



Rhizaria in the oligotrophic ocean exhibit clear temporal and vertical variability

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ABSTRACT

Recently studies have shown that Rhizaria, a super-group of marine protists, have a large role in pelagic ecosystems. They are unique in that they construct mineral tests out of silica, calcium carbonate, or strontium sulfate. As a consequence, Rhizaria can have large impacts on the ocean's cycling of carbon and other elements. However, less is known about Rhizaria ecology or their role in the pelagic food-web. Some taxa, like certain Radiolarians, are mixotrophic, hosting algal symbionts. While other taxa are flux-feeders or even predatory carnivores. Some prior research has suggested that Rhizaria will partition vertically in the water column, likely due to different trophic strategies. However, very few studies have investigated their populations over extended periods of time. In this study, we present data investigating Rhizaria abundance and vertical distribution from over a year of monthly cruises in the Sargasso Sea. This study represents the first quantification of Rhizaria throughout the mesopelagic zone in an oligotrophic system for an extended period of time. We use this data to investigate the hypothesis that Rhizaria taxonomic groups will partition due to trophic mode. We also investigate how their abundance varies in accordance with environmental parameters. Rhizaria abundance was quantified using an Underwater Vision Profiler (UVP5), an in-situ imaging device. Ultimately, we show that different Rhizaria taxa will have unique vertical distribution patterns. Models relating their abundance to environmental parameters have mixed results, yet particle concentration is a common predictive variable, supporting the importance of heterotrophy amongst many taxa.

1. Introduction

Rhizaria are an extremely diverse super-group of single-celled organisms which occupy a wide range of habitats. Planktonic Rhizaria include Foraminifera, Radiolaria (including Acantharea and Collodaria) and Phaeodaria, all of which are observed throughout the global ocean (Biard et al., 2016; Laget et al., 2024). While the taxonomy of these organisms has recently undergone several reclassifications (Biard, 2022b), their presence in ocean ecosystems has been long known to oceanographers. Some of the earliest records of their existence are from oceanographic expeditions in the 19th century (Haeckel, 1887). Rhizaria are unique members of the plankton and protist community because they can reach large sizes (up to several mm in diameter) and they construct intricate mineral skeletons out of either silica, strontium, or calcium carbonate (Kimoto, 2015; Nakamura and Suzuki, 2015; Suzuki and Not, 2015; Biard, 2022b). Despite their noticeable morphology and global distribution, Rhizaria were largely understudied throughout the 20th century. Fragile organisms like Rhizaria are difficult to adequately

study because they can be destroyed through standard zooplankton sampling techniques and preserve poorly. A number of studies in the late 1900s did employ alternative techniques to quantify Rhizaria including diaphragm pumps (Michaels, 1988) or blue-water SCUBA collections (Caron and Be, 1984; Bijma et al., 1990; Caron et al., 1995). However, the bulk of Rhizaria research was constrained to sediment traps or paleontological studies of sediment (Takahashi et al., 1983; Boltovskoy et al., 1993).

Recently, the advent of molecular techniques and *in situ* imaging tools have ignited a renewed focus on Rhizaria in pelagic ecosystems (Caron, 2016). Molecular tools have shed light on Rhizaria taxonomy (Biard, 2022b), distribution (Blanco-Bercial, 2020; Sogawa et al., 2022), ecology (Decelle et al., 2012; Nakamura et al., 2023), biogeochemical roles (Gutierrez-Rodriguez et al., 2019; Laget et al., 2024), and metabolism (Cohen et al., 2023). Yet, despite the excellent taxonomic resolution provided by molecular approaches, they do not provide a truly quantitative metric for estimating Rhizaria abundance

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or biomass. *In situ* imaging tools however, offer the ability to observe organisms in the natural state and quantify their abundance (Ohman, 2019; Barth and Stone, 2024). Biard et al. (2016) utilized *in situ* imaging at a global scale to suggest large (>500 μm diameter) Rhizaria were substantial contributors to the ocean carbon standing stock. While more recent calculations suggest lower carbon contribution (Laget et al., 2024), Rhizaria nonetheless have substantial influences on biogeochemical cycling. This influence is due to their unique mineral skeletons and ability to repackage small particles into larger, fast sinking ones (Ikenoue et al., 2019). Thus, some Rhizaria may play substantial roles in ocean export through their interception of sinking particles (Stukel et al., 2018; Laget et al., 2024). However, Rhizaria ecology is poorly understood (Biard, 2022b) and their ecological roles are likely more than diverse than simple particle interception.

The ecological role of Rhizaria in plankton communities is complicated due to the fact different taxa can exhibit very different trophic modes. Many Rhizaria are strictly heterotrophic (Biard, 2022b), yet their feeding modes can be quite varied. Phaeodaria are largely thought to be flux-feeders, collecting and feeding on sinking particles (Gowing, 1989). Alternatively, Retaria can be either exclusively heterotrophic or mixotrophic, utilizing photosynthetic algal symbionts (Anderson, 2014; Decelle et al., 2015). Mixotrophic Foraminifera host a variety of endosymbiont partners (Lee, 2006; Decelle et al., 2015), which are thought to support early and adult life stages and significantly contribute to total primary productivity (Kimoto, 2015). Still, Foraminifera are omnivorous, possibly even predominately carnivorous, with several studies suggesting that they can be effective predators (Anderson and Bé, 1976; Gaskell et al., 2019), mainly consuming live copepods (Caron and Be, 1984). Radiolaria have several lineages, many of which host symbionts (Biard, 2022a). Amongst Radiolaria, arguably the most widespread are Collodaria who can be either large solitary cells or form massive colonies, up to several meters in length (Swanberg and Anderson, 1981). All known Collodaria species host dinoflagellate symbionts (Biard, 2022a) and can contribute substantially to primary productivity, particularly in oligotrophic ocean regions (Caron et al., 1995; Dennett et al., 2002). This Collodaria-symbiont association has been suggested as a reason for their high abundances throughout the photic zone of oligotrophic environments globally (Biard et al., 2016, 2017). A few Acantharea (Radiolaria order) clades host algal symbionts (Decelle et al., 2012; Biard, 2022a), notably with two clades forming an exclusive relationship with *Phaeocystis*. However, globally, Acantharea are less abundant than Collodaria (Biard, 2022b) and contribute less to total primary productivity (Michaels et al., 1995). This may be due to the fact several clades of Acantharea are cyst-forming and strictly heterotrophs (Decelle et al., 2013; Biard, 2022a). Furthermore, Mars Brisbin et al. (2020) documented apparent predation behavior in Acantharea near the surface, suggesting that there may be a larger reliance on carnivory.

Given the high abundances, yet diverse trophic strategies found among Rhizaria taxa, it is reasonable to expect some form of niche partitioning. A number of studies do suggest evidence for vertical zonation between Rhizaria groups according to various trophic strategies. Taxon-specific studies of Radiolaria suggest they may be restricted to the euphotic zone (Michaels, 1988; Boltovskoy, 2017). Although some studies report Acantharea in deeper waters (Decelle et al., 2013; Gutiérrez-Rodríguez et al., 2022), Phaeodaria, alternatively, are generally found beyond depths where light can sustain photosynthesis, but they can feed on sinking particles (Stukel et al., 2018; Laget et al., 2024). In an imaging-based study of the whole Rhizaria community, Biard and Ohman (2020) noted clear patterns in vertical zonation which largely corresponded to different trophic roles. Protists communities, including Rhizaria, have been described to partition along autotroph-mixotroph-heterotroph gradients with increasing depth in the water column (Blanco-Bercial et al., 2022; Laget et al., 2024). Yet, few studies have made direct attempts to relate Rhizaria abundances to environmental factors (Biard and Ohman, 2020). In part, this is

due to the fact few studies have been able to sample Rhizaria in the same location over a consistent timeframe (Michaels, 1988; Boltovskoy et al., 1993; Michaels et al., 1995; Hull et al., 2011); (Gutiérrez-Rodríguez et al., 2022). Furthermore, very few studies have utilized *in situ* imaging, arguably the best method for quantifying Rhizaria, to consistently throughout the full mesopelagic (Laget et al., 2024). Given this lack of information, there are many unknowns with respect to Rhizaria ecology, seasonality and phenology across different groups.

