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Estimating Biogenic Silica Production of Rhizaria in the Global Ocean

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Key Points:
- Polycystines and phaeodarians are highly silicified cells (up to 11.6 μmol bSi mm^{-3})
- Over a large size spectrum, Si content of these siliceous rhizarians is associated with their cell size
- These protists are able to consume dissolved Si and could contribute from 1% to 19% of the biogenic silica production of the global ocean

Supporting Information:
- Supporting Information S1
- Table S1
- Table S2
- Table S3

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Abstract Siliceous polycystines and phaeodarians are open-ocean planktonic protists found throughout the water column and characterized by complex siliceous skeletons that are formed, at least partly, through the uptake of silicic acid. These protists contribute to the marine organic carbon (C) and biogenic silica (bSi) pools, but little is known about their contribution to the silica (Si) biogeochemical cycle. Here we report the first measurements of the Si uptake rate of polycystine and phaeodarian cells from samples collected in the Mediterranean Sea using the ^{32}Si-based method. The elementary composition (bSi, particulate organic carbon and nitrogen) of these organisms was also measured. Combining our results with published data on the distribution and abundance of Polycystina and Phaeodaria in the global ocean, we conclude that these organisms could contribute from 0.2 to 2.2 mmol Si m^{-2} of the marine standing stock of bSi and from 2 to 58 Tmol Si yr^{-1} (1% to 19%) of the global oceanic biogenic silica production. The implications for the global marine Si cycle are discussed.

1. Introduction

Rhizarians are eukaryotic, mostly heterotrophic single-celled organisms, ranging in size from tens to hundreds of micrometers, although some are capable of forming gelatinous colonies up to over 1 m in length (Boltovskoy et al., 2017; Suzuki & Not, 2015). These protists are globally distributed, dwelling chiefly in the open ocean, from the surface down to bathypelagic depths. Their distribution and abundance are controlled by environmental factors, such as temperature, salinity, productivity, and nutrient availability (Boltovskoy, 2017a, 2017b; Boltovskoy et al., 2017; Boltovskoy & Correa, 2016). Some rhizarian taxa produce mineral skeletons of strontium sulfate (e.g., subclass Acantharia), calcium carbonate (e.g., order Foraminifera), and opaline silica (e.g., orders Spumellaria and Nassellaria and superorder Phaeodaria).

Silicifying organisms are a critical component of the global oceanic Si cycle. Diatoms, silicoflagellates, sponges, and siliceous rhizarians are all capable of using the silicic acid available in seawater to build elaborated skeletons that are believed to improve essential functions, such as mechanical protection for the cell (Hamm et al., 2003), an armor against predators (Finkel & Kotrc, 2010), an effective pH buffer (Milligan, 2002), or an improvement for the uptake or storage of bioessential elements (Suzuki & Not, 2015). Other studies have suggested that the frustule could confer diatoms an advantage due to its peculiar optical properties (Leynaert et al., 2018). Diatoms are considered the world's largest contributors to the Si cycle, dominating both the standing stock of water column biogenic silica (bSi) and its production rate (Ragueneau et al., 2000, 2006; Tréguer & De La Rocha, 2013). A number of studies ranging from sediment traps to environmental molecular surveys have emphasized the importance of rhizarians in biogeochemical cycles and export of C and bSi to the deep ocean (Biard et al., 2018; Guidi et al., 2016; Gutierrez-Rodriguez et al., 2019; Lampitt et al., 2009). Moreover, recent studies combining genomic and in situ imaging approaches have shown that the contribution of large Rhizaria to the biomass of zooplankton has been largely underestimated (Biard et al., 2016), with their abundance correlating with carbon export fluxes at 150-m depth in oligotrophic oceanic regions (Guidi et al., 2016). Globally, in terms of numbers, some rhizarian taxa can represent approximately 33% of large zooplankton (>600 μm) in the upper water column and up to 5% of the overall oceanic biota carbon reservoir (Biard et al., 2016; Stukel et al., 2018). These new findings suggest an unsuspected role of these organisms in the biological carbon pump, as well as in the Si

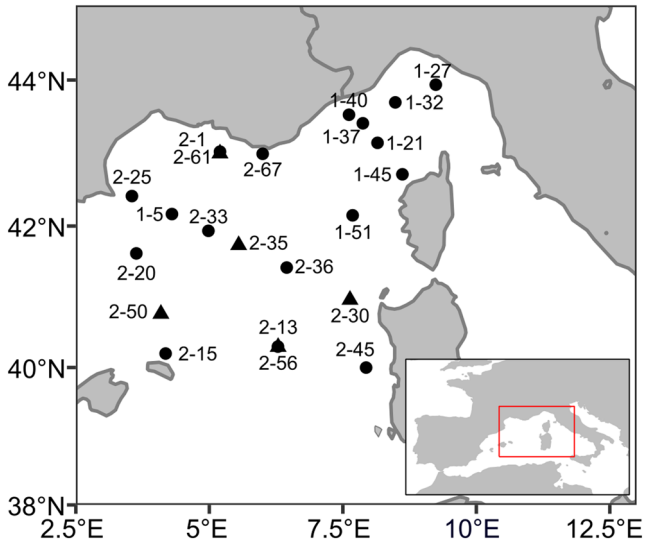

Figure 1. Sampling sites (Leg-Station) of the MOOSE-GE cruises, ● sites sampled in 2017 and ▲ sites sampled in 2018. Net was deployed between 0 and 500 m. The samples of the AMT cruise were collected at 3.69°S 24.98°W at depths between 0 and 200 m.

cycle, especially in oligotrophic areas where diatoms are poorly represented while siliceous rhizarians may be dominant in the tropical sediments (Dutkiewicz et al., 2015; Lisitzin, 1974).

