveolate	1.54	1.61	0.17
7741	Stramenopile	1.99	0.94	0.00
12357	Pseudocalanus/Scaphocalanus copepod	0.00	4.54	0.00
4398	Picozoa	2.24	0.21	0.00
15339	Sticholonche radiolarian	0.22	3.90	0.01
13812	Karlodinium-like alveolate	0.79	0.88	0.68
1770	Heliospora	0.00	0.05	1.62

Table 2: 20 most individually abundant OTUs, with taxonomic identity, and the percent each OTU made up of the total from surface waters, deep waters, and sediment samples.

Group	Surface water	Deep Water	Sediments
Alveolates (other)	20.77	43.00	3.19
Amoebas	0.00	0.01	0.18
Apicomplexa	0.03	0.08	2.62
Centrohelids	0.00	0.01	0.04
Cerocozoa	0.70	0.30	11.57
Chlorophytes	1.84	0.06	0.17
Chloroplastida	0.01	0.05	0.03
Ciliates	9.11	4.60	1.48
Cryptophytes	1.49	0.20	0.11
Diatom	17.56	2.36	31.40
Dinoflagellates	3.37	1.55	0.18
Excavates	0.10	2.18	0.41
Foraminifera	0.06	1.23	1.09
Fungus	0.05	0.07	0.51
Haptophytes	3.17	0.17	0.01
Holozoa	1.60	0.78	0.10
Metazoa	1.56	16.47	11.48
Picozoa	6.58	1.14	0.00
Radiolarians	0.56	8.56	0.11
Rhodophytes	0.01	0.00	0.00
SAR - unspecified	0.07	0.11	0.06
Stramenopiles (non-diatoms)	20.00	5.17	3.52
Telonema	2.20	0.24	0.11
Other	1.39	1.35	0.36
Unknown	7.76	10.32	31.26

Table 3: Percent of each group of sequences within each sample type. Alveolates includes all alveolates except the separately described dinoflagellates, ciliates, and apicomplexa

Figures

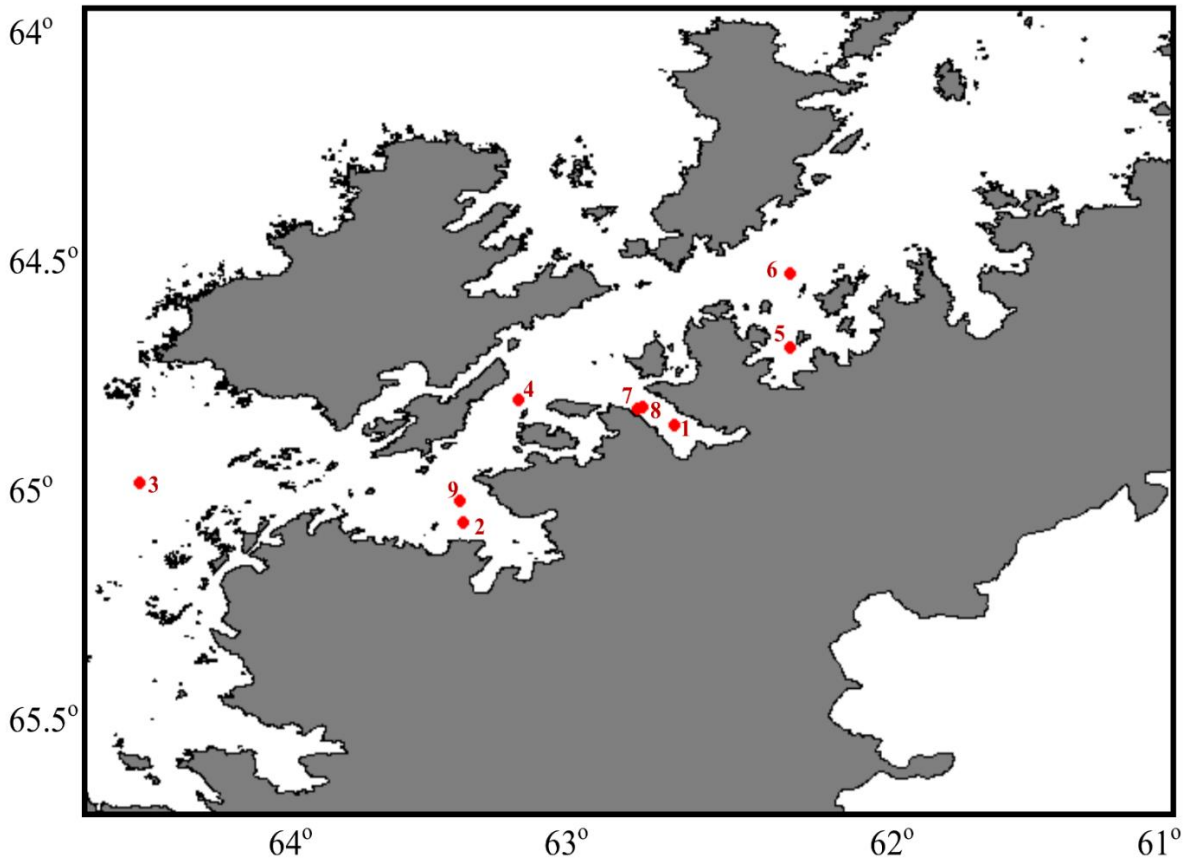

Figure 1: Map of the study region indicating sampling locations. Numbers indicate sampling sites, the corresponding samples can be found in Table 1. Latitude and Longitude are expressed in degrees south and west, respectively.

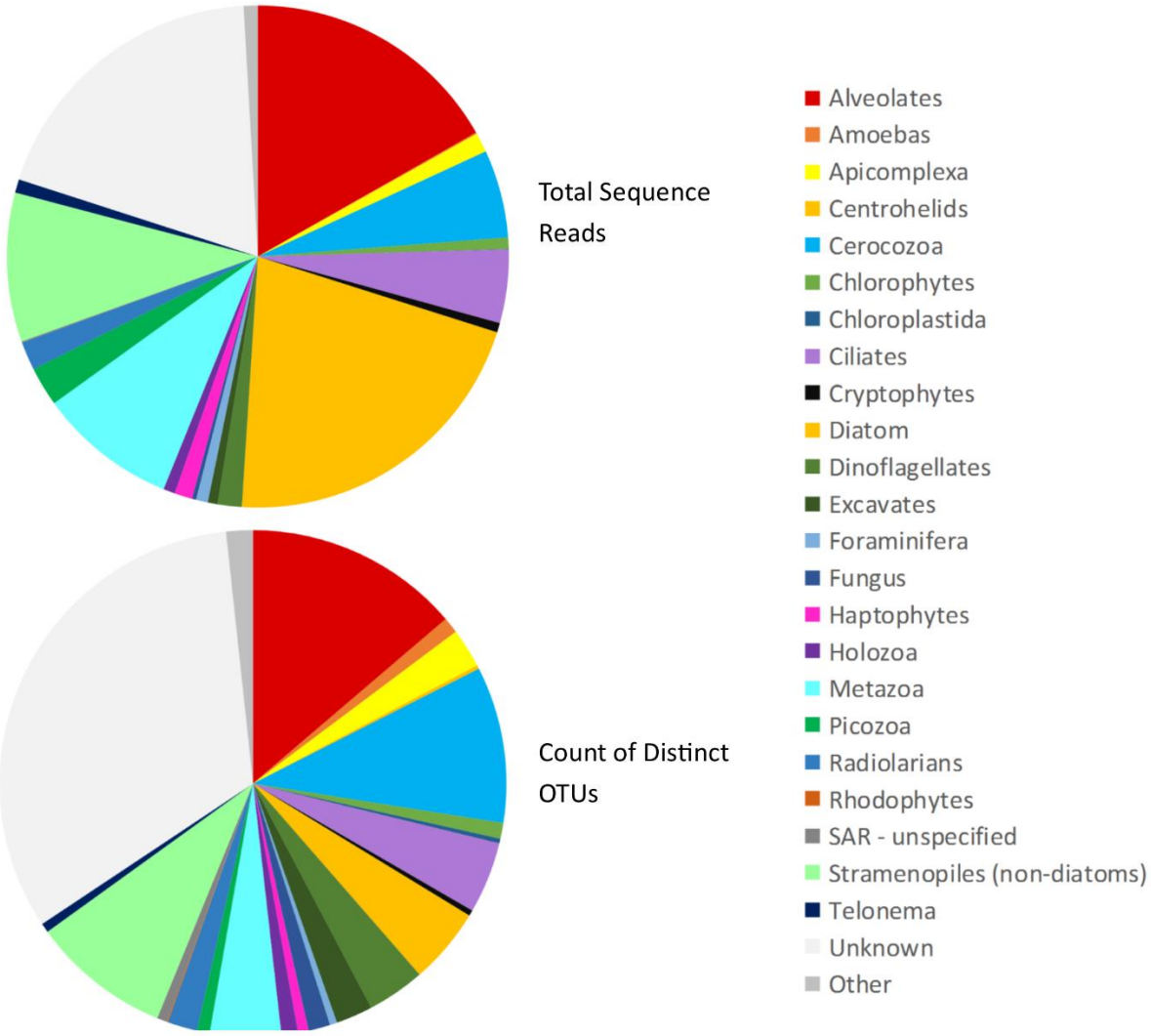

Figure 2: Pie charts of all sequence read abundances in each category (above) and the number of distinct OTUs in each category (below)

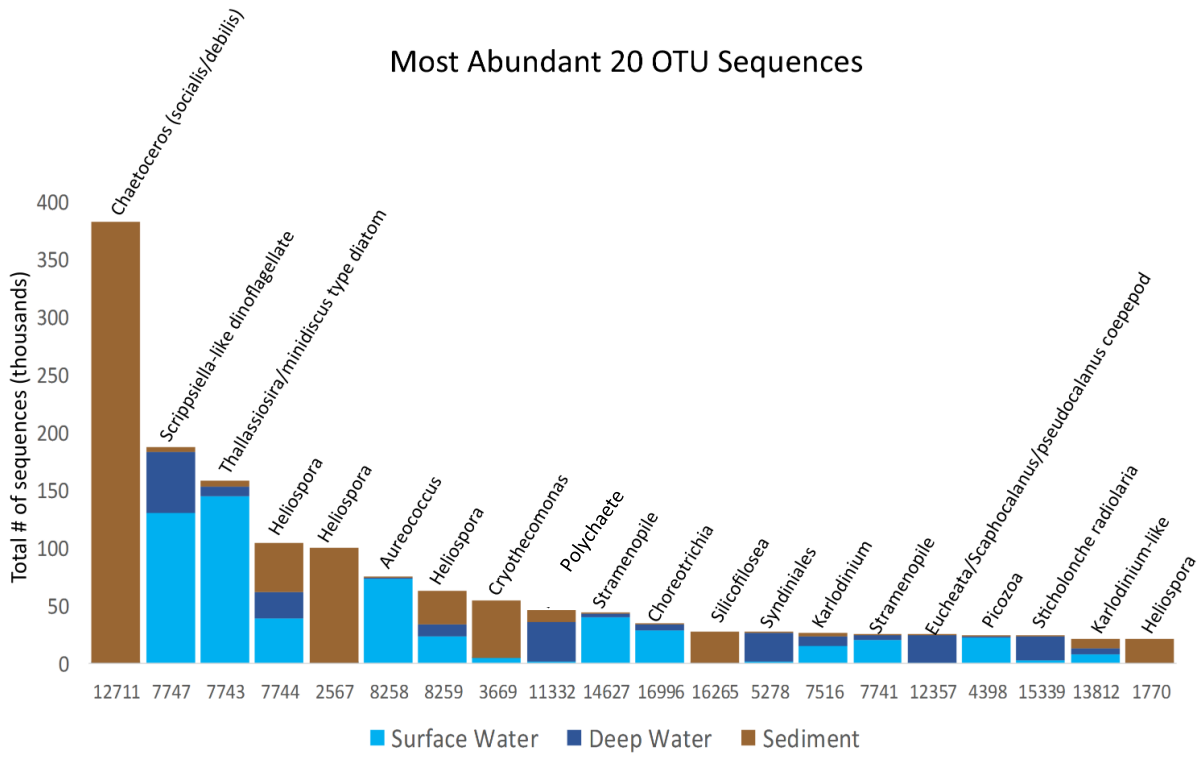

Figure 3: Rank Abundance Curve showing the total sequence abundance of the 20 most abundant OTUs overall. Taxonomic identity for each OTU is indicated above the bar, with OTU number along the x-axis. Bars are colored by the source of each sequence, as surface water, deep water, or sediment.

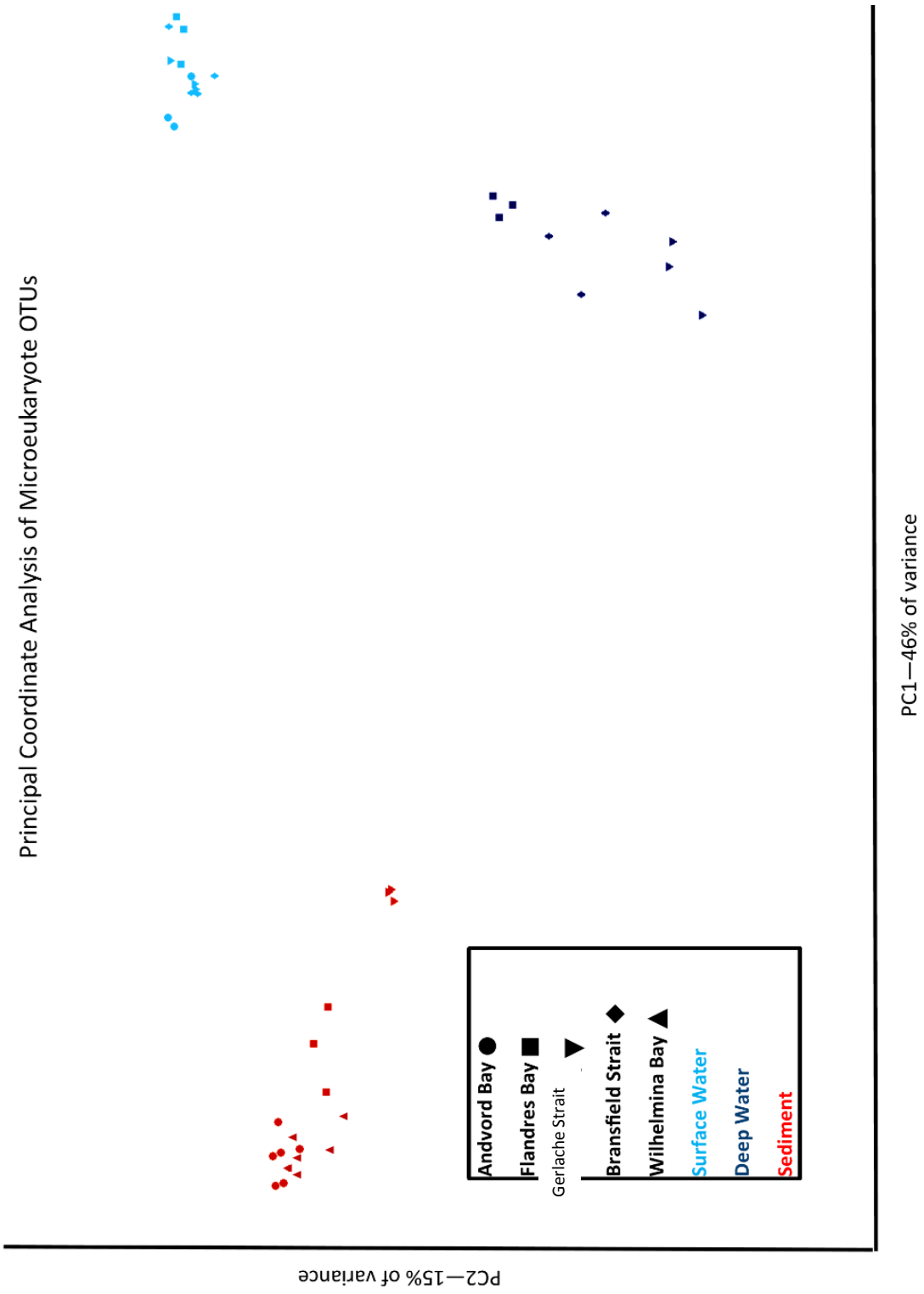

Figure 4: Principal Coordinates Plot of microeukarytoe assemblages in each sample – Colors indicate sample type, surface water, deep water, or sediment, and shape indicates sampling locations