In this study, we present a comprehensive assessment of large Rhizaria abundance measured for over a year from regularly occurring cruises at monthly intervals. We utilized an *in situ* imaging approach to facilitate abundance calculations. With this dataset, we address two critical aims. (1) Quantifying of large Rhizaria throughout the epipelagic (0–200 m) and mesopelagic (200–1000 m) over the course of an annual cycle. These data were collected in the Sargasso Sea, and represents the first study of its kind in an oligotrophic system; and (2) We aim to test the hypothesis that Rhizaria exhibit niche partitioning according to trophic roles. This hypothesis has several implications, including vertical zonation, as seen in prior studies, but also that environmental variables related to trophic strategy will explain abundance patterns. Specifically, autotrophic/mixotrophic taxa will correspond to variables related to autotrophy (chl-*a* concentration, primary productivity, local DO maxima) and other rhizaria will correspond to factors which promote heterotrophy (particle concentration, flux, and local DO minima).

2. Methods

2.1. Oceanographic sampling

Data were collected in collaboration with the Bermuda Atlantic Time-series Study (Michaels and Knap, 1996; Lomas et al., 2013) on board the R/V Atlantic Explorer. Cruises were conducted at approximately monthly intervals. Rhizaria individuals were sampled using the Underwater Vision Profiler 5 (UVP) (Picheral et al., 2010). The UVP5 is an in-situ camera used to capture plankton and particle images and has been well established to accurately quantify large Rhizaria abundance (Biard et al., 2016; Stukel et al., 2018, 2019; Biard and Ohman, 2020; Barth and Stone, 2022; Drago et al., 2022; Llopis Monferrer et al., 2022; Panaïotis et al., 2023). The UVP5 was mounted to the sampling rosette and collected data autonomously on routine casts, from which only the downcast data are utilized. The UVP5 was deployed from June–September 2019 then from October 2020–January 2022, during which time the BATS region was sampled for 3–5 days at monthly intervals (see sampling details in Supplemental Table 1). Casts were filtered to only include data collected in the BATS region, far offshore of Bermuda in the Sargasso Sea (approximately 31.0°N–32.5°N, 64.25°W–63°W; Supplemental Figure 1). In general, casts extended to either 200 m, 500 m, or 1200 m deep, with a few extended into the bathypelagic (4500 m). However, Rhizaria were only typically found in large abundances throughout the epipelagic and mesopelagic zones. As such, we limit this study to results from the upper 1000 m of the water column.

A variety of biotic and abiotic data were collected during each BATS cruise. The UVP5 provided particle count data at a high-frequency from each cast. Particle concentration, as a proxy for prey field (Whitmore and Ohman, 2021), was calculated from this data for all particles 184 μm –900 μm . The lower size range was set by what could be reliably sampled by the UVP5's pixel resolution (>2px; 0.092 mm per pixel) and the upper size range was set to below the size of observable Rhizaria yet large enough to be inclusive of particles on which some Rhizaria may feed. For each UVP cast supporting continuous profiles of the CTD parameters salinity, temperature, and auxiliary CTD channels; Dissolved Oxygen (DO), *in situ* chlorophyll fluorescence were measured at 24 Hz using the BATS CTD package. On select casts, Niskin bottles were used to collect bacterial abundance estimates (via epifluorescence microscopy) as well as measure inorganic nutrients (NO_3^- , and Si as

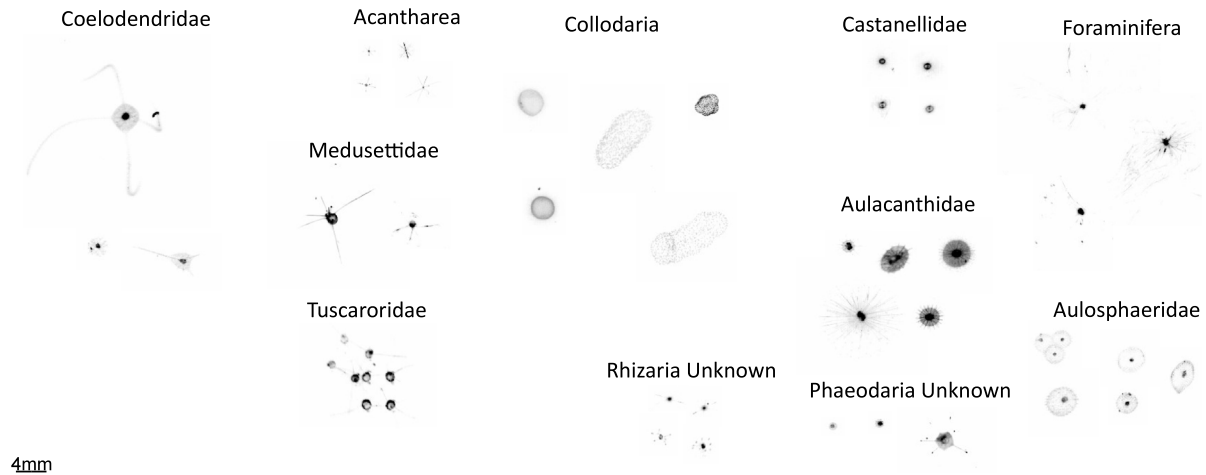


Fig. 1. Example images of different Rhizaria taxa as collected by UVP5 imaging. 4 mm scale bar shown in lower right. All vignettes are same scale.

silicate/silicic acid) at discrete depths. On each cruise, flux estimates of total mass, organic carbon, and nitrogen were also collected using sediment traps; in the present study we utilized flux to the mesopelagic as the flux at 200 m. Also primary productivity was estimated through measuring ^{14}C uptake rates from *in situ* incubations. For full descriptions of the BATS sampling program and methods, see Knap et al. (1997) and Lomas et al. (2013) for a review. Additionally, data can be viewed online (<https://bats.bios.asu.edu/bats-data/>).

Environmental data were processed to match the format of the Rhizaria abundance estimates (see below). CTD data were collected at higher frequency than the UVP (24 Hz vs 15 Hz respectively), so these data were averaged within matching bins to the UVP5 data. Data from Niskin bottles were first linearly interpolated in depth at 1 m resolution then time averaged over the cruise, then subsequently averaged into matching bins as the UVP5 data. Primary productivity estimates were depth integrated throughout the euphotic zone (0–140 m).

2.2. Rhizaria imaging processing and abundance quantification

Individual vignettes of Rhizaria images were identified using the classification platform EcoTaxa (Picheral et al.). Data were pre-sorted utilizing a random-forest classifier and pre-trained learning set. Taxonomic classification were done based on morphological parameters measured in Zooprocess (Gorsky et al., 2010), which includes parameters such as major axis, equivalent spherical diameter (ESD), and grey values. While there are sparse taxonomic guides for *in situ* images of Rhizaria, identification largely relied on descriptions in (Nakamura and Suzuki, 2015; Suzuki and Not, 2015; Biard and Ohman, 2020). Using the aforementioned sources and publicly available EcoTaxa projects, we constructed a guide accessible at: https://thealexbarth.github.io/med ia/Project_Items/Oligotrophic_Community/ecotaxa_UVP-guide-stone-1 ab.pdf. Broadly, Rhizaria were classified as Foraminifera, Radiolaria (Acantharea or Collodaria), or as a variety of Phaeodaria families (Fig. 1). When identification could not be confidently made between a few candidate taxa, a less specific label was used. As a result, we have data from “unidentified Rhizaria”, which typically were vignettes not distinguishable between Aulacanthidae or Acantharea or “unidentified Phaeodaria”, which are clearly Phaeodaria but not distinguishable into a family.

