



21/07/16

Groningen, the Netherlands

Bachelor thesis

Marine biology

Silica use as a strategy in diatoms and its contribution to their ecological succes

To what extent has the use of silica in diatoms contributed to their ecological rise and spread?

Ruben Rosen Jacobson
S2323672

Supervisor: Jacqueline Stefels

Abstract

It is safe to say that diatoms are highly ecologically successful among all phytoplankton groups. They are found all over the planet's oceans in a wide variety of environments, from iron-limited polar oceans to tropical coasts, and are the most abundant and diversified phytoplankton group. Unique to nearly all diatoms is that they use the element silica to build glass cases called frustules. One could easily argue that it is because of this unique use of silica that diatoms have become so successful over a relatively short time. This thesis aims to determine to what extent the use of silica in diatoms has been a contributor to their ecological success. Regardless of the origin of silica use, it is evident that diatoms have adapted actively to be able to maintain and even optimize their highly sophisticated silica processing and frustule production mechanisms. There is much evidence for the many benefits provided by frustules in defense, resource acquisition, etc. that explains this positive selection. Relating diatom diversity and abundance since their origin to geological changes provides evidence that diatoms have benefited from increased bioavailable silica levels to radiate, and that they have optimized their silica uptake and use over time as availability grew smaller and smaller. On the other hand, it is evident that a large part of the radiation of diatoms can be attributed to other strategies in nutrient uptake and usage efficiency, among others, to adapt to stressful environments where they dominate as major opportunistic players. Most of these adaptations are the result of the flexible and ever-changing genome uniquely characteristic of diatoms. Although many of these strategies have directly allowed diatoms to become as ecologically successful as they now are, they have been facilitated through the silica use of diatoms as they benefited from increasing or sudden large natural availabilities of the element and also optimized their uptake and use to provide minimal constraints and enhanced benefits in spread and survival.

Contents

Abstract.....	1
Introduction	3
1. Silica use in diatoms	4
1.1 Diatom frustules	4
1.2 The mechanism of silicification.....	5
1.3 The origins of silicification	6
1.4 Frustule functions	7
1.5 Why silica?	8
2. Silica use over time	9
2.1 Diatom evolution.....	9
2.2 Silica over time.....	11
2.3 Silica depletion and diatom response	13
3. Other diatom adaptations.....	15
3.1 Resource uptake and metabolism strategies	15
3.2 Chemical signaling in diatoms	18
3.3 The diatom genome: the ultimate adaptation?	18
Conclusion and discussion	21
References.....	23
Acknowledgements	26

Introduction

Diatoms are a remarkable group of microalgae. They are of major importance to the world's oceans and biogeochemical cycles. Together, they account for one-fifth of the earth's primary productivity (Armbrust *et al*, 2009) and they generate about as much organic carbon through photosynthesis as all the terrestrial rainforests combined. They therefore serve of major importance to marine food webs, and play a massive role in carbon sequestration in the open ocean and thus in the global carbon cycle and climate change. Referring to diatoms as ecologically successful is not an understatement: they inhabit oceanic environments all over the world, from tropical coastal regions to the open ocean and along the polar ice. Diatoms currently consist of between 30,000 to an estimated 200,000 different species, making them the most abundant and diversified group, ranging in size over three orders of magnitude and exist as single cells, chains or colonies (Bowler *et al*, 2010).

However, it has not always been like this in the world's oceans. Diatoms found their origins during the Cretaceous era, along with two other major groups of eukaryotic phytoplankton – the dinoflagellates and the calcareous Coccolithophores. Parallel with these groups, diatoms rapidly radiated in the oceans, but what sets them apart is that in the past 66 million years, during the Cenozoic era, diatoms are the only group to show a massive evolutionary expansion as they increased in abundance and diversity (Cermeño *et al*, 2015). The most unique feature of diatoms is that they biomineralize silica in their cell walls, giving them the 'glass houses' referred to as frustules. Other organisms, such as corals and radiolarians, are also known to silicify, yet diatoms have largely displaced them in the battle for silica as their affinity is much higher. It is the combination of these features that makes diatoms so intriguing. One could easily assume that it is because of their unique silica utilization that they have been able to become so ecologically successful, as it is the major feature setting them apart from other groups of phytoplankton. In this thesis, I intend to investigate to what extent the use of silica by diatoms has truly contributed to their ecological success as a group. The research question central in this thesis is:

“To what extent has the use of silica in diatoms contributed to their ecological success?”

In this context, I define ecological success as the immense diversification and increase in abundance that diatoms have undergone as they occupied every corner of the oceans and became a major player in the world's biogeochemical cycles in a relatively short period of time. I will mostly investigate marine diatoms in the context of oceans, which I will simply refer to as diatoms.

To answer this question, I will investigate the mechanism, origin and function of silicification, the rise of diatoms over time in relation to silica availability and other adaptations of diatoms allowing their ecological success.

1. Silica use in diatoms

1.1 Diatom frustules

The key feature distinguishing diatoms from other phytoplankton species is the use of silica – or, to be more specific, silicic acid, its dissolved form. The requirement of silica for growth is a distinctive feature of all diatom species. Although many other organisms – such as radiolarians, sponges and land plants like rice – use silica as a biomineral and have been doing so even before diatoms originated, genetic analyses of silicic acid transporter (SITs) systems suggest that it has evolved independently in diatoms shortly after their origin (Finkel & Kotrc, 2010).

Diatoms use available silicic acid to create their mineralized cell walls. The resulting “glass houses” around the cell are named frustules. Their walls consist of one or two layers of amorphous silica, perforated by pores in species-specific arrangements. The diatom plasmalemma is surrounded by two frustule halves, with one half overlapping the other like a Petri dish (Figure 1, left). Girdle bands form between the two halves, to close the gaps during the cell division cycle. According to their shape, diatoms can be divided into four major groups (Figure 1, right) radial centrics, polar centrics, raphid pennate (containing a raphe) and araphid pennate (not containing a raphe). This group distinction in shape was not present from the beginning, but split off over time from the original radial centric group (Armbrust, 2009).

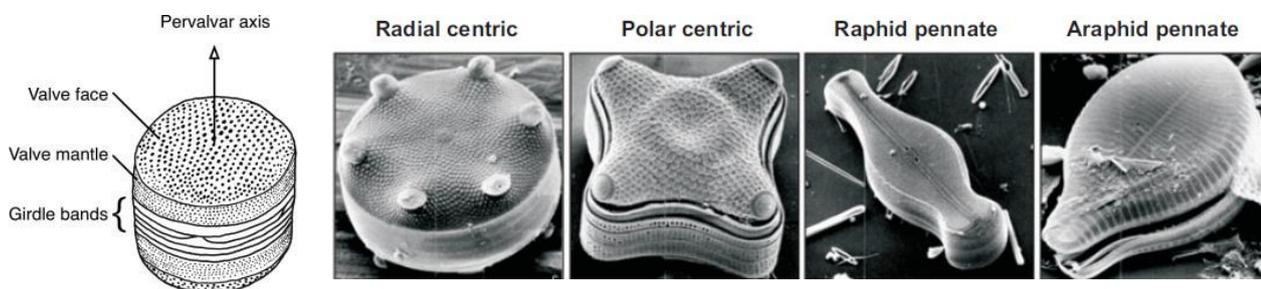


Figure 1. Left: basic morphology of the diatom frustule. Right: The four major groups of diatoms. The radially symmetrical radial and polar centric diatoms are ancestral to the bilaterally symmetrical pennates, with (raphid) and without (araphid) raphe. From Finkel and Kotrc, 2010 (left) and Kröger and Poulsen, 2008 (right).

