Plankton dynamics and anthropogenic changes

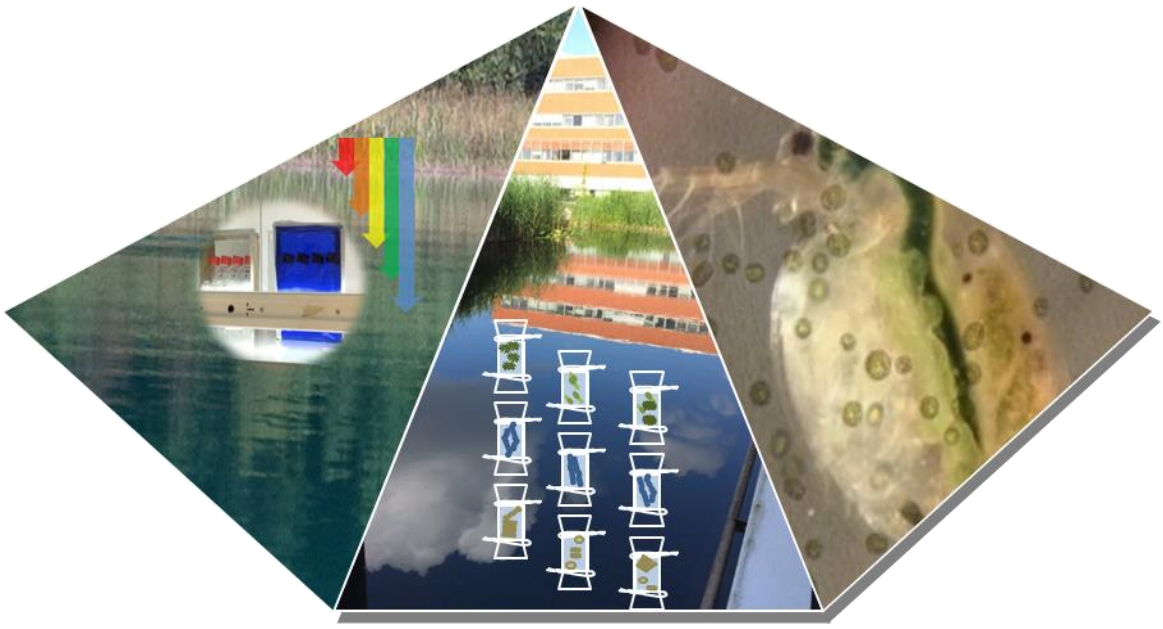

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Table of contents

Summary

Community assembly is a well-studied topic in ecology with a research history of more than 100 years. Over time, multiple theories describing the assembly of a community have been developed. However, many questions about the underlying mechanisms of community assembly processes remain unclear. Additionally, anthropogenic effects on natural environments, including climate change or fast industrial development, challenge natural communities and, ultimately, human well-being on Earth. Among other aspects, scientists developed an approach to identify the extent of human impact on the global ecosystem. This approach defines planetary boundaries for the nine most important environmental processes (climate change, ocean acidification, stratospheric ozone depletion, nitrogen/phosphorus cycle, global freshwater use, land system change, the rate of biodiversity loss, atmospheric aerosol loading, and chemical pollution). Within these boundaries, these processes sustain ecosystem services that enable ecosystem functioning for sustainable life on Earth. Ecosystem services are all naturally provided resources from which humans benefit. Anthropogenic changes to any of these processes can lead to a transgression of the recommended planetary boundary. Other features (e.g., novel entities such as substances or organisms, which were so far unknown to specific natural systems) could not yet be quantified. Thus, here I aimed to provide some further insights into the mechanisms of community dynamics under the influence of anthropogenic changes to the environment.

Phytoplankton communities are well suited to the analysis of anthropogenic environmental changes on community dynamics. Phytoplankton is a diverse taxonomic group of photosynthetic, mostly unicellular organisms with usually short generation times. Phytoplankton plays an important role in aquatic ecosystems, representing the base of nearly all aquatic food webs and being responsible for about 50% of global primary production.

In my thesis, I studied the impact of induced anthropogenic environmental changes on plankton community dynamics by performing experimental manipulations on artificial and natural phytoplankton communities and zooplankton. One of the investigated induced anthropogenic environmental changes, climate change, can alter water temperature and thereby also light climate in the water column. Light is one of the most important resources for photosynthetically active phytoplankton. Light quantity and quality can have an impact on the photosynthetic efficiency and can have consequences for algal growth. Quantity and quality of light are depended on water depth. With increasing depth light quantity and the light spectrum available for photosynthesis decrease. Through rising temperatures, water column stratification increases and reduces the depth of the upper mixed water layer (epilimnion). Thus,

phytoplankton in a water column, with lower mixing depth, will be exposed to higher light amounts but also to a different (larger) light spectrum than phytoplankton in deeply mixed water columns. In general, phytoplankton can use different light spectra through group and species-specific pigment composition. Here, under controlled laboratory conditions, I performed a light quality *in situ* experiment to investigate the responses of laboratory and field phytoplankton communities to a light quality gradient: from a reduced spectrum (only blue light, representing light spectrum conditions in deeper waters) to a full photosynthetically active irradiance (PAR) light spectrum (white light, shallow water). Diversity and evenness of natural phytoplankton communities were negatively affected by increasing exposure to blue light, whereas species richness remained unaffected. In natural communities, Cyanophyta benefited from exposure to the full PAR spectrum (decreasing mixing depth), whereas Bacillariophyta (diatoms) benefited from exposure to blue light (increasing mixing depth). These findings indicate a shift of dominant species by changing light quality, resulting in changes in phytoplankton community composition, diversity, and algal growth dynamics. My results suggest that climate change related to increasing water temperatures will also affect natural phytoplankton communities by changing mixing depths and, thereby, the underwater light climate.

In addition to abiotic environmental changes, such as climate change, biotic factors such as invading species can also change community compositions. The interactions of invading species with resident species can influence species abundances within the community. Invading species can enter new habitats by multiple ways (e.g., active or passive transport). Human activities provide additionally multifaceted ways for how species are globally distributed and can cause opening of habitat borders due to numerous increasing transport activities. However, not every invasion of new environments by organisms is successful. It seems likely that most invasion attempts are unsuccessful, and the question arises about the potential strength of ecological effects of such unsuccessful invasion events. Such *unsuccessful* species will interact with species of the resident community for a certain time. If they cannot establish themselves, they may influence the whole community (e.g., by changing resident species composition). To investigate such mechanisms, I performed a mesocosm experiment with natural phytoplankton communities to which I added different laboratory phytoplankton species from all major freshwater taxonomic groups. All added species were unsuccessful at invading the natural phytoplankton community. However, the community composition of the invaded treatments showed clear differences to non-invaded controls; having higher diversity and different

community composition. This finding indicates the importance of unsuccessful invasions (which might happen regularly) for community composition and biodiversity dynamics.

Multiple induced anthropogenic changes often co-occur under natural conditions. Therefore, species have to cope with a variety of environmental changes simultaneously. The effect on community composition might be different when multiple environmental changes co-occur compared to effects of single environmental change. Additionally, these effects probably cannot be easily predicted from the effects of single investigated induced anthropogenic environmental changes. To investigate the effect of combined abiotic and biotic induced anthropogenic environmental change on a natural phytoplankton community, I performed a laboratory experiment in which, as an indicator of climate change induced anthropogenic environmental change, I altered the abiotic resource light (red light, blue light, and white light). The biotic environmental change was represented by a Cyanophyta species (laboratory strain, *Anabaena cylindrica*) as a potential invader. Invasion affected the chlorophyll-*a* content of the community in the short-term. Invaded communities had a sustained increase in community diversity. The different light quality conditions showed no long-term effects on chlorophyll-*a* content or the diversity of phytoplankton communities, either with or without invasion.

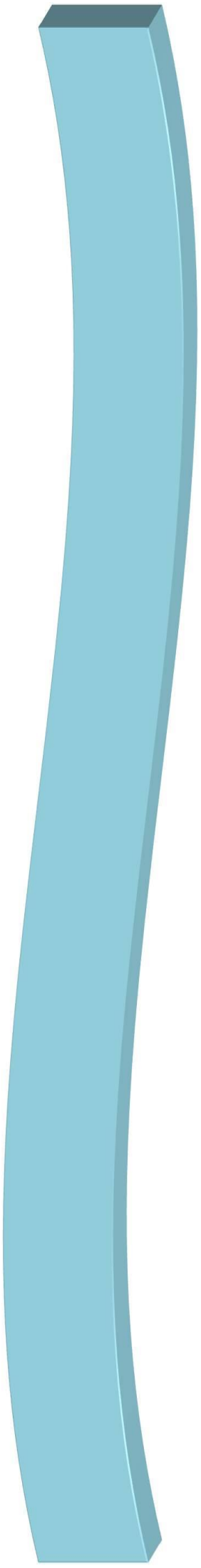
Additionally, to test the effect of the combined light-manipulations and invasion treatments on the higher trophic level, I experimentally analyzed somatic growth rates of *Daphnia magna*, a herbivorous zooplankton. The phytoplankton communities resulting from my experimental manipulations were provided as food for *Daphnia. Daphnia* showed more stable somatic growth rates when fed on invaded phytoplankton communities than on non-invaded communities.

A new form of potential invasions into aquatic environments is related to the increasing use of genetically modified algae for biotechnical purposes. Microalgae species, especially members of the eukaryotic algae, provide, amongst other things, a feasible and cost-effective way for the production of recombinant proteins. However, genetically modified organisms (GMO) can affect other species from the same trophic level, as well as organisms from higher trophic levels, thereby affecting ecosystem services. One potential hazard of GMOs is that their consumption could have an adverse impact on the life history of the consumers. To analyze such potential influences of genetically modified microalgae (GMM) on its predator, I performed a feeding experiment with the herbivore *Daphnia magna*. I used different strains of *Chlamydomonas reinhardtii* as a food source, including a wild type, a cell wall deficient

(CWD) mutant (often used to produce genetically modified strains), and a genetically modified strain (GM) with two different secreting level of a recombinant human protein. The CWD and the GM algae had an impact on *Daphnia* life history performances. I detected differences in the time until *Daphnia* release their first clutch, the first clutch neonate size, fecundity, and fitness. *Daphnia* fed on GM *C. reinhardtii* produced higher numbers of smaller offspring, indicating that feeding the GM algae resulted in some life history shifts.

My results show that induced anthropogenic changes are not only affecting phytoplankton abundance, but also phytoplankton community composition. These phytoplankton community dynamics are affecting higher trophic levels and, furthermore, can affect food web and ecosystem dynamics. Induced anthropogenic changes are becoming critical drivers of aquatic ecosystem dynamics and a detailed understanding of underlying mechanisms is urgently needed to make better predictions about how aquatic systems will respond to these induced anthropogenic changes in the future.

1. Introduction



1.1 General Introduction

The study of community assembly goes back to the early 20th century. One of the first community ecologists was the botanist Frederic Clements, who described a plant succession pattern by temporal and spatial environmental variation (Clements 1916). He developed a holistic concept and defined a community as a discrete unit, a super-organism. However, Clements' view of a community was challenged by other plant community ecologists; e. g. Henry Gleason described a plant community as an assembly of species with particular characteristics, which influence their migration ability, as well as their appearance in certain environmental gradients (Gleason 1926). This individualistic concept focused on the distribution of species, which leads to discrete communities, as well as to continuum.

In the second half of the 20th century, Harper (1967) developed a third concept, which was needed to describe communities in different habitats. His Darwinistic concept emphasizes the fact that species modify their environment and, therefore, becomes the *environment* for other species. In this concept, the community is more than a random collection of different species. Since then, researchers have studied community composition from a manifold of different viewpoints: Robert Whittaker (1975), for example, focused on both the abiotic proximity of community members and their various interactions, which, together, form a distinctive living system. In contrast to this, the concept of Robert Ricklefs (1990) does not mainly focus on species interactions but emphasizes that communities are often marked by dominant species or the physical environment, which influence the abundance of organisms.

All of the above-mentioned concepts describing community assembly are based on ecologically relevant differences between species. Hubbel (2006) introduced a new aspect, the neutral theory: his concept focuses on the functional equivalence of species in a community, not on differences. Therefore, similar species in a community should have evolved towards minimizing their differences in environmental needs, growth rate, and competitive ability. This is plausible in an evolutionary context because minimizing differences maximizes the time for competitive exclusion of these species.

The long history (more than 100 years) of research into study community assembly, as well as the existence of a wide variety of theories and definitions, is strong evidence for the importance of this topic in ecology. However, community assembly definitions varied over time and with it the perspective of the ecologist, although most share the view that a community is seen as the sum of species found in a particular place (Morin 2012). Additionally, all concepts share a

focus on species interactions or the species-specific functional traits, which maintain the species ability for interaction.

Species adaptations depend on the variable abiotic and biotic environment. The adaptation to abiotic conditions (e.g., usage of resources for survival, growth, and reproduction) is a one-sided process, where a species reacts to the abiotic circumstance. The species might change the environment by its adaptation and, therefore, modify the environment for other species. Contrary, the adaptation to a biotic environment is a two-sided process. In such a process more species are interacting with each other, such as competition between species for resources, space or predator-prey interactions (Lampert and Sommer 2007) and finally co-evolve. Interactions with the abiotic and biotic environment depend on the species itself and their adaptation abilities to survive, grow, and reproduce. The biotic interactions are more complex than the adaptation to abiotic environmental changes. They are multi-factorial by more species interacting with each other and their interactions response. Therefore, the adjustment on the biotic environment depends on all interactions of its members, their competitive ability, and also on their phenotypical ability to live and reproduce under certain circumstances. As a result of their competition abilities, individuals can survive in a certain environment and evolve further. Therefore, species interactions are subsequently the basis for each community assemblage.

In this thesis, I focus on the community composition of freshwater phytoplankton under different conditions. I studied the influence of changes in light quality (an abiotic factor) and analyzed subsequent shifts in phytoplankton communities. Additionally, I investigated the influences of biotic factors. Therefore, I examined impacts of potential invading phytoplankton species on a resident phytoplankton community under natural conditions. Furthermore, I analyzed the impact of combined abiotic and biotic changes on phytoplankton communities and their effect on higher trophic levels. Finally, I studied the influences of a genetically modified phytoplankton species on a non-genetically modified consumer of a higher trophic level.

1.2 Missing knowledge in community assembly

Community ecology and community assembly is, despite all previously described concepts and theories, a difficult subject to understand in its entity. Each studied pattern seems to be uncertain due to the complexity of changeable environmental factors and species interactions (Vellend 2010). Lawton (1999) called community ecology "a mess" based on contrary results of many empirical studies, "with so much contingency that useful generalizations are hard to find". Additionally, even theoretical community ecology can be seen as a mess for the same reason, as each little detail added to theoretical models seems to matter (Vellend 2010). As an example: adding two species to a Lotka-Voltera model with different characteristics of the seemingly most relevant factors (spatial heterogeneity, temporal heterogeneity, stochasticity, immigration, functional relationships between species, age/size structure, a third species, interactions with third species) results in more than 2300 different model solutions (Vellend 2010). Therefore, Vellend (2010) suggested only four classes of processes: selection, drift, speciation, and dispersal to categorize community dynamics, as they fully represent the logically distinct categories of important processes in community ecology.

Mittelbach (2012) supported Vellend's suggestion and mentioned that community ecologists are challenged with the illumination of the interior of "the black box of community ecology" (Figure 1). This is needed to better understand how the four basic processes of community ecology interact to determine patterns of biodiversity (Mittelbach 2012). "The black box of community ecology" is a metaphor, illustrating the missing knowledge in community ecology and highlights the need for further studies on the unknown mechanisms at play within the interior of the black box.

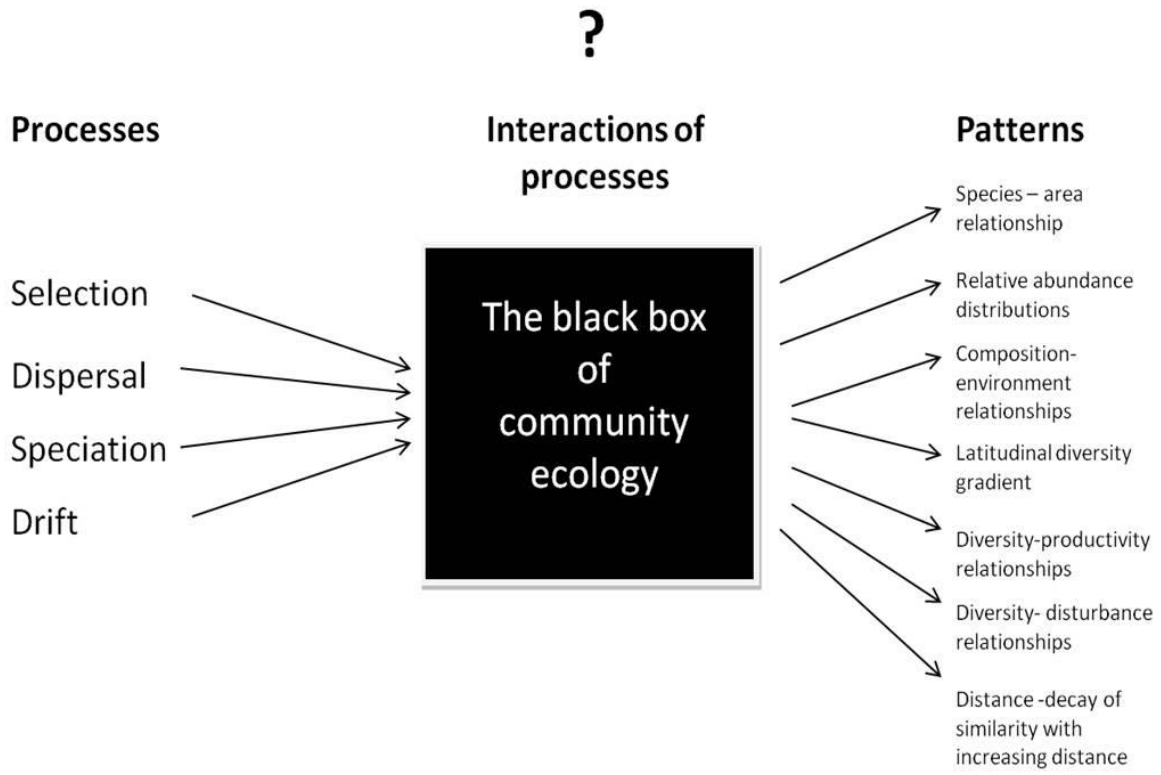


Figure 1: "The Black Box of Community Ecology". The four suggested processes (left side) of Vellend (2010) and their unknown interactions lead to several patterns in nature (right side). Modified after Vellend (2010) and Mittelbach (2012).

1.3 Global change challenges community assemblages

Communities are strongly coupled to interactions between species, or species and the environment, which are shaping community assemblages. The global impact of anthropogenic actions is changing the biotic and abiotic environment. Rising temperatures due to climate change, further and faster developments of industry, and new advances in pharmacy are just a few of the manifold impacts on communities.

Rockström et al. (2009) summarized nine processes (climate change, ocean acidification, stratospheric ozone depletion, nitrogen/phosphorus cycle, global freshwater use, land system change, the rate of biodiversity loss, atmospheric aerosol loading, and chemical pollution) influencing the functioning of the global ecosystem. They defined thresholds, called planetary boundaries, for these nine processes. Anthropogenic environmental changes can influence each of these nine processes and can cause a crossing of a critical threshold for human life on Earth. The boundaries show to which extent a sustainable life for humanity on earth is possible. The

authors state that, even if there is still a need for qualification and quantification for the planetary boundaries, humanity has already transgressed planetary boundaries for at least three processes (climate change, nitrogen cycle, and rate of biodiversity loss; Rockström et al. 2009).

In an updated version of this planetary boundaries framework, Steffen et al. (2015) adapted the processes to the current knowledge and introduced two new processes: novel entities (substances or organisms, which were unknown so far to the natural system) and functional diversity (Figure 2). Both are not yet categorized in the defined boundaries (Steffen et al. 2015). Therefore, the importance of understanding these processes and their influence on communities is beyond question. This again emphasizes the need for understanding community assembly and the mechanisms behind it. For this reason, my work focuses mainly on anthropogenic environmental changes, such as the introduction of novel entities in artificial and natural plankton communities, the impact of climate change on phytoplankton communities, and the influence of these effects on higher trophic levels.

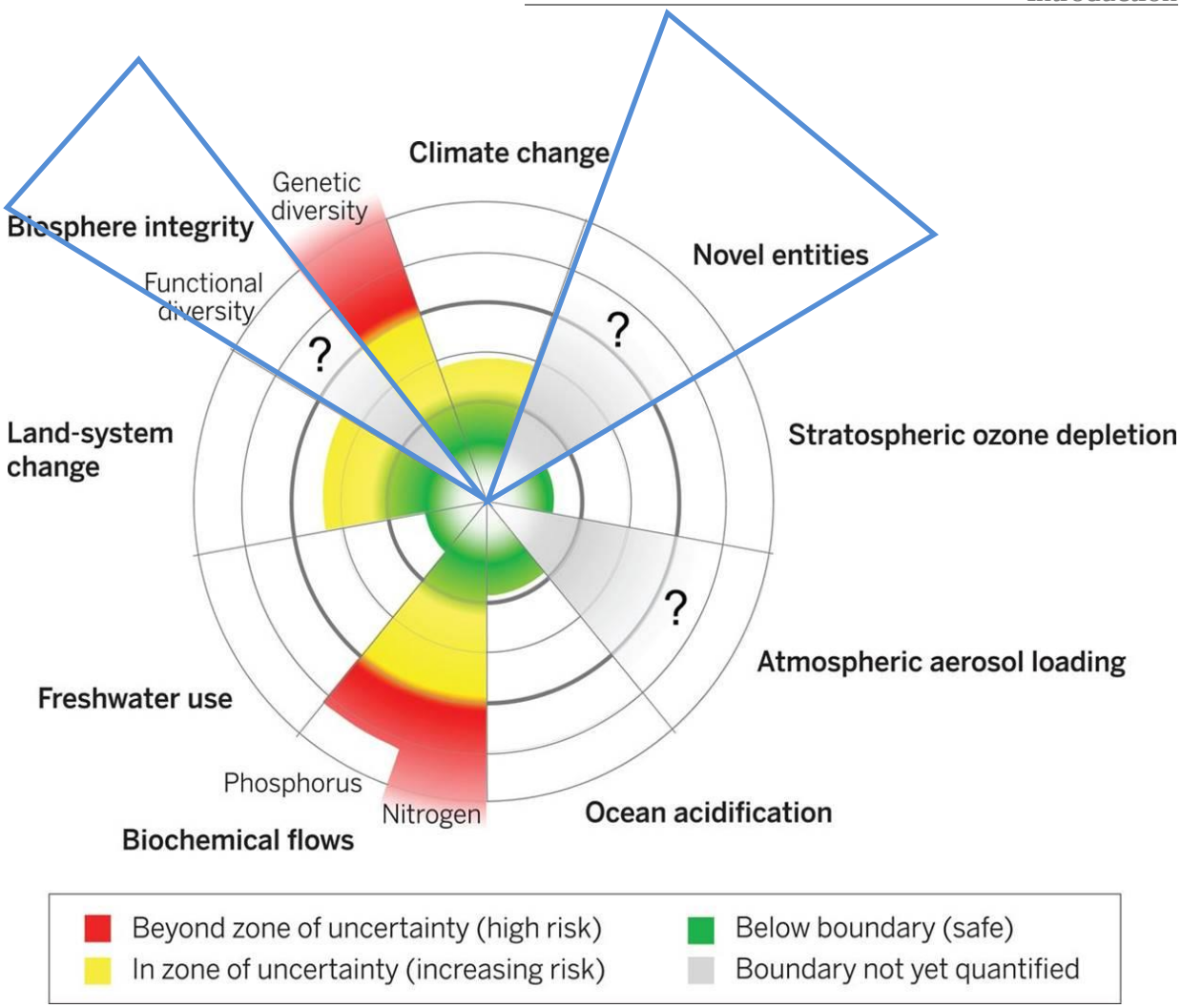


Figure 2: The seven planetary boundaries, modified after Steffen et al. (2015): green zone marks safe operating space, yellow represents a zone of uncertainty, and the red zone is high-risk zone. The planetary boundary itself lies at the intersections between green and yellow zones. Processes for which global boundaries are not yet quantified are represented by gray zones. Blue triangles show the two topics chosen for my thesis.

