

**Trait shift history: trait shifts
in phytoplankton
communities induced by
grazing and light affect the
responses to nutrients**

Dissertation

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".... as soon as you have entered into the pelagic
wonderland you will see that you cannot leave it."

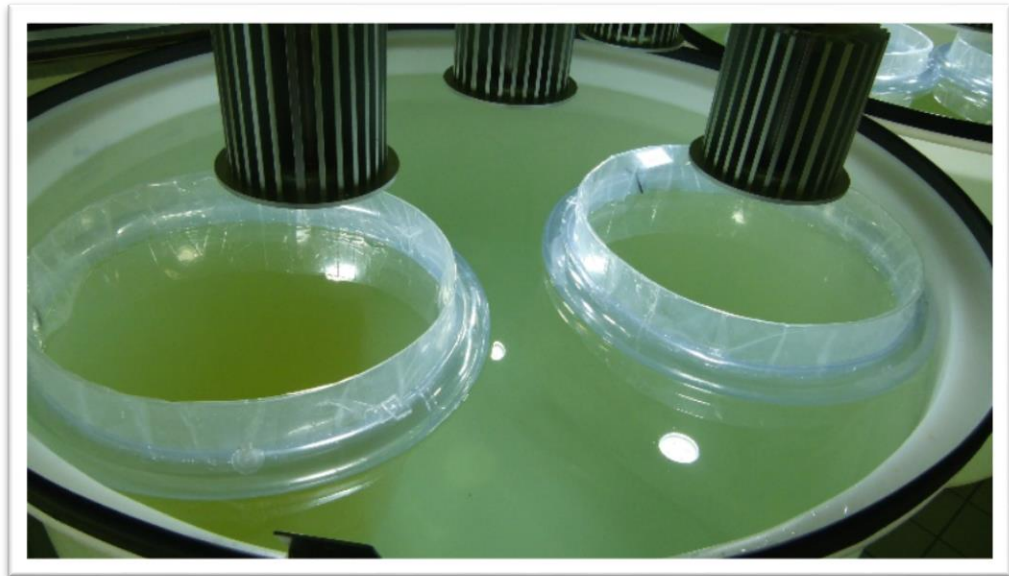
Johannes Müller to Ernst Haeckel (1854)

To my family

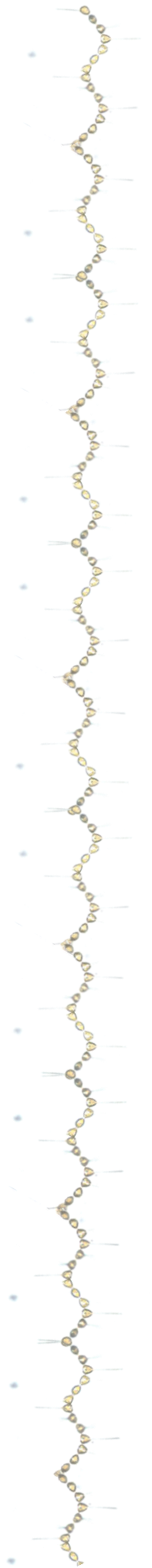
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Summary/ Zusammenfassung



Summary

Recent studies on the global primary production showed a decline in cell size and productivity of marine phytoplankton in response to climate warming. Reorganization of phytoplankton communities under global change can change community interactions and energy flows through the whole marine food web. Therefore, being able to predict phytoplankton responses to environmental drivers based on size structure could help to establish an intermediate level of complexity between the almost unachievable goal of reconstructing community behavior from single species and the overly simplistic approach of reducing phytoplankton to a single functional category. Because of strong correlations with other eco-physiological traits phytoplankton size has been considered as an ecologically significant “master” trait. In addition, a major challenge in climate change research on phytoplankton succession is to understand how multiple factors, which drive ecological changes in phytoplankton communities, shape the size distribution of the phytoplankton communities and the way this size alteration affects the performance to another stressor. Therefore, my work highlights the potential of trait based approaches to understand ecological complex processes in phytoplankton communities. It also indicates that cell size can be used as indicator trait predicting phytoplankton responses to several stressors as effect and response trait. However, its use should be based on a good knowledge of the characteristics of the studied communities and interactions between the different trophic levels should not be neglected.

The aim of this study was to examine whether cell size can be used as indicator trait for phytoplankton community responses to growth (nutrients, inhibiting light) and loss (grazing, limiting light) factors. I conducted three indoor mesocosm experiments with natural plankton communities from the Kiel Bight, Baltic Sea. In the first experiment I used sieving with three different mesh sized gauzes (100, 64 and 20 μm) to create three different sized communities. Subsequently, I treated the resulting communities with different nutrient conditions (N- limited and saturated) to test whether the known relationships between cell size and growth rates (negative slope in double log data transformation) derived from single species culture experiments also apply to natural phytoplankton communities of different cell size distributions. I also examined how the community cell size and these responses interacted with the heterotrophic populations in the community (ciliates and heterotrophic dinoflagellates). I found that the mean phytoplankton community cell size as well as the cell size of species negatively related to the maximal growth

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rates. However, the responses to nutrients of the size classes were not as clear as expected. I concluded that grazing by ciliates and heterotrophic dinoflagellates acted, which negatively correlated with the growth of the smaller sized phytoplankton, influenced the response of natural phytoplankton communities to nutrients and should not be neglected.

The experiments presented in chapters 2 and 3, focus on combined sequential stressors in order to be able to test how the response to one stressor can influence the response to a second stressor. In chapter 2, the stressors grazing by copepods (loss factor) and nutrients (growth factor) were chosen as subsequent stressors because of their importance in the natural environment and also because of their well-known effect on trait diversity. I observed that copepod grazing is able to change the taxonomic composition and cell size distribution of the plankton community. Initial stress, induced by copepod grazing, changed the response of the phytoplankton communities to nutrient availability and limitation. Cell size in the pre-grazed communities was negatively related to community, size classes and species growth rates. Also, cell size negatively related with the instantaneous nutrient utilization ability and the ability to preserve growth under nutrient limitation.

In chapter 3, I describe the results of my last experiment, where I used three different light levels (limiting, saturating and slightly inhibiting) as foregoing stressor and nutrient availability as second stressor. Light and nutrients are essential resources for phytoplankton growth and considered to shape the size structure and other morphometric traits of phytoplankton community. Low light favored smaller phytoplankton cells, higher surface to volume ratios and stronger deviations from a spherical cell shape. High light favored larger cell size, lower surface to volume ratios and more spherical cells. These trait shifts were seen both at the community level and within species. The trait shifts induced by different light levels had strong influence on the ability of the resultant communities to utilize nutrients. More precisely, the communities resulting from the low-light pre-treatment responded to a saturating nutrient pulse with faster growth, but less resource use efficiency. The opposite was the case with the communities pre-treated by higher light. The results indicate that changes in trait distributions induced by one environmental factor can influence the ability to cope with other environmental factors. Finally, the results accentuate the usefulness of morphometric traits in predicting environmental abilities and requirements of phytoplankton communities.

Zusammenfassung

Jüngste Studien über die globale Primärproduktion zeigten einen Rückgang der Zellgröße und Produktivität des marinen Phytoplanktons als Reaktion auf die Klimaerwärmung. Die Reorganisation von Phytoplanktongemeinschaften im Zuge des globalen Wandels kann die Wechselwirkungen zwischen den Nahrungsnetzkomponenten und den Energiefluss durch das gesamte marine Nahrungsnetz verändern. Während eine Reduktion des Phytoplanktons auf einen Summenparameter (z.B. Chlorophyll) zu simplistisch ist, ist das Ziel, das Gesamtverhalten und Zusammensetzungsänderungen des Phytoplanktons aus den ökophysiologischen Parametern Arten unzähliger Einzelarten vorherzusagen fast unmöglich. Daher könnte die Möglichkeit, Phytoplanktonreaktionen auf Umweltveränderungen basierend auf der Größenstruktur vorherzusagen, könnte eine Methode mittlerer Komplexität sein, die der Fragestellung angemessen ist. Aufgrund der starken Korrelationen mit anderen ökophysiologischen Merkmalen wurde die Phytoplanktongröße als ökologisch bedeutsames "Master"-Merkmal betrachtet. Darüber hinaus besteht eine große Herausforderung in der Klimaforschung zur Phytoplankton Sukzession darin zu verstehen, wie mehrere Faktoren, die ökologische Veränderungen in Phytoplanktongemeinschaften bewirken, die Größenverteilung der Phytoplanktongemeinschaften beeinflussen und wie sich diese Größenänderung auf die Stabilität ökologischer Funktionen gegenüber einem anderen Stressor auswirken. Daher überprüft meine Arbeit das Potenzial von merkmalsbasierten Ansätzen, ökologische komplexe Prozesse in Phytoplanktongemeinschaften zu verstehen. Die Ergebnisse unterstützen die Annahme, dass die Zellgröße als Indikatoreigenschaft verwendet werden kann, um Phytoplanktonreaktionen auf mehrere Stressoren als Effekt- und Reaktionsmerkmal vorherzusagen. Die Verwendung dieses Ansatzes sollte jedoch auf einer guten Kenntnis der Merkmale der Studiengemeinschaften beruhen, und die Wechselwirkungen zwischen den verschiedenen trophischen Ebenen sollten nicht vernachlässigt werden.

Ziel dieser Studie war es, zu untersuchen, ob die Zellgröße als Indikatoreigenschaft für die Reaktionen der Phytoplanktongemeinschaft auf Wachstum (Nährstoffe, hemmendes Licht) und Verlust (weidende, hemmende Lichtfaktoren) verwendet werden kann. Ich habe drei Indoor-Mesokosmos-Experimente mit natürlichen Planktongemeinschaften aus der Kieler Bucht in der Ostsee durchgeführt. Im ersten Experiment habe ich das Phytoplankton mit von drei verschiedenen Maschenweiten (100, 64 und 20 μm) fraktioniert, um drei unterschiedlich große Gemeinschaften

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zu erzeugen. Ich setzte die resultierenden Gemeinschaften dann unterschiedlichen Nährstoffbedingungen (N-limitiert und gesättigt) aus, um zu testen, ob die bekannten Zusammenhänge zwischen Zellgröße und Wachstumsraten (negative Steigung nach der doppellogarithmischer-Datentransformation) aus Ein-Spezies-Kultivierungsversuchen auch für natürliche Phytoplanktongemeinschaften mit unterschiedlichen Zellgrößenverteilungen gelten. Ich untersuchte auch, wie die durchschnittliche Zellgröße der experimentellen Phytoplanktongemeinschaften mit den heterotrophen Populationen (Ciliaten und heterotrophe Dinoflagellaten) interagierten. Ich fand heraus, dass die durchschnittliche Zellgröße der Phytoplanktongemeinschaft sowie die Zellgröße der Arten negativ mit den maximalen Wachstumsraten zusammenhängen. Die Reaktionen auf Nährstoffe der Größenklassen waren jedoch nicht so klar wie erwartet, da die Beweidung durch Ciliaten und heterotrophe Dinoflagellaten sich selektiv zum Nachteil der kleineren Phytoplanktonartne auswirkten.

Die in den Kapiteln 2 und 3 vorgestellten Experimente beruhen auf der Verwendung sequentiell kombinierter Stressoren, um testen zu können, wie die Reaktion auf einen Stressor die Reaktion auf einen zweiten Stressor beeinflussen kann. In Kapitel 2 wurden die Beweidung durch Copepoden (Verlustfaktor) und Nährstofflimitation vs. -suffizienz (Wachstumsfaktor) aufgrund ihrer Bedeutung für die natürliche Umwelt und ihrer bekannten Wirkung auf die Merkmalsvielfalt als aufeinanderfolgende Stressoren ausgewählt. Ich beobachtete, dass die Grazing durch Copepoden die taxonomische Zusammensetzung und Zellgrößenverteilung der Planktongemeinschaft verändert. Die durch das Grazing der Copepoden hervorgerufene Veränderung des Phytoplankton, veränderte ihrerseits die Reaktion der Phytoplanktongemeinschaften auf die Verfügbarkeit bzw. Mangel von Nährstoffen. Die Zellgröße war auf der Ebene einzelner Arten, einzelner Größenklassen und der ganzen Phytoplanktongemeinschaft negativ mit der Wachstumsrate korreliert. Ebenso zeigte sich eine negative Korrelation zwischen Zellgröße und der Kurzzeit-Fähigkeit Nährstoffpulse zu verwerten und der Fähigkeit das Wachstum danach unter Nährstofflimitierung aufrecht zu erhalten.

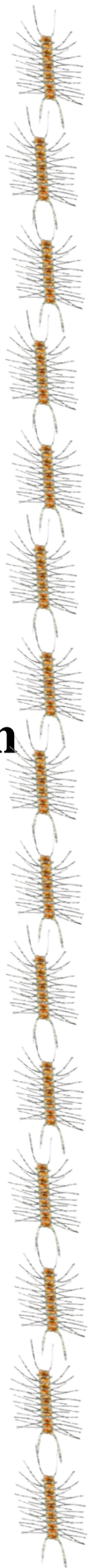
In Kapitel 3 beschreibe ich die Ergebnisse meines letzten Experiments, bei dem ich drei verschiedene Lichtniveaus (limitierend, sättigend und leicht hemmend) als Stressor und Nährstoffverfügbarkeit als zweiten Stressor verwendet habe. Licht und Nährstoffe sind essentielle Ressourcen für das Phytoplanktonwachstum und beeinflussen sowohl die Größenstruktur als auch

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andere andere morphometrische Merkmale der Phytoplanktongemeinschaft. Schwachlicht begünstigte kleinere Phytoplanktonzellen, höhere Oberflächen-Volumen-Verhältnisse und stärkere Abweichungen von einer sphärischen Zellform. Starkes Licht begünstigte eine größere Zellgröße, ein geringeres Oberflächen-Volumen-Verhältnis und mehr kugelförmige Zellen. Diese Veränderungen morphometrischer Eigenschaften wurden sowohl auf Gemeinschaftsebene als auch innerhalb der Arten beobachtet. Die durch unterschiedliche Lichtverhältnisse hervorgerufenen Merkmalsverschiebungen hatten einen starken Einfluss auf die Fähigkeit der resultierenden Gemeinschaften, Nährstoffe zu verwerten. Die Gemeinschaften, die aus der Schwachlicht-Vorbehandlung resultierten, auf einen sättigenden Nährstoffimpuls mit schnellerem Wachstum, aber weniger Ressourcennutzungseffizienz. Das Gegenteil war der Fall bei den Gemeinschaften, die mit Starklicht vorbehandelt wurden. Die Ergebnisse deuten darauf hin, dass Veränderungen in der Merkmalsverteilung, die durch einen Umweltfaktor hervorgerufen werden, die Fähigkeit beeinflussen können, mit anderen Umweltfaktoren umzugehen. Schließlich unterstützen die Ergebnisse den Nutzbarkeit morphometrischer Merkmale bei der Vorhersage von ökophysiologischen Fähigkeiten und Ansprüchen von Phytoplanktongemeinschaften.

Zusammenfassung

General Introduction



The paradox of the plankton

Phytoplankton form the most diverse photosynthetic community on the planet and consist of single celled, drifting photosynthetic organisms belonging to several phyla (Falkowski et al., 2004). This brings them in contrast with terrestrial plants that belong mostly to Embryophyta (Falkowski et al., 2004). The higher taxonomic level (phylum) diversity is followed by an even greater diversity at the species level, more than 5000 species (Tett and Barton, 1995), with different characteristics and ecological roles (Sommer et al., 2017a). Hutchinson (1961) already in the 1960's described this diverse complex of organisms co-occurring at the same time on just a few resources as “Plankton paradox”. The understanding of the paradox became one of the most fruitful challenges in community ecology. Since then, it has been an inspiring question for many scientists to discover the general rules that support this vast diversity.

The diversity of the species created a necessity to create metrics to express the observed community composition. The most popular and widely used diversity metrics are based on species richness, species evenness and heterogeneity. Some examples of the most commonly used are those of Gleason, Simpson, Shannon and Pielou's Evenness (McGurran, 2004). However, all these indices that were developed and are still widely used are usually based on species richness and species abundance or biomass, describing the communities in a “functionally blind” way and as such giving no weight to phylogenetic and trait differences and/or similarities between the species making up a community.

Global change, species loss and implications on marine food webs

The importance of disentangling the more general rules that support phytoplankton species coexistence lies in the fact that phytoplankton form the basis of marine and freshwater food-webs and in total contribute 50% to primary production (Field et al., 1998; Falkowski and Raven, 2007), supporting the higher trophic levels which in turn depend on their performance. However, the importance of phytoplankton goes beyond the food web structure since they are one of the most important links in carbon dioxide (CO₂) transfer to the deep ocean. Thus, changes in phytoplankton community structure can have severe impact on the atmospheric CO₂. Rapid changes in the severity and frequency of anthropogenic stressors such as climate warming, acidification, pollution, exploitation, and changes in salinity continue for many decades now, altering the

environment in which species perform. Consequences of global warming have been documented already and predict a decline in oceanic phytoplankton biomass and primary productivity (Boyce et al., 2010; Behrenfeld et al., 2006; Sommer and Lengfellner, 2008; Lewandowska et al., 2012), but also in community and species size (Peter and Sommer, 2012; 2013). These declines are mostly the result of the change in water column stratification, since warmer sea surface water will cause a strongly stratified water column (Doney, 2006) which will result in lower nutrient supply from the deeper ocean. The stronger stratification and resulting reduced mixed layer depth of the upper water layer might in turn shift the competition among phytoplankton species that are not only adapted to less available nutrients but also to higher light intensity. The community size shifts predicted from experiments (Peter and Sommer, 2012, 2013) and the field observations that in warmer regions, with oligotrophic and stratified water column, smaller species occur (Acevedo Trejos et al., 2018) will undoubtedly impact the global biological carbon pump, which in turn in combination with other physical alterations will on longer time scales further affect phytoplankton stoichiometry (Litchman et al., 2015a). However, different changes are expected for coastal ecosystems since increases in riverine runoff and upwelling are expected to increase nutrient concentration. Nevertheless, the implications of climate change are not restricted to the examples given above and can be broadly expanded even further to consequences driven by ice melting (salinity change, light availability etc.) for example but also to indirect effects on the metabolism of species i.e. respiration rates, increased nutrient uptake rates, faster generation times and also changes in other trophic levels such as increased grazing rates due to higher temperatures (Sommer and Lengfellner, 2008; O'Connor et al., 2009). All these environmental alterations may lead to severe changes in the structure and composition of ecological communities, either due to shifts or even losses of species or due to the introduction and establishment of invading species in the newly formed environments (Hooper et al., 2005; Loreau et al., 2005), and create the necessity to develop useful tools in order to be able to predict environmental deteriorations and better manage them.

Traits and trait based ecological approaches

Because of their important roles both phytoplankton and species diversity play for ecosystem functions, they have been under focus of numerous studies until now. The necessity to understand the factors driving species dynamics were brought up by Ramon Margalef already in 1978. He was among the first ones making an effort to explain shifts between major functional groups of species

and published his famous “mandala” providing a road map through the causes and the variations of phytoplankton composition in time and space (Wyatt 2014). Of course, this was among the first efforts to provide more general rules to understand communities and Margalef (1978) dealt only with two of the many factors, nutrients and turbulence, making no distinction between different nutrients. The resource competition theory of Tilman (1977, 1982) and Tilman et al. (1982) was initially developed for phytoplankton and focused on interspecific differences in requirements for different nutrients. Reynolds extended the focus from competition to also include stress and disturbance tolerance and went beyond taxonomic classification to functional groups based on species physiology and morphology (Reynolds 1984a,b, 2006; Reynolds et al., 2002) which paved the way for efforts to understand patterns in growth, defense or seasonality of species (Sommer, 1988; Sommer et al., 1986). These efforts were and are based on more or less arbitrarily defined functional groups, because physiological data needed to understand the ecological performance will always be too scarce to describe and analyze the behavior of naturally species rich communities. The practical impossibility of a species-by-species analysis of community dynamics and the arbitrary and sometimes subjective nature of preconceived functional groups led to the attempt to replace taxonomic composition by the analysis of trait distributions as an objective and measurable simplified descriptor of communities. It allows to search for rules how trait distributions respond to the environment and predict the performance of a community in a particular environment (Litchman et al., 2010). The most systematic consideration to achieve a trait based approach in order to get a mechanistic understanding of how species will reorganize in the future is by identifying the traits that are associated with specific functioning (Litchman et al., 2010).

The major components of trait-based approaches, which have started to be used broadly in ecology, are traits that associate species with environmental gradients, interactions between them and also with their performance (McGill et al., 2006) linking them with the ecological axes that define ecological niches of phytoplankton (physical environment, resources, and natural enemies) (Margalef, 1978; Reynolds, 1984a, 1994, 2006; Tilman et al., 1982). The majority of traits can be grouped in two main categories depending on if the trait associates with function (reproduction, resource acquisition, and predator avoidance) or specific type (morphological, physiological, behavioral, and life history) (Litchman and Klausmeier, 2008) and they can be identified as response traits (to a disturbance or change in environment) or effect traits (determine how species

affect ecosystem properties) (Lavorel and Garnier, 2002; Hooper et al., 2005; Suding et. al., 2008). For phytoplankton the most important and significant traits are the ones that deal with the factors that determine species niches. These factors are light, nutrients, susceptibility to grazers, and temperature (Litchman and Klausmeier, 2008).

Light utilization traits are of the most important ones for phytoplankton because of their autotrophic nature. Being underwater means that light is not constant in time and space. Light attenuation by pigments and suspended particles creates a vertical gradient in the water column (Kirk, 1994) where surface irradiance shows ideal and seasonal patterns. Traits related to light acquisition can be calculated by models that incorporate the relationship between either photosynthesis or growth and irradiance, capturing the low – high light adaptation of the species (Litchman and Klausmeier, 2008). The adaptation depends on whether increasing light becomes saturating or inhibiting for the species (Litchman and Klausmeier, 2008). In addition to the physiology related traits, species can have other characteristic traits like motility to optimize light capture by swimming/floating (toward or away) or differences in pigmentations in order to use different parts of the light spectrum.

Nutrients are the second essential factor for phytoplankton growth together with light. Nutrient related traits are really important characteristics of species and have been broadly studied until now. The most commonly reported ones are maximal growth rates, maximal uptake rates (for N and P), minimum and maximum cellular requirements and the half-saturation constant for nutrient uptake. These traits are correlated (Litchman et.al., 2007) and provide at the same time important information for species performance. Nitrogen fixation and mixotrophy are also significant traits for nutrients acquisition.

In planktonic food webs, one more trait category is needed to complete the understanding of growth and losses. Grazing related traits are the dominant ones not related with growth but with loss. These traits can relate to different aspects of effort that a grazer has to invest in order to catch and digest its food. That can be different morphological adaptations like size or morphology, or low nutritional quality, toxin production etc. (Reynolds 2006, Huisman et al. 2005, Litchman and Klausmeier 2008). However, these traits are specific to particular pairs of phytoplankton and grazers (Litchman and Klausmeier 2008) and cell size seems to be one of the most straight forward

to use for grazing susceptibility. In aquatic food webs size in general has been identified as the most important factor determining who-eats-whom (Sommer et al 2017).

Cell size - a master trait

Because of the vast number of measurable traits, replacing species composition by trait distribution does not necessarily lead to the required reduction of complexity in community analysis. However, positive and negative (trade-offs) correlations between traits offer the potential to identify a manageable set of master traits with known correlation to numerous secondary traits. Among them, body size is a premier choice because of its well-known correlations with a multitude of physiological, demographic, behavioral and predation related traits (Peters, 1983). Cell size (expressed as cell volume) among phytoplankton species can vary by 9 orders of magnitude (Finkel et al., 2010) and affects numerous functional traits and core metabolic rates (Peters, 1983; Chisholm, 1992; Brown et al., 1993; Marba et al., 2007; Naselli-Flores et al., 2007). Litchman et al. (2010) suggested cell size as “master trait” offering a very good approach to reduce taxonomic complexity. The choice of cell size is also quite practical since taxonomically resolved studies on phytoplankton traits include considerations of cell/organism size.

Defining the size however of a species requires some considerations. Mostly it is expressed as cell length, cell volume or as cellular carbon content (Sommer et al., 2017a). Among linear measurements the largest linear axis and the diameter of a sphere with equal volume are most often used. A further issue of consideration is the choice between cell size and colony size metrics. When colony or filament formations are loose cell size is the metric that better predicts metabolic properties, while colony size can better explain perception, prey choice and rejection by grazers (Sommer et al., 2001). Instead, compact colonies should be treated like single cells (Gaedke, 1992), except when calculating cellular carbon content from cell volumes. There is a tendency of larger cells to have a lower carbon content per unit volume than smaller ones and this tendency is stronger in diatoms, because of the large vacuole they include, than in other taxa. These effects are accounted for by the transformation equations suggested by Menden-Deuer and Lessard (2000) and modified later by Sommer et al. (2012b).

When scaling up to community cell size different methods have also been used in literature. The simplest one is the one retrieved from the equation that divides total biomass (i.e. summed

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biovolume) with total number of cells/colonies (Sommer et al., 2017a). Another approach is using biomass weighted means that give also an idea of the community dominance by larger or smaller cells. In addition to these methods the size abundance spectra can be used (Cermeño et al., 2008) which provide a slope between spaced size classes and abundance (log- log relationship) indicating, depending on the slope of this relationship, if the community is dominated by smaller or larger species (Sommer et al., 2017a).

Aim of the study

Understanding links among functional responses and effect traits remains a significant challenge, but are critical to understand the dynamics of ecosystem functioning in a changing world (Lavorel and Garnier 2001; Hooper et al., 2002). This link can become more powerful if master traits are used to estimate the effect of a specific stressor on a community mean / species trait and its response to another stressor/trait.

In this thesis, an attempt will be made to transfer the multiple stressor concept of Vinebrooke et al. (2004) within the domain of trait based ecology. His model predicts different effects of stressor induced diversity loss on the ability of a community to cope with further stressors, depending on the correlations between stressor resistances of species making up a community. According to this model, there are two options when applying sequential stressors to a community either: a) species tolerances to the different stressors will be positively correlated and the species sensitive to the first stressor will become extinct and the remaining species will resist the second stressor and recover quickly, or b) species tolerances to the different stressors will be negatively correlated and the extinction of species by one stressor should lead to a remaining community of species also sensitive to the second stressor. Note, that the term stressor is used in the widest sense here, not only to include different forms of environmental deterioration, but also naturally occurring stresses like resource limitation, predation, etc.

Despite the fact that this concept was initially built for species diversity it can also be extended to trait-based approaches, because stress induced reduction of diversity can also be translated to a reduction in trait-diversity. This means, that stressors can either truncate the distribution of a trait at the lower or at the higher end or delete organism with intermediate levels of that trait, which in any case will further affect community performance under a second stressor (Fig 1).

Aim of the study

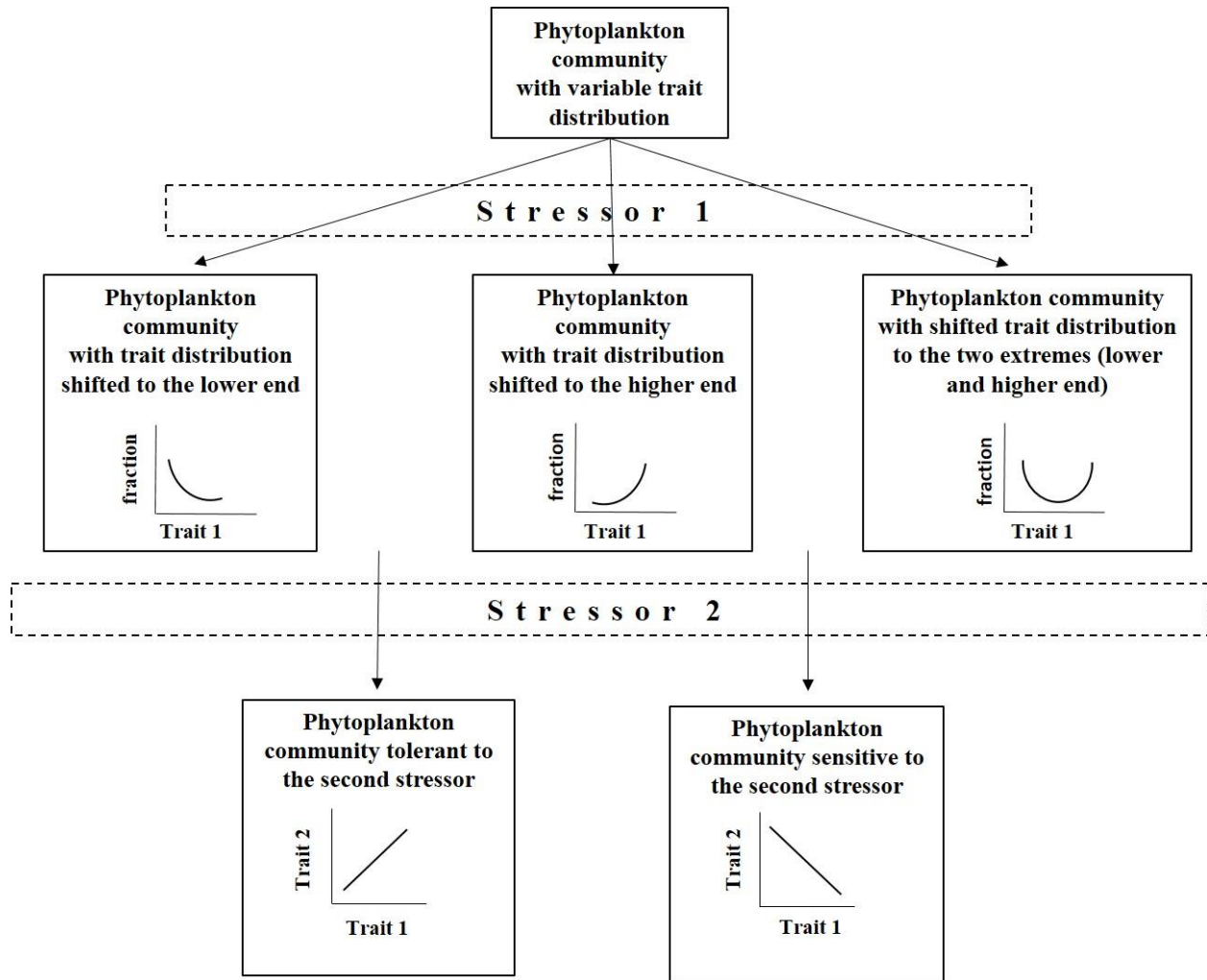


Fig 1. Conceptual model of possible trait distribution under the effect of one stressor and the effect on community performance under a second stressor.

The aim of this study, inspired of trait based ecology and Vinebrooke's et al. (2004) conceptual model, is to experimentally analyze how sequential stressors with strong impact on trait diversity, specifically cell size, will act on the resistance and resilience of communities. To reach this goal I conducted three independent mesocosm experiments with natural plankton (autotrophic phytoplankton and heterotrophic protists) of the Baltic Sea. The first experiment was conducted at the end of September 2015 and focused on the response to nutrient addition and depletion of three different sized communities. The second experiment was conducted in February 2016 addressing the effect of copepod grazing, as temporally fore-going stressor, on phytoplankton size diversity and at a second step nutrient conditions as subsequent stressors acting on plankton with a pre-

conditioning history by grazing. The third experiment was conducted in November 2016 addressing the effect of light limitation and inhibition, as temporally fore-going stressor, on phytoplankton size diversity and at a second step the nutrient conditions as subsequent stressors acting on plankton with a pre-conditioning history by different light levels.

Chapter I

In the first chapter I present the results of a mesocosm experiment performed with natural autumn phytoplankton communities with different size characteristics. The communities were exposed to different nutrient regimes, N-replete and limited, to test the effect of community size distribution on the response to nutrients. The autumn community in the Kiel Fjord is rich in heterotrophic protists which could not be excluded. For that reason, I also analyzed the grazing effect of microzooplankton and nanograzers in each community. I hypothesized that during the first three days of the experiment the small sized communities would show higher growth rates after nutrient addition but would not be able to preserve their growth until day 10. On the contrary, I expected that communities dominated by big cells would show lower growth rates after nutrient addition during the first days of the experiment but produce more biomass. I also expected these communities to continue growing at the following days because of better nutrient storage capacity that allows them to support more subsequent cell divisions under nutrient shortage. I moreover hypothesized that the responses of individual size classes in the communities would be independent of the community species composition and that species responses to nutrient availability would be predictable from size. Lastly, I aimed to test if alterations from the hypothesized outcome can be explained by microzooplankton and nanograzers.

