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Research Article

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Depth-dependent bacterial colonization on model chitin particles in the open ocean

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Abstract

Sinking particles transport carbon from the surface to the deep ocean. Microbial colonization and remineralization are important ecosystem services constraining ocean biogeochemistry by recycling and redistributing nutrients from the surface to the deep ocean. Fragmentation of particles by zooplankton and the resulting colonization by microorganisms before ingestion, known as 'microbial gardening', allows for trophic upgrading and increased microbial biomass for detritivorous zooplankton. Using model chitin particles incubated with seawater collected from the surface, mesopelagic and bathypelagic depths in the Northeast Atlantic Ocean, we determined particle-attaching bacterial communities to identify general and depth-specific candidates of particle colonization. Comparison of particle-attached communities at the amplicon sequence variant level showed that bacteria found on surface particles were also colonizers in the bathypelagic, in line with sinking particles promoting vertical connectivity. Bathypelagic particle-attached communities were most diverse. We propose that some particle colonizers attach to the surface and sink out with the particle, whilst other colonizers are depth-specific. This suggests that candidates for particle colonization differ with depth, which may be important when considering the implications for the delivery of ecosystem services, including carbon cycling and the role they play for zooplankton grazers.

Impact Statement

Microbial colonization on particles is an important ecosystem service impacting carbon flow in the deep open ocean via trophic upgrading and increased particle microbial biomass before ingestion by zooplankton in the deep ocean where particles can be nutritionally devoid. Using a model chitin particle approach, we show that particle colonizers differ across depth horizons, but some taxa are consistent across all, attaching to the surface and potentially sinking with the particle, and flourishing at depth. The diversity of particle colonizing bacteria is therefore key when considering the ecosystem services they play in marine carbon cycling, including providing essential nutrition for zooplankton.

Keywords: bacteria; environmental microbiology; biogeochemical cycles; marine microbiology; microbial diversity

Introduction

Sinking particles transport carbon from the surface to deep ocean and form a key component of the biological carbon pump, acting as a mode of organic carbon transport to mesopelagic and bathypelagic ecosystems (Honjo et al. 2014). Particles are microbial hotspots because of higher density of organic carbon and nutrients (Alldredge and Silver 1988). As attachment capability is a factor for colonizing microbes in addition to metabolic capability (Grossart et al. 2006), particle-attached microbial communities are distinct from surrounding bacterioplankton (Alldredge and Silver 1988, Fenchel and Blackburn 1999).

Microbial communities associated with particles result in rapid and efficient turnover of particulate organic carbon (POC) (Simon et al. 2002). Particles support communities with higher metabolic activity and different metabolic strategies from the surrounding water column (Shanks and Trent 1979). Since differences in metabolic activity can potentially be attributed to differences in microbial community composition (Balmonte et al. 2018), understanding the identity of particle colonizers can be useful in understanding major players in remineralization of carbon.

Particles are grazed on by detritivorous zooplankton, e.g. copepods (Jackson 1993, Wilson et al. 2008). In a process referred to as 'microbial gardening', grazing zooplankton fracture particles exposing new surface areas, promoting microbial attachment, and improving the trophic value of particles for subsequent grazers (Mayor et al. 2014). Microbial gardening allows zooplankton to utilize enzymatic repertoires of bacteria, which degrade refractory compounds (e.g. chitin), that they are unable to degrade themselves. Transformation of larger to smaller particles richer in microbial biomass provides an increased nutritional value available for metazoan growth (Mayor et al. 2014).

In temperate waters, zooplankton can fragment and ingest ~50% of sinking particles and stimulate microbial degradation (Giering et al. 2014). Microbial gardening is likely an important ecosystem service in the deep ocean where resources are scarce; however, knowledge on ecological processes