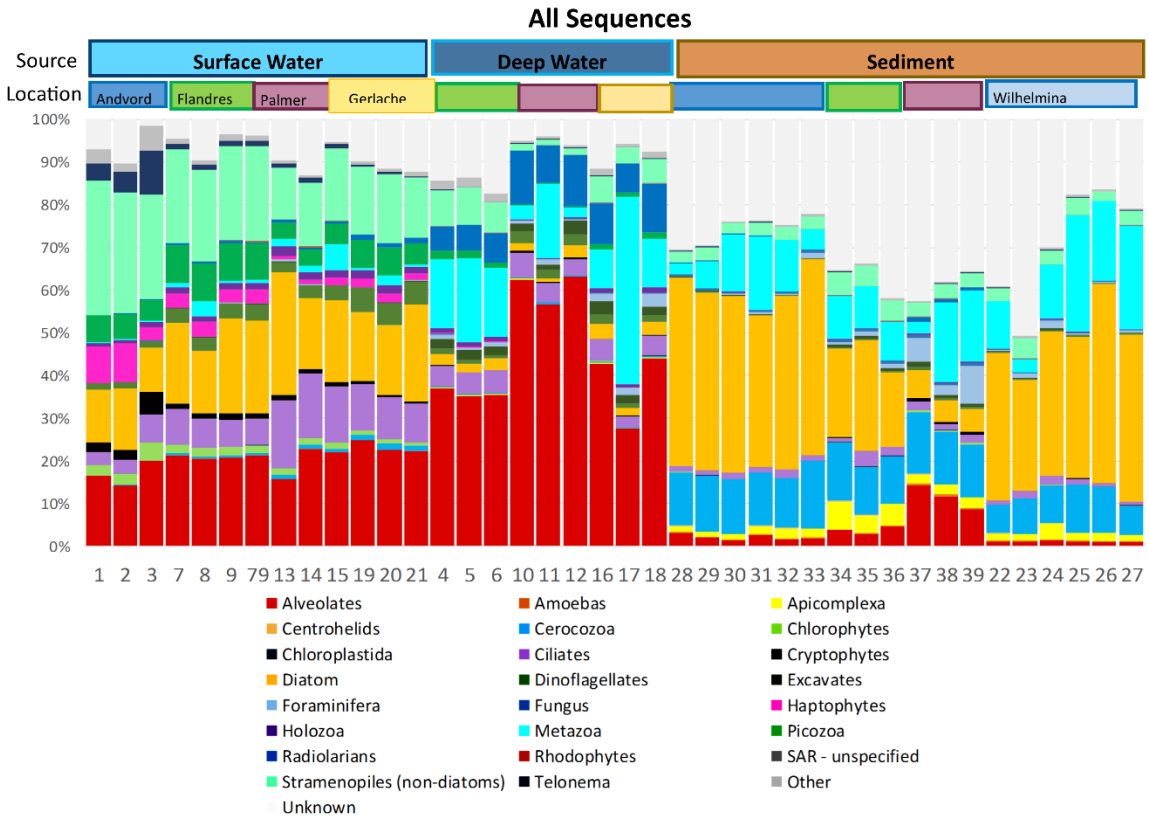

Figure 5: Overall bar graph showing the distribution of all types of OTUs across all samples. Other includes diverse groups which are of such low abundance they cannot be individually visualized at this scale, while unknown OTUs have no assigned taxonomic identity.

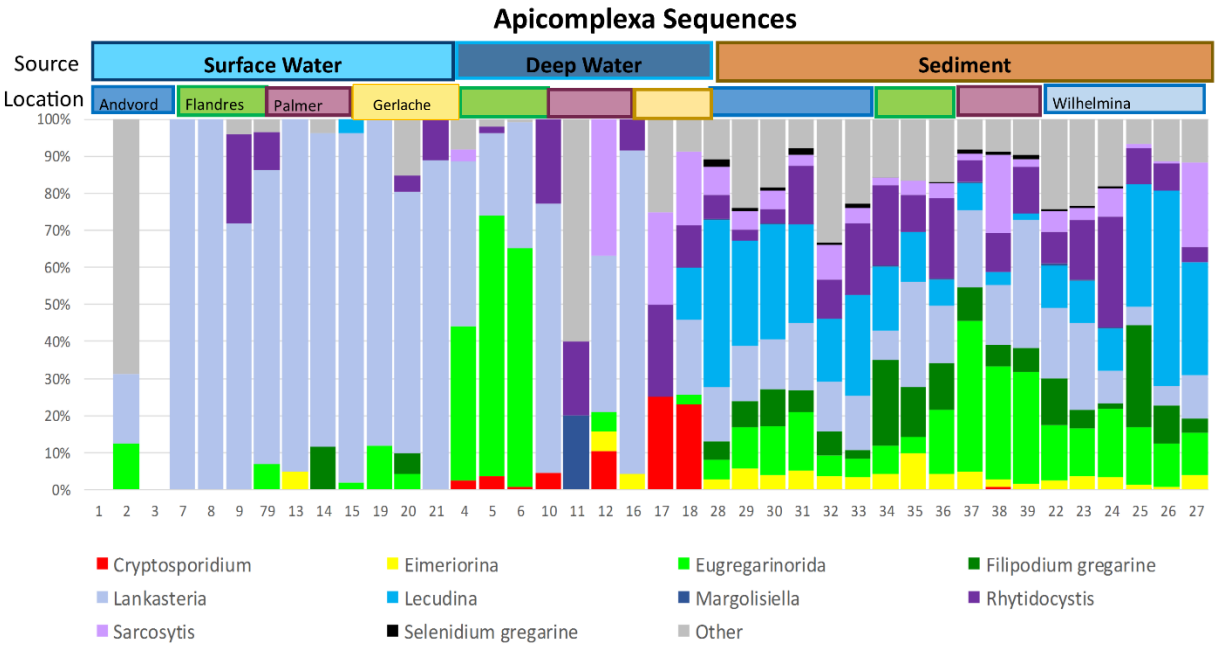

Figure 6: Apicomplexa bar graph – very few apicomplexa sequences were present in the water column and that data is not necessarily representative.

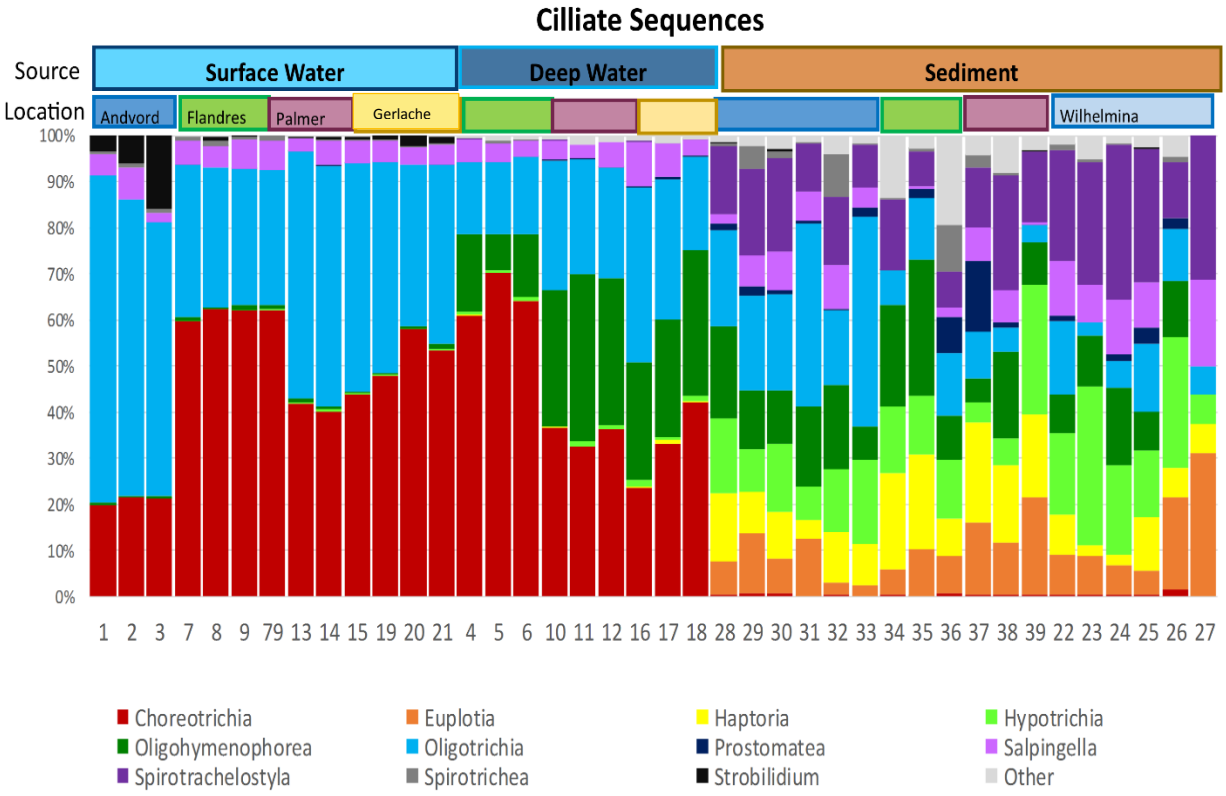

Figure 7: Cilliate bar graph showing the distribution of ciliate sequence reads across all samples.

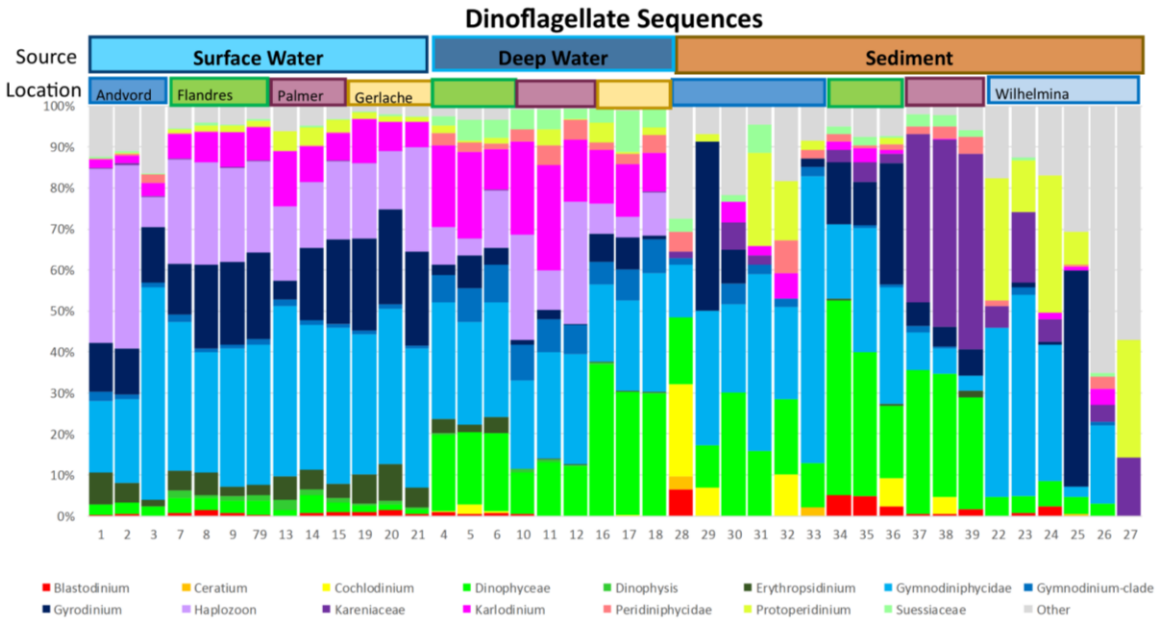

Figure 8: Dinoflagellate bar graph showing the distribution of dinoflagellate sequencing reads across all samples

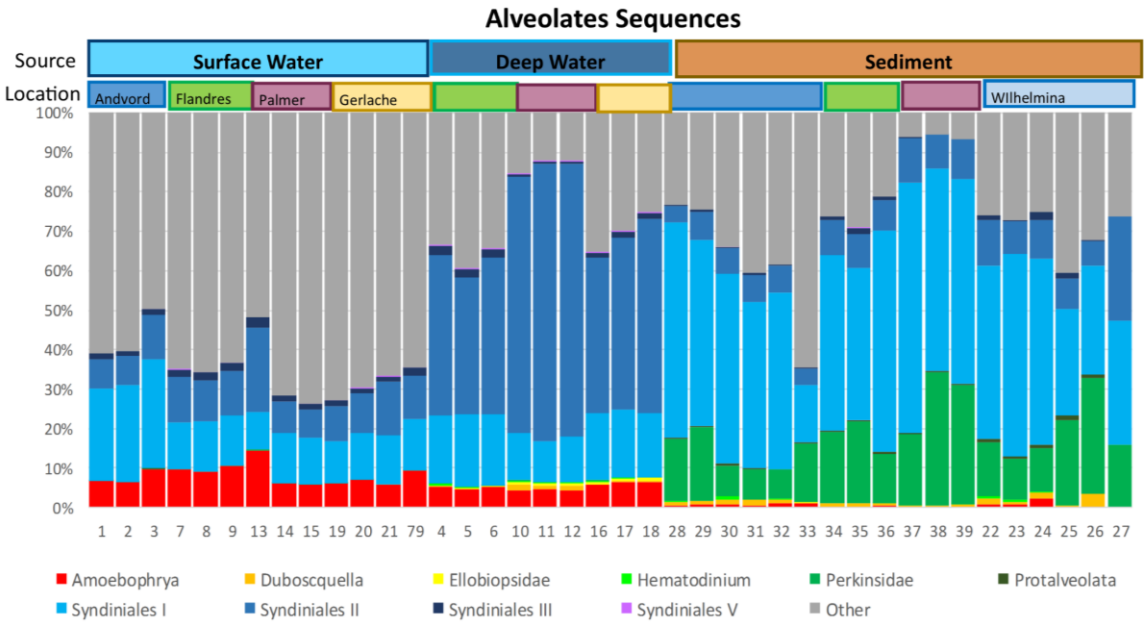

Figure 9: Other Alveolates bar graph showing the distribution of alveolate sequences across all samples. Syndiniales sequences in blue make up a large proportion of the alveolates.

