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Carbon and nitrogen content to biovolume relationships for marine protist of the Rhizaria lineage (Radiolaria and Phaeodaria)

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Abstract

Rhizaria are large protistan cells that have been shown to be a major component of the planktic community in the oceans and contribute significantly to major biogeochemical cycles such as carbon or silicon. However, unlike for many other protists, limited data is available on rhizarian cellular carbon (C) and nitrogen (N) content and cell volume. Here we present novel C and N mass to volume equations and ratios for nine Rhizaria taxa belonging to Radiolaria (i.e., *Collozoum*, *Sphaerozoum*, *Collosphaeridae*, Acantharia, Nassellaria, and Spumellaria) and Phaeodaria (i.e., *Aulacantha*, *Protocystis*, and *Challengeria*). The C and N content of collodarian cells was significantly correlated to cell volume as expressed by the mass : vol equations ng C cell^{-1} = −13.51 + 0.1524 × biovolume (μm^3) or ng N cell^{-1} = −4.33 + 0.0249 × biovolume (μm^3). Significant C and N content to volume correlations were also identified, and corresponding equations are proposed, for C : vol and N : vol of collodarian colonies (Radiolaria), and C : vol of the genus *Protocystis* (Phaeodaria). Furthermore, average C and N densities (mass per volume) are given for all studied Rhizaria. The densities and mass : vol equations established here could show that, with the exception of *Aulacantha*, biomass of most Rhizaria would have been underestimated using previously published generic protist C : vol ratios. We measured up to 35 times more C content for Acantharia than otherwise estimated, and between 1.4 and 21.5 times more for other taxa. Our mass : vol data will prove critical for model input and quantitative ecological studies of oceanic ecosystems.

Rhizaria are single-celled eukaryotes (i.e., protists) that are a key component of planktic communities in the ocean (Not et al. 2007; Amacher et al. 2009; Xu et al. 2018). Traditionally, the phylum Radiolaria (Rhizaria) included the orders Acantharia, Nassellaria, Spumellaria, and Phaeodaria (Haeckel 1887). However, Phaeodaria are now considered Cercozoa of the supergroup Rhizaria (Polet et al. 2004). Marine Rhizaria can represent up to 33% of large zooplankton community (> 600 μm) in the upper water column (Biard et al. 2016), and are also abundant in deeper layers (Biard and Ohman 2020). Rhizaria are fundamental to many biogeochemical cycles, including silicon (Biard et al. 2018; Llopis Monferrer et al. 2020), strontium (Bernstein et al. 1987), carbon through calcification (Erez 2003), and the sinking of particulate organic matter known as the biological carbon pump process (Lampitt et al. 2009; Stukel et al. 2018; Gutierrez-Rodriguez et al. 2019). The lack of success in culturing Rhizaria and their poor preservation when using conventional sampling methods (Anderson 1983; Suzuki and Not 2015) have limited our basic knowledge about ecology and physiology of these organisms. Their role in the food web is still unclear, but Rhizaria have been shown to consume a variety of prey (Gowing 1989; Swanberg and

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Additional Supporting Information may be found in the online version of this article.

Author Contribution Statement: J.S.M. and F.N. designed and planned the research. J.S.M. and A.N. performed the sampling, experiments, and image analysis for Collodaria & Acantharia. J.S.M. performed elemental and data analysis for Collodaria & Acantharia, as well as phylogenetic analyses. N.L.M. and F.N. performed the sampling and experiments for Phaeodaria, Nassellaria and Spumellaria. N.L.M. performed image analysis and prepared the data for Phaeodaria, Nassellaria and Spumellaria. S.L. performed elemental analysis and data preparation for Phaeodaria, Nassellaria and Spumellaria. J.S.M. processed all data and wrote the manuscript. All authors contributed to or commented on the manuscript.

Caron 1991). Among Rhizaria, many Retaria (e.g., Radiolaria, Foraminifera) harbor algal symbionts to supplement their metabolic needs (Stoecker et al. 2009).

Carbon (C) and nitrogen (N) content and C : N ratios of planktic organisms are fundamental information for constraints of oceanic ecosystem dynamics or biogeochemical models (Flynn and Mitra 2009; Flynn et al. 2019), as well as performing quantitative ecological studies of plankton (e.g., Biard et al. 2016; Stukel et al. 2018). Biovolume is often used for the calculation and estimation of C mass of planktic organisms, or in the estimation of relative (primary) production (Caron et al. 1995). These parameters are needed to accurately estimate nutrient and C and N fluxes in ecosystems (Fasham et al. 1990; Franks 2002). However, current biogeochemical models often neglect larger protists such as Rhizaria (Hood et al. 2006). In large part, this omission occurs because the parameters controlling such predictive models are difficult to evaluate, since quantitative physiological information (e.g., regarding the position in the food web; the functional classification, and the occurrence of mixotrophy) is extremely limited.

Regarding C to volume (C : vol) conversions, in contrast to smaller sized planktic groups (estimated spherical diameter, ESD < 17 μm, 3000 μm^3), no published measurements of C : vol specific to larger protists are available (Menden-Deuer and Lessard 2000). The use of more general values for conversion of biovolume to cellular C and N content (e.g., Michaels 1991; Biard et al. 2016; Stukel et al. 2018) could yield inaccurate or overestimated biomass estimations (Stukel et al. 2018; Ikenoue et al. 2019). For instance, data collected by Menden-Deuer and Lessard (2000) showed that the large dinoflagellate *Noctiluca scintillans* (ESD > 250 μm) is an outlier in regards to C : vol among dinoflagelattes, likely due to its aberrant size. Ikenoue et al. (2019) thus directly measured cellular C of >1 mm Phaeodaria, showing that the actual measured C content of their Phaeodaria specimen was several orders of magnitude lower than when estimated with available C : vol conversions for protists. Carbon or N content of Radiolaria has to our knowledge only been directly measured on two occasions, by Michaels et al. (1995) and Swanberg (1983), though no relationship to volume has been resolved. It is clear that future plankton studies and ecosystem modeling will benefit from improved data for protists such as Rhizaria.

The goal of this study is to fill the gap of biovolume to cellular carbon and nitrogen content data for marine Rhizaria. We focused on single-cell isolations of colonial Collodaria, as well as solitary Rhizaria (i.e., Collodaria, Acantharia, Nassellaria, Spumellaria, *Aulacantha*, *Protocystis*, and *Challengeria*) no larger than 1 mm. We provide empirical data on C and N content and biovolume for conversion of measured sizes to cellular mass, as well as a set of constants for the equations describing this conversion.

Methodology

Study sites and sampling

The study sites encompassed environmentally diverse oceanic ecosystems. We collected symbiotic Collodaria and Acantharia in the bay of Villefranche-sur-Mer (France, 43°41′10″N, 7°18′50″E) (Fig. 1) during September 2018, April 2019 and October 2019. This bay is characterized by a steep decline and upwelling from deep water from 1 km offshore, this allows the sampling of these oceanic protists near the shore. Collodaria colonies (Fig. 2 GHJK) were collected using a plankton net of 2 mm mesh size, Acantharia (Fig. 2F) with nets of 64 and 150 μm mesh size, by slowly towing the nets at

