Bacterial epibiont communities of panmictic Antarctic krill are spatially structured

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Short title: Krill microbiomes are spatially structured

Abstract

Antarctic krill (*Euphausia superba*) are amongst the most abundant animals on Earth, with a circumpolar distribution in the Southern Ocean. Genetic and genomic studies have failed to detect any population structure for the species, suggesting a single panmictic population. However, the hyper-abundance of krill slows the rate of genetic differentiation, masking potential underlying structure. Here we use high-throughput sequencing of bacterial 16S rRNA genes to show that krill bacterial epibiont communities exhibit spatial structuring, driven mainly by distance rather than environmental factors, especially for strongly krill-associated bacteria. Estimating the ecological processes driving bacterial community turnover indicated this was driven by bacterial dispersal limitation increasing with geographic distance. Furthermore, divergent epibiont communities generated from a single krill swarm split between aquarium tanks under near identical conditions suggests physical isolation in itself can cause krill-associated bacterial communities to diverge. Our findings show that Antarctic krill-associated bacterial communities are geographically structured, in direct contrast with the lack of structure observed for krill genetic and genomic data.

Introduction

Antarctic krill (*Euphausia superba*) is a keystone species of Southern Ocean food webs (Croxall *et al.* 1999), with an estimated biomass of 379 million tonnes (Atkinson *et al.* 2009). The commercial Antarctic krill fishery catch is the largest in the Southern Ocean and has been expanding in recent years (Nicol & Foster 2016). However, management of the krill fishery is hampered by lack of knowledge regarding krill population structure and the number of effective stocks.

Krill have a circumpolar, but non-uniform, distribution, with highest densities in the South Atlantic near the Antarctic Peninsula and Scotia Arc (Siegel & Watkins 2016). The presence of persistent areas of high krill density has led to suggestions that there may be between three and six separate krill stocks in the Southern Ocean (Latogurski 1979; Mackintosh 1973), but this has not been supported by genetic

and genomic studies over the last four decades (Deagle *et al.* 2015). The current consensus is that the genetic and genomic data are consistent with a single panmictic circumpolar population, however, any demographic structure may be obscured by the hyper-abundance of Antarctic krill (Jarman & Deagle 2016). Genetic drift in neutral markers is inversely related to population size, and the vast number of individual krill would slow genetic differentiation between subpopulations, even in the absence of homogenising gene flow (Deagle *et al.* 2015).

All eukaryotes carry a characteristic bacterial microbiome. Bacterial populations often exhibit more genetic structuring than those of higher eukaryotes, as large bacterial populations accumulate diversity that tends to create barriers to recombination between lineages (Yang *et al.* 2019). Spatial structuring of microbiomes has been demonstrated in several marine invertebrates (Cregeen 2016; Zwirglmaier *et al.* 2015), and can arise as a result of either environmental (including host) heterogeneity combined with selection, and/or dispersal limitation combined with ecological drift (Stegen *et al.* 2015). In a recent study, we showed that four krill swarms separated by 260-1270 km each supported distinct microbiomes, with the strongest differences observed in bacteria associated with the exoskeleton (Clarke *et al.* 2019). In this study, we expand the number of swarms and the number of krill sampled per swarm, and examine a greater range of geographic distances to test whether krill-associated bacteria show spatial structuring, and whether the observed structure is more closely related to geographic or environmental distance. In supporting experiments, we investigate whether divergent krill microbiomes can be generated in aquaria under near identical conditions. Our results demonstrate krill microbiomes in different areas represent distinct communities, and may also be functionally different; these different microbe-host associations in different spatial areas may be meaningful as conservation units.

Results

We analysed the bacterial communities on 155 krill moults collected from krill from 13 swarms (n=11-12 moults per swarm) separated by 4-3481 km (Fig. 1a). Following filtering and quality control, this dataset included 4.49 million Illumina paired-end sequencing reads (10,787 to 87,547 reads per sample) representing 3,808 zero-radius OTUs (zOTUS). Moult bacterial communities were dominated by Gammaproteobacteria (mean relative abundance 53%), followed by Bacteroidia (27%) and Alphaproteobacteria (7%). Swarm membership (trawl) explained half the variance in moult microbiome composition based on weighted UniFrac distance, and more than 40% for unweighted UniFrac distance (weighted UniFrac: $F_{12,142}$=11.85, R^2=0.500, P<0.001, unweighted UniFrac: $F_{12,142}$=8.18, R^2=0.409, P<0.001). Ecological (unweighted UniFrac) distance between moult bacterial communities increased with increasing geographic distance between trawls (Fig. 2a). An UPGMA tree based on unweighted UniFrac distance showed bacterial communities clustered by geographic region (Fig. 1a and b), with 3-5 swarms sharing similar microbiomes near each of Mawson, Casey and Dumont d'Urville stations in East Antarctica. A single swarm near BANZARE Bank north-east of Mawson station (K29) supported a distinct microbiome, though most closely related to the swarms sampled off Mawson (Fig. 1b). The spatial structuring of krill microbiomes from the vicinity of Casey and Mawson is in direct contrast to the lack of structure observed in SNP markers for krill collected from the same two regions (Deagle *et al.* 2015) (Fig. 3).