1.2 The mechanism of silicification

Since the discovery of their unique glass casing, diatoms are considered 'masters of bio-nanotechnology' (Groeger *et al*, 2009) and their highly efficient process of silica biomineralization, on a biochemical and genetic level, has raised an increasing amount of attention over time. Much progress has been made in unfolding the process. Because opaline silica, the form deposited by diatoms, is undersaturated with respect to dissolved silicic acid in nearly all natural waters, silica deposition requires the accumulation of monomeric silicic acid in a compartment (Raven and Giordano, 2008). This makes the deposition of opal thermodynamically favored, and relaxes any kinetic constraints preventing deposition. The mechanism of monosilicic acid uptake varies between species, but there are common observations. As with many nutrients, the uptake of monosilicic acid depends on surrounding concentrations (Shrestha & Hildebrand, 2015; Groeger *et al*, 2009). At high concentrations, uptake takes place through diffusion, whereas active transport by SITs is required at low concentrations. This requires silica concentration sensing and uptake regulation by SITs, as becomes evident in the rapid monosilicic acid uptake after silica starvation in diatoms (Groeger *et al*, 2009; Shrestha and Hildebrand, 2015). Diatoms respond to the ambient range of silicic acid concentrations through the induction of a variety of silicic acid transporters (Finkel and Kotrc, 2010). Martin-Jézéquel *et al* (2000) demonstrated evidence for internal regulation between silica uptake and incorporation in addition to several models for uptake regulated by surrounding silica levels. Besides clearly being under physiological control, silica uptake in diatoms is also greatly under genetic control due to its role in the cell division cycle. Cell wall patterns are reproduced during each cycle, so silica deposition inside the cell is an obligatory step before cell division (Martin-Jézéquel *et al*, 2000). This is demonstrated by maximum uptake rates during cell wall formation (Groeger *et al*, 2009) and an interruption of the cell division cycle under silicic acid deficiency (Finkel and Kotrc, 2010). Intracellular silicon processes are less well understood than uptake, and the presence of silicon storage is still not clear despite much research. Martin-Jézéquel *et al* (2000) argued that, whereas nitrogen and phosphorus limitation in diatoms can be overcome by accessing internal storage pools or breaking down intracellular compounds containing these elements, there is no such strategy in place for silica. Instead, silica starvation results in diatom growth inhibition – much faster compared to nitrogen and phosphorus. Since then, many studies have provided evidence of some form of internal silica storage pools in certain diatom species (Groeger *et al*, 2009), for example by stabilizing silicic acid with organic material. The adaptation in terms of silica uptake and utilization efficiency plays a major role in the evolution of diatoms and available silica over time, as will be discussed later. Constraints of silica metabolism are reduced by the fact that the energy for silica uptake and deposition process in diatoms is supplied solely by aerobic respiration, meaning that the silica metabolism does not require light – as opposed to metabolism of other major nutrients such as nitrogen or phosphorus (Martin-Jézéquel, 2000).

1.3 The origins of silicification

It is evident that diatoms are dependent on silica for growth and survival. The preservation of their frustules gives a great fossil record that can tell us when this function evolved, but now how. The preference for silica is not difficult to argue – it is the second most abundant element in the earth's crust (Martin-Jézéquel *et al*, 2000), so is generally present in sufficient concentrations in natural waters and was even more so at the time of diatom origins. How diatoms came to utilize silica in the first place is not clear, apart from the fact that it evolved independently from other silicifying organisms. Multiple theories have been proposed concerning the origins of diatom and their silica usage, as outlined in Sims *et al* (2006) and Medlin *et al* (2016). The diploid nature of the diatom (explained below) could provide insight into the origin of frustules. For one, the fact that the auxospore stage of many diatom species is covered in silica scales suggest that the earliest diatom developed from a scaly ancestor, evolving more scales to protect the entire cell (Medlin *et al*, 2016). Also, as explained in Sims *et al* (2006), a silicified cyst stage of a scale-covered heterokont may have become the dominant phase in the life cycle. The silica cell wall possibly developed in a terrestrial ancestor as protection against desiccation and to facilitate a temporary cell resting state (Medlin *et al*, 2016). Another theory suggests that internal silicon metabolism evolved in a simple naked biflagellate cell for its aiding survival in a prolonged resting state, leading to external deposition of silica that had polymerized internally and had thus become inaccessible for metabolism (Sims *et al*, 2006). This would explain the first origin of a silicified cell wall and the constant need for silica replenishment by uptake in diatoms. In general, the theories tend to agree that the earliest silicifying diatoms developed not in pelagic, but in shallow marine environments, limiting them to a benthic lifestyle in the world's oceans.

In any case, over time, the existence of a permanent glass cell wall placed a fundamental constraint on cell division (Finkel and Kotrc, 2010; Kröger and Poulsen, 2008). In each round of asexual division from the parental cell, a decrease in the size of daughter cells takes place. To prevent permanent loss of the ability of subsequent generations to divide, a system of sexual reproduction has evolved in diatoms. This form of reproduction is crucial to diatom survival on the long run, and requires communication and sensing (Armbrust, 2009). The essential requirement of silica generally has a large price tag in diatoms (Martin-Jézéquel *et al*, 2000; Finkel and Kotrc, 2010). As silica concentrations in the world's natural waters decreased over time, a need for active uptake of the element arose. This requires constant energy expenditure and biosynthesis of molecules involved in the process by the cell. The fact that diatoms have retained their capacity to take up and metabolize silica and the ability to produce a silicified cell wall despite the costs and restrictions, suggests a net fitness advantage of this remarkable property. So how exactly has silica use aided diatom growth, survival and eventually ecological persistence?

1.4 Frustule functions

Starting from the top-down perspective, the silica frustule has long been hypothesized to play a role in diatom defense. The most obvious function is a mechanical defense against grazing. The intricate structure of diatom frustules largely increases the mechanical pressure required to fracture them, protecting the cell inside from crushing tools of predators (Kröger and Poulsen, 2008; Lopez *et al*, 2005). Common phytoplankton predators such as copepods have coevolved with diatoms' defenses by developing siliceous teeth, in spite of which many diatoms still pass through such predators unharmed. Friedrichs *et al* (2013) showed a negative correlation between mechanical strength of diatom frustules and the amount of grazing upon them. In addition, experimental studies show a higher Si:C ratio in diatoms grown in the vicinity of grazing copepods, suggesting silicification is a phenotypically plastic, inducible defense against herbivory (Finkel and Kotrc, 2010). The silica frustule shows less protection against dinoflagellates and foraminifera, that seem to be able to fracture them, but studies suggest handling time is increased (Raven and Giordano, 2008; Finkel and Kotrc, 2010). The frustule has also been suggested to provide defense against pathogens (Raven and Giordano, 2008; Finkel and Kotrc, 2010) and enzymatic attacks (Sims *et al*, 2006). The function of the frustule as defense is not 'waterproof' as some organisms can bypass it, but it clearly provides protective benefits on the whole. Another important potential function of the siliceous frustule is that it greatly increases the density of diatoms. This increases sinking rate, which can be countered by density reduction mechanisms inside the cell, to give diatoms buoyancy control. Moving down, out of the photic zone, has a survival benefit in terms of grazing evasion at the end of a diatom bloom (Finkel and Kotrc, 2010). In addition, vertical migration allows diatoms to adjust to nutrient availability (Finkel and Kotrc, 2010; Armbrust, 2009). Experimental results (Finkel and Kotrc, 2010) show nutrient-depleted diatoms to increase their Si:C ratio, resulting in an increased sinking rate towards deeper, nutrient-rich waters. Lastly, parasitized diatoms that lose buoyancy control mechanisms will sink out of the population due to their silica content, protecting the rest of the population (Raven and Waite, 2004). Another important physical benefit provided by the frustule is the development of a raphe. This is a slit in the cell wall unique to a relatively recently emerged group of benthic diatoms – the raphid pennates – that allows them to glide along surfaces. While it probably originally developed as an adaptation to a benthic lifestyle, the raphe also allows movement in planktonic pennates, contributing to their diversification (Bowler *et al*, 2010). Armbrust (2009) describes the impact on diatom diversification of the raphe in terms of available niche expansion “as profound...as the evolution of flight had on birds”. Lastly, frustules have allowed for the formation of diatom chains and colonies, as certain morphology features facilitate diatom-diatom connections (Finkel and Kotrc, 2010).

The frustule also provides potential benefits for diatom resource acquisition – apart from the buoyancy control function described above. Due to its optical properties, certain parts of the glass casing of the diatom has often been hypothesized to aid in light harvesting for photosynthesis – as it does in, for example, rice plants (Raven and Giordano, 2008). Although no empirical evidence exists, some feasible models for this 'crystal' function have been provided (Finkel and Kotrc, 2010; Kröger and Poulsen, 2008; Lopez *et al*, 2005). Milligan and Morel (2002) demonstrated a role of the cell wall of one diatom species as a proton buffer, which is believed to aid in CO₂ acquisition via an extracellular carbonic anhydrase. This anhydrase is a component of the carbon concentrating mechanisms in some diatoms. In terms of nutrient diffusion and uptake, a thick silica cell wall would at first seem like a major limitation, mainly because of its reduction in effective cell surface area (Finkel and Kotrc, 2010). Nonetheless, diatoms have among the largest growth rates of all phytoplankton groups. It seems that the surface area not covered by silica is sufficient to maintain high uptake rates. Compensation for a reduced nutrient flux to the cell potentially happened by a shift towards a more elongated frustule shape to increase overall surface area (Finkel and Kotrc, 2010) – this shift in shape will be discussed later –, as well as by the enhanced nutrient uptake as a result of the microtopography of certain frustule surfaces. Whether this uptake-enhancing structure is a primary function of the biogenetic silica or a compensation for reduced surface area is difficult to say.