1.4 Phytoplankton communities and their importance for community ecology

Phytoplankton communities are an excellent model system to study community ecology and community assemblage, because of their small size, short generation time, large population numbers, and their potential easy manipulation in controlled conditions (Litchman and Klausmeier 2008). Additionally, phytoplankton is a highly diverse polyphyletic group of organisms that differ in their origin, evolutionary age, and modern distribution (Falkowski et al. 2004, Litchman et al. 2007). Finally, major taxonomic groups of eukaryotic phytoplankton can be classified into distinct functional groups (Iglesias-Rodriguez et al. 2002)

Phytoplankton covers only 1% of the photosynthetically active biomass on Earth, but maintain almost 50% of the Earth's primary production (Falkowski and Raven 2007). Furthermore, the assembly of phytoplankton communities affects the biogeochemical cycles of many elements, such as carbon, nitrogen, and phosphorus. This effect is based on the different requirements of the major phytoplankton taxonomic groups and their acquisition modes of these elements (Falkowski 2004). For example, nitrogen availability in the water column can be mainly determined by cyanobacteria, which can fix atmospheric nitrogen (Howarth et al. 1988, Capone et al. 1997, Herrero and Flores 2008). Additionally, phytoplankton as primary producers represent the basis of nearly all aquatic food webs and, therefore, has a major role for primary consumers (e.g., zooplankton; Elser et al. 2001, Felpeto and Hairston 2013) and, subsequently, higher trophic levels (Schoo et al. 2012). Different phytoplankton groups vary in edibility and nutritional value for higher trophic levels (Strener and Elser 2002). Finally, toxin-producing phytoplankton species can have direct effects on higher trophic levels or water quality (Anderson et al. 1998, Huisman et al. 2005).

The composition of the phytoplankton community affects the functioning of aquatic ecosystems. Therefore, it is crucial to understand the processes driving phytoplankton community assembly and dynamics (Litchman and Klausmeier 2008). Human-induced global change will alter the phytoplankton community structure (Huisman et al. 2005, Heino et al. 2009). Thus, the mechanistic understanding of the anthropogenic changes influencing phytoplankton community composition is an urgent task.

Due to its manifold individual traits, phytoplankton are nows also the focus of biotechnical and bioengineering applications: as a possible producer of alternative energy forms, such as biofuels (Mata et al. 2010) or an eukaryotic alternative (in contrast to conventionally used prokaryotes) for manufacturing complex recombinant proteins (Griesbeck et al. 2006). This led to intensified use of phytoplankton, including native and genetically modified species, in bioreactors, factories, and open ponds. The risks of an escape and the effects of an invasion for natural phytoplankton communities have not yet been quantified.

1.5 Aims of the thesis

Motivated by the open research questions of the impact of anthropogenic changes on ecosystems, I focused this thesis on two different anthropogenic changes, climate change and novel entities and their impact on phytoplankton communities and zooplankton.

Climate change as investigated, anthropogenic environmental change, influences, amongst other things, light quality (an abiotic factor). Light quantity is a main limiting resource for the growth of phytoplankton, as it is the driver of photosynthetic activity. However, light quality affects phytoplankton performances. Thus, a change in light quality might have significant consequences for the composition phytoplankton communities.

In my work, I examine anthropogenic change by "novel entities" in two ways. First, I investigated various potential invaders and their impact on resident phytoplankton communities and, subsequently, zooplankton (*Daphnia*). Second, I studied a biotechnologically established organism, a genetically modified microalgae (GMM), to analyze its influences on herbivorous zooplankton (*Daphnia*).

1.6 Research questions

Based on the missing knowledge of impacts of anthropogenic environmental changes on plankton communities, I set up a variety of laboratory and field experiments to analyze the impact of induced anthropogenic environmental changes on plankton communities to answer the following research questions:

I) Can changes in the light spectrum, naturally accompanying changes in mixing depth, influence phytoplankton communities?

In the last century, the temperature of Earth's has risen by about 0.7 °C (Brohan et al. 2006) and changes in climate conditions have exceeded any in at least a thousand years (IPCC 2007). Such changes can have strong effects on water temperatures in aquatic ecosystems. Rising water temperatures will lead to changes in water column stratification (Winder and Schindler 2004) and mixing depths. For phytoplankton in lakes and oceans, vertical mixing is a key variable, as it affects the supply of critical resources, such as nutrients and light (Tilzer and Goldman 1978, Berger et al. 2010, Winder and Sommer 2012). Higher water temperatures increase thermal stratification and water column stability (Straile et al. 2003). The dimension of the surface layer (epilimnion) is ecologically very important because it influences the availability of two important resources: light and nutrients. Mixing depth will determine the mean photosynthetically active irradiance (PAR) to which phytoplankton is exposed (Fee et al. 1996). The mixing depth of water columns must be lower than the "critical mixing depth" to provide sufficient light for phytoplankton growth (Sverdrup 1953). Decreased mixing depth increases the light availability for phytoplankton and potentially results in higher phytoplankton biomass production. However, the loss of nutrients bound in particular matter increases with decreasing mixing depth (Reynolds 1984). The altered mixing depth is, therefore, a critical mechanism by which global change can affect the functioning of pelagic ecosystems (Berger et al. 2010, Winder et al. 2012).

Most studies examining the effects of shifts in stratification on phytoplankton consider changes in light intensity as the primary driver of phytoplankton growth responses (Brand and Guillard 1981, Sunda and Huntsman 1997, Bittar et al. 2013). However, one aspect that is usually ignored is the heterogeneity in the spectral composition that also accompanies stratification. Light of wavelength 620–750 nm (red) is absorbed very fast in the water column, whereas blue light (430–500 nm) penetrates to greater depths. With increased mixing depth, the mean light

spectrum experienced by phytoplankton is shifted towards blue light. If increased water temperatures reduce mixing depth, phytoplankton is not only exposed to higher light intensities, but also to a different and much broader light spectrum.

Most phytoplankton species have no or very limited motility and are transported passively by currents. Species must, therefore, adapt to regular changes in available light. Phytoplankton species can react physiologically to both quantitative and qualitative spectral light changes (Falkowski and Owens 1980, Brunelle et al. 2012). For example, many phytoplankton species can alter the content of their primary photosynthetic pigment (chlorophyll-*a*) in response to light intensity shifts (Falkowski and Raven 2007). Low light conditions result in higher per-cell pigment concentrations, which increases light absorption efficiency (package effect; Falkowski and Owens 1980, Falkowski et al. 1985). Furthermore, phytoplankton can adjust the concentrations of secondary photosynthetic pigments to adapt to changes in the available light spectrum (Stomp et al. 2004). For example, when exposed to green light, *Tolypothrix tenius* can shift its phycoerythrin to phycocyanin ratio by promoting the synthesis of more phycoerythrin; thereby also enhancing the light use efficiency for green light (Stomp et al. 2004).

However, the species composition of the phytoplankton community plays an important role in the observed responses to light shifts. Phytoplankton species differ in their pigment composition, and different pigments absorb different wavelengths of light. Changes in the light spectrum may invoke a response in community composition by favoring species with optimal pigment compositions for those conditions. Alternately, a broader range of wavelengths, such as those experienced in shallowly mixed water columns, provides the opportunity for niche differentiation in pigment content, possibly leading to coexistence of several species and thereby higher diversity (Stomp et al. 2004).

As it is difficult to disentangle the coupled effects of light quantity and light quality *in situ*, I investigated the response of laboratory and field phytoplankton communities to changes in spectral light availability under controlled laboratory conditions by manipulating available light wavelengths, while holding light intensity constant. I analyzed population growth, species composition, species diversity, and photosynthetic efficiency of natural and laboratory algal communities along a temporal light gradient from permanent (24 h) blue (430–470 nm) to permanent (24 h) full white light (full PAR spectrum [400–700 nm]) supply.

II) Are phytoplankton species from different taxonomic groups able to invade a natural phytoplankton community and, if not, do they still influence the resident community?

For decades, invasion dynamics of non-native species has been an important topic in ecological research (Elton 1958). Especially today, the alteration of habitats by invading species is of major interest caused by rising awareness of globalization and climate changes. Invaders can have major effects on resident ecosystems, such as the loss of biodiversity, changes in nutrient cycles, and the alteration of the community composition and dynamics (Mack et al. 2000, Ehrenfeld 2003, Levine et al. 2003). For example, the giant hogweed (*Heracleum mantegazzianum*), invasive to northern Europe (Pyšek 1991), caused a 50–60% decrease of vascular plant diversity in central Europe in invaded habitats (Hejda et al. 2009). Especially resident species, such as grass or other low-growing species, were excluded from the resident communities by shading (Thiele and Otte 2007). Another example of lasting effects by invasion was shown by Vitousek et al. (1987), who found a change of nutrient cycles in volcano areas by the nitrogen fixing tree *Myrica faya*. The nitrogen fixation ability of *M. faya* increased the nitrogen availability for other plants. Initially, nitrogen-limited plants were supported in their growth, which resulted in an altered community composition. However, the invasion is not limited to terrestrial ecosystems. Aquatic ecosystems experience invasions of non-native species with an equal intensity as terrestrial ecosystems (Simberloff et al. 2013, Gallardo et al. 2016). One of the best-known examples is the invasion of the crayfish *Orconectes rusticus*, which caused a strong alteration of the community structure and trophic pathways in North American lakes. In the presence of the crayfish, the species richness and abundance of benthic macro-invertebrates decreased (Nilsson et al. 2012).

These examples show the high impact of successful invaders on ecosystems. However, to influence an ecosystem, each invading species must successfully pass different invasion stages. These stages are: transport to a new habitat, introduction into the new habitat, establishment by surviving and reproduction in the new habitat, and, finally, spreading by dispersal (Lockwood 2007, Blackburn et al. 2011). While the described invasion process is a theoretical and empirical well-studied topic (Elton 1958, Lockwood 2007, Blackburn et al. 2011), a mechanistic understanding of the different stages of invasion on the species level remains unclear. A more detailed invasion concept based on species interactions during the invasion process is needed. Valery et al. (2008) described invasion as a mechanism where a species acquires a dominant role compared to a resident species of the established community, which

allows it to proliferate and rapidly spread to new habitats. In general, all species (invaders and residents) interact with the abiotic environment (e.g., by nutrient uptake or release, as well as with the biotic environment through species interactions).

These species interactions could be direct or indirect interactions, both with effects on the species development in a community. Through direct interaction, a species could be excluded by another species (predation, competition) or both species could coexist in the same habitat (Tilman 1977). However, natural communities usually contain a large number of different species. The direct interactions between two species will, therefore, result in indirect effects on other species (Tilman 1987). The outcome of such interactions is influenced by species interaction abilities, which depend on their traits. Species traits are manifold and give species the ability to move, survive, reproduce and compete (Litchman and Klausmeier 2008).

Previous research resulted in a discrepancy of the role of invading species, convergent or divergent to resident species (Tilman 2004, Cleland 2011). On the one hand, Tilman (2004) found that divergent invading species (compared to residents) have an invasion advantage, thus allowing a better use of available resources by the slight overlap of common resource requirements. On the other hand, Case et al. (2016) hypothesized that convergent invading species (compared to residents) have an advantage in invading the resident community because they are similarly well adapted to the specific environment as resident species are.

Both assumptions incorporate a failure of the invading species, as not every invading species might be successful in establishing itself in the new habitat (Zenni and Nuñez 2013). However, the competitive advantage of an invading species may influence the resident community, even if the invading species fails to establish, as it interacts (for a certain time during the invasion process) with the resident species and the environment. Such transient effects of a failed invasion on a resident community were shown in a theoretical approach by Miller et al. (2009), who concluded that also a transient species (unsuccessful invader) could influence the resident community composition in more than 50% of the theoretically analyzed communities. However, empirical evidence for this scenario is still missing, and most studies focus on the consequences of successful invasion for the resident community and the invading species (Mooney and Cleland 2001, Molnar et al. 2008, Hejda et al. 2009).

In a recent meta-analysis, Zenni and Nuñez (2013) analyzed why invaders fail to establish and mentioned the necessity for more detailed knowledge of how unsuccessful invaders are affect resident communities. To my knowledge, there is a major lack of empirical data on the effect

of unsuccessful invading species in natural communities. To analyze the transient effects of invading species, phytoplankton communities are an appropriate study system. These communities face constant competition for space and nutrients with invading species, due to a consistent exchange of phytoplankton species between different water bodies resulting from the high dispersal of phytoplankton species (Kristiansen 1996). Phytoplankton has a short generation time (Reynolds 2006), allowing investigation of numerical responses within communities. Additionally, some phytoplankton traits are already well described (Reynolds 2006, Litchman and Klausmeier 2008). Among others, cell size (between 1 µm and 1 mm) is a key trait in phytoplankton, which influences growth, metabolism, access to resources, and sinking rates (Samayda 1970, Litchman and Klausmeier 2008, Litchman et al. 2010) and is, therefore, sensitive to species interactions such as competition. Furthermore, cell size in phytoplankton correlates with other eco-physiological traits, such as nutrient utilization and grazer resistance (Banse 1976, Shuter 1978, Sterner 1989, Litchman 2007). Traits of both the resident and invasive species are important for the success of the invasion, as it depends on the niche similarity of the residents. In other words, a large niche separation in the resident community will probably create open niches for invaders (Hui and Richardson 2017).

Here I use natural phytoplankton communities composed of many species to examine the effects of attempted invasion by additional phytoplankton species on community composition. I conducted experiments in which freshwater phytoplankton species were allowed to invade intact phytoplankton communities in small mesocosms in the field. I ran separate experiments simultaneously, that differed (taxonomic group, single or a combination of species) in the additional experimental species added to the natural phytoplankton community. I investigated the following questions: 1) What are the transient effects of unsuccessful invaders on the resident community?; 2) How important is the similarity or dissimilarity of the invading species to the dominant resident species?; and 3) Are there additive effects when two invaders are combined?

III) How far do anthropogenic changes interact in affecting natural phytoplankton communities and can these interactions affect higher trophic levels?

A widely accepted principle of ecology is that biodiversity affects ecosystem stability (Tilman 1996, Loreau et al. 2001). Resistance, the ability of a community to withstand changes due to disruption, is one facet of stability (Connell and Sousa 1983). Such disruptive forces can be abiotic, such as excessive resource load, but can also be biotic, such as an invasive species (Carpenter and Cottingham 1997, Stachowicz et al. 1999). When these perturbing forces arise, a diverse and stable environment is resistant to this pressure that could endanger biodiversity (Elton 1958).

In recent decades, there has been growing concerns over biotic homogenization (the decrease of species biodiversity) due to non-native species (Sax and Gaines 2003). The threat of biotic homogenization is exacerbated by human-induced habitat destruction, which opens up niches for invading species (Mack et al. 2000). Humans also directly introduce alien species, both intentionally and unintentionally, through the rapid movement of people and goods and population growth (Pimentel et al. 2005). The introduction of exotic species can facilitate the decline of native species populations by initiating species interactions that lead to decreases in the abundance and distribution of native species (McKinney and Lockwood 1999). A diverse community, however, can present strong direct competition between resident species and potential invaders, while also displaying indirect competition via fewer unoccupied niches and more limiting resources (Stachowicz and Byrnes 2006). Whereas communities low in diversity are characterized by stronger fluctuations in population density than diverse communities (McCann 2000). With habitat alteration and spread of non-native species becoming a global ecological and conservation crisis, it has become increasingly important to understand how invasive organisms are altering terrestrial and aquatic communities worldwide.

The majority of research on the effects of invasive species on biodiversity and stability has been conducted in terrestrial plant communities. Experimental and observational terrestrial studies have shown that communities with high species richness were more buffered against invasive species than those showing lower species richness (Tilman 1996, McCann 2000, Baez and Collins 2008). However, Stachowicz et al. (1999) have shown, in their empirical study on marine ecosystems, similar results in an aquatic system, using exotic species that had an actual impact on the natural aquatic system. Increased species diversity allowed for greater resistance to the invasion of *Botrylloides spec.* (colonial ascidian) on the grounds of space availability;

more diversity left fewer vacant niches for non-native species (Stachowicz et al. 1999). However, studies on phytoplankton communities are still limited. A previous study assessing the impacts on invasive phytoplankton in the Baltic Sea revealed that most non-native phytoplankton species (eleven out of twelve species, within an observation period of nearly 30 years) had a negligible effect on native communities (Olenina et al. 2010). Another study of planktonic bacteria in freshwater systems showed that the introduction of invasive bacteria preserved species diversity in the community (Hornak and Corno 2012). The invasive species was initially able to cause a strong decrease in abundance of the dominant resident species; however, this allowed the other resident species, formerly unable to compete within the community, to increase their abundances. The invading species only had a short-term presence in the community, and overall species richness was preserved. Miller et al. (2009) suggested with their theoretical study that the characteristics of species which failed to establish in a community (ghost species) are important in understanding how successful species were able to form the final stable community. These *ghost species* have weak competitive effects compared to the successful resident species, but their presence could affect a few other resident species. By disrupting a few species, unsuccessful invader species might alter any downstream effects these resident species may have had on the final community and, therefore, indirectly contribute to the final community themselves. This shows that unsuccessful invasive species that have only a temporary presence in a community can still have lasting effects on these communities.

Abiotic disturbances can also have drastic effects on community structure. Phytoplankton communities rely on water column mixing to distribute resources such as light and nutrients. Water column mixing strongly depends on temperature differences between different depths. The effects of global warming can alter the water temperature profiles and water column zones (Hondzo and Stefan 1993). Zones within the water column that are poorly mixed can affect species composition in pelagic communities by changing nutrient availability, light quality, and stratification (Klausmeier and Litchman 2001). Climate-related changes in stratification patterns can influence the population dynamics of algal groups that require sustained water column mixing (Jäger et al. 2008). Restricted water column mixing prevents free-floating phytoplankton from accessing various wavelengths of light available in different depths of the water column. For example, in clear waters, blue light penetrates to the greatest waters depths while red light is limited to only the upper layer (Jerlov 1951).

Variable light use is a form of niche partitioning, which allows a greater number of algal groups to coexist (Stomp et al. 2004). Therefore, a diverse phytoplankton community is more efficient due to their ability to maintain a stable coexistence by partitioning their use of the visible light spectrum (Ptacnik et al. 2008). For example, Cyanophyta has evolved some acclimation strategies to maximize their photosynthetic efficiency in changing light conditions (Gan et al. 2014 a). Some species have been shown to acclimate to far-red light by extensively remodeling their photosystem, utilizing a range of the light spectrum that cannot be used by other algal types (Gan et al. 2014 b). Therefore, restricting light quality to only narrow ranges of wavelengths should diminish phytoplankton biodiversity. Cyanophyta contain, in addition to chlorophyll-*a*, phycoerythrin and phycocyanin. These pigments allow them to absorb light in parts of the visible light from 500 nm to 600 nm (the "green gap"), which chlorophyll-*a* does not use efficiently (Larkum and Barrett 1983). Because of their ability to utilize the "green gap" and far-red light (Gan et al. 2014 b), Cyanophyta might have an advantage under certain light conditions. When environmental changes such as light restrictions arise, it could represent a huge advantage for certain Cyanophyta species over other algae.

The composition of phytoplankton communities has far reaching implications since phytoplankton serve as the basis of nearly all aquatic food webs and account for more than half of global primary production (Falkowski 2007). The abundance and community composition of phytoplankton have, therefore, a major influence on trophic interactions and nutrient fluxes in aquatic ecosystems (Falkowski et al. 2004). They are also the main food source for zooplankton such as *Daphnia magna*, which depend heavily on phytoplankton availability for growth and reproduction. Unselective feeders like *Daphnia* are strongly dependent on their quality of food. It has been shown that two different green algal species, as well as the algal growth conditions (nutrient-limited or nutrient-sufficient), have different effects on the life history of *Daphnia* (Lürling and Van Donk 1997). *Daphnia* can avoid feeding on less quality food by changing their carapace opening. Lampert (1981) reported that when in the presence of high concentrations of the Cyanophyta *Anabaena cylindrica*, *D. magna* narrowed their carapace to avoid feeding on the filaments. If Cyanophyta is ingested, they are usually of poor nutritional value to *Daphnia* species (de Bernardi and Giussani 1990). Since well-fed *D. magna* grow faster, feeding on high concentrations of Cyanophyta will reduce their growth, reproduction, and survival (Ingle et al.1937, Lampert 1987). Therefore, growth rates of *D. magna* are good indicators of phytoplankton community quality.

To investigate the effect of biotic and abiotic disturbances on algal communities, a microcosm experiment was conducted with freshwater algal communities. First, I examined the influence of the invasion of the Cyanophyta *A. cylindrica* under various light quality conditions on the biomass and diversity of a natural phytoplankton community. Second, to investigate the effects of altered phytoplankton community composition due to the previous addition of an invading species on zooplankton, I conducted a somatic growth experiment to assess *D. magna* growth rates.

IV) Can genetically modified microalgae (GMM) for biotechnological purposes affect consumers from higher trophic levels when invading plankton communities?

In the last decades, the use of genetically modified organism (GMO) increased substantially (Ma et al. 2003, James 2011, Bawa and Anilakumar 2013, Zhang et al. 2016). Organisms can be modified to combine genes from two completely unrelated species in one organism, which subsequently gains new abilities (Bawa and Anilakumar 2013). Thus, such engineered organisms can have multiple uses (e.g., in agriculture, biotechnology, pharmaceutical or industrial applications). For example, agriculture GMOs are used to provide an efficient and cheap food source for humans through higher biomass productivity, high nutritional quality, and can be resistant to parasites or diseases (Zhang et al. 2016). Furthermore, GM crops were proposed to reduce the environmental footprint of agriculture, as well as the use of pesticides (James 2011). Besides terrestrial GMOs, the use of aquatic GMOs such as microalgae increased due to their potential of mass cultivation and high production rates through short generation times.

Genetically modified microalgae (GMM) are used pharmaceutically and industrially as a cheap alternative to other GMOs to produce high amounts of recombinant proteins (Gong et al. 2011). One example for a GMM producing a transgenic protein is *Chlorella ellipsoidea* expressing the flounder growth hormone (FGH). This GMM is used as fish food to increase fish growth. Flounder fry increased 25% in growth after 30 days feeding on the transgenic *C. ellipisoidea*, (Kim et al. 2002). Other microalgae, such as *Chlamydomonas reinhardtii,* are often used as GMM expressing proteins for therapeutically and biotechnical reasons (Griesbeck et al. 2006, Specht et al. 2010). One example is the use of GM *C. reinhardtii* expressing an antigenic protein, as an additional fish food component, to increase the immune

response in trout. Therefore, an epitope of the pathogenic bacterium (*Rennibacterium salmoninarum*) of farmed salmonids was targeted to the plasma membrane in GM *C. reinhardtii* (Patent application US020030022359).

However, in addition to the advantages of such GMOs, their interference with other organisms can cause potentially harmful interactions in natural ecosystems. A study of GM crops showed that a natural consumer organism could be harmed by feeding on a GMO. Butterfly larvae of the monarch butterfly, *Danaus plexippus*, showed higher mortality rates after feeding on plant leafs (their natural food) covered with GM pollen, compared to feeding on leafs without GM pollen (Losey et al. 1999). Additionally, it is known that GM maize express invertebrate toxic crystalline proteins from the bacterium *Bacillus thuringiensis* in all plant tissues and persists in left over harvested plant parts (Zwahlen et al. 2003). Another terrestrial example is the uncontrolled dispersal of GM pollen of land plants. The spread of GM creeping bentgrass (*Agrostis stolonifera L.*), which built hybrids with the wild forms, has already demonstrated in the USA (Reichman et al. 2006).

Aquatic organisms can also be harmed due to feeding on terrestrial GMOs. Chambers et al. (2010) found slower growth rates in an aquatic invertebrate (trichopteran, *Lepidostoma liba*) fed on GM maize detritus compared to species fed with non-GM maize detritus. However, negative influences of a GMM on natural ecosystems have so far mainly been hypothesized (Snow and Smith 2012, Gressel et al. 2013, Henley et al. 2013, Usher et al. 2014, Hewett et al. 2016). A recent study empirically investigated the dispersal and invading potential of an outdoor cultivated GMM in a phytoplankton community (Szyjka et al. 2017). The direct effect of the next consumer level remains unclear.