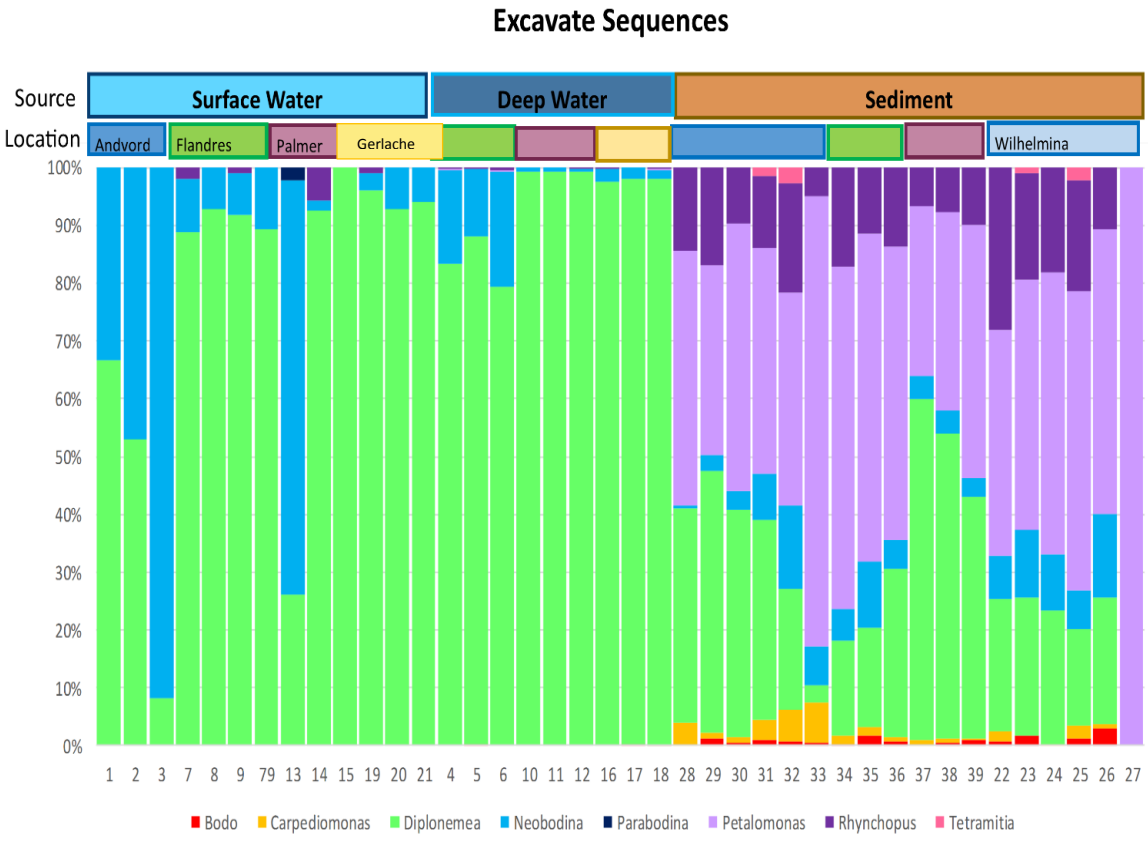

Figure 10: Excavate bar graph showing the distribution of excavate sequences, mainly types of euglena, across all samples.

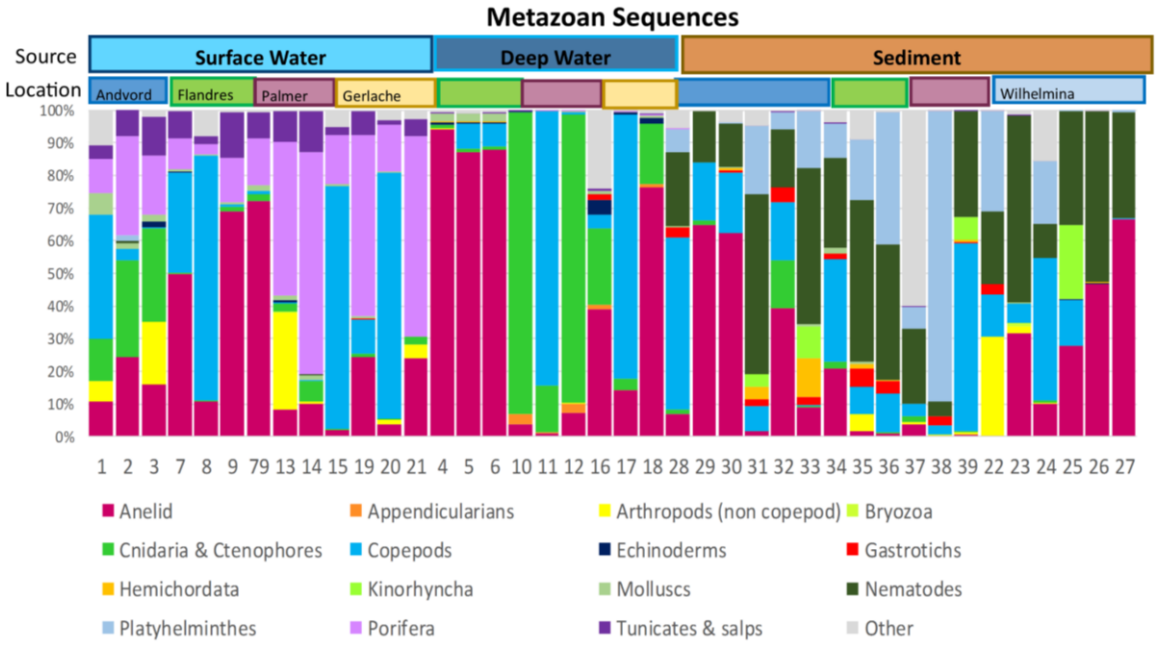

Figure 11: Metazoan bar graph showing the distribution of metazoan sequence reads across all samples.

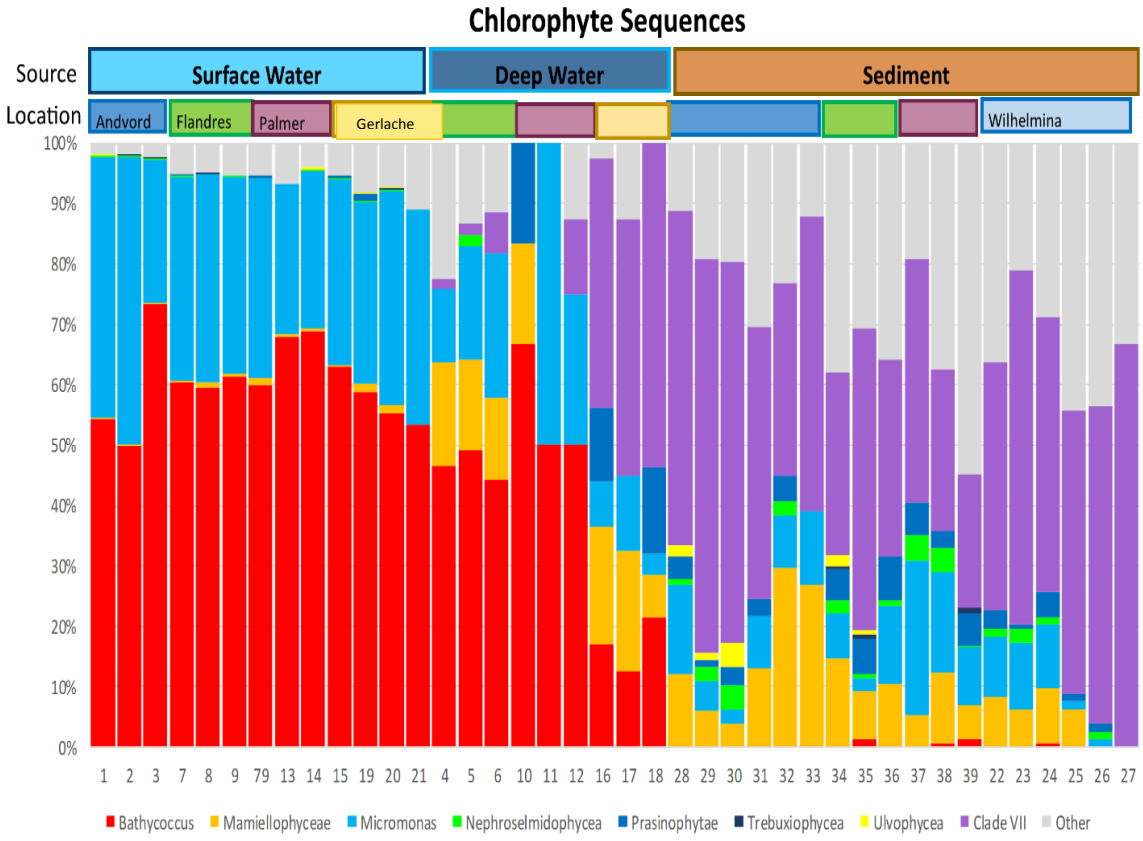

Figure 12: Chlorophyte bar graph showing the distribution of chlorophyte sequences across all samples.

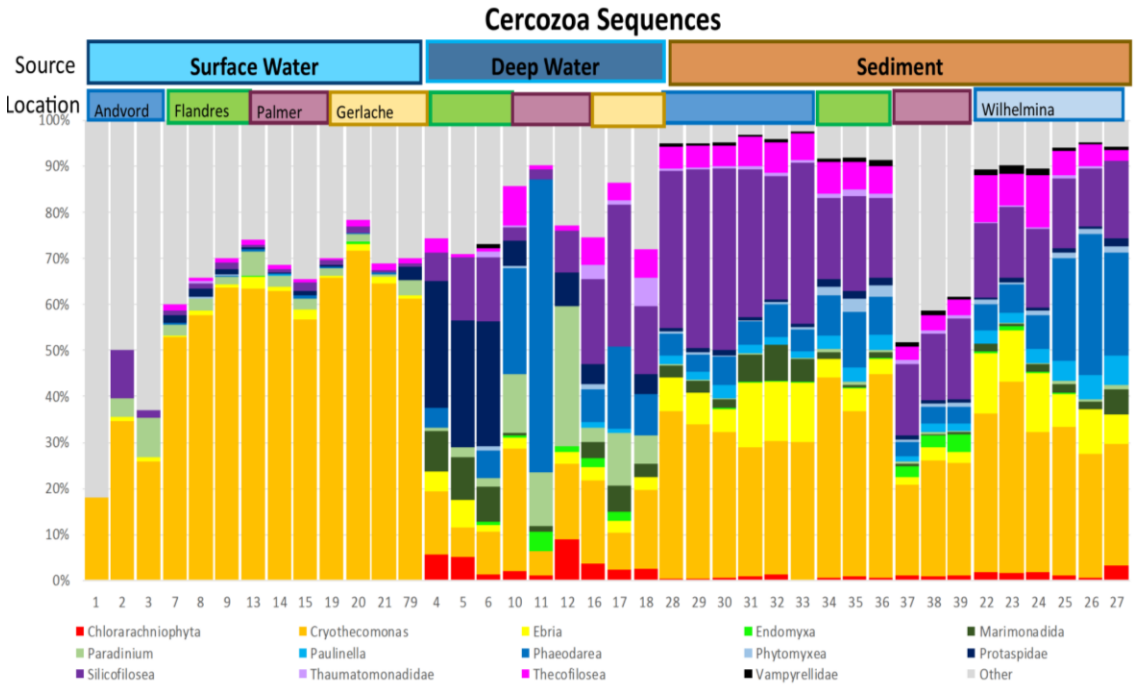

Figure 13: Cercozoa bar graph showing the distribution of cercozoa sequences across all samples.

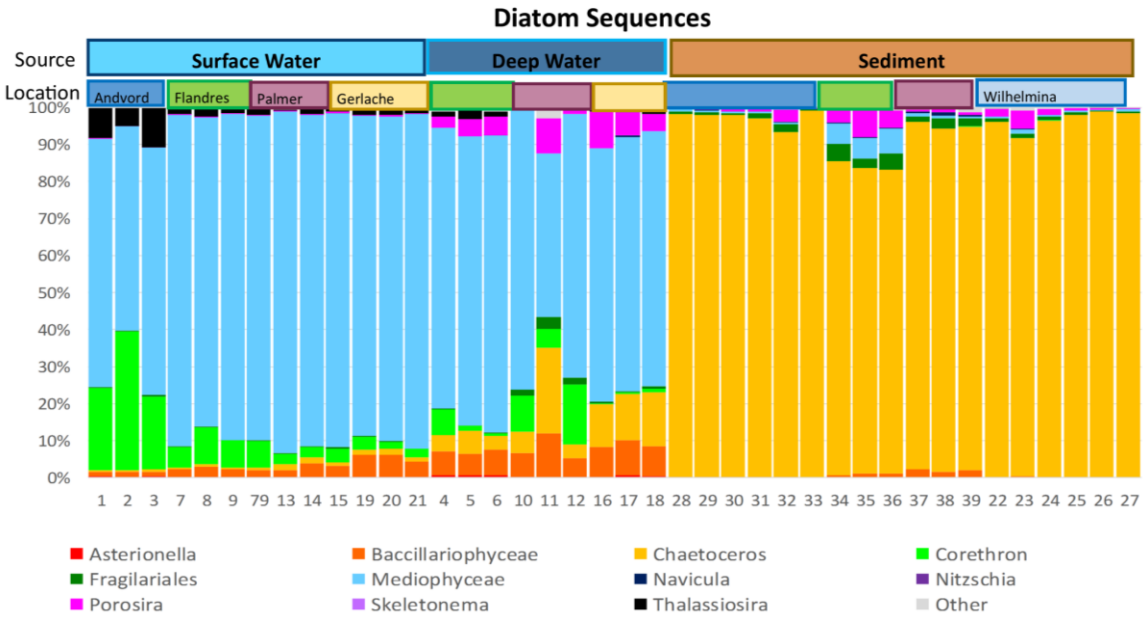

Figure 14: Diatom bar graph showing the distribution of diatom sequence reads across all samples.

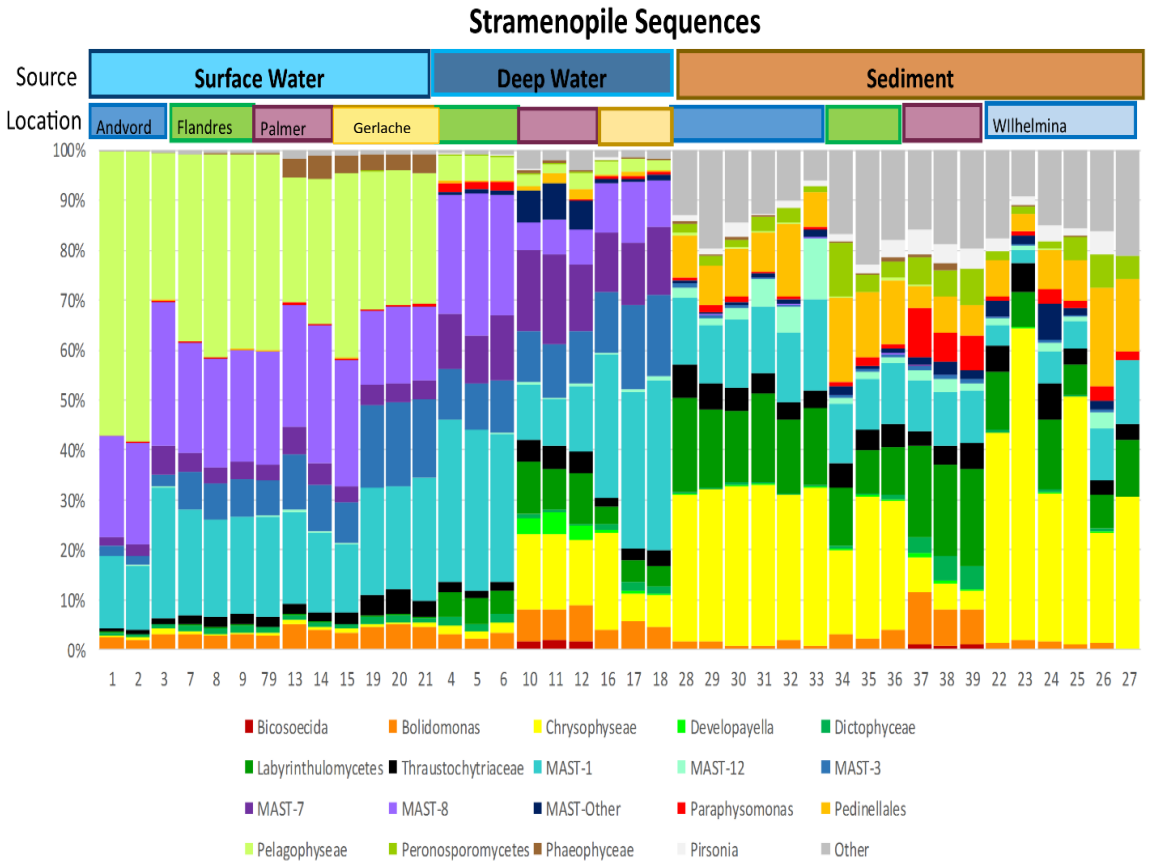

Figure 15: Stramenopile bar graph showing the distribution of all non-diatom strameopile sequence reads across all samples.