Although we have previously demonstrated that krill moult bacterial communities are distinct from seawater bacterial communities (Clarke *et al.* 2019), moult bacterial communities are an open system that can exchange bacteria with the surrounding seawater. To investigate if variation in seawater bacteria could be driving the observed geographic structuring, we repeated the above analyses using only strongly krill-associated bacteria (present in ≥50% of samples) to reduce the potential influence of local environmental bacteria. This reduced the dataset from 3,808 to 267 zOTUs but, as these were the more common bacteria, still represented 73.3% of reads. The entire moult and strongly krill-associated bacterial communities were taxonomically very similar (see Supporting Information Text)

and strongly krill-associated bacterial communities clustered by region as per Fig. 1b (Fig. S1). Swarm membership explained approximately half the variance in strongly krill-associated bacterial community composition (weighted UniFrac: R^2=0.469, unweighted UniFrac: R^2=0.529, P<0.001 for both). Unweighted UniFrac distance between strongly krill-associated bacterial communities also increased with geographic distance between trawls (Fig. S2a).

Identity of dominant core zOTUs differs between regions

The core microbiome (zOTUs present in 85% or more of the moult samples) comprised only 21 zOTUs representing 30.9% of reads. The core microbiome was dominated by zOTUs from the families Colwelliaceae (13 zOTUs), Rhodobacteraceae (4 zOTUs, 2 *Sulfitobacter*) and Flavobacteriaceae (3 zOTUS, 2 *Polaribacter*). Most of the Colwelliaceae zOTUs (11/13) had 97-99% identity to two closely related (99% identity) *Colwellia* species isolated from Antarctic sea-ice assemblages (Bowman *et al.* 1998) (Table S1). Other core zOTUs were highly similar (99-100% pairwise identity) to bacterial isolates from polar marine invertebrates (*n*=3 zOTUs) and to Antarctic sea ice bacteria (*n*=3 zOTUs), indicating these high nutrient surface environments in polar oceans are commonly colonised by similar bacterial assemblages. Interestingly, despite being present in the majority of samples and representing a high proportion of the total reads, the identity of dominant core zOTUs varied between geographic regions (Fig. 4). Four core zOTUs (three *Colwellia* and one *Sulfitobacter*) were identified as having significantly different relative abundances between regions (LEfSe, LDA > 3, $\alpha < 0.01$). Inferred metagenomic traits also suggest krill moult bacterial communities from different regions are functionally distinct (Fig. S3 and 4).

Environmental and spatial effects

We used linear mixed-effects (LME) modelling to test whether spatial (geographic distance) and/or environmental variables (ocean temperature, salinity, sea-ice melt and productivity) explained variation in krill microbial community composition between trawl sites. Geographic distance (LME

model coefficient estimate: 0.065±0.003 [0.060–0.070 95%CI]) and salinity (0.009±0.003 [0.003–0.014 95%CI]) were the only significant predictors for moult community composition (z = 23.01, $p < 0.001$ and z = 3.19, $p = 0.0014$, respectively, Table S4), based on model-averaging over the top-ranked models (n=8) using ΔAICc ≤ 10. In contrast, geographic distance (0.066±0.003 [0.059-0.073 95%CI]) was the only significant predictor for strongly krill-associated bacterial communities (z = 18.8, $p < 0.001$, Table S6), with weaker evidence for an effect of salinity depending on the random-effects specification (custom covariance structure: z-value=1.48, *p*-value=0.14, Table S6; crossed effects z-value=2.21, *p*-value=0.027, Table S10). Results were very consistent overall using the two different random-effects specifications (Table S3-10). All top-ranking (i.e., lower AICc) models included geographic distance (rather than only environmental variables) for both the entire moult community and strongly krill-associated bacteria.

Krill microbiome differentiation driven by dispersal limitation over large geographic distances

We used a combination of null models to estimate the contribution of homogeneous or variable selection, homogenising dispersal and dispersal limitation to krill microbiome community turnover (Stegen *et al.* 2013; Stegen *et al.* 2015). In our data, bacterial communities on krill with increased geographic separation showed increased dispersal limitation and decreased influence of homogenising dispersal, homogeneous selection and undominated processes. Undominated processes were the major influence for moult communities sampled from krill within a trawl (52%, Fig. 2b). Comparing samples between trawls within a cluster (e.g., Mawson, Casey and Dumont d'Urville), the influence of dispersal limitation increased from 9 to 29%, with decreased influence of homogenising dispersal and undominated processes. Dispersal limitation was the main factor when comparing communities between clusters (57%), with a further decrease in the influence of undominated processes (18%). The contribution of homogeneous selection and homogenising dispersal also decreased from 11 to 2% and 11 to 0.3%, respectively, with increasing geographic

144 separation. Strongly krill-associated bacteria showed similar patterns of increasing dispersal limitation
145 with increasing geographic distance (Fig. S2b).
146
147 ***Krill in identical aquarium conditions develop divergent microbiomes***
148 To investigate the process of microbiome differentiation, krill from a single swarm off Casey station
149 (T04, see Fig. 1a) were split across four 200 L aquarium tanks, with approximately 400 krill per tank,
150 and with consistent conditions for seven months prior to sampling. Krill from each tank developed
151 significantly different epibiont bacterial communities, with twice the proportion of variation explained
152 by tank of origin when relative abundance was taken into account, compared to presence/absence of
153 taxa (weighted UniFrac (WU): $F_{3,44}$=12.09, R^2=0.452, P<0.001, unweighted UniFrac (UU): $F_{3,44}$=4.57,
154 R^2=0.237, P<0.001, Fig. 5a and b). Bacterial communities in water within the four tanks were clearly
155 distinct from krill communities (Fig. S5, WU: $F_{1,54}$=46.56, R^2=0.463, P<0.001, UU: $F_{1,54}$=11.91, R^2=0.181,
156 P<0.001), but were also significantly different between tanks (WU: $F_{3,4}$=2.87, R^2=0.682, P=0.015, UU:
157 $F_{3,4}$=1.65, R^2=0.553, P=0.024). However, this was largely driven by tank 2A, as tank water bacterial
158 communities were not significantly different when this tank was excluded (2B, C and D, WU: $F_{2,3}$=2.78,
159 R^2=0.650, P=0.13, UU: $F_{2,3}$=1.56, R^2=0.510, P=0.067), whereas epibiont communities were distinct
160 (WU: $F_{2,33}$=11.50, R^2=0.411, UU: $F_{2,33}$=4.91, R^2=0.229, P<0.001 for both).
161
162 Comparing the ecological processes contributing to community turnover within versus between tanks,
163 the contribution of homogenising dispersal decreased from 17 to less than 1%, whereas dispersal
164 limitation increased from 9 to 20% (Fig. 5c). Interestingly, the influence of variable selection (reflecting
165 different environments) decreased from 7% within tanks to 1% between tanks, supporting the notion
166 that the tanks represented highly similar environments.
167

