

Declining silicon to nitrogen ratios: effects on phytoplankton and plankton food webs

Dissertation

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To my family

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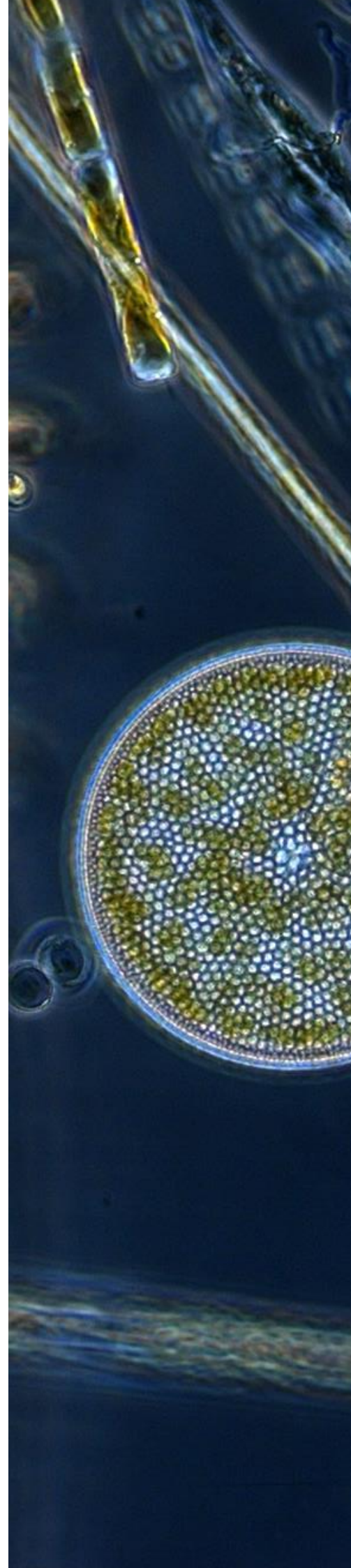
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Summary

Zusammenfassung



Summary

Increasing human activities on land, such as intensive farming, fossil fuel burning and river flow modifications alter nutrient cycles with implications for both terrestrial and marine ecosystems. The nitrogen cycle has been particularly affected: the amount of nitrogen available for primary producers has doubled due to artificial atmospheric nitrogen fixation. Consequently, more reactive nitrogen is reaching oceans via river run-off and atmospheric deposition. The silicon cycle, on the other hand, has been affected in an opposite direction and to a lesser extent: with increasing river damming, more silicate is biologically fixed in dam reservoirs and less of it is reaching the coastal oceans in a reactive form. These changes result in a decline in silicon to nitrogen (Si:N) ratios and can alter the composition of phytoplankton - small, but numerous organisms, providing the base of pelagic marine food webs.

Si:N ratios affect phytoplankton composition because nitrogen is required by all phytoplankton and silicon is essential only for certain groups, such as diatoms. Diatoms use silicate to build their porous cell walls and thus silicon availability can limit their growth. These organisms are abundant, especially in nutrient rich waters, and account for as much carbon fixed as the rainforests on land. Multiple experimental studies have shown that diatom proportion declines with decreasing Si:N ratios. Yet further knowledge of how this change in phytoplankton composition may affect the functioning of entire plankton communities is needed to ultimately understand and estimate the impacts of nutrient alterations on higher trophic levels and the marine carbon pump.

In this thesis, I experimentally assessed the impacts of changes in Si:N ratios on the complex interactions in the lower pelagic food web. Two mesocosm experiments were conducted where natural Baltic Sea plankton communities were exposed to a range of Si:N ratios and varying copepod grazing pressure. The results showed that a lowered Si:N ratio not only lowers the proportion of diatoms within the phytoplankton community, but also increases the abundance and biomass of non-silicifying groups of plankton, with implications for the quality and quantity of food available for mesozooplankton. Two conceptual models were developed in Chapter I to illustrate food web structures in low and high Si:N environments, concluding that lowered Si:N ratios result in more complex plankton food webs, which are known to lower energy transfer efficiency. An unexpected finding was that some diatom species were not affected by grazing, indicating that the efficiency of the “diatom-copepod” food chain may be

Summary

moderated by diatom edibility. In Chapter II, this aspect was investigated further, by assessing the effects of altered Si:N ratios on the nutritional value of plankton in terms of fatty acid and particulate nutrient indicators. The results showed that while high Si:N environments can be characterized by higher availability of essential fatty acids, ratios between particulate nutrients and selected fatty acids are more suitable for mesozooplankton when Si:N ratio is lowered.

Changes in phytoplankton composition with declining Si:N ratios observed in this thesis are in line with Tilman's Resource Ratio Theory, which states that ratios of limiting resources can determine the outcome of species competition. The applicability of this theory, however, has been questioned as it does not account for varying concentrations of resources. In Chapter III, this thesis presents evidence from natural communities that plankton composition responds to lowered Si:N ratios in a similar way both when nitrogen and when silicon concentrations are manipulated. However, while nutrient ratios are critical in determining community composition, absolute concentrations largely control the total biomass of phytoplankton. These findings contribute to the discussion on the ecological importance of nutrient ratios and concentrations and stress that resource ratios, in particular the ratio between silicon and nitrogen, can be used in predicting and modelling the outcome of species competition in natural phytoplankton communities.

In conclusion, anthropogenic manipulations of nitrogen and silicon cycles can have strong effects on plankton composition, biomass and trophic interactions. This thesis underlines that implications of these changes on higher trophic levels and ecosystem functioning are complex and future studies are needed to understand the role of selective grazing, phytoplankton quality and defense mechanisms in marine food webs.

Zusammenfassung

Die zunehmende Nutzung von Land und Ressourcen durch den Menschen, wie intensive Landwirtschaft, der Einsatz fossiler Brennstoffe oder der Umbau von Fließgewässern, haben einen direkten Einfluss auf die Nährstoffkreisläufe sowohl terrestrischer als auch mariner Ökosysteme. Insbesondere der Stickstoffkreislauf ist einer starken Veränderung durch menschliche Einflüsse unterworfen: Die Menge an Stickstoff, welcher den autotrophen Organismen zur Verfügung steht, wurde durch die künstliche Fixierung atmosphärischen Stickstoffs mittlerweile verdoppelt. Entsprechend nahm auch die Menge reaktiven Stickstoffs zu, der die Ozeane entweder durch Fließgewässer oder atmosphärischen Niederschlag erreicht. Der Siliziumkreislauf wurde im Gegensatz dazu durch menschliche Einflüsse zu einem geringerem Maße und in die entgegengesetzte Richtung beeinflusst: Durch die stärkere Verbauung von Flüssen, insbesondere mit Staudämmen, wird mehr Silizium bereits im Flusslauf biologisch gebunden und erreicht nicht die Ozeane. Diese Veränderungen anthropogenen Ursprungs bewirken, dass das Verhältnis der Elemente Silizium und Stickstoff (Si:N) abnimmt, was wiederum einen entscheidenden Einfluss auf die Zusammensetzung des Phytoplanktons haben kann. Die kleinen, aber äußerst zahlreichen Organismen des Phytoplanktons sind die Basis der Mehrzahl der marinen Nahrungsketten.

Das Si:N Verhältnis beeinflusst die Zusammensetzung des Phytoplanktons, da Stickstoff für alle Organismen essentiell ist, während Silizium nur für bestimmten Gruppen wie die Kieselalgen wesentlich ist. Kieselalgen benötigen Silizium um ihre porösen Zellwände zu bilden – entsprechend kann die Verfügbarkeit von Silizium deren Wachstum limitieren. Kieselalgen sind äußerst zahlreich, insbesondere in nährstoffreichen Gewässern, und zusammen sind sie für die Fixierung derselben Menge an Kohlenstoff verantwortlich wie die Regenwälder der Erde. Mehrere experimentelle Arbeiten konnten zeigen, dass die relative Häufigkeit der Kieselalgen im Phytoplankton mit abnehmendem Si:N Verhältnis ebenfalls abnimmt. Es ist jedoch von größerer Wichtigkeit zu verstehen, wie diese Änderung der Zusammensetzung des Phytoplanktons die Funktion des gesamten Planktons beeinflusst – um letztendlich abschätzen zu können, wie sich die anthropogene Änderung des Nährstoffeintrags auf die höheren Ebenen der Nahrungskette und die Funktion der essentiellen marinen Kohlenstoffpumpe auswirkt.

Zusammenfassung

In dieser Doktorarbeit untersuchte ich den Einfluss des sich wandelnden Si:N Verhältnisses auf die komplexen Interaktionen des unteren pelagischen Nahrungsnetzes. In zwei Mesokosmos Experimenten wurde das natürliche Plankton der Ostsee verschiedenen Si:N Verhältnissen sowie verschiedenen Beweidungsdrücken durch Copepoden ausgesetzt. Durch diese Experimente konnte gezeigt werden, dass ein reduziertes Si:N Verhältnis nicht nur den Anteil der Kieselalgen im Phytoplankton reduziert, sondern auch die Häufigkeit und die Biomasse von Planktongruppen, welche nicht auf Silizium angewiesen sind, erhöht. Diese Verschiebung beeinflusst wiederum die Qualität und Menge an Nahrung, welche dem Mesozooplankton zur Verfügung steht. In Kapitel 1 dieser Arbeit wurden zwei konzeptionelle Modelle entwickelt, welche die Struktur der Nahrungskette in Milieus mit niedrigen und hohen Si:N Verhältnissen illustrieren. Daraus konnte abgeleitet werden, dass reduzierte Si:N Verhältnisse in komplexeren planktonischen Nahrungsnetzen resultieren. Diese sind für ihre geringere Effizienz bzgl. des Energietransfers zu höheren Ebenen des Nahrungsnetzes bekannt. Es konnte jedoch die unerwartete Beobachtung gemacht werden, dass einige Kieselalgenspezies durch die Beweidung nicht beeinflusst wurden. Dies spricht wiederum dafür, dass die Beweidung der Kieselalgen durch Copepoden auch von der Fressbarkeit der Kieselalgen abhängt. Dieser Aspekt wurde in Kapitel II eingehender untersucht. Dazu wurde der Einfluss von veränderten Si:N Verhältnissen auf den Nahrungswert der Phytoplanktonbiomasse (Gehalt an bestimmtem Fettsäuren und mineralischen Nährstoffen) analysiert. Die Ergebnisse zeigten, dass während höhere Si:N Verhältnisse durch die bessere Verfügbarkeit essentieller Fettsäuren gekennzeichnet sind, das Verhältnis zwischen ausgewählten Fettsäuren und spezifischen Nährstoffen bei geringerem Si:N günstiger ist.

Die in dieser Arbeit beobachteten Änderungen der Zusammensetzung des Phytoplanktons mit abnehmendem Si:N Verhältnis stimmen mit Tilman's Resource Ratio Theory überein, welche besagt, dass Verhältnisse zwischen limitierenden Ressourcen das Ergebnis des Konkurrenzkampfes verschiedener Spezies bestimmen können. In Kapitel III dieser Arbeit konnte gezeigt werden, dass die Zusammensetzung des Phytoplanktons lediglich vom Si:N Verhältnis abhängt, unabhängig davon, ob dieses über die Zugabe von Silizium oder Stickstoff eingestellt wurde. Dieses Ergebnis ist von Bedeutung, da die Anwendbarkeit der Theorie aufgrund ihrer mangelnden Berücksichtigung von sich ändernden Nährstoffkonzentrationen mitunter umstritten ist.

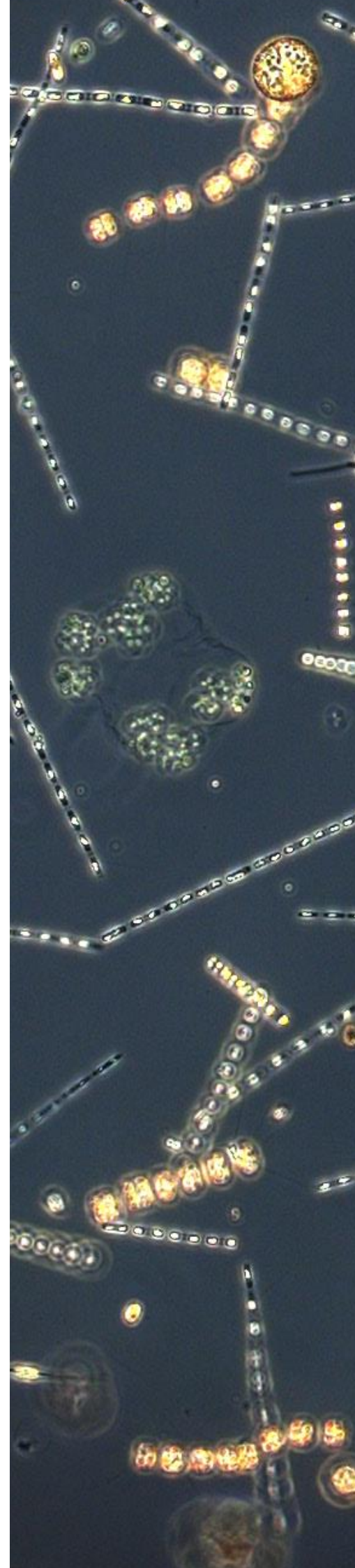
Obwohl die Nährstoffverhältnisse damit die Zusammensetzung des Phytoplanktons bestimmen, bestimmt ihre absolute Konzentration wesentlich seine Gesamtbiomasse. Dieses

Zusammenfassung

Ergebnis trägt zu der laufenden Diskussion bei, inwieweit Verhältnisse und Konzentrationen die Konkurrenz beeinflussen. Dabei konnte bestätigt werden, dass insbesondere das Verhältnis von Silizium zu Stickstoff dazu verwendet werden kann, die Zusammensetzung des natürlichen Phytoplanktons zu prognostizieren.

Zusammengefasst konnte gezeigt werden, dass die anthropogene Beeinflussung der Stickstoff- und Silizium-Kreisläufe einen profunden Effekt auf die Zusammensetzung, die Biomasse und die Nahrungsnetze des Planktons haben kann. Diese Arbeit betont, dass die Auswirkungen dieser Veränderungen für die höheren Ebenen der Nahrungsketten komplex sind und weiterführende Arbeiten angebracht sind, um die Rolle selektiver Beweidung, der Qualität des Phytoplanktons sowie deren Verteidigungsmechanismen in marinen Nahrungsketten besser verstehen zu können.

General Introduction



Introduction

Human activities are causing planetary scale changes in the Earth's system, including alterations of nutrient cycles. Changes in both the availability and stoichiometry of reactive nutrients reaching the ocean affect the biomass and composition of phytoplankton - phototrophic protists and cyanobacteria - responsible for half of the world's primary production. Taxonomic and functional diversity of phytoplankton is immense, which makes projecting the general direction of phytoplankton response to increasingly altered nutrient concentrations and ratios complicated. Phytoplankton provides the base of many marine food webs, yet the mechanistic understanding of how changes in phytoplankton composition affect plankton trophic interactions and the availability and quality of food for higher trophic levels is still limited. This thesis aims to advance our understanding of how changing availability of dissolved silicon, nitrogen and consequently altered silicon to nitrogen (Si:N) ratios affect phytoplankton and the structure and functioning of plankton communities. Additionally, this study will contribute to entangling the complexity of planktonic food webs in systems with varying plankton composition. Understanding the complexity of a system will allow for the simplification that is needed to derive realistic modeling and predictions of future conditions in the oceans.

Changing nutrient conditions in marine systems

With increasing human populations and consumption of resources, anthropogenic activities on land are affecting ecosystems in the oceans from the bottom up. Multiple nutrient cycles have been altered by increasing emissions related to intensified farming, wastewater management, energy production and landscape alterations (Farmer, 2018, Stevens, 2019, Treguer & De La Rocha, 2013). Changes in nitrogen and phosphorus cycles are especially important, as these elements are essential nutrients for all living organisms. While freshwater primary production is often limited by dissolved phosphorus availability, nitrogen limitation is considered to be more important for marine primary producers (Howarth & Marino, 2006, Vitousek & Howarth, 1991).

Nitrogen makes up 78.1% of Earth's atmosphere, yet most of it is found in an unreactive form (N_2) which is unavailable for the majority of autotrophic organisms (Stevens, 2019). Dinitrogen can be turned to bioavailable forms, such as nitrates, nitrites and ammonia, among others, via natural or anthropogenic fixation. Natural nitrogen fixation in the oceans is mainly performed

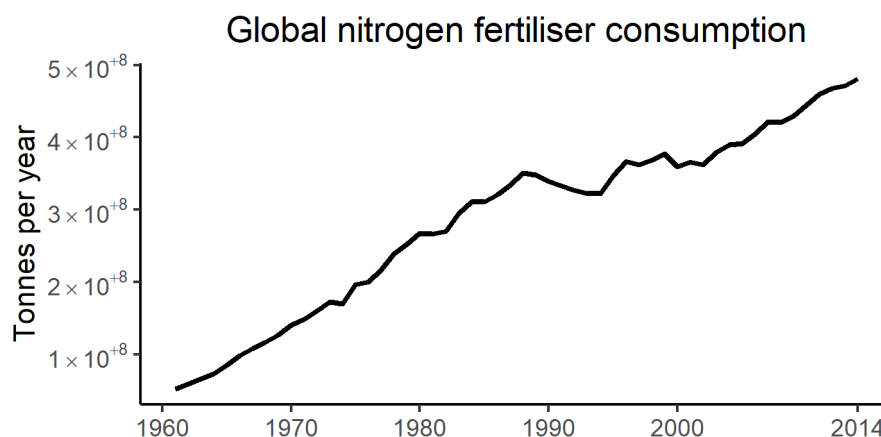


Figure I Nitrogen fertilizer use globally from 1961 to 2013. Modified from Roser and Ritchie (2013)

by diazotrophic bacteria, which are able to turn dinitrogen gas into ammonia using the enzyme nitrogenase (Zehr & Capone, 2020). The majority of anthropogenically produced reactive nitrogen originates in the Haber–Bosch process, which converts dinitrogen gas to ammonia by a reaction with hydrogen under high temperatures and pressures (Appl, 2011). Over the past century, anthropogenic nitrogen fixation exceeded natural nitrification and has doubled the global cycling of nitrogen (Fowler *et al.*, 2013, Stevens, 2019). With increasing demand for nitrogen-based fertilizers (Fig. I), the production of reactive nitrogen will likely further increase. As only ~40% of nitrogen applied to farmer’s fields is recovered in agricultural plants (Conant *et al.*, 2013), nitrogen pollution to inland water bodies, coastal oceans and the atmosphere are increasing. Further nitrogen pollution is caused by the release of animal and human waste and combustion of fossil fuels (Stevens, 2019). While reactive nitrogen compounds from agriculture and waste-water reaches coastal oceans via river run-off and usually have only local impacts, oxidized forms of nitrogen from burning fossil fuels can travel far with the air masses and be deposited in the open ocean. Atmospheric deposition of reactive nitrogen to the ocean is thought to have tripled over the last 100 years (Duce *et al.*, 2008, Lamarque *et al.*, 2013) and despite successful efforts to reduce the emissions of oxidized nitrogen in some parts of the world, global emissions continue to increase (Yang & Gruber, 2016).

Silicon is another important element in marine ecosystems. Most organisms require trace concentrations of silicon but for some, such as diatoms, radiolarians and siliceous sponges, silicon is an essential nutrient needed to build their cell walls and other silicified structures. Silicon, just as nitrogen, is an abundant element, yet it’s bioavailable form – silicic acid (Si(OH)_4) – can be scarce. The main source of dissolved silica is rock weathering and over

70% of the oceanic silica originates from river run-off (Treguer & De La Rocha, 2013). Smaller amounts of reactive silicon reach the ocean via dust deposition, glacial melt water, groundwater discharge and hydrothermal input (Hawkings *et al.*, 2017, Rahman *et al.*, 2019, Treguer & De La Rocha, 2013).

While the silicon cycle has not been affected by human activities as severely as the nitrogen cycle, modifications in river networks are thought to have lowered silicic acid loads reaching the oceans (Laruelle *et al.*, 2009). With increasing damming of rivers, the rivers' flow gets slower and sedimentation in dam reservoirs is prolonged, meaning that more silicon is fixed biologically by diatoms in rivers and sinks down before entering coastal oceans. Several regions have indeed reported a decline in silicic acid concentrations (Fig. II). While decreases in reactive silicon in river run-off affect mainly coastal and estuarine systems, declines in silicic acid concentrations have also been observed in several open ocean areas in the North Atlantic regions (Hátún *et al.*, 2017, Macovei *et al.*, 2019, Rey, 2012). These declines are likely attributed to natural multi-decadal variability in winter convection depths and a retraction of the nutrient rich subpolar gyre, which might have been amplified by global warming (Hátún *et al.*, 2017).

With increasing loads of reactive nitrogen and decreasing or stable supply of silica, not only the concentrations of these nutrients change but also the stoichiometric ratio between them.

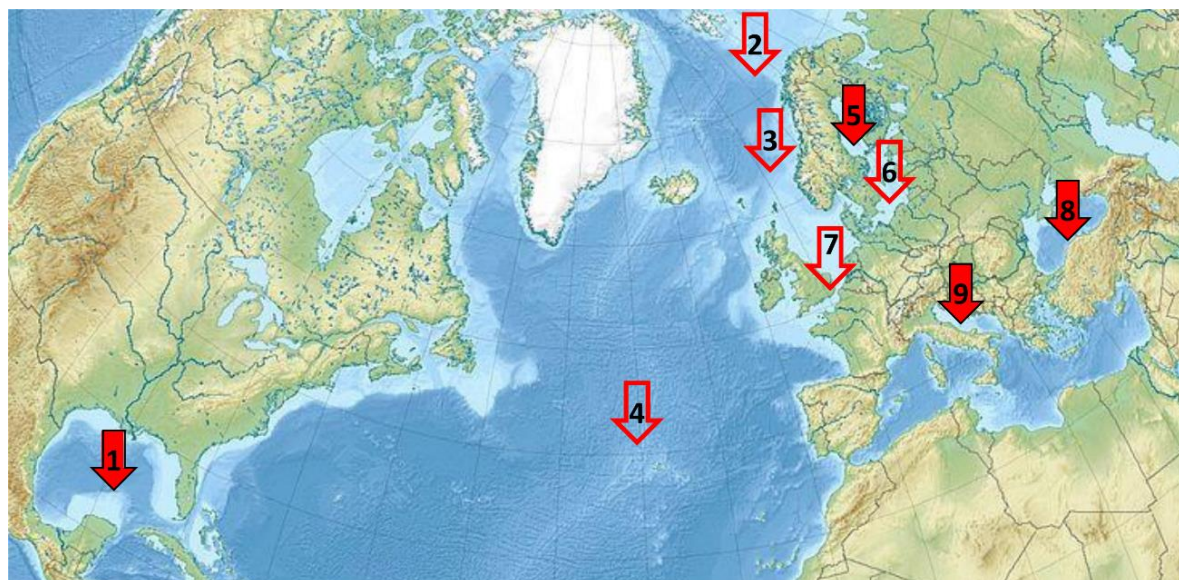


Figure II Cases of declines in Si:N ratios (filled arrows) or silicate concentrations (open arrows) in the North Atlantic region. Observations reported by: 1 - Justić *et al.* (1995), Parsons and Dortch (2002); 2 - Rey (2012); 3 - Hátún *et al.* (2017); 4 - Macovei *et al.* (2019); 5 - Humborg *et al.* (2000), Wasmund *et al.* (2013); 6 - Papush and Danielsson (2006); 7 - Wiltshire *et al.* (2010), 8 - Humborg *et al.* (1997), Yunev *et al.* (2007); 9 - Justić *et al.* (1995).

This is of importance, as resource ratios can determine the outcome of species competition and consequently community structure (Tilman, 1982a, Tilman, 1982b). Therefore, when the Si:N ratio declines, one could predict that diatoms and other silicified groups of plankton can have a competitive disadvantage to other phytoplankton, which do not require silicon.

The optimal ratio between silicon and nitrogen which diatoms require is thought to be close to 1:1 (Brzezinski, 1985). Almost a hundred years ago Redfield (1934) observed that the ratios between essential nutrients within phytoplankton biomass across different regions of the ocean are rather constant and they coincide to the nutrient ratios in the deep ocean. When updated to include silicon concerning diatoms, this ratio equals to C:Si:N:P = 106:15:16:1 (Brzezinski, 1985). While deviations from this ratio occur, and each species may have specific optimal ranges, Redfield's ratio can be a useful indicator for nutrient limitation. Consequently, if Si:N ratio declines below 15:16 this may induce silicate limitation in diatoms.

Diatoms – major primary producers

In the ocean, phytoplankton are the major consumers of dissolved nutrients. These protists, drifting in the water column, use inorganic nutrients to perform photosynthesis and build up their biomass which makes phytoplankton responsible for roughly half of the global primary production (Field *et al.*, 1998). Around one third of this contribution is provided by diatoms - a group of phytoplankton particularly abundant in nutrient rich and temperate waters (Malviya *et al.*, 2016).

Diatoms are the most diverse group of phytoplankton with estimated 200 000 different species (Kooistra *et al.*, 2007). Their size ranges from a few micrometers to several millimeters, they can be either radially (centric diatoms) or bilaterally symmetric (pennate diatoms), occur in colonies or solitary cells, and in many different shapes (Fig. III). Diatoms are not only morphologically, but also genetically diverse. The first genome sequences of diatoms showed that two diatom species can be genetically as different as humans and fish (Bowler *et al.*, 2008, Gross, 2012).

One feature which unites all diatom species is their silicified cell walls – called frustules. Diatoms use silicic acid which reaches their cells either via diffusion or silicic acid transporters. Here, it is efficiently precipitated into opaline silica to form their porous cell walls. The encrusted cell walls offer diatoms some protection from grazers (Pancic *et al.*, 2019). Additionally, it is suggested, that silica in diatom cell walls acts as an effective pH buffer,

enabling to convert bicarbonate to CO₂ more efficiently (Milligan & Morel, 2002). Thus, diatoms are important in linking global silicon and carbon cycles. While recycling of biogenic silica is a slow process comparing to nitrogen, it is estimated that each silicon atom reaching the ocean is incorporated into diatom frustules around 39 times before sinking out to the sea floor (Treguer *et al.*, 1995).

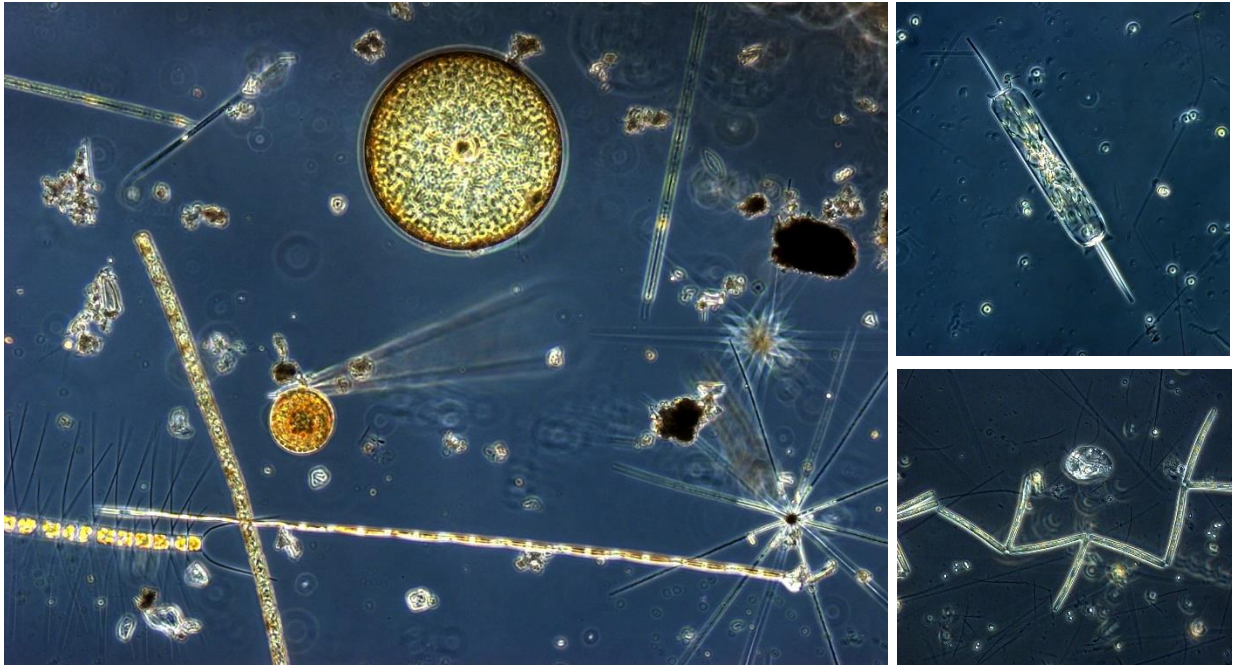


Figure III Diverse shapes in a diatom dominated phytoplankton community.

Plankton trophic interactions

Historically, trophic interactions in the marine microbial world were thought to resemble those on land: chlorophyll containing protists were considered as miniature “plants” and the microorganisms able to feed on them – as “animals”. Thus, aquatic food chains were first depicted as direct linear links from phytoplankton to nekton via zooplankton. A classic example of such chain would be a diatom-copepod-planktivorous fish chain. Yet in the 1980s, it was observed that bacteria can be important consumers of dissolved carbon, and consequently, important food source for bacterivorous protists (Azam *et al.*, 1983, Pomeroy, 1974). The term “microbial loop” was introduced to describe the pathway of how dissolved organic carbon is being transferred to higher trophic levels via bacterial biomass (Azam *et al.*, 1983). It is now recognized that the microbial loop is an important part of complex microbial food webs (Sherr & Sherr, 1988).

Another aspect, adding complexity to our understanding of plankton trophic interactions is the widespread mixotrophy among planktonic organisms. Mixotrophy refers to an organism's ability to both perform photosynthesis and consume prey. Contrary to the life on land, where mixotrophy is represented by only roughly a dozen of carnivorous plant genera, mixotrophic nutrition among protists in the ocean appears to be a rule rather than an exception (Caron, 2016, Flynn *et al.*, 2012). Planktonic organisms that exhibit mixotrophy belong to diverse taxonomic groups whose sizes range over four orders of magnitude (Flynn *et al.*, 2019). The implications of widespread mixotrophy in marine systems to plankton dynamics and nutrient fluxes are still to be understood.

Diatoms are one of the few groups of plankton which are strictly autotrophic (i.e. unable of phagotrophy). They form blooms in nutrient rich waters, such as upwelling areas or temperate and boreal seas after winter stratification, where silicon is not limiting. These blooms provide a major food source for zooplankton, such as copepods and krill. Diatom based food chains are thought to be short and efficient – with just two links between primary producers and fish, little energy is lost in the transfer between trophic levels (Sommer *et al.*, 2002).

In nutrient rich eutrophicated waters, silicon is often limiting relative to other macro-nutrients and non-diatom phytoplankton dominate (Sommer *et al.*, 2002). Most of the toxic, harmful or nuisance species do not belong to diatoms and it is thought that harmful algal blooms are more frequent under eutrophic conditions (Smayda & Reynolds, 2003). Among the few toxic diatoms, lightly silicified *Pseudo-nitzschia* spp. have been found to also increase under eutrophic conditions, when silicate declines relative to nitrogen (Parsons & Dortch, 2002). Even though copepods, the most abundant mesozooplankton in the ocean, are able to feed selectively and avoid toxin producing phytoplankton (Schultz & Kiørboe, 2009), it is likely that the overall suitable food availability under high nutrient, low Si:N conditions is lower (Sommer *et al.*, 2002). Uneaten phytoplankton are decomposed and enter the microbial food web: dissolved organic carbon is taken up by bacteria which are consumed by mixotrophic and heterotrophic microplankton and nanoflagellates, which may later be consumed by mesozooplankton (Sommer *et al.*, 2002). As a result, plankton food webs under eutrophicated and silicate limited conditions have more trophic levels compared to non-eutrophicated food webs, and hence are less efficient in transferring energy from primary producers to secondary consumers.

General Introduction

In recent decades, however, it became apparent that not only the quantity of accessible food for grazers is important, but also its quality. Diatoms in this aspect may not be superior to other groups of plankton (Ragueneau *et al.*, 2006). Dinoflagellates, for instance, contain more organic matter per volume than diatoms (Hitchcock, 1982) and may have more favorable ratios between essential fatty acids (Jónasdóttir, 2019). Copepods are able to feed selectively, for instance it has been observed that they would preferentially feed on ciliates or dinoflagellates in a mixture with diatoms (Jones & Flynn, 2005, Saage *et al.*, 2009). Additionally, evidence has accumulated that diatoms produce secondary metabolites – oxylipins – which interfere with copepod reproduction and embryonic development; however, the effects on plankton communities and trophic interactions remain controversial (Russo *et al.*, 2018).

The work presented in this thesis improves the mechanistic understanding of how changing Si:N ratios affect plankton communities and trophic interactions among them. This knowledge is needed to understand and respond to the impacts of increasingly altered nutrient conditions on major contributors to global carbon cycling and the productivity of fisheries - phytoplankton. Furthermore, understanding how changes in nutrient availability and composition are transferred up the planktonic food web is essential for modelling and predicting the future conditions in the oceans.

Aim of the study and thesis outline

The aim of this thesis is to advance the understanding of how changing availability of dissolved silicon, nitrogen and consequently altered silicon to nitrogen (Si:N) ratios affect phytoplankton composition and the structure and functioning of plankton communities. In detail, this thesis addresses the following questions:

- 1) How does plankton composition respond to changing Si:N ratios under varying grazing pressures?
- 2) Does the response of plankton communities differ depending on which component of the Si:N ratio is manipulated?
- 3) Do changes in plankton communities affect plankton food web structure and mesozooplankton abundance?
- 4) Do lowered Si:N ratios affect phytoplankton quality as a food source for higher trophic levels?

To answer these questions, I employed mesocosm experiments using summer Baltic Sea plankton communities and manipulated silicon and nitrogen concentrations to achieve a range of Si:N ratios. As phytoplankton communities under natural conditions are rarely affected only by bottom-up controls, I additionally applied two different copepod densities to account for the top-down effects on the response of plankton communities to changing nutrient ratios. The response was followed over several trophic levels, including bacteria, phytoplankton, microzooplankton, potential mixoplankton and mesozooplankton.

The doctoral thesis is presented in three chapters, addressing how different supply ratios of silicon and nitrogen affect different aspects of the structure and functioning of plankton communities. Each chapter presents an individual study, published or prepared for publication in peer-reviewed scientific journals.

Chapter 1

The first thesis chapter provides insights into how declining Si:N ratios due to a decrease in silicic acid concentrations affect phytoplankton community and plankton food webs. While it is known that a decline in Si:N ratios can cause a switch from diatom to flagellate dominated communities, it is not fully understood what effects this has on plankton trophic interactions and major phytoplankton grazers – copepods. In this chapter, I provide answers to the following questions: How do changing Si:N ratios affect individual groups of plankton? Do shifts in

phytoplankton community structure affect trophic relationships between planktonic organisms? Does this have an effect on copepod abundance?

Chapter 2

In the second chapter of the thesis, I question if changes in Si:N ratios in nutrient supply affect the nutritional quality of phytoplankton as a food source for higher trophic levels. I hypothesize that changes in phytoplankton taxonomic composition due to a decline in Si:N ratios have effects on the chemical composition of seston, particularly on the compounds important for the nutrition of mesozooplankton. I present the results based on particulate carbon to nitrogen and carbon to phosphorus ratios, essential fatty acids concentrations and the ratios between them as nutritional value indicators. Subsequently, an evaluation of changes in nutritional quality due to altered supply of Si:N ratio is presented.

Chapter 3

Si:N ratio in nutrient supply can change either due to a decrease in silicate, or an increase in reactive nitrogen (or both). In the first two chapters, I show in detail how plankton communities and their chemical composition change when the concentration of dissolved silicon declines. Yet in nature, increasing nitrogen loads might be a more important reason behind the decrease in Si:N ratios. While silicon availability affects mainly diatoms, nitrogen is important for all groups of plankton and thus a change in nitrogen concentration can have a direct effect on total phytoplankton biomass. In this chapter, I present results from a mesocosm experiment where nitrogen concentration was manipulated to achieve the same Si:N ratios as reported in the previous chapters, where silicon concentration was manipulated. I discuss if and how the response of plankton biomass and community composition differs based on whether Si:N ratio is altered due to silicate decline or nitrogen increase. Consequently, this chapter advances the understanding of how ratios and concentrations of limiting resources affect community structure.

Chapter I

Decrease in diatom dominance at
lower Si:N ratios alters plankton
food webs



Decrease in diatom dominance at lower Si:N ratios alters plankton food webs

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Abstract

Many coastal oceans experience not only increased loads of nutrients but also changes in the stoichiometry of nutrient supply. Excess supply of nitrogen and stable or decreased supply of silicon lower silicon to nitrogen (Si:N) ratios, which may decrease diatom proportion in phytoplankton. To examine how Si:N ratios affect plankton community composition and food-web structure we performed a mesocosm experiment where we manipulated Si:N ratios and copepod abundance in a Baltic Sea plankton community. In high Si:N treatments diatoms dominated. Some of them were likely spared from grazing unexpectedly resulting in higher diatom biomass under high copepod grazing. With declining Si:N ratios dinoflagellates became more abundant under low and picoplankton under high copepod grazing. This altered plankton food-web structure: under high Si:N ratios, edible diatoms were directly accessible food for copepods, while under low Si:N ratios microzooplankton and phago-mixotrophs (mixoplankton) were a more important food source for mesograzers. The response of copepods to changes in the phytoplankton community was complex and copepod density-dependent. We suggest that declining Si:N ratios favor microzoo- and mixoplankton leading to increased complexity of planktonic food-webs. Consequences on higher trophic levels will, however, likely be moderated by edibility, nutritional value or toxicity of dominant phytoplankton species.

