Antarctic krill fecal pellets – a unique bacterial habitat and mediator of carbon export

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Abstract

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The global climate is strongly regulated by the oceans, which store carbon away from the atmosphere for long periods. In an effort to understand the role of the oceans in the carbon cycle, it is necessary to understand the nuances of specific regional and functional marine ecosystems. The continental shelf of the West Antarctic Peninsula (WAP) is one particularly important regional ecosystem that plays a vital role in the Southern Ocean carbon export. Within the seasonally productive marginal ice zone of the WAP, I sought to identify the long-term drivers of particulate organic carbon (POC) flux. The vast majority of exported POC on the WAP was previously found to be made up of krill fecal pellets. I provide evidence that supports the hypothesis that the inherent life cycle of krill drives the observed 5-year oscillation in POC export. At the end of their life cycle, when krill are at their largest body size, the WAP experiences anomalously high POC export events through the production and sinking of large, carbon-rich krill fecal pellets. Conversely, when krill are young and small, POC export is anomalously low. This pattern shows that ecology exerts a first-order control on the the biogeochemical cycles of the WAP. Upon identifying the source and driver of POC export on the WAP, I set out to determine the role heterotrophic bacteria play in POC flux attenuation. I collected krill fecal pellets on the WAP over three years and measured bacterial metabolic activity in terms of bacterial production and respiration, thereby identifying the amount of organic carbon within the sinking fecal pellets that is lost due to bacteria. Overall, fecal pellet POC turnover rate by bacteria is very low. The relationship between bacteria and POC is complex with each having an affect on the other. Despite varied reactions of the free-living bacterial populations to the presence of krill fecal pellets, a consistent pattern emerged in
the concentration of nucleic acid within each bacterial cell. Access to fecal pellets increased the metabolic activity of the free-living bacterial population. This finding shows that the egestion of krill fecal pellets metabolically stimulates the surrounding bacterial community, even though bacteria play a minor role in fecal pellet POC flux attenuation. Though bacteria were found to play a minimal role in organic carbon uptake on krill fecal pellets, they are still vital members of the WAP ecosystem and biological pump. I next sought to identify which bacteria in particular were responsible for colonizing and consuming the fecal pellet POC. Krill fecal pellets were genetically sequenced after timed exposure to the free-living water column bacterial community. I found that there is an endemic population of bacteria that are associated with each population of krill and their fecal pellets. This community of fecal pellet-associated bacteria does not change over time, indicating little colonization by free-living bacteria. Krill fecal pellets, aside from being good agents of POC export, seem to be selective environments for certain specialized copiotrophic bacteria. Further, I find that only a small subset of these endemic copiotrophs actively partake in carbon consumption on krill fecal pellets. Overall, these results show that a small endemic, specialized bacterial community play an outsized role in krill fecal pellet POC degradation and flux attenuation, but that krill fecal pellets remain efficient agents of carbon export to the deep ocean.
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For Rachael Trinh – forever a cornerstone of all that I do
Taoist saying:

混兮其若濁。孰能濁以靜之徐清。孰能安以久動之徐生。

Trying to understand is like straining through muddy water.
Have the patience to wait. Be still and allow the mud to settle.

— Lao Tzu.
Chapter 1

Introduction:

Particulate organic carbon export on the West Antarctic Peninsula

1. Introduction

The carbon cycle is one of the core modulators of Earth’s climate. It is strongly regulated by the oceans, which store carbon away from the atmosphere for long periods. In an effort to understand the role of the oceans in the global carbon cycle, it is necessary to understand the particular nuances of specific regional marine ecosystems. The continental shelf of the West Antarctic Peninsula (WAP) is one particularly important regional ecosystem that plays a vital role in the Southern Ocean carbon cycle. It is a polar ecosystem, characterized by extreme seasonality, where austral winters occur under complete darkness and pronounced sea ice cover. Austral summers occur under 24 hours of full sunlight and low sea ice concentration. Ecosystems along the WAP have adapted to these conditions, and go through brief cycles of extraordinary productivity followed by months of inactivity. Further, carbon production is not nutrient limited in the WAP, and large pulses of particulate organic carbon (POC) are delivered to the deep ocean each summer. This summer peak in POC export is overwhelmingly dominated by Antarctic krill (Euphausia superba) fecal pellets (FPs). The remoteness and hazardous conditions of the WAP have made it difficult to study long term carbon cycle patterns and sources and sinks of particulate organic carbon (POC). Here, we present an unprecedented multi-decadal analysis of POC export on the WAP, identifying, for the first time, ecosystem drivers of carbon export. We additionally present multi-year and multi-seasonal data on the role the bacterial community plays on POC flux attenuation. Together, these data and analyses of biological sources, environmental modulators, and bacterial sinks of
POC help to answer key questions about the biological pump in extreme environments such as the WAP.

Specifically, my thesis addresses knowledge gaps in two categories: controls on POC flux, sources and sinks, and how these are both affected by the physical environmental condition of this unique region. In the subsequent sections, I give an introduction to these processes and then I delve into each of these elements specifically through the lens of the WAP, where I focus on the unique role that Antarctic krill play in carbon sequestration and bacteria play in krill FP carbon attenuation.

1.1. The biological pump

The global oceans play a crucial role in the modulation of atmospheric CO$_2$, thereby affecting global climate, which, in turn, has longstanding impacts on the function and health of ecosystems and societies world wide. Through numerous processes, such as air-sea gas exchange, deep water formation, and biological activity, CO$_2$ is taken up and stored by the ocean (Sabine et al., 2004), generating a reservoir that is about 50 times larger than the atmosphere. About 70% of deep ocean carbon storage occurs through biological processes, termed the biological pump (Gruber and Sarmiento, 2002). The biological pump integrates the primary production (PP), grazing, particle aggregation, and export of POC from the euphotic zone to the ocean interior (Knox and McElroy, 1984; Honjo et al., 2014). It also includes the attenuation of POC export due to biological consumption and uptake by zooplankton and bacteria, which recycle the organic carbon in POC through the upper ocean food web and microbial loop.
Long-term sequestration of POC flux is primarily dependent on the balance between POC production at the surface and remineralization of organic matter in the mesopelagic zone (Passow and Carlson, 2012). Carbon flux from the base of the euphotic zone approximately equals new PP from the use of new nutrients, such as nitrate input to the euphotic zone due to upwelling from below, allochthonous sources such as riverine input, or nitrogen fixation (Dugdale and Goering, 1967; Eppley and Peterson, 1979). Consumption of particulate matter originating in the epipelagic by mesopelagic zooplankton and decomposition by bacteria are responsible for the bulk of POC flux attenuation. Most of the net 50 Pg C fixed into phytoplankton biomass each year (Field et al., 1998) is remineralized in the upper few hundred meters of the ocean (Bishop et al., 1978; Suess, 1980; Martin et al., 1987). Between 5% and 25% (Martin et al., 1987; Buesseler, 1998; Schlitzer, 2000) of net PP is exported from the euphotic zone, with only about 1–3% reaching the deep sea and sediments. The bulk of net PP is consumed or respired in the water column, with approximately 50% being processed by bacteria (Ducklow, 2000a). Globally, modern-day carbon export through the biological pump to the deep ocean is estimated to be 5–15 Pg C y$^{-1}$ (Siegel et al., 2014). This small fraction of exported POC can be sequestered away from the atmosphere for hundreds to thousands of years. The biological pump process keeps atmospheric CO$_2$ about 200 ppmv lower than if the biological pump ceased entirely (Falkowski et al., 2000).

There is considerable regional and temporal variability in the amount of POC exported through the biological pump. In central gyres, only 1–10% of net PP is exported from the euphotic zone (Buesseler, 1998; Neuer et al., 2002), while anywhere between 30% and 100% of the net PP may be exported from polar regions and upwelling areas (Buesseler, 1998). The variability is predominantly biological in origin, stemming from the ways in which phytoplankton, zooplankton,
and microbial communities and food-web structures interact (Legendre and Rivkin, 2002). Likewise, the vertical flux of POC is higher in productive continental margin regions than in central gyres (Jahnke, 1996), where a greater supply of suspended minerals on continental margins may promote increased efficiency of the biological pump (De La Rocha and Passow, 2008).

The PP that is exported is not only fundamental for carbon sequestration, but also for the delivery of organic material to support benthic communities (Smith et al., 2008). Zooplankton grazing of phytoplankton and microzooplankton helps to biologically aggregate POC through the production and egestion of FPs. Zooplankton FPs can be large, rapidly sinking aggregates of POC that can efficiently export organic carbon to the deep ocean, thereby sequestering carbon from the atmosphere and providing carbon and nutrients to the deep ocean ecosystem (Knox and McElroy, 1984; Honjo et al., 2014). Further, FPs can serve as localized microhabitats, highly enriched in a variety of micronutrients, and as potential homes for particle-associated microbial assemblages (Alldredge and Silver, 1988). As POC sinks through the water column, biological and chemical transformations occur through processes such as bacterial production (BP) of biomass, respiration (BR), and remineralization. These processes influence the quantity and quality of carbon reaching the deep sea, affecting the export efficiency of the biological pump. An increased proportion of PP that is packaged and exported to the deep ocean yields an increase in export efficiency, minimizing the amount of remineralization and recycling of POC in the surface waters.

1.2. Zooplankton fecal pellet POC

The global variation in FP export, in terms of type, phenology, and total proportion of overall POC flux, demonstrates the importance of understanding regional ecology and ecosystem dynamics.
Without this consideration, it would be difficult to understand the pattern, trends, and importance of FPs to carbon export. Globally, the proportion of the estimated overall sinking particulate carbon flux contributed by zooplankton FPs is highly variable, ranging from less than 1% to 100%, with most values <40% (Ducklow et al., 2001; Dubischar and Bathmann, 2002; Fortier et al., 2002; Gleiber et al., 2012; Turner, 2015). The variability is a product of location, physical parameters, such as seasonality and upper ocean mixing, and the biological community present.

As an illustration of the importance of, and variation in, FP export, zooplankton FPs comprised up to 94% of the total particulate export during the productive spring period in the California Current, but much lower proportions of total flux during the fall (Stukel et al., 2013). Conversely, during periods of lower overall POC fluxes, the contribution of FP POC increased in the northeast Pacific, suggesting that there is a negative correlation between total POC flux and FP POC contribution (Wilson et al., 2013). The species composition of euphotic zone zooplankton also determines the type of FPs exported to depth. In years with large salp blooms off Bermuda, the maximum annually averaged salp FP carbon export equated to 60% of POC flux (Stone and Steinberg, 2016). In the Amundsen Sea Polynya, FP production contributed 10–40% of the total flux and was dominated by smaller FPs from copepods, with some FPs from the small ice krill *Euphausia crystallarophias* (Ducklow et al, 2015).

The main determinant of POC export composition and magnitude of flux to depth is the overlying epipelagic ecosystem. Zooplankton size and species composition affect pellet sinking rates, which range from tens of meters per day for copepod FPs (Yoon et al., 2001), hundreds of meters per day for euphausiid pellets (Atkinson et al., 2012), to more than 1,000 meters per day for salps (Phillips
et al., 2009). Part of the motivation for my thesis is to understand why such variability exists. I start from the premise that there is a balance between FP production and microbial decomposition of FPs by bacteria. This balance is essentially a competition between FP production and sinking rate and the rate of microbial activity on sinking pellets, including BP and BR by both fecal pellet-attached bacteria and free-living bacteria that have colonized FPs. Measurements of metabolic activity of the surrounding free-living bacteria that are dependent on dissolved organic matter released from sinking POC are needed to understand how egested FPs may stimulate activity of the surrounding water column population and contribute to water column metabolism. Concurrent information on community structure of these two groups of bacteria is central to linking the sources and sinks of POC.

1.3. **Bacterial ecology of fecal pellets**

The interactions between marine bacteria and their environment ultimately shape net ecosystem productivity and carbon sequestration through the microbial loop and biological pump. In terms of their numerical abundance, bacteria are the most important biological component involved in the transformation and mineralization of organic matter in the biosphere (Cho and Azam, 1988). Grossart et al. (2003) state that bacterial growth and species-specific interactions have to be taken into account to adequately describe bacterial colonization of marine particles. These small-scale processes, such as changes in colonization pattern and carbon consumption, may have profound effects on the transformation and fluxes of particulate organic matter in the ocean.

Particles, such as zooplankton FPs, and ambient water represent two distinct habitats for bacterial assemblages in aquatic environments (Zobell, 1943). Both particle-associated and free-living
bacteria perform two major functions in the transformation of organic carbon, impacting the global carbon cycle. Bacterial cells produce new bacterial biomass through BP and they respire organic carbon to CO$_2$ through BR. POC hydrolysis and organic carbon consumption by bacteria lead to flux attenuation, decreasing the amount of carbon exported and stored in the deep ocean. The organic carbon consumed by bacteria through BP may be recycled in the upper ocean via bacterivory in the microbial loop within the biological pump.

Some bacterial groups adapt to nutrient rich, particulate microhabitats, i.e., copiotrophic particle specialists (Riemann et al., 2000), whereas other groups adapt to nutrient-poor, ambient water, i.e., oligotrophic free-living planktobacteria specialists (Sieburth, 1979; Miki and Yamamura, 2005), with some taxa able to take advantage of both life styles (generalists). Particle-associated and free-living bacteria often exhibit different physiological traits, as indicated by differences in BP and growth (Unanue et al., 1992; Friedrich et al., 1999), ectoenzymatic activity (Karner and Herndl, 1992; Smith et al., 1992; Riemann et al., 2000), and kinetics of substrate uptake (Ayo et al., 2001). Copiotrophs tend to have prevalent methods of sugar uptake and transport, while oligotrophs are exhibit reliance on binding proteins as an energy source. While particle-attached bacteria may have higher per cell rates of BP, free-living cells generally contribute more to overall BP in the water column due to greater overall cell abundance (Turley and Mackie, 1994; Rieck et al., 2015).

The differences between particle-associated and free-living bacterial habitat partitioning may be further reflected in differences in community composition between the particle attached and free-living communities (DeLong et al., 1993; Ortega-Retuerta et al., 2013; Rieck et al., 2015). However,
some studies have found no taxonomic differences between particle-associated bacteria and free-living bacteria in the water column (e.g. Hollibaugh et al., 2000).

There have long been questions as to the comparative importance in terms of POC flux attenuation by bacteria contained within FPs prior to egestion versus bacteria from the surrounding water that colonize FPs after egestion (e.g. Turner, 2002). Some studies showed that colonization by free-living bacteria in the water column after FP egestion is important for FP degradation (e.g. Jing et al., 2012). Other studies found that carbon uptake by bacteria on FPs was mainly facilitated by fecal pellet-attached bacteria, where attached bacteria had higher ectoenzyme activity (e.g. Thor et al., 2003). Still other studies found that both free-living and fecal pellet-attached bacteria are equally important for POC consumption (Cnudde et al., 2013). In the Arctic, most of the bacteria attached to particles exported to depth come from near-surface suspended bacteria that attached to sinking particles (Tamelander, 2013). Conversely, a study on copepod FPs found that the bacteria associated with their FPs mainly originated from the copepod’s gut, and that the composition of bacterial communities depended on the initial food sources ingested by the copepods (De Troch et al., 2010). Similarly, Grossart and Ploug (2001) found that decomposition of particulate organic matter by attached microbes was substantial, and that the vertical flux of particles in the ocean was greatly reduced during sinking due to bacterial carbon uptake.

1.4. Southern Ocean biological pump

Biological dynamics take place against the backdrop of regional environmental controls. Despite comprising only 20% of the global ocean surface area, the Southern Ocean is a region of major global biogeochemical relevance on decadal to millennial timescales, affecting both paleo and
modern climate (Broecker and Henderson, 1998; Sarmiento et al., 2004; Marinov et al., 2006). The Southern Ocean plays an outsized role in the global biological carbon pump, in which it accounts for an estimated 30% of the global annual oceanic carbon export, about 1.5–3 Pg y\(^{-1}\) (Arteaga et al., 2018). Additionally, the Southern Ocean is the largest reservoir of unused surface ocean macronutrients, and thus has the greatest capacity for increased biological pump efficiency and atmospheric CO\(_2\) drawdown (Siegenthaler and Wenk, 1984; Anderson et al., 2002; Blain et al., 2007).

The marginal ice zone (MIZ) over the continental shelf of the WAP in the Southern Ocean is of particular importance in terms of carbon export. Globally, continental shelves are regions of intensified biological activity, high sedimentation, and deep seasonal draw down of CO\(_2\) (Walsh, 1991; Lampitt and Anita, 1997; Muller-Karger et al., 2005). The unique ecosystem of the WAP, characterized by the seasonal growth of sea ice during austral autumn and winter and ice retreat in spring and summer, has a profound impact on food web processes and nutrient availability and governs the efficiency of carbon export. The summertime bacterial assemblage in WAP shelf waters results from selective growth of relatively few heterotrophic populations, transforming a high-diversity winter community dominated by chemolithoautotrophs into a lower-diversity, mostly heterotrophic assemblage (Grzymski et al., 2012). PP is high during the summer ice-free period, supporting a rich ecosystem of consumers, specifically Antarctic krill (Euphausia superba). Despite a relatively short phytoplankton growth period (Nov-March) in this Antarctic shelf region, the overall annual PP is high relative to the open waters of the Southern Ocean (Arrigo et al., 1998, Vernet et al., 2008).
The WAP MIZ is a crucial habitat for *E. superba*, during both summer and winter (Atkinson et al., 2009). *E. superba* is a key species linking primary producers, such as diatoms, to apex predators such as penguins, seals, and whales (Loeb et al., 1997). Not only is *E. superba* a central species in the WAP food web, but *E. superba* also play an outsized role in the WAP biogeochemical cycles.

*E. superba* is one of the largest and longest-lived epipelagic zooplankton, reaching an average of ~60 mm in length by the end of their 5–6-year life cycle (Fig. 1.1). As such, krill play a critical role in Antarctic biogeochemical cycles through their grazing, and egestion (Cavan et al., 2019). Of particular importance to carbon storage is the ability of *E. superba* to produce large, carbon-rich, fast sinking, FPs (Cadée et al., 1992). Krill FPs can sink hundreds of meters per day, making them readily exported out of surface waters with low attenuation rates. This trait allows krill FPs to be biogeochemically significant couriers of carbon from the euphotic zone to the ocean interior (McDonnell and Buesseler, 2010; Atkinson et al., 2012; Gleiber et al., 2012). *E. superba* has been found to form large swarms in the upper water column extending up to an area of about 100 km², containing two million tonnes of krill (Tarling and Fielding, 2016). This swarming behavior may further increase the efficiency of krill FP POC export by producing high levels of organic carbon that far exceeds heterotrophic bacterial carbon demand.
North of the WAP, in the South Orkney Islands region of the western Scotia Sea, krill FPs comprised 60–80% of total POC flux (Belcher et al., 2017). In the Anvers Island region of the WAP (near our sediment trap), 67% of summer POC flux came from zooplankton FPs, of which 82% were *E. superba* krill FPs. During the winter, FPs comprised 34% of POC flux, with the remainder comprised of phytodetritus (Gleiber et al., 2012). One model suggests that, across the full Southern Ocean MIZ, seasonal krill FPs export about 0.04 GT C, corresponding to 17–61% (mean 35%) of satellite-derived PP estimates (Belcher et al., 2019). These data show the outsized impact a singular species can have on Southern Ocean carbon export simply through FP production.
1.5. Sediment traps collect exported POC

One of the central data sets of this thesis comes from a sediment trap moored to the sea floor off the coast of the WAP. The sediment trap presents an integrated picture of the biological and ecological processes that deliver POC to depth. Sediment traps are widely used for this purpose (reviewed in Gardner, 2000). Many of the previous studies have used short-term, drifting sediment traps to capture exported particles (e.g. Martin et al., 1987; McDonald and Buesseler, 2010; Belcher et al., 2017). But benthic moored sediment traps provide one of the few sources of continuous year-round time series on ecosystem and biogeochemical dynamics in the ocean interior, especially in Arctic and Antarctic MIZ, where seasonal sea ice cover frequently limits the use of other in situ sensors. Because of this, most observations of biological production, consumption, and sedimentation in the Southern Ocean are limited to ice-free seasons and to individual years, with few winter, or multiyear records (e.g., von Bodungen et al., 1986; Palanques et al., 2002; Manno et al., 2020; Weston et al., 2013). The lack of observations and measurements limit our understanding of the seasonal and interannual variability and long-term trends of POC export (Honjo et al., 2014). Long-term sediment trap timeseries observations of the sinking particle flux are an irreplaceable component of the assessment of the drivers of the biological pump (e.g. Karl and Lukas, 1996; Conte et al., 2001; Wynn-Edwards et al., 2020).

Long-term moored sediment traps have been deployed to study POC export in several key areas. The longest continuous running sediment trap time series began in 1978 and evolved into part of the Ocean Flux Program (OFP), measuring particle fluxes in the deep Sargasso Sea in the North Atlantic subtropical gyre with three conical sediment traps located at 500m, 1500m and 3200m depths. There is a 35+year flux record at 3200-m depth, a 27+ year flux at 1500-m depth, and a
24+ year record at 500-m depth (Conte and Weber, 2014). The Bermuda Atlantic Timeseries Study (BATS) commenced monthly sampling in October 1988 near the OFP sediment traps, as part of the US Joint Global Ocean Flux Study (JGOFS) program. A companion timeseries station located off Hawaii, the Hawaii Ocean Timeseries (HOT), was set up with an aim to understand the time-varying flux of carbon and associated elements in an oligotrophic ocean (Karl and Lukas, 1996; Steinberg et al., 2001). The ecosystems of the BATS and HOT time series vary drastically from that on the WAP, and sediment trap studies in these regions have yielded insights into these different systems. For example, an examination of POC flux collected by the OFP sediment trap time series off Bermuda found that FPs averaged only 7% of the organic carbon flux (Shatova et al., 2012), in contrast, drifting sediment traps at HOT found that FPs comprised 14% to 35% of POC in the summer (Wilson et al., 2008). BATS has a diatom dominated winter-spring bloom but resembles a central gyre oligotrophic system in the summer. HOT is persistently oligotrophic and is dominated by small celled primary producers and small microzooplankton. Both of these findings in FP contribution and main source of POC export vary from of our WAP sediment trap, where zooplankton FPs comprise about 70% of summer POC flux and about 35% of winter POC flux (Gleiber et al., 2012).

North of the Antarctic Polar Front, in the Subantarctic Zone there is a long-term sediment trap effort to study deep particle flux to the abyssal plain as part of the Australian Southern Ocean Time Series (SOTS), with traps first deployed in 1997 and running near-continuously through 2017 at different depths (1000–3800 m) (Trull et al., 2001; Wynn-Edwards et al., 2020). There, they find minimal interannual variability in POC export, which is in contrast to previous analysis of POC export on the WAP (Ducklow et al., 2008).
The WAP sediment trap of the Palmer Long-term Ecological Research (PAL-LTER; Fig. 1.2A) program has been continuously collecting exported POC with a moored sediment trap for about three decades, from 1992 to 2021. This record is the longest time series of POC export in the Southern Ocean, with year-round collection of sinking particles including during austral winter and under sea ice cover (Fig. 1.2B), thereby recording fundamental seasonal to decadal ecological processes. With these unprecedented data, it is possible to quantify seasonal and interannual patterns in POC flux, and the effects of the sea ice environment and overlying food web on carbon export. A study analyzing the PAL-LTER sediment trap POC data from 1992 to 2007 found high seasonal and interannual variability in the amount of exported POC and further suggested a shift in the timing of peak summer POC flux (Ducklow et al., 2008). This first look at the POC export on the WAP is a crucial foundation, though it did not identify any mechanisms for the observed patterns. In our work, we analyze an additional six years of the PAL-LTER sediment trap POC data, in total from 1992–2013, and find a mechanistic link between the surface ocean ecology and the interannual patterns and trends found in POC export on the WAP.
1.6. Bacterial metabolism, growth, and POC breakdown on fecal pellets

On the WAP, the amount of exported POC, which is dominated by *E. superba* FPs (Fig. 1.2B), is a function of both the amount of source material from the surface and POC decomposition at depth by heterotrophic bacteria. In addition to an analysis of the material that is collected by the sediment trap, I also analyze the bacterial ecosystem associated with sinking FP particles (Fig. 1.3). Despite the importance of bacterial remineralization at depth, the balance of processes determining POC flux attenuation are poorly understood (Giering et al., 2014). Further, the relative roles of fecal
pellet-attached copiotrophic and free-living oligotrophic bacteria on POC degradation is still an open question. These measurements are especially lacking from the Antarctic environment.

Figure 1.3. The Southern Ocean biological pump that is the main focus of this thesis. Phytoplankton take up CO$_2$ through primary production and are then grazed on by krill. This ingested carbon is either incorporated into krill biomass or egested as fecal pellets. The fast-sinking fecal pellets transfer carbon to the ocean interior. During this process, heterotrophic bacteria break down the organic carbon locked inside the fecal pellets, incorporating a portion of the carbon into bacterial biomass and respiring the rest of the organic carbon back to CO$_2$. 
Krill FPs are patches of concentrated substrates in an otherwise nutrient and DOC desert. Krill FPs are thus a niche microenvironment for fecal particle-associated bacterial assemblage. Egested FPs are exposed to the surrounding water column, where FP POC has influence on free-living bacteria. This tiny ecosystem has important implications for carbon flux and bacterial ecosystem dynamics. To understand the different roles fecal pellet-associated and free-living bacteria play in organic carbon uptake from sinking FP POC, *in situ* measurements of the bacterial populations, in terms of carbon demand, growth efficiency, and taxonomy on and around FPs are needed.

Bacterial biomass production can be calculated from rates of protein synthesis as protein comprises a large, fairly constant fraction of bacterial biomass. BP averages about 10% of PP in the subarctic North Pacific (Kirchman et al., 1993) and Antarctic South Atlantic (Kuparinen and Björnsen, 1992), 15% of PP in the equatorial Pacific (Kirchman et al., 1995) and 10-15% in the Sargasso Sea (Carlson et al., 1996; Ducklow, 1999). On the continental shelf of the WAP, mean summer-time BP was found to be about 4% of PP (Ducklow et al., 2008; Kim and Ducklow, 2016). Though the ratio of BP to PP is low on the WAP, BP in this region still represents a substantial flow of organic carbon through the Antarctic marine ecosystem (Kirchman et al. 2009a; Ducklow et al., 2012). For example, if bacteria convert organic matter to biomass at 10% efficiency, they will respire $\frac{0.04}{0.10} = 40\%$ of the carbon they consume back to CO$_2$. A yet unknown proportion of the PP consumed by bacteria is routed through the grazing of phytoplankton by krill and subsequent production of FPs.

The precise magnitude of organic carbon that flows through the microbial loop remains largely unknown. One reason is that measurements of BP are seldom accompanied by measurements of
BR (del Giorgio and Cole, 1998). BR is the major component of total respiration in most aquatic systems, so changes in BR have profound effects on the overall carbon and gas balance in aquatic ecosystems. Given that BR commonly accounts for a major fraction of the total bacterial carbon demand, it represents a master variable when trying to unravel carbon budgets in marine microbial ecology (Jahnke and Craven, 1995; del Giorgio and Cole, 1998; Ducklow, 2000a). As such, I specifically incorporate direct measurements of BR of fecal pellet-associated and free-living bacteria in my study of bacteria metabolism in association with POC consumption.

In terms of bacteria metabolism, bacterioplankton assemblages are composed both of highly “active” and growing bacteria and cells that are dead, dormant, or slowly growing (Lebaron et al., 2001; Servias et al., 2003), where highly active cells comprise only a small proportion of the total bacterial population. I hypothesize that access to krill FPs will stimulate a switch from dormant to active metabolic state in free-living bacterial cells as they are introduced to elevated amounts of organic carbon and nutrients from the egested pellets. The magnitude and regulation of bacterial growth efficiency (BGE), that is the partition of organic carbon through BP versus BR, is of interest well beyond the realm of microbial ecology as the value of BGE can greatly affect how one construes models of the carbon cycle in aquatic systems (del Giorgio and Cole, 1998; Kim et al., 2022). Understanding the relationship of BP, BR, and BGE of both particle-associated and free-living bacteria in marine ecosystems is needed to calculate the total flux of organic carbon through the microbial loop, FP POC turnover rate, and the POC flux attenuation due to the bacterial population.
1.7. Bacterial taxonomy

With modern gene sequencing techniques, it is now possible to discern bacterial species and strains present in the two different habitats offered by sinking POC and the water column. Of particular interest is whether *E. superba* FPs host an endemic microbiome of bacteria and whether exposure to the water column bacterial community during the sinking process promotes colonization by free-living bacteria. A switch in fecal pellet-attached bacterial community composition over time may be indicative of species interactions such as colonization, decolonization, commensalism, and competition.

In terms of free-living taxa, previous studies suggest that, although gammaproteo-bacterial abundance is often low in temperate waters, this group may be abundant in polar systems (Glöckner et al., 1999; Pommier et al., 2007; Schattenhofer et al., 2009). In terms of polar ecosystems, the Gammaproteobacteria were found to be the third-most abundant group in the western Arctic, following the Sphingobacteria-Flavobacteria and Alphaproteobacteria (Elifantz et al., 2007; Alonso-Saez et al., 2008). In the Antarctic Scotia Sea, the bacterioplankton was composed predominantly of Alphaproteobacteria followed by Sphingobacteria-Flavobacteria and Gammaproteobacteria (Topping et al., 2006). On the WAP shelf, substrate utilization by the free-living summer community was dominated by relatively few taxa of Sphingobacteria–Flavobacteria, Gammaproteobacteria, and Alphaproteobacteria, where bacterial groups differed in use and uptake of different proteins, amino acids, and sugars (Straza et al., 2010).

An analysis of bacterial assemblages associated with *E. superba* in the Indian sector of the Southern Ocean found that between 33 and 63% of the taxa sampled in and on *E. superba*
represented exclusively krill-associated bacteria. This late summer sampling from 2016 identified that krill body tissue and gastro-intestinal tract supported distinct phyla to those found in the surface seawater samples, including *Actinobacteria*, *Campylobacterota*, *Firmicutes*, and *Tenericutes*, suggesting krill are a major source of Southern Ocean microbial diversity (Clarke et al., 2019). Clarke et al. (2019) found that in krill guts, *Candidatus Hepatoplasma crinochetorum* made up 83% of the taxa from the order *Mycoplasmooidales*. However, they found no significant differences between krill FP and seawater. This study had very few FP samples, in contrast to the 22 krill FP experiments I present here. I hypothesize that there is indeed niche partitioning between copitrophic and oligotrophic bacteria in association with krill FPs and the surrounding WAP water column. Further, I investigate which of the specific taxa associated with krill FPs dominate POC decomposition as the pellets sink through the water column.

1.8. POC flux in a changing WAP

The magnitude of the biological pump is predicted to decline in response to global climate change, resulting in reduced ocean carbon storage and increased atmospheric CO$_2$ levels (Matsumoto et al., 2010; Steinacher et al., 2010; Manizza et al., 2010). The WAP is experiencing rapid warming, especially during the winter months. In conjunction with stronger winds from the north/northwest, the ecologically fundamental sea ice season is deteriorating (Stammerjohn et al., 2008; Montes-Hugo et al., 2009). If the WAP continues to warm and its sea ice cover diminishes, all aspects of the biological carbon pump may be affected. Ocean warming already appears to be progressively altering the Antarctic food web, and the flow of carbon, transforming it from a diatom-krill-dominated to a microbe-dominated system, where the ecosystem may switch from large, rapidly sinking particles to smaller particles that are likely to remain in the surface for longer periods,
thereby increasing the likelihood of POC loss due to respiration (Legendre and Rassoulzadegan, 1996; Ducklow et al., 2012; Sailley et al., 2013; Kim and Ducklow, 2016). The effects of continued climatic warming could not only alter the phenology and makeup of exported POC but could also enhance microbial remineralization of organic matter in the water column and benthos, thereby decreasing and altering POC flux, deep carbon storage, and food availability. It is necessary to understand how the microbial ecosystem is affecting fundamental biogeochemical cycles, such as POC export through krill FPs, and bacterial consumption of exported carbon in the Southern Ocean.

