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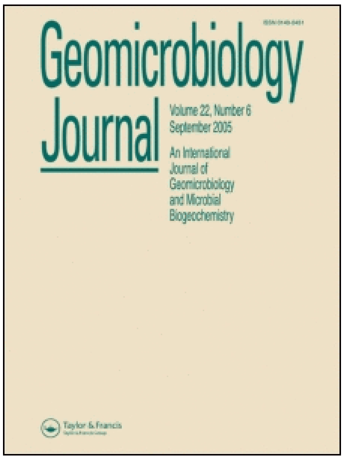

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Silica Use Through Time: Macroevolutionary Change in the Morphology of the Diatom Fustule

Zoe V. Finkel[a]; Benjamin Kotrc[b]
[a] Environmental Science Program, Mount Allison University, Sackville, New Brunswick, Canada [b] Department of Earth and Planetary Sciences, Harvard University, Cambridge, Massachusetts, USA

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Silica Use Through Time: Macroevolutionary Change in the Morphology of the Diatom Fustule

Zoe V. Finkel[1] **and Benjamin Kotrc**[2]
[1]*Environmental Science Program, Mount Allison University, Sackville, New Brunswick, Canada*
[2]*Department of Earth and Planetary Sciences, Harvard University, Cambridge, Massachusetts, USA*

Diatoms have evolved an obligate requirement for silica for their ornamented cell wall. Diatom productivity and subsequent burial of their siliceous remains on the ocean floor is a major control on the marine silica cycle. The ecological success of the diatoms over the Mesozoic and Cenozoic is associated with biogeographic shifts in siliceous sponges, decreasing silicification in the radiolarians, and a global decrease in oceanic silicic acid concentrations. We review what is known about the evolutionary advantages of the silica frustule and how selection pressures, including decreasing silicic acid concentrations, changes in growth limiting nutrients other than Si, CO_2, irradiance, predation and viral pressures have shaped the morphology of the frustule over the Mesozoic and Cenozoic.

Keywords biomineralization, diatom, macroevolution, silicon, silicic acid

INTRODUCTION

The diatoms are a diverse group of unicellular eukaryotic protists that cluster within the chromalveolates based on a combination of morphological, molecular, cytological and biochemical characteristics (Keeling et al. 2005). A diverse fossil assemblage of marine diatoms was present by the mid-Mesozoic (Harwood and Nikolaev 1995: Harwood et al. 2004), consistent with the early Mesozoic estimate for the origin of the group generated from internally calibrated molecular clocks (Kooistra and Medlin 1996; Sorhannus 2007). The fossil record indicates that the global diversity of the diatoms has increased through the Cenozoic, especially in the Neogene (Spencer-Cervato 1999), although see Rabosky and Sorhannus (2009).

It is estimated that there are currently between approximately 10,000 to over 100,000 species of diatoms and 1400–1800 planktonic marine species (Sournia et al. 1991; Hasle and Syvertsen 1997). Many of these species are freshwater or marine photosynthetic plankton but they also inhabit aquatic benthic environments, and even terrestrial environments with episodic moisture (Bold and Wynne 1978). They are responsible for nearly half of the primary production in the oceans and close to a quarter of the carbon fixed globally (Smetacek 1999). In addition, relative to most other phytoplankton groups diatoms disproportionately export organic carbon into the ocean depths, removing carbon out of contact from the atmosphere, due in part to their high growth rates, high sinking rates, relatively large cell size and density, and their ability to form large blooms (Egge and Aksnes 1992; Dugdale et al. 1995; Dugdale and Wilkerson 1998; Laws et al. 2000; Laws 2004).

The diatoms' most conspicuous distinguishing character is a mineralized cell wall, termed a frustule, made of amorphous nanometer-sized spheres of $SiO_2 \cdot nH_2O$ deposited in diverse, regular geometric patterns (Figure 1). The frustule surrounds the plasmalemma and is composed of two valves that fit together like a petri dish. Each valve has a valve face (the surface parallel to the plane dividing the two valves) and a mantle (the surface perpendicular to that plane, see Figure 1). The two valves are joined by overlapping bands of silica termed girdle bands. Valve shape, wall structures (for example, pore arrangement) and processes or the arrangement of processes such as spines and other distinguishing protrusions have traditionally been used to identify diatom taxa. The wall of the frustule generally consists of one or two layers of amorphous silica, perforated by pores (also termed areolae) in species-specific arrangements. The pores are often circular on the valve face, and on the inner side of the wall the pore is often occluded by a mesh of silica with smaller pores termed a sieve plate or velum. The pores in two-layered walls can vary in cross-section due to the curvature of the cross-walls, which are often arranged in a six-sided honeycomb pattern.

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Address correspondence to Zoe V. Finkel, Environmental Science Program, Mount Allison University, Sackville, New Brunswick E4L 1A7, Canada. E-mail: zfinkel@mta.ca

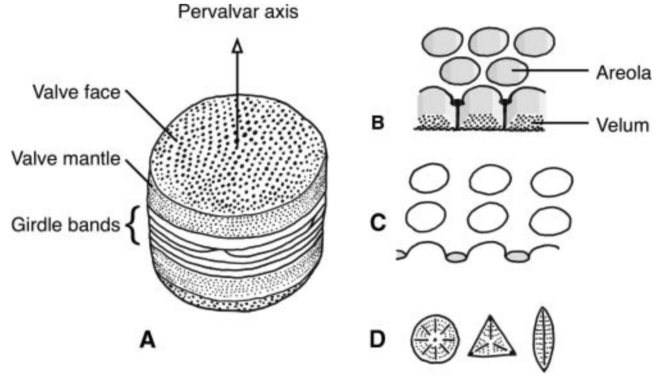

FIG. 1. (A) Basic morphology of the diatom frustule. (B) Cross-section of typical double-layered pseudoloculate wall. (C) Cross-section of single-layer of silica. (D) The three basic valve shapes: the circular centrics, multipolar centrics (can be bipolar, triangular, and even star-shaped), and bilateral pennates.

The gross symmetry of the valve face is a taxonomic feature at a higher level and separates diatoms into three major groups: the centrics, multipolar centrics and pennates (see Figure 1D). Centric diatoms tend to be circular in outline and have an identifiable central area from which pores radiate out towards the mantle edge. In multipolar centrics the valve outline can be elongated, triangular, rectangular, or even star-shaped and pores are often clustered at the edges of the valve face. The third major group is the pennates, which are elongated and bilaterally symmetric in outline and pore arrangement. Often they have a primary rib along the length of valve face from which many perpendicular mid-ribs extend. The pennates are further divided into two groups based on the presence (Raphids) or absence (Araphids) of a slit, termed a raphe. For more details about diatom taxonomy and terms for the various types of frustule structures see Ross et al. (1979), Round et al. (1990), Medlin and Kaczmarska (2004) and Kooistra et al. (2007).

Very little is known about the evolutionary origins of silicification and the diatom frustule (for recent reviews see Harwood and Nikolaev (1995), Medlin (2002), Sims et al. (2006), and Kooistra et al. (2007)). Silicification is found in most but not all the of the eukaryotic lineages: in the some of the opisthokonts (arthropods, brachiopods, demosponges and glass sponges), green algae and many of the plant groups, cercozoans (radiolaria, filose amoebans), alveolates (a few dinoflagellate cyst forming species), and in many of the heterokonts including the xanthophytes, chrysophytes, haptophytes and diatoms (Knoll 2003). Molecular comparisons of known silicic acid transporter systems in rice, a demosponge. and diatoms, suggest no sequence similarities among these groups, and therefore indicate there are multiple evolutionary histories of silicification within the eukaryotes (Schroder et al. 2004; Ma et al. 2006; Thamatrakoln et al. 2006; Ma et al. 2007: Raven and Giordano 2009). The silicon transporters in rice are related to water channel proteins (aquaporins) and have sequence similarity with a variety of plant species (Ma et al. 2006, 2007). The putative silicon transporter in the demosponge is an unrelated NBC Na^+/HCO_3^- transporter (Schroder et al. 2004). Diatom silicic acid transporters (SITs) co-transport silicic acid with sodium in marine species and may use both sodium and potassium in freshwater species. Little is known about their structure due to low sequence similarity with previously characterized transporters (Thamatrakoln et al. 2006).

Within the diatoms there is surprisingly little sequence similarity between the species examined or even different SITs within species, although there is some commonality of sequence within the centrics and pennates (Thamatrakoln et al. 2006). *Phaeodactylum tricornutum,* an unusual diatom that produces a siliceous bands embedded in its cell wall, or a single siliceous valve depending on its morphotype, and exhibits little or no requirement for Si for growth (Lewin et al. 1958; Borowitzka and Volcani 1978: Nelson et al. 1984), has SITs that are not extraordinarily different from other diatoms characterized (Thamatrakoln and Hildebrand 2005; Thamatrakoln et al. 2006). *Phaeodactylum tricornutum* and the prasinophyte *Platymonas sp.,* which also does not require Si for growth, have low-affinity silicic acid transport systems with very inefficient half saturation constants in the range of 80–100 μM Si (Nelson et al. 1984; Brzezinski et al. 1990).

Raven and Giordano (2009) suggest that biomineralization may have evolved only once in the Bacillariophyceae, Chrysophytes and Synurophytes, but there is currently not enough evidence to determine if the silicifying members of the wider Ochrista (that also includes the Parmophyceae, Silicoflagellata, and Prymnesiophyceae) and other groups such as the Prasinophyceae share common mechanisms for silicic acid uptake. Recent evidence that diatoms have a large number of prasinophyte-like derived genes in their nuclear genome constituting ~16% of nuclear coding potential (Moustafa et al. 2009) suggest this may be worth examining (Raven and Giordano 2009).