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Introduction

Silicon is one of the most common elements on land, yet can be a limiting nutrient for some phytoplankton functional groups (e.g. diatoms, silicoflagellates) in the ocean. Dissolved silicon is mainly produced by rock weathering and transported via land run-off and rivers to the ocean. Coastal eutrophication and river damming can decrease silicon loads reaching the ocean due to enhanced biogenic silica sedimentation (Laruelle *et al.*, 2009, Ragueneau *et al.*, 2006). Meanwhile supply of other nutrients, such as nitrogen and phosphorus, has increased due to nutrient pollution from a range of anthropogenic sources (Howarth *et al.*, 1996). In addition, biologically available forms of dissolved nitrogen and phosphorus are less prone to sedimentation and are recycled faster than silicon (Parsons & Harrison, 1983). This alters not only overall nutrient availability but also silicon to nitrogen (Si:N) and silicon to phosphorus (Si:P) stoichiometry in nutrient supply. Diatoms, often the dominant group among abundant eukaryotic phytoplankton and major contributors to oceanic primary production, require silicic acid for building their cell walls called frustules (Benoiston *et al.*, 2017, Malviya *et al.*, 2016). As diatom biomass Si:N:P ratios are relatively stable and on average equal to 15:16:1 (Redfield-Brzezinski ratio, Brzezinski (1985)), decreases in silicate concentrations and Si:N and Si:P ratios in coastal waters may induce silicon limitation in diatoms.

The response of diatoms to silicon limitation has been studied since the beginning of the 20th century (Officer & Ryther, 1980, Pearsall, 1932, Richter, 1904). However, the environmental impact of Si:N decline is not yet fully understood. During Si-limitation non-silicifying groups of phytoplankton have a competitive advantage over diatoms. The switch from diatom-dominated to flagellate-dominated communities has been documented both in experimental studies (Sommer, 2009, Sommer, 2004) and field observations (Smayda, 1990). Sommer *et al.* (2002) proposed a food web scheme where food chains in eutrophic environments with lowered Si:N were less efficient and fueled heterotrophically due to a high proportion of inedible algae. However, the authors did not account for the role of microzooplankton and mixoplankton (*sensu* Flynn *et al.* (2019)) in transferring nutrients from small phytoplankton inedible to copepods while simultaneously increasing their biochemical value (trophic upgrading, (Klein Breteler *et al.*, 1999)) or the fact that some non-silicifying plankton (e.g. dinoflagellates) can be nutritious food for copepods (Flynn *et al.*, 2019, Sommer *et al.*, 2005). Additionally, while eutrophic conditions are related to toxic or nuisance algal blooms, it is less clear whether this is due to nutrient enrichment or changing stoichiometric ratios among nutrients.

Traditionally, it has been assumed that diatom dominated communities provide the best food for copepods (Kleppel, 1993) and that the direct trophic link between copepods and diatoms ensures the highest energy transfer efficiency (Sommer *et al.*, 2002). This view has been challenged by accumulating evidence from laboratory-scale experiments, suggesting that diatoms can release biotoxins in response to grazing which interferes with copepod growth and reproduction (Ianora *et al.*, 2003, Poulet *et al.*, 1994) or may simply be nutritiously insufficient (Jones & Flynn, 2005). Subsequent mesocosm experiments and field observations have yielded contradicting results: some confirmed the deleterious effect of diatoms on copepods (Jenkins & Black, 2019, Nejstgaard *et al.*, 2001) while others reported no or even positive impacts (Irigoien *et al.*, 2002, Sommer, 2009). Also, while currently there is scientific consensus that some diatoms are toxic to some copepods, toxins acting on development and reproduction cannot not be considered as a defense mechanism against copepods (Flynn & Irigoien, 2009). Thus, it is yet to be understood to what extent diatom toxicity shapes plankton communities and plankton food webs in the wild.

To better understand the mechanisms behind how silicon limitation affects planktonic food webs in nutrient rich environments we performed a mesocosm experiment manipulating Si:N ratios and mesozooplankton grazing pressure. Our working hypotheses were:

- 1) In high Si:N waters, diatoms dominate phytoplankton biomass and correlate negatively with copepod abundance, indicating that the “traditional” diatom-copepod food chain prevails
- 2) In low Si:N waters, non-silicifying phytoplankton dominates. Microzoo- and mixoplankton is more abundant making food chains more complex and energy transfer less efficient due to additional trophic levels.
- 3) Consequently, copepod abundance increases with increasing Si:N ratios

Materials and Methods

Study area and experimental design

The experiment was conducted in a mesocosm system set up at the dock of GEOMAR (54°19'46.9"N 10°08'59.4"E) in the Kiel Fjord, Western Baltic Sea between 17 June and 6 July, 2016. We used twenty 20 L transparent plastic water-bags as experimental units which were filled with near-surface Baltic Sea water which was prefiltered with 125 µm mesh size sieves to exclude the majority of adult mesozooplankton grazers. In order to maintain ambient

temperature, the bags floated in water baths. They were randomly distributed over four larger (1.4 m³) containers each equipped with a flow through system and a plastic lid. We fully crossed five Si:N ratio treatments with two grazing regimes and had two replicates for each treatment. While nitrogen (NaNO₃) and phosphorus (KH₂PO₄) were added to all treatments at the beginning of the experiment to reach a constant concentration of 40 µmol L⁻¹ dissolved nitrogen (sum of NO₃⁻, NO₂⁻ and NH₄⁺) and 2.5 µmol L⁻¹ phosphate (PO₄³⁻), silicon was added as silicate (Na₂SiO₃·9H₂O) to achieve the following Si:N ratios: 0.25:1, 0.4:1, 0.55:1, 0.7:1 and 0.85:1 (at target concentrations of 10, 16, 22, 28 and 34 µmol L⁻¹ respectively).

Copepods for grazing manipulation were collected in the Kiel canal (54°22'15.7"N 10°06'22.4"E) on May 19, 2016 and kept in culture until the start of the experiment. Adult copepods of *Eurytemora affinis* (filter feeder for which selective feeding has been documented (Merrell & Stoecker, 1998, Tackx *et al.*, 2003)) were added to half of the bags at a target concentration of 30 individuals per liter (ind L⁻¹) once the phytoplankton bloom had established (on the day 5 of the experiment, when measured relative fluorescence values stopped growing exponentially) to mimic natural copepod densities at this time of year (high copepod treatments). Aliquots of pre-filtered copepod culture water were added to the remaining bags which had lowered grazer numbers due to prior sieving (low copepod treatments). Due to an experimental failure one experimental unit (with copepods, Si:N = 0.85:1) had to be excluded from the analysis.

Sampling and sample analysis

Water samples to determine the starting conditions (day 1 of the experiment) were taken from the water tank after filtering but before filling the experimental bags in duplicates for plankton and in triplicates for nutrient and bacteria analysis. Samples for dissolved nutrient content were also taken from each individual treatment unit after the nutrient addition.

During the experiment, water temperature, salinity, pH and relative fluorescence were measured daily. Experimental bags were turned over manually several times before sampling, ensuring mixing within experimental units. Water samples for plankton and nutrient analyses were taken three times per week (200-400mL, Monday, Wednesday and Friday). To measure dissolved nutrient (NO₃⁻/NO₂⁻, NH₄⁺, SiO₄⁻ and PO₄³⁻) concentrations, samples were filtered using pre-washed (10% HCl) cellulose acetate filters immediately and frozen at -20°C until nutrient measurement according to protocols by Hansen and Koroleff (1999) with an auto-

analyser (Skalar, SANPLUS; Breda/Netherlands). Relative fluorescence was measured directly after sampling using a fluorometer 10-AU (Turner Design).

Samples for micro- ($>20\ \mu\text{m}$) and larger nanoplankton ($>5\ \mu\text{m}$) counts were fixed with Lugol's iodine solution, stored and counted according to Utermöhl's (1958) inverted microscope method. Where possible, at least 100 individuals per species were counted giving $\pm 20\%$ accuracy (Andersen, 2005) and 20 randomly selected cells were measured in order to estimate species' biovolume. Biovolume of nano- and microplankton was estimated after approximation to the closest geometric form according to Hillebrand et al. (1999) and then converted to carbon biomass based on the allometric conversion suggested by Menden-Deuer & Lessard (2000). While phytoplankton was counted in all samples collected, microzooplankton (ciliates and larger dinoflagellates) were quantified microscopically at the start of the experiment, during the phytoplankton bloom and at the end of the experiment. Lugol's fixation can affect biovolume of the plankton stored and we acknowledge that this might have affected the estimation of cryptophytes and some flagellates. For small flagellates, small photosynthetic nanoplankton ($2\text{--}5\ \mu\text{m}$) and picoplankton this limitation was avoided by counting samples immediately after sampling using flow-cytometry (FACScalibur, Becton Dickinson). Bacteria abundance was estimated by flow-cytometer as well. 3mL of water sample was analyzed for autotrophic picoplankton and small nanoplankton counts and 2mL for bacteria counts. The flow rates for flow-cytometry were $35\ \mu\text{L min}^{-1}$ and $8\ \mu\text{L min}^{-1}$ for phytoplankton and bacteria respectively. Picoplankton biovolume was converted to carbon biomass using modified Menden-Deuer & Lessard (2000) method with a conversion factor of $0.157\ \text{pg C}\ \mu\text{m}^3$ (Sommer *et al.*, 2012b) to avoid overestimation of the biomass of small phytoplankton. Total biomass of different plankton functional groups was converted to relative biomass – a percent composition of each group to the total phytoplankton biomass (or microplankton biomass in the case of ciliates).

For copepod numeric abundance estimation, mesozooplankton was collected at the end of the experiment using $125\ \mu\text{m}$ mesh size filter, immediately Lugol-fixed and stored in the dark. The sample was split using Motodo plankton splitter and a quarter of each stored sample was analysed. Copepod adults were counted and identified to species level, copepodites and nauplii to family level.

Statistical analysis

To assess how community composition changed in relation to Si:N ratios and grazing pressure applied, relative biomass data of different plankton groups was analyzed using beta regression models with the *betareg* package in R (Cribari-Neto & Zeileis, 2010). Beta regression was chosen, as it provides more accurate variation and distribution rates for relative biomass data as it is bound to the interval between 0 and 1 (Ferrari & Cribari-Neto, 2004). The strength of predictors (initial Si:N ratios and copepod grazing level) was then further examined using likelihood ratio tests (*lrtest* from *lmtest* package (Zeileis & Hothorn, 2002) and *anova* function in R). To examine treatment effects on total plankton biomass, copepod and bacteria abundance we used generalized linear models (GLM) with a gamma distribution as this data was continuous, non-negative and slightly positive-skewed. For plankton biomass and copepod abundance data a log link was used and for bacteria abundance an identity link.

The effects were assessed for “bloom” and “post-bloom” periods. “Bloom period” was defined as the period at and around the biomass maximum (days 6 to 11) and “post-bloom period” as the period where phytoplankton biomass reached a plateau after declining following the bloom (days 15 to 20 of the experiment). We used mean biomass and numeric abundance values over three sampling days for the respective periods

To examine the strength of trophic interactions between different groups of plankton we calculated correlation coefficients using data across the treatments. We used Spearman correlation coefficients, assuming positive correlation to represent bottom-up and negative correlation top-down control (Boyce *et al.*, 2015). For correlation analysis during the bloom peak period, we used data from day 6 of the experiment due to limited availability of microzooplankton data. For the post-bloom period we used copepod data from the last day of the experiment and the average values over the course of the post bloom period for the remaining plankton groups. All statistical analysis and data visualizations were conducted in R version 3.2.4 (R Core Team, 2019).

Results

Dissolved nutrient concentrations

Dissolved nutrient concentrations declined sharply during the first six days of the experiment and remained largely below detection limits until the end of the experiment (Fig. 1.1). Silicate concentrations in the lowest Si:N treatments were exhausted by the day 4 and by the day 6 in the remaining treatments. Dissolved inorganic nitrogen (DIN) at the start of experiment was

composed of $0.14 \mu\text{mol L}^{-1} \text{NO}_2^-$, $33.7 \mu\text{mol L}^{-1} \text{NO}_3^-$ and $4.7 \mu\text{mol L}^{-1} \text{NH}_4^+$. DIN was exhausted by the day 6 of experiment. Si:N ratios increased in all but the lowest Si:N treatments on day 4 of the experiment. Phosphate was exhausted by the day 4 of the experiment: at the start of the experiment phosphate was $0.5 \mu\text{mol L}^{-1}$ lower than the target concentrations ($2 \mu\text{mol L}^{-1}$ instead of $2.5 \mu\text{mol L}^{-1}$). This resulted in slightly elevated N:P ratios (20:1 instead of 16:1) which could have possibly induced phosphorus stress. Similarly, N:P ratios increased in most of the treatments, followed by a decline on day 6 of the experiment.

Plankton abundance, biomass and composition

Phytoplankton composition at the start of experiment was diverse, with diatoms contributing close to 30%, cryptophytes and unidentified small flagellates 40% and autotrophic picoplankton around 20% to the total phytoplankton biomass (Table 1). Microzooplankton was mostly represented by ciliates (dominant genera: *Strobilidium*, *Codonellopsis*, *Leegardiella*,

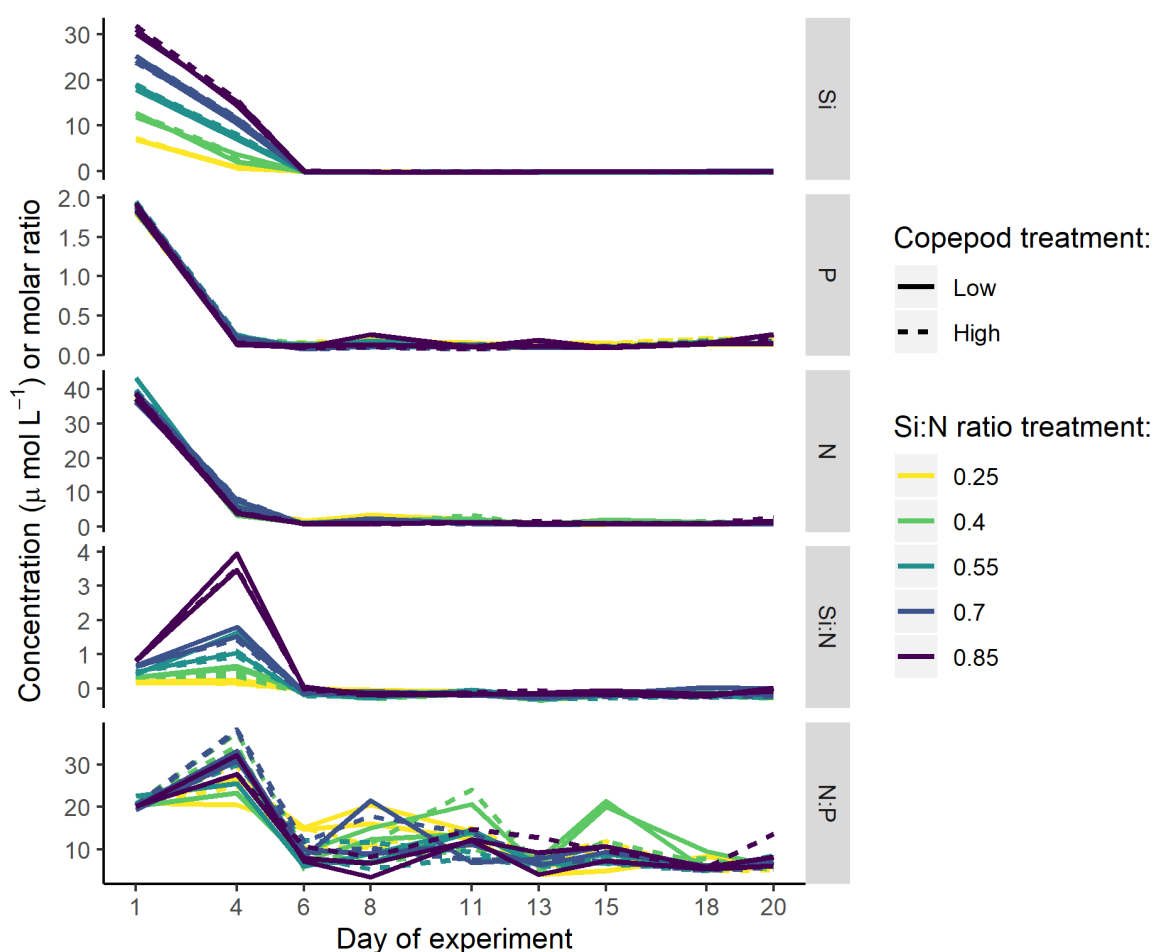


Figure 1.1 Dissolved inorganic nutrient concentrations over the course of experiment. P - PO_4^{3-} , N - sum of NO_3^- , NO_2^- and NH_4^+ , Si - SiO_4^- ; Si:N and N:P represent molar ratios between the elements.

Strombidium), with a biomass of 6.09 (sd=1.52) $\mu\text{g C L}^{-1}$, which made up a quarter of the total plankton biomass.

After nutrient addition, total phytoplankton biomass rapidly increased in all treatments, reaching peak biomass levels at days 6–11 (102–335 $\mu\text{g C L}^{-1}$, Bloom period, Fig. 1.2a). During the bloom phytoplankton biomass significantly increased with increasing initial Si:N ratios ($\chi^2=2.621$, $\text{df}=1$, $p<0.001$, Fig. 1.2b). During this period, the phytoplankton community was strongly dominated by diatoms, which contributed more than 80% of the total biomass in all treatments (Fig. 1.2d), with *Skeletonema* sp., *Cerataulina pelagica* and *Thalassionema nitzschioides* being the most common species (Fig. 1.3). With increasing Si:N ratio treatments diatom relative biomass increased while all remaining planktonic functional groups declined (Table 1.2, Fig. 1.2e, h, k, n, t, w). Diatom carbon biomass increased with increasing initial Si:N ratios also in absolute terms, while chlorophyte and picoplankton carbon biomass decreased under high copepod grazing and cyanobacteria and dinoflagellate biomass decreased in low copepod grazing treatments (Fig.S1.1).

Copepod grazing did not affect the total phytoplankton biomass and relative biomass of any plankton groups significantly during the bloom period (Fig. 1.2), with the exception of the lowest Si:N ratio treatment where picoplankton relative biomass was significantly but slightly higher in the high copepod treatments compared to low-copepod treatments (Table 1.2, Fig. 1.2k).

Table 1.1 Biomass of different plankton functional groups at the starting day of the experiment. Results represented as mean (standard deviation), ($n_{\text{bacteria}}=3$, $n_{\text{remaining groups}}=2$).

Functional group	Carbon biomass ($\mu\text{g C L}^{-1}$)	Relative phytoplankton biomass
Diatoms	5.21 (0.16)	0.29
Unidentified nanoflagellates	4.12 (0.35)	0.23
Cryptophytes	4.05 (0.10)	0.23
Picoplankton	3.66 (0.15)	0.21
Dinoflagellates	0.33 (0.11)	0.03
Chrysophytes	0.22 (0.05)	0.01
Total phytoplankton biomass	17.59 (0.92)	
Ciliates	6.09 (1.52)	
	Abundance (ind. L^{-1})	
Bacteria	2.93×10^6 (2.94×10^4)	

Chapter I

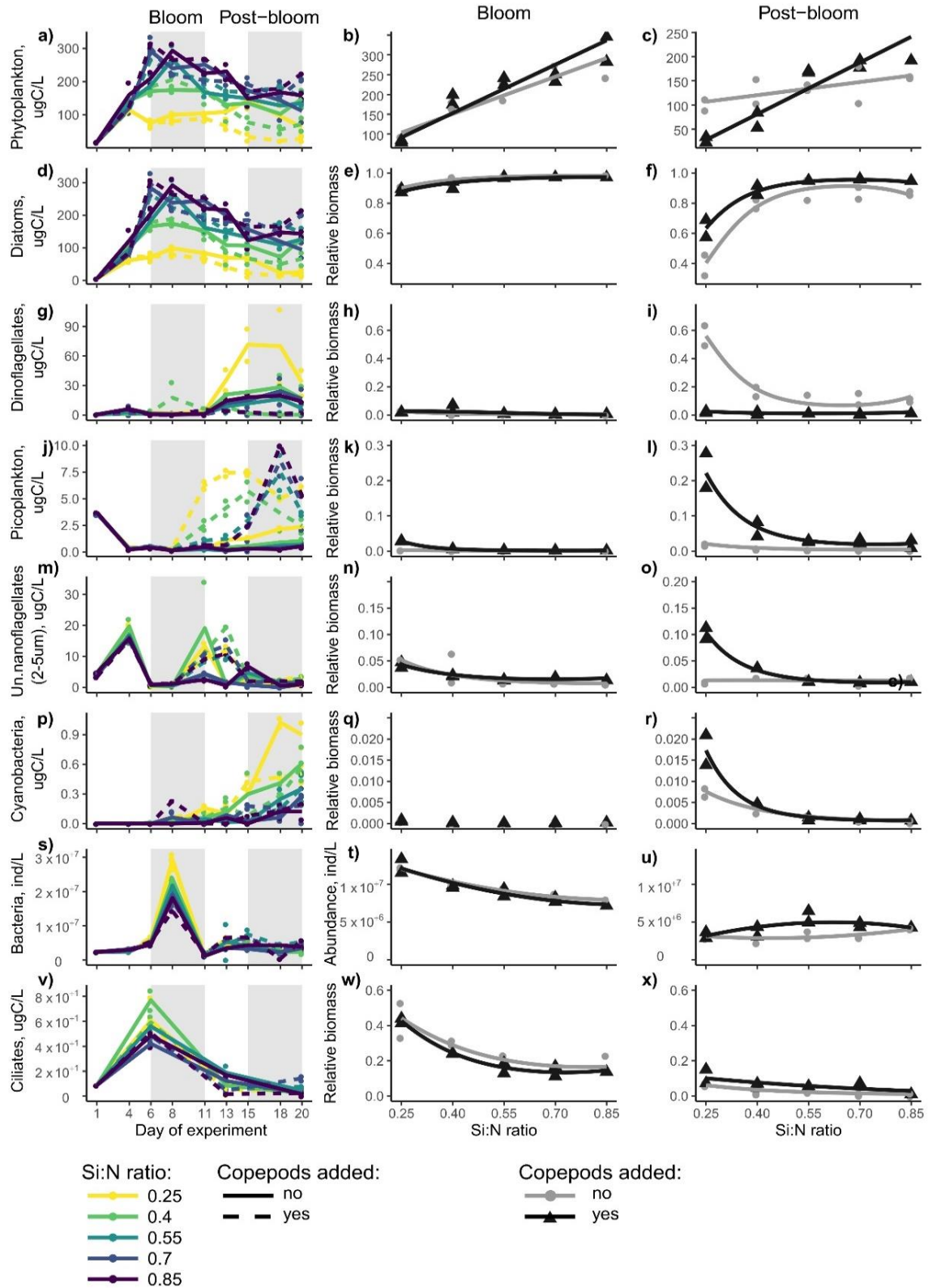


Figure 1.2 Plankton biomass during the course of the overall experiment, and the bloom and post bloom periods: (a–c) total phytoplankton, (d–f) diatoms, (g–i) dinoflagellates, (j–l) picoplankton, (m–o) unidentified nanoflagellates, (p–s) cyanobacteria, (s–u) bacteria, (v–x) ciliates. Values represented for bloom period correspond to averages over days 6–11 and post bloom to days 15–20, with an exception to ciliates where one sampling on the day 6 represents bloom period and on the day 20 post bloom.

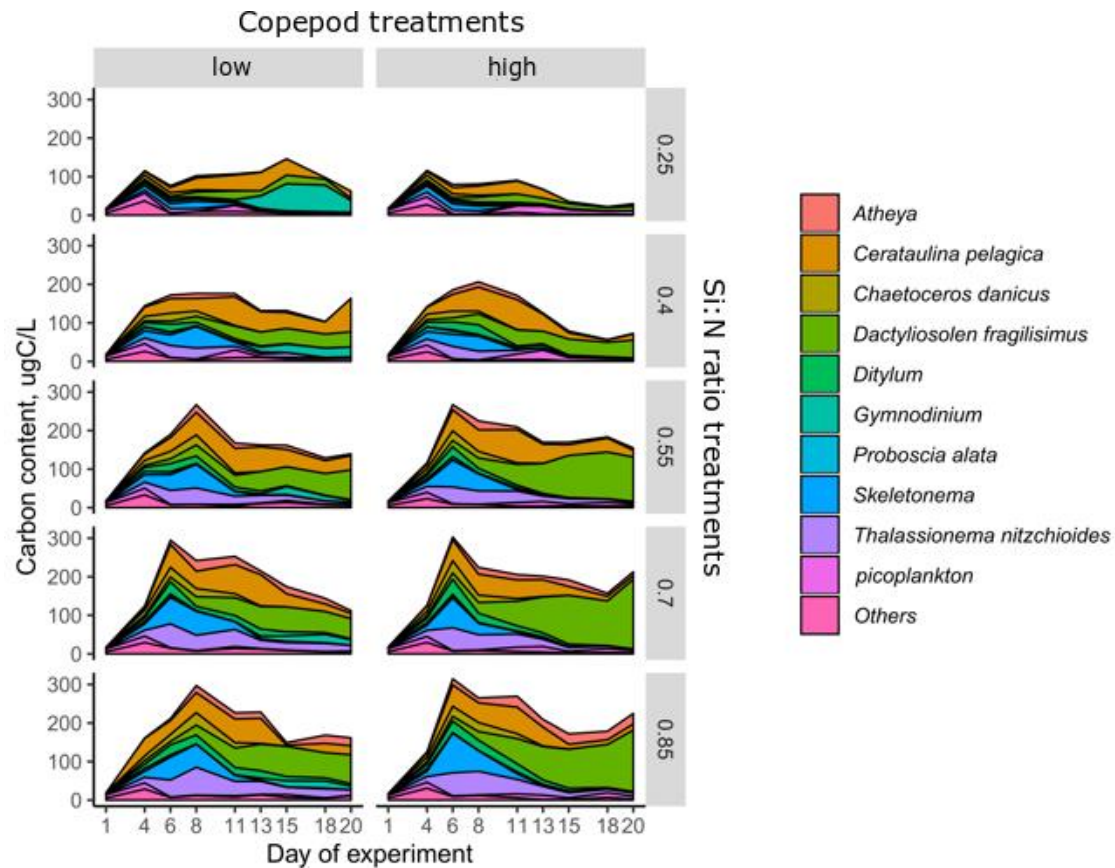


Figure 1.3 Phytoplankton community structure over the course of experiment at different Si:N ratios (from top to bottom) and without (left) and with (right) copepods added.

After the bloom period, total phytoplankton biomass slightly decreased and reached a plateau (Fig. 1.2a). During this post-bloom period (days 15-20 of experiment), plankton biomass and composition was strongly affected both by initial manipulation of Si:N ratios and grazing pressure. Total phytoplankton biomass increased with increasing Si:N treatments; however, the slope of the biomass increase depended on grazing and was steeper with than without addition of copepods (Table 1.2, Fig. 1.2c). Even under post-bloom conditions, diatoms dominated in all but the lowest Si:N ratio treatments (Fig. 1.2d), with *Dactyliosolen fragilissimus* and *Cerataulina pelagica* contributing most to the total biomass (Fig. 1.3). In the lowest Si:N treatments without copepod addition, dinoflagellates (mainly mixotrophic *Gymnodinium sp.*) contributed ~60% to the phytoplankton biomass (Fig. 2i). In high copepod treatments, relative dinoflagellate biomass was significantly lower ($\chi^2=11.940$, $df=1$, $p<0.001$, Fig. 1.2i, Fig.S1.1) and picoplankton and small photosynthetic nanoflagellates were more abundant (Table 1.2, Fig. 1.2o, Fig.S1.1). Bacteria abundance increased with initial Si:N ratios and was higher in high copepod treatments by the end of experiment (Table 1.2, Fig. 1.2u). Ciliates at this stage of the experiment consisted mostly of *Euplotes sp.* and their biomass was marginally higher under high copepod grazing (Table 1.2, Fig. 1.2x).

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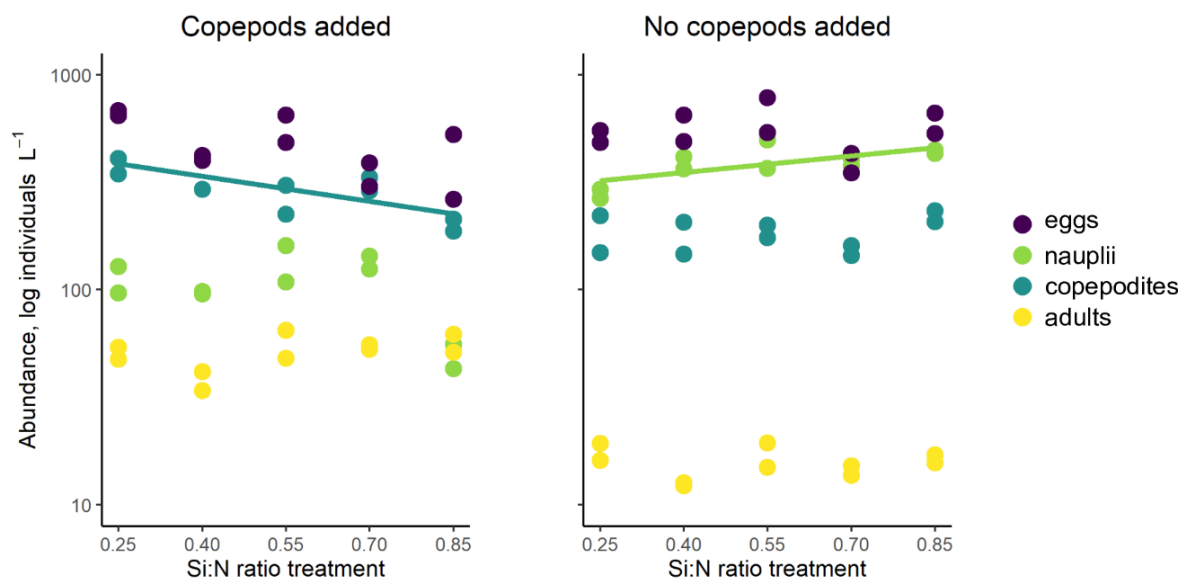


Figure 1.4 Abundance of different copepod stages at the end of experiment. Regression lines are fitted where Si:N ratio effects were significant (Copepodites, where copepods added: $\chi^2=0.187$, $df=1$, $p=0.012$; Nauplii, where no copepods added: $\chi^2=0.146$, $df=1$, $p=0.10$).

Adult copepod abundance averaged 50.9 ind L^{-1} ($sd=9.6$) by the end of the experiment in the treatments with copepod addition (Fig. 1.4). As the 125 μm mesh could not remove early stages

of copepods, by the end of experiment there were on average 15.6 (sd=2.4) adult copepods in the treatments without initially added copepods (Fig. 1.4). While changing initial Si:N ratio did not have significant effects on the abundance of adults, copepodite numbers declined with increasing initial Si:N in high copepod treatments (Fig. 1.4) and nauplii abundance increased with increasing initial Si:N ratios in low copepod treatments (Fig. 1.4).

Table 1.2 Results of likelihood ratio tests for generalized linear models (for total biomass and abundance data) and gamma regressions (for relative biomass data) testing for the effects of copepod grazing (Cop), initial silicon:nitrogen ratio (SiN) and the interaction between these terms (Cop x SiN). ^2 marks where the function for SiN is second-order polynomial instead of linear. Significant results are in bold. * = $p \leq 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Response variable	Bloom period					Post bloom period			
	factor	df	χ^2	p		df	χ^2	P	
Phytoplankton biomass ($\mu\text{gC L}^{-1}$)	Cop	1	0.0001	0.951		1	0.044	0.314	
	SiN	1	2.297	<0.001	***	1	3.269	<0.001	***
	Cop x SiN	1	0.042	0.199		1	0.948	<0.001	***
Diatoms, relative biomass	Cop	1	1.559	0.212		1	2.016	0.156	
	SiN^2	1	27.422	<0.001	***	1	25.086	<0.001	***
	Cop x SiN^2	2	5.996	0.050	*	2	15.345	<0.001	***
Dinoflagellates, relative biomass	Cop	1	1.495	0.221		1	11.940	0.001	***
	SiN^2	1	8.857	0.003	**	1	9.650	0.002	**
	Cop x SiN^2	2	2.393	0.302		2	27.240	<0.001	***
Picoplankton, relative biomass	Cop	1	2.829	0.093		1	10.186	0.001	**
	SiN^2	1	5.165	0.023	*	1	4.605	0.032	*
	Cop x SiN^2	2	36.365	<0.001	***	2	37.532	<0.001	***
Small nanoplankton, relative biomass	Cop	1	10.186	0.001	**	1	1.633	0.201	
	SiN^2	1	4.605	0.032	*	1	3.391	0.066	
	Cop x SiN^2	2	37.532	<0.001	***	2	17.242	<0.001	***
Ciliates, relative biomass	Cop	1	0.271	0.602		1	6.209	0.013	*
	SiN^2	1	33.307	<0.001	***	1	3.775	0.052	
	Cop x SiN^2	2	0.347	0.841		2	0.420	0.811	
Cyanobacteria, relative biomass, 10x transformed	Cop					1	0.389	0.533	
	SiN^2					1	30.555	<0.001	***
	Cop x SiN^2	2				2	10.924	0.004	**
Bacteria, abundance	Cop	1	0.559	0.634		1	0.825	0.001	***
	SiN^2	2	0.041	<0.001	***	2	0.619	0.038	*
	Cop x SiN^2	2	0.038	0.602		2	0.407	0.034	*

Trophic interactions

During the bloom period (day 6, Fig. 1.5a) ciliates were negatively correlated with small nanoplankton ($r = -0.53$, $p < 0.05$), indicating top-down control of the latter by the former. Cryptophytes and unidentified nanoflagellates were negatively correlated with bacteria ($r = -0.63$, $p < 0.01$ and $r = -0.72$, $p < 0.001$, respectively). During the post bloom period, copepods were negatively correlated with dinoflagellates and nauplii (Fig. 1.5b), indicating top down

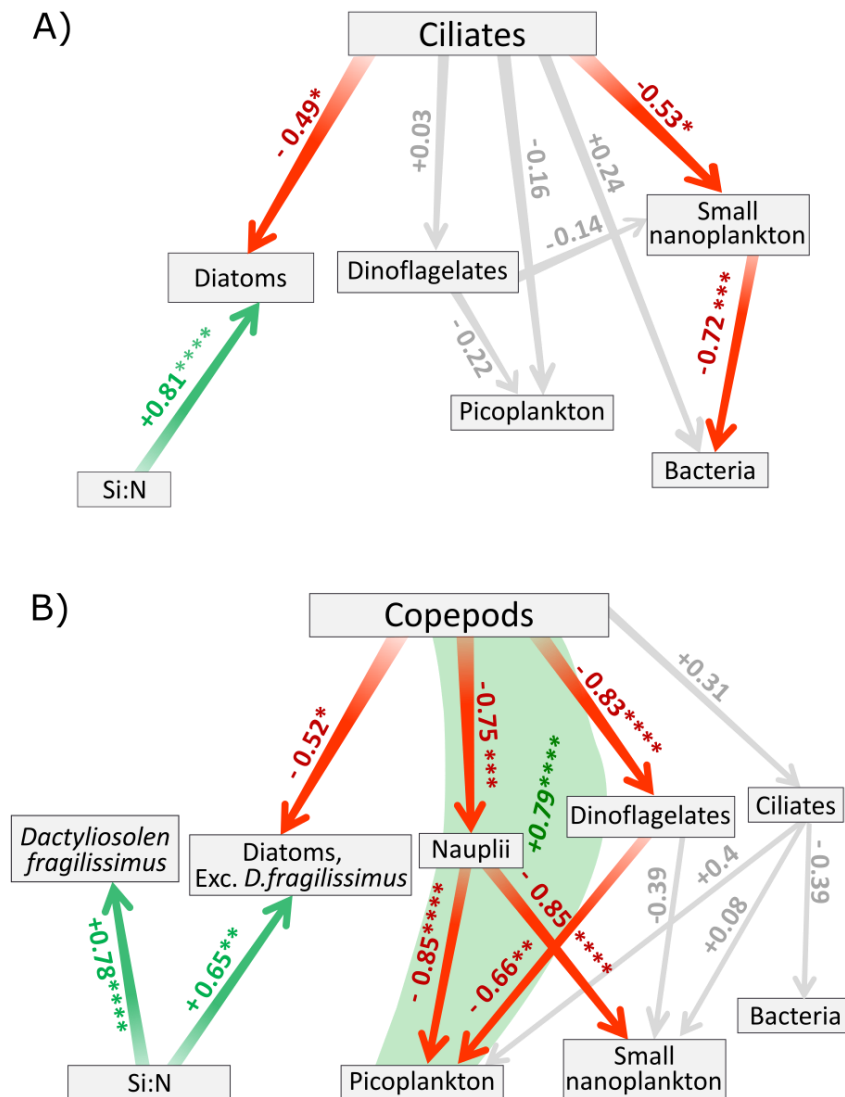


Figure 1.5 Possible trophic interaction scheme in the plankton community at the beginning of the phytoplankton bloom (day 6, A)) and during the post bloom period (days 15-20, B)). Green arrows represent significant bottom-up and red arrows significant top-down effects, while grey arrows are non-significant interactions. Shaded green area indicates possible trophic cascade effect. Numbers represent the Spearman correlation coefficient r , significance codes are as follows: $**** = p < 0.0001$, $*** = p < 0.001$, $** = p < 0.01$, $* = p < 0.05$. Copepods represent the sum of adults and copepodites. Diatoms were considered as pure autotrophs; dinoflagellates, picoplankton, small nanoplankton and ciliates as possible mixotrophs; and copepods and nauplii as strict heterotrophs.

control of copepods on dinoflagellates and possible cannibalism on nauplii. There were no significant correlations between copepods and total diatom biomass ($r = -0.02$, $p > 0.05$). As we observed higher diatom biomass in high copepod treatments, we explored diatom-copepod interactions further and checked for the effects on dominant diatom species. When we tested for the correlations separately for the most common diatom species at this stage of the experiment (*Dactyliosolen fragilissimus*) and the remaining diatoms, there was a significant negative correlation between diatoms excluding *D. fragilissimus* and copepods (Fig. 1.5b). Nauplii and dinoflagellates correlated negatively with picoplankton biomass likely indicating top-down control. Additionally, nauplii and small nanoplankton were strongly negatively correlated. Copepods and picoplankton correlated positively, indicating a possible trophic cascade effect via nauplii or dinoflagellates (Fig. 1.5b). Ciliates were less abundant in all treatments at this stage of the experiment and there were no significant correlations between them and the remaining plankton groups.