Chapter II

In the second chapter I used first a naturally occurring stressor, i.e. grazing by copepods, to achieve the size shift in the plankton communities (autotrophic phytoplankton and heterotrophic protists) in order to test whether the size shift would in turn influence the response to nutrient addition and depletion. Specifically, I predicted that the grazing vulnerability of species would be affected by cell size and in consequence copepod grazing would shift protist size distributions

Aim of the study

to a dominance of very small and very big cells. After the communities were size shifted, I hypothesized that the ones dominated by small celled individuals would respond faster to nutrient addition and that communities dominated by bigger cell would grow slower but would better cope with prolonged nutrient shortage. I tested also in these communities if species responses to nutrient availability would be predictable from size and if size classes would respond similarly independently of the grazing pre-history of the communities.

Chapter III

For the last chapter of my thesis I continued the same experimental concept. This time however I used another important environmental factor, specifically light, that shows selective impact in morphometry of species. I used three different levels of irradiance, and in a second step exposed the resulting communities to sustained nutrient limitation or a growth saturating nutrient spike and compared their responses in growth and resource use efficiency. I hypothesized that low light will lead to a community with smaller mean cell size, higher mean surface to volume ratio and a higher Shape Index (Reynolds, 1997). I would also try to detect, similar to the community, changes at species level. As for the response to the nutrient spike, my hypothesis was that the low- light pre- conditioned communities would show a higher initial growth rate after nutrient addition and that the high-light pre-conditioned communities would show a bigger conversion of nutrient spikes into biomass when reaching stationary phase.

Chapter I



Cell size drives the response of natural plankton communities to nutrients

Abstract

Several studies until now reveal a relationship between the “master trait” cell size and the maximal growth rates of phytoplankton species. As a consequence of lower maximal growth rates, it is expected that species with bigger cells will respond slower to nutrient addition than small cells. On the other hand, they are expected to better cope with subsequent nutrient shortage after consumption of a nutrient pulse. We conducted an indoor mesocosm experiment to test if these expected relationships derived from single species culture experiments apply to natural phytoplankton communities of different cell size distributions. We also examined how the community cell size and these responses interact with the heterotrophic taxa in the community (ciliates and heterotrophic dinoflagellates). Eighteen mesocosms of 120 L each were filled with a natural coastal plankton community (from the Kiel Bight, Western Baltic Sea). Three differently sized communities (0-20, 20-64 and 64-100 μm) were created by sieving with three different gauzes (mesh sizes 100, 64 and 20 μm) and treated with two different nutrient pulses (19.2 $\mu\text{mol/L}$ NO_3 ; 1.2 $\mu\text{mol/L}$ PO_4 ; 13 $\mu\text{mol/L}$ SiO_4 and control?) in a full-factorial design. The highest mesh size was used to exclude mesograzers (e.g. copepods). Six treatments were created (triplicated) as a combination of the three sized communities (0-20, 20-64 and 64-100 μm) and a nutrient pulse (19.2 $\mu\text{mol/L}$ NO_3 ; 1.2 $\mu\text{mol/L}$ PO_4 ; 13 $\mu\text{mol/L}$ SiO_4) given to half of the treatments (nutrient treatments). Overall the community growth rates responded similarly to the different nutrient treatments. On short term basis (i.e. 3 days), however, the control communities (i.e. no nutrient pulse) showed higher growth rates than those treated with a nutrient pulse. The small sized community (0-20 μm) showed the weakest short term response to nutrient addition, but that changed on the mid-term until the end of the experiment (i.e. 10 days). In the communities, the species between 10-100 μm^3 showed the highest growth rates in all the nutrient treatments until the end of the experiment. Among functional groups, ciliates showed a strong response in the first three days of the experiment, which can correlate with the very weak response of the picoplankton in all treatments. We conclude that cell size influence phytoplankton community responses to nutrients but heterotrophic taxa in the community can alter the expected responses.

Introduction

Since many decades, size is considered as an ecologically significant trait and scientists tried to understand the advantages and disadvantages that a specific size gives to a species (Haldane, 1926; Pirie, 1973; Sommer et al, 2017a,b) and also how size affects species metabolic rates (Peters, 1986). Notably for phytoplankton, cell size has been characterized as “master” trait (Litchman et al., 2010) affecting edibility, sinking, motility, growth rates, nutrient uptake rates (Sommer et al., 2017a). Hence the great span in size (4-5 orders of magnitude in linear dimensions) (Finkel et al., 2010) among species creates a mosaic of phytoplankton with different ecological roles and environmental requirements (Sommer et al., 2017a). In both marine and freshwater food webs phytoplankton holds a key role since its responses to various environmental changes (i.e. temperature, nutrients, turbidity, light) drive the responses of the associated species and functional groups (Lewandowska and Sommer, 2010; Sommer and Lewandowska, 2011; Peter & Sommer, 2012). Among different geographic regions, marine phytoplankton community mean size shows a minimum at the equator and increases in both average and variability with latitude (Acevedo-Trejos et al., 2018). This difference is attributed to latitudinal differences in bottom up (nutrient availability) and top down (grazing) control and the emerging trade-off between high nutrient affinity of smaller cells and predation avoidance by larger cells (Acevedo-Trejos et al., 2018). Moreover, the projected seawater surface warming is expected to favour smaller species/cells since temperature has been shown to be a major driver of community mean cell size and also species “shrinkage” (Lewandowska and Sommer, 2010; Polovina and Woodworth, 2012; Peter and Sommer, 2013, 2015). For the prediction of the performance of natural phytoplankton assemblages it would be of critical importance to understand whether the well-known size-dependencies of physiological rates of individual species (Banse, 1982; Sommer, 1989; Tang, 1995; Finkel et al., 2010; Edwards et al., 2012) translate into aggregated responses of communities with different mean cell size. If yes, then it would actually provide a useful simplistic tool to foresee changes in the in marine food web function, it could help to establish an intermediate level of complexity between the almost unachievable goal of reconstructing community performance from single species and the overly simplistic approach of reducing phytoplankton to a single functional category.

Several researchers have studied the size dependence of resource saturated growth rates (μ_{\max}) (Banse, 1982; Sommer, 1989; Tang, 1995; Finkel et al., 2010; Edwards et al., 2012) by which they demonstrated that smaller cells show higher maximal growth rates than larger cells. Despite the earlier findings, Marañon et al. (2013) found that intermediate sized cells of ca. $100 \mu\text{m}^3$ cell volume showed the highest μ_{\max} . Prior to Marañon et al. (2013), the relationship between μ_{\max} and cell size had been explored using single species cultures of nano- and microphytoplankton species and the extrapolation to picoplankton (smaller) sizes gave in most cases unrealistic responses (Sommer et al., 2017a). Besides differences in maximal growth rates, size has also strong effects on the utilization of limiting nutrients and storage of nutrients. Growth is directly dependent on intracellular nutrient concentration, as implemented in the Droop model (1973, 1983). Many nutrient utilization traits, i.e maximum nutrient uptake rate, half-saturation constant for uptake and uptake affinity have been shown to scale allometrically with cell volume (Grover, 1989; Litchman et al., 2007; Tambi et al., 2009; Edwards et al., 2012; Marañon et al., 2013). Marañon et al. (2013) provided full equations of Q_{\max} and Q_0 relationships with cell size, and analyzed the advantages and disadvantages of being smaller or bigger for nutrient utilization ability. Bigger cells have a better nutrient storage capacity ($Q_{\max}:Q_0$ higher), are able to take up nutrients faster when available in large concentrations and keep up their uptake rates for longer periods. However, Marañon et al. (2013) noticed that larger cells had a smaller ability to convert nutrients into biomass than smaller ones, probably because of the larger distance from cell surface to metabolic processing sites into the cell. On the contrary, smaller cells have higher surface:volume ratios, facilitating biomass specific uptake rates. In contrast, smaller cells have not the same nutrient storage capacity and therefore their growth is related to the rate of nutrient supply. Sommer et al. (2017a) based on the equations provided by Marañon et al. (2013) calculated the ability of different sized species to divide in nutrient replete conditions and found that bigger cells can support more subsequent cell divisions than smaller ones, if there is no new uptake.

Besides the size dependence of growth parameters, also grazing losses are highly size dependent. Cell or colony size is the dominant factor that determines the vulnerability of phytoplankton to the different grazer types (Sommer et al., 2017a). While mesograzers and their feeding strategies have long time occupied the scientific community due to their direct link to higher trophic levels, microzooplankton (ciliates, heterotrophic dinoflagellates and foraminifera) got later into the focus of research, although they are an important link in biogeochemical cycles in marine ecosystems

(Calbet and Laundry, 2004). In their studies Calbet (2001) and Calbet and Laundry (2004) demonstrated the importance of microzooplankton grazing and estimated that they can consume 60-75% of particulate primary production depending on the ecosystem type although this consumption is not always translated into a biomass decline of phytoplankton. Microzooplankton prey on species of a size range 1-100 μ m and form together with heterotrophic nanoflagellates an important regulator of community size distribution (Peter and Sommer, 2012). Although ciliates act as important trophic intermediate (Johansson et al., 2004). Their role is understudied because of their fragile structure and difficult preservation (Stoecker et al., 1994). In addition, the wide range of dinoflagellate prey size in combination with the complexity of mixotrophy in several species, make research on their feeding impact in natural systems more complex but in the same time more critical.

Working hypothesis

In order to test the effect of phytoplankton community size distribution on the response to nutrients we designed an experiment where three plankton communities with different size structure were exposed to different nutrient treatments. Since our size manipulation (sieving by plankton gauze) could not exclude grazing by heterotrophic protists we also analyzed the grazing effect of microzooplankton and nanograzers in each community.

Specifically, we expected:

1. Communities dominated by small cells to show higher growth rates after nutrient addition during the first days of the experiment (first three days).
2. Communities dominated by big cells to show lower growth rates after nutrient addition during the first days of the experiment but also to continue growing at the following days (from day 3 until day 10)
3. Communities dominated by big cells to produce a higher biomass in response to nutrients
4. Responses of individual size classes to be independent of the community composition
5. Species responses to nutrient availability to be predictable from size.
6. Grazing by microzooplankton and nanograzers to affect the expected responses of phytoplankton to nutrients.

Methods

Experimental design

A natural autumn plankton community (23.09.2015) containing bacteria, phytoplankton, microzooplankton, was taken from the Kiel Fjord, Western Baltic Sea. Using a peristaltic pump, the plankton community was filled into a 250 L bucket after being pre-filtered with a 100µm plankton net in order to remove mesograzers (i.e. mainly copepods).

Size fractionation: In order to create three different sized communities two plankton nets with mesh sizes of 20µm and 64µm were used to produce three size fractions with the nominal ranges 0-20µm, 20-64µm, 64-100µm, called small (S), medium (M), and large (L) below. In order to equalize biomass between the treatments fluorescence (Fluorometer 10-AU, Turner Design) was used as a proxy for estimating the biomass. Subsequently, the difference in the measured biomass was adjusted by diluting the M and L communities with pre-filtered (0.2 µm) Baltic Sea water in order to have the same starting biomass in all treatments. After dilution, 18 plastic bags (6 per size fraction), 120 L each, floating with swimmers in 9 different tanks, were filled gradually, in order to achieve similar communities in all bags. The tanks containing the bags were distributed over 3 temperature controlled rooms, each room containing one of each size fraction, randomized for the position of each size fraction in the room.

Nutrient treatment: Prior to the manipulation the different size fractions (S, M and L) were tested for the initial nutrient concentration because of the dilution. To create nutrient supplemented communities, 3 of the 6 bags of each size treatment got a nutrient spike with a target concentration of 19.2 µmol L⁻¹ NO₃; 1.2 µmol L⁻¹ PO₄; 13 µmol L⁻¹ SiO₄ (nutrient treatments, abbreviated as N). The remaining bags served as controls and were all adjusted to an equal initial nutrient concentrations of 4.3 µmol/L NO₃; 1.2 µmol/L PO₄; 13 µmol/L SiO₄ (control treatments, abbreviated as C), leading to six triplicated treatment combinations to which in the following we refer to S_N, S_C, M_N, M_C, L_N, L_C.

Abiotic conditions: The temperature was set tot 15 °C, like the fjord water temperature at filling day, and monitored daily. Above each tank (1m depth, 1.5m³ volume) a light system was installed that mimicked the natural light conditions at the time of the experiment (almost 12h light and 12h darkness with a smooth transition) based on the Brock (1981) astronomic model dimmed for 10m depth.

Sampling and sample analysis: Twice per day the bags were mixed by moving slowly up and down a Secchi-disk. After the first mixing samples (50 mL) were taken to measure fluorescence as a proxy for biomass in order to monitor the daily changes in the different treatments. Samples for the other measurements were taken on day 1, 3 (days 1-3: first period nutrient effect), and 10 (days 3-10: second period nutrient effect). A silicone tube was used to withdraw a 5 L sample from the bags, which were split into sub-samples for dissolved inorganic nutrients, particulate organic matter (POP, POC, PON) and plankton analysis. The plankton samples were kept in 200mL brown glass bottles and fixed with 2mL acid Lugol (Rott, 1981). Picoplankton was measured directly with a flow cytometer (FACScalibur, Becton Dickinson). The Utermöhl (1958) sedimentation method was used to estimate the abundance of phytoplankton $>5\mu\text{m}$ and heterotrophic micrograzers, i.e. ciliates and dinoflagellates. Samples for dissolved nutrients were filtered with pre-washed (10% HCl) cellulose acetate filters and then stored in -20°C immediately until measurement. Particulate organic matter was filtered onto GF/F pre-washed and pre-combusted filters, dried and stored in a desiccator until measurement. Cell size measurements (linear dimensions) were made with the use of the AxioVision program (Zeiss, Oberkochen, Germany) and converted into cell volumes (μm^3) according to the geometric models of Hillebrand et al. (1999). Sizes for species too rare or too complex to capture in a photo were supplemented from literature (Olenina et al., 2006). Plankton taxa were separated in functional groups: autotrophs, mixotrophic dinoflagellates, heterotrophic dinoflagellates and ciliates. Total phytoplankton biomass and size classes' biomass were calculated from autotrophic taxa and mixotrophic dinoflagellates. In each size fractionated community, taxa were grouped in five size classes: picoplankton ($<10\mu\text{m}^3$), small ($10-100\mu\text{m}^3$), medium ($100-1000\mu\text{m}^3$), big ($1000-10000\mu\text{m}^3$) and large ($>10000\mu\text{m}^3$) based on their cell volume.

To estimate the growth of the communities, size classes and species growth rates (μ) were calculated as $\mu = (\ln b_2 - \ln b_1) / (t_2 - t_1)$,

where b is the biomass in a specific time point and t the time.

Community mean cell volume ($V_{\text{mean community}}$) was calculated based on the relative contribution of each phytoplankton taxon to total phytoplankton biomass ($p_i = B_i / B_{\text{tot}}$) multiplied by the taxon specific cell volume (V_i): $V_{\text{mean community}} = p_i * V_i$

Statistical analysis

ANOSIM analysis was used to test if the starting experimental communities were similar in total biomass concentration and statistically different in the dominant size classes contribution to total biomass. Two factorial ANOVA was used to test the effect of treatment size fractionation (S, M and L) and nutrient treatment (N or C) on the biomass of total phytoplankton, heterotrophic dinoflagellates and ciliates and also on the growth rate of total phytoplankton community, size classes and species at each experimental period. Multiple regression analysis (lm,) was used to assess the effect of the factors ciliate and heterotrophic dinoflagellates biomass on size classes growth rates. The effect was calculated only for the first experimental period since the starting conditions for all treatments were almost equal. Two factorial ANOVA was used to test the effect of treatment size fractionation (S, M and L) and nutrient treatment (N or C) on N:P, C:N and C:P ratios at the end of the experiment. The effect of cell size (log) on the first and second period responses of total phytoplankton community, size classes and species growth rates was tested with linear regression analysis (lm). For species the effect was tested on the highest growth rate (μ_h) observed in the experimental treatments. R version 3.4.1 (2017-06-30) was used for statistical analysis.

Results

Community size fractionation. Sieving and dilution resulted in starting treatments with similar biomass (ANOSIM: $R=0.233$, $r^2=0.006$). Autotrophic phytoplankton taxa dominated all experimental communities (S:75.9±SD4.5%, M:64±SD9.5% and L:64±SD2.4% contribution to total plankton community) (Fig. 1-1a), however the share of taxa differed among the size fractions. Picoplankton, Cryptophytes, Chlorophytes and Prymnesiophytes dominated the S community (92.07±SD2.44% contribution to total biomass of autotrophs) while diatoms dominated the M (61.92±SD5.99% contribution to total biomass of autotrophs) and L (63.32±SD6.69% contribution to total biomass of autotrophs) size fractions. Mixotrophic taxa (S:18.36±SD4.11%, M:29±SD7.2% and L:33±SD2.1% contribution to total plankton community) followed (Fig 1-1a) and the Heterotrophs (ciliates and dinoflagellates) were the least abundant in all communities (S:5.65±SD1.47%, M:7±SD2.65% and L:3±SD1.27% contribution to total plankton community) (Fig. 1-1a). Sieving successfully resulted in fractionated communities with different size class characteristics (ANOSIM: $R=0.986$, $r^2=0.001$). Specifically, picoplankton and small cell size

classes (<10 and $10-100\mu\text{m}^3$) dominated ($73\pm\text{SD}5.03\%$) the S phytoplankton communities' biomass, while the size class of big cells ($1.000-10.000\mu\text{m}^3$) contributed only $16\pm\text{SD}4.79\%$ to total biomass (Fig 1-1b). An intermediate size class contribution to total biomass was successfully achieved in the M communities, where except for the picoplankton ($<10\mu\text{m}^3$) which contributed $9.37\pm\text{SD}1.1\%$ to total phytoplankton biomass, the remaining size classes showed an almost equal contribution to total biomass (small: $18.8\pm\text{SD}2.16\%$, medium: $25\pm\text{SD}9\%$, big: $26.69\pm\text{SD}7.66\%$ and large $20\pm\text{SD}5.52\%$) (Fig 1-1b). In L communities, on the contrary, large cells ($>10.000\mu\text{m}^3$) dominated ($48\pm\text{SD}7.8\%$) the phytoplankton community biomass whereas the rest of the size classes contributed similarly (picoplankton $10.56\pm\text{SD}1.81\%$, small: $16.59\pm\text{SD}3.61\%$, medium: $11.97\pm\text{SD}1.86\%$ and big: $12.85\pm\text{SD}3.47\%$) (Fig 1-1b).

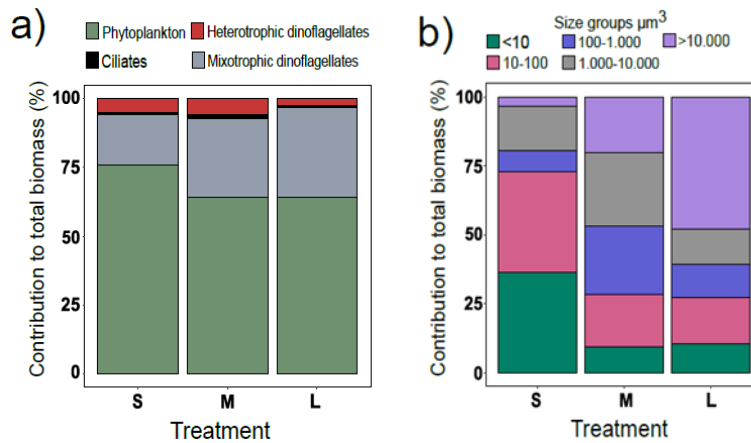


Fig. 1-1: a) Initial contribution of functional groups (%) (phytoplankton, mixotrophic dinoflagellates, heterotrophic dinoflagellates and ciliates) in the starting communities (S, M, L). b) Contribution to total phytoplankton biomass of the different size classes to total phytoplankton biomass (<10 , $10-100$, $100-1000$, $1000-10000$, $>10000\mu\text{m}^3$)

Functional groups' biomass. Phytoplankton growth occurred in all treatments independent of the nutrient treatment (Fig. 1-2). Through the experiment, nutrient treatment (N and C) did not affect total phytoplankton (ANOVA; day 1-3: $F=0.34$, $p=0.57$; day 3-10: $F=0.34$, $p=0.57$) or ciliate (ANOVA; day 1-3: $F=0.5$, $p=0.49$; day 3-10: $F=1.47$, $p=0.24$) biomass, but significantly affected the biomass of heterotrophic dinoflagellates (ANOVA; day 1-3: $F=0.55$, $p=0.47$; day 3-10: $F=11.89$, $p=0.004$) during the second period. On the other hand, size fractionation of the

communities affected total phytoplankton (ANOVA; day 1-3 $F=12.98$, $p<0.001$; day 3-10: $F=5.95$, $p=0.01$) and ciliate (ANOVA; day 1-3 $F=9.31$, $p=0.003$; day 3-10: $F=3.28$, $p=0.07$) biomass but not the biomass of heterotrophic dinoflagellates (ANOVA; day 1-3 $F=0.3$, $p=0.74$; day 3-10: $F=1.84$, $p=0.2$). The interaction of the factors (size fractionation and nutrients) did not show any significant effect on total phytoplankton, heterotrophic dinoflagellates or ciliate biomass.

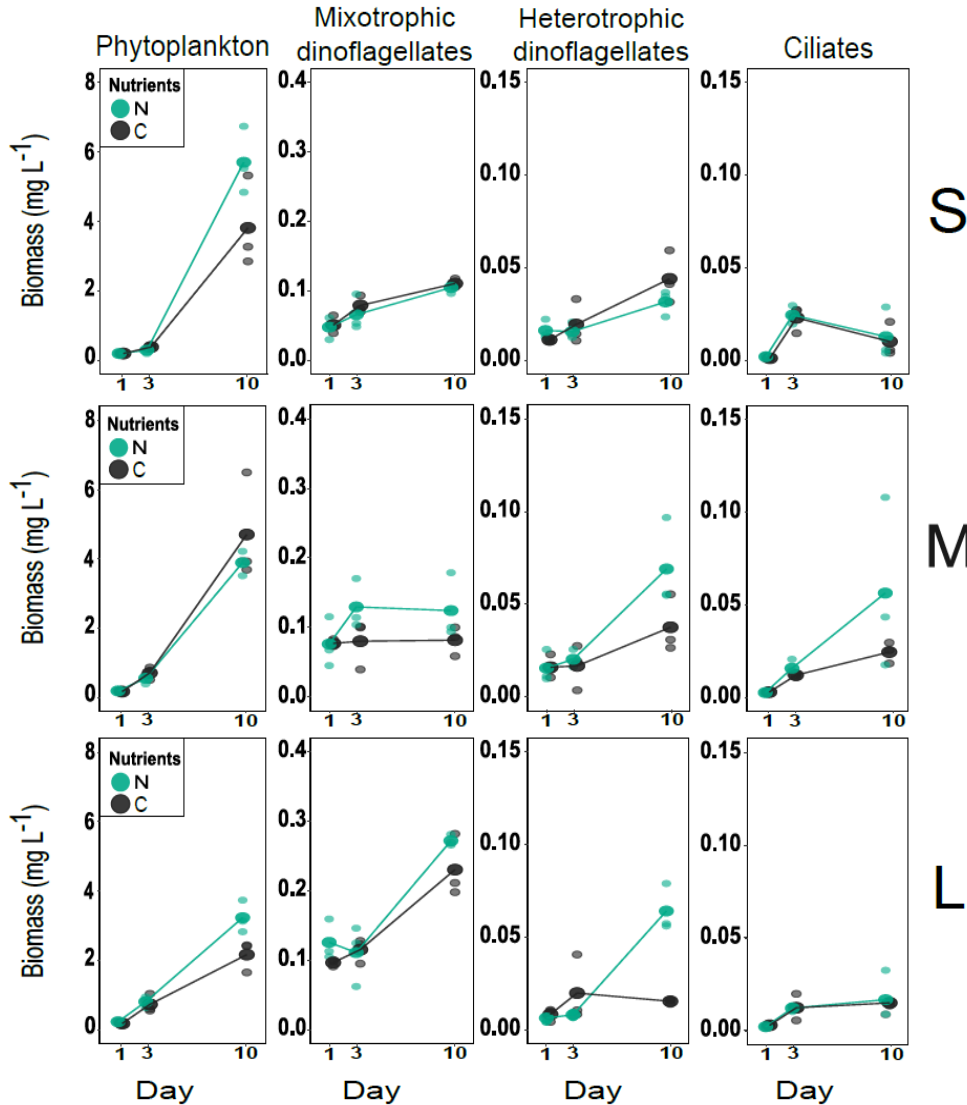


Fig. 1-2. Biomass change of the different functional groups (phytoplankton, mixotrophic dinoflagellates, heterotrophic dinoflagellates and ciliates) in each treatment (S_N , S_C , M_N , M_C , L_N , L_C) through the experiment. Small circles indicate the biomass of the replicates while big circles indicate the average between the treatments.

Nutrient and size fraction effects on phytoplankton growth rates. Initial size fractionation significantly influenced total phytoplankton growth rate (Table 1-1) during the first and second experimental period (Table 1-1) although the effect changed direction during the experiment (Appendix 1-1A and Appendix 1-1B). During the first three days the M and L fractions showed higher growth rates under both nutrient conditions (N and C), while from day 3 until 10 higher growth rates were observed at the S (N and C) fraction (Appendix 1-1). Added nutrients affected the growth rate of size classes of picoplankton ($<10 \mu\text{m}^3$) (highest growth rates in the C treatments) and medium cells ($100\text{-}1000 \mu\text{m}^3$) (higher growth rates in the N treatments) during the second period (Table 1-1) (Appendix 1-1B). On the other hand, initial community size affected the growth rate of picoplankton ($<10 \mu\text{m}^3$) (higher growth rates at L), small ($10\text{-}100 \mu\text{m}^3$) (higher growth rates at L) and large ($>10000 \mu\text{m}^3$) (higher growth rates at M) cells during the first period (Table 1-1) (Appendix 1-1A). In addition, initial community size fractionation affected the size classes of picoplankton ($<10 \mu\text{m}^3$) (higher growth rates at S), small ($10\text{-}100 \mu\text{m}^3$) (higher growth rates at S), medium ($100\text{-}1000 \mu\text{m}^3$) (higher growth rates at S) and large ($>10000 \mu\text{m}^3$) (higher growth rates at S) cells (Table 1-1) at the second period (Appendix 1-1B). The interaction of nutrients and community size fractionation affected only the growth of picoplankton ($<10 \mu\text{m}^3$) during the second period (Table 1-1).

Community size fractionation affected the growth rate of most species (Table 1-1). Specifically, from day 1 to 3 the highest growth rates were found in the L communities (Table 1-1) (Appendix 1-2A) while the pattern reversed (higher at S) from day 3 to 10 (Table 1-1) (Appendix 1-2B). Nutrients affected only the growth rate of *Thalassiosira levanderi* (higher growth rate at C) (Table 1-1) (Appendix 1-2A) during the first period and the growth rate of *Cerataulina pelagica* and *T. levanderi* (higher growth rate at N) during the second period (Table 1-1) (Appendix 1-2B). The interaction of nutrients and community size fractionation affected the growth rates of *T. levanderi*, *Thalassiosira sp.*, *Ceratium fusus* and *Polycricos cofoidii* on the first and of *Pseudonitzschia sp.2* on the second experimental period (Table 1-1) (Appendix 1-2A and Appendix 1-2B).

Table 1-1. ANOVA results showing the short and mid-term effects of the factors community size, nutrient addition and the interaction between them on total biomass, size groups biomass and species biomass. Numbers designate $F_{1,2}$ from ANOVA analysis; Significant results ($p < 0.05$) are in bold. *: $p < 0.05$, **: $p < 0.01$;

first period				second period			
	Nutrients	Community size fraction	Nutrients *Community size fraction		Nutrients	Community size fraction	Nutrients *Community size fraction
Growth rate	F	F	F	Growth rate	F	F	F
Total Community	2.6	7.44** M	0.07	Total Community	4.92	31.44** S	0.65
Size classes(μm^3)				Size classes(μm^3)			
<10	0.21	7.09**M & L	0.15	<10	70.85**	104.5**S	14.2**
10-100	0.79	40.75**L	1.2	10-100	3.34	9.84**S	0.92
100-1000	1.85	2.78	0.57	100-1000	12.03**	12.79**S	0.083
1000-10000	0.15	0.78	1.85	1000-10000	0.14	1.16	1.91
>10000	0.13	12.38**M	1.15	>10000	4.48	10.46**S	1.03
Species				Species			
<i>Cerataulina pelagica</i>	1	4.9*	1	<i>Cerataulina pelagica</i>	10.88**	9.5**S	0.222
<i>Chaetoceros sp.</i>	0.1	3.9*L	2.1	<i>Leptocylindrus danicus</i>	0.56	6.4*S	0.156
<i>Pseudosolenia calcar-avis</i>	1.9	4.4*L	0.54	<i>Pseudonitzschia sp.1</i>	0.99	4.1*M	0.547
<i>Skeletonema sp.</i>	3.6	6.4*L	0.7	<i>Pseudonitzschia sp.2</i>	4.59	1.8	5.89*
<i>Thalassiosira sp.</i>	0.4	5.5*L	6.7*	<i>Skeletonema sp.</i>	4.1	28.3**S	1.314
<i>Thalassiosira levanderi</i>	5.5*	2.8	5.02*	<i>Thalassiosira levanderi</i>	13.4**	10.1**S	2.709
<i>Ceratium fusus</i>	0.1	0.5	8.2**	<i>Ceratium tripos</i>	2.35	22.1**L	1.742
<i>Polykrikos kofoidii</i>	0.006	0.9	5.6*	<i>Dinophysis sp.</i>	0.18	18.1**M	0.281
				<i>Prorocentrum minimum</i>	0.44	4*S	0.711

Ciliates and heterotrophic dinoflagellate effects on phytoplankton size classes

Through the experimental days all size classes exhibited positive growth except picoplankton ($<10 \mu\text{m}^3$) in S communities. This however could be explained by the grazing activities of ciliates and heterotrophic dinoflagellates which were found to have negative effects on the growth rates of picoplankton ($<10 \mu\text{m}^3$) and small ($10-100 \mu\text{m}^3$) cells. Specifically, the increase of the biomass of ciliates and heterotrophic dinoflagellates during the first three days of the experiment negatively affected the growth rates of picoplankton ($<10 \mu\text{m}^3$) (Table 1-2).

Table 1-2. Overview of the significant results of linear models (lm,) testing for the effects of ciliate biomass (Ciliates) and heterotrophic dinoflagellates (Het. Din.) biomass on picoplankton ($<10\mu\text{m}^3$) and small cells ($10\text{-}100\mu\text{m}^3$) growth rates during the first experimental period.

Response variable	Factor	Estimate	Std. Error	t-value	p
Growth rate of picoplankton ($<10\mu\text{m}^3$ size class) $F_{2,15}=7.19, p=0.006, r^2=0.42$	Ciliate biomass	-35.58	12.9	-2.8	0.01
	Het. Din. biomass	-11.98	6.6	-1.8	0.08
Growth rate of small cells ($10\text{-}100\mu\text{m}^3$ size class) $F_{2,15}=5.18, p=0.019, r^2=0.32$	Ciliate biomass	-48.49	17.2	-2.82	0.012
	Het. Din. biomass	-6.79	8.8	-0.8	0.45

Nutrient analysis - The temporal patterns of dissolved nutrient concentrations showed the typical decline resulting from algal uptake for biomass built-up. Until the end of the experiment, SiO_4 and NO_3 were depleted to the detection limit, while there was still some detectable PO_4 , particularly in the nutrient treatments of the M- and L-fractions (Appendix 1-3). NH_4 concentrations remained relatively high ($>0.5 \mu\text{mol L}^{-1}$) in all treatments (Appendix 1-3). The exhaustion of silicate indicates Si-limitation of diatoms. Otherwise, particulate nutrients analysis indicated a different nature of nutrient limitation at the end of the experiments between controls and nutrient treatments. Controls seemed to be P-sufficient (C:P = $117.43 \pm \text{SD } 30.14:1$ to $137.02 \pm \text{SD } 8:38:1$), but N-limited (C:N $> 14.1 \pm \text{SD } 0:44:1$; N:P $< 9.3 \pm \text{SD } 0.4:1$) (Appendix 1-4). With one exception in the S-fraction, nutrient treatments in contrast seemed to be P-limited (C:P $> 242 \pm \text{SD } 90.64:1$; N:P $> 15:99 \pm \text{SD } 3:45:1$) (Appendix 1-4). Size fractionation of the treatments had a marginally significant effect only on the C:N ratio (highest value at L) (Table 1-3) (Appendix 1-4) whereas the nutrient treatment affected the N:P and C:P ratios (Table 1-3). Carbon to limiting nutrient ratios (C:N in the controls, C:P in the nutrient treatments) were highest in the L-fractions (Appendix 1-4).