The UVP5 samples at ~15 Hz rate as it descends the water column and records the exact position of each particle larger than 600 μm (ESD). However, identified rhizaria ranged from a 934 μm ESD Aulacanthidae cell to a Collodarian colony over 10 mm in diameter. To confirm that the UVP5 was sampling adequately across all size ranges, an normalized biomass size spectrum (NBSS) slope was constructed to identify a drop-off which would indicate poor-sampling at the small size

range (Lombard et al., 2019; Barth and Stone, 2024). However, it was evident from this analysis that all size ranges were adequately sampled across the size range (Supplemental Figure 2) so no data were excluded. The UVP5 reports the exact depth at which a particle is recorded, however to estimate abundance, observations must be binned over fixed depth intervals. Our deployments had variable descent depths and speeds with more casts descending to 500 m than 1000 m and descents quicker through the epipelagic than the mesopelagic (see Barth and Stone (2022) for an extended discussion of UVP5 data processing). For the present study, Rhizaria abundances were estimated in 25 m vertical bins, which offer a moderate sampling volume per bin (average 0.948 m^3 in the epipelagic and 0.589 m^3 in the mesopelagic; Supplemental Table 1) while still maintaining ecologically relevant widths. However, concentrations in a 25 m bin would need to be greater than 2.428 ind. m^{-3} and 3.912 ind. m^{-3} , in the epipelagic and mesopelagic respectively, to fall below a 10% non-detection risk (Benfield et al., 1996; Barth and Stone, 2024). Because we typically observed many Rhizaria taxa below these concentrations, we present the 25 m binned data to visualize broad-scale average distributions. For quantifying and modeling Rhizaria abundances, we present integrated abundance estimates, with each cast. Due to the variable descent depths of the UVP, data are categorized as epipelagic (0–200 m), upper mesopelagic (200–500 m), and lower mesopelagic (500–1000 m). The average sampling volume integrated through these regions were 7.59 m^2 , 7.06 m^2 , and 11.77 m^2 , with non-detection thresholds at 0.30 ind. m^{-2} , 0.33 ind. m^{-2} , and 0.20 ind. m^{-2} respectively. All UVP data processing was done using the EcotaxaTools package in R (<https://github.com/TheAlexBarth/EcotaxaTools>).

2.3. Modeling environmental controls of Rhizaria abundance

Generalized Additive Models (GAMs) were used to assess the relationship between integrated Rhizaria abundance and different environmental factors. GAMs offer the ability to model non-linear and non-monotonic relationships, which can be particularly useful in assessing ecological relationships (Wood, 2017) and have been successfully applied to Rhizaria ecology (Biard and Ohman, 2020). The mgcv package (Wood, 2001) was used to construct models relating environmental parameters to each taxonomic group's integrated abundance estimates from each cast. To select the most parsimonious model for each analysis, a backwards step-wise approach was taken. First, a full model was fit using any term which may be ecologically relevant. Terms were fit using maximum likelihood with a double penalty approach on unnecessary smooths (Marra and Wood, 2011). The smoothness parameter was restricted ($k = 6$) to prevent over fitting the models. Then, the full model was checked for concurvity (a metric indicating

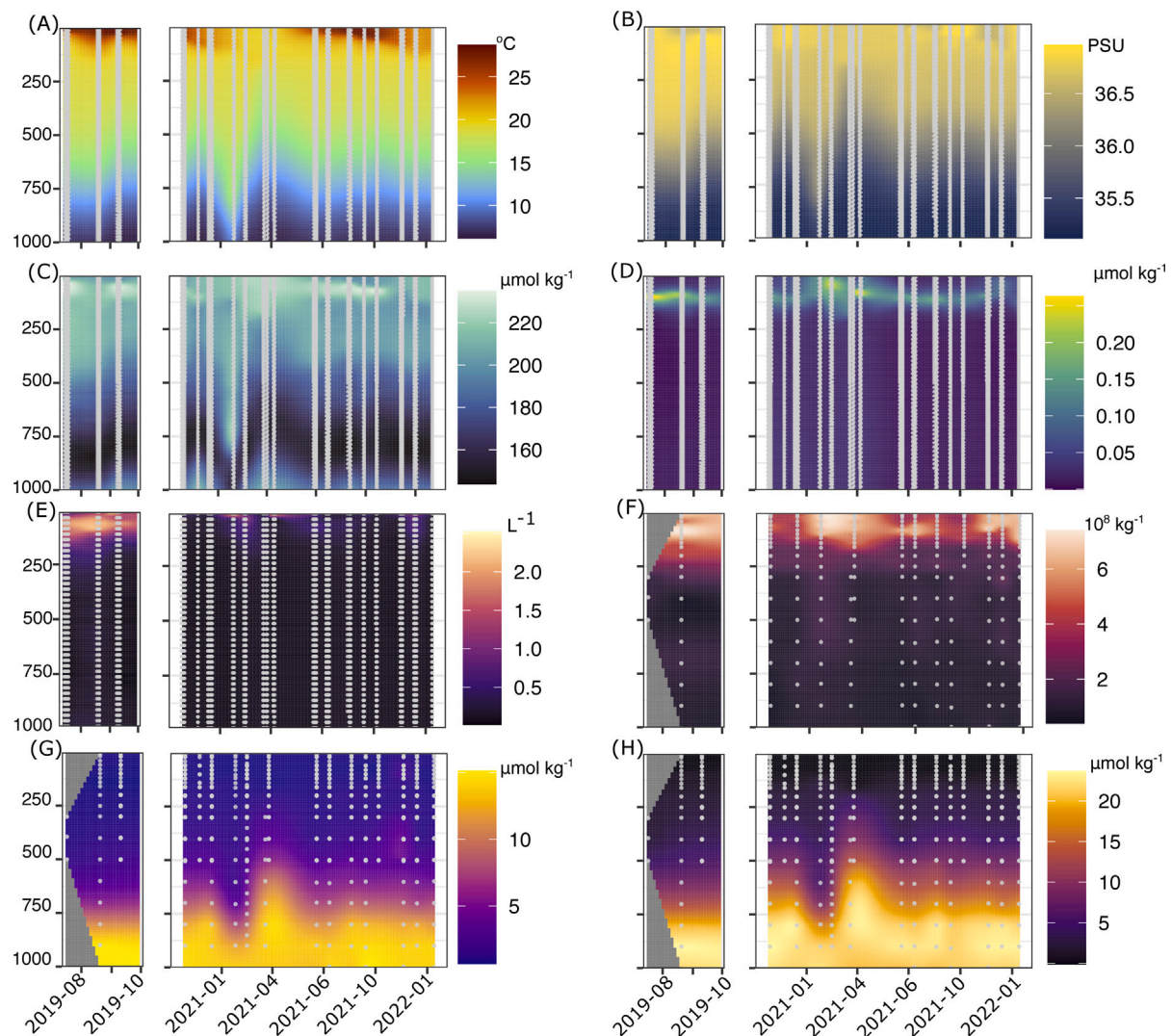


Fig. 2. Environmental profiles across time-series of study period. Y axis shows depth in meters. (A) Temperature. (B) Salinity. (C) Dissolved Oxygen. (D) in situ chlorophyll fluorescence. (E) Particle concentration (184–900 μm). (F) Bacteria Abundance. (G) Silica. (H) Nitrate. Points represent sampling data with linear interpolation inbetween.

high parameter relatedness in GAMs). If two terms had a high concurrency value (>0.8), then two alternative models were constructed each leaving out one of the related terms. The more parsimonious model of these two was selected using AIC. Once concurrency of terms was reduced, then the model was subjected to the backwards step-wise procedure. At each iteration, the model term with the lowest F score (least statistically significant) was removed. This was repeated until all model terms were statistically significant or the R^2_{adj} was substantially reduced. Models were fit for each water column region; epipelagic, upper mesopelagic, and lower mesopelagic. In cases where observations were too sparse for a given taxonomic grouping, models were not run. All code, full models, and model selection tools are available in open-source scripts, as well as intermediate data products at <https://github.com/TheAlexBarth/RhizariaSeasonality>.

3. Results

3.0.1. Environmental variability

While the environmental conditions were generally low in variation and oligotrophic, there is considerable influence from deep winter-mixing and summer stratification as well as secondary influences from mesoscale eddies which result in spatiotemporal heterogeneity (McGillicuddy et al., 1998; Lomas et al., 2013). Variability in the

water column structure was visible during the study period (Fig. 2). This is best evidenced through the temperature profiles; In the late summer and early fall there was a stratified water column with high temperatures in the surface (<75 m) (Fig. 2 A) and slightly elevated salinity (Fig. 2B). This warm, stratified period appeared more intense during the few months sampled in 2019. In 2021, we observed the stratified layer slowly dissipated into the winter months following mixed layer entrainment. There was a consistent oxygen minimum zone (OMZ) located at about 800 m deep (Fig. 2C). February 2021 saw a notable downwelling event, likely due to a passing anti-cyclonic eddy which impacted the local region during early 2021. During this phase, warmer, oxic water was significantly depressed deeper into the mesopelagic. This process was reversed in the spring months (March, April) primary due to the interaction of convective mixing and the passing of a strong cyclonic eddy resulting in a deep cold mixed layer. Primary production was highest during the spring mixing period, evidenced both by *in situ* fluorescence (Fig. 2D) and productivity incubation experiments (Fig. 3 A). Originating near the surface, the productivity peak moved deeper throughout the spring and declined into the summer (Fig. 2D). However, there was a notable, yet smaller productivity bump in the late summer and early fall (Fig. 3 A) which occurred deeper in the epipelagic (Fig. 2D). The particle concentration (184 μm –900 μm) was closely coupled to chlorophyll-a patterns.

