

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.

² Introduction

³ Rhizaria are an extremely diverse super-group of single-celled organisms which occupy a wide range of
⁴ habitats. Planktonic Rhizaria include Foraminifera, Radiolaria (including Acantharea and Collodaria) and
⁵ Phaeodaria, all of which are observed throughout the global ocean (Biard *et al.*, 2016; Laget *et al.*, 2024)
⁶. While the taxonomy of these organisms has recently undergone several reclassifications (Biard, 2022b),
⁷ their presence in ocean ecosystems has been long known to oceanographers. Some of the earliest records
⁸ of their existence are from oceanographic expeditions in the 19th century (Haekel, 1887). Rhizaria are
⁹ unique members of the plankton and protist community because they can reach large sizes (up to several
¹⁰ mm in diameter) and they construct intricate mineral skeletons out of either silica, strontium, or calcium
¹¹ carbonate (Kimoto, 2015; Nakamura and Suzuki, 2015; Suzuki and Not, 2015; Biard, 2022b). Despite their
¹² noticeable morphology and global distribution, Rhizaria were largely understudied throughout the 20th
¹³ century. Fragile organisms like Rhizaria are difficult to adequately study because they can be destroyed
¹⁴ through standard zooplankton sampling techniques and preserve poorly. A number of studies in the late
¹⁵ 1900s did employ alternative techniques to quantify Rhizaria including diaphragm pumps (Michaels, 1988)
¹⁶ or blue-water SCUBA collections (Caron and Be, 1984; Bijma *et al.*, 1990; Caron *et al.*, 1995). However, the
¹⁷ bulk of Rhizaria research was constrained to sediment traps or paleontological studies of sediment (Takahashi
¹⁸ *et al.*, 1983; Boltovskoy *et al.*, 1993).

¹⁹ Recently, the advent of molecular techniques and *in situ* imaging tools have ignited a renewed focus on
²⁰ Rhizaria in pelagic ecosystems (Caron, 2016). Molecular tools have shed light on Rhizaria taxonomy (Biard,
²¹ 2022b), distribution (Blanco-Bercial, 2020; Sogawa *et al.*, 2022), ecology (Decelle *et al.*, 2012; Nakamura *et*
²² *al.*, 2023), biogeochemical roles (Gutierrez-Rodriguez *et al.*, 2019; Laget *et al.*, 2024), and metabolism (Cohen
²³ *et al.*, 2023). Yet, despite the excellent taxonomic resolution provided by molecular approaches, they do not
²⁴ provide a truly quantitative metric for estimating Rhizaria abundance 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 ($>500\mu\text{m}$ 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

48 Collodaria-symbiont association has been suggested as a reason for their high abundances throughout the
49 photic zone of oligotrophic environments globally (Biard *et al.*, 2016, 2017). A few Acantharea (Radiolaria
50 order) clades host algal symbionts (Decelle *et al.*, 2012; Biard, 2022a), notably with two clades forming an
51 exclusive relationship with *Phaeocystis*. However, globally, Acantharea are less abundant than Collodaria
52 (Biard, 2022b) and contribute less to total primary productivity (Michaels *et al.*, 1995). This may be due to
53 the fact several clades of Acantharea are cyst-forming and strictly heterotrophs (Decelle *et al.*, 2013; Biard,
54 2022a). Furthermore, Mars Brisbin *et al.* (2020) documented apparent predation behavior in Acantharea
55 near the surface, suggesting that there may be a larger reliance on carnivory.

56 Given the high abundances, yet diverse trophic strategies found among Rhizaria taxa, it is reasonable to
57 expect some form of niche partitioning. A number of studies do suggest evidence for vertical zonation
58 between Rhizaria groups according to various trophic strategies. Taxon-specific studies of Radiolaria suggest
59 they may be restricted to the euphotic zone (Michaels, 1988; Boltovskoy, 2017). Although some studies
60 report Acantharea in deeper waters (Decelle *et al.*, 2013; Gutiérrez-Rodríguez *et al.*, 2022), Phaeodaria,
61 alternatively, are generally found beyond depths where light can sustain photosynthesis, but they can feed
62 on sinking particles (Stukel *et al.*, 2018; Laget *et al.*, 2024). In an imaging-based study of the whole Rhizaria
63 community, Biard and Ohman (2020) noted clear patterns in vertical zonation which largely corresponded
64 to different trophic roles. Protists communities, including Rhizaria, have been described to partition along
65 autotroph-mixotroph-heterotroph gradients with increasing depth in the water column (Blanco-Bercial *et*
66 *al.*, 2022; Laget *et al.*, 2024). Yet, few studies have made direct attempts to relate Rhizaria abundances to
67 environmental factors (Biard and Ohman, 2020). In part, this is due to the fact few studies have been able
68 to sample Rhizaria in the same location over a consistent timeframe (Michaels, 1988; Boltovskoy *et al.*, 1993;
69 Michaels *et al.*, 1995; Hull *et al.*, 2011; Gutiérrez-Rodríguez *et al.*, 2022). Furthermore, very few studies
70 have utilized *in situ* imaging, arguably the best method for quantifying Rhizaria, to consistently throughout

71 the full mesopelagic (Laget *et al.*, 2024) . Given this lack of information, there are many unknowns with
72 respect to Rhizaria ecology, seasonality and phenology across different groups.

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

84 Methods

85 Oceanographic sampling

86 Data were collected in collaboration with the Bermuda Atlantic Time-series Study (Michaels and Knap, 1996;
87 Lomas *et al.*, 2013) on board the R/V Atlantic Explorer. Cruises were conducted at approximately monthly
88 intervals. Rhizaria individuals were sampled using the Underwater Vision Profiler 5 (UVP) (Picheral *et al.*,
89 2010). The UVP5 is an in-situ camera used to capture plankton and particle images and has been well
90 established to accurately quantify large Rhizaria abundance (Biard *et al.*, 2016; Stukel *et al.*, 2018; Stukel *et*
91 *al.*, 2019; Biard and Ohman, 2020; Barth and Stone, 2022; Drago *et al.*, 2022; Llopis Monferrer *et al.*, 2022;

92 Panaïotis *et al.*, 2023). The UVP5 was mounted to the sampling rosette and collected data autonomously on
93 routine casts, from which only the downcast data are utilized. The UVP5 was deployed from June-September
94 2019 then from October 2020 - January 2022, during which time the BATS region was sampled for 3-5 days
95 at monthly intervals (see sampling details in Supplemental Table 1). Casts were filtered to only include data
96 collected in the BATS region, far offshore of Bermuda in the Sargasso Sea (approximately $31.0^{\circ}N$ - $32.5^{\circ}N$,
97 $64.25^{\circ}W$ - $63^{\circ}W$; Supplemental Figure 1). In general, casts extended to either 200m, 500m, or 1200m deep,
98 with a few extended into the bathypelagic (4500m). However, Rhizaria were only typically found in large
99 abundances throughout the epipelagic and mesopelagic zones. As such, we limit this study to results from
100 the upper 1000m of the water column.