1.5 Why silica?

Silicate is not the only mineral that is used in biogenesis as a key strategy in phytoplankton. Calcification in Coccolithophores (calcareous nannoplankton) is a perfect example of parallel evolution of biomineralization (Raven and Giordano, 2008). Instead of silica they take up calcium and inorganic carbon and precipitate crystalline forms of calcium carbonate. Calcification as such shows multiple origins in taxa, reminiscent of silicification, and most importantly, has similar potential functions as a grazing and pathogen defense, mechanical support, buoyancy control through density increase, light harvesting and carbon acquisition. This raises the question as to why silicate would be more beneficial for actively and genetically regulated biomineralization in a cell wall. As mentioned before, silica is the second most abundant element in the earth's crust, so its availability was not a limiting factor – at least not at the time of diatom origins. A major factor in addition to silica abundance is that silica deposition is a thermodynamically favored process. This means that constructing a cell wall from silica instead of carbohydrate results in a significantly decreased energy expenditure (Kröger and Poulsen, 2008; Sims *et al*, 2006). This would allow diatoms to not only fit into a previously unexploited ecological niche but to also outgrow other species (Martin-Jézéquel *et al*, 2000). Finkel and Kotrc (2010) argue that the energy expenditure involved in the regulation of the silica metabolism, such as synthesis of required molecules and active uptake, come with costs that could offset the energetic advantage of silica precipitation. Nonetheless,

the theoretical low energetic cost of precipitation in combination with high abundance at the time of diatom origin explains the preference for silica. The deposition of calcium carbonate as a biomineral is also a thermodynamically favored process, due to its supersaturation in the surface ocean (Raven and Giordano, 2009), again accounting for a similar advantage. Coccolithophores and diatoms both belong to eukaryotic phytoplankton red lineages that thrived over a similar time period, but show different patterns in diversification and ecological success. Focusing on these patterns in relation to the geological record of their biomineral substrates will give us more insight into how the use of silica sets diatoms apart.

2. Silica use over time

2.1 Diatom evolution

The evolutionary history of diatoms begins about 230 million years ago (Mya), in the middle Triassic. Their origin is preceded by the End-Permian extinction event, after which a group of eukaryotic phytoplankton referred to as the 'red' lineage, due to secondary endosymbiosis of red algae, began to replace green algal groups on a large scale (Medlin, 2016; Falkowski *et al*, 2004). These three major phytoplankton groups – dinoflagellates, calcifying Coccolithophores and diatoms – expanded in abundance and diversity throughout the Mesozoic era (Figure 2). Many estimates of diatom origin have been made based on molecular clocks, with the earliest estimate lying close to the Permian-Triassic boundary, with a maximum at 240 Mya (Medlin, 2016; Sims *et al*, 2006). However, the silicified remains of diatoms, and especially their spores, have allowed for extensive fossil records. It is important to note that diatom frustules are more soluble than other siliceous remains, so reliability is difficult. The earliest frustule fossils reported are from the Jurassic era, estimated around 190 Mya (Sims *et al*, 2006). Not only did diatoms originate much later in the Mesozoic than dinoflagellates and Coccolithophores, they only started radiating in the early Cretaceous, around 125 Mya, according to the fossil record (Katz *et al*, 2005) – by then, the other two groups were already on the rise in diversity and abundance. The consistent ecological success in the Mesozoic of the three phytoplankton groups was paralleled to a long-term increase in sea level with an accompanying expansion of flooded continental shelf area, as can be seen in figure 2 (Falkowski, 2004), opening up habitable coastline and increasing nutrient availability (Katz *et al*, 2005). By the end of the Cretaceous, diatoms had diversified throughout oceans and even into non-marine environments. Apart from the different period over which they radiated and diversified, another thing stands out when we compare evolutionary history of diatoms to dinoflagellates and Coccolithophores. The aftermath of a bolide impact marking the end of the Cretaceous era removed large portions of all phytoplankton

groups (Armbrust, 2009; Falkowski *et al*, 2004). Coccolithophore and dinoflagellate diversity recovered but then slowly underwent a long-term decline throughout the Cenozoic until the present, parallel to the drop in sea level. Diatoms, on the other hand, increased steadily in diversity throughout this same period as they colonized offshore areas, with two major pulses in diversification, as can be seen in figure 2. Because the main feature setting diatoms apart from the two other groups is the use of silica as a biomineral, it is definitely worth investigating to what extent this pattern of diversity 'domination' can be attributed to silicification.

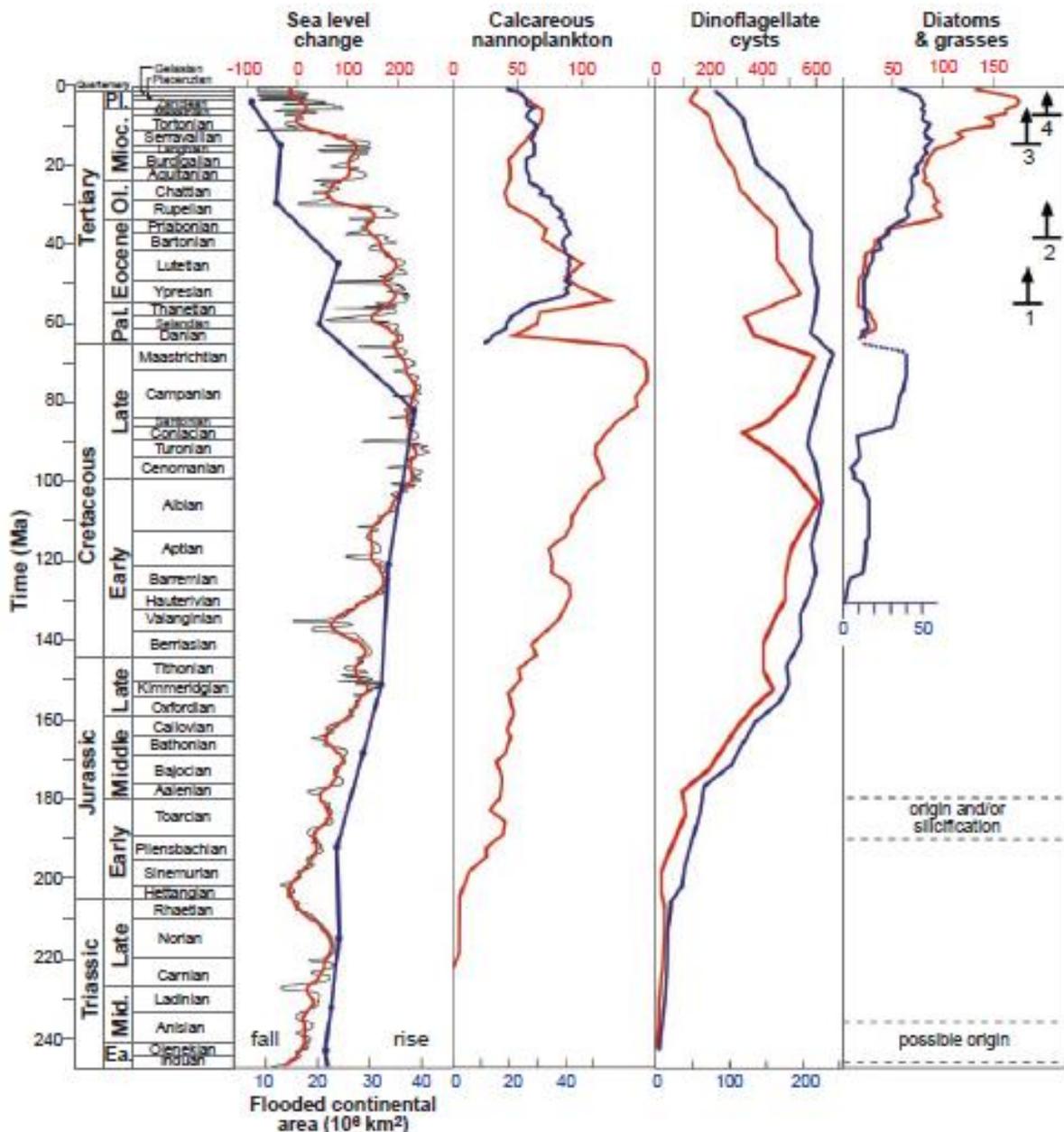


Figure 2. Comparison of eukaryotic phytoplankton diversity curves with sea-level change (red), flooded continental areas (blue), and the evolution of grasses. Phytoplankton species diversities are red, phytoplankton genus diversities are blue. Key to grass panel: (1) First conclusive occurrence of C3 grass pollen, phytoliths first appear in the marine record; (2) C3 grasslands expand, phytolith diversity and abundance increase ; (3) first macrofossil evidence of C4 grass evolution; (4) grassland expansion is coupled with a shift in dominance from C3 to C4 grasses. From Falkowski, 2004.