Table 1-3. ANOVA results showing the effects of the factors size fractionation, nutrient addition and the interaction between them on N:P, C:N and C:P ratios at the end of the experiment. Significant results ($p < 0.5$) in bold.

Response variable	Factor	Df	F-value	p
N:P	Nutrients	1	48.8	<0.000***
	Size fraction	2	2.06	0.16
	Nutrients * Size fraction	2	0.8	0.46
C:N	Nutrients	1	0.12	0.72
	Size fraction	2	2.94	0.09
	Nutrients * Size fraction	2	0.22	0.8
C:P	Nutrients	1	33.5	<0.000***
	Size fraction	2	2.58	0.11
	Nutrients * Size fraction	2	0.8	0.47

Cell size effects on phytoplankton growth rates

Community. Mean community cell size positively affected the growth rate of the phytoplankton communities (Fig 1-3, panels A & B) during the first period of the experiment (day 1 to 3) whereas the direction of the effect changed to negative at the second period (day 3 to 10) (Fig. 1-3, panels C & D). The relationship between community mean cell volume and growth rate follows the equations:

First period (Fig 1-3 A & B)

$$N: \mu = -0.88 + 0.34 \log_{10} V \quad (F = 11.3, p = 0.015, r^2 = 0.59)$$

$$C: \mu = -0.42 + 0.24 \log_{10} V \quad (F = 8.58, p = 0.022, r^2 = 0.48)$$

Second period (Fig 1-3 C & D)

$$N: \mu = 1.04 - 0.2 \log_{10} V \quad (F = 19.28, p = 0.003, r^2 = 0.69)$$

$$C: \mu = 0.53 - 0.08 \log_{10} V \quad (F = 2.03, p = 0.19, r^2 = 0.11)$$

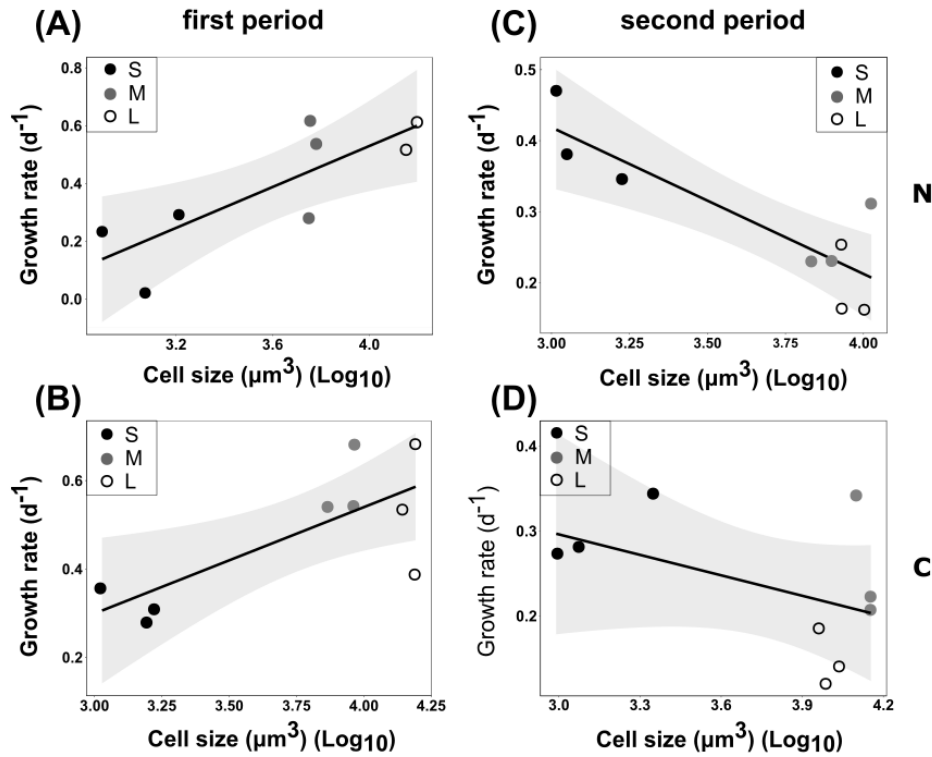


Fig. 1-3. Regression (lm) of phytoplankton community growth rate for the first experimental period (experimental days 1-3) (A & B) and the second experimental period (experimental days 3-10) (C & D) on community mean cell volume for the nutrient saturated communities (N) (A & C) and the control communities (C) (B & D). Grey area: 95% confidence interval.

Size classes. We also analyzed the effect of cell size on the growth rates of the size classes in each treatment for the first (Fig. 1-4) and second (Fig 1-5) period. The relationships, fitting polynomial equations between second and fourth degree, are described below:

First period (Fig. 1-4)

$$\mu_{SN} = -0.19 + 0.44 \log_{10} V - 0.08 (\log_{10} V)^2 \quad (F_{2,12}=9.35, p=0.003, r^2=0.54)$$

$$\mu_{SC} = -0.4 + 0.9 \log_{10} V - 0.19 (\log_{10} V)^2 \quad (F_{2,12}=15.7, p=0.000, r^2=0.67)$$

$$\mu_{MN} = -0.05 + 1.56 \log_{10} V - 0.83 (\log_{10} V)^2 + 0.11 (\log_{10} V)^3 \quad (F_{3,11}=5.38, p=0.018, r^2=0.47)$$

$$\mu_{MC} = -0.13 + 2.32 \log_{10} V - 1.3 (\log_{10} V)^2 + 0.18 (\log_{10} V)^3 \quad (F_{3,11}=6.132, p=0.01, r^2=0.52)$$

$$\mu_{LN} = -0.04 + 1.62 \log_{10} V - 0.72 (\log_{10} V)^2 + 0.08 (\log_{10} V)^3 \quad (F_{3,11}=17.59, p=0.000, r^2=0.78)$$

$$\mu_{LC} = -0.06 + 1.5\log_{10}V - 0.66 (\log_{10}V)^2 + 0.07(\log_{10}V)^3 \quad (F_{3,11}=9.89, p= 0.001, r^2=0.65)$$

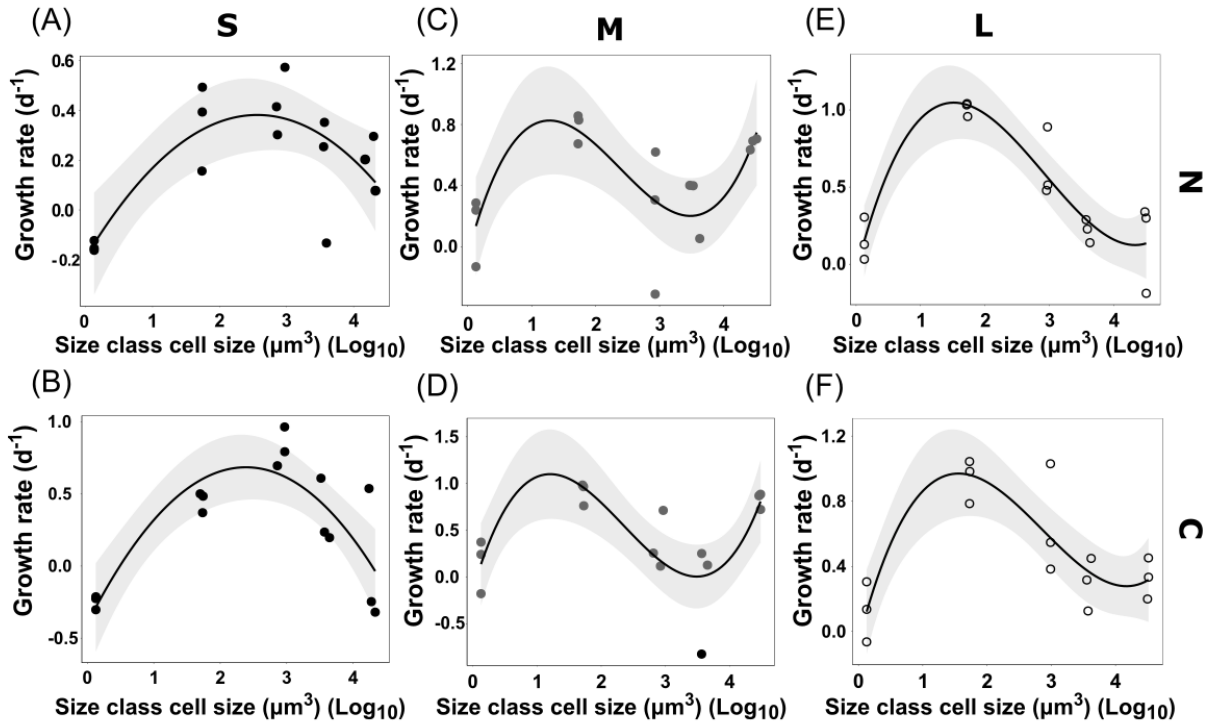


Fig. 1-4. Regression (lm) of size groups growth rate (first period - experimental days 1-3) on size groups mean cell volume for the S (panels A & B), M (panels C & D) and L (panels E & F) treatments with added nutrients (panels A, C & E), and the control treatments (panels B, D & F). Grey area: 95% confidence interval.

Second period (Fig. 1-5)

$$\mu_{SN} = 0.11 + 0.55\log_{10}V - 0.24 (\log_{10}V)^2 - 0.03(\log_{10}V)^3 \quad (F_{3,11}=3.709, p= 0.04, r^2=0.36)$$

$$\mu_{SC} = 0.15 + 0.42\log_{10}V - 0.22 (\log_{10}V)^2 + 0.02(\log_{10}V)^3 \quad (F_{3,11}=4.449, p= 0.028, r^2=0.42)$$

$$\mu_{MN} = -0.11 + 0.68\log_{10}V - 0.31 (\log_{10}V)^2 + 0.04(\log_{10}V)^3 \quad (F_{3,11}=7.76, p= 0.00, r^2=0.59)$$

$$\mu_{MC} = -0.18 + 2.65\log_{10}V - 1.43 (\log_{10}V)^2 + 0.75(\log_{10}V)^3 - 0.07(\log_{10}V)^4 \quad (F_{4,10}=7.02, p= 0.005, r^2=0.63)$$

$$\mu_{LN} = -0.26 + 0.61\log_{10}V - 0.22 (\log_{10}V)^2 + 0.02(\log_{10}V)^3 \quad (F_{3,11}=16.04, p= 0.000, r^2=0.76)$$

$$\mu_{LC} = -0.15 + 1.52\log_{10}V - 1.31(\log_{10}V)^2 + 0.38(\log_{10}V)^3 - 0.03(\log_{10}V)^4 \quad (F_{3,11}=6.94, p= 0.006, r^2=0.63)$$

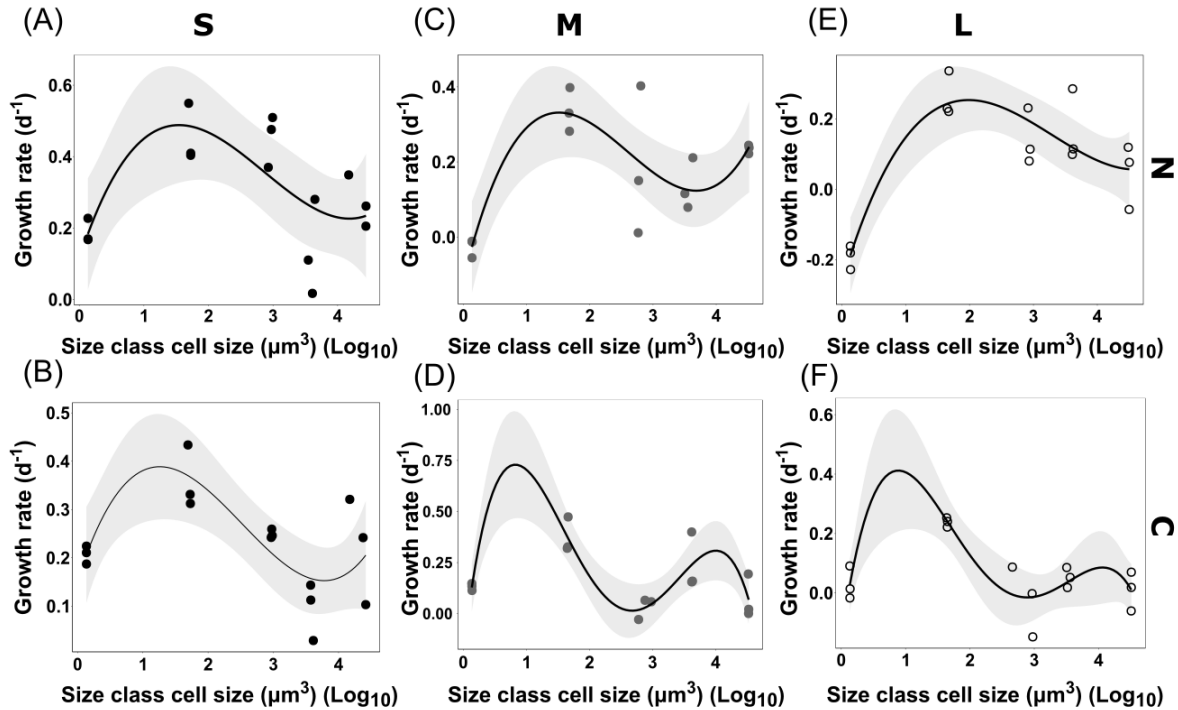


Fig. 1-5. Regression (lm) of size class growth rate (second period - experimental days 3-10) in response to size class mean cell volume for the S (panels A & B), M (panels C & D) and L (panels E & F) treatments with added nutrients (panels A, C & E), and the control treatments (panels B, D & F). Grey area: 95% confidence interval.

Species response:

The highest apparent growth rate estimated for each species were defined as best estimate of maximal growth rates (μ_h). Most dinoflagellates of the same size as diatoms showed lower μ_h than diatoms of the same size. Maximal growth rates (μ_h) showed a significant but highly variable negative relationship with cell size (Fig 1-6) following the equation:

$$\mu_h = 1.89 - 0.248 \log_{10} V \quad (F=7.85, p=0.008, r^2=0.18)$$

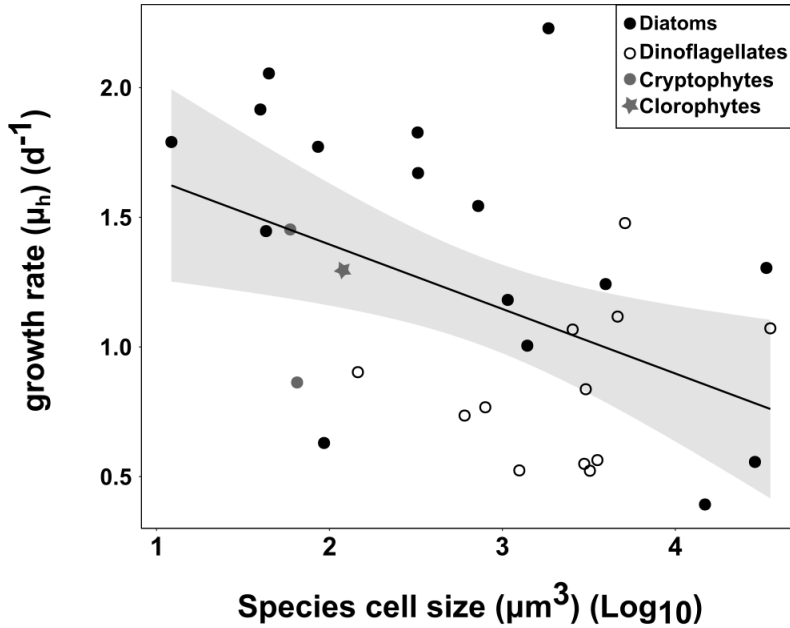


Fig. 1-6. Regression (lm) of species highest apparent growth rate (μ_h) observed during the experiment in response to species cell volume. Grey area: 95% confidence interval.

Discussion

With our experiment we wanted to explore the suitability cell size as a “master” trait indicating the responses of natural phytoplankton communities with different size properties and different nutrient conditions. While size fractionation had a significant effect on community growth rates, hypotheses 1 and 2 could not be confirmed in our experiment. It had been expected the smaller sized community (S) to show a faster response to the nutrient spike (days 1 to 3) but the observed response was the opposite of the expected. A contradiction to the expected, was observed also for the experimental communities dominated by big cells (L) that showed a higher growth rate than the smaller ones when nutrients were added. The unexpected responses of the communities during the first three days of the experiment might be either due to an extended lag phase that the communities needed in order to recover from the sieving manipulation procedure, which probably impaired the health of the cells, or due to top-down control. However, top down control would need further consideration, since heterotrophic dinoflagellates and ciliates, with the exception of ciliates biomass decline in S treatments from day three until day ten, continued to increase their biomass during the second nutrient experiment phase. The missing link of heterotrophic

nanoflagellates might would be able to make our speculation stronger. Hypothesis 3 could also not be confirmed, since the small sized fraction produced the highest biomass in response to nutrients.

Despite the unexpected response at the community level, a less surprising effect of cell size was observed at the size class level, although deviations from the expected were also observed. Specifically, picoplankton ($<10 \mu\text{m}^3$), which was expected to show the fastest response to nutrients, in fact showed the slowest. Another deviation from the expected result was observed in the size class of large cells ($>10000 \mu\text{m}^3$) which showed a stronger response to nutrients than the two smaller size classes (medium and big cells) in almost all M experimental communities, while in the L communities the response to nutrients was almost equal to the next smaller size class (big cells). The effect of size class mean cell size on the size class growth rates was similar in all treatments, although the strength of the response was significantly affected by the experimental size fractionation.

Both at the community and at the size class level, the nutrient spike did not provide any significant advantage to the experimental communities during the first period (day 1 to 3). Similarly, the dissolved nutrient concentrations in the controls indicate, that nutrients had not yet been limiting at the time of the nutrient spike. While not stimulating growth instantaneously, as it would have been in line with the planning of the experiment, the nutrient spike just provided an extended nutrient pool, thus permitting an extended period of non-limited growth. As a result, a positive nutrient effect on final biomass levels in S- and L-communities was found, while this effect did not show up in the M-communities. However, in the M-communities heterotrophic and mixotrophic dinoflagellate's final biomass profited from the nutrient spike. We can therefore speculate that the extra phytoplankton production fuelled by the nutrient spike was consumed by the grazers.

A further deviation of the actual course of the experiments from the underlying design lies in the fact that obviously silicate became the first nutrient to limit the growth of the diatom dominated phytoplankton. If the response of phytoplankton biomass to nutrients was mainly driven by diatoms, it is no surprise that hypothesis 3 was not supported. The predicted higher conversion of incorporated nutrients by larger phytoplankton derives from the tendency, that larger phytoplankton tend to have lower cell volume specific minimal cell quotas of N and P. While, to the best of our knowledge, there is no comparable study about Si-quotas, there are reasons to

assume that it might also be true for Si, mainly because of the higher surface:volume ratio of smaller diatoms. However, the cell quota effect underlying hypothesis 3 could be seen in the stoichiometry of particulate matter. In P-limited nutrient treatments, final C:P ratios were highest in the L-fraction and in the N-limited controls C:N ratios were highest in the L-fraction.

Grazing by ciliates and heterotrophic dinoflagellates is also a factor that had a significant effect on the community response. Ciliates and heterotrophic dinoflagellates prey on species of a size span from 1 to around 100 μm (Azam et al., 1983; Jacobsen, 1999). All species in the experimental treatments were between these size limits since the communities were initially filtered through a gauze with mesh size 100 μm . To our surprise, picoplankton (<10 μm^3) instead of having the highest growth rate among size classes when nutrients were added at the first period, its biomass either declined or responded to nutrients with a growth rate similar to the one of the big cells. This trend was similar also during the second period. The negative relationship between the growth rates of phytoplankton size classes and ciliates' and heterotrophic dinoflagellates' biomass through the experiment reveals a strong grazing impact of these two functional groups to the biomass of specific size classes. This interaction has possibly caused not only the decline in the biomass of picoplankton but also resulted in lower growth rates of the small size class (10-100 μm^3). In addition to intense micro-zooplankton grazing negative effects, the Kiel Fjord experienced a bloom of the zooplanktivorous ctenophore *Mnemiopsis leidyi* bloom in September 2015 which probably caused a trophic cascade with the removal of meso-zooplankton from the food web. This resulted in a rich microzooplankton community and possibly also quite rich in heterotrophic nanoflagellates. Heterotrophic nanoflagellates prey on bacterioplankton and nanoplankton of almost equal size as their own, and could also cause additional negative grazing impact on the low growth rate of picoplankton (size class <10 μm^3).

Taking the grazing effect of ciliates and heterotrophic dinoflagellates on the two small size groups (picoplankton and small cells) into account we recalculated the growth rates of these two size groups for the second period of the experiment, considering that the observed growth rate is a function of the maximal growth rate minus the grazing effect, specifically:

$$\mu = \mu_{\max} - (aB_{\text{cil}} + bB_{\text{Het. Din.}})$$

where $aB_{\text{cil}} + bB_{\text{Het. Din.}}$ describe the grazing effect factor that decelerated growth rate (a and b are the regression estimates calculated (Table 1-2) for ciliates and heterotrophic dinoflagellates). The

corrected growth rates now show a more linear relationship with cell size (Fig. 1-7) and the new growth rate – cell size relationships are now:

$$\mu_{SN} = 1.37 - 0.27 \log_{10} V \quad (F_{1,13} = 24.34, p < 0.001, r^2 = 0.62)$$

$$\mu_{SC} = 1.32 - 0.29 \log_{10} V \quad (F_{1,13} = 46.37, p < 0.001, r^2 = 0.76)$$

$$\mu_{MN} = 2.07 - 0.47 \log_{10} V \quad (F_{1,13} = 14.64, p < 0.002, r^2 = 0.49)$$

$$\mu_{MC} = 1.28 - 0.28 \log_{10} V \quad (F_{1,13} = 29.51, p < 0.001, r^2 = 0.67)$$

$$\mu_{LN} = 0.93 - 0.21 \log_{10} V \quad (F_{1,13} = 19.67, p < 0.001, r^2 = 0.56)$$

$$\mu_{LC} = 0.87 - 0.21 \log_{10} V \quad (F_{1,13} = 22.13, p < 0.001, r^2 = 0.60)$$

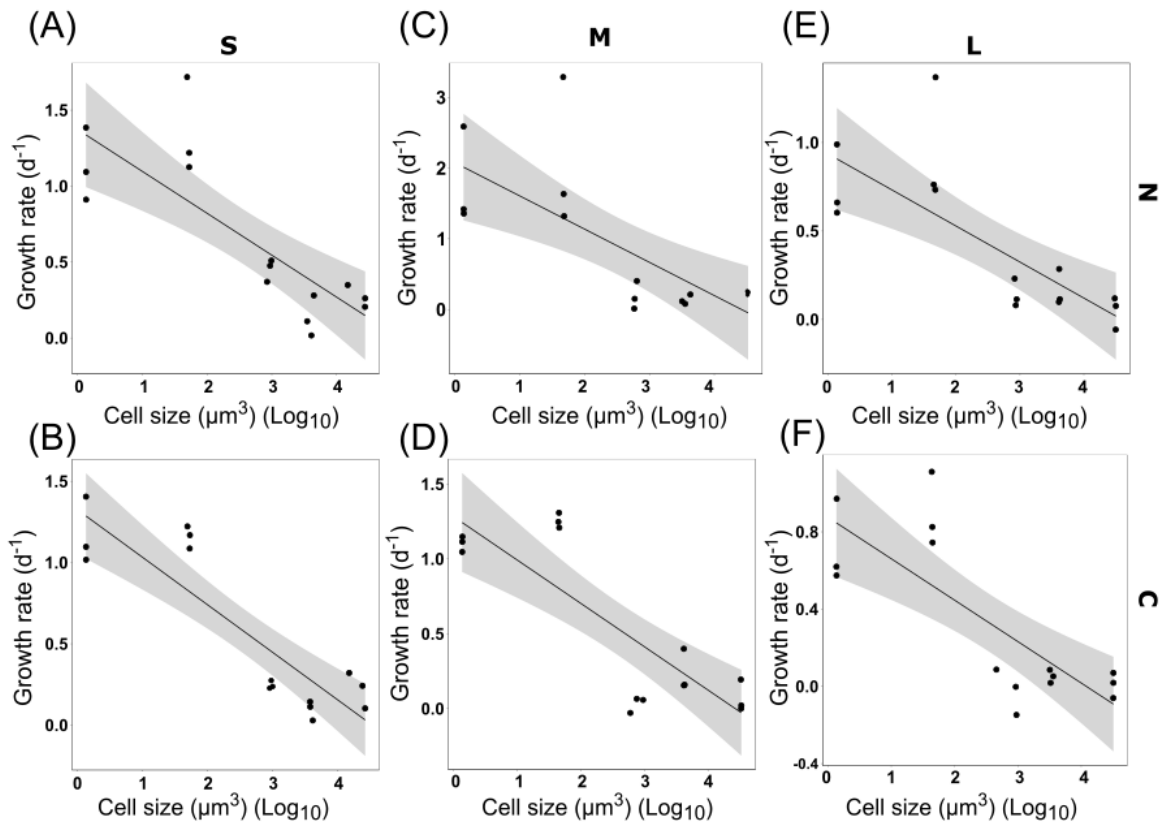


Fig. 1-7. Regression (lm) of corrected for grazing by ciliates and heterotrophic dinoflagellates size classes growth rates (second period - experimental days 3-10) in response to size classes mean cell volume for the S (panels A & B), M (panels C & D) and L (panels E & F) treatments with added nutrients (panels A, C & E), and the control treatments (panels B, D & F). Grey area: 95% confidence interval.

On species level cell size had a significant negative effect on the highest observed growth rate between species of different size confirming hypothesis 5. At the same time, species showed the

expected differences in the responses, with diatoms showing higher growth rates than dinoflagellates, due to taxa characteristics differences, as also observed in previous research (Banse, 1982; Edwards et al., 2012; Charalampous et al., 2018).

The allometries between cell size and growth rates that were retrieved in our experiment were mostly between -0.2 and -0.29 with one of them exceeding to -0.47 (recalculated allometry for size group M_N). In order to be able to compare it with previous studies, a double log transformation of the relationships, transforms the relationships to:

$\mu = 0.53 * V^{-0.3}$ ($F_{1,7}=13.75$, $p=0.007$, $r^2=0.61$) (from community growth rates at the second period)

$\mu_{SN} = 0.21 * V^{-0.23}$ ($F_{1,13}=11.25$, $p=0.005$, $r^2=0.42$) (from size class growth rates at the second period in S_N community)

$\mu_{MN} = 0.29 * V^{-0.27}$ ($F_{1,13}=9.7$, $p=0.008$, $r^2=0.38$) (from size class growth rates at the second period in M_N community)

$\mu_{LN} = 0.034 * V^{-0.24}$ ($F_{1,12}=25.55$, $p<0.001$, $r^2=0.65$) (from size class growth rates at the second period in L_N community)

$\mu_h = 0.38 * V^{-0.13}$ ($F_{1,29}=12.78$, $p=0.001$, $r^2=0.28$) (from species data)

The recalculated allometries are really close to the ones of Peters (1982), -0.25, and Edwards et al. (2012), -0.24, except the one retrieved by species highest observed growth rates (-0.13) that is close to the results of -0.13 of Banse (1982) and -0.15 of Marañón (2013). This lower value compared to the rest retrieved in this experiment might be a result of the grazing pressure that was clearly observed at the size class level. As a result, the smaller sized species even if they showed higher growth rates than the bigger species their growth rates do not represent the full potential of growth of these species in the communities.

Conclusions

Our experiment confirms our hypothesis that phytoplankton community size shifts affect the response of phytoplankton communities to nutrients and that cell size is a trait capable to indicate these responses. More precisely, communities with smaller mean cell size showed a faster growth after the nutrient pulse and achieved a higher biomass built up, while the communities with bigger

mean cell size showed lower growth rates and lower biomass built up until the end of the experiment. However, due to the initial size fractionation of the treatments, the phytoplankton communities initially did not show the expected response to nutrient addition. In addition, grazing by ciliates, heterotrophic dinoflagellates and HNF can suppress the growth rates of picoplankton and small sized species. The grazing effect is not detectable at total phytoplankton community level or species level but becomes clear when size class responses to nutrients are examined independently of the initial community mean cell size. To conclude, cell size is a promising “master” trait to act as indicator of phytoplankton response to nutrients and in addition, it is able to indicate losses due to grazing activities.

Chapter II



Grazing induced size shifts affect phytoplankton response to nutrients

Abstract

Phytoplankton cell size is important for a multitude of functional traits, such as maximal growth rates, nutrient utilization efficiency and resistance to grazing. Thus, size selective effects of one environmental factor should impact the ability to cope with other factors. In order to test this assumption, we performed a controlled experiment with natural unicellular plankton from the Baltic Sea which was first subject to grazing by six different copepod (*Acartia tonsa*) densities. Thereafter, three of the experimental communities were subject to a saturating nutrient pulse to test, how the changes in size structure influenced the growth stimulation by the nutrient pulse in a short term (day 1 to 3) and a mid-term (day 3-10) perspective. As expected, copepod grazing reduced the medium phytoplankton size classes and increased the share of the smallest ($<10 \mu\text{m}^3$) and the largest ($>100\,000 \mu\text{m}^3$) size class. The responses of different species to the nutrient pulse showed the same pattern as the responses of the size classes. The short term nutrient effect confirmed the growth advantage of smaller phytoplankton at the community, the size class and the species level with one exception. Within the communities that were not pre-grazed by copepods the short term growth rates showed a U-shaped response to size, possibly reflecting a superseding effect of protist grazing. From day 3 to day 10, biomass of all communities decreased, but the decrease was slower for larger phytoplankton, thus confirming that larger phytoplankton are better able to preserve their biomass in the post-bloom phase. Our findings show that exposure of the phytoplankton community to a specific “stressor” affects the performance of the community exposed to other environmental factors, however the performance can be indicated by cell size.

Introduction

Human induced global change is under study and well documented since decades now. There is now ample evidence that phytoplankton community structure will rapidly be altered (Huisman et al., 2005; Litchman et al., 2006) with consequences for the structure of the whole food web. Therefore, the need to understand the way how phytoplankton communities organize themselves is increasing (Litchman & Klausmeier, 2008). Trait based approaches are believed to be a key tool for that. While they simplify the vast complexity of phytoplankton communities, they provide a more mechanistic approach to phytoplankton group performance.

Cell size is considered one of the most significant traits of phytoplankton species. In fact, it has been characterized as a “master” trait by Litchman and Klausmeier (2008) since it relates to several further traits i.e. growth related, resource acquisition, grazer resistance traits (Litchman and Klausmeier, 2008; Irwin and Finkel, 2017). Cell size in phytoplankton in fact ranges from less than a micrometer up to several millimeters (Finkel et al., 2010).

Already in the late 1970s to early 1980s Tilman (Tilman, 1977, 1982; Tilman et al., 1982) started to develop a theory on resource competition theory in phytoplankton communities which was followed later on by many researchers trying to predict species performance under specific nutrient conditions and relate this performance to functional or taxonomic traits. The importance of cell size for the ability of species to use the available nutrients and the relationship between them has been examined using culture experiments several times over the years. The most consistent trend was a decline of maximal (nutrient saturated) growth rates (μ_{\max}) with cell size (Banse, 1982; Sommer, 1989; Tang, 1995; Finkel et al., 2010; Edwards et al., 2012; Marañon et al., 2013). The only exception from this general rule was postulated by Marañon et al. (2013) showing a positive μ_{\max} – cell size relationship for very small species ($<100\mu\text{m}^3$), after including hitherto neglected pico- and small nanoplankton into their analysis. However, cell size is not only important for the ability of species to grow but also for a better ability to immediately use (small cells) (Edwards et al., 2011; Litchman et al., 2015b) or store nutrients (big cells) (Edwards et al., 2011; Marañon et al., 2013; Sommer, 1985; Sommer et al., 2017a) and consequently (for the big cells) preserve the gained biomass for longer despite their slower growth.