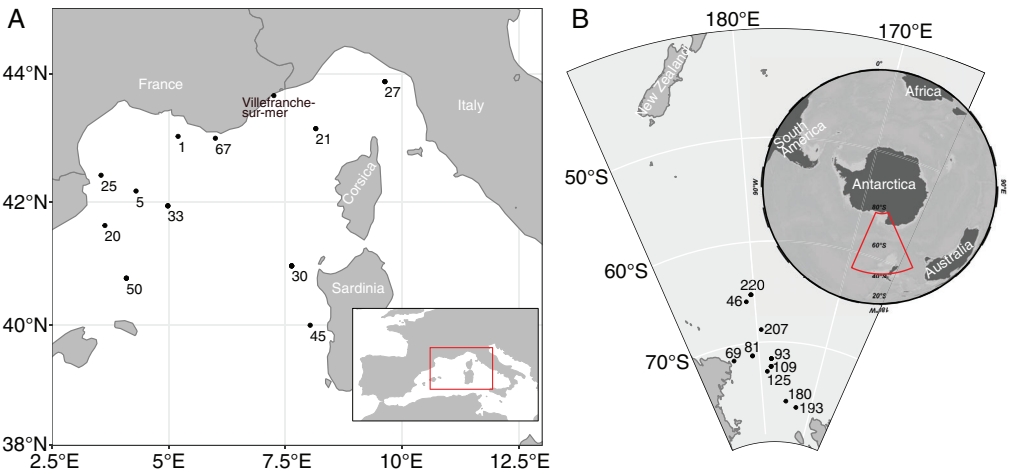

Fig 1. Sampling sites of the MOOSE-GE cruises, and the sampling location of Villefranche-sur-Mer, with an inlay of the Mediterranean Sea (**A**), modified from Llopis Monferrer et al. 2020. Estimating biogenic silica production of Rhizaria in the Global Ocean. Global Biogeochem. Cycles 34. Doi:10.1029/2019GB006286 (CC BY-NC 4.0). Sample sites of the TAN1901 cruise; inlay shows the south polar region (**B**). Not shown are the samples of the AMT cruise that were collected at 3.69°S 24.98°W at depths between 0 and 200 m. Numbers correspond to the sampling station of each sample as indicated in the raw data table https://doi.org/10.17632/j9262jxgt8.1.

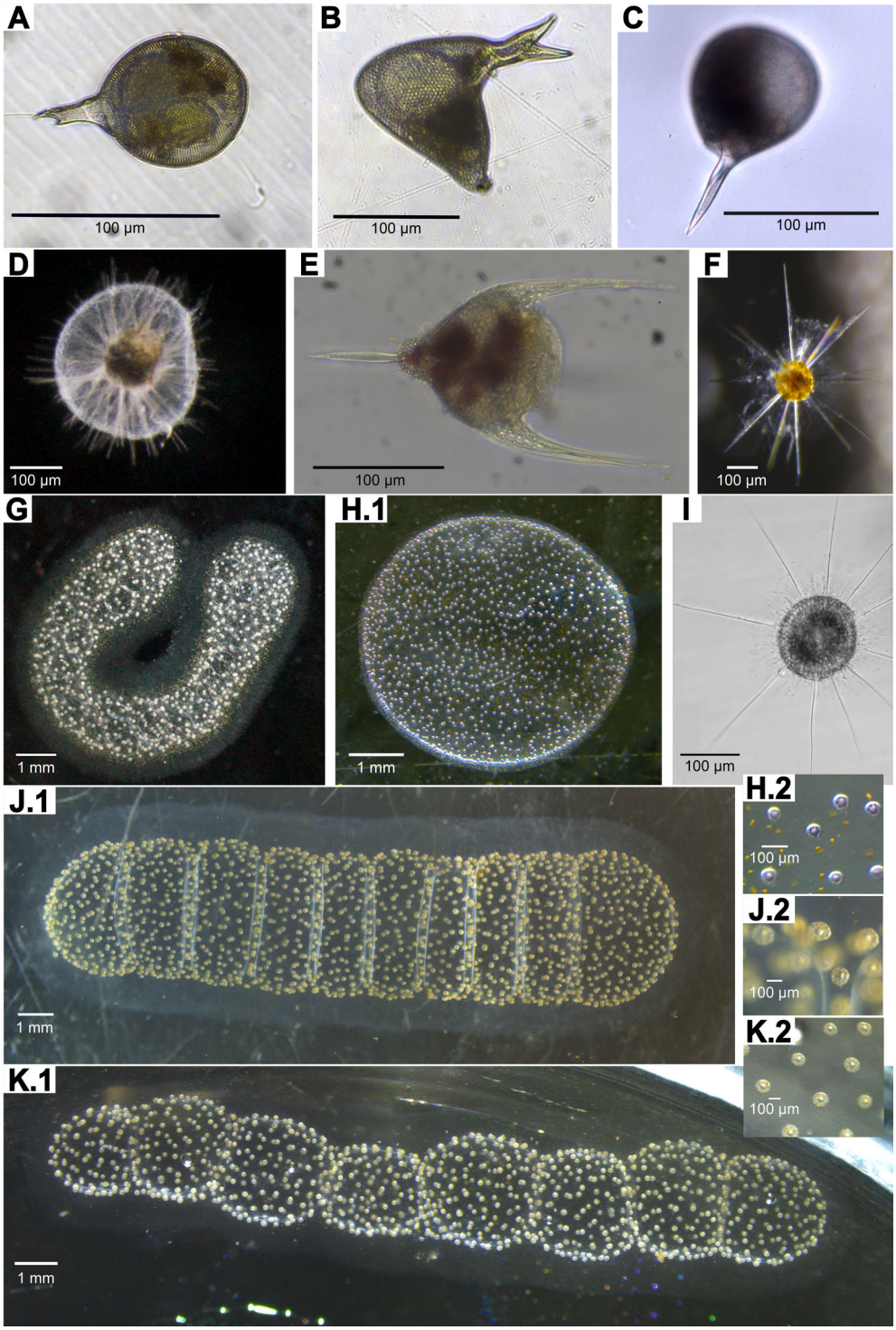

Fig 2. Light microscopy image examples of the Rhizaria studied. (**A**) *Protocystis tridens*; (**B**) *Protocystis triangularis*; (**C**) *Challengeria xiphodon*; (**D**) *Aulacantha scolymantha*; (**E**) Nassellaria (stack of four images); (**F**) Acantharia; (**G**) *Sphaerozoum* sp.; (**H**) Collosphaeridae; (**I**) Spumellaria (black & white image); (**J** and **K**) two *Collozoum pelagicum* colonial morphologies. Collodarian colonies (**HJK.1**) are shown alongside a higher magnification image (**HJK.2**), zooming in on the central capsules. The dinoflagellate symbionts can clearly be seen as the golden cells. A scale is indicated separately in each image, note the different units for Collodaria colonies.

the subsurface. Phaeodaria (Fig. 2A–D) were collected at numerous study sites during three different cruises (Fig. 1), using a triple net (mesh size of 64, 120, and 200 μm). The genera *Aulacantha* (primarily *Aulacantha scolymantha*, Fig. 2D) and *Challengeria* (primarily *Challengeria xiphodon*, Fig. 2C) were collected in the Western Mediterranean basin during the Mediterranean Ocean Observing System on Environment - Grande Echelle (MOOSE-GE) 2017 cruise. Carbon data on these specimens were previously reported in Llopis Monferrer et al. (2020), but have been recalculated to be consistent with the biovolume calculations in this study (e.g., using the same geometric shape formulas for the same groups). *Protocystis* species *P. tridens* (PhaeoA, Fig. 2A), *P. harstoni*, and *P. triangularis* (PhaeoC, Fig. 2B) were collected during the TAN1901 expedition along the Pacific sector of the Southern Ocean during the austral summer (January and February 2019). Polycystine Radiolaria of the orders Spumellaria (Fig. 2I) and Nassellaria (Fig. 2E) were collected during both cruises. Spumellaria were further collected during the Atlantic Meridional Transect (AMT28) in October 2018 at 3.69°S 24.98°W from depths between 0 and 200 m.

Collected samples were immediately diluted in buckets with fresh surface seawater. Specimens were handpicked and transferred into filter-sterilized seawater (FSW, 0.2-μm-pore-size). Collected and isolated specimens were incubated minimally 1 h in FSW, after which they were transferred again to fresh FSW. This washing process was repeated at least three times, allowing the self-cleaning of particles attached to the specimen and dilution to extinction of any organisms accidentally taken with (*see* Single-cell isolation procedure: dx.doi.org/10.17504/protocols.io.bqvrmw56).

Even though Phaeodaria were identifiable during sampling, Rhizaria taxonomy is largely based on skeletal characteristics that are difficult to observe accurately under low magnification when individuals still contain tissue. Consequently, Radiolaria were grouped by overall shape characteristics and specimen were identified solely on the order level, i.e., Acantharia, Nassellaria, Spumellaria, or on the genus level for Collodaria. Acantharia and Collodaria identification was verified and complemented with molecular data comparison.