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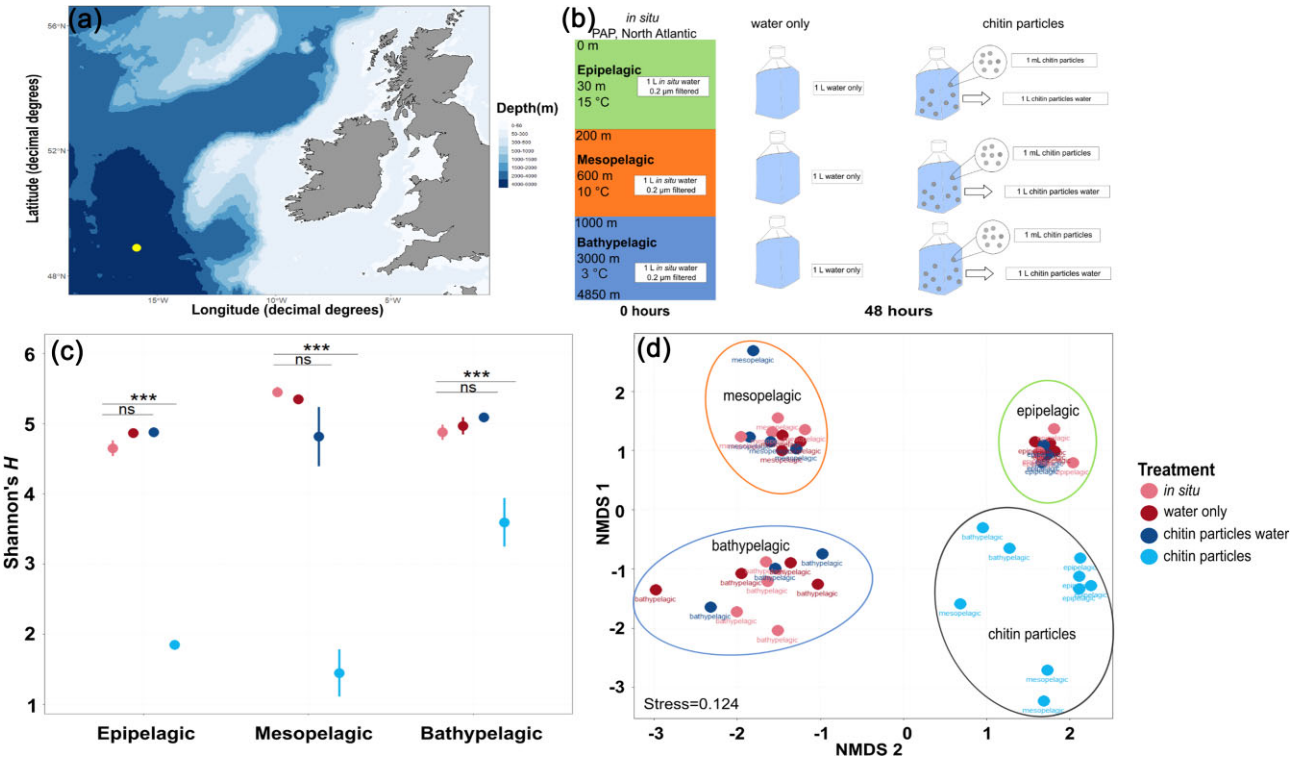

Figure 1. (a) Sampling site of PAP-SO. PAP-SO site marked by yellow point. (b) Experimental incubation setup. (c) Alpha diversity of bacterial community across all depths. Bars represent standard error and asterisks denote level of significance (***$P < 0.001$). (d) Nonmetric multidimensional scaling (NMDS) visualization of community structure across all treatments. Ellipses not statistically calculated but used to aid guidance of differences between depths and incubated chitin particle community.

involved in processing organic material in the so-called 'twilight zone' is limited (Martin et al. 2020). At present, taxa involved in microbial gardening (i.e. microbial taxa that attach to new particle surfaces) are poorly understood with few studies characterizing key members of the community involved (Kong et al. 2021) and with a limited focus on the mesopelagic and bathypelagic zones.

To address these knowledge gaps, we incubated model chitin particles in seawater collected from the epipelagic, mesopelagic, and bathypelagic depths at the Porcupine Abyssal Plain Sustained Observatory (PAP-SO) in the Northeast Atlantic Ocean. Chitin is one of the most abundant polysaccharides in the marine environment (Kirchner 1995) and a structural component of many marine organisms and associated debris (e.g. faecal pellets) (Weiner and Traub 1984, Yoshikoshi and Kô 1988, Latgé 2007, Durkin et al. 2009), forming an important part of the sinking flux (Souza et al. 2011). Observational and modelling-based estimates indicate that the Northeast Atlantic Ocean is the largest ocean sink for atmospheric CO_2 in the Northern Hemisphere, taking up $\sim 0.7 \pm$ Pg C year^{-1} (Gruber et al. 2002). The North-East Atlantic Ocean is an area with a high flux of organic carbon (Billett and Rice 2001, Lampitt et al. 2010), with chitinous sinking material estimated to comprise of 40% faecal pellets and 10% zooplankton carcasses (Lampitt et al. 1993, 2001). Despite chitin being a significant fraction of sinking material (Souza et al. 2011), many studies pool observations of sinking particles together, meaning the identities of microbes contributing to the remineralization of and redistribution of nutrients associated with chitinous particles specifically are missing. We aimed to explore the community composition on model chitin particles across the three major depth zones in the open ocean and identify cosmopolitan (i.e. throughout the water column) and depth-specific candidates colonizing on chitinous particles.