2.2 Silica over time

From the fossil record of silicifying organisms, it has become clear that diatoms have a competitive advantage in silica uptake and thus a negative effect on other silicifiers (Raven and Giordano, 2009; Finkel and Kotrc, 2009). The increase in diatom presence has been paralleled by a decrease in silicifying corals and radiolarians – except for deep sea corals, that have thrived on the dissolving frustules of dead and sunken diatoms. Cermeño *et al* (2015) used fossil evidence of decreasing silicification in radiolarian throughout the Cenozoic – as a response to their competitive disadvantage – to create a model where diatoms and radiolarians compete for silica. The competition model did not accurately predict the observed changes found in the fossils, deeming competition alone is insufficient to explain the ecological success of marine diatoms". However, the model was found to be more accurate when taking into account the continental weathering and erosion over time, suggesting that increased continental silicate weathering fluxes facilitated the rise of diatoms to ecological prominence. This is evident in two clear pulses in diatom diversity – the first at the Eocene/Oligocene border and a second halfway through the Miocene (Figure 2 and 3) –, which the study attributes to the uplift of the Himalayas, resulting in 20% and 80% increase in silica flux to the oceans, respectively. Previous evidence for increased weathering rates as a result of increased physical erosion supports not only these trends seen in the past 50 My, but also the rapid diversification of diatoms in the late Cretaceous. The rise in sea level and increase in flooded continental shelves (Figure 2) resulted in increased erosion and release of silica and other nutrients into the growing ocean (Armbrust, 2009). Of course, erosion is not limited to silica but also increases calcium release, so why is diversification clearly more present in diatoms than it is in Coccolithophores after the extinction event and the Cretaceous era that followed? Katz *et al* (2005) have gathered evidence combining evolution on land, the silica cycle and diatom rise to present a theory that has been much debated since. Grasses store silica as opal phytoliths, and the dissolution of these phytoliths releases soluble silica two times more efficiently than chemical weathering, so grasses can be considered to speed up the flux of silica to

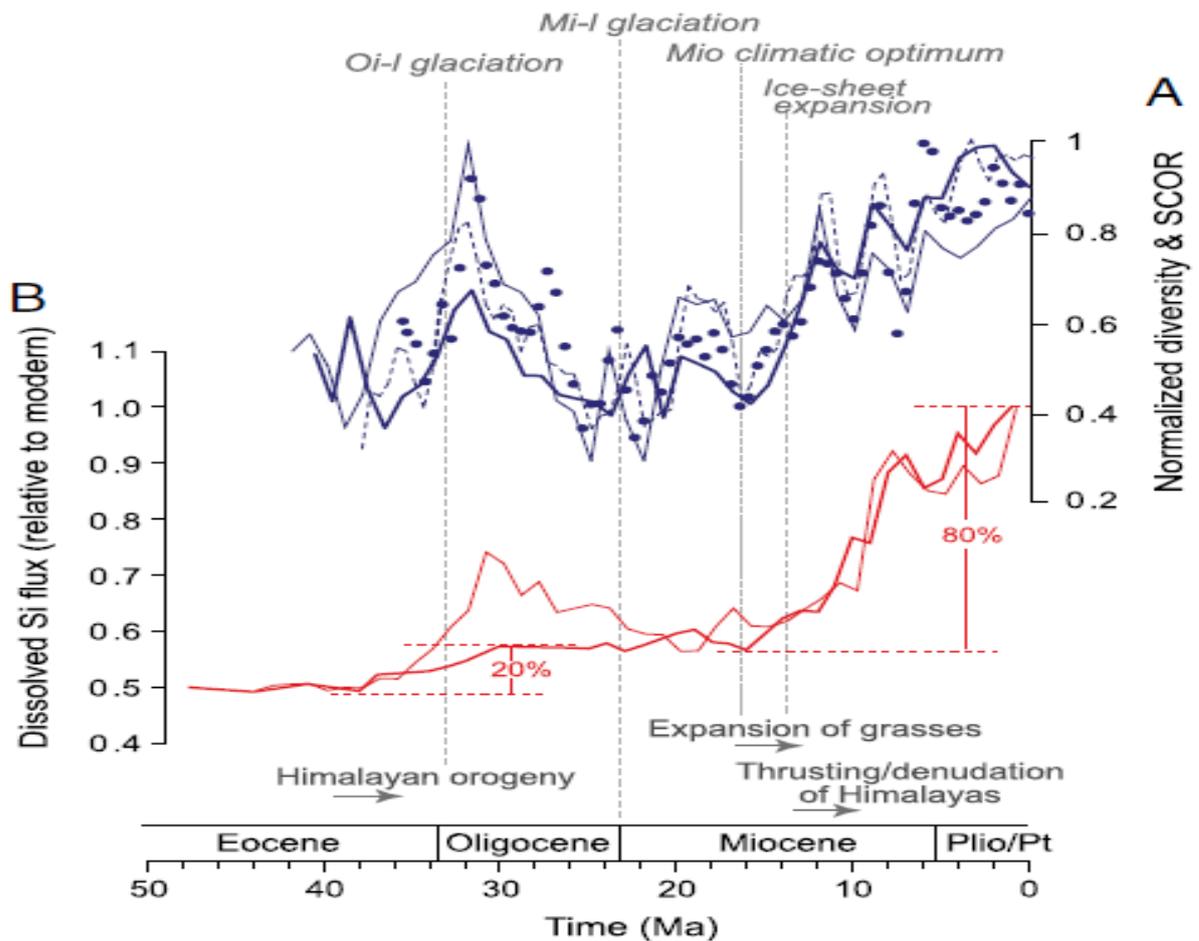


Figure 3. Sedimentary records of marine planktonic diatoms and silica flux to the oceans based on global ocean data compilations. (A) Normalized diversity and Summed Common species Occurrence Rate (SCOR). Diversity curves are generated using two different methods of sampling standardization, the unweighted lists subsampling method (UW, solid thin line) and the Shareholder Quorum Subsampling method (SQS, dashed thin line). Also represented is the consensus diversity curve (LBR, solid thick line) and SCOR (dots). (B) Fluxes of H_4SiO_4 to the global ocean based on the lithium isotope record (red thick line) and to the Southern Ocean based on marine sedimentary clays (red thin line) are both relative to modern fluxes. From Cermeño et al, 2015.

the ocean. This biologically catalyzed weathering process was enhanced when browsing grazers were displaced by ungulates, whose grazing strategy enhanced phytolith release (Vandevenne, 2013). As such, the radiation of grasses at the Eocene/Oligocene boundary and late Miocene mobilized significant amounts of silica, resulting in the diatom diversity and abundance pulses observed. As accurately as this theory could explain the observed fossil records, criticism exists. Raven and Giordano (2009) consider biomineralization on the land and in inland waters to be “delays in the transport of weathering products into the ocean” and find the role of grasses in the radiation of diatoms unjustified, unless grasses are shown to increase the extent of abiogenic weathering. One could reason against this and state that silica stored up in phytoliths over time could cause a sudden release when grazed upon in large amounts. Nonetheless, they find that “it is continental elevation, rather than biology that determines fluxes of solutes from the continents to the oceans”. Rabosky and Sorhannus (2009) found the fossil record to be unreliable and created a model with reconstructed diatom diversity. The temporal mismatch between this diatom diversity and the timing of grassland expansion argues strongly against the view that a relationship exists.

2.3 Silica depletion and diatom response

It is clear that diatom abundance and diversity respond to increases in available silica. As Finkel and Kotrc (2010) state, "...their evolutionary success depends on the Si cycle and their ability to acquire silicic acid from the environment". But how has available silica responded to diatom increase over time? And what effect does this have on diatoms, in return – what feedback exists? Before the evolution of diatoms, dissolved silicon (DSi) was close to saturation in the oceans (Martin-Jézéquel, 2000). Since then, diatoms have depleted the oceans of DSi in a stepwise manner (Raven and Giordano, 2009), such that its concentration is now consistent with the minimum requirement for diatom growth (Finkel and Kotrc, 2010). As Raven and Giordano (2009) state, silicifying organisms – diatoms mostly – have had a larger impact on the availability of silica than calcifying organisms have had on their available substrates. The result is a competition for silicon as a cell wall material in the modern ocean (Martin-Jézéquel, 2000). Occurrences of diatom blooms as a response to sudden bioavailable silica are observed in aquatic waters such as Lake Malawi (Brown, 2011), where silica concentration are less buffered than in the ocean. Yet in every ocean environment examined, limitation of diatom silicification rates have been observed at times (Martin-Jézéquel, 2000). The most obvious example is the commonly occurring spring blooms in temperate and subpolar regions, where diatoms bloom first and decline once the bioavailable silicate is depleted (Bowler *et al*, 2010). In fact, it could explain the slight decrease in diatom diversity between the diversity pulses in the Cenozoic (Figures 2 and 3), as bioavailable silica pools were probably not increasing in these periods. The fact that diatoms pump silicate to the ocean bottom thanks to their sinking features, effectively prolonging the recycling of silica and sequestering a small part of it constantly, does not aid bioavailable silica levels in the euphotic zone. As a consequence of the depletion caused by their domination of the oceans, silica availability in the oceans now has a strong effect on the distribution and abundance of diatoms, because of their absolute requirement for the element. This also leads to a certain amount of evolutionary pressure: the decreasing saturation of monosilicic acid was a major driver of increased effectiveness of silica use, involving high-affinity transporters and, in some cases, a decreased degree of silicification (Raven and Giordano, 2009). Laboratory studies (Finkel and Kotrc, 2010) have already shown diatom frustules to become more heavily silicified with increasing silicic acid concentrations, and ancient diatom morphotypes to be more heavily silicified than younger ones under the same silicic acid concentrations. This selective pressure is also hypothesized to lead to morphologies that would increase nutrient diffusion but require less silica per cell. Finkel and Kotrc (2010) examined extensive fossil record to study the change in frustule morphology over time. They did indeed find a slight decrease in silicification in diatom species from the early origins to the modern oceans (figure 4), suggesting an adaptation to bioavailable silica, among other nutrients, becoming more limited.