The major taxa of silicifying rhizarians are represented by the Phaeodaria (Cercozoa, Thecofilosea) and by Spumellaria, Nassellaria, and Collodaria (Retaria, Radiolaria, Polycystinea) (Adl et al., 2018). The two groups differ in the robustness of their skeletal structures. While most polycystines possess solid and dense skeletons (Takahashi, 1983), phaeodarian skeletons are porous (Nakamura et al., 2018). The ballast produced by this skeleton causes them to sink toward the ocean floor where they can be incorporated into the sediment if their bSi has not been affected by the dissolution and recycling into $Si(OH)_4$. Polycystine bSi is more resistant to dissolution and often remains well preserved in the sediment, whereas that of Phaeodaria is rarely found in bottom deposits (Takahashi, 1983). Among Polycystina, Spumellaria and Nassellaria have been widely used for paleoceanographic reconstructions (Matsuzaki et al., 2014; Moore, 1978). However, essentially due to the difficulty of culturing planktonic rhizarians, our knowledge of their ecology, physiology, and biogeochemistry is very limited, especially with regard to the processes associated with Si sources, uses, stocks, and fluxes. Marron et al. (2016) reported the presence of Si transporters in rhizarians, which enable the uptake of Si (mainly in the form of silicic acid) from the environment.

In this study, we analyze the Si, carbon (C), and nitrogen (N) content of isolated rhizarian cells collected during two oceanographic cruises in the Mediterranean Sea (Mediterranean Ocean Observing System on Environment Grande Echelle, MOOSE-GE) and from the Atlantic Ocean (Atlantic Meridional Transect, AMT28) cruise. We also measured their bSi production rates using the ^{32}Si isotope, an approach successfully applied until now only to estimate diatom bSi production rates. We first discuss the relationship between rhizarians elemental composition and their cell size, and combining these measurements with available data on rhizarian abundances worldwide, we assess their potential contribution to the global marine Si cycle.

2. Material and Methods

2.1. Sampling

Samples were collected at 22 sampling sites in the western Mediterranean basin (Figure 1) during the MOOSE-GE expedition in September 2017 and June 2018 aboard the R/V *Atalante* and at one site during the AMT28 in October 2018 aboard the RRS James Clark Ross.

Plankton samples were obtained at discrete depth intervals using vertical tows. Upon recovery, samples were immediately diluted in 0.2-μm filtered seawater and observed onboard under a stereomicroscope or an inverted microscope. Rhizarians were handpicked using a Pasteur pipette and transferred to 20-ml glass vials filled with filtered seawater. Cells were sorted according to targeted taxonomic groups, namely, Nassellaria, Spumellaria, and Collodaria, chiefly represented by Pterocorythidae, Hexastyloidea, and Sphaerozoidae, respectively. Among the Phaeodaria, because of their high abundances, we differentiated the genera *Aulacantha*, primarily represented by *Aulacantha scolymantha,* and *Challengeria*.

In each glass vial, we stored from 1 to 50 individuals, depending on cell size and abundance of the target organisms in the sample. Individual pictures were taken prior the experiments to obtain morphometric measurements using the ImageJ software. For each specimen, we measured length, width and area, and subsequently calculated the biovolume associated using the most similar simple geometric shape (e.g., sphere, ellipsoid, and cone). Due to logistic problems, for some of the stations, no pictures were available for biovolume estimates. However, samples from all the stations were analyzed using the Flowcam (preserved with Lugol's solution) and the Zooscan (preserved with formaldehyde) immediately after the cruise. We also compared cell size from living and preserved individuals, and we did not observed differences in cell dimensions between them. Therefore, we used the vignettes obtained with these imaging technologies

Table 1
Biogenic Silica Stocks, Production Rates, and POC Content of the Rhizarians Analyzed

	bSi				bSi Production			POC					Si:C
	n	^{a}n	ESD ± SD (μm)	Mean ± SD (nmol Si cell^{-1})	Mean ± SD (mg bSi mm^{-3})	n	^{a}n	Mean ± SD (nmol Si cell^{-1} day^{-1})	n	^{a}n	ESD ± SD (μm)	Mean ± SD (μmol C cell^{-1})	Mean ± SD (mg C mm^{-3})	
Polycystina														
Collodaria	6	378	150 ± 34	1.24 ± 0.79	0.05 ± 0.04	6	378	0.60 ± 0.72	—	—	—	—	—	
Nassellaria	3	64	80 ± 1	2.16 ± 1.07	0.53 ± 0.25	3	64	0.17 ± 0.05	1	33	102	0.07	1.56	0.03
Spumellaria	4	44	143 ± 51	6.09 ± 5.80	0.20 ± 0.09	4	44	1.59 ± 1.35	3	99	144 ± 38	0.09 ± 0.05	1.00 ± 1.03	0.07
Phaeodaria														
Aulacantha	17	249	835 ± 119	26.09 ± 11.80	0.01 ± 0.01	13	198	1.78 ± 3.10	17	302	695 ± 122	0.26 ± 0.13	0.02 ± 0.02	0.10
Challengeria	5	97	124 ± 66	3.09 ± 3.34	0.24 ± 0.19	5	97	0.34 ± 0.23	3	73	151 ± 39	0.09 ± 0.08	1.62 ± 1.35	0.03

Note. ESD, equivalent spherical diameter. n denotes the number of samples analyzed and ^{a}n the number of specimens analyzed. SD: Standard Deviation.

to complete our set of measurements. For Collodaria (mostly colonial radiolarians comprising hundreds to thousands of siliceous shells or spicules embedded in a gelatinous matrix), the entire colony was measured, but the results are reported per individual. Capsules were counted and measured using ImageJ software.

Seawater samples for the incubations were collected at each sampling location and at different depths using Niskin bottles.

2.2. Elementary Composition

We analyzed the bSi (in nmol Si cell^{-1}) and the particulate organic carbon and nitrogen (POC/PON in μmol cell^{-1}) in 35 and 24 samples, respectively (Table 1). In total, 1,339 cells were isolated and analyzed.