Discussion

Diatoms contribute significantly to the Earth's primary productivity (Field *et al.*, 1998, Nelson *et al.*, 1995) and to the carbon sequestration in the biological carbon pump (Jin *et al.*, 2006). It is therefore crucial to understand their role in planktonic trophic interactions and how resilient they are to changing nutrient conditions. Results of this study demonstrate that a decrease in Si:N ratios lowered diatom dominance leading to complex effects on planktonic food web structure and mesozooplankton grazers. These bottom-up effects dominated in the phytoplankton bloom period, whereas in the post-bloom period, we also detected top-down control of copepods on diatoms, dinoflagellates and nauplii, as well as from dinoflagellates and nauplii on picoplankton and small nanoplankton.

Diatoms contributed most to the phytoplankton biomass in the highest Si:N ratio treatments suggesting that most of the energy was likely transferred via the diatom-copepod trophic link. This supports our first hypothesis, that in high Si:N waters "traditional" diatom-copepod food chains prevail. However, diatom (and consequently phytoplankton in general) biomass was higher in high copepod treatments at high Si:N ratios. Higher phytoplankton biomass under increased grazing is counter-intuitive, however it has been previously observed in several diatom dominated communities (Moriceau *et al.*, 2018, Pree *et al.*, 2017, Sommer & Sommer, 2006). In our study, the higher biomass was mainly caused by the higher abundance of the dominant diatom species *D. fragilissimus*, indicating that it may have been spared from grazing

and possibly benefited from reduced competition with other diatoms and/or a copepod nutrient remineralization (Miller & Glibert, 1998). *Dactyliosolen* is one of the top five genera responsible for most of the diatom carbon standing stock in the oceans (Leblanc *et al.*, 2012); however, little is known about its ecology. *D. fragilissimus* is a medium sized diatom (length of pervalvar axis = 42-300 μm) with a characteristic short central spine; it occurs in single cells or loose chains (Karlson, 2018) and can produce enhanced amounts of transparent exopolymer particles (TEP) (Taucher *et al.*, 2015). To our knowledge, there are no reports of any *Dactyliosolen* species producing toxins or possessing specific defense mechanisms, yet increases in its biomass under grazing pressure have been observed before (Jepsen, 2014, Sommer & Sommer, 2006). In general, it is known that higher Si:N ratios can affect the degree of diatom silicification and their ability to excrete secondary metabolites positively (Flynn & Martin-Jézéquel, 2000, Van Nieuwerburgh *et al.*, 2004) which in turn can enhance diatom defense against grazers (Liu *et al.*, 2016, Pancic *et al.*, 2019, Pohnert, 2005). As a result, a fraction of diatom biomass may be unavailable for copepod grazing.

At low Si:N ratio treatments in our experimental system, non-silicifying plankton, such as picoplankton and dinoflagellates, dominated. Of these only dinoflagellates were likely directly available as food for copepods (Fig. 1.5b). Considering a wide spread of mixotrophy among dinoflagellates (Munn, 2011) and our observed negative correlation between dinoflagellate and picoplankton biomass (Fig. 1.5b), it is likely that there was a trophic cascade from picoplankton to copepods via mixotrophic dinoflagellates. This is in line with previous observations, that mixoplankton often dominates in systems where nutrients are provided in unbalanced proportions (Flynn *et al.*, 2019, Mitra *et al.*, 2014b). Due to their small size, picoplankton and small nanoplankton first had to be consumed by ciliates or dinoflagellates (Fig. 1.5), thus planktonic food chains in low Si:N would have an additional trophic level. More trophic levels may lead to decrease in energy transfer efficiency, as only a fraction of the energy can be transferred to the next higher trophic level. While the classic assumption of a 10 percent transfer efficiency (Lindeman, 1942) had to be corrected upwards to accommodate the microbial food web (Azam *et al.*, 1983) and may need further corrections to include the effects of mixoplankton, any food chain elongation between primary production and fish production (Sommer *et al.*, 2018) still means 70% to 80% energy loss at each step.

Additionally, Sommer *et al.* (2002) suggested that in low Si:N nutrient rich seas more energy would flow through the detritus food chain via dissolved organic carbon release and subsequent bacterial uptake due to high accumulation of inedible and toxic microplankton. In our study,

bacteria numbers peaked during the bloom period and they were indeed inversely proportional to initial Si:N ratios (Fig. 1.2s) indicating that more decomposition processes could have taken place in low Si:N (Bauerfeind, 1985). Dominant copepod *E.affinis* could have consumed detritus directly but living plankton has likely contributed more to its diet due to preferential feeding on living matter (Tackx *et al.*, 2003). Bacteria during the bloom period were likely consumed by mixotrophic nanoflagellates which in turn were consumed by ciliates (Fig. 1.5a), further illustrating the complexity of food web interactions in lowered Si:N ratios. Conversely, during the post-bloom period, dominant dinoflagellates were strongly grazed by copepods (Fig. 1.2i), and bacteria numbers were increasing with Si:N likely due to more biomass, including poorly edible

diatoms, accumulating at high Si:N ratios. Thus, the exact response of planktonic food webs to lowered Si:N ratios would highly depend on which species dominate the system, yet the complexity of food chains would increase either due to increased importance of detritus food chains, enhanced mixotrophy in case of dinoflagellate dominance, or extra trophic levels in case of prevalence of small flagellates and ciliates.

We hypothesized that copepod abundance would increase with Si:N due to more efficient trophic transfer in a food chain with fewer trophic levels. However, our results revealed that the response of copepods to changing Si:N, and therefore changing diatom proportion, is more complex. The numbers of copepodites were decreasing with increasing start Si:N ratios in high copepod treatments, while in low copepod treatments nauplii numbers increased with Si:N. The decrease of copepodites are in line with several studies reporting negative effect of diatoms on copepods (Ianora *et al.*, 2004, Poulet *et al.*, 1994, Van Nieuwerburgh *et al.*, 2004). The increase of nauplii with Si:N ratios, however, has also been observed before (Sommer, 2009). We can only speculate why we (and previous studies) observed these seemingly contradicting results. There could be several reasons for the observed decrease in copepodite numbers with increasing Si:N in high copepod treatments: 1) Jones and Flynn (2005) suggested that copepods require some non-diatom food to maintain their growth and reproduction. Under high copepod abundance, this alternative food may be selectively “eaten out” and the increasing lack of it with increasing Si:N would restrain egg production and nauplii growth to copepodites. Additionally, copepod cannibalism could have been more pronounced under higher adult densities, especially when nutritious food was not available at high Si:N ratio treatments; 2) Increasing consumption of heterotrophic prey in lower Si:N ratios may have overcompensated the decrease in trophic transfer efficiency via trophic upgrading (Klein Breteler *et al.*, 1999);

3) With higher copepod densities, the imprint of chemical cues such as polar lipids copepodamides may have been more distinct and could have triggered a stronger defense response in diatoms (Selander *et al.*, 2019). In low copepod treatments, there were likely sufficient alternative food sources even in the highest Si:N ratio treatments, therefore copepods could have benefited from higher biomass available in high Si:N ratios. 4) Diatoms may affect different copepod stages differently (Flynn & Irigoien, 2009): while in high copepod treatments majority of copepods during phytoplankton bloom were adults, in low copepod treatments they were in earlier developmental stages. Though further studies are needed to understand the role of copepod density and demography on phytoplankton defense and their response, our results further stress the complexity in copepod-diatom interactions.

Our observed sensitivity of copepods to the composition of their prey also indicates the need to improve the accuracy of diatom description and to review and update copepod link in ecosystem models (Flynn, 2005, Mitra *et al.*, 2014a). Additionally, the high importance of mixotrophs in low Si:N treatments illustrates the wide spread of mixotrophic organisms within plankton and stresses that in addition to phyto- and zooplankton, a separate guild of mixoplankton, as defined by Flynn *et al.* (2019), is useful (Flynn *et al.*, 2012, Mitra *et al.*, 2016). Furthermore, this indicates the increased complexity of plankton food webs when mixoplankton guild is considered (Flynn *et al.*, 2019, Leles *et al.*, 2018).

In an attempt to summarize the results of this and previous studies (Roberts, 2003, Sommer, 2009, Sommer *et al.*, 2005, Sommer *et al.*, 2004), we propose conceptual food web models for low and high Si:N nutrient rich seas (Fig. 1.6). In high Si:N environments (Fig. 1.6a), such as temperate or boreal seas after seasonal mixing or upwelling regions, diatoms are the dominant primary producers and the main source of energy for copepods and higher trophic levels. However, a part of diatom biomass is inaccessible for grazers due to their chemical, morphological or structural defense. In low Si:N environments (Fig. 1.6b), such as eutrophicated coastal seas, plankton food webs are more complex. Mixoplankton, such as dinoflagellates and small, potentially nuisance phytoplankton dominate. While some dinoflagellates can be nutritious food for copepods, many others may be toxic or inaccessible due to their size, shape or thick cell walls. Picoplankton are first consumed by ciliates and dinoflagellates, and only then accessible for copepods. Uneaten algae enter a heterotrophic food chain on their death via dissolved inorganic carbon (DOC) and result in increased numbers of bacteria, which are consumed by heterotrophic and mixotrophic nanoflagellates and

picoplankton (Hartmann *et al.*, 2013), which in turn provide food for dinoflagellates and ciliates and eventually copepods.

The findings of this study have to be interpreted in light of some limitations. Firstly, our experimental units were closed. In nature, slow but constant nutrient exchange occurs and this may have an effect on phytoplankton composition as even small inputs of silicate can have strong effects on diatoms. On the other hand, dead plankton and fecal pellets could not sink out of the closed system, possibly allowing nitrogen, phosphorus and to a smaller extent silicon to be recycled. Secondly, we reconstructed food webs based on biomass and abundance data and assumed that trophic relationships and energy flows would be strongest where negative correlations and biomass of different plankton groups were highest. However, productivity

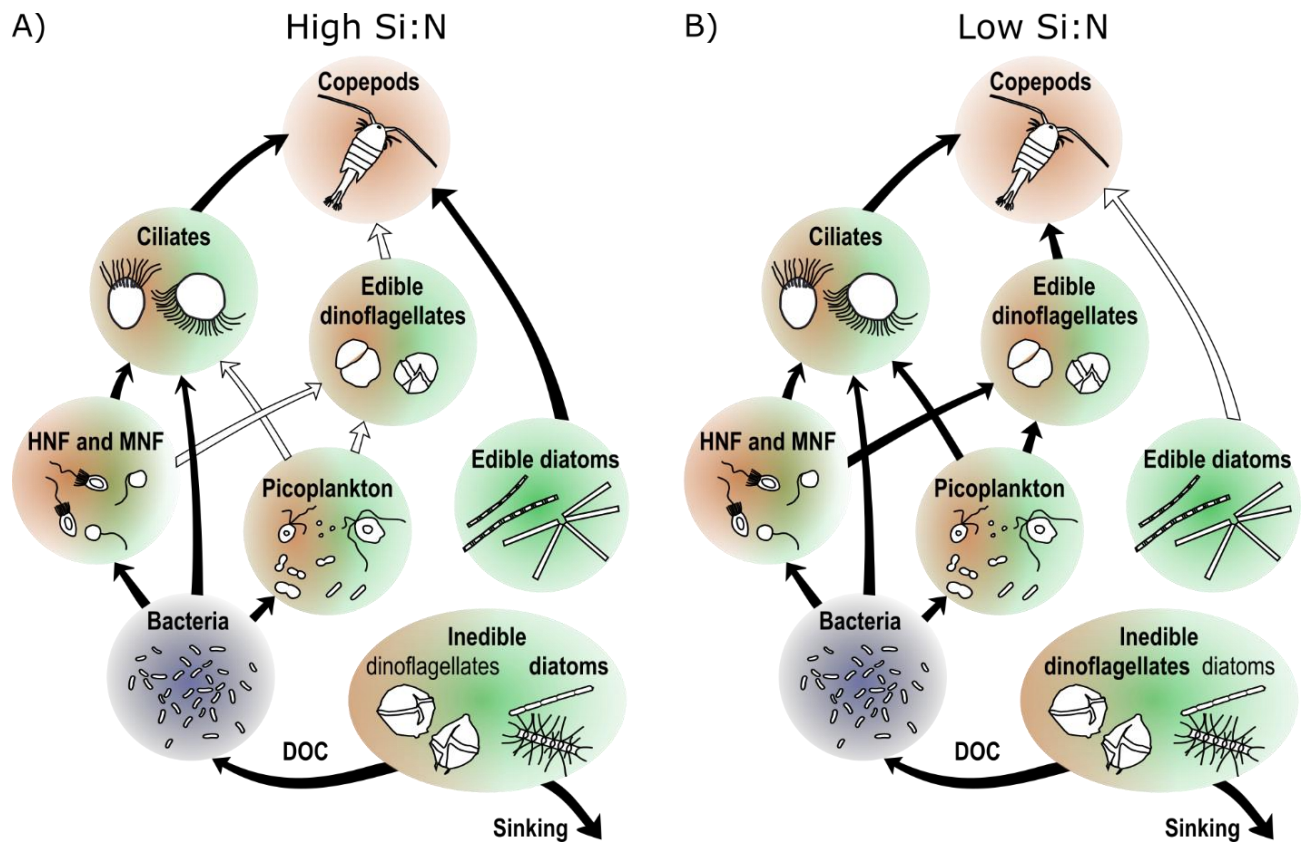


Figure 1.6 Plankton trophic interactions in different Si:N conditions in nutrient-rich seas. (a) In Si:N ratios close to 1:1 (Redfield-Brzezinski ratio), phytoplankton is dominated by diatoms and they provide the main energy source for copepods and consequently higher trophic levels. However, a part of the diatom biomass is inaccessible for grazers and presents a dead end in the system. (b) In severely lowered Si:N ratios, nonsilicifying phytoplankton replace diatoms. Some dinoflagellates are directly consumed by copepods, smaller plankton are first eaten by ciliates and heterotrophic dinoflagellates. Toxic and nuisance algae are decomposed and enter the food chain via bacteria. Solid arrows represent dominant, empty – minor pathways of energy transfer. Autotrophic functional groups of plankton are shown in green, heterotrophic in brown, mixotrophic – in brown/green and decomposing bacterioplankton in dark blue. DOC stands for dissolved organic carbon, HNF – heterotrophic nanoflagellates, MNF – mixotrophic nanoflagellates.

does not always correspond to biomass and this may affect the relative importance of the plankton groups in the energy transfer. Measuring productivity of different plankton groups in a natural community, which are often of similar size, is complicated and further efforts are needed to improve the methods and enhance our understanding of plankton food webs.

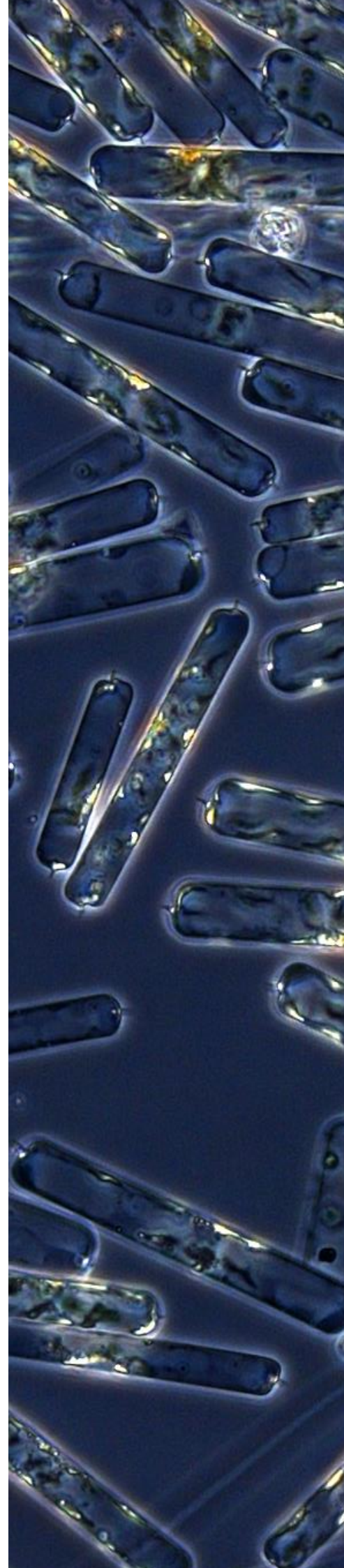
Conclusions

Our results demonstrate that phytoplankton communities respond strongly to changes in Si:N ratios and this response is moderated by copepod grazing pressure. Changes in the composition of phytoplankton species or functional groups may have far reaching consequences on the structure and functioning of planktonic food webs in nutrient rich environments, specifically:

- 1) In high Si:N environments, diatoms dominate and classical “diatom → copepod” food chains with high energy transfer efficiency prevail. However, some diatom species may be inaccessible for copepod grazing.
- 2) In low Si:N environments, planktonic food webs are more complex with more trophic levels and microzooplankton playing a more important role.
- 3) The effects of these changes on copepod recruitment are complex and may be copepod density dependent.

Chapter II

Phytoplankton nutritional quality
is altered by shifting Si:N ratios
and selective grazing



Phytoplankton nutritional quality is altered by shifting Si:N ratios and selective grazing

Kriste Makareviciute-Fichtner, Birte Matthiessen, Heike K. Lotze, Ulrich Sommer

Abstract

Diatoms often dominate phytoplankton in temperate, polar and upwelling regions. Decreases in silicate availability or silicon to nitrogen (Si:N) ratios may lower their proportion within phytoplankton communities. The effects of such shift on the nutritional quality of phytoplankton for primary consumers is not well understood. To examine how changing Si:N ratios affect plankton nutritional value we applied a range of Si:N ratios on a natural plankton community and manipulated grazing pressure to assess copepod selective grazing. Diatom proportion in phytoplankton increased with increasing Si:N ratios and so did phytoplankton nutritional quality in terms of major fatty acid (FA) concentrations, such as polyunsaturated fatty acids (PUFA), docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids. However, stoichiometric indicators (carbon to nitrogen, C:N, carbon to phosphorus ratios, C:P), DHA:EPA and omega 3:6 (ω 3: ω 6) ratios declined with increasing Si:N ratios, suggesting that proportions between essential compounds in copepod diet may be more favorable in lowered Si:N ratios. Copepods had a negative effect on DHA contents, DHA:EPA and ω 3: ω 6 ratios, indicating possible selective grazing on more nutritious plankton. Our findings show that changes in phytoplankton due to declining silicate concentrations can affect nutrient and fatty acid availability to copepods which they can likely moderate by selective grazing.

Introduction

Phytoplankton communities are diverse and respond quickly to both natural and anthropogenic perturbations. One major factor shaping phytoplankton communities is changing supply of nutrients and their stoichiometry. Many aquatic systems face increased supply of nitrogen and phosphorus (Beusen *et al.*, 2016, Howarth *et al.*, 1996, Kroon *et al.*, 2012). The availability of other nutrients, such as silicon, has not changed or even decreased (Hátún *et al.*, 2017, Laruelle *et al.*, 2009, Ragueneau *et al.*, 2006). Declining silicon to nitrogen ratios (Si:N) can lower the proportion of diatoms in phytoplankton communities as silicon is an essential nutrient for diatoms and silicoflagellates, but not for other groups of phytoplankton.

Changes in phytoplankton composition may have far reaching consequences as phytoplankton provides the base of many aquatic food webs. Different phytoplankton functional groups have different nutritional value for primary consumers due to variable chemical composition. For instance, the composition and amount of long chain polyunsaturated fatty acids (PUFAs) are specific for different phytoplankton taxa (Parrish, 2009). Cyanobacteria and ochrophytes generally contain low levels of PUFAs, while dinoflagellates contain high PUFA levels, particularly in docosahexaenoic acid (DHA) (Jónasdóttir, 2019). Diatoms contain high amounts of fatty acids (FAs) per carbon content and roughly 10% of them belong to long chain eicosapentaenoic acid (EPA). However, around 40% of FAs in diatoms belong to 16 carbon saturated or monounsaturated FAs such as palmitoleic acid (Jónasdóttir, 2019). Cryptophytes are characterized by a high proportion of PUFAs, which can constitute up to 60% of the total FAs (Jónasdóttir, 2019). FA composition of the diet is important for consumers, as many PUFAs are essential for membrane and bioactive compound synthesis and cannot be synthesized by most of consumers themselves (Parrish, 2009).

The sum of PUFAs, selected essential FAs, DHA, EPA, and ratios between omega 3 and omega 6 ($\omega 3:\omega 6$) are frequently used as indicators of plankton nutritional value as they correlate to primary consumer growth and reproduction metrics (i.e. copepod egg production, hatching rate, maturation rate, female length (Arendt *et al.*, 2005, Jónasdóttir & Kiørboe, 1996, Payne & Rippingale, 2000, Shin *et al.*, 2003)). Stoichiometric ratios between phytoplankton carbon and nitrogen (C:N) or phosphorus (C:P) are also used as nutritional value indicators (Bi *et al.*, 2017, Jones & Flynn, 2005) .

Phytoplankton communities in temperate, boreal, polar and upwelling ecosystems are often dominated by diatoms (Medlin & Priddle, 1990, Tréguer *et al.*, 2018). The nutritional value of

diatoms has been under debate for the last three decades (Ianora *et al.*, 2003, Jones & Flynn, 2005, Poulet *et al.*, 1994). Initially thought as an ideal food source for secondary consumers in marine systems (Kleppel, 1993), many diatoms were shown to have deleterious effects on copepod ontogeny and reproduction in laboratory scale studies (Ianora *et al.*, 2003) and in some natural communities (Jenkins & Black, 2019, Nejstgaard *et al.*, 2001). Nutritional inadequacy was suggested as one possible reason behind this (Jones & Flynn, 2005), possibly due to relatively low contents of some essential fatty acids, such as DHA (Arts *et al.*, 2009). Diatoms have rather low C:P and N:P relative to other phytoplankton classes (Quigg *et al.*, 2003) but there is high variability of C:P, N:P, and C:N among diatom species (Garcia *et al.*, 2018). However, copepods can feed selectively, and in natural communities there are almost always alternative food sources available. Evidence from laboratory experiments suggests that copepods can select for more nutritious food items in terms of C:N and C:P ratios (Cowles *et al.*, 1988, Jones & Flynn, 2005) but studies on FAs and copepod selective feeding are scarce. Additionally, it is not clear if selective grazing can affect nutritional value of the whole phytoplankton community under more natural conditions.

Here we present results from a mesocosm experiment in which we manipulated natural Baltic Sea phytoplankton community composition by applying a range of Si:N ratios. The Baltic Sea is a semi-enclosed eutrophic sea and a decline in Si:N ratios in some of its regions has been reported (Danielsson *et al.*, 2008, Humborg *et al.*, 2008). We asked if changing Si:N would affect phytoplankton nutritional quality and if yes, whether a diatom dominated community would be more or less nutritious according to FA and C:N/C:P indicators? Additionally, we manipulated copepod grazing pressure to examine if copepods select for more nutritious food in natural communities. Our study provides new insights into how bottom-up and top-down forces shape food availability and quality in natural plankton foods webs.

Methods

Experimental design and location

To address our research questions, we manipulated Si:N ratios and copepod grazing pressure on Baltic Sea plankton communities in an outdoor mesocosm system at the dock of GEOMAR (54°19'46.9"N, 10°08'59.4"E) in the Kiel Fjord, Germany. The experiment took place between 17 June and 6 July, 2016. We fully crossed five Si:N ratio treatments with two grazing levels, which resulted in ten unique treatments carried out in duplicates.

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Twenty 20 L plastic bags served as experimental units. The bags were randomly distributed in four larger tanks equipped with flow-through systems where constant flow of sea water kept the temperatures in the tanks close to ambient. On the starting day, near-surface fjord water was pumped to a larger tank, pre-filtered using plankton gauze with 125 μm mesh size and distributed into the experimental units. Nutrients were added to reach the following Si:N ratios: 0.25:1, 0.4:1, 0.55:1, 0.7:1, 0.85:1; with constant target nitrogen concentration of 40 $\mu\text{mol L}^{-1}$, variable silicon concentrations and constant phosphorus concentrations of 2.5 $\mu\text{mol L}^{-1}$ at the start of the experiment. Nitrogen was added as nitrate (NaNO_3), phosphorus as phosphate (KH_2PO_4) and silicon as silicate ($\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$).

For the grazing treatment, *Eurytemora affinis* copepods were collected in the Kiel canal (54°22'15.7"N, 10°06'22.4"E) on May 19, 2016 and kept in culture until the start of the experiment. Adult copepods were added to half of the experimental units at a target concentration of 30 individuals per liter (ind L^{-1}) once the phytoplankton bloom had established on day 5 of the experiment (high copepod treatments). Aliquots of filtered copepod culture water were added to the remaining bags (low copepod treatments). Because of an experimental failure one experimental unit (with copepods, Si:N = 0.85:1) had to be excluded from the analysis.

Sampling and analysis

Samples for phytoplankton counts, dissolved nutrients, and seston particulate organic carbon, nitrogen, and phosphorus (POC, PON, POP) analyses were collected three times per week (Monday, Wednesday, Friday). Microzooplankton was counted twice during the experimental period: on day 6 and day 20. Fatty acid composition was analyzed on the last day of the experiment (day 20).

Plankton samples were fixed with Lugol's solution and counted microscopically according to Utermöhl's (1958) inverted microscope method. Picoplankton and small nanoplankton (2-5 μm) were enumerated immediately after sampling using flow-cytometry (FACScalibur, Becton Dickinson). Relative biomass of phytoplankton was calculated after converting biovolume to carbon content according to Hillebrand *et al.* (1999) and Menden-Deuer and Lessard (2000).

Samples for dissolved nutrient analysis (nitrate, nitrite, ammonium, silicate and phosphate) were filtered with pre-washed (10% HCl) cellulose acetate filters and stored at -20°C until further analysis according to Hansen and Koroleff (1999) with a Skalar auto-analyzer (SANPLUS; Breda/the Netherlands). Plankton carbon biomass and dissolved inorganic

nutrient data from this experiment was reported in the Journal of Plankton Research (Makareviciute-Fichtner *et al.*, 2020) and is available in PANGAEA data base (<https://doi.pangaea.de/10.1594/PANGAEA.912157>).

For particulate organic carbon and nitrogen (POC, PON) analysis, 30-100 mL of sample water (volume depending on biomass) were filtered onto pre-washed (in 5-10% HCl) and precombusted (6 h, 550°C) Whatman GF/F filters. Filters were dried and stored in desiccators until further analysis. POC and PON were determined in an elemental analyser (Thermo Flash 2001, Thermo Fisher Scientific Inc., Schwerte, Germany) using gas chromatography (Sharp, 1974). POP was determined calorimetrically after conversion to orthophosphate following Hansen and Koroleff (1999).

For fatty acid analysis, 100-200 ml of each sample (volume depending on biomass) were filtered onto GF/F filters (Pore size: 0.7 µm) and stored at -80°C until further analyses. FAs were measured as FA methyl esters (FAMES) using a gas chromatograph (Thermo Fisher Scientific, Germany). FAMES were prepared according to a modified procedure by Christie (1989). The extraction process is described in detail in Chi *et al.* (2018). After gas chromatography, peaks were integrated using Chromcard software (Thermo Fisher Scientific, Germany) and identified with reference to Supelco 37 FAME mixture (Sigma-Aldrich, USA). Consequently, fatty acid content per carbon was calculated using seston particulate carbon data for equivalent water volume. Relative FA content for individual FAs and FA groups were calculated as percentage contribution to total FAs.

Statistical analysis

Analysis of covariance (ANCOVA) was used to assess the possible effects of changing Si:N ratios, copepod grazing and the interaction between these variables. The effects were assessed for bloom and post-bloom periods for stoichiometric indicators and the last day of the experiment for FA data. We defined “bloom” as the period where, after an initial rapid increase, seston POC, PON, and POP reached peak values in most of the treatments (days 14-11). “Post-bloom” period was defined as the period where POC declined or plateaued following the “bloom” (days 13-20). Copepod grazing level was used as a categorical variable (2 levels: high, low) and Si:N ratio as a covariate. Parametric assumptions of linear models were verified using plots of residuals for normality and homoscedasticity. Statistical analyses were conducted in R with R studio (R Core Team, 2019, R Studio Team, 2019), graphs were constructed using ggplot2 package (Wickham, 2016).

Results

Phytoplankton composition

Phytoplankton composition at the start of experiment was diverse, with high abundance of diatoms, cryptophytes and unidentified pico- and nano-plankton (Fig. 2.1). After nutrient manipulation, diatoms became dominant in all treatments. During the second half of the experiment, the relative biomass of dinoflagellates increased, and they became dominant at the lowest Si:N treatment under low copepod grazing. In high copepod treatments, diatoms continued to dominate. Here picoplankton contributed a higher share to the total phytoplankton biomass, especially in the lower Si:N ratios (Fig. 2.1).

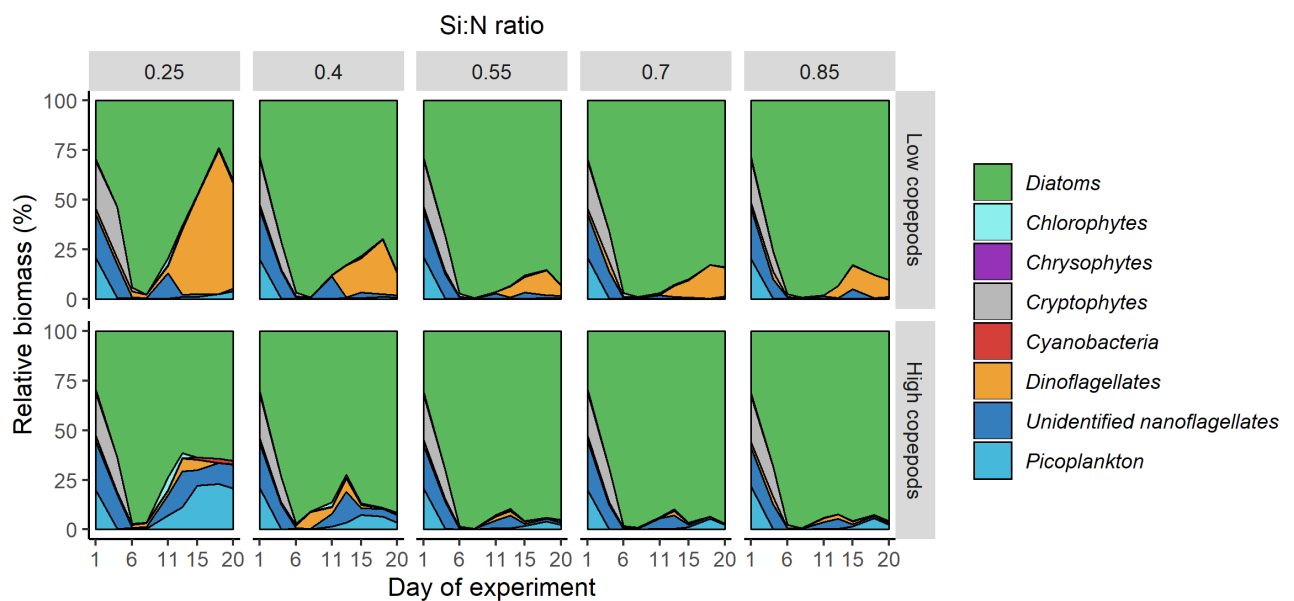


Figure 2.1 Phytoplankton functional group composition over the course of experiment in relative biomass (%) based on carbon content.

During the peak of the bloom period, on day 6 of the experiment, ciliates, dominated by *Strobilidium* sp. significantly contributed to total plankton biomass, especially in the lowest Si:N treatments (Fig. 2.2). During the post-bloom period, on day 20 of the experiment, the diatom *Dactyliosolen fragilisimus* dominated biomass in high copepod treatments. Under low copepod grazing, the dinoflagellate *Gymnodinium* sp. and the diatom *Cerataulina pelagica* had the highest biomass in low Si:N ratios (Fig. 2.2).

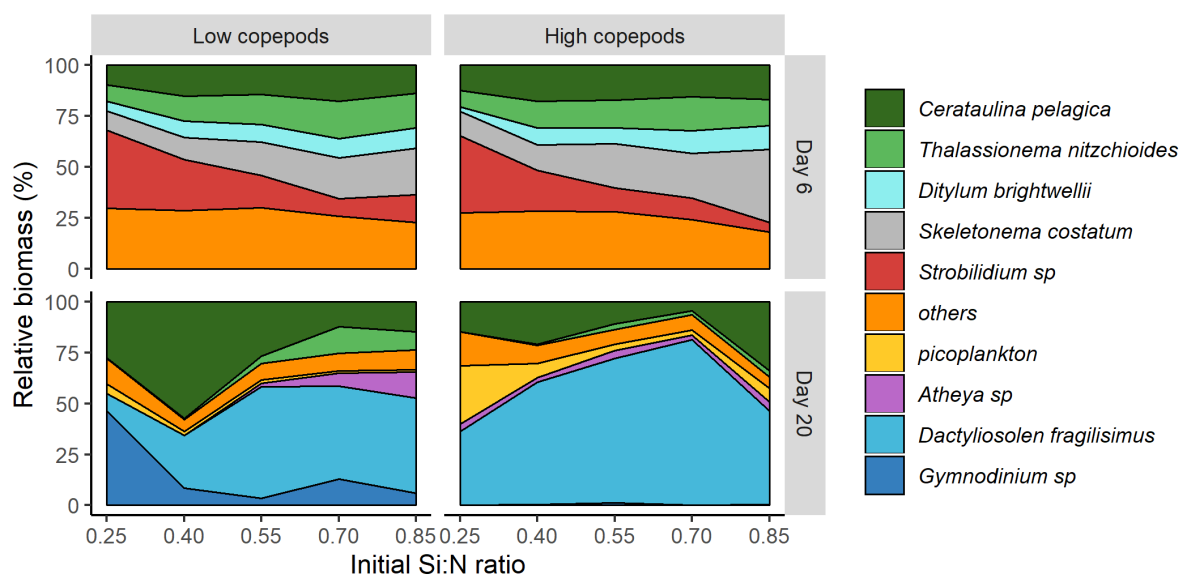


Figure 2.2 Phyto- and microzooplankton composition during days 6 and 20 of the experiment over initial Si:N ratio treatments.

Particulate nutrients

Seston particulate carbon, nitrogen and phosphorus increased rapidly after nutrient addition and reached peak values in most of the treatments during days 4-11 of the experiment (bloom period) (Fig. 2.3). Particulate carbon then declined (high copepod treatment) or plateaued (low copepod treatment) in days 13-20 (post-bloom period). Both during bloom and post-bloom periods, particulate carbon content increased with increasing Si:N ratio and was lower in high copepod treatments (Table 2.1, Fig. 2.3).

Particulate nitrogen and phosphorus concentrations slightly increased again during the post-bloom period in high Si:N treatments, and decreased in the lowest Si:N in high copepod treatments. Both during bloom and post-bloom periods particulate nitrogen and phosphorus concentrations increased with increasing Si:N ratios and generally were lower in high copepod treatments (Table 2.1, Fig. 2.4). Copepod grazing affected particulate organic phosphorus concentrations stronger in low Si:N ratios than in high Si:N during the post-bloom period ($F=10.52$, $p=0.005$, Fig. 2.4).