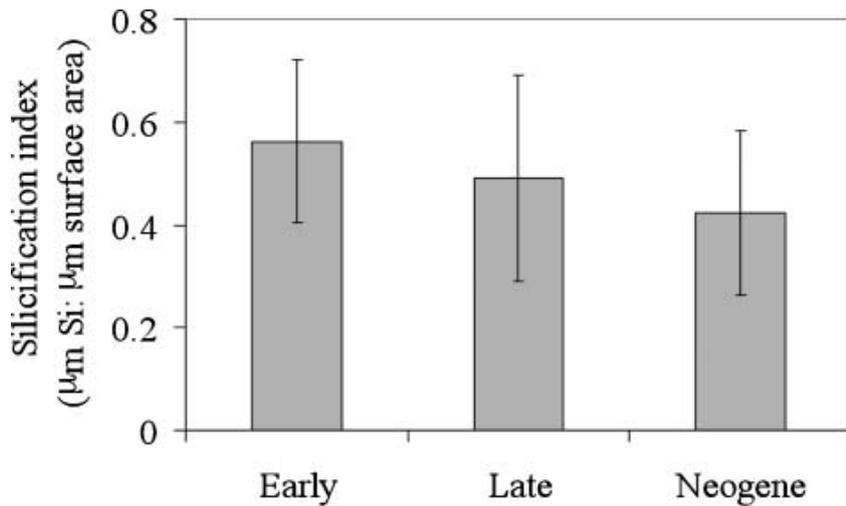


Figure 4. Qualitative estimate of average (\pm s.e.) silicification ($\mu\text{m Si}:\mu\text{m surface area}$) for Early Cretaceous, Late Cretaceous and Neogene assemblages measured from selected frustule wall fragments. From Finkel and Kotrc, 2010.

Evolution of diatoms towards an overall smaller frustule and a longer and thinner shape increases nutrient flux and decreases silica requirements. In studying diatom frustule morphology, it is important to remember that the diatoms have undergone major evolutionary radiation since their early beginnings. From the extensive fossil record

(Sims *et al.*, 2006) it is evident that diatoms have benefited from ecological niches opening up – after the Cretaceous/Tertiary boundary extinction event – to radiate. This has led biologists to distinguish between certain diatom groups that split off from each other over time (see figure 1, right), and silicate is just one of many factors influencing adaptation and thus morphology change between and within those groups. Nonetheless, there is evidence of morphology, as well as metabolic (uptake) adaptations to bioavailable silica decrease over time. Because diatoms can only adapt to this specific change up to a certain point, their success can be argued to be 'self-limiting' (Finkel and Kotrc, 2010). Important to note here is that early diatoms had relatively large and heavy shells limiting them to a benthic existence. The building of thinner walls and advanced structures such as spines and even the presence of a raphe would have allowed them to diversify into the open ocean as they adapted a planktonic existence, contributing to their worldwide spread (Bowler *et al.*, 2010). Therefore, one can also regard the change in frustule morphology as an increased 'sophistication' in silicon utilization pushing their ecological success, while also adapting to decreasing bioavailable silica concentrations.

Looking back at the diversification pattern of diatoms, there may be more explanations for the overall and time-specific increases in diatom diversification and success, in comparison to other eukaryotic phytoplankton. They are partially results of the geological events over time, such as continental shelf flooding and climate change, and the factors other than silica availability and use that contribute to diatom success.

3. Other diatom adaptations

3.1 Resource uptake and metabolism strategies

One of the most important adaptations of diatoms responsible for their ecological success is their nutrient uptake strategy. Like any other phytoplanktonic organism, diatoms require nutrients such as nitrogen and phosphorus for photosynthetic reactions and growth. As discussed previously, diatoms possess a certain amount of buoyancy control, partly thanks to their silica cell walls. Moving up and down through the water column allows them to move between the well-lit but nutrient-depleted surface waters in which they photosynthesize, and nutrient-rich waters where they take up and store the nutrients necessary for growth (Armbrust, 2009). This strategy has facilitated the diatoms' colonization of the open ocean. More importantly, pelagic diatoms have uniquely evolved a nutrient storage vacuole, occupying about 40% of the cell and allowing them to retain sufficiently high concentrations of nitrate and phosphate to undergo several cell division cycles without requiring external macronutrients (Falkowski, 2004). In addition, their silica cell walls make it possible for diatoms to exist as spores at the ocean floor, remaining dormant until nutrients are restored at a level that stimulates them to massively photosynthesize, grow and divide again (Suto *et al.*, 2012; Bowler *et al.*, 2009). This resource acquisition strategy results in the massive diatom blooms we can observe today and is exemplary of diatoms' adaptation to an environment of unstable and sporadic upwelling of nutrients, especially in coastal habitats. It also largely explains diatom diversification throughout the Cenozoic, as multiple researches (Suto, 2005; Falkowski *et al.*, 2004; Cermeño *et al.*, 2015; Suto *et al.*, 2012) have correlated geological events to diatom evolution. The Mesozoic was characterized by quiescent conditions in the oceans with a stable water column with a constant nutrient supply, where Coccolithophores and dinoflagellates thrived, but the onset of polar ice caps, thermohaline circulation and upper-ocean turbulence caused vertical mixing and nutrient upwelling at the Eocene/Oligocene boundary along with increased weathering and river inflow. In these new conditions, diatoms thrived, competitively displacing other phytoplankton groups and explosively diversifying (see figure 3). Global cooling and nutrient mixing followed by decreasing nutrient supply in some regions may have resulted in a second increase in diversity and abundance, but to a lesser extent. Whereas Coccolithophores and dinoflagellates decreased in diversity and abundance as long-term sea level and the extent of flooded continental area decreased, diatoms responded to an increased coastal niche area along with other environmental factors (Katz *et al.*, 2005). Certain species of diatoms coevolved with these conditions by reducing their size to optimize nutrient intake (Katz *et al.*, 2005; Finkel and Kotrc, 2010; Suto, 2005), adding some explanation to the changes in morphology discussed previously. As with the grass-silica-loop theory, Rabosky and Sorhannus (2009) use a reconstructed diatom diversity to statistically reject the theory that competitive advantages in terms of nutrient uptake

and storage can account for diatom patterns across the Cenozoic. Lazarus *et al* (2014) use the same reconstructed data set to argue that the primary reason for diatom diversification across the Cenozoic is “not that diatoms are better adapted to increasing Cenozoic eutrophic environments, but that they are better adapted to Cenozoic environments that are geographically distinct”, claiming that the geographical isolation of the waters dominated by diatoms also plays a major role in creating the varying stressful environments that diatoms rapidly adapted to.

Diatoms are adapted to times of nutrient shortage not only by their uptake strategy, but also by their metabolic pathways. Genomic studies of certain diatom species have revealed the presence of all proteins involved in the urea cycle (Nunn *et al*, 2009) – a feature normally found in metazoans used to excrete nitrogenous waste from the catabolism of proteins and amino acids – as well as the enzyme urease and a urea transporter. Diatoms seem to use this metabolic cycle in order to recycle nitrogen internally when it is limited externally, using or storing urea as a primary nitrogen source instead of excreting it like animals do. It is speculated that this efficient recycling of nutrients was a major selective force for the evolution of the secondary symbiont, as it prevented the original heterotrophic host cell from losing a valuable nutrient, while photosynthesis in the new protoplast provided a steady supply of organic carbon (Vardi *et al*, 2008). In addition to nitrogen recycling, diatoms can convert fat into sugar and sugar into fat, another adaptation to nutrient shortage (Karthick, 2009).