Material and methods

Study site and experimental setup

Sampling took place on board the *RRS Discovery* (cruise DY103) between 21 June and 9 July 2019 at the PAP-SO (49° N 16.5° W) (Fig. 1a). Seawater properties (salinity, temperature, oxygen, and fluorescence) throughout the water column were measured using a conductivity–temperature–depth rosette sampler (CTD SeaBird) (Supplementary Fig. 1a–f). Seawater was collected from epipelagic termed 'surface' (30 m), mesopelagic (600 m), and bathypelagic (3000 m) waters using Niskin bottles on the CTD rosette.

To determine the microbial communities in the water column (termed '*in situ*'), seawater (1 l) was filtered through 0.2 µm cellulose filters. Magnetic chitin microbeads (New England Biolabs; Ø100 µm) were used for incubations using protocols adapted from (Datta et al. 2016, Roberts et al. 2020). Incubations were carried out in 1 l polycarbonate bottles (Nalgene) containing seawater only (i.e. no microbeads) and seawater plus chitin microbeads (1125 ± 222 beads per ml) with four replicates for each treatment (Fig. 1b). Bottles were kept at *in situ* temperatures (30 m 15°C, 600 m 10°C, and 3000 m 3°C) in the dark with twice daily inversion (Fig. 1b). After 48 h, all bottles were harvested. Bottles containing seawater only were filtered on 0.2 µm cellulose

nitrate filters (Cole-Parmer). For bottles containing microbeads, seawater was passed through a 40-μm sterile cell strainers (Corning) to collect the microbeads. The strainer was inverted and washed with 0.2 μm filtered seawater from the respective depth into a sterile Petri dish (Sarstedt), from which the microbeads were harvested into a sterile centrifuge tube (Eppendorf). A magnet was used to collect the microbeads at the side of the tube and excess water was removed and discarded. The seawater that passed through the strainer was filtered onto 0.2-μm cellulose nitrate filters. All samples were preserved in a DNA/RNA Shield (Zymo Research, USA) and stored at −80°C. Some replicates from the mesopelagic and bathypelagic were lost during processing.

DNA extraction and metabarcoding

DNA was extracted using the ZymoBIOMICS DNA/RNA Miniprep Kit (Zymo Research, USA) following the manufacturer's instructions, including a blank extraction (e.g. no sample) to act as a 'kitome' to enable identification of potential reagent contaminants (Salter et al. 2014), which was also sequenced. Partial 16S rRNA gene (v4 region) was amplified using primers 515F (Parada) (Caporaso et al. 2011, Parada et al. 2016) and 806R (Apprill) (Apprill et al. 2015) followed by sequencing on the Illumina MiSeq platform and processed using the DADA2 pipeline to determine amplicon sequence variants (ASVs) (Callahan et al. 2016) in R Studio (R Core Team 2019). Demultiplexed reads were filtered and trimmed to remove primers and low-quality sequences. Paired-end reads were merged to obtain full denoised sequences. Chimeric sequences were removed and taxonomy was assigned using the naïve Bayesian classifier method via the *assignTaxonomy* function in DADA2 using the SILVA database (release 128) (Quast et al. 2013) and species level assignment using the SILVA species assignment database (release 128) (Quast et al. 2013). Chloroplast and mitochondria sequences were removed. ASV table, taxonomic assignment, and metadata were combined into a phyloseq object using the *phyloseq* package (McMurdie and Holmes 2013) and sequences were rarefied to 1614 reads before further analysis. Low rarefaction was required to retain enough replication of samples.