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Table 2.1 ANCOVA results testing for the effects of changing Si:N ratio (SiN), copepod grazing (Cop) and the interaction between these variables (Cop x SiN) on particulate organic carbon (POC), particulate organic phosphorus (POP) and particulate organic nitrogen (PON) in the bloom and post-bloom periods. * $p \leq 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Response variable	Bloom period				Post-bloom period			
	Factor	df	F	p	df	F	P	
POC	SiN	1	41.779	0.000	***	1	68.332	0.000 ***
	Cop	1	4.802	0.045	*	1	49.396	0.000 ***
	Cop x SiN	1	0.062	0.806		1	0.770	0.394
POP	SiN	1	9.361	0.008	**	1	110.307	0.000 ***
	Cop	1	5.625	0.032	*	1	50.217	0.000 ***
	Cop x SiN	1	0.000	0.984		1	10.520	0.005 **
PON	SiN	1	11.743	0.004	**	1	11.033	0.005 ***
	Cop	1	7.191	0.017	*	1	5.643	0.031 *
	Cop x SiN	1	0.002	0.968		1	1.738	0.207

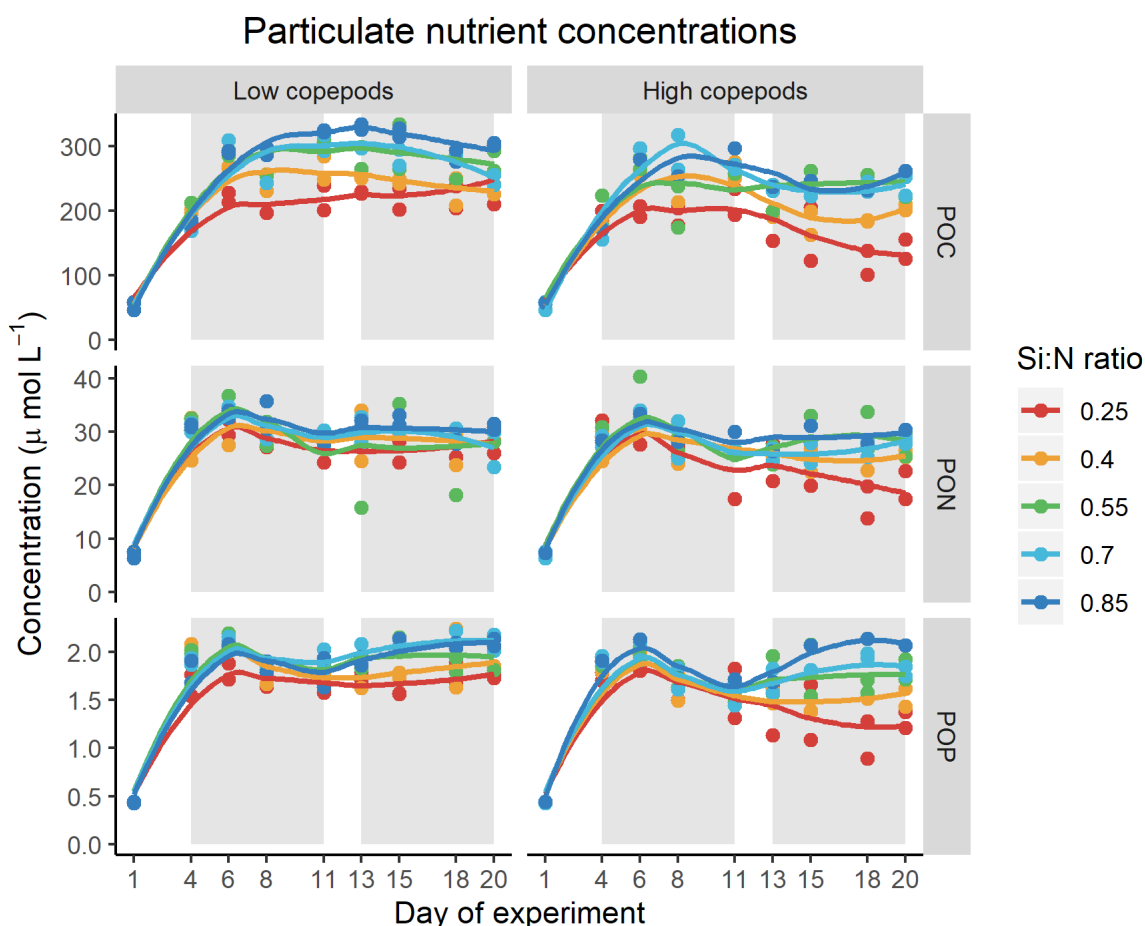


Figure 2.3 Particulate nutrient concentrations over the course of the experiment under different Si:N ratios. Grey shaded areas represent bloom peak (days 4-11) and post-bloom (days 13-20) periods. POC – particulate organic carbon, PON – particulate organic nitrogen, POP – particulate organic phosphorus.

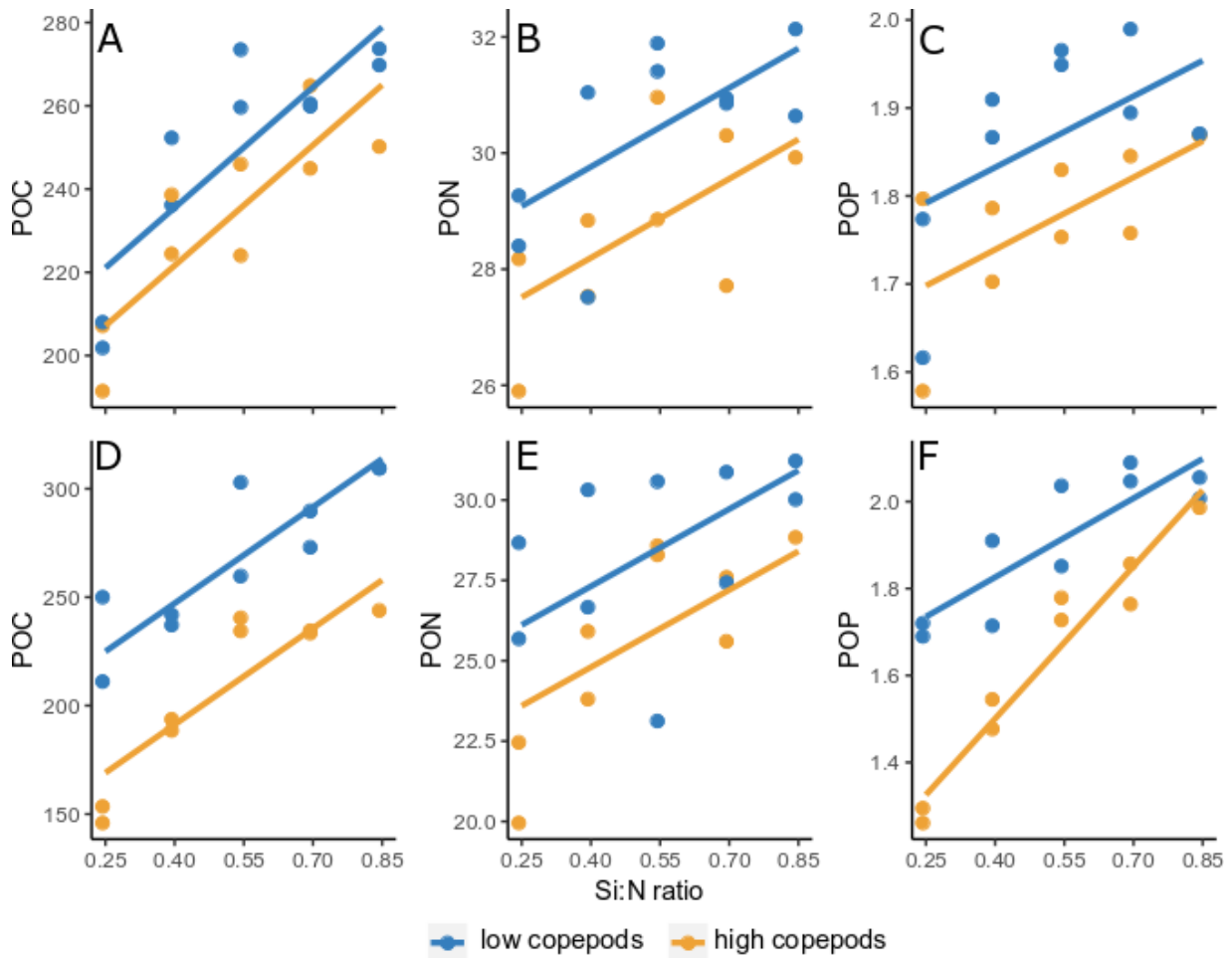


Figure 2.4 Particulate organic nutrient concentrations ($\mu\text{mol L}^{-1}$) across different Si:N ratios and copepod grazing treatments during bloom peak (graphs A, B, C) and post-bloom (graphs D, E, F) periods. POC – particulate organic carbon, PON – particulate organic nitrogen, POP – particulate organic phosphorus.

Stoichiometric ratios

Seston C:P and C:N increased with increasing Si:N during most of the experimental period (Fig. S1), including both in bloom and post-bloom periods (Fig. 2.5, Table 2.2). During the post-bloom period, C:N and C:P were significantly lower in high copepod treatments ($F=20.141$, $p<0.001$ and $F=12.547$, $p=0.003$, respectively). N:P was not affected by the experimental manipulation during the bloom period but decreased with increasing Si:N during the post-bloom ($F=9.081$, $p=0.009$, Fig. S2.2)

Fatty acids

By the end of the experiment total fatty acid content per seston carbon increased with increasing Si:N (Fig. 2.6A, Table 2.3) and so did the contents of all functional fatty acid groups (PUFAs, monounsaturated fatty acids (MUFA), saturated fatty acids (SFA)) and selected essential fatty

acids (DHA, EPA, arachidonic (ARA) and alpha-linoleic (ALA) acids) whereas the ratios of DHA:EPA and $\omega 3:\omega 6$ decreased (Fig. 2.6, Table 2.3, Fig. S2.3). Relative concentration (% of total FA) of PUFA was not affected by changing Si:N and grazing (Table 2.3, Fig. S2.3A). ALA absolute concentration was higher in low copepod treatments, but relative concentration was lower, especially in the lowest Si:N treatment (Fig. 2.6H, Table 2.3, Fig. S2.3H). DHA concentrations, DHA:EPA and $\omega 3:\omega 6$ ratios were higher in low copepod treatments (Fig. 2.6C, D, E) and ARA relative concentration was slightly higher in high copepod treatments (Fig. S2.3).

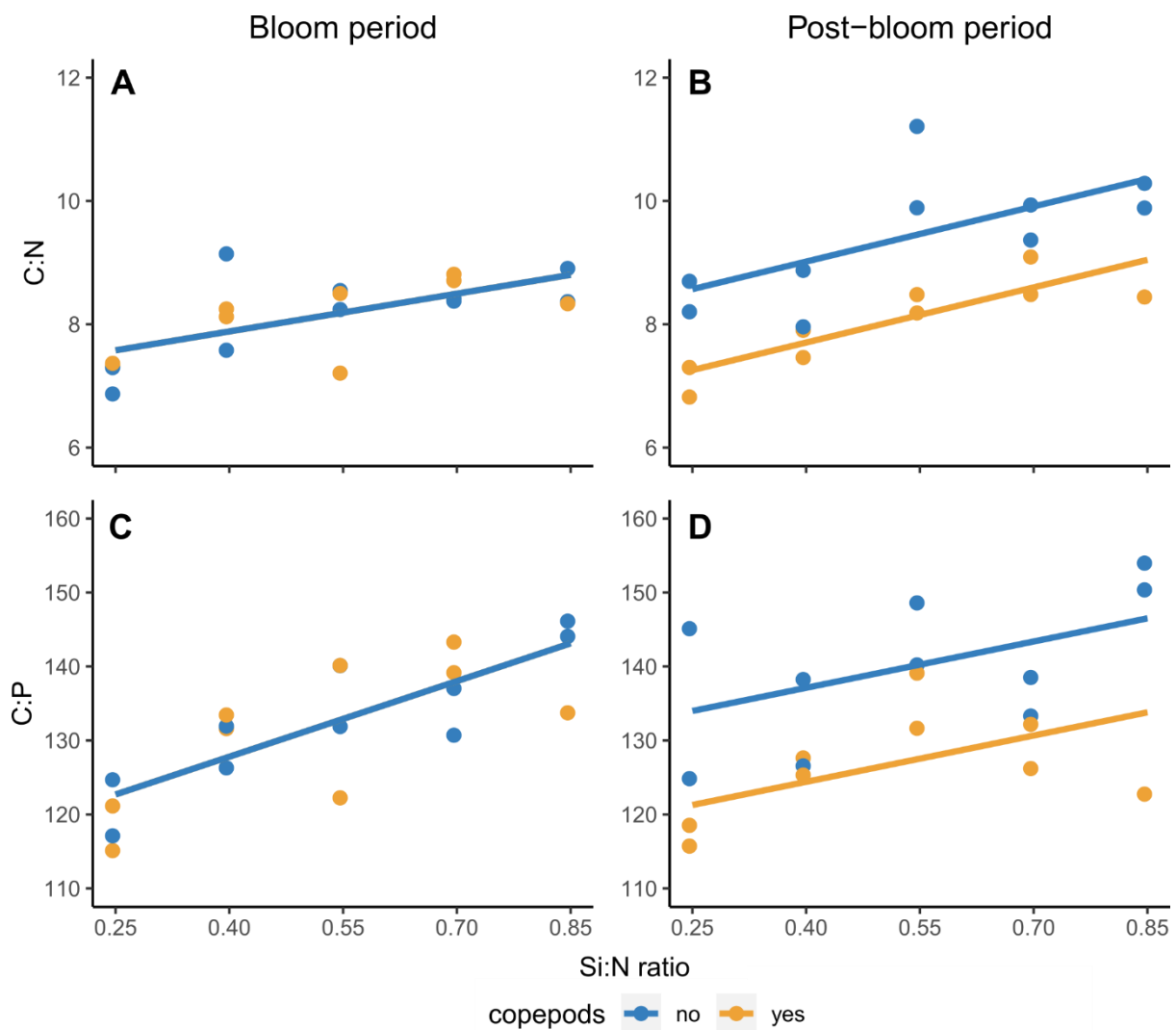


Figure 2.5 Seston particulate nutrient ratios across Si:N ratios during bloom (graphs A, B) and post-bloom (graphs C, D) periods. C:N - carbon to nitrogen, C:P - carbon to phosphorus molar ratios.

Chapter II

Table 2.2 ANCOVA results testing for the effects of changing Si:N ratio (SiN), copepod grazing (Cop) and the interaction between these variables (Cop x SiN) on seston nutrient ratios in the bloom and post-bloom periods. C:N - carbon to nitrogen, C:P - carbon to phosphorus, N:P – nitrogen to phosphorus molar ratios. * $p \leq 0.05$, ** $p < 0.01$, *** $p < 0.001$. Positive increase with increasing Si:N is indicated with \uparrow , negative with \downarrow .

Bloom period					Post bloom period				
Response variable	factor	df	F	P		df	F	P	
C:N	SiN	1	11.721	0.004	* \uparrow	1	20.975	0.000	*** \uparrow
	Cop	1	0.022	0.884		1	20.141	0.000	***
	Cop x SiN	1	0.012	0.913		1	0.001	0.973	
C:P	SiN	1	25.280	0.000	*** \uparrow	1	7.256	0.017	* \uparrow
	Cop	1	0.075	0.789		1	12.547	0.003	**
	Cop x SiN	1	0.064	0.803		1	0.326	0.576	
N:P	SiN	1	0.000	0.988		1	9.081	0.009	** \downarrow
	Cop	1	0.034	0.856		1	3.567	0.078	
	Cop x SiN	1	0.009	0.924		1	0.658	0.430	

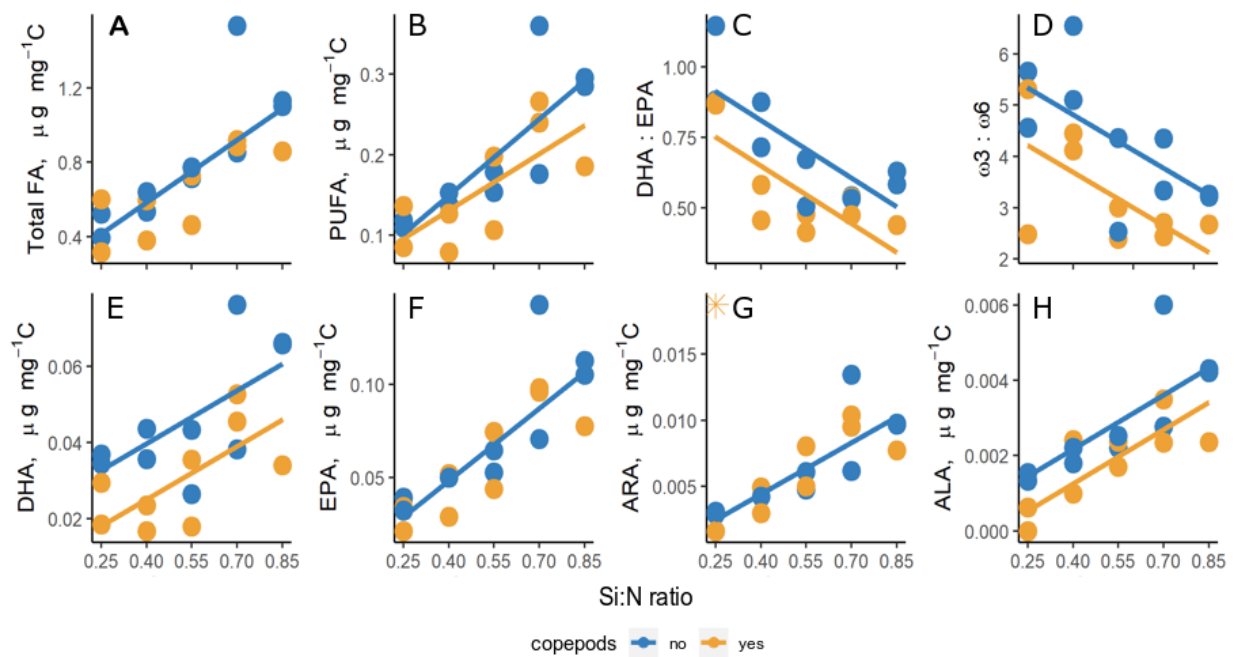


Figure 2.6 Seston fatty acid parameters on the last day of the experiment across different Si:N ratio in copepod treatments. Asterisk in ARA graph (G) marks an outlier which was not included in the statistical analysis.

Chapter II

Table 2.3 ANCOVA results testing for the effects of changing Si:N ratio (SiN), copepod grazing (Cop) and the interaction between these variables (Cop x SiN) on seston fatty acids. * $p \leq 0.05$, ** $p < 0.01$, *** $p < 0.001$. Positive increase with increasing Si:N is indicated with ↑, negative with ↓.

Response variable	factor	df	F	p
Total fatty acids ($\mu\text{g gC}^{-1}$)	SiN	1	30.892	0.000 *** ↑
	Cop	1	3.005	0.104
	Cop x SiN	1	1.103	0.310
PUFA ($\mu\text{g gC}^{-1}$)	SiN	1	25.991	0.000 *** ↑
	Cop	1	1.595	0.226
	Cop x SiN	1	0.537	0.475
PUFA (% of total FA)	SiN	1	0.065	0.802
	Cop	1	0.045	0.835
	Cop x SiN	1	0.304	0.590
DHA ($\mu\text{g gC}^{-1}$)	SiN	1	14.491	0.002 ** ↑
	Cop	1	7.489	0.015 *
	Cop x SiN	1	0.252	0.623
EPA ($\mu\text{g gC}^{-1}$)	SiN	1	37.074	0.000 *** ↑
	Cop	1	1.111	0.308
	Cop x SiN	1	0.172	0.685
ARA ($\mu\text{g gC}^{-1}$)	SiN	1	30.995	0.000 *** ↑
	Cop	1	0.029	0.867
	Cop x SiN	1	0.022	0.884
ALA ($\mu\text{g gC}^{-1}$)	SiN	1	27.824	0.000 *** ↑
	Cop	1	5.530	0.033 *
	Cop x SiN	1	0.439	0.518
DHA (% of total FA)	SiN	1	3.332	0.088
	Cop	1	6.639	0.021 *
	Cop x SiN	1	1.754	0.205
EPA (% of total FA)	SiN	1	16.004	0.001 *** ↑
	Cop	1	0.537	0.475
	Cop x SiN	1	3.330	0.088 *
ARA (% of total FA)	SiN	1	10.826	0.005 ** ↑
	Cop	1	7.809	0.014 *
	Cop x SiN	1	1.905	0.189
ALA (% of total FA)	SiN	1	19.088	0.001
	Cop	1	5.682	0.031 *
	Cop x SiN	1	6.703	0.021
DHA:EPA	SiN	1	20.061	0.000 ** ↓
	Cop	1	7.261	0.017 *
	Cop x SiN	1	0.063	0.805
ω 3: ω 6	SiN	1	9.685	0.007 ** ↓
	Cop	1	6.713	0.020 *
	Cop x SiN	1	0.134	0.719

Discussion

Phytoplankton communities are changing globally and both increases and decreases in the relative abundance of diatoms have been reported in different areas of the ocean (e.g. increase: (Ajani *et al.*, 2014, Hinder *et al.*, 2012), decrease: (Leterme *et al.*, 2005, Leterme *et al.*, 2006)). However, the effects of changing diatom proportion in phytoplankton on consumers, such as copepods, in terms of food quantity and quality is not well understood. Evaluating essential fatty acid and particulate nutrient indicators in phytoplankton communities shaped by different Si:N ratios can provide insight into how phytoplankton nutritional quality is changing across different levels of diatom dominance. Our study shows that essential fatty acid and particulate nutrient quantities and composition are strongly affected by changing Si:N ratios. Furthermore, we present indications that nutritional quality of phytoplankton is likely moderated by selective grazing of copepods.

Fatty acid indicators

The majority of fatty acid indicators, namely the sum of PUFAs, DHA and EPA per seston carbon unit, increased with increasing Si:N ratios. As diatom relative biomass increased with Si:N, this can indicate that diatom dominated communities contained more essential fatty acids than communities in which non-silicifying plankton were more abundant. This may have a positive effect on copepod egg production, hatching success and development (Arts *et al.*, 2009, Evjemo *et al.*, 2008). Further, essential fatty acids ARA and ALA also increased with increasing Si:N. ARA and ALA are important precursors to EPA and DHA and even though it is not yet fully understood to what extent copepods are able to convert them, high amounts of these FAs in copepod eggs signal about their importance (Brett *et al.*, 2009, Evjemo *et al.*, 2008).

Whereas the increase in essential fatty acid absolute concentrations indicates better food quality in high Si:N environments, the relative proportion of certain FAs may be more beneficial for consumers at lowered Si:N. The ratios $\omega 3:\omega 6$ and DHA:EPA are important indicators of phytoplankton food quality (Shin *et al.*, 2003) and in our study they significantly decreased with increasing Si:N. Dinoflagellates are rich in DHA (Jónasdóttir, 2019) and their dominance in low Si:N treatments likely caused elevated $\omega 3:\omega 6$ and DHA:EPA in low copepod and low Si:N treatments. The reported optimal $\omega 3:\omega 6$ ratio for copepod egg production varies between 6 and 25 (Jónasdóttir, 1994, Jonasdottir *et al.*, 1995). The ratio between $\omega 3$ and $\omega 6$ fatty acids in our highest Si:N treatments were between 2 and 5 indicating lowered food quality. This

shows that even in natural communities dominated by diatoms, seston fatty acid composition may be suboptimal which supports the hypothesis that diatoms may be nutritionally insufficient (Jones & Flynn, 2005).

Stoichiometric indicators

Seston C:N and C:P ratios, frequently used indicators of stoichiometric food quality, were higher than the Redfield ratio of approximately 7:1 and 106:1, respectively, in most of the treatments, indicating relatively low food quality. Additionally, the food quality deteriorated with increasing Si:N ratios. While the difference between Redfield C:N and the ratios in the lowest Si:N ratio was marginal, C:N values reached values close to 10 in high Si:N ratios during the post-bloom period. This can be considered as a strong limitation as Van Nieuwerburgh *et al.* (2004) showed that a limitation in phytoplankton C:N ratios of 8 to 13 can be transmitted to copepods. Our results are in line with Gilpin *et al.* (2004) who also reported lowered C:N ratios under silicon limitation.

While seston C:P molar ratio increased with increasing Si:N treatments, it was not in a range which would affect metazoans negatively. In our study, C:P values varied between 120 and 140 and even though this is slightly above the Redfield ratio (106), it is within a range which Boersma and Elser (2006) reported to result in maximum secondary production (125-250).

The role of copepods

Copepods can feed selectively – multiple studies have shown that when copepods are offered artificial microalgal mixtures, they feed preferentially on non-toxic, more nutritious or less silicified algae (Cowles *et al.*, 1988, Liu *et al.*, 2016, Schultz & Kiørboe, 2009). Evidence of selective feeding based on phytoplankton nutritional quality in natural communities is, however, scarce. In our study, fatty acid quality indicators were higher in low copepod treatments, with significant differences in DHA and ALA contents per seston carbon unit and DHA:EPA and $\omega 3$: $\omega 6$ ratios. This indirectly indicates that more nutritious plankton was removed more efficiently in high copepod treatments.

The relative content of ALA and ARA were positively affected by increased copepod grazing in some Si:N ratios. ALA is an important biosynthetic precursor to EPA and DHA, however most of copepod species are not able to synthesize significant amounts of these FAs from ALA (Bell *et al.*, 2007). ARA is an $\omega 6$ FA, which, together with EPA, is an ultimate precursor for eicosanoid synthesis. Higher relative abundance of ALA and ARA in high copepod treatments

could be explained by the fact that seston of smaller size fractions has been reported to contain more ALA and ARA (Kainz *et al.*, 2009). In our study, picoplankton relative biomass was higher in high copepod treatments. Small phytoplankton, such as picoplankton, is often inaccessible food for copepods due to their small size (Katechakis *et al.*, 2004). Consequently, a proportion of ALA and ARA was likely not accessible for copepods.

Seston nutrient stoichiometry can be affected by grazers in several ways. We hypothesized, that a signal of selective grazing on more nutritious plankton could be seen in higher C:N and C:P ratios in high copepod treatments. An increase of C:N with increasing grazer abundance has indeed been previously observed (Sommer *et al.*, 2005). However, our results showed the opposite, indicating that other impacts of copepods could be more important on seston stoichiometry. Grazers could increase seston C:N and C:P in two ways: first, by making nitrogen and phosphorus directly available via release of remineralized nutrients; second, by reducing phytoplankton abundance and therefore indirectly increasing nutrient availability “per capita” (Urabe, 1995).

Phytoplankton quality across Si:N ratios

Both C:P, C:N ratios and fatty acid indicators are used to assess microalgae nutritional value (Bi *et al.*, 2017, Boersma *et al.*, 2001, Jones & Flynn, 2005). In the present study, however, the two indicators showed contrasting results: with increasing Si:N ratios the phytoplankton nutritional quality was increasing according to most of the FA indicators yet decreasing according to stoichiometric indicators and DHA:EPA and $\omega 3:\omega 6$ ratios. One explanation could be that reduced C:N and C:P ratios affected algal biochemistry. For instance, it is known that lipid contents can increase when nutrients are limiting (Lynn *et al.*, 2000, Malzahn *et al.*, 2007, Sterner & Hessen, 1994). Malzahn *et al.* (2007) showed that nitrogen- and phosphorus-limited *Rhodomonas salina* cultures had higher content of total and unsaturated fatty acids but phosphorus-limited food resulted in poor condition of larval fish despite high fatty acid contents. Contradictory, Park *et al.* (2002) demonstrated that essential fatty acids affect algal food quality for *Daphnia* much more than phosphorus limitation. It is likely that the interplay between nutrient and fatty acids requirements depends on the condition of phytoplankton and the grazer: Anderson and Pond (2000) compared the roles of essential fatty acids, carbon, and nitrogen in the nutrition of marine copepods and concluded that limitation by fatty acids is the strongest when zooplankton use a monospecific diet. In natural communities, grazers may have higher contents of food available in high Si:N environments - both in terms of total carbon

content and FA availability. However, the quality per food item in terms of optimal ratios between FAs and nitrogen/phosphorus availability might be higher in lowered Si:N ratios.

Potential limitations of the study

The results of this study need to be interpreted in the light of some limitations. We used natural plankton communities, however, the closed nature of the experimental units did not allow for the exchange of organisms and nutrients as it would have occurred in the nature. This could have enhanced nutrient remineralization rates and lower C:N and C:P ratios as seston particles could not leave the system via sinking. Additionally, fatty acid data in seston were sampled at a single time point at the end of the experiment and it is known that fatty acid composition can change over the course of phytoplankton blooms in nature (Reuss & Poulsen, 2002). The time point for fatty acid sampling was selected to represent post-bloom conditions, which are very important for copepod recruitment (Miralto *et al.*, 1999, Sommer *et al.*, 2012a). However, these results may not represent full natural conditions in the bloom or pre-bloom periods.

Conclusions

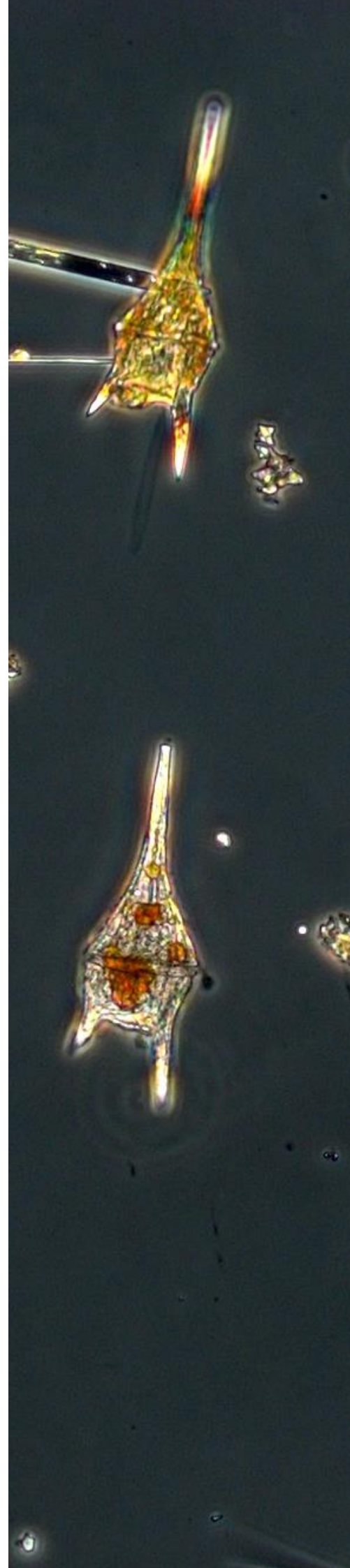
Our results confirm that changing Si:N ratios affect not only phytoplankton community structure but also its nutritional value for consumers in terms of fatty acid composition and nutrient stoichiometry. This suggests that altered nutrient supply in coastal waters, whether due to natural or anthropogenic perturbations, is affecting plankton food webs through changes in food quantity and quality. Additionally, we show that grazers are important in shaping seston stoichiometry and fatty acid composition in natural phytoplankton communities. We suggest that copepods feed selectively on phytoplankton rich in essential fatty acids and this can affect FA composition of seston. Our study adds to our growing understanding of how various changes in bottom-up and top-down factors alter the structure and functioning of plankton food webs in the ocean.

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Chapter III

Changing Si:N ratios due to
nitrogen enrichment:
Effects on plankton biomass and
community composition



Changing Si:N ratios due to nitrogen enrichment: effects on plankton biomass and community composition

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Abstract

Despite many environmental efforts, anthropogenic nutrient enrichments continue to enhance phytoplankton blooms worldwide. Nitrogen and phosphorus compounds released to inland and coastal waters not only provide surplus of nutrients for aquatic primary producers but also disbalance the stoichiometry of nutrient supply. Silicon is an essential element for diatoms but not required by most other groups of plankton; thus, nitrogen and phosphorus enrichment leads to lowered silicon to nitrogen (Si:N) and silicon to phosphorus ratios. Using a mesocosm experiment, we studied the effects of increasing nitrogen concentrations and thereby decreasing Si:N ratio on natural plankton communities from the Baltic Sea and their trophic interactions. We used five different concentrations of nitrogen and phosphorus (balanced according to Redfield stoichiometry) to create a range of Si:N ratios. Additionally, we manipulated copepod abundance to understand the top-down effects on communities experiencing nutrient enrichment. While plankton biomass during the bloom period was higher in high nitrogen (low Si:N) treatments, during the post-bloom period this trend switched and diatom dominated low nitrogen (high Si:N) communities contained higher biomass. Lowered Si:N ratios affected most phytoplankton groups positively during the bloom period, with an exception to several abundant diatom species. High Si:N ratios seem to be crucial in sustaining diatom biomass when nutrients are limited: during the post-bloom period, when all nutrients were exhausted, there was an increasing trend in diatom biomass with increasing Si:N. Copepods shape plankton communities via feeding on dinoflagellates, chlorophytes and a diatom *Skeletonema costatum*, yet we detected no significant effects of altered Si:N ratios on copepod abundance. In agreement with the resource ratio theory, results of this study confirm that plankton community composition respond to the decrease in Si:N ratios due to nitrogen enrichment in a similar way as they responded to Si:N decline due to silicate decrease in a previous experiment. Consequently, while nutrient concentrations define the total biomass of phytoplankton bloom, resource ratios are important in determining phytoplankton community structure and composition in eutrophic conditions.

Introduction

Water bodies around the world face increasing inputs of nitrogen (N) and phosphorus (P) compounds from anthropogenic sources such as agriculture, waste-water and fossil fuels burning (Howarth *et al.*, 2002, Spokes & Jickells, 2005). Climate change might further accelerate nutrient pollution due to predicted increase in precipitation in many regions (Sinha *et al.*, 2017). The supply of dissolved silicon (Si), on the other hand, originates mainly from natural sources, such as rock weathering. Silicon supply has not changed or even decreased over the last decades due to damming-related increase in sedimentation (Treguer & De La Rocha, 2013), because with increased N and P concentrations and slower river flow more of the bioavailable Si is taken up by silicifying phytoplankton and exported to sediments before reaching coastal waters. Consequently, both the reduction in Si availability and increase in N and P concentrations result in lowered Si:N ratios in coastal and marine waters.

Changes in nutrient supply ratios are important as they can affect community composition of primary producers. As mechanistically explained in Tilman's resource ratio theory, ratios between limiting resources can predict the outcome of species competition (Tilman, 1982a). Thus, when Si:N ratios are lowered, non-silicifying phytoplankton have the potential to outcompete silicifiers, such as diatoms. This has been shown in several experiments where silicate concentration was manipulated to achieve reduction in Si:N ratios (Egge & Aksnes, 1992, Makareviciute-Fichtner *et al.*, 2020, Moriceau *et al.*, 2018, Sommer, 1998, Sommer, 2009, Sommer *et al.*, 2005).

Yet in nature, increasing nitrogen concentrations rather than declining silicate may be the more important reason behind lowered Si:N ratios in coastal areas (Downing *et al.*, 2016). Nitrogen, as opposed to silicon, is an essential nutrient for all phytoplankton groups and increasing nitrogen (and phosphorus) concentrations are known to increase total phytoplankton biomass (Smith, 2006). While nitrogen fixing cyanobacteria can use N_2 as nitrogen source, all other phytoplankton have to rely on combined nitrogen sources, mainly nitrate and ammonium. High phytoplankton biomass can increase shading and as a result light rather than nutrients may become the limiting resource which structures phytoplankton community composition (Brauer *et al.*, 2012). While several experiments have manipulated nitrogen concentrations to achieve different Si:N ratios (Gilpin *et al.*, 2004, Granéli, 1999, Kuosa *et al.*, 1997, Schöllhorn & Granéli, 1996), there is limited knowledge on how phytoplankton composition, micro- and zooplankton abundance changes over a gradient of Si:N ratios under nitrogen enrichment.

This study aims to assess how diatoms, non-silicifying phytoplankton, microzooplankton and copepods respond to a decline in Si:N ratios due to nitrogen enrichment and asks whether this response can be altered by changing top-down control. Additionally, we discuss the importance of ratios and concentrations of nutrient components while comparing the results of this study with an analogous experiment (Makareviciute-Fichtner *et al.*, 2020) where the same Si:N ratios were achieved by changing silicate concentrations instead of nitrogen.

Methods

Experimental design

The experiment was conducted between June 19th and July 7th 2017 in a mesocosm system in Kiel Fjord Germany (54°19'46.9"N 10°08'59.4"E). Natural Baltic Sea water was filtered using 125µm mesh size filters to exclude major mesozooplanktonic grazers and distributed among twenty 20L transparent plastic water-bags which served as experimental units. We manipulated nutrient concentrations to achieve five different Si:N ratios (Table 1) by adding nitrogen as nitrate (NaNO₃). Phosphorus was added as phosphate (KH₂PO₄) to keep N:P ratio according to Redfield proportions (16:1) (Redfield, 1934) in all treatments. The experimental units were placed in four bigger containers equipped by a flow through system with seawater to keep the ambient temperatures and a lid to reduce harmful UV light levels at the surface.