Elemental analysis

Carbon and nitrogen content was measured from bulk samples or entire Collodaria colonies. Specimens were filtered onto precombusted (450°C, 4 h) Whatman GF/F filters. For colonial Collodaria one or two colonies were used per filter. Samples of solitary specimen were composed of multiple cells, i.e., 30 Acantharia, a mean (range) of 18 (1–44) *Aulacantha*, 40 (30–46) Nassellaria, 35 (25–40) *Protocystis*, 24 (22–26) *Challengeria*, 33 (25–40) Spumellaria. A mix of taxa were combined on a single filter for Acantharia, Spumellaria and Nassellaria out of necessity to acquire sufficient biomass, though care was taken to use same-sized specimen. Blank filters were prepared for each sample by filtering a volume of FSW, similar to that used for the sample, onto a precombusted GF/F filter. Filters were dried at 60°C for a minimum of 24 h and subsequently kept in sealed containers in the dark for transport and subsequent analysis.

Filters were dried at 55°C for a minimum duration of 24 h shortly before the analysis. Analysis of particulate C and N content of Collodaria and Acantharia were done at the METABOMER facility at the Station Biologique de Roscoff, France. All other specimens were analyzed at the LEMAR laboratory in Brest, France. The C and N content of entire filters was determined as CO_2 and N_2 released by flash combustion using a Flash 1112 series EA (Thermo Fisher). Samples of acetanilide (in Roscoff) or atropine (in Brest) of different mass were used to calibrate the analyzer and determine C and N content of the samples. Standard deviations were 0.11 and 0.06 μg for C and N, respectively. Carbon and N signal of the samples was corrected by subtracting the signal of the related blank sample. The detection limit was thus at the blank level, plus 10 times the standard deviation in order to avoid false positives. Data was processed in ISODAT 2.0 software and Microsoft Excel.

Image analyses

Pictures of specimen were taken after sampling for size inference, cell counts, and biovolume estimates (Zeiss Stemi SV11 stereoscope with Olympus DP21 camera in 2018 and a Leica S8AP0 with a Leica MC170HD camera in 2017 and 2019). Total volume of Collodaria colonies and individual cells were estimated from pictures of live cells. The diameter of at least 20 collodarian cells (i.e., central capsules, from here on also referred to as cells) was measured per colony and were assumed a spherical shape for biovolume calculations. Colony volume was measured excluding the outer edges of the gelatinous matrix of the colony (Supplemental Fig. 1), because this material is variable with physiological state (Swanberg 1979). Colonies were assigned a simple standardized shape for these calculations: either sphere, prolate spheroid, or cylinder with two half spheres (Supplementary Methodology 2). Biovolume of other Rhizaria was calculated in similar fashion using the closest geometric shape (e.g., truncated cone for Nassellaria, prolate spheroid for *Protocystis*, *Challengeria* and Spumellaria, and sphere for *Aulacantha*). For Spumellaria and *Aulacantha* biovolume calculations, the radiate spines were not considered in the calculations, only the central body shape, as suggested by Stukel et al. (2018) and Ikenoue et al. (2019). Additionally, the number of collodarian cells (the central capsules, Fig. 2HJK.2) per Collodaria colony was estimated from images, either counting all cells or counting an area with a minimum of 200 cells and extrapolating this to the surface area (excluding outer gelatinous matrix). All image analysis was done using ImageJ 1.52a for Windows (Abràmoff et al. 2004).

Molecular identification

Collodaria colonies of identical morphology as the ones used for elemental analyses were used for genetic

identification. They were collected and photographed in Villefranche-sur-Mer in 2016. For Acantharia similarly, representative specimens were preserved in 96% EtOH for molecular identification. Ribosomal gene sequences of the Collodaria were retrieved from transcriptome data. DNA of Acantharia, and one Collodaria, was extracted and ribosomal 18S was amplified and sequenced. Subsequently, phylogenetic trees were constructed using the maximum likelihood method for molecular identification (further details in Supplemental Methodology 1). Amplified 18S ribosomal sequences are deposited under Bioproject PRJNA658429, and sequences extracted from transcriptome data under accession numbers MT985517-MT985527.

Acantharia and Collodaria used for elemental analysis were identified by 18S rDNA phylogenetic placement, in combination with photographic comparison of our specimens to those linked to reference sequences in the works of Decelle et al. (2012) and Biard (2015).

Data analysis

Measured C and N content was normalized to cell count for bulk samples and central capsule counts for colonies. Linear least squares regression analysis was used to determine the C or N content to biovolume relationships using JMP for Windows (Version 1.19.4; SAS Institute Inc., Cary, NC). The residuals were analyzed to examine deviations from normality and equal variance. Data regarding total Collodaria colonies were $\log_{10}$ transformed to fit the assumptions of the linear regression analysis. The regression coefficients (slope, b) were consistently found to be different from zero as tested with a t-test. Cellular mass estimations can be made following the standard regression equation (Eq. 1). When regression was performed on transformed data, the regression parameters are shown in $\log_{10}$ format, and cell mass can be estimated from Eq. 2 for nonlogarithmic results. Where b is the slope, and a the y-intercept of the regression equation. Statistics are shown as mean with standard deviation.

$$Mass = a + b \times biovolume \quad (1)$$

$$Mass = 10^{a} \times biovolume^{b} \quad (2)$$

Validation of prediction equations

The regression equations for collodarian biovolume to C content were validated on elemental data from samples of two types of Collodaria colonies not included in the regression analysis (April 2019 samples, data available at https://doi.org/10.17632/j9262jxgt8.1). Predictions of the total C content of the colony acquired by using our regression equations was compared to C content predictions acquired using the mean C density, and per cell C content. Mean Absolute Percentage Error (MAPE) was subsequently calculated for the different prediction methods according to Eq. 3, where Obs_i is the observed mass, and Pre_i the predicted mass.

$$\text{MAPE} = mean\left(\frac{|Obs_i - Pre_i|}{|Obs_i|}\right) \times 100 \quad (3)$$

Results

Colonial Collodaria

Colonies collected during October 2019 (Fig. 2J,K) were identified as *Collozoum pelagicum* (Supplemental Fig. 2 and 4). Blue/violet colonies (Fig. 2H) were verified as Collosphaeridae (Supplemental Figs. 2 and 4), which form blue pigments in reproductive stages (Swanberg 1979). The identification of *Sphaerozoum* sp. (Fig. 2G) was not confirmed by molecular data, but was based on the morphological features including the vacuoles and segmentation of the colonies, as compared to images of previous studies (Biard 2015).

Carbon and nitrogen content and C : N ratios

The C content of *Collosphaeridae* cells ranged from 32.73 to 251.49 ng C cell^{-1} (75.43 ± 74.12 ng C cell^{-1}; mean ± SD; n = 8), and N content from 4.17 to 24.54 ng N cell^{-1} (9.10 ± 7.68 ng N cell^{-1}, n = 6). *Sphaerozoum* and *Collozoum* had a higher C and N content per cell with a range of 15.74 to 597.91 ng C cell^{-1} (152.90 ± 130.46, n = 22) and 9.56 to 100.84 ng N cell^{-1} (29.66 ± 22.0, n = 20) for *Sphaerozoum*, and a range of 90.31 to 215.85 ng C cell^{-1} (148.01 ± 37.29, n = 16) and 8.49 to 22.73 ng N cell^{-1} (15.35 ± 4.07, n = 16) for *Collozoum* (Table 1). The average C : N ratio for *Collosphaeridae* was close to Redfield ratio (6.6) with an average of 6.3 (± 1.7) (Table 1). C : N ratio of *Sphaerozoum* was lower with a mean of 5.9 and *Collozoum* samples were higher with a mean C : N of 9.7, similar to Michaels et al. (1995). Carbon and N content and biovolume was also measured for a sample of *Thalassicolla* sp., a solitary Collodaria (Table 1). Total biomass was not always sufficient to measure N hence the C : N ratio could not be calculated for all samples.

The cellular C content for all Collodaria taxa combined ranged from 15.73 to 597.89 ng C cell^{-1} (137.73 ± 100.50 ng C cell^{-1}; n = 46). Nitrogen content showed a similar wide range from 4.17 to 100.84 ng N cell^{-1} (21.27 ± 17.83 ng C cell^{-1}; n = 42). The C : N ratio averaged 7.4 (± 2.5).