The entire content of each vial with isolated cells in filtered seawater was filtered onto polycarbonate membrane (Nuclepore 47 mm, 0.6 μm pore size) for bSi determination and onto 25-mm GF/F precombusted filters (at 450 °C for 4 hr) for POC and PON analysis. Nuclepore membranes were four-folded in a petri dish, dried, and stored at ambient temperature until later analysis in the laboratory. GF/F filters were folded in a petri dish and stored at −20 °C until analysis.

The bSi of Polycystina and Phaeodaria was quantified by colorimetric determination of the orthosilicic acid after leaching. A single digestion in hydrofluoric acid (HF) was performed, since the samples only contained isolated rhizarians, that is, there was no possible interference with lithogenic Si as there might be when filtering raw seawater (see Ragueneau et al., 2005). We added 0.2 ml of HF 2.5 N to the polymethylpentene (PMP) tubes containing the filters. The filter was then compressed until submerged in HF, and air bubbles were removed. The tube was tightly covered with a cap and kept under a fume hood at room temperature for 48 hr to allow for digestion of the bSi. We then added 9.8 ml of saturated H_3BO_3 solution. The standards used for calibration were prepared with the same matrix as for the samples (HF/H_3BO_3), before analysis by colorimetric methods on a Technicon Auto-Analyzer II (Aminot & Kérouel, 2007; Brzezinski & Nelson, 1989).

Concentrations of POC and PON were measured with a mass spectrometer (Delta plus, ThermoFisher Scientific) coupled to a C/N analyzer (Flash EA, ThermoFisher Scientific). Standard deviations (*SD*) were 0.009 and 0.004 μM for POC and PON, respectively. In order to avoid false positives, the detection limit was set at the control level plus 10 times the standard deviation. Although POC and PON analyses were performed simultaneously using an elemental analyzer, N was often close to the detection limit, in which case, the values were discarded, thus yielding more results for C than for N. The POC/PON measurements of Collodaria were rejected for the same reason.

2.3. Assessment of bSi Production Rates (ρ_P)

For the measurements of bSi production rates (ρ_P), we used the radioisotope of silicon (^{32}Si) (Leynaert et al., 1996; Tréguer et al., 1991). Immediately after isolation, glass vials containing between 1 and 50 cells of the same taxonomic group were incubated on deck with 800 becquerel (Bq) of high specific activity ^{32}Si for 24 hr in a flowing-seawater incubator to maintain constant water temperature. The light in the incubation baths was attenuated by means of neutral screen to 50% of the incident light. The ^{32}Si additions increased silicic acid concentrations in the incubation bottles by less than 10 nM, a negligible value compared to the

Table 2
Mean Abundances in cells m^{-3} and [cells m^{-2}] of Polycystina and Phaeodaria, as Reported in 22 Publications Based on Plankton Materials

	40°N–40°S				>40°N/S			
	0–200 m		200–1,000 m		0–200 m		200–1,000 m	
N° of data points	523		91		383		194	
N° of sources	14		7		12		9	
Polycystina								
Collodaria	52	[10439]	10	[7756]	0	[1]	0	[13]
Spumellaria	235	[46993]	72	[57223]	79	[15876]	37	[29588]
Nassellaria	892	[178316]	105	[83742]	103	[20645]	69	[55288]
Phaeodaria								
Phaeodaria	20	[3940]	4	[3217]	5	[957]	1	[478]

Note. Values include all cells recorded (i.e., living and dead shells).

dissolved silica (DSi) concentration in seawater. A split of the seawater sample used for the incubation was stored for subsequent analyses of silicic acid concentration using the automated method of Aminot and Kérouel (2007).

After incubation, samples were filtered by gentle (<150 mmHg) vacuum filtration onto 47-mm diameter, 0.6-μm pore-size polycarbonate membrane filters (Nuclepore) and rinsed twice with filtered seawater to wash away nonparticulate ^{32}Si. Each filter was then placed in a clean 20-ml polypropylene liquid scintillation vial, and the vial was capped loosely to allow the sample to dry at room temperature for 48 hr. The vials were then capped tightly and returned to the laboratory for counting.

The activity of ^{32}Si in the samples from the incubation experiments was determined using the Cerenkov counting method (Leynaert, 1993) 3 months after the samples were filtered, allowing ^{32}Si and its daughter isotope ^{32}P to return to secular equilibrium. Although this method is less sensitive than some others (Brzezinski & Phillips, 1997), it was chosen because it allows using the materials for further bSi analyses. Twenty-four hours before assessing the activity on the filter, HF (2.0 ml of 2.5 N) was added to each sample to dissolve all bSi. Samples were assayed using a Wallac Model 1414 scintillation counter. Because ^{32}Si does not produce Cerenkov emissions, the procedure allowed quantifying the amount of ^{32}P only. However, as ^{32}Si and ^{32}P are in secular equilibrium, the activities of the two isotopes are equal, and the ^{32}Si activity can be deduced from that of ^{32}P (Leynaert, 1993). Triplicate 40-min counts were performed on each sample. Counting precision (95% confidence interval) was less than ±1%, except for a few very low-activity samples yielding <250 CPM (counts per minute), for which counting precision was ±2–5%. Counts yielding less than three times the background (8 CPM) were discarded. Collodarians without siliceous spicules or shells (e.g., *Collozoum spp*.) were incubated in parallel to obtain a production blank, which yielded production rates close to the detection limit, that is, below 0.02 nmol Si cell^{-1} day^{-1} in all cases.

2.4. Extrapolation of Siliceous Rhizarians to the Global Ocean: bSi Stocks and Production Rates

We performed an estimate of rhizarian abundances based on the compilation of worldwide data of Boltovskoy et al. (2010), supplemented with more recent studies (see supporting information). Our database contained 1,191 data points of Polycystina and Phaeodaria densities (cells m^{-3}) in plankton samples collated from 22 publications. Most of the studies used plankton nets to collect rhizarians, including vertical and horizontal tows. Other studies sieved Niskin bottle samples to quantify abundances of these organisms (see details in supporting information).