Table 3.1 Nutrient concentrations before experimental manipulation (ambient) and target concentrations for achieving a range of Si:N ratios. N - dissolved nitrogen as sum of NO₃, NO₂ and NH₄, P - PO₄, Si - SiO₄.

Concentrations	N, µmol/L	P, µmol/L	Si, µmol/L	Si:N ratio
<i>Ambient</i>	1.85	0.26	11.70	6.32:1
<i>Target</i>	46.88	2.93	11.70	0.25:1
	29.30	1.83	11.70	0.4:1
	21.21	1.33	11.70	0.55:1
	16.76	1.05	11.70	0.7:1
	13.83	0.86	11.70	0.85:1

Once a phytoplankton bloom had established (22nd of July), copepods were added to half of the experimental units at a target concentration of 30 ind/L (high copepod treatments). Remaining bags had lowered copepod concentrations due to filtration at the start of experiment (low copepod treatments). All treatments were realized in duplicates. Copepods dominated by *Eurytemora affinis* species were collected in Kiel canal and kept in culture until experimental manipulation.

Sampling

Fluorescence, temperature, pH and salinity were measured six times per week to follow the development of the experiment. Experimental bags were shaken before taking samples to ensure mixing. Relative fluorescence was measured using a fluorometer 10-AU (Turner Design) immediately after sampling.

Samples for nutrient, pico-, nano- and microzooplankton (further referred to as “plankton”) and bacteria analyses were taken three times per week (Monday, Wednesday, and Friday). Water samples for dissolved nutrient analysis ($\text{NO}_3^-/\text{NO}_2^-$, NH_4^+ , SiO_4^- and PO_4^{3-}) were filtered using pre-washed (10% HCl) cellulose acetate filters, frozen and kept at -20°C until nutrient measurement according to protocols by Hansen and Koroleff (1999) with an auto-analyzer (Skalar, SANPLUS; Breda/Netherlands).

Photosynthetic picoplankton and bacteria were counted using flow-cytometer (FACScalibur, Becton Dickinson). For picoplankton enumeration, 3mL of water were analysed at a flow rate of $35\mu\text{L min}^{-1}$; for bacteria counts – 2mL at $8\mu\text{L min}^{-1}$. Water samples for nano- and mikroplankton counts were fixed with Lugol’s iodine solution and later counted according to Utermöhl’s (1958) inverted microscope method. For most common species at least 100 individuals were counted giving minimum 20% accuracy (Andersen, 2005). For each species, 20 randomly selected cells were measured and biovolume was calculated according to their closest geometric forms (Hillebrand *et al.*, 1999). Biovolume was converted to carbon according to the allometric conversion suggested by Menden-Deuer & Lessard (2000) for all plankton groups except picoplankton, where conversion a factor of $0.157\text{ pg C }\mu\text{m}^3$ was used in order to avoid overestimation of small species biomass (Sommer *et al.*, 2012b).

For copepod counts, water from experimental bags at the end of the experiment was filtered by $125\mu\text{m}$ mesh size filter, fixed using Lugo iodine solution and stored in the dark. Copepods were counted using dissecting microscope and identified to species level (adults) or family level (copepodites and nauplii).

Statistical analysis

To examine treatment effects on total plankton carbon content and biomass of different plankton groups we performed analysis of covariance (ANCOVA). The effects were assessed during two periods of the experiment: “bloom” – which we defined as the period including and surrounding peak of plankton biomass (days 5-10) and “post-bloom”, where plankton biomass

decline after the peak slowed down in most of the treatments (days 15-19). Due to inhomogeneous variances in bacteria data, generalized linear models (GLM) with Gamma family and log link were used instead of ANCOVAs. Statistical analysis and graphical representations were performed in R version 3.2.4 (R Core Team, 2019).

Results

Dissolved nutrients

Dissolved nutrients were depleted fast and reached low levels already by day 5 (Fig. 3.1). Only in the lowest Si:N treatments dissolved nitrogen (N) and phosphorus (P) remained relatively high until day 8, while in the remaining treatments N and P concentrations were low and decreased further with increasing Si:N ratios on day 5 of the experiment (Fig. 3.2). Silicate was

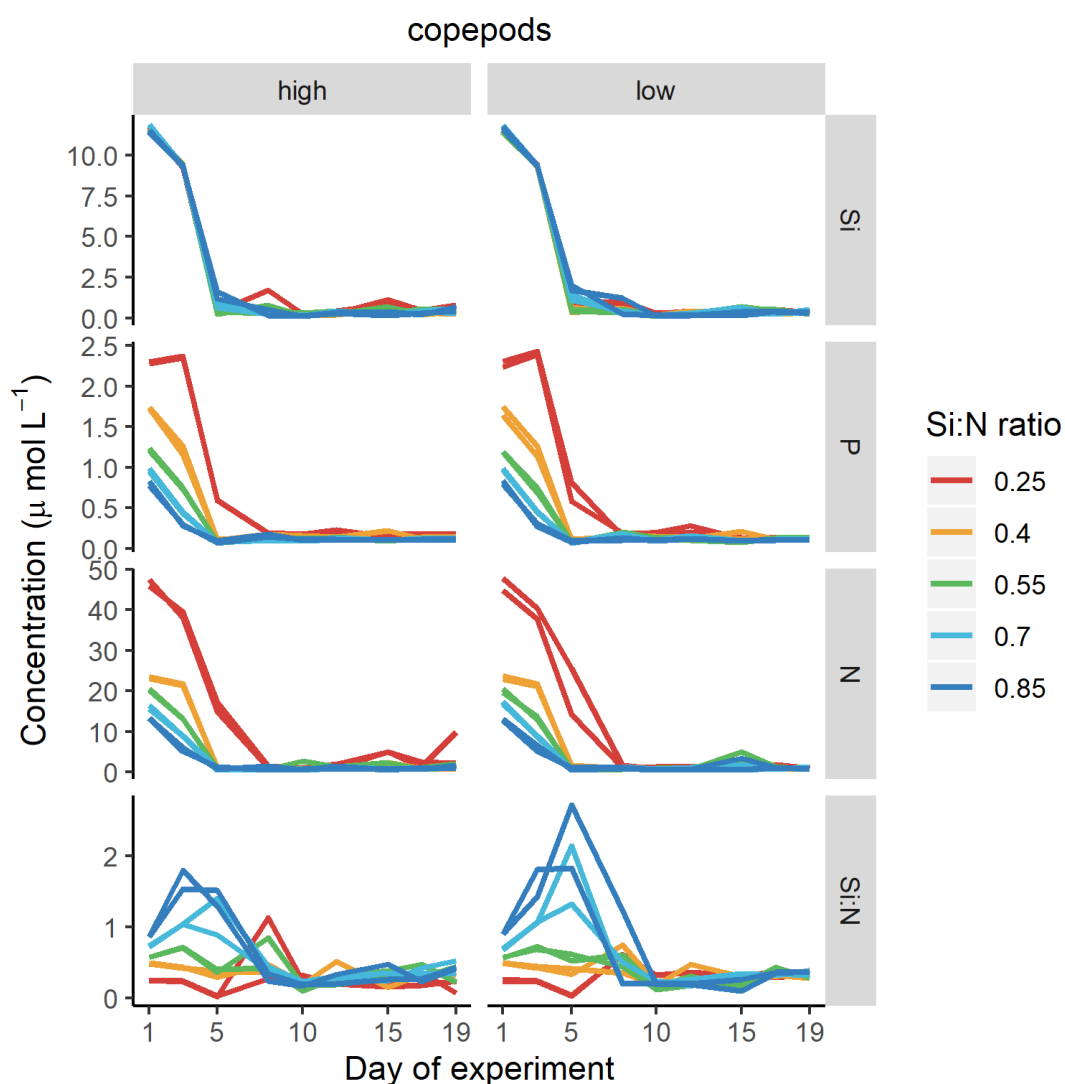


Figure 3.1 Dissolved nutrient concentrations and Si:N ratio values over the course of experiment. Si - SiO_4^- , P - PO_4^{3-} , N - sum of NO_3^- , NO_2^- and NH_4^+ , Si:N - molar ratio between Si and N.

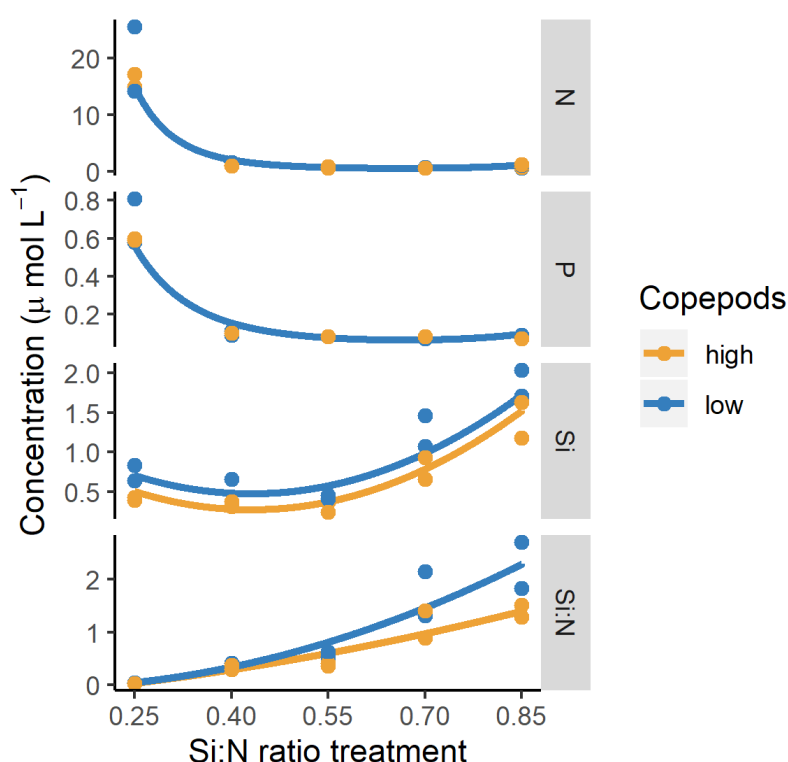


Figure 3.2 Dissolved nutrient concentrations and Si:N ratios across Si:N ratio treatments. Statistical results are available in Table S 3.1.

taken up a little slower than nitrogen and small amounts were still available on day 5 (Fig.3.1). This led to Si:N ratios becoming even higher in high Si:N treatments (Fig. 3.1, Fig. 3.2). Dissolved silicon was taken up faster in high copepod treatments (Fig. 3.2, $df=1$, $\chi^2=7.191$, $p=0.001$). The ratio between dissolved silicon and dissolved nitrogen was higher under low copepod treatments in high Si:N ratio treatments, while the difference in the lowest Si:N treatments was marginal (Fig. 3.2, Table S 3.1).

Plankton biomass and composition

Unicellular plankton biomass increased sharply after nutrient manipulation and reached peak values between days 5 and 10 of the experiment (bloom period) (Fig. 3.3 A). After the bloom period, plankton biomass declined first sharply and then slowly (post-bloom period) (Fig. 3.3 A). During the bloom period plankton biomass decreased with increasing initial Si:N ratios ($df=1$, $F=86.52$, $p<0.001$, Fig. 3.3 B). However, during the post-bloom period this trend switched and there was a slight increase in plankton carbon concentrations with increasing Si:N ratios ($df=1$, $F=7.51$, $p=0.015$, Fig. 3.3 C). High copepod grazing had a negative effect on

plankton biomass during the bloom period ($df=1$, $F=19.15$, $p<0.001$, Fig. 3.3 B) but not during the post-bloom ($df=1$, $F=0.87$, $p=0.36$, Fig. 3.3 C).

Plankton composition was relatively diverse at the start of the experiment, but by day 5 diatoms became dominant and formed the major part of the bloom biomass in all treatments (Fig. 3.4). Diatom biomass during the bloom period decreased only slightly along the Si:N gradient while the biomass of picoplankton, dinoflagellates and chlorophytes decreased more strongly (Fig. 3.5, Table 3.2). High copepod grazing did not affect diatom and ciliate biomass but resulted in lowered biomass of dinoflagellates, chlorophytes and picoplankton during the bloom period (Fig. 3.5, Table 3.2).

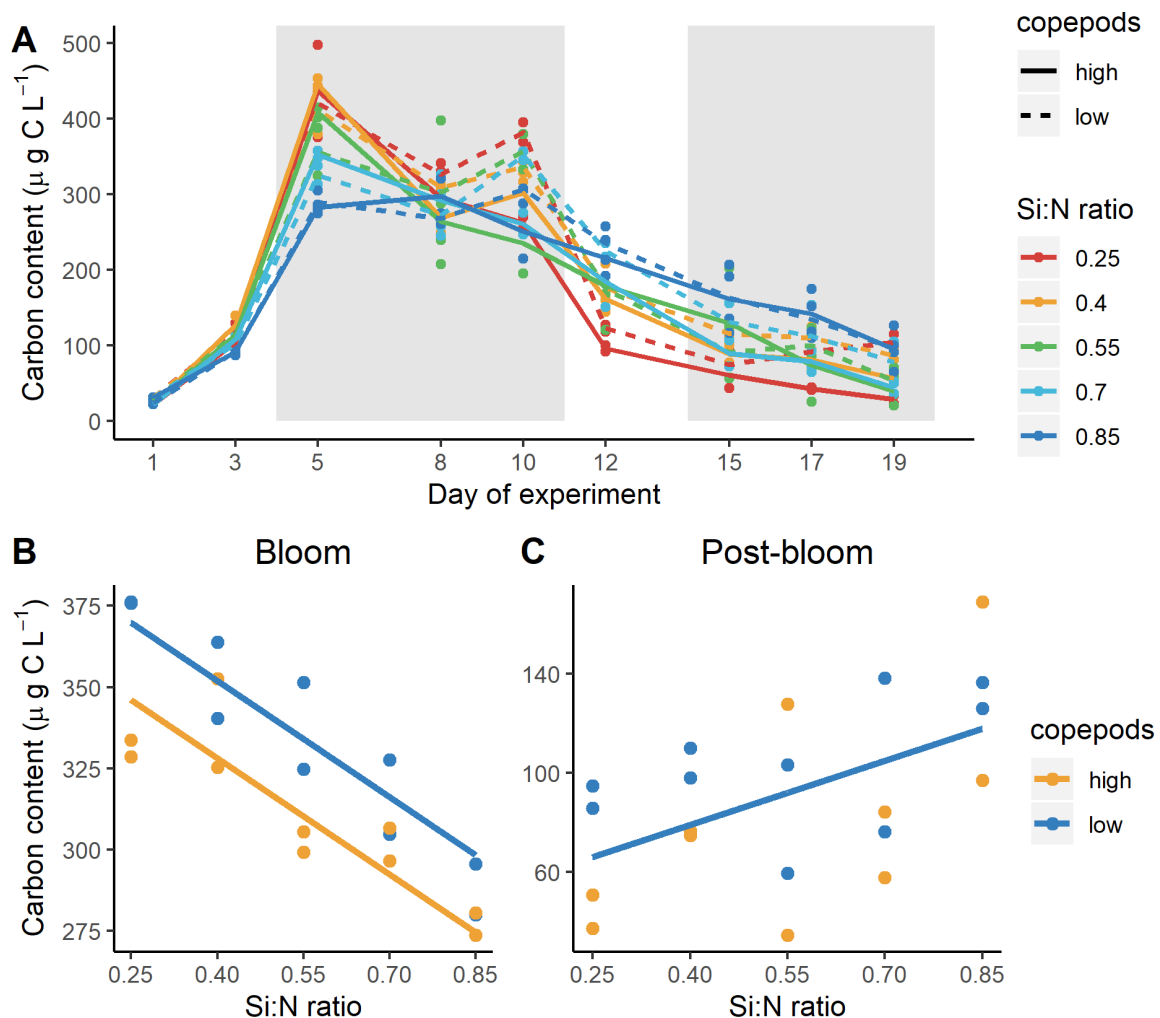


Figure 3.3 Total plankton biomass change over the course of experiment (A), and as a function of Si:N ratio during the bloom (B) and post-bloom (C) periods. Grey shaded areas in (A) indicate bloom (days 5-10) and post-bloom (days 15-19) periods.

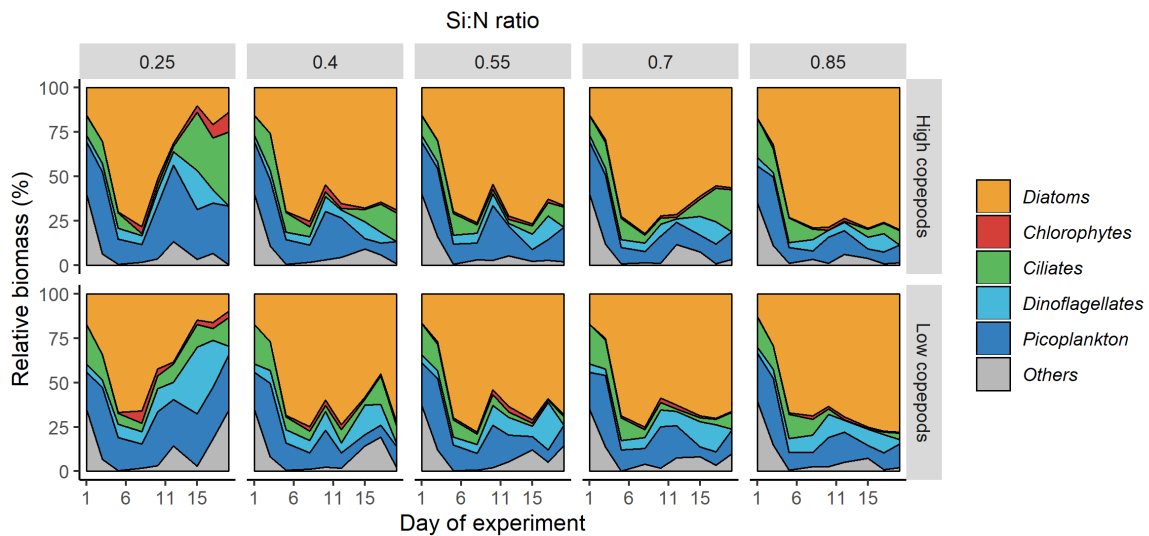


Figure 3.4 Plankton functional group composition over the course of experiment

After the bloom, diatoms remained relatively abundant in all but the lowest Si:N treatment (0.25:1), where photosynthetic picoplankton, dinoflagellates and ciliates formed a large part of plankton biomass (Fig. 3.4). Total diatom biomass during post-bloom period increased with increasing Si:N ratio, while ciliates, chlorophytes and sum of less common plankton groups (others, under low copepod grazing) decreased with increasing Si:N ratio (Fig. 3.5).

During the bloom period, high copepod grazing significantly affected dinoflagellate, chlorophyte and picoplankton biomass negatively but had no statistically significant effects on diatoms, ciliates and other plankton (Fig. 3.5, Table 3.2). During the post-bloom period, ciliate biomass was higher under high copepod grazing, the biomass of other plankton (dominated by unidentified ameboids) was lower, and diatom, chlorophyte, dinoflagellate and picoplankton biomass was not affected by grazing during this stage of the experiment.

The responses of individual diatom species to nutrient addition and resulting changes in Si:N ratios were not uniform. The biomass of *Skeletonema costatum* increased sharply after nutrient manipulation and declined afterwards relatively fast in all Si:N treatments (Fig. 3.6). During bloom period, it's biomass was highest in the lowest Si:N treatments, and decreased with Si:N ratio (Fig. 3.7). The biomass of *Cerataulina pelagica* increased sharply after the start of the experiment and declined fast in low Si:N ratio treatments, yet it sustained moderate biomass until the end of experiment at high Si:N ratios (Fig. 3.6). *Dactyliosolen fragilissimus* was the most dominant diatom and its biomass increased slower but remained high also during the post-bloom period in most of the treatments with the exception of the lowest Si:N ratio (Fig. 3.6).

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The average biomass of *D. fragilissimus* and *C. pelagica* increased with initial Si:N ratios already during the bloom period (Fig. 3.7). The bloom of *Thalassionema nitzschioides* occurred later than of the other species and, while its biomass declined sharply in the lowest Si:N ratios, it sustained its biomass in higher Si:N treatments until the end of the experiment (Fig. 3.6). The average biomass of *T. nitzschioides* was not affected by Si:N manipulation during the bloom period, but during post-bloom, it's biomass increased with increasing Si:N ratios, just as the biomass *D. fragilissimus* and *S. costatum* (Fig. 3.7).

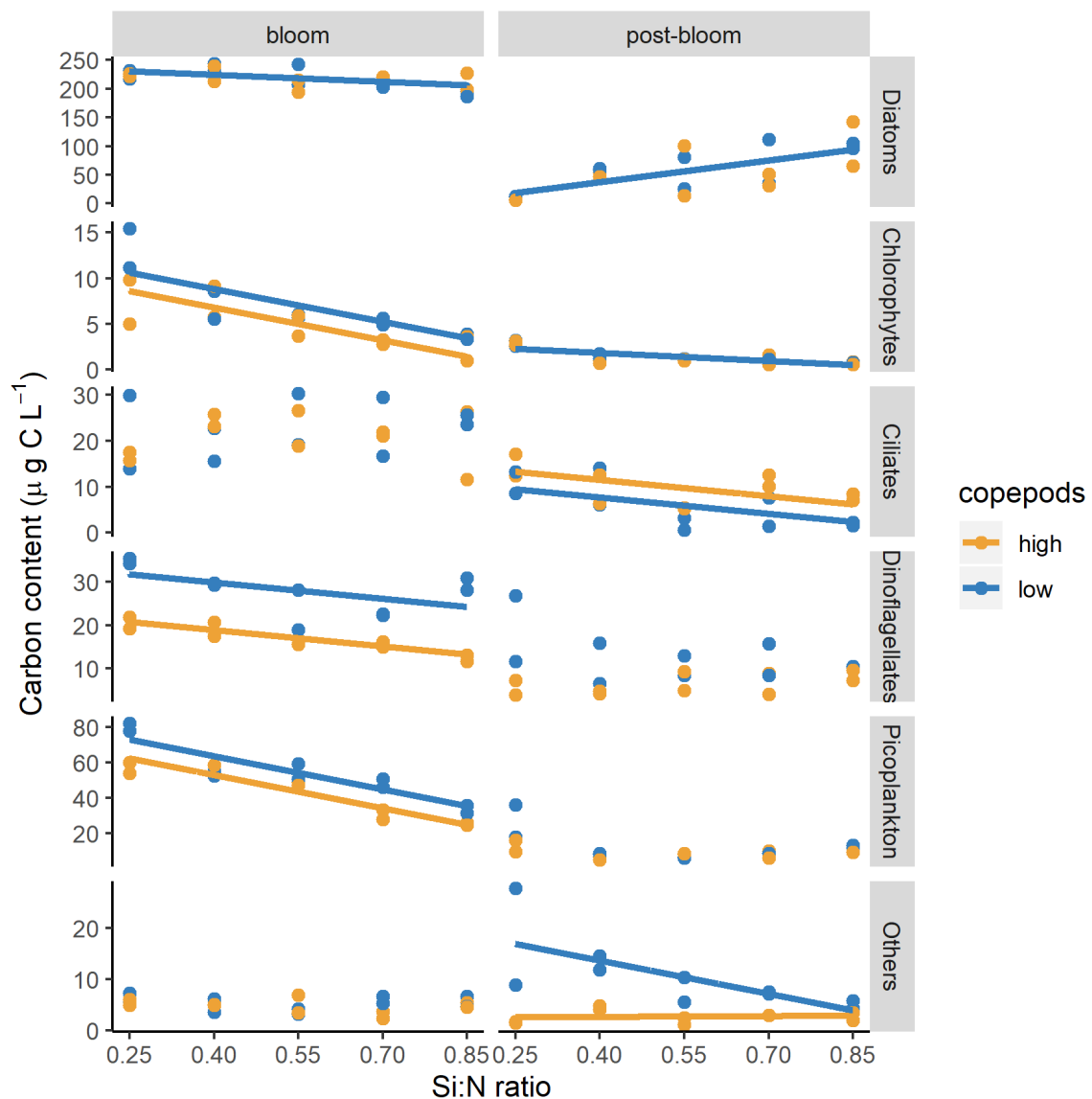


Figure 3.5 Average biomass of major plankton groups during bloom and post-bloom periods as a function of initial Si:N ratios. Lines indicate where Si:N ratio treatments significantly affected carbon content of different groups. See Table 2 for all statistical results.

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Table 3.2 ANCOVA results for the effects of changing Si:N ratio (Si:N), copepod grazing (Cop) and the interaction between these variables (Cop x Si:N) on carbon biomass of different groups of plankton during bloom and post-bloom periods. * $p \leq 0.05$, ** $p < 0.01$, *** $p < 0.001$.

	Bloom period					Post-bloom period			
	Factor	Df	F value	Pr(>F)		Df	F value	Pr(>F)	
<i>Diatoms</i>	Si:N	1	7.457	0.014	*	1	14.779	0.001	**
	cop	1	0.001	0.974		1	0.320	0.579	
	Si:Nxcop	1	2.339	0.145		1	0.002	0.961	
		16				16			
<i>Chlorophytes</i>	Si:N	1	32.820	3.11x10 ⁻⁵	***	1	23.249	0.0001	***
	cop	1	5.262	0.035	*	1	0.170	0.685	
	Si:Nxcop	1	1.022	0.326		1	0.059	0.809	
		16				16			
<i>Ciliates</i>	Si:N	1	0.350	0.562		1	9.474	0.007	**
	cop	1	0.489	0.494		1	5.478	0.039	*
	Si:Nxcop	1	0.168	0.688		1	1.001	0.331	
		16				16			
<i>Dinoflagellates</i>	Si:N	1	10.832	0.004	**	1	0.453	0.510	
	cop	1	46.25	4.28x10 ⁻⁶	***	1	11.147	0.004	**
	Si:Nxcop	1	0.036	0.851		1	3.358	0.085	
		16				16			
<i>Picoplankton</i>	Si:N	1	96.800	3.44x10 ⁻⁸	***	1	2.806	0.113	
	cop	1	15.503	0.001	*	1	1.788	0.199	
	Si:Nxcop	1	0.199	0.660		1	1.390	0.255	
		16				16			
<i>Others</i>	Si:N	1	0.6308	0.438		1	7.147	0.016	*
	cop	1	0.890	0.359		1	21.038	0.0003	***
	Si:Nxcop	1	0.364	0.554		1	7.819	0.012	*
		16				16			

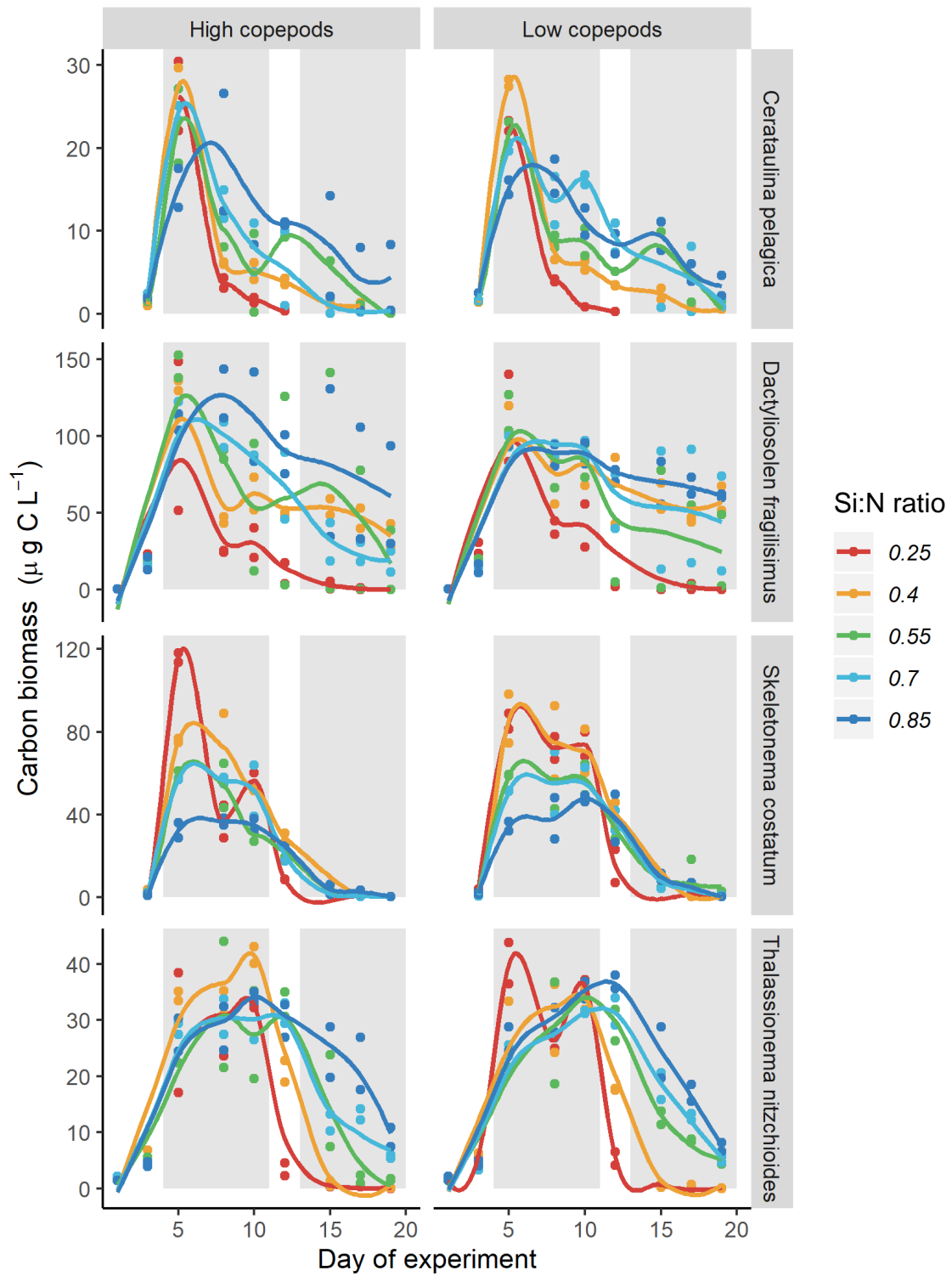


Figure 3.6 Carbon biomass development of four most common diatom species over the course of experiment. Gray shaded areas indicate bloom and post-bloom periods.

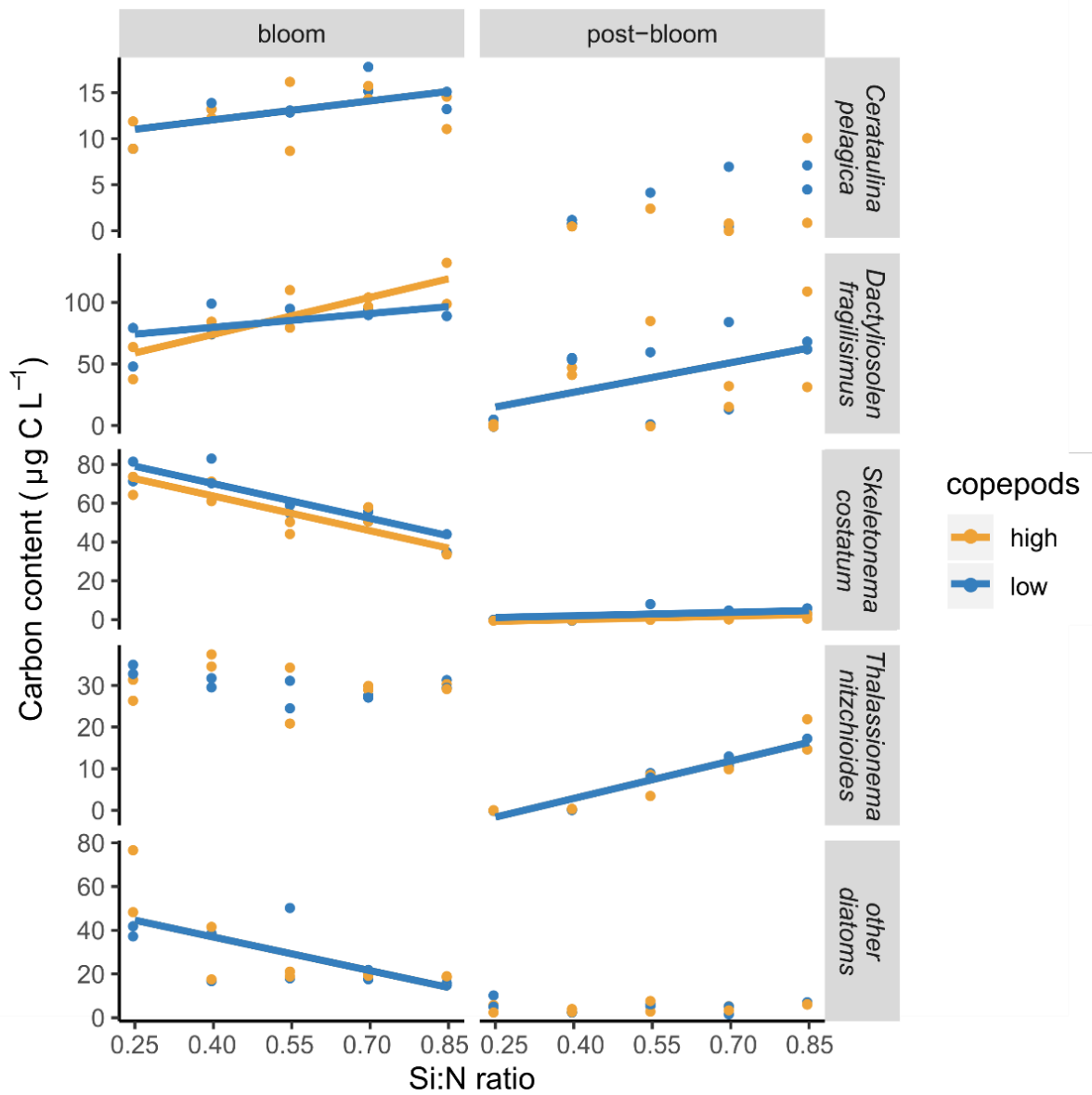


Figure 3.7 The change in the biomass of most common diatom species over a range of Si:N treatments during bloom and post-bloom periods. Lines indicate where Si:N ratio treatments significantly affected carbon content of different groups. See Table 3 for all statistical results.

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Table 3.3 ANCOVA model for the effects of initial Si:N ratio manipulation, copepod grazing and interaction between these terms on most common diatom species.

	Bloom period					Post-bloom period			
	Factor	Df	F value	Pr(>F)		Df	F value	Pr(>F)	
<i>Cerataulina pelagica</i>	Si:N	1	8.291	0.011	*	1	4.347	0.063	
	cop	1	0.274	0.608		1	0.804	0.391	
	Si:Nxcop	1	0.786	0.389		1	0.017	0.898	
		16				16			
<i>Dactyliosolen fragilisimus</i>	Si:N	1	22.194	0.000	***	1	5.894	0.027	*
	cop	1	0.352	0.561		1	0.080	0.780	
	Si:Nxcop	1	4.660	0.046	*	1	0.000	0.990	
		16				16			
<i>Skeletonema costatum</i>	Si:N	1	65.386	0.000	***	1	9.394	0.007	**
	cop	1	4.148	0.059	*	1	5.948	0.027	*
	Si:Nxcop	1	0.495	0.492		1	2.040	0.172	
		16				16			
<i>Thalassionema nitzschioides</i>	Si:N	1	1.456	0.245		1	126.348	0.000	***
	cop	1	0.030	0.865		1	0.098	0.759	
	Si:Nxcop	1	0.194	0.665		1	0.100	0.755	
		16				16			
<i>Other diatoms</i>	Si:N	1	15.082	0.001	**	1	0.358	0.558	
	cop	1	0.238	0.632		1	0.302	0.590	
	Si:Nxcop	1	1.090	0.312		1	0.658	0.429	
		16				16			

Bacterial abundance increased first during the bloom period and after a slight decline increased even stronger during the post bloom period (Fig. 3.8A). Lowered Si:N ratios affected bacterial abundance positively both during bloom ($\chi^2=2.2$, df=2, $P<0.001$, Fig. 3.8B) and post-bloom periods ($\chi^2=1.8$, df=2, $P<0.001$, Fig. 3.8C). During the bloom, bacteria abundance was higher in high copepod treatments ($\chi^2=0.02$, df=1, $P<0.001$), yet during post-bloom copepods affected bacteria abundance negatively, especially in low Si:N treatments ($\chi^2=0.79$, df=2, $P<0.001$, Fig. 3.8C). Neither nauplii, copepodites nor adult copepod abundance responded to Si:N ratio treatments (Fig. S3.1, Table S3.2).