Overall, particle concentration was high near the surface during the 2021 spring bloom, then moved deeper throughout the water column attenuating throughout the lower epipelagic (Fig. 2E). Similarly, heterotrophic bacteria abundance was closely linked to overall productivity, although there was a more consistent moderate-abundance layer near the top of the mesopelagic (~250 m) (Fig. 2F). Concurrent with the secondary fall production peak, there was also higher particle concentration and bacterial abundance in the later summer and early fall. Interestingly, while primary productivity estimates from July–August were not that different between 2019 and 2021 (Fig. 3 A), chlorophyll-a fluorescence, particle concentration, and bacterial abundance were much higher in 2019's summer/fall (Figure 2D–F). Inorganic nutrients (Si and NO₃) were generally well stratified, with low concentrations in the epipelagic and increasing throughout the mesopelagic. However, both nutrients did vary vertically in accordance with the 2021 February downwelling and spring mixing period (Fig. 2G–H). Additionally, in the late fall of 2021, Si concentrations were slightly elevated in the mid-mesopelagic (Figure 2G).

Overall mass flux to the mesopelagic was highest during the 2021 February downwelling (Fig. 3B). Generally, export was similarly high during March, declining in April then increasing slightly throughout the summer and early fall. While magnitude was slightly different, this pattern was consistent with total mass, carbon and nitrogen fluxes (Fig. 3B–D). Higher mass, carbon and nitrogen flux also occurred in the 2019 late summer - early fall period.

3.0.2. Rhizaria abundance and distribution

Across all imaged mesozooplankton (>900 µm), Rhizaria comprised a considerable fraction of the total community. Considering the total abundances of the observational period, Rhizaria comprised on average, 42.6% of all mesozooplankton abundance (Supplemental Figure 3). Copepods were the second most abundant, comprising 35.5% and all other living mesozooplankton were 22%. The large contribution of Rhizaria to the mesozooplankton community is most prominent in the epipelagic, where they accounted for 47% of all mesozooplankton abundance. In the mesopelagic Rhizaria were a smaller fraction, at 38% in the upper layers (200–500 m) and 37% in the deeper mesopelagic (500–1000 m).

Average abundance of all Rhizaria had a bimodal distribution with respect to depth. Total abundance was highest just below the surface (0–100 m), with secondary, wider peak occurring in the mid mesopelagic (Fig. 4 A). Variation in depth binned abundance was large, likely due to seasonal variability but also increased from the detection-risk described in the methods. The vertical distribution pattern and abundance varied considerably across taxonomic groups. Radiolaria were some of the most abundant taxa observed, particularly in the epipelagic (Fig. 4, Fig. 5 A). Acantharea displayed a bimodal distribution accounting for a large portion of the total Rhizaria pattern (Fig. 4B, Fig. 5). The surface layers were largely comprised of Collodaria, whose colonies were abundant (Fig. 4C). The most abundant Phaeodaria, Aulacanthidae, also had a bimodal pattern with the density was highest in the lower mesopelagic (Fig. 4D). Foraminifera had a similar bimodal distribution, yet their overall average densities were much lower (Fig. 4E). Aulosphaeridae had low average density and was nearly homogeneously distributed throughout the water column, although slightly lower in the epipelagic (Fig. 4F). Castanellidae were the only Phaeodaria who appeared to be effectively restricted to the photic zone (Fig. 4G). Alternatively, Coelodendridae primarily occurred in the lower mesopelagic (Fig. 4H). A few individuals from the families Tuscaroridae and Medusettidae were also observed in the mesopelagic, yet they were much rarer (data not shown).

Between the monthly cruises, Rhizaria integrated abundance varied in the epipelagic. Highest average abundance occurred in June 2021 and was lowest during the winter months (Fig. 5 A). The 2019 later summer - fall period also had much higher integrated abundance than similar months in 2021. While the majority of integrated abundance

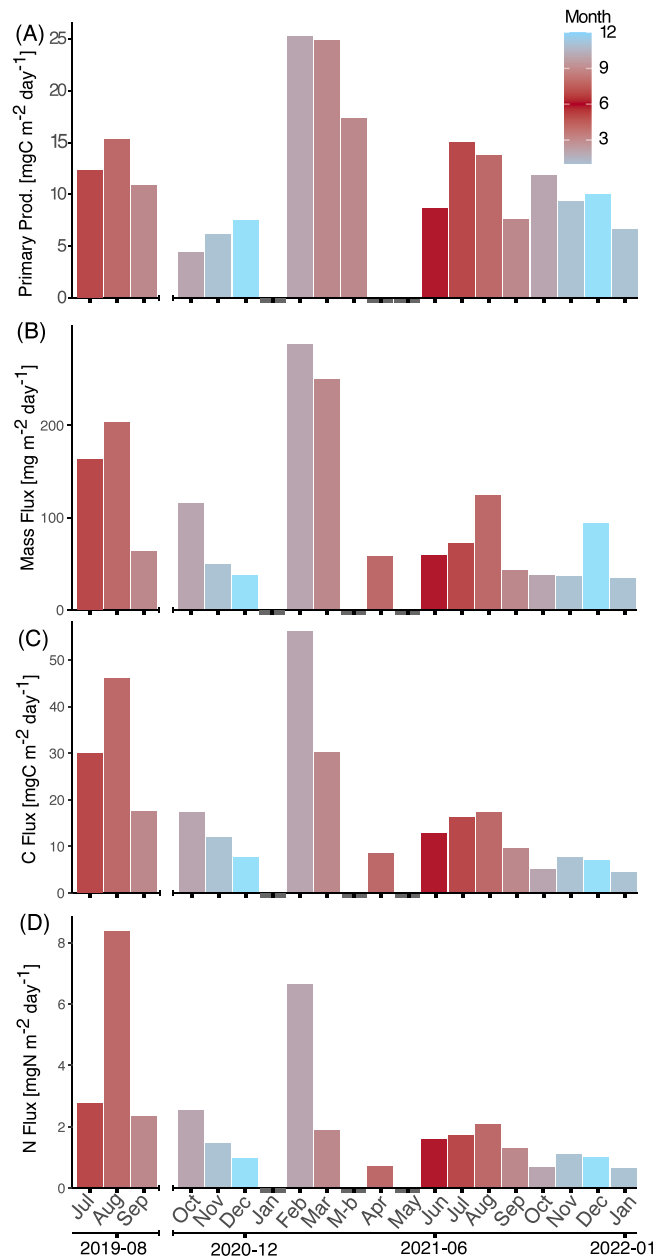


Fig. 3. Primary productivity (A) and flux estimates from total mass (B), carbon (C), and nitrogen (D). Values are from monthly cruises with month displayed in corresponding colors. Absent data are shown by gray bar.

in the epipelagic was consistently attributable to Collodaria, Acantharea abundance occurred sporadically and could account for a large portion of the total in some months (Fig. 5 A). The mesopelagic integrated abundance was much more consistent across monthly cruises, although average abundance was notably higher in 2019 (Fig. 5B, 5C). The community composition in the mesopelagic was more diverse, mostly comprised of Phaeodaria families. However, Acantharea and unidentified Rhizaria also were common members of the community (Fig. 5B, C).