These data were averaged over two bins: tropical-subtropical (40°N to 40°S) and colder waters (>40°N or S), each in turn was subdivided into two depth layers: 0–200 m and below 200-m depth (Table 2). For the Phaeodaria, where the literature information is scarcer, all species were pooled in a single group (Phaeodaria). For the Polycystina, densities of Spumellaria and Nassellaria were estimated separately (and also used separately in subsequent calculations).

To convert abundance values reported in literature into bSi stocks (nmol Si m^{-2}) and production rates (ρ_P μmol Si m^{-2} day^{-1}), we used the estimates obtained in our cruises. For each taxonomic group, we

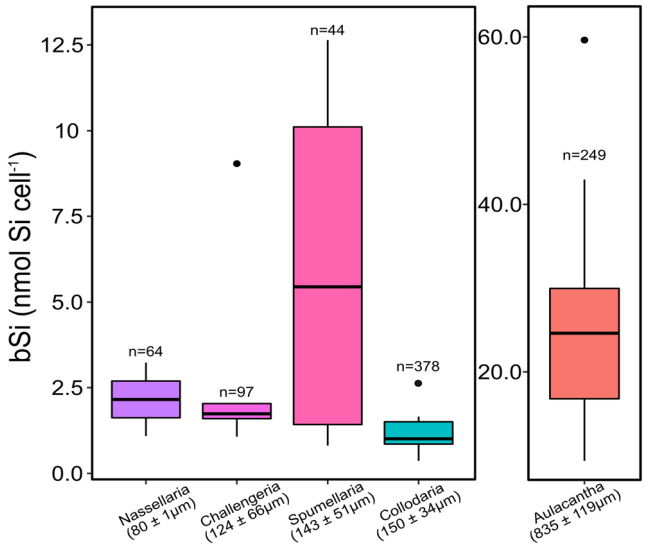

Figure 2. bSi content and size of the taxa studied. *n* denotes the number of specimens (or individual cells, for the Collodaria *n**) analyzed. Colors correspond to the taxonomic groups identified in Figure 3.

established ranges based on the minimum and maximum values for both bSi stock and ρ_P (Table 1). To obtain global estimates, we considered an area of $2.68E^{14}$ m^2 (40°N to 40°S) for the warm waters and an area of $9.3E^{13}$ m^2 (>40°N or S) for the cold waters.

3. Results

3.1. Elemental Composition: bSi, POC, and PON

3.1.1. bSi Content

Overall, bSi per cell varied over one order of magnitude, from 1.24 ± 0.79 nmol Si $cell^{-1}$ (mean ± *SD*) for Collodaria to 26.09 ± 11.80 nmol Si $cell^{-1}$ for *Aulacantha*. The bSi content differed significantly between *Aulacantha* (Kruskal-Wallis, $P < 0.05$) and the other groups (Figure 2). The largest cells (i.e., genus *Aulacantha*) have the highest Si content. In order for our data to be comparable with the units reported in the literature, Si concentrations were converted from nmol Si $cell^{-1}$ to µg Si $cell^{-1}$ assuming a molecular weight of 67 g mol^{-1} for hydrated amorphous silica (Mortlock & Froelich, 1989).

We evaluated the log-log relationship between the Si content and cell size of the rhizarians surveyed. Based on the Si contents of three samples of Nassellaria (64 individuals), four samples of Spumellaria (65 individuals), six samples of Collodaria (378 individuals), five samples of *Challengeria* (97 individuals), and 17 samples of *Aulacantha* (249 individuals), Si content (Q_{bSi}) was significantly associated with the cell's equivalent spherical diameter (ESD) ($R^2 = 0.8$, $F(1, 33) = 129$, $P < 0.001$) according to the following equation:

$$\log_{10}(Q_{bSi}) = [-3.61 \pm 0.29] + [1.30 \pm 0.11] \cdot \log_{10}(\mathrm{ESD}), \quad (1)$$

where Q_{bSi} is in µg Si $cell^{-1}$ ± standard error (SE) and ESD in µm ± SE.

3.1.2. POC/PON Content and C:N Ratios

The POC and PON content of the rhizarians analyzed differed significantly between taxa (ANOVA, $P < 0.001$; excluding the Nassellaria, for which no data were available). Nitrogen measurements were obtained for 24 samples including a total of 507 siliceous rhizarians cells. Overall, PON concentrations ranged between 0.001 and 0.02 µmol N $cell^{-1}$.

In these 24 samples, the POC content (Q_C) varied between 0.09 ± 0.05 and 0.26 ± 0.13 µmol C $cell^{-1}$. The highest Q_C was found for *Aulacantha* cells. However, the relationship between Q_C and ESD was not significant. The average carbon:nitrogen ratio was 12.

3.2. Rhizarian bSi Production Rates

We successfully measured, for the first time, the silicic acid uptake (ρ_P) rates of 31 rhizarian samples (781 individuals). Rates of bSi production ranged from 0.17 ± 0.05 nmol Si $cell^{-1}$ day^{-1} for Nassellaria to 1.78 ± 3.10 nmol Si $cell^{-1}$ day^{-1} for *Aulacantha* (Table 1). Production rates seem to be related to cell size.

In order to estimate specific uptake rate (V_P, in day^{-1}), ρ_P were normalized to the concentration of bSi (ρ_P/bSi) (Tables 1 and 3). The largest cells (*Aulacantha*) present the lower V_P, while Nassellaria, the smallest cells found in this study, had the highest V_P.

3.3. Worldwide Si Standing Stocks and Production Rates (ρ_P)

According to the literature data collated, densities of Polycystina and Phaeodaria peak in the upper layers where their abundances (i.e., cells L^{-1}) are ~1.5–8.5 times higher than below 200-m depth, with higher contrasts in the warm waters. Nevertheless, low densities integrated throughout large depth intervals often yield larger standing stocks below 200 m than in the 0- to 200-m layer, especially at low latitudes (Table 2). Our estimates suggest that polycystine and phaeodarian bSi standing stocks in the water column range from 0.3 to 2.2 mmol of bSi m^{-2} (Table 4). For ρ_P, we obtained a tentative range of 5 to 58 Tmol yr^{-1} (Table 4). The ρ_P are of more consequence in temperate waters than in cold waters, which reflects differences in abundances. Production rates throughout the water column are similar in the two oceanic zones considered.