Cell size is also relevant for the resistance to factors which remove cells from the community, i.e. loss factors. One of the most important loss factors is grazing, which not only is responsible for

species reduction through specialized grazing but also for mean community size shifts since specific grazers feed on specific size classes. Generally, predator and prey sizes are positively correlated in aquatic systems (Wirtz, 2012; Sommer et al., 2017a), however, exceptions exist also here, since many zooplankton guilds overlap in their food size spectra. Copepods are one of the zooplankton groups that strongly impact the size distribution of the plankton communities (Sommer et al., 2017a) both by size selective grazing (direct path) but also by suppression of micrograzers (indirect path) (Sommer et al., 2001; Calbet and Saiz, 2005; Sommer and Sommer, 2006). Many species however, have developed morphological and physiological mechanisms in order to defend themselves against grazing or other loss factors, such as sinking. Among these are toxin production, colony formation to increase their size, flagella to help them move and escape or keep them in the water column or spine formation that helps species to better utilize the available nutrients by increasing the surface of the cells, slow down the sinking velocity and also cause difficulty to possible grazers.

Sommer et al. (2016) reviewed the ecological implications of a change in phytoplankton size structure, as it might be driven by global warming. Earlier studies on the effects of climate change in phytoplankton communities examined temperature effects alone or in combination with other stressors on the communities (Peter & Sommer, 2012, 2013; Sommer et al., 2007; Sommer & Lengfellner, 2008; Sommer & Lewandowska, 2011; Sommer et al., 2013). Since environmental stressors have strong effects on phytoplankton size structure, it might be further questioned how the effect of one particular stressors might influence the sensitivity or tolerance to another stressor (Vinebrooke et al., 2004).

In order to test the impact of one stressor on the response of the plankton community to another stressor we designed a mesocosm experiment with natural plankton. Specifically, we wanted to test whether shifts in protist plankton (autotrophic phytoplankton and heterotrophic protists) composition and trait distribution due to mesozooplankton grazing in turn influence the response to nutrient addition and depletion. We hypothesized, that this influence primarily should be mediated by cell size. We used plankton from the Baltic Sea as experimental model community in a mesocosm experiment. Zooplankton grazing as initial stressor and nutrient limitation as subsequent stressor were chosen because of their importance in the natural environment and because of their well-known effect on trait diversity.

Specifically, our working predictions are shaped as:

- 1) Grazing vulnerability of species is affected by cell size and in consequence copepod grazing will shift protist size distributions to a dominance of very small and very big cells.
- 2) Communities dominated by small celled individuals will respond faster to nutrient addition.
- 3) Species responses to nutrient availability are predictable from size. In particular, small sized species will show higher growth rates than bigger ones when nutrients will be available.
- 4) Size classes will respond similarly in the differently pre-grazed treatments
- 5) Bigger cells and in consequence communities dominated by big cells, will better cope with prolonged nutrient shortage.

Materials and methods

Experimental design

An indoor mesocosm experiment with natural Baltic Sea plankton communities was conducted in temperature and light controlled climate rooms from 23rd of February until the 11th of March 2016. Prior to the experimental onset (22nd of Feb) eighteen mesocosms of 250 L each were filled with seawater containing the plankton community from the Kiel Bight (Baltic Sea). The mesocosms were randomly distributed across three temperature and light controlled climate rooms each equipped with three LED light system units (Paul et al., 2015; Sommer et al., 2015) and set in pairs under one light system unit. Daily irradiance patterns stayed constant over the course of the experiment and were computer controlled (GHL, Prometheus). The light–dark cycle was 11 h 50 min:12 h 10 min including dusk and dawn. Light supply and day length were aligned to the seasonal light patterns calculated by the astronomic model of Brock (1981) for an approximated cloudless 21 March reduced to 60% of solar irradiance in order to account for underwater light attenuation.

Mesocosms were filled with a peristaltic pump taking water from 2m depth in the Kiel Fjord (54°19'47.0"N, 10°08'59.6"E) and distributing it equally trough a hose system into each of the mesocosms in the three climate rooms. According to previous experiments with the same system (Lewandowska and Sommer, 2010; Paul et al., 2015, 2016; Sommer et al., 2015) unicellular plankton well represented in situ conditions while copepods were heavily underrepresented.

Water temperature at the day of the filling was 4°C (in situ) and was gradually shifted to 10°C in order to provoke growth in the plankton communities and to mimic the mean water temperature

expected for March in agreement with the programmed light conditions. For this purpose, the room temperature was set at 7°C on the 22nd of February and at 10°C on the 23rd. The water in the mesocosms reached the desired temperature on the 25th of February.

To minimize sedimentation and to achieve a good distribution of the plankton communities the mesocosms were gently stirred manually using a paddle every 12 hours.

In order to address our scientific questions, the experiment consisted of two subsequent phases. The first one (i) included the size shift of the plankton communities caused by zooplankton grazing (grazing phase). The second one (ii) included the community response to nutrients (nutrient phase).

Grazing phase - The mesocosms were treated with a triplicated gradient of 6 different copepod (*Acartia tonsa*) densities (Ambient, 10, 20, 40, 80, 160 ind/L). The copepods originated from cultures and net catches (54°20'32.7"N 9°58'05.7"E). The Ambient treatment level did not involve additional grazers to serve as ambient treatment control. The mesocosms were exposed to grazing for 7 days.

After the seventh day of the grazing phase the mesocosms were sampled and the water was microscopically examined with regard to the predicted size effects of the grazing treatments. Accordingly, the two mesocosms showing the maximum difference in community size structure and one intermediate one were chosen to provide the experimental source communities for the nutrient phase. The selected mesocosms were of the Ambient, the 40 ind/L and of the 160 ind/L pre-grazed treatments. In the following text the new treatments will be referred to as Ambient, Low (40 ind/L) and High (160 ind/L) pre-grazed treatments depending on the grazing pressure they had experienced.

Nutrient Phase – Eighteen smaller (30L) mesocosms were filled with water from the three designated pre-grazed (Ambient, Low and High) source communities (6 of each) after the removal of the grazers. Grazers were removed by sieving of the community through a 200 µm mesh size plankton net. The new mesocosms were filled gradually in order to achieve a good distribution of the plankton communities. Two different nutrient treatments, nutrient enriched (N) and nutrient limited (C) that served as control, were applied to each of the three pre-grazed communities and replicated three-fold which resulted in six different treatment combinations of pre-grazed and nutrient treated communities.

The target concentration of the enriched nutrient treatment was 16 µmol/L Si, 16 µmol/L NO₃ and 1 µmol/L PO₄, i.e. Redfield ratio (16N/1P). Nutrients were added according to the pre-measured

concentration at the end of the grazing phase in each of the three chosen pre-grazed mesocosms. The nutrient limited treatments (C) did not receive any additional nutrients.

Sampling and measurements

Salinity, pH, temperature and relative fluorescence as a proxy for phytoplankton biomass were measured daily in order to monitor the experiment. The relative fluorescence was measured using a fluorometer 10-AU (Turner Design) immediately after the sampling. Sampling was performed directly after mixing and water was syphoned with a tube to a 5L container. Then, subsamples were taken for phytoplankton analysis (phytoplankton counts, cell measurements and flow cytometry) and dissolved inorganic nutrients from the container. The sampling dates were the beginning of the experiment, the end of the grazing phase/start of nutrient phase, then the third and tenth day of the nutrient experiment in order to test the short (3 days) and mid-term response (10 days) of the communities to nutrients.

Dissolved nutrients were filtered through pre-washed (10% HCl) cellulose acetate filters and stored in -20°C immediately. Samples for microscopic phytoplankton analysis were collected in brown bottles (200 ml) and fixed with Lugol's iodine (Rott, 1981). Phytoplankton ($>5\mu\text{m}$) was counted according to the Utermöhl (1958) method with an inverted microscope. In every sample at least 400 individuals were counted in total and at least 100 of the most abundant taxa. Directly after the sampling phytoplankton smaller than $5\mu\text{m}$ was counted with a flow cytometer (FACScalibur, Becton Dickinson). Cell size measurements (linear dimensions) were made with the use of the AxioVision program (Zeiss, Oberkochen, Germany) and converted into cell volumes according to the geometric models of Hillebrand et al. (1999). Species biomass was calculated from the species total abundance multiplied with the individual cell volume. In order to test the suitability of a size based "classification" instead of a taxonomic characterization of phytoplankton, similar sized species were grouped in six size classes. Specifically, the size classes were: picoplankton ($<10\mu\text{m}^3$), small ($10-100\mu\text{m}^3$), medium ($100-1000\mu\text{m}^3$), big ($1000-10000\mu\text{m}^3$), very big ($10000-100000\mu\text{m}^3$), and large ($>100000\mu\text{m}^3$) sized species. Size class biomasses were determined based on taxa cell volume.

Vulnerability of species and size classes to grazing was inferred from by the regression slopes of the abundance of each species and size class in response to grazer density. Positive values indicate

that a species was not grazed and negative values indicate that a species was among the preferred as food source. The more negative the slope the stronger is the vulnerability to grazing.

Biomass increase during the first three days of the nutrient phase was calculated by the difference of biomass (b_i) between day three and one $b_{inc}=b_3-b_1$. Growth rates (μ) were calculated by the difference of the logarithmically transformed biomass (b_i) between the three different sampling dates divided by the time (t_i) difference: $\mu = (\ln b_2 - \ln b_1) / (t_2 - t_1)$.

Community mean cell volume ($V_{\text{mean community}}$) was calculated based on the % contribution of each taxon to total abundance (N_i) multiplied by the taxon biovolume (V_i): $V_{\text{mean community}} = N_i * V_i$

The ability of the communities to preserve their biomass under extended nutrient shortage ($\Delta_{B_{3\text{to}10}}$) was calculated as the absolute value of the increment of the biomass at the end of the experiment (B_{10}) and the biomass build up each community achieved after the addition of the nutrients (B_3) (nutrient phase, day 3): $\Delta_{(B_{3\text{to}10})} = B_{10} - B_3$.

Statistical analysis

The effect of grazing intensity on total biomass and total community, size class and species abundance (independent variable) was analyzed through GLMs (family Gamma, link=log), which allowed us to deal with heteroscedasticity in the data. ANOSIM was used to test if the pre-grazed communities were significantly different in size class contribution to total biomass after the grazing phase before and after the sieving for copepods. After sieving Simper analysis was used to test which size class shifts were responsible for the differentiation of the treatments at the onset of the nutrient phase. Two factorial ANOVA was used to assess the effect of pre-grazing and nutrient availability on the growth rate of the differently sized plankton communities in nutrient phase. One factorial ANOVA was used to assess the effect of pre-grazing to particulate organic nutrients ratios (C/N, C/P, N/P) at the end of the experiment. The effect of cell size on the growth rate of the nutrient added communities, the size classes, the single species, the biomass loss from day 3 to 10 and the particulate organic nutrients ratios (C/N, C/P, N/P) was analyzed through a simple linear regression model (LM). All statistics were performed using R version 3.4.1 (2017-06-30).

Results

Grazing phase - Effect of grazing on community cell size structure

Phytoplankton started to grow with the increased light supply and temperature in the climate chambers (Appendix 2-1A, Grazing phase). Grazer density did not affect total biomass ($F_{1,16}=1.8$; $p=0.71$ GLM) (Appendix 2-2A) but total abundance ($F_{1,16}=17.831$; $p<0.000$ GLM) (Appendix 2-2B) significantly. Community size structure was different between the different grazing treatments (ANOSIM: $R: 0.2296$, $p: 0.023^{***}$ for biomass-based and $R: 0.1942$, $p: 0.029^{***}$ for cell abundance-based). The size structure differences between the communities selected for the second phase of the experiment were even more pronounced (ANOSIM: $R=0.67$, $p= 0.001^{***}$, biomass-based). Grazer density (in particular high densities) also affected mean community cell size in a negative way. The Ambient pre-grazed treatments showed a mean community cell size of $183.3 \mu\text{m}^3$ (average between the replicates), the Low pre-grazed $220.3 \mu\text{m}^3$ (average between the replicates) and the High pre-grazed treatments $9.4 \mu\text{m}^3$ (average between the replicates) (Appendix 2-3).

While copepod grazing reduced the medium phytoplankton size classes it increased both the share of the picoplankton ($<10 \mu\text{m}^3$) and the large ($>100\ 000 \mu\text{m}^3$) size classes (Fig 2-1B). Precisely, grazer density showed a positive effect on the abundance of the picoplankton ($<10\mu\text{m}^3$) ($F=25$, $p=0.001$) and on the large cells in the community ($>100000 \mu\text{m}^3$) ($F=3.8$, $p=0.069$) (Fig 2-1A) before sieving for copepods. The size classes of small ($10-100 \mu\text{m}^3$) ($F=9.1$, $p=0.008$), medium ($100-1000 \mu\text{m}^3$) ($F=10.3$, $p=0.009$) and very big ($10000-100000 \mu\text{m}^3$) ($F=25$, $p=0.03$) cells were negatively affected whereas the size class big ($1000-10000 \mu\text{m}^3$) ($F=0.32$, $p=0.57$) was not affected by grazer density (Fig 2-1A). Most species were affected by grazing in the same way as their respective size class (compare Fig 2-1A and Appendix 2-4). This was also the case for species not affected by grazing. For example, the species *Chaetoceros danicus* and *Chaetoceros sp.*, which were the most abundant species of the size class big cells ($1000-10000 \mu\text{m}^3$; $>90\%$ contribution to size class biomass in all treatments), showed no response to grazer density and neither did the size class (compare Fig 2-1A and Appendix 2-4). All detailed results of the model analysis of grazer densities on the single species and size classes are shown in Appendix 2-4 and Fig 2-1A, respectively. Regarding community composition before sieving in the Ambient and Low pre-grazed treatments (10 ind/L) the small species ($10-100\mu\text{m}^3$) contributed more than 20% in total

biomass in most of the replicates (Fig 2-1B), whereas the biomass of this size class declined with increasing grazer density. The relative biomass of the medium and big cells ($100-1000\mu\text{m}^3$ and $1000-10000\mu\text{m}^3$) appears to be unaffected by grazer density except for the High pre-grazed treatment (160 ind/L). The relative biomass of the large cells ($>100000\mu\text{m}^3$) was favored by severe copepod grazing.

The resulting subset of the three remaining pre-grazed treatments for the following nutrient phase after sieving for copepods did not contain large cells (removed by sieving procedure) but kept the distinctive altered size distribution that was achieved through the grazing experimental phase (Fig 2-1C). Specifically, the size classes medium ($100-1000\mu\text{m}^3$) and big ($1000-1000\mu\text{m}^3$) cells together contributed most to the differentiation of the pre-grazed communities (Simpson Analysis; Ambient vs Low: 97.32%, Ambient vs High: 76.76% and Low vs High: 65.02%; Appendix Table 2-1). In addition, the responses of picoplankton ($<10\mu\text{m}^3$) to pre-grazing also contributed more than 10% to the differentiation of the High pre-grazed community from the others (Simpson Analysis; Ambient vs High: 13.73% and Low vs High: 21.87%). The change in large cells ($>10000\mu\text{m}^3$) contributed also more than 11.11% to the differentiation of the two pre-grazed communities (Low vs High) (see Appendix Table 2-1).

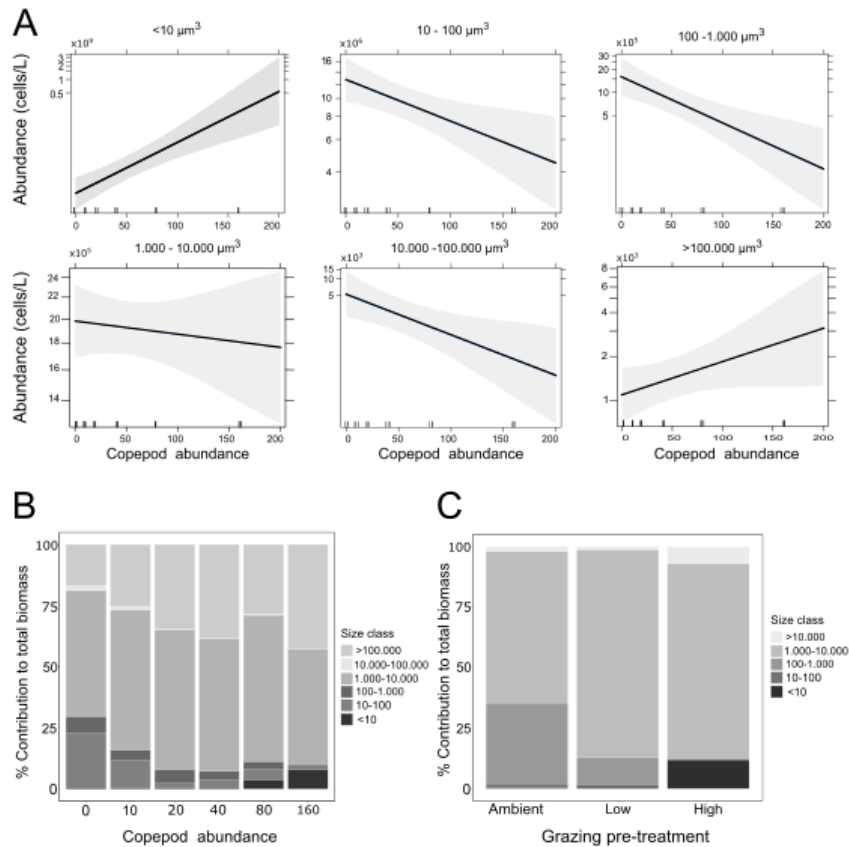


Fig 2-1: A- Effect of grazer density to the abundance of different size classes (<math><10, 10-100, 100-1,000, 1,000-10,000, 10,000-100,000, >100,000 \mu\text{m}^3</math>). The grey area shows the 95% C.I. Effect modeled by GLM (family GAMMA, link=log). B- Contribution of different size classes to total biomass in the different pre-grazed treatments after seven days of grazing. C- Contribution of different size classes to total biomass in the different pre-grazed treatments after sieving for copepods.

Response to nutrients

Biomass- Biomass increased in all nutrient treatments (N) in the nutrient phase during the first three days (Appendix 2-1B (N)). The highest biomass increase (b_{inc}), increment between day one and three, occurred in the High pre-grazed treatment ($5.4 \pm \text{SD} 0.92 \text{ mm}^3 \text{L}^{-1}$) and the lowest in the Low pre-grazed treatment ($3.0 \pm \text{SD} 0.59 \text{ mm}^3 \text{L}^{-1}$). Also the Ambient pre-grazed in the nutrient control treatments (C) showed a small biomass increase ($1.5 \pm \text{SD} 0.33 \text{ mm}^3 \text{L}^{-1}$) (Appendix 2-1B (C)) although the available nutrients were depleted almost to the same level as in the other control (C) treatments (Appendix 2-5). The Low pre-grazed ($0.38 \pm \text{SD} 1.03 \text{ mm}^3 \text{L}^{-1}$) and the High pre-grazed ($0.58 \pm \text{SD} 0.51 \text{ mm}^3 \text{L}^{-1}$) of the (C) treatments showed almost no growth (Appendix 2-1B

(C)). Fluorescence measurements, on contrary, indicated that Ambient pre-grazed treatments reached the highest biomass followed by the Low pre-grazed treatments (Appendix 2-1A). The High pre grazed treatments reached the lowest fluorescence values (Appendix 2-1A).

From day three until day ten biomass decreased in all nutrient treatments (N and C) (Appendix 2-1B). Among the three nutrient treatments (N) the High pre-grazed showed the biggest decline in biomass between the third and tenth day of the experiment while the Ambient and Low pre-grazed communities managed to better preserve the biomass attained until day 3 (Appendix 2-1B). From the control treatments (C) the Ambient pre-grazed was the one with the most biomass loss from day 3 until day 10.

Growth rate - While nutrients but not pre-grazing positively influenced community growth rates (Table 2-1) (Appendix 2-6), there was no interaction between the two factors. Nutrient addition had also a significantly positive effect on the growth rates of all size classes (Table 2-1) (Appendix 2-6). Pre-grazing negatively affected the growth rate of the size classes of picoplankton ($<10 \mu\text{m}^3$) and large ($>10000 \mu\text{m}^3$) cells (maximum growth rate at Ambient pre-grazed treatments) (Table 2-1) (Appendix 2-6), and positively the size class of small cells ($10-100 \mu\text{m}^3$) (maximum growth rate at High pre-grazed treatments) (Table 2-1) (Appendix 2-6). Regarding the major species in these experimental communities, pre-grazing positively affected the growth rates of the species *Chaetoceros danicus*, *Skeletonema marinoi* (highest growth rate at Low-not present at High pre-grazed treatments) and *Atheya septentrionalis* (highest growth rate at High pre-grazed treatments) (Appendix 2-7, Table 2-1). The pre-grazing effect was in contrast negative for the growth rates of *Pseudonitzschia sp.* (highest growth rate at Ambient pre-grazed treatments). *Chaetoceros sp.* showed a different response to the treatments with the lowest growth rate observed at Low and highest growth rate at Ambient pre-grazed treatments (Appendix 2-7, Table 2-1). Nutrients affected significantly both *Chaetoceros* species, *S. marinoi.*, and *A septentrionalis* while for *Pseudonitzschia sp.* and *S. marinoi* (Appendix 2-7, Table 2-1) the combination of the factors was found significant. *Pseudonitzschia sp.* showed an additive negative effect from pre-grazing and nutrient addition while *S. marinoi* pre-grazing effect was positive for the nutrient treatments (N) but negative for the control nutrient treatments (C) (Appendix 2-7, Table 2-1).

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Table 2-1. ANOVA results showing the short term effects (days 1-3) of the factors pre- grazing, nutrient addition and the interaction between them on total community growth rate, size groups growth rate and species growth rate.

Growth rate	Factor	Df	Sum Sq	Mean Sq	F	p
Total Community	pre-Grazing	2	0.06	0.03	2.8	0.102
	Nutrients	1	0.49	0.49	43.3	<0.000***
	pre-Grazing*Nutrients	2	0.04	0.02	1.6	0.24
Size classes(μm^3)						
<10	pre-Grazing	2	1.31	0.65	66.4	<0.000***
	Nutrients	1	0.18	0.18	18.5	0.001**
	pre-Grazing*Nutrients	2	0.01	0.01	0.6	0.551
10-100	pre-Grazing	2	1.52	0.76	20.5	<0.000***
	Nutrients	1	0.78	0.78	21.1	<0.000***
	pre-Grazing*Nutrients	2	0.13	0.07	1.8	0.211
100-1000	pre-Grazing	2	0.32	0.16	1.4	0.274
	Nutrients	1	0.72	0.72	6.6	0.024*
	pre-Grazing*Nutrients	2	0.30	0.15	1.4	0.289
1000-10000	pre-Grazing	2	0.09	0.05	1.8	0.2
	Nutrients	1	0.68	0.68	27.5	<0.000***
	pre-Grazing*Nutrients	2	0.06	0.03	1.2	0.347
>10000	pre-Grazing	2	3.25	1.63	19.8	<0.000***
	Nutrients	1	0.66	0.66	8	0.015*
	pre-Grazing*Nutrients	2	0.12	0.06	0.7	0.501
Species						
<i>Chaetoceros sp.</i>	pre-Grazing	2	0.43	0.22	4.2	0.042*
	Nutrients	1	0.87	0.87	16.7	0.002**
	pre-Grazing*Nutrients	2	0.02	0.01	0.2	0.84
<i>Chaetoceros danicus</i>	pre-Grazing	2	0.19	0.10	3.9	0.053 .
	Nutrients	1	0.54	0.54	21.6	<0.000***
	pre-Grazing*Nutrients	2	0.18	0.09	3.6	0.064 .
<i>Pseudonitzschia sp.</i>	pre-Grazing	2	0.24	0.12	13.8	0.001*
	Nutrients	1	0.00	0.00	0.2	0.63
	pre-Grazing*Nutrients	2	0.07	0.03	4	0.049*
<i>Guinardia delicatula</i>	pre-Grazing	1	0.26	0.26	3.8	0.087 .
	Nutrients	1	0.18	0.18	2.6	0.146
	pre-Grazing*Nutrients	1	0.03	0.03	0.5	0.507
<i>Skeletonema marinoi</i>	pre-Grazing	2	0.82	0.41	8.8	0.004**
	Nutrients	1	0.60	0.60	12.9	0.004**
	pre-Grazing*Nutrients	2	1.00	0.50	10.6	0.002**

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<i>Thalassiosira sp.1</i>	pre-Grazing	1	0.07	0.07	0.3	0.63
	Nutrients	1	0.45	0.45	1.5	0.252
	pre-Grazing*Nutrients	1	0.02	0.02	0.1	0.797
<i>Thalassiosira sp.2</i>	pre-Grazing	2	0.99	0.49	1.7	0.228
	Nutrients	1	0.21	0.21	0.7	0.415
	pre-Grazing*Nutrients	2	0.49	0.25	0.8	0.457
<i>Dactyliosolen fragilissimus</i>	pre-Grazing	2	1.53	0.77	3.3	0.074 .
	Nutrients	1	0.34	0.34	1.4	0.252
	pre-Grazing*Nutrients	2	0.39	0.19	0.8	0.46
<i>Teleaulax sp.</i>	pre-Grazing	2	0.17	0.09	0.4	0.691
	Nutrients	1	1.02	1.02	4.5	0.054 .
	pre-Grazing*Nutrients	2	0.25	0.12	0.6	0.591
<i>Atheya sp.</i>	pre-Grazing	2	1.52	0.76	18.6	<0.000***
	Nutrients	1	0.70	0.70	17	0.001**
	pre-Grazing*Nutrients	2	0.15	0.08	1.9	0.198

Growth rates of heterotrophic protists during the first three days varied widely between positive and negative values. Among the N treatments positive growth was observed only in the Ambient grazer treatment (growth of heterotrophic dinoflagellates). In the nutrient control treatments positive growth was observed in the Ambient and Low grazer treatments (growth of heterotrophic dinoflagellates) (Appendix 2-8).

Nutrient analysis - Except for NH_4 , the available nutrients in the nutrient treatments (N) were almost exhausted already on the third day of the Nutrient phase in all treatment combinations (Appendix 2-5). NH_4 increased in all treatments until day 10 of the nutrient phase to not more than $2 \mu\text{mol}\cdot\text{L}^{-1}$. Chemical analyses of particulate organic nutrients at the end of the experiment indicated that all nutrient treatments (N) were in the transition between N- and P- limitation at day 10, reflected by a N:P ratio of 16.7 ± 1.52 . Both C:N (ANOVA: $F_{2,6}=14.14$, $p=0.005$) and C:P (ANOVA: $F_{2,6}=13.07$, $p=0.006$) ratios showed the highest value ($22.3\pm\text{SD}0.34$ and $388.1\pm\text{SD}33.4$ accordingly) at the Ambient pre-grazed treatments and the lowest at the High pre-grazed ($15.03\pm\text{SD}2.4$ and $248.3\pm\text{SD}38.68$ accordingly).

Cell size effect on phytoplankton traits

Cell size effects on grazing vulnerability - The grazing vulnerability, as indicated by the regression slopes of abundance responses of size classes and species to grazer density in Fig. 2-1a and Appendix 2-2, given as

$$\text{Vulnerability}_{\text{species}} = 0.03 - 0.027 \log_{10}(V_{\text{species}}) + 0.0042 \log_{10}(V_{\text{species}})^2 \quad (F_{2,16} = 6.88, p = 0.007, r^2 = 0.39) \text{ and}$$

$$\text{Vulnerability}_{\text{sizeclass}} = 0.04 - 0.029 \log_{10}(V_{\text{sizeclass}}) + 0.0037 \log_{10}(V_{\text{sizeclass}})^2 \quad (F_{2,3} = 3.678, p = 0.15, r^2 = 0.51),$$

showed a parabolic-shaped response to cell size (Fig 2-2).

Medium sized cells showed the highest vulnerability to grazing (Fig 2-2).

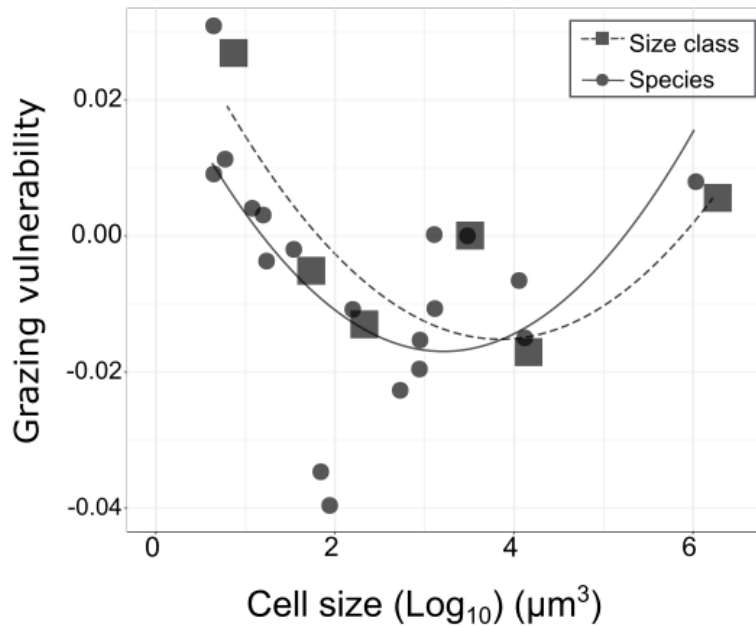


Fig 2-2: Grazing vulnerability, expressed as the regression slope of cell abundance on copepod abundance, on size classes and on species.

Cell size effect on growth rates - Mean community cell size affected the communities' growth rates negatively (Fig 2-3). The relationship between community mean cell size (μm^3) and community growth rate is described as:

$$\mu_{\text{mean community size}} = 0.68 - 0.13 \log_{10} V \quad (F_{1,7} = 7.5, p = 0.028, r^2 = 0.44)$$

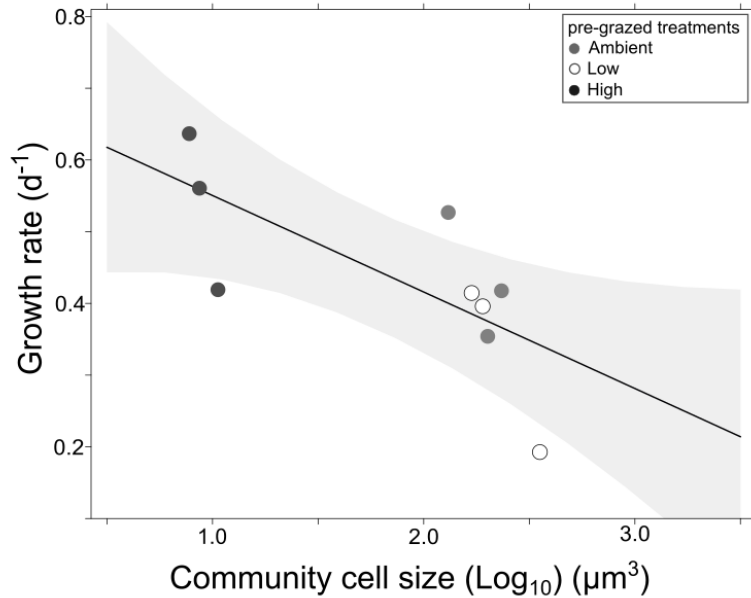


Fig 2- 3: Regression of community growth rate (short term response, experimental days 1-3) in response to community mean cell size. Grey area: 95% confidence intervals.