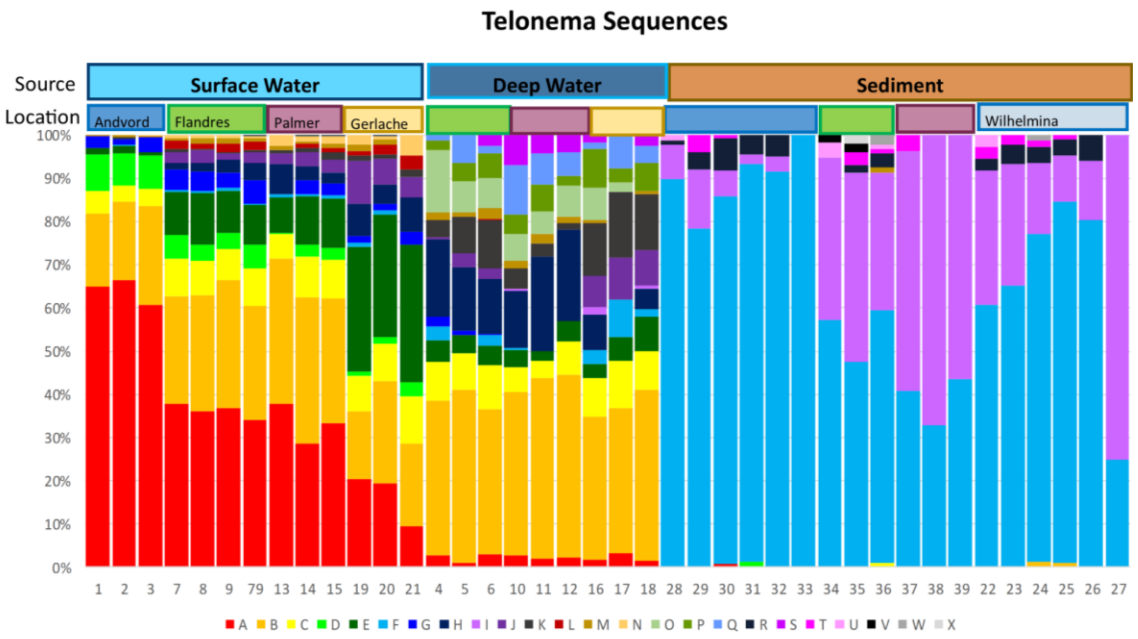

Figure 16: Telonema bar graph showing the distribution of sequences from each telonema type across all samples.

Unexpected prevalence of parasite sequences amongst Antarctic marine protists

Alison Cleary*, Edward Durbin

This manuscript was prepared in response to discussion in the defense of this dissertation and is formatted for submission to PLoS One. The idea was conceived by AC and ED, AC analyzed the data. AC wrote the manuscript with contributions from ED.

Unexpected prevalence of parasite sequences amongst Antarctic marine protists

Alison Cleary*, Edward Durbin

Graduate School of Oceanography, University of Rhode Island, Narragansett, Rhode Island, USA

* Corresponding author: acleary@my.uri.edu, (415) 300-6556

Abstract

Parasites are not typically considered to be important components of polar marine ecosystems. Environmental 18S rDNA surveys of the diversity and distribution of eukaryotes in the West Antarctic Peninsula in winter revealed surprisingly high abundances of sequences associated with parasitic protist groups. Parasite sequences made up on average over half (52%) of the sequence reads in samples from deep water. Surface water and sediment samples containing fewer parasite sequences (14% and 11% respectively), but these abundances still suggest potential ecological importance of parasites. One thousand and forty two distinct parasite Operational Taxonomic Units were observed, with the largest abundances and diversities within the avleolate groups, particularly the *Syndiniales* and related *Amoebophrya*. Less abundant parasite sequences included those associated with *Apicomplexa*, *Blastodinium*, *Chytriodinium*, *Cryptocaryon*, *Ichthyosporea*, *Paradinium*, *Perkinsidae*, and *Pirsonia*. While it is possible some of this abundance of parasite sequences may result from methodological artifacts, this abundance may suggest the role of diverse lifestyles within parasite groups, and also suggests it may be worth considering more closely the role of parasites in the West Antarctic Peninsula marine ecosystem. Higher abundances of parasites within this productive ecosystem has potential implications for the role of the microbial loop, carbon flows, and ecosystem responses to ongoing anthropogenic climate change.

Key Words: 18S rDNA, parasites, protists, plankton, West Antarctic Peninsula, Syndiniales

Introduction

The West Antarctic peninsula has traditionally been thought of as a simple ecosystem, with the food web dominated by large phytoplankton being consumed by krill, which were in turn consumed by penguins, seals, and whales (Huntley et al. 1991). More recently, the roles of smaller phytoplankton, microzooplankton, bacteria, and the ensuing microbial loop have been increasingly recognized (Sailley et al. 2013). This microbial loop is now known to play an important role in carbon cycling and food web dynamics in the region (Sailley et al. 2013). Even as the role of many of these smaller organisms in the WAP marine ecosystem has been increasingly recognized, one group of small marine organisms which continues to escape attention are the parasitic protists.

The abundance of protistan parasites is thought to be under-accounted for in marine planktonic systems generally (Skovgaard 2014). Analysis of metazoan parasites in estuaries showed they accounted for 2-3% of the total biomass (Kuris et al. 2008). Protistan parasites can be difficult to observe, since they are small and spend much of their lives hidden within their hosts. These parasites may be invisible when analyzing preserved plankton samples (Skovgaard & Daugbjerg 2008).

Protistan parasite diversity is also poorly known, particularly in the marine environment. For many parasitic protist groups there are only rough estimates of the total diversity and abundance. It has been suggested that the biodiversity of parasites as a whole may be comparable to that of all non-parasite groups combined (Hudson et al. 2006). Many groups which have been identified as obligate parasites include only a handful of described species (Chambouvet et al. 2014). Yet, molecular surveys suggest

these groups may be highly diverse and contain hundreds or more species (Chambouvet et al. 2014, Skovgaard 2014).

Although these protistan parasites are poorly known, they may play important roles in ecosystem dynamics. Protistan parasites can crop the abundances of their hosts, affecting their population dynamics (Coats & Park 2002). Such reductions in the populations of hosts can also have potential impacts on the predators of these hosts. In the process of killing, or reducing the fitness, of hosts in a species specific and density dependent manner, selective protistan parasites can also play a role in maintaining ecosystem diversity (Hudson et al. 2006). Protistan parasite activity can also lead to increased release of dissolved and particulate organic matter (DOM & POM) from their hosts as they put them under increased physiological stress, or cause them to lyse (Skovgaard 2014). Such organic matter can be taken up by bacteria or small eukaryotes, fueling the microbial loop. Thus, understanding the roles of these parasites is important to our overall understanding of carbon flows and trophic interactions in the west Antarctic Peninsula marine ecosystem.

New data from the West Antarctic Peninsula marine ecosystem offered an opportunity to investigate the diversity, distributions and relative abundance of protistan parasites. Millions of 18S DNA barcode sequences from the microeukaryote communities of the water column and sediments were used to obtain a first glimpse into the types of parasites present in the region, how these parasites are distributed in this ecosystem, and how much of the protist DNA in this ecosystem can be attributed to parasites.

Materials and Methods

Field Collections - Samples were collected between May 18 and June 3 on RVIB Nathaniel B Palmer cruise NBP1304. All samples were collected in triplicate. Water samples were collected in 12L niskin bottles on a CTD rosette. Surface water was collected from the surface mixed layer at 20 m depth. Deep water was collected at 10m above the seafloor within bays, and at 600m in Palmer Deep (bottom depth 1345m) (table 1). For each water sample, 2L of whole seawater were filtered by peristaltic pump onto a 0.2 µm membrane filter thus collecting all organisms or pieces of organisms between 0.2 µm and approximately 5 mm diameter. Surface and deep water samples were collected from Flandres Bay, Gerlache Strait, and Palmer Deep; surface water only was collected in Andvord Bay (Figure 1, Table 1). Filters were placed in individual cryovials and immediately frozen at -80^{o}C. Temperature and salinity were recorded simultaneously with sample collection with a SBE 911plus CTD (SeaBird).

Sediment samples were collected with a megacorer (Ocean Scientific Instruments Limited). In order to expose the sediment surface, overlaying water was gently removed by peristaltic pump and cores were extruded to just below the level of the sediment surface. The surface-most layer of sediment was sampled using sterile scrapers, placed in cryovials, and immediately frozen at -80^{o} C. Sediment samples collected all organisms less than approximately 5 mm. Sediment was sampled from two locations in Wilhelmina Bay, two locations in Andvord Bay, one location in Flandres Bay and one location in Palmer Deep (Figure 1, Table 1). Samples were collected from 3 separate cores from one of the corings in Andvord Bay, and from a single core in each of the other corings. All

samples were shipped from Chile to Rhode Island on dry ice and stored at -80^{o} until analysis. Bottom depth was recorded by shipboard Chirp 3260 echo sounder (Knudsen).

Laboratory Processing – Total DNA was extracted from water filters with the DNeasy blood and tissue kit (Qiagen). Volumes of the initial lysis buffers were all doubled to ensure the filter was submerged and all material was lysed. Total DNA was extracted from 0.25 grams of each sediment sample using the PowerSoil kit (MoBio) with the bead-vortex lysis option as per manufacturer's instructions. Only samples of the same type (water/sediment) were extracted on the same day. All extractions were conducted in a sterilized laminar flow hood with project-dedicated pipettes, tips, and chemicals, to minimize possible contamination.

18S rDNA was amplified using universal eukaryotic primers (Gast et al. 2004) modified by the addition of a variable position in the reverse primer to improve priming of ciliates (Cleary et al. in press), and to include adaptors for Illumina sequencing, and a variable number (0-3) of ambiguous bases to offset the amplicons and increase the variability at each read position for improved base calling. Each reaction contained a final concentration of 1x Pfu Ultra II clear buffer (Agilent), 1x Bovine Serum Albumin (New England Biolabs), 0.25 mM equimolar mixture of all four deoxynucleotide triphosphates (dNTPs) (Promega), 0.1 µM each primer (forward and reverse), 1x Pfu Ultra II polymerase (Agilent), and 20% by volume DNA template at extracted concentrations. Thermocycling consisted of 95^{o} C for 30s, followed by cycles of 94^{o} C for 30s, 58^{o} C for 45s, and 72^{o} C for 30s, with a final extension of 72^{o} C for 5 min. Samples were amplified for the minimum number of cycles necessary to obtain sufficient DNA for sequencing in order to reduce amplification biases and over-representation of abundant targets. Water

samples were all amplified for 35 cycles, as were sediment samples 28, 29, 30, 35, and 37, while the remaining sediment samples were amplified for 30 cycles. Amplicon presence and size was confirmed with gel electrophoresis and UV visualization. Amplifications of no-template blanks included in each PCR showed no signs of contaminating DNA in gel images.

Amplicon purification and sequencing were done at the URI Genomics and Sequencing Center. Amplicons were AmPure cleaned, re-amplified to add sample identification tags, and quantified on a BioAnalyzer (Agilent). Amplicons were pooled into one half Illumina Miseq run, and sequenced for 500 cycles, allowing for almost complete overlap of the amplicon.

Data Analysis – Paired ends of reads were joined if the entire overlap region was identical in both read directions; if the overlap region was not identical, both reads were discarded. Amplicons were then assigned sample-specific names and pooled for further analysis. Primers, and any sequence data beyond the end of the amplicons were trimmed, and any sequences in which the exact primer sequence was not found were again discarded. This fairly stringent approach to quality control likely eliminated most sequencing errors, as such errors are unlikely to occur identically in the two directions of sequencing. Amplicons were clustered into 97% sequence identity Operational Taxonomic Units (OTUs), which have been commonly used as a proxy for species (Sogin et al. 2006). Any OTU with only a single sequence in it was discarded. OTUs with chimeric sequences were detected with the blast_fragments approach (Altschul et al. 1990), and removed from the data. All of the above analyses were conducted in Qiime v.1.8 (Caporaso et al. 2010). Because multicellular organisms are unlikely to be

quantitatively sampled in the small volumes of water and sediment analyzed here, these OTUs were also removed from the final data set.

Taxonomic identity was assigned to each OTU through automated comparison in Qiime with the Silva database v. 111 (Wang et al. 2007, Quast et al. 2013). BLAST searching was used to confirm and in some cases refine taxonomic assignments (Altschul et al 1990). OTUs were classified as parasitic, non-parasitic, or unknown based on literature. To keep estimates conservative those organisms for which a lifestyle could not be determined were included with the free-living organisms in all calculations.

A dissimilarity matrix of all samples was constructed using the Bray-Curtis metric (Bray & Curtis 1957) base on the sequence read counts for each of the parasite OTUs, normalized by the overall (parasite & free-living) total sequence read for each sample. Likewise, a dissimilarity matrix was constructed for all free-living OTUs. Principal coordinates were calculated and visualized in MatLab. Parasite and free-living communities were compared in a side-by-side cluster analysis based on Bray-Curtis distances. Interactions between parasite OTUs and potential host OTUs were explored using an OTU-wise Bray-Curtis metric with read counts normalized by OTU, and simple linear correlations with read counts normalized by sample. Parasite-host interaction analysis was limited to the 500 most abundant OTUs overall to avoid artifacts due to stochasticity at low abundance. Parasite-host analysis also excluded organisms with poor taxonomic resolution, or with very phylogenetically close parasites and hosts to avoid erroneous correlations due to potentially imperfect OTU picking.

Results

A total of 1042 parasite-associated OTUs which encompassed 363,135 sequence reads were recovered from the Antarctic coastal environment. 400 of these OTUs were found in surface water samples, 594 were found in deep-water samples, and 576 were found in sediment samples. Sequence reads from parasite-associated OTUs made up between 5.6% of the total sequence reads in sample 22 (Wilhelmina Bay sediment) and 73.0 % of the total sequence reads in sample 11 (Palmer Deep deep-water), with an overall average of 21.4% of the sequence reads in a sample. Deep-water samples showed the highest percentages of parasite-associated OTU reads, with 52.4% of the deep-water reads falling into these groups. 13.9% of surface water sequences were classified as parasite-associated OTUs. Sediments showed the lowest relative abundance of parasite reads, with 10.8% of total reads classified into parasite-associated OTUs (Table 2).

Parasite OTUs included a diverse range of organisms. Most of the parasite OTUs belonged to the alveolata. The most abundant group of sequences belonged to the syndiniales, making up 11.3% of the total sequences, and 62.2% of the parasite sequences. Within the syndiniales, organisms associated with syndiniales group II made the greatest contributions to both number of reads and number of OTUs. Also within the syndiniales, *Amoebophyra* were present mainly in water samples, but also in sediments, *Duboscquella* were found mainly in deep-water, and haplozoons were found most abundant in surface waters (Table 2, Figure 2). Dinoflagellate parasites were also observed; *Hematodinium* were found mainly in surface water samples, *Chytriodinium* was found mainly in water samples, and *Blastodinium* was found in all sample types (Table 2, Figure 2). Other alveolate parasites found included apicomplexa, which were mainly observed in sediments, cryptocaryon ciliates which were observed in all sample

types, ellobiopsis which was found mainly in water samples, and perkinsidae which had their highest relative abundance in sediments (Table 2, Figures 2 & 3).