Before a GMO can have a potential impact on a natural environment, it needs to pass various steps: reach it, survive within it, and invade it. Thus, an active or passive transport of the GMOs is necessary to spread between habitats. It could be shown within a meta-analysis of structuring meta-communities of aquatic organisms that body size and the dispersal ability are key traits. Furthermore, the authors suggest, based on their finding in a category of passive dispersers, that an increasing body size causes an increasing dispersal limitation (De Bie et al. 2012). Small organisms such as microalgae are often passively transported between habitats, whereas other species such as fish are mobile and spread in a broader spatial scale, depending on the connectivity of habitats. Snow and Smith (2012) hypothesized that GMMs have the same potential risk to spread as pollen of GM plants and to become invasive. Microalgae are

mass cultivated in factories, laboratories, and open pond systems. Thus, they potentially come into contact with natural ecosystems. Although GMMs are enclosed in production factories with escaping precautions (Griesbeck et al. 2006), an escaping risk remains as they might be released unintentionally (Snow and Smith 2012). After the release, GMM can be transported like wild type (WT) microalgae into another habitat through air or water bodies, or attached to animals or humans (Kristiansen 1996, Figuerola et al. 2003, Ingle 2003). Thus, GMMs are potentially able to enter a natural habitat.

Due to the not negligible risk of a GMM entering natural ecosystems, it is important to study possible impacts on natural aquatic ecosystems and food webs (Henley et al. 2013), especially in the context of the global relevance of Earth primary production, to which phytoplankton (including microalgae) contribute almost 50% (Falkowski and Raven 2007). Additionally, phytoplankton is the base of aquatic food webs and, therefore, the main food resource for primary consumers like zooplankton (Elser et al. 2001, Felpeto and Hairston 2013) and can subsequently impact higher trophic levels (Schoo et al. 2012), including humans.

Because of the less empirically studied effects of a GMM on natural aquatic systems, I conducted a laboratory feeding experiments with the herbivore zooplankton *Daphnia magna* and with the most common used GMM, the model organism *C. reinhardtii* (Harris 2001, Griesbeck et al. 2006, Specht et al. 2010). The unmodified wild-type (WT) algae is a common member of freshwater phytoplankton communities and is of high food quality for zooplankton (Porter and Orcutt 1980, Alghren 1990). Thus, *C. reinhardtii* is well-suited to investigations into the impacts of GMM food on zooplankton. In my experiments, I used different strains of *C. reinhardtii* to examine the influences on *D. magna* performance. I compared two non-genetically modified *C. reinhardtii* strains as food for *D. magna*; whereas one is a non-manipulated wild-type (WT), similar to a natural *C. reinhardtii* and a good food source for *D. magna* (Mitchell et al. 1992), the second *C. reinhardtii* strain has a cell wall deficient (CWD) and is often used to produce transgenic *C. reinhardtii* (Griesbeck et al. 2006). I aimed to answer the question: does a CWD *C. reinhardtii*, as the basis for the production of a GMM, has a different influence on *D. magna* life history performances than a WT *C. reinhardtii*?

Additionally, I used a GM *C. reinhardtii* strain that was established to support the use of engineered tissues for wound healing by the ability to synthesize and secrete the human vascular endothelial growth factor VEGF-165 (VEGF). Through the photosynthesis of *C. reinhardtii*, oxygen is available within the tissue, and the secretion of the growth factor VEGF-

165 by the algae stimulates endothelial blood vessels to grow into the tissue (Chávez et al. 2016).

In general, all VEGFs belong to the VEGF/PDFG (platelet-derived growth factor) group of the cystine-knot superfamily of hormones and extracellular signaling molecules (Vitt et al. 2001). These vertebrate molecules are important for embryogenesis and angiogenesis, and some are important for the development of lymph vessels in juvenile and adult stages. Furthermore, VEGF-like molecules are highly conserved. This is supported by the finding of VEGF-related molecules in invertebrates without a vascular system, like *Drosophila* or *C. elegans* (Holmes and Zachary 2005).

The existence of VEGF-165 related molecules in invertebrates opens the question of whether invertebrate zooplankton would be affected by feeding on such modified *Chlamydomonas* species. Therefore, I analyzed the impacts on the life history parameters and fitness of the cladoceran zooplankton model organism *Daphnia* fed with two GM *C. reinhardtii strains*, able to secrete the human VEGF-165 at two different secreting levels (high and low). I followed the responses of individually kept *Daphnia* to answer the question whether the GMM has different effects on zooplankton performance compared to the WT and CWD *C. reinhardtii*.

2. Methods

2.1 Light quality change and community assembly

Experimental design

I established two different phytoplankton community types in semi-continuous batch conditions (exchange rate 5% of culture volume per day). One community type was assembled from lab-cultured algal strains (laboratory communities). The other community type was taken directly from natural samples (natural communities). I created laboratory communities by mixing eleven species from five major freshwater algal classes: Chlorophyta, Cyanophyta, Bacillariophyta, Cryptophyta and Chrysophyta (Table 1). Each algal strain was pre-cultured in monoculture in modified Woods Hole growth medium (WC-medium; Guillard and Lorenzen 1972) for several months or longer before initiating the experiment. For natural communities, I sampled phytoplankton from Lake Brunnensee located in Bavaria, Southern Germany. I took a 1 L water sample from the well-mixed epilimnion and filtered samples through a 200 µm mesh to remove macro- and mesozooplankton. I acclimated samples in a climate chamber at 20 °C for ten days prior to the start of the experiment.

I used 200 mL of phosphorus reduced WC growth medium as a culture medium for each treatment. The initial total algal biovolume in all replicate cultures was identical ($2.4 \cdot 10^6$ fL mL^{-1}) at the beginning of the experiment. For laboratory communities, biomass was split evenly among the eleven species. Treatments containing natural phytoplankton were diluted to the same initial algal biovolume as laboratory communities.

During the experiment, I exposed cultures to 65 µMol photons m^{-2} s^{-1} for 24 h d^{-1}. I varied the light quality experienced by cultures by varying the number of hours. I exposed cultures to blue and white light in five treatments ranging from a blue (h):white (h) light ratio of 0:24, over 6:18, 12:12, 18:6, and 24:0. These light variations simulate variations in mixing depth in a very simple way. Shallow mixing is associated with longer daily exposition to a more full (white) PAR spectrum and deep mixing with proportionally longer daily exposition to a reduced and mainly blue PAR spectrum. I provided blue light using cool white light lamps covered with blue plastic screen (transmission: 430-470 nm). I established four replicates (laboratory) and two replicates (natural) of each light treatment x community type (N = 30).

Measurements

I used an AquaPen AP 100 (Photon Systems Instruments, Czech Republic) fitted with white and blue light emitting diodes (LEDs) (see also Kromkamp et al. 2009) to take fluorescence measurements. A 3 mL sample of each treatment was analyzed to estimate chlorophyll-*a* content and the efficiency of photosystem II (PSII). I used transient fluorescence of PSII, or OJIP measurements (Kromkamp et al. 2009), to estimate the probability that an absorbed photon will move an electron into the electron transport chain (φ_{Eo} = ET_0 / ABS). The minimum fluorescence after 50 µs (F_o) and the maximum fluorescence (F_m) of the OJIP analyses were used as measures of the phenomenological absorption flux per excited cross section (see Strasser et al. 2000). All samples were dark adapted before measurements and with both white actinic and blue actinic excitation light of the same intensity.

To estimate the initial and final phytoplankton species composition in laboratory (0 and 20 days) and field communities (0 and 20 days), I counted 5 mL samples fixed with Lugol´s iodine using the standard Utermöhl technique (Utermöhl 1958). I counted a minimum of 100 individuals by scanning a minimum of five perpendicular transects or 20 randomly distributed distinct fields to keep the counting error at less than 10% (Lund et al. 1958) to determine species presence and abundance. Diversity (H') and evenness (E) of phytoplankton functional groups were calculated based on the Shannon- Index (Krebs 1989). I used linear regressions to examine the effect of the contribution of blue light on each phytoplankton variable.

Table 1: List of algal strains in laboratory communities

Class	Algal strain (strain number; culture collection)
Chlorophyta	*Scenedesmus obliquus* Kützing (276-10; SAG, Göttingen)
	Staurastrum tetracerum (Meyen) Ralfs (7.94; SAG, Göttingen)
	Planktosphaeria gelatinosa G.M. Smith (262-1b; SAG, Göttingen)
Cyanophyta	*Anabaena cylindrica* Lemmermann (1403-2; SAG, Göttingen)
	Planktothrix rubescens Anagnostidis et Komárek (5.89; SAG Göttingen)
	Chroococcus minutus Kützing (41.79; SAG Göttingen)
Cryptophyta	*Cryptomonas phasedus* Skuja (2013; SAG, Göttingen)
Crysophyta	*Synura petersenii* (Korshikov) (24.86; SAG, Göttingen)
Bacillariophyta	*Nitzschia palea* Kützing; W. Smith (1052-3a; SAG, Göttingen)
	Asterionella formosa Hassall (2339; SAG Göttingen)
	Cyclotella meneghiniana Kützing (2136; SAG, Göttingen)

2.2 Invaders as induced anthropogenic environmental biotic change for a natural phytoplankton community

Experimental setup

The experiment was carried out in a mesotrophic pond (TP = 13.06 μgL^{-1}; 11 °C, pH of 8.4 ± 0.2 SE; Martinsried (Bavaria, Germany) and set up in dialysis bags (Nadir®, Microdyn-Nadir GmbH, Wiesbaden, Germany), which allow diffusion of molecules up to a size of 10-20 kDa. The dialysis membrane enables natural nutrient conditions for phytoplankton communities within the bags for a certain amount of time (four days, Sommer et al. 2005). The tubes were formed to bags and filled with 250 mL pond water containing the resident phytoplankton community. To avoid macro- and mesozooplankton within the dialysis bags, the pond water was previously filtered through 225 µm gauze.

As potential invaders, I chose six different laboratory phytoplankton species with individual biovolume ranging from 100-700 μm^3, deriving from three different common phytoplankton

groups (Table 2). The resident community consisted of 40.6% Bacillariophyta with 34% of *Synedra spec.* (dominant species of the community), 32% Chrysophyta, 26% Chlorophyta (mainly small coccal Chlorophyta) and 0.5% Cyanophyta, which was estimated by microscopic counting (Utermöhl 1958).

All introduced species were cultivated in monoculture in modified Woods Hole Combo growth medium (WC-Medium, Guillard and Lorenzen 1972) under laboratory conditions (12/12 h day and night rhythm; 90 µMol photons m^{-2} s^{-1}; 20 °C).

The control treatment contained only the resident phytoplankton community of the pond. The experimental treatments additionally included the respectively added laboratory phytoplankton species (single species or a combination of two species of the same taxonomic group). The added biovolume of the added species equaled 10% of the resident community biovolume, for the combination of two added species each species contributed equally to the 10%. The biovolume was measured using a cell counter (CASY®1 Cell Counter and Analyzer system TTC, Schärfe System GmbH, Germany). All treatments were set up in triplicates, which resulted in a total number of N = 30 dialysis bags.

The dialysis bags were incubated in the pond 40 cm below the water surface at the deepest point of the pond (4 m). After four days, dialysis bags were exchanged to avoid a closure of the dialysis membrane by bacterial growth. The experiment lasted for nine days in total.

At the end of the experiment, samples of each phytoplankton communities were preserved with Lugol´s iodine. For analyses of species diversity and phytoplankton community compositions, samples were counted using an inverted light microscope (M40, WILD; Heerbrugg, Switzerland) according to Utermöhl (1958). If possible, at least 100 individuals (or colonies) per category were counted in two perpendicular transects to maintain the counting error below 10% (Lund et al. 1958). To estimate the added species´ biovolume, measurements of the respective laboratory cultures were used. Individual biovolume of the resident community species was estimated according to Kremer et al. (2014). Additionally, I categorized the community in three cell volume-size classes: nanoplankton (10-10^3 µm^3), microplankton (10^3-10^6 µm^3) and mesoplankton (10^6-10^9 µm^3) according to Ignatiades (2016). Based on this, all my potential invader species belong to nanoplankton.

Data analysis

To test whether the experimental species were successful at invading the resident communities during the experiment, I calculated invasion success modified after Sperfeld et al. (2010). The invasion success was calculated as follows:

$$\text{Invasion success} = \log 2 \left(\frac{ASP_{end,i} - EASP_{end,i})}{ASP_{start,i}} \right) \quad \text{(I)}$$

Where, $ASP_{end,\,i}$ represent the added species (% biovolume abundance) *i* at the end, $EASP_{end,\,i}$ the equivalent phytoplankton species to species *i* (% biovolume abundance) and $ASP_{start,\,i}$ represented added phytoplankton species *i* at the start (% biovolume abundance). Furthermore, the arithmetic mean and the standard error (SE) from replicates (n = 3) were calculated, except for one case, where no experimental species could be found in two of the three replicates. If the invasion success estimate is greater than zero, the invader was successful, whereas invasion successes estimate lower than zero or equal to zero indicates an unsuccessful invader.

Whenever I found similar species to my potential invader species in the resident community and I was unable to distinguish them, I corrected these values with the abundance of the corresponding species in the control treatment. Additionally, I also calculated Shannon diversity (H') and Pielou's evenness (E) of the final phytoplankton communities (Krebs 1985).

Statistical analyses were performed with SigmaPlot (Version 11, Systat Software Inc., United States), Primer (OLIGO Primer Analysis Software Version 7, Molecular Biology Insights, Inc., Cascade, CO, United States) and PAST (Version 2.17c; Hammer et al. 2001). To analyze the diversity and evenness differences (based on taxonomic groups) between experimental treatments and controls a t-test was conducted. The differences of similarities between experimental and control treatments were calculated using the Bray-Curtis similarity coefficient (Bray and Curtis 1957) based on species log (x + 1) transformed biovolume proportion data.

The species richness was estimated for each treatment. To analyze differences between the start community and control treatment as well as control treatment and experimental treatments, the arithmetic mean and the 95% confidence intervals were calculated (mean ± 1 CI, n = 3). Differences in community composition in taxonomic group and species composition between each pair of experimental treatment and control treatment were calculated. To analyze differences between experimental treatments and control treatment the arithmetic mean and the

95% confidence intervals were calculated (mean ± 1 CI, n = 3). The multivariate similarity percentages analyses (SIMPER) were used to estimate the percentage contribution and ranking of each species to the average dissimilarity among start, control and experimental (invaded) communities.

Table 2: Experimental species as potential invaders with their taxonomic group classification

Taxonomic group	**Added species**
Chlorophyta	*Chlamydomonas reinhardtii*
Chlorophyta	*Pediastrum simplex*
Cyanophyta	*Anabaena cylindrica*
Cyanophyta	*Pseudoanabaena galeata*
Bacillariophyta	*Fragilaria crotonensis*
Bacillariophyta	*Cyclotella meneghiniana*

2.3 Combined light quality and invasion effects on community assembly and their effects on zooplankton

Experimental Setup

To estimate the effect of different light quality on the success of an invading species in a natural phytoplankton community, a laboratory experiment was conducted. First, light quality was manipulated using different wavelengths of light (red, blue and full-spectrum), and second, presence or absence of a potential invader, *A. cylindrica* (Cyanophyta).

Phytoplankton samples were acquired from the eutrophic Lake Bansee (TP 62.4 ± 7 µg L^{-1}), Bavaria, Germany, in spring. Collected water samples were filtered through a 250 µm gauze to remove macro- and mesozooplankton. For each replicate, 200 mL of filtered Lake Bansee water was filled in a translucent cell culture flask (BD Falcon, BD Biosciences, San Jose, USA). Three times a week, a 20% medium exchange (40 mL) was done (semi-batch culture)

with media consisting of 0.7 µm-filtered (fiberglass, GF/F Whatmann, UK) water from Lake Bansee. Flasks were separated into three light treatments (red, blue, and full-spectrum) in the presence and absence of invaders. Each treatment was conducted in triplicates (N = 18). The different light conditions were created with a red filter (transmission of 650-800 nm), blue filter (transmission of 450-480 nm), or no filter (full-spectrum), also referred to as white.

The light quality of each treatment was set at 90 µMol photons m^{-2} s^{-1}. Half of the communities were inoculated with the potentially invasive species *A. cylindrica* two times during the experiment (day 1 and 7). The inoculums were equivalent to 5% of the total chlorophyll-*a* content in each community. The other half communities were kept as controls. The chlorophyll-*a* content was measured with the Algae LabAnalyser (bbe moldaenke GmbH, Germany). The flasks with the phytoplankton communities were kept in a controlled climate chamber set at 18 ± 2 °C with a 12/12 h day and night rhythm. The total duration of the experiment was 41 days.

Phytoplankton measurements

Algal measurements were conducted with the Algae LabAnalyser. Measurements from the Algae LabAnalyser provided analyses of phytoplankton community composition based on the different pigments present in the sample (bbe moldaenke GmbH 2016). Using LED lights, it can determine how the different pigment systems of various algal groups interact with chlorophyll-*a*. This interaction creates an excitation spectrum specific to each phytoplankton functional group (Chlorophyta, Cyanophyta, Bacillariophyta, and Cryptophyta). Measurements for total chlorophyll-*a* and distribution between phytoplankton functional groups, were collected twice a week. Diversity of phytoplankton communities was determined using the functional group distribution according to the Shannon Index (H'; Krebs 1985).

At the end of the experiment, samples of each phytoplankton community were preserved with Lugol´s iodine. To further investigate population structure of the phytoplankton communities, algal counts were conducted to determine community composition. Each community sample was counted using an inverted light microscope (M40, WILD; Heerbrugg, Switzerland) according to Utermöhl (1958). If possible, at least 100 individuals (or colonies) per category were counted in two perpendicular transects to maintain the counting error below 10% (Lund et al. 1958).

Somatic growth experiment

A supplemental experiment testing the effect of the different phytoplankton composition (resulted from invasion and control treatments under various light conditions as described before) on *Daphnia magna* growth was conducted. For this, I used the phytoplankton communities collected at the end of the experiment (day 41) as food source. Previous of the experiment *D. magna*, which originates from a laboratory stock culture, were kept for several generations in growth medium (SSS; Jeschke and Tollrian 2000), which was fully exchanged three times per week. The *Daphnia* were kept under constant laboratory conditions (climate chamber with constant conditions of 20 ± 2 °C with a 12/12 h day and night rhythm) and were fed daily *ad libitum* with *Scenedesmus obliquus.*

Experimental setup

The experiment was conducted with the third clutch of 12 h age-synchronized *D. magna* neonates, which were washed prior to the experiment (Van Donk et al. 1997, Porter 1973). The experiment was set up in a climate chamber with constant conditions of 20 ± 2 °C with a 12/12 h day and night rhythm.

Five synchronized *D. magna* individuals (three days old) were added to 100 mL of each phytoplankton community from the previous experiment. They were all kept in flasks and under full-spectrum light to eliminate the effect of different light quality on somatic growth. After four days, the *D. magna* were preserved in a sugar formalin solution (final concentration of 4% formalin – 40% sucrose; according to Haney and Hall 1973). Pictures and measurements of *D. magna* length (distance between the upper edge of the carapace and the base of the tail spine) were performed using the Dinolite Capture 2.0 software (AnMo Electronics Corporation, Taipei, Taiwan). Biomass was calculated from the length (mm) of *D. magna* using the following equation (Stibor 1995):

$$\text{Biomass}_{end} = 13.87 \times \text{length}^{2.452} \qquad \text{(II)}$$

Starting biomass (mg) was calculated from an aliquot of five individuals that were preserved in a sugar formalin solution (final concentration of 4% formalin – 40% sucrose) on the start day. Growth rate was calculated with the following equation:

$$\text{somatic growth rate} = \frac{(\ln(\text{biomass}_{start}) - (\ln(\text{biomass}_{end}))}{t} \qquad \text{(III)}$$

where t represents the number of days.

Data analyses

Five days were selected for statistical analysis for the phytoplankton manipulation experiment. These specific days are day 6, day 10, representing days after the addition of the potential invaders, day 20 and day 24 in the middle of the experiment, and day 41, as the end of the experiment. All statistical analyses were completed with R (R Development Core Team 2016). To analyze the effects of light manipulation and invasion on phytoplankton communities, two-way ANOVAS with Tukey *post hoc* tests were used. Coefficients of variance were used to compare *D. magna* somatic growth rates. To analyze relationships between *D. magna* somatic growth rates and phytoplankton functional group contents, the correlation coefficients were calculated.

2.4 Life history of *Daphnia magna* when feeding on genetically modified microalgae

Cultivation of WT C. reinhardtii

I used *C. reinhardtii* (strain number 54.72) from the SAG Culture Collection of Algae, Göttingen, Germany as a non-manipulated wild type (WT). The strain has been cultivated for several years in Woods Hole Combo medium (Guillard and Lorenzen 1972) under constant laboratory conditions (20 °C, with a 12/12 h day and night rhythm). During the experiment, the WT *C. reinhardtii* culture was 24 h exposed to 90 µMol photons m^{-2} s^{-1} PAR with a three times medium exchange (30%) per week.

Processing and cultivation of CWD and GM C. reinhardtii

Additionally, I used a *C. reinhardtii* characterized by a cell wall deficiency (CWD; Neupert et al. 2009). This CWD *C. reinhardtii* was the basis to produce a genetically modified GM *C. reinhardtii*. We produced a GM *C. reinhardtii* allowing to secret the human vascular endothelial growth factor VEGF-165 (VEGF; according to Chávez et al. 2016). I used two GM *C. reinhardtii* strains secreting the VEGF, whereas one strain showed a lower (low) secreting level of VEGF and the other showed a high-secreting level (high) of VEGF. To provide optimal growth conditions, all three strains (CWD, GM low, GM high) were cultivated in TAPS-medium supplemented with 1% (w/v) sorbitol (Harris 2009) with the same laboratory condition and medium exchange as described for WT *C. reinhardtii*.

Cultivation of Daphnia magna

As consumer of the different *C. reinhardtii* food sources, I used a single clone of the cladoceran *Daphnia magna*, which originates from a stock culture of the LMU Munich. To exclude maternal effects, previous to the experiments, *Daphnia* were kept for several generations in growth medium (SSS; Jeschke and Tollrian 2000), which was fully exchanged three times per week. The *Daphnia* were kept under constant laboratory conditions (climate chamber of 20 ± 2 °C with a 12 /12 h day and night rhythm) and were fed daily *ad libitum* with *Scenedesmus obliquus.*

Experimental setup

The experiment was conducted with the third clutch of 12 h age-synchronized *D. magna* neonates, which were washed prior to the experiment (Porter 1973; Van Donk et al. 1997) with fresh medium to remove food left over's on the carapace and to discharge the gastrointestinal tract of old food. The neonates were kept individually in glass jars in 200 mL of SSS growth medium (see above). The experiment was set up in a climate chamber with constant conditions of 20° C with a 12/12 h day and night rhythm.

The *D. magna* were fed every second day with fresh prepared algae solution. In total the feeding amount was 0.5 mg C L^{-1} daily to create a more natural environmental scenario (Agatz et al. 2012). Each algae culture was centrifuged (1137 rpm for six minutes) and their pellet

resuspended in decocted water to avoid input of the two different algal growth media in the experimental glass jars. Afterward, algal abundances were measured with a cell counter (Casy 1, Schärfe Systems, Germany) and algal solutions of equal biovolume were established. Once per week, half of the medium was exchanged, and the glass jars were replaced by new ones, to minimize bacterial growth and provide a potential accumulation of the VEGF respective growth hormone of GM *C. reinhardtii*. The WT and CWD treatments were replicated five times and the GM low, and GM high treatments were replicated three times resulting in a total number of 16 replicates.

Record parameters

Each glass jar was screened daily for potential mortality and reproduction related record. I collected the following characteristics of each *D. magna:* age and size at first reproduction the number of neonates within each clutch of the first three reproductions and the neonate body length at the first clutch. To measure the body length, I took alive pictures of *D. magna* after the release of the first clutch, within the first egg stage (Ebert 2005) and measured the length of each individual (distance between the upper edge of the complex eye and the base of the tail spine). Neonates of each *D. magna* were immediately fixed after birth (< 8 h) in a 4% sugar-formol solution according to Haney and Hall (1973). Afterward, the lengths of up to five randomly chosen neonates, per *Daphnia*, from the first clutch were measured as described above. The experiment was individually terminated for each *D. magna* with the release of the third clutch from the brood chamber.