1.9. Dissertation overview and objectives

Chapter 2 – Ecology matters

My first objective was to identify the principal drivers of POC flux in the WAP. Using the unprecedented PAL-LTER sediment trap long-term time series, I identify a 5-year cycle in POC export and tie this to physical and biological mechanisms that drive seasonal, interannual, and interdecadal POC flux variability. I present evidence of sea ice impact on mixed layer depth and the resulting change in particle residence time in the surface ocean using satellite-derived sea ice cover and in situ water column measurements over the two decades studied. As the vast majority of exported POC on the WAP was previously found to be made up of krill FPs, I further compare these data and the in situ sediment trap POC data to measurements of krill abundance and size from net tows and penguin diets. This comprehensive analysis is the first to identify the importance of krill life cycle, in particular their age-size cycle, on FP size and export, likely through the production of large, fast sinking FPs by older krill.
Chapter 3 – Bacterial decomposition of fecal pellets

Upon understanding the source and driver of POC export on the WAP, I set out to determine how much of the FP POC flux is consumed by heterotrophic bacteria as the fecal pellets sink through the water column. I collected krill FPs on the WAP over three years and measured the rates of BP and BR on the FPs to determine the proportion of POC that was assimilated into cellular biomass and the proportion that was respired back into CO₂. With these measurements, I was able to identify whether access to carbon-rich krill FPs increased BGE. Overall, the calculated FP POC turnover rate by bacteria was found to be very low. The effect of FP on water column free-living bacteria was mixed in that in some instances, the presence of FPs increased free-living BP, while in other cases, FPs appeared to depress free-living BP, or free-living BP remained stable. Though there was a complex reaction in free-living BP to exposure of krill FPs, I found a consistent pattern of increased nucleic acid content in bacterial cells that were exposed to FP POC relative to those that did not have access to FPs. This finding indicates that the egestion of krill FPs metabolically stimulates the surrounding bacterial community, but bacteria play a minor role in FP POC flux attenuation, supporting previous findings that krill FPs are good agents of carbon export.

Chapter 4 – Bacterial community structure associated with fecal pellets

Though bacteria were found to play a minimal role in organic carbon uptake on krill FPs, they are still vital members of the WAP ecosystem and biological pump. I next sought to identify which bacteria in particular were responsible for colonizing and consuming the FP POC. Krill FPs were collected over several years and genetically sequenced after timed exposure to the free-living water column bacterial community. I find that there is an endemic population of bacteria that are associated with each population of krill and their FPs and that this community does not change
over time, indicating little colonization by free-living bacteria. Krill FPs, aside from being good agents of POC export, seem to be selective environments for certain specialized copiotrophic bacteria. Further, I find that only a small subset of these endemic copiotrophs actively partake in carbon uptake via BP on the FPs. Overall, these results show that a small endemic, specialized bacterial community play an outsized role in krill FP POC degradation and flux attenuation.

Chapter 5 – Overall conclusion

I end this thesis with a summary and concluding remarks on POC export in the WAP and the important mechanisms that are necessary to take into account when trying to understand and predict carbon sequestration in the Southern Ocean.
Chapter 2

Size Does Matter: larger Antarctic krill play an outsized role in driving seasonal and interannual variability of particulate organic carbon flux along the West Antarctic Peninsula
Chapter 2: Size Does Matter: larger Antarctic krill play an outsized role in driving seasonal and interannual variability of particulate organic carbon flux along the West Antarctic Peninsula

Abstract
The West Antarctic Peninsula (WAP) experiences some of the largest summer particulate organic carbon (POC) export rates in the world’s oceans. To understand the patterns and mechanisms that drive carbon export over seasonal and interannual time scales, POC fluxes were measured from 1992–2013 using a moored sediment trap on the WAP continental shelf. This 21-year record is the longest time series of particle export in the Southern Ocean. Winters of anomalously low sea ice preceded summers with reduced upper water-column stratification, delaying seasonal flux termination, and increasing seasonal flux duration. Integrated annual POC flux varied, from 0.31 to 5.31 g C m$^{-2}$ (mean 2.3±1.3 g C m$^{-2}$) and did not correlate with flux duration. A significant 5-year periodicity in POC flux magnitude was positively correlated with mean summertime Antarctic krill body size (length) which also peaked every 5–6 years. Krill body size likely alters POC flux through the production and export of size-varying fecal pellets, which is the dominate proportion of total flux. During years of anomalously high POC flux, on average 63%±12% of the krill population was comprised of large adults ≥ 41 mm in length. These findings indicate that sea ice plays an important role in modulating the seasonality of POC flux, and that krill body size, not abundance, is the dominant driver of POC flux magnitude on the WAP shelf, likely due to the production and export of larger fecal pellets by larger, older adult krill.
2.1. Introduction

The biological production, aggregation, and export of sinking particulate organic carbon (POC) from the euphotic zone to the ocean interior are important processes in global biogeochemical cycles (Knox and McElroy, 1984; Honjo et al., 2014). The export of POC to depth integrates physical and biological processes throughout the water column, including upper water column mixing, primary production, zooplankton consumption and microbial decomposition, net community metabolism, and particle aggregation and diagenesis. This biological pump (BP) (Volk and Hoffert 1985) redistributes carbon and nutrients within the ocean and plays an integral role in modulating atmospheric carbon dioxide (CO$_2$) concentrations (e.g., Sarmiento et al., 1998; Marinov et al., 2008; Studer et al., 2018).

Globally, modern-day carbon export through the BP to the deep ocean is estimated to be 5–15 Pg C y$^{-1}$ (Siegel et al., 2014). Despite comprising only 20% of the global ocean surface area, the Southern Ocean is a region of major global biogeochemical relevance on decadal to millennial timescales, affecting paleo- and modern climate, through modification in circulation, air-sea interactions, and changes in BP efficiency (Broecker and Henderson, 1998; Sarmiento et al., 2004; Li et al., 2020). The Southern Ocean plays an outsized role in the global BP, in which it accounts for an estimated 30% of the global annual oceanic carbon export, about 1.5–3 Pg C y$^{-1}$ (Arteaga et al., 2018). Therefore, it is critical to understand how climate change will alter Southern Ocean atmospheric CO$_2$ uptake, nutrient utilization, food webs, and POC export and remineralization through the BP (Bopp et al., 2001; Passow and Carlson, 2012). In order to answer this question, it is essential to understand the drivers and fates of sinking carbon (Sigman and Boyle, 2000) in the Southern Ocean, and how these factors may change due to ocean warming and changes in sea ice.
To determine the biological sources of POC, *in situ* measurements that capture sinking particles are needed. Despite their limitations as tools to quantify the full magnitude of POC export (Gardner, 2000; Buessler et al., 2007), sediment traps are widely used for collecting sinking particles that comprise POC flux. Moored sediment traps provide one of the few sources of continuous year-round time series on biogeochemical dynamics in the ocean interior, especially in Arctic and Antarctic marginal ice zones (MIZ), where seasonal sea ice cover limits ship operations and the use of many sensors. In spite of a long history of investigation of Southern Ocean ecology, most observations of biological production, consumption, and sedimentation are limited to ice-free seasons and to individual years, with few winter, or multiyear records (e.g., Nöthig and von Bodungen, 1989; Palanques et al., 2002; Manno et al., 2020). This lack of measurements limits our understanding of seasonal and interannual variability and long-term trends in POC export. Long-term sediment trap time series of sinking particle flux are thus an irreplaceable component of the assessment of the drivers of the BP, and potential changes to carbon export and sequestration (e.g., Karl and Lukas, 1996; Conte et al., 2001; Wynn-Edwards et al., 2020).

Globally, continental shelves are regions of intensified biological activity, high sedimentation, and deep seasonal draw down of CO$_2$ (Walsh, 1991; Muller-Karger et al., 2005). The MIZ over the continental shelf of the West Antarctic Peninsula (WAP) in the Southern Ocean is one such region. The unique ecosystem of the WAP is tied to the seasonal growth of sea ice during austral autumn and winter, and ice melt in spring and summer. Primary productivity is high during the summer ice-free period, supporting a rich ecosystem of consumers, such as Antarctic krill (*Euphausia superba*). The WAP MIZ is a crucial habitat for *E. superba*, during both summer and winter (Atkinson et al., 2009). *E. superba* comprises the highest concentration of animal biomass of a
singular species in the world (Atkinson et al., 2009) and is a key species in the Antarctic food web (Loeb et al., 1997). Further, *E. superba* is one of the largest and longest-lived epipelagic zooplankton, reaching an average of ~60 mm in length by the end of their 5–6-year life cycle. As such, krill play an outsized role in Antarctic biogeochemical cycles through their grazing, and egestion (Cavan et al., 2019). Of particular importance to carbon storage is the ability of *E. superba* to produce large, carbon-rich, fast sinking, fecal pellets (FPs) (Cadée et al., 1992). Krill FPs can sink hundreds of meters per day, making them readily exported out of surface waters with low attenuation rates, thereby making them important couriers of carbon from the euphotic zone to the ocean interior (McDonnell and Buesseler, 2010; Atkinson et al., 2012; Gleiber et al., 2012).

To understand how the overlying biology and physical environment of the MIZ affect POC export over time, we analyze a multi-decadal sediment trap time series (1992–2013) of POC flux on the continental shelf of the WAP, as part of the Palmer Antarctica Long-Term Ecological Research (PAL-LTER) project. This record is the longest time series of POC export in the Southern Ocean, with year-round collection of sinking particles including during austral winter and under sea ice cover, thereby recording ecologically important seasonal to decadal processes. We quantify seasonal and interannual patterns in POC flux, and find that winter sea ice has lasting effects, modulating summertime POC flux phenology and that krill life-history and body size (length), as opposed to abundance and numerical density, are integral parts of the WAP BP through their production and subsequent export of size-varying, carbon-rich FPs.
2.2. Methods

2.2.1. Study region

The PAL-LTER has been studying the marine ecosystem of the WAP since 1992, with long-term measurements conducted during the austral summer annually each Jan–Feb within the PAL-LTER offshore sampling grid aboard the MV Polar Duke (1993–1997) and ARSV Laurence M. Gould (1998–2020). The PAL-LTER sampling grid covers a 170,000 km² area of the Bellingshausen Sea (Fig. 2.1), extending approximately 700 km from Palmer Station, Anvers Island (64.77°S, 64.05°W) to Charcot Island (69.45°S, 75.15°W). Grid lines are numbered from -100 to 600 and spaced 100 km apart, and stations within each line are spaced 20 km apart, beginning from the coast to approximately 200 km offshore at the slope/shelf break (Waters and Smith, 1992).

Figure 2.1. Bathymetric map of the Antarctic Peninsula and bathymetry with location of PAL-LTER sampling stations (black circles) within gridlines labeled –100-600, Palmer Station on Anvers Island (yellow triangle), long-term sediment trap (red diamond), Marguerite Bay, and Charcot Island. White dashed lines separate geographical coast, shelf, and slope regions of the West Antarctic Peninsula (WAP).
2.2.2. Sediment trap

As part of the PAL–LTER effort to understand the marine ecology and biogeochemistry of the WAP, a sediment trap (PARFLUX Mark 78H 21-sample traps; McLane Research Labs, Falmouth, MA) has been deployed since Nov 1992 to collect sinking particles from the biologically productive continental shelf euphotic zone (Ducklow et al., 2008). The sediment trap is moored at 64.5°S, 66.0°W in ~350 m water depth. It is located about 130 km offshore, on the outer continental shelf, and between the 500 and 600 PAL grid lines (Fig. 2.1). The trap consists of a large conical funnel, 0.5 m\(^2\) in opening area with a plastic baffle mounted in the opening to prevent large-sized organisms from swimming into the trap. The trap continuously intercepts sinking particles at 170 m depth. Below the funnel are 21 collection cups filled with a high-density solution, 7.5 g NaCl 1\(^{-1}\) and 2\% borate-buffered formalin in filtered seawater (34 ppt), with a final concentration of 41 ppt, to retain and preserve sinking material. The trap autonomously collects sinking particles throughout the year into each cup in intervals varying from 4 to 61 days, with shorter intervals during the expected peak flux in austral summer, and longer intervals during low biological productivity in the winter. The deployment location was seasonally covered with sea ice 105±37 days (mean±SD) per year during the 21-year study period. The trap was recovered and redeployed each Jan–Feb during the annual cruise.

The bottom water mass at the sediment trap location is typically modified Circumpolar Deep Water (mCDW), a relatively warm and salty subsurface water mass sourced from the Antarctic Circumpolar Current (ACC). The mCDW is overlain by a thick (50–100 m) layer of Winter Water (WW), a cold and salty water mass that in summer, as a result of sea ice melt, becomes capped by a relatively shallow (5–50 m) but strongly stratified surface fresh layer of Antarctic Surface Water.
(AASW). Although WW is initially formed at the freezing point in winter, by summer it can become highly modified and eroded (thinned) through mixing processes with both mCDW from below and AASW from above (Moffat and Meredith, 2018).

### 2.2.3. Trap sample processing and data analysis

Upon recovery each year, the trap samples were sealed and held at 5°C until return to the USA for processing. Samples from heavy sediment flux periods were split up to 1/512 for subsequent analysis. Zooplankton swimmers were removed from each trap sample prior to being assayed. Total mass dry weight, POC, and particulate nitrogen were measured from each sample. Detailed methods for zooplankton removal are described in Ducklow et al. (2008) and sediment trap chemical analyses are described in the JGOFS Protocols (Knap et al., 1994). Briefly, after removal of zooplankton swimmers and splitting, samples were dried, homogenized, and fumed with dilute HCl to remove inorganic carbon. POC was measured on CHN Analyzers. Due to the multi-decadal nature of the PAL–LTER, POC was measured at several institutions on several different CHN Analyzers: Perkin-Elmer 2400 (1992–May 1994), Europa Scientific SL (Jun 1994–1997), Exeter Analytical Elemental Analyzer (1997–2003), Carlo Erba EA 1108 (2003–2007), Perkin Elmer 2400 and Thermo Scientific Flash 2000 (2007–2013). Samples were all run against the same standard, acetanilide (C₈H₉NO) (Karl et al., 1991; Ducklow et al., 2008). Up to three replicate analyses of chemical properties were performed on each sample and the analytical replicates were averaged for each sample. When multiple traps were simultaneously deployed adjacent to each other during the same time frame, samples were averaged for corresponding intervals (see Ducklow et al., 2008).
We report daily POC flux from each year’s 21 collection cups from 1992–2013 (Fig. 2.2). To compute monthly POC flux, the data from the sediment trap cups that were open and collecting for more than 31 days during the winters were linearly interpolated to monthly time steps. In order to understand interannual variability in POC flux, and its ecological drivers, the sediment trap POC flux data were integrated in July–June year format, starting in July 1 of year one, and spanning to June 30 the following year, to avoid systematically splitting up the spring and summer peak flux period (Nov–Mar) in which the vast majority of POC flux occurs (Ducklow et al., 2008). All annual POC flux data are analyzed and presented graphically in this July–June format (Fig. 2.3 onward). The sediment trap measurements did not start until Nov 1992 and there were two years in which the sediment trap either malfunctioned (2001) or was lost at sea (2009) (Fig. 2.2). After formatting the time series into July–June years, there was a total of five incomplete/missing years (1992, 2000, 2001, 2008, and 2009), yielding 16 POC flux years to analyze for the 1993–2013 period. Full results from 2014–2020 period were not available at the time of this analysis.

To evaluate the phenological timing of POC flux, an annual peak flux episode (which typically occurs during austral summer months) was defined to be a period of flux where daily POC flux was greater than the annual (July–June) mean flux for at least 14 consecutive days (Ducklow et al., 2008). With this criterion, it was possible to objectively and automatically identify annual peak pulse periods in POC flux, as well as the initiation and termination dates, and the duration of the annual seasonal pulse.

To determine how POC flux varied over the 21-year interval, an empirical orthogonal function (EOF) analysis was performed on the sediment trap timeseries data to find the principal
components that best explain the variance in the carbon flux within and between years. EOF analysis is a method widely used for quantifying the interannual variability about the climatological means of a variable of interest (Martinson et al., 2008). The POC timeseries matrix was set up as individual (July–June) years by monthly POC flux of each year and eigen-decomposed to explore the seasonal–interannual co-variability, i.e., the interannual variability of the seasonal progression of POC flux captured by the sediment trap. The power spectral density of the full annual July–June time series was also analyzed to detect any periodicity in POC flux and to help identify ecological parameters driving the annual cycle.

2.2.4. Comparison with environmental and biological parameters

To understand the mechanistic drivers of POC flux, individual environmental and biological factors affecting the region were compared to temporal patterns in POC flux. Variables analyzed include the monthly multivariate El Niño-Southern Oscillation (ENSO) Index (MEI.v2), indices from the NOAA Physical Sciences Laboratory website (https://psl.noaa.gov/enso/mei/), and the monthly Southern Annular Mode (SAM) index from the British Antarctic Survey (BAS, http://www.nerc-bas.ac.uk/icd/gjma/sam.html).

Daily sea ice cover data from 1992–2013 were extracted from the NASA Scanning Multichannel Microwave Radiometer (SMMR) and the Defense Meteorological Satellite Programs (DMSP) Special Sensor Microwave/Imager (SSM/I) satellite record for the gridded area corresponding to the sediment trap location, following protocols described in Stammerjohn et al., (2008). August was found to be the month in which sea ice cover is at its maximum over the sediment trap. The
median percentage of August sea ice cover over this region of interest was calculated for each year and compared to sediment trap POC flux phenology.

All other oceanographic and biological data analyzed in this study are part of the ongoing PAL–LTER time series (https://pallter.marine.rutgers.edu/data/). Each year, data are collected along the WAP during the austral summer (roughly between 01 Jan and 10 Feb) when sea ice cover is near its annual minimum, daylengths are long (with nearly 24 hours of light), and biological activity is at its annual maximum. Relevant summer-time parameters measured during the annual research cruise are compared to the corresponding annual (July–June) POC flux data.

Water column temperature (\(T; \degree C\)), salinity, and pressure (dbar) were measured with a SeaBird 911+ CTD deployed at sampling stations along the PAL grid (Fig. 2.1). At each station, sampling consisted of one or more casts with the Seabird CTD-rosette system from the surface to just above the ocean bottom. Sigma-theta (\(\sigma_\theta\), potential density; kg m\(^{-3}\)) was calculated at each depth bin (binned to 1 dbar) using CTD data acquired along the shelf region of the 500 and 600 PAL grid lines (Fig. 2.1), to roughly match the area analyzed for sea ice cover. \(\sigma_\theta\) was used to calculate mean summer upper water column stability over the shelf for each year. The temperature minimum (\(T_{\text{min}}\)) and its depth (meters) were determined for each cast, ensuring that they represented the remnant cold WW from the previous winter. The \(\sigma_\theta\) gradient to \(T_{\text{min}}\) (\(\Delta \sigma_\theta T_{\text{min}} – 0; \text{kg m}^{-3} \text{m}^{-1}\)) is defined as \(\sigma_\theta\) at the depth of \(T_{\text{min}}\) minus \(\sigma_\theta\) at the surface divided by the depth difference between the two (Martinson and Iannuzzi, 1998; Saba et al., 2014). Using the interquartile range (IQR) method to find the difference between the third (\(Q_3\)) and first (\(Q_1\)) quartiles, and using the 1.5 x IQR and 3 x IQR rules to define inner and outer outlier fences, respectively, the \(\Delta \sigma_\theta T_{\text{min}} – 0\) value
for 2005 ($\Delta\sigma_\theta_{T\min-0} = 0.010$) proved to be a major outlier, as it was greater than $Q_3 + 3 \times \text{IQR}$, and was an order of magnitude larger than all other $\Delta\sigma_\theta_{T\min-0}$ values in the time series. Therefore, the 2005 outlier was removed from subsequent $\Delta\sigma_\theta_{T\min-0}$ analysis.

Depth integrated phytoplankton chlorophyll-a (chl-a) measurements from the top 100 m of the water column were spatially averaged across the PAL–LTER sample grid points each year and used as a proxy for phytoplankton biomass during peak POC flux periods. Seawater was collected from vertical profiles along the 500 and 600 PAL grid lines in Go-Flo bottles at different depths and was filtered onto GF/F filters and flash frozen for fluorometric phytoplankton chl-a analysis (mg chl-a m$^{-3}$) (Schofield et al., 2017).

*E. superba* and *Salpa thompsoni* (salp) abundances and *E. superba* body size were measured on animals collected using a 2 m-square frame Metro net (700 μm mesh) towed obliquely to a depth of 120 m along the 500 and 600 PAL grid lines (Steinberg et al., 2015). A General Oceanics flow meter positioned in the center of the net mouth was used to calculate the volume of seawater filtered (Steinberg et al., 2015). A random subsample of up to 100 *E. superba* individuals was measured for standard body length (distance between tip of rostrum and blunt end of uropod) for each net tow. For tows containing fewer than 100 individual krill, all *E. superba* were measured for body size (length). The body lengths (mm) were assigned to 1-mm length bins and the median size in each 1-mm length bin was used in conjunction with the total annual number of krill in each length bin to calculate the weighted summer mean krill body size each year.
E. superba body size was also measured on krill recovered in Adélie penguin diet samples during the summer breeding season in Jan–Feb from colonies surrounding Palmer Station. Adélie penguin diet samples were obtained from adult penguins by using the water off-loading method (Fraser and Hoffman, 2003). Krill from diet samples were measured from the base of the eye to the tip of the telson and placed in 5-mm length bins. As krill grow >5 mm per year (Siegel 1987), these binning methods resolve changes in krill population size class structure that occur between years (Siegel and Loeb, 1994; Fraser and Hoffman, 2003; Saba et al., 2014). Seabird diets have been shown to be good proxy indicators of the spatial and temporal variance and structure (length–frequency distribution) of their prey populations (Reid and Brierley, 2001). Though Adélie penguin diets represent a high-quality snapshot of the size-structure of the krill population each summer, they may not accurately reflect overall krill abundance; hence we paired these data with scientific net tow data for krill body size.

To examine annual trends and patterns in the data, we calculated annual standardized anomalies of each environmental and biological factor (Z-scores) to reduce the confounding effects of outlier values and extreme variability. For annual summer-time zooplankton abundance anomalies, abundance values were log10-transformed prior to calculating annual mean abundance and then Z-scores were calculated (Steinberg et al., 2015). Each standardized variable was then compared with the POC flux over the time series through linear regression to determine the potential drivers of POC flux.
2.3. Results

2.3.1. Variability in POC flux phenology

Sediment trap deployments in the WAP over the 21-year period showed a consistent seasonal pattern, with each year characterized by peak POC flux initiation during the austral spring-summer, following sea ice retreat, when there is near 24 hours of sunlight and biological activity is high. POC flux then increased sharply above the overall mean flux rate (9.1±16.2 mg m\(^{-2}\) d\(^{-1}\)). Peak POC flux is then followed by a large drop during late autumn, with low flux during the winter, when there is near 24 hours of darkness, the sea surface is covered by sea ice, and primary production is low (Fig. 2.2). Though each year can be broadly characterized by these seasonal patterns, there is a high degree of variation in the magnitude, timing, and duration of POC flux. The highest daily POC flux occurred in the summer of 2006 (124 mg C m\(^{-2}\) d\(^{-1}\)), 25-times greater than the highest POC flux measured in the summer of 2008 (4.8 mg C m\(^{-2}\) d\(^{-1}\)) which was the lowest peak daily flux in the time series (Fig. 2.2).

Figure 2.2. Full POC flux time series from 1992 to 2013 in blue. Black dashed line shows mean POC flux rate over the timeseries; x-axis ticks mark Jan. 1 of each year. See text for the years with no data.
POC flux was ongoing during the winter but usually minimal (mean 0.71±1.1 mg C m$^{-2}$ d$^{-1}$), and began to increase after the ice-edge retreated in spring. On average, the initiation of peak POC flux began 80±32 days after the retreat of winter sea ice from the sediment trap location. Interannual variability in sea ice duration is high and POC flux peak initiation began as early as 35 days, or as late as 149 days after ice retreat.

The date of peak flux initiation is relatively stable year to year, while the date of peak flux termination shows higher interannual variability. On average, the initiation of peak POC flux began on 16 Jan±16 days, and terminated on 10 Mar±33 days (Fig. 2.3A). Although it appears that the date of POC flux termination is being pushed later into austral summer and autumn, this relationship is nonsignificant (Fig. 2.3a; $R^2 = 0.20$, $p = 0.08$). Similarly, the date of maximum daily POC flux appears to occur later in the season, however this was also nonsignificant ($R^2 = 0.11$, $p = 0.20$). Peak POC flux duration ranged from 21 to 114 days, with a mean of 54±27 days, with no long-term trend over the time series (Fig. 2.3B). An average of 80%±23% of the annual POC flux occurs within the peak initiation and termination period, showing that the vast majority of annual flux accumulation occurred during less than one-sixth of the year, specifically, during the spring-summer period. Interannual variation in peak POC flux duration is not correlated with the timing of peak flux initiation (Fig. 2.3C; $R^2 = 0.023$, $p = 0.57$), but is correlated with the date of flux termination (Fig. 2.3D; $R^2 = 0.77$, $p < 0.001$).
Over 76% of the seasonal POC flux variability is captured by the first EOF mode (Fig. 2.4A), further substantiating the extreme seasonality of POC flux in the WAP. It shows a pronounced summer peak in POC flux, with the pulse increasing from near zero in Nov, rising sharply in Dec, peaking in Feb, decreasing sharply in Mar, and declining to near zero in Apr Mode 1 of the EOF defines the annual flux climatology. Though this pronounced summer-time pulse in POC flux is the dominant pattern in this region of the WAP, where peak POC flux typically occurs in Feb,
there is a high degree of interannual variability in flux timing, duration, and integrated annual flux over the 21-year period. The majority of seasonal variability occurred during peak summer when the largest standard deviation in monthly POC flux, 33.1, 59.3, and 64.3 mg m$^{-2}$ month$^{-1}$, occurred in Dec, Jan, and Feb, respectively. For comparison, the mean standard deviation during the winter months of June–Sep was 0.97 mg m$^{-2}$ month$^{-1}$.

EOF modes 2 and 3 (Fig. 2.4B, & C, respectively) captured the variability in seasonal timing and duration, together respectively describing 17% and 4% of variance in the POC flux time series. Together, the first three modes account for ~97% of the seasonal and interannual variability in POC flux (Fig. 2.4A–C). The three EOF modes represent distinct patterns in the variability of the seasonal progression of POC flux. While mode 1 of the EOF analysis described the magnitude and typical seasonal shape and climatology of POC flux each year, modes 2 and 3 described shifts in the timing and duration of the peak flux within austral spring, summer, and autumn.

When occurring with positive annual Principal Components (PC; described below), EOF mode 2 would indicate a shift of the peak POC flux, or a broadening in flux duration, toward late austral summer and early autumn, typified by a decrease in flux in Dec–Jan and increase in POC flux in Feb–Apr (Fig. 2.4B). When coinciding with positive PC, EOF mode 3 would indicate a shift of the peak POC flux or a broadening in flux duration earlier in the austral spring and summer, increasing flux in Dec and decreasing flux in Jan–Apr, with no change to Feb (Fig. 2.4C), contrasting the phenology of the POC flux captured by mode 2. Opposite characteristics occur when the sign of each respective PC is reversed.
Figure 2.4. EOF decomposition analysis of POC flux time series. A) EOF mode 1, B) EOF mode 2, and C) EOF mode 3, show seasonal modes of variability throughout the year. Together, the three modes account for 97.3% of the variance in the POC flux timeseries. D) Principal Component time series of mode 1 (PC 1), E) Principal Component time series of mode 2 (PC 2), and F) Principal Component time series of mode 3 (PC 3), showing how their respective modes of seasonal variability changed over the 21-year timeseries; dashed line marks y = 0.

The PC time series show how these three seasonal patterns (EOF modes) in POC flux vary interannually over the 21 years. The PC time series of EOF 1 (PC 1, Fig. 2.4D) oscillates between high and low positive magnitudes, indicating the high interannual variability in integrated POC flux magnitude, with maxima 5–6 years apart. The PC time series of modes 2 and 3 are shown in Fig. 2.4E and 2.4F (PC 2 and PC 3), respectively. Years characterized by positive PC 2 values and near-zero or negative PC 3 values (e.g., July–June years 1997–1998, 1998–1999, 2003–2004) were typically years in which peak POC flux decreased in Dec and Jan, and increased in Mar–May, effectively pushing peak POC flux later into autumn, causing peak flux termination to be later than average (Fig. 2.3A).
2.3.2. Environmental parameters and POC flux phenology

Sea ice cover significantly impacts the seasonal timing of peak POC flux by affecting when flux termination occurs through modulation of upper water column stratification. Using the anomaly of median August sea ice cover over the sediment trap area, we can estimate the duration of peak POC flux (Fig. 2.5A). August sea ice anomaly was significantly negatively correlated with flux duration (Fig. 2.5A; \( R^2 = 0.26, p = 0.045 \)) and significantly negatively correlated to POC flux termination (Fig. 2.5B; \( R^2 = 0.49, p = 0.003 \)). Further, winter sea ice anomaly is significantly correlated with summer upper water column stratification (\( \Delta \sigma_{\theta, T_{\text{min}} - 0} \) values), where low winter sea ice cover tended to precede summers with weaker upper water column stratification (Fig. 2.5C; \( R^2 = 0.35, p = 0.01 \)).
Reduced stratification leads to a more unstable water column, preconditioned toward greater vertical mixing for the same energy input. Summer-time $\Delta \sigma_{T_{\text{min}} - 0}$ was significantly negatively correlated with POC flux duration (Fig. 2.6A; $R^2 = -0.37$, $p = 0.02$) and termination date of peak POC flux (Fig. 2.6B; $R^2 = -0.53$, $p = 0.003$). High winter sea ice cover preceded summers with increased $\Delta \sigma_{T_{\text{min}} - 0}$, upper water column stratification was strong, and peak POC flux terminated earlier, with a shorter flux duration period, whereas decreased stratification was associated with peak termination dates pushed later into Mar, Apr, and May (Fig. 2.5 and 2.6).
Figure 2.6. Relationship between water column stratification ($\Delta \sigma_T$) and A) peak POC flux duration, and B) the timing of peak POC flux termination ($p = 0.02$ & 0.003, respectively).

2.3.3. Interannual variability in POC flux

The mean integrated annual (July–June) POC flux rate over the 21-year time series was $2.3 \pm 1.3 \text{ g C m}^{-2} \text{ y}^{-1}$, but varied over an order of magnitude, between $0.3$ and $5.3 \text{ g C m}^{-2} \text{ y}^{-1}$ during anomalously low and high POC flux years, respectively (Fig. 2.7A). Integrated annual POC flux accounted for an average of $13.6 \pm 10.8\%$ of annual integrated total mass flux, and ranged from $4\%$ in 1993 to $49\%$ in 2012.

There is no long-term, unidirectional trend in annual POC flux over the two decades ($R^2 = 0.04, p = 0.43$). However, integrated annual POC flux (Fig. 2.7A) further illustrates the high interannual variability in carbon flux in the WAP, as also indicated in the PC 1 time series of the POC EOF analysis (Fig. 2.4D). Integrated annual POC flux oscillated between strongly positive and strongly negative anomalies, with the time series dominated by anomalously low flux years (10 years), mostly occurring in the latter half of the time series, and with six anomalously high flux years, (Fig. 2.7B). The positive anomalies reach a larger magnitude than the negative anomalies (Fig.
Mean annual POC flux anomalies showed peak positive anomalies occurred 5–6-year apart (1994, 1999, and 2005), with strongly-positive POC flux anomaly peaks followed by 4–5 years of weakly-positive or strongly negative POC flux (Fig. 2.7C). Spectral analysis of integrated annual POC flux confirmed a significant 5-year periodicity in the time series (Fig. 2.7D; \( p < 0.001 \)).