101 A variety of biotic and abiotic data were collected during each BATS cruise. The UVP5 provided particle
102 count data at a high-frequency from each cast. Particle concentration, as a proxy for prey field (Whitmore
103 and Ohman, 2021), was calculated from this data for all particles $184\mu m$ - $900\mu m$. The lower size range
104 was set by what could be reliably sampled by the UVP5's pixel resolution ($>2px$; 0.092mm per pixel) and
105 the upper size range was set to below the size of observable Rhizaria yet large enough to be inclusive of
106 particles on which some Rhizaria may feed. For each UVP cast supporting continuous profiles of the CTD
107 parameters salinity, temperature, and auxiliary CTD channels; Dissolved Oxygen (DO), *in situ* chlorophyll
108 fluorescence were measured at 24Hz using the BATS CTD package. On select casts, Niskin bottles were
109 used to collect bacterial abundance estimates (via epifluorescence microscopy) as well as measure inorganic
110 nutrients (NO_3 , and Si as silicate/silicic acid) at discrete depths. On each cruise, flux estimates of total
111 mass, organic carbon, and nitrogen were also collected using sediment traps; in the present study we utilized
112 flux to the mesopelagic as the flux at 200m. Also primary productivity was estimated through measuring ^{14}C
113 uptake rates from *in situ* incubations. For full descriptions of the BATS sampling program and methods,
114 see Knap *et al.* (1997) and Lomas *et al.* (2013) for a review. Additionally, data can be viewed online

115 (<https://bats.bios.asu.edu/bats-data/>).

116 Environmental data were processed to match the format of the Rhizaria abundance estimates (see below).

117 CTD data were collected at higher frequency than the UVP (24Hz vs 15Hz respectively), so these data were

118 averaged within matching bins to the UVP5 data. Data from Niskin bottles were first linearly interpolated

119 in depth at 1m resolution then time averaged over the cruise, then subsequently averaged into matching

120 bins as the UVP5 data. Primary productivity estimates were depth integrated throughout the euphotic zone

121 (0-140m).

122 Rhizaria imaging processing and abundance quantification

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

136 The UVP5 samples at ~15Hz rate as it descends the water column and records the exact position of each

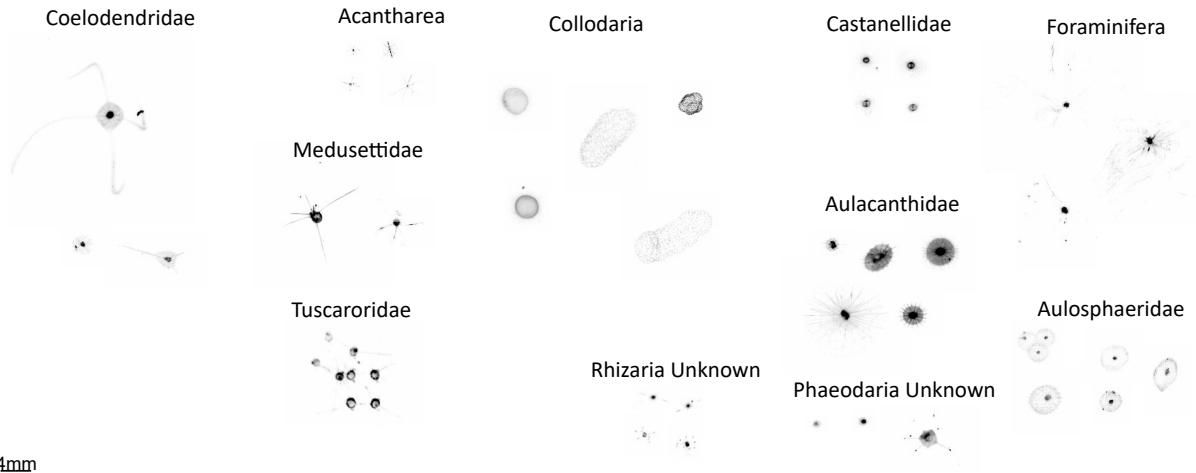


Figure 1: Example images of different Rhizaria taxa as collected by UVP5 imaging. 4mm scale bar shown in lower right. All vignettes are same scale

137 particle larger than $600\mu\text{m}$ (ESD). However, identified rhizaria ranged from a $934\mu\text{m}$ ESD Aulacanthidae
 138 cell to a Collodarian colony over 10mm in diameter. To confirm that the UVP5 was sampling adequately
 139 across all size ranges, an normalized biomass size spectrum (NBSS) slope was constructed to identify a drop-
 140 off which would indicate poor-sampling at the smaller end of the size range (Lombard *et al.*, 2019; Barth
 141 and Stone, 2024). However, it was evident from this analysis that all size ranges were adequately sampled
 142 across the size range (Supplemental Figure 2) so no data were excluded. The UVP5 reports the exact depth
 143 at which a particle is recorded, however to estimate abundance, observations must be binned over fixed
 144 depth intervals. Our deployments had variable descent depths and speeds with more casts descending to
 145 500m than 1000m and descents quicker through the epipelagic than the mesopelagic (see Barth and Stone
 146 (2022) for an extended discussion of UVP5 data processing). For the present study, Rhizaria abundances
 147 were estimated in 25m vertical bins, which offer a moderate sampling volume per bin (average 0.948m^3 in
 148 the epipelagic and 0.589m^3 in the mesopelagic; Supplemental Table 1) while still maintaining ecologically
 149 relevant widths. However, concentrations in a 25m bin would need to be greater than $2.428 \text{ ind. m}^{-3}$

150 and 3.912 ind. m^{-3} , in the epipelagic and mesopelagic respectively, to fall below a 10% non-detection
151 risk (Benfield *et al.*, 1996; Barth and Stone, 2024). Because we typically observed many Rhizaria taxa
152 below these concentrations, we present the 25m binned data to visualize broad-scale average distributions.
153 For quantifying and modelling Rhizaria abundances, we present integrated abundance estimates, with each
154 cast. Due to the variable descent depths of the UVP, data are categorized as epipelagic (0-200m), upper
155 mesopelagic (200-500m), and lower mesopelagic (500-1000m). The average sampling volume integrated
156 through these regions were $7.59m^2$, $7.06m^2$, and $11.77m^2$, with non-detection thresholds at 0.30 ind. m^{-2} ,
157 0.33 ind. m^{-2} , and 0.20 ind. m^{-2} respectively. All UVP data processing was done using the `EcotaxaTools`
158 package in R (<https://github.com/TheAlexBarth/EcotaxaTools?tab=readme-ov-file>).

159 Modelling environmental controls of Rhizaria abundance

160 Generalized Additive Models (GAMs) were used to assess the relationship between integrated Rhizaria abun-
161 dance and different environmental factors. GAMs offer the ability to model non-linear and non-monotonic
162 relationships, which can be particularly useful in assessing ecological relationships (Wood, 2017) and have
163 been successfully applied to Rhizaria ecology (Biard and Ohman, 2020). The `mgcv` package (Wood, 2001)
164 was used to construct models relating environmental parameters to each taxonomic group's integrated abun-
165 dance estimates from each cast. To select the most parsimonious model for each analysis, a backwards
166 step-wise approach was taken. First, a full model was fit using any term which may be ecologically relevant.
167 Terms were fit using maximum likelihood with a double penalty approach on unnecessary smooths (Marra
168 and Wood, 2011). The smoothness parameter was restricted ($k = 6$) to prevent over fitting the models.
169 Then, the full model was checked for concurvity (a metric indicating high parameter relatedness in GAMs).
170 If two terms had a high concurvity value (>0.8), then two alternative models were constructed each leaving
171 out one of the related terms. The more parsimonious model of these two was selected using AIC. Once

172 concurvity of terms was reduced, then the model was subjected to the backwards step-wise procedure. At
173 each iteration, the model term with the lowest F score (least statistically significant) was removed. This
174 was repeated until all model terms were statistically significant or the R^2_{adj} was substantially reduced. Mod-
175 els were fit for each water column region; epipelagic, upper mesopelagic, and lower mesopelagic. In cases
176 where observations were too sparse for a given taxonomic grouping, models were not run. All code, full
177 models, and model selection tools are available in open-source scripts, as well as intermediate data products
178 at <https://github.com/TheAlexBarth/RhizariaSeasonality>.