Discussion

We show that Antarctic krill-associated bacterial communities are geographically structured, in direct contrast with the lack of structure observed for krill genetic and genomic data (Deagle *et al.* 2015). Individual taxa and communities within the human microbiome can be used as markers of migration and ancestry (Dominguez-Bello & Blaser 2011; Henne *et al.* 2014), allowing resolution of migration events previously indistinguishable with human genetic markers (Moodley *et al.* 2009; Wirth *et al.* 2004). The potential for members of the human microbiome to accurately trace human migrations is dependent on the degree to which they are vertically transmitted (Dominguez-Bello & Blaser 2011). In contrast, structuring of krill-associated bacterial communities is driven by homogenising dispersal within a krill swarm (horizontal transmission), but increasing dispersal limitation with increasing geographic distance (Fig. 2). Our findings support the conclusion from a study of bat fur microbiomes (Kolodny *et al.* 2018) that for species experiencing high levels of homogenising dispersal between individuals, the colony or swarm rather than the individual may be the meaningful biological unit on which selection influences the microbiome. Krill microbiome dynamics can thus be understood best in light of metacommunity theory, being influenced by both processes within the community (e.g., competition) and dispersal between communities (Burns *et al.* 2017; Miller *et al.* 2018).

The significant effect of spatial distance on both moult and strongly krill-associated bacterial communities, but limited evidence for environmental influence on the latter suggests different environments are not essential for the development of distinct bacterial communities. This is further supported by development of divergent microbiomes in aquarium krill populations under near-identical conditions. The contribution of variable selection, reflecting community differences arising due to selection in different environments, showed a modest increase comparing bacterial communities within a swarm versus those in separate bacterial geographic clusters (17 to 21% for entire moults communities, 23 to 26% for strongly krill-associated bacteria). In contrast, the contribution of dispersal limitation showed a much larger increase from 9 to 57% (Fig. 2b). If

194 environmental factors were driving variation in krill microbiomes, we would expect swarms in
195 different water masses to have distinct bacterial communities, as the Southern Ocean fronts separate
196 waters with distinct physical and chemical properties. However, the Casey and Dumont d'Urville
197 clusters include swarms north and south of one or more oceanographic fronts (Fig. 1a). Similarly, LEfSe
198 analysis showed that swarms north or south of the southern boundary of the Antarctic Circumpolar
199 Current were not enriched in particular zOTUs (Table S11). Rather, clusters were mainly distributed
200 west to east rather than north to south, consistent with meso-scale circulation in the region. In
201 particular, Mawson (and BANZARE Bank) clusters correspond to the Prydz Bay gyre, and the Casey
202 cluster corresponds to recirculation within the Australian-Antarctic basin (Bindoff *et al.* 2000). A series
203 of cyclonic eddies off the Adelie Coast that terminates near 130° E may link krill swarms in the Dumont
204 d'Urville cluster, whilst isolating them from sites further west (Aoki *et al.* 2007). However, the
205 influence of circulation patterns, environmental factors and variable selection on krill microbiomes
206 warrants further investigation. The difference observed for aquarium populations between distance
207 measures including or excluding relative abundance suggests ecological drift may be driving
208 divergence in isolated populations in the absence of environmental selection. Weighted UniFrac
209 distance (including relative abundance) explained twice the proportion of variation between tanks
210 compared to unweighted UniFrac distance (a presence/absence metric), suggesting changes in
211 relative abundance precede changes in community membership.
212
213 Ecological drift in krill epibiont communities is likely exacerbated by the regular moulting of the
214 exoskeleton, which occurs every 12-30 days during summer (Reiss 2016). Characterising bacteria from
215 moulted exoskeletons means samples represent bacterial communities at the same stage of the moult
216 cycle. However, this was not the case for the aquarium samples. The occurrence of ciliate epibionts
217 decreases from 66% in pre-moult individuals to 0% in post-moult krill (Tarling & Cuzin-Roudy 2008),
218 with bacterial epibionts likely to experience a similar, but potentially less extreme, drop in abundance.
219 Subsequent colonisation may then occur essentially at random by whichever species gets there first,

following the “competitive lottery model” (Burke *et al.* 2011; Hubbell 2001), although the presence of protozoan epibionts could also influence bacterial moult community composition. The existence of a core microbiome is consistent with the need for species to have similar ecologies to compete for the same vacant niche. Given krill swarms can exceed densities of 1000 individuals m^{-3} (Nowacek *et al.* 2011; Tarling *et al.* 2009), colonisation is most likely to occur from epibionts on neighbouring krill, hence the greater influence of homogenising dispersal for bacterial communities within a swarm (Fig. 2b). Homogenising dispersal should have less influence in smaller or less dense swarms, leading to faster rates of drift. Although bacteria on model marine (chitin) particles exhibit rapid and reproducible succession patterns (Datta *et al.* 2016), further aquarium studies are required to better understand krill epibiont colonisation dynamics.