Some parasites were also observed from the rhizaria. Rhizarian parasites included *Cryothecomonas* which was found most abundantly in sediments, *Paradinium* spp. which was observed across all sample types, and phytomyxea which was found mainly in sediments. Two groups of parasites belonging to the stramenopiles were observed; *Pirsonia* spp. was found mainly in deep-water and sediments, and *Solenicola* spp. was found mainly in surface waters. The only parasite group found which did not fall within the Stramenopile-Alveolate-Rhizaria (SAR) complex was the holozoa *Ichtyosporea* spp. (Table 2, Figures 2 & 3).

Parasites showed different assemblages in surface waters, deep-waters, and sediments. Surface waters contained large fractions of syndiniales and sub-groups, as well as noticeable contributions from cryothecomonas and haplozoons (Figure 3). Deep-water sample parasite assemblages were dominated by syndiniales and subgroups, particularly syndiniales II, with relatively low contributions from other groups. Sediment parasite assemblages showed much lower abundances of syndiniales than was observed in the water column, with the parasite assemblage composed mainly of cryothecomonas and apicomplexa, with contributions from perkinsidae as well as small contributions from syndiniales and sub-groups (Figure 3). The notable exception to this trend is Palmer Deep, where samples showed relatively more syndiniales, particularly group I. These differences in assemblages are evident in a Principal Coordinates Analysis (Figure 4). Samples cluster most strongly by sample type, but within sample type also show clustering by location. Parasite assemblages are closely tied to non-parasite assemblages.

A clustering analysis dendrogram indicated very similar clusterings for parasites and for non-parasite OTUs (Figure 5).

Considering relationships between specific parasites and potential hosts, the strongest correlations observed were between syndiniales and radiolarians. Twelve syndiniales OTUs correlated with $r^2>0.9$ with individual radiolarian OTUs (figure 6). Considering all syndiniales and all radiolarians, the positive linear correlation between the percent abundances of these groups over all samples showed a slope of 9.4 and an r^2 of 0.94 (Figure 6).

Discussion

The high proportions of parasite sequences reads observed in these samples from the Antarctic environment begs the question: Are these observed high abundances of parasite OTU sequences indicative of high parasitic activity in Antarctic marine ecosystems? There are several potential explanations for the observed high abundances: 1) The observed sequence abundances are an artefact of the DNA barcoding approach 2) Groups classified as parasitic in fact contain organisms with other lifestyles 3) Parasites have long lived spores or resting stages 4) Parasite abundance in the Antarctic is high. Each of these explanations likely plays a part in explaining the overall results, and the merits and likely impact of each are discussed below.

Pyrosequencing does not provide data on the overall abundance of organisms, but it has traditionally been used to infer relative abundances of different organisms and groups of organisms (Not et al. 2009). There is potential for biases in preservation efficiency, 18S copy numbers per cell, DNA extraction efficiency, PCR primer binding,

polymerase extension, amplicon purification, sequencing, and quality control. Despite these potential sources of bias, studies with bacterial mock communities have shown close resemblances of sequence read proportions with true cell abundance proportions (Jumpstart consortium 2012). Steps were taken to minimize the effect of all of these potential sources of bias in this analysis. PCR primers used have been optimized to efficiently amplify all major groups of eukaryotes (Cleary et al. in press).Differences in the number of copies of the 18S gene per cell or per unit carbon have been observed in some groups (Zhu et al. 2005). However, some of the groups notorious for high copy numbers were observed at low abundances, such as the free living dinoflagellates, which only made up 1.5% of the total sequences. This suggests that while 18S copy variations are certainly present, they are unlikely to be the full explanation for the observed high proportions of parasite sequences.

Many of the groups of parasites found in this study are very poorly known. Little is known about their diversity, abundances, distributions, morphology or ecology (Skovgaard 2014). This is particularly true of the largest group of parasite sequences observed, the syndiniales, for which there are many uncertainties (Bråte et al. 2012). Here organisms were classified as parasites if literature described the group to which they belong as obligately parasitic, or if all described organisms within the narrowest group to which the OTU could be identified were obligate parasites. However, it may be possible that some of these groups contain as yet undiscovered species with other lifestyles, such as free-living or mutualistic symbiosis. Including such hidden free-living organisms might explain some of the abundance of parasite sequences observed here.

Many parasitic organisms have some form of spore or infective stage, allowing them to spend time outside of a host in the process of finding a new host (Hudson et al. 2006). It is possible that some fraction of the parasite sequences observed here came not from active parasites but from some type of such a spore. However, many of these spores are very short lived, suggesting they would be unlikely to form a large reservoir of DNA sequences in the environment. For example, syndiniales and related organisms are thought to have very short lived spores, and to require new hosts within a matter of days (Coats & Park 2002). *Amoebophyra* spp. (syndiniales) spores show exponential declines in abundance, with most spores disintegrated within 3 to 13 days, an even amongst surviving spores, ability to infect declined rapidly over time since production (Coats & Park 2002). It may be possible, however, that some of the parasite organisms found in this study have longer lived spores, potentially as an adaptation to the extreme seasonality in the abundance and biomass of potential hosts in Antarctic marine ecosytems. In austral winter in the Antarctic Peninsula, phytoplankton biomass is very low, with measured values during the time of sampling consistently less than 0.5 µg chlorophyll a L^{-1}, whereas in summer in these same regions, chlorophyll a concentrations can exceed 30 µg L^{-1} (data not shown). It might thus potentially be advantageous for protistan parasites which rely on planktonic hosts to have the capacity to survive as a spore during over the winter period with low abundances of hosts.

Correlations between syndiniales OTUs and radiolarian OTUs suggest an ecological interaction, however, arguing against resting cysts as the major source of parasite sequences. Previous analysis of perkinsidae parasite sequences have been shown to represent ribosomally active cells in marine sediments, suggesting their DNA

abundance is indicative of an ecologically important role, and not derived from resting spores or cysts (Chambouvet et al. 2014).

It appears likely that protist parasites are more abundant, and more ecologically important than we have traditionally given them credit for in the West Antarctic Peninsula coastal waters, even given all of the above potential secondary explanations and caveats. Models of Antarctic marine food webs have not typically included parasites (Melbourne-Thomas et al. 2013, Skovgaard 2014), yet they are potentially important. Parasitism can divert carbon and energy out of the classic phytoplankton-krill-whale food chain, and into the microbial loop as particulate and dissolved organic matter released from ailing and dying hosts. The microbial food web is thought to be increasing in importance in this northern West Antarctic Peninsula region, as a result of ongoing anthropogenic change (Sailley et al. 2013). Parasitism has traditionally been considered to be more important in warmer ecosystems (Rhode 1984), and thus as temperatures continue to increase, parasitism may play a role in the increasing importance of the microbial loop.

Parasites can also have effects on the population dynamics of hosts and the diversity of the ecosystem more broadly. *Syndinium* infestation is estimated to cause copepod mortality comparable to predation mortality with rates as high as 42% mortality per day reported (Konovalova 2008), and *Amoebophrya* spp. have even been suggested as a biocontrol on harmful algal blooms as they can remove over 50% of their hosts daily (Coats & Clark 2002, Skovgaard 2014). Many parasites are highly host specific, parasitizing only a single species (Hudson et al. 2006, Skovgaard 2014). Such high-specificity parasites may play a role in maintaining diversity within Antarctic marine

ecosystems, by causing mortality or reducing fitness of an abundant species, parasites may create opportunities for other species within the ecosystem. Thus, incorporating parasites and their roles in regulating plankton populations into ecosystem models may allow for better predictions of the trends in species dynamics in the West Antarctic Peninsula.

Parasitism may be more important than commonly considered in marine ecosystems more broadly. When considering metazoan parasites on larger organisms such as fish, the Antarctic has been found to have lower parasite loads than other parts of the world ocean, (Rhode 1984). Here we presented data on protistan parasites for a limited area from the West Antarctic Peninsula in winter. Yet, comparable data for other regions of the world ocean are still sparse; DNA sequencing technologies have been rapidly improving, and with the public availability of reference databases for eukaryotes (Quast et al. 2013) it has only very recently become feasible to conduct and analyse broadscale surveys of eukaryote communities. Limited data from pyrosequencing and clone libraries in other regions of the world ocean suggest the unexpectedly high prevalence of parasite sequences observed here may be a wider phenomenon. Metanalyses of clone libraries suggest syndiniales make up over half of the dinoflagellate sequences observed in marine samples (Guillou et al.2008). Clone library sequences of Antarctic deep-water samples north of our sampling region in austral summer were composed 65-76% by unclassified alveolates, which are related to the syndiniales parasite groups (López-García et al. 2001). At the other end of the earth, clone libraries in the high arctic also contained high abundances of syndiniales, with various syndiniales groups making up 44% of the clones (Sørensen et al. 2012). Radiolarians sampled in the

Arctic were associated with alveolates, as observed here in the Antarctic (Figure 6) (Bråte et al. 2012). Similar syndiniales-like alveolates have also been observed near hydrothermal vent systems in both the Atlantic and Pacific along with more well-known parasites such as *Perkinsidae* spp. (Edgcomb et al. 2002, López-García et al. 2003, Moreira & López-García 2003). As new observations over diverse areas of the world ocean become available it will be interesting to see how the importance of these parasite-associated sequence groups varies globally, and begin to understand the magnitude of their ecological roles more widely.

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Tables

Samples	Type	Date	Time (local)	Depth	Latitude	Longitude	Location	Map Symbol	Temp C	Salinity (psu)
1,2,3	Water	24-May	23:05	20	-64.847	-62.611	Andvord Bay	1	-0.892	33.655
4,5,6	Water	29-May	11:15	250	-65.047	-63.301	Flanders Bay	2	0.718	34.474
7,8,9,79	Water	29-May	11:15	20	-65.047	-63.301	Flanders Bay	2	-0.767	33.636
10,11,12	Water	1-Jun	10:40	600	-64.967	-64.355	Palmer Deep	3	1.427	34.661
13,14,15	Water	1-Jun	10:40	20	-64.967	-64.355	Palmer Deep	3	-0.864	33.646
16,17,18	Water	3-Jun	00:50	300	-64.795	-63.121	Gerlache Strait	4	-0.236	34.507
19,20,21	Water	3-Jun	00:50	20	-64.795	-63.121	Gerlache Strait	4	-0.581	33.838
22,23,24	Sediment	18-May	18:25	520	-64.686	-62.235	Wilhemina Bay (in krill)	5		
25,26,27	Sediment	22-May	18:00	628	-64.535	-62.235	Wilhemina (out of krill)	6		
28,29,30	Sediment	26-May	20:20	545	-64.812	-62.733	Andvord Bay	7		
31,32,33	Sediment	27-May	16:00	356	-64.811	-62.718	Andvord trawl line	8		
34,35,36	Sediment	30-May	16:30	725	-65.003	-63.311	Flanders Bay	9		
37,38,39	Sediment	1-Jun	13:20	1345	-64.967	-64.355	Palmer Deep	3		

Table 1: Sampling locations and metadata. Sample numbers correspond to independent water filters or sediment scrapings, except in the case of 79 which is a technical replicate for 9. All dates are in 2013, time is in Chilean local 24 hr time, depth is in meters, map symbol corresponds to figure 1. Salinity and temperature data are not available for sediment samples.

Group	**# OTUs**	**% in Surface**	**% in Deep**	**% in sediment**	**Known hosts**	**Ref**
Amoebophrya	124	2.766	3.687	0.013	Dinoflagellates	1
Apicomplexa	216	0.058	0.130	3.004	crustaceans	3,1
Blastodinium	4	0.047	0.008	0.003	Diatoms, crustaceans	1,11
Chytriodinium	3	0.001	0.001	0.000	Copepod eggs	1
Cryothecomonas	14	0.744	0.068	4.486	Diatoms	1,5
Cryptocaryon	17	0.002	0.015	0.032	Fish	7
Duboscquella	5	0.003	0.377	0.024	Tintinids	1
Ellobiopsidae	4	0.002	0.340	0.000	Crustaceans	1,11
Haplozoon	9	1.185	0.390	0.000	Marine worms	6
Hematodinium	9	0.006	0.210	0.002	Crustaceans	11
Ichtchyosporea	8	0.004	0.020	0.027	Fish eggs	4, 1
Paradinium	19	0.029	0.036	0.055	Copepods	1,12
Perkinsidae	88	0.003	0.062	0.589	Mollusks, dinoflagellates	2, 3
Phytomyxea	19	0.001	0.002	0.132	Diatoms	9
Pirsonia	7	0.001	0.032	0.037	Diatoms	8,1, 5
Solenicola	1	0.001	0.000	0.000	Diatoms	1
Syndiniales, unclassified	54	0.683	1.401	0.463	Copepods, radiolarians	10,1
Syndiniales I	137	4.835	9.562	1.645		10
Syndiniales II	304	3.499	36.063	0.277		10
Totals:	**1042**	**13.869**	**52.405**	**10.789**		

Table 2: Parasite groups encountered in 18S rDNA sequences from the West Antarctic Peninsula. # of OTUs is the total number of observed distinct OTUs for each group. % in indicates the percent of the total sequences in each sample type which were attributable to each group. References: 1) Skovgaard 2014 2) Chambouvet et al. 2014 3) Moreira & López-García 2003 4) Glockling et al. 2013 5)Tillmann et al. 1999 6) Leander et al. 2002 7) Wright & Colorni 2002 8) Kühn et al. 2004 9) Neuhauser et al. 2011 10)Guillou et al. 2008 11)Konovalova 2008 12)Skovgaard & Daugbjerg 2008