179 Results

180 Environmental Variability

181 While the environmental conditions were generally low in variation and oligotrophic, there is considerable
182 influence from deep winter-mixing and summer stratification as well as secondary influences from mesoscale
183 eddies which result in spatiotemporal heterogeneity (McGillicuddy *et al.*, 1998; Lomas *et al.*, 2013). Vari-
184 ability in the water column structure was visible during the study period (Figure 2). This is best evidenced
185 through the temperature profiles; In the late summer and early fall there was a stratified water column with
186 high temperatures in the surface (<75m) (Figure 2A) and slightly elevated salinity (Figure 2B). This warm,
187 stratified period appeared more intense during the few months sampled in 2019. In 2021, we observed the
188 stratified layer slowly dissipated into the winter months following mixed layer entrainment. There was a
189 consistent oxygen minimum zone (OMZ) located at about 800m deep (Figure 2C). February 2021 saw a
190 notable downwelling event, likely due to a passing anti-cyclonic eddy which impacted the local region during
191 early 2021. During this phase, warmer, oxic water was significantly depressed deeper into the mesopelagic.
192 This process was reversed in the spring months (March, April) primary due to the interaction of convective

193 mixing and the passing of a strong cyclonic eddy resulting in a deep cold mixed layer. Primary production
194 was highest during the spring mixing period, evidenced both by *in situ* fluorescence (Figure 2D) and produc-
195 tivity incubation experiments (Figure 3A). Originating near the surface, the productivity peak moved deeper
196 throughout the spring and declined into the summer (Figure 2D). However, there was a notable, yet smaller
197 productivity bump in the late summer and early fall (Figure 3A) which occurred deeper in the epipelagic
198 (Figure 2D). The particle concentration ($184\mu m - 900\mu m$) was closely coupled to chlorophyll-a patterns.

199 Overall, particle concentration was high near the surface during the 2021 spring bloom, then moved deeper
200 throughout the water column attenuating throughout the lower epipelagic (Figure 2E). Similarly, het-
201 erotrophic bacteria abundance was closely linked to overall productivity, although there was a more con-
202 sistent moderate-abundance layer near the top of the mesopelagic (~250m) (Figure 2F). Concurrent with
203 the secondary fall production peak, there was also higher particle concentration and bacterial abundance in
204 the later summer and early fall. Interestingly, while primary productivity estimates from July-August were
205 not that different between 2019 and 2021 (Figure 3A), chlorophyll-a florescence, particle concentration, and
206 bacterial abundance were much higher in 2019's summer/fall (Figure 2D-F). Inorganic nutrients (*Si* and
207 NO_3) were generally well stratified, with low concentrations in the epipelagic and increasing throughout the
208 mesopelagic. However, both nutrients did vary vertically in accordance with the 2021 February downwelling
209 and spring mixing period (Figure 2G-H). Additionally, in the late fall of 2021, *Si* concentrations were slightly
210 elevated in the mid-mesopelagic (Figure 2G).

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

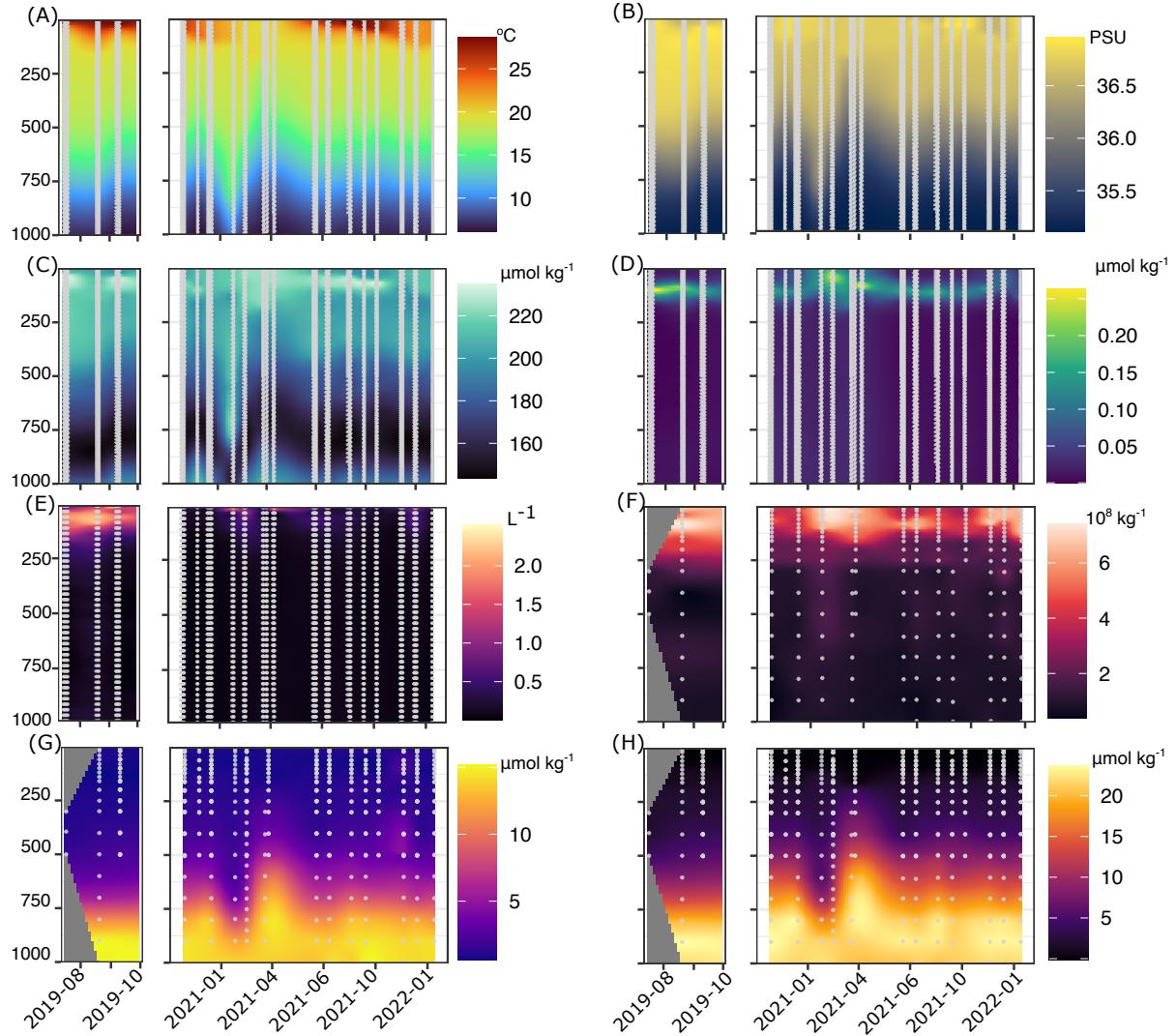


Figure 2: Environmental profiles across time-series of study period. Y axis shows depth in meters. (A) Temperature. (B) Salinity. (C) Dissolved Oxygen. (D) in situ chlorophyll fluorescence. (E) Particle concenctrion ($184 - 900 \mu\text{m m}$). (F) Bacteria Abundance. (G) Silica. (H) Nitrate