The fishery for Antarctic krill is the largest by tonnage in the Southern Ocean (Nicol & Foster 2016). The krill fishery is managed using regional catch limits, but whether there is a single circumpolar krill stock or if there are distinct populations within each region is unclear (Jarman & Deagle 2016). We have shown that krill microbiomes in different regions represent distinct communities, indicating a non-homogeneous mixture throughout their range. Microbiomes may also be functionally different and furthermore affect host fitness. It seems plausible that different microbe-host associations in different regions may be meaningful as conservation units. Current spatial management of the krill fishery is focussed within the southwest Atlantic, where small-scale management units have been established. Our results suggest that the Indian Sector of the Southern Ocean contains at least two distinct spatial clusters of krill bacterial communities, reflecting different microbe-host associations, near Casey and Dumont d’Urville which both lie within a single large management division. A better understanding of how microbiomes vary at different temporal scales, including throughout the moult cycle, and both seasonal and interannual variation, will be necessary to understand how to apply microbe-host associations to inform regional krill fishery management.

There are many ways population connectivity can be measured; from ancient linkages through phylogeography, to more recent linkages with population genetics, and contemporary movement though animal tracking. Several studies have now demonstrated links between host population genetic structure and host-associated microbiome composition in marine species (Díez-Vives *et al.* 2020; Easson *et al.* 2020; Fietz *et al.* 2018). Our study does not explicitly test whether there is a direct association between krill moult microbiomes and krill population dynamics; indeed this is not possible given that we have little independent information about krill population dynamics. However, we consider that some link between krill microbiomes and population connectivity is a reasonable hypothesis given the apparent lack of an environmental driver and observed isolation by distance. Future studies should explore and ideally test whether there is an association between krill microbiomes and krill population dynamics, especially in the key krill fishery areas of the Antarctic Peninsula and Scotia Arc. Supporting aquarium experiments that investigate horizontal transmission rates between krill with distinct microbiomes, and between krill and the water column, would also assist establishing the scope of inference.

The potential for long-distance dispersal in many marine taxa leads to high population connectivity, making it difficult to resolve population structure with classic genetic approaches (Kelley *et al.* 2016). An improved view of host-microbiome structuring could expand our understanding of system connectivity in the marine environment. Studying the structure of the bacterial communities that effectively hitch a ride with their host may allow previously unseen linkages and barriers to dispersal to be identified.

Materials and Methods

Sample collection

Samples were collected on board the *RSV Aurora Australis* during voyage 3 between 31 January and 19 February 2016 (Kerguelen Axis voyage), and voyage 2 between 16 December 2016 and 16 January

2017 (Totten Glacier voyage). Antarctic krill were sampled from 13 swarms across the Indian sector of the Southern Ocean (Fig. 1a) using targeted trawls with a Rectangular Mid-water Trawl 8+1 (RMT-8+1 metre square) net. Trawls were made on acoustically identified targets at depths between the surface and 60-70 m. The pairwise distance between trawls ranged from 4 to 3481 km.

In order to isolate moults, live krill were transferred immediately after capture to 250 mL jars (one krill per jar) which were ventilated with small holes to allow seawater exchange as per Virtue et al. (2010). The jars were incubated in a large (1600 L) flow-through seawater tank close to ambient ocean temperature (approx. 1 °C) with no additional food provided (Kawaguchi *et al.* 2006). Jars were inspected for moults at 12 hour intervals, with the first 12 animals to moult from each trawl sampled for microbial community profiles (all collected within 48 hours). Moults were removed from the jar, rinsed with 0.22 µm-filtered seawater and stored separately in liquid nitrogen before being stored at -86 °C on return to Australia.

Aquarium samples

Krill from a single swarm caught off Casey station mid-December 2016 (T04 in Fig. 1a) were split across four 200 L krill aquaria tanks at the end of January 2017, with approximately 400 krill per tank. Krill in each tank experienced identical conditions for seven months, including light environment, food (phytoplankton), with the same filtered and UV-sterilised water supply for each tank (Kawaguchi *et al.* 2010; King *et al.* 2003). In September 2017, 12 krill per tank were individually netted and both lateral surfaces swabbed with Epicentre Catch-All™ sample collection swabs for approximately 10 seconds. Krill were then sexed and staged. Swabs were transferred to sterile Eppendorf tubes and stored at -86 °C. Nets were treated with 1% bleach then 0.5% sodium thiosulphate and rinsed with reverse-osmosis and deionised water between individuals. Tank water bacterial communities were sampled by filtering 1 to 1.6 L onto 0.22 µm Sterivex™ filters, with two samples per tank. Filters were transferred to tubes prior to extraction.

298

299 ***DNA extraction, PCR amplification and high-throughput sequencing***

300 Swabs (n=48) were sent to the Australian Genome Research Facility (AGRF, Adelaide, Australia;
301 http://www.agrf.org.au) on dry ice, and moults (n=155), Sterivex filters (n=8) and extraction controls
302 (1 ml ethanol) were sent in ethanol on dry ice. DNA was extracted from all samples using the QIAGEN
303 DNeasy PowerLyzer PowerSoil kit. DNA concentrations were quantified using a NanoDrop ND-8000
304 Spectrophotometer (ThermoFisher Scientific). PCR amplification, amplicon purification and high-
305 throughput sequencing of bacterial 16S V1-3 rRNA (primers 27F: AGAGTTTGATCMTGGCTCAG, Lane
306 1991; and 519R: GWATTACCGCGGCKGCTG, Lane *et al.* 1985) were carried out at the Ramaciotti
307 Centre for Genomics (Sydney, Australia) on an Illumina MiSeq following the Australian Marine
308 Microbes protocol (Brown *et al.* 2018).