The total fecundity (F) was calculated as the number of neonates per female within three clutches. As fitness parameter I calculated the intrinsic rate of increase, r (day^{-1}), using the Euler- Lotka equation (Krebs 1985):

$$1 = \sum_{i=1}^{n} l_x m_x e^{-rx} \qquad \text{(IV)}$$

Where x represents age class (days), l_x indicate the probability of surviving to the next age class x + 1, m_x represent the number of new born at age class x. In my experiment l_x was set to one, as no specific survivorship could be estimated due to the use of individual animals. To get *r*, the equation was iteratively solved with Microsoft Excel according to (Löffler et al. 2004).

The reproductive effort (RE) was characterized as percentage of total investment of *Daphnia* into the reproduction within the first three clutches. To calculate this parameter, I converted the body size into a somatic body weight (bw) with the length-weight relationship ($W = 13.87x\ L^{2.406}$) according to Stibor and Macháček (1998) and used the equation,

$$RE = \frac{\text{mean neonate bw [µg]} * F}{(\textit{Daphnia}\ \text{bw} + \text{mean neonate bw [µg]} * F)} \qquad \text{(V)}$$

where F represents the fecundity within three clutches. One individual from the treatment of CWD died within the first experimental week and was excluded from further analyses.

Data analyses

All data analyses were performed with Sigmaplot 11.0 (Systat Software 2008, Germany) software. Both experiments were analyzed together. I performed one-way ANOVAS, to assess effects of different *C. reinhardtii* strains on the age and size at the first reproduction, size of neonates at first reproduction, fecundity, fitness and reproductive effort of different treated *D. magna,* when applicable with Holm Sidak *post hoc* tests for multiple pair wise comparisons.

3. Results

3.1 Light quality change and community assembly

Chlorophyll-a

The proportion of exposure to blue light had a significant influence on chlorophyll-*a* production. Increasing blue light supply was associated with increased total chlorophyll-*a* content of the phytoplankton communities in both laboratory and natural communities (data not shown). The specific chlorophyll-*a* content per cell also increased significantly in laboratory and natural communities with increasing contribution of blue light (linear regression laboratory: $R^2 = 0.8$; $p < 0.0001$; linear regression natural: $R^2 = 0.6$; $p = 0.008$; Figure 3 A, B).

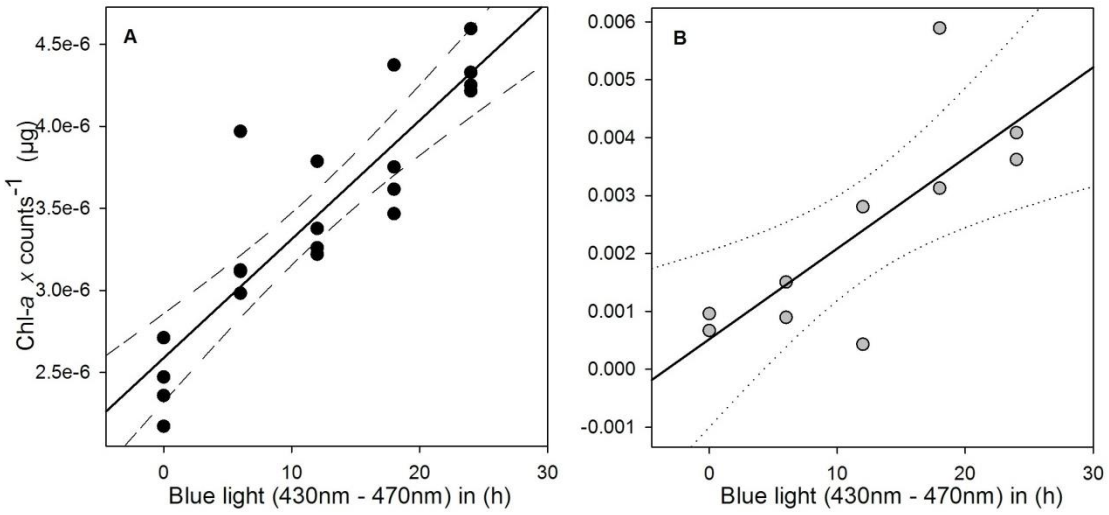


Figure 3: Cell specific chlorophyll-*a* content (Chl-*a* μgL^{-1} (Counts $L^{-1})^{-1}$ in communities as a function of increasing blue light (430nm – 470nm) in h in A) laboratory communities: linear regression: y = 7.2E-008x + 2.6E-006; $R^2 = 0.8$; $p < 0.0001$ and B) natural communities: linear regression: y = 0.0002x + 0.0005; $R^2 = 0.5$; $p = 0.008$.

Photosynthetic efficiency of PSII and spectral light use efficiency

The total photosynthetic efficiency of electron transport chain in PSII increased with increasing blue light contribution in laboratory and natural communities (Figure 4 A, B). Values ranged from 0.19 to 0.33 in laboratory communities (linear regression: $R^2 = 0.76$; $p < 0.0001$; slope: $a = 0.004$) and from 0.18 to 0.35 in natural communities (linear regression: $R^2 = 0.78$; $p = 0.0006$; slope: $a = 0.005$). F_o, the initial fluorescence, and F_m, the maximum fluorescence of the transient fluorescence analyses, both serve as a measure of the phenomological absorption flux

(ABS) per excited cross section (CS) as long as samples are in their dark-adapted state (Strasser et al. 2000). I measured these parameters with blue and white actinic light excitation. The ratios of these absorption fluxes (ABS/CS white:ABS/CS blue) in natural phytoplankton communities show a significant increase with increasing contribution of full spectrum PAR (linear regression F_o: R^2 = 0.71; p = 0.002; linear regression F_m: R^2 = 0.66; p = 0.004; Figure 4 C, B). Communities cultured at 24 h of full PAR spectrum showed, on average, a higher absorption flux per excited cross section (ratio > 1) for white light than for blue light, whereas for communities growing only under blue light the opposite was observed (ratio ~0.5). Results from laboratory communities showed a different pattern of spectral light use efficiency in response to light quality. Communities of all treatments had a much higher absorption flux per excited cross section for white light than for blue light (linear regression F_o: R^2 = 0.36; p = 0.006; linear regression F_m: R^2 = 0.21; p = 0.04; Figure 4 C, D). Flux ratios were > 2 in all treatments; I could even observe a weak increase of flux ratios with increasing blue light contribution. This observation is indicating a potential better physiological status of communities with higher contribution of blue light, as communities did not differ in species composition between treatments.

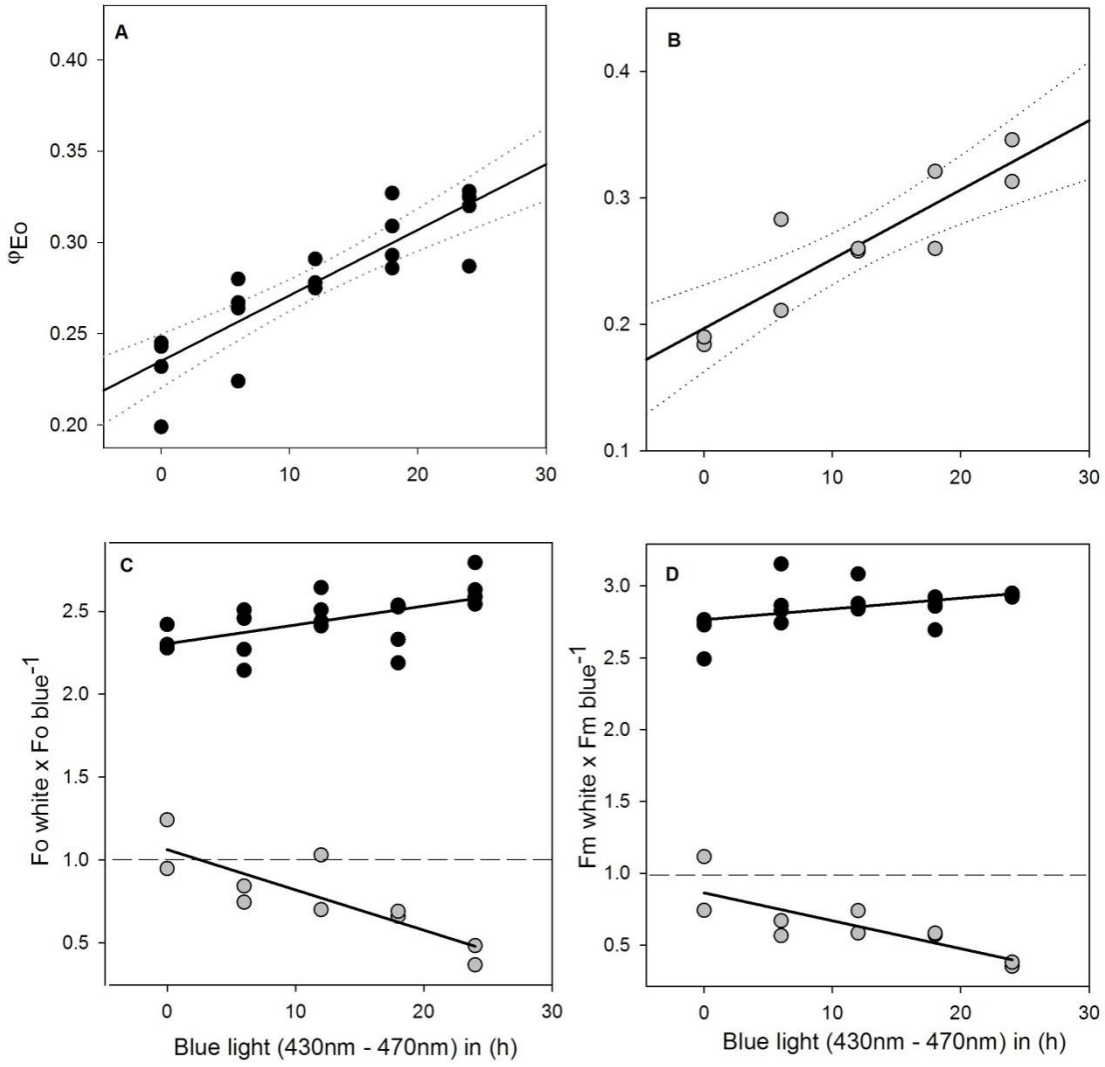


Figure 4: Total photosynthetic efficiency of electron transport chain in PSII (φ_{Eo}) as a function of increasing blue light (430nm – 470nm) in h in A) laboratory communities: linear regression: y = 0.004x + 0.24; R^2 = 0.76; p < 0.0001 and in B) natural communities: linear regression: y = 0.005x + 0.2; R^2 = 0.78; p = 0.0006. C) Initial fluorescence (Fo) of the transient fluorescence analyses as a function of increasing blue light (430nm – 470nm) in laboratory communities (black dots): linear regression: y = 0.01x + 2.3; R^2 = 0.36; p = 0.006 and in natural communities (grey dots): linear regression: y = -0.02x + 1.06; R^2 = 0.71; p = 0.002. D) Maximum fluorescence (Fm) the transient fluorescence analyses as a function of increasing blue light (430nm – 470nm) in laboratory communities (black dots): linear regression: y = 0.008x + 2.8; R^2 = 0.21; p = 0.04 and in natural communities (grey dots): linear regression: y = -0.02x + 0.86; R^2 = 0.66; p = 0.004.

Phytoplankton community composition

The phytoplankton composition in the natural communities from Lake Brunnensee was initially dominated by Bacillariophyta (99%), the rest was split into Chlorophyta, Chrysophyta, and Cyanophyta. The community composition of the natural communities changed drastically, depending on the spectral light regime. Cultures exposed to the full PAR spectrum were dominated by Cyanophyta, followed by Bacillariophyta, Cryptophyta, and Chlorophyta, in descending order (Table 3). A shift in the light spectrum towards more exposure to blue light resulted in a significant increase in abundance of Bacillariophyta (R^2 = 0.9; p = 0.02) and a decrease in abundance of Cyanophyta (R^2 = 0.9; p = 0.01). Abundances of Chlorophyta and cryptophyta showed no significant differences across (blue) light treatments. Diversity (H'), calculated based on the functional groups (Bacillariophyta, Chlorophyta, Cyanophyta, Cryptophyta) in natural phytoplankton communities, decreased with increasing blue light exposure (R^2 = 0.9; p = 0.01). Evenness (E) showed a similar decrease with increased exposure to blue light (R^2 = 0.9; p = 0.01; Figure 5). Species richness did not change significantly depending on the treatment with a maximum of twelve species in white light and six h blue light treatments and eight species in only blue light treatments. With increasing duration of blue light exposure, Bacillariophyta dominated the community with five different species out of maximum eight species. However, in treatments with increasing white light exposure time, the functional group of Cyanophyta contained mainly (~80%) one species: *Anabaena cylindrica*.

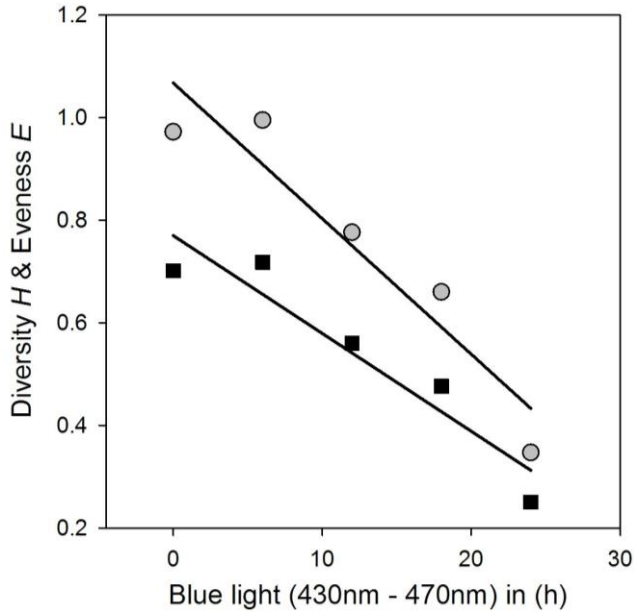


Figure 5: Diversity H' (grey dots) and Evenness (black squares) as a function of increasing blue light (430nm – 470nm) in natural communities. Diversity H': linear regression: y = -0.03x + 1.1; R^2 = 0.9; p = 0.01. Evenness: linear regression: y = -0.02x + 0.8; R^2 = 0.9; p = 0.01.

Table 3: Functional group distribution under increasing blue light supply in natural communities:

Field community

Community composition after 20 days (%)

(Brunnensee)

	Blue light (430nm-470 nm) in h					**Linear regression y = ax + b**			
Functional group	0	6	12	18	24	a	R^2	p	signif. Level
Chlorophyta	0.18	0.77	0.88	0.57	0.19	-0.00	0.00	0.9	ns
Cyanophyta	58.58	56.84	7.76	5.81	0.25	-0.19	0.9	0.01	**
Bacillariophyta	23.27	27.83	73.32	79.12	89.96	0.12	0.9	0.02	*
Cryptophyta	17.97	14.56	18.04	14.50	9.60	-0.02	0.6	0.12	ns

In laboratory communities, phytoplankton groups were initially distributed as follows (based on biovolume): Bacillariophyta (49%), Chlorophyta (31%), Cyanophyta (11%), Cryptophyta (8%) and Chrysophyta (1%). I observed no significant changes in the distribution of functional algal groups during the experiment. Algal composition did not show significant changes in response to the contribution of blue light to the daily light supply (Table 4).

Table 4: Functional group distribution under increasing blue light supply in laboratory communities:

Laboratory communities	*Community composition after ten days (%)*								
	Blue light (430nm-470 nm) in h					**Linear regression y = ax + b**			
Functional group	0	6	12	18	24	a	R^2	p	signif. Level
Chlorophyta	45.00	51.31	40.08	41.58	46.48	-0.01	0.06	0.70	ns
Cyanophyta	13.49	13.90	19.64	13.50	10.86	-0.01	0.09	0.60	ns
Bacillariophyta	38.91	32.08	38.32	42.44	39.53	0.01	0.23	0.40	ns
Cryptophyta	1.90	2.05	1.63	1.33	2.02	0.00	0.07	0.70	ns
Chrysophyta	0.70	0.65	0.33	1.16	1.11	0.01	0.30	0.40	ns

3.2 Invaders as induced anthropogenic enviormental biotic changes for a natural phytoplankton community

Invasion success of introduced species

None of the added species were successful at invading the resident community, which was indicated by a negative value for the calculated invasion success (Figure 6). However, added *Anabaena cylindrica* (Cyanophyta) showed the lowest negative invasion success, in the treatment with two combined added Cyanophyta (-15.94 ± 0.65) compared to all other unsuccessful invading species. Single added *Fragilaria crotonensis* (Bacillariophyta; -8.62 ± 0.20) showed, among the unsuccessful invading species, the highest negative invasion success. Furthermore, I found that some added species showed a lower abundance when they were added in combination with the second species of the same taxonomic group. Added *A. cylindrica* decreased more strongly when they were added in combination with the second Cyanophyta group compared to when added as single species. Added *Cyclotella meneghiniana* (Bacillariophyta) showed the opposite pattern; they decreased more strongly in communities to which they were added as single species rather than in combination with the second Bacillariophyta (*F. crotonensis*). On average, the two single added Chlorophyta (*Chlamydomonas reinhardtii*, *Pediastrum simplex*) showed a lower negative invasion success than the two single added Bacillariophyta (*F. crotonensis*, *C. meneghiniana*) and the two single added Cyanophyta (*A. cylindrica*, *Pseudonanabaena galeata)*. In treatments where algae were added in combination of two species of the same taxonomic group, Bacillariophyta showed the lowest negative invasion success (Figure 6).

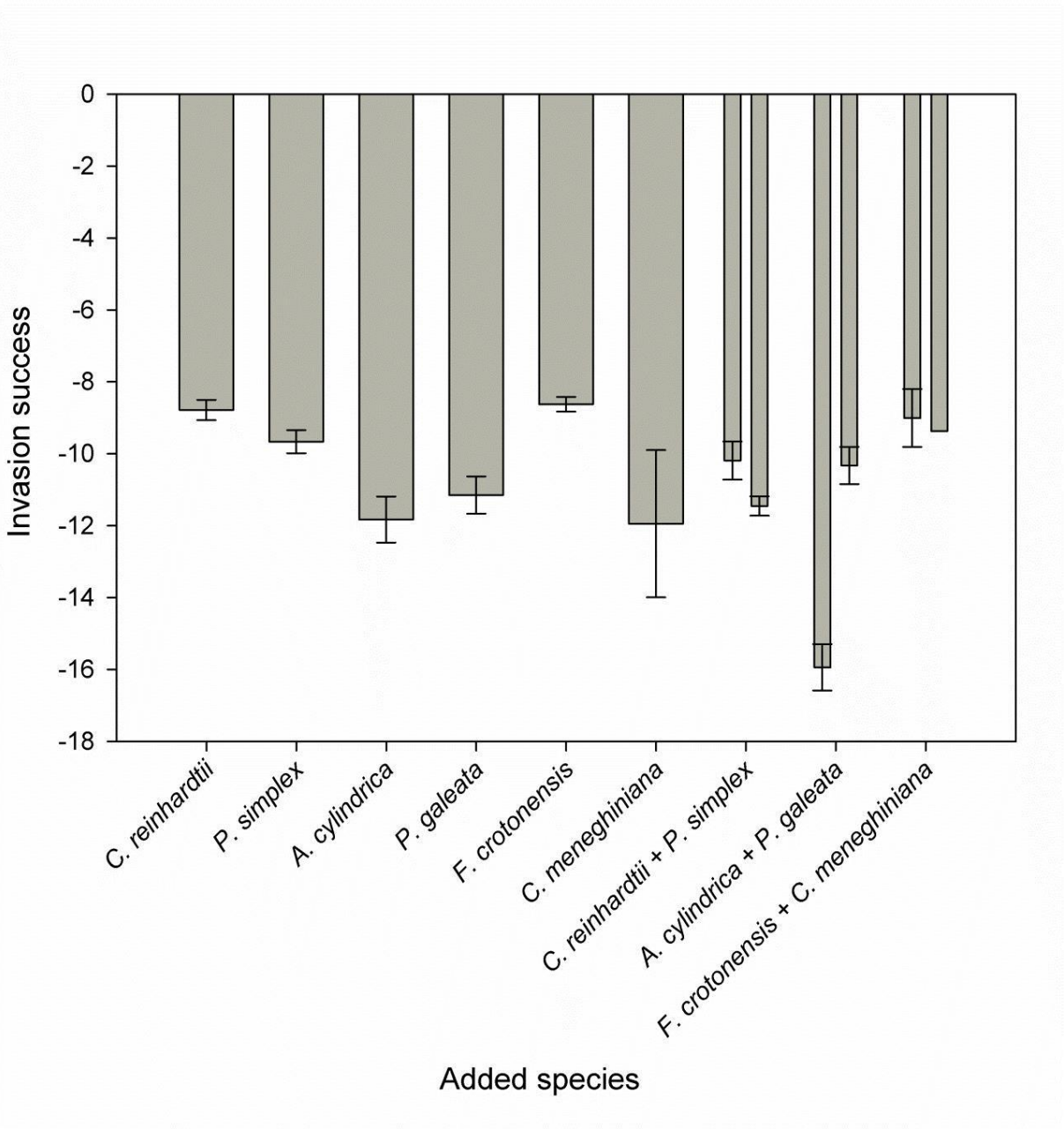


Figure 6: Invasion success of added species at the end of the experiment (mean ± 1 SE, n = 3). In the combined added species groups, the first bar represents the species stated first.

Diversity, evenness and species richness

Final diversity (H') and evenness (E) were lower in all experimental and control treatments compared to the initial community at the beginning of the experiment. However, treatments with experimental added species showed significant higher H´ and E than the control treatment without experimental added species (Figure 7). Especially, treatments with added *F.*

crotonensis, *C. meneghiniana* (Bacillariophyta) or respectively *A. cylindrica*, *P. galeata* (Cyanophyta) showed significantly higher values of H´ and E compared to the control treatment. Communities to which experimental Chlorophyta species (*C. reinhardtii*, *P. simplex*) were added showed no significant differences to control treatments (Figure 7, Table 5).