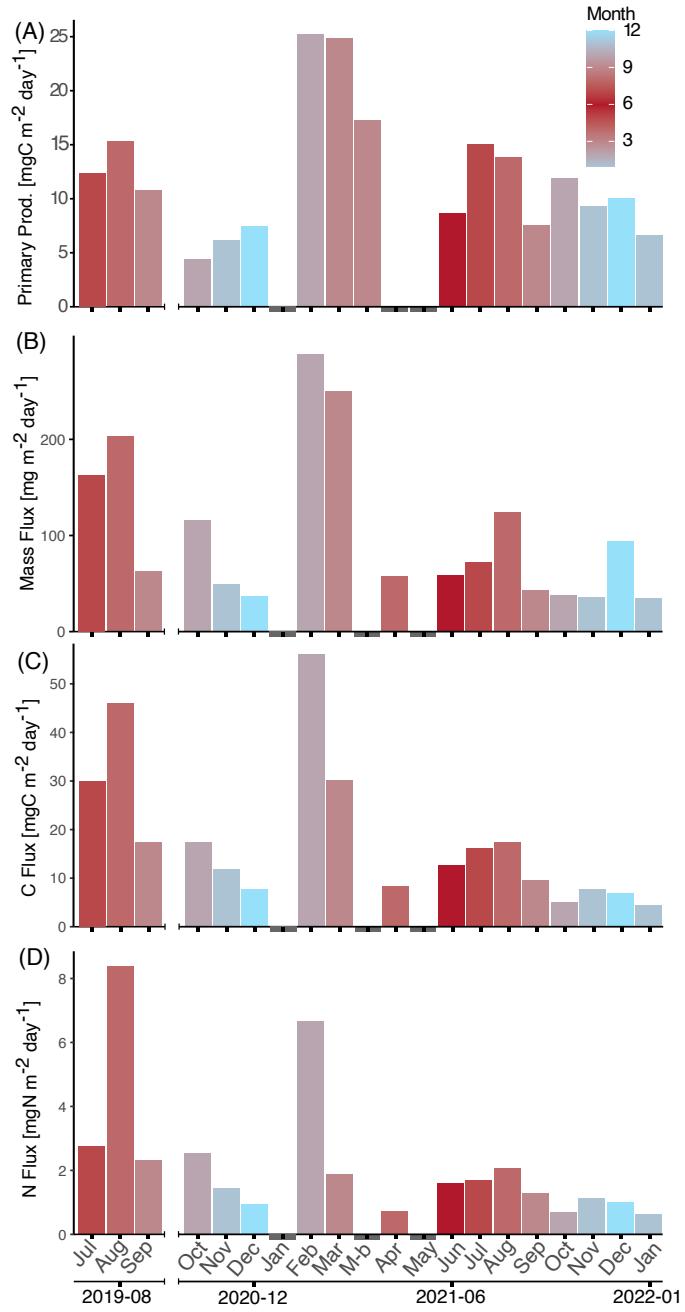


Figure 3: Primary productivity (A) and flux estimates from total mass (B), carbon (C), and nitrogen (D). Values are from monthly cruises with month displayed in corresponding colors. Absent data are shown by grey bar.

216 Rhizaria abundance and distribution

217 Across all imaged mesozooplankton ($>900\mu m$), Rhizaria comprised a considerable fraction of the total com-
218 munity. Considering the total abundances of the observational period, Rhizaria comprised on average, 42.6%
219 of all mesozooplankton abundance (Supplemental Figure 3). Copepods were the second most abundant, com-
220 prising 35.5% and all other living mesozooplankton were 22%. The large contribution of Rhizaria to the
221 mesozooplankton community is most prominent in the epipelagic, where they accounted for 47% of all meso-
222 zooplankton abundance. In the mesopelagic Rhizaria were a smaller fraction, at 38% in the upper layers
223 (200-500m) and 37% in the deeper mesopelagic (500-1000m).