309

310 ***Data analysis***

311 DNA sequence processing and taxonomic assignment followed the Australian Marine Microbial
312 Biodiversity Initiative workflow (Brown *et al.* 2018), with data presented as amplicon sequence
313 variants, or zero-radius operational taxonomic units (zOTUs, Edgar 2016), to maximise potential
314 phylogenetic resolution. In brief, paired-end reads were merged, short sequences (<400 base pair, bp)
315 and sequences containing N's or homopolymer runs >8 bp were removed. Sequences were de-
316 replicated and those with <4 representatives removed. Chimeras were removed and zOTUs identified
317 using the UNOISE3 algorithm(Edgar 2016). Quality-filtered sequences (including those <4
318 representatives) were mapped to the zOTUs to create a sample-by-read abundance matrix. Taxonomy
319 was assigned to each zOTU using the RDP Bayesian classifier (Wang *et al.* 2007) based on the SILVA
320 v132 database (Yilmaz *et al.* 2014) and 60% probability cut-off. Lastly, zOTUs present in only one
321 sample or with less than 10 reads across the dataset were removed. The number of reads per sample
322 was 10,787-87,547 for moults, 13,083-72,384 reads for swabs, and 5,107-10,215 for the Sterivex
323 filters, with the number of observed zOTUs approaching saturation at the minimum read depth for

each sample type (Fig. S6). DNA sequence data for this study can be found in the NCBI database under BioProject ID: PRJNA505226. The zOTU table and mapping file are available on the Dryad data repository (Clarke *et al.* 2020).

Differences in entire moult and strongly krill-associated (present in ≥50% of samples) bacterial community composition between swarms were explored using weighted and unweighted UniFrac distances (Lozupone & Knight 2005) in QIIME v1.8.0 (Caporaso *et al.* 2010) (beta_diversity_through_plots.py) based on a rarefied zOTU table (10,000 reads or 5,000 reads for entire moult and strongly krill-associated bacteria, respectively), with strength and significance of swarm assessed using the Adonis method (Anderson 2001) (compare_categories.py, 999 permutations). The same method was used to characterise differences between krill microbiomes from separate aquarium tanks, with the zOTU table rarefied to 5,000 reads for analyses including the Sterivex filters. The phylogenetic tree used for the UniFrac analysis and to estimate the contribution of selection and dispersal processes to microbiome turnover (see below) was generated in QIIME using PyNAST (Caporaso *et al.* 2009) to align sequences against the Greengenes (v13_8) core set (McDonald *et al.* 2012), then filtering the alignment (removing 0.0005% most variable positions and those that were >80% gaps) and building the tree using FastTree 2.1.3 (Price *et al.* 2010).

We defined core moult microbiome membership as zOTUs present in 85% or more moult samples (>131/155 samples) using QIIME (compute_core_microbiome.py). Ecological dissimilarity between krill moult bacterial communities was visualised by an UPGMA tree based on unweighted UniFrac distance created using the jackknifed_beta_diversity.py workflow in QIIME, with subsampling repeated 100 times. The Linear Discriminant Analysis (LDA) Effect Size (LEfSe, Segata *et al.* 2011) method was used to highlight zOTUs that showed different abundances between bacterial geographic clusters (Fig. 1). Default settings were used except the LDA threshold was increased to 3.0 and the α-value reduced to 0.01 to highlight the most significant taxa discriminating between clusters.

We tested whether spatial (geographic distance) and/or environmental variables explained variation in krill microbial community composition between trawl sites using a linear mixed-effects (LME) modelling approach (R package 'lme4', Bates *et al.* 2015). Analyses were performed separately for both the entire moult community and strongly krill-associated bacteria. The response variable was calculated as the mean unweighted UniFrac distance across all pairwise krill samples for each pair of trawl sites (see Fig. 2a). We included a random-effects term for the non-independent error structure of pairwise datasets (Clarke *et al.* 2002; Row *et al.* 2017) by setting up the covariance structure such that a proportion (ρ_τ) of the total variance (σ^2) is due to the correlation between data points that share a common site. Thus, the covariance for *n* pairwise data points that share a common site is $\rho_\tau\sigma^2$ and zero for those that do not (Clarke *et al.* 2002; Van Strien *et al.* 2012). These results were compared against LME models that more straightforwardly specified the two trawl sites as crossed random effects.

Predictor variables considered as fixed-effects included geographic distance (km) between trawl sites and a suite of environmental variables obtained from both ship-based underway (sea surface temperature [°C] and salinity [on the practical salinity scale, PSS]) and satellite-derived data (time [weeks] since sea-ice melt, surface chlorophyll-*a* [mg m^{-3}]). Full descriptions of these environmental data are given in the Supporting Information. Euclidean distances were calculated for all environmental variable pairs between sites. We centred and scaled all predictor variables and ensured that the maximum correlation between predictor variables (all Pearson correlations <0.66) and variance inflation factors (all VIFs ≤ 1.52) were acceptably low prior to model fitting (Zuur *et al.* 2010).