3.0.3. Body size throughout the water column.

Very few taxa had consistent distributions throughout the water column. Only Acantharea, Foraminifera, Aulacanthidae, and Aulosphaeridae were consistently abundant in the epipelagic and mesopelagic. To investigate if the populations or morphologies shifted throughout

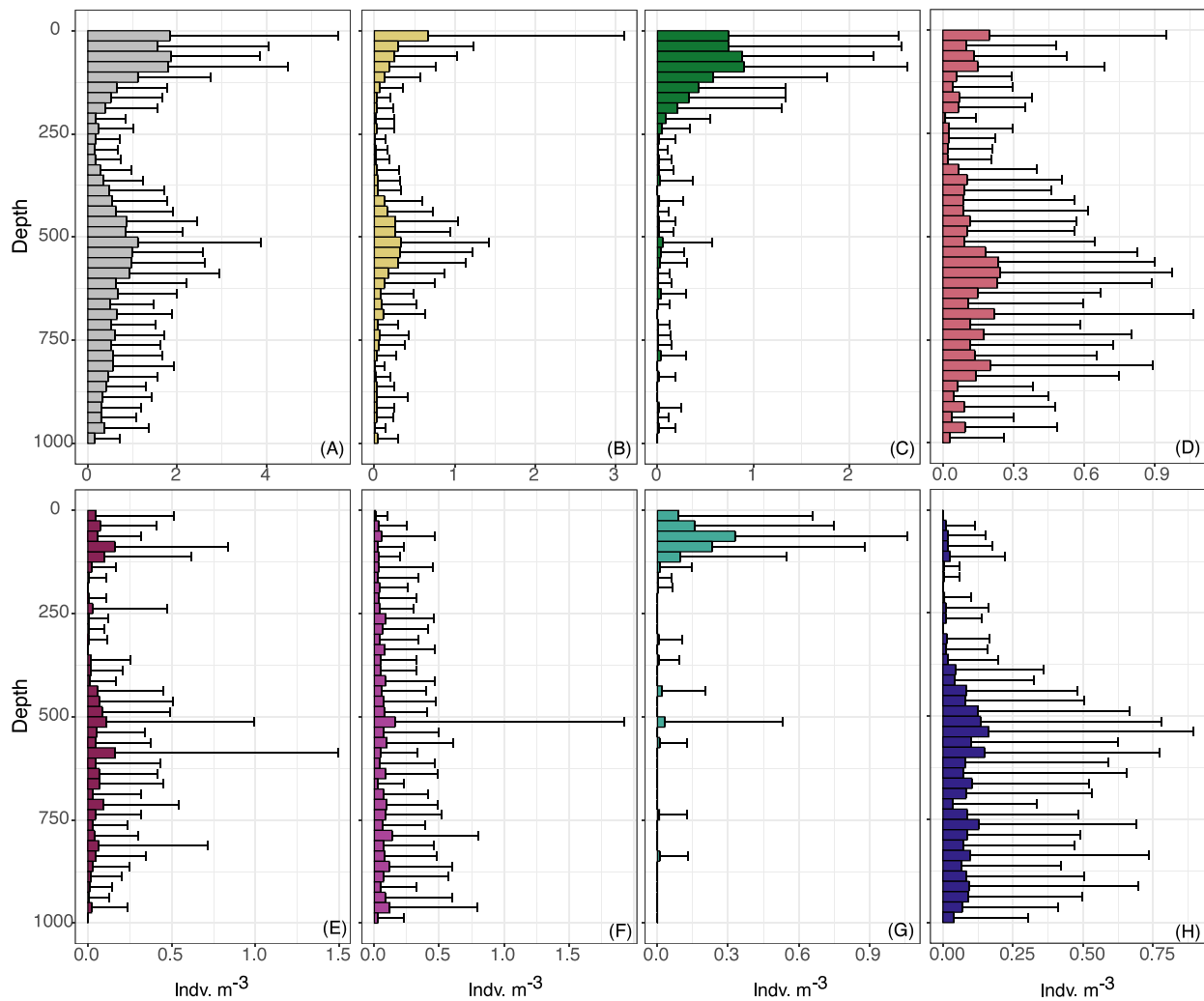


Fig. 4. Average abundance of Rhizaria in 25 m bins, across entire study period. Shown are total Rhizaria (A), Acantharea (B), Collodaria (C), Aulacanthidae (D), Foraminifera (E), Aulosphaeridae (F), Castanellidae (G), Coelodendridae (H). Error bars shown are standard deviation.

the water column, we compared the sizes (ESD) between mesopelagic and epipelagic groups for each taxa. All groups were significantly different on average (Wilcox Rank Sum p -value < 0.001). Acantharea were smaller, on average in the mesopelagic while all other taxa tended to be larger (Fig. 6).

3.0.4. Environmental drivers of Rhizaria abundance

For integrated abundance of all Rhizaria, the GAMs produced moderate fits ($R^2_{adj} = 0.406$ – 0.603) (Table 1). In the epipelagic, there were several significant predictor variables (Fig. 7A–7G). This included terms which are likely indicators of changing water masses; Salinity (7E) and Temperature (7G). The model also had parameters related to both heterotrophy (Particle concentration, 7A) and autotrophy (Primary production, 7B). However the relationship to particle concentration is weakly increasing trend while the primary productivity effect appears to be influenced by a cruise with a high outlier for increased primary productivity. The models for the mesopelagic regions were much more reduced and the only significant effects were particle-related terms (particle concentration and mass flux). In the upper mesopelagic (Fig. 7H–7J), final model included N flux, particle concentration and a non-significant effect for Silica. The lower mesopelagic (Fig. 7K, 7L) only had model effects kept of total mass flux and particle concentration. For both the upper and lower mesopelagic, particle concentration had a clear, strong positive relationship (Fig. 7I, 7L).

GAMs for individual taxa were much less consistent in their fits (Table 2). This is likely in part due to the high number of non-observations for certain taxa. Note that due to low abundances, GAMs were not constructed for Tuscaroridae or Medusettidae. Furthermore no, significant terms were found for a model with Aulosphaeridae in the epipelagic nor Foraminifera in the mesopelagic.

Epipelagic Acantharea were explained by several predictor variables and had a good fit ($R^2_{adj} = 0.539$, Table 2, Supplemental Figure 4). Foraminifera had a good fitting GAM in the epipelagic ($R^2_{adj} = 0.543$). There were several significant explanatory variables, although the clearest pattern was observed of high temperatures associated with more Foraminifera abundance (Table 2, Supplemental Fig. 4U). Epipelagic Aulacanthidae similarly had several predictor variables which were significant, including both water quality parameters and particle/flux predictors (Table 2). There was a weak fit for Collodaria in the epipelagic ($R^2_{adj} = 0.16$), although there was a logit-like relationship where higher abundances tended to occur during higher DO conditions in the surface waters (Supplemental Figure 4). Castanellidae also had a weak model fit in the epipelagic ($R^2_{adj} = 0.228$), although particle concentration was the strongest predictor term (Table 2, Supplemental Figure 4).

In the upper mesopelagic (200–500 m), abundances were generally low (Fig. 4) so GAMs were only constructed for Acantharea, Aulacanthidae, Aulosphaeridae, and Coelodendridae (Table 2). All these models had generally poor fits ($R^2_{adj} < 0.20$). Yet, for all upper mesopelagic