Carbon and nitrogen to biovolume relationships

To allow for extrapolation and usability in modeling and ecological (in situ) studies, we determined C : vol and N : vol relationships of all Collodaria taxa clustered. The strong correlation of C content per cell to the average cell volume in a colony (ng C cell^{-1} = −13.51 + 0.0001524 × volume (μm^3), $F_{1,38}$ = 139.70, R^2 = 0.79, $p < 0.0001$, Fig. 3B, Table 2) largely explains the variation among C content. Yet, N content is only moderately correlated with the average cell volume by the regression equation ng N cell^{-1} = −4.33 + 0.000249 ×

Table 1. Cell volume and carbon (C) and nitrogen (N) data for Rhizaria from this study and from the literature. Carbon or N per volume (i.e., mass density) of Collodaria (i.e., Collosphaeridae, *Collozoum* and *Sphearozoum*) is indicated both per colony (in μg mm^{-3}) and per cell (central capsule) (in pg mm^{-3}). Data of Collodaria are also separately shown as combined for all Collodaria. C content indicated per cell, implies central capsules for Collodaria. The total number of samples analyzed (*n*) differs for N and C content, because N mass was not always above the detection limit. Additionally, the total number of cells of all samples is given. Data of this study is highlighted in bold. Data is given ± standard deviation. Further data on central capsules per colony, volume measurements, as well as data ranges are summarized in Supplemental Table 1. Full raw data table is accessible at https://doi.org/10.17632/j9262jxgt8.1.

		Carbon				Nitrogen					
Taxa	Cells	*n*	Ng C cell^{-1}	*n*	pg C μm^{-3}, or μg C mm^{-3} for colonies	*n*	ng N cell^{-1}	*n*	pg N um^{-3}, or μg N mm^{-3} for colonies	C : N	Reference
Colonial Collodaria											
Collosphearidae	**8632**	**8**	**75.43 ± 74.12**	**7**	**0.168 ± 0.083**	**6**	**9.1 ± 7.68**	**5**	**0.0348 ± 0.0253**	**6.3 ± 1.73**	**This study**
Acrospheara spinosa	2456	6	172 ± 94		–		–		–	7.7 ± 0.3	Michaels et al., 1995
Acrospheara spinosa	480	13	100		–		–		–	8.3 ± 1.7	Swanberg 1983
Collosphaera Huxleyi	340	1	172		–		–		–		Michaels et al., 1995
Sphaerozoum	**10,194**	**22**	**152.9 ± 130.46**	**17**	**0.139 ± 0.042**	**20**	**29.66 ± 22**	**15**	**0.0278 ± 0.011**	**5.89 ± 2.13**	**This study**
Sphaerozoum punctatum	2210	5	146 ± 31		–		–		–	6.7 ± 1.1	Michaels et al., 1995
Sphaerozoum punctatum	500	3	64		–		–		–	9.8 ± 0.98	Swanberg 1983
Collozoum	**23,396**	**16**	**148.01 ± 37.29**	**16**	**0.139 ± 0.035**	**16**	**15.35 ± 4.07**	**16**	**0.0144 ± 0.0037**	**9.72 ± 0.98**	**This study**
Collozoum pelagicum	5224	5	131 ± 104		–		–		–	8.5 ± 1.2	Michaels et al., 1995
Collozoum pelagicum	1500	4	107		–		–		–	13 ± 1.7	Swanberg 1983
Collozoum radiosum	350	13	200		–		–		–	8.4 ± 0.76	Swanberg 1983
Collozoum longiforme	–	3	67 to 96		–		–		–	8.6	Swanberg and Harbison 1980
Collozoum inerme	7700	3	51 ± 10		–		–		–	9.7	Michaels et al., 1995
Collozoum inerme	2500	11	50		–		–		–	11 ± 3.2	Swanberg 1983
Rhaphidozoum acuferum	2230	5	115 ± 28		–		–		–	9.4 ± 1.7	Michaels et al., 1995
All colonial Collodaria	**22,102**	**46**	**137.73 ± 100.5**	**40**	**0.144 ± 0.049**	**42**	**21.27 ± 17.53**	**36**	**0.0228 ± 0.0138**	**7.41 ± 2.49**	**This study**
All colonial Collodaria	20,160	25	133 ± 73				–			8.2 ± 1.5	Michaels et al., 1995
Collosphearidae (colony)	**8632**		**NA**	**8**	**0.629 ± 0.413**		**NA**	**8**	**0.0836 ± 0.0568**		**This study**
***Collozoum* (colony)**	**23,396**		**NA**	**16**	**1.21 ± 0.516**		**NA**	**16**	**0.1254 ± 0.0572**		**This study**
***Sphaerozoum* (colony)**	**10,194**		**NA**	**22**	**1.423 ± 0.94**		**NA**	**20**	**0.2637 ± 0.1179**		**This study**
All colonial Collodaria (colony)	**42,222**		**NA**	**46**	**1.211 ± 0.781**		**NA**	**42**	**0.1853 ± 0.1181**		**This study**
Solitary Collodaria											
***Thalassicolla* sp.**	**16**	**1**	**21,577.46**	**1**	**0.189**		**3723.60**	**1**	**0.03**	5.79	**This study**
Thalassicolla melanogaster	1	1	–		0.01		–		–	4.70	Michaels et al., 1995
Thalassicolla nucleata	15	11	–		0.28 ± 0.25		–		–	6.4 ± 2.1	Michaels et al., 1995

(Continues)

Table 1. Continued

Taxa	Cells	Carbon				Nitrogen				C : N	Reference
		n	Ng C cell^{-1}	*n*	pg C μm^{-3}, or μg C mm^{-3} for colonies	*n*	ng N cell^{-1}	*n*	pg N um^{-3}, or μg N mm^{-3} for colonies		
Spumellaria	**74**	**2**	**799.38 ± 345.79**	**2**	**0.328 ± 0.127**	**2**	**94.96 ± 5.54**	**2**	**0.0456 ± 0.0325**	**8.33 ± 3.16**	**This study**
Physematium muelleri	17	12			0.009 ± 0.003		–		–	4.8 ± 0.06	Michaels et al., 1995
Nassellaria	**241**	**6**	**458.45 ± 249.26**	**6**	**1.472 ± 0.73**	**6**	**52.97 ± 27.64**	**6**	**0.1713 ± 0.0853**	**8.79 ± 1.43**	**This study**
Acantharia (clade F3)	**540**	**18**	**168.59 ± 151.15**		**0.04 to 0.939***		–		–		**This study**
Acantharia mix	370	12	–		0.0026 ± 0.0036†		–		–		Michaels et al., 1995
Foraminifera											
Orbulina universa (spherical chambers)	36	12	–		0.018 ± 0.008		–		–	6.1 ± 1.7	Michaels et al., 1995
Orbulina universa (trochospiral chambers)	19	2	–		0.18 ± 0.13		–		–	6.6	Michaels et al., 1995
Hasugerina pelagica	0	0	–		0.092 ± 0.014		–		–	5.7 ± 1.4	Michaels et al., 1995
Globigerinoides ruber	8	1	–		0.06		–		–	–	Michaels et al., 1995
Mixed assemblage	24	4	–		0.045 ± 0.007		–		–	–	Michaels et al., 1995
All foraminifera (excl. spherial *O. universa*)	112	22	–		0.089 ± 0.055		–		–	5.8 ± 1.3	Michaels et al., 1995
Phaeodaria											
Phaeodaria (size >1 mm)			7200 to 25,000								Ikenoue et al. 2019
Aulacantha	**302**	**17**	**3075.48 ± 1559.57**	**17**	**0.018 ± 0.015**	**17**	**305.7 ± 174.83**	**17**	**0.0018 ± 0.0016**	**10.61 ± 2**	**This study**
Protocystis	**414**	**12**	**721.92 ± 450.34**	**12**	**2.224 ± 1.283**	**12**	**82.8 ± 42.45**	**12**	**0.2593 ± 0.1314**	**8.61 ± 1.76**	**This study**
Challengeria	**48**	**2**	**1226.84 ± 1360.87**	**2**	**0.24 ± 0.113**	**2**	**64.89 ± 61.29**	**2**	**0.0146 ± 0.0017**	**16.25 ± 5.62**	**This study**

*Estimated assuming a cell size of 200 and 70 μm for min and max, *see* Results for further explanation.
†Note that these values are based on biovolume calculated from the spicule extremes.

volume (μm^3) ($F_{1,34}$ = 56.44, R^2 = 0.62, $p < 0.0001$, Fig. 3A, Table 2).