Figures

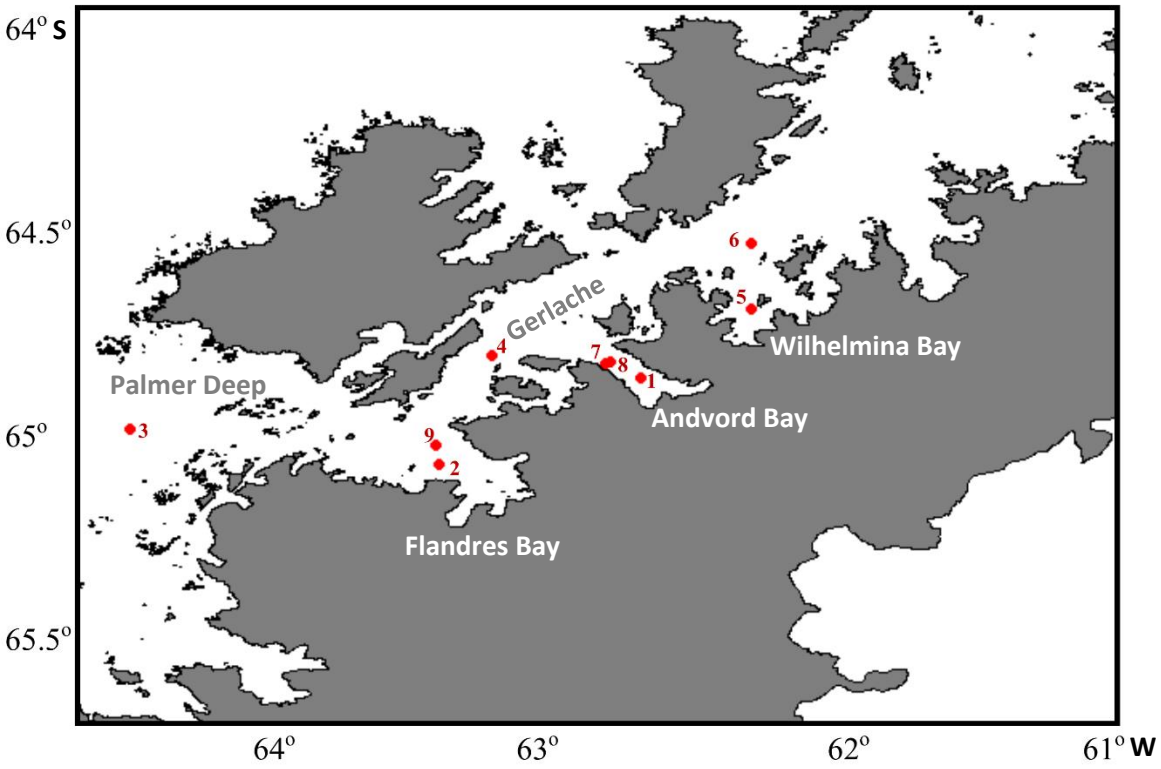


Figure 1: Map of sampling locations. Numbers correspond to positions on Table 1

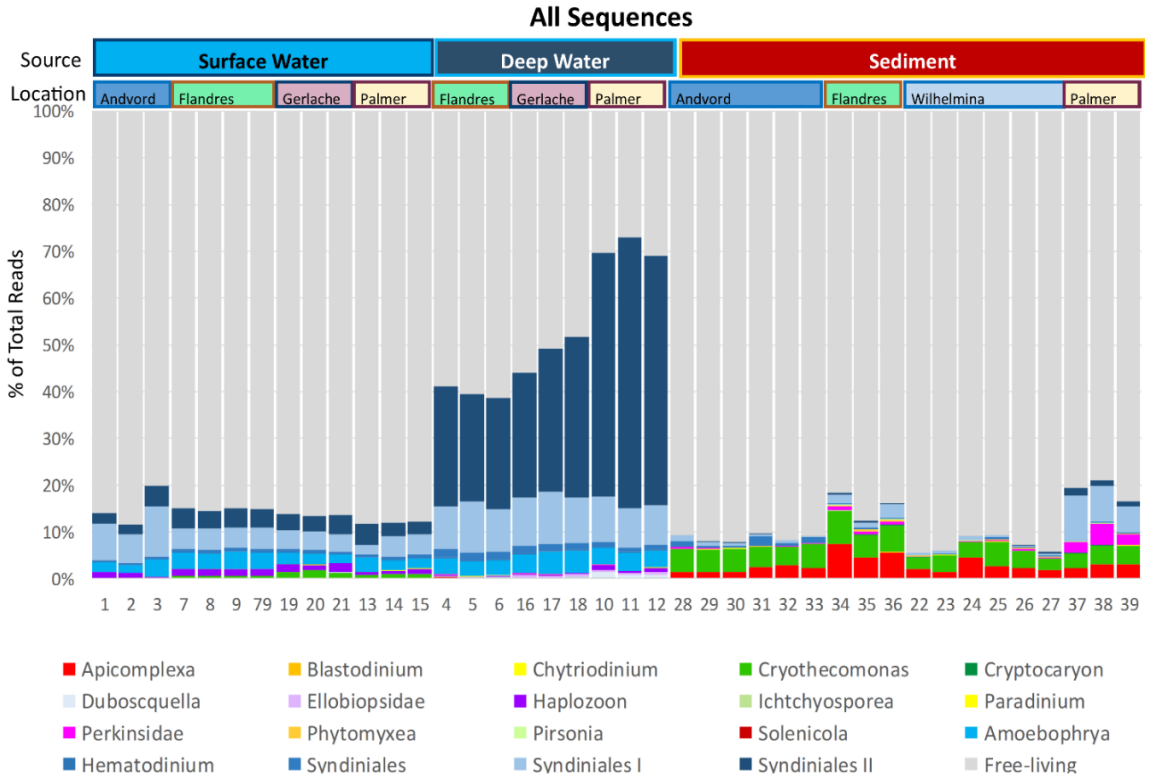


Figure 2: Distribution of sequence reads for parasite and free-living organism OTUs across all samples. Upper rectangles indicate sample source; lower rectangles indicate sample location as Andvord Bay, Flandres Bay, Gerlache Straight or Palmer Deep; sample numbers along the x-axis correspond to table 1 and are arranged within sample type by increasing bottom depth. The high percentage of parasites in sequencing reads from deep-water samples is striking.

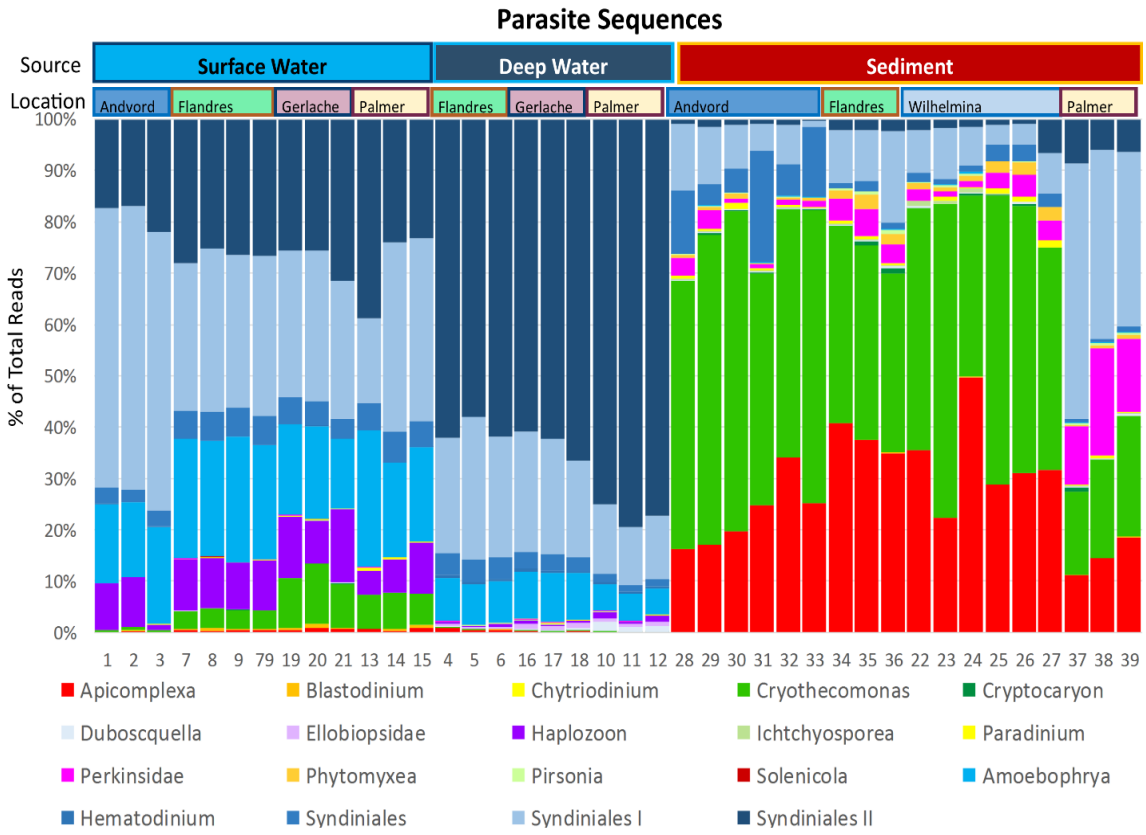


Figure 3: Parasite sequence reads across all samples. Formatting follows Figure 2. The high contribution from Syndiniales and related organisms (Amoebophrya & Hematodinium) all shown in blue is apparent, particularly in water samples.

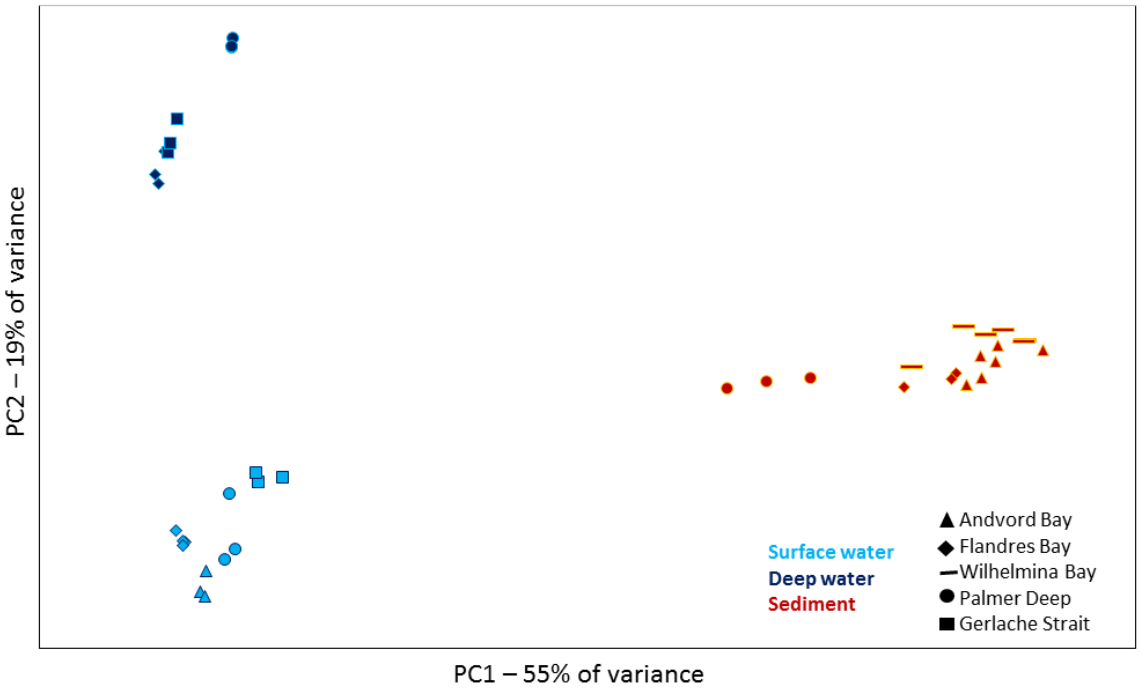


Figure 4: Principal Coordinates analysis of the parasite communities sampled. Shape indicates the location of each sample, with color indicating the sample type. Sample type is clearly the dominant structuring factor amongst these assemblages.

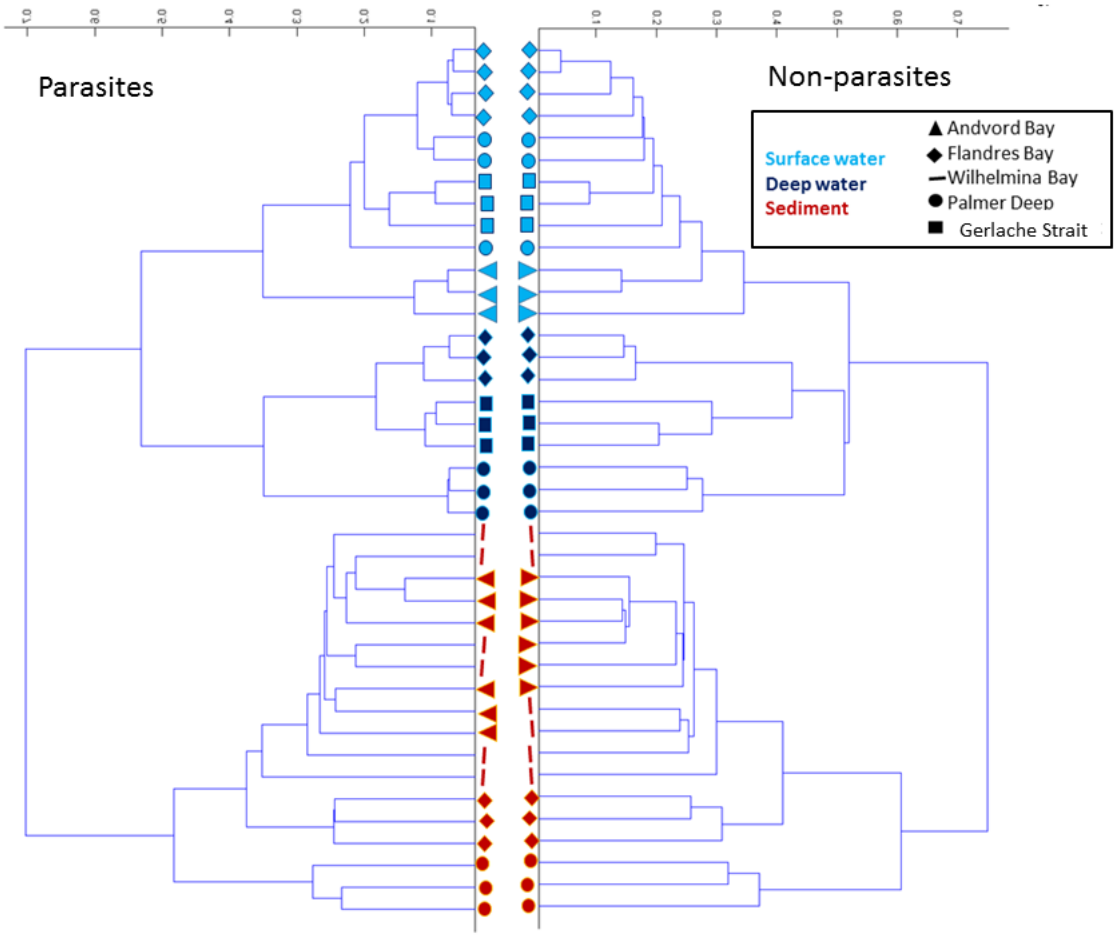


Figure 5: Cluster analysis of all samples for both parasite and non-parasite OTUs show strong clustering by sample type (surface water, deep-water, sediment), as well as clustering by location.