Almost all diatoms require silica for growth; most species will stop growing at silicic acid (the dissolved form of silica, dominantly $Si(OH)_4$) concentrations below about 2 μM (Figure 2), a level approached over large areas of the ocean surface (Egge and Aksnes 1992; Sarmiento and Gruber 2006). Silicic acid concentrations in the oceans have declined throughout the same episode in Earth history that saw the diatoms' rise to prominence (Harper and Knoll 1975; Maliva et al. 1989; Siever 1991). Silicification likely became more difficult as the diatoms became increasingly successful (Harper and Knoll 1975). The persistence and success of the diatoms implies that their siliceous frustule must confer some benefit (Knoll 2003). In this review we explore the selection pressures hypothesized to contribute to the success of the diatoms, the diversity of diatom frustule morphostructure, and changes in silicification strategies over the Mesozoic and Cenozoic. We also consider the potential consequences of future climate change on the morphology of the diatom frustule and the ecological success of the diatoms.

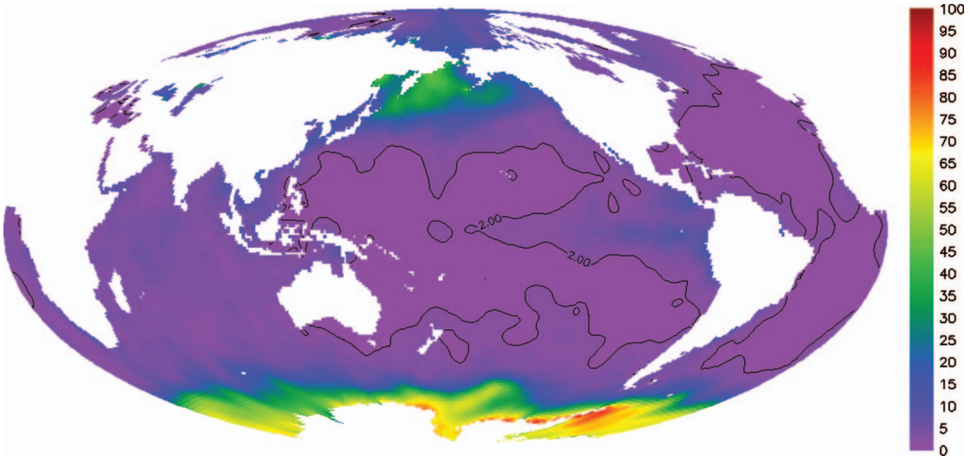

FIG. 2. Global ocean average surface (integrated 0–50 m) silicate concentrations (μM) from the World Ocean Atlas (Garcia et al. 2006). The 2 μM contour is marked by a black line. (Figure is provided in color online.)

COSTS AND BENEFITS ASSOCIATED WITH THE DIATOM FRUSTULE

Most diatom species have evolved an obligate requirement for silica for growth and reproduction and therefore their evolutionary success depends on the Si cycle and their ability to acquire silicic acid from the environment. In most diatoms silicic acid deficiency will stop the cell division cycle at two key transition points (the first and second gap phases), either preventing DNA synthesis or cell division (Brzezinski et al. 1990; Brzezinski 1992; Brzezinski and Conley 1994). Growth and silicic acid uptake kinetics have been shown to follow a Michaelis-Menten-type saturation below ~60 μM (Paasche 1973a; Martin-Jezequel et al. 2000; Leynaert et al. 2009). As a result when silicic acid concentrations are low ($<$1–2 μM) diatom growth is limited and other phytoplankton groups may proliferate (Egge and Aksnes 1992; Yool and Tyrrell 2003). Over more moderate ranges of silicic acid concentration where other nutrients or factors (such as irradiance) limit growth, silicic acid uptake can become decoupled from growth increasing the stoichiometry of Si to C and limiting elements such as N and P in the diatom cell (Paasche 1973b; Martin-Jezequel et al. 2000). Diatoms respond to the ambient range of silicic acid concentrations through the induction of a variety of silicic acid transporters (Thamatrakoln and Hildebrand 2007; Mock et al. 2008; Thamatrakoln and Hildebrand 2008; Leynaert et al. 2009). The silica frustule acts as a fundamental constraint on Si requirements and physical cell division. The rigidity of the silica frustule restricts division of daughter cells within the parental valve, resulting in a several fold decrease in the size of daughter cells with consecutive rounds of asexual reproduction, which is only restored after sexual reproduction (Round et al. 1990). As a result if sexual reproduction is not triggered within a specific size range the diatom may permanently lose its ability to divide (Mann et al. 2003).

One of the strongest, although indirect, sources of evidence indicating that the frustule provides a net fitness advantage to the diatoms is its cost. The average concentration of silicic acid in the modern ocean is more than an order of magnitude below the solubility of amorphous silica, and over most of the surface ocean the difference is around two orders of magnitude (Sarmiento and Gruber 2006). As a consequence diatoms must use an active uptake system to acquire and accumulate silica into low-pH silica deposition vesicles. Phosphoprotiens termed silaffins, long-chain polyamines, and cytoskeleton elements then deposit the amorphous $SiO_2 \cdot nH_2O$ spheres and small amounts of organic material into the highly organized silica frustule. More is known about the detailed sub-cellular and biochemical processes involved in frustule formation than can be summarized here, but see Hildebrand and Wetherbee (2003; Perry (2003), and Mock et al. (2008) and references therein for more detail. It is sufficient for the question at hand to point out that the process is highly complex and involves many different specialized proteins that require both capital and running costs to produce the silica frustule.

Raven (1983) suggested that the energetic cost of silica precipitation is low, requiring at most the expenditure of one and likely 0.5 mol ATP per mole of silica, so that for a diatom composed of 25% silica by dry weight, silicification consumes ~2% of the cell's energy budget. By comparison, the energetic costs of forming polysaccharides or lignin, on a volume basis are 10- to 20-fold the cost of a wall of silica (Raven 1983). However, this quantification of cost accounts only for the ATP cost of silicic acid transport across the plasma membrane, and considers the rest of the biomineralization process to carry no energetic cost. From the advances in our understanding of silica biomineralization, the concentration of silica in vesicles, the cytoskeletal arrangement of these vesicles into precise locations will all require energy expenditure (Perry 2003). A recent study found over 8000 genes associated with silica metabolism in the marine diatom *Thalassiosira pseudonana* (Mock et al. 2008). Perhaps most importantly, measuring costs purely in terms of energy ignores the costs of materials. Clearly, the assembly of the many transporters, silaffms, long-chain polyamines, low-pH vesicles, and so forth, require material investments that will consume resources at the expense of reproductive fitness.

For a structure with such cost to be maintained through natural selection suggests it must also carry some fitness benefit. A number of functional hypotheses have been proposed for the frustule and a variety of its morphological features. These hypotheses can be broken down into three major roles: I) the acquisition of growth-limiting resources (light, nutrients, CO_2); II) a direct impediment to grazing, parasitoid or viral attack, and III) changing sinking rate and turbulent flow around the cell which can moderate the availability or resources and potential interaction with predators, parasitoids and viruses (benefits I and II).

Light

The frustule, like a glass window, may influence the light field, either as shading to screen out UV-B or as a waveguide to focus photons. Scattering and absorption studies on different frustule morphologies are scarce but a theoretical model suggests that cells with a frustule will have greater backscattering and attenuation than naked cells (Kitchen and Zaneveld 1992). There is some evidence that the silica frustule may have very slightly greater absorption in the UV range, however species-specific differences in the UV-absorption characteristics of the frustule could not be correlated to differences in survival in response to UV-B exposure (Davidson et al. 1994). Alternatively there is evidence that the intricate patterning of the frustule can act to focus incident light into the cell inside the frustule. De Stefano et al. (2007a, 2007b) suggest that the focusing of light results from the coherent superposition of light due to scattering by the surface areolae, while the smaller homogenously distributed pores in the velum likely act to broaden the path of the focused light. If the specific microstructure of the cell wall (the number of silica layers, the orientation, size, and shape of the openings in the walls, etc.) act to control light incident on the diatom cell (Fuhrmann et al. 2004; De Stefano et al. 2007a, 2007b), then changes in light absorption and changes in growth might act to shape wall structure through natural selection. The size and shape of phytoplankton are also known to influence light absorption and scattering. Smaller phytoplankton cells are more efficient at absorbing light than larger cells with the same concentration and type of photosynthetic pigment and long and thin cells are much more efficient light absorbers than rounded forms of the same volume (Kirk 1983; Finkel 2001). Much more work is required to understand the relationship between the optical properties of the diatom frustule and changes in silicification and morphology.

Carbon Assimilation

Over normal ranges of oceanic pH the concentration of $CO_{2(aq)}$ in the ocean surface is often below half saturation for RUBISCO, the enzyme that fixes inorganic carbon in photosynthetic organisms (Giordano et al. 2005). It has been hypothesized that diatom biosilica might have the capacity to act as buffer for high catalytic rate external carbonic anhydrase activity, a component of the carbon concentrating mechanisms in (some) diatoms (Milligan and Morel 2002). Carbonic anhydrase catalyses the inter-conversion of bicarbonate (HCO_3^-) to $CO_{2(aq)}$. In response to low concentrations of dissolved $CO_{2(aq)}$ some diatoms catalyze bicarbonate protonation to increase concentrations of dissolved $CO_2(_{aq})$ in the immediate area surrounding the cell (Giordano et al. 2005).

Milligan and Morel (2002) demonstrated that diatom biosilica appears to act as a pH buffer, increasing the reaction rate of external carbonic anhydrase. There are two potential caveats: (1) these experiments used acid-cleaned diatom frustules while living diatom frustules are covered by an organic membrane with a presumably lower permeability to protons, and (2) not all diatoms use extracellular carbonic anhydrase, although Milligan and Morel (2002) hypothesize that the buffering capacity of the frustule may catalyse a diversity of other chemical reactions.