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

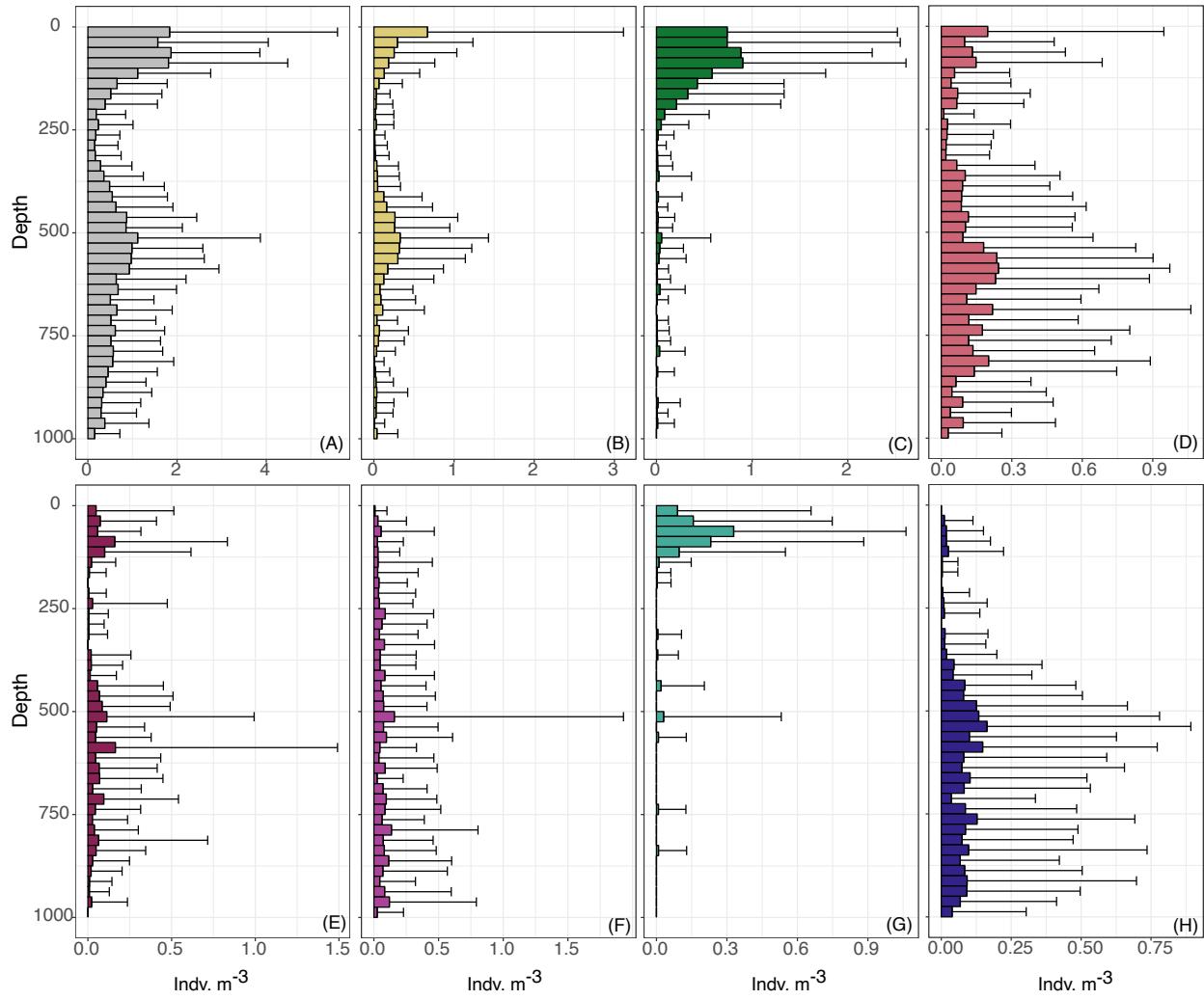


Figure 4: Average abundance of Rhizaria in 25m bins, across entire study period. Shown are total Rhizaria (A), Acantharea (B), Collodaria (C), Aulacanthidae (D), Foraminifera (E), Aulosphaeridae (F), Castanellidae (G), Coelodendridae (H).

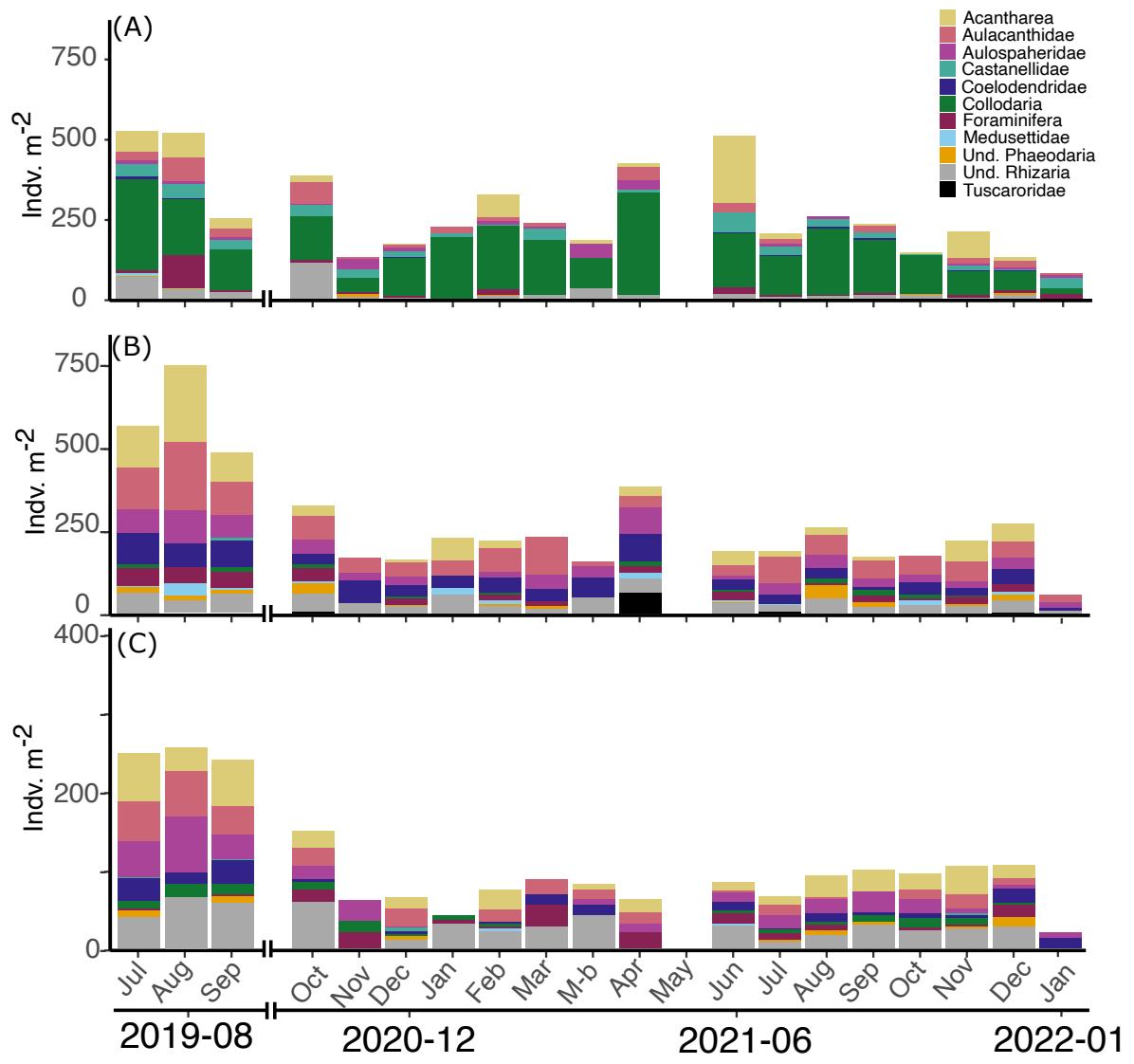


Figure 5: Seasonality of Rhizaria integrated abundance for the epipelagic (0-200m) (A), upper mesopelagic (200-500m) (B), lower mesopelagic (500-1000m) (C). Data shown are the cruise mean integrated abundance in the water column region colored for each taxon.

239 Between the monthly cruises, Rhizaria integrated abundance varied in the epipelagic. Highest average abun-
240 dance occurred in June 2021 and was lowest during the winter months (Figure 5A). The 2019 later summer
241 - fall period also had much higher integrated abundance than similar months in 2021. While the majority
242 of integrated abundance in the epipelagic was consistently attributable to Collodaria, Acanthrea abundance
243 occurred sporadically and could account for a large portion of the total in some months (Figure 5A). The
244 mesopelagic integrated abundance was much more consistent across monthly cruises, although average abun-
245 dance was notably higher in 2019 (Figure 5B, 5C). The community composition in the mesopelagic was more
246 diverse, mostly comprised of Phaeodaria families. However, Acantharea and unidentified Rhizaria also were
247 common members of the community (Figure 5B, C).

248 **Body size throughout the water column.**

249 Very few taxa had consistent distributions throughout the water column. Only Acanthrea, Foraminifera,
250 Aulacanthidae, and Aulosphaeridae were consistently abundant in the epipelagic and mesopelagic. To invest-
251 igate if the populations or morphologies shifted throughout the water column, we compared the sizes (ESD)
252 between mesopelagic and epipelagic groups for each taxa. All groups were significantly different on average
253 (Wilcox Rank Sum p-value <0.001). Acantharea were smaller, on average in the mesopelagic while all other
254 taxa tended to be larger (Figure 6).