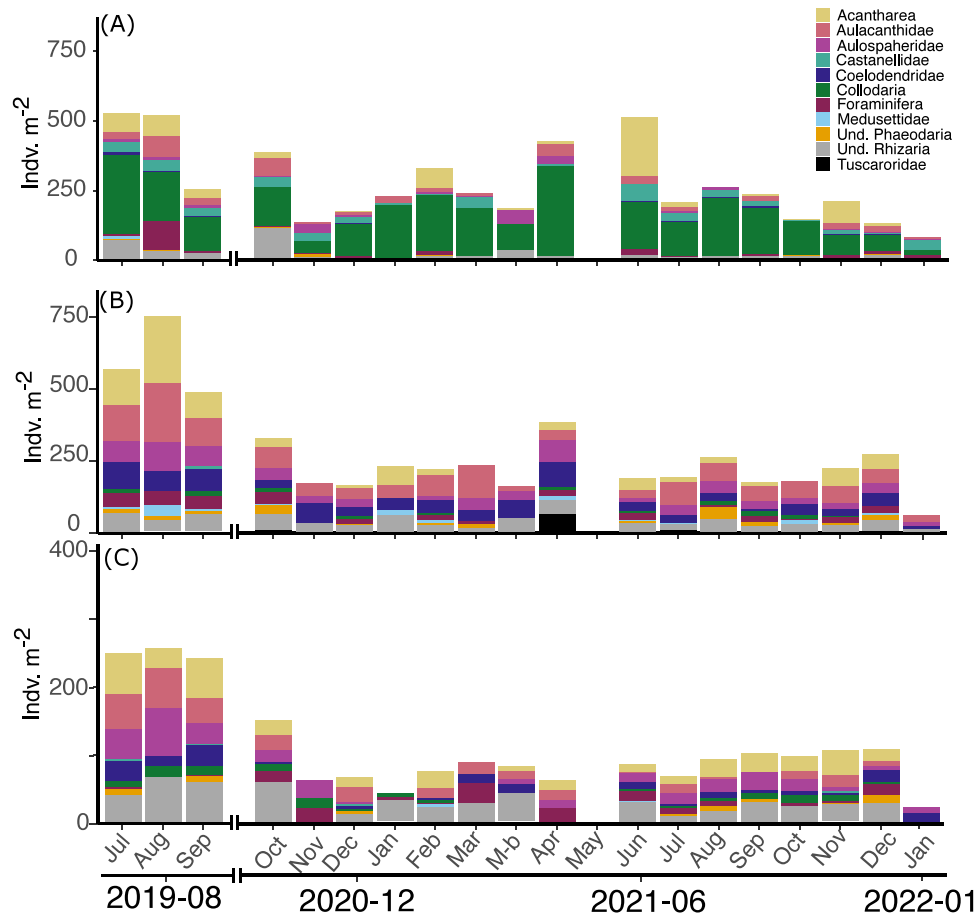


Fig. 5. Seasonality of Rhizaria integrated abundance for the epipelagic (0–200 m) (A), upper mesopelagic (200–500 m) (B), lower mesopelagic (500–1000 m) (C). Data shown are the cruise mean integrated abundance in the water column region colored for each taxon.

Table 1				
Generalized Additive Model results for integrated total Rhizaria abundance in different regions of the water column. edf = estimated degrees of freedom, F = F-statistic, p = p-value. Models were selected using a backwards-stepwise approach with a retention threshold of p = 0.1 or a large enough reduction in model fit.				
Model	Term	edf	F	p
All Rhizaria Epipelagic $R^2_{adj} = 0.48$	Temperature	0.7950	0.492	0.0440
	Salinity	2.4117	5.113	<0.001
	Silica	2.1511	8.601	<0.001
	Bacteria Concentration	0.8870	1.544	<0.001
	Avg Mass Flux	1.4107	0.802	0.0211
	Primary Productivity	1.9280	2.736	<0.001
	Particle Concentration	0.9151	2.136	<0.001
All Rhizaria Upper Mesopelagic $R^2_{adj} = 0.40$	Silica	0.6702	0.404	0.0814
	Avg N Flux	2.9524	3.172	<0.001
	Particle Concentration	3.5568	11.82	<0.001
All Rhizaria Lower Mesopelagic $R^2_{adj} = 0.61$	Avg Mass Flux	2.036	2.953	<0.001
	Particle Concentration	3.819	27.43	<0.001

models, particle concentration was a significant explanatory variable (Table 2, Supplemental Figure 5). The lower mesopelagic also had generally poor GAM fits for taxon specific models ($R^2_{adj} < 0.31$), with the exception of Acantharea ($R^2_{adj} = 0.496$). Acantharea in the lower mesopelagic was most clearly positively associated with particle concentration (Supplemental Figure 6). For all Phaeodaria with a significant model, particle concentration was a main predictor variable (Table 2, Supplemental Figure 6).

4. Discussion

4.0.1. Overall Rhizaria abundance and patterns

In the epipelagic Rhizaria exhibited a notable seasonal pattern. Rhizaria abundances were higher in the summer months and lower during the winter. During a prior time period, Blanco-Bercial et al. (2022) noted that there is considerable seasonality in the community composition of all protists. Despite the seasonality of Rhizaria

Table 2

Taxa-specific generalized additive models for different regions of the water column. edf = estimated degrees of freedom, F = F-statistic, p = p-value. Models were selected using a backwards-stepwise approach with a retention threshold of $p = 0.1$ or a large enough reduction in model fit.

Model	Term	edf	F	p
Acantharea Epipelagic $R^2_{adj} = 0.539$	Temperature	0.868	0.380	0.0935
	Salinity	2.349	4.646	<0.001
	O ₂	2.501	5.493	<0.001
	Avg Mass Flux	4.815	22.59	<0.001
	Bacteria	1.608	1.360	0.00945
Acantharea Upper Mesopelagic $R^2_{adj} = 0.197$	Particle Concentration	0.877	1.414	0.00449
	Avg Mass Flux	1.866	1.220	0.0166
	Avg N Flux	2.208	3.100	<0.001
	Primary Productivity	2.382	2.497	<0.001
	Particle Concentration	0.724	0.521	0.0437
Acantharea Lower Mesopelagic $R^2_{adj} = 0.496$	Temperature	1.958	2.986	<0.001
	Avg N Flux	1.513	1.565	0.0037
	Primary Productivity	0.856	1.153	0.0045
	Particle Concentration	1.661	8.667	<0.001
Aulacanthidae Epipelagic $R^2_{adj} = 0.394$	Temperature	2.591	5.003	<0.001
	Salinity	1.523	1.789	0.002
	RFU	0.872	1.315	0.0042
	Bacteria	0.808	0.833	0.0160
	Primary Productivity	2.622	9.160	<0.001
	Particle Concentration	1.937	2.751	<0.001
Aulacanthidae Upper Mesopelagic $R^2_{adj} = 0.132$	Avg C Flux	1.675	1.408	0.0109
	Particle Concentration	2.308	2.476	0.0013
Aulacanthidae Lower Mesopelagic $R^2_{adj} = 0.308$	Avg Mass Flux	1.476	1.418	0.0076
	Particle Concentration	2.457	7.756	<0.001
Aulosphaeridae Upper Mesopelagic $R^2_{adj} = 0.196$	Particle Concentration	2.778	12.76	<0.001
Aulosphaeridae Lower Mesopelagic $R^2_{adj} = 0.148$	Particle Concentration	2.012	6.306	<0.001
	Particle Concentration	2.012	6.306	<0.001
Castanellidae Epipelagic $R^2_{adj} = 0.228$	Salinity	0.915	0.400	0.0935
	RFU	0.912	2.052	<0.001
	Si	2.018	3.656	<0.001
	Bacteria	1.128	0.883	0.0170
	Particle Concentration	2.278	4.028	<0.001
Coelodendridae Upper Mesopelagic $R^2_{adj} = 0.09$	Avg N Flux	0.734	0.551	0.0527
	Particle Concentration	0.965	5.102	<0.001
Coelodendridae Lower Mesopelagic $R^2_{adj} = 0.157$	Particle Concentration	3.051	6.768	<0.001
Collodaria Epipelagic $R^2_{adj} = 0.16$	Salinity	0.926	2.267	<0.001
	O ₂	2.015	2.217	<0.001
	Bacteria	1.843	2.100	0.0024
Foraminifera Epipelagic $R^2_{adj} = 0.543$	Temperature	4.420	7.624	<0.001
	O ₂	0.799	0.788	0.023
	Bacteria	0.000	0.000	0.523
	Avg N Flux	1.847	3.022	<0.001
	Primary Productivity	0.472	0.139	0.183
	Particle Concentration	1.685	2.955	<0.001

abundance, community composition was relatively consistent, with Collodaria representing the bulk of the community. It should be noted that the overall taxonomic resolution of the UVP5 is fairly low, so there may be a switching of species within the broad groups identified in this study which were not captured. Throughout the mesopelagic, month-to-month variation in 2021 was relatively low. Again, this is consistent with observations from metabarcoding of the whole protist community in the same study region (Blanco-Bercial et al., 2022). This finding is not surprising as the overall seasonal variation in environmental conditions in this region were low.