Nutrient Diffusion and Uptake

How does the presence of the diatom frustule, its size, shape, and micro-morphological features affect nutrient diffusion and nutrient uptake capacity? The siliceous cell wall greatly reduces the effective cell surface area across which nutrient diffusion or active uptake can take place relative to a naked cell. Despite this handicap, the diatoms have among the largest growth rates (when normalized for cell size) of all the phytoplankton groups (Furnas 1990; Tang 1995; Irwin et al. 2006; Raven et al. 2006), indicating that the surface area not covered by silica provides more than enough surface area for the number of transporters required to maintain high uptake rates (Berg and Purcell 1977). The increase in cellular radius due to the often <1 to ~ 3 μm layer of silica associated with the frustule will act to decrease the diffusive flux per unit of cell carbon or cell volume. The thickness of the diffusive boundary layer, which is approximately proportional to the cell's equivalent spherical radius, may further reduce silicic acid uptake and growth of larger diatoms under low silicic acid concentrations (Wischmeyer et al. 2003).

For spherical or nearly spherical cells, including cylinders with radii not too much larger than their longest linear dimension, larger cells will have lower diffusive flux. Shifts in frustule shape, such as an elongation of the pervalvar axis, can act to increase overall surface area and increase diffusive nutrient flux to the cell, reducing or even counteracting any reduction in competitive ability for nutrients (Grover 1989).

Cells in chains will generally have lower diffusive flux per surface area than a similarly sized solitary cell due to the restriction of water flow between closely spaced cells and their linking structures (Pahlow et al. 1997). Turbulent flow has the potential to increase nutrient transport to the cell surface for more elongated versus more spherical cells of the same size (Pahlow et al. 1997), although in general the size range of the microalgae is significantly smaller than the smaller turbulent eddies occurring the surface layer of the ocean (Wolf-Gladrow and Riebesell 1997; Wischmeyer et al. 2003). Chains with widely spaced cells are the most likely to get a net increase in nutrient flux under turbulent flow relative to solitary cells, especially under high nutrient conditions (Pahlow et al. 1997).

The increase in surface area due to small-scale features of the frustule (pores, ridges, small spines, etc.) has a negligible effect on nutrient flux (Pahlow et al. 1997). However, Hale and Mitchell (2001) suggest that micro- and nano-scale patterning on the valve face of diatom frustules can alter the diffusion and advection of materials around the frustule independent of surface area. Using high-speed microphotography of micron-sized beads interacting with diatom frustules, they observed that the microtopography of the frustule surface alters the diffusion of Brownian particles (Hale and Mitchell 2001a, 2001b). The micro-topography of the frustule surfaces examined tended to concentrate particles at the ridges of pores in the frustule, although the degree of concentration varies with species and particle size.

Hale and Mitchell (2001) suggest that surface features might enhance nutrient uptake by lengthening the residence time of colloidal particles in their diffusive or advective transect on the frustule surface. The micro-topography of diatom frustules may control size-selective sorting of particles at the frustule surface perhaps acting to concentrate specific nutrient particles and deflect other particles (such as viruses, parasitoids and bacteria) based on size. If this is the case, particle processing may be one of the primary functions of the silica frustule and may be a driving factor responsible for the arrangement of pore fields and other wall features associated with the diversity of diatom frustules. Although this functional hypothesis can explain the morphology of the frustule at the exterior valve face surface, it leaves the many other components of frustule morphology unexplained: if the frustule functions primarily to facilitate nutrient diffusion, what is the purpose of the finely perforate plate (velum) present in many taxa on the protoplast side of the cell wall? A further problem is that while diffusive and advective particle paths are shown to be reduced relative to a glass surface, it is not demonstrated that nutrient uptake is enhanced relative to a naked cell.

Sinking

The siliceous frustule increases the sinking rate of planktonic diatoms because silica has a greater density than water (quartz has a density of 2.6 g cm^{-3}, amorphous silicas are generally below 2.2 g cm^{-3}, depending on the degree of hydration). Sinking is a significant loss process that can shape community composition and viability of planktonic species (Smol et al. 1984; Smetacek 1985). Diatoms are able to reduce their cellular density and prevent sinking by reducing cellular concentrations of heavy ions and increasing their accumulation of lipids or quaternary ammonium derivatives (Boyd and Gradmann 2002; Raven and Waite 2004). The shape of the frustule, the presence of spines or other protuberances as well as chain and colony formation can moderate sinking rate. Form resistance, the ratio of sinking velocity of a particle of interest relative to a sphere of the same density and volume, is high for cells with larger length-to-diameter ratios and for long chains (Pasisak et al. 2003). Small projections or topography on cell surfaces are not expected to alter form resistance, but spines are an effective mechanism to increase form resistance; for example the spines of *Thalassiosira fluviatilis* increase form resistance by two-fold (Walsby and Xypolyta 1977; Pasisak et al. 2003).

Although sinking out of the euphotic zone will reduce growth rate and perhaps lead to death, there are potential benefits including shifts in the light field, nutrient and carbon availability, predation and viral pressure. Grazing is often most intensive in surface mixed layers and at the end of a diatom bloom (Vogel 1994). It may be advantageous for the diatoms to sink rapidly out of the mixed layer, perhaps to isopycnal surfaces, for sexual reproduction and resting stage formation. The ability to sink out of the photic zone may be a survival strategy in the transition from the vegetative to the resting stage of the life cycle (Smetacek 1985).

Many diatom species persist for significant portions of the year at relatively low populations, and reproduce rapidly in blooms only under very specific conditions. Raven and Waite (2004) suggest that sinking may be an effective strategy to remove diatoms infected by viruses or parasitoids, protecting the rest of the closely related population. Sinking can alter fluid flow and the availability of nutrients (Pahlow et al. 1997). As nutrients such as N, P, or Fe become depleted, cells lose their ability to regulate buoyancy, and tend to form cells with higher Si to C ratios, especially under iron deficiency (Boyle 1998; Takeda 1998; Martin-Jezequel et al. 2000). These nutrient-stressed, heavily silicified cells will have higher sinking rates, taking them towards the nutrient-enriched dark waters of the deep ocean (Raven and Waite 2004).

Defense

Most of the functional hypotheses for the frustule presented above emphasize the importance of resource acquisition for growth rate, competition, and fitness. Predation, parasitoid and viral attack can also control net growth rate and community composition (Smetacek 2001). From this top-down point of view, the evolutionary history of the marine plankton can be interpreted as a series of innovations for avoiding being attacked by predators or viruses: tough organic cell walls, mineral cell coverings, extreme cell size, chain formation, coloniality, and the production of noxious chemicals (see Hamm and Smetacek (2007) for more information). A number of lines of evidence strongly indicate that the diatom frustule acts as anti-predatory armor. The material properties and architecture of the diatom frustule lend it remarkable compressive strength, making it able to withstand the considerable forces exerted by predators that feed using a crushing mechanism (Hamm et al. 2003)

These prominently include copepods, the most abundant zooplankton in the ocean (Smetacek et al. 2004), which possess mandibles lined with siliceous teeth suggested to have coevolved with diatoms (Sullivan et al. 1975) and euphausiid crustaceans (krill), whose gastric mill bears similar teeth, though it is not known whether these are silicified (Hamm and Smetacek 2007). In spite of these features of the predators, a portion of the diatoms ingested by both copepods and euphausiids remain uncrushed and can survive passage through the predator's gut unscathed (Fowler and Fisher 1983). Copepods and euphausiids are the major predators on diatoms but salps, ciliates, dinoflagellates, and other protists also feed on diatoms (Smetacek 1999).

To these organisms, crushing resistance is of little consequence; pallium feeders such as dinoflagellates can digest the cell contents regardless of the frustule (Raven and Waite 2004), although the frustule may increase handling time (Smetacek 1999). There is also some evidence that benthic foraminifera extracellularly crack diatom frustules and extract their intracellular contents (Austin et al. 2005). Perhaps the strongest evidence in favor of a protective function for the diatom frustule is the observation that the degree of its silicification is inducible, much like chemical and even siliceous defenses in grasses (McNaughton and Tarrants 1983). Diatoms grown in artificial seawater media in which copepods were grazing on diatoms have a significantly higher Si:C ratio than those grown in the control media without copepods, suggesting that silicification is a phenotypically plastic, inducible defense against herbivory (Pondaven et al. 2007).

The implications of frustule morphology for mechanical strength from an engineering perspective have not been explored extensively. However, it is likely that both the overall diatom bauplan and the detailed structure of pores, ribs, wall chambers, and nodules affect the performance of the frustule under compression (as might be experienced during crushing by copepods). A finite element model of the pennate diatom *Fragilariopsis kerguelensis,* for example, suggested that the transverse ribs in this species deflected stress when loaded; when these ribs were removed from the model and the corresponding amount of silica redistributed among the rest of the frustule, significantly less force (∼60%) was required to bring this structure to failure (Hamm et al. 2003). Because the energy required to create a fracture increases with the fracture surface area, features of frustule architecture affecting wall cross-sectional area (such as wall height or pore size) should affect the frustule's strength, and this should be true at all scales.

Observation of a fractured frustule at the nanoscale, for example, revealed that the crack traveled around, rather than through, the ∼40 nm spherical silica particles (Crawford et al. 2001), substantially increasing the fracture surface area and thus the energy required to break the frustule (Hamm et al. 2003). While porous materials may have less cross-sectional surface area, porosity can also serve to increase resistance to breakage: the sharper a crack tip is relative to the length of the crack, the more force is concentrated at the tip; in other words, the longer a crack gets, the more stress is concentrated at its tip and thus the more easily it will propagate. In porous materials, propagating cracks are likely to terminate in a pore before they get too long, and when they do so the round edges of the pore will concentrate stress far less than the tip of a crack and thus inhibit the crack propagation (Vogel 2003).