We adopted a multi-model inference and model averaging approach (Burnham & Anderson 2002; Burnham *et al.* 2011) using the R package 'MuMIn' (Barton 2019) to generate a set of models with all combinations of fixed-effects. We calculated the marginal (R_m^2) and conditional (R_c^2) coefficient of

determination (Nakagawa & Schielzeth 2013) for all models, and for model evaluation used the second-order Akaike Information Criterion (AICc) suitable for small sample sizes, ranking the models via Akaike weights. For model averaging we included all models with a ΔAICc ≤ 10 from the top-ranked model. We calculated the 95% confidence interval (CI) of the model-averaged regression coefficients (full average) and considered those which did not straddle zero to have a significant effect on the response under investigation. Results are presented in text as LME coefficient estimate ± SE [95% CI].

We used the procedures described by Stegen et al. (2013; 2015) to estimate the contribution of selection and dispersal processes to krill microbiome assembly. Variable selection generates divergent communities due to distinct selective environments, whereas homogeneous selection generates convergent communities due to their presence in similar selective environments. Similarly, divergent communities arising primarily due to low rates of dispersal, which causes communities to drift apart, is referred to as 'dispersal limitation', whereas high dispersal preventing drift is referred to as 'homogenising dispersal'. The fraction of community turnover where neither selection nor dispersal is the primary driver of community turnover (e.g., due to the combination of moderate dispersal and weak selection) is referred to as 'undominated' (called 'drift' in Stegen *et al.* 2013; Stegen *et al.* 2015).

Firstly, we computed the between-community mean nearest taxon distance (βMNTD), the mean phylogenetic distance between each zOTU in one community and its closest relative in a second community. A null-model distribution of this parameter was generated by randomly shuffling zOTUs across the tips of the phylogeny (999 permutations). β-nearest taxon indices (βNTI) were calculated as the difference between the observed βMNTD and the mean of the null distribution, expressed in units of standard deviations; βNTI values < -2 or > +2 were deemed significant deviations and indicative of homogeneous selection or variable selection, respectively. Where pairwise comparisons showed |βNTI|< 2, we used the Bray-Curtis-based Raup-Crick metric (Chase *et al.* 2011; Stegen *et al.* 2013) to compare observed and expected turnover without using phylogenetic information to infer the

contribution of homogenising dispersal, dispersal limitation, or undominated processes (moderate dispersal and weak selection, referred to as 'drift' in Stegen *et al.* 2013). For pairwise comparisons where $|\beta NTI| < 2$, $RC_{bray} < -0.95$ represented homogenising dispersal, $RC_{bray} > +0.95$ represented dispersal limitation, and $RC_{bray} < |0.95|$ suggested no single ecological process dominated compositional turnover. We compared the relative contribution of each process for pairwise comparisons of krill bacterial communities within a swarm, between swarms within a bacterial geographical cluster (see Fig. 1), and between swarms in separate clusters; and within and between tanks for the aquarium populations.

Data Acessibility

DNA sequence data for this study can be found in the NCBI database under BioProject ID: PRJNA505226.

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590 Scotia Ridge (Antarctica) hydrothermal vents. *MicrobiologyOpen* **4**, 136-150.

591

592 **Author contributions**

593 LC, LS, RK, and BD conceived the experiments and collected the samples. LC, AB and SB performed the
594 data analysis. LC wrote the manuscript. All authors contributed to discussing results and the final
595 version of the manuscript.