Data processing and statistical analysis

Shannon's index (H) was used to calculate alpha diversity, and the effect of depth and treatment on alpha diversity was determined using two-way ANalysis Of VAriance (ANOVA) with Tukey's Honestly Significant Difference (HSD) in R Studio (R Core Team 2019). Differences in community structure between samples were calculated using a Bray–Curtis dissimilarity matrix and visualized through NMDS ordination. Permutational multivariate analysis of variance (PERMANOVA) (Anderson 2001) was used to test the effect of depth and treatment on differences in community structure using the *adonis* function in the R package *vegan* (Oksanen et al. 2015).

Determining vertical microbial connectivity

To determine vertical connectivity of chitin particle colonizers, the number of ASVs that were shared or unique on incubated particles between depths were compared using the rarefied ASV table. Individual ASVs were categorized as shared or unique for each depth as follows (Supplementary Fig. 2). Individual ASVs that were detected only on incubated particles and not detected in the respective *in situ* bacterioplankton (potentially due to detection limits) were termed 'unique to particle' ASVs. Individual ASVs detected on both incubated particles and in the *in situ* bacterioplankton for the respective depth were termed 'shared *in situ*' ASVs. Assuming unidirectionality, i.e. the potential flow of ASVs is from surface to the bathypelagic only, individual ASVs detected on particles incubated in the mesopelagic and bathypelagic water, as well as in the surface, were termed 'shared with epipelagic incubated particles' and those not detected on surface incubated particles but detected on mesopelagic and bathypelagic incubated particles were termed 'shared with mesopelagic incubated particles'. In the case where there was overlap between categories e.g. an individual ASV was 'shared with epipelagic incubated particles' and 'shared *in situ*' in the bathypelagic, the ASV was defined as 'shared with epipelagic incubated particles' under the assumption of unidirectionality of an individual ASVs transport in the water column. Individual replicates were compared against pooled replicates of bulk communities to overcome differences in replication. Individual ASVs were grouped at the order level to determine vertical microbial connectivity. A two-way ANOVA with Tukey's HSD was used to determine significant enrichments of select particle-attached taxa incubated in bathypelagic water in comparison to particles incubated in surface water.

Results and discussion

Using model chitin particles incubated with seawater collected from the surface, and mesopelagic and bathypelagic depths in the Northeast Atlantic Ocean, we determined the diversity of particle-attaching bacterial communities to help identify depth-specific and general key taxa.

The diversity of particle-attached microbial communities was reduced in comparison to the surrounding bacterioplankton communities across all depths (Fig. 1c; Tukey's HSD $P < 0.05$), as previously reported of microbial communities colonizing chitin particles in marine and freshwater ecosystems (Datta et al. 2016, Roberts et al. 2020, Suominen et al. 2021). This suggests that there is a selection for distinct particle-attaching communities (Datta et al. 2016), potentially as a result of *r* strategists (e.g. fast- growing populations) in the surrounding water column responding rapidly to substrate enrichment on encountering a particle. While bacterioplankton alpha diversity remained relatively high across the three depth zones, the alpha diversity of particle-attaching communities was highest in the bathypelagic region, in agreement with previously reported at 2000 m on chitin particles (Suominen et al. 2021). This suggests a mechanism enriching bacterial ASVs on particles at depth.

Community structure varied between the depths and treatments, with a significant difference between these treatments (Fig. 1d; PERMANOVA $P < 0.001$). *In situ*, water only and particle water treatment from each depth were broadly similar, dominated by Flavobacteriales (particularly in the surface), Oceanospirillales, Pelagibacterales (SAR11), SAR324 clade (Marine group B), Rhodobacterales, Planctomycetales, and the Archaea order Thermoplasmatales (Fig. 2). The similarity of community profiles of the *in situ* and water only (i.e. 0 h vs. 48 h) highlights that bottling the communities did not have a profound impact on the respective community. Particle communities were more similar to each other than their respective treatments but distinct with depth,