Table 3
Specific Si Uptake Rates for Polycystina, Phaeodaria, and Diatoms

Groups	V_P (day^{-1})	Reference	Area of study
Polycystina			
Collodaria (individual)	0.48	This study	Mediterranean Sea
Nassellaria	0.26	This study	Mediterranean Sea
Spumellaria	0.08	This study	Mediterranean Sea
Phaeodaria			
Aulacantha	0.07	This study	Mediterranean Sea
Challengeria	0.11	This study	Mediterranean Sea
Diatoms			
	0.96	Leynaert et al. (2001)	Equatorial Pacific
	0.40	Nelson and Brzenzinski (1990), Brzezinski and Nelson (1996)	Sargasso Sea
	0.62	Nelson and Dortch (1996)	Mississipi
	0.06	Nelson and Tréguer (1992)	Ross Sea
	1.80	Goering et al. (1973)	Peru upwelling
	0.19	Leblanc et al. (2003)	Northwest Mediterranean Sea
	0.38	Kristiansen et al. (2000)	Southern Norway
	1.02	White and Dugdale (1997)	California
	1.06	Bonnet (2001)	Bay of Brest
	0.21	Brzezinski et al. (1998)	Central North Pacific
	0.31	Nelson et al. (2001)	Southern Ocean

Note. Values for diatoms are from Claquin et al. (2006) and references therein.

4. Discussion

4.1. Filling the Gaps in Size-Si Relationship: From Small to Giant Protists

Our work on the assessment of the elemental composition of siliceous rhizarians included several small-sized Polycystina (Collodaria, Nassellaria, and Spumellaria), as well as the larger Phaeodaria, encompassing a wider size spectrum than that covered by previous studies. Takahashi (1981) was the first to report the Si content, weight, length, width, projected area, and biovolume of polycystines and phaeodarians.

Table 4
Estimated Values of bSi Stock and Production as Derived from Our Experimental Data Using the Minimum and the Maximum Values for Each Group and the Information Detailed in Table 2

	Stock (μmol Si cell^{-1} m^{-2})			
	0–40°N–40°S		>40°N/S	
	0–200 m min–max	>200 m min–max	0–200 m min–max	>200 m min–max
Collodaria	3.9–27.5	2.9–20.4	0.0–0.0	0.0–0.0
Spumellaria	38.1–594.0	46.4–723.3	12.9–200.7	24.0–374.0
Nassellaria	192.6–576.0	90.44–270.49	22.3–66.7	59.7–178.4
Phaeodaria	4.2–234.9	3.4–191.8	1.0–57.1	0.51–28.5
Total	238.7–1,432.3	143.1–1,206.0	36.2–324.4	84.12–580.9
	Production (μmol Si m^{-2} day^{-1})			
	0–40°N–40°S		>40°N/S	
	0–200 m min–max	>200 m min–max	0–200 m min–max	>200 m min–max
Collodaria	0.6–20.6	0.5–15.3	0.0–0.0	0.0–0.0
Spumellaria	5.6–159.3	6.9–194.0	1.9–53.8	3.6–100.3
Nassellaria	19.6–35.7	9.2–16.8	2.3–4.1	6.1–11.1
Phaeodaria	0.4–46.7	0.4–38.1	0.1–11.3	0.1–5.7
Extrapolation (Tmol Si yr^{-1})	2.6–25.7	1.7–25.8	0.1–2.4	0.3–4.0

Note. We estimated for each oceanic area a value of production in Tmol Si yr^{-1}.

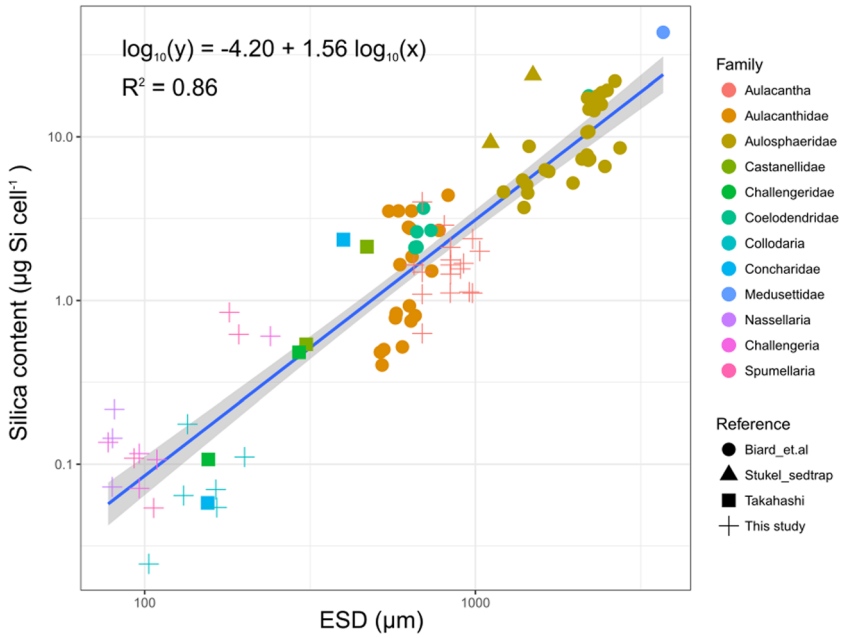

Figure 3. Relationship between the Si content of Polycystina and Phaeodaria and their ESD (µm) across all the specimens assessed. Regression (blue line) and 95% confidence interval (gray shading). Combined data from Biard et al. (2018) and this study.