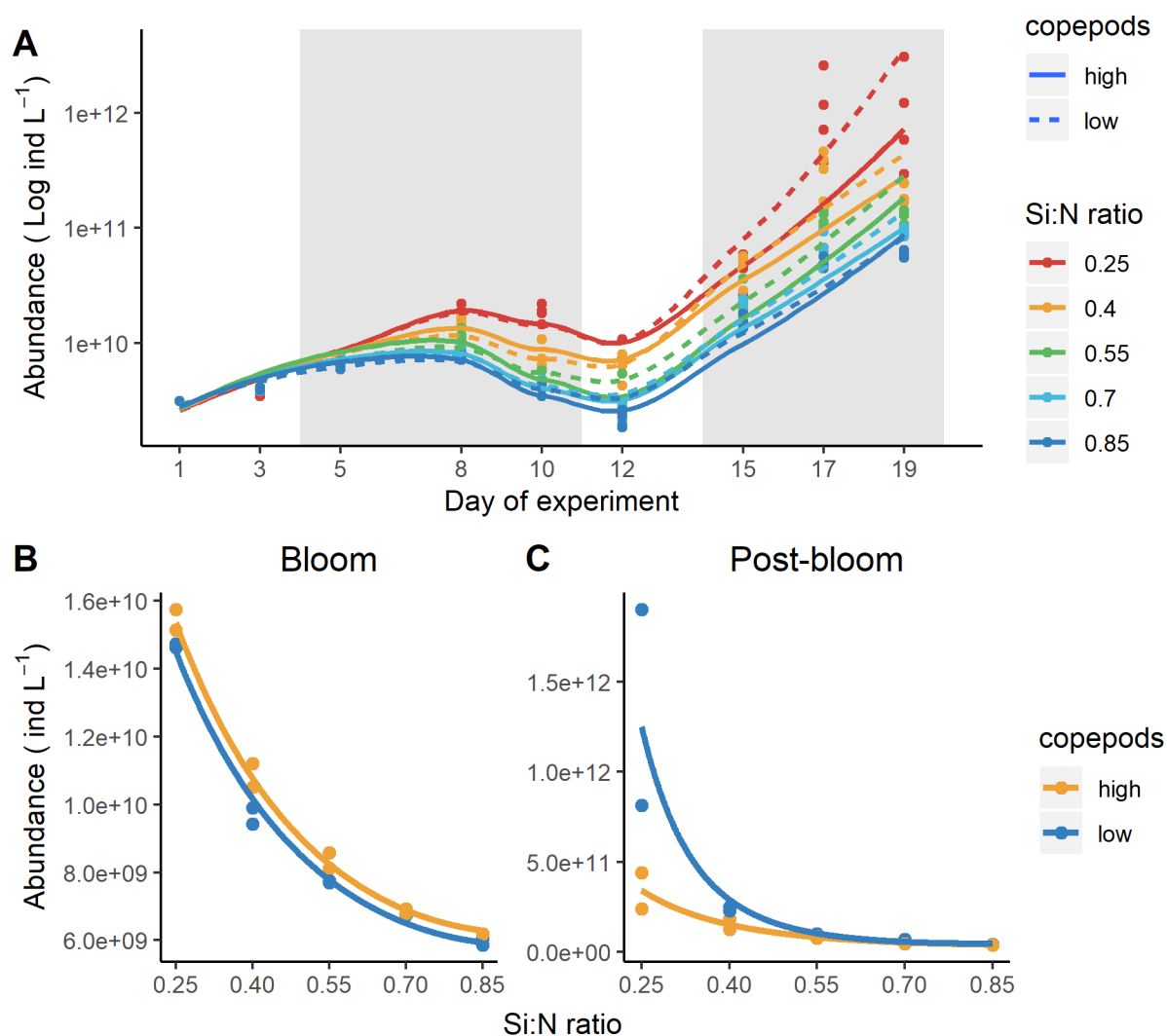


Figure 3.8 Bacteria abundance over the course of experiment (A) and average abundance during bloom (B) and post-bloom (C) periods. Gray-shaded areas mark plankton bloom and post-bloom periods.

Discussion

It is well known that nitrogen and phosphorus enrichment can lead to eutrophication and degradation of aquatic systems (Nixon & Fulweiler, 2009). The role of changing nutrient ratios, however, is debated (Brauer *et al.*, 2012). Our results show, that lowered Si:N ratios due to nitrogen addition have strong effects on diatoms with consequences on total plankton biomass and likely other groups of plankton, especially during the period after a phytoplankton bloom.

Plankton response to changing Si:N due to an increase in nitrogen supply

Nitrogen enrichment, as expected, led to an increase in the phytoplankton bloom. Most of phytoplankton groups benefited from nutrient addition during the bloom period, yet total diatom biomass responded less to nitrogen enrichment than other phytoplankton groups.

Coinciding with the build-up of the bloom (day 5) silicate was used up most rapidly in high nitrogen (low Si:N) treatments. This indicates that already during the bloom period diatoms were affected by silicon limitation in high nitrogen treatments (low Si:N).

However, individual diatom species responded to nutrient addition differently. *S. costatum* did benefit from nitrogen and phosphorus addition: during the bloom period, its biomass was higher in high nitrogen treatments and decreased with increasing Si:N ratios. The growth of *S. costatum* was characterized by a fast increase followed by a sharp decrease of its biomass once the nutrients were depleted. The biomass of two other common species - *D. fragilissimus* and *C. pelagica*, in contrast, increased with increasing Si:N already during the bloom and they both grew slower and their biomass declined later. This could possibly be explained by different nutrient uptake and growth rates. *S. costatum* is smaller than the latter two species and therefore has likely higher growth rates and could take up nutrients more rapidly and grow faster, which coincides with its ability to form blooms under nutrient surplus (Hu *et al.*, 2011). *D. fragilissimus* and *C. pelagica* are likely able to store nutrients (Phlips *et al.*, 2010) which would explain their persistent abundance until the end of experiment under high Si:N ratios. In general, biomass of all dominant diatom species persisted longer in higher Si:N treatments. This is in accordance with observations in nature where diatom blooms can be terminated by low silicate concentrations (Krause *et al.*, 2019). Consequently, silicate availability and high Si:N ratios in nutrient supply seem to be essential in sustaining diatoms and in the case of this experiment also total phytoplankton biomass under nutrient limitation.

Silicate is also thought to play a substantial role in diatom defense against copepod grazing as there is experimental evidence from single-species studies that grazing is reduced when cells are more heavily silicified (Liu *et al.*, 2016, Pancic *et al.*, 2019). Additionally, Grønning and Kiørboe (2020) reported that diatom cell silicification increases when copepod cues are present and suggested that this could lead to earlier silica limitation of diatom blooms under high copepod grazing pressure. Our study supports this with evidence from more natural phytoplankton communities – when dissolved nutrients started to be limiting (day 5 of the experiment), silicon was indeed taken up faster under high copepod grazing (Fig. 3.2). This could suggest that diatoms would be more susceptible to grazing under low Si:N ratios. However, grazing did not affect total biomass of diatoms in the current study. Only fast-growing *S. costatum* was negatively affected by grazing during bloom period (Fig. 3.7, Table 3.3). The absence of significant differences between biomass of other diatom species in low and high copepod treatments and higher diatom proportion in plankton under high copepod

grazing (Fig. 3.10) indicate that in general diatoms were not preferential food sources for copepods. Diatoms were originally thought to be crucial and nutritious food for mesozooplankton (Mann, 1993), yet in the last two decades evidence has accumulated that diatom diet can cause reduction in copepod egg production, hatching success and post-embryonic development (Carotenuto *et al.*, 2002, Ianora *et al.*, 2003). While copepods do feed on diatoms (Olson *et al.*, 2006), an avoidance of several dominant diatom species has also previously been observed (e.g. *Thalassiosira* spp. and *Dactyliosolen fragilissimus*) (Leising *et al.*, 2005, Makareviciute-Fichtner *et al.*, 2020, Olson *et al.*, 2006). This illustrates further need to investigate copepod preferential feeding on a species level, diatom defense mechanisms and their role in copepod nutrition.

Our results show that non-silicifying phytoplankton groups benefited from higher nitrogen and phosphorus availability and possibly lowered competition with diatoms in low Si:N treatments during the bloom period. However, during post-bloom either the slope of biomass decline with increasing Si:N was lower (chlorophytes) or not significant (picoplankton, dinoflagellates) anymore. High copepod abundance had a negative effect on dinoflagellate, chlorophyte and picoplankton biomass. Dinoflagellates are a nutritious food source for copepods (Jónasdóttir, 2019), chlorophytes were also a suitable size prey, thus their lower biomass in high copepod treatments could indicate preferential feeding on these groups. Lower picoplankton biomass under high grazing pressure is, however, counter-intuitive. Picoplankton is usually considered poorly accessible food source for copepods due to their small size (Berggreen *et al.*, 1988) and an increase in picoplankton abundance has been observed under high copepod grazing due to trophic cascade effects (copepods feed on major picoplankton grazers – microzooplankton) (Makareviciute-Fichtner *et al.*, 2020, Sundt-Hansen *et al.*, 2006, Zhao *et al.*, 2020). Heterotrophic nanoplankton (HNF) are also important grazers of picoplankton and bacteria (Sherr & Sherr, 2002, Zöllner *et al.*, 2009), however, their abundance was not assessed in our study. HNF can present an additional trophic level between microzooplankton and picoplankton or bacteria (Zöllner *et al.*, 2009) and consequently this could explain an increase of picoplankton and bacteria abundance under high copepod grazing pressure. Direct feeding of copepods on picoplankton, however, has also been reported by some studies, presumably via agglomeration or colony formation (Motwani & Gorokhova, 2013, Wilson & Steinberg, 2010). While we are not able to conclude whether higher picoplankton abundance resulted from more complex trophic interactions or due to direct feeding, our results show that increasing Si:N ratios lower the availability of preferential prey for copepods.

Bacterial abundance was positively affected by nitrogen and phosphorus enrichment (lower Si:N ratios) possibly due to more decomposition processes occurring under high biomass (bloom-period) or higher proportion of detritus (post-bloom period). We could speculate that different copepod effects during bloom and post-bloom periods could be either due to trophic or resource control. During bloom, bacterial growth could have been released from grazing pressure by dinoflagellates due to enhanced copepod grazing in high copepod treatments. The increase of bacterial abundance with decreasing Si:N ratios during post-bloom is likely related to accumulation of organic matter, of which the amount of detritus was likely highest in low-copepod, low Si:N treatments. A switch in microbial food-web structure could have also played a role: as Zöllner *et al.* (2009) has shown, bacteria abundance can increase with increasing copepod numbers due to trophic cascade effects via ciliates and HNF.

Increasing nitrogen or decreasing silicate – does it matter which component of the ratio changes?

Nutrient stoichiometry is defined by ratios among potentially limiting nutrients. While nutrient stoichiometry and resource ratio theory are well established ecological concepts, they do receive criticism, in particular as the importance of the absolute concentrations of nutrients is not accounted for (Brauer *et al.*, 2012). For instance, Flynn (2010) wrote: “*Ratios can be dangerous tools; they may be elevated because the denominator is low or the numerator is high*”. Here we discuss the evidence from two experiments where we applied the same Si:N ratios while manipulating either silicon (Makareviciute-Fichtner *et al.*, 2020) or nitrogen (this study) concentrations (Fig 3.9) and argue, that while absolute concentrations do play a role, the general trends of changing Si:N ratio on plankton community structure are similar when either silicon or nitrogen are manipulated.

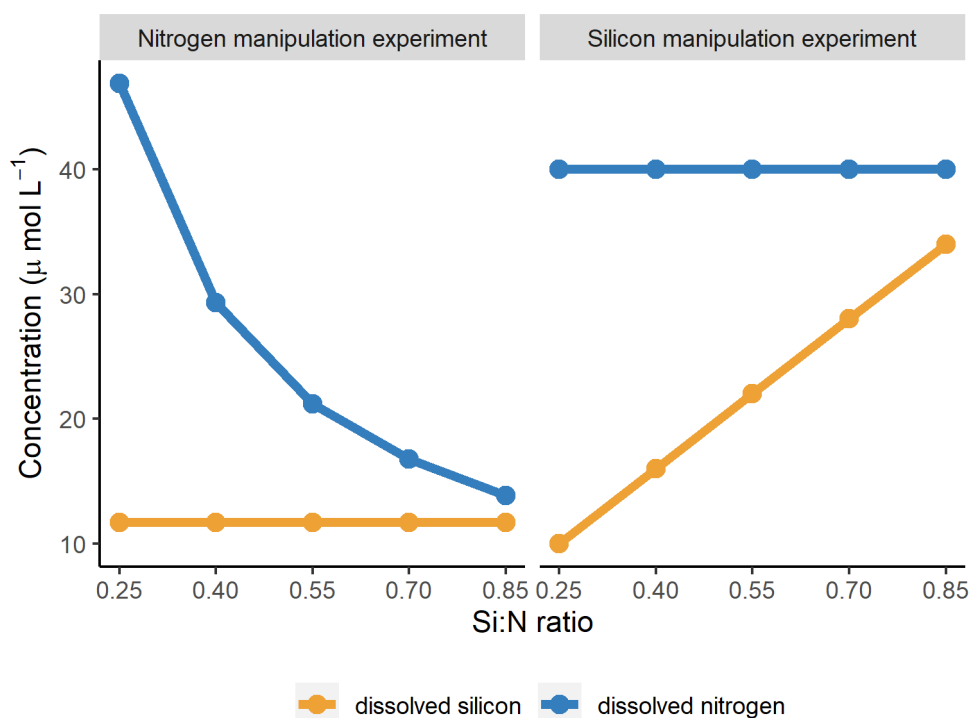


Figure 3.9 Experimental nutrient manipulations in two experiments to achieve the following Si:N ratios: 0.25:1, 0.4:1, 0.55:1, 0.7:1, 0.85:1. The nitrogen experiment refers to this study and the silicon experiment to Makareviciute-Fichtner *et al.* (2020).

In the current study, we manipulated nitrogen concentrations and showed that with increasing Si:N ratio, diatom proportion increases (Fig. 3.10). In an experiment where we manipulated silicate concentration to achieve the same Si:N ratios (Makareviciute-Fichtner *et al.*, 2020), a similar increase of diatom proportion with increasing Si:N ratio could be observed (Fig. 3.10). While experiments were conducted in two different years meaning that starting plankton communities, including the relative contribution of diatoms, were not identical, the slopes of diatom proportion over Si:N ratios were similar both during bloom and post-bloom periods (with an exception to the high copepod treatments during bloom period in the nitrogen manipulation experiment) (Fig. 3.10). The increase of diatom proportion with increasing Si:N ratios is, however, not linear: the decrease of diatom proportion with decreasing Si:N ratio is first slow or even non-significant, but when the ratios get lower than 0.4:1, diatom relative biomass drops strongly. For instance, during post-bloom period, diatom relative carbon biomass was around 25% lower in the lowest Si:N ratio treatment of 0.25:1 comparing to the remaining Si:N treatments. This suggests that Si:N ratio of 0.25:1 is critical for sustaining

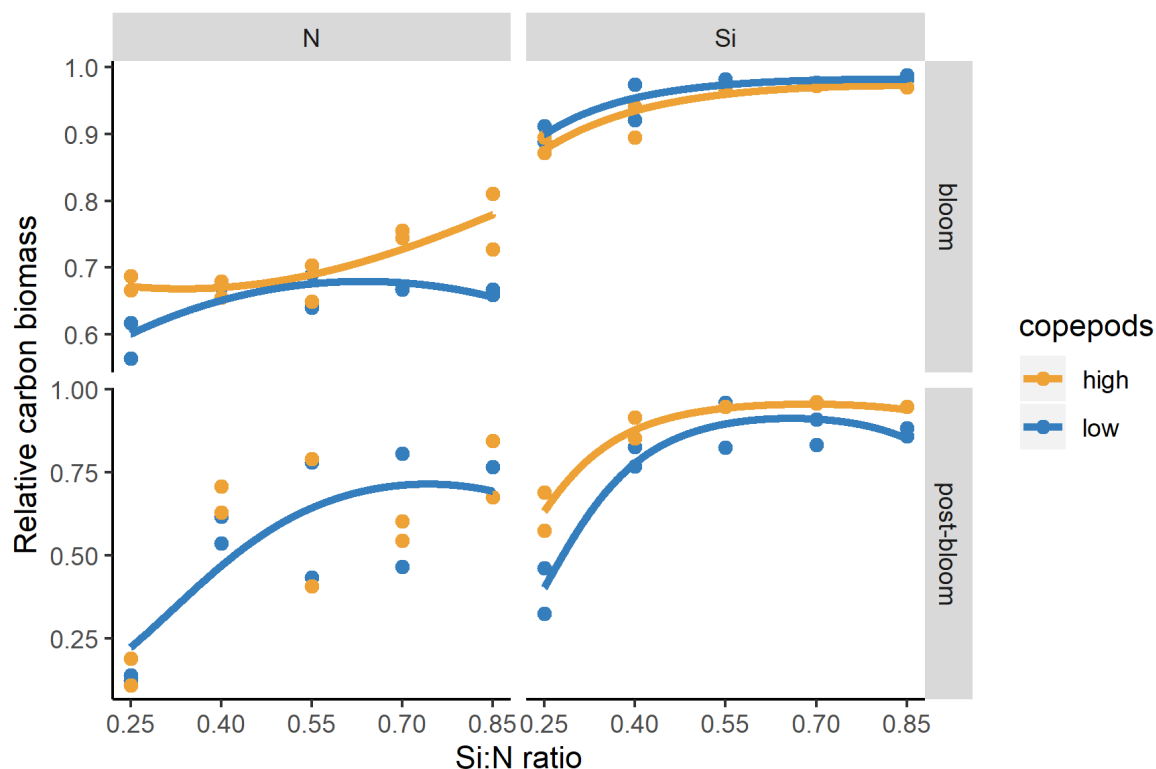

















Figure 1 Diatom proportion in nitrogen (N) and silicon (Si) manipulation experiments during bloom and post-bloom periods. Figure is partially adapted from Makareviciute-Fichtner *et al.* (2020) (for silicon manipulation experiment).

diatom biomass within phytoplankton communities. A similar trend has also been observed by Gilpin *et al.* (2004) who noted that experimental bags with N:Si of 4:1 were experienced silicon exhaustion before nitrate which resulted in substantial silicon limitation.

Additionally, changing Si:N ratio affected not only diatoms but also the other major plankton groups (dinoflagellates, picoplankton, ciliates and bacteria) in the same direction both when silicon and nitrogen were manipulated (Table 3.4). Yet, as anticipated, the manipulation of N versus Si in Si:N ratios can have opposite effects on total phytoplankton biomass during bloom period (Table 3.4). Nitrogen is an essential component of the biomass of all phytoplankton, while silicate is essential only for diatoms, silicoflagellates and radiolarians. Therefore, nitrogen enrichment leads to higher biomass of phytoplankton blooms, which may include diatoms, as long as silicate is not limiting. However, after the phytoplankton bloom, nitrogen concentration seems to play a less important role and both diatom and total phytoplankton biomass increased with increasing Si:N ratios in both experiments (Table 3.4, Fig. 3.5).

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Table 3.4 Response of different plankton parameters to increasing Si:N ratio in an experiment where nitrogen or silicon concentrations were manipulated. Red arrows indicate a decrease, green arrows an increase. Filled arrows mark when the trend is valid through both bloom and post-bloom periods in both copepod treatments. N.s. indicate where no significant relation was found. Data supporting trends in Si manipulation experiment is reported by Makareviciute-Fichtner *et al.* (2020).

Plankton parameter	With increasing Si:N ratio	
	N manipulation experiment	Si manipulation experiment
Phytoplankton biomass	 (bloom)  (post-bloom)	
Diatom %		
Dinoflagellate %	 (bloom)	
Picoplankton %		
Ciliate %	 (post-bloom)	
Bacteria, ind./L		 (bloom)
Nauplii, ind./L	n.s.	 (low cop treatment)
Copepodites+adults, ind./L	n.s.	 (high cop treatment)

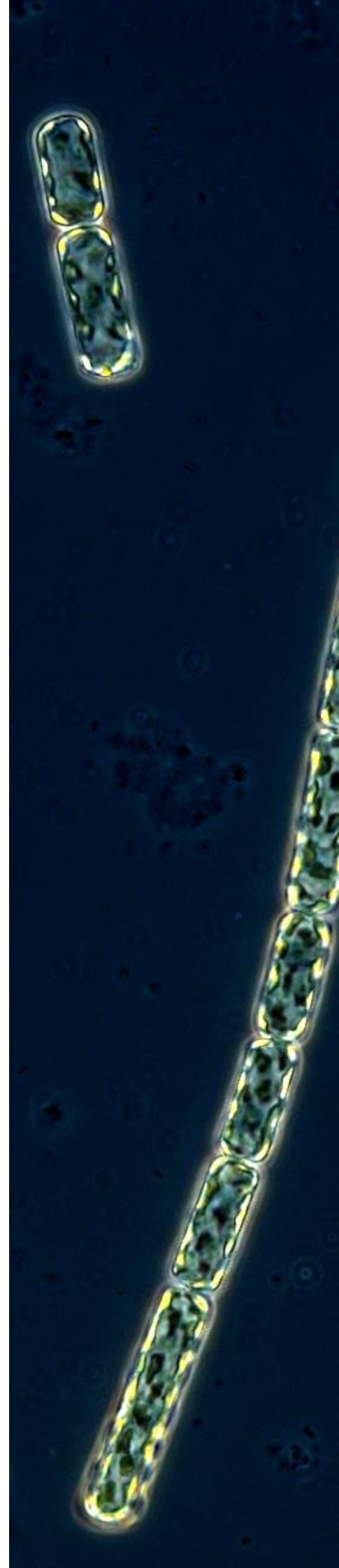
Conclusions

Decline in Si:N ratio due to nitrogen enrichment resulted in an increase of phytoplankton bloom biomass, but a decrease in diatom proportion within it. High copepod abundance caused an earlier silicon limitation, likely due to enhanced diatom silicification under higher grazing pressure.

Non-silicifying groups, such as dinoflagellates, picoplankton, ciliates and bacteria benefited from nitrogen enrichment and lowered Si:N ratios. However, these changes in plankton composition and biomass did not lead to significant effects on copepod abundance.

Si:N ratio can decline either due to increase in nitrogen availability or decline in silicon concentrations (or both). Here we show, that while nitrogen and silicon manipulation can have opposite effects on phytoplankton bloom biomass, plankton community composition responds to the decline in Si:N ratios in a universal way no matter which of the nutrients is changing.

General Discussion



General Discussion

Human activities continue to alter nutrient supply to marine ecosystems, leading to increased loads of reactive nitrogen and a decrease in silicon supply in many regions worldwide. The work presented in this thesis provides important clues to the mechanistic understanding of how altered Si:N ratios in nutrient supply shape plankton communities and trophic interactions among them. While it is generally known that diatom relative abundance declines with decreasing Si:N ratios, this study provides evidence that changes in Si:N ratios affect not only silicifying phytoplankton but the whole plankton community structure with implications for the quantity and quality of food available for higher trophic levels. Additionally, results of this thesis advance the understanding of the role diatoms play in plankton food webs and contribute to answering general ecological questions on how changes in resource ratios and concentrations affect the composition and biomass of primary producers. In the following paragraphs, I will discuss the outcomes of the research questions raised in this thesis, draw conclusions and provide an outlook for future research directions.

1) How does plankton composition respond to changing Si:N ratios under varying grazing pressures?

Decline in Si:N ratios can induce silicon limitation in silicifying phytoplankton, such as diatoms. In line with several previous experiments and field observations (Roberts, 2003, Smayda, 1990, Sommer, 2009, Sommer, 2004), I report a decline in diatom relative abundance with decreasing Si:N ratios, both with a decline of silicon (Chapter I) and an increase of nitrogen (Chapter III) concentrations. Further, among diatoms, the responses of individual species differ: when Si:N is lowered by increased inputs of nitrogen (Chapter III), weakly silicified fast growing *Skeletonema costatum* benefit from increased nitrogen in low Si:N treatments, but larger species such as *Dactyliosolen fragilissimus* and *Cerataulina pelagica* grow higher biomass under high Si:N ratios. Individual diatom species are adapted to grow under different nutrient conditions (McNair *et al.*, 2018, Tilman, 1981) and, additionally, diatoms are able to reduce silicon requirements by lowering silicification of the cells under silicon limitation (McNair *et al.*, 2018). Thus, diatoms, especially when represented by a diverse community of species, can tolerate silicon limitation to some extent. In the experiments presented in this thesis, 0.25:1 was the critical Si:N ratio, where diatom proportion in phytoplankton communities declined sharply (Chapters I and III). Thus, while diatoms are able to tolerate silicon limitation either by changes in morphology or species composition, once

Si:N ratio is lowered below a critical threshold, diatoms are replaced by non-silicifying phytoplankton.

The composition of phytoplankton groups replacing diatoms in lowered Si:N ratios may depend on multiple factors, including initial plankton composition, overall nutrient levels, physical environment and grazing pressure among other forces (Bergquist *et al.*, 1985, Jurgensone *et al.*, 2011, Moline & Prézelin, 1996). The results of this thesis stress the importance of top-down control by copepods in determining phytoplankton composition in lowered Si:N waters. As shown in Chapter I, dinoflagellates dominated when copepod abundance was low, and they were replaced by autotrophic picoplankton under enhanced copepod grazing. This corresponds to previous observations that copepods feed selectively on dinoflagellates and ciliates (Chen & Liu, 2011) – major consumers of picoplankton – creating a trophic cascade. Bacteria abundance was also strongly positively affected by a decrease in Si:N ratios, yet copepod effects on their abundance differed depending on the stage of the phytoplankton bloom and whether silicon or nitrogen was manipulated (Chapters I and III).

2) Does the response of plankton communities differ depending on which component of the Si:N ratio is manipulated?

The importance of resource ratios in determining community composition has been questioned due to the fact that ratios do not consider absolute concentrations of nutrients (Brauer *et al.*, 2012, Flynn, 2010). This thesis provides support for the resource ratio theory which claims that ratios between limiting resources structure community composition (Tilman, 1982a). Our results show that while absolute biomass of phytoplankton can be affected oppositely by the same ratio, depending on which component of it changes, the phytoplankton community structure, as expressed in relative biomass, responds to changes in Si:N ratios in a similar way no matter if Si or N is manipulated (Chapter III, Table 4). This is of importance, as changes in phytoplankton community structure have effects on the quality of food available for grazers as well as the trophic efficiency of the transfer of this food (Chapters I and II). Having evidence that changes in Si:N ratios affect different groups of plankton the same way when Si, N or both of them are manipulated allows for using Si:N ratio in modelling and predicting the response of plankton communities with global change of nutrient supply.

Nonetheless, total phytoplankton biomass response is ecologically important, and it highly depends not only on nutrient ratios, but also on the concentrations of each ratio component. Increasing nitrogen concentrations and simultaneously decreasing Si:N ratios are expected to

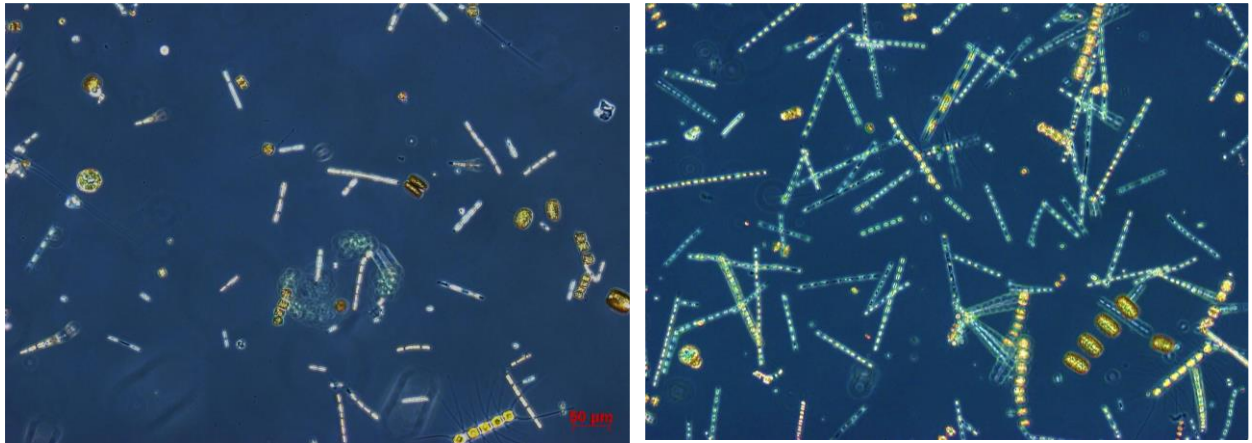


Figure IV Different magnitude of spring phytoplankton bloom cell densities in a mesocosm experiment where silicate concentration was manipulated to achieve Si:N ratios of 0.25:1 (A) and 1:1 (B). Makareviciute et al, unpublished.

result in higher phytoplankton biomass as nitrogen is an essential element for all organisms and it has a fertilizing effect on photoautotrophs in general. In Chapter III, I show that the phytoplankton bloom peak is indeed determined by nitrogen concentrations, i.e. the peak is highest when Si:N ratios are lowest. However, Si:N ratios also have a positive effect on sustaining phytoplankton biomass following the phytoplankton bloom, even when initial nitrogen concentrations are lower (Chapter III). Furthermore, lowered silicon concentrations result in lower phytoplankton biomass (Chapter I, Fig. IV).

To summarize the observed effects of changes in Si:N ratio, and Si and N concentrations on phytoplankton biomass, I developed a conceptual graph of phytoplankton bloom formation under different Si:N ratio manipulations (Fig. V). Based on the results of this thesis,

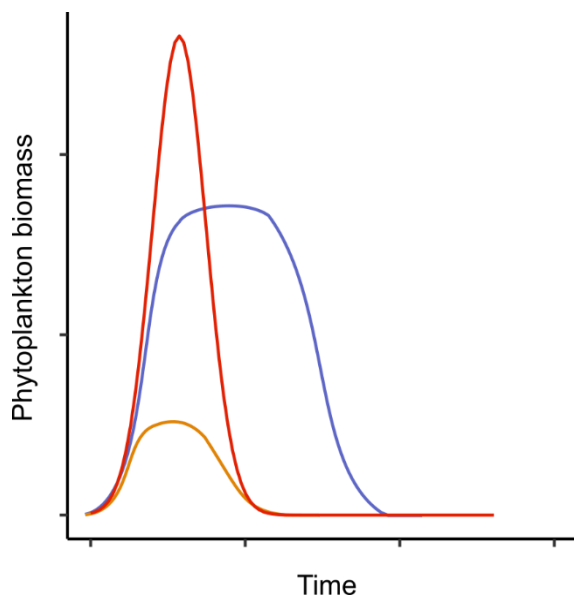


Figure V Conceptual diagram of phytoplankton bloom formation after nutrient supply at different Si:N ratios.

phytoplankton biomass grows exponentially after nutrient enrichment (or increased light availability in the case of vernal blooms) until one or several nutrients become limiting. First this occurs if silicate concentrations are reduced, for instance in the case of enhanced damming (Fig. V, yellow line). Phytoplankton bloom is highest if Si:N ratio is lowered by an increase in nitrogen inputs due to, for example, intensified fertilizer use (Fig. V, red line). Si:N ratios in Redfield

proportions result in a lower bloom peak, than under nitrogen fertilization, but phytoplankton biomass is sustained for longer time (Fig. V, blue line). While more studies are needed to confirm the suggested conceptual trends and understand the mechanisms behind them, if they do occur in nature, changes in phytoplankton bloom dynamics with declining in Si:N ratios would have implications on a variety of marine processes, including carbon storage and sedimentation and food availability for different stages of mesozooplankton, among others.

3) Do changes in plankton communities affect plankton food web structure and mesozooplankton abundance?

Results of this thesis show that altered Si:N ratios in nutrient supply result in changes in phytoplankton, mixo- and microzooplankton abundance and composition which affect trophic interactions in the associated lower marine food webs. In high Si:N environments, where diatoms dominate, the results of this thesis underline that a direct diatom-copepod food chain prevails as suggested by Sommer *et al.* (2002). However, this food chain may not be as efficient as previously thought: results of this thesis reveal that only some species of diatoms are affected negatively by grazing (Chapters I and III). Copepods seem to avoid grazing on several dominant diatom species, such as *D. fragilissimus* and *C. pelagica*. Uneaten diatoms may be decomposed and support heterotrophic food chains via bacteria or increase carbon export via sinking. Additionally, Chen and Liu (2011) reported that copepod selective grazing on dinoflagellates increases with increasing concentration of diatoms. Thus, while most energy is likely transferred via the classical diatom-copepod food chain in high Si:N environments, alternative trophic pathways may also be playing an important role as not all diatoms represent preferred food.

In low Si:N environments, such as eutrophicated coastal seas, non-silicifying phytoplankton groups dominate. Under nutrient rich conditions, they can be represented by dinoflagellates, chlorophytes or small flagellates, among others. In this thesis, I show that food web structure is more complex when silicon is limiting (Chapter I). With bacteria abundance increasing with decreasing Si:N ratios (Chapters I and III), more carbon is likely transferred via the heterotrophic link: from dissolved organic matter via bacteria to bacterivores, such as nanoflagellates and ciliates, up to a point where it is available for copepods. Additionally, low Si:N plankton communities were characterized by higher proportion of dinoflagellates, of which the majority are mixotrophs (Munn, 2011). This is in agreement with previous observations that mixotrophic plankton is more abundant when nutrient ratios deviate from the

Redfield ratio (Flynn *et al.*, 2019, Mitra *et al.*, 2014b). Thus, copepods gain their energy not directly from autotrophic phytoplankton, but by feeding on mixotrophic and heterotrophic protists. This likely negatively affects energy transfer efficiency within a food web, as part of the energy is lost between trophic levels.

The response of copepods to Si:N ratio manipulations during the experiments presented in this thesis were inconclusive and copepod density dependent (Chapters I and III). Under high copepod abundance, copepodite numbers seem to be decreasing with increasing Si:N ratios (significantly when Si was manipulated, and not statistically significantly under N manipulation). Yet, when copepod abundance was low, no such trend was apparent for copepodites and adults, and nauplii numbers were even increasing with increasing Si:N ratios in the Si manipulation experiment (Chapter I). One could speculate that this inconsistent response is due to more alternative food available when copepod abundance is lower and that copepods could have benefited from higher food availability in high Si:N treatments.

4) Do lowered Si:N ratios affect phytoplankton quality as a food source for higher trophic levels?

Results of this thesis, together with previous studies, suggest that high Si:N environments are characterized by diatom dominated phytoplankton which should ensure high energy transfer efficiency via direct trophic links between primary producers and copepods. However, not only the energy available but also the quality of the food matter. In the last decades, evidence has accumulated that some diatoms interfere with copepod reproduction and early development (Ianora *et al.*, 2003, Russo *et al.*, 2018). This has been related to oxylipins produced by diatoms, yet the role of nutritional inadequacy is also assumed to play a role (Jones & Flynn, 2005). The results of this thesis (Chapter II) show that diatom dominated communities are rich in essential fatty acids, however, some nutritional value indicators, such as ratios between and DHA:EPA, $\omega 3:\omega 6$ fatty acids and stoichiometric ratios between particulate carbon, nitrogen and phosphorus were higher in low Si:N treatments. Additionally, my results indicate that copepods feed selectively on plankton rich in essential fatty acids. Thus, while changes in Si:N alter both the quantity and optimal ratios among nutritional indicators, the copepods can show resilience to these changes via selective feeding. This is in line with previous observations that the biggest harm to copepods was caused when fed by single species diatom cultures and negative impacts were avoided when a mixture of diatoms and dinoflagellates were offered (Jones & Flynn, 2005).

Conclusions

Ever increasing human populations and their demand for food and energy will likely further decrease Si:N ratios in coastal oceans. This thesis shows that lowered Si:N ratios result in a decline in diatom proportion within phytoplankton, leading to more complex food webs with flagellates, ciliates and bacteria playing a more important role. The quantity of food available for copepods, and ultimately fish, can thus be lowered due to food web elongations under silicon limitation. The quality of food in eutrophicated coastal seas, where Si:N ratios are low, may also be lower, as this study provides evidence that phytoplankton contain less essential fatty acids under silicon limitation.

However, highly diatom dominated plankton communities are not as ideal feeding grounds for mesozooplankton as previously thought. This thesis demonstrates, that several species of diatoms are avoided by copepod grazers; meanwhile dinoflagellates are fed upon selectively. Moreover, while diatom dominated communities contain more essential fatty acids, the ratios between selected fatty acids, particulate carbon, nitrogen and phosphorus are more suitable under low Si:N ratios. This indicates, that diverse phytoplankton community structure is of importance in sustaining high productivity of marine ecosystems.

Overall, my thesis shows that changes in Si:N ratio in nutrient supply have strong effects on phytoplankton composition, nutritional value and food web structure. The observed universal response of plankton community composition to changes in Si:N ratio provides opportunities for simplifications, which is essential for modelling future changes in the oceans. However, the implications of the altered plankton structure on higher trophic levels and broader ecosystem processes, such as the biological carbon pump, are complex and require further assessment of the systems resilience to nutrient change.

Outlook

This study provides important contributions to the basic understanding of how altered Si:N ratios affect plankton communities, their structure and functioning. In addition to the research questions addressed in the thesis, new questions arose which require further studies to enhance our understanding of the complex relationships in planktonic food webs.