Overall Rhizaria were the most commonly identified group of mesoplankton throughout the study period. We do note that the UVP5

commonly captures *Trichodesmium* colonies, yet these were excluded in this comparison as they are strictly autotrophs. It should be noted that previous work has suggested that avoidance behavior with the UVP is possible, at times likely, for visual and highly mobile zooplankton (Barth and Stone, 2022). Thus, the percent contribution reported here (42.7%) of Rhizaria to the total mesozooplankton community may be inflated due to under sampling of organisms such as Euphausiids and Chaetognaths which have quick escape responses. Regardless, it is worth noting that in the same region, with data collected in 2012 and 2013 using similar calculation methods, Biard et al. (2016) estimated large Rhizaria only contribute 15% of the total mesozooplankton community in the upper 500 m. Likely, Rhizaria display considerable

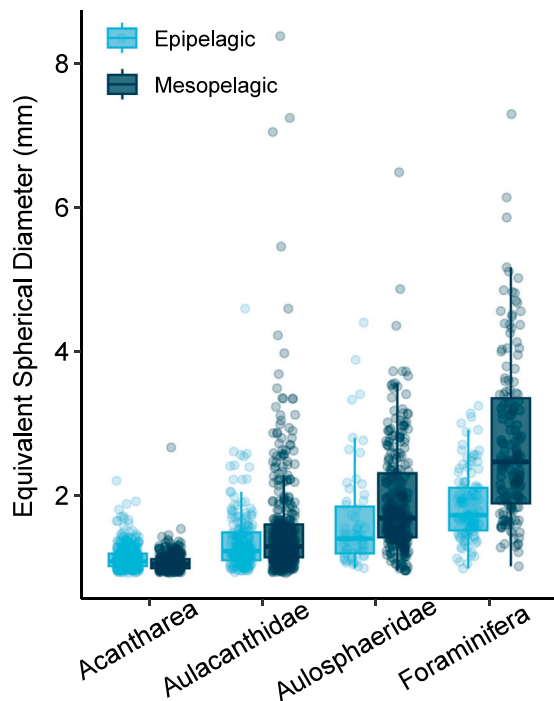


Fig. 6. Comparison of average sizes (ESD) amongst Rhizaria taxa which occurred throughout the water column.

interannual variability. In the present study, we noticed considerably higher Rhizaria abundance throughout the water column in 2019 compared to 2021. Similarly, in the California Current a multi-year study of Aulosphaeridae saw considerable variation between years (Biard et al., 2018). To truly understand the drivers of interannual variability however, sustained observations of Rhizaria over longer time periods are required.

4.0.2. Relationship to environmental parameters

In general, the fit of most GAMs were moderate to poor. One possible reason for the poor fits may have been that for some taxa, conditions were not variable enough to capture a range of conditions at which they may exist. For instance, Collodaria were the most abundant taxa observed, yet the fit of their GAM was particularly poor. In studies which covered a wider range of parameters, Collodaria has been shown to strongly vary with changes in parameters such as temperature, chlorophyll-*a*, mixing, and water clarity (Biard et al., 2017; Biard and Ohman, 2020). Alternatively, Acantharea had relatively good fitting GAMs. These taxa also had some of the largest variation from month to month on cruises. Thus, it may be that in the oligotrophic, the relatively stable conditions can support certain taxa while others are more sporadic. It should also be noted that due to the challenge of adequately sampling enough volume to overcome low-detection issues, GAMs were run on integrated data. However, variation with environmental parameters throughout the water column are likely, just not captured in the modeling aspect of this study. Future studies which are able to account for detection biases in finer vertical resolution of Rhizaria may improve ecological models of their abundance. One consistent parameter which had significant positive associations was particle concentration. This observation is not surprising as most Rhizaria likely to some extent engage in flux feeding.

4.0.3. Vertical structure and trophic roles

In this study we present a clear pattern of vertical zonation between different Rhizaria groups. Largely, the taxonomic composition and

vertical positioning were similar to Rhizaria zonation in the California Current Ecosystem (Biard and Ohman, 2020). It should be noted however, that the secondary abundance peak reported in the present study is lower. This is likely due to the more oligotrophic nature of the study region, where the euphotic zone penetrates deeper into the water column. Most prevalent in the epipelagic were Collodaria. These mixotrophic Radiolaria have long been reported to contribute to primary productivity in the euphotic zone (Michaels et al., 1995; Dennett et al., 2002). Collodaria are thought to be particularly successful globally in oligotrophic regions due to their photosymbiotic relationships (Biard et al., 2016, 2017). We observed the highest abundance of Collodaria during June 2021, supporting the notion they can thrive during the typically low-nutrient conditions of summer stratification. However, Collodaria also increased during the spring mixing period, suggesting that they can thrive during conditions which may typically be thought to favor autotrophs. Furthermore, while Collodaria were primarily absent from below 250 m, there were a few instances of deeper colonies being observed. Global investigations of polycystine flux, suggest that deep-Collodaria in oligotrophic regions may be a consequence of isothermal submersion (Boltovskoy, 2017). Alternatively, surface waters at BATS often mix into the mode water during the seasonal mixing, so Collodaria in the deeper waters may be a result of diapycnal mixing. Another effectively exclusively epipelagic Rhizaria was the Phaeodaria family of Castanellidae. All Phaeodaria are thought to be fully heterotrophic (Nakamura and Suzuki, 2015), nonetheless a number of studies, including this one, report Castanellidae to be typically found in the lower epipelagic (Zasko and Rusanov, 2005; Biard et al., 2018; Biard and Ohman, 2020). It should be considered that perhaps Castanellidae specializes in feeding on sinking particles directly at the base of the epipelagic. Given it is smaller size (Nakamura and Suzuki, 2015), Castanellidae does not need a large diameter to efficiently flux feed at the typically particle rich region of the lower epipelagic.

The mesopelagic generally was home to known heterotrophic organisms, particularly for those which were constrained to exclusively occupy deeper waters. This is consistent with Blanco-Bercial et al. (2022)'s observation of an auto-/mixotroph to heterotroph gradient in local protist community as well as global patterns in Rhizaria ecology (Laget et al., 2024). The upper mesopelagic interestingly had relatively low total abundance. This low-abundance region likely reflects the dynamics of productivity and export throughout the water column. While productivity and thus sinking particles for flux feeders are high in the euphotic zone, much of this is attenuated throughout the epipelagic. So, while the base of the epipelagic may provide a rich feeding environment for Castanellidae, smaller protists, or heterotrophic bacteria (Fig. 2F), the region from 200–500 m might be otherwise food poor. Perhaps it is more advantageous for Rhizaria to situate deeper, in darker regions of the twilight zone where visual predators and vertically migrating organisms may not feed on them. Also it should be noted that Phaeodaria utilize silica to build their opaline tests, and silica concentrations started to increase around 500 m (Figure 2G). However, Si was not a significant term for any taxon-specific model in the mesopelagic. Aulosphaeridae was only found to have significant relationships, although weak fits, to particle concentration in the mesopelagic. In our study, while consistently observed, overall abundances of Aulosphaeridae were very low. In the Pacific Ocean, on California's Coast, much higher abundances of Aulosphaeridae have been reported (Zasko and Rusanov, 2005; Biard and Ohman, 2020) and they have massive potential to impact silica export (Biard et al., 2018). Coelodendridae were also seemingly restricted to the deeper section of the mesopelagic. This is interesting given that in the California Current, (Biard and Ohman, 2020) found a bimodal distribution in Coelodendridae. There are several morphotypes corresponding to different taxa of Coelodendridae (Nakamura and Suzuki, 2015; Biard and Ohman, 2020). So it may be that only a few types of Coelodendridae were