Diatoms have also developed a strategy to cope with the limitation of another nutrient: iron. Three major areas in the oceans (see figure 5), known as ‘High nutrient

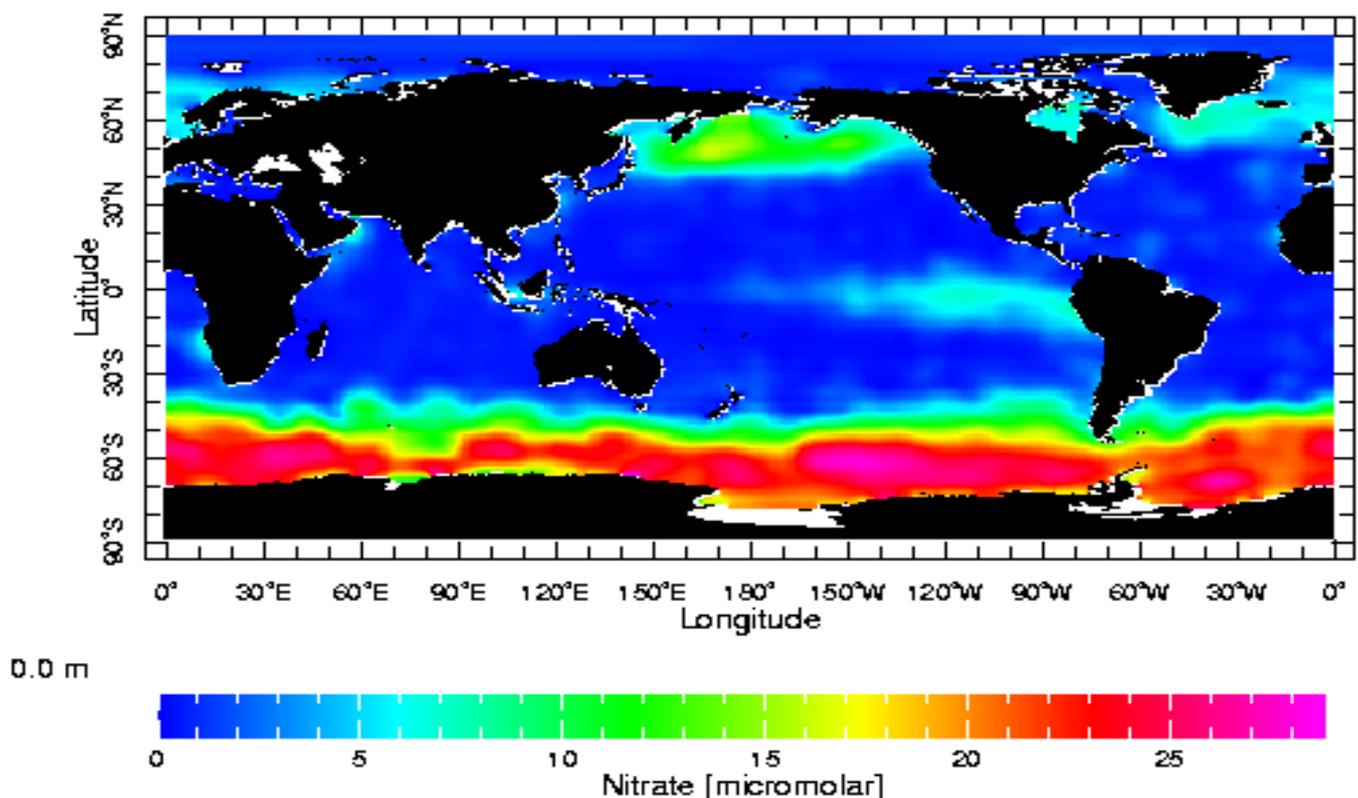


Figure 5. World map highlighting areas of high nitrate concentrations that contrastingly show low primary productivity. These areas, called High Nutrient low Chlorophyll (HNLC) are characterized by low iron concentrations, limiting primary productivity. From <http://klimat.czn.uj.edu.pl/>.

low chlorophyll' (HNLC), are rich in nutrients but limited in primary productivity by iron—a micronutrient required for photosynthesis. Diatoms are adapted to these environments by reducing their iron requirements under iron-limiting conditions (Armbrust, 2009). In all experiments where iron was artificially added to these regions, pennate diatoms were the first group of organisms to bloom (Vardi *et al*, 2008), suggesting that somehow diatoms dominate these regions of low iron conditions by seizing their chance when the smallest amount of iron becomes available. The reason for this is that these diatoms all produce ferritin, an iron-storage molecule that also protects against oxidative stress (Armbrust, 2009), allowing them to be highly tolerant to iron limitation and to bloom as soon as iron becomes available (Bowler *et al*, 2009). Other diatom species show different adaptations to iron limitation. For example, oceanic diatoms have been found to contain low levels of iron-rich photosystem I complexes and substituting the iron-rich cytochrome C_6 with the copper-containing plastocyanin in the photosynthetic electron chain. Some diatom species, on the other hand, respond to iron limitation by down-regulating their entire carbon-metabolism, thus reducing their iron requirement (Bowler *et al*, 2009). All of these adaptations have allowed diatoms to dominate oceanic habitats of constant or fluctuating iron limitation. Just as iron fertilization results in massive pennate diatom blooms, we would expect a sudden natural increase in iron availability to allow such blooms. And indeed, Suto *et al* (2012) correlate the Himalaya Mountain uplift and aridification on land, resulting in increased iron-rich dust supply to the oceans, to the increased diatom diversification in the late Miocene—a sudden availability in the micronutrient that they require so little but can store for later had given diatoms an evolutionary chance to seize. Armbrust (2009) suggests that the result was not only diversification in these iron-poor waters, but also in other regions: as iron and silicon pathways are linked in diatoms, they use less silicon relative to nitrogen under iron-rich conditions, leaving excess silicate in surface waters which then circulates out of the Southern Ocean and fuels diatom productivity in the subtropics.

Diatoms show some unique properties in their photosynthesis components. First of all, Bailleul *et al* (2015) have recently showed that diatoms are able to optimize their photosynthetic carbon assimilation rate by sharing reducing equivalents and ATP between mitochondria and plastids, a mechanism not found in other taxa. Many diatoms inhabit polar regions where light is inconsistent throughout the year with certain periods of darkness. They show tolerance to long periods of darkness and rapid growth resumption upon return to light (Bowler *et al*, 2010; Armbrust, 2009), presumably through the storage of lipids as metabolic intermediates and for ATP production. As mentioned previously, silicification processes are dependent on energy from aerobic respiration and not from photosynthesis, which allows diatoms to continue these processes under dark conditions. Sea ice proposes another challenge in terms of light and temperature, but diatoms show extremophilic properties through which they have optimized their carbon assimilation and survival, such as adaptation to the light conditions in ice (Wilhelm *et al*, 2006) and antifreeze proteins (Janech *et al*, 2006). Some of these 'extremophilic' species thrive under very different conditions as well, suggesting metabolic flexibility that allows them to

acclimate to an extremely wide range of environmental conditions (Wilhelm *et al*, 2006). Decades of research has unveiled another optimization of the carbon assimilation process in diatoms. Diatoms as a photosynthetic unit appear to have a higher affinity for inorganic carbon than the enzyme Rubisco itself and in addition, a complete set of genes involved in C₄-metabolism in land plants has been identified, all suggesting that diatoms have evolved some form of carbon-concentrating mechanism (Bowler *et al*, 2010). Carbon concentrating mechanisms that can saturate the carboxylase reaction of Rubisco, along with the ability to take up HCO₃⁻ would give diatoms a competitive advantage over microalgae that rely solely on the passive diffusion of CO₂ for photosynthesis. Among species, diatoms probably employ a diversity of CCMs (Bowler *et al*, 2010; Vardi *et al*, 2009) but their existence has not yet been conclusively demonstrated.

3.2 Chemical signaling in diatoms

Some species of diatoms are unique in that they appear to be fine-tuned and responsive to their surroundings, using sophisticated mechanisms to monitor and adapt appropriately to changing environmental conditions, further enhancing their evolutionary and ecological success (Bowler *et al*, 2010). These mechanisms would allow for resting spores to respond to optimal blooming conditions. Recurring blooming and spore sinking patterns in response to yearly light patterns suggest the use of internal clocks able to measure light quantity, quality and duration of incident light, similar to mechanisms in higher plants. Nutrient or grazing stress in diatom populations induces biosynthesis of diatom-derived unsaturated aldehydes (DD), which have been found to be used as chemical agent in control of grazing copepod populations (Armbrust, 2009). In addition, research has identified calcium and nitric oxide (NO) to act as important second messengers in diatom perception and transduction of stress conditions (Vardi *et al*, 2008). These signaling mechanisms have intriguing and important roles in cell-to-cell communication and programmed cell death in diatom communities (Bowler *et al*, 2010). One role for this could be differential susceptibility to these infochemicals within diatom populations, facilitating the elimination of deleterious mutations in stressed cells containing high levels of mutagenic reactive oxygen species (ROS). These diatom features on a community level are most likely an adaptation to the blooming strategy they implore, allowing them to maximize opportunistic strategies.