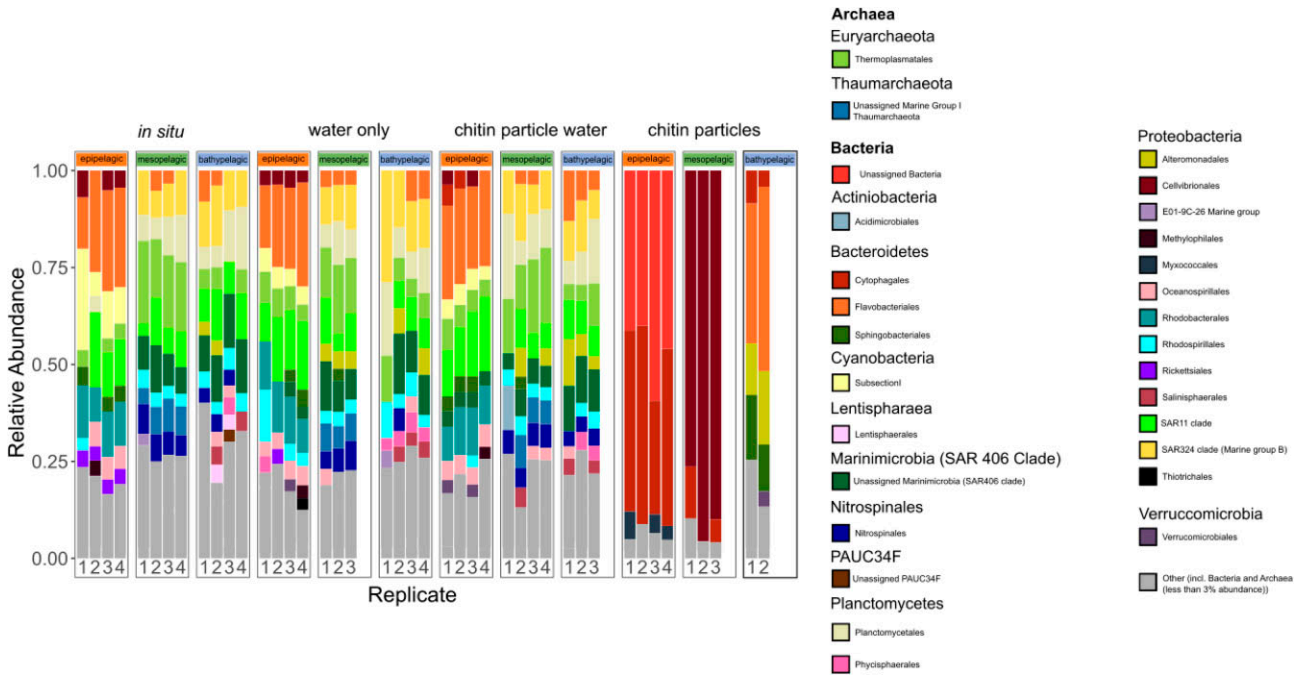

Figure 2. Order-level relative abundance of bacterial and archaeal ASVs across all treatments conducted across the three depths, epipelagic, mesopelagic, and bathypelagic for individual replicates grouped by Phyla. Relative abundances of >3% are grouped under 'other'.

indicated by separation on the NMDS plot for chitin particles by depth, although this trend was not statistically significant (Fig. 1d, Tukey's HSD $P = 0.1$). This is likely because of loss of replication the mesopelagic and bathypelagic incubations, resulting in low statistical power.

Particles exposed to surface water were dominated by Cytophagales and a large proportion of an unassigned ASV that the SILVA database could only assign to the kingdom of bacteria (Figs 2 and 3). Further BLAST(Basic Local Alignment Search Tool) searches of the ASV sequence against the NCBI nr database (Altschul et al. 1990) and the Microbe Atlas Project tool (Matias Rodrigues et al. 2017) showed high-similarity hits against previously recorded uncultured bacteria in the phylum Fibrobacteres (99% query cover; >83.96% identity cover, accession number: FJ753112.1) and the class Fibrobacteria (82% identity cover, MAP taxon ID:90_15 625) from each database, respectively. Fibrobacteres isolated from hypersaline soda lakes have been identified as chitin degraders (Rahman et al. 2016). Given the dominance of this taxon in the surface, further work should be done to uncover its identity and potential role as a candidate for chitin particle microbial gardening. Mesopelagic particles were dominated by Cellvibrionales and to a lesser extent also Cytophagales (Figs 2 and 3). Bathypelagic particles were more diverse than the surface and mesopelagic, namely Flavobacteriales along with Alteromonadales, Cellvibrionales, Cytophagales, and Sphingobacteriales (Figs 2 and 3). ASVs belonging to the Archaea were found attached to particles across the depth ranges, but these did not form greater than 0.12% of the relative abundance on particles (Supplementary Table 1), so were not considered further.