Assessing the relationship of entire colony C (or N) content with biovolume showed a similar significant positive correlation by the regression equations $\log_{10}$ μg C colony^{-1} = 0.692 + 0.649 × $\log_{10}$ volume (mm^3), and $\log_{10}$ μg N colony^{-1} = 0.597 + 0.297 × $\log_{10}$ volume (mm^3) ($F_{1,44}$ = 58.76, R^2 = 0.57, $p < 0.0001$, and $F_{1,40}$ = 26.73, R^2 = 0.40, $p < 0.0001$, for C and N respectively, Fig. 4, Table 2), though this correlation was less strong than on the cell level. Further analysis shows a significant negative correlation of C and N density (mass per biovolume) with colonial volume ($F_{1,44}$ = 17.15, R^2 = 0.28, $p < 0.0002$, and $F_{1,40}$ = 149.74, R^2 = 0.79, $p < 0.0001$, respectively) (Supplemental Fig. 5). Carbon and N density per colony and per cell are given in Table 1.

Validation of Collodaria biovolume to carbon content equations

We compared the total colonial Collodaria C content results of four different prediction methods, to identify the most accurate approach. (1) Colony C content (ng C) was predicted using the average C content per cell of 137.73 ng C cell^{-1} (C content$_{cell}$), and multiplied by the total number of cells counted in the colony (No$_{cells}$) of which C content was to be predicted (Eq. 4).

$$\text{Predicted colonial C content} = \text{average}(\text{C content}_{\text{cell}}) \times \text{No}_{\text{cells}} \quad (4)$$

(2) Colony C content (μg C) was predicted based on the average colonial C density of 1.21 μg C mm^{-3} (C density$_{colony}$), and multiplied by the colony's volume (V, mm^3) (Eq. 5).

$$\text{Predicted colonial C content} = \text{average}\left(\text{C density}_{\text{colony}}\right) \times \text{V} \quad (5)$$

(3) Colony C content (ng C) was estimated using our collodarian cell biovolume to C content equation (Fig. 3B; Table 2), with V (μm^3) being the average biovolume of the cells. The predicted C content per cell was multiplied by the total number of cells counted in the colony (No$_{cells}$) to acquire colony C content (Eq. 6).

$$\text{Predicted colonial C content} = (-13.51 + 0.0001524 \times \text{V}) \times \text{No}_{\text{cells}} \quad (6)$$

(4) Lastly, our equation for colonies was used to predict colony C content (μg C) with V in mm^3 (Fig. 4B; Eq. 7), thereby not having to account for cell biovolume and quantity.

$$\text{Predicted colonial C content} = 10^{(0.6922)} \times \text{V}^{(0.6492)} \quad (7)$$

In order of lowest to highest Mean Absolute Percentage Error (MAPE) we found: method 3 (MAPE = 37.12), method 4

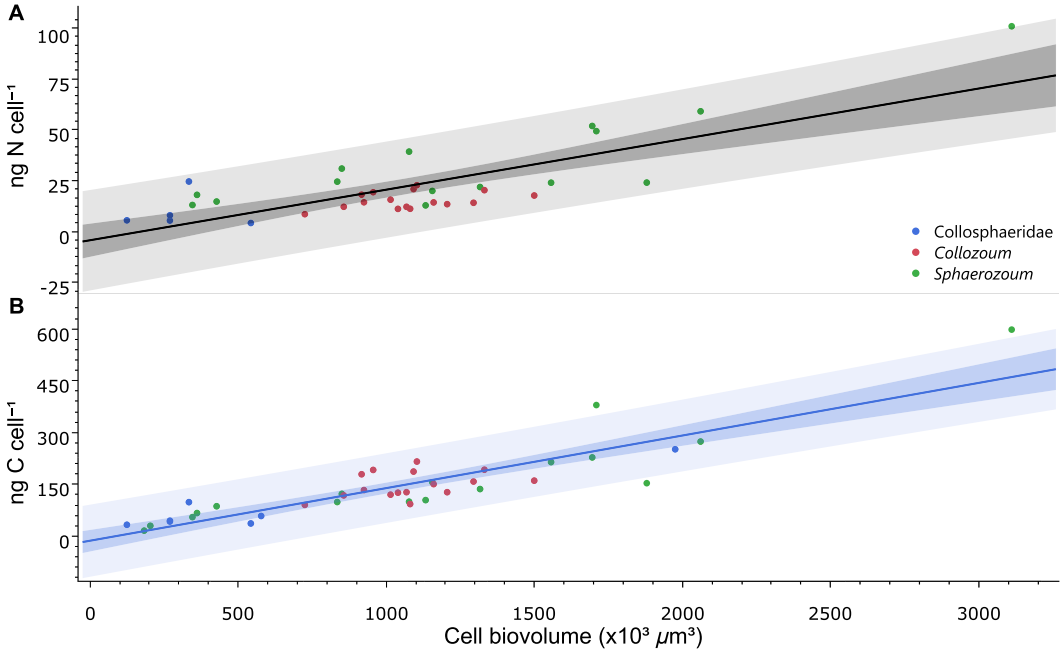

Fig 3. Relationships between cell volume (μm^3) and (**A**) nitrogen (ng) per cell, and (**B**) carbon (ng) per cell for central capsules of colonial Collodaria. The different Collodaria genera investigated in this study are indicated by color, Collosphaeridae (blue), *Collozoum* (red), and *Sphaerozoum* (green). The line of best fit is shown as a solid black/blue line with the 95% confidence interval of the fit in dark gray/blue shading and 95% prediction interval in lighter gray/blue shading. Regression parameters are shown in Table 2.

Table 2. Results of significant least-squares regression analyses of C and N to biovolume. Presented are the slope (regression coefficient, *b*) and y-intercept (*a*) of the regression equations; the standard deviation (SD); the square of the correlation coefficient *r* (R^2), and the number of data points (*n*). All slopes are significantly different from zero ($p < 0.05$). For collodarian colonies the data was $\log_{10}$-transformed. The cellular C (or N) content is thus determined as μg C (or μg N) colony^{-1} = $10^a \times$ (volume (mm^3))^b, Eq. 2. All other data is untransformed, and mass is thus determined by linear regression as ng C (or ng N) cell^{-1} = $a + b \times$ volume (μm^3), Eq. 1.

Data	Intercept (*a*)	SD	Slope (*b*)	SD	R^2	*n*
Collodaria central capsule C	−13.51	15.40	0.0001524	0.0000129	0.786	40
Collodaria central capsule N	−4.33	4.01	0.0000249	0.0000033	0.624	36
Collodaria colony C*	0.692	0.17	0.649	0.0847	0.572	46
Collodaria colony N*	0.597	0.118	0.297	0.0575	0.401	42
Protocystis C	128.31	278.8	0.00176	0.000759	0.349	12

*Data was $\log_{10}$ transformed.

(MAPE = 45.17), method 2 (MAPE = 55.69), and method 1 (MAPE = 64.16) (Supplemental Table 2).

Nassellaria and Spumellaria

Carbon content of Nassellaria varied between 178.40 and 876.27 ng C cell^{-1} (458.44 ± 249.25 ng C cell^{-1}; $n = 6$), and N content ranged from 22.39 to 88.61 ng N cell^{-1} (52.97 ± 27.64 ng N cell^{-1}; SD; $n = 6$), with an average C : N ratio of 8.8 (Table 1). The C : vol relationships for Nassellaria cells showed no significant correlations ($F_{1,4} = 0.54$, $p = 0.5022$), nor did it for N : vol ($F_{1,4} = 0.30$, $p = 0.6108$) (Supplemental Fig. 6). Hence, the best mass estimates can only be made by the average C (or N) density, 1.47 ± 0.73 pg C μm^{-3} (or 0.17 ± 0.09 pg N μm^{-3}, Table 1).