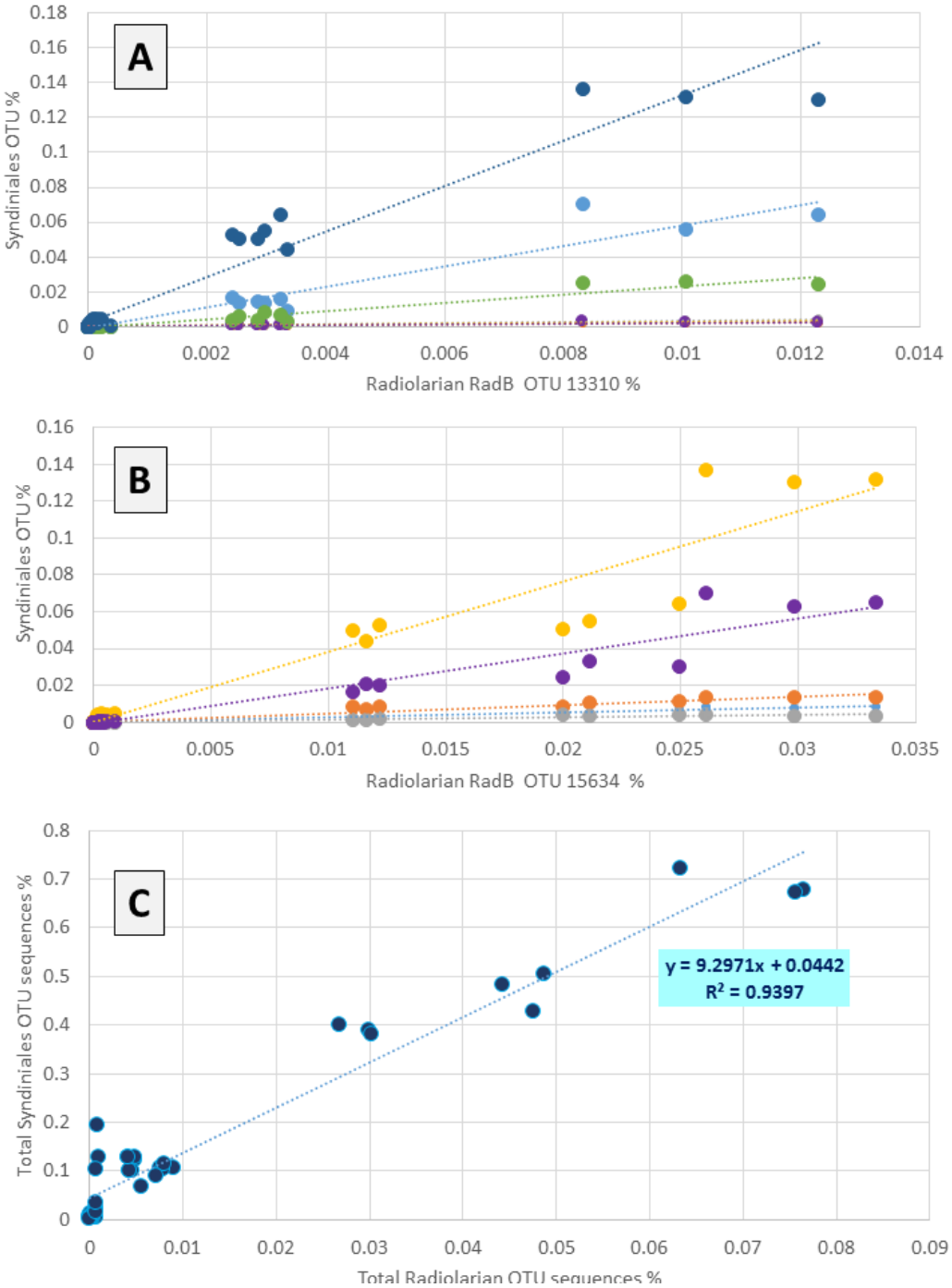


Figure 6: Correlations between radiolarians and syndiniales. All plots show radiolarian hosts on the x axis, with syndiniales parasites on the y axis, with both axis indicating sequence read abundance as a percent of total sequence reads per sample. A) various syndiniales OTUs correlated with radiolarian OTU 13310 B) various syndinales OTUs correlated with radiolarian OTU 15634 C) All syndiniales OTUs as compared to all radiolarian OTUs

Appendix A: *Pseudocalanus* gut contents OTUs

GenBank accession	OTU	Pie chart category	Narrowest ID	Total abundance	Count
KC952737	0	Microzooplankton	Oomycete	5	1
KC952738	1	Diatom	Chaetoceros	379	11
KC952805	2	Gelatinous	Chaetognath	2	2
KC952823	4	Microzooplankton	Oligohymenophorea	59	4
KC952847	7	Fungus	Tetracladium	4	1
KC952864	9	Fungus	Pezizomycotina	12	1
KC952748	11	Terrestrial	Pine tree	115	14
KC952761	13	Fungus	Ustillaginomycotina	11	2
KC952770	14	Gelatinous	Pantachogon	20	6
KC952783	16	Fungus	Saccharomycotina	23	2
KC952799	18	Microzooplankton	Thaumatomastix	17	5
KC952806	20	Other Alga	Prasinococcus-like	3	2
KC952807	21	Diatom	Chaetoceros	255	14
KC952808	22	Mesozooplankton	Euphausiid	14	6
KC952809	24	Fungus	Pucciniomycotina	4	2
KC952810	25	Mesozooplankton	Plomid rotifer	5	2
KC952811	26	Microzooplankton	Kinetoplastid	2	1
KC952812	27	Microzooplankton	Labyrinthulid	41	11
KC952813	28	Fungus	Pezizomycotina	21	4
KC952814	29	Diatom	Attheya	8	5
KC952815	30	Other Alga	Dunaliella	4	1
KC952816	31	Other Alga	Chlorarachnea	6	1
KC952817	32	Fungus	Pezizomycotina	6	2
KC952818	33	Diatom	Fragilariopsis	867	27
KC952819	34	Microzooplankton	Colpodea	14	3
KC952820	35	Mesozooplankton	Brittle Star	857	2
KC952821	36	Other Alga	Gyrodinium	8	2
KC952822	38	Gelatenous	Pseudosagitta	16	3
KC952824	40	Diatom	Porosira	77	4
KC952825	41	Unknown	?	2	1
KC952826	43	Gelatinous	Mertensia	4	3
KC952827	44	Mesozooplankton	Conchecia	2	1
KC952828	45	Mesozooplankton	Barnacle	49	2
KC952829	48	Fungus	Agarimycotina	10	1
KC952830	49	Terrestrial	Camellia	3	1

KC952831	51	Fungus	Sistotrema	7	4
KC952832	52	Gelatinous	Cnidarian	2189	30
KC952833	53	Diatom	Chaetoceros	452	18
KC952834	54	Other Alga	Chlorellaceae	8	2
KC952835	56	Microzooplankton	Oomycete	31	7
KC952836	57	Gelatinous	Mertensia	18	3
KC952837	58	Diatom	Chaetoceros	349	22
KC952838	59	Other Alga	Chrysophyte	6	4
KC952839	60	Fungus	Chytridiomycetes	7	2
KC952840	62	Unknown	?	20	1
KC952841	63	Gelatinous	Anthomedusae/lepto medusea	26	2
KC952842	64	Other Alga	dinoflagellate	2	1
KC952843	65	Fungus	Pezizomycotina	57	14
KC952844	66	Diatom	Thalassiosiraceae	4	2
KC952845	67	Fungus	Knufia	26	1
KC952846	68	Mesozooplankton	Artemia	5	1
KC952848	70	Heterotrophic dinoflagellate	Polykrikos	4239	28
KC952849	71	Unknown	?	8	3
KC952850	72	Unknown	?	9	3
KC952851	73	Unknown	?	7	1
KC952852	74	Fungus	Cryptococcus	16	4
KC952853	76	Fungus	Pucciniomycotina	21	3
KC952854	79	Fungus	Ustillaginomycotina	14	5
KC952855	80	Fungus	Lanspora	19	4
KC952856	81	Heterotrophic dinoflagellate	Amoebophrya	20	6
KC952857	82	Diatom	Fragilaria	1003	19
	83	Unknown	?	5	1
KC952858	84	Diatom	Chaetoceros	6	1
KC952859	85	Microzooplankton	Cercozoan	4	2
KC952860	86	Gelatinous	Sagitta/Krohnitta	495	36
KC952861	87	Fungus	Ochroconis	28	2
KC952862	88	Mesozooplankton	Euphausiid	8	2
KC952863	89	Microzooplankton	Cryothecomonas	45	10
KC952865	90	Unknown	?	3	2
KC952866	91	Fungus	Saccharomycea	10	2
KC952867	93	Diatom	Navicula	91	10
KC952868	94	Fungus	Chytridiomycetes	33	10

KC952869	97	Diatom	Achnanthales	6	1
KC952870	98	Heterotrophic dinoflagellate	Polykrikos	5	1
KC952871	99	Mesozooplankton	Squid	53	1
KC952739	100	Fungus	Candida	8	4
KC952740	101	Microzooplankton	Cercozoan	9	2
KC952741	102	Diatom	Stauroneis	16	2
KC952742	104	Fungus	Chytridiomycetes	3	1
KC952743	105	Terrestrial	Spider	14	2
KC952744	106	Other Alga	Chlamydomonadacea e	3	1
KC952745	107	Fungus	Pezizomycotina	230	26
KC952746	108	Fungus	Taphrina	8	2
KC952747	109	Gelatinous	Eukrohnia	30	13
KC952749	113	Fungus	Rhizochaete	94	16
KC952750	114	Gelatinous	Ctenophore	2	1
KC952751	118	Gelatinous	Mertensia	1340	31
KC952752	119	Mesozooplankton	Metridea	124	10
KC952753	120	Gelatenous	Beroe	8	4
KC952754	121	Microzooplankton	Heterophrys	2	1
KC952755	122	Other Alga	Gyrodinium	34	3
KC952756	123	Gelatinous	Semaeostomae	76	3
KC952757	125	Other Alga	Klebsormidiaceae	19	2
KC952758	126	Mesozooplankton	Hyperiid Amphipod	16	4
KC952759	127	Other Alga	dinoflagellate	34	4
KC952760	129	Microzooplankton	Cercomonas	5	4
KC952762	131	Diatom	Chaetoceros	2	1
KC952763	132	Unknown	?	4	1
KC952764	133	Unknown	?	7	4
KC952765	134	Fungus	Birch tree	205	21
KC952766	135	Diatom	Thalassiosiraceae	9451	44
KC952767	136	Fungus	Agarimycotina	93	17
KC952768	137	Other Alga	dinoflagellate	20	3
KC952769	138	Gelatinous	Parasagitta	23	11
KC952771	141	Microzooplankton	Gymnophrys	5	2
KC952772	143	Other Alga	Phaeocystis	454	21
KC952773	144	Diatom	Thalassiosiraceae	11	4
KC952774	145	Microzooplankton	Oligohymenophorea	3	1
KC952775	146	Other Alga	dinoflagellate	9	2
KC952776	147	Diatom	Amphiprora	9	1

KC952777	149	Other Alga	dinoflagellate	70	8
KC952778	150	Other Alga	dinoflagellate	33	6
KC952779	153	Mesozooplankton	Euphausiid	723	37
KC952780	155	Mesozooplankton	Bdelloid rotifer	3	1
KC952781	158	Terrestrial	Springtail	7	1
KC952782	159	Diatom	Rhizosolenia	33	6
KC952784	160	Diatom	Achnanthales	12	2
KC952785	162	Diatom	Chaetoceros	11	4
KC952786	163	Microzooplankton	Cercozoan	3	1
KC952787	164	Unknown	?	11	1
KC952788	165	Mesozooplankton	Euphausiid	7	1
KC952789	167	Fungus	fungus	3	2
KC952790	168	Heterotrophic dinoflagellate	Polykrikos	508	15
KC952791	169	Microzooplankton	Cryothecomonas	63	1
KC952792	170	Diatom	Thalassiosiraceae	11	5
KC952793	171	Heterotrophic dinoflagellate	Amoebophrya	2	1
KC952794	172	Gelatinous	Polypodium	24	4
KC952795	175	Gelatinous	Siphonophore	40	3
KC952796	176	Microzooplankton	Colpodea	4	2
KC952797	177	Mesozooplankton	Pteropod	5	2
KC952798	178	Diatom	Proboscia	35	13
KC952800	181	Unknown	?	4	1
	184	Unknown	?	3	1
KC952801	185	Mesozooplankton	Conchecia	2	1
KC952802	186	Terrestrial	Grain (wheat/rice)	212	15
KC952803	189	Heterotrophic dinoflagellate	Amoebophrya	1974	39
KC952804	190	Microzooplankton	Protostelium	2	1

Appendix B: Size fractionated krill biomass from MOCNESS tows

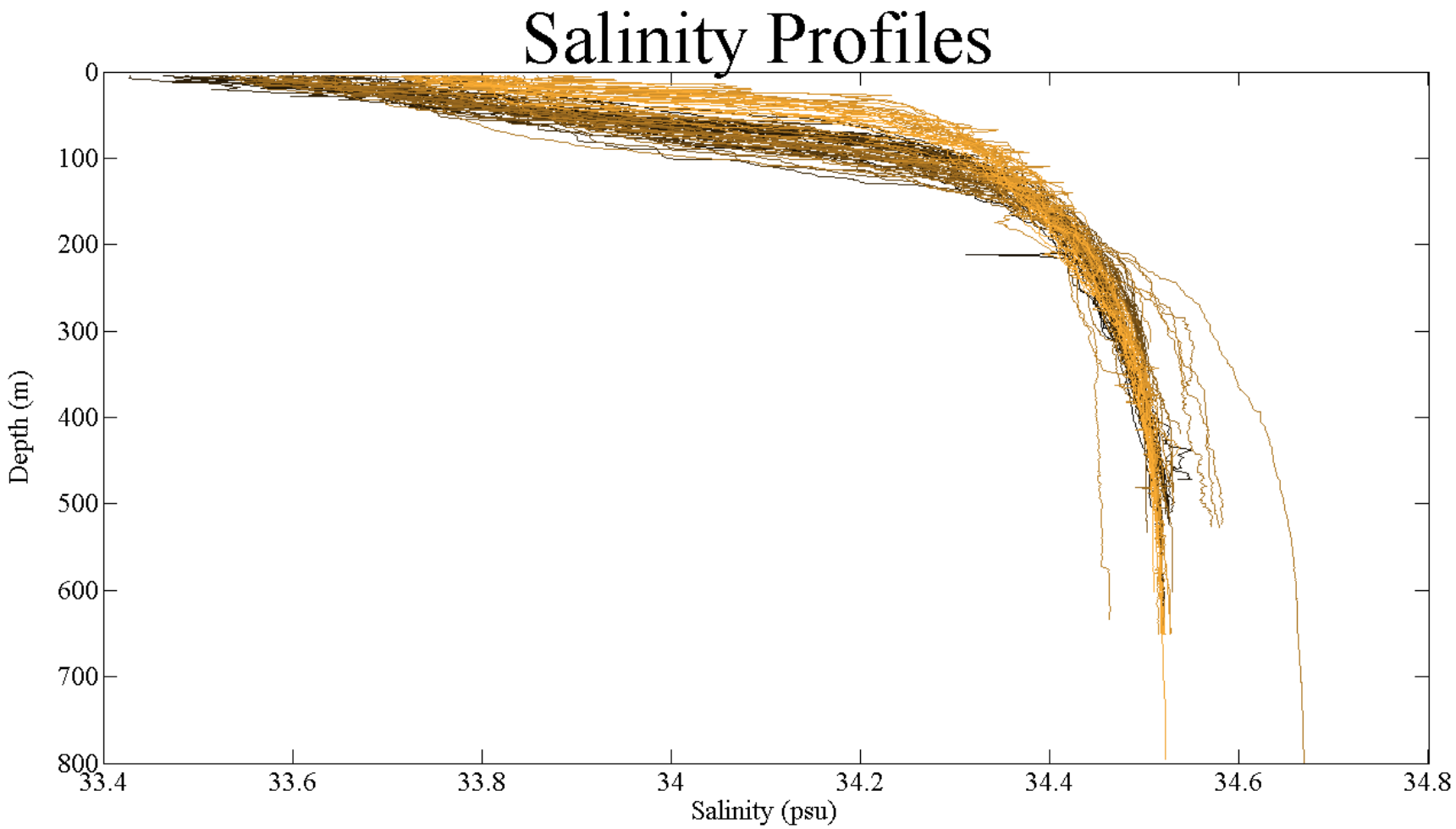
Size fractionated krill biomass from MOCNESS tows. Minimum and maximum depths sampled are in meters. Volume filtered is in m^3. Split indicates the factor by which counts were multiplied to account for splitting at sea and in the laboratory. Size bins are 0.25 cm Standard Length and are indicated by the upper limit on the size bin, krill of unknown size were removed for other analyses prior to measuring and were assigned the overall mean weight. Biomass in each size bin for each net is given as grams formalin-preserved wet weight m^{-3}.