Our findings indicate that some members of particle-attached communities in surface waters are also found attached to particles in the mesopelagic and bathypelagic region of the open ocean, while others are depth-specific (Table 1). In the bathypelagic, 19.18% (±8.02 SD) of ASVs on particles were shared with ASVs found on particles in the surface with a further 8.96% (±0.89 SD) shared with particles from the mesopelagic (Table 1). In the mesopelagic, 29.60% (±6.78) of the of ASVs on particles were shared with particles in the surface (Table 1). In contrast, the number of ASVs shared between the particle-attached and reciprocal *in situ* communities decreased with depth (Table 1, $P < 0.05$). The surface particle-attached community shared a 51.20% (±4.43 SD) ASV similarity with the *in situ* community, whilst the mesopelagic and bathypelagic particles shared a reduced ASV similarity with the reciprocal *in situ* communities at 13.91% (±8.96 SD) and 8.65% (±1.19 SD), respectively. Evidence for identical bacterial ASVs found on chitin particles across the three depth zones suggests in part, observed elevated diversity in the bathypelagic may therefore be explained by particles promoting vertical connectivity in the open-ocean microbiome (Mestre et al. 2018, Ruiz-González et al. 2020). Furthermore, bathypelagic particles could be more diverse due to increased functional diversity of bacteria required to degrade highly refractory material, which often comprises a large proportion of the sinking material and simultaneously high molecular weight polysaccharides such as chitin.

Identical ASVs belonging to the orders Flavobacteriales, Cytophagales, Alteromonadales, and Sphingobacteriales were found on particles in the surface as well as the bathypelagic incubations (Fig. 3, dotted pattern), suggesting that they may be cosmopolitan colonizers. However, as these orders significantly increased in their abundance in the bathypelagic (all $P < 0.01$), with the exception of Cytophagales, they may be of greater importance in the bathypelagic. The reported increase at depth on particles for some of these taxa, including Flavobacteriales, Alteromonadales, and Sphingobacteriales has previously been shown and is suggested to be as a result of being dormant or slow-growing ASVs in the surface, which flourish as conditions because more favourable, or their ability to withstand pressure and temperature changes below