In discussing the role of frustule silicification in protecting diatoms from grazing, it is worth considering briefly what is meant by mechanical strength from a fitness perspective. Rather than rigidity, which is a tempting view when imagining more heavily silicified frustules evolving in response to crushing predators, the proximally relevant factor is the force required to break the frustule—and this may be greater in a flexible, as opposed to a stiff, material. Indeed, the measured material properties of a girdle band suggest that diatom silica deforms elastically to very high degrees of strain (Hamm et al. 2003).

It has also been suggested that the frustule protects diatoms from attack by pathogens. While most diatom taxa have at least some pores large enough to permit entry by viruses and some bacteria, the surface area susceptible to attack is decreased and in many cases entry by eukaryotic parasites may be discouraged by the velum (Smetacek 1999). The observation that diatoms can still be infected by parasites in spite of the frustule has been raised in criticisms of the hypothesized protective function of the frustule (Raven and Waite 2004). However, it is important to keep in mind that a feature need not perform perfectly to be maintained in a population. If the mortality rate can be decreased by even a small factor, then as long as this benefit offsets the costs of producing the structure, it can be maintained.

MACROEVOLUTIONARY PATTERNS IN THE DIATOM CELL WALL

Patterns in the diatom frustule from the fossil record can be used to evaluate the proposed functional benefits associated with the frustule and its various morphological structures over geological time. If the frustule, or a particular structural feature associated with the frustule, provides a fitness advantage to the diatoms, then we should observe macroevolutionary changes in frustule morphology corresponding to shifts in resource availability, predation, and viral pressure, associated with changes in climate and ocean biology that change these selection pressures over geological time. The fossil record of the dominant extant diatom predators such as the copepods and euphausiids is extremely weak but if evolutionary patterns in these groups correspond to the overall escalation of marine predator-prey interactions recorded over the Mesozoic (Vermeij 1977) then there have been large evolutionary changes in the diversity and composition of diatom predators over the Mesozoic and Cenozoic (Van Valen 1973; Rigby and Milsom 2000). In addition to the likely changes in predation pressure there have been significant shifts in CO_2, ocean temperature, and circulation, altering resource availability and the light field in the surface ocean since the Mesozoic (Cerling 1991; Wilson and Norris 2001; Zachos et al. 2001).

There is significant evidence that there has been a secular decrease in the availability of silicic acid in the surface ocean over the course of the Phanerozoic due to an increasing biological uptake flux. Geological evidence for a Phanerozoic decline in surface water silicic acid concentrations includes mass balance calculations based on chert nodule formation from remobilized sponge spicules (Siever 1991), secular changes in the facies distribution of sedimentary cherts, the geological end-products of the marine silica cycle, and their correlation with the evolutionary rise of siliceous sponges, radiolarians, and diatoms (Maliva et al. 1989), changes in the rare earth element composition and Ge/Si ratio of volcanic jasper deposits (Grenne and Slack 2003), and the relatively high abundance of Eocene versus current deep-sea cherts (Muttoni and Kent 2007).

The disappearance of heavily silicified sponges from the euphotic zone after the Mesozoic and the decreasing silicification observed in Cenozoic radiolarian assemblages have been attributed to the rise of the diatoms and the consequently growing scarcity of silicic acid (Harper and Knoll 1975; Maldonado et al. 1999; Lazarus et al. 2009). Maintaining the high growth rates and the integrity of the silica frustule in response to decreasing silicic acid concentrations might have acted as a selective pressure towards morphologies that would increase nutrient diffusion (for silicic acid and as well as other nutrients), yet require less silica per cell or surface area. An analysis of frustule size in the dominant fossilized marine planktonic diatoms over the Cenozoic indicates that there has been a disproportionately large increase in the richness of smaller species, which are better equipped to deal with lower nutrient concentrations and less likely to become nutrient limited than larger cells (Finkel et al. 2005). In laboratory experiments diatom frustules become more heavily silicified with increasing silicic acid concentrations, and under the same silicic acid concentrations more ancient diatom morphotypes tend to be more heavily silicified than younger morphotypes (Finkel et al. 2010). Next, we attempt to determine if there is any evidence for macroevolutionary changes in silicification and morphology of the diatom frustule over geological time that are consistent with natural selection in response to decreases in silicic acid through time and contemporaneous changes in other environmental and biotic factors.

There is no quantitative analysis of changes in diatom wall structure through time, although anecdotal evidence for decreases in silicification and frustule robustness in the diatom frustule over the Mesozoic and Cenozoic has been noted (Bauldauf and Barren 1990; Round et al. 1990; Armstrong and Brasier 2005). Below we present a preliminary semi-quantitative survey of the major changes in the diatom frustule structure from three points over the Mesozoic and Cenozoic based on a scanning electron microscope survey of a few well-preserved diatom fossil assemblages from the Deep Sea Drilling Project and subsequent Ocean Drilling Project cores (Figures 3–7). We focus on changes in wall thickness, degree of silicification (μm silica: μm surface area of the wall), the % silica by volume, pore sizes, and the size of the small pores in the velum. These morphological features are likely to respond to changes in selection pressure due to changes in grazing, viral pressure and silicic acid concentrations. For an analysis of the better analyzed structures such as the labiate and strutted processes and raphe, see Gersonde and Harwood (1990), Round et al. (1990), and Medlin and Kaczmarska (2004).

Some of the earliest best-preserved, well-documented diverse Mesozoic diatom assemblages come from the Early Cretaceous (Aptian/Albian transition) from a Weddell Sea, Antarctica site (Gersonde and Harwood 1990; Harwood and Gersonde 1990; Harwood and Nikolaev 1995). There is no evidence of pennate diatoms from this time; most of the species in the assemblage have a simple centric morphology and only 2 of the 22 new species identified show evidence of bipolar symmetry (Gersonde and Harwood 1990). The majority of the species present are circular in valve view and elongated along the pervalvar axis (Figure 3). A remarkably similar two-layered siliceous frustule wall (termed "pseudoloculate", described next) is associated with the mantle of the majority of species from the Early Cretaceous. In contrast the wall structure of the valve face is much more variable across species; some are characterized by the

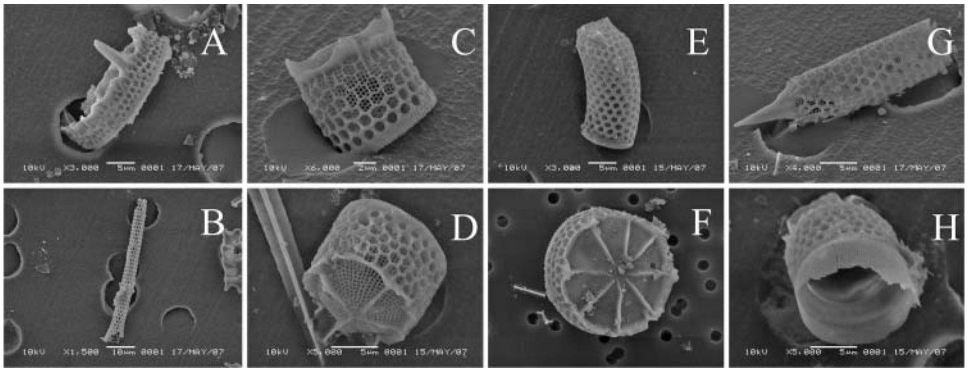

FIG. 3. Typical Early Cretaceous diatom frustules from ODP 113-693A-44R-1, 14-18 cm. Panels A-G illustrates typical features of the frustule wall as discussed in the text. Scanning electron micrographs taken by a JEOL JSM-5600 at Mount Allison's Digital Microscopy Facility. Some images are taken at a 45° tilt, scale bars as shown.

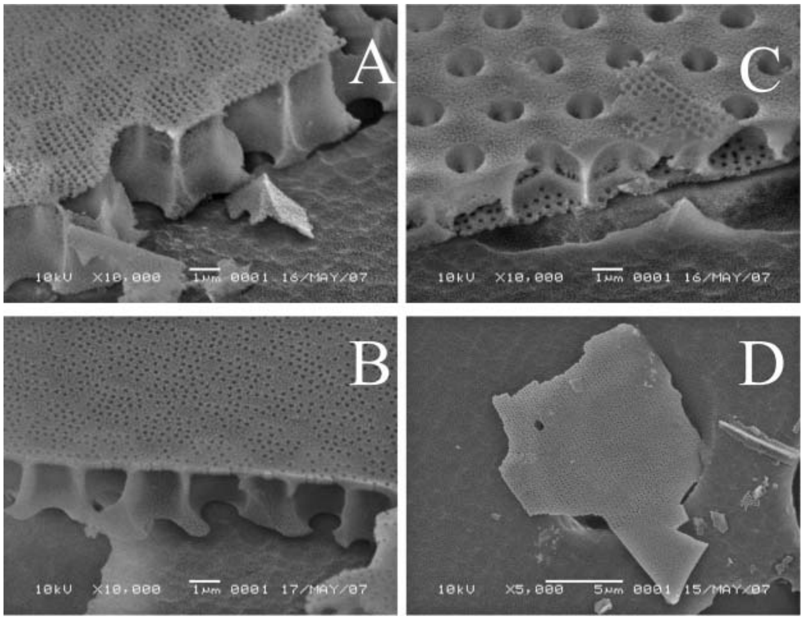

FIG. 4. Cross-sections of Early Cretaceous diatom walls of various thicknesses (A-D) from ODP 113-693 A-44R-1, 14–18 cm. Scanning electron micrographs taken by a JEOL JSM-5600 at Mount Allison's Digital Microscopy Facility. Some images are taken at a 45° tilt, scale bars as shown.