**Figure 2.7.** A) Annual (July–June) POC flux time series from 1993–2012. B) Histogram of annual cumulative POC flux anomaly; dashed line separates negative and positive anomalies. C) Mean POC flux anomaly each year. D) Spectral analysis of annual POC flux data. Analysis indicates a 5-year periodicity (\( p < 0.001 \), Bartlett's Kolmogorov–Smirnov statistic, Fuller,1996); dashed line at 5 years is for reference.
The amount of annual POC flux was not driven by flux duration. Increased flux duration did not correlate with higher integrated annual POC flux (Fig. 2.8; $R^2 = 0.025$, $p = 0.54$). Years of high integrated annual POC flux and anomalously high annual mean flux rate (1994, 1999, and 2005) had relatively shorter flux duration, each lasting less than 58 days. An exception is the high integrated annual POC flux in 1998, which in conjunction with its low mean flux rate relative to other positive anomaly years indicates the increased integrated annual POC flux is likely a result of an increase in flux duration (77 days; Fig. 2.3B).

![Figure 2.8. Annual POC flux as a function of peak POC flux duration. The regression is non-significant ($p = 0.54$).](image)

**2.3.4. Environmental and Biological parameters and annual POC flux**

Although sea ice cover significantly impacts the seasonal timing of peak POC flux, sea ice cover was not significantly correlated with the magnitude of POC exported out of the upper water column (Supplementary Table 2.1). Environmental parameters including sub-decadal climate oscillation indices such as ENSO and SAM, and biological parameters such as phytoplankton biomass (chl-a)
and salp abundance, also were not significantly correlated with the magnitude of POC flux (Supplementary Table 2.1).

As the magnitude of integrated annual POC flux is driven by the summer-time pulse (on average 80% of the annual POC flux), and krill FPs dominate summer POC flux in the WAP (Gleiber et al., 2012), we hypothesized that krill abundance would be significantly correlated with POC flux. However, summer total krill abundance (E. superba juveniles and adults combined) was not significantly correlated with integrated annual POC flux (Fig. 2.9A & B), and although they appear antiphased (Fig. 2.9A), the negative relationship between total E. superba abundance and POC flux was not significant (Fig. 2.9B; $R^2 = 0.15$, $p = 0.13$). Larger, adult krill that migrate over the shelf to deeper waters during the summer may be more likely to contribute to the summer peak in POC flux on the outer shelf, producing larger FPs than juveniles. However, there was also no significant correlation between adult (>30 mm) krill abundance and annual POC flux (Supplementary Fig 1A, b; $R^2 = 0.063$, $p = 0.35$).

Rather, adult krill body size (length) was the most significant factor relating to integrated annual POC flux. Annual POC flux oscillates in synchrony with the mean summer-time adult E. superba body size, peaking every 5–6 years (Fig. 2.9C), and with POC flux significantly positively correlated with adult body size (Fig.2.9D; $R^2 = 0.34$, $p = 0.018$). Thus, when the krill population was dominated by larger (older) adult krill, where 63.5%±12% of the krill population was comprised of large adults ≥ 41 mm in length, integrated annual POC flux was higher. Years of anomalously low POC flux coincided with years following high juvenile krill recruitment, with < 26±26% of krill ≥ 41 mm in length.
Figure 2.9. A) Annual POC flux (blue circles) and total krill abundance anomaly (black diamonds) time series from 1993–2012. B) Annual POC flux as a function of total krill abundance anomaly ($p = 0.13$). C) Annual POC flux (blue circles) and annual mean adult *E. superba* body size (black diamonds) time series from 1993–2012. D) Annual POC flux as a function of annual mean adult krill body size and ($p = 0.018$). All krill data are from the northern-most sector of the PAL-LTER sampling grid (500 and 600 lines; see Fig. 2.1).

Analysis of krill body size from Adélie penguin diets over the same 21-year study period also indicates krill body size oscillates over a 5-year cycle, in synchrony with both net-tow krill body size and POC flux in the WAP (Supplementary Fig. 2.2) and was also significantly positively correlated with POC flux (Supplementary Fig. 2.3; $R^2 = 0.36, p = 0.01$).
POC flux in the WAP over this 21-year study period is thus linked to the 5–6-year krill life cycle, wherein the mean size class of the adult krill within the population is the dominant driver of POC flux, as opposed to total krill abundance. Annual total *E. superba* abundance is significantly negatively correlated to annual mean krill body size when all size classes of krill are taken into account in both net tows ($R^2 = 0.50$, $p < 0.001$) and Adélie diets ($R^2 = 0.45$, $p = 0.001$), Supplementary Fig. 2.A & B, respectively, indicating that during years of high krill abundance, the population is made up of mostly young, small krill. This helps to explain why there is a no relationship between total *E. superba* abundance and integrated annual POC flux (Fig. 2.9 A & B).

![Figure 2.10. A) Annual POC flux (blue circles) and krill biomass anomaly (black diamonds) time series from 1993–2012. B) Annual POC flux as a function of krill biomass anomaly ($p = 0.26$. All krill data are from the northern-most sector of the PAL-LTER sampling grid (500 and 600 lines; see Fig. 2.1](image)

Overall krill biomass in the water column was not significantly correlated with annual POC flux. Although the time series of POC flux and krill biomass appear antiphased (Fig. 2.10A), the negative relationship between total *E. superba* biomass and POC flux was not significant (Fig. 2.10B; $R^2 = 0.09$, $p = 0.26$). Taken all together, it is shown that the size of the krill, not the amount
of krill, either via total abundance or total biomass, that is correlated with annual POC export on the WAP.

2.4. Discussion

2.4.1. Comparison of Antarctic POC sediment trap export

The seasonal and the semi-decadal episodic nature of POC flux that characterizes the WAP continental shelf would be impossible to capture without long-term sediment trap measurements. The PAL sediment trap provides the longest running time series of POC flux in Antarctica and the only year-round, ongoing in situ biogeochemical record of any kind for this remote and ice-covered region. For comparison, the majority of Southern Ocean trap datasets ranged from seasonal to two years (Table 2.1). Such a long-lived sediment trap record has helped us to understand the complex dynamics of POC export and the role of the overlying sea ice and zooplankton community. In our study, peak summer POC flux ranged from 4.76 to 124 mg C m$^{-2}$ d$^{-1}$ with a mean daily flux over the entire time series of 9.11 mg C m$^{-2}$ d$^{-1}$, peaking around Jan–Feb. Integrated annual (July–June) POC flux over the 21-year time series varied over an order of magnitude, from 0.31 to 5.31 g C m$^{-2}$ with a mean of 2.3 g C m$^{-2}$. Only about 1% of organic carbon export makes it to the deep ocean (>2000 m; Lampitt and Anita, 1997). Using this factor, and our maximum annual flux, about 0.05 g C m$^{-2}$ would be exported to the deep ocean of the WAP. A comparison of our observations with other mid-water and abyssal sediment trap POC fluxes shows considerable variability in the Southern Ocean (Table 2.1). The majority of trap datasets ranged from days to two years and ranged from enclosed bays to pelagic regions, epipelagic to abyssal plains and open waters to under the sea ice. In the South Orkney Islands region of the western Scotia Sea, about 600 km north of the Antarctic peninsula, short-term trap deployments during the austral summer showed that krill
FPs comprised 60–80% of total POC flux in the upper 200 m (Belcher et al., 2017), comparable to krill FP contribution in our WAP sediment trap. In the nearby Strait of the Northern Antarctic Peninsula (NAP), mean flux was 0.98 mg C m\(^{-2}\) d\(^{-1}\) (Palanques et al., 2002), much lower than our trap, likely due to increased attenuation in deeper waters. In contrast to the low winter fluxes in our study, a study at Deception Island showed high POC fluxes in the winter (Baldwin and Smith, 2003). In Marguerite Bay, annual POC fluxes were similar to that of our region (Weston et al., 2013). In the Amundsen Sea Polynya, two distinct flux peaks, in Jan and Dec, were measured. POC flux was higher than on the WAP, but was dominated by *Phaeocystis antarctica*. Zooplankton FP contributed less than on the WAP, at 10–40% of the total flux and were dominated by smaller copepods (*Metridia gerlachei, Calanoides acutus*), and some small ice krill *Euphausia crystallarophias*, FP (Ducklow et al., 2015). In the Terra Nova Bay Polynya, small ovoid pteropod *Limacina helicina* FP contributed 19% of the POC flux, reaching maximum values in Mar–Apr (Manno et al., 2010). In the central Ross Sea, POC flux peaked at 60 mg C m\(^{-2}\) d\(^{-1}\) in Apr–May and was also dominated by *L. helicina* (Collier et al., 2000). In the East Antarctic, POC flux phenology was similar to our region, though fluxes were lower and dominated by diatoms (Pilskaln et al., 2004).

In the Subantarctic, the Australian Southern Ocean Time Series (SOTS) deployed deep long-term traps from 1997 to 2017. The deep traps measured POC flux that ranged from 0.6 to 1.9 g C m\(^{-2}\) y\(^{-1}\) at three depths (Wynn-Edwards et al., 2020). Though their average fluxes were lower than those found on the WAP, their traps are much deeper, which likely led to increased attenuation and the low interannual flux variability found in their traps, in contrast to the high interannual variability that characterized our region on the WAP. These results illustrate the large variability in the
patterns and ecology of flux in the Southern Ocean. The composition of the overlying species assemblage, sea ice conditions or lack thereof, and proximity to land modify the composition, concentration of carbon, and phenological timing of flux. It is likely that other regions of the Southern Ocean dominated by *E. superba* may experience similar 5-year cycles in POC export through FP production. Unfortunately, there are no long term sediment trap measurements of export flux in these regions and this hypothesis warrants further investigation.
### Table 2.1. PAL–LTER long-term WAP sediment trap POC flux comparison with other Southern Ocean sediment trap POC fluxes and their dominant flux material.

<table>
<thead>
<tr>
<th>Location</th>
<th>Timeframe</th>
<th>Trap Depth (m)</th>
<th>Bottom Depth (m)</th>
<th>Daily POC Flux (mg C m⁻² d⁻¹)</th>
<th>Annual POC Flux (g C m⁻² y⁻¹)</th>
<th>Peak Period</th>
<th>Mean %POC</th>
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<td>Bransfield Strait - NAP</td>
<td>Mar 1995 - Feb 1996</td>
<td>500</td>
<td>1000</td>
<td>0.01 - 9.8 (0.98)</td>
<td>0.35</td>
<td>Nov</td>
<td>8.7</td>
<td>Fecal pellets</td>
<td>Palanques et al., 2002</td>
</tr>
<tr>
<td>West Antarctic Peninsula</td>
<td>1992 - 2013</td>
<td>175</td>
<td>350</td>
<td>&lt;0.01 - 124 (9.11)</td>
<td>0.31 - 5.31 (2.3)</td>
<td>Dec - Feb</td>
<td>13.6</td>
<td>Euphausia superba fecal pellets</td>
<td>This study</td>
</tr>
<tr>
<td>Marguerite Bay - WAP</td>
<td>2005 - 2006</td>
<td>200</td>
<td>520</td>
<td>&lt;0.02 - 180</td>
<td>1.47</td>
<td>Nov - April</td>
<td>4.6</td>
<td>Phaeocystis antarctica phytodetritus</td>
<td>Weston et al., 2013</td>
</tr>
<tr>
<td>Amundsen Sea Polynya</td>
<td>Dec 2010 - Dec 2011</td>
<td>350</td>
<td>800</td>
<td>&lt;0.01 - 96 (16.56)</td>
<td>3.78 (1.38)</td>
<td>Nov - Jan</td>
<td>12</td>
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<td>Manno et al., 2010</td>
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<td>1998 - 2001</td>
<td>180</td>
<td>998</td>
<td>-</td>
<td>0.51 - 0.88</td>
<td>Mar–Apr</td>
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<tr>
<td>Northern Ross Sea</td>
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<td>565</td>
<td>0.43 - 16.7</td>
<td>6</td>
<td>Apr - May</td>
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<td>Foraminifera Neo globorquadrina pachyderma</td>
<td>Collier et al., 2000</td>
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<td>581</td>
<td>0.36 - 60.1</td>
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<td>Limacina helicina</td>
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<tr>
<td>Weddell Sea</td>
<td>1 Feb - 12 Feb 1985</td>
<td>80</td>
<td>&gt;1000</td>
<td>61</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>Protozoan fecal pellets</td>
<td>Nöthig and von Bodungen, 1989</td>
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<tr>
<td></td>
<td></td>
<td>350</td>
<td>73</td>
<td></td>
<td></td>
<td>-</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>East Antarctica</strong></td>
<td></td>
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<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Prydz Bay</td>
<td>Dec 1998 - Dec 1999</td>
<td>1400</td>
<td>4000</td>
<td>0.2 - 14</td>
<td>0.9</td>
<td>Jan - Feb</td>
<td>3.6</td>
<td>Diatoms</td>
<td>Pilskaln et al., 2004</td>
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<tr>
<td>Pierre Lejay Bay</td>
<td>Nov - Dec 2001</td>
<td>3</td>
<td>53</td>
<td>10.6 - 62.1</td>
<td>&gt;7</td>
<td>Dec</td>
<td></td>
<td>Diatoms</td>
<td>Riaux-Gobin et al., 2013</td>
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<tr>
<td></td>
<td></td>
<td>47</td>
<td>53</td>
<td>15.2 - 78.6</td>
<td></td>
<td>Dec</td>
<td></td>
<td>Diatoms</td>
<td></td>
</tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Australian Southern Ocean Time Series</td>
<td>1997–2017</td>
<td>1000</td>
<td>4600</td>
<td>4 - 2298</td>
<td>0.5 - 1.9 (1.2)</td>
<td>Oct - Nov</td>
<td>17.1</td>
<td>Coccolithophore-carbonate</td>
<td>Wynn-Edwards et al., 2020</td>
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<td></td>
<td></td>
<td>2000</td>
<td></td>
<td>67 - 2457</td>
<td>1 - 1.8 (1.4)</td>
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<td>13.3</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>3800</td>
<td></td>
<td>54 - 1312</td>
<td>0.6 - 1.4 (1)</td>
<td>Jan</td>
<td>10.4</td>
<td></td>
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<td>South Georgia</td>
<td>Apr 1975 - Mar 1977</td>
<td>16</td>
<td>18</td>
<td>70 - 520</td>
<td>60</td>
<td>Nov - Jan</td>
<td>1.7</td>
<td></td>
<td>Platt, 1979</td>
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<tr>
<td>Kerguelen Plateau - Indian sector</td>
<td>Oct 2011 - Sep 2012</td>
<td>289</td>
<td>527</td>
<td>0.5 - 19</td>
<td>1.2</td>
<td>Dec - Feb</td>
<td>3.3 - 17.4</td>
<td>Diatoms</td>
<td>Rembauville et al., 2015</td>
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53
2.4.2. POC flux phenology in the marginal ice zone

The drastic seasonal transformation of the MIZ from winter to summer shapes the phenology in POC flux. POC flux on the shelf of the WAP is characterized by a pronounced summer-time peak, as shown by our EOF analysis. This summer peak in flux is due to the melting of sea ice, increase in primary production under ~24 hours of sunlight, and the summer-time migration of adult krill on to the continental shelf (Siegel, 1988; Nicol, 2006) with their associated production of large FPs. The single pronounced peak in POC flux on the WAP shelf during summer varies in timing, where some years are characterized by sharp short-lived pulses, and others by broad peaks lasting until late summer. These differences in timing and duration are apparent in the EOF analysis that indicates how the summer flux is pushed earlier or later in the season, especially through the modification of flux termination date.

Peak POC flux duration and termination date were correlated with maximum winter sea ice cover. During the winter, sea ice insulates the water column from high winter winds, thus preventing the formation of a deep winter mixed layer and provides melt water in the spring, which further strengthens the density gradient in the upper water column (Venables et al., 2013). Reduced sea ice cover leads to increased mixing and heat loss in the winter and reduction in stratification, as reflected by low $\Delta \sigma_\theta T_{\text{min}} - 0$, persists into the following summer. Low $\Delta \sigma_\theta T_{\text{min}} - 0$ preconditions the water column for greater vertical extent of surface-driven mixing, pushing the summer mixed layer depth (MLD) below 100 m (Venables and Meredith, 2014). We found that low $\Delta \sigma_\theta T_{\text{min}} - 0$ correlated with delayed peak POC flux termination and increased peak flux duration. Conversely, when winter sea ice cover was high, summer $\Delta \sigma_\theta T_{\text{min}} - 0$ was increased, and POC flux termination occurred relatively earlier in the summer, likely due to the occurrence of a shallower mixed layer.
The number of particles in a convecting fluid decreases exponentially with time, with a decay rate equal to the settling velocity of the particles divided by the thickness of the layer (Kerr and Kuiper, 1997). A deeper MLD (thicker mixed layer) would increase the residence time of a given particle in the upper water column before it is exported (Gardner et al., 1999). Deeper convective mixing increased the time that particles circulated in the layer, increasing their residence time. Therefore, when summer stratification is low and the MLD deepens, following low winter sea ice, it is likely krill FPs exhibit a longer residence time before being exported, leading to the increased flux duration and later peak flux termination seen in our analysis.

The phenology of \textit{E. superba}, and other taxa, also affects POC flux patterns. Zooplankton FPs are the dominant particle type contributing to annual POC export in the WAP, with the cylindrical FPs of \textit{E. superba} contributing the most in terms of absolute number and total POC (Gleiber et al., 2012). During summer, \textit{E. superba} FPs constituted 82\% of total FP POC flux, while small ovoid copepod FPs and tabular salp FPs contributed significantly less, 17 and 0.8\%, respectively (Gleiber et al., 2012). Other Southern Ocean regions experience both differing POC material and export rates, as well as different flux phenology, influenced by the composition of the overlying species assemblage, presence of sea ice, and proximity to land. For example, in the Terra Nova Bay Polynya, small pteropod \textit{Limacina helicina} FPs comprised the bulk of the POC flux, reaching maximum values in Mar–Apr (Table 2.1; Manno et al., 2010).

During the summer months, krill in the WAP are found in the upper 120 m of the water column, exhibiting no to limited diel vertical migration (DVM) within the upper 100 m (Conroy et al., 2020a). When the summer MLD is deep, \textit{E. superba} are more likely to be found within the mixed
layer. Likewise, krill FPs are more likely to be produced within the mixed layer, as opposed to below it, as in years in which the MLD is shallow. Decreased stratification and a deepening of the MLD likely keep FPs suspended in the upper water column for longer, increasing their residence time (Lande and Wood, 1987; Gardner et al., 1999). These physical conditions likely lead to the broadening of POC flux duration into late summer and autumn and a later termination date for peak flux. Additionally, in the summer, small juvenile krill are found mostly over the inner shelf, while the larger, sexually mature adults make a longer ontogenetic migration from coastal inlets, congregating over the outer shelf and continental shelf break (Kawaguchi, 2016). Further, large gravid adult females move offshore of the rest of the adult population, from mid-Dec to early Mar, to access deep waters that are critical for hatching success of eggs and larval development (Nicol, 2006).

The seasonal age-specific, and therefore size-specific, habitat preferences of *E. superba* are especially amplified during the summer reproductive period. The effect of these differential distribution patterns is that the krill in younger stages are separated from the adults, and adult krill play a larger role in summer POC flux over the outer continental shelf. The summer-time arrival of large adult krill over the outer shelf and their grazing and egestion of large, carbon rich FPs (Gleiber et al., 2012) leads to the intense and episodic peak in POC flux during the summer.

During the autumn and winter months, light limitation and growth of sea ice limit primary production and prompt the seasonal abandonment of offshore waters by krill as they begin their ontogenetic migration to coastal inlets and canyons (Nicol, 2006). These ecological shifts limit the production of phytodetrital material and FPs that contribute to POC flux on the shelf during the
winter. Interestingly and in contrast to our study, POC flux at Deception Island to the north of our trap location showed high export rates in the winter (Table 2.1; Baldwin and Smith, 2003). The winter MIZ is essential for successful *E. superba* larval survival and successful recruitment (Siegel, 1988, Meyer et al., 2017), which helps to set up the 5-year cycle of the next krill cohort abundance and body size.

### 2.4.3. The 5-year cycle in POC flux and Antarctic krill

Our study suggests that the pattern of high and low interannual POC flux magnitude in the WAP follows a 5-year cycle and is mechanistically linked to the summer-time body size (length) of the adult *E. superba*. The consistency of the correlation between krill body size and annual POC flux across multiple data collection methods, net tows and Adélie penguin foraging, supports our conclusion that krill body size drives POC flux. When the krill population is dominated by larger, older adults, integrated annual POC flux increases, likely due to the production and export of larger FPs. In summer these adults migrate over the outer continental shelf (Nicol 2006), where the PAL-LTER sediment trap is located. When POC flux was highest, the majority of the krill population was comprised of large adults ≥ 41 mm in length, matching the length-frequency distribution of a population comprised of age classes 4 years and older (Siegel et al., 2013; Reiss, 2016). Additionally, larger (longer) krill have higher FP carbon production rates (Pauli et al., 2021). Anomalously low annual POC flux coincided with years following high juvenile krill recruitment, with the majority of krill matching the length-frequency distribution of a population comprised of age classes 2 years and younger (Siegel et al., 2013; Reiss, 2016).
The krill within a cohort continue to grow throughout each year of their life span, with faster growth rates during spring and summer when environmental conditions are favorable and food quality and quantity are high (Reiss, 2016). This continued annual growth, combined with the longevity of *E. superba* of 5–6 years, means that individual krill within the large cohort grow in body length in near synchrony over the 5-year cycle. Further, krill possess simple, tube-like, linear guts, and thus produce long cylindrical strings that grow in size relative to their body length (Cadée et al., 1992). The larger, longer FPs produced by older adults are more likely to sink out of the upper mixed layer faster than the small FPs produced by smaller, younger krill and be found in deeper sediment traps (Atkinson et al., 2012; Cadée et al., 1992). The swarming and schooling behavior of *E. superba* could further lead to an increase and synchrony in export of POC, which would be amplified when krill are larger. Given the relationship shown between krill body size and POC flux, it is predicted that the missing 2009 data would constitute a fourth peak-year of anomalously high POC flux, coinciding with the large body size of krill found that summer. Regardless of the missing data, we were able to resolve the 5-year periodicity in integrated annual POC flux over the 21 years and relate this cycllicity to that of *E. superba* body size.

Though krill and their FPs are a major contributor to POC flux in the WAP, there is no significant relationship between krill abundance and POC flux. In our study, the size of the krill within the cohort correlated with POC export, where increased krill body size, as opposed to increased abundance of krill, corresponded to increased POC flux. A number of studies are increasingly showing the importance of *E. superba* in the biogeochemical cycles of the Southern Ocean through their production of FPs and exoskeleton molts, excretion of dissolved organic carbon and nitrogen, and the subsequent stimulation and redistribution of the microbial loop (Aristegui et al., 2014;
Belcher et al., 2017; Cavan et al., 2019; Manno et al., 2020). A recent study used *E. superba* abundance densities (individuals m$^{-2}$) from 1926–2016, across the Southern Ocean MIZ, and applied a constant krill body size of 34 mm to model krill FP POC flux. The study suggests that seasonal krill FP export flux is about 0.039 GT C, about 17–61% (mean 35%) of satellite-derived export estimates (Belcher et al., 2019). Using krill abundance densities to model FP flux does not take into account changing krill body size during population cycles, which we have shown to be the main predictor of POC flux in the WAP. Further, there is a significant negative correlation between krill abundance and krill body length. The consideration of krill body size is especially critical over a long-lived timeseries, such as the one presented in their study, where many cycles in krill body size (and abundance) have likely taken place. These model estimates likely overestimate the contribution of krill FP POC export during years when the krill abundance is high but the population is dominated by small krill, and may underestimate FP contribution during years when abundance is high but the population is dominated by large krill, as acknowledged by the authors (Belcher et al., 2019). An additional study by Pauli et al. (2021) likely also under- and overestimated the contribution of krill FP POC by using krill abundances as the main driver in their equation for FP production.

The 5-year cycle in POC flux in our analysis matches the 5-year cycle in krill size-class (Fraser and Hofmann 2003) and is antiphased with the 5-year cycle of krill abundance (Steinberg et al., 2015). The negative relationship between *E. superba* abundance and annual mean krill body size has been shown previously (e.g., Ross et al., 2014). Thus, one consequence of the unique life history of *E. superba* is that annual POC flux is at a minimum when krill abundance and biomass are peaking, when the cohort is comprised of many young, small juveniles after successful
recruitment. Annual POC flux increases as the krill cohort ages and grows over the course of the next 4 to 5 years. Strong recruitment of the next cohort has been found to coincide with several environmental factors such as a 4–5-year cycle of high winter sea ice extent (Fraser and Hofmann, 2003), increased summer stratification and shallower MLD (Saba et al., 2014), and following years of increased primary production or chl-a concentration (Quetin and Ross, 2003; Ross et al., 2014; Saba et al., 2014; Steinberg et al., 2015). This 5-year cycle in POC flux might also be found in other MIZ locations with similar krill populations, such as along the Northern Antarctic Peninsula (Reiss, 2016) and South Georgia (Fielding et al., 2014).

The large summer-time pulse of POC flux dominated by fast sinking krill FPs has implications for transfer of organic carbon and nutrients to the benthic ecosystem on the Antarctic shelf. Although prior studies find no coupling between summer surface primary production, POC export, and benthic ecological processes, it is suggested that the accumulation of a sediment food bank buffers the benthic ecosystem from high seasonal variability of the upper water column (Smith et al., 2006). The persistence of high organic-matter concentrations in the benthos of the WAP has been hypothesized to be due to the very low temperatures typical of the Antarctic shelf, causing reduced microbial consumption of POC (Arnosti and Jorgensen, 2003). This temperature-induced limitation on microbial activity may cause organic matter to build up to relatively high levels before community respiration can balance the sinking flux of labile POC (Mincks et al., 2005). Therefore, it is likely that the intense pulse of krill FPs and POC export every summer builds up on the shelf and helps to buffer the benthic ecosystem over the autumn and winter; the major pulse of summer POC flux every five years, when the krill population is mostly comprised of older, large adults, may help to further buffer variability in food availability for benthic organisms. A
substantial portion of the summer-time POC flux is thus likely exported and stored in the benthos over relatively long timescales, as benthic organisms carbon demand is lower than the supply of export flux, making the WAP an efficient carbon sink.

2.5. Conclusions

The extreme seasonality and interannual variability of physical conditions in the WAP, along with the seasonal ontogenetic horizontal and vertical migrations of krill, combine to drive the phenological variability from extremely low POC flux during the winter to a pronounced summer-time pulse, with interannual variability in flux duration. As an anomalously long-lived zooplankton species, *E. superba* integrates the seasonal and annual interactions of sea ice, water column stratification, primary production, and spatial migrations over a semi-decadal timescale. The 5-year cycles in POC flux magnitude and the seasonality of POC flux have different scales of variability, however they are likely working in concert in driving ecosystem variability on different timescales.

The warming of the WAP climate may also have implications for krill abundance and life cycle phenology, and thus POC export. The WAP has experienced a 6°C increase in mean atmospheric temperature since 1950 during the critical winter months (Vaughan et al., 2003), leading to the deterioration of the ecologically fundamental sea ice season (Parkinson, 2004; Stammerjohn et al., 2012). *E. superba* abundance has decreased long-term in the far northern Antarctic Peninsula, and their range is contracting southward, which is hypothesized to be due to warming and increased positive SAM anomalies (Atkinson et al., 2019). *E. superba* in the far northern Antarctic Peninsula also exhibit a 4–6-year cycle in post-larval krill abundance and strong cohort dynamics, similar to
that of our region in the WAP (Fielding et al., 2014, Reiss, 2016, Conroy et al., 2020b). As the Antarctic Peninsula continues to warm and sea ice cover diminishes, a pole-ward shift in the krill population and a lack of successful recruitment of juveniles to replenish the local aging population (Atkinson et al., 2019) could lead to changes in phenology, the loss of local krill populations, and ultimately changes in timing and magnitude of POC flux and deep carbon storage. Two decades of POC export timeseries data highlight the importance of understanding the complex life history strategies of the organisms that comprise the BP and the synergistic interplay between them and their sea ice environment. The resident krill population and their dependence on sea ice make the MIZ of the WAP a region of intense export in the Southern Ocean and an important region for carbon storage.
2.6. Supplementary Material:

**Supplementary Table 2.1.** Least-squares linear regressions of environmental and biological parameters against annual POC flux magnitude over the 21-year timeseries which showed no significant correlation.

<table>
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Supplementary Figure 2.1. A) Annual POC flux (blue circles) and adult-only krill abundance anomaly (black diamonds) time series from 1993–2012. B) Integrated annual POC flux as a function of adult krill abundance anomaly ($p = 0.35$).
Supplementary Figure 2.2. Annual POC flux (blue circles), annual mean adult-only krill body size from net tows (black diamonds), and annual mean adult-only krill body size from Adélie diets (red squares) timeseries from 1993–2012.
Supplementary Figure 2.3. Annual POC flux as a function of annual mean adult-only krill body size from Adélie diet ($p = 0.10$).
Supplementary Figure 2.4. A) Annual mean total krill body size from net tows as a function of annual krill abundance ($p < 0.0010$). B) Annual mean total krill body size from Adélie diets as a function of annual total krill abundance anomaly ($p = 0.0010$).
Chapter 3

Bacterial metabolic activity on krill fecal pellets and attenuation of carbon export
Chapter 3: Bacterial metabolic activity on krill fecal pellets and attenuation of carbon export

Abstract

Antarctic Krill (Euphausia superba) fecal pellets (FPs) are large aggregates of particulate organic carbon (POC) that can efficiently export organic carbon to the deep ocean. Further, FPs can potentially serve as localized sources of a variety of nutrients and organic carbon, and as potential homes for microbial assemblages. Biological and chemical transformations occur on sinking particles, through processes such as bacterial production (BP), and respiration (BR) and nutrient remineralization, influencing the quantity and quality of organic matter reaching the deep sea. It is therefore necessary to determine the impact of the microbial ecosystem on important biogeochemical cycles, such as POC export, in the Southern Ocean. We conducted studies over two years, measuring BP, BR, and cell abundance of free-living bacteria in the seawater, free-living bacteria that have exposure and access to krill FPs, and krill-associated bacteria themselves on the West Antarctic Peninsula (WAP). From these data we were able to derive bacterial growth efficiency and cell abundances of free-living bacteria with and without access to FP POC, and overall POC turnover rate of FPs by bacteria. The egestion of krill FPs into the water column and exposure of these FPs to the free-living community lead to complex patterns of change in the microbial community. The presence of FPs did not significantly impact water column BR and can both increase or decrease BP, thereby altering the growth efficiency of the bacterial population. The exposure of free-living bacteria to FP POC altered the metabolic activity of free-living cells, shifting the population from a metabolically dormant to a more active state. All together, this indicates a switch from respiration to biomass production in the presence of large, organic carbon-
rich particles. Though the free-living bacteria in the water column may become more metabolically active in the presence of FP POC, overall organic consumption rate by the bacterial population and FP POC turnover rate were low. These results suggest that fecal pellet-associated bacteria in the euphotic zone are not a major sink for FP carbon, and are not responsible for the bulk of POC flux attenuation in the upper 150 m of the WAP marine system, but krill FPs nonetheless have significant impacts on the ecology of free-living bacteria.
3.1. Introduction

The production and export of marine particulate organic carbon (POC) to depth redistributes carbon and nutrients within the ocean and plays an integral role in modulating atmospheric carbon dioxide (CO$_2$) concentrations (e.g., Sarmiento et al., 1998; Marinov et al., 2008; Studer et al., 2018). Zooplankton fecal pellets (FPs) constitute an important pool of POC in the epipelagic ocean. Feces of marine zooplankton act as particulate patches of nutrients and can be sites of high bacterial abundance (Honjo and Roman, 1978; Pomeroy and Deibel, 1980; Alldredge and Youngbluth, 1985). Biological breakdown of FPs is, in part, driven by hydrolytic activity of these attached bacteria. Much of the organic matter originating from FPs is cycled through the microbial loop of the biological pump in the upper ocean and remains part of the long-lived organic carbon in the epipelagic (Legendre and Michaud, 1998). This consumption and reduction in POC flux with depth (attenuation) can be particularly rapid in the upper mesopelagic, driven not only by microbial remineralization, but also by zooplankton grazing (coprophagy) and fragmentation of FPs (coprohexy). In this study we focus on Antarctic krill FPs, the main source of POC export on the West Antarctic Peninsula (WAP; Gleiber et al., 2012; Trinh et al., in review), and the role heterotrophic bacteria (here meaning heterotrophic members of the domains Bacteria and Archaea) play in FP carbon consumption and POC flux attenuation.