255 **Environmental Drivers of Rhizaria Abundance**

256 For integrated abundance of all Rhizaria, the GAMs produced moderate fits ($R^2_{adj} = 0.406-0.603$) (Table
257 1). In the epipelagic, there were several significant predictor variables (Figure 7A-7G). This included terms
258 which are likely indicators of changing water masses; Salinity (7E) and Temperature (7G). The model
259 also had parameters related to both heterotrophy (Particle concentration, 7A) and autotrophy (Primary
260 production, 7B). However the relationship to particle concentration is weakly increasing trend while the

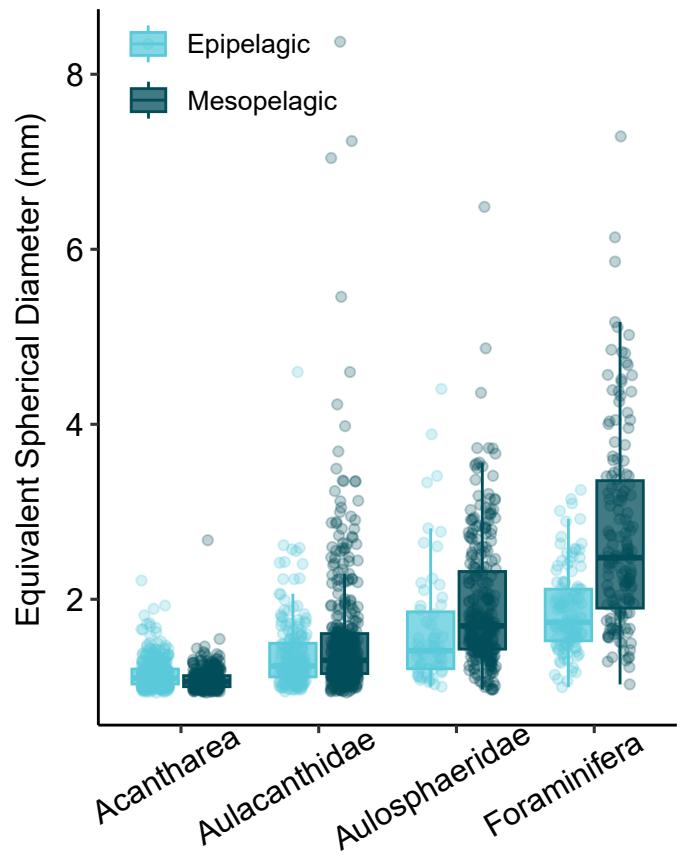


Figure 6: Comparison of average sizes (ESD) amongst Rhizaria taxa which occurred throughout the water column.

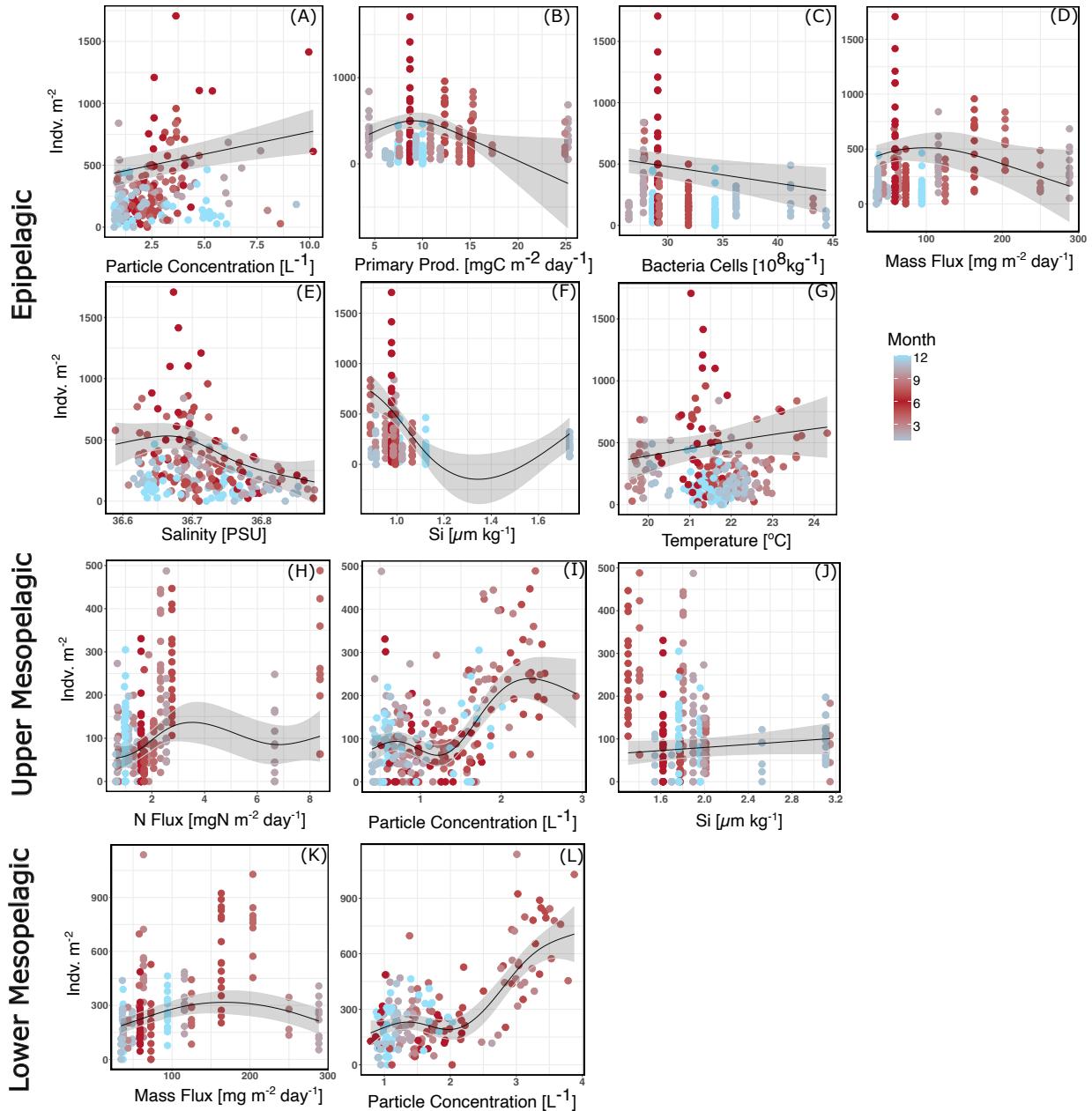


Figure 7: Partial effect plots from Generalized Additive Models (GAMs) of all Rhizaria abundance. Models were constructed separately for depth integrated abundance through the Epipelagic (0-200m) (A-G), Upper Mesopelagic (200-500m) (H-J), and the Lower Mesopelagic (500-1000m) (K-L). Each panel represents 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.

261 primary productivity effect appears to be influenced by a cruise with a high outlier for increased primary
 262 productivity. The models for the mesopelagic regions were much more reduced and the only significant
 263 effects were particle-related terms (particle concentration and mass flux). In the upper mesopelagic (Figure
 264 7H-7J), final model included N flux, particle concentration and a non-significant effect for Silica. The lower
 265 mesopelagic (Figure 7K, 7L) only had model effects kept of total mass flux and particle concentration. For
 266 both the upper and lower mesopelagic, particle concentration had a clear, strong positive relationship (Figure
 267 7I, 7L).