Cell size affected the growth rates of the different size classes differently in the differently pre-grazed treatments. In the Ambient pre-grazed communities, the growth rates of the size classes showed a U-shaped response (Fig 2-4) to increasing cell size. In the Low and High pre-grazed communities the growth rates of the different size classes declined with increasing cell size (Fig 2-4). The equations describing the effect of cell size on the size classes growth rate, in the differently pre-grazed treatments, are described as:

$$\text{Ambient: } \mu_{\text{Ambient}} = 1.92 - 1.16 \log_{10} V + 0.19 \log_{10}(V^2) \quad (F=, p=0.0008, r^2=0.64)$$

$$\text{Low: } \mu_{\text{Low}} = 1.24 - 0.24 \log_{10} V \quad (F=, p=0.0007, r^2=0.56)$$

$$\text{High: } \mu_{\text{High}} = 1.35 - 0.35 \log_{10} V \quad (F=, p=0.0001, r^2=0.66)$$

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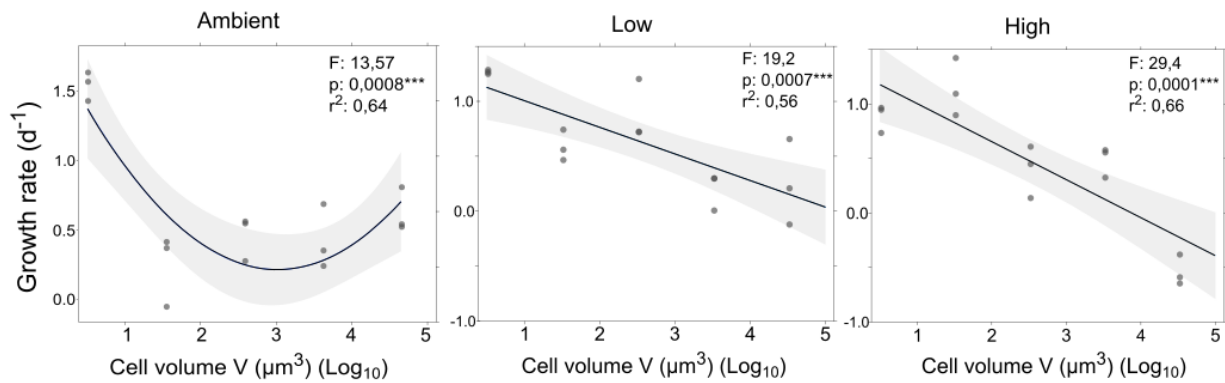


Fig 2-4: Regression of size classes growth rate (short term response, experimental days 1-3) on size classes volume for the Ambient, Low and High pre-grazed treatments. Grey area: 95% confidence interval.

The highest growth rates (μ_h) observed for each species observed after nutrient addition showed a negative relationship with cell size (Fig 2-5).

$$\mu_h = 1.89 - 0.3 \log_{10} V \quad (F_{1,9}: 9.6, p = 0.002, r^2 = 0.1)$$

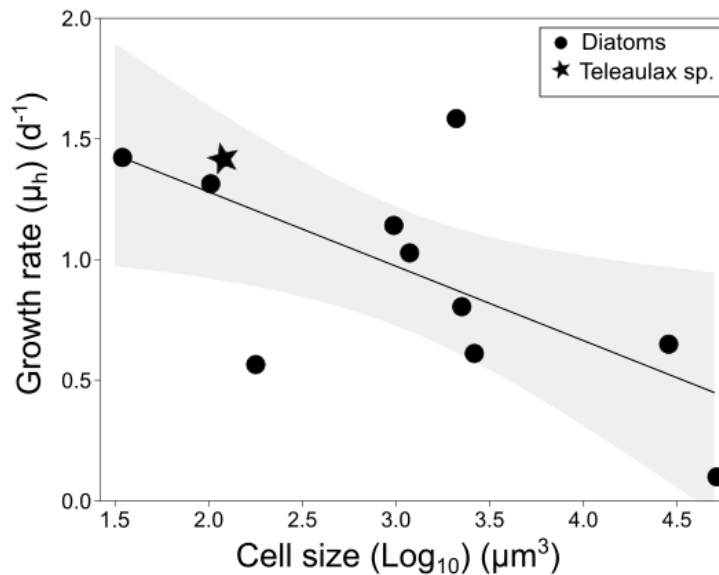


Fig 2-5: Regression of single species growth rate (short term response to nutrient addition - experimental days 1-3) on cell volume.

Cell size effect on biomass preservation from day 3 to 10 -. The relationship of community mean cell size and the biomass loss under extended nutrient shortage was indicated weaker losses and a better biomass preservation with increasing cell size (Fig 2-6).

$$\Delta_{B3\text{to}10} = -5.64 + 2.06 \log_{10} V \quad (F_{1,7}=19.59, p=0.003, r^2=0.69)$$

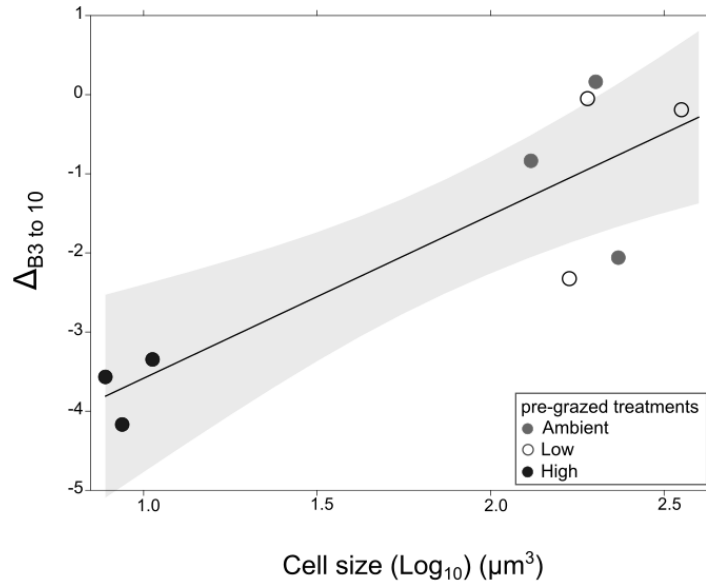


Fig 2-6: Regression of biomass preservation under nutrient shortage (biomass loss under nutrient shortage, $\Delta_{B3\text{to}10}$) in response to cell size. Grey area: 95% confidence interval.

Cell size effect on C/N and C/P ratios-The relationship of community mean cell size and the C/N and C/P ratios was found positive (Fig 2-7) following the equations:

$$\log_{10}(\text{C:N}) = 1.08 + 0.1 \log_{10} V \quad (F_{1,7}=15.03, p=0.006, r^2=0.63) \text{ and}$$

$$\log_{10}(\text{C:P}) = 2.28 + 0.11 \log_{10} V \quad (F_{1,7}=14.94, p=0.006, r^2=0.63),$$

respectively.

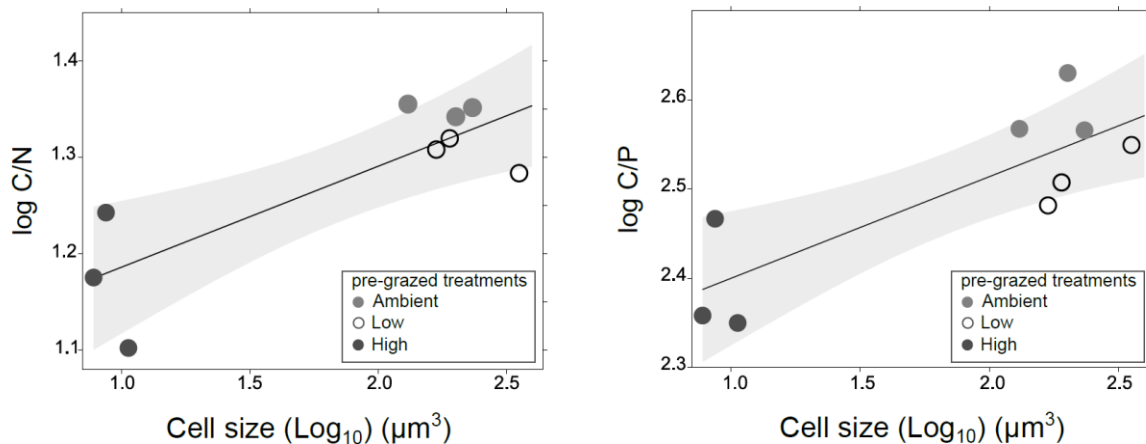


Fig 2-7: Regression of C/N and C/P ratios in the nutrient treatments (N) at day 10 of the nutrient phase in response to mean community cell size. Grey area: 95% confidence interval.

Discussion

Our mesocosm experiment was based on the assumed double nature of phytoplankton traits, first as an indicator of a particular environmental stress and, second, as an attribute to cope with environmental stress. In agreement with Litchman and Klausmeier (2008) we chose cell size as the core trait of our study. We exposed a natural community of unicellular plankton first to grazing and then to nutrient stress in order to test whether the sensitivity to the first stressor affects the response, i.e. growth rate and the ability to preserve the gained biomass, to the second.

Previous experiments with natural plankton communities showed that copepod grazing can affect community size structure (Sommer et al. 2001, Sommer & Sommer 2006) mainly benefiting the small and the very large phytoplankton taxa. Recently, Stamieszkin et al. (2017) used a variety of mesograzers and densities of them in mesocosm experiments and did not find community size shift but only particle abundance changes. Our findings however, indicate a clear size shift in the communities described by the response of the size classes to grazing and the % contribution of size classes to total biomass. The benefit for small phytoplankton is explained partly by suppressing protozoan grazers and partly by removal of their larger competitors. However, also very big or well defended cell share the exclusion from grazing such as *Coscinodiscus wailesii* and hard-shelled dinoflagellates (Sommer et al., 2005, Sommer & Lewandowska, 2011). Our findings

agree with these results. By increasing copepod abundance, we managed to suppress the abundance of all size classes except the picoplankton ($<10\mu\text{m}^3$) and the large cells ($>100\ 000\ \mu\text{m}^3$). This dominance of size extremes in the communities indicated that a classical calculation of mean community cell size did not fully capture the size shift, while the use of abundance data in the calculation proved to be a better descriptor. The same response (lower or higher abundance) applied also to the individual taxa within the size classes. Taxa with cell size from around $100\ \mu\text{m}^3$ to $1000\ \mu\text{m}^3$ were the most vulnerable to grazing pressure, with the two diatoms *Nitzschia longissima* and *Skeletonema sp.* being the most vulnerable. On the contrary, the almost neutral (not favored or negatively affected) size class response of medium sized cells ($1000\text{-}1000\ \mu\text{m}^3$), with *Chaetoceros* as the dominant species can be explained by the copepods not preferring them as food source. This “diet exclusion” of *Chaetoceros* species was also observed at the highly grazed treatments where the majority of taxa were eaten and *Chaetoceros* was among the few if not the only abundant species that did not belong to picoplankton. We assume that their shape, especially the presence of long spines, was the determining factor for not being grazed.

The cell size effect on growth rates revealed a clear negative relationship between cell size and growth rates in all examined levels of community size organization confirming our hypotheses 2 and 3. The cell size effect as measured by the slopes of the regressions as a measure of effect strength, was strongest at the size class level and weakest at the community level. In order to compare our results with the literature we recalculated the growth rate - cell size relationship according to a double \log_{10} -transformation. In addition, the new relationships were corrected with Eppley's (1972) Q10-value of 1.88 to account the difference in growth between 10 and 20 °C. The new relationships describing the growth rate – cell volume relationship were:

for total community growth: $\mu = -0.2 V^{-0.14}$ ($F_{1,7}=5.48$, $p=0.05$, $r^2=0.36$)

which is close to the -0.15 slope described by Marañón (2013) for species of a volume larger than $40\mu\text{m}^3$ and for highest observed growth rates of individual species:

$\mu = -0.96 V^{-0.075}$ ($F_{1,7}=8.016$, $p=0.019$, $r^2=0.41$)

a relationship much weaker than the one of Marañón et al. (2013). Both, for community and for species growth rates, the slopes of the relationships are much weaker than the one described by Edwards et al. (2012) for marine species, but within the range of some earlier studies (Banse, 1982;

Blasco et al., 1982; Sommer, 1989). This weaker relationship could be partly explained because the highest observed growth rates in our study were not necessarily the μ_{\max} of each species or maybe not even close to it, since the time frame of 3 days given to the communities to grow after nutrient addition was probably too big and thus we missed the phase of maximal growth of some species. In addition, the species involved in the equation consist of only diatoms and one flagellate (Cryptophyte: *Teleaulax sp.*). Dinoflagellates which in general have lower growth rates than diatoms (Tang, 1996; Edwards et al., 2012; Marañón et al., 2013; Sommer et al., 2017a; Charalampous et al., 2018) were not included.

Contrary to hypothesis 4, size classes from the Ambient pre-grazing treatments did not show the same relationship between growth rates and cells size as the communities that were subject to Low or High copepod pre-grazing. The U-shaped response, as opposed to the monotonous decline, might have been the consequence of microzooplankton grazing which could not be excluded by sieving after the first phase of the experiment, because of the ample size overlap between phytoplankton and microzooplankton. In the Ambient pre-treatment mesozooplankton grazing was excluded as in earlier experiments using the same system (see Sommer et al., 2012a) where mesozooplankton (mainly copepods) either could not survive the pumping and filling procedure or escaped from the inlet of the pumping system and had to be added from net catches or cultures, as also in this experiment. In the pre-grazed treatments (i.e. with actively added mesograzers) the consumer pressure of micro-grazers was in turn weakened if not totally eliminated because ciliates and dinoflagellates are often preferred prey items for copepods. In addition, picoplankton showed higher growth rate in the Ambient community, which indicates that grazing by ciliates and dinoflagellates also removed heterotrophic nanoflagellates (HNF), which mainly graze on picophytoplankton (Azam et al., 1983). This way grazing by HNFs on picophytoplankton was also reduced and as consequence the negative effect on picophytoplankton growth. The lower numbers of micro-grazers in the pre-grazed by copepods communities create different and more complicated trophic relationships in the communities (Moustaka et al., 2016) resulting in lower growth rates of small species that we observed.

The timing of our sampling schedule was not optimal for testing Hypothesis 5 (bigger cells will better cope with prolonged nutrient shortage). Based on the temporal dynamics of previous experiments in the same experimental systems, we expected that until day 3 small phytoplankton

would display their faster growth, while thereafter the larger phytoplankton would surpass them because of their longer maintenance of nutrient saturated growth. Unfortunately, bloom dynamics were faster than expected and all types of phytoplankton had reached their peak already with day 3 (or possibly shortly afterwards, but clearly before day 10). Nevertheless, the outcome of the experiment revealed some interesting size related trends. The communities with bigger mean cell size achieved less biomass loss after day 3 than the communities dominated by smaller cells. However, the smaller cell sized communities show a lower C/N and C/P ratio at day 10 which indicates that they can preserve a healthier state since they can continue their ribosomal metabolic activities, produce proteins and continue growing for longer. Smaller cells, because of their low surface to volume ratios might not have the same storage capacities as the bigger cells but they showed a better ability to respond to a nutrient spike and also better nutrient utilization ability in low nutrient concentrations, as shown already during the first three days of the experiment. The slower decline of the larger phytoplankton should, therefore, be attributed to a higher resistance to the prevalent loss factors, in our case most probably grazing by mesozooplankton.

Conclusions

Our experiment confirms our hypothesis that grazing vulnerability of species is driven by cell size, although the shape of a species can alter the sensitivity to specific grazers. Picoplankton and very big cells can profit from copepod grazing. The extreme size difference of abundant species in a phytoplankton community caused by copepod grazing reveals a weakness of mean cell size as a metric to reveal the size shift of the community, because copepods remove the medium sized algae and benefit both extremes of the size spectrum. Community trait shift induced by copepod grazing affected the response of the communities to nutrient addition. More precisely, the communities resulting from intense copepod grazing (High treatment) showed a faster growth after the nutrient pulse and achieved a higher biomass built up, however, it was also the community that preserved the least biomass until day ten. Our results indicate that the trait shifts induced by one environmental factor impact the ability of the community to respond to other environmental factors. To conclude, cell size is a trait that can indicate the responsiveness of natural phytoplankton communities to environmental changes.

Chapter II

Chapter III



Light effects on phytoplankton morphometric traits influence nutrient utilization ability

Abstract

Light and nutrients are essential resources for phytoplankton growth and considered to shape the size structure and other morphometric traits (surface:volume ratio, deviation from spherical shape) of phytoplankton communities. If morphometric traits influence growth and resource use, shifts by one of the two factors should influence the capability to utilize the other factor. We performed a two-step experiment, where a natural phytoplankton community was first exposed to three different light levels (supposed to be limiting, saturating and slightly inhibiting for the majority of species) and grown until stationary phase. Then, the pre-conditioned communities were split into two nutrient treatments (control and saturating nutrient pulse) and again grown until stationary phase under the medium light intensity. During the experimental light phase, community mean cell-size increased with light, but surface:volume ratio and deviation from spherical shape decreased. Moreover, in response to the following nutrient pulse the low light pre-conditioned communities showed the highest initial growth rates in response to the nutrient pulse. The high light pre-conditioned communities showed the highest conversion of the nutrient pulse into biomass during the stationary phase. These results demonstrate how the imprint of one environmental factor on trait distribution influences the ability to cope with another.

Introduction

Size is considered a dominant trait in comparative ecophysiology in general (Peters, 1983) and in phytoplankton ecology specifically (Litchman et al., 2010; Marañón, 2015; Sommer et al., 2017a,b). While all phytoplankton appear “small” for ecologists working with macroflora or – fauna, they span 4 to 5 orders of magnitude in linear cell- or colony dimensions, i.e. from $<1\mu\text{m}$ picophytoplankton cells to cm sized colonies (Finkel et al., 2010). The relationship of physiological properties to size is often described by the allometric equation

$$Y = aX^b$$

where X is a three-dimensional metric of body size (volume or mass) and a is a constant. The exponent (“allometry coefficient”) b is usually positive but <1 (often 0.75) for absolute physiological rates (rate per individual) and <0 (often -0.25) for specific rates (rate per unit mass) including growth rates (general: Peters, 1983; Brown et al., 2004: phytoplankton: Tang, 1995; Edwards et al., 2012). However, there is also some evidence, that phytoplankton maximal growth rates show a unimodal response to cell size with a peak at a cell volume of ca. $100\mu\text{m}^3$ (Marañón et al., 2013; Marañón, 2015).

In terms of resource acquisition (nutrients, light), low availability usually favors smaller phytoplankton, which exhibit higher acquisition and growth rates at low resource availability (“affinity”). For recent examples regarding uptake at low nutrient concentrations see Edwards et al. (2011) and Litchman et al. (2015b), and for light utilization at low intensities Edwards et al. (2015, 2016) and Schwaderer et al. (2011). On the contrary, larger phytoplankton, , have better storage capacities, as indicated by higher ratios between the maximal and the minimal cell quota of limiting nutrients (Edwards et al., 2011; Marañón et al., 2013). Based on data in Marañón et al. (2013), Sommer et al. (2017) calculated that a nitrogen replete mother cell of $10\mu\text{m}^3$ could support only one cell division without any new uptake of N, while cells $>23000\mu\text{m}^3$ could support two subsequent cell divisions. Since minimal cell quotas of N scale with the 0.88th power of cell volume (Edwards et al., 2012), 1000 times bigger cells should be able to build the 2.3 fold biovolume, if phytoplankton are permitted to grow until complete exhaustion of the internal N pool. Thus, larger cells can build up a higher biomass from the same amount of the limiting nutrient because of lower biomass specific minimal cell quotas (Edwards et al., 2011; Marañón et al., 2013).

In terms of resistance to loss factors, smaller cells are more sensitive to grazing, due to the dominance of small, protistan grazers while larger cells are more sensitive to sinking (Reynolds,

1984; Sommer, 1984), except for giant diatoms with buoyancy regulation (Villareal, 1988; Villareal et al., 2014). However, under pre-dominance of copepod grazing the medium size range, from 5 to 10 μm cell length to ca. 200 μm , will experience the strongest grazing pressure (Katechakis et al., 2004; Sommer et al., 2005; Sommer and Sommer, 2006).

While the influence of size on resource competition and loss factors is well documented, the role of cell shape needs deeper consideration. On resource shortage conditions the advantage of being small is usually seen in higher surface:volume (S:V) ratios (Chisholm, 1992; Raven, 1998), in the case of light limitation also in higher surface:cross sectional area ratios and in a smaller package effect (Kirk, 2010). While S:V is inversely proportional to linear dimensions for bodies of identical shape, there is a tendency towards increasingly non-spherical morphologies among larger phytoplankton, thus constrainig S:V in a narrower range of values than predicted from volumes alone (Lewis, 1976). Reynolds (1997) suggested the dimensionless product of the longest linear axis (L) with S:V as shape index (SI) to characterize the deviation from sphericity. Reynolds (1988) postulated that strongly non-spherical shapes (needles or complex morphologies) are typical for the “ruderal” life-form type thriving under deep mixing conditions, which combine low light with high nutrient availability.

If size or other morphometric traits determine the capability of phytoplankton to cope with various environmental pressures, it is no surprise that environmental pressures leave an imprint on morphometric properties of phytoplankton communities. Indeed, a number of repeatable patterns in the response of phytoplankton size to environmental pressures have been identified (for a review see: Sommer et al., 2017a). Cell size tends to decrease with warming (Atkinson et al., 2003; Daufresne et al., 2009; Sommer and Lewandowska, 2011), and nutrient limitation (field observations: Marañón, 2015; experiment: Peter and Sommer, 2013, 2015). Grazing by mesozooplankton (i.e. copepods) tends to decrease cell size (Sommer et al., 2005), and grazing by microzooplankton (and in lakes also by *Daphnia*) has the opposite effect (Sommer et al., 2005; Sommer and Sommer, 2006). Zooplankton and nutrient effects on phytoplankton size structure were found to be stronger at higher temperatures (Sommer and Lewandowska, 2011; Peter and Sommer, 2012a, 2013, 2015). Kruk et al. (2015) found S:V ratio to increase with the vertical attenuation coefficient and supposedly the extent of light limitation in a subtropical estuary.

While the vast majority of trait measurements in the ecophysiological literature and in trait based ecological studies have assigned fixed traits to species, there is an increasing awareness to also

consider intraspecific trait variation (Violle, 2012; Litchman et al., 2015b). Wherever trait shifts within species were compared to traits shifts at the phytoplankton community level, the sign of the change was the same while the magnitude of the intraspecific effect was smaller (Peter and Sommer, 2012, 2013, 2015).

If environmental stressors can change trait distributions in a community, it may be expected that a particular stressor can influence the community level ability to cope with another stressor. This means that the shift in trait distributions can either reduce the ability to cope with the second stressor (SICS = “stressor induced community sensitivity” sensu Vinebrooke et al. (2004)) or can increase this ability (SICT = “stressor induced community tolerance”). Inspired by this concept, we performed a mesocosm experiment with marine phytoplankton, in which phytoplankton were exposed to pressure by two environmental factors, i.e. nutrients and light. Both of these factors are considered dominant limiting growth factors in situ and have been shown repeatedly to have selective impacts on morphometric traits, in particular size. In the experiment, we first exposed a natural phytoplankton community from the Western Baltic Sea to three different levels of irradiance, and in a second step exposed the resulting communities to sustained nutrient limitation or a growth saturating nutrient spike and compared their response in growth and resource use efficiency.

Our working hypotheses were:

- 1) Low light will lead to a smaller community mean cell size, a higher mean S:V ratio and a higher SI.
- 2) If detectable at all, intraspecific morphometric changes will have the same direction as community level changes.
- 3) Low-light pre-conditioned communities will show a higher initial growth rate after nutrient addition because of the shift towards lower cell sizes brought about by low light.
- 4) When reaching stationary phase, high-light pre-conditioned communities will show a bigger conversion of nutrient spikes into biomass because of lower biomass specific minimal cell quotas of larger cells.

Materials and methods

Experimental design. Near-surface seawater containing the natural mix of phytoplankton, protozoa and bacteria was pumped from Kiel Fjord, Baltic Sea, on 31 October 2016 and distributed through a hose system into 9 mesocosms (volume 1500 L, 1.5m in diameter and 1m high) which were evenly placed in three walk-in climate chambers. Thereafter, the mesocosms were exposed to three different constant irradiance levels at a 9:15 hrs light:dark cycle, conforming to the seasonal day length calculated in the astronomic model of Brock (1981). Light was supplied by computer controlled (GHL, Prometheus) LED light units, similar to the former BIOACID experiments (Sommer et al., 2015; Paul et al., 2015). Each light treatment was triplicated and evenly distributed among the climate chambers. The three different light intensities were assumed to represent low light stress (LL: $31 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$), moderately limiting to saturating conditions (ML: $72 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$), and saturating to slightly inhibiting conditions (HL: $139 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$) for the majority of species (Reynolds, 1984; Kohl and Nicklisch, 1988). The experimental temperature was 11°C , representing in situ conditions. Fluorescence measurements were done every day in the lab at the same time using a subsample from the mesocosms. These measurements were used as a proxy to monitor the biomass change through time (Appendix 3-1). The different light treatments were applied until fluorescence measurements indicated stationary phase of growth (day 7 for the ML and HL treatments, day 11 for the LL treatment), at which time cellular nutrient quotas are assumed to approach the structural minimum (Q_0 sensu Droop, 1973, 1983). An automatic propeller was used to mix the mesocosms gently, representing natural water waving to avoid sedimentation and achieve homogeneity of the plankton community.

After reaching stationary phase, the light pre-conditioned communities were split into two nutrient treatments. One of them received a saturating nutrient pulse and the other was not modified as it served as control. For the new treatments 120 L^{-1} volume floating plastic bags were used (0.5m in diameter and 0.6m high), set in pairs (one nutrient and one control treatment) inside the bigger mesocosms and under each light unit (maximum distance from light: 1m). The plastic bags containing the new treatments were filled gradually using a 10 L bucket to achieve the same initial plankton assemblage. The content of the bags was stirred manually twice per day. Light intensity for the second experimental phase was set at the moderately limiting to saturating conditions (ML: $72 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$) for all treatments, to have minimal low or high light stress for the majority

of species. The split into nutrient treatments and controls took place at day 11 for LL and at day 8 for ML and HL, respectively (see bifurcation of growth curves in Fig. 3-1). The nutrient pulse targeted final concentrations of $2 \mu\text{mol L}^{-1}$ phosphate, $32 \mu\text{mol L}^{-1}$ nitrate, and $32 \mu\text{mol L}^{-1}$ silicate in each mesocosm. Growth was followed until the nutrient augmented treatments had reached the stationary phase.

Biological sampling. Phytoplankton samples were taken on days 1, 4, 7, 8, 9, 10, 11, 12, 14, and 17 of the experiment (Fig. 3-1) and fixed by Lugol's iodine for microscopic analysis of nano- and microplankton. Picoplankton were measured directly using flow cytometry (FACScalibur, Becton Dickinson). The one-day sampling intervals at the beginning of the second phase were chosen to get realistic estimates of nutrient saturated net growth rate (μ_{nsat}), which is often maintained only during 1 or 2 days while the approach towards the stationary phase is a slower process.

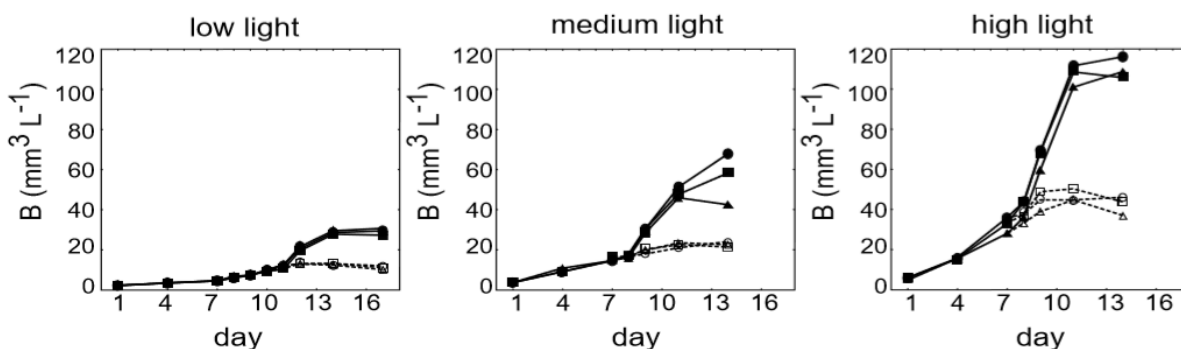


Fig. 3-1 Time course of total phytoplankton biomass (B) ($\text{mm}^3 \text{L}^{-1}$) in low light-, medium light- and high-light pre-treated mesocosms. Solid line: mesocosms before nutrient addition and nutrient treatments, broken line: controls; circles, triangles and squares label the different replicates.

Microscopic phytoplankton counts were performed according to Utermöhl's (1958) sedimentation method. For the dominant species, at least 400 cells were counted in each sample and for all other species that were sufficiently abundant it was attempted to count at least 100 cells. However, species too rare to count 100 cells were also included in calculations of community properties. Linear dimensions for calculating cell morphometric properties were measured for 20 randomly selected cells of each species at the end of the light phase of the experiment. These measurements

were taken separately for each mesocosm for 11 species which together contributed more than 94% to total phytoplankton biomass. For the remaining species a pooled sample was taken. Geometric proxies according to Hillebrand et al. (1999) were used to calculate cell volumes (V ; in μm^3) and surface areas (S ; in μm^2). In addition, we calculated surface : volume ratios ($S:V$; μm^{-1}) and the dimensionless shape index (SI) sensu Reynolds (1997) which indicates the deviation from sphericity

$$SI = L * S * V^{-1}$$

where L is the longest linear axis (e.g. diameter of a sphere and a discoid cylinder, height of an elongate cylinder, longest axis of an ellipsoid, etc.). Based on the equations for volume and surface area, $SI = 6$ for spheres. Spines were only included into calculations if they contained protoplasm, such as the chloroplast containing spines of the diatom *Chaetoceros danicus*. Total phytoplankton biomass (B ; $\text{mm}^3 \text{mL}^{-1}$) was calculated from cell numbers (N_i ; cells mL^{-1}) and cell volumes (V_i) of the individual species.

$$B = \sum N_i * V_i$$

Community means of morphometric properties (X_c) were calculated as biomass weighted means:

$$X_c = \sum p_i * X_i$$

where X is the parameter of interest (V , $S:V$, SI) and p_i the relative biomass of species i ($p_i = N_i * V_i * B^{-1}$).

Short term nutrient effects were measured as nutrient saturated net growth rates (μ_{nsat}) during the first day immediately following nutrient additions, because samples taken afterwards already showed some decline in growth rates (Fig. 3-1). Growth rates were calculated for B ($\mu = \ln(B_1 - B_0) t^{-1}$) and for individual species. As a measure of resource use efficiency, the net effect of nutrient addition on B (NEB: nutrient effect on biomass) was calculated as phytoplankton biomass increment from the start of the nutrient addition (B_{N0}) to biomass at stationary phase ($B_{N\text{max}}$) minus the biomass increment in the controls at the same time:

$$NEB = (B_{Nmax} - B_{N0}) - (B_{Cmax} - B_{C0})$$

The correction by the increment in the control treatments was made, because residual internal and dissolved nutrient stores might have been different between different light pre-treatments.

Chemical sampling. Samples for dissolved inorganic nutrients were taken together with the phytoplankton samples. The samples were filtered through pre-washed (10% HCl) cellulose acetate filters and frozen immediately until analysis (Appendix 3-2). As an additional measure of nutrient utilization in the nutrient addition treatments we also analysed particulate matter stoichiometry at the end of the experiment. Samples for POC, PON and POP measurements were filtered onto prewashed (in 5–10% HCl) and precombusted (6 h, 550 °C) Whatman GF/F filters. POC and PON was determined by gas chromatography (Sharp, 1974) in an elemental analyser (Thermo Flash 2001, Thermo Fisher Scientific Inc., Schwerte, Germany). POP was converted to orthophosphate and determined colorimetrically (Hansen and Koroleff, 2007).

Statistics. Prior to analysis, B and morphometric parameters were log-transformed and p_i was logit-transformed. A Pearson correlation was used to test whether the three morphometric traits (V to S:V; V to S:I; S:V to SI) at community and individual species level are strongly connected. The effect of the light treatments on community morphometric traits, species morphometric traits and species relative biomass at the end of the light treatment phase were analysed by ANOVA. For the second experimental phase, when nutrients were added, an ANOVA was also used to test the effect of light pre-treatment on the communities' apparent short term response to nutrients (μ_{nsat}) and the final conversion of the nutrient into biomass (NEB, C:N, C:P ratios). The relationships between community morphometric traits and nutrient responses (μ_{nsat} , NEB, C:N and C:P ratios) and between species specific maximal growth rates and morphometric variables were analysed by linear regressions of using V, S:V and SI as predictor variables. To test if the μ_{nsat} response is affected only by V or also by species type (diatom or flagellate) an ANCOVA was calculated using V as continuous and species type as categorical factor on μ_{nsat} . A 2-way ANOVA with the factors light pre-treatment and nutrients and their interaction was calculated at the end of the experiment to understand the effects on the morphometric traits (V, SI, S:V).