same pseudoloculate wall structure as on the mantle, while others are covered with a uniform basal layer, or have a variety of thickenings or linking structures (Figure 3 D–H). The basal layer, which would have been closest to the plasmalemma, is a uniform sheet of silica a fraction of a micron thick with radially arranged punctuate striae (small pores) ∼0.15–0.2 μm in diameter (Figure 3, 4A–C). This internal layer is overlaid by a 6-sided honeycomb structure often 1–3 microns thick in height but only ∼0.1–0.2 μm in width (Figure 4A–C). The honeycomb walls are often thickened at the top of the wall layer leaving a series of

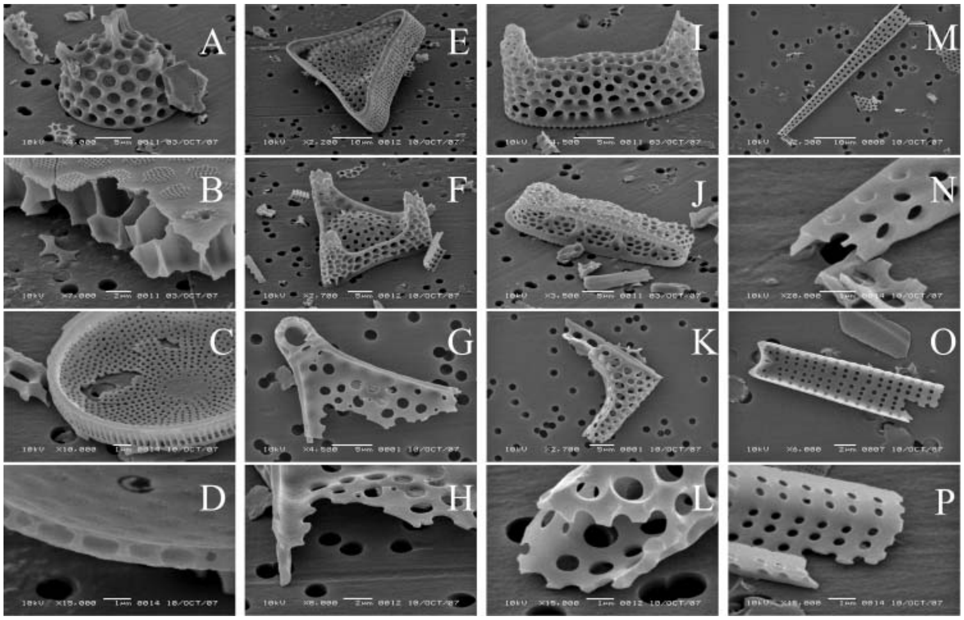

FIG. 5. Typical Late Cretaceous diatom frustules from DSDP 29-275-2R-5, 109-111 cm. (A–D) Examples of thick and thin-walled centric species. (E–H) Tripolar frustule walls, (G, H) Images of the same wall fragment at 2 angles. (I–L) Bi-polar centrics; K and L are images of the same wall fragment at 2 angles. (M–P) Bilateral pennates, and are images of the same wall fragment at 2 angles. Scanning electron micrographs taken by a JEOL JSM-5600 at Mount Allison's Digital Microscopy Facility. Some images are taken at a 45° tilt, scale bars as shown.

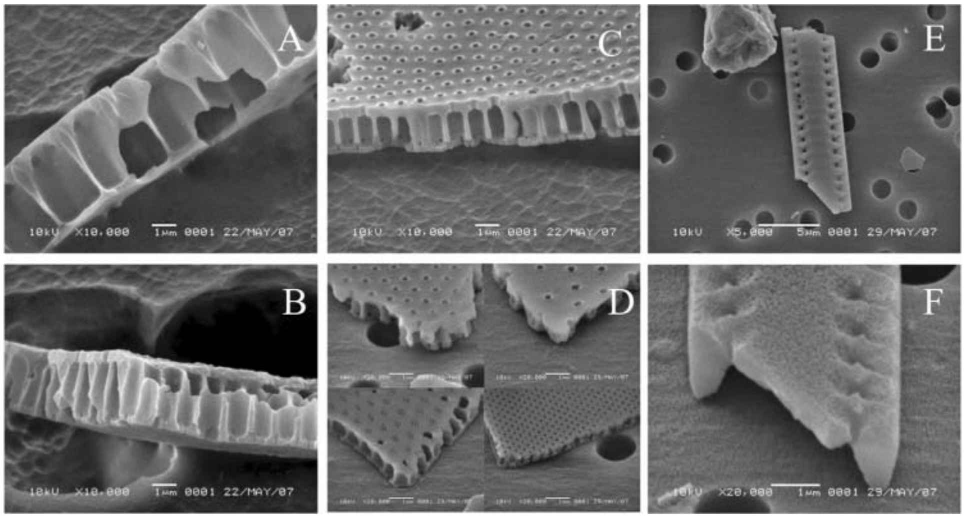

FIG. 6. Typical Neogene diatom frustules from DSDP 35-323-1R-4, 72-72 cm. (A–D) Typical double-layered walls of various thicknesses. (E–F) Pennate wall fragment at 2 angles. Scanning electron micrographs taken by a JEOL JSM-5600 at Mount Allison's Digital Microscopy Facility. Some images are taken at a 45° tilt, scale bars as shown.

closely spaced aerolae (pores) on the valve surface that are frequently ~1 μm in diameter (Figure 4A, C). As a result there is an empty chamber inside the siliceous, thin honeycomb walls with a diameter somewhat larger than the surface aerolae. A variety of other types of wall structures are present in the assemblage, for instance, the predominately solid sheets of silica associated with diatom resting stages and a variety of single-layered sheets of silica with fine pores. Some of these thin sheets are girdle bands, while others are the single-layered walls of diatom valves not frequently observed (Figure 4D). There are some much rarer (in diversity and abundance) weakly silicified species present in the assemblage, Gersonde and Harwood described 2 genera *(Trochus* and *Microorbis),* representing 3 species, with weakly silicified valves that appear to be made of a single thin layer of silica. Based on a few fragments we estimate that the wall height of these weakly silicified species can be <0.2–0.3 μm (Figure 4D).

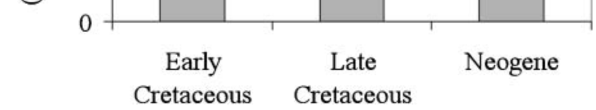

FIG. 7. Qualitative estimate of average (± s.e.) silicification (μm Silica:μm surface area) for Early Cretaceous (4 fragments: 4A–D), Late Cretaceous (4 fragments: 5B, D, L, P) and Neogene (5 fragments: C, D (top and bottom quadrant), F, and one thin pennate not shown) assemblages measured from selected frustule wall fragments depicted in Figures 3–6.

Modern species grown under low silicic acid concentrations tend to have lower Si:C and abnormal valve morphology (Paasche 1975; Booth and Harrison 1979). The early Cretaceous diatoms are noted for the fairly uniform, robust, and highly silicified appearance of their frustule wall. A highly silicified wall may have been a consequence of the extremely high concentrations of silicic acid in the Mesozoic ocean; silicic acid concentrations may have been over an order of magnitude larger than present (Siever 1991). Gersonde and Harwood (1990) hypothesize that the robust pseudoloculate wall structure and the elongated pervalvar axis may be a structurally strong design for strongly silicified chain formers where the heavily silicified linking spines and matching sockets are attached on the valve face in a central or sub-central position.

In support of this hypothesis they point out that most of the species with pseudoloculate-type frustule walls have highly silicified linking structures on the valve face while the single-layered lightly silicified species lack heavily silicified linking structures. They suggest the evolution of processes to link diatom cells may have driven the change in wall structure (or vice-versa), decreasing the degree of silicification. The Early Cretaceous diatoms assemblages appear to be restricted to shallow continental and interior seas. This observation in conjunction with high abundances of resting stages and chrysophytes as well as the robustness of the majority of the valves, has been used to infer that these early diatoms were not completely planktonic in habit (Gersonde and Harwood 1990; Harwood and Gersonde 1990; Kooistra et al. 2007). If this is the case one would expect that these older diatoms would have taller frustule walls in cross-section and more silica per unit of surface area than younger planktonic diatoms.

In the Later Cretaceous and Neogene there is an increase in the diversity of valve shapes and wall structures, with centric frustule valve morphologies ranging from those with elongated pervalvar axes (similar to the Early Cretaceous forms) to more button-shaped and flattened valve shapes (Figure 5A–D). The increased diversity of frustules with less elongated shapes, which are less efficient for light and nutrient acquisition, indicates that these are not the only factors driving the evolutionary changes in the morphology of the frustule. The centrics continue to exhibit a range of two-layered walls, many as thick as those in the Early Cretaceous but there are a large number of species with thinner walls and a range of species with single layered walls that are variable in height (Figure 5C, wall height <0.25 μm), perhaps indicating an evolution towards less silicified species. There is an increase in diversity of the multi-polar centrics, many triangular or ovoid in valve view (Figure 5E–L). Many of the multi-polar centric species, for example the *Triceratium*, *Trinacria*, and *Hemiaulus* spp., have thick robust linking spines and a single moderately thick single-wall of silica that tend to be >0.6 μm thick with pores of ∼0.7 to 2 μm in diameter (Figure 5I–L). Extraordinarily well preserved specimens have been found with a sieve plate (Figure 5E), indicating that the sieve plate in these species may be extremely sensitive to dissolution and breakage. *Hemiaulus* includes predominately highly silicified species in the Cretaceous and only lightly silicified species in the Cenozoic (Round et al. 1990), supporting the contention that there may be a macroevolutionary trend toward more lightly silicified species over the Mesozoic and Cenozoic.