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	Temperature	0.7950	0.492	0.0440
$R^2_{adj} = 0.48$	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	Silica	0.6702	0.404	0.0814
$R^2_{adj} = 0.40$	Avg N Flux	2.9524	3.172	<0.001
	Particle Concentration	3.5568	11.82	<0.001
All Rhizaria Lower Mesopelagic	Avg Mass Flux	2.036	2.953	<0.001

Model	Term	edf	F	p
$R^2_{adj} = 0.61$	Particle Concentration	3.819	27.43	<0.001

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

272 Epipelagic Acantharea were explained by several predictor variables and had a good fit ($R^2_{adj} = 0.539$,
 273 Table 2, Supplemental FIgure 4). Foraminifera had a good fitting GAM in the epipelagic ($R^2_{adj} = 0.543$).
 274 There were several significant explanatory variables, although the clearest pattern was observed of high
 275 temperatures associated with more Foraminifera abundance (Table 2, Supplemental Figure 4U). Epipelagic
 276 Aulacanthidae similarly had several predictor variables which were significant, including both water quality
 277 parameters and particle/flux predictors (Table 2). There was a weak fit for Collodaria in the epipelagic
 278 ($R^2_{adj} = 0.16$), although there was a logit-like relationship where higher abundances tended to occur during
 279 higher DO conditions in the surface waters (Supplemental Figure 4). Castanellidae also had a weak model
 280 fit in the epipelagic ($R^2_{adj} = 0.228$), although particle concentration was the strongest predictor term (Table
 281 2, Supplemental Figure 4).

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	Temperature	0.868	0.380	0.0935

Model	Term	edf	F	p
$R_{adj}^2=0.539$	Salinity	2.349	4.646	<0.001
	O2	2.501	5.493	<0.001
	Avg Mass Flux	4.815	22.59	<0.001
	Bacteria	1.608	1.360	0.00945
	Particle Concentration	0.877	1.414	0.00449
Acantharea Upper Mesopelagic	Avg Mass Flux	1.866	1.220	0.0166
$R_{adj}^2=0.197$	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	Temperature	1.958	2.986	<0.001
$R_{adj}^2=0.496$	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	Temperature	2.591	5.003	<0.001
$R_{adj}^2=0.394$	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	Avg C Flux	1.675	1.408	0.0109
$R_{adj}^2=0.132$	Particle Concentration	2.308	2.476	0.0013
Aulacanthidae Lower Mesopelagic	Avg Mass Flux	1.476	1.418	0.0076

Model	Term	edf	F	p
$R_{adj}^2=0.308$	Particle Concentration	2.457	7.756	<0.001
Aulosphaeridae Upper Mesopelagic	Particle Concentration	2.778	12.76	<0.001
$R_{adj}^2=0.196$	Particle Concentration	2.012	6.306	<0.001
Aulosphaeridae Lower Mesopelagic	Particle Concentration	2.012	6.306	<0.001
$R_{adj}^2=0.148$	Salinity	0.915	0.400	0.0935
Castanellidae Epipelagic	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	Avg N Flux	0.734	0.551	0.0527
$R_{adj}^2=0.09$	Particle Concentration	0.965	5.102	<0.001
Coelodendridae Lower Mesopelagic	Particle Concentration	3.051	6.768	<0.001
$R_{adj}^2=0.157$	Salinity	0.926	2.267	<0.001
Collodaria Epipelagic	O2	2.015	2.217	<0.001
	Bacteria	1.843	2.100	0.0024
Foraminifera Epipelagic	Temperature	4.420	7.624	<0.001
$R_{adj}^2=0.543$	O2	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

Model	Term	edf	F	p
	Particle Concentration	1.685	2.955	<0.001

282 In the upper mesopelagic (200-500m), abundances were generally low (Figure 4) so GAMs were only con-
 283 structed for Acantharea, Aulacanthidae, Aulosphaeridae, and Coelodendridae (Table 2). All these models
 284 had generally poor fits ($R^2_{adj} < 0.20$). Yet, for all upper mesopelagic models, particle concentration was a
 285 significant explanatory variable (Table 2, Supplemental Figure 5). The lower mesopelagic also had generally
 286 poor GAM fits for taxon specific models ($R^2_{adj} < 0.31$), with the exception of Acantharea ($R^2_{adj} = 0.496$).
 287 Acantharea in the lower mesopelagic was most clearly positively associated with particle concentration (Sup-
 288 plemental Figure 6). For all Phaeodaria with a significant model, particle concentration was a main predictor
 289 variable (Table 2, Supplemental Figure 6).

290 Discussion

291 Overall Rhizaria abundance and patterns

292 In the epipelagic Rhizaria exhibited a notable seasonal pattern. Rhizaria abundances were higher in the
 293 summer months and lower during the winter. During a prior time period, Blanco-Bercial *et al.* (2022) noted
 294 that there is considerable seasonality in the community composition of all protists. Despite the seasonality
 295 of Rhizaria abundance, community composition was relatively consistent, with Collodaria representing the
 296 bulk of the community. It should be noted that the overall taxonomic resolution of the UVP5 is fairly
 297 low, so there may be a switching of species within the broad groups identified in this study which were not
 298 captured. Throughout the mesopelagic, month-to-month variation in 2021 was relatively low. Again, this is
 299 consistent with observations from metabarcoding of the whole protist community in the same study region

300 (Blanco-Bercial *et al.*, 2022). This finding is not surprising as the overall seasonal variation in environmental
301 conditions in this region were low.

302 Overall Rhizaria were the most commonly identified group of mesoplankton throughout the study period.
303 We do note that the UVP5 commonly captures *Trichodesmium* colonies, yet these were excluded in this
304 comparison as they are strictly autotrophs. It should be noted that previous work has suggested that
305 avoidance behavior with the UVP is possible, at times likely, for visual and highly mobile zooplankton
306 (Barth and Stone, 2022). Thus, the percent contribution reported here (42.7%) of Rhizaria to the total
307 mesozooplankton community may be inflated due to under sampling of organisms such as Euphausiids and
308 Chaetognaths which have quick escape responses. Regardless, it is worth noting that in the same region,
309 with data collected in 2012 and 2013 using similar calculation methods, Biard *et al.* (2016) estimated large
310 Rhizaria only contribute 15% of the total mesozooplankton community in the upper 500m. Likely, Rhizaria
311 display considerable interannual variability. In the present study, we noticed considerably higher Rhizaria
312 abundance throughout the water column in 2019 compared to 2021. Similarly, in the California Current a
313 multi-year study of Aulosphaeridae saw considerable variation between years (Biard *et al.*, 2018). To truly
314 understand the drivers of interannual variability however, sustained observations of Rhizaria over longer time
315 periods are required.

316 **Relationship to environmental parameters**

317 In general, the fit of most GAMs were moderate to poor. One possible reason for the poor fits may have
318 been that for some taxa, conditions were not variable enough to capture a range of conditions at which
319 they may exist. For instance, Collodaria were the most abundant taxa observed, yet the fit of their GAM
320 was particularly poor. In studies which covered a wider range of parameters, Collodaria has been shown
321 to strongly vary with changes in parameters such as temperature, chlorophyll-a, mixing, and water clarity

322 (Biard *et al.*, 2017; Biard and Ohman, 2020). Alternatively, Acantharea had relatively good fitting GAMs.
323 These taxa also had some of the largest variation from month to month on cruises. Thus, it may be that
324 in the oligotrophic, the relatively stable conditions can support certain taxa while others are more sporadic.
325 It should also be noted that due to the challenge of adequately sampling enough volume to overcome low-
326 detection issues, GAMs were run on integrated data. However, variation with environmental parameters
327 throughout the water column are likely, just not captured in the modelling aspect of this study. Future
328 studies which are able to account for detection biases in finer vertical resolution of Rhizaria may improve
329 ecological models of their abundance. One consistent parameter which had significant positive associations
330 was particle concentration. This observation is not surprising as most Rhizaria likely to some extent engage
331 in flux feeding.