Low effects of copepod grazing on diatom biomass observed in this study were unexpected and call for further investigations into the reasons behind these results. Diatoms are known to produce secondary metabolites which interfere with copepod development and reproduction, but it is debated if these chemicals can offer protection from grazers. While there is some evidence that polyunsaturated aldehydes produced by diatoms repel grazers and thus could protect other cells in a colony from further grazing (Jüttner, 2005), others claim that the release of these metabolites should not be considered as a defense strategy as they affect only the upcoming generation and offer no immediate benefit for diatoms (Flynn & Irigoien, 2009). Thus, it is important to assess the role of secondary metabolites in deterring grazing, especially in species such as *Dactyliosolen fragilissimus*, which seems to benefit from higher copepod grazing pressure (Chapter I and II). Further, increasing diatom concentrations affect selective grazing of copepods (Chen & Liu, 2011), yet the reasons behind this are not understood. Thus, the effects of increasing diatom proportion on nutritional inadequacies and possible chemical deterrence of grazers should be studied simultaneously. As shown in this study, experiments manipulating Si:N ratio can create semi-natural plankton communities with varying dominance of diatoms and could be used for further studies of the role of diatoms in plankton food webs and chemical interactions between planktonic organisms.

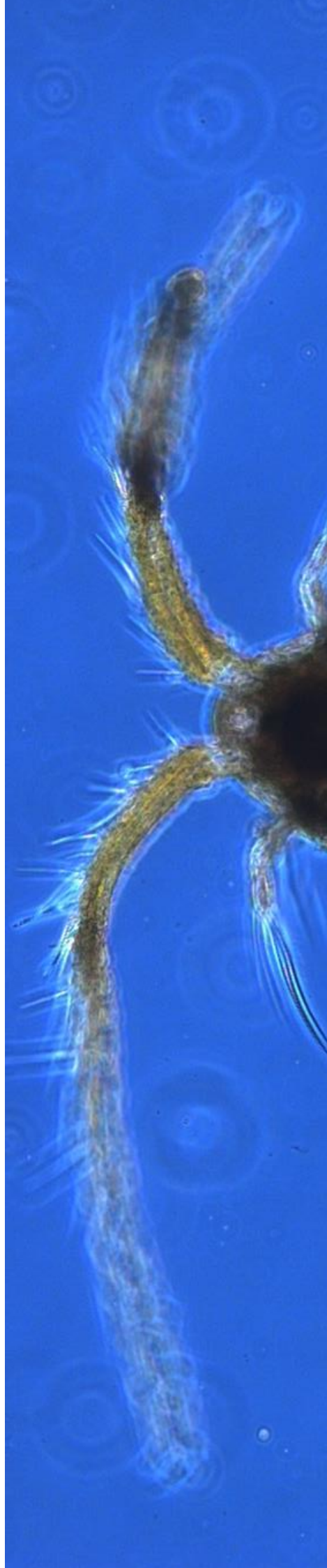
In this thesis, I concentrated on the response of phytoplankton to changing Si:N ratios and what this could mean for higher trophic levels. The results indicate that changes in Si:N ratios affect not only the organisms feeding on phytoplankton but also the bacterial community. Bacteria abundance decreased with decreasing Si:N ratios, both when nitrogen increased (Chapter III) and when silicate declined (Chapter I). Bacteria play an important role in plankton dynamics and nutrient cycling and I believe that this universal response deserves to be addressed further. To fully understand the impacts of the bacteria response, the type and function of these organisms should be identified. Additionally, viruses are important players in marine biogeochemical cycles and it was recently observed that diatom susceptibility to viral

General Discussion

infections increase when silicon becomes limiting (Kranzler *et al.*, 2019). Thus, addressing the response of the smallest compartments of life to changes in plankton systems under silicon limitation would help to further entangle the complexity of interactions within plankton communities.

Lastly, this thesis presents results from two mesocosm experiments on summer phytoplankton communities. Conditions in spring, when major phytoplankton blooms occur in the Northern hemisphere may be different, as low light and cold temperatures may limit the growth of some species and functional groups of phytoplankton. Thus, additional experiments using spring plankton communities could advance the applicability of the findings of this thesis. Additionally, further mesocosm experiments together with time-series data analysis could contribute to our understanding on the role of Si:N ratios on spring phytoplankton biomass and plankton dynamics.

References



References

- Ajani, P. A., Allen, A. P., Ingleton, T. and Armand, L. (2014) A decadal decline in relative abundance and a shift in microphytoplankton composition at a long-term coastal station off southeast Australia. *Limnology and oceanography*, **59**, 519-531.
- Andersen, R. A. (2005) *Algal culturing techniques*. Vol. 1, Academic Press Burlington, Mass.
- Anderson, T. R. and Pond, D. W. (2000) Stoichiometric theory extended to micronutrients: Comparison of the roles of essential fatty acids, carbon, and nitrogen in the nutrition of marine copepods. *Limnology and Oceanography*, **45**, 1162-1167.
- Appl, M. (2011) Ammonia *Ullmann's Encyclopedia of Industrial Chemistry*. Wiley, Electronic Release.
- Arendt, K., Jónasdóttir, S., Hansen, P. J. and Gärtner, S. (2005) Effects of dietary fatty acids on the reproductive success of the calanoid copepod *Temora longicornis*. *Marine Biology*, **146**, 513-530.
- Arts, M. T., Brett, M. T. and Kainz, M. (2009) *Lipids in aquatic ecosystems*. Vol. 1, Springer Science & Business Media.
- Azam, F., Fenchel, T., Field, J., Grey, J., Meyer-Reil, L. and Thingstad, F. (1983) The ecological role of water-column microbes. *Marine Ecology Progress Series*, **10**, 257-263.
- Bauerfeind, S. (1985) Degradation of phytoplankton detritus by bacteria: estimation of bacterial consumption and respiration in an oxygen chamber. *Marine ecology progress series Oldendorf*, **21**, 27-36.
- Bell, M. V., Dick, J. R., Anderson, T. R. and Pond, D. W. (2007) Application of liposome and stable isotope tracer techniques to study polyunsaturated fatty acid biosynthesis in marine zooplankton. *Journal of Plankton Research*, **29**, 417-422.
- Benoiston, A.-S., Ibarbalz, F. M., Bittner, L., Guidi, L., Jahn, O., Dutkiewicz, S. and Bowler, C. (2017) The evolution of diatoms and their biogeochemical functions. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **372**, 20160397.
- Berggreen, U., Hansen, B. and Kiørboe, T. (1988) Food size spectra, ingestion and growth of the copepod *Acartia tonsa* during development: Implications for determination of copepod production. *Marine biology*, **99**, 341-352.
- Bergquist, A. M., Carpenter, S. R. and Latino, J. C. (1985) Shifts in phytoplankton size structure and community composition during grazing by contrasting zooplankton assemblages 1. *Limnology and Oceanography*, **30**, 1037-1045.
- Beusen, A. H., Bouwman, A. F., Van Beek, L. P., Mogollón, J. M. and Middelburg, J. J. (2016) Global riverine N and P transport to ocean increased during the 20th century despite increased retention along the aquatic continuum. *Biogeosciences*, **13**, 11.
- Bi, R., Ismar, S., Sommer, U. and Zhao, M. (2017) Environmental dependence of the correlations between stoichiometric and fatty acid-based indicators of phytoplankton nutritional quality. *Limnology and Oceanography*, **62**, 334-347.
- Boersma, M. and Elser, J. J. (2006) Too much of a good thing: on stoichiometrically balanced diets and maximal growth. *Ecology*, **87**, 1325-1330.
- Boersma, M., Schöps, C. and Mccauley, E. (2001) Nutritional quality of seston for the freshwater herbivore *Daphnia galeata* × *hyalina*: biochemical versus mineral limitations. *Oecologia*, **129**, 342-348.

References

- Bowler, C., Allen, A. E., Badger, J. H., Grimwood, J., Jabbari, K., Kuo, A., Maheswari, U., Martens, C., Maumus, F. and O'tillar, R. P. (2008) The *Phaeodactylum* genome reveals the evolutionary history of diatom genomes. *Nature*, **456**, 239-244.
- Boyce, D. G., Frank, K. T. and Leggett, W. C. (2015) From mice to elephants: overturning the 'one size fits all' paradigm in marine plankton food chains. *Ecology Letters*, **18**, 504-15.
- Brauer, V. S., Stomp, M. and Huisman, J. (2012) The Nutrient-Load Hypothesis: Patterns of Resource Limitation and Community Structure Driven by Competition for Nutrients and Light. *The American Naturalist*, **179**, 721-740.
- Brett, M. T., Müller-Navarra, D. C. and Persson, J. (2009) Crustacean zooplankton fatty acid composition *Lipids in aquatic ecosystems*. Springer, pp. 115-146.
- Brzezinski, M. A. (1985) The Si:C:N ratio of marine diatoms: interspecific variability and the effect of some environmental variables. *Journal of Phycology*, **21**, 347-357.
- Caron, D. A. (2016) Mixotrophy stirs up our understanding of marine food webs. *Proceedings of the National Academy of Sciences*, **113**, 2806-2808.
- Carotenuto, Y., Ianora, A., Buttino, I., Romano, G. and Miralto, A. (2002) Is postembryonic development in the copepod *Temora stylifera* negatively affected by diatom diets? *Journal of Experimental Marine Biology and Ecology*, **276**, 49-66.
- Chen, M. and Liu, H. (2011) Experimental simulation of trophic interactions among omnivorous copepods, heterotrophic dinoflagellates and diatoms. *Journal of experimental marine biology and ecology*, **403**, 65-74.
- Chi, X., Javidpour, J., Sommer, U. and Mueller-Navarra, D. (2018) Tracking Fatty Acids From Phytoplankton to Jellyfish Polyps Under Different Stress Regimes: A Three Trophic Levels Experiment. *Frontiers in Ecology and Evolution*, **6**, 118.
- Christie, W. W. (1989) *Gas chromatography and lipids: A practical guide*. Vol. 1, The Oily Press, Bridgwater, Somerset.
- Conant, R. T., Berdanier, A. B. and Grace, P. R. (2013) Patterns and trends in nitrogen use and nitrogen recovery efficiency in world agriculture. *Global Biogeochemical Cycles*, **27**, 558-566.
- Cowles, T. J., Olson, R. J. and Chisholm, S. W. (1988) Food selection by copepods: discrimination on the basis of food quality. *Marine Biology*, **100**, 41-49.
- Cribari-Neto, F. and Zeileis, A. (2010) Beta regression in R. *Journal of Statistical Software*, **34**, 1-24.
- Danielsson, Å., Papush, L. and Rahm, L. (2008) Alterations in nutrient limitations — Scenarios of a changing Baltic Sea. *Journal of Marine Systems*, **73**, 263-283.
- Downing, J. A., Cherrier, C. T. and Fulweiler, R. W. (2016) Low ratios of silica to dissolved nitrogen supplied to rivers arise from agriculture not reservoirs. *Ecol Lett*, **19**, 1414-1418.
- Duce, R. A., Laroche, J., Altieri, K., Arrigo, K. R., Baker, A. R., Capone, D. G., Cornell, S., Dentener, F., Galloway, J., Ganeshram, R. S., Geider, R. J., Jickells, T., Kuypers, M. M., Langlois, R., Liss, P. S., Liu, S. M., Middelburg, J. J., Moore, C. M., Nickovic, S., Oschlies, A., Pedersen, T., Prospero, J., Schlitzer, R., Seitzinger, S., Sorensen, L. L., Uematsu, M., Ulloa, O., Voss, M., Ward, B. and Zamora, L. (2008) Impacts of Atmospheric Anthropogenic Nitrogen on the Open Ocean. *Science*, **320**, 893-897.
- Egge, J. K. and Aksnes, D. L. (1992) Silicate as regulating nutrient in phytoplankton competition. *Marine ecology progress series*, **83.2**, 281-289.
- Evjemo, J. O., Tokle, N., Vadstein, O. and Olsen, Y. (2008) Effect of essential dietary fatty acids on egg production and hatching success of the marine copepod *Temora longicornis*. *Journal of Experimental Marine Biology and Ecology*, **365**, 31-37.

References

- Farmer, A. (2018) Phosphorus: Polluter and Resource of the Future: Motivations, Technologies and Assessment of the Elimination and Recovery of Phosphorus from Wastewater. In: C. Schaum (ed) *Phosphate pollution: a global overview of the problem*. Vol. 35. IWA Publishing.
- Ferrari, S. and Cribari-Neto, F. (2004) Beta regression for modelling rates and proportions. *Journal of Applied Statistics*, **31**, 799-815.
- Field, C. B., Behrenfeld, M. J., Randerson, J. T. and Falkowski, P. (1998) Primary Production of the Biosphere: Integrating Terrestrial and Oceanic Components. *Science*, **281**, 237-240.
- Flynn, K. J. (2005) Modelling marine phytoplankton growth under eutrophic conditions. *Journal of Sea Research*, **54**, 92-103.
- Flynn, K. J. (2010) Do external resource ratios matter?: Implications for modelling eutrophication events and controlling harmful algal blooms. *Journal of Marine Systems*, **83**, 170-180.
- Flynn, K. J. and Irigoien, X. (2009) Aldehyde-induced insidious effects cannot be considered as a diatom defence mechanism against copepods. *Marine Ecology Progress Series*, **377**, 79-89.
- Flynn, K. J. and Martin-Jézéquel, V. (2000) Modelling Si-N-limited growth of diatoms. *Journal of Plankton Research*, **22**, 447-472.
- Flynn, K. J., Mitra, A., Anestis, K., Anschütz, A. A., Calbet, A., Ferreira, G. D., Gypens, N., Hansen, P. J., John, U., Martin, J. L., Mansour, J. S., Maselli, M., Medić, N., Norlin, A., Not, F., Pitta, P., Romano, F., Saiz, E., Schneider, L. K., Stolte, W. and Traboni, C. (2019) Mixotrophic protists and a new paradigm for marine ecology: where does plankton research go now? *Journal of Plankton Research*, **41**, 375-391.
- Flynn, K. J., Stoecker, D. K., Mitra, A., Raven, J. A., Glibert, P. M., Hansen, P. J., Granéli, E. and Burkholder, J. M. (2012) Misuse of the phytoplankton–zooplankton dichotomy: the need to assign organisms as mixotrophs within plankton functional types. *Journal of Plankton Research*, **35**, 3-11.
- Fowler, D., Coyle, M., Skiba, U., Sutton, M. A., Cape, J. N., Reis, S., Sheppard, L. J., Jenkins, A., Grizzetti, B. and Galloway, J. N. (2013) The global nitrogen cycle in the twenty-first century. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **368**, 20130164.
- Garcia, N. S., Sexton, J., Riggins, T., Brown, J., Lomas, M. W. and Martiny, A. C. (2018) High Variability in Cellular Stoichiometry of Carbon, Nitrogen, and Phosphorus Within Classes of Marine Eukaryotic Phytoplankton Under Sufficient Nutrient Conditions. *Front Microbiol*, **9**, 543-543.
- Gilpin, L. C., Davidson, K. and Roberts, E. (2004) The influence of changes in nitrogen: silicon ratios on diatom growth dynamics. *Journal of Sea Research*, **51**, 21-35.
- Granéli, E., Carlsson, P., Turner, J. T., Tester, P. A., Béchemin, C., Dawson, R., & Funari, E. (1999) Effects of N:P:Si ratios and zooplankton grazing on phytoplankton communities in the northern Adriatic Sea. I. Nutrients, phytoplankton biomass, and polysaccharide production. *Aquatic Microbial Ecology*, **18**, 37-54.
- Grønning, J. and Kiørboe, T. (2020) Diatom defence: Grazer induction and cost of shell-thickening. *Functional Ecology*, **34**, 1790-1801.
- Gross, M. (2012) The mysteries of the diatoms. *Current Biology*, **22**, R581-R585.
- Hansen, H. P. and Koroleff, F. (1999) Determination of nutrients. In: K. Grasshoff, K. Kremling and M. Ehrhardt (eds) *Methods of seawater analysis*. 3 ed. Wiley, pp. 159-228.
- Hartmann, M., Zubkov, M. V., Scanlan, D. J. and Lepère, C. (2013) In situ interactions between photosynthetic picoeukaryotes and bacterioplankton in the Atlantic Ocean: evidence for mixotrophy. *Environmental microbiology reports*, **5**, 835-840.

References

- Hátún, H., Azetsu-Scott, K., Somavilla, R., Rey, F., Johnson, C., Mathis, M., Mikolajewicz, U., Coupel, P., Tremblay, J. É., Hartman, S., Pacariz, S. V., Salter, I. and Ólafsson, J. (2017) The subpolar gyre regulates silicate concentrations in the North Atlantic. *Scientific Reports*, **7**, 14576.
- Hawkings, J. R., Wadham, J. L., Benning, L. G., Hendry, K. R., Tranter, M., Tedstone, A., Nienow, P. and Raiswell, R. (2017) Ice sheets as a missing source of silica to the polar oceans. *Nature Communications*, **8**, 14198.
- Hillebrand, H., Dürselen, C. D., Kirschtel, D., Pollinger, U. and Zohary, T. (1999) Biovolume calculation for pelagic and benthic microalgae. *Journal of phycology*, **35**, 403-424.
- Hinder, S. L., Hays, G. C., Edwards, M., Roberts, E. C., Walne, A. W. and Gravenor, M. B. (2012) Changes in marine dinoflagellate and diatom abundance under climate change. *Nature Climate Change*, **2**, 271.
- Hitchcock, G. L. (1982) A comparative study of the size-dependent organic composition of marine diatoms and dinoflagellates. *Journal of plankton research*, **4**, 363-377.
- Howarth, R. W., Billen, G., Swaney, D., Townsend, A., Jaworski, N., Lajtha, K., Downing, J. A., Elmgren, R., Caraco, N., Jordan, T., Berendse, F., Freney, J., Kudeyarov, V., Murdoch, P. and Zhao-Liang, Z. (1996) Regional Nitrogen Budgets and Riverine N & P Fluxes for the Drainages to the North Atlantic Ocean: Natural and Human Influences. *Biogeochemistry*, **35**, 75-139.
- Howarth, R. W. and Marino, R. (2006) Nitrogen as the limiting nutrient for eutrophication in coastal marine ecosystems: evolving views over three decades. *Limnology and Oceanography*, **51**, 364-376.
- Howarth, R. W., Sharpley, A. and Walker, D. (2002) Sources of nutrient pollution to coastal waters in the United States: Implications for achieving coastal water quality goals. *Estuaries*, **25**, 656-676.
- Hu, H., Zhang, J. and Chen, W. (2011) Competition of bloom-forming marine phytoplankton at low nutrient concentrations. *Journal of Environmental Sciences*, **23**, 656-663.
- Humborg, C., Conley, D. J., Rahm, L., Wulff, F., Cociasu, A. and Ittekkot, V. (2000) Silicon Retention in River Basins: Far-reaching Effects on Biogeochemistry and Aquatic Food Webs in Coastal Marine Environments. *AMBIO: A Journal of the Human Environment*, **29**, 45-50.
- Humborg, C., Ittekkot, V., Cociasu, A. and Bodungen, B. V. (1997) Effect of Danube River dam on Black Sea biogeochemistry and ecosystem structure. *Nature*, **386**, 385-388.
- Humborg, C., Rahm, L., Conley, D. J., Tamminen, T. and Von Bodungen, B. (2008) Silicon and the Baltic Sea. *Journal of Marine Systems*, **73**, 221-222.
- Ianora, A., Miralto, A., Poulet, S. A., Carotenuto, Y., Buttino, I., Romano, G., Casotti, R., Pohnert, G., Wichard, T., Colucci-D'amato, L., Terrazzano, G. and Smetacek, V. (2004) Aldehyde suppression of copepod recruitment in blooms of a ubiquitous planktonic diatom. *Nature*, **429**, 403.
- Ianora, A., Poulet, S. A. and Miralto, A. (2003) The effects of diatoms on copepod reproduction: a review. *Phycologia*, **42**, 351-363.
- Irigoin, X., Harris, R. P., Verheye, H. M., Joly, P., Runge, J., Starr, M., Pond, D., Campbell, R., Shreeve, R. and Ward, P. (2002) Copepod hatching success in marine ecosystems with high diatom concentrations. *Nature*, **419**, 387.
- Jenkins, G. P. and Black, K. P. (2019) Contrasting impact of diatoms on dominant calanoid and cyclopoid copepods in a temperate bay. *Estuarine, Coastal and Shelf Science*, **217**, 211-217.
- Jepsen, P. M. (2014) *Copepods as live feed: optimisation and use in aquaculture*. PhD thesis, Roskilde Universitet.
- Jin, X., Gruber, N., Dunne, J., Sarmiento, J. L. and Armstrong, R. (2006) Diagnosing the contribution of phytoplankton functional groups to the production and export of particulate organic carbon,

References

- CaCO₃, and opal from global nutrient and alkalinity distributions. *Global Biogeochemical Cycles*, **20**.
- Jónasdóttir, S. (1994) Effects of food quality on the reproductive success of *Acartia tonsa* and *Acartia hudsonica*: laboratory observations. *Marine Biology*, **121**, 67-81.
- Jónasdóttir, S. and Kiørboe, T. (1996) Copepod recruitment and food composition: do diatoms affect hatching success? *Marine Biology*, **125**, 743-750.
- Jónasdóttir, S. H. (2019) Fatty acid profiles and production in marine phytoplankton. *Marine drugs*, **17**, 151.
- Jonasdottir, S. H., Fields, D. and Pantoja, S. (1995) Copepod egg production in Long Island Sound, USA, as a function of the chemical composition of seston. *Marine ecology progress series Oldendorf*, **119**, 87-98.
- Jones, R. H. and Flynn, K. J. (2005) Nutritional status and diet composition affect the value of diatoms as copepod prey. *Science*, **307**, 1457-9.
- Jurgensone, I., Carstensen, J., Ikauniece, A. and Kalveka, B. (2011) Long-term Changes and Controlling Factors of Phytoplankton Community in the Gulf of Riga (Baltic Sea). *Estuaries and Coasts*, **34**, 1205.
- Justić, D., Rabalais, N. N. and Turner, R. E. (1995) Stoichiometric nutrient balance and origin of coastal eutrophication. *Marine Pollution Bulletin*, **30**, 41-46.
- Jüttner, F. (2005) Evidence that Polyunsaturated Aldehydes of Diatoms are Repellents for Pelagic Crustacean Grazers. *Aquatic Ecology*, **39**, 271-282.
- Kainz, M. J., Perga, M.-E., Arts, M. T. and Mazumder, A. (2009) Essential fatty acid concentrations of different seston sizes and zooplankton: a field study of monomictic coastal lakes. *Journal of Plankton Research*, **31**, 635-645.
- Karlson, B., Andreasson, A., Johansen, M., Karlberg, M., Loo, A., Skjevik, a-T. (2018) Nordic Microalgae *World-wide electronic publication*.
- Katechakis, A., Stibor, H., Sommer, U. and Hansen, T. (2004) Feeding selectivities and food niche separation of *Acartia clausi*, *Penilia avirostris* (Crustacea) and *Doliolum denticulatum* (Thaliacea) in Blanes Bay (Catalan Sea, NW Mediterranean). *Journal of Plankton Research*, **26**, 589-603.
- Klein Breteler, W. C. M., Schogt, N., Baas, M., Schouten, S. and Kraay, G. W. (1999) Trophic upgrading of food quality by protozoans enhancing copepod growth: role of essential lipids. *Marine Biology*, **135**, 191-198.
- Kleppel, G. S. (1993) On the Diets of Calanoid Copepods. *Marine Ecology Progress Series*, **99**, 183-195.
- Kooistra, W., Gersonde, R., Medlin, L. and Mann, D. (2007) The Origin and Evolution of the Diatoms: Their Adaptation to a Planktonic Existence. In: P. Falkowski and A. Knoll (eds) *Evolution of primary producers in the sea*. Academic Press, pp. 207-249.
- Kranzler, C. F., Krause, J. W., Brzezinski, M. A., Edwards, B. R., Biggs, W. P., Maniscalco, M., Mccrow, J. P., Van Mooy, B. a. S., Bidle, K. D., Allen, A. E. and Thamatrakoln, K. (2019) Silicon limitation facilitates virus infection and mortality of marine diatoms. *Nature Microbiology*, **4**, 1790-1797.
- Krause, J. W., Schulz, I. K., Rowe, K. A., Dobbins, W., Winding, M. H. S., Sejr, M. K., Duarte, C. M. and Agustí, S. (2019) Silicic acid limitation drives bloom termination and potential carbon sequestration in an Arctic bloom. *Scientific Reports*, **9**, 8149.
- Kroon, F. J., Kuhnert, P. M., Henderson, B. L., Wilkinson, S. N., Kinsey-Henderson, A., Abbott, B., Brodie, J. E. and Turner, R. D. (2012) River loads of suspended solids, nitrogen, phosphorus

References

- and herbicides delivered to the Great Barrier Reef lagoon. *Marine pollution bulletin*, **65**, 167-181.
- Kuosa, H., Autio, R., Kuuppo, P., Setälä, O. and Tanskanen, S. (1997) Nitrogen, Silicon and Zooplankton Controlling the Baltic Spring Bloom: An Experimental Study. *Estuarine, Coastal and Shelf Science*, **45**, 813-821.
- Lamarque, J. F., Dentener, F., McConnell, J., Ro, C. U., Shaw, M., Vet, R., Bergmann, D., Cameron-Smith, P., Dalsoren, S., Doherty, R., Faluvegi, G., Ghan, S. J., Josse, B., Lee, Y. H., Mackenzie, I. A., Plummer, D., Shindell, D. T., Skeie, R. B., Stevenson, D. S., Strode, S., Zeng, G., Curran, M., Dahl-Jensen, D., Das, S., Fritzsche, D. and Nolan, M. (2013) Multi-model mean nitrogen and sulfur deposition from the Atmospheric Chemistry and Climate Model Intercomparison Project (ACCMIP): evaluation of historical and projected future changes. *Atmos Chem Phys*, **13**, 7997-8018.
- Laruelle, G. G., Roubex, V., Sferratore, A., Brodherr, B., Ciuffa, D., Conley, D. J., Dürr, H. H., Garnier, J., Lancelot, C., Le Thi Phuong, Q., Meunier, J. D., Meybeck, M., Michalopoulos, P., Moriceau, B., Ní Longphuirt, S., Loucaides, S., Papush, L., Presti, M., Ragueneau, O., Regnier, P., Saccone, L., Slomp, C. P., Spiteri, C. and Van Cappellen, P. (2009) Anthropogenic perturbations of the silicon cycle at the global scale: Key role of the land-ocean transition. *Global Biogeochemical Cycles*, **23**.
- Leblanc, K., Arístegui, J., Armand, L., Assmy, P., Beker, B., Bode, A., Breton, E., Cornet, V., Gibson, J., Gosselin, M. P., Kopczynska, E., Marshall, H., Peloquin, J., Piontkovski, S., Poulton, A. J., Quéguiner, B., Schiebel, R., Shipe, R., Stefels, J., Van Leeuwe, M. A., Varela, M., Widdicombe, C. and Yallop, M. (2012) A global diatom database – abundance, biovolume and biomass in the world ocean. *Earth System Science Data*, **4**, 149-165.
- Leising, A. W., Pierson, J. J., Halsband-Lenk, C., Horner, R. and Postel, J. (2005) Copepod grazing during spring blooms: does *Calanus pacificus* avoid harmful diatoms? *Progress in Oceanography*, **67**, 384-405.
- Leles, S. G., Polimene, L., Bruggeman, J., Blackford, J., Ciavatta, S., Mitra, A. and Flynn, K. J. (2018) Modelling mixotrophic functional diversity and implications for ecosystem function. *Journal of Plankton Research*, **40**, 627-642.
- Leterme, S. C., Edwards, M., Seuront, L., Attrill, M., Reid, P. and John, A. (2005) Decadal basin-scale changes in diatoms, dinoflagellates, and phytoplankton color across the North Atlantic. *Limnology and Oceanography*, **50**, 1244-1253.
- Leterme, S. C., Seuront, L. and Edwards, M. (2006) Differential contribution of diatoms and dinoflagellates to phytoplankton biomass in the NE Atlantic Ocean and the North Sea. *Marine Ecology Progress Series*, **312**, 57-65.
- Lindeman, R. L. (1942) The Trophic-Dynamic Aspect of Ecology. *Ecology*, **23**, 399-417.
- Liu, H., Chen, M., Zhu, F. and Harrison, P. J. (2016) Effect of Diatom Silica Content on Copepod Grazing, Growth and Reproduction. *Frontiers in Marine Science*, **3**.
- Lynn, S. G., Kilham, S. S., Kreeger, D. A. and Interlandi, S. J. (2000) Effect of nutrient availability on the biochemical and elemental stoichiometry in the freshwater diatom *Stephanodiscus minutulus* (Bacillariophyceae). *Journal of Phycology*, **36**, 510-522.
- Macovei, V. A., Torres-Valdés, S., Hartman, S. E., Schuster, U., Moore, C. M., Brown, P. J., Hydes, D. J. and Sanders, R. J. (2019) Temporal Variability in the Nutrient Biogeochemistry of the Surface North Atlantic: 15 Years of Ship of Opportunity Data. *Global Biogeochemical Cycles*, **33**, 1674-1692.
- Makareviciute-Fichtner, K., Matthiessen, B., Lotze, H. K. and Sommer, U. (2020) Decrease in diatom dominance at lower Si:N ratios alters plankton food webs. *Journal of Plankton Research*, **42**, 411-424.

References

- Malviya, S., Scalco, E., Audic, S., Vincent, F., Veluchamy, A., Poulain, J., Wincker, P., Iudicone, D., De Vargas, C., Bittner, L., Zingone, A. and Bowler, C. (2016) Insights into global diatom distribution and diversity in the world's ocean. *Proceedings of the National Academy of Sciences*, **113**, E1516-E1525.
- Malzahn, A. M., Aberle, N., Clemmesen, C. and Boersma, M. (2007) Nutrient limitation of primary producers affects planktivorous fish condition. *Limnology and Oceanography*, **52**, 2062-2071.
- Mann, K. (1993) Physical oceanography, food chains, and fish stocks: a review. *ICES Journal of Marine Science*, **50**, 105-119.
- Mcnair, H. M., Brzezinski, M. A. and Krause, J. W. (2018) Diatom populations in an upwelling environment decrease silica content to avoid growth limitation. *Environmental Microbiology*, **20**, 4184-4193.
- Medlin, L. K. and Priddle, J. (1990) *Polar marine diatoms*. Vol., British Antarctic Survey.
- Menden-Deuer, S. and Lessard, E. J. (2000) Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. *Limnology and Oceanography*, **45**, 569-579.
- Merrell, J. R. and Stoecker, D. K. (1998) Differential grazing on protozoan microplankton by developmental stages of the calanoid copepod *Eurytemora affinis* Poppe. *Journal of Plankton Research*, **20**, 289-304.
- Miller, C. A. and Glibert, P. M. (1998) Nitrogen excretion by the calanoid copepod *Acartia tonsa*: results of mesocosm experiments. *Journal of Plankton Research*, **20**, 1767-1780.
- Milligan, A. J. and Morel, F. M. M. (2002) A Proton Buffering Role for Silica in Diatoms. *Science*, **297**, 1848-1850.
- Miralto, A., Barone, G., Romano, G., Poulet, S. A., Ianora, A., Russo, G. L., Buttino, I., Mazzarella, G., Laabir, M., Cabrini, M. and Giacobbe, M. G. (1999) The insidious effect of diatoms on copepod reproduction. *Nature*, **402**, 173-176.
- Mitra, A., Castellani, C., Gentleman, W. C., Jónasdóttir, S. H., Flynn, K. J., Bode, A., Halsband, C., Kuhn, P., Licandro, P. and Agersted, M. D. (2014a) Bridging the gap between marine biogeochemical and fisheries sciences; configuring the zooplankton link. *Progress in Oceanography*, **129**, 176-199.
- Mitra, A., Flynn, K. J., Burkholder, J. M., Berge, T., Calbet, A., Raven, J. A., Granéli, E., Glibert, P. M., Hansen, P. J., Stoecker, D. K., Thingstad, F., Tillmann, U., Våge, S., Wilken, S. and Zubkov, M. V. (2014b) The role of mixotrophic protists in the biological carbon pump. *Biogeosciences*, **11**, 995-1005.
- Mitra, A., Flynn, K. J., Tillmann, U., Raven, J. A., Caron, D., Stoecker, D. K., Not, F., Hansen, P. J., Hallegraeff, G. and Sanders, R. (2016) Defining planktonic protist functional groups on mechanisms for energy and nutrient acquisition: incorporation of diverse mixotrophic strategies. *Protist*, **167**, 106-120.
- Moline, M. A. and Prézelin, B. B. (1996) Long-term monitoring and analyses of physical factors regulating variability in coastal Antarctic phytoplankton biomass, in situ productivity and taxonomic composition over subseasonal, seasonal and interannual time scales. *Marine Ecology Progress Series*, **145**, 143-160.
- Moriceau, B., Iversen, M. H., Gallinari, M., Evertsen, A.-J. O., Le Goff, M., Beker, B., Boutorh, J., Corvaisier, R., Coffineau, N., Donval, A., Giering, S. L. C., Koski, M., Lambert, C., Lampitt, R. S., Le Mercier, A., Masson, A., Stibor, H., Stockenreiter, M. and De La Rocha, C. L. (2018) Copepods Boost the Production but Reduce the Carbon Export Efficiency by Diatoms. *Frontiers in Marine Science*, **5**, 82.
- Motwani, N. H. and Gorokhova, E. (2013) Mesozooplankton grazing on picocyanobacteria in the Baltic Sea as inferred from molecular diet analysis. *PLoS One*, **8**, e79230.

References

- Munn, C. (2011) *Marine microbiology: Ecology & applications*. Vol.1, Garland Science, New York.
- Nejstgaard, J. C., Hygum, B. H., Naustvoll, L. J. and Båmstedt, U. (2001) Zooplankton growth, diet and reproductive success compared in simultaneous diatom- and flagellate-microzooplankton-dominated plankton blooms. *Marine Ecology Progress Series*, **221**, 77-91.
- Nelson, D. M., Tréguer, P., Brzezinski, M. A., Leynaert, A. and Quéguiner, B. (1995) Production and dissolution of biogenic silica in the ocean: Revised global estimates, comparison with regional data and relationship to biogenic sedimentation. *Global Biogeochemical Cycles*, **9**, 359-372.
- Nixon, S. and Fulweiler, R. (2009) Nutrient pollution, eutrophication, and the degradation of coastal marine ecosystems. *Global loss of coastal habitats: rates, causes and consequences*, 23-58.
- Officer, C. and Ryther, J. (1980) The possible importance of silicon in marine eutrophication. *Marine Ecology Progress Series*, **3**, 83-91.
- Olson, M. B., Lessard, E. J., Wong, C. H. J. and Bernhardt, M. J. (2006) Copepod feeding selectivity on microplankton, including the toxigenic diatoms *Pseudo-nitzschia* spp., in the coastal Pacific Northwest. *Marine Ecology Progress Series*, **326**, 207-220.
- Pancic, M., Torres, R. R., Almeda, R. and Kiorboe, T. (2019) Silicified cell walls as a defensive trait in diatoms. *Proceedings of the Royal Society B*, **286**, 20190184.
- Papush, L. and Danielsson, Å. (2006) Silicon in the marine environment: Dissolved silica trends in the Baltic Sea. *Estuarine, Coastal and Shelf Science*, **67**, 53-66.
- Park, S., Brett, M. T., Müller-Navarra, D. C. and Goldman, C. R. (2002) Essential fatty acid content and the phosphorus to carbon ratio in cultured algae as indicators of food quality for *Daphnia*. *Freshwater Biology*, **47**, 1377-1390.
- Parrish, C. C. (2009) Essential fatty acids in aquatic food webs. *Lipids in aquatic ecosystems*. Springer, pp. 309-326.
- Parsons, M. L. and Dortch, Q. (2002) Sedimentological evidence of an increase in *Pseudo-nitzschia* (Bacillariophyceae) abundance in response to coastal eutrophication. *Limnology and Oceanography*, **47**, 551-558.
- Parsons, T. and Harrison, P. (1983) Nutrient cycling in marine ecosystems. *Physiological Plant Ecology IV*. Springer, pp. 85-115.
- Payne, M. and Ripplingale, R. (2000) Evaluation of diets for culture of the calanoid copepod *Gladioferens imparipes*. *Aquaculture*, **187**, 85-96.
- Pearsall, W. (1932) Phytoplankton in the English lakes: II. The composition of the phytoplankton in relation to dissolved substances. *The Journal of Ecology*, **20**, 241-262.
- Phlips, E. J., Badylak, S., Christman, M. C. and Lasi, M. A. (2010) Climatic trends and temporal patterns of phytoplankton composition, abundance, and succession in the Indian River Lagoon, Florida, USA. *Estuaries and Coasts*, **33**, 498-512.
- Pohnert, G. (2005) Diatom/copepod interactions in plankton: the indirect chemical defense of unicellular algae. *Chembiochem*, **6**, 946-59.
- Pomeroy, L. R. (1974) The Ocean's Food Web, A Changing Paradigm. *BioScience*, **24**, 499-504.
- Poulet, S., Ianora, A., Miralto, A. and Meijer, L. (1994) Do diatoms arrest embryonic development in copepods? *Marine Ecology Progress Series*, **111**, 79-86.
- Pree, B., Larsen, A., Egge, J. K., Simonelli, P., Madhusoodhanan, R., Tsagaraki, T. M., Våge, S., Erga, S. R., Bratbak, G. and Thingstad, T. F. (2017) Dampened copepod-mediated trophic cascades in a microzooplankton-dominated microbial food web: A mesocosm study. *Limnology and Oceanography*, **62**, 1031-1044.