By the later Cretaceous there are a few species of the bilateral pennate valves (Figure 5M–P), and their diversity and abundance increases especially through the Neogene. The dominant Late Cretaceous pennates have a single layered cell wall, commonly the thinnest walls observed (∼0.2 μm) with pores often ∼0.4 um in diameter (Figure 5N–P). Although the majority of the marine pennates observed have walls constructed with a single layer of silica, there are pennates with 2 layered walls (Thaler and Kaczmarska 2009). An increasing number of thinner walled centric and pennate species with small pores appear through the Neogene (Figure 6). Although many diatoms have thin perforate layers of silica across their larger areola (pores) only the pennates are known to have a rica, an extremely thin perforate layer of silica where the perforations are not more than 15 nm in diameter (Ross et al. 1979). The small pore size in pennates may indicate an evolutionary tendency towards smaller minimum pore sizes, perhaps an evolutionary response to keep out certain types of parasitoids and viruses.

We estimated the degree of silicification (μm silica: μm surface area and % silica by volume μm^3) of select wall of fragments depicted in Figures 3–6 using scanning electron microscopy with a rotating stage; we corrected our morphometric measurements for the 45° tilt angle in some of the images. Our semi-quantitative analysis (small sample size, not statistically significant) of the Early Cretaceous, Late Cretaceous and Neogene diatoms indicates that the diversity of both thin and thick walled, and single and doubled layered siliceous walls increases from the Cretaceous to modern time. There appears to be an increase in the relative abundance of thin single-layered walls and walls with small pores contributing to a slight decrease in the degree of silicification over time (Figure 7). In general, thinner walls are very dense, ∼99% silica by volume, but as the wall height increases the density of silica in the wall decreases to ∼15% of volume (Figure 8). These estimates of silicification and % silica by volume does not include the heavily silicified linking structures, thickenings of the wall at the mantle, etc., or velum in many of the multi-polar species, and therefore likely underestimates silica use especially the bi and tri-polar centries from the Late Cretaceous. The decrease in the minimum pore size may be an evolutionary response to selection pressures from grazers, parasitoids, and viruses. Thinner valve walls may reflect selection for more efficient use of silica in wall construction in response to ever decreasing silicic acid concentrations. Although a macroevolutionary change in silicification in the diatoms is consistent with evidence of decreasing silicic acid concentrations through time, all the potential macroevolutionary changes in the cell wall identified here need to be confirmed by a careful quantitative analysis of change in wall structure and silicification through time and must account for potential biases due to sampling, sample processing, and preservation.

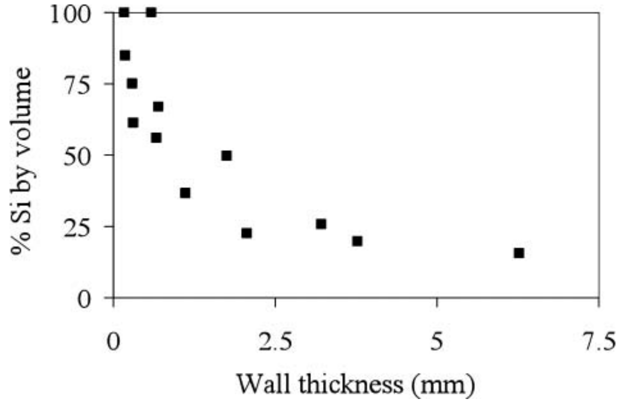

FIG. 8. Percent silica density of the frustule wall as a function of wall height (μm). Percent silica density was estimated for selected wall fragments depicted from Figures 3–6, and as listed in the caption for Figure 7.

FEEDBACKS BETWEEN DIATOM FRUSTULE MORPHOLOGY AND THE SILICA CYCLE

Geological and biological evidence suggests that silicic acid use by diatoms and to a lesser extent radiolarians, silicoflagellates and sponges has resulted in a general decrease in surface silicic acid concentrations, especially over the Mesozoic and Cenozoic. Siliceous sponges have been pushed into deep waters and radiolarians have decreased their silica use in response to this decrease in silicic acid concentration. Current average surface water silicate concentrations are ≤ 2 μM (Figure 2), consistent with the current minimum requirement for diatom growth, indicating that diatoms not only regulate the ocean silica cycle but that their success is self-limiting (Harriss 1966; Egge and Aksnes 1992; Yool and Tyrrell 2003). The past success of the diatoms has resulted in increasingly widespread low surface silicic acid concentrations, limiting their future competitive success (Egge and Aksnes 1992; Yool and Tyrrell 2003). The silicic acid and other nutrient concentrations required for diatom growth varies with cell size (Eppley et al. 1969; Hein et al. 1995; Sommer 1998) and shape (Marchetti and Harrsion 2007) as well as species-specific differences in the degree of silicification (Brzezinski 1985). This suggests that the diatoms can adapt or acclimate to the secular, widespread decreases in silicate concentrations, up to a point. Decreases in frustule size and increase in aspect ratio (long and thin) will both increase nutrient flux and decrease obligate silica requirements.

How will current changes in climate influence the diatoms, their frustule morphology and the silica cycle? There is some evidence that as large areas of the ocean are becoming more stratified there has been a decrease in chlorophyll standing stock and primary production (Behrenfeld et al. 2006; Irwin and Oliver 2009). It seems likely that such an increase in stratification might result in a decrease in silicate flux into the euphotic zone in these regions, reducing the availability of silicic acid. In the past, over millions of years, decreases in nutrient availability have resulted in an increasing number of small-sized (Finkel et al. 2005) and perhaps lightly silicified, thin-walled diatoms, but it is unclear whether modern diatoms will become continue to become smaller or less silicified in response to current warming.

A time-series analysis from the Bermuda Atlantic Time-series station in the Sargasso Sea found a ∼40% decline in particulate silica (decline in diatoms) associated with increasing water column stratification over the last 15 years (Krause et al. 2009). Further increases in warming and water column stratification may contribute to further decreases in silicic acid availability and more widespread decreases in diatom abundance over decades to centuries. From a geological standpoint, increases in CO_2 should increase temperature, which should increase rates of silicate weathering (Walker et al. 1981), increasing the supply of dissolved silica to the oceans. Large uncertainties remain in our understanding of how both the silica cycle and the diatoms and their frustule will respond to changes in climate.

Distinct morphological features of the diatom silica frustule confer a range of functional benefits in response to contrasting selection pressures. Some frustule ornamentation may be neutral, conferring no selective advantage. From an engineering perspective, the diversity of morphological traits could either reflect distinct functions or multiple, equally fit solutions that satisfy many functional needs (Niklas 1994). Often the optimal morphotype for one function is not the same as for another resulting in trade-offs (Marshall 2003), for example highly silicified frustules might be best suited for mechanical strength to reduce grazing pressure, while lightly silicified frustules might be best suited for maximizing growth and survival under low silicic acid concentrations. Specific combinations of functional morphological traits (cell size, shape, pore size and arrangement, wall height, silicification, spines, etc.) will be selected under different environmental regimes, resulting in a diverse continuum of evolutionarily stable frustule morphologies. We expect the relative benefits of different frustule morphologies will continue to change with abiotic and biotic conditions, especially silica availability and the predation-defense arms race.

REFERENCES

Armstrong H, Brasier MD. 2005. Microfossils. Maiden, MA: Wiley-Blackwell. 296 p.

Austin HA, Austin WEN, Paterson DM. 2005. Extracellular cracking and content removal of the benthic diatom *Pleurosigma angulatum* (Quekett) by the benthic foraminifera *Haynesina germanica* (Ehrenberg). Marine Micropalaeontol 57:68–73.

Bauldauf JG, Barren JA. 1990. Evolution of biosiliceous sedimentation patterns—Eocene through Quaternary: paleoceanographic response to polar cooling. In: Bliel U, Thiede J, editors. Geological history of the Polar Oceans: Arctic versus Antarctic. Netherlands: Kluwer Academic Publishers. P 575–607.

Behrenfeld MJ, O'Malley R, Siegel D, McClain C, Sarmiento J, Feldman G, Milligan A, Falkowski P, Letelier R, Boss E. 2006. Climate-driven trends in contemporary ocean productivity. Nature 444:752–755.

Berg HC, Purcell EM. 1977. Physics of chemoreception. Biophys J 20;193–219.

Bold HC, Wynne MJ. 1978. Introduction to the Algae—Structure and Reproduction. Englewood Cliffs: Prentice-Hall.

Booth B, Harrison PJ. 1979. Effect of silicate limitation on valve morphology in *Thalassisira* and *Coscinodiscus* (Bacillariophyceae). J Phycol 15:326–329.

Borowitzka MA, Volcani BE. 1978. The polymorphic diatom *Phaeodactylum tricornutum:* ultrastructure of its morphotypes. J Phycol 14:10–21.

Boyd CM, Gradmann D. 2002. Impact of osmolytes on buoyancy of marine plankton. Mar Biol 141:605–618.

Boyle E. 1998. Pumping iron makes thinner diatoms. Nature 393:733–734.

Brzezinski MA. 1985. The Si:C:N ratio of marine diatoms: Interspecific variability and the effect of some environmental variables. J Phycol 21:347–357.

Brzezinski MA. 1992. Cell-cycle effects on the kinetics of silicic acid uptake and resource competition among diatoms. J Plankton Res 14:1511–1539.

Brzezinski MA, Conley DJ. 1994. Silicon deposition during the cell cycle of Thalassiosira weissflogii (Bacillariophyceae) determined using dual rhodamine 123 and propidium iodide staining. J Phycol 30:45–55.

Brzezinski MA, Olson RJ, Chisholm SW. 1990. Silicon availability and cell-cycle progression in marine diatoms. Mar Ecol Prog Ser 67:83–96.

Cerling TE. 1991. Carbon dioxide in the atmosphere; evidence from Cenozoic and Mesozoic Paleosols. Am J Sci 291:377–400.

Crawford SA, Higgins MJ, Mulvaney P, Wetherbee R. 2001. Nanostructure of the diatom frustule as revealed by atomic force and scanning electron microscopy. J Phycol 37:543–554.