3.3 The diatom genome: the ultimate adaptation?

Overall, it has become clear that diatoms have on many levels adapted strategies unique to their species, facilitating their ecological expansion and evolutionary success. There is one general feature that have allowed for diatoms to adapt all these attributes, and that is their genome. Research has shown evidence that

diatoms are derived from a serial secondary endosymbiosis (Fig. 6), with a green algal endosymbiont preceding the red algae and with many of the green algal genes being retained and incorporated into the red algal endosymbiont, giving rise to diatoms (Bowler *et al*, 2010; Armbrust, 2009). This explains the combination of plant-like photosynthetic features with animal-like attributes – which can be traced back to the union between heterotrophic host and red algae – such as the urea cycle and fat storage and breakdown abilities, unique to diatoms. Another unique

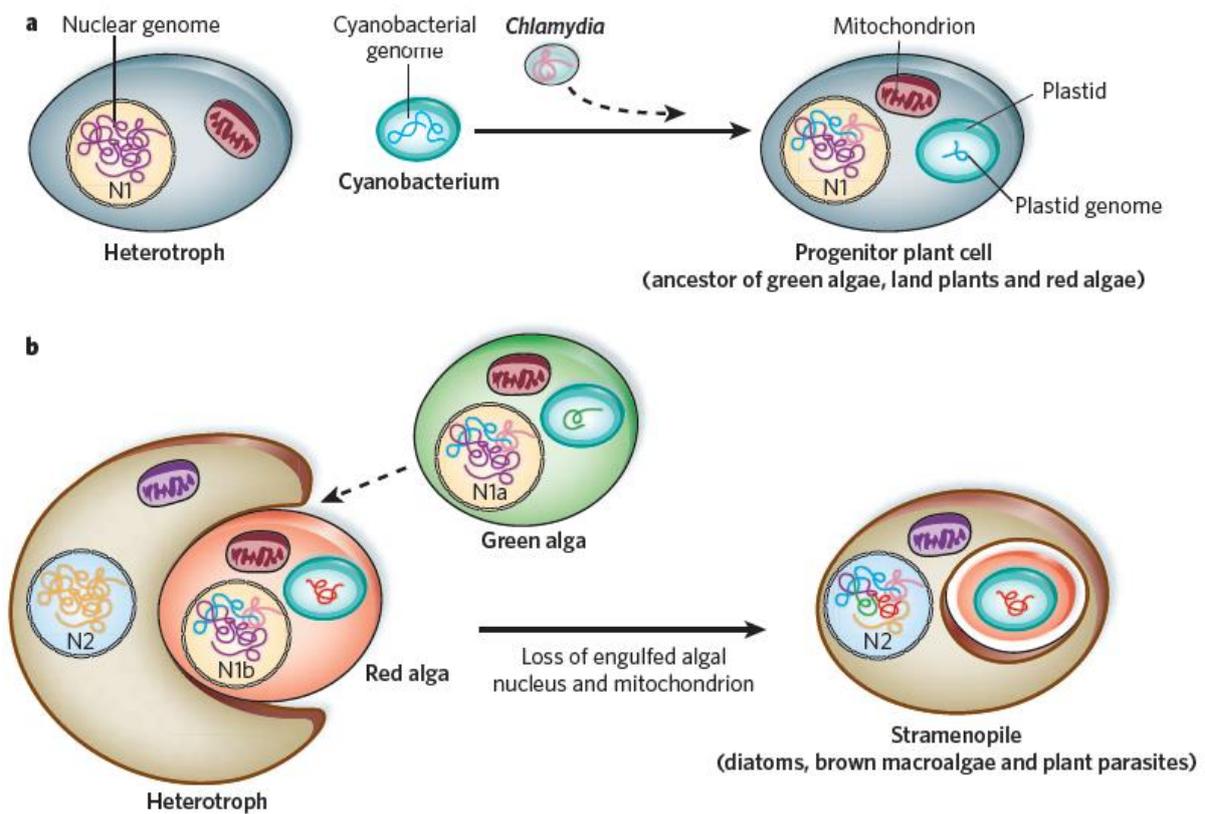


Figure 6: Representation of the origin of diatom plastids through sequential primary (a) and secondary (b) endosymbioses, and their potential effects on genome evolution. From Armbrust (2009)

feature of diatom genomes is that in many species, large parts of the genome are of bacterial origin, made possible through multiple events of horizontal gene transfer over time. Horizontal gene transfer with bacteria is usually rare in eukaryotes, but contrastingly pervasive and of higher frequency in diatoms (Karthick, 2009; Bowler *et al*, 2005). These genes do not seem to be derived from any one specific source, but from a range of origins including proteobacteria, cyanobacteria and archaea, and most interestingly, an early endosymbiosis event with Chlamydia prokaryotes (Fig. 6) (Armbrust, 2009; Vardi *et al*, 2009). The high amount of horizontal gene transfer that has shaped and is still shaping diatom genomes has most likely been allowed by the age-old symbioses with bacteria. Numerous examples exist of bacteria and diatoms living in close harmony (Armbrust, 2009; Bowler *et al*, 2010; Amin *et al*, 2012), with bacteria thriving in the crevices of diatom cell walls or even between the third and fourth outermost membranes of diatom plastids. With viruses as another source of new genes (Finazzi *et al*, 2010; Vardi *et al*, 2009; Bowler *et al*, 2010), diatoms have

picked up genetic pieces from all around them through horizontal gene transfer, facilitating their unique adaptation to certain conditions. Many of the attributes discussed above and others in diatoms are the result of horizontal gene transfer, including the 'internal clock', the genes coding for the iron-storing ferritin, carbon and nitrogen utilization, enzymes, functioning of the diatom urea cycle and diatom cell-wall silicification (Karthick, 2009; Bowler *et al*, 2010). This 'mixed bag of diatom genes' (Bowler *et al*, 2010) and incorporation of bacteria genes is likely to have also helped the optimization of silica use in diatoms, allowing them to move into the open ocean through the development of organic coating on the frustule allowing them to become thinner and lighter without dissolving, and the development of buoyancy control mediated by cellular lipid content and frustule morphology that increased its surface area (Bowler *et al*, 2010).

Another novel feature of diatom genomes is the rates at which they diversify, making diatoms one of the most rapidly evolving eukaryotic taxa on earth (Vardi *et al*, 2009). A prime example of this is that the two most genetically researched diatom species, *T. pseudonoma* and *T. tricoratum* are more divergent than fish and humans, having diverged as much in 90 million years' time as metazoans have in 550 million years (Bowler *et al*, 2010). This rapid diversification seems to have been permitted by the presence of diatom-specific mobile genetic elements that increase the rate of insertions, deletions and recombination to alter the diatoms' genetic structure. Some of these elements are actively transcribed in response to environmental stress such as nitrogen starvation (Bowler *et al*, 2010; Vardi *et al*, 2009). Diatom diversification in response to environmental cues is thus partially the result of genome arrangements and gene loss and expansions during niche adaptation. Furthermore, Bowler *et al* (2010) suggest that their fluctuating environments have driven selection towards epigenetic-based mechanisms in diatoms that create phenotypic variation.

Overall, it is clear that different species of diatoms are characterized by a complex combination of genes and pathways acquired from a variety of sources. The diatoms' overall capabilities originate from the heterotrophic ancestor and green and red algae via endosymbiotic gene transfer, along with the ability to genetically diversify at remarkable rates in order to adapt to stressful environments, mostly through transposable elements. Horizontal gene transfer with bacteria and possibly viruses throughout time has allowed for the subsequent gains and loss of specific genes which has played a major role for diatoms in adapting to new ecological niches. It is important to note that so far, only the genomes of several diatom species have been studied, which contrasts strongly with the massive amount of potential species today. The genomic insights discussed here are not necessarily relevant to the majority of diatoms, but they do provide insights into the evolutionary origins and underlying mechanisms of the diversification rate of diatoms.

Conclusion and discussion

In this thesis, I set out to investigate the relative contribution that silica use has had in the ecological and evolutionary success of marine diatoms. First of all, I reviewed the mechanism by which diatoms use silica, how it possibly originated and what constraints and benefits are provided for diatoms by this unique feature. I then investigated the evolutionary history of diatoms since their origin, together with changes in bioavailable silica over time. Lastly, I reviewed the many other adaptations of diatoms that have allowed their survival and diversification, and the underlying genetic mechanisms that have facilitated them.