The marine ecosystem is home to a diverse array of bacteria, not only in terms of taxonomic species, but also behavioral and metabolic capabilities and niche occupation. Within this diverse ecosystem, bacteria span a spectrum from obligate free-living lifestyle to obligate particle attached lifestyle, with generalists that are able to switch among states in between. Large fractions of marine free-living bacteria have the capability to be motile (Grossart et al., 2001), and many of them have
chemotactic behavior (Keegstra et al., 2022) that allows them to move and cluster around and stay within patches of dissolved organic matter supplied by particles. Free-living bacteria with these behavioral capabilities may be able to rapidly colonize particulate organic matter (Kiørboe et al., 2002). Chemotactic bacteria may grow up to 50% faster when clustering within patches of nutrients from detrital particles such as zooplankton FPs (Blackburn et al., 1997; Stocker and Seymour, 2012). Thus, motility and foraging strategies of free-living bacteria can be important adaptations for taking advantage of dissolved organic carbon (DOC) and colonization of POC (Grossart et al., 2003), setting up bacterial interactions between particle-associated and free-living motile bacterial cells that may be commensal, antagonistic, or a mix of both.

Ecologically, there is an important commensal relationship between particle-attached and free-living heterotrophic bacteria in marine systems. Bacteria associated with organic particles play a role in the initial degradation of POC, hydrolyzing polymers and releasing DOC that can be utilized by free-living bacteria in the water column (Stocker, 2012; Dang and Lovell, 2016). The hydrolytic activity on particle surfaces, such as zooplankton FPs, can be high, while the rate of consumption of hydrolysate by particle-associated bacterial cells is often low. This leads to an uncoupling between DOC production and uptake by particle-attached bacteria (Smith et al., 1992, Grossart and Simon, 1998, Grossart et al., 2003), potentially resulting in increased DOC availability, consumption, and increased bacterial production (BP) of the free-living assemblage (Cho and Azam, 1988; Friedrich et al., 1999; Grossart et al., 2003). This process illustrates the positive influences of particle-associated cells on free-living cells (Miki and Yamamura, 2005).
Alternatively, since organic particles such as FPs can act as stable, non-nutrient-limiting habitats for microbes, it may be beneficial for attached bacteria to invest in defending against free-living newcomers by releasing antibiotic substances (Long et al., 2005). Antagonistic interactions may play important roles in structuring bacterial communities, where the evolutionary advantages afforded by an effective chemical defense may be crucial for survival of certain taxa (Wietz et al., 2013). Many particle-associated bacteria exhibit antagonistic behavior against other bacteria (Long and Azam, 2001; Gossart et al., 2003). Microbial metabolic interactions between community members on particles are well documented, with various mechanisms responsible for these effects, ranging from direct cell death through an antibiotic, to the removal of an essential nutrient through the use of an iron chelating siderophore. Antagonism between cells and taxa can also result from the production of small organic acids or other compounds that render the environment unsuitable for the growth of competing bacteria (Schnürer and Magnusson, 2005). Studies on bacterial growth frequently show inhibition of growth in the presence of other strains of bacteria due to production of repellents, antibiotics, toxins, and inhibition of quorum sensing ability (Gram et al., 2002; Wilson et al., 2011; Kempnich and Sison-Mangus, 2020). These bacterial interactions are a key component of the microbial ecosystem and ocean biogeochemistry.

Both particle-associated and free-living heterotrophic bacteria perform two major functions in the transformation of organic carbon that have significant impact in the global carbon cycle. Bacterial cells produce new bacterial biomass through BP and they respire organic carbon to CO₂ through bacterial respiration (BR). While particle-attached bacteria may have higher per cell rates of BP, free-living cells generally contribute more to overall BP in the water column due to greater overall cell abundance (Turley and Mackie, 1994; Rieck et al., 2015). The majority of marine microbial
metabolic studies have focused solely on BP as a measure of biological activity and have overlooked BR or derived BR through model calculations and estimations (Jahnke and Craven, 1995; del Giorgio and Cole, 1998; Ducklow et al., 2002). The lack of knowledge about BR limits our ability to understand the full role of bacteria in the biological pump of aquatic ecosystems (del Giorgio and Cole, 1998; Robinson and Williams, 2005).

The sum of biomass production through BP and metabolic activity through BR determines the total bacterial carbon demand (BCD), while the ratio of BP to BCD defines the bacterial growth efficiency (BGE) of each cell. BGE, the amount of bacterial biomass produced per unit of DOC consumed, is a basic property that determines the net biogeochemical and ecological role of bacterioplankton (del Giorgio and Cole, 1998, del Giorgio and Williams, 2005; Carlson et al., 2007). BGE for any given system has been shown to vary in time (Lemée et al., 2002) and space (Griffith et al., 1990), depending on many factors such as temperature (Bjørnsen, 1986; Daneri et al., 1994) and quantity and quality of organic matter, more specifically, the molecular weight of organic compounds, as different bacteria are specialized at breakdown and uptake of different compounds (Amon and Benner, 1996; Briand et al., 2004).

In terms of their numerical abundance, heterotrophic bacteria are the most important biological component involved in the transformation and mineralization of organic matter in the biosphere (Cho and Azam, 1988). However, not all bacterial cells are the same. Not only are bacteria different in terms of species, niche occupation, and functional role, but also in terms of their cell-specific metabolic activity at any given point in space and time. The nucleic acid content of individual bacterial cells can be used to discriminate between sub-populations within natural marine bacterial
communities (Marie et al., 1997, Troussellier et al., 1999). Cells with a high nucleic acid (HNA) content are proposed to be the most active cells, and that they contribute to the most important part of community activity such as BP and BR (Gasol et al., 1999; Lebaron et al., 2001; Gasol et al., 2002). In contrast, the cells with a low nucleic acid (LNA) content are suggested to represent inactive, moribund, or dead cells (Lebaron et al., 2002). Other studies suggest that LNA cells may be active and ecologically important members of the microbial loop (Zubkov et al., 2001), or that LNA cells can transition to HNA states under certain conditions (Servais et al., 2003; Bouvier et al., 2007; Sharuddin et al., 2018). Factors influencing the relative importance of HNA to LNA cells within marine systems are uncertain. It is unclear if the addition of nutrients, such as the introduction of zooplankton FPs into the water column will alter the biological activity of surrounding free-living bacteria, changing their nucleic acid content by increasing growth or by decreasing their nucleic content and metabolic activity through antagonistic behavior.

FPs are unique in terms of other POC in that when they are first egested, they are essentially pristine of free-living bacteria and home only to bacteria associated with zooplankton guts and the food they have ingested (Chapter 4). This allows us to study the dynamics between the endemic particle-associated bacteria, their role in hydrolysis of POC to DOC, and their effect on free-living bacterial physiology in the surrounding environment. These physiological changes may include changes in BP, BR, BGE, and nucleic acid content.

POC in various forms represent micro-patches of concentrated carbon and nutrients that may create micro-niches or hot-spots of bacterial processes. These particles are patches of concentrated substrates, in an otherwise virtual desert of resources, that pelagic bacteria may preferentially
exploit. The microscale mucus-rich region around phytoplankton cells and its sphere of influence in the microbial ecosystem has been termed the ‘phycosphere’ (Bell and Mitchell, 1972; Azam and Ammerman, 1984), while the microenvironment of degrading phytoplankton detritus has been termed the ‘detritospheres’ (Biddanda and Pomeroy, 1988). We propose to advance these concepts further and call the microenvironment influenced by FPs the ‘fecalsphere’. The fecalsphere encompasses the localized FP microhabitat enriched in a variety of micronutrients and POC, which acts as a niche for the fecal particle-associated microbial assemblage, as well as its influence on free-living bacteria in the surrounding water. We specifically analyze the ecological impact of krill FPs and changes in the fecalsphere over time in waters along the West Antarctic Peninsula (WAP).

Antarctic krill (Euphausia superba) FPs represent nutrient-rich microenvironments in the Southern Ocean, possibly supporting bacterial abundances several orders of magnitude higher than the surrounding seawater (Donachie and Zdanowski, 1998; Clarke et al., 2019). The FPs of krill are the dominant source of POC that is exported from the surface ocean of the WAP (Gleiber et al., 2012; Chapter 2). Krill FPs can have export rates of hundreds of meters per day (Atkinson et al., 2012). This sinking rate is balanced by heterotrophic consumption of the organic nutrients and carbon within the FPs. One important group of consumers is heterotrophic bacteria, which consume organic carbon through BP and BR.

Though low relative to temperate oceans, bulk BP still represents a substantial flow of carbon in the Antarctic marine ecosystem (Kirchman et al., 2009a; Ducklow et al., 2012) where carbon flow was once believed to be dominated by larger organisms such as diatoms and krill (Hart, 1934; Karl et al., 1996). But little is known about BR and BGE in polar waters, especially in relation to specific
types of POC, such as krill FPs. Climate change appears to be further shifting these food webs from diatom-krill-dominated to microbe-dominated systems (Legendre and Rassoulzadegan, 1996; Ducklow et al., 2012; Sailley et al., 2013; Kim and Ducklow, 2016), increasing the importance of understanding the links between members of the microbial community and carbon flux through the microbial loop and carbon export to depth.

With the practical difficulties of making measurements in the hostile Southern Ocean region, despite the importance of bacterial remineralization at depth to atmospheric CO₂ levels (through upwelling processes), the balance of processes determining POC flux attenuation is poorly understood (Giering et al., 2014). Our main goal was to bridge this gap by experimentally testing metabolic changes to the Antarctic microbial community with the introduction of krill FPs, relating microbial carbon uptake to POC flux attenuation. In the first study of its kind, we experimentally measured both BP and BR of free-living and fecal pellet-associated bacteria and were thus able to directly derive BCD and BGE of the free-living bacteria and those exposed to FP POC. We also measured HNA and LNA cell abundance over timed incubations in the presence and absence of *E. superba* FPs. Understanding the relationship of BP, BR, and BGE of both particle-associated and free-living bacteria in marine ecosystems is needed to calculate the total flux of organic carbon through the microbial loop, the export of POC from surface waters and the flux attenuation with depth. This study allowed us to determine potential stimulation of free-living bacteria within the fecalsphere. We address three broad questions concerning the role of bacteria in particle degradation. First, do bacteria associated with krill FPs have enhanced productivity compared to free-living bacteria? Second, does the addition of FPs to seawater increase biological activity and
cell abundance of free-living bacteria? Third, what is the relative importance of bacterial activity on FP POC flux attenuation?

3.2. Methods

3.2.1. Study region

The Palmer Antarctica Long-term Ecological Research (PAL-LTER) program has been studying the marine ecosystem of the WAP since 1992, with long-term measurements conducted during the austral summer annually each Jan–Feb. This study was performed on three PAL-LTER research cruises aboard the ARSV Laurence M. Gould between 2018 and 2020 within the PAL-LTER sampling grid, which covers a 170,000 km² area of the Bellingshausen Sea (Fig. 3.1), extending approximately 700 km from Palmer Station, Anvers Island (64.77°S, 64.05°W) to Charcot Island (69.45°S, 75.15°W). Grid lines are numbered from −100 to 600 and spaced 100 km apart, and stations along each line are spaced 20 km apart, extending from the coast to approximately 200 km offshore at the slope/shelf break (Waters and Smith 1992). The PAL-LTER grid is divided into latitudinal sub-regions based on hydrographic and sea ice conditions (Martinson et al., 2008; Stammerjohn et al., 2008), with the ‘North’ defined as lines 600, 500, and 400, the ‘South’ as lines 300 and 200, and the ‘Far South’ as lines 100 and below. In addition to the three summer cruises, an additional spring cruise was undertaken in Nov. 2018 to sample bays and inlets during sea ice cover in the north of the PAL-LTER grid (Fig. 3.1). Spring, summer, and late summer sampling also took place on smaller Rigid Hull Inflatable Boats (RHIBs) in coastal areas surrounding Palmer Station (Fig. 3.1) The area surrounding Palmer Station on Anvers Island, is relatively shallow (~75–200 m), compared to the offshore shelf-slope region (400–3500 m).
Figure 3.1. Bathymetric map of the West Antarctic Peninsula, showing the PAL-LTER survey grid (black circles), Palmer Station (yellow triangle), and experiment locations (red circles).

3.2.2. Sample collection

In total, 21 experiments were conducted along the WAP from Jan 2018–Feb 2020 (Table 3.1), where 17 experiments had coincident BP and bacterial abundance measurements, and four of which had additional BR measurements. Freshly egested krill FPs were analyzed and placed within the free-living bacterial community in collected seawater to determine changes in community structure and carbon uptake.

*E. superba* was collected using a 2 m-square frame Metro net (700 μm mesh) gently towed obliquely to a depth of 120 m at each station along the PAL-LTER grid along the WAP or down to about 15–60 m in coastal regions near Palmer Station (Steinberg et al., 2015). A General
Oceanics flow meter positioned in the center of the net mouth was used to calculate the volume of seawater filtered (Steinberg et al., 2015).

Collected krill were placed in clean 5-gallon mesh-bottom buckets within another bucket of about 15 liters of 0.2 μm filtered seawater, to ensure krill would egest their FPs in sterile seawater, thereby preserving the original community of bacteria associated with the FPs (the endemic population). The lidded two-tiered buckets were placed in flow-through tanks of surface seawater, matching ambient temperature as closely as possible to that in which the krill were caught. The two-tiered bucket system with mesh separation allowed for FPs to fall through and prevent krill from swimming down and ingesting their own FPs (coprophagy). Krill were allowed to swim and egest FPs for 1–4 hours before being removed. This time point was selected to ensure FPs were as fresh and pristine as possible, while maximizing FP production by ensuring adequate time for gut evacuation, which has been shown to be 0.1–0.4 pellets h⁻¹ (Perissinotto and Pakhomov, 1996) with a gut passage time of 3.7–6.3 hours (Atkinson and Snyder, 1997).
Table 3.1. Date and location of each experiment conducted over three years on the WAP.

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Collected FPs were carefully removed from the 5-gallon buckets and rinsed with 0.2 µm filtered seawater and separated into 4 treatments: 0-hour (initial), 12-hour, 24-hour, and 48-hour incubations in seawater collected from concurrent vertical CTD profiles. The seawater used for the incubations were collected in Niskin bottles at about 10-meter intervals, from surface to about 150 meters and homogenized, as net-tows for krill were deployed between the surface and 120 m. The seawater for the incubations was gently filtered at 3 µm to limit the presence of meso- and microzooplankton grazers.

In order to compare the results of this study to that of Chapter 2, wherein a sediment trap collected sinking krill FPs at 175 m depth for 22 years, 48-hour incubations were carried out. As krill FPs sink at a rate of hundreds of meters per day (Atkinson et al., 2012), and the PAL-LTER sediment trap was located at 175 m depth, it was calculated that two days (48 hours) would be the amount
of time the FPs would be exposed to the water column bacterial community before reaching the sediment trap and being preserved in formalin. One goal of this study was to determine if the FP POC caught over the 22 years in the sediment trap experienced high flux attenuation due to the microbial loop. Therefore, tracking the BP and BR on krill FPs over 48 hours elucidates the amount of organic carbon FPs likely lose through bacterial consumption as they sink through the water column before reaching the sediment trap. Further, analysis of bacteria-particle interactions on artificial particles showed that bacterial colonization can occur on short timescales (hours) and long timescales (~2 days), supporting our methodology (Grossart et al., 2003).

At each incubation time point, triplicate samples of FPs were removed and frozen at –80°C for subsequent DNA extraction and sequencing (discussed in Chapter 4). A further triplicate set of FPs was removed for BP measurements. A triplicate sample of seawater without exposure to FPs (Water) and a triplicate sample of seawater exposed to FPs (WaterFP) from each of the four time points (0, 12, 24, and 48 hours) were also removed for BP analysis. A sample of Water was removed at each time point for free-living bacterial abundance analysis through a flow cytometer, along with a sample of WaterFP. The Water acted as a control, and we tested the dual hypotheses that FPs may be colonized by bacteria from the seawater free-living community, increasing both bacterial diversity on FPs (Discussed in Chapter 4) and fecal pellet-associated BP; and that the presence of FPs and fecal pellet-associated bacteria would increase nutrients and organic carbon in the seawater and therefore increase the free-living bacterial community BP, abundance, and growth rate in WaterFP. Four of these experiments included BR measurements with concurrent BP and bacterial abundance. Pairwise differences between Water and WaterFP and differences in
metabolic parameters over time were tested with a one-way ANOVA followed by Tukey’s Honest Significant Difference (HSD) test.

### 3.2.3. Environmental sampling

Sea ice concentration data were extracted from the NASA Scanning Multichannel Microwave Radiometer (SMMR) and the Defense Meteorological Satellite Programs (DMSP) Special Sensor Microwave/Imager (SSM/I) satellite record for the gridded area along the WAP, following protocols described in Stammerjohn et al. (2008).

Concurrent water column temperature ($T; ^{\circ}C$), salinity, and pressure (dbar) were measured with a SeaBird 911+ CTD deployed at sampling stations along the PAL grid (Fig. 3.1). At each station, sampling consisted of one or more casts with the Seabird CTD-rosette system from the surface to just above the ocean bottom. Sigma-theta ($\sigma_\theta$, potential density; kg m$^{-3}$) was calculated at each depth bin (binned to 1 dbar) using CTD data to obtain mixed layer depth (MLD). Surface phytoplankton chlorophyll-a (chl-a) was measured by filtering CTD-collected seawater onto GF/F filters, which were flash frozen for fluorometric phytoplankton chl-a analysis (mg chl-a m$^{-3}$) (Schofield et al., 2017). Surface BP was also measured for each station to compare against experimental depth-homogenized water BP and FP BP.

### 3.2.4. Bacterial production

The rate of $^3$H-leucine incorporation into bacterial protein on collected water samples was measured to derive BP rates. We followed a modified protocol of the original leucine assay proposed by Smith and Azam (1992), as described in Ducklow et al. (2012). The $^3$H-leucine
incorporation rate (pmol l$^{-1}$ h$^{-1}$) was converted to carbon-based BP rates (mg C l$^{-1}$ h$^{-1}$) using the factor 1.5 kgC mol$^{-1}$ leucine incorporated (Ducklow et al., 2000a). We recognize that conversion factors may vary (Kirchman et al., 2009b; Giering and Evans, 2022); but adopt a constant factor to facilitate comparison with other studies (e.g., Ducklow et al., 2012).

Briefly, triplicate 1.5 ml samples of Water, WaterFP, and FP samples were incubated in the dark for ~3 h with $^3$H-leucine (MP Biomedical, Santa Ana, CA; >100 Ci/mmol, 20–25 nM final concentration) in 2.0 ml microcentrifuge tubes (Axygen SCT-200, Union City, CA). One killed control was prepared for each assay by adding 0.1 ml of 100% trichloroacetic acid (TCA). Incubations were maintained within 0.5 °C of the in situ temperature in refrigerated circulator baths and terminated by the addition of 0.1 ml of 100% TCA. Samples were concentrated by centrifugation, rinsed with 5% TCA and 70% ethanol and air-dried overnight prior to radioassay by liquid scintillation counting in Ultima Gold cocktail (Perkin-Elmer, Waltham, MA). Blank values of TCA-killed samples were subtracted from the average of the triplicates for each discrete sample. Outlier values for BP within each material type (Water, WaterFP, FP) were removed via the interquartile range method.

### 3.2.5. Bacterial respiration

Oxygen consumption by free-living bacteria in seawater and by free-living bacteria in water in the presence of krill FPs, was determined using FireSting fiber-optic oxygen meters, fiber-optic cables, and OXP5 optical spots (Pyro Sciences, Aachen, Germany) attached to the inner surface of 60 ml gas-tight BOD bottles with optically transparent silicon glue (Pyro Sciences, Aachen Germany). The use of these optode spots eliminated the need for drawing of aliquots from the sample bottles.
and avoids gas and water exchange through capillary holes in bottle lids for probe sensors. The FireSting fiber-optic oxygen meters were calibrated (average standard uncertainty ± 0.1 mg O₂ L⁻¹) using air saturated seawater and zeroed using a 2% sodium sulfite solution at the corresponding temperatures for the experiment (± 0.5°C SST).

A negative control with sodium sulfite was incubated along with triplicate Water and triplicate WaterFP bottles. Oxygen concentration was measured in triplicates for each BOD bottle and then averaged for each timepoint. Measurements were made every two hours for 48 hours. Oxygen concentration data versus time were then fitted to a logarithmic regression, and the time course of respiration was then calculated from the slope of the fitted function. Carbon respiration calculated assuming a respiratory quotient of 1 mol O₂ to 1 mol CO₂, where typical values applied range from 0.7–1.2 for respiration of carbohydrates and lipids (Berggren et al., 2012).

### 3.2.6. Bacterial growth efficiency and POC turnover

BGE is a fundamental constraint on both bacterial growth and on microbial food web pathways. BGE was calculated for both Water and WaterFP samples to determine if the addition of krill FPs enhanced BGE of WaterFP samples relative to Water samples. BGE can be defined generally as the balance between BP and BR:

\[
BGE = \frac{BP}{BP+BR} \quad \text{(Equation 1)}
\]

Due to Covid-19, we were unable to process samples for FP number, length and POC content. We therefore use literature values that are our best estimate for our experiments to calculate the amount
of POC available via FPs in each experiment. We use the mean adult krill FP diameter (radius; r) for adult krill (Cadee et al., 1992) and length (l) of adult krill FP (Atkinson et al., 2012) to compute FP volume, using the formula for the volume of a cylinder, as krill FP are long cylinders (Gleiber et al., 2012). We then use the minimum POC content per pellet volume (Suzuki et al., 2001; Belcher et al., 2016), in order to obtain a conservative estimate, to calculate the average carbon content per FP (C$_{FP}$). We assume there were 75 pellets per sample, which is our best average estimate given the circumstances (Table 3.2). The amount of POC available via FPs in each experiment was:

\[ POC_{bottle} = \pi r^2 l * C_{FP} * 100 \]  

(Equation 2)

Using these values, along with measured BR rates, we were able to calculate the FP POC turnover rate, with the turnover rate (k) being expressed as a rate per day. To compute k, we use measured BR$_W$ and BR$_{WFP}$, which are the volumetric respiration rates from the water column samples and water with FP samples, respectively, along with V$_{inc}$ which is the incubation volume (20 ml, as FPs did not take up much volume in bottles), RQ$_{C,O_2}$, the molar respiratory quotient for the remineralization of organic matter at depth, which we selected to be a value of 1 mol O$_2$ to 1 mol of CO$_2$ (Trimmer et al., 2012), and POC$_{bottle}$ (equation 2) is the quantity of POC used in the incubation:

\[ k(d^{-1}) = \frac{(BR_W - BR_{WFP}) * V_{inc} * RQ_{C,O_2} * 24}{POC_{bottle}} \]  

(Equation 3)
3.2.7. Bacterial abundance

Bacterial abundance samples were analyzed by flow cytometry following the protocol of Gasol and del Giorgio (2000) on an Accuri C6 flow cytometer (BD Biosciences, San Jose, CA). To identify clusters of HNA and LNA bacteria, total bacteria concentrations were determined by adding 1 μm microspheres (Polysciences, Warrington, PA) and 5 μM final concentration of SYBR-Green I stain (Invitrogen, Carlsbad, CA, USA) to 0.5 ml of samples. The samples were then measured for 2-min at a low flow rate. The absolute concentration of stained cells was calculated using the total sample volume analyzed, as determined by the count of the added microspheres.

All flow cytometry events (detected cells) for both Water and WaterFP samples at every timepoint were concatenated, and a training set of 50,000 events was selected at random. The training set was used to build an Self-Organizing Map (SOM) using the Kohonen package (Wehrens and Buydens, 2007) in R v. 3.6 following Bowman et al. (2017). The SOM represents sample similarity with topography, where samples that are part of the same topographic feature are more similar. Parameters used to construct the map included forward scatter, side scatter, FL1: 488/533 nm, and FL3: 488/675 nm after log transformation of signal height. Clusters of nodes, identifying separate clusters of bacterial cells and bead standards, were identified using k-means clustering, with a reasonable value for k chosen through the evaluation of a scree plot of within-clusters sum of squares. A k value of 8 was selected. The resulting model was used to classify the complete data set into the identified clusters of cell size and fluorescence. The fractions of cells belonging to the HNA and LNA populations were calculated by dividing the number of HNA and LNA cells, respectively, by the total number of cells, excluding presumed non-cellular material. Two of the clusters were largely defined by their fluorescent signal at 533 nm and interpreted as an HNA.
population and a LNA population. Five populations that varied widely in fluorescence at 533 nm were assumed to be non cellular, with one additional cluster defined by the bead standard added as controls to each sample.

### 3.2.8. Bacterial cellular growth rate

To estimate whether the addition of FPs to water would increase cell specific growth rate, the growth rate of HNA and LNA bacterial populations in each experiment was calculated by dividing BP of Water and WaterFP by their corresponding HNA and LNA cell biomass. Growth rate of the total bacterial population was calculated using total cell abundances. Cell abundances, as measured through flow cytometry, were converted to cell biomass by estimating there to be 10 fg C per cell (Fukuda et al., 1998; Kirchman, 2001). Bulk BP was used for each sample as we were unable to separate out the fraction of leucine incorporation by HNA and LNA cell fractions, but this served to elucidate what growth rates might be if total BP was driven by the HNA population only, and conversely, the LNA population only.

### 3.3. Results

#### 3.3.1. Bacterial production

Within the 21 incubation experiments, the ecological changes in microbial activity and abundance were tracked over 48 hours for Water, FP and, WaterFP, created through the addition of FPs to Water. When all incubation experiments are considered together, at To, Water and WaterFP BP were very similar in terms of mean and variance, while initial FP BP was lower, though this difference is non-significant (Fig. 3.2). However, taken all together, there was no significant difference in BP between material type (Water, WaterFP, and FP). In regards to incubation time,
there was a significant difference in BP over the 48 hours of the experiments (Fig. 3.2). BP at 48 hours, of all of samples combined, is significantly different than BP at 0 hours and 24 hours ($p = 0.022$, $p = 0.031$, respectively). BP of all Water samples linearly increased over the 48 hours of incubation ($p = 0.0062$). There was no significant increase in WaterFP over the 48 hours of incubation when all experiments are analyzed together ($p = 0.089$). FP BP increased significantly logarithmically over the 48 hours of incubation ($p = 0.0001$).

![Figure 3.2. Bacterial production of Water (black), WaterFP (blue), and FP (red) samples over 48 hours of incubation. Bacterial production of all samples together is significantly different after 48 hours from 0 hours and 24 hours ($p = 0.022$ and $p = 0.031$, respectively).](image)

Though the ratios of BP to PP are low on the WAP (Bowman et al., 2016), BP can exceed 0.04 mg C m$^{-3}$ hr$^{-1}$ in productive surface waters (Ducklow et al., 2012; Kim and Ducklow, 2016), values typical of eutrophic temperate marine ecosystems (Cottrell and Kirchman, 2003). In our study, the mean BP of freshly egested FP was found to be close to this elevated BP value found in productive surface, at 0.045 mg C m$^{-3}$ hr$^{-1}$, with a maximum of 0.89 mg C m$^{-3}$ hr$^{-1}$, while the mean free-living Water BP was about 0.023 mg C m$^{-3}$ hr$^{-1}$, with a maximum of 0.13 mg C m$^{-3}$ hr$^{-1}$, and mean WaterFP BP was about 0.02 mg C m$^{-3}$ hr$^{-1}$, with a maximum of 0.28 mg C m$^{-3}$ hr$^{-1}$.
There were three main scenarios in patterns of BP in Water, WaterFP, and FPs. Examples of these different scenarios are illustrated in Fig. 3.3. In the first scenario, in line with our initial hypothesis, FP had increased BP relative to Water and WaterFP and WaterFP BP had increased above that of Water (Fig. 3.3A). In the second scenario, FP may have elevated BP, however the addition of FP to water does not increase BP of WaterFP (Fig. 3.3B). In the third scenario, FP have significantly lower BP relative to Water, and the addition of FP to water suppresses WaterFP BP (Fig. 3.3C). In 18% of the experiments, the exposure of seawater to FPs increased WaterFP BP relative to Water BP by the end of the 48 hours. In 59% of the experiments, the addition of FPs depressed WaterFP BP relative to Water BP. In 24% of experiments, the addition of FPs did not have an impact on the BP of WaterFP relative to Water BP. Though we see these three different patterns, when averaged all together (Fig. 3.3D), there is no significant difference in BP between Water, WaterFP, and FP.
Free-living Water BP did not significantly differ by latitudinal regimes along the WAP or by sampling season. However, BP was significantly different on FPs based on sampling latitude, where BP of FPs from the South (purple; Fig. 3.4) was significantly different from all other latitudes (Far North: $p = 0.0011$; North: $p < 0.00001$; Far South: $p = 0.00016$). BP rates on the FPs did not significantly differ by season. Neither free-living bacterial samples nor fresh FPs at time 0 were correlated with days since sea ice retreat, MLD, SST, chl-a concentration, or BP in surface waters.
Figure 3.4. Fecal pellet bacterial production at each incubation time point, along with that of the initial free-living community, broken down by sampling latitude, where bacterial production of fecal pellets from the South (purple) was significantly different from all other latitudes (Far North: $p = 0.0011$; North: $p <0.00001$; Far South: $p = 0.00016$).

3.3.2. Bacterial respiration, growth efficiency, and POC turnover rate

Maximal BR occurred at the beginning of each experiment and the minimal BR occurred after 48 hours of incubation. Mean BR of Water and WaterFP significantly decreased over time in a logarithmic fashion ($p = 5.64 \times 10^{-4}$). WaterFP had elevated BR relative to Water, though this difference was nonsignificant over the incubation time (Fig. 3.5A). Water BR ranged from 3.05 to 26.81 mg C m$^{-3}$ hr$^{-1}$, with a mean of 11.56 mg C m$^{-3}$ hr$^{-1}$. WaterFP BR ranged from 7.99 to 29.63 mg C m$^{-3}$ hr$^{-1}$, with a mean of 14.21 mg C m$^{-3}$ hr$^{-1}$. Mean FP specific BR was calculated to be 2.69 mg C m$^{-3}$ hr$^{-1}$.
The linear relationship of BR and BP contained an intercept that was statistically different from 0 ($p = 2.17 \times 10^{-7}$), meaning that when BP = 0, there were still measurable rates of BR consistent with a metabolic threshold for bacterial growth. BCD and BGE were calculated using the concurrent BP and BR measurements. As BR was about two orders of magnitude higher than corresponding BP, respiration made up the bulk of BCD, with BCD trends mirroring BR closely.