Results

Time course of growth. Phytoplankton already started to grow during the first sampling interval, which was reflected in an immediate increase of B with obvious differences between the light treatments. Immediately after nutrient addition, nutrient and control treatments diverged from each other, with conspicuous growth in the nutrient treatments and only little growth in the controls (Fig. 3-1). However, growth rates of nutrient treatments also began to decline after 1 day already, thus validating to choose the first day after nutrient addition as time interval for μ_{nsat} -calculations.

Response to light. The experimental communities were dominated by diatoms which made up >99% of B in the final samples of all light treatments. Mixotrophic dinoflagellates followed with <0.6%, cryptophytes with <0.3% and picoplankton with 0.1%. Microzooplankton (ciliates and heterotrophic dinoflagellates) formed always <0.4% of autotroph (incl. mixotroph) biomass. B increased with light and community mean cell size increased with light (Table 3-I; Fig. 3-2A and 2B, respectively), whereas mean surface to volume ratio and the mean shape index decreased with light (Table 3-I; Fig. 3-2C and 2D, respectively). At the community level (i.e. among species), the three morphometric traits were strongly correlated: V to S:V: $r = -0.97$ ($n = 9$; $p < 0.0001$), V to SI: $r = -0.95$ ($n = 9$; $p < 0.0001$), S:V to SI: $r = 0.99$ ($n = 9$; $p < 0.0001$). Morphometric traits of the individual species (means across all treatments) were less strongly correlated among each other than at the community level, V to S:V: $r = -0.44$ ($n = 20$; $p < 0.05$), V to SI: $r = 0.313$ ($n = 20$; ns), S:V to SI: $r = 0.44$ ($n = 20$; $p < 0.05$).

The changes of community mean morphometry resulted both from species shifts and from shifts within species. Eleven species (all diatoms; Table 3-I) were abundant enough to be sized separately for each mesocosm. Together they made up >94% of B. Species showing the highest relative biomass at low light were thin needle- or spindle- shaped (*Pseudo-nitzschia pungens*, *Pseudo-nitzschia delicatissima*, *Rhizosolenia pungens*, *R. setigera*, *Thalassionema nitzschioides*) or morphologically complex (*C. danicus*, see also below). The small, stout cylindrical *Skeletonema marinoi* was the only species achieving its maximal p_i at medium light. Species with a high light optimum were discoid cylinders (both *Thalassiosira*-types) and the very large, elongated cylindrical *Ditylum brightwellii*. The majority of species showed a significant response in morphometric traits to increasing light and most of the significant cases followed the general pattern of increasing V, and decreasing S:V and SI with light intensity (Table 3-I). The exception

was the SI of *S. marinoi* and *T.* “small” (unidentified species, called *T.* “small”), for which SI increased with light. However, these two species never exceed 3% of B, thus having only a negligible impact on community mean traits.

Table 3-I ANOVA results of the response of relative biomass (p_i), the mean cell volume (V), the mean surface to volume ratio (S:V), and the shape index (SI) to light treatments. p_i values were logit transformed, V, S:V and SI log-transformed before analysis. Numbers designate $F_{2,6}$ from ANOVA analysis; Significant results ($p < 0.05$) are in bold. *: $p < 0.05$, **: $p < 0.01$; letters L, M and H indicate the light treatment with the highest values of p_i , V, S:V and SI, respectively.

Species	Total phytoplankton biomass ($\text{mm}^3 \text{ L}^{-1}$); p_i	V (μm^3)	S:V (μm^{-1})	SI
Community mean response	126.6**	61.1**	287.1**	245.6**
<i>Cerataulina pelagica</i>	2.47	305.4**H	527.5**L	48.1**L
<i>Chaetoceros danicus</i>	368.6**L	21.1**H	14.3**L	11.7**L
<i>Ditylum brightwellii</i>	52.1**H	89.3**H	191.2**L	11.4**L
<i>Pseudo-nitzschia delicatissima</i>	1110**L	0.11	0.08	0.10
<i>Pseudo-nitzschia pungens</i>	82.5**L	0.03	0.02	0.02
<i>Rhizosolenia pungens</i>	172.5**L	0.20	0.19	0.23
<i>Rhizosolenia setigera</i>	18.6**L	0.43	0.62	0.09
<i>Skeletonema marinoi</i>	17.7**M	64.7**H	42.3**L	54.9**H
<i>Thalassionema nitzschioides</i>	64.8**L	43.0**H	40.0**L	22.2**L
<i>Thalassiosira</i> big“	71.2**H	37.3**H	41.5**L	2.24
<i>Thalassiosira</i> “small“	0.85	53.0**H	43.5**L	123.0**H

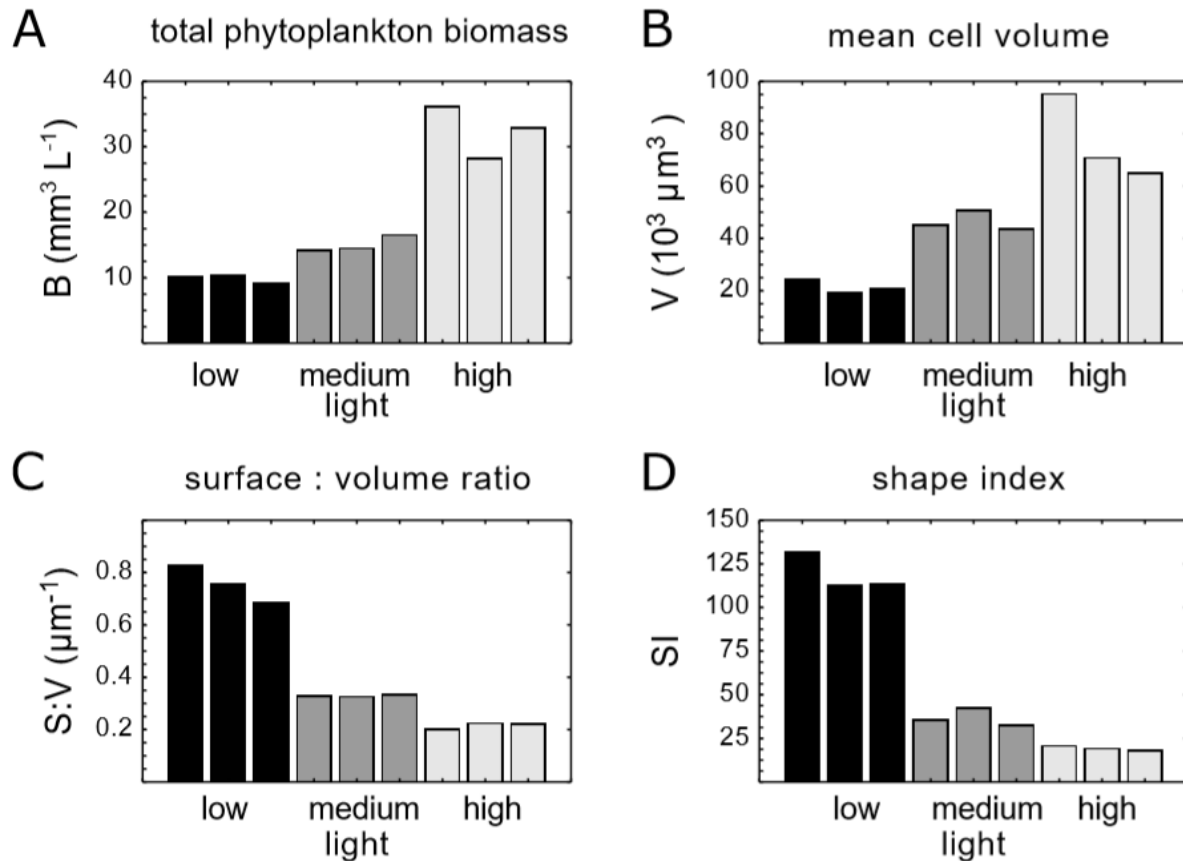


Fig. 3-2 Response of phytoplankton community properties to low, medium, and high light pre-treatment: A: total phytoplankton biomass (B) ($\text{mm}^3 \text{L}^{-1}$), B: mean cell volume ($10^3 \mu\text{m}^3$), C: surface : volume ratio (μm^{-1}), D: shape index (SI) (dimensionless).

Five species achieved individually a relative biomass of 10% in at least one treatment (Fig. 3-3). Among them *Chaetoceros danicus* and *Rhizosolenia setigera* were dominant in the low-light treatments (Fig. 3-3 A, E). *Chaetoceros danicus* is a medium sized ($5\,000$ to $15\,000 \mu\text{m}^3$) diatom owing its high S:V (0.5 to $1 \mu\text{m}^{-1}$) and SI (140 to 300) to its long spines which contain chloroplasts. Its morphometric traits responded similarly to light as the community mean traits (Fig. 3-3 B-D; Table I). *R. setigera* is a large ($40\,000$ to $130\,000 \mu\text{m}^3$) diatom with an elongate, cylindrical, almost needle like shape (SI = 100 to 150) and a moderate S:V ratio (0.25 to $0.35 \mu\text{m}^{-1}$). While its relative biomass declined with light (Fig. 3-3E; Table 3-I), its morphometric traits showed no response to light (Fig. 3-3F-H; Table 3-I).

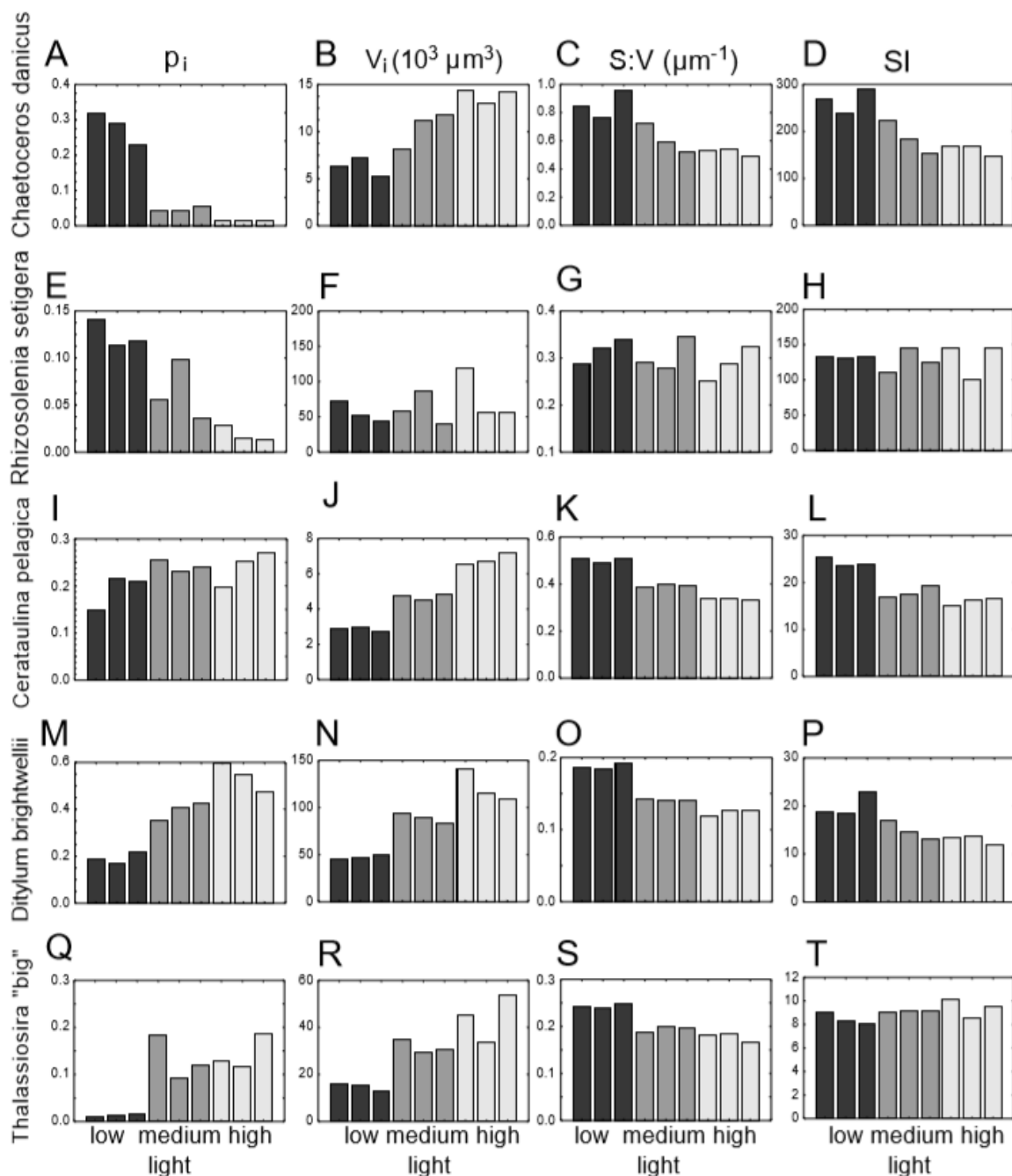


Fig. 3-3 Response of the five most important phytoplankton species (*Chaetoceros danicus*: A, B, C, D; *Rhizosolenia setigera* E, F, G, H; *Cerataulina pelagica*: I, J, K, L; *Ditylum brightwellii*: M, N, O, P; *Thalassiosira* "big": Q, R, S, T) to low, medium, and high light pre-treatment: 1st column: relative biomass (p_i) (dimensionless), 2nd column: cell volume (V_i) ($10^3 \mu\text{m}^3$), 3rd column: surface:volume ratio (S:V) (μm^{-1}), 4th column: shape index (SI) (dimensionless).

Cerataulina pelagica was the only major species showing no significant relationship between relative biomass and light, but intraspecific trait changes followed the same pattern as community means (Fig. 3-3I-L; Table 3-I). It is a cylinder-shaped, medium sized (2500 to 7700 μm^3) diatom with a moderate S:V ratio (0.33 – 0.51 μm^{-1}) and SI (14 – 26). Two of the major species were dominant at high light, the diatoms *Ditylum brightwellii* and the big type of *Thalassiosira* (unidentified species, called *T. “big”* henceforth) (Fig. 3-3 M, Q). *D. brightwellii* is very large (40000 - 150000 μm^3) and characterized by elongate cylindrical cells with a low S:V ratio (0.10 – 0.20 μm^{-1}) and a moderate SI (12 - 23). Its morphometric traits showed the same response to light as the average community traits (Fig. 3-3 N-P; Table 3-I). The cells of *T. “big”* are flat, disc-shaped cylinders of moderately large size (15000 – 55000 μm^3), a low S:V ratio (0.15 – 0.25 μm^{-1}) and a low SI (8 – 10). Its cell volume and S:V ratio followed the general community trend in response to light (Fig. 3-3 R, S), while there was no significant response of SI (Fig. 3-3 T; Table 3-I).

Response to nutrients. Biomass in the nutrient treatments reached a plateau after 4 d, which was 2.4 to 4 times (mean: 2.84) higher than the biomass before nutrient addition (Fig. 3-1). In the control treatments, biomass increased slowly and only reached the 1.1 to 1.5 (mean 1.3) fold of the initial value. The estimate of community μ_{nsat} (nutrient saturated net growth rate during 1st day after nutrient addition) depended on the light pre-treatment (ANOVA: $F_{2,6} = 12.2$; $p < 0.01$), with the communities originating from the low-light pre-treatments having the highest and communities originating from the high-light treatments the lowest μ_{nsat} (Fig. 3-4A). For the NEB built-up the pattern was vice-versa, with the highest values obtained for the high-light pre-treated communities and the lowest values for the low-light pre-treated communities (ANOVA: $F_{2,6} = 24.5$; $p < 0.001$) (Fig. 3-4B). Since phytoplankton growth in the control treatments was much smaller than in the nutrient treatments, omission of the correction term ($B_{\text{Cmax}} - B_{\text{C0}}$) would have had almost no influence on the calculated NEB. The regression analyses of μ_{nsat} and NEB on morphological traits showed a negative effect of cell volume on μ_{nsat} and a positive one of S:V and SI, while the reverse was found for NEB (Table 3-II). The highest r^2 values were found for the μ_{nsat} - V and for the NEB – SI relationships (Fig. 3-4).

Table II Regression of μ_{nsat} and NEB (nutrient effect on biomass) on community means of morphological traits (V: cell volume in μm^3 ; S:V: surface to volume ratio; μm^{-1} ; SI: shape index, dimensionless); F_{df} stands for ANOVA F-statistic; Significant results ($p < 0.05$) are in bold. *: $p < 0.05$, **: $p < 0.01$

Equation	$F_{2,6}$	r^2
$\ln \mu_{\text{nsat}} = 1.082 - 0.162 \ln V$	13.88**	0.62
$\ln \mu_{\text{nsat}} = -0.488 + 0.149 \ln (S:V)$	11.78*	0.57
$\ln \mu_{\text{nsat}} = -1.061 + 0.111 \ln SI$	12.37**	0.59
$\ln \text{NEB} = -0.253 + 0.994 \ln V$	36.47**	0.82
$\ln \text{NEB} = 9.351 - 0.958 \ln (S:V)$	46.9**	0.85
$\ln \text{NEB} = 13.061 - 0.723 \ln SI$	62.6**	0.89

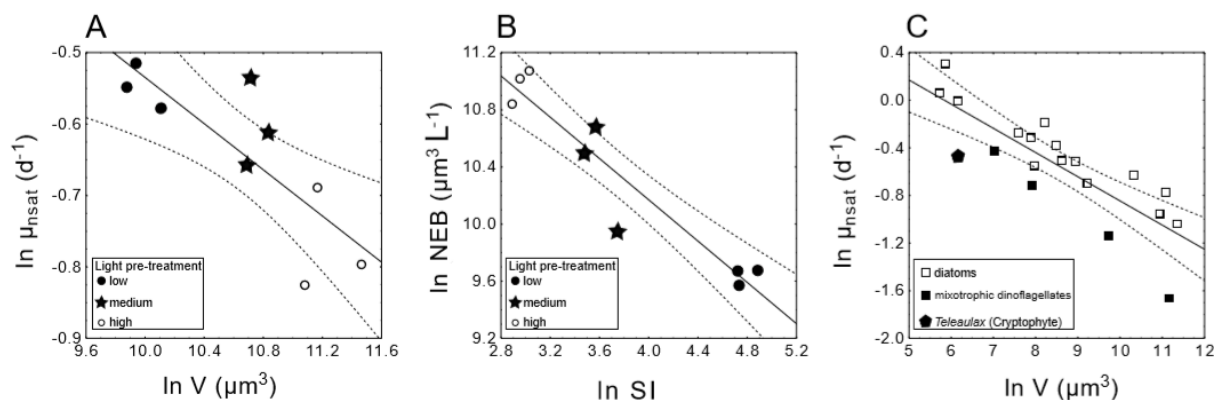


Fig. 3-4 A: regression of community μ_{nsat} on community mean cell volume (V); B: regression of the nutrient effect on biomass built-up (NEB) on the community mean shape index (SI); and C: regression of single species μ_{nsat} (means across all nutrient treatments) and cell volume (V). Continuous line: regression line; broken line: 95% confidence limits.

The chemical analyses supported the increased nutrient conversion into biomass after high light pre-treatment. Particulate matter N:P ratios in the nutrient addition treatments were around the Redfield-ratio of 16:1 (14.9+2.9 SD), indicating that the phytoplankton community was in the transition zone between N- and P-limitation. C:N ratios increased from the LL to the HL pre-

treated communities (ANOVA: $F_{2,6} = 12.59$, $p < 0.01$). The same trend was found for C:P ratios (ANOVA: $F_{2,6} = 17.83$, $p < 0.005$). Regressions on C:N and C:P ratios with morphometric traits showed the significant positive relationships with community V and negative ones with community S:V and SI (Table 3-III).

Table III Regression of C:N and C:P ratios on community means of morphological traits (V: cell volume in μm^3 ; S:V: surface to volume ratio; μm^{-1} ; SI: shape index, dimensionless); F_{df} stands for ANOVA F-statistic; Significant results ($p < 0.05$) are in bold. *: $p < 0.05$, **: $p < 0.01$

Equation	$F_{2,6}$	r^2
$\ln(\text{C:N}) = -0.82 + 0.26 \ln V$	13.37**	0.61
$\ln(\text{C:N}) = 1.75 - 0.23 \ln(\text{S:V})$	8.63*	0.49
$\ln(\text{C:N}) = 2.63 - 0.17 \ln \text{SI}$	8.78*	0.49
$\ln(\text{C:P}) = -0.44 + 0.49 \ln V$	44.05**	0.85
$\ln(\text{C:P}) = 4.22 - 0.45 \ln(\text{S:V})$	37.72**	0.81
$\ln(\text{C:P}) = 5.89 - 0.32 \ln \text{SI}$	27.38**	0.77

Individual species μ_{nsat} (means across all nutrient treatments) were related to V (Fig. 3-4C), S:V and SI (Table 3-IV), but picoplankton had to be removed from the analysis because of negative μ_{nsat} -values, possibly caused by nanoflagellate grazing. V was clearly the strongest predictor of μ_{nsat} while the relationship between SI and μ_{nsat} was non-significant. Nutrient saturated growth rates of flagellates (the cryptophyte *Teleaulax* and 4 mixotrophic dinoflagellates) were lower than the growth rates of similar sized diatoms (Fig. 3-4C). An ANCOVA showed a significant effect of V ($F_{1,1,17} = 97.5$; $p < 0.00001$) and of type (diatom vs. flagellate, $F_{1,1,17} = 28.47$; $p < 0.0001$) on μ_{nsat} while no significant interaction effect was found. Separate $\ln \mu_{\text{nsat}} - \ln V$ regressions for diatoms are also shown in Table 3-IV.

Table IV Regressions of μ_{nsat} of individual species morphological traits (V: cell volume in μm^3 ; S:V: surface to volume ratio; μm^{-1} ; SI: shape index, dimensionless; means across all mesocosms); F_{df} stands for ANOVA F-statistic; Significant results ($p < 0.05$) are in bold. *: $p < 0.05$, **: $p < 0.01$

Equation	$F_{1,17}$	r^2
$\ln \mu_{\text{nsat}} = 1.186 + 0.203 \ln V$	38.9**	0.67
$\ln \mu_{\text{nsat}} = 0.364 + 0.294 \ln (S:V)$	8.703*	0.29
$\ln \mu_{\text{nsat}} = -0.467 - 0.022 \ln SI$	0.067	0.05
only diatoms	$F_{1,13}$	
$\ln \mu_{\text{nsat}} = 1.92 - 0.191 \ln V$	67.9**	0.83
only flagellates	$F_{1,3}$	
$\ln \mu_{\text{nsat}} = 1.27 - 0.253 \ln V$	31.7**	0.88

At the end of the experiment (mean of last two samples) the nutrient treatments had no significant effect on the community means of morphological traits while light pre-history still had significant effects: $\ln V$: $F_{2,12} = 46.4$; $p < 0.0001$, $\ln SI$: $F_{2,12} = 122$; $p < 0.0001$; $\ln (S:V)$: $F_{2,12} = 5.23$; $p < 0.05$. The nutrient-light interactions were non-significant.

Discussion

Our results confirm hypothesis 1 (community mean cell size increased, but S:V ratios and SI decreased with increasing light). For the majority of species, hypothesis 2 (the morphometric effects of light supply on phytoplankton had largely the same direction on the intraspecific and on the interspecific level) was also supported. Our experimental design does not directly distinguish between phenotypic response and clonal selection. However, the specific mechanism of diatom cell growth and division impose restrictions on phenotypic responses. During the growth of individual cells, only the length of the axis perpendicular to the division plane (perivalvar axis) can increase. Thus, the only mechanism to obtain larger cells would be cell division at bigger length of the perivalvar axis. This means, if elongate cylinders (e.g. *Ditylum*, *Cerataulina*) become bigger, V and SI should change in the same direction, i.e. they should become even more elongate in proportions. Conversely, for discoid cylinders V and SI should change in opposite directions, i.e. they should become thicker. Our results, however, were just the opposite. *Ditylum* and *Cerataulina*

became stouter under high light, while *Thalassiosira* became proportionately flatter, i.e. the diameter grew stronger than the height. There is one additional, though very weak, mechanism of size change related to cell divisions. The cross-sectional area perpendicular to the pervalvar axis of one of the daughter cells decreases, though only slightly, with each cell division. Thus, the size of the cross-sectional area should decrease faster at higher growth rates, in our case at higher irradiance. Again, the opposite was observed in our experiment. Therefore, we conclude that intraspecific morphometric changes more likely resulted from clonal selection than from phenotypic responses.

Hypothesis 3 (higher community level μ_{nsat} after low-light pre-treatment) was also supported by our results. However, the tight correlation between V, S:V and SI makes it impossible to discern which of the morphological traits was responsible of transmitting the effects from the light-phase of the experiment to the nutrient phase. The slope of the $\ln \mu_{\text{nsat}} - \ln V$ -regression is within the usual range from -0.06 to -0.28 (Tang et al., 1995; Finkel et al., 2010). Comparing our result with literature results, however, needs a correction for temperature, because most of the reported regressions are based on data measured at or near 20°C while our experiments were conducted at 11°C. Using Eppley's (1972) Q_{10} -value of 1.88 for correction would lead to the equation

$$\ln \mu_{\text{nsat}} = 1.754 - 0.203 \ln V$$

which is remarkably close to the relationship by Edwards et al. (2012) with an intercept of 1.61 and a slope of -0.24. The vertical offset between the V - μ_{nsat} relationships of diatoms and flagellates is a known phenomenon for dinoflagellates (Tang, 1996) and can be explained by a lower chlorophyll to carbon ratio and additional investments into the cellular machinery needed for the ability to feed both autotrophically and heterotrophically.

Hypothesis 4 (higher biomass yield of high light pre-treated communities following the nutrient spike) was also supported by our experiment and exceeded the expectation based on a 0.88-power scaling of minimal N-quotas (QN_0) with cell volume (Edwards et al., 2012). Ratios of the mean individual cell volumes between the HL and the LL pre-treated communities were 3.58, which would lead to a QN_0 -ratio of 3.07, and thus to 1.17 times higher NEB for the HL pre-treated communities. However, for unknown reasons, the NEB was actually 3.82 times higher.

In community level experiments focusing on resource effects (light and nutrients), there is always the question, how much the outcome might have been influenced by top down factors. We can

exclude mesozooplankton (mainly copepods) grazing, because mesozooplankton cannot survive the filling procedure or they escape from the inlet of the pumping system. In numerous previous experiments using the same system (e.g. Sommer et al., 2012a), mesozooplankton had to be added from net catches if the experimental design required these organisms to be present. We cannot entirely exclude the possibility of microzooplankton grazing (ciliates, heterotrophic dinoflagellates), because they are transported into the mesocosms together with the phytoplankton. However, throughout the experiment they never comprised more than 0.4% of biomass, thus making grazing impacts on nano- and microphytoplankton negligible. Moreover, we cannot exclude grazing impacts of heterotrophic nanoflagellates on picophytoplankton, which might have been the cause for the negative growth rates of picoplankton at the start of the nutrient phase of the experiment. Our calculation of the NEB rests on the assumption that phytoplankton growth at the end of phase 1 stopped because of nutrient limitation, i.e. because cell quotas of the limiting were approaching Q_0 . This assumption is supported by the immediate start of phytoplankton growth after nutrient addition in comparison to the almost absence of growth in the controls. The possibility of cell quotas slightly above Q_0 at the end of the light phase is accounted for by correcting the biomass increment in the nutrient treatments by the biomass increment in the controls when calculating NEB.

The extremely tight correlations between the community level morphometric traits V , $S:V$ and SI make it impossible to decide which one would be most influential. While the tight correlation between V and $S:V$ is almost a geometric necessity, the size effect being much bigger than the shape effect, no such necessity exists for SI . There is a general tendency, that shapes deviating strongly from linearity are more common among larger phytoplankton, as demonstrated already for in situ-lake plankton by Lewis (1976). However, large phytoplankton also contains stout cylindrical shapes, i.e. short elongate or thick discoid cylinders, such as the larger *Thalassiosira sp.* in our study (SI : 8:0 to 10.1). In fact, the tight correlations between the different morphometric traits became much weaker when extending the analysis from community means to species traits. Therefore, concentrating on cell volume alone might simplify future trait based analysis, but might nevertheless be premature. Moreover, there is field evidence that medium sized phytoplankton retaining a relatively high $S:V$ ratio because of a needle-shaped morphology (high S) occupy a distinct niche characterized by deep mixing, low light and high nutrients (Reynolds, 1988, 1997; Kruk et al., 2002, 2010; Reynolds et al., 2014) as opposed to the widely known dominance of

picoplankton in chronically nutrient deficient waters. While these are all freshwater examples, a recent estuarine study by Kruk et al. (2015) showed that S:V ratios were more strongly correlated to the vertical attenuation coefficient (an indicator of light limitation) than cell volumes. Oceanic studies relating other morphometric properties than cell volume to environmental pressures are lacking so far. A further adaptive function of deviating from sphericity might be seen in reducing handling success by grazers in enlarging one or two linear axes (Katechakis et al., 2004; Sommer et al., 2006) without sacrificing too much of the metabolic advantage brought about by high S:V ratios.

Conclusions

Our experiment supports the hypothesis, that low light favours smaller phytoplankton cells, higher surface to volume ratios and stronger deviations from a spherical cell shape. High light favors larger cell size, lower surface to volume ratios and more spherical cells. These trait shifts were seen both at the community level and within species. The trait shifts induced by different light levels had strong influence on the ability of the resultant communities to utilize nutrients. More precisely, the communities resulting from the low-light pre-treatment responded to a saturating nutrient pulse with faster growth, but less resource use efficiency. The opposite was the case with the communities pre-treated by higher light. More generally speaking, our results indicate that changes in trait distributions induced by one environmental factor can influence the ability to cope with other environmental factors. Finally, our results accentuate the usefulness of morphometric traits in predicting environmental abilities and requirements of phytoplankton communities.

General discussion



General discussion

The results of my thesis provide an understanding on the importance of trait based research and the potential of master traits as community change explanatory trait. The overall aim of my thesis was to investigate if changes in phytoplankton communities can be tracked using a trait based community description. Specifically, I tested the use of the “master” trait cell size as replacement of the classical taxonomic/functional groups approaches. My experiments were based on Vinebrooke’s (2004) conceptual model of sequential stressors which had initially been coined for the issue of biodiversity effects on community stability. According to this model different effects of stressor induced diversity loss on the ability of a community to cope with further stressors are predicted. The ability to cope with the second stressor depends on the correlations between stressor resistances of species making up a community. Here, his model was adapted to the issue of trait diversity. While cited quite frequently, Vinebrooke’s et al. (2004) concept is not widely used in experiments. Recently, Stefanidou et al. (2018) implemented experiments with a heat shock and salinity changes as sequential stressors with a focus on the effects on species diversity. Resistance and resilience of communities to environmental stressors are also recently coming on the frontline (Feckler et al., 2018; Garnier et al., 2017; Remy et al., 2017) but to my knowledge my work is the first approach where trait diversity is explicitly considered in the context of Vinebrooke’s et al. (2004) model.

The grazing selection of copepods also include ciliates and heterotrophic and mixotrophic dinoflagellates. Thus, to test for alterations of the expected community response to nutrients because of micro-grazing (Chapter I), I initially applied my hypothesis of the effect of size shifted communities on different nutrient treatments on an autumn community, naturally rich in heterotrophic microzooplankton (ciliates, heterotrophic dinoflagellates and possibly HNF). As a second step, I used different complex experimental settings (Chapter II and Chapter III) and performed experiments by sequentially applying environmental stressors (grazing – Chapter II, light – Chapter III). I based my hypotheses, that I would manage to shift the size distribution of the communities with the application of the initial stressors, on the fact that the factors used are essential components of loss and growth of phytoplankton. I attempted to study the way they shape the trait distribution (cell size) in the phytoplankton community and then I assessed the

consequences of these alterations to the performance of the phytoplankton communities in different nutrient treatments (second stressor).