332 **Vertical Structure and Trophic Roles**

333 In this study we present a clear pattern of vertical zonation between different Rhizaria groups. Largely, the
334 taxonomic composition and vertical positioning were similar to Rhizaria zonation in the California Current
335 Ecosystem (Biard and Ohman, 2020). It should be noted however, that the secondary abundance peak
336 reported in the present study is lower in magnitude. This is likely due to the more oligotrophic nature
337 of the study region, where the euphotic zone penetrates deeper into the water column. Most prevalent
338 in the epipelagic were Collodaria. These mixotrophic Radiolaria have long been reported to contribute
339 to primary productivity in the euphotic zone (Michaels *et al.*, 1995; Dennett *et al.*, 2002). Collodaria are
340 thought to be particularly successful globally in oligotrophic regions due to their photosymbiotic relationships
341 (Biard *et al.*, 2016, 2017). We observed the highest abundance of Collodaria during June 2021, supporting
342 the notion they can thrive during the typically low-nutrient conditions of summer stratification. However,
343 Collodaria also increased during the spring mixing period, suggesting that they can thrive during conditions

344 which may typically be thought to favor autotrophs. Furthermore, while Collodaria were primarily absent
345 from below 250m, there were a few instances of deeper colonies being observed. Global investigations of
346 polycystine flux, suggest that deep-Collodaria in oligotrophic regions may be a consequence of isothermal
347 submersion (Boltovskoy, 2017). Alternatively, surface waters at BATS often mix into the mode water during
348 the seasonal mixing, so Collodaria in the deeper waters may be a result of diapycnal mixing. Another
349 effectively exclusively epipelagic Rhizaria was the Phaeodaria family of Castanellidae. All Phaeodaria are
350 thought to be fully heterotrophic (Nakamura and Suzuki, 2015), nonetheless a number of studies, including
351 this one, report Castanellidae to be typically found in the lower epipelagic (Zasko and Rusanov, 2005; Biard
352 *et al.*, 2018; Biard and Ohman, 2020). It should be considered that perhaps Castanellidae specializes in
353 feeding on sinking particles directly at the base of the epipelagic. Given it's smaller size (Nakamura and
354 Suzuki, 2015), Castanellidae does not need a large diameter to efficiently flux feed at the typically particle
355 rich region of the lower epipelagic.

356 The mesopelagic generally was home to known heterotrophic organisms, particularly for those which were
357 constrained to exclusively occupy deeper waters. This is consistent with Blanco-Bercial *et al.* (2022)'s
358 observation of an auto-/mixotroph to heterotroph gradient in local protist community as well as global
359 patterns in Rhizaria ecology (Laget *et al.*, 2024). The upper mesopelagic interestingly had relatively low total
360 abundance. This low-abundance region likely reflects the dynamics of productivity and export throughout
361 the water column. While productivity and thus sinking particles for flux feeders are high in the euphotic
362 zone, much of this is attenuated throughout the epipelagic. So, while the base of the epipelagic may provide
363 a rich feeding environment for Castanellidae, smaller protists, or heterotrophic bacteria (Figure 2F), the
364 region from 200-500m might be otherwise food poor. Perhaps it is more advantageous for Rhizaria to situate
365 deeper, in darker regions of the twilight zone where visual predators and vertically migrating organisms may
366 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 500m (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 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 sur-

390 face layers of the water column Zasko and Rusanov (2005). This would support the paradigm that large
391 Acantharea abundances may be supported by their mixotrophic abilities (Michaels *et al.*, 1995; Suzuki and
392 Not, 2015). While the UVP5 images cannot distinguish between mixotrophic and heterotrophic Acantharea,
393 the GAMs constructed for Acantharea abundance found positive associations with particle concentration
394 and mass flux, suggesting a higher reliance on heterotrophy. Recently Mars Brisbin *et al.* (2020) described
395 apparent predator behavior amongst near-surface Acantharea. Thus it is likely that epipelagic Acantharea
396 may commonly be heterotrophic or mixotrophic with an increased reliance on heterotrophy. Yet, it should
397 be noted in the Sargasso Sea, both heterotrophic and symbiotic lineages of Acantharea have been reported
398 (Blanco-Bercial *et al.*, 2022). Additionally, Michaels (1988) noted that the majority of Acantharea (by abun-
399 dance) were smaller than $160\mu m$. While that estimate may be inflated due to inability to capture larger
400 cells, small Acantharea were not captured in the present study. Thus, trophic strategy may shift based on
401 sizes of Acantharea.

402 Decelle *et al.* (2013) proposed a hypothetical life cycle for cyst-bearing (strictly heterotrophic) Acantharea.
403 This hypothesized life cycle suggests that epipelagic Acantharea are adult populations, which form cysts
404 that sink into the mesopelagic, then reproduce and rise. Furthermore, given that horizontal transfer of
405 symbionts between generations of Acantharea is unlikely due to their spawning behavior, the newly spawned
406 mesopelagic Acantharea are not necessarily required to rapidly return to the photic zone (Decelle *et al.*,
407 2012, 2013). This hypothesis predicts that Acantharea in the mesopelagic would be smaller (Decelle *et al.*,
408 2013). Mars Brisbin *et al.* (2020) provided some support for this hypothesis, with a significant decrease in
409 Acantharea sizes with depth. Although the authors also observed low abundances in the mesopelagic and
410 noted that the smaller sizes may be due to lower food availability (Mars Brisbin *et al.*, 2020). Since food is
411 more scarce in the mesopelagic, nutritional quality lower (Kim *et al.*, 2018), yet flux feeders would likely grow
412 larger to increase their feeding range (Stukel *et al.*, 2018; Biard and Ohman, 2020). In the data collecting in

413 this study, Acantharea in the mesopelagic were significantly smaller than the epipelagic, despite the other
414 bimodal taxa (Foraminifera and Aulacanthidae) being significantly larger with depth. This provides added
415 support for the hypothesis that cyst-forming Acantharea may utilize different sections of the water column
416 throughout their life cycle. However to further investigate this, more work is needed with higher temporal
417 and taxonomic resolution.

418 **Conclusions and Considerations**

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

427 The vertical partitioning documented in this study do support the hypothesis that mixotrophic rhizaria will
428 occupy shallower waters while deeper waters are dominated by heterotrophy. However the degree to which
429 mixotrophic Rhizaria in the euphotic zone rely on heterotrophy versus symbiosis is uncertain. Collodaria
430 were recorded as consistent and dominant members of the near surface region. These organisms have the
431 potential to contribute considerably to the otherwise low productivity of oligotrophic regions. However, their
432 role in food webs is not well understood. While this study represents a step forward in our understanding of
433 Rhizaria, further research is needed to better characterize their ecology and role in biogeochemical cycling.
434 To fully understand the functioning of the oceans, not only carbon or nitrogen, but other elements such as

435 silica or strontium, extended work addressing the interannual patterns in Rhizaria, their biotic interactions,
436 and the key biological rates that impact ocean biogeochemistry, will be required.

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