References

- Quigg, A., Finkel, Z. V., Irwin, A. J., Rosenthal, Y., Ho, T.-Y., Reinfelder, J. R., Schofield, O., Morel, F. M. and Falkowski, P. G. (2003) The evolutionary inheritance of elemental stoichiometry in marine phytoplankton. *Nature*, **425**, 291-294.
- R Core Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- R Studio Team (2019) RStudio: Integrated Development for R. *RStudio, Inc.*, Boston, MA.
- Ragueneau, O., Conley, D. J., Leynaert, A., Longphuirt, S. N. and Slomp, C. P. (2006) Responses of coastal ecosystems to anthropogenic perturbations of silicon cycling *The Silicon Cycle: Human Perturbations and Impacts on Aquatic Systems*. Island Press Washington, DC, pp. 197-213.
- Rahman, S., Tamborski, J. J., Charette, M. A. and Cochran, J. K. (2019) Dissolved silica in the subterranean estuary and the impact of submarine groundwater discharge on the global marine silica budget. *Marine Chemistry*, **208**, 29-42.
- Redfield, A. C. (1934) *On the proportions of organic derivatives in sea water and their relation to the composition of plankton*. Vol. 1, Liverpool: university press of Liverpool.
- Reuss, N. and Poulsen, L. (2002) Evaluation of fatty acids as biomarkers for a natural plankton community. A field study of a spring bloom and a post-bloom period off West Greenland. *Marine Biology*, **141**, 423-434.
- Rey, F. (2012) Declining silicate concentrations in the Norwegian and Barents Seas. *ICES Journal of Marine Science*, **69**, 208-212.
- Richter, O. (1904) Über Reinkulturen von Diatomeen und die Notwendigkeit der Kieselsäure für Nitzschia Pale (Kütz) W.Sm. *Verhandlungen der Gesellschaft deutscher Naturforscher und Ärzte*, **76**, 249.
- Roberts, E. C. (2003) Response of temperate microplankton communities to N:Si ratio perturbation. *Journal of Plankton Research*, **25**, 1485-1495.
- Roser, M. and Ritchie, H. (2013) Fertilizers. OurWorldInData.org.
- Russo, E., Ianora, A. and Carotenuto, Y. (2018) Re-shaping marine plankton communities: effects of diatom oxylipins on copepods and beyond. *Marine Biology*, **166**, 9.
- Saage, A., Vadstein, O. and Sommer, U. (2009) Feeding behaviour of adult *Centropages hamatus* (Copepoda, Calanoida): Functional response and selective feeding experiments. *Journal of Sea Research*, **62**, 16-21.
- Schöllhorn, E. and Granéli, E. (1996) Influence of different nitrogen to silica ratios and artificial mixing on the structure of a summer phytoplankton community from the Swedish west coast (gullmar fjord). *Journal of Sea Research*, **35**, 159-167.
- Schultz, M. and Kiørboe, T. (2009) Active prey selection in two pelagic copepods feeding on potentially toxic and non-toxic dinoflagellates. *Journal of Plankton Research*, **31**, 553-561.
- Selander, E., Berglund, E. C., Engstrom, P., Berggren, F., Eklund, J., Harethardottir, S., Lundholm, N., Grebner, W. and Andersson, M. X. (2019) Copepods drive large-scale trait-mediated effects in marine plankton. *Science Advances*, **5**, eaat5096.
- Sharp, J. H. (1974) Improved analysis for "particulate" organic carbon and nitrogen from seawater 1. *Limnology and Oceanography*, **19**, 984-989.
- Sherr, E. and Sherr, B. F. (1988) Role of microbes in pelagic food webs: a revised concept.
- Sherr, E. B. and Sherr, B. F. (2002) Significance of predation by protists in aquatic microbial food webs. *Antonie van Leeuwenhoek*, **81**, 293-308.
- Shin, K., Jang, M.-C., Jang, P.-K., Ju, S.-J., Lee, T.-K. and Chang, M. (2003) Influence of food quality on egg production and viability of the marine planktonic copepod *Acartia omorii*. *Progress in Oceanography*, **57**, 265-277.

References

- Sinha, E., Michalak, A. M. and Balaji, V. (2017) Eutrophication will increase during the 21st century as a result of precipitation changes. *Science*, **357**, 405-408.
- Smayda, T. J. (1990) *Novel and nuisance phytoplankton blooms in the sea: evidence for a global epidemic*. Vol. 1, RWS-North Sea Directorate.
- Smayda, T. J. and Reynolds, C. S. (2003) Strategies of marine dinoflagellate survival and some rules of assembly. *Journal of Sea Research*, **49**, 95-106.
- Smith, V. H. (2006) Responses of estuarine and coastal marine phytoplankton to nitrogen and phosphorus enrichment. *Limnology and Oceanography*, **51**, 377-384.
- Sommer, U. (1998) From algal competition to animal production: Enhanced ecological efficiency of *Brachionus plicatilis* with a mixed diet. *Limnology and Oceanography*, **43**, 1393-1396.
- Sommer, U. (2009) Copepod growth and diatoms: insensitivity of *Acartia tonsa* to the composition of semi-natural plankton mixtures manipulated by silicon:nitrogen ratios in mesocosms. *Oecologia*, **159**, 207-15.
- Sommer, U., Adrian, R., De Senerpont Domis, L., Elser, J. J., Gaedke, U., Ibelings, B., Jeppesen, E., Lüring, M., Molinero, J. C., Mooij, W. M., Van Donk, E. and Winder, M. (2012a) Beyond the Plankton Ecology Group (PEG) Model: Mechanisms Driving Plankton Succession. *Annual Review of Ecology, Evolution, and Systematics*, **43**, 429-448.
- Sommer, U., Charalampous, E., Scotti, M. and Moustaka-Gouni, M. (2018) Big fish eat small fish: implications for food chain length? *Community Ecology*, **19**, 107-115.
- Sommer, U., Hansen, T., Blum, O., Holzner, N., Vadstein, O. and Stibor, H. (2005) Copepod and microzooplankton grazing in mesocosms fertilised with different Si:N ratios: no overlap between food spectra and Si:N influence on zooplankton trophic level. *Oecologia*, **142**, 274-83.
- Sommer, U., Hansen, T., Stibor, H. and Vadstein, O. (2004) Persistence of phytoplankton responses to different Si:N ratios under mesozooplankton grazing pressure: a mesocosm study with NE Atlantic plankton. *Marine Ecology Progress Series*, **278**, 67-75.
- Sommer, U., Hansen, T., Stibor, H., Vadstein, O. (2004) Persistence of phytoplankton responses to different Si:N ratios under mesozooplankton grazing pressure: a mesocosm study with NE Atlantic plankton. *Marine Ecology Progress Series*, **278**, 67-75.
- Sommer, U., Lengfellner, K. and Lewandowska, A. (2012b) Experimental induction of a coastal spring bloom early in the year by intermittent high-light episodes. *Marine Ecology Progress Series*, **446**, 61-71.
- Sommer, U. and Sommer, F. (2006) Cladocerans versus copepods: the cause of contrasting top-down controls on freshwater and marine phytoplankton. *Oecologia*, **147**, 183-94.
- Sommer, U., Stibor, H., Katechakis, A., Sommer, F. and Hansen, T. (2002) Pelagic food web configurations at different levels of nutrient richness and their implications for the ratio fish production:primary production. *Hydrobiologia*, **484**, 11-20.
- Spokes, L. J. and Jickells, T. D. (2005) Is the atmosphere really an important source of reactive nitrogen to coastal waters? *Continental Shelf Research*, **25**, 2022-2035.
- Sterner, R. W. and Hessen, D. O. (1994) Algal nutrient limitation and the nutrition of aquatic herbivores. *Annual review of Ecology and Systematics*, **25**, 1-29.
- Stevens, C. J. (2019) Nitrogen in the environment. *Science*, **363**, 578-580.
- Sundt-Hansen, L. E., Olsen, Y., Stibor, H., Høldal, M. and Vadstein, O. (2006) Trophic cascades mediated by copepods, not nutrient supply rate, determine the development of picocyanobacteria. *Aquatic microbial ecology*, **45**, 207-218.

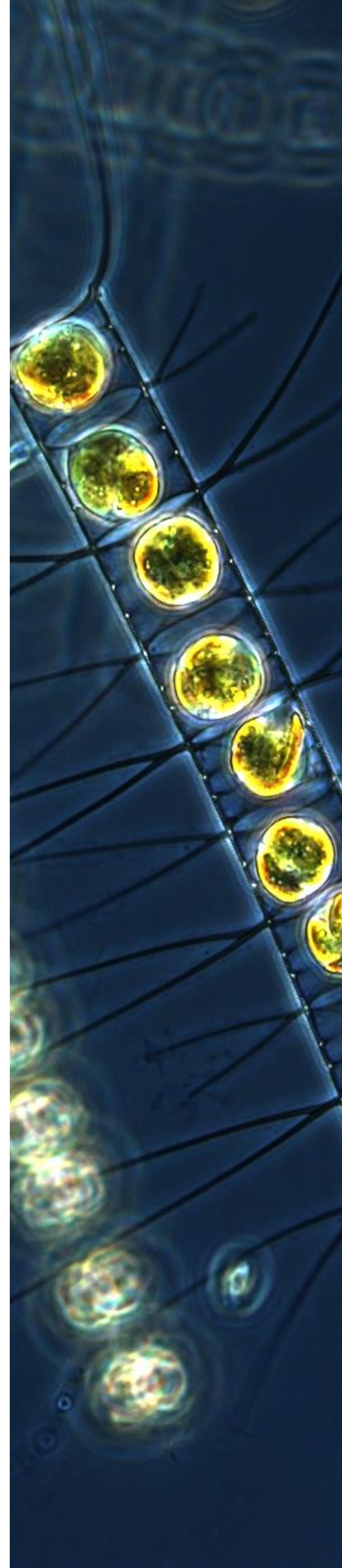
References

- Tackx, M. L. M., Herman, P. J. M., Gasparini, S., Irigoien, X., Billiones, R. and Daro, M. H. (2003) Selective feeding of *Eurytemora affinis* (Copepoda, Calanoida) in temperate estuaries: model and field observations. *Estuarine, Coastal and Shelf Science*, **56**, 305-311.
- Taucher, J., Jones, J., James, A., Brzezinski, M. A., Carlson, C. A., Riebesell, U. and Passow, U. (2015) Combined effects of CO₂ and temperature on carbon uptake and partitioning by the marine diatoms *Thalassiosira weissflogii* and *Dactyliosolen fragilissimus*. *Limnology and Oceanography*, **60**, 901-919.
- Tilman, D. (1981) Tests of Resource Competition Theory Using Four Species of Lake Michigan Algae. *Ecology*, **62**, 802-815.
- Tilman, D. (1982a) *Resource competition and community structure*. Vol., Princeton university press.
- Tilman, D., Kilham, S.S. And Kilham, P. (1982b) Phytoplankton community ecology: the role of limiting nutrients. *Annual Review of Ecology and Systematics* **13**, 349-372.
- Tréguer, P., Bowler, C., Moriceau, B., Dutkiewicz, S., Gehlen, M., Aumont, O., Bittner, L., Dugdale, R., Finkel, Z. and Iudicone, D. (2018) Influence of diatom diversity on the ocean biological carbon pump. *Nature Geoscience*, **11**, 27-37.
- Treguer, P., Nelson, D. M., Van Bennekom, A. J., Demaster, D. J., Leynaert, A. and Queguiner, B. (1995) The silica balance in the world ocean: a reestimate. *Science*, **268**, 375-379.
- Treguer, P. J. and De La Rocha, C. L. (2013) The world ocean silica cycle. *Ann Rev Mar Sci*, **5**, 477-501.
- Urabe, J. (1995) Direct and indirect effects of zooplankton on seston stoichiometry. *Écoscience*, **2**, 286-296.
- Utermöhl, H. (1958) Zur Vervollkommnung der quantitativen Phytoplankton-Methodik: Mit 1 Tabelle und 15 abbildungen im Text und auf 1 Tafel. *Internationale Vereinigung für theoretische und angewandte Limnologie: Mitteilungen*, **9**, 1-38.
- Van Nieuwerburgh, L., Wänstrand, I. and Snoeijs, P. (2004) Growth and C:N:P ratios in copepods grazing on N- or Si-limited phytoplankton blooms. *Hydrobiologia*, **514**, 57-72.
- Vitousek, P. M. and Howarth, R. W. (1991) Nitrogen limitation on land and in the sea: How can it occur? *Biogeochemistry*, **13**, 87-115.
- Wasmund, N., Nausch, G. and Feistel, R. (2013) Silicate consumption: an indicator for long-term trends in spring diatom development in the Baltic Sea. *Journal of Plankton Research*, **35**, 393-406.
- Wickham, H. (2016) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York
- Wilson, S. and Steinberg, D. (2010) Autotrophic picoplankton in mesozooplankton guts: evidence of aggregate feeding in the mesopelagic zone and export of small phytoplankton. *Marine Ecology Progress Series*, **412**, 11-27.
- Wiltshire, K. H., Kraberg, A., Bartsch, I., Boersma, M., Franke, H.-D., Freund, J., Gebühr, C., Gerdt, G., Stockmann, K. and Wichels, A. (2010) Helgoland Roads, North Sea: 45 Years of Change. *Estuaries and Coasts*, **33**, 295-310.
- Yang, S. and Gruber, N. (2016) The anthropogenic perturbation of the marine nitrogen cycle by atmospheric deposition: Nitrogen cycle feedbacks and the 15N Haber-Bosch effect. *Global Biogeochemical Cycles*, **30**, 1418-1440.
- Yunev, O. A., Carstensen, J., Moncheva, S., Khaliulin, A., Ærtebjerg, G. and Nixon, S. (2007) Nutrient and phytoplankton trends on the western Black Sea shelf in response to cultural eutrophication and climate changes. *Estuarine, Coastal and Shelf Science*, **74**, 63-76.
- Zehr, J. P. and Capone, D. G. (2020) Changing perspectives in marine nitrogen fixation. *Science*, **368**, eaay9514.
- Zeileis, A. and Hothorn, T. (2002) Diagnostic Checking in Regression Relationships. *R News*, **2**, 7-10.

References

- Zhao, Y., Dong, Y., Li, H., Lin, S., Huang, L., Xiao, T., Gregori, G., Zhao, L. and Zhang, W. (2020) Grazing by microzooplankton and copepods on the microbial food web in spring in the southern Yellow Sea, China. *Marine Life Science & Technology*, **2**, 442-455.
- Zöllner, E., Hoppe, H.-G., Sommer, U. and Jürgens, K. (2009) Effect of zooplankton-mediated trophic cascades on marine microbial food web components (bacteria, nanoflagellates, ciliates). *Limnology and Oceanography*, **54**, 262-275.

Appendix



Chapter I

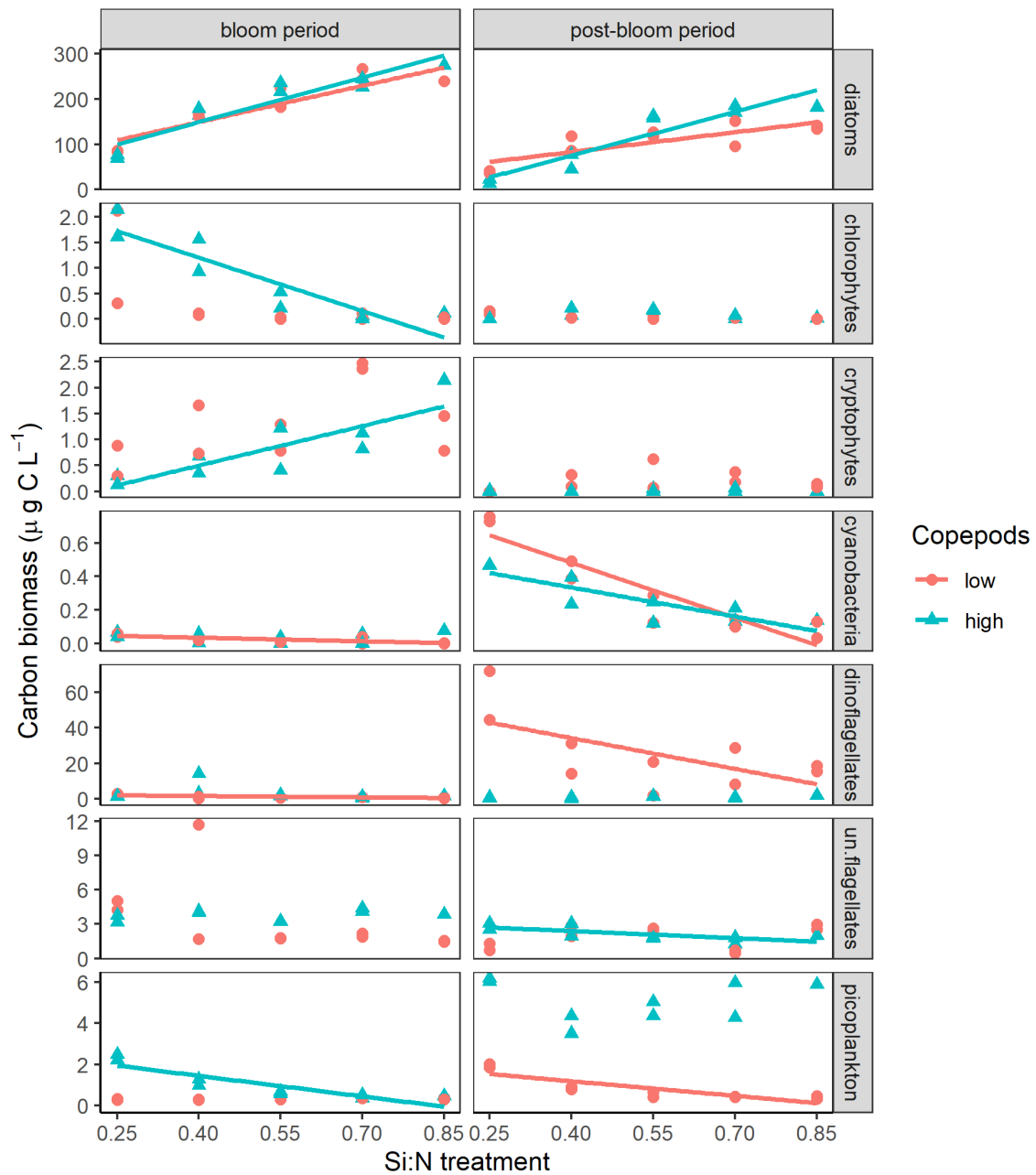


Figure S1.1 Carbon biomass of different phytoplankton groups during bloom and post-bloom periods over Si:N ratio treatments. Un.flagellates – unidentified small (2-5μm) flagellates containing chlorophyll. Regression lines are fitted where Si:N effects were significant.

Appendix - Chapter I

Table S1.1 Results of likelihood ratio tests for generalized linear models testing for the effects of Si:N ratio on the abundance of different copepod stages. Significant results are in bold. * = $p \leq 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Response variable		Df	χ^2	p	
<i>Adults</i>	cop +	1	0.075	0.124	
	cop -	1	0.0004	0.909	
<i>Copepodites</i>	cop +	1	0.187	0.012	*
	cop -	1	0.012	0.560	
<i>Nauplii</i>	cop +	1	0.027	0.627	
	cop -	1	0.146	0.010	*
<i>Eggs</i>	cop +	1	0.129	0.163	
	cop -	1	0.0001	0.962	

Chapter II

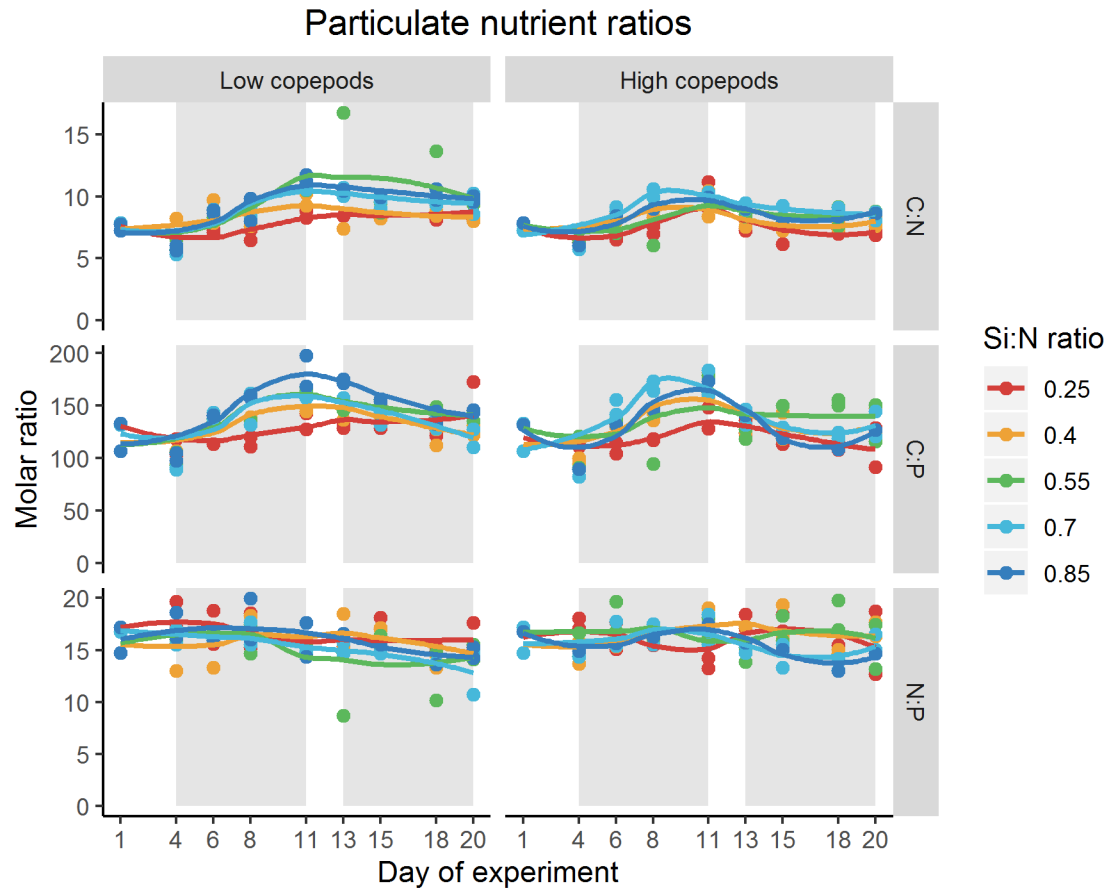


Figure S2.1 Seston nutrient ratios over the course of the experiment. Grey shaded areas represent bloom peak (days 4-11) and post-bloom (days 13-20) periods. C:N - carbon to nitrogen, C:P - carbon to phosphorus, N:P – nitrogen to phosphorus molar ratios.

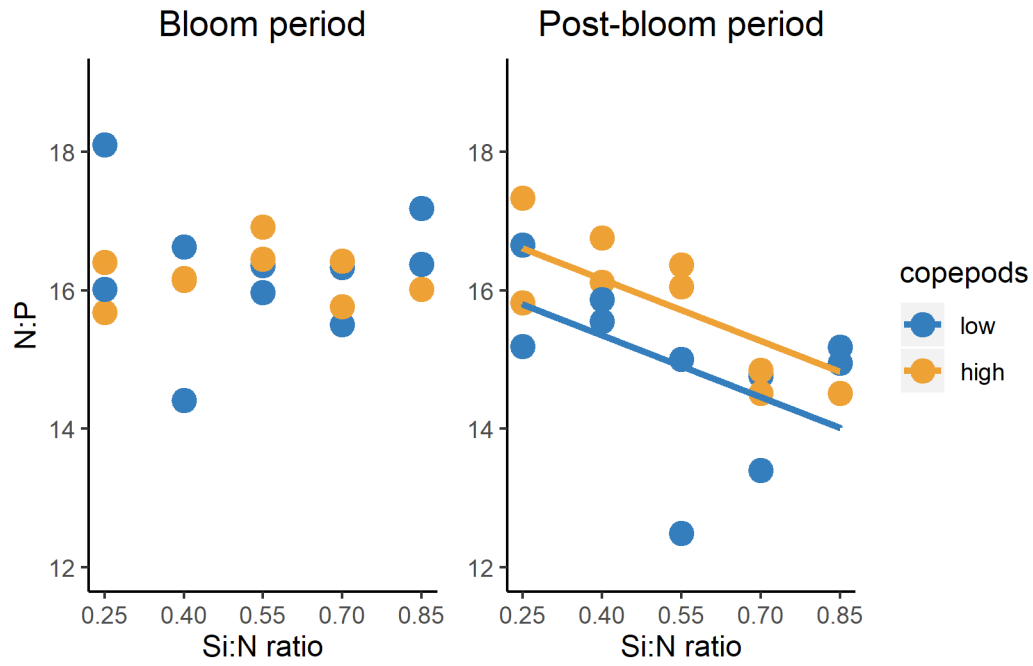


Figure S2.2 Seston N:P ratios over Si:N ratio during bloom peak (days 4-11) and post-bloom (days 13-20) periods.

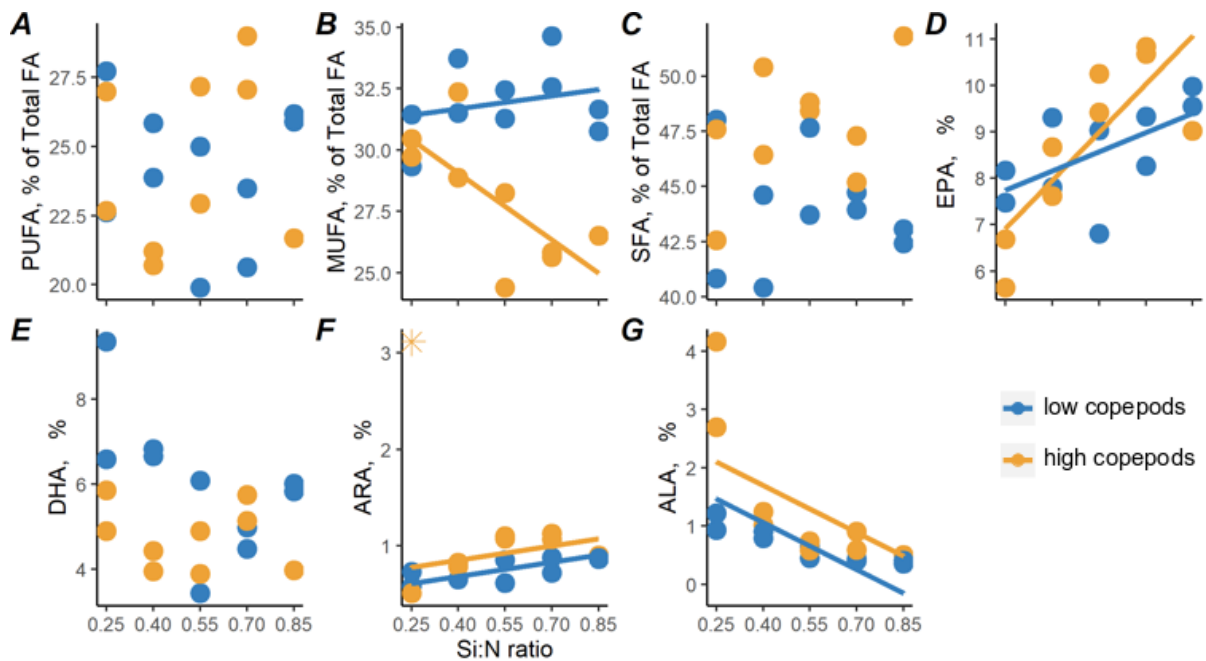


Figure S2.3 Seston fatty acid relative (percentage of total fatty acids) contents over Si:N ratio

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Table S3.1 Treatment effects on dissolved nutrient concentrations and Si:N ratio on day 5 of the experiment. ² indicates that Si:N function was quadratic, arrows mark where the parameter decreased (↓) or increased (↑) with Si:N treatments. GLM model with Gamma link with log link was used for dissolved nitrogen and phosphorus data and identity link was used for estimating effects on dissolved silicon Si:N ratio.

Day 5 of the experiment					
	Factor	Df	χ^2	Pr(> χ^2)	
<i>Dissolved Nitrogen</i>	Si:N ²	2	2.440	0.000	*** ↓
	cop	1	39.837	0.288	
	Si:N ² xcop	2	2.375	0.800	
<i>Dissolved Phosphorus</i>	Si:N ²	2	1.092	0.000	*** ↓
	cop	1	17.591	0.411	
	Si:N ² xcop	2	1.064	0.812	
<i>Dissolved Silicon</i>	Si:N ²	2	1.119	0.000	*** ↑
	cop	1	7.191	0.001	**
	Si:N ² xcop	2	0.942	0.281	
<i>Si:N ratio</i>	Si:N ²	2	1.705	0.000	*** ↑
	cop	1	26.667	0.001	**
	Si:N ² xcop	2	1.204	0.052	*

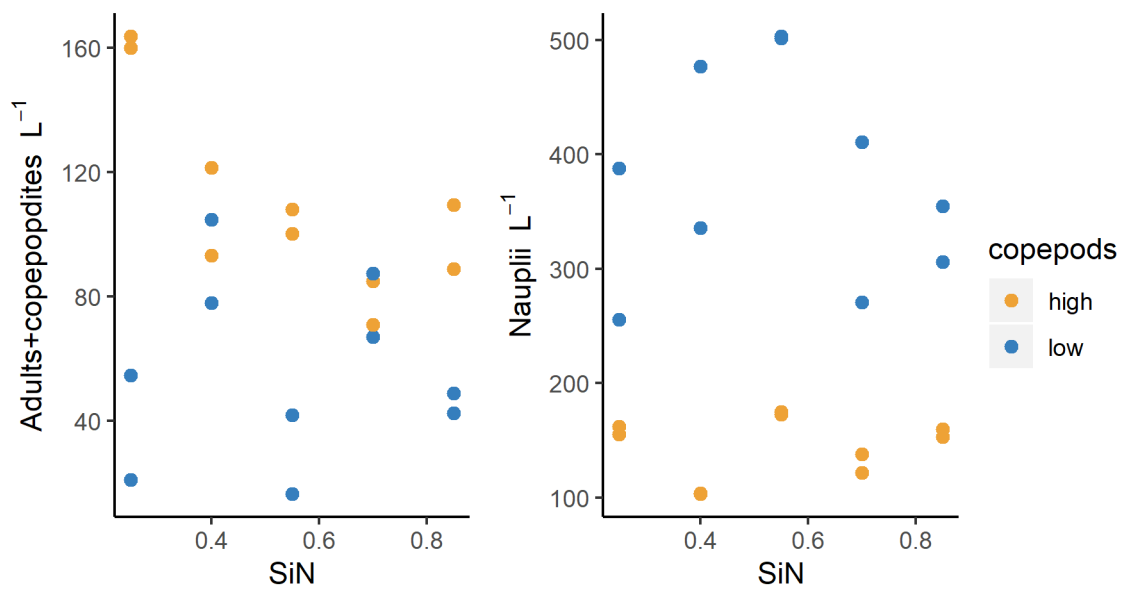


Figure S3.1 Abundance of copepods (A – adults and copepodites, B – nauplii) at the end of the experiment as a function of initial Si:N ratio.

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Table S3.2 ANCOVA results for Si:N ratio effects on copepod abundance

	factor	Df	F value	Pr(>F)	
Copepods+adults	Si:N ratio	1	3.446	0.082	
	Cop	1	21.252	0.000	***
	Si:N X cop	1	3.591	0.076	
	Residuals	16			
Nauplii	Si:N ratio	1	0.013	0.909	
	Cop	1	54.460	0.000	***
	Si:N X cop	1	0.095	0.761	
	Residuals	16			

Table S3.3 Effects of treatment manipulations on relative biomass (%) of plankton groups.

Functional group	Factor	Bloom period				Post-bloom period			
		Df	F value	Pr(>F)		Df	F value	Pr(>F)	
Diatoms	Si:Nv2	2	9.599	0.002	**	2	12.326	0.001	***
	Cop	1	17.640	0.001	***	1	0.008	0.932	
	Si:N^2 X cop	2	4.408	0.033	*	2	0.071	0.932	
Dinoflagellates	Si:N^2	2	3.528	0.057		2	1.315	0.300	
	Cop	1	52.711	0.000	***	1	2.800	0.116	
	Si:N^2 X cop	2	6.188	0.012	*	2	0.330	0.725	
Picoplankton	Si:N^2	2	26.062	0.000	***	2	9.393	0.003	**
	Cop	1	6.483	0.023	*	1	0.139	0.715	
	Si:N^2 X cop	2	0.924	0.420		2	0.315	0.735	
Ciliates	Si:N^2	2	2.335	0.133		2	8.617	0.004	**
	Cop	1	0.003	0.960		1	11.578	0.004	**
	Si:N^2 X cop	2	0.845	0.450		2	1.287	0.307	

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Qu, Y., Wu, N., Guse, B., **Makarevičiūtė, K.**, Sun, X., & Fohrer, N. (2019). Riverine phytoplankton functional groups response to multiple stressors variously depending on hydrological periods. *Ecological Indicators*, 101, 41-49.

Qu, Y., Wu, N., **Makarevičiūtė, K.**, Guse, B., & Fohrer, N. (2018). Effects of land-use pattern and physiochemical conditions on phytoplankton communities in a German lowland catchment. *Fundamental and Applied Limnology/Archiv für Hydrobiologie*, 191(3), 175-187.

Wu, N., Qu, Y., Guse, B., **Makarevičiūtė, K.**, To, S., Riis, T., & Fohrer, N. (2018). Hydrological and environmental variables outperform spatial factors in structuring species, trait composition, and beta diversity of pelagic algae. *Ecology and evolution*, 8(5), 2947-2961.

CONFERENCE PRESENTATIONS

Makarevičiūtė-Fichtner, K., Matthiessen, B., Lotze, H.K., Sommer, U. (2020) Declining silica availability-a challenge in the North Atlantic region? EGU General Assembly 2020. Vienna, Austria. [Poster presentation]

Chua, A., Bertlich, J., **Makareviciute-Fichtner, K.**, Rakshit, S. (2020) Interdisciplinary and intercultural approaches for addressing scientific and socio-economic challenges in the North Atlantic region. EGU General Assembly 2020. Vienna, Austria [Convener of the Session: ITS1.11/OS1.14]

Makarevičiūtė-Fichtner, K., Matthiessen, B., Lotze, H.K., Sommer, U. (2018) . Mesozooplankton shapes phytoplankton response to changing Si:N stoichiometry. ASLO (Association for the Sciences of Limnology and Oceanography) 2018 Summer Meeting. Victoria, BC, Canada. [Oral presentation]

Description of Author contributions

Chapter I: Decrease in diatom dominance at lower Si:N ratios alters plankton food webs

Authors: Kriste Makareviciute-Fichtner, Birte Matthiessen, Heike K. Lotze, Ulrich Sommer

Published in the Journal of Plankton Research (2020), DOI: 10.1093/plankt/fbaa032

Contributions: US, KMF, HL and BM designed the experiment; KMF conducted the experiment and analyzed the data. US, KMF, HL and BM discussed the results; KMF wrote the manuscript with input from the other authors.

Chapter II: Phytoplankton nutritional quality is altered by shifting Si:N ratios and selective grazing

Authors: Kriste Makareviciute-Fichtner, Birte Matthiessen, Heike K. Lotze, Ulrich Sommer

Under review in the Journal of Plankton Research

Contributions: US, KMF, HL and BM developed the idea for this study; KMF collected the samples and analyzed the data. US, KMF, HL and BM discussed the results; KMF wrote the manuscript.

Chapter III: Changing Si:N ratios due to nitrogen enrichment: effects on plankton biomass and community composition

Authors: Kriste Makareviciute-Fichtner, Birte Matthiessen, Heike K. Lotze, Ulrich Sommer

Under preparation to be submitted

Contributions: US, KMF and BM designed the experiment; KMF implemented the experiment and analyzed the data. US, KMF, HL and BM discussed the results; KMF wrote the manuscript with contributions from the co-authors.

Declaration

I, Kristė Makarevičiūtė-Fichtner, hereby declare that the dissertation submitted, entitled “Declining silicon to nitrogen ratios: effects on phytoplankton and plankton food webs” was written independently by me and only using the sources listed. The content and design of this thesis, apart from the supervisor’s guidance, is my own work. The thesis has not been submitted either partially or wholly as a part of a doctoral degree to another examining body and is my first and only doctoral procedure. Chapter I of this thesis was published in the scientific Journal of Plankton Research in 2020. The authors' share of the manuscripts is explained in the section "Author contributions" (page 127). This work has been prepared respecting the Rules of Good Scientific Practice of the German Research Foundation. I have not been deprived of an academic degree.

Kiel, 07.03.2021



Kristė Makarevičiūtė-Fichtner