There is no doubt that biomineralization of silica, whatever the first function or mechanism was, had great implications for diatoms. Regarding the element's abundance, especially at the time of their origin, silicon was an easy choice and potentially even energetically favorable. Despite the constraints that the frustules placed on diatom life history, silica use remained under active physiological and genetic control and diatoms adjusted, partially to changing silica levels, by optimizing their silica uptake and use. Frustule optimization as diatoms diversified increased the benefits it brought to them, including a physical defense against grazing predators and pathogens, the formation of diatom colonies and optimization of light and nutrient acquisition. Enhanced efficiency in frustule design and the development of a raphe allowed for the increased colonization of both pelagic and benthic habitats. It spurred a rapid diversification in diatoms over the Cretaceous era, facilitated by an increasing sea level and a steady flow of available silica through continental erosion. Although diatoms heavily depleted the ocean's silica over time, burying large parts in the ocean floor and thus slowing down the element's recycling, it is safe to say that diatoms got lucky in the Cenozoic. Increased chemical weathering of silica as a result of continental erosion events and possibly the evolution of grasslands and grazers gave diatoms a huge competitive advantage, allowing them to optimize and diversify. Were this not the case, diatoms could possibly have faced the same fate as the eukaryotic phytoplankton that paralleled their rise – dinoflagellates and Coccolithophores – and decreased in diversity and abundance along with a retreating sea level. Although diatoms have adapted to silica level decrease and occasional limitation through silica use efficiency and uptake efficiency, the element remains a major limiting factor for diatoms.

However, it is not only silica use that is responsible for the diatoms' ecological success we see today. Diatoms show multiple adaptations to nutrient-poor and extreme conditions, such as nutrient storage and nitrogen recycling features that have allowed them to thrive in coastal regions with regular upwellings, iron-limitation coping mechanisms allowing them to occupy iron-poor regions such as the Southern Ocean, and light and temperature stress that allows their survival in polar regions. Other events in the Cenozoic corresponding to diatom diversity pulses, such as a

massive change in ocean thermodynamics and iron supply, have probably facilitated the adaptation and subsequent radiation of diatoms. Genomic studies reveal that diatom genomes are uniquely programmed for rapid change, providing an underlying mechanism for these adaptations to stressful environments – including optimization of silica use for survival and radiation.

Silica has facilitated many features of diatoms that explain their current success. Silica frustules provide an anti-grazing mechanism which, together with massive growth and nutrient depletion and chemical weapons against grazers, allows blooms to be as successful as they are. Silica aids nutrient and light uptake and life history strategies by facilitating buoyancy during vertical migration, nutrient uptake-optimizing frustule morphology and the thick frustules of spores allowing them to stay dormant on the ocean floor during nutrient-poor times. Light harvesting strategy is potentially a combination of frustule optics and optimized photosystems. There has been much investment by diatoms over time in not only maintaining silica processing and use but also in frustule optimization for enhanced adaptation to a range of environments.

On a larger scale, we observe that the use of silica has allowed the explosive rise of diatoms during the Cretaceous. In the Cenozoic, diatoms adapted to a wide array of stressful environments, thanks to the underlying genomic mechanisms allowing enhanced optimization to the large environmental changes shaping the modern oceans. During this period, increased availability of silica facilitated the diversification and ecological success of diatoms, while they also optimized silica use in harmony with other features.

In conclusion, we cannot attribute the ecological success of diatoms directly to silica use, as their adaptations to the wide range of environments they inhabit are mostly thanks to several stress-tolerating and opportunistic strategies which are the result of their uniquely flexible and rapidly changing genomes. However, the use of silica has given diatoms a major advantage in their radiation as availability increased in the Cretaceous and showed major pulses in the Cenozoic, facilitating the rise in numbers and radiation of the opportunistic diatoms, as well as through the optimization of silica use to provide minimal constraints and enhanced benefits in spread and survival.

References

- Amin, S. A., Parker, M. S., & Armbrust, E. V. (2012). Interactions between diatoms and bacteria. *Microbiology and Molecular Biology Reviews*, 76(3), 667-684.
- Armbrust, E. (2009). The life of diatoms in the world's oceans. *Nature*, 459(7244), 185-192.
- Bailleul, B., Berne, N., Murik, O., Petroustos, D., Prihoda, J., Tanaka, A., et al. (2015). Energetic coupling between plastids and mitochondria drives CO₂ assimilation in diatoms. *Nature*, 524(7565), 366-369.
- Bowler, C., Vardi A FAU - Allen, Andrew, E., & Allen, A. E. (2010). Oceanographic and biogeochemical insights from diatom genomes. *Annual Review of Marine Science*, 2, 333-365.
- Brown, E. T. (2011). Lake Malawi's response to "megadrought" terminations: Sedimentary records of flooding, weathering and erosion. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 303(1-4), 120-125.
- Brunner, E., Gröger, C., Lutz, K., Richthammer, P., Spinde, K., & Sumper, M. (2009). Analytical studies of silica biomineralization: Towards an understanding of silica processing by diatoms. *Applied Microbiology and Biotechnology*, 84(4), 607-616.
- Cermeño, P., Falkowski, P. G., Romero, O. E., Schaller, M. F., & Vallina, S. M. (2015). Continental erosion and the Cenozoic rise of marine diatoms. *Proc Natl Acad Sci U S A*, 122(14), 4239-4244.

- Falkowski, P., Katz, M., Knoll, A., Quigg, A., Raven & J., Schofield, O. (2004). The evolution of modern eukaryotic phytoplankton. *Science*, 305(5682), 354-60.
- Finkel, Z. V., & Kotrc, B. (2010). Silica use through time: Macroevolutionary change in the morphology of the diatom frustule. *Geomicrobiology Journal*, 27(6-7), 596-608.
- Friedrichs, L., Hörnig, M., Schulze, L., Bertram, A., Jansen, S., & Hamm, C. (2013). Size and biomechanic properties of diatom frustules influence food uptake by copepods. *Mar Ecol Prog Ser*, 481, 41-51.
- Janech, M. G., Krell, A., Mock, T., Kang, J., & Raymond, J. A. (2006). Ice-binding proteins from sea ice diatoms (bacillariophyceae). *Journal of Phycology*, 42(2), 410-416.
- Karthick, B. (2009). Genome sequencing of cells that live inside glass cages reveals their past history. *Current Science*, 96, 334–337.
- Kröger, N., & Poulsen, N. (2008). Diatoms-from cell wall biogenesis to nanotechnology. *Annual Review of Genetics*, 42, 83-107.
- Lazarus, D., Barron, J., Renaudie, J., Diver, P., & Türke, A. (2013). Cenozoic planktonic marine diatom diversity and correlation to climate change. *PloS One*, 9(1), e84857.
- Lopez, P. J., Descles J FAU - Allen, Andrew,E., FAU, A. A., & Bowler, C. (2005). Prospects in diatom research. *Current Opinion in Biotechnology*, 16(2), 180-186.
- Martin-Jézéquel, V., Hildebrand, M., & Brzezinski, M. A. (2000). Silicon metabolism in diatoms: Implications for growth. *Journal of Phycology*, 36(5), 821-840.

- Medlin, L. K. (2016). Evolution of the diatoms: Major steps in their evolution and a review of the supporting molecular and morphological evidence. *Phycologia*, 55(1), 79-103.
- Milligan, A. J., & Morel, F. M. M. (2002). A proton buffering role for silica in diatoms. *Science*, 297(5588), 1848-1850.
- Nunn, B. L., FAU, A. J., FAU, S. S., Tsai S FAU - Strzeppek, Robert,F., FAU, S. R., FAU, B. P., et al. (2009). Deciphering diatom biochemical pathways via whole-cell proteomics. *Aquatic microbial ecology*, 55(3), 241-253.
- Rabosky, D. L., & Sorhannus, U. (2009). Diversity dynamics of marine planktonic diatoms across the cenozoic. *Nature*, 457(7226), 183-186.
- Raven, J. A., & Giordano, M. (2009). Biomineralization by photosynthetic organisms: Evidence of coevolution of the organisms and their environment? *Geobiology*, 7(2), 140-154.
- Shrestha, R. P., & Hildebrand, M. (2015). Evidence for a regulatory role of diatom silicon transporters in cellular silicon responses. *Eukaryotic Cell*, 14(1), 29-40.
- Vandevenne, F. I., FAU, B. A., Schoelynck, J. F., Smis, A. F., Ryken N FAU,- Van Damme, Van Damme, S. F., et al. (2013). Grazers: Biocatalysts of terrestrial silica cycling. *Proc Biol Sci*, 280(1772), 20132083.
- Vardi, A., Thamatrakoln K FAU - Bidle, Kay,D., FAU, B. K., & Falkowski, P. G. (2008). Diatom genomes come of age. *Genome Biology*, 9(12), 245.

Wilhelm, C., Buchel, C. F., Fisahn, J. F., Goss, R. F., Jakob, T. F., Laroche, J. F., et al. (2006). The regulation of carbon and nutrient assimilation in diatoms is significantly different from green algae. *Protist*, 157(2), 91-124.

Acknowledgements

Many thanks to Jacqueline Stefels of the department of Ecophysiology of Plants for the support and insight provided during the writing of this thesis.