As BR declined, BGE increased for both Water and WaterFP over the 48-hour incubations. BGE of Water and increased logarithmically over time ($p = 0.040$; Fig. 3.5B) and there was a significant difference in BGE at 48 hours relative to all other time points. There was a significant difference in BGE between Water and WaterFP, where WaterFP has a lower BGE relative to Water ($p = 0.041$). The trend in BGE mirrored the trends of corresponding BP measurements. Water BGE ranged from $4.90 \times 10^{-4}$ to 0.011, with a mean of 0.0034. WaterFP BGE ranged from $2.63 \times 10^{-4}$ to 0.0056, with a mean of 0.0019.

**Figure 3.5.** A) Mean bacterial respiration of Water (black) and WaterFP (blue) samples over 48 hours of incubation. B) Mean bacterial growth efficiency over 48 hours of incubation of Water and WaterFP.
POC turnover rate by fecal pellet-associated bacteria was calculated using equations 2 and 3. Using literature values and our best estimates, we calculated a POC turnover rate of 0.0012 day\(^{-1}\) (Table 3.2). This would indicate that it would take about two and a half years for heterotrophic bacteria attached to krill FP to fully consume the POC within a sinking pellet without the help of free-living bacterial colonization.

Table 3.2. Krill fecal pellet POC turnover rate by heterotrophic bacteria calculated from a combination of measured values and literature-derived values.

<table>
<thead>
<tr>
<th>FP Characteristics</th>
<th>Mean Values</th>
<th>Derivation of Values</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>FP diameter (mm)</td>
<td>0.25</td>
<td>Mean FP diameter for adult krill</td>
<td>Cadee et al., 1992</td>
</tr>
<tr>
<td>FP length (mm)</td>
<td>15</td>
<td>Estimated mean</td>
<td>Atkinson et al., 2012; Conservative estimate of average length in our experiments</td>
</tr>
<tr>
<td>FP volume (mm(^3))</td>
<td>0.74</td>
<td>Calculated as volume of a cylinder</td>
<td>Within range measured by Atkinson et al., 2012</td>
</tr>
<tr>
<td>FP carbon content (mg C/mm(^3))</td>
<td>0.020</td>
<td>Minimum</td>
<td>Belcher et al., 2016</td>
</tr>
<tr>
<td>FP carbon content (mg C/pellet)</td>
<td>0.015</td>
<td>Calculated</td>
<td></td>
</tr>
<tr>
<td>Number of pellets per bottle</td>
<td>75</td>
<td>Estimated</td>
<td>Conservative estimate of number in our experiments</td>
</tr>
<tr>
<td>FP carbon content per bottle (mg C)</td>
<td>1.10</td>
<td>Calculated</td>
<td></td>
</tr>
<tr>
<td>FP Respiration + Production (mg C hr(^{-1}))</td>
<td>0.000054</td>
<td>Measured</td>
<td>Measured in this study</td>
</tr>
<tr>
<td>FP POC turnover rate (hr(^{-1}))</td>
<td>.000049</td>
<td>Calculated</td>
<td></td>
</tr>
<tr>
<td>FP POC turnover rate (day(^{-1}))</td>
<td>0.0012</td>
<td>Calculated</td>
<td></td>
</tr>
</tbody>
</table>

3.3.3. Bacterial cell abundance and growth rate

Total bacterial cell abundance and the fraction of HNA and LNA cells that make up the total population of each Water and WaterFP sample were compared over the 48 hours of incubation via
flow cytometry (Fig. 3.6). It was not technically possible to measure the abundance of fecal pellet-attached bacterial cells in this study. In terms of total bacterial cell abundance, there was no significant difference between Water and WaterFP. Further, there was no significant change in total cell abundance over time for either material type (Fig. 3.7A). However, the proportion of HNA and LNA that made up each bacterial population did show differences. There was a significant difference between Water and WaterFP bacterial populations in that the percentage of HNA bacteria that make up the total population does not change over time in Water, but increased after 48 hours in WaterFP (Fig. 3.7B; $p = 2.04 \times 10^{-5}$). The fraction of LNA in the population does not change over time in Water samples, but significantly decreased over time in WaterFP samples (Fig. 3.7C), commensurate with the increase in HNA. The increase in HNA in WaterFP is logarithmic ($p = 0.035$).

**Figure 3.6.** Flow cytometry cytogram of bacterial cell size (forward scatter) and log of green emission by cells stained with SYBR-Green. Each point is one detected cell. LNA and HNA cells can be discerned, as well as non-cellular particulate material and added beads.
Figure 3.7. A) Total bacterial cell abundance of Water (black) and WaterFP (blue) samples over 48 hours of incubation. B) Percentage of HNA bacterial cells within the population of Water and WaterFP over 48 hours. C) Percentage of LNA bacterial cells within the population of Water and WaterFP over 48 hours.

The addition of FPs to Water, thereby creating WaterFP, consistently significantly increased the HNA cell abundance in WaterFP relative to its initial abundance, as measured in Water at time 0 (Fig. 3.8A; p = 0.04). Conversely, the addition of FPs to Water did not create a consistent pattern of change in LNA cell abundance in experiments. In some cases, the addition of FP decreased LNA abundance in WaterFP relative to Water, and in other instances, it increased LNA abundance (Fig. 3.8B).
Figure 3.8. A) HNA cell abundance of Water (black) and WaterFP (blue) for each experiment. HNA abundance in WaterFP is consistently higher than that of Water ($p = 0.04$). B) LNA cell abundance of Water and WaterFP for each experiment conducted. There is no consistent pattern in LNA abundance between Water and WaterFP throughout the experiments.

Total cell abundance for Water increased significantly with corresponding BP measurements ($p = 0.021$; Fig. 3.9A), as did HNA cell abundance ($p = 0.0017$; Fig. 3.9B). However, LNA cell abundance did not significantly increase with BP (Fig. 3.9C). Total cell abundance increased significantly with increasing BP in WaterFP samples (Fig. 3.9D; $p = 0.0071$;), as did HNA cell abundance ($p = 0.042$; Fig. 3.9E), and LNA cell abundance (Fig. 3.9F; $p = 0.024$;). The slope ($m$) of each figure (Fig. 3.9) indicates the per cell BP rate, also known as the growth rate. The growth rate of the total bacterial community in WaterFP ($6.25 \times 10^{-8}$) is greater than that of the Water community ($5.56 \times 10^{-8}$). The BP plotted is the BP of the total population of bacteria, however, it would be illuminating to identify what the growth rate of HNA and LNA cells may be if they were the bulk of the population. The LNA growth rate of WaterFP ($1.27 \times 10^{-7}$) is an order of magnitude greater than that of the HNA growth rate ($5.63 \times 10^{-8}$), possibly indicating the transition of LNA cells to HNA cells. Additionally, the LNA growth rate of WaterFP is an order of magnitude greater.
than the LNA growth rate of Water ($5.44 \times 10^8$), potentially indicating that access to the POC in krill FPs help facilitate faster growth rate in the free-living bacterial community.

There was a significant difference in total cell growth rate between Water and WaterFP ($p = 0.019$), where cell specific growth rate was significantly higher after 48 hours than initial growth rate at time 0 (Fig. 3.10A). Mean Water total cell growth rate at time 0 was $0.26 \text{ day}^{-1}$, and increased significantly to $0.56 \text{ day}^{-1}$ at 48 hours. Mean WaterFP total cell growth rate at time 0 was similar to that of Water, at $0.30 \text{ day}^{-1}$, decreased over 24 hours to $0.16 \text{ day}^{-1}$, and increased after 48 hours to $0.36 \text{ day}^{-1}$. WaterFP HNA growth rate significantly decreased over the 48 hours of incubation ($p = 1.15 \times 10^{-4}$), while Water HNA growth rate did not significantly change over time (Fig. 3.10B). There was no significant difference in growth rate of LNA between Water and WaterFP bacteria (Fig. 3.10C).

Figure 3.9. A-C) Bacterial production of Water as a function of bacterial cell abundance. D-F) Bacterial production of WaterFP as a function of bacterial cell abundance.
Figure 3.10. A) Mean total bacterial cell growth rate of Water (black) and WaterFP (blue) over 48 hours of incubation. B) Mean HNA growth rate of Water and WaterFP samples over 48 hours of incubation. There is a significant difference in HNA growth rate between Water and WaterFP ($p = 1.15 \times 10^{-4}$). C) Mean LNA growth rate of Water and WaterFP over 48 hours of incubation. Error bars denote standard error.

3.4. Discussion

Rates of POC flux attenuation in the mesopelagic vary widely throughout the global oceans and a number of mechanisms have been proposed to explain these variations, such as zooplankton grazing and bacterial decomposition. The quantity and quality of POC and DOC, and the balance between the rate of POC hydrolysis and the release of DOC, determine the coexistence between free-living generalists and the particle-associated specialists in the bacterial community of the fecalssphere. Grossart and Ploug (2001) found that decomposition of sinking diatom aggregates by attached microbes was substantial, and that the vertical flux of particles in the ocean was greatly reduced during sinking. However, on the WAP, the main source of POC export is *E. superba* FPs. Our findings are in line with other arthropod FP studies, where bacterial decomposition of FPs varied with zooplankton food source, but overall decomposition rates remained low regardless (Lee and Fisher, 1992; Thor et al., 2003; Ploug et al., 2008; Morata and Seuthe, 2014). Where many of these studies measured only BP or BR or bacterial abundance, with some using these
values to then estimate the remaining parameter, in this study we measured each of these parameters experimentally to obtain a more accurate picture of bacterial population dynamics associated with Antarctic POC export. As stated by Belcher et al. (2016), studies combining direct measurements of particle-associated FP respiration with FP incubations in the presence of free-living bacteria are needed to better balance the budget of microbial-driven degradation processes on POC export and attenuation in the Southern Ocean. Our study will help to fill this gap.

For this study, a two-day timescale was chosen in order to be able to compare these results to that found in Chapter 2. Chapter 2 used a sediment trap to collect sinking POC on the WAP over 22 years, finding that the bulk of POC that sunk out of the top couple of hundreds of meters were krill FPs (Gleiber et al., 2012) and tightly tied to the oscillation in krill body size. As the sediment trap intercepted particles at ~200 m depth, and krill FPs sink at about a hundred meters per day, it would take about two days for the FPs produced in surface waters to reach the sediment trap. Over those two days, the krill FPs are exposed to the free-living bacterial community. This exposure may result in bacterial colonization onto the sinking FPs, increasing fecal pellet-associated BP and BR. An increase in bacterial metabolism associated with the FPs would likely increase POC flux attenuation. If POC flux attenuation is high, the sediment trap data presented in Chapter 2 likely does not reflect the amount of POC produced in the surface ocean of the WAP.

Krill fecal pellet-attached bacteria exhibit a wide range of metabolic behavior. The growth of attached bacteria is dependent on the specific microenvironment created by the marine particles (Simon et al., 2002). The environment of a FP may be related to the type of food the krill have consumed and the associated bacteria, which are then incorporated into FPs. Further, krill FPs are
encased in a peritrophic membrane that allows for structural stability of FPs as they sink through the water column, making them efficient agents of POC export (Gleiber et al., 2012). The peritrophic membrane surrounding krill FPs is made up of chitin. Certain particle-associated bacteria, mainly copiotrophs, are specialized in chitinase production and hydrolysis of chitin, playing an important role in breaking down the peritrophic membrane of FPs and giving access to POC and DOC to other bacteria (Lauro et al., 2009). The dynamic habitat of a krill FP is likely a selective environment where certain bacteria may thrive and others are outcompeted.

The egestion of FPs and the affect they have on the water column free-living bacterial community was complex and varied over the two years of study. The variation in bacterial response to FP POC may be due to many different environmental factors such as season, location, and temperature. Food type and bacterial populations in the water column are known to vary over space and time on the WAP (Wilkins et al., 2013; Luria et al., 2016), as discussed in Chapter 4. Biological factors such as community structure and gene expression of the bacterial population also affect the response of the free-living bacterial community (Baty et al., 2000). Attached bacteria modify the surrounding microenvironment, thereby affecting the behavior and physiology of other microbes (Møller et al., 1998). Studies on bacterial populations have shown inhibition of growth in the presence of another strain of bacterium due to production of repellents, antibiotics, or toxins while other studies indicate increased bacterial growth in the presence of other bacteria due to production of probiotics and quorum sensing molecules (Madigan et al., 2000; Grossart et al., 2003).

The rate of leucine incorporation is a measure of bacterial biomass production through protein synthesis, as leucine is an essential amino acid. Overall, BP increased over the 48-hour incubations
when all incubations were analyzed together. The addition of FP to Water caused varying changes to WaterFP BP over time, wherein WaterFP BP increased, stayed the same, or decreased relative to Water BP. In 34% of the experiments, FP BP was higher than Water BP. In 34% of the experiments FP BP was lower than Water BP and in 32% of the experiments FP BP is the same as Water BP. This indicates there was no consistent pattern for WaterFP BP relative to Water BP. The taxa of bacteria responsible for the fecal pellet-associated BP are discussed in Chapter 4. The increase in WaterFP BP may be due to an uncoupling between fecal pellet-attached bacteria ectoenzyme activity, DOC solubilization from POC, and DOC uptake by particle-attached bacteria, thereby increasing DOC availability for the free-living bacteria (Grossart et al., 2003). The suppression of WaterFP BP may be due to antagonistic behavior of fecal pellet-associated bacteria towards the free-living bacteria, (Long and Azam, 2001; Gossart et al., 2003; Beyersmann et al., 2017). Additionally, the concentration of secondary metabolites contained within the FPs themselves, such as oxylipins from ingested food sources may suppress bacterial activity and alter bacterial community structure (Ribalet et al., 2008). Surprisingly, Miki and Yamamura (2005) found that a high level of POC hydrolysis by particle-associated bacteria, thereby increasing available DOC, excluded free-living DOC specialists from the community. This may account for the lack of increase of WaterFP BP. Mean Water, and mean WaterFP BP were equivalent to each other; however, the maximum WaterFP was double that of the maximum measured Water BP. Mean FP BP was double the mean of Water and WaterFP BP and the maximum FP BP exceeds the maximum BP of Water and WaterFP. The mean FP BP is equivalent to the mean measured free-living BP in summer-time surface waters of the WAP, while the maximum FP BP exceeds the highest measured free-living BP of the same study (Ducklow et al., 2012). Mean Water BP in our study is about half that of surface water values found by Ducklow et al. (2012). This may be
due to the fact that our samples included the bacterial community at depths up to 150 m. Our mean Water BP was slightly higher than most of the BP rates found in the Indian sector of the Southern Ocean at 60 m depth, though of similar magnitude (Christaki et al., 2021). We found that FP BP varied by latitude, where FPs from the South were significantly different than other regions of the WAP. As the climate along the WAP undergoes a transition from a cold-dry polar ecosystem, to a more warm-humid sub-Antarctic ecosystem, due to continued warming, there has been a distinct separation and change in the marine ecology between the North and South of the WAP (Hugo et al., 2009). These latitudinal shifts suggest krill may be consuming different assemblages of bacteria from different regions, where the bacteria have different rates of BP. This is further supported by taxonomic findings discussed in Chapter 4.

BR for both Water and WaterFP started high and decreased over the incubation time, opposite of the increasing trend of mean BP. It has been shown that if the supply of organic matter is low, whatever its nature, a large fraction of this substrate will be catabolized and used primarily for BR energy requirements rather than for growth, resulting low relatively low BP in comparison (Russell and Cook, 1995; del Giorgio and Cole, 1998). Over time, the bacterial population in our study switched from preferential carbon uptake for BR and began to increase their biomass through BP, though BR continues to account for most of the organic carbon consumption in the Water and WaterFP. The addition of FP to Water did not significantly alter WaterFP BR over the incubation period, where BR decreased logarithmically over time at the same rate for both Water and WaterFP. BR in our study was within the range of other water column BR rates measured in Antarctic waters, as compared by Ducklow et al. (2000a). Specifically looking at summer-time BR on the WAP, our BR range matched well with range of BR found by Aristegui et al. (1996), which
ranged from 5-65 mg C m\(^{-3}\) hr\(^{-2}\). BR of Water found in the Indian sector of the Southern Ocean was about an order of magnitude lower than that found in our study (Christaki et al., 2021).

Though not significant, the addition of FP to Water did elevate BR of WaterFP above that of Water. Estimated FP BR, using values shown in Table 3.2, was lower than the surrounding water column. Our FP BR was low compared to that found on krill FPs by Cavan et al. (2021) and Pauli et al. (2021). This may be due to the fact that the Cavan et al. (2021) study was carried out at elevated temperatures, as opposed to in situ temperatures representative of the Antarctic region, as we did. Further, in their study, there was exposure of FPs to the surrounding free-living community before experiments were carried out, possibly altering the bacterial community and activity on the FPs. However, our FP BR was similar to the minimum FP BR value found by Belcher et al. (2016).

BCD mirrored the pattern and magnitude of BR for both Water and WaterFP, indicating that most of the organic carbon used by the bacteria was for respiration as opposed to increasing biomass. At the population level, numerous factors can affect BGE, including temperature (Apple et al., 2006), inorganic nutrient limitation (Goldman and Dennett, 2000), energetic quality, such as oxidation state, of the organic substrate (del Giorgio and Cole, 1998), viral interaction (Middelboe et al., 2003), and diversity of bacteria (Reinthaler and Herndl, 2005). BGE for both Water and WaterFP increased over time, mirroring the increase in BP over the incubation period. The addition of FP to Water decreased WaterFP BGE relative to Water BGE, wherein BGE increases logarithmically over the incubation time for both material types, but the rate of increase by WaterFP is lower. The overall increase in growth efficiency may be due to the lack of nutrient and DOC limiting conditions in the experimental bottles. The exposure to FP may increase BP of free-living bacteria in WaterFP, but in most cases we found that the addition of FP to Water suppressed
free-living WaterFP BP or did not significantly change it. Other Southern Ocean studies have calculated BGEs of 0.03-0.40, with overall global marine BGE ranging from 0.010 to 0.69 (del Giorgio and Cole, 1998; Carlson et al., 1999; Christaki et al., 2021). Our maximum measured BGE was within this range, though our mean BGE was an order of magnitude lower. This is likely due to relatively high BR and low BP measured in our study. BGE of WaterFP was lower than that of Water, indicating that the free-living bacterial population had decreased growth efficiency in the presence of FPs. As discussed in Chapter 4, krill FPs on the WAP host only a small community of attached bacteria and there is little colonization by free-living bacteria over the time scale of days. And only a small subset of the fecal pellet-associated bacteria are metabolically active, responsible for the bulk of FP BP (as discussed in Chapter 4). Low FP BP may result in low DOC solubilization and availability for free-living bacteria in WaterFP and may help to explain the low BGE of bacteria associated with krill FPs. Further, antagonistic behavior by fecal pellet-associated bacteria may inhibit BGE of WaterFP bacteria (Wietz et al., 2013). Overall, bacteria of the WAP was found to have low growth efficiency.

Our krill FP POC turnover rate was an order of magnitude lower than turnover rates found by Belcher et al. (2016) and Cavan et al. (2020), where they studied krill FPs from the Scotia Sea and FPs from captured aquarium krill, respectively. This may be due to the lack of bacteria attached to pristine, freshly egested FPs that have not been exposed to the water column, as compared to FPs that were egested into whole seawater, as in the afore mentioned studies. Morata and Seuthe (2014) found that copepod FP carbon-specific decomposition rate by bacteria varied based on whether they were pristinely egested in 0.2 μm filtered seawater, as performed in our study, or exposed to water from depth and the chlorophyll maximum. There may be varying patterns in carbon uptake
in space and time, as indicated by the variation in BP patterns we found in FP and WaterFP. Further, there may be inaccuracies in our estimated values and values taken from the literature used to calculate POC turnover rate. Our krill FPs were larger and our FP BR was lower than those reported on by Belcher et al. (2016) and Cavan et al. (2021). Our POC turnover rate illustrates that breakdown and consumption of organic carbon from FPs would be slow without colonization by additional bacteria from the water column as the pellets sink. Low carbon-specific respiration rates suggest that particle associated microbial respiration plays a minimal role in the attenuation of FP POC flux in the upper mesopelagic. Low bacterial POC turnover rate was similarly found in a different study on the WAP on bulk water column POC, relative to temperate regions (McDonnell et al., 2015), though their rates were an order of magnitude higher than in our study.

Total cell abundance did not change over time for either Water or WaterFP, indicating the addition of FP to Water did not alter total cell abundance, nor was there bacterial replication within the Water conditions. However, the addition of FP to Water did significantly increase the proportion of HNA cells in the WaterFP free-living population and decreased the proportion of LNA cells in the population. With this shift in cell nucleic acid content, and with total cell abundance remaining the same, this may indicate a metabolic shift from small, inactive bacterial cells (LNA) to more active and larger cells (HNA) due to the presence of POC and nutrients associated with FPs. The increase in nucleic acid content may reflect the increases in both cellular RNA and DNA content, where increases in RNA are indicative of increased protein synthesis. Further, both Water and WaterFP HNA cell abundance increased with increasing BP, indicating that it is the HNA cells that are actively partaking in BP through protein synthesis. This finding is in line with those of many other marine microbial studies, where HNA cells are responsible for the majority of BP (e.g.}
Lebaron et al., 2001; Servais et al., 2003; Morán et al., 2011). When a total cell count is made up of growing and nongrowing, or fast and slow-growing populations, the carbon incorporation rates increase faster than total cell abundance (Ducklow, 2000b), as seen in our study. These results are further supported by evidence that HNA and LNA bacterial populations are made up of the same dominant species which can switch physiological states in the presence or absence of ideal conditions (Servais et al., 2003). The addition of FP to Water caused a significant decrease in WaterFP HNA cell specific growth rate, while it caused no change in WaterFP LNA growth rate relative to Water. Global cell specific growth rate has been shown to range from ca. 0.1 to 1 day\(^{-1}\) (Ducklow, 2000b). In our study, the total cell growth rate in Water by the end of 48 hours was lower than those found in temperate conditions such as in the Gulf Stream (Ducklow and Hill, 1985), but are in line with those found in free-living bacteria of the Australian sector of the Southern Ocean (Church et al., 2000), and about an order of magnitude higher than those measured in the Indian sector of the Southern Ocean (Christaki et al., 2021). Both Water and WaterFP total cell growth rate were within the range of rates found in coastal waters of East Antarctica (Pearce et al., 2010).

Overall, bacterial activity associated with the fecalsphere does not seem to lead to large amounts of FP POC flux attenuation. The low rate of POC turnover on krill FPs by attached bacteria, and lack of colonization by new free-living bacteria, suggest that bacteria are not a major sink of FP carbon, making krill FPs especially good agents of organic carbon export to the deep ocean. The large size, fast sinking rates, up to over 1000 m d\(^{-1}\) (Atkinson et al., 2012), and peritrophic membrane of krill FPs likely allow them to avoid high magnitudes of bacterial degradation as they
sink through the water column. FP flux attenuation is likely driven by lateral advection and fragmentation and consumption by zooplankton (Suzuki et al., 2003; Steinberg et al., 2008).

The creation, through FP production, and the alteration of the krill fecalsphere over time have important implications for the microbial loop and biological pump. Krill FPs have been found to be home to a dynamic ecosystem of bacteria. Depending on the state and composition of the FP, it may support increased BP relative to the water column, as it may act as a refuge and a carbon-rich habitat for increased bacterial activity. Fecal pellet-associated bacteria may work commensally with free-living bacteria, increasing their abundance and metabolic activity through solubilization of POC to DOC. Conversely, FPs may act as a hostile environment for newcomers, limiting colonization by free-living bacteria and decreasing their overall metabolic activity. Though there are varying responses by free-living bacteria to FP POC in terms of BP and BGE, there is a consistent shift from LNA to HNA cell-state in the presence of krill FPs, while total cell abundance remains constant. Bacterial consumption of organic carbon associated with krill FPs was low. Therefore, krill FPs are not only ecologically significant as a source of carbon export and sequestration, but are significant drivers of changes in the microbial ecosystem.

3.5. Conclusion

The egestion of krill FPs into the water column leads to complex patterns of change in the microbial community. The presence of FPs does not significantly impact water column BR and can either increase or decrease BP, thereby altering the growth efficiency of the bacterial population. The exposure of free-living bacteria to FP POC alters the metabolic activity of free-living cells, shifting the population from a metabolically dormant to a more active state. All
together, this may indicate a switch from respiration to biomass production in the presence of large, organic carbon-rich particles. Though the free-living bacteria in the water column may become more metabolically active in the presence of FP POC, overall organic consumption rate by the bacterial population within the fecalsphere is low. These results are in line with the previous findings that *E. superba* FPs are the main component of POC export from the euphotic zone of the WAP, as there is little microbial remineralization and carbon uptake within the timeframe the FPs would be in the upper ocean. This makes krill FPs an excellent source of organic carbon that is efficiently exported to the deep ocean with low bacterial-induced flux attenuation.
Chapter 4

The Krill Fecal Pellet Microbiome: community size, species composition and carbon metabolism
Chapter 4: The Krill Fecal Pellet Microbiome: community size, species composition and carbon metabolism

Abstract:
The fecal pellets (FPs) of Antarctic Krill (*Euphausia superba*) are large aggregates of particulate organic carbon (POC) that can efficiently export organic carbon to the deep ocean. Further, FPs can serve as localized microhabitats, highly enriched in a variety of nutrients and organic carbon, and as potential homes for microbial assemblages. Biological and chemical transformations occur on sinking particles, through processes such as bacterial production (BP), respiration, and remineralization, influencing the quantity and quality of organic matter reaching the deep sea. Therefore, it is necessary to understand how the microbial ecosystem is affecting important biogeochemical cycles, such as POC export, in the Southern Ocean. To determine the relationship between FP POC export and carbon uptake through the microbial loop by particle-associated and free-living bacteria, we conducted studies over two years, using high-throughput sequencing of the bacterial 16S rRNA gene to characterize seawater and krill-associated bacteria from 17 locations on the West Antarctic Peninsula (WAP). Krill FPs act as a microhabitat, facilitating a unique fecal pellet-associated community that was distinct from the surrounding seawater. This fecal pellet-associated bacterial community contained overall lower species richness and alpha diversity relative to the free-living water column bacterial community, indicating that krill do not have a highly diverse fecal-microbiome. Exposure of FPs to free-living bacteria over 48 hours identified little change in fecal pellet-associated taxa, with little colonization by free-living bacteria, suggesting that krill FPs offer a exclusive habitat for the specialized bacterial community with which it is initially colonized. A smaller subset of these bacteria was responsible for the majority of carbon uptake from the FPs through BP. BP on fresh FPs did not significantly differ
from that of the surrounding water column community, indicating that the relatively low diversity of bacteria on FPs had elevated specific production rates relative to the higher species diversity in the free-living community. These results suggest that krill FPs host an endemic population of copiotrophic bacteria, unique from the free-living oligotrophic population in the surrounding water column. A small subset of these copiotroph taxa likely contain further adaptations that allow for initial proliferation of POC hydrolysis and carbon uptake on FPs, making them an important group in terms of POC flux attenuation.
4.1. Introduction:

The interactions between marine bacteria (here meaning heterotrophic members of the domains Bacteria and Archaea) and their environment ultimately shape net ecosystem productivity and carbon sequestration through the microbial loop and biological pump. Aggregates of particulate organic carbon (POC) are important agents in the flux of biogenic carbon from the sea surface to the deep ocean. Biological and chemical transformations occur on sinking particles, through processes such as bacterial production (BP) of biomass, respiration, and remineralization. These processes influence the quantity and quality of carbon reaching the deep sea, affecting the export efficiency of the biological pump. Zooplankton fecal pellets (FPs) are large, rapidly sinking aggregates of POC that can efficiently export organic carbon to the deep ocean, thereby sequestering carbon from the atmosphere, helping to modulate atmospheric CO$_2$ (Knox and McElroy, 1984; Honjo et al., 2014). Further, FPs can serve as localized microhabitats, highly enriched in a variety of micronutrients and as potential homes for particle-associated microbial assemblages (Alldredge and Silver, 1988).

Many marine bacteria have evolved to grow optimally at either high (copiotrophic) or low (oligotrophic) organic carbon and nutrient concentrations, enabling different species to thrive in distinct habitats in the oceans (Lauro et al., 2009). Free-living communities are dominated by phototrophs and oligotrophs (Lauro et al., 2009; Yooseph et al., 2010), which tend to have stable populations based on the carrying capacity of limiting resources, such as dissolved organic carbon (DOC) and tend to have streamlined genomes. Particle-associated communities tend to be composed of copiotrophs with a larger genetic repertoire for adaptation to motility and particle attachment. (DeLong et al., 1993; Agis et al., 1998; Cottrell and Kirchman, 2016). Both particle-
associated copiotrophic and free-living oligotrophic bacteria are integral participants in the microbial loop. Heterotrophic bacteria utilize and remineralize POC and DOC in the water column, mobilizing carbon through the microbial loop and recycling carbon back through primary production (PP) and upper trophic levels via bacterivory by microzooplankton. The microbial loop is an integral part of the larger biological pump, catalyzing carbon fluxes and cycling in the ocean (Azam et al., 1983; Ducklow, 1983).

Particles and ambient water represent two distinct habitats for microbial assemblages in aquatic environments (Zobell, 1943). Particle-associated copiotrophs and free-living oligotrophs often exhibit different physiological traits, as indicated by differences in BP and growth (Unanue et al., 1992, Friedrich et al., 1999), ectoenzymatic activity (Karner and Herndl, 1992; Smith et al., 1992; Riemann et al., 2000), and kinetics of substrate uptake (Ayo et al., 2001). Cellular uptake rates of sugars and amino acids by particle-attached bacteria are sometimes greater than those of surrounding, free-living bacteria (Kirchman and Mitchell, 1982). The differences between particle-associated and free-living bacterial habitat partitioning are further reflected in differences in community composition between the particle-attached copiotrophs and free-living oligotrophic communities (DeLong et al., 1993; Ortega-Retuerta et al., 2013; Rieck et al., 2015).

However, particle-associated and free-living communities sometimes show common features in terms of phylogenetic composition (Hollibaugh et al., 2000; Riemann and Winding, 2001) and metabolic functions (Worm et al., 2001). Between 40 to 70% of marine bacteria are estimated to be motile (Grossart et al., 2001), and have chemotactic behavior that allows them to cluster around patches of dissolved organic matter or to rapidly colonize POC (Blackburn et al., 1998; Kiørboe
et al., 2002). These free-living generalists are able to switch metabolic states to take advantage of high organic carbon or nutrient sources such as FPs (Riemann et al., 2000). Some free-living bacterial taxa are genetically amendable to colonize and attach to particles through gliding motility and surface adhesion (Dang and Lovell, 2016).

Questions remain about the role of particles in microbial ecology and the role bacteria play in POC decomposition. Some studies have found that FPs are rapidly colonized by free-living bacteria, quickly increasing particle-attached microbial abundance, leading to increased remineralization of POC (Jing et al., 2012). In contrast, other studies suggest that degradation by fecal pellet-associated bacteria already attached to the FPs are equally important in the consumption of POC (Cnudde et al., 2013). Some reports suggest that particles are inimical to bacteria altogether (Karl et al., 1988), while others suggest that they act as refugia (Cho and Azam, 1988) or as sources of organic carbon for the surrounding water through solubilization of POC; either through passive diffusion or through hydrolytic activity by particle-associated bacteria (Jumars et al., 1989; Smith et al., 1992; Hollibaugh et al., 2000).

Considering the important and dominant role that zooplankton FPs play in the transfer of carbon out of the upper ocean to the deep sea, an improved understanding of the balance between FP production and microbial decomposition of FPs by bacteria is needed (Steinberg et al., 2008; Iversen et al., 2010; Giering et al., 2014; Trinh et al., in review). This requires not only knowledge of dominant sources of FPs and POC flux, but also the rates of microbial activity, such as BP on sinking particles by both particle-attached, free-living bacteria that have colonized FPs, and free-living bacteria that may be dependent on DOC released from sinking POC. Concurrent information
on community structure of these two groups of bacteria would further help identify the key players in the fecal pellet-associated microbial loop and POC flux attenuation.