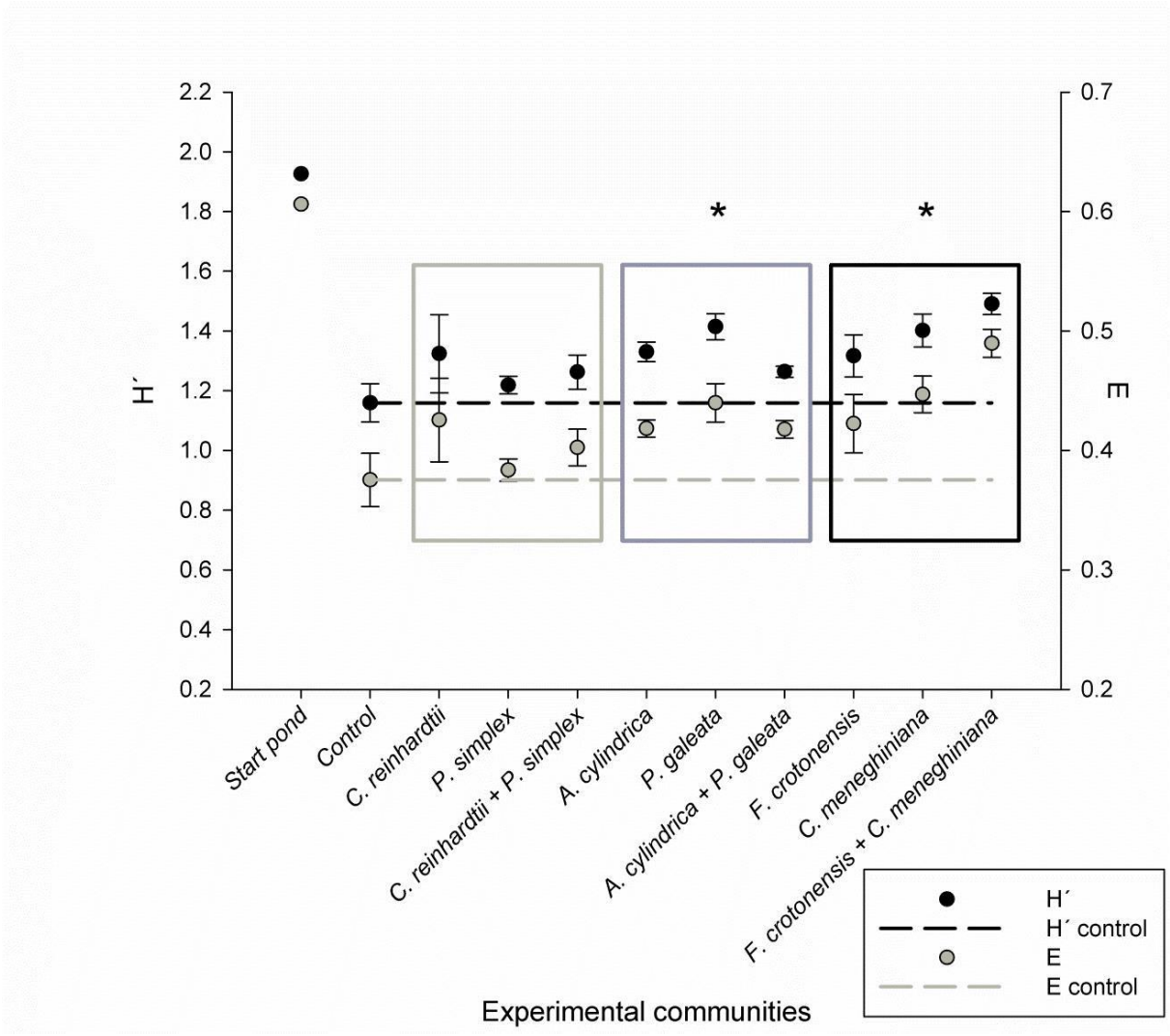


Figure 7: Diversity (H') and evenness (E) of start, control and experimental treatments (mean ± 1 SE, n = 3) at the end of the experiment. Asterisks indicate significant differences of experimental treatments with added species from the same taxonomic group (light gray box: Chlorophyta, dark gray box: Cyanophyta, black box: Bacillariophyta) versus control treatment without added species (t-test; $p < 0.017$).

Table 5: Differences in diversity (H') and evenness (E) between experimental treatments with added species (within a taxonomic group) and control treatment without added species t values with degrees of freedom (lowered number) and p-values are shown (t-test, significant level: $p < 0.017$ after Bonferroni correction for multiple testing). Significant differences from controls are indicated by asterisks. (Abbreviations: Add.spec. = Added species; tax. = taxonomic).

Add.spec. treatments within a tax. group	Chlorophyta		Bacillariophyta		Cyanophyta	
	t_{10}	p	t_{10}	p	t_{10}	p
H'	1.26	0.24	2.99	0.014*	3.27	0.008*
E	1.10	0.30	3.03	0.013*	2.93	0.015*

Species richness decreased in all treatments during the experiment, except in treatments where *P. simplex* was added. In these treatments, the mean species richness was significantly higher (indicated by 95% confidence intervals not overlapping) compared to the control (Figure 8).

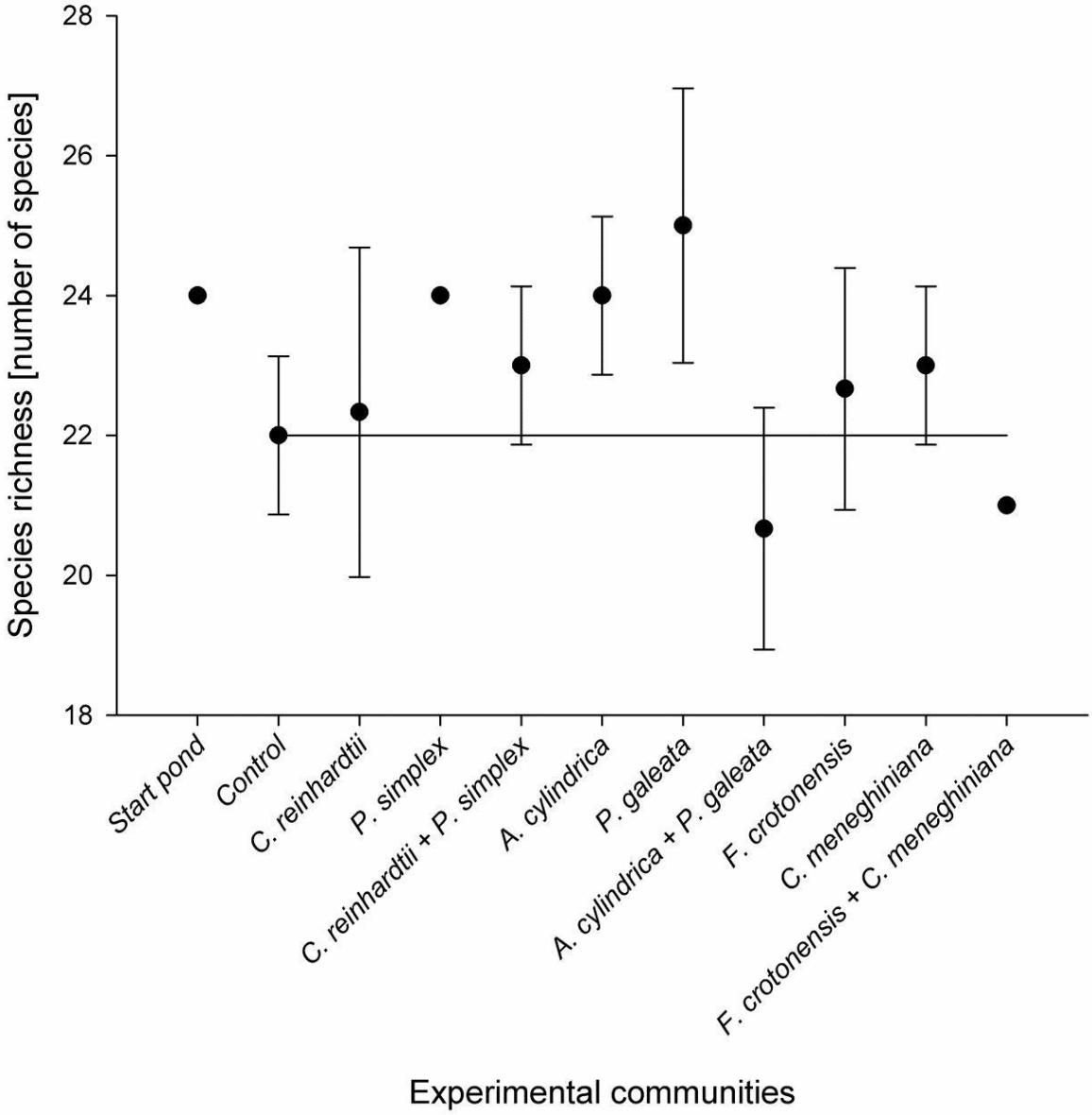


Figure 8: Species richness as number of species of start, control and experimental treatments with added species (mean ± 1 CI, n = 3) at the end of the experiment. The Black line shows mean species richness of the control treatment. Missing significant differences between experimental treatments and control treatment were indicated by non-crossing of 95% confidence intervals.

Community group composition

At the end of the experiment, the control phytoplankton communities consisted of mainly Bacillariophyta (67.3% ± 1.3), followed by Chlorophyta (31.2% ± 1.2), Cyanophyta (0.6% ± 0.08) and Chrysophyta (0.4% ± 0.2). This result indicates an increase in Bacillariophyta and a decrease in Chrysophyta. However, there was an overall loss of Chrysophyta in all treatments in the experiment.

A similar pattern was obtained when control and experimental treatments (Figure 9) were compared. The group composition of treatments with added species was still very similar to the control. The treatment with a combination of two added Bacillariophyta species (combined *F. crotonensis, C. meneghiniana* treatment) showed the lowest (77.5% ± 4.2) similarity, whereas the treatment with two combined added Chlorophyta species (combined *C. reinhardtii, P. simplex* treatment 92.4% ± 0.5) showed the highest similarity with the control (Figure 9).

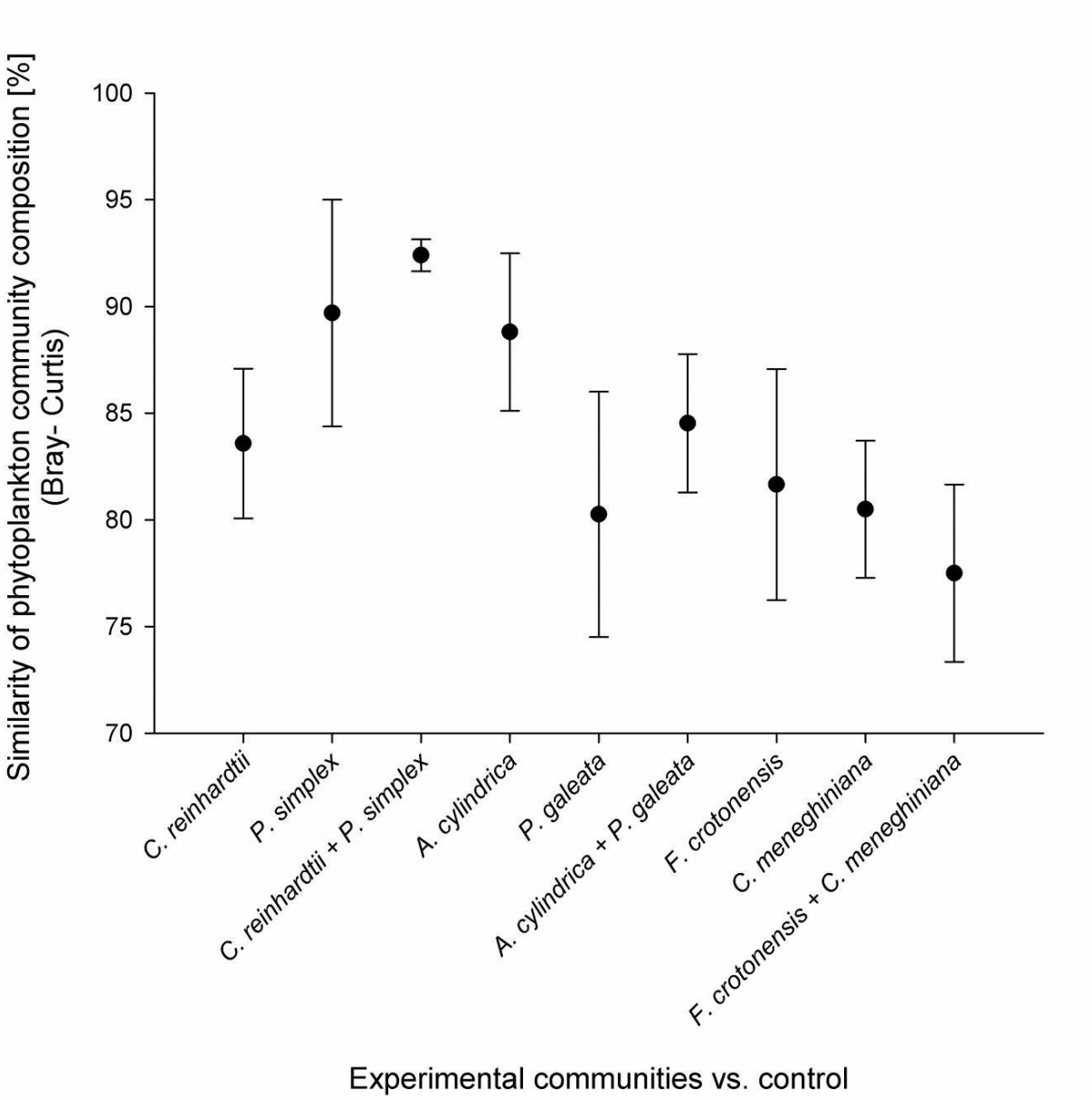


Figure 9: Comparison of phytoplankton communities' composition by Bray-Curtis similarity (100% = total similar; 0% = total dissimilar) based on log (x + 1) transformed species biovolume (mean ± 1 CI, n = 3). Data show similarity (%) of experimental treatments with added species compared to control treatment without added species.

However, the differences in the taxonomic group composition observed in the experimental treatments already show some significant (indicated by 95% confidence intervals not overlapping with zero; Figure 10) differences compared to the control. For instance, experimental treatments with added Bacillariophyta showed a higher abundance of resident Chlorophyta (*C. meneghiniana* treatment*:* 10.5% ± 2.7, combined *F. crotonensis*, *C.*

meneghiniana treatment: 10.53 ± 8.7), but a lower abundance of resident Bacillariophyta (*C. meneghiniana* treatment:-10.42% ± 2.5, combined *F. crotonensis, C. meneghiniana* treatment: -12.17 ± 8.3) compared to the control (Figure 10). Very similar patterns were found in treatments with added experimental Cyanophyta (single and combination species). In treatments added with single *P. galeata* and a combination of two Cyanophyta species, the resident Chlorophyta abundance was higher (*A. cylindrica* treatment*:* 10.3% ± 9.3; *P. galeata* treatment: 7.6% ± 6.9) compared to the control. Accordingly, resident Bacillariophyta abundance was lower in these experimental treatments (*A. cylindrica* treatment: -11.5% ± 10.3; *P. galeata* treatment: -9.2% ± 7.2) in comparison to the control. The abundance of resident Cyanophyta only changed significantly in treatments with added Cyanophyta (single and combination species *A. cylindrica* treatment: 0.8% ± 0.4, *P. galeata* treatment: 0.9% ± 0.7, *A. cylindrica* and *P. galeata* combination treatment: 1.8% ± 0.6) and one treatment with added Chlorophyta (*C. reinhardtii* treatments: 0.4% ± 0.12) compared to the control.

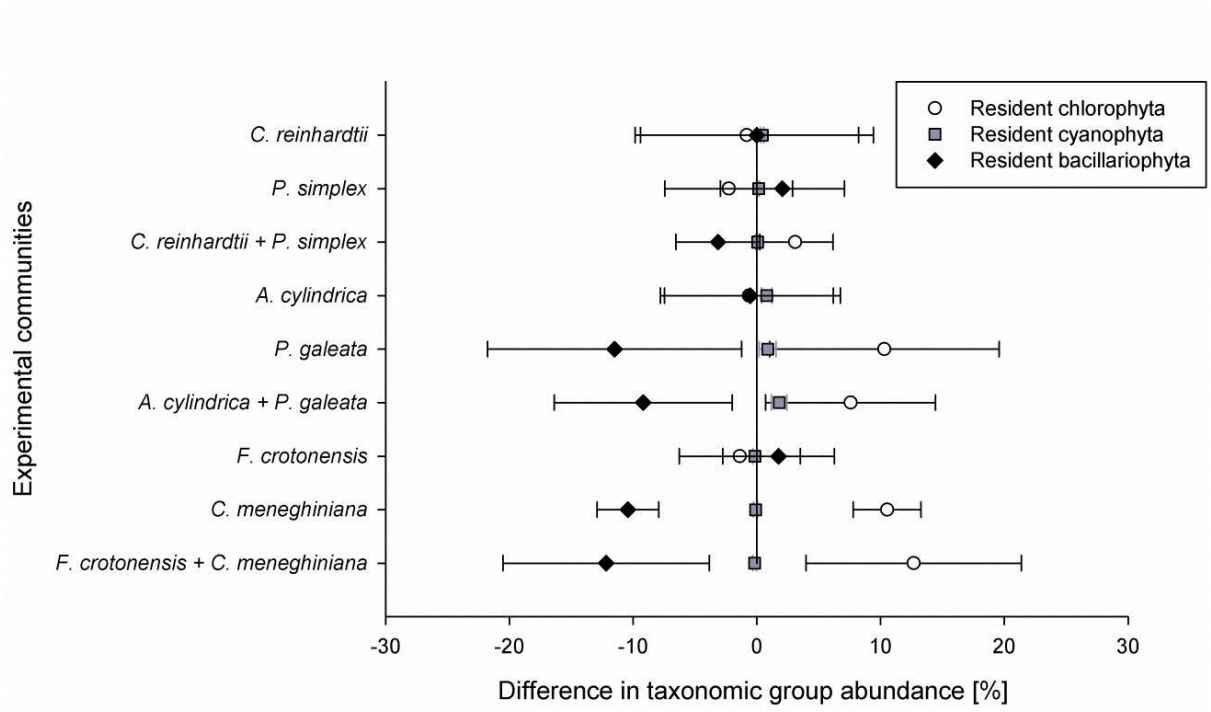


Figure 10: Differences in abundance (% on total phytoplankton biovolume) of the most abundant resident taxonomic groups between control and experimental treatments (mean ± 1 CI, n = 3). Significant differences were indicated by 95% confidence intervals not overlapping with the zero line (black straight line).

Species composition changes in resident communities

Not all experimental treatments with added species showed major changes in their resident species composition. Four of the treatments showed only very minor changes (about 0.1%). However, five (up to 15.8%) of the nine treatments showed significant changes in their resident species composition, mainly regarding their dominant species (Figure 11).

In all treatments where I added Bacillariophyta species, either added as single species or in combination of both additional Bacillariophyta species, four different resident species, *Synedra spec.*, *Ankistrodesmus spec*., small coccal Chlorophyta, *Fragillaria spec.* were affected. Two of these resident species were dominant in the resident community (*Synedra spec.*, small coccal Chlorophyta). SIMPER was used to determine the species contribution to the dissimilarity between experimental treatments and control. *Synedra spec.* (microplankton) was one of the major contributors to the overall dissimilarity (18.0% (*F. crotonensis* treatment), 24.3% (*C. meneghiniana* treatment), 32.1% (combined *F. crotonensi*, *C. meneghiniana* treatment) to the controls. *Synedra spec.* showed lower abundances in all treatments where I added Bacillariophyta (*F. crotonensis* treatment: -3.5% ± 1.7, *C. meneghiniana* treatment: -9.2% ± 0.8, combined *F. crotonensis*, *C. meneghiniana* treatment: -15.8% ± 5.9) compared to the control (64.0% ± 1.8). The resident Chlorophyta *Ankistrodesmus spec*.; nanoplankton, was according to the SIMPER analysis also a main contributor to dissimilarity (17.6% in *F. crotonensis* treatment, to 38.0% in *C. meneghiniana* treatment and 28.5% in combined *F. crotonensis*, *C. meneghiniana* treatment). Different to *Synedra spec.*, *Ankistrodesmus spec.* showed higher abundances after adding Bacillariophyta species. (*F. crotonensis* treatment: 6.4% ± 3.6, *C. meneghiniana* treatment: 14.7% ± 2.6, combined *F. crotonensis*, *C. meneghiniana* treatment: 14.0% ± 5.5). Similar to *Ankistrodesmus spec.*, adding Bacillariophyta species resulted in higher abundances of *Fragilaria spec.; nanoplankton* (*F. crotonensis* treatment: 8.5% ± 1.4, combined *F. crotonensis* and *C. meneghiniana* treatment: 6.3 ± 0.9) compared to control. Based on the SIMPER analyses *Fragilaria spec.* was the main species contributing to the dissimilarity between the control and added single Bacillariophyta treatment (*F. crotonensis* treatments) with 23.3%, and in combined *F. crotonensis* and *C. meneghiniana* treatment with 12,7%. In treatments with single added *F. crotonensis* and single added *C. meneghiniana*, the abundance of resident small coccal Chlorophyta (< 5 μm, nanoplankton) was lower (-8.4% ± 6.6 and -7.1% ± 3.9) in comparison to controls. According to the SIMPER analyses the reduction of small coccal Chlorophyta abundance in treatments

where I added Bacillariophyta contributed with 23.2% (*F. crotonensis* treatment) and 18.2% (*C. meneghiniana* treatment) to dissimilarity to the control.

In treatments with added Cyanophyta species, only one resident species was strongly affected especially by the addition of *P. galeata* and the combination of both Cyanophyta species (*A. cylindrica and P. galeata)*. The abundance of *Ankistrodesmus spec*. was higher (14.7% ± 3.9 *P. galeata* treatment and 10.5% ± 0.5 combined *A. cylindrica, P. galeata* treatments when compared to control treatment and was the major contributor (37.2% *P. galeata* treatment; 35.4% combined *A. cylindrical, P. galeata* treatment) for dissimilarity to the control.

Two resident species were affected by adding Chlorophyta species. SIMPER analysis showed a main contribution of *Chlamydomonas spec*. (17.6% *C. reinhardtii* treatment; 12.3% combined *C. reinhardtii, P. simplex* treatment) and small coccal Chlorophyta (30% *C. reinhardtii* treatment; 24.1% combined *C. reinhardtii*, *P. simplex* treatment) to the dissimilarity compared to control. The abundance of *Chlamydomonas spec*. was higher (5.1% ± 3.2 *C. reinhardtii* treatment) compared to the control, whereas the abundance of small coccal Chlorophyta was lower (-8.7% ± 7.7 *C. reinhardtii* treatment).

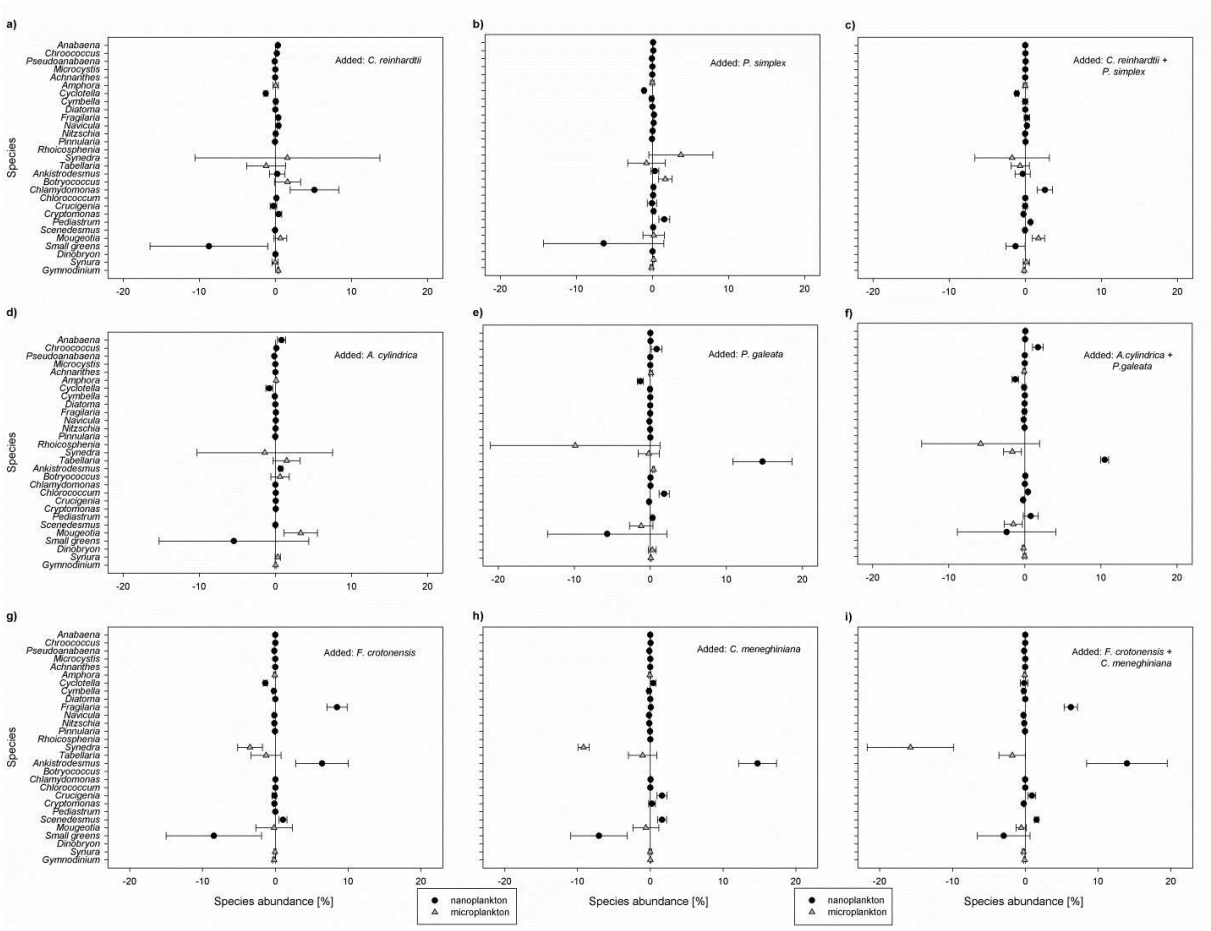


Figure 11: Differences in abundance (% of total phytoplankton biovolume) and cell size class of the most abundant resident species in experimental treatments with added species compared to control treatment without added species. a - c) shows community compositions of treatments with added Chlorophyta d - f) shows community compositions of treatments with added Cyanophyta communities and g - i) shows community compositions of treatments with added Bacillariophyta (mean ± 1 CI, n = 3). Significant differences between experimental treatments (added species) and control treatment (without added species) were indicated by non-overlapping of 95% confidence intervals with the zero line (black straight line). Gaps indicate that no individual of the species was found in the treatment.