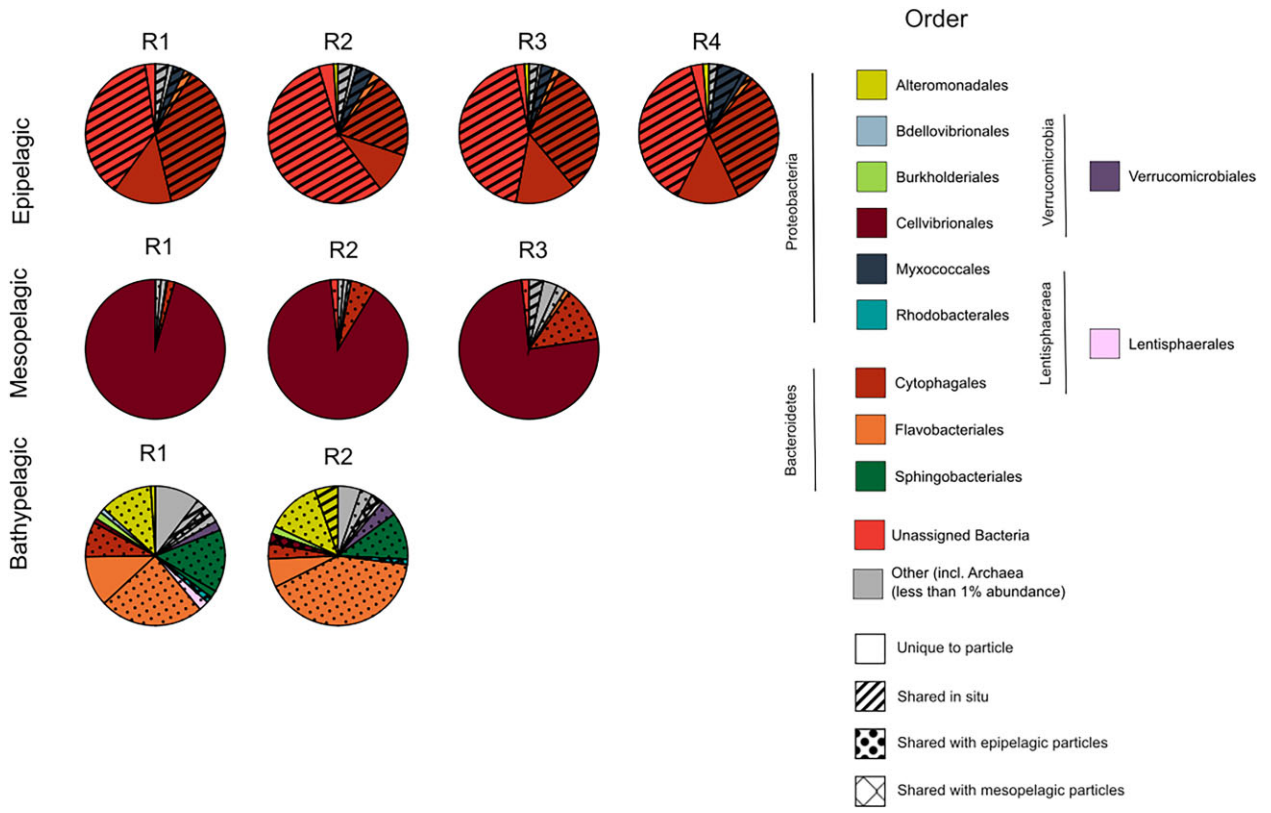

Figure 3. Order-level relative abundance of bacterial ASVs shared and unique found on particles incubated in epipelagic, mesopelagic, and bathypelagic water by individual replicates. Orders are grouped by Phyla. Values reported for percentage of ASVs shared are percentage of relative abundance shared. Relative abundances of less than 1% are grouped under 'other' categories.

Table 1. ASVs shared between particles and *in situ* communities.

Depth	Replicate	Total ASVs on particle	Unique to particle		Shared *in situ*		Shared epipelagic		Shared mesopelagic	
			Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
Epipelagic (30 m)	1	61	28	45.90	33	54.09	–	–	–	–
	2	74	32	43.24	42	56.75	–	–	–	–
	3	60	31	51.67	29	48.33	–	–	–	–
	4	57	31	54.39	26	45.61	–	–	–	–
Surface mean				48.80 ± 4.49		51.2 ± 4.43				
Mesopelagic (600 m)	1	46	31	67.39	4	8.69	11	23.91	–	–
	2	46	25	54.35	3	6.52	18	39.13	–	–
	3	132	63	47.73	35	26.52	34	25.76	–	–
Mesopelagic mean				56.49 ± 8.17		13.91 ± 8.96	–	29.60 ± 6.78		
Bathypelagic (3000 m)	1	161	93	62.11	12	7.45	43	26.70	13	8.07
	2	122	60	49.18	12	9.84	13	10.66	12	9.84
Bathypelagic mean				55.65 ± 6.47		8.65 ± 1.195		19.18 ± 8.02		8.96 ± 0.885

Values were calculated by counting the total number of ASVs found on particles for each replicate at each depth and then counting the number of ASVs that were also found *in situ*. This number was then divided by the total number of ASVs on the particles to identify the percentage of ASVs shared with the *in situ* community.

the photic zone (Lennon and Jones 2011, Mestre et al. 2018) in comparison to surface dominant ASVs. These taxa were not at high abundances in the corresponding *in situ* data, and as niche partitioning between free living and particle attached at a phylogenetic level has been previously reported (Salazar et al. 2015, Leu et al. 2022), this may also be responsible for the more pronounced enrichment on particles.

As particles sink and conditions change, some bacteria may not be adapted to changes and detach (Mestre et al. 2018), potentially supporting the recorded higher diversity of the bacterioplankton around them and provide a mechanism in which new depth-specific microbial gardening candidates can colonize the particle under more optimal conditions. For example, the Cellvibrionales, as a group were not detected on surface particles but dominated on mesopelagic particles where they may have newly colonized (Fig. 3, dark green, no pattern).

In this study, we did not control pressure during the incubation period, which alongside low temperatures may constrain colonization and growth of communities at depth and drive differences in diversity observed in our incubations, as a result of delayed non-specific colonization of particles (Datta et al. 2016, Suominen et al. 2021). However, the similarity

in diversity and distinct community structure across the additional treatments suggests not. Nevertheless, as temperature and hydrostatic pressure can influence the physiology of microorganisms in the deep ocean (Whitman et al. 1998, Tamburini et al. 2002), future studies could consider this. Furthermore, only a single incubation time point was sampled (i.e. after 48 h) and therefore successional changes within depth treatments will not be captured and could be considered in future work. Collecting inorganic nutrient data alongside experimental incubations may also help to account for metabolic requirements of taxa and further constraints on abundance of particle-associated organisms (Azam and Malfatti 2007).