Davidson AT, Bramich D, Marchant HJ, McMinn A. 1994. Effects of UV-B irradiation on growth and survival of Antarctic marine diatoms. Mar Biol 119:507–515.

De Stefano L, De Stefano M, Maddalena P, Moretti L, Rea I, Mocella V, Rendina I. 2007a. Playing with light in diatoms: small water organisms with a natural photonic crystal structure. Proc SPIE, Inter Soc Optical Eng 6593:1–9.

De Stefano L, Rea I, Rendina I, De Stefano M, Moretti L. 2007b. Lensless light focusing with the centric marine diatom *Coscinodiscus walesii*. Optics Exp 15:1–7.

Dugdale RC, Wilkerson FP. 1998. Silicate regulation of new production in the equatorial Pacific upwelling. Nature 394:270–273.

Dugdale RC, Wilkerson FP, Minas HJ. 1995. The role of a silicate pump in driving new production. Deep Sea Res I: Oceanogr Res Pap 42:697–719.

Egge JK, Aksnes DL. 1992. Silicate as regulating nutrient in phytoplankton competition. Mar Ecol Prog Ser 83:281–289.

Eppley RW, Rogers JN, McCarthy JJ. 1969. Half-saturation constants for uptake of nitrate and ammonium by marine phytoplankton. Limnol Oceanogr 14:912–920.

Finkel ZV. 2001. Light absorption and size scaling of light-limited metabolism in marine diatoms. Limnol Oceanogr 46:86–94.

Finkel ZV, Katz M, Wright J, Schofieid O, Falkowski PG. 2005. Climatically driven macroevolutionary patterns in the size of marine diatoms over the Cenozoic. Proc Natl Acad Sci USA 102:8927–8932.

Finkel ZV, Matheson KA, Regan KS, Irwin AJ. 2010. Genotypic and phenotypic variation in diatom silicification under paleooceanographic conditions. Geobiology. 1–10. 10.1111/j.1472-4669.2010.00250.x

Fowler SW, Fisher NS. 1983. Viability of marine phytoplankton in zooplankton fecal pellets. Deep-Sea Res 130:963–969.

Fuhrmann T, Landwehr S, El Rharbi-Kucki M, Sumper M. 2004. Diatoms as living photonic crystals. Appl Phys B 78:257–260.

Furnas MJ. 1990. In situ growth rates of marine phytoplankton: approaches to measurement, community and species growth rates. J Plankton Res 12: 1117–1151.

Garcia HE, Locarnini RA, Boyer TP, Antonov JI. 2006. Nutrients (phosphate, nitrate, silicate). In: Levitus S, editor. World Ocean Atlas 2005 Washington, DC U.S. Government Printing Office, P 396.

Gersonde R, Harwood DM, editors. 1990. Lower Cretaceous diatoms from ODP Leg 113 Site 693 (Weddell Sea). Part I: vegetative cells. College Station, TX: Ocean Drilling Program. P 365–402.

Giordano M, Beardall J, Raven JA. 2005. CO_2 concentrating mechanisms in algae: mechanisms, environmental modulation, and evolution. Ann Rev Plant Biol 56:99–131.

Grenne T, Slack J. 2003. Paleozoic and Mesozoic silica-rich seawater: evidence from hematitic chert (jasper) deposits. Geology 31:319–322.

Grover JP. 1989. Influence of cell shape and size on algal competitive ability. J Phycol 25:402–405.

Hale M, Mitchell J. 2001a. Functional morphology of diatom frustule microstructures: hydrodynamic control of Brownian particle diffusion and advection. Aqua Microb Ecol 24:287–295.

Hale M, Mitchell J. 2001b. Motion of submicrometer particles dominated by Brownian motion near cell and micrfabricated surfaces. Nano Lett 1:617–623.

Hamm CE, Merkel R, Springer O, Jurkojc P, Maier C, Prechtel K, Smetacek V. 2003. Architecture and material properties of diatom shells provide effective mechanical protection. Nature 421:841–843.

Hamm CE, Smetacek V. 2007. Armour: why, when and how. In: Falkowski PG, Knoll AH, editors. Evolution of aquatic photoautotrophs. San Diego: Academic Press.

Harper HE, Jr., Knoll AH. 1975. Silica, diatoms, and Cenozoic radiolarian evolution. Geology 3:l75–177.

Harriss RC. 1966. Biological buffering of the oceanic silica. Nature 212: 275–276.

Harwood DM, Chang KH, Nikolaev VA. 2004. Late Jurassic to earliest Cretaceous diatoms from Jasong Synthem, Southern Korea: evidence for a terrestrial origin (Abstract). In: Witkowski A, Radziejewska T, Wawrzyniak-Wydrowska B, Daniszewska-Kowalczyk G, Bak M, editors; Miedzyzdroje, Poland. 18th International Diatom Symposium.

Harwood DM, Gersonde R, editors. 1990. Lower Cretaceous diatoms from ODP Leg 113 Site 693 (Weddell Sea). Part 2: Resting spores, chrysophycean cysts, an endoskeletal dinoflagellate, and notes on the origins of the diatoms. College Station, TX: Ocean Drilling Program. P 403–425.

Harwood DM, Nikolaev VA. 1995. Cretaceous diatoms: morphology, taxonomy, biostratigraphy. In: Blome CD, Whalen PM, Katherine R, editors. Siliceous microfossils: Paleontological Society, Knoxville, Tennessee, USA. P 81–106.

Hasle GR, Syvertsen EE. 1997. Marine diatoms. In: Tomas CR, editor. Identifying marine phytoplankton. San Diego: Academic Press. P 5–386.

Hein M, Folager Pedersen M, Sand-Jensen K. 1995. Size-dependent nitrogen uptake in micro- and macroalgae. Mar Ecol Prog Ser 118:247–253.

Hildebrand M, Wetherbee R. 2003. Components and control of silicification in diatoms. Prog Mol Subcell Biol 33:1l–57.

Irwin AJ, Finkel ZV, Schofield OME, Falkowski PG. 2006. Scaling-up from nutrient physiology to the size-structure of phytoplankton communities. J Plankton Res 28:459–471.

Irwin AJ, Oliver MJ. 2009. Are ocean deserts getting larger? Geophys Res Lett 36:10.1029/2009GL039883.

Keeling PJ, Burger G, Durnford DG, Lang BF, Lee RW, Pearlman RE, Roger AJ, Gray MW. 2005. The tree of eukaryotes. Trends Ecol Evol 20:670–576.

Kirk JTO. 1983. Light and Photosynthesis in Aquatic Ecosystems. Cambridge: Cambridge University Press. 401 p.

Kitchen JC, Zaneveld RV. 1992. A three-layer sphere model of the optical properties of phytoplankton. Limnol Oceanograph 37:1680–690.

Knoll AH. 2003. Biomineralizatin and evolutionary history. Revie in Mineral Geochem 54:329–356.

Kooistra WHCF, Gersonde R, Medlin LK, Mann DG. 2007. The origin and evolution of the diatoms: their adaptation to a planktonic existence. In: Falkowski PG, Knoll AH, editors. Evolution of aquatic photoautotrophs. San Diego: Academic Press, P 207–249.

Kooistra WHCF, Medlin LK. 1996. Evolution of the diatoms (Bacillariophyta). IV. A reconstruction of their age from small subunit rRNA coding regions and the fossil record. Mol Phylogen Evol 6:391–407.

Krause JW, Lomas M, Nelson DM. 2009. Biogenic silica at the Bermuda Atlantic Time-series study site in the Sargasso Sea: temporal changes and their inferred controls basedon a 15-year record. Global Biogeochem Cycles 23: doi:10.1029/2008GB003236, 2009.

Laws E. 2004. Export flux and stability as regulators of community composition in pelagic marine biological communities: Implications for regime shifts. Prog Oceanog 60:343–354.

Laws EA, Falkowski PG, Smith WOJ, Ducklow H, McCarthy JJ. 2000. Temperature effects on export production in the open ocean. Global Biogeochem Cycles 14:1231–1246.

Lazarus DB, Kotrc B, Wulf G, Schmidt DN. 2009. Radiolarians decreased siliciflcation as an evolutionary response to reduced Cenozoic ocean silica availability. Proc Natl Acad Sci USA 106:9333–9338.

Lewin JC, Lewin RA, Philpott DE. 1958. Observations on *Phaeodactylum tricornutum*. J Gen Microbiol 18:418–426.

Leynaert A, Longphuirt SN, Claquin P, Chauvaud L, Ragueneau O. 2009. No limit? The multiphasic uptake of silicic acid by benthic diatoms. Limnol Oceanog 54:571–576.

Ma JF, Tamai K, Yamaji N, Mitani N, Konishi S, Katsuhara M, Ishiguro M, Murata Y, Yano M. 2006. A silicon transporter in rice. Nature 440:688–691.

Ma JF, Yamaji N, Mitani N, Tamai K, Konishi S, Fujiwara T, Katsuhara M, Yano M. 2007. An efflux transporter of silicon in rice. Nature 448:209–213.

Maldonado M, Carmona MC, Uriz MJ, Cruzado A. 1999. Decline in Mesozoic reef-building sponges explained by silicon limitation. Nature 401:785–788.

Maliva RG, Knoll AH, Siever R. 1989. Secular change in chert distribution: a reflection of evolving biological participation in the silica Cycle. Palaios 4:519–532.

Mann DG, Chepurnov VA, Idei M. 2003. Mating system, sexual reproduction, and auxosporulation in the anomalous raphid diatom Eunotia (Bacillariophyta). J Phycol 39:1067–1084.

Marchetti A, Harrsion PJ. 2007. Coupled changes in cell morphology and the elemental (C,N, and Si) composition of the pennate diatom *Pseudo-nitzchia* due to iron deficiency. Limnol Oceanog 52:2270–2284.