3.3 Combined light quality and invasion effects on community assembly and their effects on zooplankton

Chlorophyll-a content

Chlorophyll-*a* of each phytoplankton community was measured biweekly (Figure 12). Both, light quality and presence of *A. cylindrica* had a significant effect on total chlorophyll-*a* content of the phytoplankton communities on day 6 (light: $F_{(2,18)} = 8.06$, $p < 0.01$; invasion: $F_{(1,18)} = 4.84$, $p = 0.05$, Table 6). On day 10, light quality had a significant effect on chlorophyll-*a* content while invasion had no significant effect (light: $F_{(2,18)} = 4.86$, $p = 0.03$; invasion: $F_{(1,18)} = 0.01$, $p = 0.93$). On all other days, neither light nor invasion had a significant effect on total chlorophyll-*a* content (Table 6, Table S1).

A peak of Cyanophyta occurred between day 8 and day 17 in communities with invasion exposed to red light (Figure S1). During peak Cyanophyta presence, there were no significant differences in chlorophyll-*a* levels between invaded and non-invaded communities.

Table 6: Two-way ANOVA results for effect of light, invasion and interaction effect between light and invasion on chlorophyll-*a* (chl-*a*) content in the phytoplankton communities. Asterisks are indicating statistically significant differences ($p \leq 0.05$).

Day	Effect of light on chl-*a*	Effect of invasion on chl-*a*	Interaction between light and invasion on chl-*a*
6	<0.01*	0.05*	0.87
10	0.03*	0.93	0.07
20	0.66	0.11	0.92
24	0.28	0.56	0.24

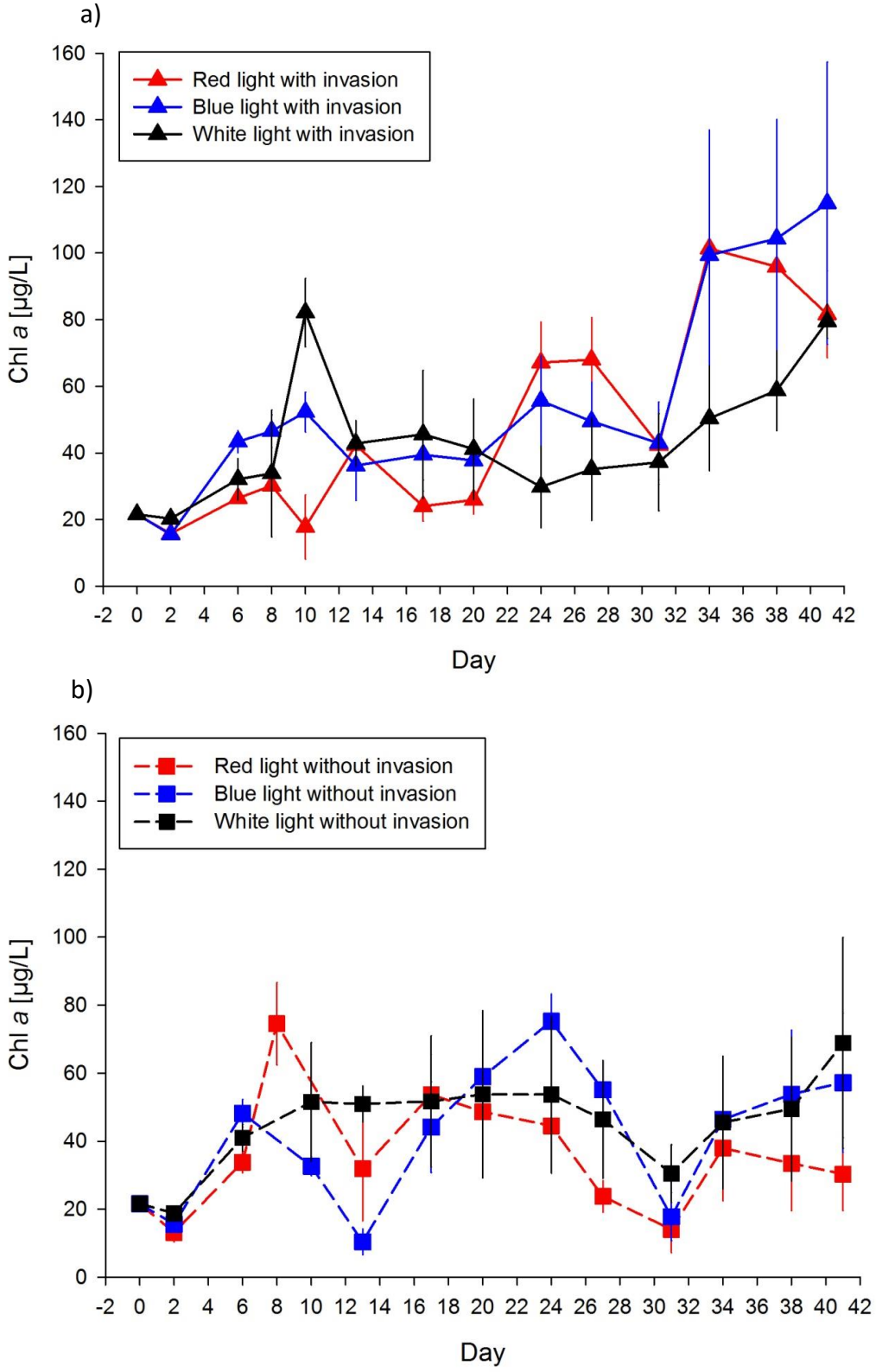


Figure 12: Total chlorophyll-*a* content of the phytoplankton communities over the time course of the experiment. Chlorophyll-*a* content with (a) and without (b) addition of potential invading *A. cylindrica* to the phytoplankton communities (mean ± 1 SE, n = 3).

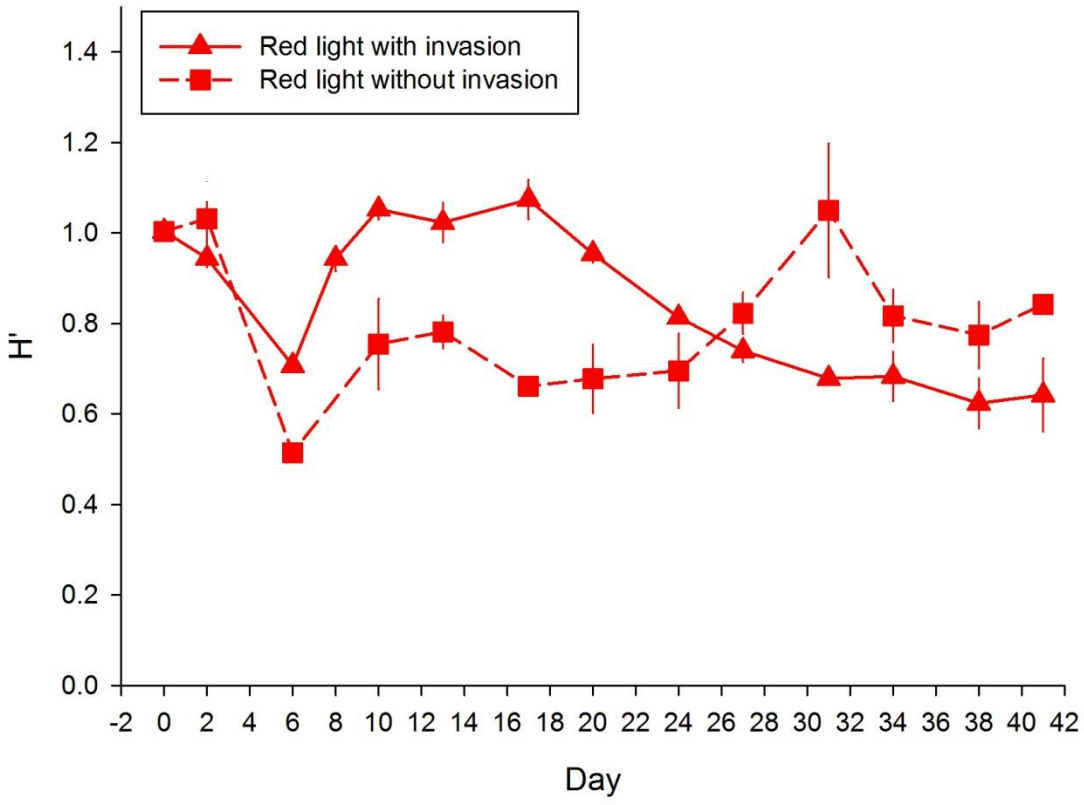
Biodiversity

Biodiversity for each study community was characterized using the Shannon Index (H'; Figure 13, Table 7). Invasion had a significant influence on the biodiversity of the phytoplankton communities on all days except day 41 (invasion: day 6 – $F_{(1,18)} = 28.34$, $p < 0.01$; day 10 – $F_{(1,18)} = 13.65$, $p < 0.01$; day 20 – $F_{(1,18)} = 27.79$, $p < 0.01$; day 24 – $F_{(1,18)} = 4.74$, $p = 0.05$). Invaded phytoplankton communities had higher biodiversity than non-invaded communities from day 6 to day 24 (Figure 13, Table S2). Light only had a significant effect on diversity of the phytoplankton communities on day 6 (light: $F_{(2,18)} = 17.05$, $p < 0.01$). Light had negligible effects on diversity of the phytoplankton communities on all other days (Table S2). A significant interaction effect in the phytoplankton communities on diversity was found on day 6 (interaction: $F_{(2,18)} = 31.86$, $p < 0.01$, Table S2).

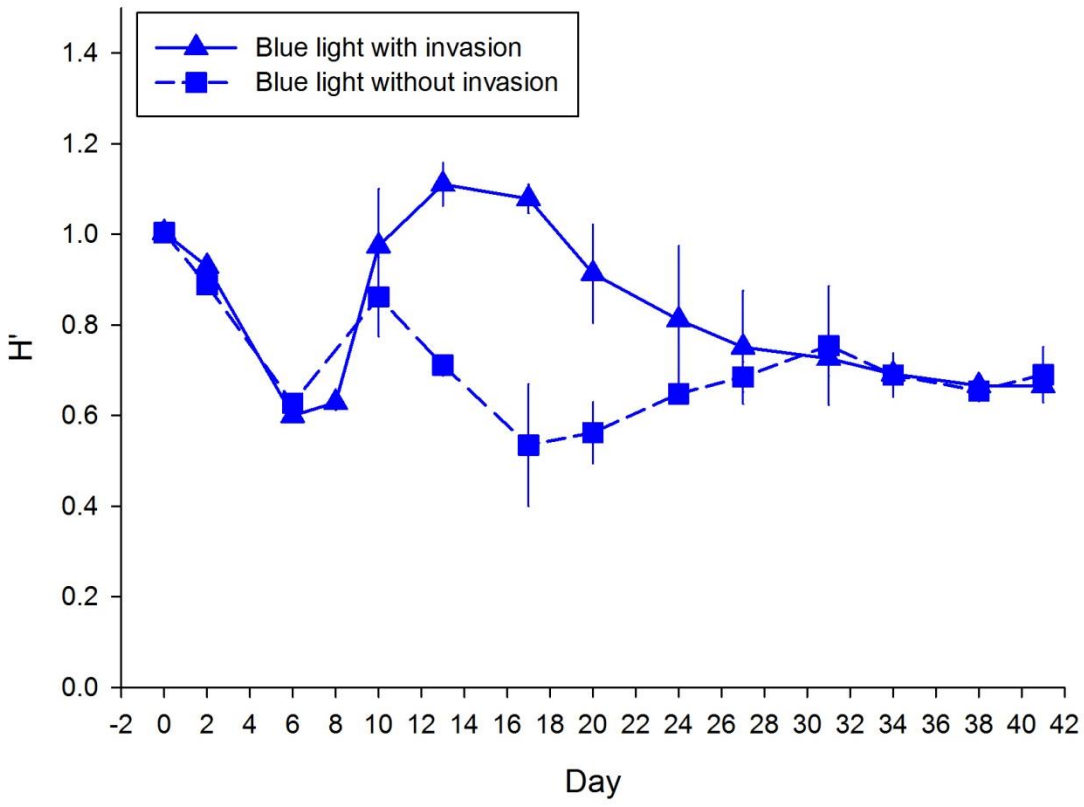
Table 7: Two-way ANOVA results for effect of light, invasion and interaction effect between light and invasion on diversity (H') in the phytoplankton communities. Asterisks indicate statistically significant differences ($p \leq 0.05$).

Day	Effect of Light on H'	Effect of Invasion on H'	Interaction between Light and Invasion on H'
6	<0.01*	<0.01*	<0.01*
10	0.41	<0.01*	0.43
20	0.49	<0.01*	0.49
24	0.89	0.05*	0.95

a)


Red light with invasion
Red light without invasion
H'
Day
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
-2 0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42

b)


Blue light with invasion
Blue light without invasion
H'
Day
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
-2 0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42

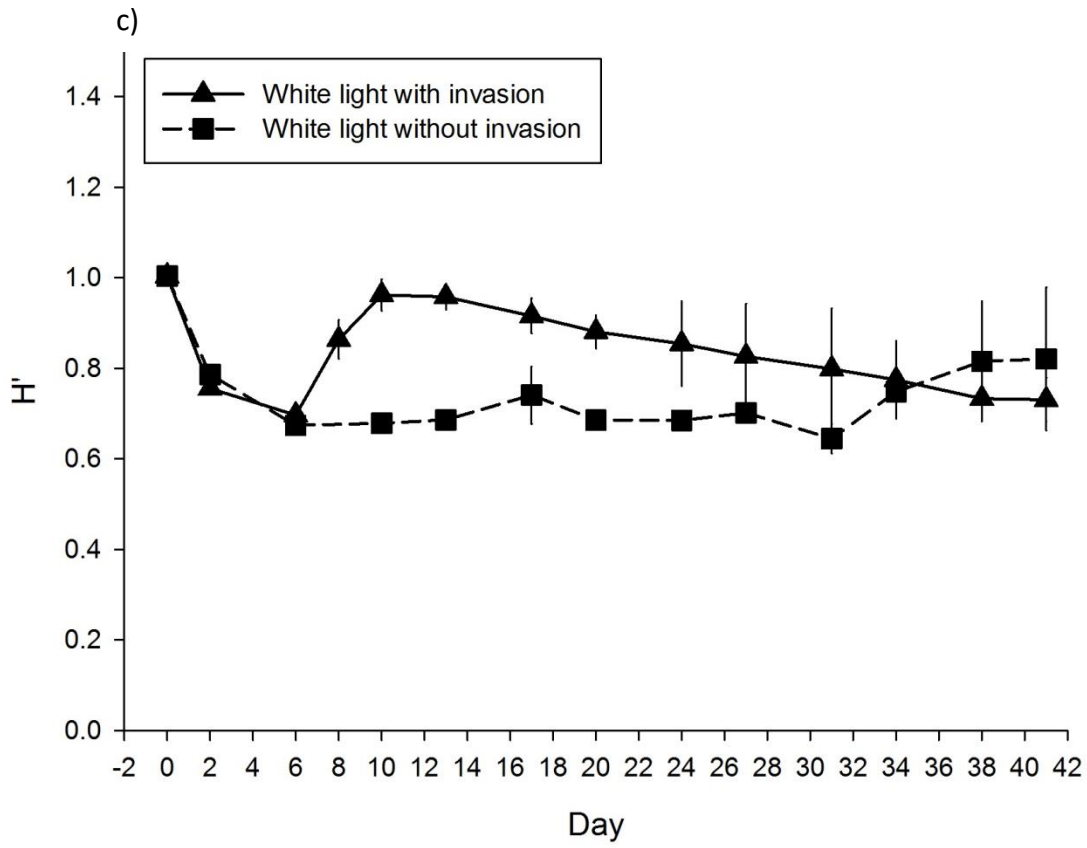


Figure 13: Shannon Diversity Index (H') of the phytoplankton communities over the time course of the experiment. H' of phytoplankton communities under red light condition with and without addition of invasive *A. cylindrica* (a), under blue light condition with and without addition of invasive *A. cylindrica* (b) and under white/full-spectrum light with and without addition of invasive *A. cylindrica* (c) (mean ± 1SE, n = 3).

Community composition

Microscopic analyses of phytoplankton at the end of the experiment show that Bacillariophyta are the dominating phytoplankton group in each treatment. Cyanophyta increase more in red light than in blue or white light, regardless of presence of invader (Table 8).

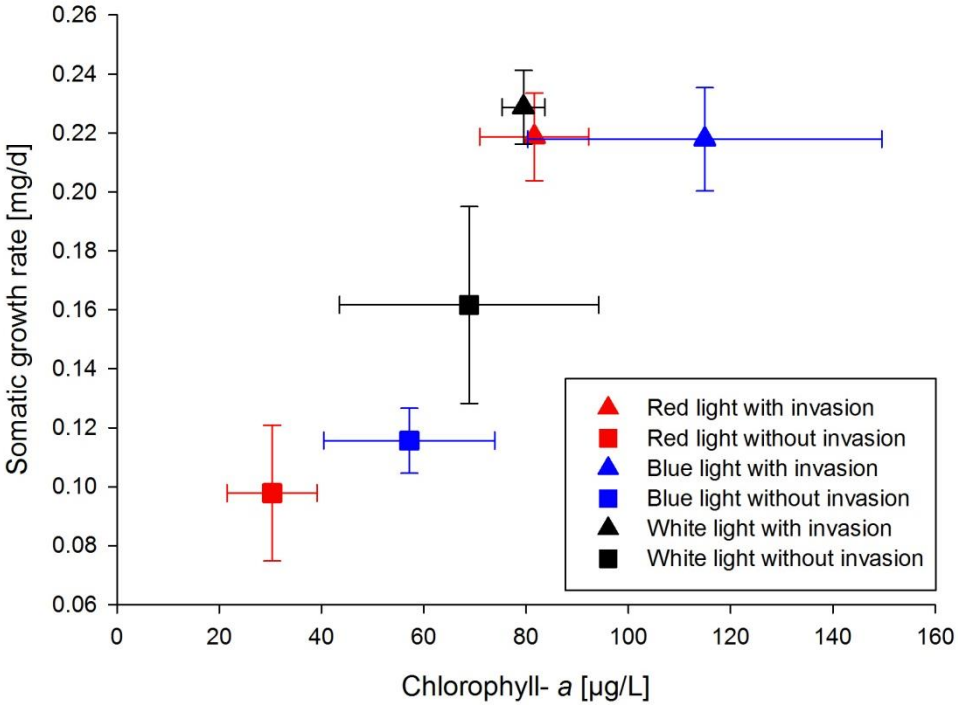
Table 8: Abundance of phytoplankton functional groups based on treatments (mean, n = 3).

Treatment	Bacillariophyta %	Chlorophyta %	Cyanophyta %
Red light with invasion	82.0	2.4	15.6
Red light without invasion	69.3	0.9	29.8
Blue light with invasion	71.3	27.4	1.3
Blue light without invasion	60.8	30.8	8.4
White light with invasion	76.9	14.3	8.8
White light without invasion	60.4	36.7	2.9

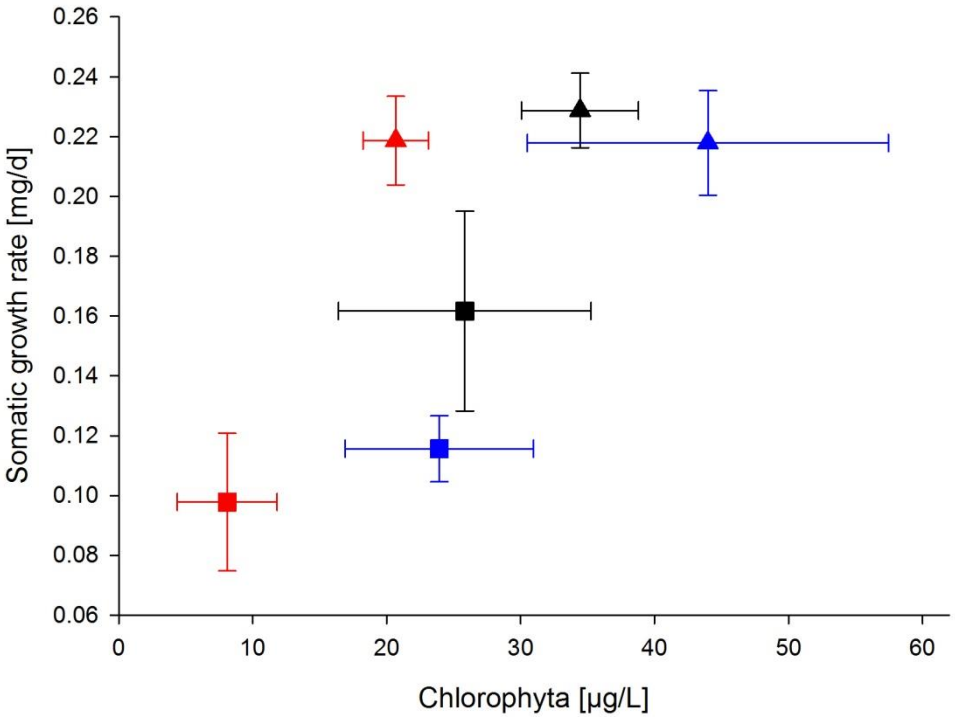
Somatic growth of Daphnia magna

The presence of an invader had a significant effect of *Daphnia* somatic growth (Two-way ANOVA, $F_{(2,18)} = 30.7$, $p = 0.03$), whereas different light conditions showed no differences in *D. magna* growth rate ($F_{(2,18)} = 1.88$, $p = 0.35$). Additionally, statistical analyses of variation within the "invaded" and "non-invaded" groups were conducted to test if the presence of invasion facilitated or degraded stability, as measured by variance. The coefficient of variance showed a greater variance in *D. magna* growth rate ($CV_{invader} = 2.7$, $CV_{control} = 26.4$) and chlorophyll-*a* content ($CV_{invader} = 21.6$, $CV_{control} = 37.9$) in non-invaded communities than those that were invaded (Figure 14 a). Between chlorophyll-*a* content and somatic growth rate of *D. magna* a significantly positive correlation was found (Correlation coefficient = 0.85, $p = 0.03$). Since Chlorophyta are the preferred diet for *D. magna* (Ebert 2005), a correlation test was used to investigate the relationship between Chlorophyta levels and somatic growth rate. A positive correlation was found between Chlorophyta levels and *Daphnia* somatic growth rate (correlation coefficient = 0.65, $p = 0.004$). Chlorophyta grow well in blue and white light relative to red light (Table 8). Chlorophyta levels were more varied in control communities without invasion than in communities with invasion ($CV_{invader} = 35.4$, $CV_{control} = 50.5$, Figure 14 b).

a)


Somatic growth rate [mg/d]
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0
20
40
60
80
100
120
140
160
Chlorophyll- a [µg/L]
Red light with invasion
Red light without invasion
Blue light with invasion
Blue light without invasion
White light with invasion
White light without invasion

b)


Somatic growth rate [mg/d]
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0
10
20
30
40
50
60
Chlorophyta [µg/L]

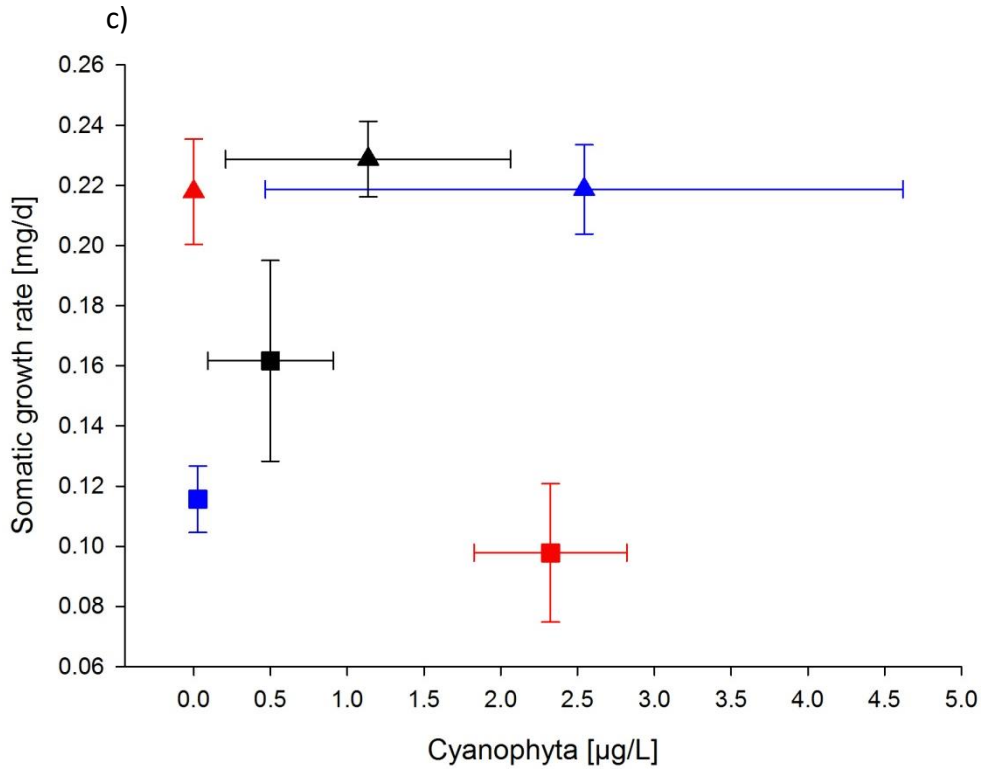


Figure 14: Coefficient of variation of *D. magna* somatic growth rate versus the chlorophyll-*a* content (a), the abundance of Chlorophyta (b) and the abundance of Cyanophyta (c) in the phytoplankton communities of different light exposure (red-, blue-, white-light) and with or without the addition of the potential invader *A. cylindrica* (mean ± 1SE; n = 3).