However, his studies were based on dead cells recovered from sediment traps. Using these data and specimens collected off the coast of California, Biard et al. (2018) established an allometric relationship that shows that the Si content of phaeodarians is closely associated with cell length and cell biovolume. These relationships were chiefly based on large specimens (0.6 to >10 mm, average: 2 mm) and have not hitherto been validated for the smaller rhizarians.

In order to investigate whether their Si content is associated with biovolume and cell size (expressed as ESD) throughout a wider size range, we combined our measurements and Si content values with the log-log linear relationship shown by Biard et al. (2018). The result of this exercise showed that over a range of 103 to 3,920 µm, rhizarian Si content is significantly associated with biovolume ($R^2 = 0.86$, F(1, 93) = 593, $P < 0.001$):

$$\log_{10}(Q_{\mathrm{bSi}}) = [-4.05 \pm 0.18] + [0.52 \pm 0.02] \cdot \log_{10}(\mathrm{biovolume}), \quad (2)$$

where Q_{bSi} is in µg Si cell^{-1} ± SE and biovolume in µm^3 ± SE.

Si content is also correlated with the ESD (Figure 3; $R^2 = 0.86$, F(1, 93) = 593, $P < 0.001$):

$$\log_{10}(Q_{\mathrm{bSi}}) = [-4.20 \pm 0.18] + [1.56 \pm 0.06] \cdot \log_{10}(\mathrm{ESD}), \quad (3)$$

where Q_{bSi} is in µg Si cell^{-1} ± SE and ESD in µm ± SE. Biard's length data were converted to ESD for this analysis.

The slope of equation (3) is similar to those of Biard et al. (2018) for ESD (ANCOVA, $P = 0.41$), confirming its validity for a wide spectrum of rhizarians sizes.

Silica content has previously been shown to be related to cell size in other siliceous organisms, such as diatoms. Conley et al. (1989) observed that the Si content of diatoms varies over five orders of magnitude depending on cell size and found a significant log-log linear relationship between Si content and biovolume. However, the relationship for diatoms gives much lower cellular bSi concentrations (100 times lower on average) than for rhizarians of comparable size. Thus, among the siliceous planktonic organisms in the ocean, rhizarians appear to be the most silicified.

Although the largest cells contain more Si, they are not necessarily the densest according to our conversion from Si biomass to density. Nassellarians, which are the smallest cells in our study, had 530 µg bSi mm^{-3},

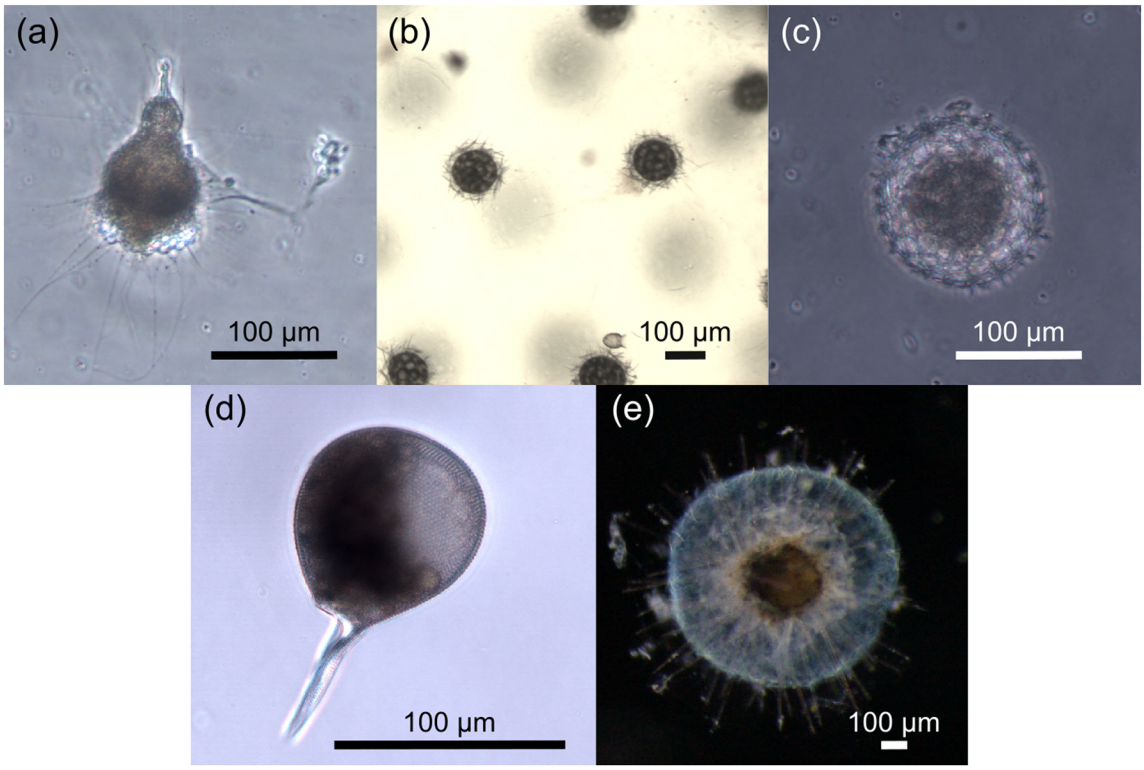

Figure 4. Images of the most abundant morphotypes surveyed in this work, including Polycystina (a–c), and Phaeodaria (d–e). (a) Nassellaria-Pterocorythidae; (b) Collodaria-Sphaerozoidae; (c) Spumellaria-Hexastyloidea; (d) Challengeridae-*Challengeria xiphodon*; (e) Aulacanthidae-*Aulacantha scolymantha*.

while *Aulacantha*, the largest cells analyzed (measurements based on the solid skeleton), barely reached 10 µg bSi mm^{-3}. As reported recently, the structure of Polycystina and Phaeodaria differs considerably. Polycystine skeletons are solid, whereas phaeodarian skeletons are porous and in some species, including *Aulacantha*, they are composed of loose spines protruding from the cell (Nakamura et al., 2018) (Figure 4). Sedimentation rates in the water column are likely to be affected by differences in density of these structures, as observed by Baines et al. (2010) for diatoms.