Along the WAP, Antarctic Krill (*Euphausia superba*) FPs are the main source of POC that is exported out of surface waters (Glieber et al., 2012, Trinh et al., in review). Krill are important grazers in the Southern Ocean, with an estimated biomass of between 300 and 500 million tonnes (Atkinson et al., 2009). Antarctic krill, like other zooplankton, are likely to support distinct bacterial assemblages compared to seawater (Tang et al., 2010; Troussellier et al., 2017). Given the high abundance and circumpolar distribution of *E. superba*, krill-associated microbiota could be a substantial proportion of Southern Ocean microbial communities (Clarke et al., 2019). Antarctic krill body tissue and their FPs may represent nutrient-rich microenvironments in the Southern Ocean, possibly supporting bacterial abundances several orders of magnitude higher than the surrounding seawater (Donachie and Zdanowski, 1998). Whether these bacterial communities are endemic to freshly produced FPs or the community ends up being comprised of free-living bacteria that were able to colonize the FPs remains an open question.

The rich summertime WAP ecosystem is characterized by high PP and POC export (Ducklow et al., 2008; Buesseler et al., 2010; Kim and Ducklow, 2016; Trinh et al., in review). Though the ratio between BP and PP is low on the WAP, a substantial part of this carbon export flows through the bacterial population (Ducklow et al., 2012). Heterotrophic bacteria on the WAP are limited by DOC availability, where sources include phytoplankton derived DOC and zooplankton derived DOC through excretion, egestion, and sloppy feeding (Ducklow et al., 2011; Kim and Ducklow, 2016). The WAP has been experiencing increased warming in the face of climate change, which
appears to be progressively altering the Antarctic food web, and flow of carbon, transforming them from diatom-krill-dominated to microbe-dominated systems (Legendre and Rassoulzadegan, 1996; Ducklow et al., 2012; Sailley et al., 2013; Kim and Ducklow, 2016). These ecological changes increase the importance of understanding the links between members of the microbial community and carbon flux through the microbial food web. Therefore, it is necessary to understand how the microbial ecosystem is affecting important biogeochemical cycles, such as POC export through krill FPs, and bacterial consumption of exported carbon in the Southern Ocean.

Bulk BP in polar regions is low compared to the rates of BP in temperate oceans (Ducklow et al., 2001; Kirchman et al., 2009a). The low rates of BP in polar regions could be due to bacteria being inactive in these perennially cold waters or to DOC limitation (Ducklow et al., 2001; Smith and del Giorgio., 2003; Kim and Ducklow, 2016). Early work suggested that bacterial growth is limited in cold temperatures, potentially requiring higher concentrations of substrate to achieve rates similar to bacterial growth in warmer, temperate waters (Pomeroy and Wiebe, 2001). Yet single-cell studies have shown that in spite of low bulk rates, the fraction of individual cells that are active in the Arctic Ocean is high (Elifantz et al., 2007; Malmstrom et al., 2007; Vila-Costa et al., 2008). Single-cell activity has been examined much less extensively in Antarctic waters (Straza et al., 2010).

Many microbial studies are of limited duration, conducted on timescales of a few days to about a month, or possibly a full season. Moreover, many studies collect particles lacking constraints on their exposure time to the water column (e.g. Hollibaugh et al., 2000; Garneau et al., 2009; Belcher
et al., 2016; Cavan et al., 2018). Our regional-scale study encompasses eight months of data collected over two years, from 17 sites along the WAP (Fig. 4.1). Further, we collected freshly formed FP particles in a sterile environment to determine the endemic bacterial population, and the rate of carbon consumption through BP by this community. To determine the nature of fecal pellet-associated bacteria and their relationship to FP POC export we used high-throughput sequencing of the bacterial 16S rRNA gene to characterize free-living water column and krill fecal pellet-associated bacteria community structure, and measured BP of each bacterial community. We further used 48-hour incubations to control for timed-exposure to the water column community to determine if any colonization by free-living bacteria occurs. This unprecedented dataset is the longest and most extensively sampled effort to study bacterial community structure and BP on and around krill FPs and relate these findings to free-living bacteria in the same samples.

We have addressed three questions concerning the role of bacteria in FP degradation. First, is there an endemic bacterial population of copiotrophs on krill FPs that is different from the surrounding free-living oligotrophs? Second, is there colonization of free-living bacteria on freshly egested FPs over time by bacterial generalists? Third, which bacterial taxa on FPs are responsible for this carbon consumption?

4.2. Methods

4.2.1. Study region

The Palmer Antarctica Long Term Ecological Research (PAL-LTER) program has been studying the marine ecosystem of the WAP since 1992, with long-term measurements conducted during the austral summer annually each Jan–Feb. Our research was performed on two PAL-LTER research
cruises aboard the ARSV Laurence M. Gould conducted in 2018 and 2019 within the PAL-LTER sampling grid, which covers a 170,000 km$^2$ area of the Bellingshausen Sea (Fig. 4.1), extending approximately 700 km from Palmer Station, Anvers Island (64.77°S, 64.05°W) to Charcot Island (69.45°S, 75.15°W). Grid lines are numbered from −100 to 600 and spaced 100 km apart, and stations within each line are spaced 20 km apart, beginning from the coast to approximately 200 km offshore at the slope/shelf break (Waters and Smith, 1992). The PAL-LTER grid is divided into latitudinal sub-regions based on hydrographic and sea ice conditions (Martinson et al., 2008; Stammerjohn et al., 2008), with the ‘North’ defined as lines 600, 500, and 400, the ‘South’ as lines 300 and 200, and the ‘Far South’ as lines 100 and below. In addition to the two summer cruises, an additional spring cruise was taken in Nov. 2018 to sample bays and inlets during sea ice cover, north of the PAL-LTER grid, considered to be the ‘Far North’ (Fig. 4.1). Austral Spring was delineated as occurring between October and November, Early Summer was defined as occurring between December through January 31, and Late Summer was defined as February to March 21. Spring, Early summer, and Late summer sampling also took place on smaller Rigid Hull Inflatable Boats (RHIBs) in coastal areas surrounding Palmer Station (Fig. 4.1). The waters surrounding Palmer Station on Anvers Island, is relatively shallow (∼75 m – 200 m), compared to the offshore shelf-slope region (400-3500 m).
4.2.2. Sample Collection

In total, 22 experiments were conducted along the WAP from Dec. 2017–Feb. 2020. However, due to COVID-19, samples from Jan.–Feb. 2020 were not processed for DNA extractions. In this study, 18 of the 22 experiments were analyzed (Table 4.1), with 17 studies having paired bacterial genomic data and BP data. In total, 162 quality-controlled samples were collected for paired DNA and BP measurements over the 48-hour time course incubations. Freshly egested krill FPs were placed within the free-living bacterial community in collected seawater and analyzed to determine changes in community structure and carbon uptake.
Table 4.1. Date and location of each DNA and BP experiment conducted over two years on the WAP.

<table>
<thead>
<tr>
<th>Experiment Number</th>
<th>Date</th>
<th>Lat</th>
<th>Lon</th>
<th>Latitude on WAP</th>
<th>Longitude on WAP</th>
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</table>

*E. superba* was collected using a 2 m-square frame Metro net (700 μm mesh) towed obliquely to a depth of 120 m at each station along the PAL-LTER grid along the WAP, or down to about 15-60 m in coastal regions near Palmer Station (Steinberg et al., 2015). A General Oceanics flow meter positioned in the center of the net mouth was used to calculate the volume of seawater filtered (Steinberg et al., 2015).

Collected krill were placed in clean 5-gallon mesh-bottom buckets within another bucket with about 15 liters of 0.2 μm filtered seawater, to ensure krill would egest their FPs into sterile, bacteria-free seawater. The two-tiered buckets were placed in the dark in flow-through tanks of surface seawater or cold rooms, matching ambient temperature as closely as possible to that in which the krill were caught. The two-tiered bucket system with mesh separation allowed for FPs to fall through while preventing krill from swimming down and ingesting their own FPs.
(coprophagy). Krill were allowed to swim and egest FPs for 1-5 hours before being removed. This time point was selected to ensure FPs were as fresh and pristine as possible, while maximizing FP production by ensuring adequate time for gut evacuation, which has been shown to be 3.7-6.3 hours (Atkinson and Snyder, 1997).

Collected FPs were removed from the 5-gallon buckets and separated into 4 treatments: 0-hour (initial), 12-hour, 24-hour, and 48-hour incubations in 30 ml of 3 µm filtered seawater collected concurrently with net-tows for krill. Control bottles of seawater without FPs were incubated side by side with FP-water bottles and sampled in the same time intervals. The seawater for each experiment was collected from concurrent vertical CTD profiles using Niskin bottles at 10-meter intervals, from the surface to about 150 meters, on the ship; and at 5-meter intervals to the same depths as net-tows around Palmer Station, and homogenized. The seawater was filtered at 3 µm to limit the presence of particles and meso- and microzooplankton grazers. Incubation bottles were kept in the dark at temperatures within 1ºC of ambient seawater temperatures. Incubation bottles were manually swirled every two hours to resuspend FP particles.

In order to compare the results of this study to that of Chapter 2, wherein a sediment trap collected sinking krill FPs at 175 m depth for 22 years, and to the bacterial metabolic analysis in Chapter 3, 48-hour incubations were carried out. As krill FPs sink at a rate of hundreds of meters per day (Atkinson et al., 2012), and the PAL-LTER sediment trap was located at 175 m depth, it was calculated that two days (48 hours) would be the amount of time the FPs would be exposed to the water column bacterial community before reaching the sediment trap and being preserved in formalin. One goal of this study was to determine if the FP POC caught over the 22 years in the
sediment trap was host to the same bacterial communities over seasons and decades. Therefore, tracking the bacterial community structure on krill FPs over 48 hours elucidates what bacterial taxa likely originate from the FPs themselves and which taxa likely colonize the FPs as they sink through the water column on the timescale of a few days. And this would serve as a baseline comparison to the long-term community analysis of the sediment trap samples. Further, analysis of bacteria-particle interactions on artificial particles showed that bacterial colonization can occur on short timescales (hours) and long timescales (~2 days), supporting our methodology (Grossart et al., 2003). However, due to COVID-19, we were unable to examine the genomic structure of the sediment trap POC samples and only present data and analysis of the fresh FPs and their change over 48 hours.

At each incubation time point, triplicate samples of FPs were removed and kept frozen at −80°C for DNA extraction and sequencing. A further triplicate set of FPs was removed for BP analysis. A triplicate sample of seawater without exposure to FPs and a triplicate sample of seawater exposed to FPs from each of the four time points were also removed for BP analysis. The seawater without FPs (Water) acted as our control, while we tested the dual hypotheses that FPs may be colonized by bacteria from the seawater free-living community, increasing both bacterial diversity on FPs and fecal pellet-associated BP; and that the presence of FPs and fecal pellet-associated bacteria would increase nutrient availability within the bottles, thereby increasing the free-living bacterial community BP.

For measurement of the initial free-living bacterial community, seawater DNA samples were taken by filtering approximately 4000 ml of seawater through a sterile 47 mm 0.2 μm Supor filter (Pall
Corporation, Port Washington, NY, USA) from the top 150 meters depth using a peristaltic pump. Seawater was collected from vertical CTD profiles along the WAP in Niskin bottles at 10-meter intervals and combined prior to filtration. The filters were immediately stored at –80 °C until extraction.

4.2.3. Environmental Sampling

Sea ice concentration data were extracted from the NASA Scanning Multichannel Microwave Radiometer (SMMR) and the Defense Meteorological Satellite Programs (DMSP) Special Sensor Microwave/Imager (SSM/I) satellite record for the gridded area along the WAP, following protocols described in Stammerjohn et al. (2008).

Concurrent water column temperature ($T$; °C), salinity, and pressure (dbar) were measured with a SeaBird 911+ CTD deployed at sampling stations along the PAL grid (Fig. 4.1). At each station, sampling consisted of one or more casts with the Seabird CTD-rosette system from the surface to just above the ocean bottom. Sigma-theta ($\sigma_\theta$, potential density; kg m$^{-3}$) was calculated at each depth bin (binned to 1 dbar) using CTD data to obtain mixed layer depth (MLD). Surface phytoplankton chlorophyll-a (chl-a) was measured by filtering CTD-collected seawater onto GF/F filters, which were flash frozen for fluorometric phytoplankton chl-a analysis (mg chl-a m$^{-3}$) (Schofield et al., 2017). Surface BP was also measured for each station to compare against experimental depth-homogenized water BP and FP BP.
4.2.4. Bacterial Production

The $^3$H-leucine incorporation rate of the collected FPs and water samples was measured to derive BP rates across the 48 hours of incubation. Further, concurrent surface water samples were measured for BP. We followed a modified protocol of the original leucine assay proposed by Smith and Azam (1992), as described in Ducklow et al. (2012). The $^3$H-leucine incorporation rate (pmol $l^{-1}$ h$^{-1}$) was converted to BP rates (mg C $l^{-1}$ h$^{-1}$) using the factor 1.5 kg C mol$^{-1}$ leucine incorporated (Ducklow et al., 2000). We recognize that conversion factors may vary (Kirchman et al., 2009b and references therein); but adopt a constant factor to facilitate comparison with other studies (e.g., Ducklow et al., 2012).

Briefly, triplicate 1.5 ml replicates of water samples, water samples exposed to FPs, and FP only samples were incubated in the dark for ~3 h with $^3$H-leucine (MP Biomedical, Santa Ana, CA; >100 Ci/mmol, 20–25 nM final concentration) in 2.0 ml microcentrifuge tubes (Axygen SCT-200, Union City, CA). Additionally, a killed control sample, using 100% TCA, for each sample type was incubated and measured. Incubations were maintained within 0.5 °C of the in situ temperature in refrigerated circulator baths and terminated by the addition of 0.1 ml of 100% trichloroacetic acid (TCA). Samples were concentrated by centrifugation, rinsed with 5% TCA and 70% ethanol and air-dried overnight prior to radioassay by liquid scintillation counting in Ultima Gold cocktail (Perkin-Elmer, Waltham, MA). Blank values of TCA-killed samples were subtracted from the average of the triplicates for each discrete sample.
4.2.5. DNA extraction and sequencing

Through March 2019, filters were extracted manually using the Qiagen DNEASY PowerWater Kit and FP samples were manually extracted using the Qiagen PowerSoil Kit (Qiagen, Venlo, Netherlands), after which point, both filters and FP samples were extracted using the KingFisher™ Flex Purification System and MagMax Microbiome Ultra Nucleic Acid Extraction kit (ThermoFisher Scientific, Waltham, Massachusetts, USA).

Extracted DNA was quantified using the Qubit HS DNA quantification kit (Invitrogen). PCR amplification of the 16S rRNA gene using primers 515F and 806R (Walters et al., 2016) for bacteria and archaea was ran and then quality checked by gel electrophoresis. High quality extracted DNA was submitted to the Argonne National Laboratory sequencing center for library preparation and sequencing with the same primer set, followed by 2 x 151 paired-end sequenced on the Illumina Miseq platform.

Reads were processed following Erazo et al. (2021). In brief, reads were filtered, denoised and merged with dada2 (Callahan et al., 2016). Merged reads were analyzed with paprica v0.7.0 (Bowman and Ducklow, 2015; https://github.com/bowmanjeffs/paprica) to determine the community structure.

4.2.6. DNA sequence analysis

Illumina Miseq reads were demultiplexed using the ‘iu-demultiplex’ command in Illumina utils. Demultiplexed reads were further quality controlled and denoised using the ‘FilterandTrim’ and ‘dada’ commands within the R package dada2 (Callahan et al., 2016), and assembled
with the ‘mergePairs’ command. The final merged reads had mean quality scores >30. The non-redundant fasta files of the generated unique reads produced by dada2 were used as input for the paprica pipeline for microbial community structure inference (https://github.com/bowmanjeffs/paprica). Paprica utilizes phylogenetic placement (Barbera et al., 2019) to place query reads on a reference tree constructed from the full-length 16S or 18S rRNA genes from all completed genomes in GenBank. All unique reads were assigned to internal branches or terminal branches on the reference tree. The paprica method for determining microbial community structure differs from operational taxonomic units (OTUs) subjective threshold clustering methods used by other studies (e.g. Campbell et al., 2011; Lima-Mendez et al., 2015) in that it places reads on a phylogenetic tree created from the 16S rRNA gene reads from all completed bacterial and archaeal genomes in Genbank (Bowman and Ducklow, 2015). These unique sequences, referred to as amplicon sequence variants (ASVs), and estimated gene abundances were normalized according to predicted 16S rRNA gene copy number prior to downstream analysis.

4.2.7. Bacterial diversity and statistics analysis

Samples with more than 5000 unique sequences, and ASVs with more than one occurrence were further analyzed. After the removal of taxa with low abundance, and samples with low reads, we obtained 162 samples totaling 8631 unique ASVs for analysis. Species richness and the alpha diversity index, inverse of Simpson, for ASVs was calculated using the ‘vegan’ package in R v. 3.6.3 (Oksanen, 2011). To assess the impact of treatment and temporal effects, and other biogeochemical factors, on the underlying shifts in overall phylogenetic community composition, a Tukey’s HSD post-hoc test was used. A two-way ANOVA and Tukey’s HSD test were used to
test differences among free-living water column bacterial communities and that of FP-associated bacterial communities and biogeochemical properties, such as incubation time, material, latitudinal location, season, sea ice, and chl-a concentration.

Non-metric multidimensional scaling (NMDS) ordination with the Bray-Curtis dissimilarity index was performed to identify patterns in microbial community structure in relationship to environmental variables. A stress function was used to assess the goodness-of-fit of the ordination. Stress values range from 0 to 0.08; values below 0.2 suggest that the ordination accurately represents the observed dissimilarity between samples (Clarke, 1993). To test for differences in groupings defined according to sample type, location, and season in the NMDS, we performed an Analysis of Similarity (ANOSIM) using Bray-Curtis dissimilarities in the R package ‘vegan’ (Oksanen, 2011). These analyses were also used for pairwise comparisons of groupings of bacterial communities from different locations, seasons, as well as free-living versus particle-associated communities. We used Analysis of Variance using Distance Matrices (ADONIS) with 999 permutations as implemented in ‘vegan’ to investigate the correlation between measured environmental parameters and bacterial community dissimilarity (BrayCurtis).

To relate subnetworks of microbial taxa on krill FPs to BP and to identify modules of highly correlated taxa we used Weighted Gene Correlation Network Analysis (WGCNA; Langfelder and Horvath, 2008), following the methods of Wilson et al. (2018). As we found no taxonomic difference between FPs at time 0, and over the 48 hours of incubation through our NMDS analysis, we proceed with only analyzing the bacterial community of FPs at time 0. WGCNA assesses the co-occurrence of taxa via an adjacency function that accounts for the degree of shared neighbors.
between two taxa. This assessment is then magnified by a power-law function in order to make
the topology of the graph scale-free. After taxa are sorted into subnetworks, the co-occurrence
profiles for each subnetwork (i.e., the first principal component of that subnetwork’s abundance
matrix) are then related to ecological variables, in this instance, BP. A Hellinger-transformed
relative abundance matrix was then limited to the 3,568 most abundant taxa. A signed adjacency
measure for each pair of features (unique ASVs) was calculated by raising the absolute value of
their Pearson correlation coefficient to the power of parameter p. The value $p = 7$ was used for
each global network to optimize the scale-free topology network fit, which gave an $R^2$ of 0.75.
This power allows the weighted correlation network to exhibit a scale free topology where key
nodes are highly connected with others. The obtained adjacency matrix was then used to calculate
the topological overlap measure (TOM), which, for each pair of features, considers their weighted
pairwise correlation (direct relationships) and their weighted correlations with other features in the
network (indirect relationships). For identifying subnetworks or ‘modules’ a hierarchical
clustering was performed using a distance based on the TOM. This resulted in the definition of
several subnetworks, each represented by its first principal component. Our minimum module
(subnetwork) size was set to 30 and the co-occurrence expression profiles for the resulting
subnetworks were then related to BP by way of a Spearman Rank Correlation. Module membership
(MM) was also calculated using a Spearman Rank Correlation. P-values were adjusted using
Bonferroni method. A subnetwork is the association between the subnetworks and a given trait
that is measured by the pairwise relationships (correlations) between the taxa. To find correlations
between subnetworks and ecological factors, Pearson’s correlation coefficients were calculated
between the BP and the respective principal components.
To identify unique reads that were differentially present between the initial FP bacterial community and that of the free-living community in the water samples, we used DESeq2 (Love et al., 2014), following the methods of Webb et al. (2019). DESeq2 performs differential abundance analysis based on the negative binomial/Gamma-Poisson distribution. The default DESeq2 settings were used to estimate size factors with the median ratio method (Love et al., 2014), followed by estimation of dispersion. The tested model looked at the differences between taxa in terms of material (FP or free-living water), season, and latitude, and the interactions between these variables. A Wald test for generalized linear model coefficients was used to test for significance of coefficients, taking into account size factors and dispersion. The p-values attained by the Wald test were corrected for multiple testing using the Benjamini and Hochberg method (Love et al., 2014). The bacterial taxa that were significantly differentially present were further examined to identify potential separation of microbial communities between krill FPs and the water column, and latitude, and season.

4.3. Results

To determine the relationship between FP POC export and carbon uptake by particle-associated and free-living bacteria, we conducted studies over two years on the WAP, using high-throughput sequencing of the bacterial 16S rRNA gene to characterize seawater and krill-associated bacteria from 17 locations. Incubation of FPs in concurrent 3 µm-filtered seawater for 48 hours allowed for detection in changes in community structure and changes in BP from both the fecal pellet-attached community and the free-living community.
4.3.1. Community structure of freshly egested fecal pellet-attached vs free-living bacteria

4.3.1.1. Alpha Diversity

Freshly egested krill FPs had a mean species richness (unique ASVs) of 133, while the free-living community had a mean of 190 species. The community of free-living water column bacteria contains significantly greater species richness (Fig. 4.2A; \( p = 0.013 \)) compared to the initial bacteria community on freshly egested krill FPs. Further, the fecal pellet-associated bacterial community contained overall lower Shannon diversity (Fig. 4.2B; \( p = 6 \times 10^{-7} \)) and inverse Simpson diversity (Fig. 4.2C; \( p = 0.00021 \)), relative to the free-living water column bacterial community. Freshly egested krill FPs do not have a highly diverse microbiome in terms of species richness and contain a community of fecal pellet-attached bacteria that was less even, containing more “rare” (relatively low abundance) taxa relative to the water column community, as shown by the lower Shannon and inverse Simpson diversity indices.

There is no correlation between species richness within the water column and that of freshly egested krill FPs from the same experiments (\( p = 0.13 \)).
4.3.1.2. Beta Diversity

The freshly egested FPs at time 0 started with a bacterial community that was 30% similar to the surrounding water in terms of taxa present in both environments. NMDS analysis (k = 8; stress = 0.041) revealed that the freshly egested FP bacterial community was distinct from the free-living community in terms of community structure (Fig. 4.3; \( p = 1 \times 10^{-4} \)), where FP communities (orange circles) cluster separately from free-living communities (blue triangles). FPs act as a microhabitat, facilitating a unique fecal pellet-associated community that was distinct from the surrounding seawater.
Figure 4.3. NMDS analysis of beta diversity (k = 8; stress = 0.041), showing separation of fecal pellet-associated bacterial community (orange circles) away from free-living bacterial community (blue triangles; $p = 1 \times 10^{-4}$).

The free-living water column bacterial community was dominated by *Candidatus* Thioglobus sp. NP1, in terms of relative abundance of the top 50 taxa, at 19%, followed by *Candidatus* Pelagibacter sp. FZCC0015 at 16%. *Nitrosopumilus maritimus* SCM1 is also found in the top 50 most abundant taxa in the free-living community. The initial FP community is dominated by *Candidatus* Hepatoplasma crinochetorum Av at over 19%, followed by *Pelagibacterales* at 18%. The taxonomic identity, ecology, and associations of the members of the two communities are addressed in the Discussion section and Appendix I.

4.3.2. Change in fecal pellet bacterial community over 48 hours

Incubations revealed little change in fecal pellet-associated taxa, implying little colonization by free-living bacteria. Krill FPs offer a unique habitat for the specialized bacterial community it is
initially colonized with, and 48 hours of exposure to free-living bacteria from the water column is not sufficient for free-living bacteria to impactfully colonize the FPs.

### 4.3.2.1. Alpha Diversity

Mean species richness on FPs after 48 hours of incubation was 151. There was no difference in alpha diversity of the bacterial community on FPs over the 48 hours of incubation in the water column community (Fig. 4.4), indicating that incubation within the free-living seawater community did not alter FPs over time. Although alpha diversity of FPs did not change over incubation time, all FPs maintained a distinct Shannon and inverse Simpson diversity index relative to the free-living water column community ($p = 4.38 \times 10^{-5}$; $p = 0.0016$, respectively).

![Figure 4.4](image)

**Figure 4.4.** Alpha diversity metrics of krill fecal pellets (orange), over incubation time points in the free-living seawater community, compared to that of the seawater free-living bacterial community (green). **A)** Species richness. **B)** Shannon diversity index ($p = 4.38 \times 10^{-5}$). **C)** Inverse Simpson diversity index ($p = 0.0016$).
4.3.2.2. Beta Diversity

The FP bacterial community was distinct from that of the free-living community in terms of community structure (NMDS k = 7; stress = 0.055) and remained so over prolonged exposure (12, 24, and 48 hours) to the free-living bacterial community (Fig. 4.5; p = 1 x 10^{-4}). There was no change in FP bacterial community over incubation time and prolonged exposure to the free-living community, indicating there was little exchange between the two communities over 48 hours. The initial FP community dictates the community found at each incubation time point. These results also indicate that incubation itself (i.e., the “bottle effect”; Hammes et al., 2010) does not affect the FP and free-living communities.

![Figure 4.5. NMDS analysis of beta diversity (k = 87; stress = 0.055), showing separation of the fecal pellet-associated bacterial community (circles) away from the free-living bacterial community (pink triangles; p = 1 x 10^{-4}). However, there is no significant clustering of fecal pellet communities based on incubation period (colored circles) within the free-living seawater bacterial community.](image)

On the class level, 25% of the fecal pellet-associated community was comprised of *Gammaproteobacteria*, 13% *Alphaproteobacteria*, 8% *Flavobacteriia*, 4% *Betaproteobacteria*,

135
4% unidentified bacteria, 2% Actinobacteria, 2% Mollicutes, 1.5% Deltaproteobacteria, 1% Bacteroidetes, and 1% Cytophagia. Similarly, the free-living community was comprised of 23% of Gammaproteobacteria, 15% Alphaproteobacteria, 11% Flavobacteria, 6% Betaproteobacteria, 4% unidentified bacteria, 1% Mollicutes, 3% Deltaproteobacteria, 0.40% Bacteroidetes, and 0.50% of Cytophagia. The abundance of Actinobacteria in the free-living community was an order of magnitude lower than in the FP community at 0.20%.

On a more in-depth analysis, when the relative abundance of all the FP incubation samples and water column community are considered together, Candidatus Hepatoplasma crinochetorum Av accounted for over 20% of the top 50 FP most abundant taxa at time 0 hour, 16% at 12 hour, 16% at 24 hour, and 16% at 48 hour time points. Pelagibacterales accounted for about 19% of the FP taxa at 0 hours, 21% at 12 hours, 18% at 24 hours, and 17% at 48 hours of incubation in seawater, but was not found in the top 50 most abundant taxa in seawater samples. These data indicate little change in the FP community over prolonged exposure to the seawater community, and demonstrate that krill FPs are stable environments for the bacteria they host. Candidatus Thioglobus s. NP1 was the most abundant taxon found in the free-living community at over 25% (Fig. 4.6).
4.3.3. Environmental effects on bacterial diversity

4.3.3.1 Alpha Diversity

Though exposure to the free-living community did not result in differences in the fecal pellet-attached bacterial community over time, the geographic latitude at which the krill were inhabiting had significant effects on FP bacterial community composition. There was significant difference in species richness between FPs collected in the North of PAL–LTER grid and the South (Fig. 4.7A; \( p = 0.0021 \)). In terms of Shannon diversity index (Fig. 4.7B), the fecal pellet-associated bacterial community in the South significantly differed from those from the North (\( p = 0.0015 \)) and the Far South (\( p = 0.018 \)). In terms of inverse Simpson diversity index, the FP community in the Far South is significantly distinct from those in the Far North, North, and South (Fig. 4.7C; \( p \))
There were no differences in alpha diversity metrics of the free-living bacterial community based on latitude.

**Figure 4.7.** Alpha diversity metrics of krill fecal pellets from different latitudinal locations along the PAL-LTER sampling grid. A) Species richness of fecal pellets from the North were significantly different than that of the South ($p = 0.0021$). B) Shannon diversity index shows fecal pellet-associated bacterial community in the South significantly differed from those from the North ($p = 0.0015$) and the Far South ($p = 0.018$). C) Inverse Simpson diversity index shows taxa diversity within the Far South is significantly different from Far North, North, and South ($p = 0.00021; p = 2.7 \times 10^{-6}; p = 9.7 \times 10^{-6}$ respectively).

Seasonality did not have a significant effect on alpha diversity of fecal pellet-associated bacteria.

There was no significant effect of seasonality on free-living bacterial alpha diversity.

**4.3.3.2. Beta Diversity**

There were distinct bacterial assemblages associated with krill FPs in different locations and seasons, for example in the North in Spring and in the South in Early Summer (Fig. 4.8).

The krill FP bacterial community structure differed between latitude locations along the WAP, with FPs collected from different latitudes clustering separately (Fig. 4.8A; $k = 8$; stress = 0.045;
$p = 1 \times 10^{-4}$). However, the water column free-living community did not significantly differ by latitude. There was no significant shift in bacterial community based on coast–offshore locality for either FP taxa or free-living water taxa.

Though there were no differences in seasonal bacterial community alpha diversity on krill FPs, the FP community composition did significantly differ between seasons (Fig. 4.8B; $k = 8$; stress = 0.045; $p = 1 \times 10^{-4}$). Further, there was a shift in community composition in the free-living fraction between different seasons ($k = 5$; stress = 0.08; $p = 0.038$).

![Figure 4.8. NMDS analysis of beta diversity of all fecal pellet bacterial communities. A) Separation and distinct clustering of bacterial communities based on sampling latitude on the PAL-LTER grid ($k = 8$; stress = 0.045; $p = 1 \times 10^{-4}$). B) Separation and distinct clustering of fecal pellet-associated bacterial community based on sampling season in which the krill were caught ($k = 8$; stress = 0.045; $p = 1 \times 10^{-4}$).](image)

An unidentified Bacteria.196 was the most abundant taxon found in the Far North, making up 22% of the top 50 most abundant FP taxa. This taxon was found at lower levels in the North and South, 3.4% and 0.62%, respectively, and was not found in the FPs from the Far South. *Oleispira*
*antarctica* RB-8 was the second most abundant taxon found in the Far North at 14%. Pelagibacterales was the most abundant taxon found in the North at over 26%, followed by *Candidatus* Hepatoplasma crinochetorum Av at 23%. FPs from the Far North, South, and Far South contained lower percentages of Pelagibacterales, at 1.9%, 0.63%, and 8.63%, respectively. A different bacterium, Bacteria.254 was the most abundant taxa found in the South, comprising 32% of the community. This taxa was not found in FPs of the Far North. *Sulfitobacter* sp. SK011 made up 14% of the community found on FPs in the South. In the Far South, *Candidatus* Hepatoplasma crinochetorum Av was the most abundant taxon, making up over 12% of the community, followed by *Kineobactrum salinum* at over 11% (Fig. 4.9A).