The correlation between Cyanophyta amounts of the phytoplankton communities and *Daphnia* somatic growth rate showed no significant correlation (Correlation coefficient = -0.10, p = 0.69). Communities of both control without invasion and communities with invasion exhibit high variations of Cyanophyta amounts among communities; the control communities showed higher variation of Cyanophyta amounts than invaded communities ($CV_{invader}$ = 103.9, $CV_{control}$ = 127.7, Figure 14 c).

3.4 Life history of *Daphnia magna* when feeding on genetically modified microalgae

As a response of *D. magna* to the different food sources (WT, CWD, GM low, GM high) of *C. reinhardtii* the age at first reproduction, size of neonates at first reproduction, fecundity, and fitness showed different performances. *D. magna* fed with GM low produced on average five days later their first clutch compared to *D. magna* fed with WT *C. reinhardtii* (Figure 15 a),

whereas *D. magna* of all treatments showed a similar response in size at first clutch (Figure 15 b, Table 9).

Table 9: One-way ANOVA of life history parameters of *D. magna* (df- degrees of freedom; FR- first reproduction; *r*- intrinsic growth rate per day). Where applicable, Holm Sidak *post-hoc* test was used to assign *D. manga* responding differences between the food sources of *C. reinhardtii*: wild type (WT), cell wall deficient (CWD), genetically modified (GM) with high and low secreting VEGF. Bold numbers indicate significant differences. Different letters indicate significant differences between different food sources of *C. reinhardtii*.

Life history trait	**df**	***F* ratio**	***P* value**	**Holm Sidak test food source**			
				WT	CWD	GM low	GM high
Age FR [day]	3,11	7.08	**0.006**	a	ab	b	ab
Size FR [mm]	3,11	1.82	0.20	--	--	--	--
Neonate size FR [mm]	3,70	51.28	**<0.001**	a	b	d	c
Reproductive effort [%]	3,11	1.13	0.38	--	--	--	--
Fecundity	3,11	9.72	**0.002**	a	ab	ac	ac
Fitness r	3,11	25.02	**<0.001**	a	b	b	b

D. magna fed with CWD *C. reinhardtii* showed on average 0.05 mm significant bigger neonate size at first reproduction compared to *D.magna* fed with WT *C. reinhardtii* Furthermore, *D. magna* fed with either GM low or GM high *C. reinhardtii* produced on average 0.06 mm to 0.07 mm smaller neonates in comparison to *D. magna* fed with CWD *C. reinhardtii*. Additionally, neonates at the first reproduction of *Daphnia* fed with GM high *C. reinhardtii* were on average 0.07 mm significant smaller than neonates of *Daphnia* fed with GM low *C. reinhardtii* (Figure 15 c, Table 9).

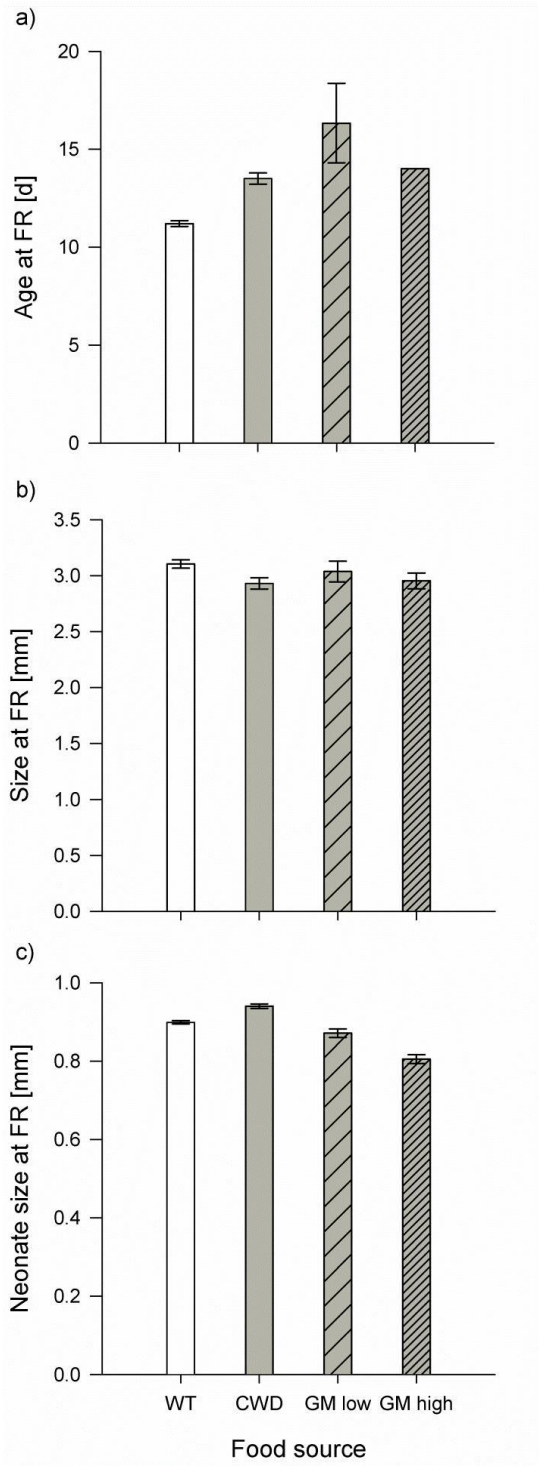


Figure 15: Life history responses at first reproduction (FR) of *D. magna* fed with different food sources of *C. reinhardtii*, wild-type (WT), cell wall deficient (CWD), genetically modified (GM) with low and high-secreting VEGF: a) *Daphnia* age, b) *Daphnia* body length, c) Neonate body length. Error bars indicate ± 1 SE.

All *Daphnia* independently of the food source put on average about 50 percent of their resource allocation in their reproduction (Figure 16 a). However, *D. magna* fed with GM low or GM high *C. reinhardtii* had a tenth higher fecundity within three clutches than *D. magna* fed with CWD *C. reinhardtii*. *D. magna* of the WT treatment showed a fecundity between the both GM *C. reinhardtii* and the CWD *C. reinhardtii* treatments (Figure 16 b).

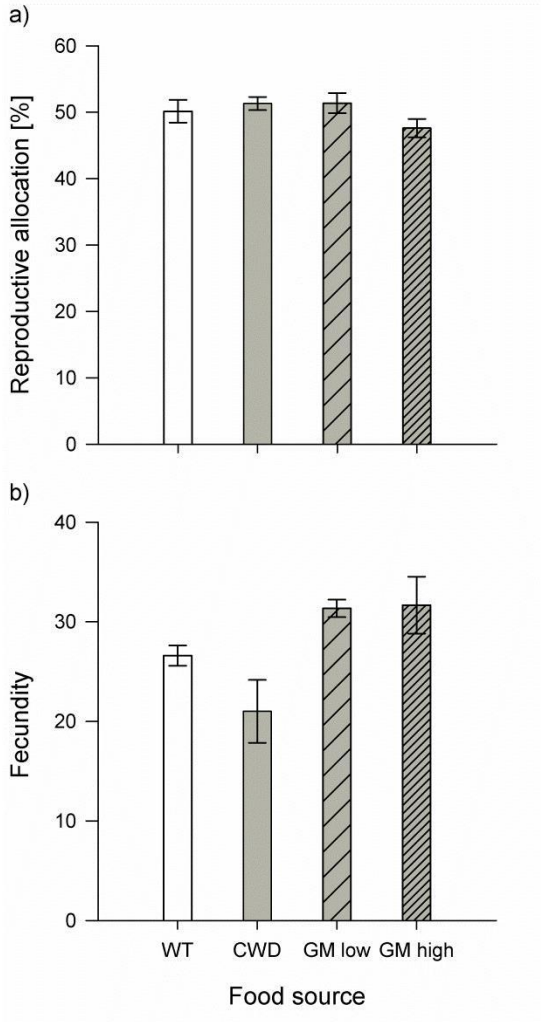


Figure 16: Reproductive effort (a) and Fecundity (b) as number of neonates within three clutches per mother of *D. magna* fed with different food sources of *C. reinhardtii*, wild-type (WT), cell wall deficient (CWD), genetically modified (GM) with low- and high-secreting VEGF. Error bars indicate ± 1 SE.

For the fitness (*r*) as intrinsic growth rate per day *D. magna* fed with CWD, GM low or GM high *C. reinhardtii* had a sixth lower fitness than *D. magna* fed with WT *C. reinhardtii* (Figure 17, Table 9).

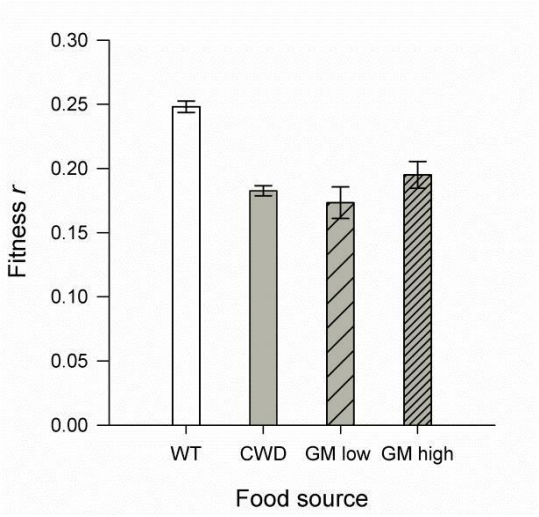


Figure 17: Fitness (*r*) of *D. magna* fed with different food sources of *C. reinhardtii*, wild- type (WT), cell wall deficient (CWD), genetically modified (GM) with low- and high-secreting VEGF within three clutches. Error bars indicate ± 1 SE.

4. Discussion

4.1 Light quality change and community assembly

Expected increasing surface water temperatures will lead to a decrease in mixing depths of water columns (Le Quéré et al. 2003, Winder and Schindler 2004). Changing water column stratification has biological consequences and can alter community dynamics of pelagic organisms (Diehl 2002, Jäger et al. 2008, Berger et al. 2010). The biological consequences of these changes have already been experimentally demonstrated in phytoplankton communities, which form the base of pelagic food webs (Diehl 2002, Jäger et al. 2008, Berger et al. 2010). These changes may be driven by several mechanisms, including mixing depth-related changes in resource supply (affecting growth) and sedimentation losses (affecting mortality). However, how spectral aspects of light linked to changing water column stratification affect phytoplankton has rarely been considered. Here, I show that expected shifts in spectral light availability (e.g., caused by shallower mixing depths) can result in large changes in phytoplankton community composition and performance.

As hypothesized, an experimental reduction of the available light spectrum for growth (and thereby the number of spectral light niches) resulted in reduced diversity (~50%) and evenness (~50%) in a natural phytoplankton community. The full white PAR spectrum allows a larger number of functional groups to coexist, probably due to niche differentiation by group-specific different secondary pigments (Stomp et al. 2004, Behl et al. 2011, Stockenreiter et al. 2013). The reduction of the available light spectrum from white to only blue resulted in strong alterations in how different functional groups were represented in the communities. For example, Cryptophyta and Cyanophyta strongly decreased in treatments with long (24 h and 18 h) blue light exposure. Mainly Bacillariophyta benefited from an exposition to a reduced PAR spectrum towards blue, whereas Cyanophyta showed increasing growth with increasing exposition to the full PAR spectrum. These results are consistent with the pigment compositions of these functional groups. Whereas all phytoplankton species carry chlorophylls, they differ in their secondary pigments, such as biliproteins in Cyanophyta (Rowan 1989, Kirk 1994). Cyanophyta can synthesize large amounts of such accessory pigments, which absorb at different wavelengths than chlorophylls. In the case of Cyanophyta, the high amounts of accessory pigments can efficiently fill parts of the so-called “green gap” of chlorophylls resulting from their absorption mainly in the blue and red spectrum. However, Cyanophyta is additionally very efficient at absorbing light of red wavelengths (Stomp et al. 2004). The white light treatment in my experiment provided a broader spectrum of wavelength accessible to accessory pigments, thereby supporting the growth of more different functional groups than the

blue light treatments. However, the functional group of Cyanophyta consisted mainly (80%) of one species, *Anabaena cylindrica*. *A. cylindrica* is known to carry allophycocyanin, with an absorbance maximum in the red part of the PAR spectrum (650 nm and 620 nm; Murakami et al. 1981). Whereas Bacillariophyta are known to reduce their photosynthetic efficiency when exposed to red light and increase it while exposed to blue and green light (Valle et al. 2014), as their pigment components are mainly chlorophylls and carotenoids (Kirk 1994). These findings are supported by the species distribution; in blue light treatments, Bacillariophyta dominated the community with several different species, whereas in white light treatments also species from other functional groups were more evenly distributed (Table 4). This also resulted in a higher light use efficiency for the full PAR spectrum, which increased linearly with higher availability of white light, as indicated by the decreasing ratio of the phenomenological absorption flux per excited cross section for white light and blue light.

However, I was surprised by the strong response of phytoplankton composition to the experimental manipulations of spectral light availability for a natural phytoplankton community. Resources and important environmental factors such as light intensity, temperature, and nutrients were held constant between treatments, but changes in light quality were able to severely influence phytoplankton community composition in a predicted and replicable way. Major algal groups were replacing each other in dominance, depending on light quality. White light supply, for example, shifted a community with initially 99% (biovolume based) dominance of Bacillariophyta towards a community with a clear dominance of Cyanophyta (Table 3). Hence, the effect size of light quality aspects for observed algal community shifts *in situ* is seldom discussed. Cyanophyta is a group of phytoplankton that is of interest for a variety of aspects related to the human use of water bodies and ecosystem services supplied by fresh and marine waters. Cyanophyta is often a nuisance or toxic and can drastically influence the use of water bodies for tourism, fisheries, or drinking water supply. Cyanophyta blooms are usually explained by eutrophication and/or increasing water temperatures (Robarts and Zohary 1987, Kosten et al. 2012, Winder 2012). However, Lürling et al. (2013) have shown in a growth experiment that Cyanophyta had no higher growth rates than Chlorophyta exposed to similar high temperatures. They suggested that Cyanophyta blooms are more based on their ability to migrate vertically when waters are strongly stratified. Hence, my results support the conclusion of Lürling et al. (2013) that increasing water temperatures might not only increase Cyanophyta abundances directly by physiological effects of temperature *per se* but also indirectly. My experiments show that temperature associated

declines in water column mixing depth and, thereby, increasing phytoplankton exposure to the full PAR spectrum, are an additional mechanism that can lead to Cyanophyta blooms. Previous studies typically manipulated temperature, nutrients, and light as a quantitative resource (Smith 1986, Blomqvist et al., 1994 Scheffer et al. 1997), but rarely has the quality of light been investigated experimentally. In their survey of 143 lakes, Kosten et al. (2012) showed that shallow lakes characterized by high light absorbance coefficients had a high proportion of Cyanophyta in their phytoplankton communities and speculated that this could be due to differences in the underwater light spectrum. My results support that idea and, to my knowledge, are the first to experimentally show that changes in light quality can contribute to Cyanophyta dominance in natural phytoplankton communities.

Shifts in phytoplankton composition were accompanied by shifts in community performance and photosynthetic parameters. The amount of chlorophyll-*a* per cell and the efficiency of PSII for blue light increased in communities grown under large blue light contribution. This resulted from a transient community shift towards groups containing more chlorophyll-*a*, such as Bacillariophyta and Chlorophyta. Indeed, Gorai et al. (2014) have shown that the chlorophyll-*a* content per unit biomass increased only in certain algal species (especially in Chlorophyta) when exposed to blue light over a longer period.

Results from the treatments with laboratory communities show that the algal species, which were used to assemble the different communities, were most probably already well adapted to use white light, indicated by the high ratio of absorption fluxes for white and blue light (>2). This seems to be a realistic assumption, as all strains were cultured under laboratory conditions for a minimum of several months, but mostly for years, meaning that algae were grown under white light illumination for a minimum of 100 generations or more. This might also be the reason why I did not observe a species shift in community composition along the spectral light gradient under laboratory communities. These findings suggest that the origin of phytoplankton strains is important for the outcome of ecological experiments (Strauss et al. 2008) and calls into question to which degree results from phytoplankton experiments are influenced by the cultivation history of the strains used.

Community composition, diversity, and algal performance of natural phytoplankton assemblages were strongly influenced by experimentally modifying spectral light quality during growth. The response patterns to changes in light quality were greater that those reported by studies investigating the effects of light intensity on phytoplankton communities

(Litchman 1998, Flöder et al. 2002). Litchman (1998) showed that high light conditions favored cyanobacterial growth, but also enhanced diversity at the same time.

These strong community shifts are likely to further influence dynamics at higher trophic levels and, thereby, affect entire pelagic food web processes. For example, Cyanophyta is known to be of minor food quality for most zooplankton species. Cyanophyta dominance related to shifts in the available PAR spectrum might, therefore, reduce food web transfer efficiencies and influence pelagic ecosystem dynamics (Karjalainen et al. 2007). In many pelagic systems, shifts in the available light spectrum that accompany changes in mixing depth may affect the phototrophic base of the food web and have far-reaching consequences, which require further experimental investigation. For example, Kokociński et al. (2010) have shown how *Cylindrospermopsis raciborskii*, an invasive Cyanophyta influences, plankton communities in shallow lakes by increasing the phytoplankton diversity, whereas the opposite was observed for high abundances of another Cyanophyta, *Planktothrix agardhii*, which resulted in decreasing phytoplankton diversity. Such different influences on plankton communities by native and invading species show the importance of effects of invasive species on plankton communities.

4.2 Invaders and their effects on a natural phytoplankton community

The impact of successful invasions on resident communities is a well-described process that can cause biodiversity loss and results in changes in the biotic and abiotic environment (Vitousek et al. 1987, Vitousek 1990, Lockwood et al. 2007). Only very few invading species can establish in a new habitat, and most of the new arriving species cannot successfully establish. However, all of them will interact (at least for a certain amount of time) with several resident species, which influence the dynamics of the resident community. Unsuccessful invasion constantly occurs, for example, by dispersal of seeds or living individuals (Kristiansen 1996, Ingle 2003, Figuerola et al. 2003). Also, phytoplankton communities constantly face potential invasion by dispersal of species between habitats. Nevertheless, the effect of an unsuccessful invader on the resident community composition is barely studied and still not well known. To my knowledge, the results described in section 3.2 show, for the first time, how unsuccessful invaders can cause changes in the community composition of a late spring, mesotrophic freshwater community.

Although none of the six added species (single or in combination) in my experiment was able to establish itself within the phytoplankton community, I found major alterations in the resident community compared to a control community without invasion. Diversity and evenness changed in communities with added species compared to the control communities and resulted in lower similarity compared to the control communities than between communities with added species. I observed shifts in the taxonomic group and species abundance caused by different species influencing the dissimilarities between the control and experimental treatments. Especially, evenness of the communities responded more rapidly as just species numbers after disturbance (Mattingliy et al. 2007). Such clear evenness shifts were visible in communities with added Bacillariophyta and Cyanophyta species. Evenness was significantly higher in invasion treatments. Although the dominant species (e.g., *Synedra spec*.) was still highly abundant, their reduced abundance due to the addition of species in experimental communities contributed the most to the dissimilarity of the communities compared to controls.

So far, potential effects of unsuccessful invaders on resident communities have been mostly neglected, and only a theoretical model of Miller et al. (2009) predicted crucial effects on resident communities. The authors assume that the effects of changes in the community are the result of indirect effects of few unsuccessful species on the community. In my study, both, direct and indirect interactions of unsuccessful invading species and resident species could lead to different community compositions compared to non-invaded ones. I suggest three possible explanations for that: 1) the direct influence of the added species on resident species belonging to the same taxonomic group by competition for same resources; 2) substitution of one resident species by another resident species, both of the same taxonomic group; and 3) the indirect influence of the added species on resident species.

Direct competition between species for the same resource will be strongly defined by the different competitive abilities of the individual species for a specific resource: thus, similar species (e.g., belonging to the same taxonomic group) are more likely to have similar resource requirements and compete for those (Tilman 1977, Sommer 1984, Kneitel and Chase 2004). However, species belonging to the same taxonomic group may still differ in other functional traits (e.g., motility) and, therefore, slightly different utilization abilities, as well as competitive abilities (Litchman 2007, Litchman and Klausmeier 2008). Looking at more detail, my results provide evidence that competition among species from the same taxonomic group and size class result in a lower abundance of dominant resident species belonging to the same taxonomic group as the experimentally added species. Such an effect is seen in the treatment

where I added *C. reinhardtii*, which lowered the abundances of other resident Chlorophyta species (Figure 11).

Substitution, where one species is replaced by another through possible better competitive abilities (Sommer 1983, Tilman and Sterner1984), also occurred in my experiment. In the treatment where *F. crotonensis* was added, a resident Chlorophyta (*Ankistrodesmus spec.*) had a higher abundance, whereas another resident Chlorophyta (small coccal Chlorophyta) showed lower abundance (Figure 11) compared to controls. The exchange of the two species abundances is only seen at the species level and hidden at the taxonomic group level, due to an almost stable total abundance of the taxonomic group. This implicates the importance to analyze the mechanism of interactions on the species level.

In my experiment, the combination of both competition mechanisms (direct and indirect species interactions) occurred. In direct interactions, species belonging to different taxonomic groups compete for possible open niches suitable for both species based on different competitive abilities (Litchman 2007). This occurred in treatments where I added Cyanophyta to the resident community. I observed a lower abundance of resident species (e.g., *Nitzschia spec*., *Cyclotella spec*.) belonging to the dominating resident taxonomic group Bacillariophyta (Figure 11).

Furthermore, the direct interaction of two species can cause an indirect effect on a third species (Wootton 1994). The indirectly affected species might use available niches (e.g., resources or space), which become available through the direct interaction of competing species, which also diminish the competitive strength of the dominant species. This mechanism can be a possible explanation for an observed higher abundance of a specific resident taxonomic group (Chlorophyta) in treatments where I added species of another taxonomic group compared to controls (Bacillariophyta, Figure 10). Similar results on community composition assemblage were found in treatments with added Cyanophyta, showing a lower abundance of resident Bacillariophyta and higher resident Chlorophyta abundance compared to controls (Figure 10).