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597 **Competing Interests**

598 The authors declare no competing interests.

599

Figures

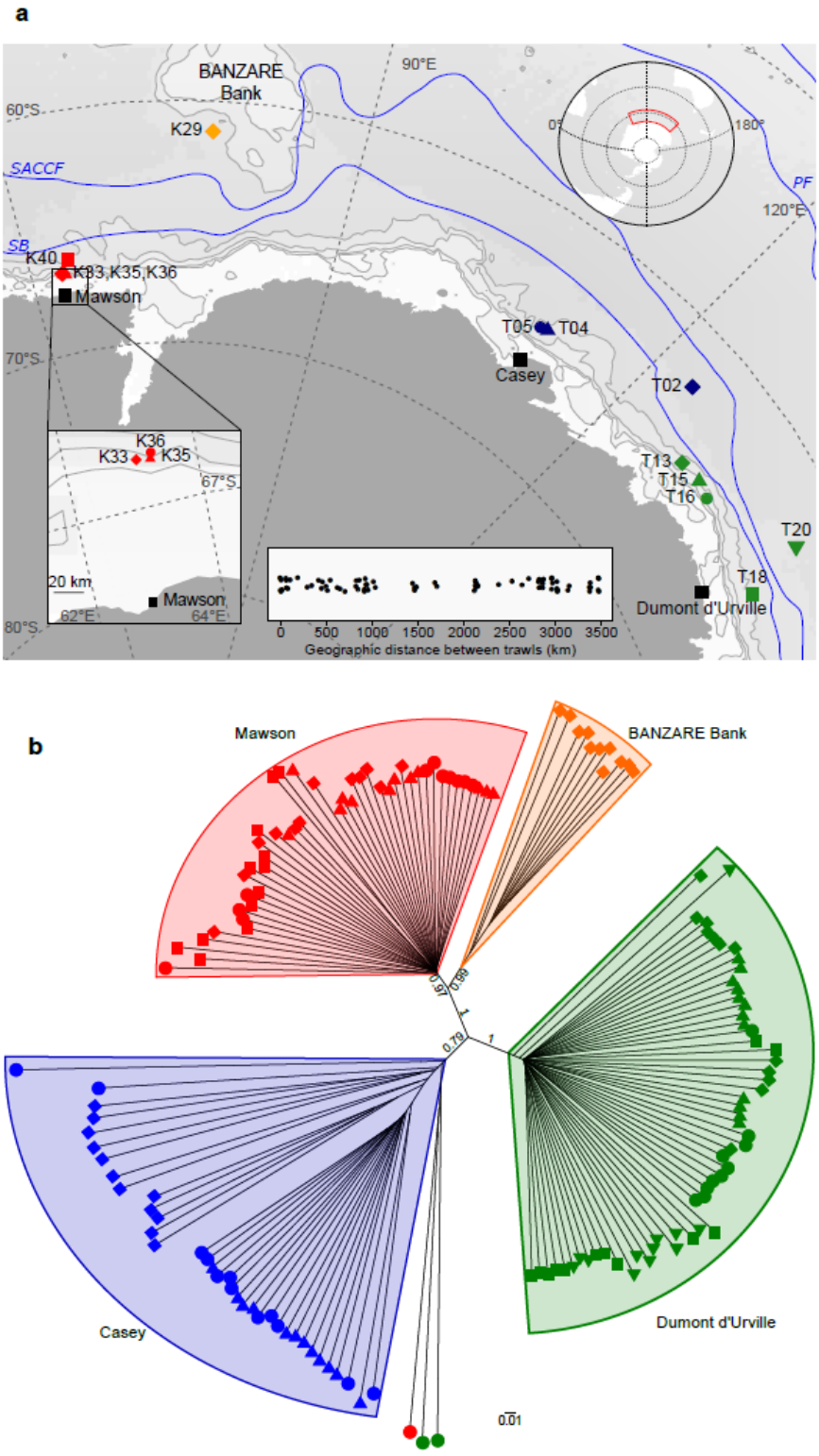


Fig. 1. Spatial structuring of krill moult bacterial communities. a Map of Southern Ocean trawl locations off the East Antarctic coast used to study krill-associated bacteria. Trawls are coloured according to clustering in (**b**). The inset shows three trawls near Mawson station separated by 4-11 km. Mean locations of the principal fronts (following Orsi *et al.* 1995) are shown as blue lines. PF – Polar Front, SACCF – southern Antarctic Circumpolar Current front, SB – Southern Boundary of ACC. **b** UPGMA clustering of krill moult bacterial communities based on unweighted UniFrac distance. Trawls within each region are differentiated by symbols. Jack-knife support is shown for central branches.

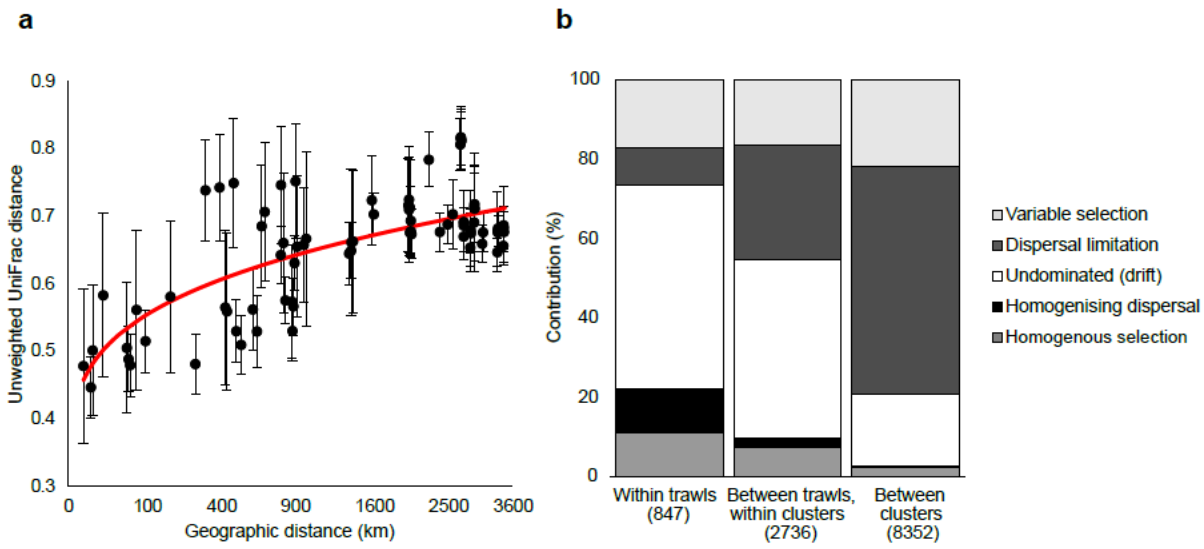


Fig. 2. Krill microbiome differentiation driven by dispersal limitation over large geographic distances. **a** Geographic vs. ecological (unweighted UniFrac) distance for krill moult bacterial communities from separate trawls. The x-axis has been square-root transformed for clarity. Equation of the fitted line: y = 1/(2.354 - 0.116 * log(x)), adjusted R^2 = 0.432, $p < 2 \times 10^{-16}$. Values are means ± SD unweighted UniFrac distance for all samples from each pair of trawls. **b** Contribution of ecological processes to krill moult bacterial community assembly at different geographical scales. The number of pairwise comparisons for each category is shown in brackets.