Cell size as indicator trait

Cell size shows a multitude of allometric relationships, e.g. to maximal growth rates, nutrient affinity, grazing resistance and sinking rates (Litchman et al., 2009; Schwaderer et al., 2011; Edwards et al., 2012; Kerimoglu et al., 2012). However, until now most of the research done to retrieve this allometries was based on data retrieved from clonal cultures of specific taxa (Banse, 1982; Sommer, 1989; Tang, 1995; Finkel et al., 2010; Edwards et al., 2012; Marañón et al., 2013). The question of the applicability of cell size as indicator trait on natural ecosystems and also the accuracy of the allometric relationships was still open. The combination of all the results of this work confirm that cell size is potentially an applicable indicator trait of growth and loss factors of phytoplankton communities. In this thesis, I conducted three mesocosm experiments, in order to test, in addition to the role of history that will be discussed below, the applicability of cell size as indicator trait. I also analyzed if taxon specific responses limit its applicability. I used communities with different characteristics (late winter, early and late autumn) in terms of species composition in order to gain as much as possible information for this study. Cell size was confirmed to be the determinant factor for species grazing vulnerability (Chapter II) and also one of the best descriptors of community characteristics after different light conditioning (Chapter I). When testing for the response of the different sized communities to nutrient stress in all experiments (Chapters I, II and III) significant relationships between cell size and growth rates were retrieved. These relationships were significant in community, size class and species level. Grazing by microzooplankton and HNF however (Chapter I and Chapter II for the Ambient community) were superimposed on the expected responses to nutrients. This was not obvious at community or species level but at the size class level (Chapter I and Chapter II). It was expected that the observed growth rates were reduced and the decreasing relationship of growth rates and cell size was less steep than it would have if no grazing activity would happen in the communities, since smaller species had more grazing losses. In all experiments the retrieved allometric coefficients were near the range of the existing literature, -0.06 to -0.28 (Tang, 1995; Finkel et al., 2010) (see also discussion Chapter I, Chapter II and Chapter III). In addition, expected differentiations among taxonomic groups, i.e. diatoms show higher growth rates compared to dinoflagellates of the same size, were also found in the experiments conducted in this thesis.

Shaping trait diversity in natural communities

As hypothesized, grazing by copepods (Chapter II) and exposure to different levels of irradiance (Chapter III) altered community taxonomic composition which was reflected in shifted cell size distributions of the communities. A similar effect was achieved by artificial sieving in the first experiment (Chapter I).

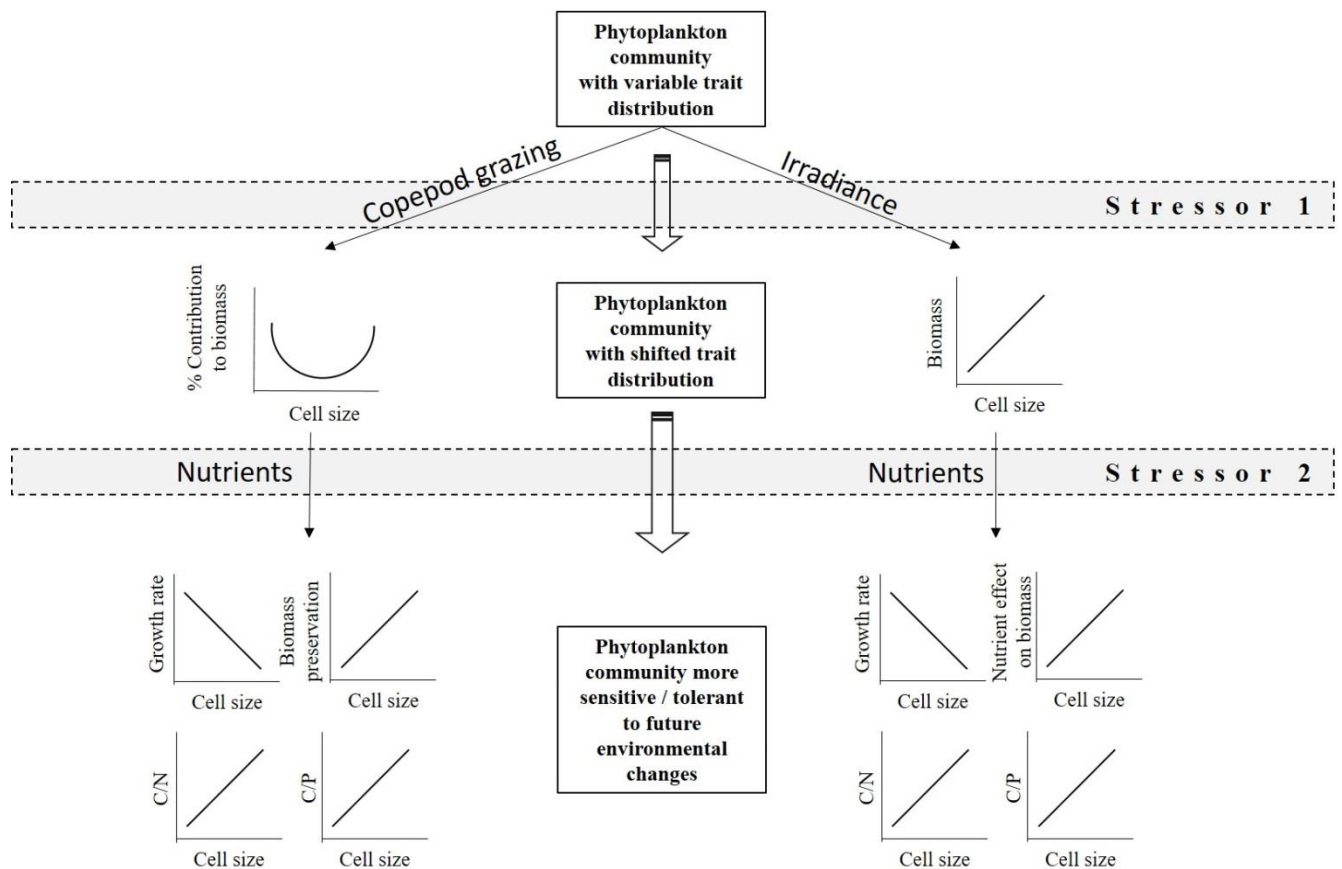
Grazing: Previous research has shown that grazing is a major factor of species loss (see also Introduction, Chapter I, Chapter II) and size selective feeding of zooplankton species is recognized since the 1980's (Gliwicz, 1980; Geller and Müller, 1981). Sommer et al. (2001) indicated that zooplankton grazing, specifically copepods and *Daphnia*, was able to shift the size distribution of the plankton communities to a dominance of smaller cells, when copepods acted on the community, or to the other end of size spectra the dominance of bigger cells, when *Daphnia* was acting as grazer. In this thesis, I confirmed again the size shift due to copepod grazing (Chapter II: Fig 2-2B & C). I found no exclusion from the copepods diet of specific species within the cell size range they target (Chapter II: Appendix 2-4), with one exception of a taxa with complex morphology (*Chaetoceros*). This means that the grazing impact had similar direction among species of similar size. Therefore, I explored the driver of species vulnerability to grazing and found a significant relationship with cell size (Chapter II: Fig 2-2). The results were similar also if exploring the grazing vulnerability of size groups instead of species (Chapter II: Fig 2-2) which allows the upscaling of the laborious detailed analysis from the phytoplankton species level to the size groups level when analyzing complex systems. Size is also the driving factor for microzooplankton grazing as observed in Chapter I, where I observed a negative effect of microzooplankton grazing on picoplankton and cells smaller than $10\mu\text{m}^3$ suppressing the growth rates of the species of this size. In the experiment described in chapter III picoplankton growth was also suppressed possibly by HNF grazing pressure. These observations agree with previous ones on the top-down impact of microzooplankton on phytoplankton size structure (Sommer et al., 2001, 2005) and the more generally used assumption of significant nanoplankton or picoplankton feeding (Sommer et al., 2017aa). The different and complex feeding strategies used by many of the heterotrophic protists (filter feeding, pallium feeding, pseudopodia etc.), however, make it difficult to assess if cell size is the driving factor also in the case of micro and nano-grazers.

Irradiance: In Chapter III, three different levels of irradiance were applied to represent moderately limiting, saturating and slightly inhibiting light conditions. Light treatment worked to shift community structures and differentiate them in species composition which was reflected in different size structures but also different morphological characteristics (Chapter III: Fig-3.2). Limiting light favored needle shaped species, with higher surface to volume ratios, which at the same time became smaller. Inhibiting light had the opposite result, with larger, more spherical cells (cylinders that became stouter) and lower surface to volume ratios, dominating the phytoplankton community. Responsible for these changes of the cells (becoming narrower or stouter) is a possible clonal selection that resulted in intraspecific morphometric changes. The light induced size structure changes during the first phase of the experiment (light treatment) led to altered time needed to use the available nutrients and reach the stationary phase of growth and to altered resource use efficiency (see also Chapter III: Fig.3-1 and Fig. 3-2). Specifically, low light treated communities needed more days to utilize all available nutrients and the conversion of these nutrients to biovolume was the lowest among the light treatments whereas the high light treated communities were faster in growth and more productive.

The role of phytoplankton trait shift history

Until today, phytoplankton responses to environmental stressors were tested either considering single stressor effects or full factorial simultaneous combinations of different stressors in order to understand the direction that plankton communities will change in the future. In this thesis, Vinebrooke's et al. (2004) original approach of sequential stressor effects on biodiversity was modified to a trait based approach. According to the modified conceptual graph of Vinebrooke et al. (2004) (Introduction-Fig. 1) and applying on it the results from the conducted experiments, with cell size as the trait of interest (Trait 1) I conclude that trait distributions depend on the ecological history of communities since both copepod grazing (Chapter II) and irradiance (Chapter III) (initial stressors) shaped the size distribution of phytoplankton communities (General Discussion-Fig 1). The application of the initially applied stressors altered the response to nutrient conditions in the differently sized communities (Chapter I, Chapter II and Chapter III). The correlations between the traits among the experiments imply that that ecological history thereby influences responsiveness to new stressors. The mean community size negatively correlated with growth rates

of the new communities, in both cases, indicating that the suppression of individuals of specific size by the first stressors lead to a remaining community of species responding differently to the second stressor. However, effects had different direction for initial growth rates after nutrient addition, C/N and C/P (positive effects of smaller cell sizes), biomass built-up and the ability to sustain the gained biomass. In addition, the combination of the knowledge retrieved from these relationships describe the advantages and disadvantages that might occur in the communities depending on the initial direction of the trait shift. For example, if the trait distribution resulting from one stressor drives the community mean cell size to smaller values then the communities will respond faster to nutrients (negative correlation with growth rate), have a healthier state in terms of metabolic activity (lower C/N and C/P ratios), but will not achieve the same nutrient conversion into biomass, as a bigger sized community would have, and also will not be able to preserve this biomass for long periods (General Discussion-Fig 1).



General Discussion-Fig 1: Trait distributions and correlations retrieved from experiments conducted in this thesis (Chapter II and Chapter III) according to modified to trait based approach of Vinebrooke's et al (2004) conceptual model on sequential stressor effects on communities.

Global change and trait based research

My findings strongly suggest cell size as a trait that can indicate phytoplankton responses to stressors in several levels (as response and effect trait). This leads to the assumption that global change scenarios can profit a lot from models that will use cell size as the driver of future responses to environmental change. The increased stratification (due to warming) and the expansion of nutrient limited ocean regions (Behrenfeld et al., 2006) in combination with the existing knowledge that small sized species (better nutrient competitors) are favored with limiting nutrient conditions will further affect the global carbon cycle. In particular, the contribution to carbon export will be limited, since small cells are less likely to sink, especially the ones without a hard cell (Litchman et al., 2015b). At the same point, the carbon and nutrient recycling in the euphotic zone will become more intense (Bopp et al., 2005). These facts, combined with increased grazing activity, due to increased metabolic rates of grazers, will increase the numbers also of the major grazers of small sized species (such as HNF and microzooplankton) and cause increased activity in the microbial loop. In addition, the energy transfer to the higher trophic levels will be limited since the increase of this parts of the food web will lead the energy transfer to the “jelly food chain” (Sommer et al., 2018), contributing less energy to nekton because of high water content and the scarcity of grazers that prefer them (Sommer et al., 2018). In contrast to the oligotrophic areas, at the highly productive northern part of the ocean, warming is already leading to ice melting, which in turn increases the euphotic zone. One, would expect that these areas would become more productive due to this change, however the nutrient supply from the deep ocean will be limited due to the changes described above. The large species that till today dominate this part of the ocean will have to deal with stressors that have contradicting effects on their growth (increased light availability, low nutrient supply). Consequently, the export of energy to the higher tropic levels, usually leading to long food web chains to the higher food web (fish and other nekton) will again be affected. All these changes to the global ocean will be supplemented by differently driven changes to the coastal systems where increased turbidity and fluctuating increased nutrient supply due to more frequent riverine runoffs will also alter the existing phytoplankton communities.

Future perspectives

This thesis is an experimental approach testing the applicability of cell size as indicator trait for natural plankton community responses to growth and loss factors. The analysis that was made was mostly in respect to this main question, however more traits that affect these responses were identified in Chapters II and III, i.e. morphometric traits as such spines (Chapter II), surface to volume ratio and shape index (Chapter III). In addition, a version of Vinebrooke's et al (2004) concept modified to include a trait based theory analysis was applied and an effort to disentangle the hardening or sensitizing of the communities resulting from to their immediate history was also made.

In order to advance reliability of my results several communities with different species composition should be tested in the future. Here, we tested three different communities but all of them originated from the same species pool, the Baltic Sea. Conducting similar experiments in different oceanic regions with communities that have different species/ traits pool (eg. including diazotrophs) and also different evolutionary history would offer a wider aspect of these responses. In addition, findings from my thesis can be further looked for in time series data analyses to draw conclusions from cell size and shapes for the development of biomass.

The environmental stressors grazing and irradiance that were tested here are factors that have strong impact on phytoplankton cell size. Due to future global change scenarios more environmental factors will be altered such as salinity, temperature, turbidity. All these changes will indefinitely change the trait distributions of phytoplankton communities, including cell size, motility, shape, etc. The application of the modified experimental scheme that was applied in this thesis needs to be applied also to other traits of interest and more trade-offs to be discovered. Putting together the knowledge retrieved from these experiments can provide reliable tools and models can be applied in order to better estimate the phytoplankton changes in the future and foresee the implications to higher trophic levels.

General discussion

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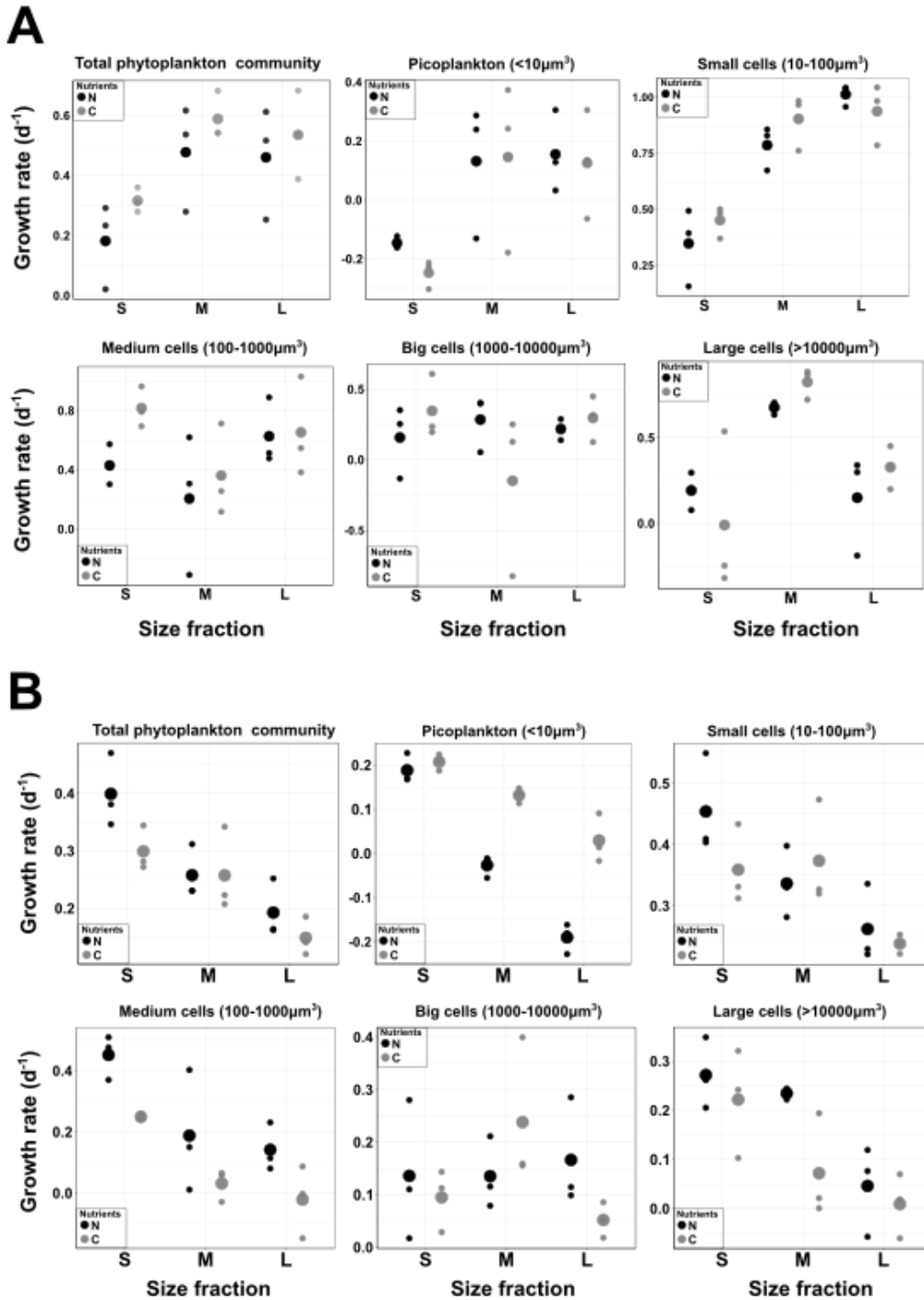
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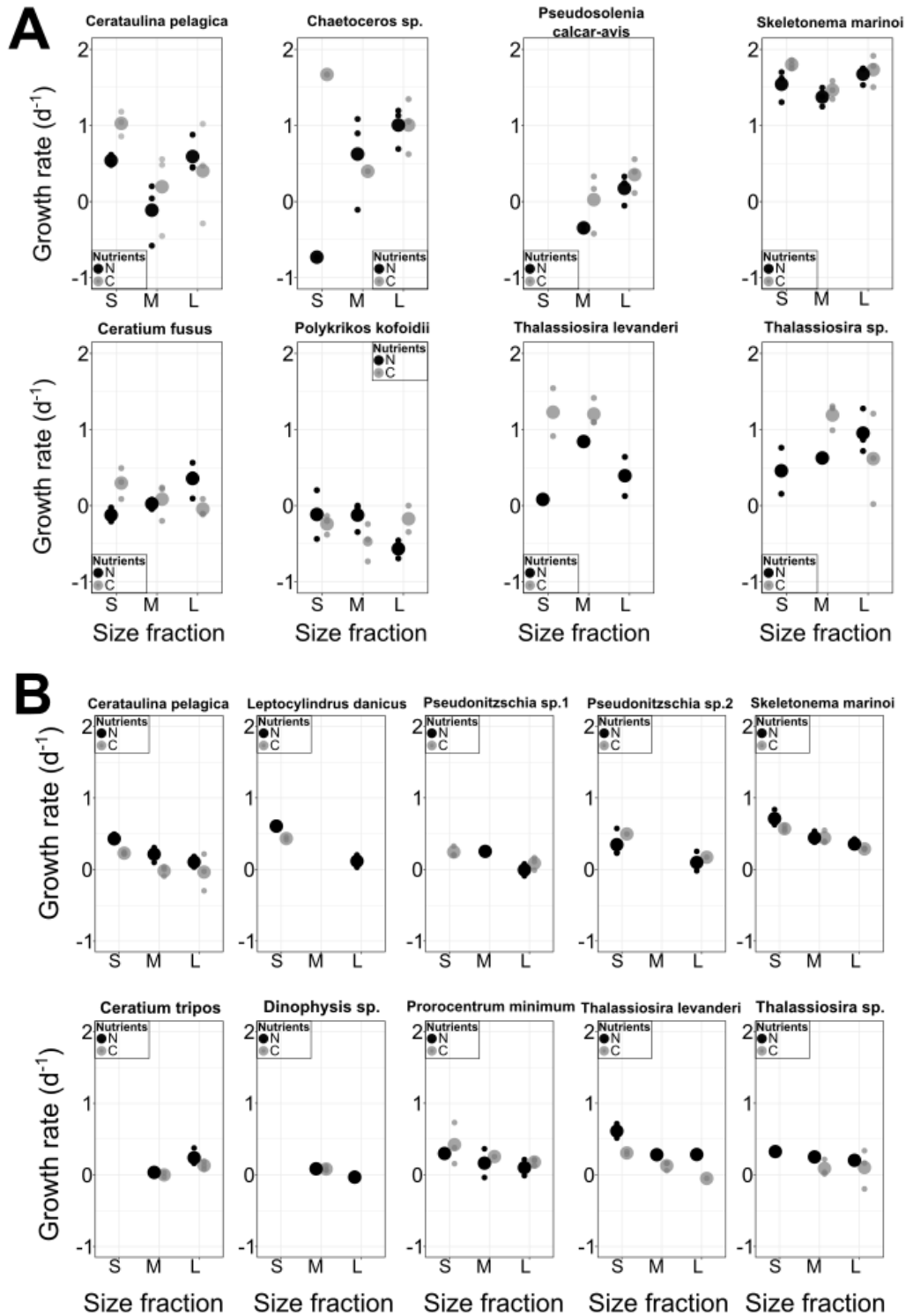
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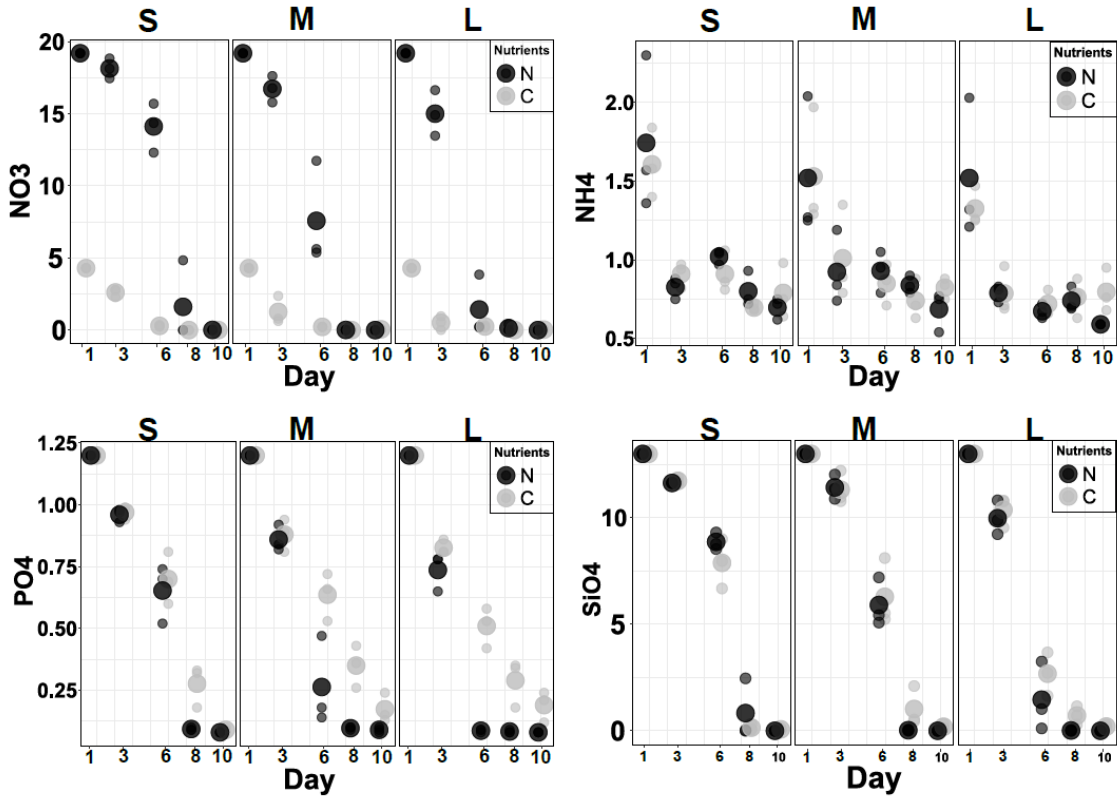
Chapter I



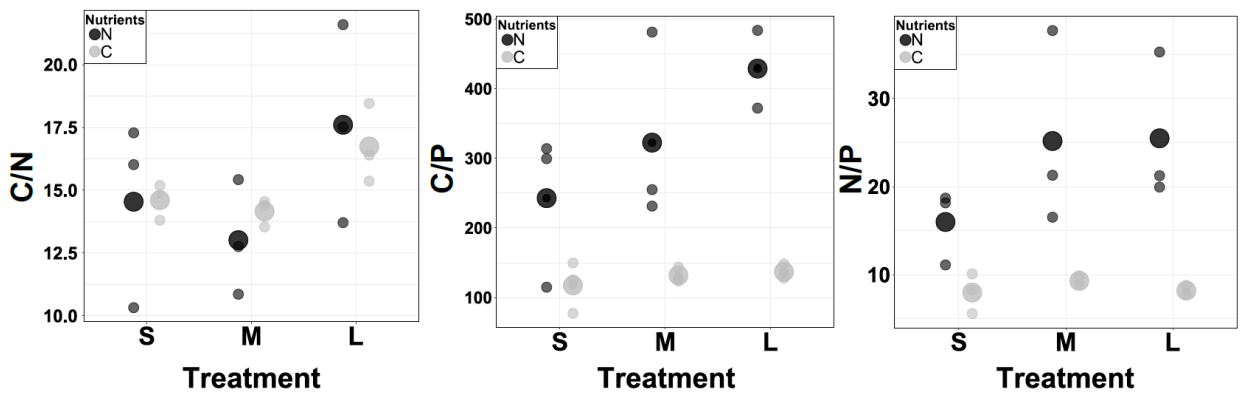
Appendix 1-1. A: Phytoplankton community and size classes growth rate of S, M, L size fractionated nutrient shifted (N) and control (C) treatments from days 1 to 3 (first period). B: Phytoplankton community and size classes growth rate of S, M, L size fractionated nutrient shifted (N) and control (C) treatments from days 3 to 10 (second period).



Appendix 1-2. A: Species growth rate of S, M, L size fractionated nutrient shifted (N) and control (C) treatments from days 1 to 3 (first period). B: Species growth rate of S, M, L size fractionated nutrient shifted (N) and control (C) treatments from days 3 to 10 (second period).

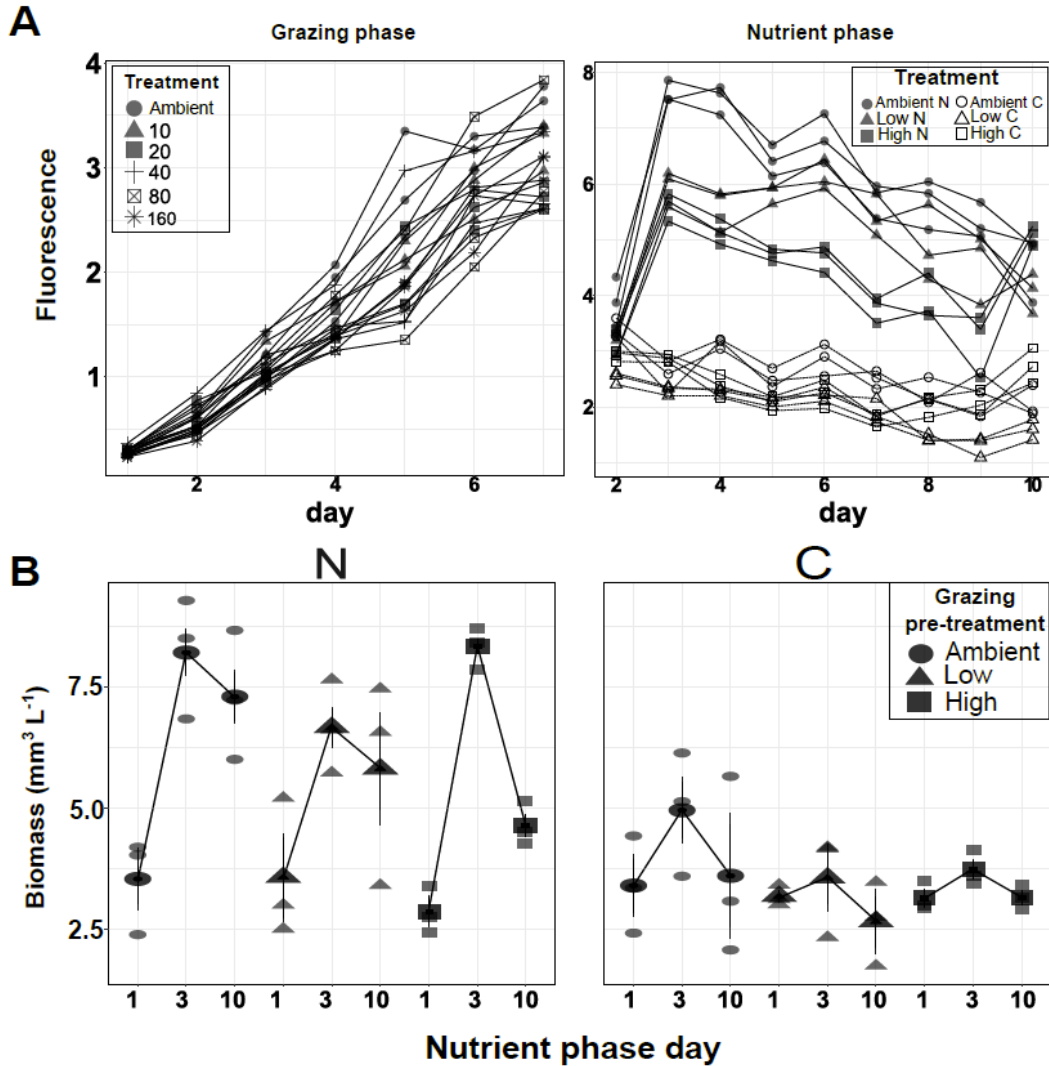


Appendix 1-3. NO₃, NH₄, PO₄ and SiO₄ concentration in S, M and L fractions and for nutrient (N) and control (C) treatments during the experiment.

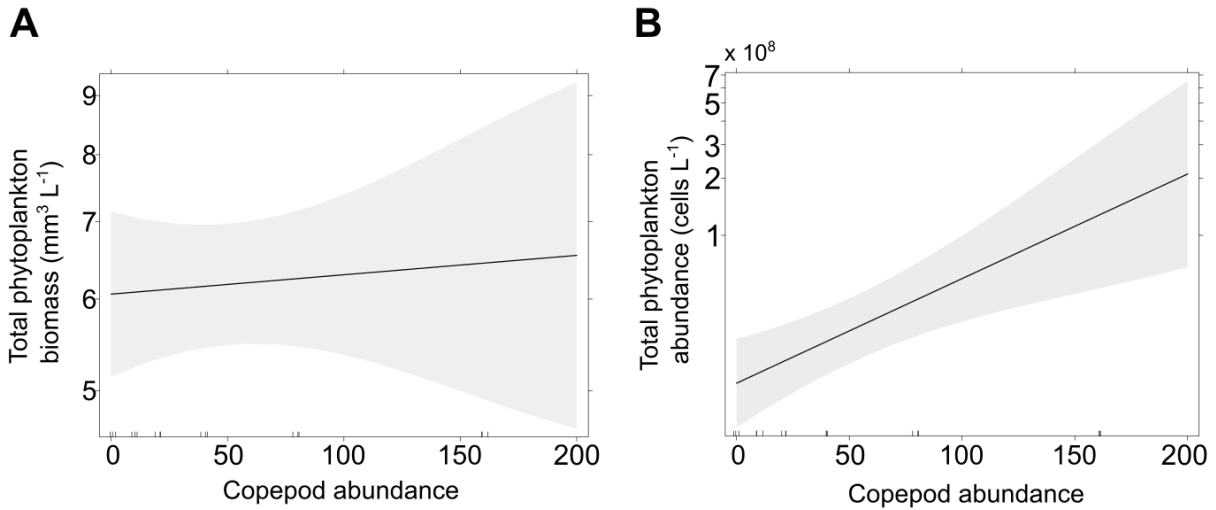


Appendix 1-4. C/N, C/P and N/P ratios at day ten of the experiment in the size fractioned (S, M and L) and nutrient (N) and control (C) treatments.