For Spumellaria we measured C content of 554.87 and 1043.89 ng C cell^{-1} ($n = 2$), and N content of 91.04 and 98.87 ng N cell^{-1}. Average carbon and nitrogen density was 0.33 ± 0.13 pg C μm^{-3} and 0.046 ± 0.033 pg N μm^{-3} (Table 1). Since we had only two samples, no regression analysis could be made.

Acantharia

Phylogenetic placement of the sequences identified the Acantharia specimen as belonging to subgroup F3b

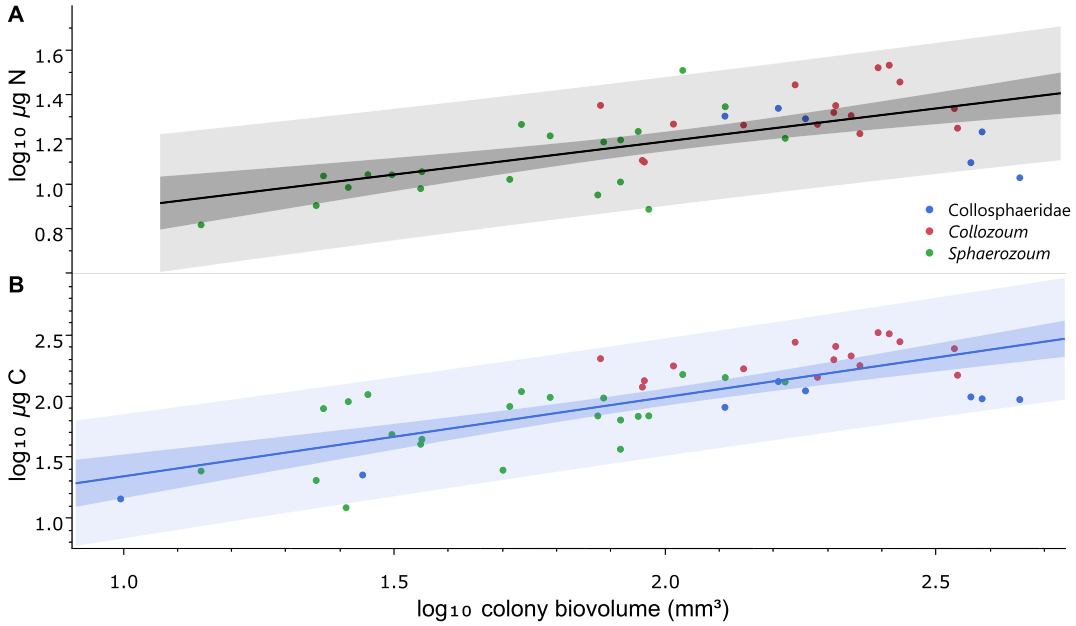

Fig 4. Relationships between total colony volume (mm^3) and (**A**) nitrogen (μg) per colony, and (**B**) carbon (μg) per colony for colonial Collodaria. Graphs show log_{10}-transformed data. The different Collodaria genera investigated in this study are indicated by color, Collosphaeridae (blue), *Collozoum* (red), and *Sphaerozoum* (green). The line of best fit is shown as a solid black/blue line with the 95% confidence interval of the fit in dark gray/blue shading and 95% prediction interval in lighter gray/blue shading. Regression parameters are shown in Table 2. The conversion of the log_{10} expressed regression equation is outlined in the methods.

(Supplemental Figs. 3 and 4). Subgroup F3 lacks molecular resolution (Decelle et al. 2012), therefore the genera of the samples could not be distinguished molecularly between the closely related taxa of clade F3. Photographic comparison show the samples Ac-6 and Ac-16 to be most similar to *Acanthostaurus*, possibly *Acanthostaurus purpurascens*, which is commonly found at the sampling location (Supplemental Fig. 4). Ac-17 is likely *Amphistaurus complanatus* (Supplemental Fig. 4). Because acantharian taxonomy is usually determined by skeletal features, it was not possible to be certain of the identification. Nonetheless, considering the specimens were molecularly clearly a mix of subgroup F3b Acantharia, this was deemed sufficient.

Overall, Acantharia had an average C content of 168.59 ± 151.15 ng C $cell^{-1}$ ($n = 18$). Unfortunately, no pictures were taken for these samples, hence average size and biovolume could not be determined *a posteriori*. However, based on the Acantharia observed during the cell isolation process, the diameter of the central capsule was estimated between the limits of 70 to 200 μm. Using those as the upper and lower limits of size range, and assuming a spheroid shape, we can calculate the C : vol ratio to be between 0.94 ± 0.85 and 0.04 ± 0.03 pg C μm^{-3} (Table 1). A tentative estimate of Acantharia size from the spicule extremes would range from 200 to 500 μm for a C : vol ratio between 0.04 ± 0.03 and 0.0026 ± 0.002 pg C μm^{-3}. Elemental analysis of Acantharia was hampered by a lack of total biomass. Consequently, we were unable to measure N content, and in some cases, neither C content, resulting in a loss of samples.

Phaeodaria

The C content of the genera *Aulacantha* and *Protocystis* ranged from 943.03 to 6003.69 and 256.87 to 1670.20 ng C $cell^{-1}$, respectively. Nitrogen content ranged from 66.70 to 700.34 and 26.95 to 158.09 ng N $cell^{-1}$ for *Aulacantha* and *Protocystis*, respectively. No significant correlation of C : vol nor N : vol was found for *Aulacantha* (respectively: $F_{1,15} = 0.90$, $p = 0.357$; $F_{1,15} = 0.86$, $p = 0.368$, Supplemental Fig. 6). *Aulacantha* C and N density is significantly negatively correlated to biovolume ($F_{1,15} = 15.04$, $R^2 = 0.501$, $p < 0.0015$ and $F_{1,15} = 12.98$, $R^2 = 0.464$, $p < 0.0026$ for C and N density respectively, Supplemental Fig. 7A). Additionally, for *Aulacantha* a wide range of cells (1–44) was used per sample, this could have influenced C and N content measurements. Concordantly, we find C and N density weakly but significantly negatively correlated to the number of cells per samples ($F_{1,15} = 4.99$, $R^2 = 0.250$, $p < 0.0412$ and $F_{1,15} = 4.87$, $R^2 = 0.245$, $p < 0.0433$ for C and N density respectively, Supplemental Fig. 7B). When a low number of cells were used per sample a higher density seems to be measured and vice versa. A significant weak correlation was found for *Protocystis* C : vol by the equation ng C $cell^{-1} = 128.31 + 0.000176 \times$ volume (μm^3) ($F_{1,10} = 5.37$, $R^2 = 0.349$; $p = 0.0430$; Table 2 and Supplemental Fig. 6), but not for N : vol ($F_{1,10} = 3.72$, $p = 0.083$,

Supplemental Fig. 6). The correlation was heavily influenced by one outlier with a high biovolume, without this sample there would be no correlation (Fig. 5). More samples of higher biovolume could possible resolve this. Thus, average C and N densities, as given in Table 1, might be better estimates for prediction.

Only three samples containing the genus *Challengeria* were procured, and for one sample the C and N content data was aberrant and thus excluded. The two samples considered had 264.56 and 2189.12 ng C cell^{-1}, and 21.55 and 108.23 ng N cell^{-1} (Table 1).

Discussion

Mean Rhizaria carbon and nitrogen densities and mass estimations

Carbon or N content and biovolume data on Rhizaria are extremely scarce, with only one study with C and N measurements for Acantharia and Phaeodaria known to us (*see* Table 1). While the rest of the available data are for the Collodaria taxa. The measured Collodaria C and N density and relatively high variation in C and N content of all our samples was similar to that reported in previous studies (Table 1). Here we have measured C and N density of several not previously investigated Rhizaria taxa, in addition to establishing new mass to biovolume relationships (Table 1; Fig. 6).