An unidentified Bacteria.166 made up over 25% of relative abundance of the bacterial community in the Spring on FPs, 0.37% of the community in the Early Summer, and 0% of the community in the Late Summer. *Oleispira antarctica* RB-8 made up over 12% of the Spring community. 28% of the FP bacterial community in the Early Summer was comprised of Pelagibacterales, which only made up 0.84% of the community in the Spring. *Paraglaciecola psychrophila* 170 increases from Spring to Early Summer and decreases in Late Summer. The Late Summer FPs were dominated by *Candidatus* Hepatoplasma crinochetorum Av at over 38% of the community (Fig. 4.9B).
Figure 4.9. A) The relative abundance of the top 50 most abundant taxa of fecal pellets based on sampling latitude. B) The relative abundance of the top 50 most abundant taxa of fecal pellet bacterial community based on sampling season.
4.3.4. Bacterial production on fecal pellets and in the water column

BP across all FP samples and free-living samples varied over three orders of magnitude and was highly variable from experiment to experiment, where the standard deviations are routinely higher than the mean BP values at each time point (Table 4.2).

Table 4.2: Bacterial production (mg C m⁻³ hr⁻¹) of fecal pellets over incubation periods within the free-living community and that of the free-living community itself.

<table>
<thead>
<tr>
<th>Incubation (hr)</th>
<th>min</th>
<th>median</th>
<th>mean</th>
<th>standard deviation</th>
<th>max</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.80E-04</td>
<td>0.0036</td>
<td>0.0360</td>
<td>0.0732</td>
<td>0.2520</td>
<td>44</td>
</tr>
<tr>
<td>12</td>
<td>7.90E-04</td>
<td>0.0106</td>
<td>0.0276</td>
<td>0.0600</td>
<td>0.3000</td>
<td>32</td>
</tr>
<tr>
<td>24</td>
<td>2.64E-03</td>
<td>0.0112</td>
<td>0.0216</td>
<td>0.0252</td>
<td>0.0984</td>
<td>33</td>
</tr>
<tr>
<td>48</td>
<td>4.08E-03</td>
<td>0.0288</td>
<td>0.0984</td>
<td>0.2040</td>
<td>0.9120</td>
<td>32</td>
</tr>
<tr>
<td>Free-living</td>
<td>7.31E-05</td>
<td>0.0132</td>
<td>0.0156</td>
<td>0.0180</td>
<td>0.0840</td>
<td>21</td>
</tr>
</tbody>
</table>

min = minimum value  
max = maximum value  
n = number of samples

The community of bacteria initially present on freshly egested FPs did not change significantly over time (Figs. 4.2 & 4.4) and was responsible for the majority of carbon uptake from the FPs through BP, as opposed to a community of free-living bacteria that have colonized the FPs, as there is little evidence of community exchange between the FPs and free-living community. BP on fresh FPs did not significantly differ from the BP rate of the surrounding free-living water column community (Fig. 4.10A), where the mean BP of the initial FP community was 0.036 mg C m⁻³ hr⁻¹ and BP of the free-living community was 0.016 mg C m⁻³ hr⁻¹, though the maximum initial FP BP was an order of magnitude higher than the maximum free-living BP.

After 48 hours of incubation within the water column community, BP on the FPs did not significantly increase relative to FPs at time 0 or the initial water column BP in a pair-wise manner. However, there was a significant step-wise increase in FP BP from 24 hours to 48 hours of
incubation in the free-living community (Fig. 4.10A; $p = 0.035$) though the bacterial community composition did not change (Fig. 4.5). Further, there was a significant logarithmic increase in BP over the 48-hour incubations (Fig. 4.10B; $p = 0.00037$).

BP of free-living bacterial community decreased with increasing bacterial species richness (Fig. 4.10C; $p = 0.020$) after the removal of outlier BP values ($BP > 0.08 \text{ mg C m}^{-3} \text{ hr}^{-1}$). BP of FPs at time 0 was not correlated with species richness on FPs. When considered all together, FP BP was negatively correlated to species richness (Fig. 4.10D; $p = 0.040$) after the removal of outlier BP values ($BP > 0.5 \text{ mg C m}^{-3} \text{ hr}^{-1}$), where BP decreased with increased species richness.
Figure 4.10. A) Bacterial production on krill fecal pellets over incubation time and in the initial free-living water column community ($p = 0.035$). B) Logarithmic increase in fecal pellet bacterial production over the incubation times within the free-living bacterial community ($p = 0.00037$). C) Free-living water column bacterial community decreases bacterial production as species richness increases ($p = 0.0029$). D) Fecal pellet bacterial community decreases bacterial production as species richness increases ($p = 0.040$).

4.3.5. Bacterial community correlated with bacterial production on fecal pellets

Weighted gene correlation network analysis (WGNA) of the initial FP community found distinct community clusters of highly correlated taxa across samples related to BP. In total, 12 distinct modules, representing 12 distinct bacterial communities, were identified. The bacterial community within the brown module was shown to be the most correlated with fresh FP BP (Fig. 4.11A; $R =$
0.65; \( p = 6 \times 10^{-6} \)). Within this brown module, 52 taxa were significantly assigned to this module community. Of those 52 taxa, 40 taxa were significantly correlated with BP. The top taxa associated with the brown module and FP BP (Fig. 4.11B; \( R = 0.95; \ p = 6.3 \times 10^{-27} \)) included *Thalassolituus oleivorans* R6-15 (\( R = 0.97; \ p = 1.65 \times 10^{-24} \)), *Flavobacteriaceae* (\( R = 0.97; \ p = 1.32 \times 10^{-25} \)), *Winogradskyella* (\( R = 0.96; \ p = 1.26 \times 10^{-23} \)), *Alcanivorax* (\( R = 0.96; \ p = 2.09 \times 10^{-22} \)), *Alteromonas mediterranea* UM7 (\( R = 0.96; \ p = 1.99 \times 10^{-23} \)), *Flavobacterium kingsejongi* (\( R = 0.88; \ p = 1.23 \times 10^{-13} \)), *Alphaproteobacteria* (\( R = 0.92; \ p = 1.28 \times 10^{-16} \)), *Colwellia* sp. MT41.2 (\( R = 0.76; \ p = 1.47 \times 10^{-8} \)), *Halobacteriovorax marinus* SJ (\( R = 0.89; \ p = 8.42 \times 10^{-15} \)), and *Finegoldia magna* ATCC 29328 (\( R = 0.85; \ p = 3.31 \times 10^{-12} \)).
Figure 4.11. WGCNA analysis of fecal pellet-associated bacterial communities correlated to fecal pellet bacterial production. 

A) Heat map showing correlation of each colored module bacterial community with bacterial production of freshly egested fecal pellets. Pearson correlation coefficients for subnetworks are shown in the top number and the number in the parentheses is the p-value for each relationship. Positive relationships are in red and negative relationships are in blue. Related to Table 4.3. 

B) Relationship between taxa within the brown module and their correlation with bacterial production on fecal pellets ($R = 0.95; p = 6.3 \times 10^{-27}$).

All these taxa were significantly correlated with FP BP, along with 30 other taxa (Table 4.3). None of these taxa were found in the top 100 most abundant taxa described above, with the majority not found in the top 1,000 most abundant taxa, indicating that relatively low-abundance taxa are the ones responsible for most of the biological activity on krill FPs.
### Table 4.3. WGCNA results of taxa within the brown module, the module most highly correlated to fecal pellet bacterial production.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Module Color</th>
<th>R (BP)</th>
<th>p (BP)</th>
<th>R (brown)</th>
<th>p (brown)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria 87</td>
<td>brown</td>
<td>0.75599</td>
<td>1.70E-08</td>
<td>0.859433</td>
<td>1.26E-12</td>
</tr>
<tr>
<td>Bacteriovoracales 4</td>
<td>brown</td>
<td>0.647434</td>
<td>6.32E-06</td>
<td>0.990972</td>
<td>8.93E-35</td>
</tr>
<tr>
<td>Thalassolituus oleivorans R6-15</td>
<td>brown</td>
<td>0.641671</td>
<td>8.12E-06</td>
<td>0.96835</td>
<td>1.65E-24</td>
</tr>
<tr>
<td>Flavobacteriaceae 10</td>
<td>brown</td>
<td>0.632635</td>
<td>1.19E-05</td>
<td>0.97234</td>
<td>1.32E-25</td>
</tr>
<tr>
<td>Winogradskyella 5</td>
<td>brown</td>
<td>0.626994</td>
<td>1.50E-05</td>
<td>0.964714</td>
<td>1.26E-23</td>
</tr>
<tr>
<td>Alcanivorax 1</td>
<td>brown</td>
<td>0.624045</td>
<td>1.69E-05</td>
<td>0.958986</td>
<td>2.09E-22</td>
</tr>
<tr>
<td>Alteromonas mediterranea UM7</td>
<td>brown</td>
<td>0.623611</td>
<td>1.72E-05</td>
<td>0.963848</td>
<td>1.99E-23</td>
</tr>
<tr>
<td>Bacteria 69</td>
<td>brown</td>
<td>0.603508</td>
<td>3.75E-05</td>
<td>0.924123</td>
<td>1.84E-17</td>
</tr>
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<td>Flavobacterium kingsejorgi</td>
<td>brown</td>
<td>0.593485</td>
<td>5.43E-05</td>
<td>0.876642</td>
<td>1.23E-13</td>
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<tr>
<td>Alphaproteobacteria 35</td>
<td>brown</td>
<td>0.591956</td>
<td>5.74E-05</td>
<td>0.91564</td>
<td>1.28E-16</td>
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<td>Colwellia sp. MT41 2</td>
<td>brown</td>
<td>0.590688</td>
<td>6.01E-05</td>
<td>0.758076</td>
<td>1.47E-08</td>
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<td>Halobacteriovorax marinus SJ 2</td>
<td>brown</td>
<td>0.585942</td>
<td>7.11E-05</td>
<td>0.89374</td>
<td>8.42E-15</td>
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<td>Finegoldia magna ATCC 29328</td>
<td>brown</td>
<td>0.575784</td>
<td>0.000101</td>
<td>0.851525</td>
<td>3.31E-12</td>
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<td>Polaribacter sp. L3A8 15</td>
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<td>0.554118</td>
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<td>Hyphomonas 2</td>
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<td>Thalassolituus oleivorans MIL-1 1</td>
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<tr>
<td>Fluvicola taffensis DSM 16823 33</td>
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<td>0.000324</td>
<td>0.846275</td>
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<td>Colwellia sp. Arc7-635 1</td>
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<td>0.536</td>
<td>0.000364</td>
<td>0.875301</td>
<td>1.49E-13</td>
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<tr>
<td>Flavobacteriaceae 8</td>
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<td>0.000762</td>
<td>0.86801</td>
<td>4.11E-13</td>
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<tr>
<td>Tenacibaculum dicentrarchi 1</td>
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<td>0.00105</td>
<td>0.78473</td>
<td>2.06E-09</td>
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<td>Owenweeksia hongkongensis DSM 17368 66</td>
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<td>0.002403</td>
<td>0.691424</td>
<td>7.82E-07</td>
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<td>Owenweeksia hongkongensis DSM 17368 7</td>
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<td>0.4594</td>
<td>0.002863</td>
<td>0.64214</td>
<td>7.95E-06</td>
</tr>
<tr>
<td>Carnobacterium</td>
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<td>0.4594</td>
<td>0.002863</td>
<td>0.64214</td>
<td>7.95E-06</td>
</tr>
<tr>
<td>Cutibacterium acnes HL096PA1</td>
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<td>0.4594</td>
<td>0.002863</td>
<td>0.64214</td>
<td>7.95E-06</td>
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<tr>
<td>Halieaceae 8</td>
<td>brown</td>
<td>0.4594</td>
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<td>7.95E-06</td>
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<td>Rhodobacteraceae 25</td>
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<td>0.4594</td>
<td>0.002863</td>
<td>0.64214</td>
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</tr>
<tr>
<td>Fluvicola taffensis DSM 16823 36</td>
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<td>0.4594</td>
<td>0.002863</td>
<td>0.64214</td>
<td>7.95E-06</td>
</tr>
<tr>
<td>Glaciecola amylolytica 2</td>
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<td>0.4594</td>
<td>0.002863</td>
<td>0.64214</td>
<td>7.95E-06</td>
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<td>0.455676</td>
<td>0.003129</td>
<td>0.737317</td>
<td>5.75E-08</td>
</tr>
<tr>
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R: correlation coefficient;  
*p*: p-value.  
BP: taxa correlation to bacterial production.  
Brown: taxa correlation to the brown module.
In terms of relative abundance, *Alcanivorax* was the most abundant taxon of the primary bacterial community associated with the BP on FPs. Within the brown module, *Alcanivorax* made up 45% of FP taxa in terms of relative abundance at time 0, 31% of FP at time 12-hour, 43% of taxa of FP at time 24-hour, 19% of FP taxa at time 48-hour, and was not present in the top 50 taxa of the free-living bacterial community (Fig. 4.12). *Glaciecola nitratireducens* FR1064 was the most abundant taxon associated with FP BP at time 48-hour, accounting for 42% of the taxa present. In terms of taxa that were responsible for the bulk of BP on FPs. *Owenweeksia hongkongensis* DSM 17368 was found in relatively low abundances on FP, but accounted for 75% of the bacterial abundance in the free-living community.

**Figure 4.12.** Relative abundance of the top 50 most abundant taxa from WGCNA brown module, which are highly correlated to bacterial production of fecal pellets. Relative abundances are shown for fecal pellets over incubation time and for the initial free-living water column community (W0).
4.3.6. Differential abundance of these bacteria between water and fecal pellets

In terms of differential expression (abundance) between FP and free-living bacterial communities, in relation to seasonal and latitudinal impacts, 122 taxa were found to be significantly different. 70 taxa were found to be significantly more abundant on FPs, while 52 taxa were more abundant in the free-living community (Fig. 4.13A; Table 4).

A total of 64 taxa was found to be significantly different in terms of abundance between the Far North and the Far South, where 29 taxa were more abundant in the Far North, and 35 taxa more abundant in the Far South (Fig. 4.13B; Table 5).

In terms of seasonal differences of fecal pellet-associated bacteria, 31 taxa were preferentially found in the Spring relative to Late Summer, whereas 32 taxa were significantly more abundant in the Late Summer relative to the Spring (Fig. 4.13C; Table 6).

Figure 4.13. Volcano plots of differential expression (abundances) of taxa between environmental parameters. A) Differential abundances between fecal pellet-associated taxa and the community of bacteria free-living in the water column. B) Differential abundances of fecal pellet bacterial taxa between latitudes Far South and Far North. C) Differential fecal pellet bacterial taxa abundances between Late Summer and Spring.
Table 4.4. Deseq2 results of differential abundances between fecal pellet bacterial taxa and free-living water column taxa. Positive log2 fold change indicates increased abundance of taxa on fecal pellets, while negative log2 fold change indicates an increased abundance of taxa in the water column. Adjusted p-value shows statistical significance.

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Table 4.5. Deseq2 results of differential abundances of fecal pellet bacterial taxa based on sampling latitude. Positive log2 fold change indicates increased abundance of taxa in the Far South, while negative log2 fold change indicates an increased abundance of taxa in the Far North. Adjusted p-value shows statistical significance.

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Table 4.6. Deseq2 results of differential abundances of fecal pellet bacterial taxa based on season. Positive log2 fold change indicates increased abundance of taxa in Late Summer, while negative log2 fold change indicates an increased abundance of taxa in the Spring. Adjusted p-value shows statistical significance.

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Candidatus Thiolobus sp. NP1, Sulfitobacter sp. SK011, Methanomassiliicoccales, Neorickettsia, and Polaribacter sp. L3A8, in their free-living life stage, were significantly more abundant in the water column during the Spring relative to the Late Summer.

These differences in FP and free-living bacterial communities, and their relational change due to seasons and latitude can be seen in Fig. 4.14. Increased taxon relative abundance is shown in warmer colors (red), while low abundances are shown in cool colors (blue). Ecological and environmental parameters, i.e. material type (FP vs free-living water), latitude, and season, are
indicated and color coded on the top, showing how different taxa and communities were found in unique ecosystem states.

**Figure 4.14.** Heat map showing differentially abundant bacterial taxa based on material type (free-living water vs krill fecal pellets), location (latitude), and season.
4.4. Discussion

Understanding the bacterial dynamics on sinking POC is crucial toward further understanding of the microbial loop, biological pump, and carbon export. A knowledge of the microbial taxa associated with sinking particle types is needed, as well as their carbon consumption rate over time. We add to this knowledge base by analyzing the microbial ecology of the dominant source of POC export in the WAP – *E. superba* FPs. To the best of our knowledge, this is the first study to identify the extent of bacterial community exchange between the endemic population on freshly produced particles and that of the surrounding water column in association with measures of bacterial metabolic activity in the two different niches.

4.4.1. Endemism on krill fecal pellets – a stable community distinct from free-living bacteria

Our results indicate that krill FPs are home to an endemic population of bacteria that are likely acquired through ingestion, or are residents of the krill gut. The population of bacteria that are part of the initial FP does not significantly change with prolonged exposure in the water column, suggesting that FPs are a stable environment for endemic populations that are likely copiotrophic specialists. Over the same amount of time, Grossart et al. (2003) found that growth of the resident bacterial community on particles dominated over colonization and decolonization.

Further, FPs may possibly be an environment that selects against non-resident taxa, such as oligotrophs. The taxa found on FPs may be optimized for life on organic particles, as discussed below. The POC and other nutrients of FPs are cycled within the resident population of bacteria.
on FPs and does not appear to be significantly taken up by the free-living community, as found in Chapter 3.

The overall species diversity within *E. superba* FPs was significantly lower than that of the surrounding free-living community. Analysis of the river prawn gut microbiome also found that it was less diverse than corresponding lake or river water bacterial diversity (Chen et al., 2017). Similarly, the bacterial communities associated with FPs from a mixture of coastal copepods was found to contain bacteria that were composed of different phylogenetic groups from those living in the natural seawater (Jing et al., 2012). In their study, bacterial communities in seawater had higher species richness than those in FPs, similar to our findings on the WAP.

There was a 30% overlap in taxa between the free-living water column community and the bacterial community on the freshly egested FPs at time 0. This reflection of the free-living community suggests that the krill are able to incorporate these bacteria into their gut through ingestion and pass them into their FPs. Further, the bacterial community associated with the krill FPs differed based on the latitude and season at which krill were caught. Changes in diet with either location and season, will alter both the bacteria ingested and the nutrient environment within the zooplankton gut and the subsequent FPs egested. De Troch et al. (2010) found that the bacteria associated with a marine copepod and its FPs mainly originated from the copepod’s gut, and that the composition of bacterial communities of the copepods’ guts depended on the initial food sources ingested by the copepods. These regional and seasonal differences in krill fecal pellet-associated bacterial community may also be reflected in different Southern Ocean regions that are dominated by *E. superba*, such as the Scotia Sea.
The bacterial communities in the Southern Ocean are known to vary between water masses (Wilkins et al., 2013) and season (Luria et al., 2016), which will influence the bacteria available to colonize internal and external surfaces (Clarke et al., 2019). However, we found no significant difference in free-living bacterial community structure by latitude, suggesting that the water column community is more spatially stable.

4.4.2. No colonization of FPs by free-living bacterial communities

The results of our study show that there is little community exchange between the free-living and fecal pellet-attached bacteria over 48 hours. It was hypothesized that free-living microorganisms may optimize their position near particle nutrient fields, leading to microbial clusters in the vicinity of detrital aggregates such as FPs (Azam and Ammerman, 1984). If there was clustering of free-living bacteria in the vicinity of the krill FPs, there was little evidence of attachment, colonization, and growth of the free-living bacteria on FPs over time. As seen by our NMDS beta-diversity results, there was no significant change in FP community structure over 48 hours of exposure to seawater containing a rich ecosystem of bacteria, indicating little colonization of FPs by the free-living bacteria. This is supported by the results of Grossart et al. (2003).

However, in contrast to our findings on krill FPs, Jing et al. (2012) found that there were major shifts in the bacterial community composition associated with copepod FPs in the first two days of experimental incubations in natural seawater. The communities of bacteria in FPs and seawater became more similar with time, indicating that bacteria from surrounding seawater colonized FPs
during experimental degradation incubations, suggested that colonization of FPs by free-living bacteria from surrounding seawater was an important part of the pellet decomposition process.

The lack of colonization by free-living bacteria on krill FPs may be due to the uniquely different ecosystem of the WAP, including its low temperatures. The extremely low temperatures of the Antarctic oceans may slow bacterial metabolism, growth, mobility, and colonization capabilities (Maayer et al., 2014). It may be the case that many of the free-living bacteria are natural oligotrophs that do not possess the genetic repertoire to take advantage of, and do not thrive in, high nutrient, high organic carbon situations, and therefore do not benefit from FP colonization. Additionally, attached bacteria are capable of modifying the surrounding microenvironment within the particle, thereby affecting the behavior and physiology of other microbes. It is also possible that growth of newcomer free-living taxa on FP particles was suppressed by the resident copiotroph population. Marine particles are physically more stable, exhibiting potentially fewer resource-limiting habitats than the water column (Grossart et al., 2003). It may therefore be beneficial for attached bacteria to invest in defending against invaders by releasing antibiotic substances. Indeed, many particle-associated bacteria exhibit antagonistic behavior against other bacteria (Long and Azam, 2001; Gossart et al., 2003). Microbial metabolic interactions between community members of particles are well documented, and studies of bacterial growth frequently show inhibition of growth in the presence of other strains of bacteria due to production of repellents, antibiotics, or toxins (Madigan et al., 2000; Gram et al., 2002; Gram et al., 2010; Wilson et al., 2010; Kempnich and Sison-Mangus, 2020). Additionally, the concentration of secondary metabolites contained within the FPs themselves, such as oxylipins, from ingested food sources
(Collins et al., 2018), may suppress bacterial activity and alter bacterial community structure (Ribalet et al., 2008).

### 4.4.3. Small subset of taxa on fecal pellets disproportionately active in bacterial production

Mean initial FP BP and free-living BP were equivalent to one another, suggesting that that the initial resident taxa on FPs were taking up DOC, hydrolyzed from the FPs, at similar rates to their free-living counterparts through biomass production. After 48 hours of incubation within the water column community, BP on the FPs did significantly increase though the community composition did not change, indicating increases in cell abundance or substantial growth in carbon demand by fecal pellet-associated bacterial cells. This growth in BP exhibited a logarithmic increase to a mean of 0.12 mg C m$^{-3}$ hr$^{-1}$ and maximum of 0.97 mg C m$^{-3}$ hr$^{-1}$, exceeding the high-end BP values of surface waters of 0.60 mg C m$^{-3}$ hr$^{-1}$ (Ducklow et al., 2012). Though FP BP increases over the 48 hours, the rate of carbon uptake was overall relatively low, suggesting heterotrophic bacteria are not a major sink of krill FP POC, and are not responsible for the bulk of POC flux attenuation in the upper water column of the WAP. Beyond 48 hours, krill FPs likely have sunk out of the euphotic zone and are efficient transporters of organic carbon into the mesopelagic, where they will likely encounter a different bacterial community with less metabolic activity (Karner, et al., 2001; Church et al., 2003; Nguyen et al., 2022).

We found a smaller subset of the already low-diversity population on FPs was associated with the majority of carbon uptake from the FPs through BP. Similarly, many other marine studies have found that only a small subset of the bacterial community is actively participating in biomass
production and BP is negatively correlated to species richness. On the WAP, water column BP was found to be significantly negatively correlated to species richness of free-living bacteria, where BP increased from winter to summer as bacterial species diversity decreased as the seasons shifted (Luria et al., 2016). In a summertime study comparing the WAP and the mid-Atlantic, Straza et al. (2010) found that only 40% of the community were actively partaking in BP, similar to what was observed in the mid-Atlantic. Ghiglione et al. (2009) found that less than half of the attached bacterial populations on particles in the euphotic zone were biologically active, with regard to the high proportion of OTUs present. These results suggest that most of the POC remineralization appeared to be mediated by a rather low number of dominant active OTUs specialized in exploiting such specific microenvironment. This small subset of copiotrophs may be better suited for a life-style on krill FPs. Free-living bacterial community composition and biomass production in the western Arctic Ocean were also found to be dominated by a few bacterial groups. Not only was BP dominated by only a few taxa, it was, at times, dominated by taxa with over all low abundances relative to the entire FP bacterial population, indicating a high taxon-specific growth rate (Malmstrom et al., 2007). We found similar results in our study on the WAP. Though they were comprised of distinct bacterial communities, BP rate on fresh FPs did not significantly differ from that of the surrounding water column community, indicating that the relatively small number of taxa of bacteria on FPs had increased taxa-specific production rates relative to the higher species diversity in the free-living community. Overall, a small subset of bacterial taxa are key players in the recycling of organic carbon from FPs through the microbial loop in the upper ocean.
Bacterioplankton are the sole consumers of DOC in the ocean. Their ecological role within the microbial loop facilitates the transformation of DOC to bacterial biomass through BP (Azam et al., 1983; Pomeroy et al., 2007) and the remineralization of DOC into inorganic CO$_2$ through respiration (Ducklow et al., 1986; Goldman and Dennett, 2000). Coastal waters of the WAP are characterized by low BP as a consequence of low bulk DOC concentrations compared to the Arctic Ocean and other coastal ecosystems due to the lack of terrestrial and allochthonous inputs of DOC (Kim and Ducklow, 2016). Therefore, bacteria must ultimately rely on in situ DOC released by phytoplankton (Morán and Estrada., 2002), cell lysis, and trophic processes that produce POC, such as zooplankton grazing, sloppy feeding and egestion of FPs (Steinberg and Landry, 2017). This in situ production of POC via egestion of FPs is an important process in the carbon cycle and microbial loop.

Krill FPs, like those of many other arthropods, are encased in a peritrophic membrane. This encasement allows for structural stability of FPs as they sink through the water column, making them efficient agents of POC export, unlike FPs of other organisms, such as salps, that are fragile and easily broken up during the sinking process (Gleiber et al., 2012). The peritrophic membrane surrounding krill FPs consists of chitin, and its breakdown appears to be facilitated by high hydrolytic activity by attached bacteria (Cottrell et al., 1999). The degradation of the peritrophic membrane allows bacteria access to the nutrients and POC contained within the FPs. Studies of marine copepods have shown time scales of FP disintegration ranging from 3 hours at 20°C to 20 days at 5°C (Honjo and Roman, 1978; Turner, 1979), while Bathelt et al. (1983) found little to no degradation of freshwater zooplankton FP peritrophic membranes over 144 hours. Thor et al. (2003) found that hydrolysis of chitin on copepod FPs ceased after 48 hours, possibly due to
complete decomposition of the peritrophic membrane by that time. Further, they found that BP on copepod pellets correlated with chitinase and glucosaminidase activity, which catalyzes the hydrolysis of chitin, reaching a maximum at 48 hours activity, concluding that chitin may play an important role in BP associated with FPs. Certain bacteria, mainly copiotrophs, are specialized in chitinase production and hydrolysis of chitin, playing an important role in breaking down the peritrophic membrane of FPs and giving access to POC and DOC to other bacteria (Lauro et al., 2009). Particle associated bacteria are likely to derive more value from chitinases, than free-living oligotrophic bacteria. This degradation of peritrophic membranes on FPs by 48 hours of exposure to the water column may be correlated with the increase in BP we measured on krill FPs on the WAP.

4.4.4. Some significant fecal pellet taxa

Prokaryotes are limited to the uptake of low molecular weight (LMW) compounds through their cell membranes via permeases, thus they must hydrolyze high molecular weight (HMW) polymeric organic matter to LMW fragments prior to transport into the cell (Christian and Karl, 1995; Arnosti, 2011). An important source of LMW DOC is through the hydrolysis of POC, such as FPs, mediated by particle-associated bacteria. Amino acids are generally incorporated more efficiently than sugars such as glucose (del Giorgio and Cole, 1998). Different bacterial taxa have different capabilities in terms of compound hydrolysis and preferences in compound uptake, making them more suitable for certain life strategies relative to others.

Copiotrophs tend to have prevalent methods of sugar uptake and transport, while oligotrophs exhibit reliance on binding proteins as an energy source. Copiotrophs contain a greater proportion
of genes associated with chemotaxis, biofilm formation, and antibiotic resistance. Copiotrophs tend to have larger diversity of proteins localized in the outer membrane, allowing an increased capacity to rapidly and tightly regulate metabolism and use energetically expensive transporters for nutrient acquisition (Lauro et al., 2009). Oligotrophs dominate the marine microbiome in terms of abundance and tend to be smaller in cell size, contain less genetic material, exhibit a “feast and famine” life strategy, and contain other adaptive traits for acquiring carbon and nutrient limiting environments, such as slow growth rate and large surface area to volume ratio, characteristic of small oceanic bacterioplankton (Lauro et al., 2009; Haggerty and Dinsdale, 2017; Norris et al., 2021).

In the Antarctic Scotia Sea, the bacterioplankton assemblage was composed predominantly of Alphaproteobacteria followed by Sphingobacteria-Flavobacteria and Gammaproteobacteria (Topping et al., 2006). The summertime bacterial assemblage in WAP shelf waters results from selective growth of relatively few heterotrophic populations, transforming a high-diversity winter community dominated by chemolithoautotrophs into a lower-diversity, mostly heterotrophic assemblage (Grzymski et al., 2012). Substrate utilization by the free-living summer community on the WAP was dominated by relatively few taxa of Sphingobacteria–Flavobacteria, Gammaproteobacteria, and Alphaproteobacteria, where bacterial groups differed in use and uptake of different proteins, amino acids, and sugars (Straza et al., 2010). We find that there is agreement between these previous studies and our results, but we go into greater taxonomic detail than the phylogenetic class and order.
### 4.4.4.1. Alphaproteobacteria

The majority of marine Alphaproteobacteria are aerobic heterotrophs that preferentially metabolize LMW compounds such as monomers, consistent with an oligotrophic mode of life (Malmstrom et al., 2005; Fernández-Gómez et al., 2013). Alphaproteobacteria in general were highly correlated to FP BP.

Several *Rhodobacteraceae* were found to be highly correlated to FP BP. Members of *Rhodobacteraceae* are often found in close association with phytoplankton blooms in either the particle attached or free-living part of the community. They generally use LMW substrates, including the secondary degradation products produced by *Flavobacteria* (Pinhassi et al., 2004; West et al., 2008; Wemheuer et al., 2015). In line with these conclusions, on the WAP, Luria et al. (2016) found that the relative abundance of free-living *Rhodobacteraceae* tripled during a phytoplankton bloom, peaking later in the bloom, indicating that they utilize secondary products of decomposing organic matter, such as LMW compounds (Voget et al., 2015).

*Phaeobacter porticola* sp. nov., an antibiotic-producing bacterium isolated from a sea harbor (Breider et al., 2017), was preferentially associated with krill FPs relative to the water column. *Phaeobacter inhibens* was found to be able to produce the broad-spectrum antibiotic tropodithietic acid (TDA), which also causes the same regulatory effects in quorum sensing molecules. Production of TDA by *P. inhibens* affects motility, biofilm formation, and antibiotic production, all important processes for settlement on new host-associated surfaces. Antibiotics can not only antagonize other bacteria (Beyersmann et al., 2017), but can also prevent new taxa from colonizing
biofilms and host particles. Further, *Phaeobacter* has high nutritional versatility, able to metabolize carbohydrates, organic acids, and all 20 proteinogenic amino acids (Wiegmann et al., 2014).

### 4.4.4.2. Bacteroidetes

The bacteria belonging to the *Bacteroidetes* division have been divided into three main groups: Bacteroides, Sphingobacteria, and Flavobacteria (Garrity and Holt, 2001; Cole et al., 2003). Many taxa within these groups have the ability to synthesize a variety of either cell-bound or extracellular degradative enzymes like cellulases, chitinases or proteases making them especially proficient in degrading various biopolymers such as cellulose, chitin, and pectin. Additionally, the presence of adhesion proteins, as well as the genes for gliding motility may give them an advantage to exploit POC (Kirchman, 2002). These *Bacteroidetes* are important decomposers of HMW organic matter in seawater, showing a preference for consuming polymers rather than monomers (Cottrell and Kirchman, 2000). Proteorhodopsin-containing *Bacteroidetes* shared two characteristics: small genome size and a higher number of genes involved in CO$_2$ fixation, where proteorhodpsin is a membrane protein that can convert light energy into available forms of biochemical energy (Kirchman et al., 2014). The ability to fix inorganic carbon is likely important in order to survive when in the free-living form in the illuminated, but nutrient-poor, ocean surface (Fernández-Gómez et al., 2013).