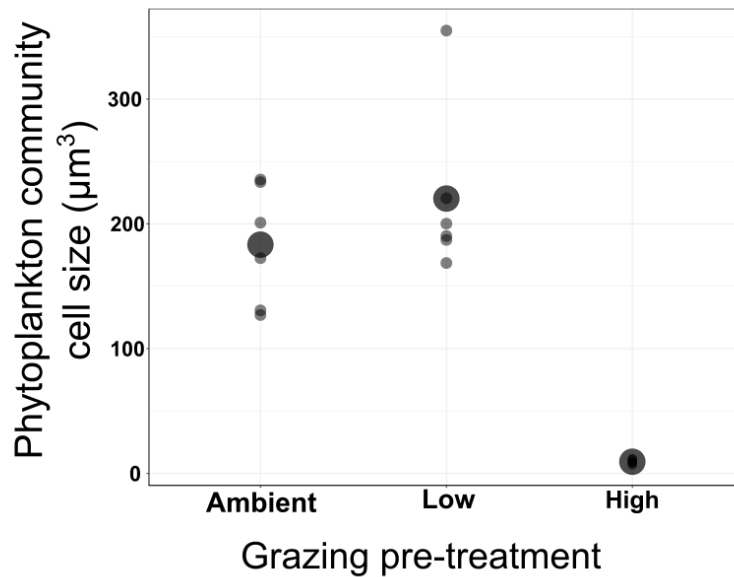
Chapter II



Appendix 2-1: A: Time course of fluorescence changes as a proxy of total phytoplankton biomass in the two experimental phases. Grazing phase: different shapes indicate the different treatments. Nutrient phase: different shapes indicate the different treatments. Grey shapes indicate the nutrient shifted treatments, empty shapes the control treatments. **B.** Time course of total phytoplankton biomass during the nutrient phase in Ambient (circles), Low (Triangles) and High (Squares) pre-grazed treatments with nutrient shift (N) and without nutrient addition (C). Lighter symbols indicate the biomass of the replicates; darker symbols indicate the average biomass of the treatments and SE.

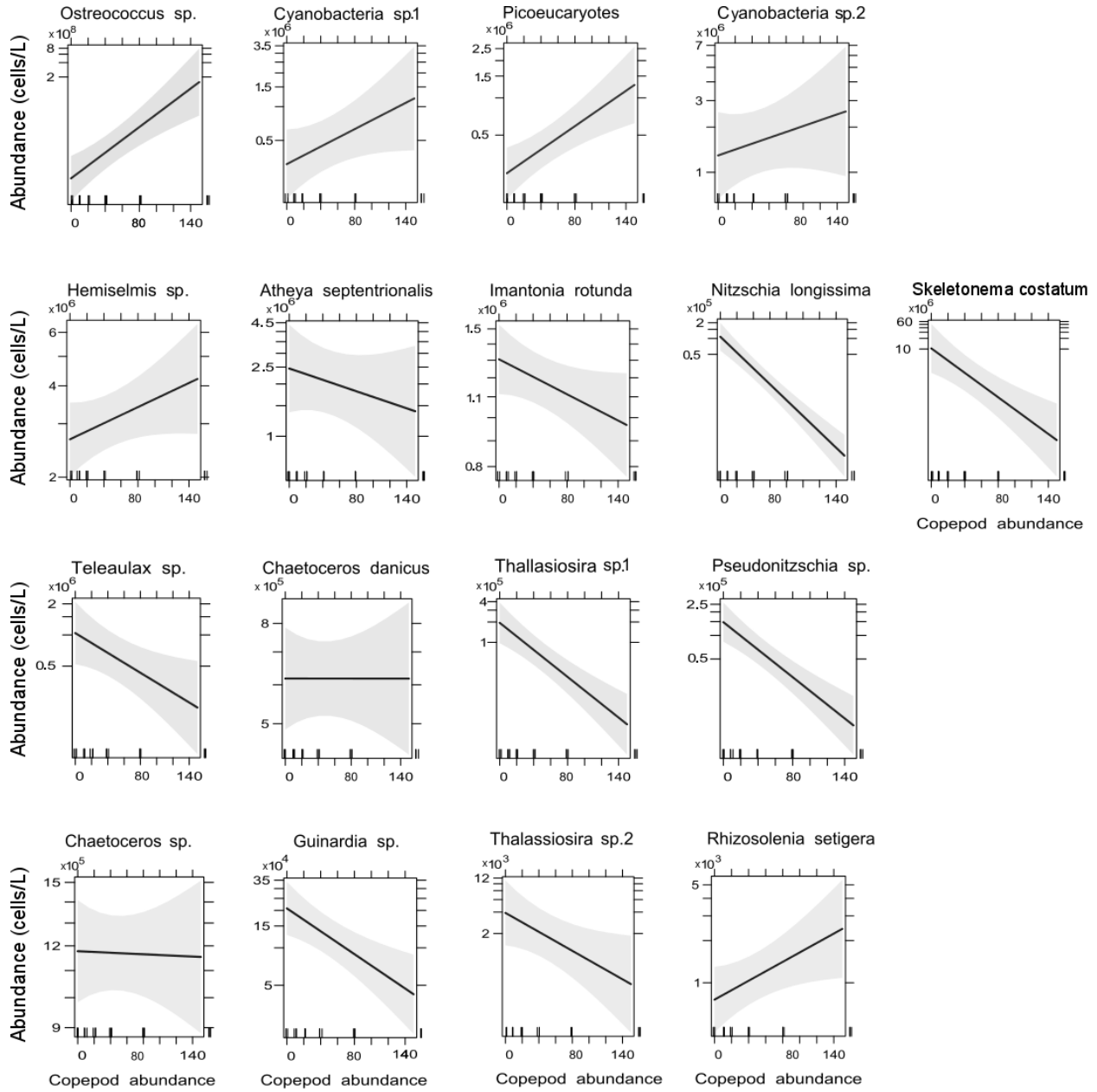


Appendix 2-2. Effect of grazing intensity to the total phytoplankton biomass (A) and abundance (B). The grey area shows the 95% C.I. Effect modeled by GLM (family GAMMA, link=log).

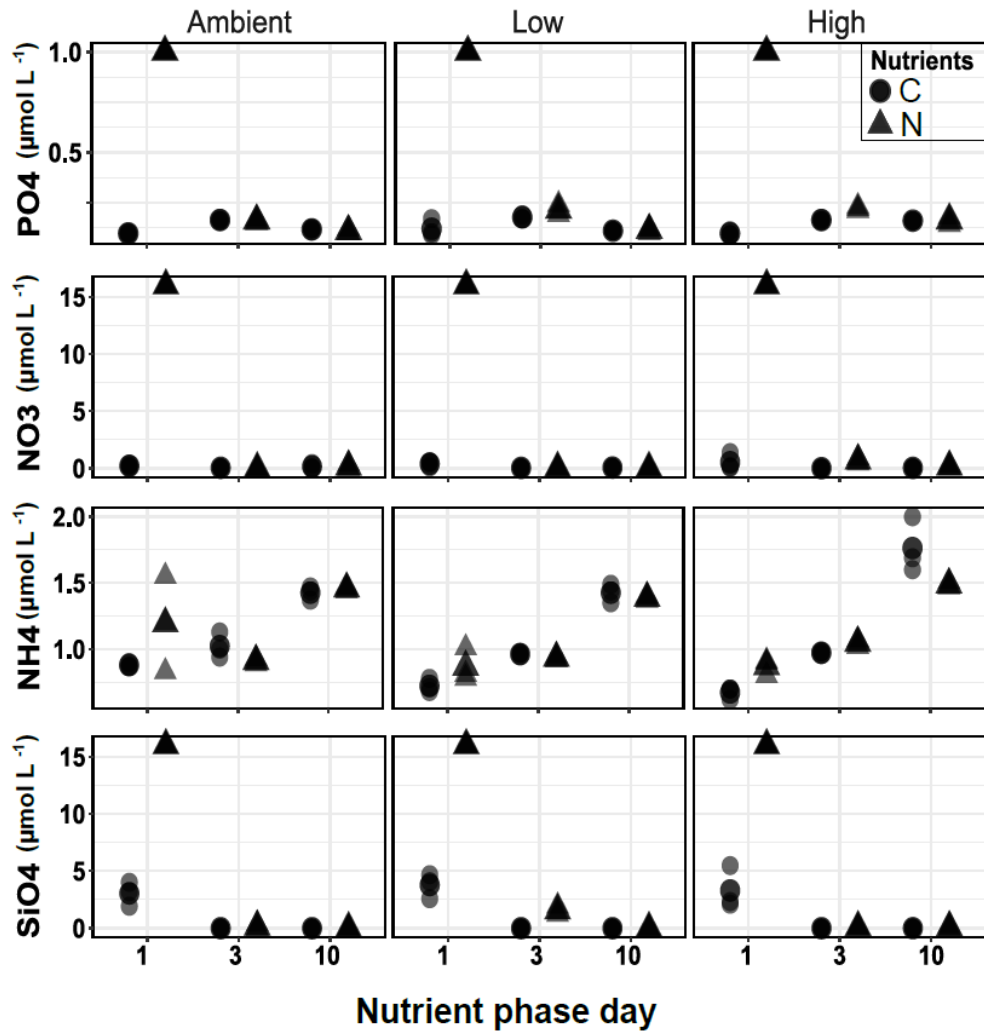


Appendix 2-3: Phytoplankton mean community cell size (μm^3) in grazing pre-treatments (Ambient, Low and High) after sieving for copepods. Smaller circles indicate the cell size of the replicates and bigger circle the mean cell size of the treatment.

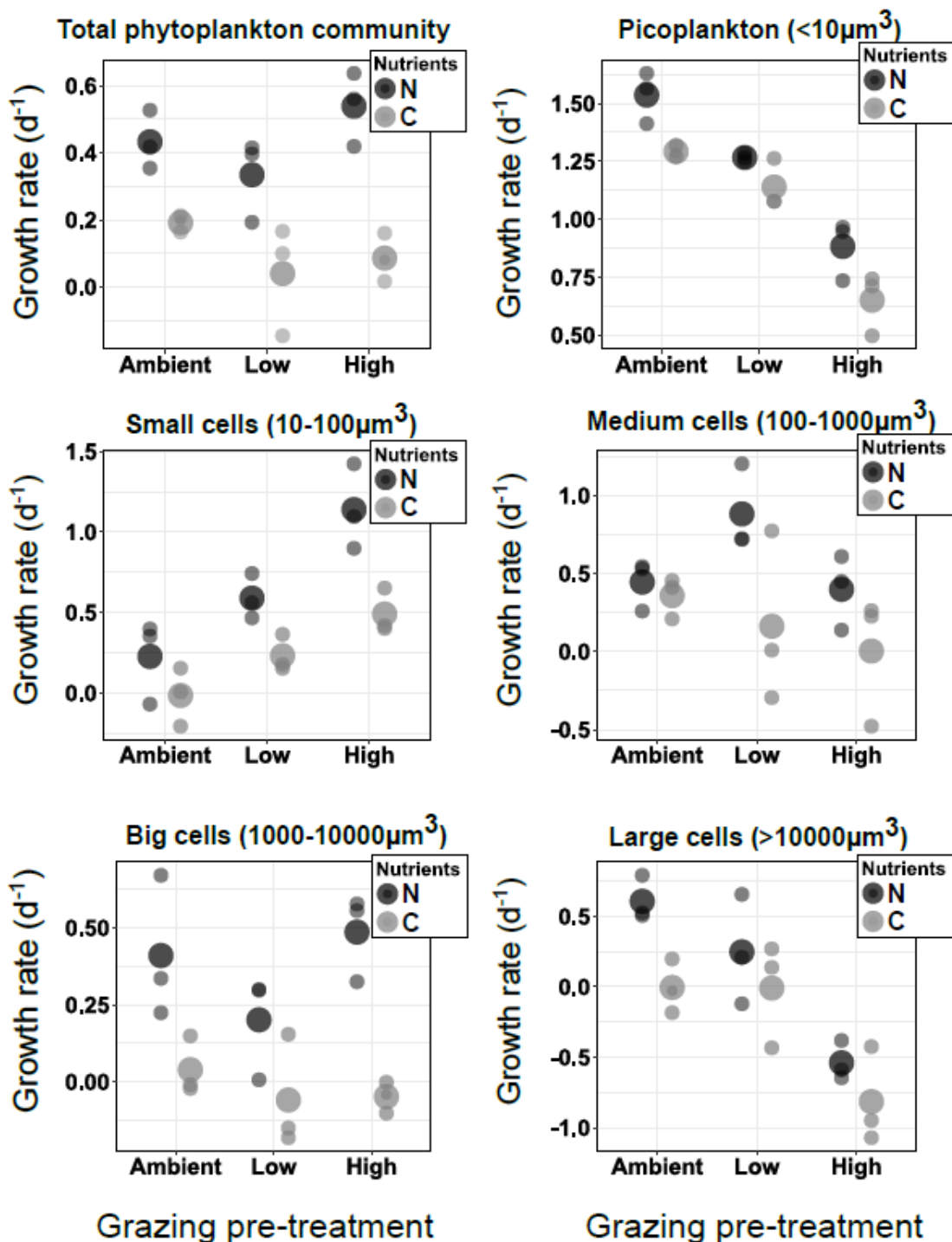
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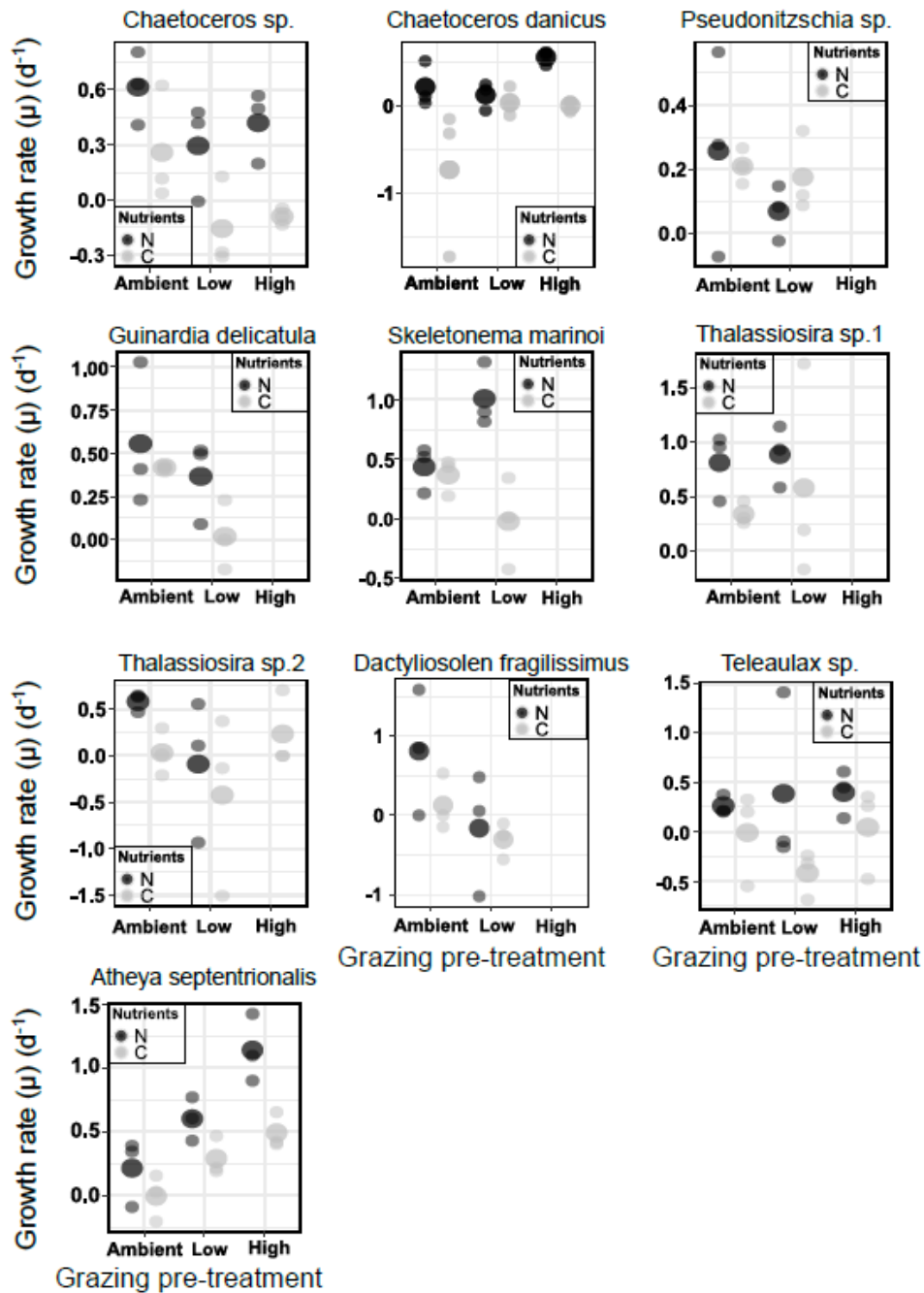
Appendix 2-4. Effect of grazing intensity to the abundance of different species. The grey area shows the 95% C.I. Effect modeled by GLM (family GAMMA, link=log).



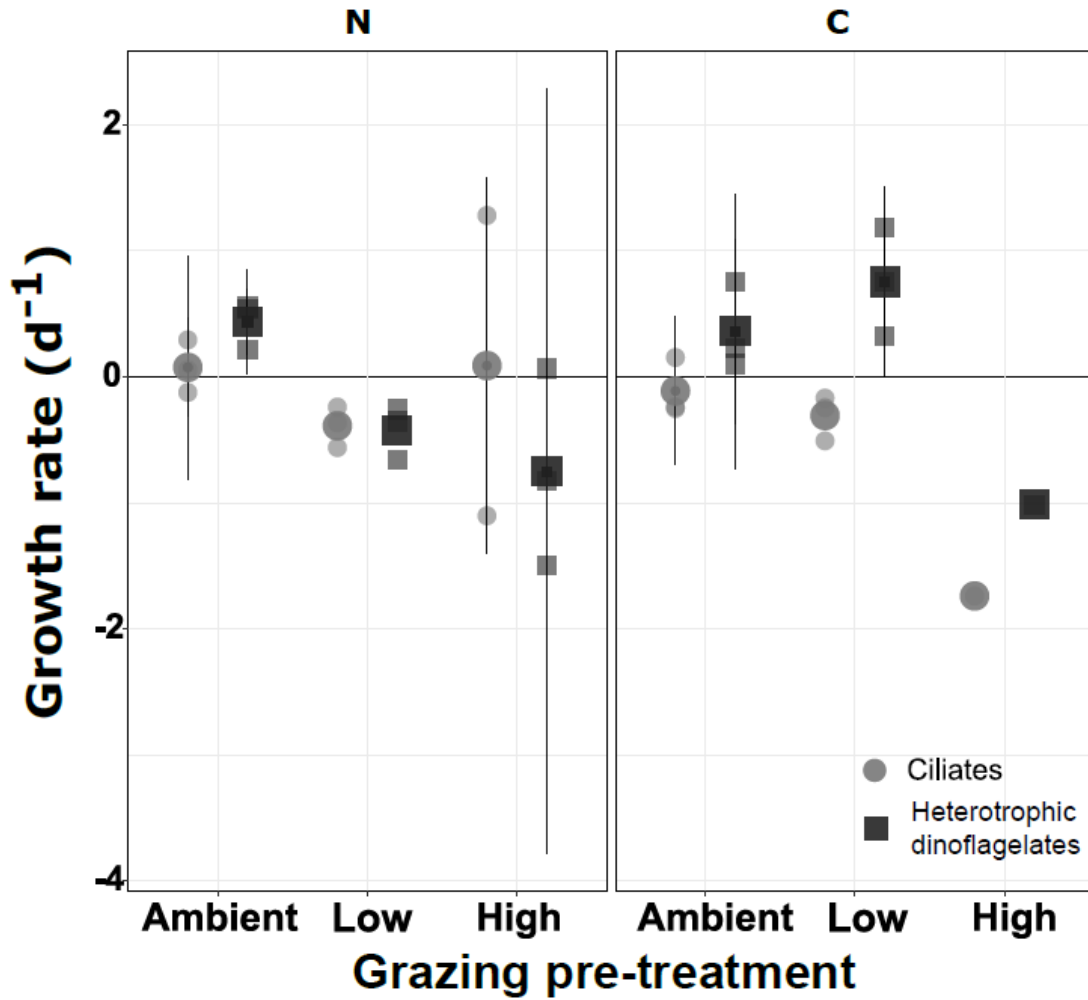
Appendix 2-5: PO₄, NO₃, NH₄ and SiO₄ concentration in Ambient, Low and High pre-grazed treatments during nutrient phase. Lighter symbols indicate the biomass of the replicates; darker symbols indicate the average nutrient concentration of the treatments.



Appendix 2-6. Total phytoplankton community and size classes (picoplankton (<10), small (10-100), medium (100-1000), big (1000-10000) and very big (>10000μm³) cells) growth rate (short term, days 1-3) for the nutrient (N) and control (C) pre-grazed treatments (Ambient, Low and High). Small circles represent the response of the replicates and big ones the mean between the replicates



Appendix 2-7 Species growth rate (short term, days 1-3) for the nutrient (N) and control (C) pre-grazed treatments (Ambient, Low and High). Small circles represent the response of the replicates and big ones the mean between the replicates

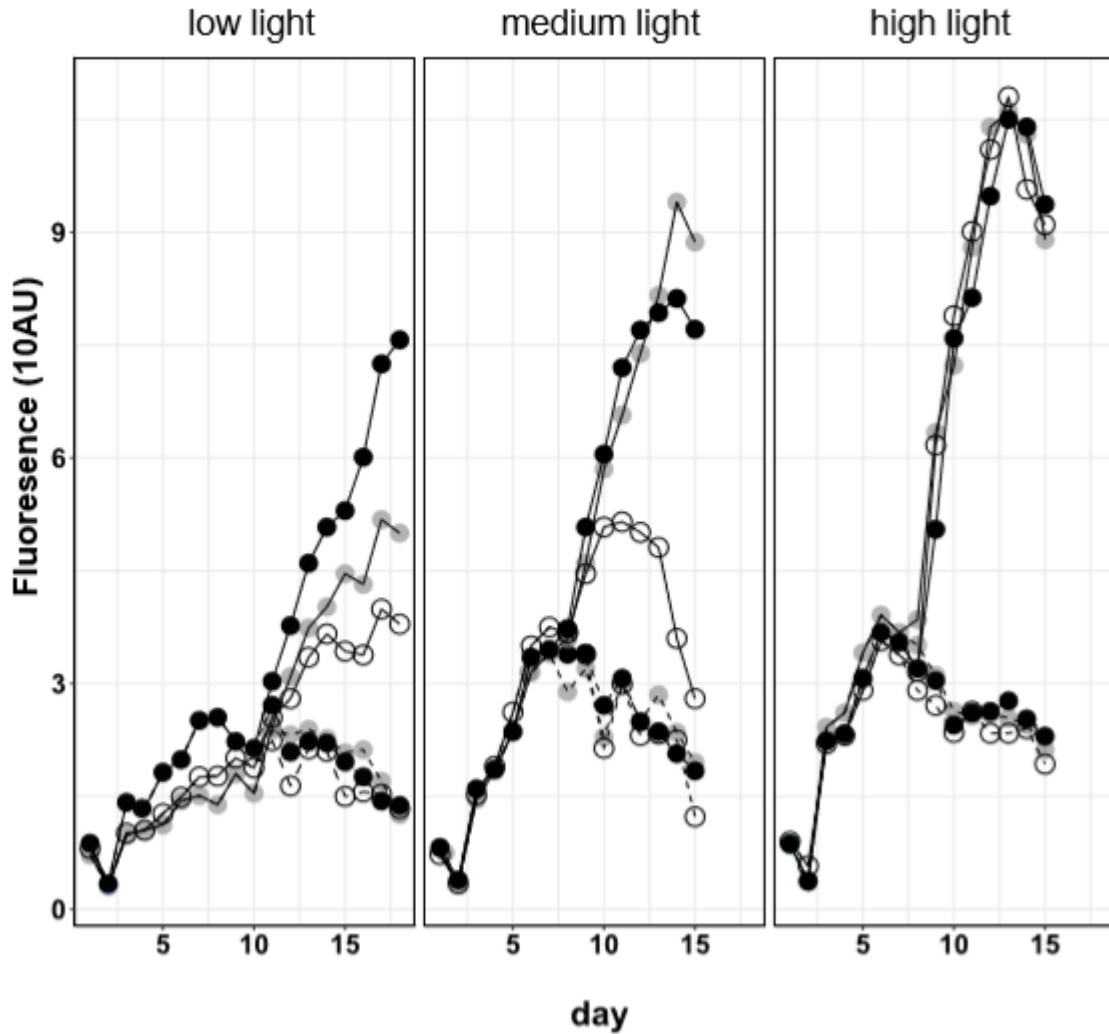


Appendix 2-8: Short term (days 1-3) growth rate of Ciliates (light grey circles) and heterotrophic Dinoflagellates (dark grey squares) for the nutrient added treatments (N) and the nutrient replete (control treatments (C)) treatments of the pre-grazed (Ambient, Low and High) treatments. Small circles and squares represent the response of the replicates and big ones the mean between the replicates with standard error lines.

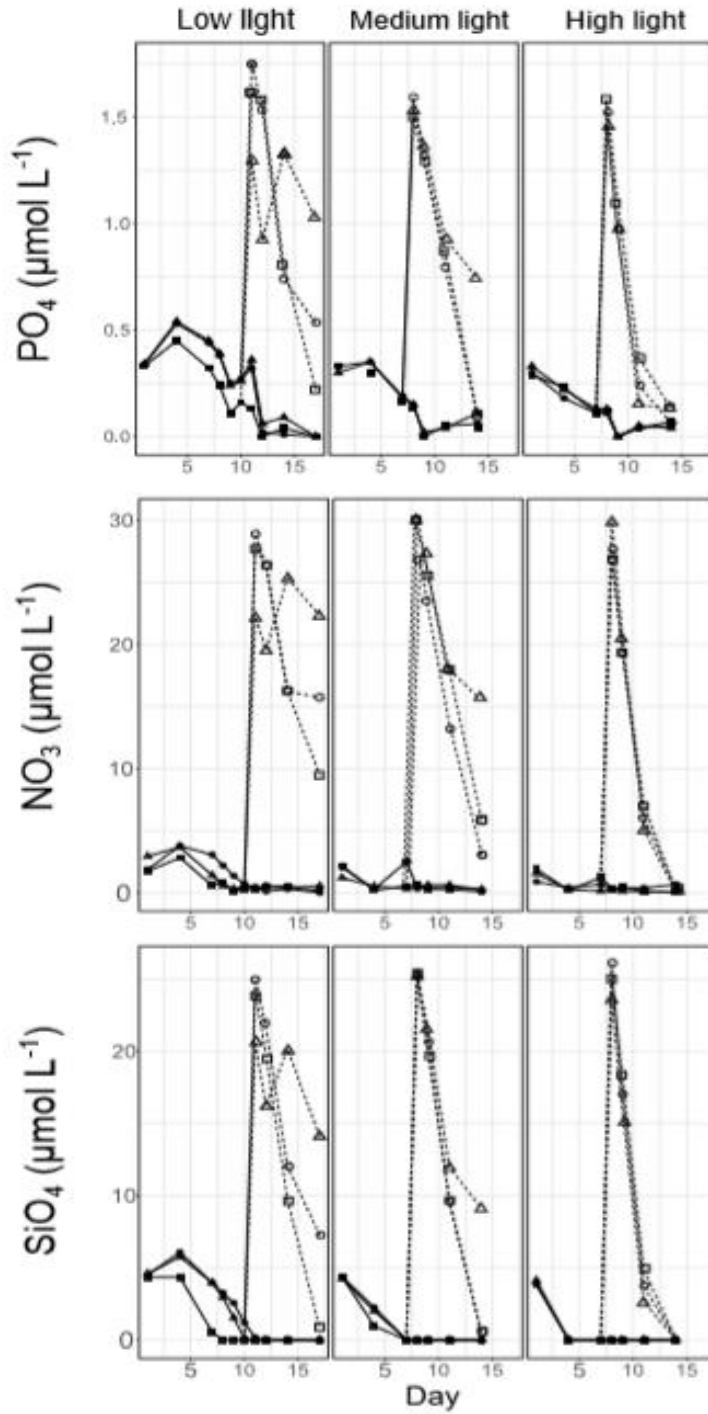
Appendix Table 2-1: Simper analysis results for the contribution of each size group to the differentiation of the pre-grazed communities.

Contrast between Ambient - Low pre-grazed communities	
Size groups (μm^3)	%
1000-10000	56.75
100-1000	40.57
>10000	1.69
10-100	0.68
<10	0.31
Contrast between Ambient -High pre-grazed communities	
Size groups (μm^3)	%
100-1000	46
1000-10000	32.76
<10	13.73
>10000	5.86
10-100	1.65
Contrast between Low - High pre-grazed communities	
Size groups (μm^3)	%
1000-10000	41.05
100-1000	23.97
<10	21.87
>10000	11.11
10-100	2

Chapter III



Appendix 3-1. Time course of fluorescence (10AU) changes as a proxy of total phytoplankton biomass in low light, medium light and high light pre-treated mesocosms. Solid line: mesocosms before nutrient addition and nutrient treatments, broken line: controls; black, grey and empty circles label the different replicates.



Appendix 3-2. Time course of dissolved nutrient concentration (PO₄; NO₃; SiO₄ (µmol L⁻¹)) in low light, medium light and high light pre-treated mesocosms. Solid line: mesocosms before nutrient addition and control treatments, broken line: nutrient treatments. Squares, circles and triangles, black and empty shapes, label the different replicates.

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Curriculum Vitae

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Publications

Charalampous, E., Matthiessen, B. and Sommer, U. (2018) Light effects on phytoplankton morphometric traits influence nutrient utilization ability. *J. Plankton Res.* 40(5), 568-579 doi: 10.1093/plankt/fby037

Sommer, U., Charalampous, E., Scotti, M. and Moustaka-Gouni, M. (2018). Big fish eat small fish: implications for food chain length? *Community Ecology*, 19(2), 107-115 doi: 10.1556/168.2018.19.2.2

Sommer, U., Charalampous, E., Genitsaris, S. and Moustaka-Gouni, M. (2017) Costs, benefits and taxonomic distribution of phytoplankton body size. *J. Plankton Res.*, 39, 494-508

Katsiapi M., Mazaris A.D., Charalampous E., Moustaka-Gouni M. (2012) Watershed land use types as drivers of freshwater phytoplankton structure. In: Salmaso N., Naselli-Flores L., Cerasino L., Flaim G., Tolotti M., Padisák J. (eds) *Phytoplankton responses to human impacts at different scales. Developments in Hydrobiology*, vol 221. Springer, Dordrecht

Description of Author contributions

Chapter I: Cell size drives the response of natural plankton communities to nutrients

Authors: Evangelia Charalampous, Ina Stoltenberg, Birte Matthiessen, Ulrich Sommer

Under preparation to be submitted

Contributions: US, EC and BM developed the idea for this study; EC conducted the experiment; EC and IS provided the data; EC performed data analysis; EC, BM and US discussed the results; EC wrote the manuscript.

Chapter II: Cell size effects on the response to nutrients depends on grazing history

Authors: Evangelia Charalampous, Birte Matthiessen, Ulrich Sommer

Under preparation to be submitted

Contributions: US, EC and BM developed the idea for this study; EC conducted the experiment; EC performed data analysis; EC, BM and US discussed the results; EC wrote the manuscript.

Chapter III: Light effects on phytoplankton morphometric traits influence nutrient utilization ability

Authors: Evangelia Charalampous, Birte Matthiessen, Ulrich Sommer

Published in Journal of Plankton Research (2018); DOI: 10.1093/plankt/fby037

Contributions: US developed the idea for this study; EC conducted the experiment; US and EC performed data analysis; EC, BM and US discussed the results; EC wrote the manuscript.

Description of individual contribution

Declaration

I, Evangelia Charalampous, hereby declare that the dissertation submitted, entitled “Trait shift history: trait shifts in phytoplankton communities induced by grazing and light affect the responses to nutrients” 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 III of this thesis was published in the scientific Journal of Plankton Research in 2018. The authors' share of the manuscripts is explained in the section "Author contributions" (page 135). 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, 22.02.2019

.....
Evangelia Charalampous