For both Radiolaria and Phaeodaria it is not the bigger cell that is most C dense. Nassellaria (Radiolaria) and *Protocystis* (Phaeodaria), on average the smallest of their representative taxa, have a C and N density an order of magnitude higher than the other taxa (Fig. 6). Nearly all Rhizaria species have elaborate skeletal structures, with the extracapsulum making up most of the soft body, yet most of the biological material is likely centrally located, in the central capsule (Haeckel 1887; Suzuki and Not 2015). Thus, from the total inferred size only a small fraction would contain plenty organic matter, and the total C (and N) density would be lower than expected from the volume of the entire body. The central capsule of these smaller taxa might be relatively bigger in relation to the total structure as compared to other Rhizaria.

Acantharia were previously reported with a very low C : vol ratio (Michaels et al. 1995, Table 1). This has been attributed to a lack of the acantharian ectoplasm outer membrane in their samples, and to biovolume measurements estimated of the spicule extremes, whereas most C would be expected in the central capsule. We did not observe lack of this outer membrane in our samples. Using the C : vol ratio of 0.0026 pg C μm^{-3}, as reported by Michaels et al. (1995), a cell of half a millimeter (from the spicule extremes) would on average have a C content of 170.2 ng C cell^{-1}, which is near our average cellular carbon content for Acantharia (168.59 ng C cell^{-1}). This C density of 0.0026 pg C μm^{-3} falls below our lowest estimate for acantharian C density, assuming the biggest cell analyzed (i.e., 200 μm). Our data thus suggest average C density is more likely to be higher than the previously reported 0.0026 pg C μm^{-3}. In agreement with the previous measurement, we find highly variable C and N content, likely caused by both intraspecific and interspecific variation as attributed to the bulk analysis on low taxonomic resolution.

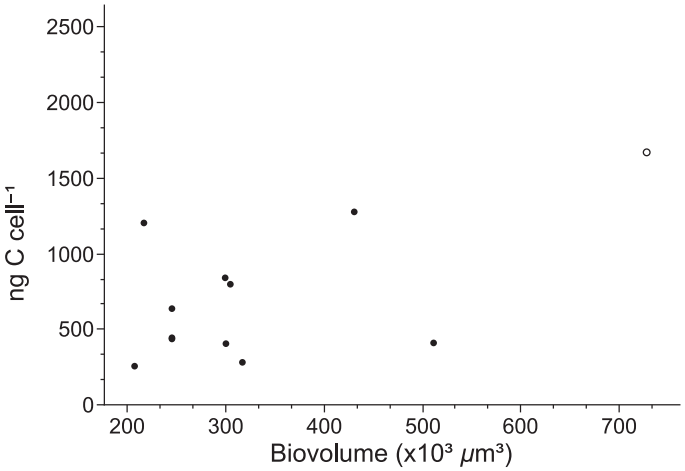

Fig 5. *Protocystis* carbon to cell volume relationship. The outlier dictating the carbon : volume relationship is shown as an open circle. The regression statistics are shown in Table 2.

It has been suggested that the C : vol conversion factors for protists reported by Menden-Deuer and Lessard (2000) overestimates biomass of larger protists considerably (Stukel et al. 2018; Ikenoue et al. 2019). The C (and N) density of the largest protist here studied (*Aulacantha*) is one to two orders of magnitude lower than that of the other studied Rhizaria taxa. If we take *Aulacantha* as an example (mean biovolume = 0.241 mm^3), the general protist plankton C : vol equation (i.e., log pg C cell^{-1} = $-0.665 + 0.939 \times \log$ (μm^3), Menden-Deuer and Lessard 2000) would give a cellular C content of 16.04 μg C cell^{-1}. This is around five times more than the average C content that we found for *Aulacantha*, i.e., 3.08 μg C cell^{-1}. Indeed, as has been suggested by Stukel et al. (2018) (for Phaeodaria > 200 μm) and Ikenoue et al. (2019) (for Phaeodaria > 1 mm), global biomass of Phaeodaria would have been overestimated due to the use of inappropriate C : vol conversions. However, this only holds for a single genus considered here. The genus *Protocystis* has the highest C density (2.22 pg C mm^{-3}) of our study. Using the mean biovolume of 0.000338 mm^3, and the same equation would give a large underestimation of C content, namely 33.60 ng C cell^{-1} instead of the actually measured mean of 721.92 ng C cell^{-1}. Based on our measurements, it turns out that C mass for all Rhizaria taxa with a size range from 0.69 to 236 μm would be underestimated, whereas *Aulacantha* (size range 529–860 μm) would indeed be overestimated with previously published generic protist C : vol equation (Table 3). Our data shows that global Rhizaria biomass might have thus in fact still been underestimated, by up to a factor of 35 (Table 3).

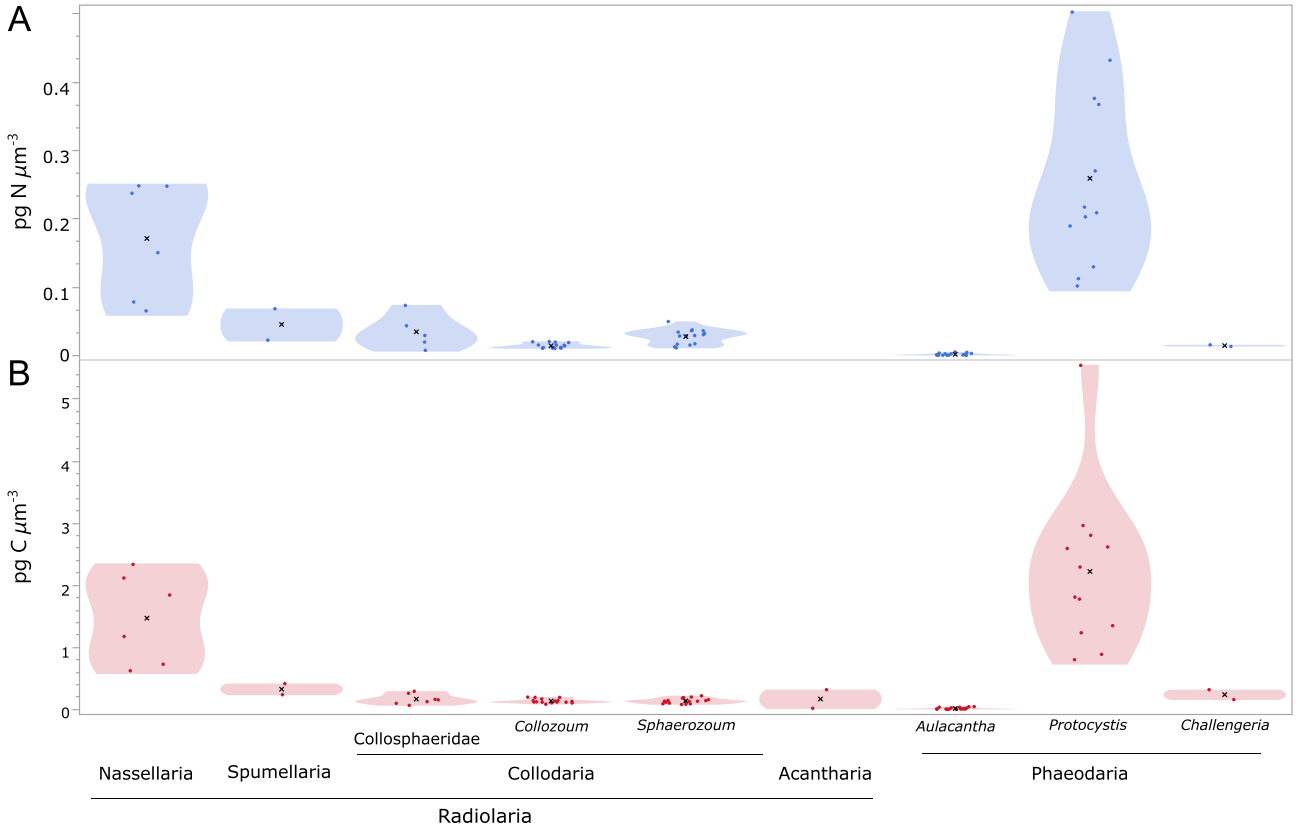

Fig 6. Violin plot for (**A**) nitrogen and (**B**) carbon density of Rhizaria. The mean is shown as an "x," the individual datapoints of pg N μm^{-3} or pg C μm^{-3}, and the distribution of the data is shown with respectively blue dots and shading or red dots and shading. All values are shown by cell and for Collodaria per central capsules. The range of C content for Acantharia has been estimated based on a suspected size of 100–200 μm.