As opposed to Si, we were not able to find any relationship between POC and PON and cell size; therefore, an average was calculated per taxon (Table 1). Data on rhizarian Q_C are scarce. To our knowledge, the first Q_C published are those of Michaels et al. (1995) where they reported extremely variable Q_C values for solitary collodarians, ranging from 0.009 to 0.28 mg C mm^{-3}. Stukel et al. (2018) estimated a Q_C for Aulosphaeridae of 0.011 mg C mm^{-3} based on a downward revision of Biard et al.'s (2016) measurements. Our data are high when compared to those in these two studies. Polycystina and Phaeodaria Si:C molar ratios (0.03 to 0.1) are lower than those of diatoms (0.13 ± 0.04), according to data reported by Brzezinski (1985) for 27 diatom species. On the other hand, our mean C:N ratio (~12) is higher than that reported by Michaels et al. (1995) for colonial collodarians (8.2) and much higher than the Redfield ratio (6.6). Higher molar ratios for the colonial radiolarians could be due to the presence of mucopolysaccharide material surrounding the colonies (Michaels et al., 1995).

4.2. The Silicic Acid Uptake Rates of Rhizarians (ρ_P)

In this study, ρ_P were measured using the radioisotope ^{32}Si, quantifying, for the first time, the ability of these protists to consume dissolved Si from seawater. Our results show consumption rates ranging from 0.17 nmol Si $cell^{-1}$ day^{-1} (for Collodaria) to up to 1.78 nmol Si $cell^{-1}$ day^{-1} (*Aulacantha*).

Comparison of these results with the ρ_P of other plankton, in particular diatoms, is complicated by the fact that the rates measured for diatoms in the field are expressed in terms of volume (i.e., per liter), rather than "per cell." For diatoms, ρ_P per cell were only assessed in culture experiments in laboratory-controlled

conditions (Del Amo & Brzezinski, 1999; Riedel & Nelson, 1985), yielding values around 0.001–21 pmol Si cell^{-1} day^{-1}. In contrast, the consumption rates of silicic acid uptake by the rhizarians assessed in this work ranged from 200 to 2000 pmol Si cell^{-1} day^{-1}, which is around 100 times higher relative to diatoms and consistent with what was observed for the Si content, also 100 times higher in rhizarians.

To gain insight into the role of rhizarians in the Si cycle, we assessed the V_P of these organisms by normalizing the silicic acid uptake rates to the bSi concentration (Table 4). Our V_P ranged from 0.07 to 0.48 day^{-1}, for *Aulacantha* and Collodaria, respectively. Despite differences in the methods used, these values are generally in line with results previously found for "larger rhizarians," whose average V_P was ~0.1–0.2 day^{-1} (Stukel et al., 2018). Our values also agree with those found for diatoms worldwide (0.06–1.80 day^{-1}) for a large temperature range (Table 4).

Based on these results, we evaluated the turnover rates of these protists ($t = \ln 2/V_P$) assuming that the rhizarians analyzed in this study reproduce by binary cell division, which might not always be the case, as sexual reproduction may also occur (Boltovskoy et al., 2017). Turnover rates range between 3 and 10 days (for *Aulacantha* and Spumellaria, respectively), which are comparatively low when compared to that of phytoplankton, which usually span from several hours to a few days (Flynn et al., 2018; Krause et al., 2017).

4.3. Potential Impact of Siliceous Rhizarians on the Si Cycle of the World Ocean

Since the 1970s, silicified rhizarians (formerly collectively designated as Radiolaria) have drawn attention for their role in the marine Si cycle (Heath, 1974). Takahashi (1983) suggested that, in some oceanic areas, the daily flux of rhizarian bSi ranges around 20% to 30% of the overall bSi. Biard et al. (2016, 2018) found that the biomass of large Rhizaria, the so-called "giant protists," could constitute a substantial fraction of the >600-μm plankton biomass representing more than a third of the bSi standing stocks in oligotrophic and high nutrient-low chlorophyll regions of the ocean. These data conflict with the fact that in most global oceanic silicon budget estimates, only diatoms (Nelson et al., 1995; Tréguer et al., 1995) and more recently sponges (Maldonado et al., 2019; Tréguer & De La Rocha, 2013), are taken into account. This standpoint, however is gradually changing. For example, Tréguer and De La Rocha (2013) suggested that up to 23% of the bSi standing stocks in the upper 120 m of the ocean could be associated with rhizarians. However, due to the lack of information at that time, estimating the contribution of these organisms to the marine Si cycle was overly speculative.

Diatoms' bSi production is restricted to the photic layer. In contrast, rhizarians inhabit (and produce bSi) throughout the entire water column. Although their concentrations decrease sharply with depth, depth-integrated values (i.e., individuals per m^2) can be as high, or even higher, at depth than in the surface (0–100 m) layers (Boltovskoy, 2017a, 2017b; Kling & Boltovskoy, 1995).

According to our results, the water column bSi standing stock due to small-sized rhizarians (average ESD ~0.1 mm for Polycystina and *Challengeria* and 0.8 mm for *Aulacantha*) ranges from 0.5 to 3.5 mmol Si m^{-2}. Although small in size, these protists would increase by up to 35% the biomass of the giant protists (Aulosphaeridae, average size ~2 mm), estimated by Biard et al. (2018) and ranging between 0.01 and 10 mmol Si m^{-2}. In order to obtain a global ocean scale estimate of biomass in the water column, we combined the two size-classes yielding a pool of bSi of 0.5 to 13.5 mmol Si m^{-2}.