Previous work has shown the abundance of particle-associated Flavobacteria correlates with chl-a concentration and periods of high primary productivity (Abell and Bowman, 2005; Murray and Grzymski, 2007). Flavobacteria are able to break down complex organic matter by direct attachment and exoenzymatic attack of phytoplankton cells and phytoplankton-derived detrital
particles (Williams et al., 2013). We found that Flavobacteria correlated with FP BP, with many strains being more abundant in FPs relative to in the water column. *Flavobacterium kingsejongi*, a novel carotenoid-producing species originally isolated from Antarctic penguin feces (Choi et al., 2018), was found to be highly correlated with krill FP BP. *F. kingsejongi* may have been incorporated into penguin feces through the ingestion of krill, their main food source.

An abundant genus of *Flavobacteria, Polaribacter*, is a copiotroph that possesses traits consistent with a life-strategy of particle attachment and polymer degradation (Fernández-Gómez et al., 2013; Cottrell and Kirchman, 2016). However, *Polaribacter* is metabolically flexible and also abundant in the free-living community, indicating that its ecological niche extends beyond particle attachment (Smith et al., 2013; Williams et al., 2013). A genomic analysis of the *Polaribacter* strain MED152, found a considerable number of genes that enable surface or particle attachment, gliding motility and polymer degradation. Additionally, *Polaribacter*, an aerobic anoxygenic phototroph (Grzymski et al., 2012; Kirchman et al., 2014), was found to contain proteorhodopsin genes, as well as other genes that could be used for carbon fixation in surface waters. *Polaribacter*, therefore, is more adept at moving towards POC and staying within patches of DOC, attaching to nutrient-rich surfaces, and, alternatively, using inorganic carbon fixation to synthesize intermediates of metabolic pathways in low nutrient, high light conditions as a free-living organism (González et al., 2008). On the WAP, Luria et al. (2016) found that the relative abundance of free-living *Polaribacter* doubled during a phytoplankton bloom and suggest that *Polaribacter* has a strong competitive advantage early in bloom formation. During the summer, *Polaribacter* was found to comprise 57% of the Sphingobacteria-Flavobacteria group.
with the preference for HMW organic compounds (Straza et al., 2010). In our study, the strain
*Polaribacter sp. L3A8* was highly correlated to FP BP.

*Winogradskyella*, part of the family *Flavobacteriaceae*, phylum *Bacteroidetes*, is a gram-negative,
yellow-pigmented, aerobic, gliding, oxidase- and catalase-positive genus of bacteria. All members
of the genus *Winogradskyella* have been isolated from marine environments. Many strains have
originated from samples associated with phytoplankton blooms, marine invertebrates, such as
mollusks, and benthic sediments (Nedashkovskaya et al., 2005; Begum et al., 2013; Park et al.,
2015). *Winogradskyella* spp. have been reported to be polysaccharide degraders that have a very
diverse array of enzymes for breaking down complex and diverse macromolecules (Alejandre-
Colomo, et al., 2020). A specific strain, *Winogradskyella* sp. PG-2, contains proteorhodopsin,
allowing it to convert light energy into available forms of biochemical energy in the absence of
organic carbon and nutrients (Kumagai et al., 2014). *Winogradskyella* sp. strain PG-2, originally
isolated from surface seawater in Japan, was preferentially found in krill FPs relative to free-living
water and in the Far South of the WAP in our study. It is one of the taxa that was significantly
correlated with krill FP BP.

The genus *Owenweeksia* in the family *Cryomorphaceae* branches between the families
*Flavobacteriaceae* and *Bacteroidaceae* (Bowman et al., 2003), has been isolated from Southern
Ocean particulates and from quartz stone. Members of *Owenweeksia* are aerobic, psychrotolerant,
rod-shaped and orange pigmented, typical for *Flavobacteria*. Strains of *Owenweeksia* contain
genes that indicate the capability of respiratory degradation of recalcitrant compounds (Riedel et
al., 2012). In our study, three strains of *Owenweeksia hongkongensis* DSM 17368 were found to
be significant members of the brown module, indicating that these are active members of fecal pellet-associated BP.

**4.4.4.3. Gammaproteobacteria**

Although the abundance of Gammaproteobacteria often averages <10% in temperate and Arctic oceanic areas (Glöckner et al., 1999; Pommier et al., 2007; Alonso-Saez et al., 2008), Straza et al. (2010) found that these bacteria were quite abundant in the WAP during the summer. We found that 25% of total abundance of both free-living and fecal pellet-associated bacteria were Gammaproteobacteria. Marine Gammaproteobacteria include free-living bacteria, biofilm formers, and obligate symbiotes. Well-known clades of marine copiotrophs are Gammaproteobacterial taxa (Lauro et al., 2009).

*Oleispira antarctica* RB-8 (DSM 14852) was isolated from a hydrocarbon-enrichment set-up from Antarctic seawater (Yakimov et al., 2004). Along with other representatives of the order *Oceanospirillales*, those from *Oleispira* spp. comprise marine obligate hydrocarbonoclastic bacteria whose metabolism is restricted to the linear and branched aliphatic, saturated and non-saturated hydrocarbons and their derivatives, fatty acids or alcohols. *O. antarctica* has unique protein folding mechanisms that allow for cold-adaptation and coping with various habitat-specific stress (Kube et al., 2013). Further, *O. antarctica* was included as a member of the brown module from our WGCNA analysis, making it highly correlated with the BP of FPs.

*Colwellia* are non-pigmented, curved rod-like cells which exhibited psychrophilic and facultative anaerobic growth and possessed an absolute requirement for sea water (Deming et al., 1988). They
are generally able to utilize amino acids and organic acids, but not many of the sugars or alcohols. *Cowellia* are capable of nitrate reduction but not iron reduction or H$_2$S production. Generally chitinolytic, *Cowellia* is able to degrade starch, and positive for oxidase, catalase, and alkaline phosphatase (Deming and Junge, 2015). Chitin is widely distributed in nature, particularly as a structural polysaccharide in the exoskeletons of arthropods, the outer shells of crustaceans, such as krill. Hydrolysis of chitin has been shown to support a substantial part of estuarine BP (Kirchman and White, 1999). High relative abundances of molt-associated *Colwelliaceae* were also found on other arthropod zooplankton, such as Calanus copepods (Moisander et al., 2015). Colwellia-related clones and isolates have been obtained from aggregates of particulate organic material and coastal marine water samples (DeLong et al., 1993; Suzuki et al., 1997) and are additionally associated with coastal Antarctic sea-ice diatom assemblages, isolated from Antarctic fast sea-ice, from an Antarctic meromictic marine salinity lake (Bowman et al., 1997a; Bowman et al., 1998a) and in gas vesicles from the ice-water interface of sea ice in McMurdo Sound, Antarctica, (Gosink and Staley, 1995). In our study, strains *Colwellia* sp. MT41 2 and *Colwellia* sp. Arc7-635 1 and an unspecified strain of Cowellia were significantly correlated with FP BP.

*Alteromonas* and *Pseudoalteromonas* have been shown to be capable of producing antibacterial compounds (Fencial, 1993; Holmstrom et al., 2002; Wagner-Dobler et al., 2002; Burgess et al., 2003). The genus *Alteromonas* contains gram-negative aerobic heterotrophic marine bacteria with one polar flagellum and a mostly free-living life strategy. (Baumann et al., 1972; Ivanova et al., 2015). With the description of the genome of *Alteromonas mediterranea* DE, it was revealed that there was considerable diversity within *Alteromonas* in terms of adaptation to utilization of particles of different size and copiotrophic adaptations. *Alteromonas* is a typical bloomer that
relies on fast growth rates to compete in marine oligotrophic waters, which likely restricts its habitat to the relatively nutrient rich and extremely diverse realm of particulate organic matter (López-Pérez et al., 2016). On the WAP, we found *A. mediterranea* UM7 to be highly correlated to FP BP.

*Graciecola* forms a novel lineage adjacent to the genus *Alteromonas*. It was first isolated from sea-ice cores from the coastal area of eastern Antarctica and is a group of pigmented, psychrophilic, strictly aerobic chemoheterotrophs (Bowman et al. 1998b). Members of the *Glaciecola* genus have since been isolated from Arctic sea ice, marine invertebrate specimens, seawater and marine sediments (Romanenko et al., 2003; Zhang et al., 2006). Members of the genus *Glaciecola* are characterized as being gram-negative and aerobic. The novel species *Glaciecola amylolytica* sp. was isolated from the East Sea off of Korea (Xiao et al., 2019). It was found to be an amylase-producing bacterium. Amylase is an enzyme that catalyzes the hydrolysis of starch into sugars. On *E. superba* FPs, *G. amylolytica* was a member of the community highly correlated with FP BP.

4.4.4.4. *Tenericutes*

Mollicutes is now the sole class in the phylum *Tenericutes*, a phylum of gram-negative bacteria consisting of cells bounded by a plasma membrane devoid of cell walls. *Tenericutes* have been detected in the gut and gonad microbiomes of fish, sea stars, oysters, mussels, and arthropods. Many mollicutes are pathogens and/or mutualistic symbionts in the gut of their host species. *Mycoplasmas* and hepatoplasmas affiliated with *Mycoplasmatales*, such as *Candidatus* Hepatoplasma crinochetorum, play an important role in degrading recalcitrant carbon sources in the stomach and pancreas of arthropods, including deep sea isopods (Wang et al., 2004;
Wang et al., 2016). By adopting a parasitic mode of life, Mollicutes were able to reduce their genetic material considerably, eliminating genes related to biosynthesis of amino acids and intermediate metabolic compounds, which must, instead, be imported from the host cytoplasm or tissue. (Wang et al., 2020) To maintain their parasitic mode of life the Mollicutes have developed sophisticated mechanisms to colonize their hosts and resist the host immune system through antigenic variation on cell surfaces (Razin et al., 1998). On the WAP, one strain of Mycoplasmataceae was found preferentially enriched in krill FPs, while a different strain was found more abundant free-living in the water column. One strain was found to be significantly more abundant in FPs in the Spring in the Far North. Three Candidatus Hepatoplasma crinochetorum Av strains were significantly more abundant in krill FPs relative to in the water column and one strain was found more significantly in FPs in the Far North of the WAP. Though highly associated with krill FPs, these taxa did not significantly contribute to FP BP.

In terms of significant differences in taxon abundance of the free-living bacteria, Candidatus Thioglobus sp. NP1, Sulfitobacter sp. SK011, Methanomassiliicoccales, Neorickettsia, and Polaribacter sp. L3A8, were significantly more abundant in the water column during the Spring relative to the Late Summer.

Overall, gene families involved in transcription and carbohydrate transport and metabolism tend to be overrepresented on copiotroph genomes relative to oligotrophs, corresponding to an overall strategy of rapid acquisition of nutrients and protein production. Conversely, gene families involved in energy production and conversion and replication, recombination, and repair are typically overrepresented on oligotroph genomes, corresponding to an overall strategy of energy
production and cell maintenance (Weissman et al., 2021). Our study found similar ecological patterns and distinctions between fecal pellet-associated bacteria and free-living oligotroph bacteria.

*E. superba* FPs potentially contained transient food-associated bacteria not permanently associated with krill, explaining differences in community structure found on pellets across seasons and latitude. However, the consistent presence of specific taxa in FPs across many locations, spanning over 700 km, seasons, and years, especially on freshly egested pellets, suggests some permanent associations. Particularly, the significantly increased occurrence of mollicutes in FPs suggest a persistence of gastro-intestinal endosymbionts across the krill population. Though we found that the free-living bacterial communities were composed of both copiotrophs and oligotrophs, there was little colonization of krill FPs over 48 hours by the free-living bacteria. We found that the bacterial community first found on pellets as they are egested tend to remain stable on pellets over 48 hours. A smaller subset of copiotrophic taxa found associated with FPs are shown to have highly specialized adaptations, such as the ability to produce chitinase and antibiotics, as well as the ability to breakdown HMW hydrocarbons, increasing their BP and allowing them to take advantage of the organic carbon on and within sinking FPs.

### 4.5. Conclusion

Taken together, our BP, species richness, and community composition data indicate strong resource partitioning between particle-associated and free-living bacteria. We find that krill FP aggregates harbor microbial assemblages that are distinct from the free-living bacterioplankton, as supported by other studies on particle-associated microbiomes and that a small subset of these taxa
are metabolically active, disproportionately partaking in BP (DeLong et al., 1993; Rath et al., 1998; Moeseneder et al., 2001; Ghiglione et al., 2009). Growth of particle-attached microbial specialists on aggregates, such as zooplankton FPs, has several biogeochemical implications. Increased hydrolysis and remineralization of sinking POC attenuates the flux at shallower depths, reducing passive carbon flux. Additionally, the release of HMW polymers via hydrolysis by particle-associated bacteria may produce slowly degraded dissolved organic matter for free-living bacteria in the vicinity of sinking POC. Attached microbial assemblages are capable of expressing proteases, phosphatases, β-glucosidase, and chitinase in unique proportions (Karner and Herndl, 1992; Smith et al., 1992; Arnosti, 2011) and at greater activity rates compared to those produced by free-living cells in the surrounding water (Carlson and Hansell, 2015), making them better adapted to breakdown and uptake of biopolymers associated with FPs. Our results vastly extend the known diversity of fecal pellet-associated bacteria by looking at krill collected across different locations and seasons over multiple years, providing the first insight into microbial diversity, colonization capabilities, and carbon uptake of sinking POC on the WAP. Overall, a low-diversity community of bacteria is responsible for organic carbon hydrolysis on krill FPs, and the low rate of carbon uptake suggests FPs efficiently export POC out of surface waters and bacteria do not play a large role in flux attenuation.
Chapter 5

Concluding remarks
5. Conclusion

The biological function of different organisms within an ecosystem has complex effects on regional biogeochemical cycles. Here, I showed that within a group of organisms, such as bacteria, different species can play different functional roles in POC uptake and flux attenuation. These functions likely evolved out of niche partitioning between carbon- and nutrient-rich environments versus carbon and nutrient depleted conditions. These species-specific adaptations are important when analyzing the flow of carbon through the biological pump. A singular species can have a disproportionate effect on the carbon cycle, particularly in extreme environments such as the Southern Ocean. Along the West Antarctic Peninsula (WAP), Antarctic krill (*Euphausia superba*) fecal pellets dominate the portion of particulate organic carbon (POC) that is exported out of the surface ocean. I have presented evidence that taking into account the evolved life history cycle of *E. superba* is crucial to answering the question as to what mechanisms drive the export of POC and deep ocean carbon sequestration over seasonal and decadal timescales. A holistic analysis of the WAP marine ecosystem shows that krill have a significant impact on POC export through the production of fast-sinking fecal pellets that are able to escape decomposition by bacteria before being sequestered in the deep ocean. The mechanistic drivers of carbon export, as well as the interactions between sinking fecal pellets and the surrounding water column organisms, that I have identified will help to better predict changes to the biological pump and oceanic carbon sequestration due to warming Antarctic temperatures.

5.1 Species composition and life history matter

Ocean biodiversity and ecology play an important role in regional POC production and export. Specifically, on the WAP I found integrated annual POC flux over the 21-year timeseries varied
by more than one order of magnitude, from 0.31 g m\(^{-2}\) to 5.31 g m\(^{-2}\). I find that this high interannual variability occurs on a 5-year cycle that aligns with the cycle in *E. superba* body size. By overlooking the unique life history of these krill, and merely examining their overall abundance, it would be impossible to discern the driving factor of POC export on the WAP and likely elsewhere in the Southern Ocean. *E. superba*, unlike many other organisms, continually grow in body size as they age from year to year. As such, the fecal pellets they produce grow in size on an annual basis, reaching peak size about every five years, when krill are at their largest and at the end of their life cycle. Paradoxically, this occurs when the krill population is at its lowest in terms of numerical abundance. However, this pattern of decrease in abundance as a population ages is not a new ecological concept and further provides evidence that abundance is not a good predictor of POC export on the WAP, which is heavily dominated by krill fecal pellets.

There is great complexity in a tiny microbial ecosystem. Epipelagic and mesopelagic bacteria consume sinking POC, where particle-attached bacteria hydrolyze POC to DOC. This process yields two separate impacts. First is the physical loss of POC with depth. Second is the biological impact of POC and DOC consumption on the bacterial community, such as species diversity, competition, and metabolism. Understanding the different functions of particle-attached and free-living bacteria on the WAP helps to determine the extent to which heterotrophic bacteria cause POC flux attenuation and the extent to which habitat partitioning occurs. I find that there is indeed a distinction between krill fecal pellet-associated bacterial species and those free living in the water column. The krill fecal pellet, the main source of POC export on the WAP, is a habitat for a low-diversity bacterial community. Of this small community, an even smaller percentage of species are responsible for the bulk of metabolic activity and carbon consumption on the fecal pellets,
illustrating the potentially large impact of a small subset of bacteria. I find that many of the fecal pellet-attached bacterial species are well adapted to life on organic carbon-rich particles, in contrast to those found in the planktonic state. In some cases, access to the carbon-rich fecal pellets does stimulate bacterial production rate of the free-living bacterial community. In other cases, the presence of fecal pellets, and their associated bacterial community, inhibits free-living bacterial growth, possibly due to antagonism and competition. Competition and species interactions of certain bacteria likely drive different behavioral responses to the presence of krill fecal pellets. In all cases, access to krill fecal pellets did not stimulate increases in total cell abundance but increased the nucleic acid content of the free-living community, indicating a switch from a dormant state to a metabolically active state.

Overall, I find that heterotrophic bacteria on the WAP do not decompose sinking krill fecal pellets to a degree that would significantly impact POC flux attenuation. The main loss in POC with depth is likely due to coprophagy of fecal pellets by other zooplankton and krill themselves. However, the large size of krill fecal pellets at the latest stage of the krill life cycle, combined with their summer swimming and swarming behavior, create large pulses of fecal pellet egestion that likely sink quickly and escape localized heterotrophic carbon demand. Taken all together, the sediment trap time series and bacterial metabolism and diversity analyses are in agreement that *E. superba* fecal pellets are efficient at transferring organic carbon from surface waters to depth. This work has demonstrated that the microbial loop on the WAP does not mobilize a significant amount of carbon from sinking POC and that there is a disproportionate importance of Antarctic krill in the WAP biological pump. Moving forward, global studies of carbon export and sequestration must take into account regional ecology and life history of the local ecosystem and accurately model the
differential importance of each organism, as opposed to merely using functional role and abundance.

5.2 Environmental modulation of POC export on the WAP

The evolved life style strategies of organisms are impacted by their environment. The extreme environment of the Antarctic region makes the interactions between the physical oceanographic environment and surrounding ecology clear. The seasonal and semi-decadal episodic nature of POC flux that characterizes the WAP, along with the composition of exported POC, would be difficult to capture without long-term sediment trap collections. With these measurements, and the long-term record on Southern Ocean sea ice, I was able to identify the effect of winter sea ice on summer POC export phenology. Anomalously low winter sea ice on the WAP correlated with deeper summer-time mixed layer depth. This in turn, coincided with a delay in summer POC export, likely due to increased particle residence time in the surface mixed layer before export.

*E. superba* life history also oscillates on a seasonal cycle, commensurate with the extreme seasons that define the WAP. The dominance of summer-time POC export on the shelf of the WAP is correlated to the summer ontogenetic horizontal migration of krill from inshore coastal regions to the deeper waters of the continental shelf. Krill move inshore during fall and winter months, where sea ice provides a refuge and food source for them. During the spring and summer, as the days grow longer and the sea ice melts, the krill move towards open water, increasing the regional production of fecal pellets, leading to summer peaks in POC export on the continental shelf. Alteration of sea ice cover will likely impact the timing of this seasonal migration, thereby effecting the timing of peak POC export on the WAP shelf.
5.3 A changing WAP ecosystem

The environmental changes experienced by the WAP over the past decades have begun to alter the ecology of the organisms that call it home. The WAP, and all life around it, are intimately tied to the formation and disintegration of winter sea ice. Warming temperatures have changed both the amount of ice produced and the timing of ice breakup. This alteration in sea ice in a once stable ecosystem has a wide range of impacts on the biological pump. Therefore, it is critical to understand how climate change will alter atmospheric CO$_2$ uptake, POC export and remineralization within the marginal ice zones of the Southern Ocean. My thesis illuminates the complex environmental and biological factors that are driving the biological pump in the Southern Ocean.
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Appendix

Appendix A: Subset of some ecologically significant bacterial taxa associated with krill fecal pellets and surrounding environment

Alphaproteobacteria

Alphaproteobacteria in general were highly correlated to FP BP and found predominantly in FPs and in the Far North.

Several Rhodobacteraceae were found to have increased abundance in krill FP, relative to free-living in the water column, and preferentially found in Late Summer.

Pseudorhodobacter sp. S12M18, a strain of Pseudorhodobacter within the Rhodobacteraceae family, is a novel species named Pseudorhodobacter turbinis sp. nov., isolated from the gut of the Korean turban shell, Turbo cornutus (Jeong et al., 2021) and was found associated with krill FPs, potentially living endemically in krill guts and being incorporated into their FPs.

Phaeobacter, a genus within the Rhodobacteraceae, was preferentially found in FPs in the Far South and in the Late Summer.

Pelagibacterales, a member of the SAR 11 clade, is a highly abundant class of marine bacteria common in oligotrophic waters (Giovannoni, 2017). Groups such as Pelagibacter are well adapted to the free-living state and non-bloom conditions (Williams et al., 2013; Buchan et al., 2014; Voget et al., 2015). Candidatus Pelagibacter is an aerobic anoxygenic phototroph, expressing proteorhodopsin proteins that can function as light-dependent proton pumps, supporting Ca.
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_Pelagibacter ubique_ metabolism during carbon starvation, a condition that is likely to occur in the extreme conditions of ocean environments (Steindler et al., 2011). Although _Ca. Pelagibacter_ is often thought of as an oligotrophic specialist, it is more accurately considered as a planktonic specialist that prefers bloom conditions. Its dominance during non-bloom periods arises from its ability to persist under relatively low concentrations of bulk DOC (Bowman et al., 2017). The growth of _Ca. Pelagibacter_ depends on exogenous sources of reduced sulfur compounds such as dimethylsulfoniopropionate (DMSP), generated by phytoplankton (Tripp et al., 2008). On the WAP, the onset of Spring saw a decline in relative abundances of free-living _Pelagibacteraceae_ (Luria et al., 2016), though SAR 11 still made up > 80% of the free-living Alphaproteobacter during the summer (Straza et al., 2010). In our study, some strains of _Pelagibacterbacter_ were more abundant in FPs in the Late Summer, while _Ca. Pelagibacter_ sp. FZCC0015, and _Ca. Pelagibacter_ ubique HTCC1062 were differentially more abundant in seawater as free-living bacterioplankton.

**Bacteroidetes**

In our study, _Cytophagales_ was found to be significantly more abundant in krill FPs from the Far South of the WAP.

The strain _Polaribacter sp. L3A8_ was preferentially found in krill FPs in the Spring and in the Far North. It was highly correlated to FP BP. The strain _Polaribacter sp. L12M9_ was found in the Late Summer and found more prevalently in the water column in its free-living life strategy.
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Winogradskyella sp. strain PG-2, originally isolated from surface seawater in Japan (Kumagi et al., 2014), was preferentially found in krill FPs relative to free-living water and in the Far South of the WAP in our study.

Two strains of Owenweeksia were found to be differentially more abundant in seawater than in krill FPs, with one strain being found FPs in the Far South and another in the Far North and in the Spring.

Fluviicola taffensis DSM 16823 bacterium of the family Cryomorphaceae in the phylum Bacteroidetes, was found to be strictly aerobic, gram-negative, yellow–orange-pigmented, motile, catalase-positive, and oxidase-negative. Cells were long flexible rods with rounded ends, with an apparent lack of nutritional versatility and an inability to utilize carbohydrates (O’Sullivan et al., 2005). Two strains of F. taffensis DSM 16823 were significantly correlated with FP BP in our study. Three strains were of F. taffensis DSM 16823 were significantly more abundant in seawater as bacterioplankton than associated with FPs. One strain was differentially more abundant on krill FPs in the Far North of the WAP and three strains were found on FPs in Late Summer.

Gammaproteobacteria

Alcanivorax is a genus of aliphatic hydrocarbon-degraders, where of alkanes are their principal carbon source. Alcanivorax borkumensis is a cosmopolitan marine bacterium that uses oil hydrocarbons as its exclusive source of carbon and energy. Although barely detectable in unpolluted environments, A. borkumensis becomes the dominant microbe in oil-polluted waters. A. borkumensis SK2 has a streamlined genome with a paucity of genes for scavenging of
nutrients, particularly organic and inorganic nitrogen and oligo-elements, biofilm formation at the oil-water interface, biosurfactant production and niche-specific stress responses (Schneiker et al., 2006). *A. borkumensis* was significantly more enriched in krill FPs than in the water column, and preferentially found in FPs from the Spring. Other strains of *Alcanivorax* were preferentially found in the Far South and during the Late Summer. *Alcanivorax* was one of the subset of fecal pellet-associated bacteria that was highly correlated with the BP on FPs.

*Oleispira antarctica* was significantly more abundant in krill FPs than in the water column and was found to be differentially abundant in terms of season and latitude, being found more in the Spring in the Far North.

Gram-negative, vibrioid to spiral, motile cells, *Thalassolituus oleivorans* R6-15 and *T. oleivorans* MIL-1 are also aerobic obligate hydrocarbon degraders (Yakimov et al., 2004) and were found to be enriched in FPs compared to seawater and highly correlated with FP BP. These strains were found in Spring on the WAP.

On the WAP, *Colwellia* sp. PAMC 21821, *Colwellia* sp. 20A7, *Colwellia psychrerythraea* 34H, and *Colwellia* sp. MT41 were significantly more abundant in krill FPs than in seawater as free-living bacteria. *Colwellia* sp. PAMC 20917, *Colwellia* sp. 20A7, and *Colwellia* sp. PAMC 21821 were found in the Far South and *C. psychrerythraea* 34H was found in the Far North. *C. psychrerythraea* 34H was found in the Spring, whereas *Colwellia* sp. PAMC 20917 and *Colwellia* sp. 20A7 were found in the Late Summer. In a different study, early February peaks in the
abundance of free-living *Colwelliaceae* corresponded to near-complete depletion of nitrate and nitrite, an unusual event in the WAP (Luria et al., 2016; Kim et al., 2016).

Phylogenetic analysis (Deming et al., 1988; DeLong et al., 1993; Gosink and Staley, 1995; Bowman et al., 1997a) has indicated that the Colwellia genus is most closely related to other cultivable gram-negative marine genera such as *Shewanella, Pseudoalteromonas* and *Alteromonas* (Gauthier et al., 1995; Rosselló-Mora et al., 1995). Cultivable bacterial strains tend to be copiotrophic and capable of growth on agar and other surfaces.

The genus *Shewanella* includes facultatively anaerobic, gram-negative, motile, rodlike cells and have been found to be characterized dissimilatory iron-reducing strains mostly isolated from Antarctic sea ice. The strains were isolated from samples of land-fast sea ice, grease ice, and ice algal biomass collected from the coastal areas of eastern Antarctica (Bowman et al., 1997b). *Shewanella livingstonensis* strains NF22T and NF24 were isolated from sediment and water, respectively, collected from Livingston Island, Antarctica (Bozal et al., 2002). *Shewanella* are capable of anaerobic growth either by fermentation of carbohydrates or by anaerobic respiration, and utilize a variety of electron acceptors, including nitrate, ferric compounds, and trimethylamine N-oxide. On the WAP, *Shewanella* was observed to increase in relative abundance mid-way through a phytoplankton bloom (Luria et al., 2016). In our study, *S. livingstonensis* was significantly more abundant in FPs and found in Far South of the WAP and Late Summer. An unidentified strain of *Shewanella* was found in Far North in the Spring and *Shewanella* sp. MEBiC00475 1 found in Late Summer.
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An unidentified strain of Alteromonadaceae was more commonly found as free-living bacteria in the water column.

*Pseudoalteromonas* sp. strain *SM9913* is an aerobic, psychrophilic, deep-sea marine gram-negative bacterium. It has gene clusters related to both polar and lateral flagella biosynthesis. Lateral flagella, which are usually present in deep-sea bacteria, are absent in the related surface bacteria. With these two flagellar systems, *Pseudoalteromonas* sp. can swim in sea water and swarm on the sediment particle surface, favoring the acquisition of nutrients from particulate organic matter and reflecting its particle-associated alternative lifestyle in the deep sea (Qin et al., 2011). In our study of FP particle-associated microbial dynamics, *Pseudoalteromonas* sp. *SM9913* found more on krill FPs than its free-living life strategy.

A strain of *Graciecola amylytica* was found to be more enriched in seawater than in FPs. An additional two separate strains were preferentially found in FPs during the Spring.

*Candidatus* Thioglobus sp. strain NP1 is an open-ocean isolate from the SUP05 clade of Gammaproteobacteria. Mixotrophic members of the SUP05 clade have the potential to oxidize sulphur and fix carbon in marine biogeochemical cycles. However, whole-genome comparisons of strain NP1 indicate that it lacks the ability to fix inorganic carbon using the Calvin-Benson-Bassham cycle (Spietz et al., 2019). When found in krill FPs, *Ca. Thioglobus* was found mostly in the Spring. Strains of *Ca. Thioglobus* were the most abundant taxon found in the water column.
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*Psychrobacter* is a genus of gram-negative, osmotolerant, oxidase-positive, psychrophilic or psychrotolerant, aerobic, non-motile bacteria. Ornithogenic soil, derived from the deposition of the fecal matter of various species of birds, is a major source of nutrient input in the Antarctic marine ecosystem. A significant proportion of the microbiota of ornithogenic soil collected from an Adélie penguin colony in eastern Antarctica consisted of bacteria identified as *Psychrobacter* strains. Phenotypic, genotypic, and 16s ribosomal DNA phylogenetic analyses revealed that the two Antarctic *Psychrobacters* strains were novel species, including *Psychrobacter urativorans* sp. (Bowman et al., 1996). *Psychrobacters cryohalolentis* K5 was found to have survived a 43,000-year burial within Siberian permafrost where it is capable of reproducing at $-10^\circ$C (Bakermans et al., 2003; Bakermans et al., 2006). On the WAP, krill are the main food source for Adélie penguins. Interestingly, *P. urativorans* was enriched in krill FPs. *P. cryohalolentis* K5 was also enriched in krill FPs relative to free-living in the water column and *Psychrobacter sp*. KCTC 72983 was found in FPs found in the Far South.

The genus *Psychromonas* includes piezophilic, halophilic, and psychrophilic adapted species that are widely distributed in aquatic environments and are important components of polar and deep-sea microbiota (Auman et al., 2010; 2Nogi et al., 2007). *Psychromonas* is commonly motile with polar flagellae. *Psychromonas antarcticus*, an aerotolerant anaerobic, halophilic psychrophile was isolated from pond sediment of the McMurdo Ice Shelf, Antarctica. Aerotolerant anaerobes use fermentation to produce adenosine triphosphate (ATP). They do not use oxygen, but they can protect themselves from reactive oxygen molecules. *Psychromonas* strain CNPT3 was isolated from a decaying amphipod collected at a depth of 5,700 m in the central North Pacific Ocean. The genome contains complete pathways for heterotrophic lifestyle such as the complete glycolytic
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and tricarboxylic acid pathways and was found to be enriched in genes for motility and chemotaxis (Lauro et al., 2013). On the WAP, *Psychromonas* sp. CNPT3 7 was enriched in krill FPs and preferentially found in the Far South.