Marshall CR. 2003. Nomothetism and understanding the Cambrian "Explosion". Palaios 18:1–2.

Martin-Jezequel V, Hildebrand M, Brzezinski MA. 2000. Silicon metabolism in diatoms: implications for growth. J Phycol 36:821–840.

McNaughton S, Tarrants J. 1983. Grass leaf silicification: natural selection for an inducible defense against herbivores. Proc Natl Acad Sci USA 80:790–791.

Medlin LK. 2002. Why silica or better yet why not silica? Speculation as to why the diatoms utilize silica as their cell wall material. Diatom Res 17:453–459.

Medlin LK, Kaczmarska I. 2004. Evolution of the diatoms: V. Morphological and cytological support for the major clades and a taxonomic revision. Phycologia 43:245–270.

Milligan AJ, Morel FMM. 2002. A proton buffering role for silica in diatoms. Science 297:1848–1850.

Mock T, Samanta MP, Iverson V, Berthiaume C, Robison M, Holtermann K, Durkin C, Splinter BonDurant S, Richmond K, Rodesch M and others. 2008. Whole-genome expression profiling of the marine diatom *Thalassiosira pseudonana* identifies genes involved in silicon bio-processes. Proc Natl Acad Sci USA 105:1579–1584.

Moustafa A, Beszteri B, Maier UG, Bowler C, Valentin K, Bhattacharya D. 2009. Genomic footprints of a cryptic plastid endosymbiosis in diatoms. Science 324:1724–1726.

Muttoni G, Kent DV. 2007. Widespread formation of cherts during the early Eocene climate optimum. Palaeo 253:348–362.

Nelson DM, Riedel GF, Millan-Nunez R, Lara-Lara JR. 1984. Silicon uptake by algae with no known Si requirement. I. True cellular uptake and pH-induced precipitation by *Phaeodactylum tricornutum* (Bacillariophyceae) and *Platymonas sp.* (Prasinophyceae). J Phycol 20:140–l47.

Niklas KJ. 1994. Morphological evolution through complex domains of fitness. Proc Natl Acad Sci USA 91:6772–6779.

Paasche E. 1973a. Silicon and the ecology of marine plankton diatoms. II. Silicate uptake kinetics in five diatoms species. Mar Biol 19:262–269.

Paasche E. 1973b. Silicon and the ecology of marine plankton diatoms. I. Thalassiosira pseudonana (*Cyclotella nana*) grown in a chemostat with silicate as limiting nutrient. Marine Biol 19:117–126.

Paasche E. 1975. Growth of the plankton diatom Thalassiosira nordenskioeldii Cleve at low silicate concentrations. J Exper Mar Biol Ecol 18:173–183.

Pahlow M, Riebesell U, Wolf-Gladrow DA. 1997. Impact of cell shape and chain formation on nutrient acquisition by marine diatoms. Limnol Oceanog 42: 1 660–672.

Pasisak J, Soroczki-Pinter E, Rezner Z. 2003. Sinking properties of some phytoplankton shapes and the relation of form resistance to morphological diversity of plankton an experimental study. Hydrobiologia 500:243–257.

Perry C. 2003. Silicification: the processes by which organisms capture and mineralize silica. Rev Mineral Geochem 54:291–327.

Pondaven P, Gallinari M, Chollet S, Bucciarelli E, Sarthou G, Schultes S, Jean F. 2007. Grazing-induced changes in cell wall silicification in a marine diatom. Protist 158:21–28.

Rabosky DL, Sorhannus U. 2009. Diversity dynamics of marine planktonic diatoms across the Cenozoic. Nature 457:183–186.

Raven JA. 1983. The transport and function of silicon in plants. Biol Rev 58:179–207.

Raven JA, Finkel ZV, Irwin AJ. 2006. Picophytoplankton: bottom-up and top-down controls on ecology and evolution. Vie et Milieu 55:209–215.

Raven JA, Giordano M. 2009. Biomineralization by photosynthetic organisms: evidence of coevolution of the organisms and their environment? Geobiology 7:140–154.

Raven JA, Waite AM. 2004. The evolution of silicification in diatoms: inescapable sinking and sinking as escape? New Phytol 162:45–61.

Rigby S, Milsom CV. 2000. Origins, evolution, and diversification of zooplankton. Annu Rev Ecol Sys 31:293–313.

Ross R, Cox EJ, Karayeva NI, Mann DG, Paddock TBB, Simonsen R, Sims PA. 1979. An amended terminology for the siliceous components of the diatom cell. Nova Hedwigia 64:513–533.

Round FE, Crawford RM, Mann DG. 1990. The diatoms: biology and morphology of the genera. Cambridge: Cambridge University Press.

Sarmiento JL, GruberN. 2006. Ocean biogeochemical dynamics. Princeton: Princeton University Press.

Schroder HC, Perovic-Ottstadt S, Rothenberger M, Wiens M, Schwertner H, Batel R, Korzhev M, Muller IM, Muller WEG. 2004. Silica transport in the demosponge *Suberites domuncula:* fluorescence emission analysis using the PDMPO probe and cloning of a potential transporter. Biochem J 381:665–673.

Siever R. 1993. Silica in the oceans: biological-geochemical interplay. In: Scientists on Gaia, Schneider, S.H. and Boston, P. (eds), MIT Press. 458 pages.

Sims PA, Mann DG, Medlin LK. 2006. Evolution of the diatoms: insights from fossil, biological and molecular data. Phycologia 45:361–402.

Smetacek V. 1985. Role of sinking in diatom life-history cycles: ecological, evolutionary and geological significance. Mar Biol 84:239–251.

Smetacek V. 1999. Diatoms and the ocean carbon cycle. Protist 150:25–32.

Smetacek V. 2001. A watery arms race. Nature 411:745.

Smetacek V, Assmy P, Henjes J. 2004. The role of grazing in structuring Southern Ocean pelagic ecosystems and biogeochemical cycles. Antarct Sci 16:541–558.

Smol JP, Brown SR, Mclntosh HJ. 1984. A hypothetical relationship between differential algal sedimentation and diatom succession. Verh lnternat Verein Limnol 22:1361–1365.

Sommer U. 1998. Silicate and the functional geometry of marine phytoplankton. J Plank Res 20:1853–1859.

Sorhannus U. 2007. A nuclear-encoded small-subunit ribosomal RNA timescale for diatom evolution. Mar Micropalaeontol 65:1–l2.

Sournia A, Chretiennot-Dinet M-J, Ricard M. 1991. Marine phytoplankton: how many species in the world ocean? J Plank Res 13:1093–1099.

Spencer-Cervato C. 1999. The Cenozoic Deep Sea mlcrofossil record: Explorations of the DSDP/ODP sample set using the Neptune database. Palaeontol Electron 2:1–268.

Sullivan BK, Miller CB, Peterson WT, Soeldner AH. 1975. A scanning electron microscope study of the mandibular morphology of boreal copepods. Mar Biol 30:175–182.

Takeda S. 1998. Influence of iron availability on nutrient consumption ratio of diatoms in oceanic waters. Nature 393:774–777.

Tang EPY. 1995. The allometry of algal growth rates. J Plank Res 17:1325–l335.

Thaler M, Kaczmarska I. 2009. *Gyrosigma orbitum* sp. nov. (Bacillariophyta) from a salt marsh in the Bay of Fundy, eastern Canada. Botan Mar 52:60–68.

Thamatrakoln K, Alverson AJ, Hildebrand M. 2006. Comparative sequence analysis of diatom silicon transporters: towards a mechanistic model of silicon transport. J Phycol 42:822–834.

Thamatrakoln K, Hildebrand M. 2005. Approaches for functional characterization of diatom silicic acid transporters. J Nanosc Nanotechnol 5:1–9.

Thamatrakoln K, Hildebrand M. 2007. Analysis of *Thalassiosira pseudonana* silicon transporters indicates distinct regulatory levels and transport activity throught the cell cycle. Eukaryot Cell 6:27l–279.

Thamatrakoln K, Hildebrand M. 2008. Silicon uptake in diatoms revisited: A model for saturable and nonsaturable uptake kinetics and the role of silicon transporters. Plant Physiology 146:1397–1407.

Van Valen L. 1973. A new evolutionary law. Evol Theory 1:1–30.

Vermeij GJ. 1977. The Mesozoic marine revolution; evidence from snails, predators and grazers. Paleobiology 3:245–258.

Vogel S. 1994. Life in moving fluids: the physical biology of flow. Princeton: Princeton. University Press.

Vogel S. 2003. Comparative biomechanics: life's physical world. Princeton: Princeton University Press. 580 p.

Walker JCG, Hays PB, Kasting JF. 1981. A negative feedback mechanism for the long-term stabilization of Earth's surface temperatures. J Geophys Res 86:9776–9782.

Walsby AE, Xypolyta A. 1977. The form resistance of chitan fibers attached to the cells of *Thalassiosira fluviatilis* Hustedt. Brit Phycol J 12:215–223.

Wilson PA, Norris RD. 2001. Warm tropical ocean surface and global anoxia during the mid- Cretaceous period. Nature 412:425–429.

Wischmeyer AG, Del Amo Y, Brzezinski M, Wolf-Gladrow DA. 2003. Theoretical constraints on the uptake of silicic acid species by marine diatoms. Mar Chem 82:13–29.

Wolf-Gladrow D, Riebesell U. 1997. Diffusion and reactions in the vicinity of plankton: a refined model for inorganic carbon transport. Marine Chemistry 59:17–34.

Yool A, Tyrrell T. 2003. Role of diatoms in regulating the ocean's silicon cycle. Global Biogeochem Cycles 7:1–21.

Zachos J, Pagani M, Sloan L, Thomas E, Billups K. 2001. Trends, rhythms, and aberrations in global climate 65 Ma to present. Science 292:686–693.