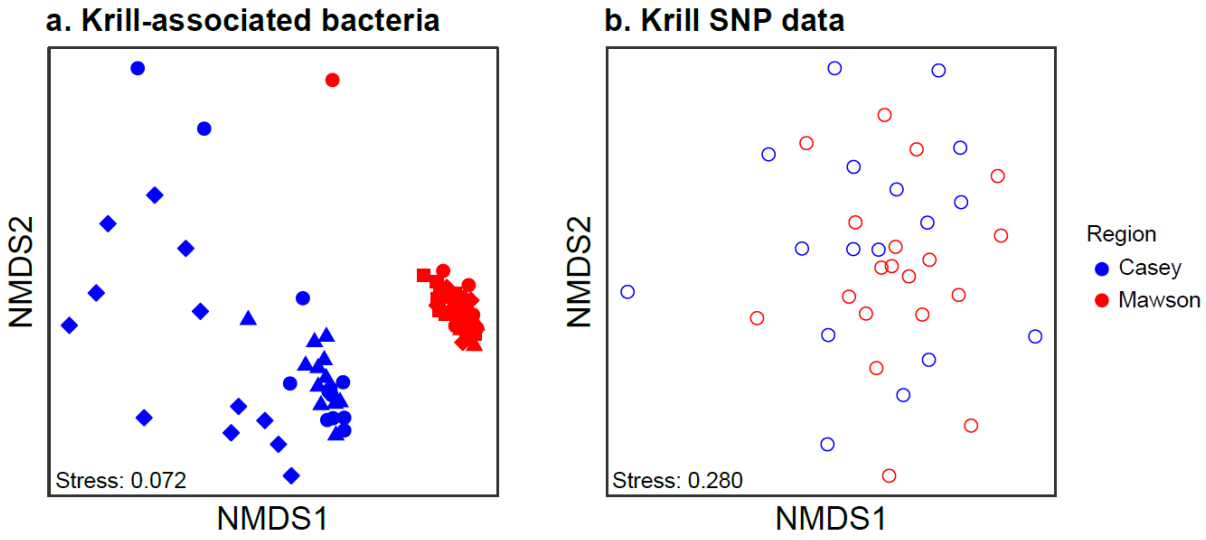


Fig. 3. Spatial structuring of krill microbiomes contrasted with lack of structure observed in SNP markers. Non-metric multidimensional scaling (nMDS) plots for krill from the vicinity of Mawson and Casey stations based on either (**a**) moult bacterial communities using unweighted UniFrac distance, or (**b**) Single Nucleotide Polymorphism (SNP) markers using Bray-Curtis dissimilarity of allelic sequence counts (data from Deagle *et al.* 2015). Krill-associated bacterial community data is from 3 and 5 trawls from the Casey and Mawson regions, respectively, coloured by bacterial geographic cluster as per Fig. 1.

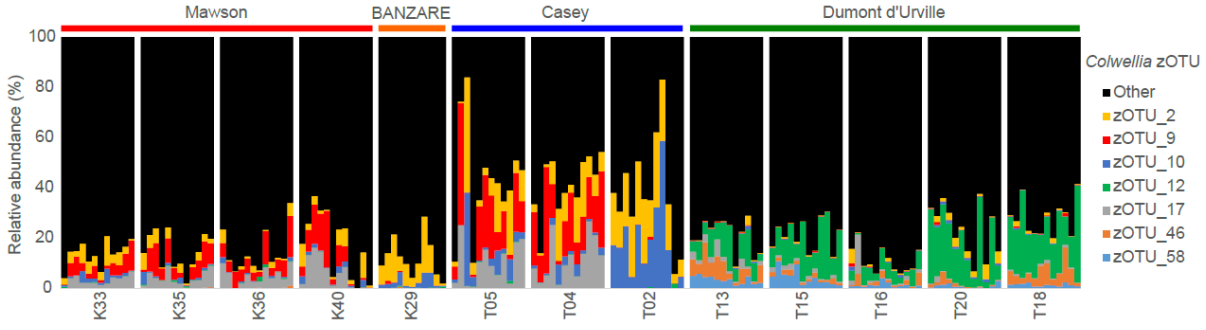
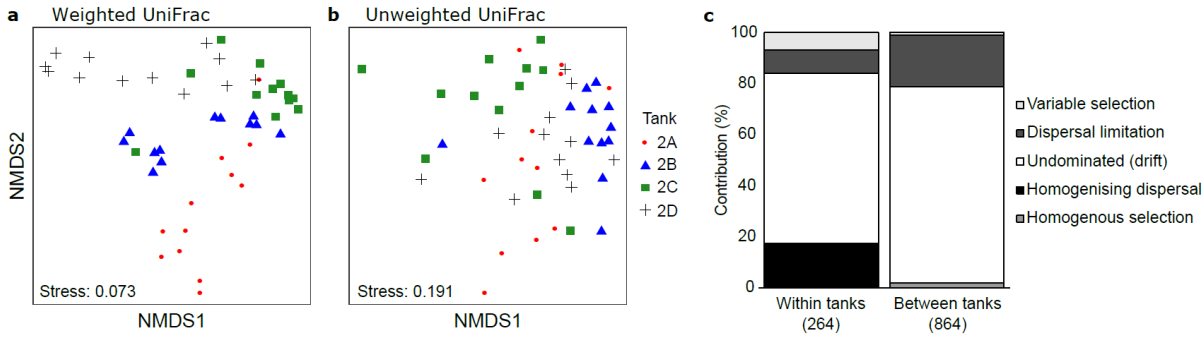


Fig. 4. Relative abundance of dominant *Colwellia* zOTUs in krill moults shown relative to all other zOTUs, highlighting variability between swarms within a single genus. Samples are grouped by trawl and arranged west to east. Each of the seven zOTUs are members of the core microbiome (present in 85% or more moult samples).



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634 **Fig. 5. Differences between krill bacterial communities originating from a single swarm split across**

635 **four aquarium tanks for seven months.** Non-metric multidimensional scaling (nMDS) plot based on

636 weighted (**a**) or unweighted UniFrac distance (**b**). **c** Contribution of ecological processes to krill

637 epibiont bacterial community assembly within versus between aquarium tanks. The number of

638 pairwise comparisons for each category is shown in brackets.

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