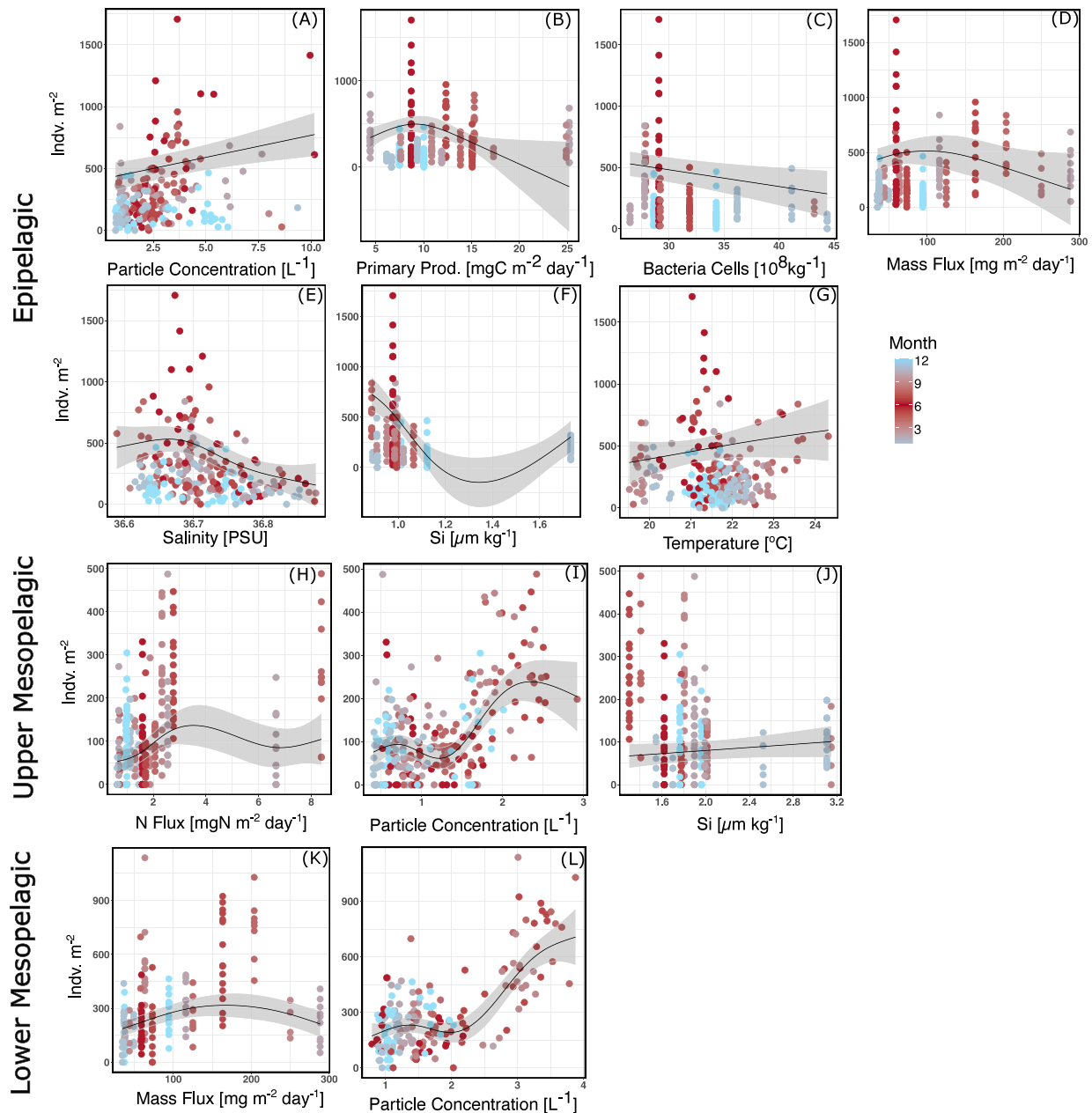


Fig. 7. Partial effect plots from Generalized Additive Models (GAMs) of all Rhizaria abundance. Models were constructed separately for depth integrated abundance through the Epipelagic (0–200 m) (A–G), Upper Mesopelagic (200–500 m) (H–J), and the Lower Mesopelagic (500–1000 m) (K–L). Each panel represents the partial effect of a GAM model term, with smoothed relationship shown shaded with 95% confidence intervals. Individual points are depth integrated values for all Rhizaria from an individual cast, colored by month.

observed in this study, while the epipelagic variety was not. Alternatively, the lower epipelagic of the extremely productive California Current may provide adequate habitat for Coelodendridae, which is not available in the oligotrophic Sargasso Sea.

A number of taxa were found to have a bimodal distribution, with sizable populations in both the epipelagic and mesopelagic. Aulacanthidae had a bimodal distribution, although abundances were highest in the lower mesopelagic. Foraminifera also had a bimodal distribution. Some lineages of Foraminifera are known to host photosymbionts (Kimoto, 2015; Biard, 2022b), however they are also efficient predators commonly seen throughout the mesopelagic (Caron and Be, 1984; Gaskell et al., 2019). Thus it is not surprising to find their presence in both locations of the water column. Foraminifera are also known to vary their vertical distribution across their life cycle in phase with lunar cycles (Bijma et al., 1990; Kimoto, 2015; Gaskell et al., 2019; Biard,

2022b). However, the sampling scheme of the BATS program does not capture this frequency and was not investigated in the present study.

Acantharea also had a bimodal distribution, with much more sizable abundances than Aulacanthidae or Foraminifera. Most prior studies of Acantharea vertical distribution found them concentrated in near surface layers of the water column (Zasko and Rusanov, 2005). This would support the paradigm that large Acantharea abundances may be supported by their mixotrophic abilities (Michaels et al., 1995; Suzuki and Not, 2015). While the UVP5 images cannot distinguish between mixotrophic and heterotrophic Acantharea, the GAMs constructed for Acantharea abundance found positive associations with particle concentration and mass flux, suggesting a higher reliance on heterotrophy. Recently Mars Brisbin et al. (2020) described apparent predator behavior amongst near-surface Acantharea. Thus it is likely that epipelagic Acantharea may commonly be heterotrophic or mixotrophic with an

increased reliance on heterotrophy. Yet, it should be noted in the Sargasso Sea, both heterotrophic and symbiotic lineages of Acantharea have been reported (Blanco-Bercial et al., 2022). Additionally, Michaels (1988) noted that the majority of Acantharea (by abundance) were smaller than 160 μm . While that estimate may be inflated due to inability to capture larger cells, small Acantharea were not captured in the present study. Thus, trophic strategy may shift based on sizes of Acantharea.

Decelle et al. (2013) proposed a hypothetical life cycle for cyst-bearing (strictly heterotrophic) Acantharea. This hypothesized life cycle suggests that epipelagic Acantharea are adult populations, which form cysts that sink into the mesopelagic, then reproduce and rise. Furthermore, given that horizontal transfer of symbionts between generations of Acantharea is unlikely due to their spawning behavior, the newly spawned mesopelagic Acantharea are not necessarily required to rapidly return to the photic zone (Decelle et al., 2012, 2013). This hypothesis predicts that Acanthareas in the mesopelagic would be smaller (Decelle et al., 2013). Mars Brisbin et al. (2020) provided some support for this hypothesis, with a significant decrease in Acantharea sizes with depth. Although the authors also observed low abundances in the mesopelagic and noted that the smaller sizes may be due to lower food availability (Mars Brisbin et al., 2020). Since food is more scarce in the mesopelagic, nutritional quality lower, yet flux feeders would likely grow larger to increase their feeding range (Stukel et al., 2018; Biard and Ohman, 2020). In the data collecting in this study, Acanthareas in the mesopelagic were significantly smaller than the epipelagic, despite the other bimodal taxa (Foraminifera and Aulacanthidae) being significantly larger with depth. This provides added support for the hypothesis that cyst-forming Acantharea may utilize different sections of the water column throughout their life cycle. However to further investigate this, more work is needed with higher temporal and taxonomic resolution.

4.0.4. Conclusions and considerations

This study provides a detailed look at Rhizaria abundance over time throughout the water column in a major oligotrophic gyre. We show that their abundances are generally related to particle concentration and flux, although lack of environmental variability may have reduced the fit of our GAMs. Considering the potential role of Rhizaria in the biological carbon pump, they may have a somewhat mixed role. In the shallower regions, they may be an attenuating force on sinking particles (Stukel et al., 2019). However, once consumed and repackaged by larger Rhizaria, they can sink quicker and contribute more to overall flux (Michaels, 1988). Thus, Rhizaria may act as an aggregation mechanism. However, to truly test this, more work is needed measuring Rhizaria flux.

The vertical partitioning documented in this study do support the hypothesis that mixotrophic rhizaria will occupy shallower waters while deeper waters are dominated by heterotrophy. However the degree to which mixotrophic Rhizaria in the euphotic zone rely on heterotrophy versus symbiosis is uncertain. Collodaria were recorded as consistent and dominant members of the near surface region. These organisms have the potential to contribute considerably to the otherwise low productivity of oligotrophic regions. However, their role in food webs is not well understood. While this study represents a step-forward in our understanding of Rhizaria ecology, continued research on Rhizaria is much needed to better understand their ecology. Particularly extended work describing interannual patterns in Rhizaria abundance. Also work defining biotic interactions, feeding rates, productivity, and life history are all rich fields of interest in Rhizaria.

CRediT authorship contribution statement

Alex Barth: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Leocadio Blanco-Bercial:** Writing – review & editing, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Rod Johnson:** Writing – review & editing, Supervision, Resources, Project administration. **Joshua Stone:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Text in the manuscript links to a github repository with all intermediate products are available: <https://github.com/TheAlexBarth/RhizariaSeasonality>. Raw data available on request.

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Appendix A. Supplementary data

Supplementary material related to this article can be found online at <https://doi.org/10.1016/j.dsr.2024.104371>.

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