With regard to global rhizarian production rates, our assessment suggests a tentative range of 5 to 58 Tmol Si yr^{-1} for the whole water column. Tréguer and De La Rocha (2013) estimated that in the photic layer of the World Ocean, the production of bSi is 240 Tmol Si yr^{-1} (mainly by diatoms; Nelson et al., 1995). According to these figures, our estimates indicate that in a steady state ocean (i.e., where inputs and losses of Si are balanced), the rhizarians analyzed (<1 mm) account for 2% to 19% of these 240 Tmol Si yr^{-1}. This suggests that the contribution of these rhizarians to the production of bSi in the euphotic layer should not be neglected even though they are orders of magnitude less abundant than diatoms. If we take into account the average bSi standing stock (5 mmol Si m^{-2}) estimated by Biard et al. (2018) for the family Aulosphaeridae ("large rhizarians") and assuming a V_P of 0.1 day^{-1} (from our measurements), giant rhizarians could produce 65 Tmol of bSi yr^{-1}, which would have to be added to the production of the smaller rhizarians.

This estimate is based on data from the Mediterranean western basin and the California current, two oceanic zones with significant hydrographic differences. Extrapolating standing stocks and production values from specific oceanic areas to the global ocean has obviously important caveats. Contrasts in Polycystina and Phaeodaria standing stocks in warm versus cold waters have been accounted for in our estimates. Abundances of these protists tend to be much lower in northern latitudes. However, physiological processes (including production and doubling rates) and seasonality, which likely depend on water temperature, have not been considered. In the contemporary global ocean, the factors controlling the distribution, abundances, and silica production of rhizarians are poorly constrained. Although in our estimates efforts were made to consider living cells only, an important limitation of the extrapolations performed is the distinction between living and dead cells, in particular concerning production estimates, for which only physiologically active cells are relevant. Researchers have tried to circumvent this problem by discriminating cells with cytoplasm attached to the skeleton, either in unstained (Boltovskoy et al., 1993; Nimmergut & Abelmann, 2002) or in stained samples (Ikenoue et al., 2015; Matsuzaki et al., 2016). However, undecayed cytoplasm can take from weeks to months to disappear completely, which renders these techniques unreliable (Grego et al., 2013). In contrast, nuclear stains yield more realistic estimates of the proportions of living rhizarians (Boltovskoy, 2017a, 2017b), but they are seldom used (Gowing, 1986; Gowing & Coale, 1989; Nöthig & Gowing, 1991). In attempting to overcome this potential bias, we estimated the proportions of living cells as a function of depth and latitude using data from studies that used the more reliable technique (nuclear stains) only (Gowing, 1986; Gowing & Coale, 1989; Klaas, 2001; Nöthig & Gowing, 1991). Using this information, we performed empirical best fit analyses of samples based on location and taxonomic group, obtaining a linear and an exponential regression. The results of this exercise suggest that in warm and temperate waters (40°N to 40°S), above 200 m, 61% of all Polycystina (i.e., all individuals retrieved in the sample, including in situ living and dead cells sinking from above) are represented by living cells, whereas below 200 m, 11% are alive. For Phaeodaria, the figures are 96% and 83%, respectively. In cold water areas (>40°N or S), above 200 m, living Polycystina are ~90% and ~75% below 200 m; for Phaeodaria, the values are 97% and 73%, respectively (See supporting information). Therefore, our estimate of 2% to 19% (5 to 58 Tmol Si yr^{-1}) of the global oceanic bSi production is indicative of maximum values; excluding purportedly dead cells from the overall polycystine and phaeodarian standing stocks decreases these figures to 1–10% (2 to 30 Tmol Si yr^{-1}).

On the other hand, there also are potential biases which may involve underestimations in our bSi production. Our data on subsurface depth-integrated abundances are based on a conservative approach, using the 200- to 1,000-m layer only, but many radiolarians are known to dwell and often reach highest abundances below 1,000 m (Kling & Boltovskoy, 1995; Suzuki & Not, 2015), which might render our integrated subsurface abundance figures too low. In addition, spicule-bearing Collodaria are not included in our abundance estimates. Collodaria include colonies composed by tens to thousands of cells, either naked (e.g., *Collozoum sp*.) or provided with siliceous spines embedded in the cytoplasm (e.g., *Sphaerozoum sp*.). There also are solitary species (single cells with or without siliceous spicules). In this study, abundance data for this group are based on shelled organisms only (e.g., Collosphaeridae), because information on densities of species with siliceous spicules scattered around the central capsule (without a solid shell; e.g., genera *Sphaerozoum*, *Rhaphidozoum*) is virtually null. However, the very few surveys that made rough estimates of the densities of shelled and spicule-bearing Collodaria suggest that, in warm waters, the latter are at least as abundant as the former (Swanberg, 1979). Some other rhizarians with siliceous spines, like *Sticholonche zanclea* (Taxopodia, sensu Adl et al., 2018), are not included in our abundance estimates due to the lack of information which is certainly associated to their delicate skeletons disintegrating rapidly after death (Takahashi & Ling, 1980).

Further, our experiments were carried out with a limited set of radiolarian species pooled into high taxonomic levels, which might represent a potential source of bias when extrapolating these results to global ocean scales. However, our measurements always included several different species (of the same higher level taxon), which presumably takes this variability into account, at least as far as tropical/subtropical waters are concerned. Their representativeness for cold water areas is conceivably lower. Nonetheless, for the Polycystina, size does not seem to be affected by temperature. Indeed, the weight of the skeleton of 48 polycystine species estimated by Jacot Des Combes and Abelmann (2009) is unrelated to their preferred sea surface temperatures (i.e., the sea surface temperatures where they peak in abundance, as estimated from worldwide distribution data by Boltovskoy & Correa, 2016). However, Lomas et al. (2019) found that Si:

biovolume ratio increased in diatoms that had adapted to cold water. Whether this effect also impacts Rhizaria is still a research focus. Thus, although these results are preliminary, we contend that the potential bias involved is marginal.

This study highlights the significant contribution of rhizarians to the standing stock and production of bSi on a global scale and challenges the current assumption that diatoms alone control the Si cycle in marine surface waters. Further studies are required on the ecology and physiology of rhizarians and the factors controlling their growth and skeleton's dissolution rate before we can fully elucidate rhizarians' role in the Si and other marine biogeochemical cycles.

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