Zooplankton selectively graze on particles and their attached microbial communities to obtain a dietary supply of essential amino acids and fatty acids, which they are unable to synthesize (Anderson et al. 2017). Regulation of poly-unsaturated fatty acids such as eicosapentaenoic acid (EPA), produced by taxa observed here, including Flavobacteriales (Shulse and Allen 2011) and Cytophagales (Nichols and McMeekin 2002), is important below the euphotic zone where high pressure and low temperature can influence the functioning of copepod cell membranes (Pond et al. 2014). EPA also has an important role in growth and fecundity of freshwater zooplankton (Muller-Navarra 1995). Therefore, diversity of particle colonizing bacterial groups may be important when considering the ecosystem service that they play in providing essential nutrition for zooplankton throughout the water column, and potentially influence the community structure of zooplankton communities, which should be explored further.

Microbial gardening on chitin-based particles allows for the increased nutritional value of material in the deep ocean, which can be nutritionally limited (Mayor et al. 2014). It also allows for the modification of chitinous carbon to a more nutritionally beneficial source (i.e. trophic upgrading) for zooplankton. Currently, the feeding dynamics of detritivorous zooplankton on chitin-based POM are not well defined. Further studies should attempt to unravel the feeding dynamics of detritivorous zooplankton on chitin-based POM, including the palatability of chitinous POM with and without associated bacterial communities and assess the trophic transfer capability of chitin carbon via microbial gardening.

Conclusions

In this short-term experimental incubation, we show the ASV-level diversity of particle-attached bacteria across the water column, with elevated diversity on particles in the bathypelagic region. We also demonstrate the cosmopolitan distribution of particle colonizers across the water column in agreement with previous observations suggesting particles are vectors of connectivity as previously recorded in environmental sampling using an operational taxonomic unit-based resolution (Mestre et al. 2018, Ruiz-González et al. 2020). We propose that high diversity on particles in the deep sea could be explained in part via sinking of particle-attached communities and colonization from the surrounding bacterioplankton at depth. We suggest that particle-colonizing taxa at depth are likely a combination of those that have attached in the surface or mesopelagic and those attaching in the bathypelagic zone. This should be explored at a finer depth resolution to determine the source of the remaining particle diversity of the bathypelagic zone, including successional dynamics on particles. This study suggests that particle-colonizing taxa differ with depth, which may be important when considering the wider implications of particles for ecosystem services such as carbon cycling and the role they play for zooplankton grazers. This is especially pertinent given the established importance of chitin specifically in POC (Souza et al. 2011). Our observations could also be investigated for other types of organic and anthropogenic particles, which may have further unknown implications for wider food web interactions. Additionally, knowledge on the identity of microbial communities involved in the degradation of chitin specifically hosts wider impact than just understanding microbial involvement in biogeochemical cycling. In an applied setting, identifying chitin-colonizing taxa provides a starting point for associated enzymatic repertoires, which may be useful in the growing problem of chitin-based waste management in the seafood industry (Santos et al. 2020).

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Author contributions

Cordelia Roberts (Formal analysis [lead], Investigation [lead], Methodology [lead], Visualization [lead], Writing – original draft [lead], Writing – review & editing [equal]), Kimberley Bird (Conceptualization [equal], Formal analysis [equal], Investigation [equal], Methodology [equal], Visualization [equal], Writing – review & editing [equal]), Nathan Chrismas (Formal analysis [supporting], Writing – review & editing [equal]), Susan Hartman (Funding acquisition [equal], Investigation [equal], Project administration [equal], Writing – review & editing [equal]), and Michael Cunliffe (Conceptualization [lead], Formal analysis [equal], Funding acquisition [lead], Project administration [equal], Supervision [lead], Writing – original draft [supporting], Writing – review & editing [lead])

Supplementary data

Supplementary data is available at *LAMBIO Journal* online.

Conflict of interest: None declared.

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Data availability

Sequence data have been deposited at the National Centre for Biotechnology Sequence Read Archive under project number PRJNA882764.

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