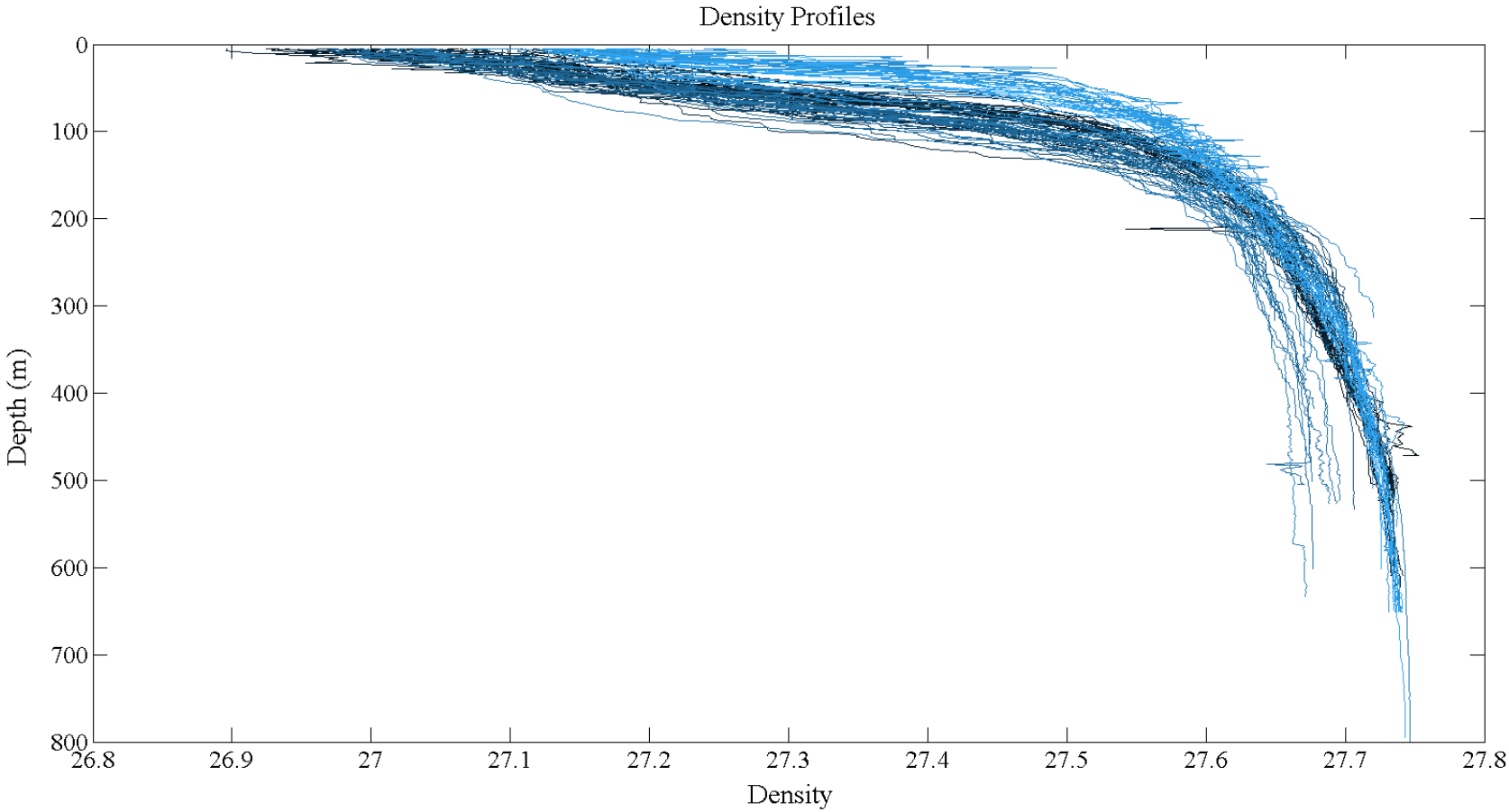
Tow	Net	Depth min	Depth max	Vol Filt	Split	<1	1.25	1.5	1.75	2	2.25	2.5	2.75	3	3.25	3.5	3.75	4	4.25	4.5	>4.5	Unknown	tot g ww m-3
7	1	300	300	106	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.010
7	2	200	300	53	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.007
7	3	150	200	130	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.016
7	4	100	150	209	4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.03	0.02	0.07	0.08	0.11	0.08	0.04	0.00	0.466
7	5	50	100	240	8	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.08	0.08	0.06	0.05	0.08	0.07	0.06	0.04	0.02	0.00	0.566
7	6	0	50	230	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.018
8	1	275	275	566	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.01	0.00	0.00	0.00	0.032
8	2	200	275	235	64	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.27	0.81	0.92	1.74	0.72	0.44	0.13	0.31	0.17	0.00	5.564
8	3	150	200	172	4	0.00	0.00	0.00	0.00	0.01	0.01	0.04	0.05	0.02	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.151
8	4	100	150	225	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.01	0.00	0.01	0.01	0.00	0.053
8	5	50	100	219	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.034
8	6	0	50	199	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.016
14	1	200	236	100.9	64	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.88	1.57	2.95	2.87	1.04	0.51	0.61	1.10	0.00	0.00	11.528
14	2	150	200	142	128	0.00	0.00	0.00	0.00	0.00	0.00	0.08	1.25	2.38	3.81	2.64	2.37	0.36	0.00	0.52	0.00	0.00	13.413
14	3	100	150	168	64	0.00	0.00	0.00	0.00	0.00	0.03	0.07	0.19	1.88	2.58	1.93	1.00	0.15	0.18	0.00	0.24	0.00	8.254
14	4	50	100	198	64	0.00	0.00	0.00	0.00	0.00	0.02	0.03	0.69	1.60	1.03	0.60	0.43	0.00	0.00	0.00	0.00	0.00	4.394
14	5	0	50	206	2	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.04	0.05	0.02	0.01	0.00	0.00	0.00	0.01	0.00	0.15	0.302
15	1	250	300	137	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.04	0.03	0.03	0.00	0.01	0.00	0.00	0.00	0.132
15	2	200	250	212	32	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.06	0.07	0.26	0.24	0.75	0.55	0.51	0.35	0.19	0.00	2.974
15	3	150	200	227	64	0.00	0.00	0.01	0.00	0.03	0.11	0.24	0.96	1.12	1.31	0.52	0.09	0.23	0.14	0.00	0.00	0.00	4.746
15	4	100	150	213	4	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.03	0.11	0.07	0.01	0.01	0.00	0.01	0.00	0.00	0.00	0.263

15	5	50	100	107	1	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.02	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.058
15	6	0	50	259	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.02	0.02	0.01	0.01	0.00	0.00	0.00	0.00	0.106
18	1	200	255	160	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.03	0.01	0.02	0.01	0.01	0.01	0.01	0.00	0.118
18	2	150	200	179	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.03	0.01	0.02	0.02	0.02	0.01	0.01	0.00	0.141
18	3	100	150	210	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.02	0.01	0.01	0.00	0.01	0.00	0.00	0.00	0.085
18	4	50	100	222	4	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.03	0.06	0.10	0.07	0.06	0.04	0.05	0.02	0.00	0.00	0.448
18	5	0	50	169	2	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.048
19	1	300	400	279	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.008
19	2	200	300	354	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.008
19	3	100	200	515	4	0.00	0.00	0.02	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.037
19	4	50	100	215	1	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.021
19	5	0	50	226	2	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.025
20	1	1000	1165	338	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.001
20	2	800	1000	637	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.002
20	3	600	800	900	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.001
20	5	300	400	306	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.002
20	6	200	300	315	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.011
20	8	0	100	420	4	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.02	0.02	0.02	0.01	0.01	0.00	0.00	0.01	0.00	0.111

Appendix C: Hydrographic profiles of NBP1304

Hydrographic profile summaries and T-S diagram for cruise NBP1304 to the West Antarctic Peninsula (May-June 2013). All CTD casts for the cruise are plotted together to give a general impression of hydrographic conditions during sampling. Line colors are shaded by sampling order, with earlier samples in darker shades and later samples in lighter shades. All data were generated by a 911plus SeaBird CTD. Depth is in meters below the surface, salinity is in practical salinity units (psu), temperature is in degrees Celsius, and density is expressed as (kg m^{-3})-1000.


Salinity Profiles
Depth (m)
0
100
200
300
400
500
600
700
800
33.4
33.6
33.8
34
34.2
34.4
34.6
34.8
Salinity (psu)


Density Profiles
Depth (m)
0
100
200
300
400
500
600
700
800
26.8
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27
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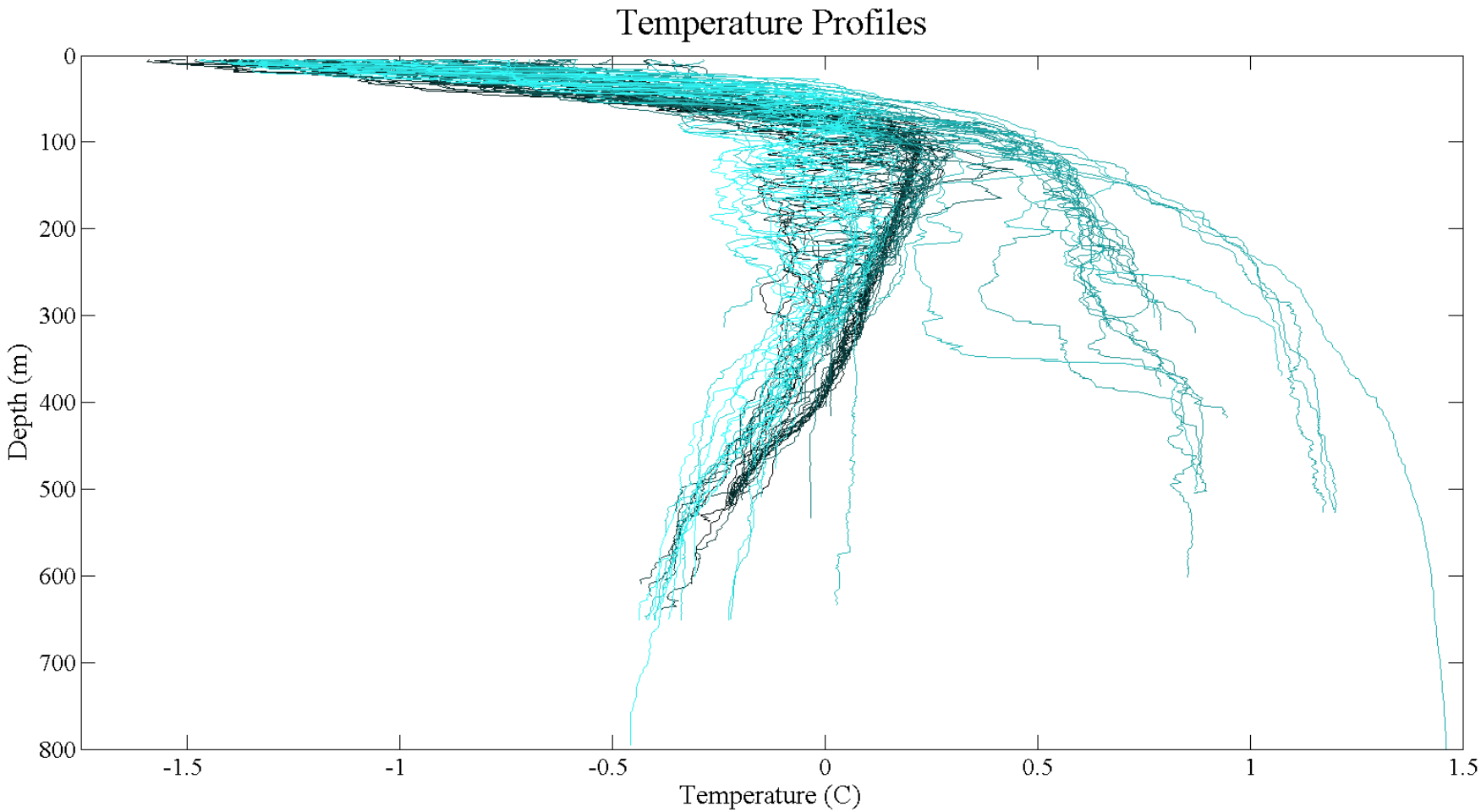
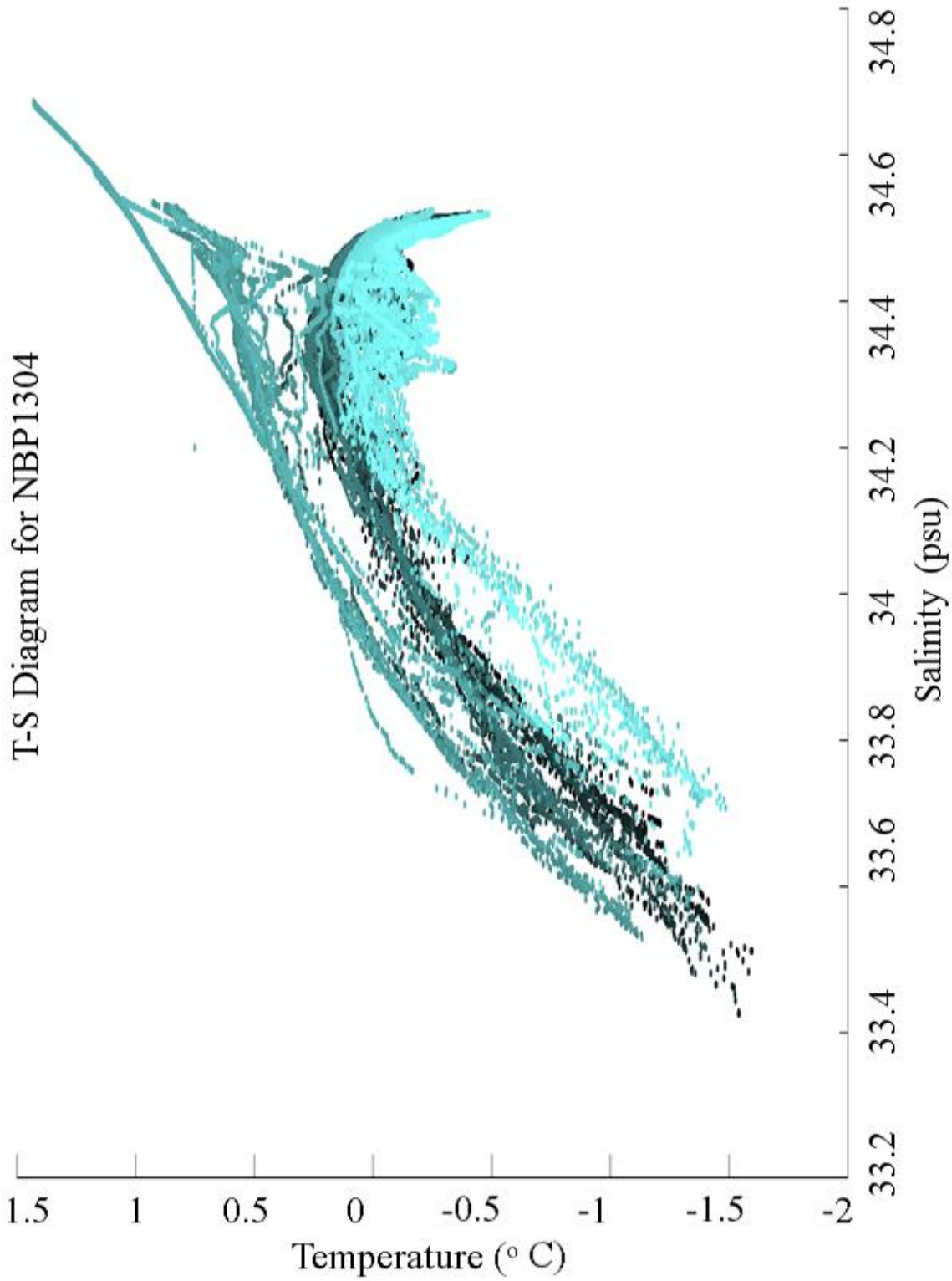
27.4
27.5
27.6
27.7
27.8
Density


Temperature Profiles
Depth (m)
0
100
200
300
400
500
600
700
800
-1.5
-1
-0.5
0
0.5
1
1.5
Temperature (C)


T-S Diagram for NBP1304
Salinity (psu)
33.2
33.4
33.6
33.8
34
34.2
34.4
34.6
34.8
Temperature (° C)
1.5
1
0.5
0
-0.5
-1
-1.5
-2