Most notable is the difference between the estimate and our measurement for Acantharia (35.8 factor of change), whereas the factor of change for Collodaria is less pronounced with C content only differing a factor of 1.4. Thereby underlining the need for direct C and N content measurements of different plankton groups and sizes, to be able to make more accurate biomass estimations. Specifically for large sized protists like Rhizaria, taxa-specific mass : vol conversions as presented here can increase the estimation accuracy.

C : vol relationship of Collodaria—Comparison of prediction methods

Carbon content of several Collodaria taxa have previously been measured and normalized based on central capsule quantity, but these values did not account for biovolume (Swanberg 1983; Michaels et al. 1995). Accordingly, C content estimations of collodarian colonies have previously been based entirely on the average C content per central capsule (Dennett et al. 2002; Villar et al. 2018). We compared the error in cellular C content estimates of four different prediction methods, illustrating that factoring in biovolume for C (or N) estimation greatly reduce the prediction error. Comparison of the prediction errors showed that C content of collodarian colonies is best predicted when taking into account the central capsule quantity and biovolume, and thus using Eq. 6 (Supplemental Table 2).

Though previous studies report C content values of Rhizaria on the species level, we opted for a lower taxonomic resolution. This not only assured higher sample quantity, it aims at being more pragmatic in future studies where it is often not possible to identify Rhizaria to species level. The regression analysis combining genera of colonial Collodaria shows a regression without extreme outliers, consenting for the analysis of Collodaria on this taxonomic level.

Furthermore, C and N measurements normalized to central capsule quantity would not always allow easy and accurate extrapolation to ecologically relevant colonies. Microscopic measurement and accurate counts of central capsules are not always practical for in situ monitoring of Collodaria. Therefore, we have additionally shown C and N content in relation to colony volume. It will be particularly useful for mass estimates in situations where three dimensionally layered central capsules cannot be accurately determined (e.g., in situ optics-based technologies, like Underwater Vision Profilers or similar plankton recorders (Nakamura et al. 2017; Biard and Ohman 2020). In these cases, despite showing weaker correlations, possibly due to the size and quantity variation of the central capsules in the colony, the equations for C : vol

Table 3. Comparison of carbon estimates by protist plankton formula of Menden-Deuer and Lessard (2000) to carbon as measured in this study. Carbon content estimates are based on the mean cell volumes found in our samples, for Acantharia a volume associated with a 100-μm cell was used instead. Collodaria carbon content is per central capsule. A green highlight indicates that the actual measurement is higher than the prediction; red highlight indicates the opposite. Furthermore, the average diameter of our samples and the factor of change between the estimated and measured mass is given.

	Average size (diameter, μm)	C estimate using protist plankton formula (ng C cell^{-1})	Mean C measured (ng C cell^{-1})	Factor of change
Collosphearidae	94.93	63.19	75.43	1.19
Collozoum	125.57	100.04	148.01	1.48
Sphaerozoum	122.65	107.97	152.90	1.42
All colonial Collodaria	118.97	97.91	137.73	1.41
Spumellaria	158.00	249.21	799.38	3.21
Nassellaria	83.67	31.47	458.45	14.57
Acantharia (F3)	70–200*	4.71	168.59	35.83
Aulacantha	733.35	16,036.31	3075.48	0.19
Protocystis	97.83	33.60	721.92	21.49
Challengeria	184.00	362.00	1226.84	3.39

*Estimated based on visual observations, *see* Results for further explanation.

(or N : vol) of colonies (Eq. 7, Table 2) can still give a more accurate prediction than other mass estimation methods for these Collodaria colonies (Supplemental Table 2).

Using the data presented here, collodarian cellular C and N content can be relatively accurately predicted, though these predictions remain subject to high variation inherent to the organism. This is also reflected in the C to biovolume relationship that has an R^2 of 0.782, whereas for other protists the R^2 mostly lies above 0.9 (Menden-Deuer and Lessard 2000). Nitrogen to biovolume relationships are less strong than those of C content, hence, cellular N content might be alternatively estimated by C : N ratio.

However, life history stages could influence the cell's C : N stoichiometry, as has been shown in animals and metazoans that undergo significant morphological changes, like copepods (Sterner and Elser 2002). The blue pigmented Collosphaeridae samples were likely all reproductive stages (Swanberg 1979). We do not have sufficient data to compare different Collosphaeridae life stage stoichiometry, though we find no reason in our Collodaria mass : vol regression analysis to consider them as outliers. The central capsules of the Collosphaeridae specimen are smaller in diameter in comparison to those of *Sphaerozoum* and *Collozoum* with average diameters of 100.5 ± 2.8 μm, 122.6 ± 3.2 and 125.6 ± 0.8, respectively. However, the C content per central capsule is not different between these species, and the smaller size of Collosphaeridae cells likely explains their lower C content per cell. It should also be noted that solitary Collodaria, which are likely an alternative life stage of colonial forms (Swanberg 1979; Biard et al. 2015), show a C and N density two orders of magnitude higher than colonies (average of 0.0012 vs. 0.189 pg C μm^{-3}, and 0.000185 vs. 0.03 pg N μm^{-3}), as well as a lower C : N ratio (7.41 vs. 5.79). Though the radiolarian life cycle is not fully understood, such high densities could allow vegetative growth and colony formation when nutrients are otherwise limited.

Furthermore, the effect of physiological status is shown to affect cellular C and N content. For example, Tada et al. (2000) showed the C and N content and C : N ratio of *Noctiluca scintillans* cells to increase for fed cells and vice versa for starved cell. Nutritional status can subsequently affect C : N ratio, as a decrease is often observed with increased particulate (prey) and/or dissolved inorganic N (exemplified in symbiotic jellyfish (Djeghri et al. 2020), symbiotic Foraminifera (Uthicke and Altenrath 2010), and symbiotic corals (Muscatine et al. 1989)). We thus expect the C : N ratio to fluctuate to the relative prevalence of autotrophy vs. heterotrophy, which will especially be influential in mixotrophic specimens like Collodaria and Acantharia. Unfortunately, it is not well known to what proportion they rely on which trophic mode for nutrition, nor about the environmental influences on switching between trophic modes.

Conclusion

We report for the first time carbon and nitrogen to biovolume relations for several groups of Rhizaria and summarized similar available data from the literature. Rhizaria are widely distributed in the world oceans (Biard et al. 2016). Symbiont bearing Rhizaria, like Collodaria and Acantharia, are most abundant in the upper epipelagic zone, whereas Phaeodaria are predominantly found in deeper water layers (Michaels 1988; Biard and Ohman 2020). Phaeodaria and Radiolaria have shown especially high abundance in locations

where the efficiency of the biological carbon pump is high, but also in oligotrophic regions such as the North Pacific and California Current Ecosystem (Stukel et al. 2018; Gutierrez-Rodriguez et al. 2019). Accordingly, knowledge and quantification of cellular C and N content of these abundant organisms is paramount in characterizing oceanic ecosystems and C and N fluxes.

The empirically measured C and N densities of Rhizaria can be used to estimate their cellular C and N content in observational or modeling studies. For Collodaria we show biovolume to mass equations that can give more accurate predictions than mean C or N density or previously available central capsule normalized values. These novel data will be invaluable for further studies involving these large protistan plankton (>100 μm). With the data presented here, improved incorporation of these larger protists into (biomass-based) eco-physiological and ecosystem models is expected, in conjunction with improved estimates. The focus on single-cell isolated specimens, of different sizes and life stages, further permits the usage of the data in individual-based models, also known as agent-based models. Such studies will allow us to formulate hypotheses on the role of these important organisms in the ecosystem and elucidate their contribution to global biogeochemical cycles.

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Conflict of Interest

None declared.

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