Also, my results give evidence for the importance of the interactions between the added species themselves and their combined influences on the resident community composition. In my study, this is seen in treatments with added Bacillariophyta (Figure 11). Resident Bacillariophyta in the treatment with combined added *F. crotonensis* and *C. meneghiniana* showed lower abundance than in the treatment were Bacillariophyta were added as single species. This supports the effect of the invasion meltdown hypothesis of Simbleroff and Holle

(1999), which suggests that one invader potentially, facilitates the establishment of another species resulting in a synergetic effect on the resident community. This effect is even stronger when the two species are added in combination. Cell size is a possible explanation for this effect, as smaller cells can have advantages over larger cells. For example, smaller cells are more efficient in the uptake of limiting nutrients due to a higher surface to volume ratio (Litchman and Klausmeier 2008). Thus, in my study, the combination of the two nanoplankton species (*F. crotonensis* and *C. meneghiniana*) reduced the abundance of microplankton species (*Synedra spec.*) through possible better resource utilization due to their smaller size. The impact of the size and species contributions to the dissimilarity between invasion treatments and controls indicate that species with smaller cell size are better competitors (Litchman and Klausmeier 2008). These results support my assumption of the importance of the trait cell size for competition mechanisms in community composition and species interactions. However, other mechanisms (e.g., limited resources) might play an additional role for my observed changes in community assemblage. Therefore, further analyses of how species are interacting might give more insight into the mentioned species interactions and how dominance might change within a community in the presence of added species.

Additionally, I found that the taxonomic similarity of the added species to the resident species could be neglected. I observed the same influences of added species independent of whether they were similar or dissimilar compared to the resident species pool. For example, adding Bacillariophyta, which was similar to the Bacillariophyta dominated resident community, resulted in comparable effects to adding Cyanophyta, which was very dissimilar to the resident species pool. This is in line with the findings of Warren et al. (2003); in their experiments, they used different resident protist communities invaded by other protist species. Independent of which protist species invaded, the same final community was reached.

The unsuccessful invasion had clear effects on community composition following some general patterns. Unsuccessful invaders had negative effects on species from the same and different taxonomic group and, at the same time, promoted species from other taxonomic groups. In particular, initially dominant resident species were negatively affected, thus enabling other species to increase in abundance. This finding is in line with Emery and Gross (2007) who hypothesized that dominant species are more likely to interact with other species because they are more likely to come into contact with other species due to their high abundance. Furthermore, all my findings reinforce the importance of species interactions.

This study examines the effects of unsuccessful invading species on community composition of a freshwater phytoplankton community and possible underlying mechanisms (direct- and indirect species interactions) under natural conditions. My experiment was limited by the number of species that could be added. However, my experiments show the importance of unsuccessful invasions resulting in clear effects on the composition of phytoplankton communities. To fully understand the dynamics of natural plankton communities, potential effects of unsuccessful invasions must be studied in more detail. Additionally, natural phytoplankton communities are often exposed to more than one environmental change at the same time. Thus, it is also important to investigate combined influences like changes in the resource light quality and the influence of invasion effects on natural plankton communities.

4.3 Combined light quality and invasion effects on phytoplankton community assembly and their effects on zooplankton

Light had a significant effect on biomass (referred to chlorophyll-*a*) at the beginning of the experiment. A *post hoc* Tukey test showed that the differences in biomass between communities in red and blue light conditions caused the significant disparity after one week of the experiment. Indeed, Cyanophyta abundance was significantly higher in red light treatments than in blue light treatments, causing the biomass difference (Figure S1). Later in the experiment, results of the white light treatments showed the same pattern as red light treatments. Although complementary light utilization has been found to be an important mechanism of diversity-productivity relationships (Stomp et al. 2004, Behl et al. 2011), results here only supported changes in biomass at the beginning of the experiment and were not consistent.

From the terrestrial primary producer studies, it is known that disturbance (e.g., by invasion) can lead to a decrease in native community abundance and biodiversity (McKinney and Lockwood 1999). In the presented study, diversity was strongly influenced by the invasion of *A. cylindrica*. However, results were contrary to what was previously found in terrestrial studies. Higher diversity was found in all invasion treatments when compared to control. Hornak and Corno (2012) already found in aquatic bacterial communities that invader species compete with dominant resident species and, therefore, opened up a niche for other bacteria which would otherwise not be able to compete. This might result in higher biodiversity. Although *A. cylindrica* was not successful in invading the communities, it initiates higher

biodiversity. The observed increase in biodiversity is maintained even after the sharp decline of Cyanophyta levels in the invaded communities (Figure S1). This phenomenon may be explained by what is called the "ghost of competition present", as proposed by Miller et al. (2009). In this scenario, species that attempt to invade a community but eventually die out still contribute significantly to the final stable community (Miller 2009).

The effects of ecological invasions are described in terms of changes of native species composition or as changes in abiotic properties of the environment (Grosholz 2002). It has often been found in terrestrial plant communities that productivity is higher with higher diversity levels, mainly due to complementary effects (Hector et al. 1999, Tilman et al. 2001, van Ruijven and Berendse 2005, Crutsinger et al. 2006). Interspecific differences in spatial and temporal resource use create complementary niches, allowing several species to cohabitate and, thus, facilitating higher productivity (Loreau and Hector 2001). Phytoplankton communities have also been found to exhibit positive complementary effects with algal group diversity (Stockenreiter et al. 2013). In my experiment, although there was increased diversity, invasion only temporarily affected overall biomass production, as shown by the total chlorophyll-*a* levels. Total chlorophyll-*a* levels did not differ between invaded and non-invaded communities beyond the early stages of the experiment. The initial inoculation of *A. cylindrica* caused an immediate effect on chlorophyll-*a* content, which decreased in the beginning, suggesting that the addition of an invasive species affected the overall productivity of the community. However, at the end of the experiment, the community biomass was no longer lower than the control community biomass (Figure S1). Even during peak Cyanophyta presence, there were no significant differences in chlorophyll-*a* levels between invaded and non-invaded communities. An explanation for this could be that even with high invasive pressure, biotic resistance from the resident community can stabilize itself and retain near control levels of chlorophyll-*a* (Britton-Simmons and Abbott 2008).

The effect of invasion on biodiversity showed that the presence of non-native *A. cylindrica* increased community diversity. Contrary to previous findings (Behl et al. 2001), the increase in diversity did not affect productivity (since chlorophyll-*a* levels stayed the same). As natural phytoplankton communities were used, it could be that the community was well complimented and already exhibited high productivity levels. Addition of a Cyanophyta algal group may have filled a niche but did not significantly contribute to total chlorophyll-*a* content.

Significant differences in phytoplankton community structure may lead to upstream effects on their consumers, such as the zooplankton *D. magna* (Striebel et al. 2012). *D. magna* relies on phytoplankton as their main food source, preferring Chlorophyta species over those of other algal groups (Ebert 2005). My results showed that there is a positive correlation between chlorophyll-*a* content and somatic growth rate of *D. magna* (Figure 14 a), which implies that they grow best when there is more abundant and available consumable phytoplankton biomass. There was a positive correlation between Chlorophyta levels and *D. magnas* somatic growth rate (Figure 14 b), which is to be expected since Chlorophyta is a better food source than Cyanophyta or diatoms for *Daphnia spec*. (Ebert 2005). Qualitative data from species distribution counts (Table 8) show higher Chlorophyta abundances in blue and white light relative to red light, which may explain why *D. magna* has the lowest somatic growth rate when fed with phytoplankton only exposed to red light. As stated above, in red light treatments, Cyanophyta was most abundant, which causes a decrease in somatic growth of *D. magna* (de Bernardi and Giussani 1990, Ebert 2005). Therefore, light quality may have an indirect effect on *D. magna* via altered growth of different phytoplankton groups. Additionally, my results show that diversity may have a positive effect on *D. magna* growth. A coefficient of variation test between growth rates of *D. magna* fed with phytoplankton from control or invaded communities showed that there is greater variance in growth rates in the control than in the invaded treatments (Figure 14). This suggests that invasion in phytoplankton communities can cause alterations in community structure and composition and have major effects on upper trophic levels than non-invaded communities.

The invasion of *A. cylindica* facilitated positive diversity changes, which was indirectly beneficial for *D. magna* growth. Reason for this might be a better food quality e.g., an abundance of metabolic biochemical, such as fatty acids and sterols (Ravet et al. 2003) of more diverse phytoplankton communities. Stockenreiter et al. (2012) showed that phytoplankton essential fatty acid content increased with increasing diversity. Lower variance in growth rates of *D. magna* can also be referred to greater stability. A coefficient of variation of *D. magna* somatic growth rate was conducted in relation to Cyanophyta and the Chlorophyta abundance. Both confirmed the previous result that *D. magna* in control communities experienced higher variation in growth rate than those which fed on invaded phytoplankton communities (Figure 14 b and 14 c). These results further support the role invaders play in affecting final phytoplankton communities in such a way that zooplankton growth is more stabilized. Zooplankton life history performances can also be affected by changing phytoplankton

community composition, due to shifts in food sources. The influence of a potential invading biotechnologically modified phytoplankton species on a filter feeder zooplankton will be discussed in the following section.

4.4 Life history of *Daphnia magna* when feeding on genetically modified microalgae

The food quality for *Daphnia* depends on the size, toxicity and cell wall consistency, as well as on the chemical composition of the microalgae. For example, the fatty acid composition of microalgae as a food source for zooplankton can be more important than proteins or carbohydrates (De Pauw and Pruder 1986, Ahlgren et al. 1990). In general, *C. reinhardtii* is a suitable food source in terms of size and nutritional composition for different *Daphnia* species (Porter and Orcutt 1980, Alghren 1990), including *D. magna* (Mitchell et al. 1992). Furthermore, it was shown that cell wall deficient *C. reinhardtii* have a higher food quality for *Daphnia* than a wild type of *C. reinhardtii* due to a more efficient ingestion and digestion by *Daphnia* (Van Donk et al. 1997). The results of my experiment, however, could not support these observations. Contrary to these observations, my results give slight evidence for a negative response due to reduced fitness (*r*) of *Daphnia* to the food source cell wall deficient (CWD) *C. reinhardtii* compared to wild type (WT) *C. reinhardtii*.

The different performances (fitness, neonate size at first reproduction) of *Daphnia* fed with WT and CWD can be an effect of the different motility modes of the two *Chlamydomonas* strains: CWD strains of *C. reinhardtii* are less mobile than WT *C. reinhardtii* (estimated by several microscopic observations). Thus, the CWD strain will sink faster, and its availability as food for *Daphnia* in the water column will be limited. Although *D. magna* can graze on the ground (Suedel et al. 1993), this behavior is observable if the food level in the water column is lower than 0.05 mg CL^{-1} (Siehoff et al. 2009). The daily food level of 0.5 mg CL^{-1} in my experiment was considerably higher than 0.05 mg CL^{-1}. Thus, grazing on the ground by *D. magna* seems unlikely. Presumably, based on the observed higher sinking rate, *Daphnia* fed on CWD had a lower food quantity in the water column and showed a lower fitness, and worse life history responses compared to *D. magna* fed on WT.

The CWD strain is the basis for the modified *C. reinhardtii* strains secreting levels of VEGF at either low and high levels. Thus, it is likely that some effects of the CWD also have consequences for the modified strains. *D. magna* in treatments with genetically modified (high or low) algae might have lower food quantities compared to *Daphnia* fed on WT due to the

observed lower motility of CWD. My observed differences in the number and size of neonates are in line with an earlier observation that *D. magna* produces more and smaller neonates at low food levels and fewer, larger neonates at high food levels (Cowgill et al. 1985, Smith 1963). These findings stand in contrast to the results described by McKee and Ebert (1996), who found in *Daphnia magna* under low food conditions heavier and less offspring. Variations in food quantity can represent a relevant environmental stressor of natural zooplankton communities, due to a direct constraint on the energy uptake and, consequently, the energy status of an organism. *Daphnia* is known to deal with resource variations in terms of varying food quantity by changing their life history traits to allocate the available resources optimally (Guisnde and Gliwicz 1992, Boersma 1999).

Besides food quantity, food quality can provoke a stress response in changing life history performances of *Daphnia*. Studies already showed that different food quality due to different food morphologies, such as cell wall thickness or food grown under nutrient limitation, affected *Daphnia* life history traits (e.g., growth and fitness), as well as age and size at first reproduction and neonate size at first reproduction (Vanni and Lampert 1992, Kilham et al. 1997). Juveniles can be more affected than adults by a relatively low food quality, resulting in a time delay of the first reproduction (Vanni and Lampert 1992). I found such a delay in the treatments where *Daphnia* fed on the GM low algal type. Other parameters, like size at first reproduction or reproductive effort, were not influenced by the different food sources. However, the different food sources affected neonate size, fecundity, and fitness. A feeding experiment with D*aphnia* by Choi et al. (2016) found variations in number and size of neonates as a response to two phytoplankton species, which were different in the stoichiometric and lipid composition supplied as a food source. The authors assume that the observed differences in *Daphnia* offspring are food quality dependent (Choi et al. 2016).

However, *Daphnia* feeding on GM algae showed lower fitness, which, in my study, was mainly influenced by the reduced fecundity and the delayed release of the first brood. De Lange and Van Reeuwijk (2003) showed a similar fitness reduction in *Daphnia* fed on UVB stressed phytoplankton and argued that this was due to a change in food quality by changes in the fatty acid composition of the algae. Based on the observations of Choi et al. (2016) and De Lange and Van Reeuwijk (2003), I assume the food quality (fatty acid- or lipid-composition or stoichiometry), rather than the food quantity, caused the observed life history performances in my experiment, as food was provided at low, but natural food levels (Agatz et al. 2012). In my experiment, a nutritional change of the GMM through the genetical modifications (Henley et

al. 2013, Snow and Smith 2012) and/or the VEGF production presumably caused the observed differences between *Daphnia* fed on GMM and WT. *D. magna* is a common model organism in toxicological and ecotoxicological research (Barry 1996, Atienzar et al. 2001, Kramer et al. 2004). *D. magna* provides a cost-effective alternative to other model organisms such as mammals to investigate long-term effects of GM plants on non-target organism through feeding tests (Bøhn 2010). Negative impacts of GM maize and soybean products, as the insecticide Cry1Ab protein and the herbicide glyphosate, fed as suspension to *Daphnia* have been shown (Bøhn et al. 2008, Cuhra 2015, Holderbaum et al. 2015, Venter and Bøhn 2016). For example, Bøhn et al. (2008) analyzed in a feeding experiment the effects of GM maize (producing the Cry1Ab protein) on *D. magna*. They found higher mortality, a lower proportion of females reaching sexual maturation, and lower egg production in *Daphnia* fed with a suspension of GM maize compared to *Daphnia* fed with unmodified maize.

In conclusion, three different possible changes of the food quality might affect the food source "GMM". First, the microalgae stoichiometry could be changed by the additional production of proteins. The production of additional proteins could result in a higher N:P ratio in the biomass of algae (Geider and La Roche 2002, Sterner and Elser 2002). Higher N:P and/or C:P ratios in algal biomass are known to influence food quality of algae negatively, and can limit growth rate for herbivorous zooplankton such as *Daphnia*, as *Daphnia* has low intrinsic N:P (~12:1) and C:P (~85:1) ratios (Andersen and Hessen 1991, Elser et al. 1996). For example, if the C:P ratio in the food microalgae increases to values over 350, it can limit *Daphnia* growth due to a serious P limitation (DeMott et al. 2001, Becker and Boersma 2003).

Second, the specific human protein VEGF produced by the GM could provoke such a response in *Daphnia*. The vertebrate origin of the protein *per se* could induce a stress response in *Daphnia*. For example, it has been shown that a protein from a foreign species, such as GM crops, affected *D. magna* life history responses (Raybould et al. 2014). The authors showed in their study a small decrease in the growth rate of *Daphnia magna* exposed to a single high concentration of Vip3Aa20, the insecticidal protein (toxic, to control a wide spectrum of lepidopteran pests) in MIR162 maize (a commercial insect resistant maize from Syngenta Seeds, Basel, Switzerland). In a follow-up study, they tested the effect of different concentrations, as well as a high level of non-toxic protein (bovine serum albumin [BSA]) on *D. magna*. From this, the authors assumed that the high concentrations of proteins (non-toxic), and not the specific protein *per se*, were responsible for the observed pattern in *D. magna* (Raybould et al. 2014).

The third possibility is that, even if the protein is human specific, it might affect receptors in *Daphnia* directly and induce stress responses in *Daphnia*. This assumption is supported by the finding of VEGF-like molecules in invertebrate organisms (Holmes and Zachary 2005). Furthermore, the finding in an *in vitro* experiment, that *D. magna* hypoxia response elements (HRE) could bind specific human hypoxia-inducible factors (Gorr et al. 2004) strengthen the possibility of a protein interaction between the VEGF and *Daphnia* system. With this study, we could show measurable effects on some life history parameters and the fitness of *D. magna* feeding on GMM. The introductions of such species can, therefore, have further implications on the food web; however, I could only show effects on fitness and life history parameters. The mechanisms behind these effects remain unknown and to be examined in further studies.

5. Conclusion and outlook

5.1 Anthropogenic induced environmental changes influencing community composition: further investigations

Anthropogenic induced changes in aquatic systems can have strong effects on community assemblages (e.g., Genner et al. 2004, Gobbi et al. 2006, Davej et al. 2013). However, some of the potential effects on communities (like species loss or environmental change) are well studied, whereas many other questions, such as how anthropogenic changes influence community compositions, remain open.

Human induced changes in the environment affect abiotic and biotic conditions, which finally resulted in altered phytoplankton community patterns (changes in diversity, or effects on zooplankton). For example, light quality, a major abiotic phytoplankton resource, can be influenced by climate change due to higher water temperatures. Shifts in the available light spectrum altered natural phytoplankton communities. The phytoplankton species specific traits (e.g., pigment composition) are the limiting factors of the ability to adapt to an altered environment (e.g., changes in light spectrum). Finally, light quality could affect community composition (e.g., in terms of biodiversity loss). This effect is possibly due to the complementary chromatic adaptation, which describes the ability of phytoplankton species to change their pigment composition ratios under a constant total amount of pigments (Tandeau de Marsac 1977, Ohki et al. 1985, Stomp et al. 2004). My results indicate the possibility of more frequent Cyanophyta blooms under a larger light spectrum exposure (full PAR light) of natural phytoplankton communities. The broader PAR spectrum is a “side effect” of temperature induced changes in mixing depth. My results highlight the need for more detailed studies about such “side effects” of anthropogenic environmental changes, which can affect essential phytoplankton resources.

Due to increased human activities (transportation), there is now a higher rate of dispersal for species from a variety of different environments than previously existed. Thus, there is a greater possibility of the introduction of new species into new environments. The addition of a potential invader to natural phytoplankton communities resulted in maintained diversity levels. The mechanism for species interactions seems to be the competition ability of the resident species, as well as the potential invading species (e.g., nutrients or space). Therefore, the invasion success is strongly dependent on the traits of both the invader and resident species. However, an inherent plasticity of traits exists. Thus, in the same species, different trait expressions are possible, depending on the environmental conditions and the acclimation

history (Litchman and Klausmeier 2008). Chromatic adaptation of phytoplankton is an example of functional trait plasticity.

Trade-offs between traits can lead to species coexistence due to differentiation of different resources. Species can have trade-offs when they compete for multiple resources (Litchman et al. 2007). For example, differentiation of resources influencing species abundances were shown under varying nutrient vs. light conditions (Husiman and Weissing 1994, Klausmeier and Litchman 2001) or for one nutrient vs. another nutrient (Tillman 1982). Thus, in my experiments, maintained biodiversity was probably based on the species functional traits. This emphasizes the importance of phytoplankton functional traits for species interactions with changing abiotic and biotic environments. Further experiments with a focus on phytoplankton functional traits could help to understand which traits and which "trait-statistics" (plasticity of traits, or trade-offs between traits) drive mechanisms of community assembly.

Additionally, the maintained biodiversity in the invaded communities demonstrates the important influences of, often underestimated, unsuccessful invaders on the community dynamics. It even supports the importance of species dispersal as a potential mechanism to maintain diversity in natural communities.

The maintained diversity in invaded communities indicates a possible effect of disturbance. According to the intermediate disturbance hypothesis of Connell (1978), diversity should be highest at an intermediate level of disturbance. In a recent study, Hammerstein et al. (2017) found in a laboratory experiment a clear pattern between disturbance and the diversity level of different phytoplankton communities. It is possible that the potential invaders could represent a disturbance at a level where biodiversity was maintained. Based on that, follow up studies on different natural phytoplankton communities, with more and different potential invader species, might give further insights of a mechanism, which can occur during the invasion process. Additionally, it might explain if the observed effects of transient effects are general mechanisms, which maintain biodiversity in natural water bodies. Under natural conditions, anthropogenic influences on ecosystems often occur in combination and species probably have to adapt simultaneously to different abiotic or biotic changes. When multiple changes occur, one change can influence the effect of the other change in different ways: for example, it could increase the effect on phytoplankton communities by an additive or multiplicative interaction or minimize the effect by out canceling the other effect. In a multi anthropogenic stressor experiment (drought, acidification, and warming), Christensen et al. (2006) have shown that

the interaction among the stressors, rather than the sum of the individual stressor effects, had significant effects on planktonic producers and consumers. Furthermore, the authors found synergistic and antagonistic effects on consumers and producers (Christensen et al. 2006). My results of the discrete influence of changing resource (light quality) and discrete change by the addition of potential invaders on phytoplankton communities give evidence for an antagonistic effect on the community in terms of biodiversity. In the combined changes (light quality and potential invasion) experiment, light spectrum shift and invasion show a similar pattern when considered separately. The combination of both induced anthropogenic changes gives evidence for an antagonistic effect. The combination of changes interaction on diversity was only observable for a short time during the first week of the experiment; over a longer period, invasion maintained diversity.

I could show that such a short time disturbance of a transient species in the community has lasting effects on the next trophic level in terms of the food quality. Phytoplankton communities exposed to invasion provided as a food source for *Daphnia* resulted in higher *Daphnia* growth rates. A positive effect of more diverse phytoplankton community level on zooplankton could be already shown (Striebel et al. 2012). Further laboratory and field experiments, including additional and combined anthropogenic changes, could bring deeper insights how the combination of induced anthropogenic changes act on phytoplankton communities and how such influences affect higher trophic levels.

5.2 Changing traits of new entities and risk management of GMM

My results show potential signatures of stress induced performances in *D. magna* as a response to feeding on genetically modified microalgae (GMM). A comparable reduced *Daphnia* fitness to my results was shown in *Daphnia* exposed to low concentration (above 0.0125 mg L^{-1}) of the genotoxic polycyclic aromatic hydrocarbon, benzo[*a*] pyrene (B*a*P; Atienzar et al. 1999) as well as exposed to the toxin producing *Microcystis aeruginosa* (Gustafsson et al. 2005). These studies support the assumption of a stress response of *D. magna* to GMM.

Additionally, my results are the first showing direct influences of a GMM on the primary consumer level. The results indicate a measurable response of *Daphnia* neonate size and fecundity to GMM food, which can result in effects on the next trophic levels. For example, invertebrate predators select for smaller *Daphnia* (Cooper 1983, Branstrator and Lehmann 1991), whereas optical predators such as fish, feed on bigger sized *Daphnia* (Galbraith 1967).

Thus, a *Daphnia* exposed to GM food changing the growth and, thereby, the size of *Daphnia* might have a strong effect on size-selective predation on *Daphnia*. Further studies could be performed with higher food web complexity (more than two trophic levels), including predators that select for differently sized prey. By such an approach, one could get a complete picture of the impact of a GMM on herbivores in natural ecosystems.

The small effect of a GMM on the life history of *D. magna* and the limited knowledge of other impacts of the GMM on natural aquatic environments demonstrates the need for further experiments. The GMM fitness effects under natural nutritional conditions, as well as the investigation of competition with other phytoplankton species, could give insights of the surviving ability of a GMM in natural ecosystems.

One recent study examined the dispersal- and invasion-potential of genetically modified microalgae, *Acutodesmus dimorphus*, which was cultivated in open ponds (Szyjka et al. 2017). The authors found a similar dispersal potential and similar effects of GM- and WT-*A. dimorphus* on five natural phytoplankton communities in terms of biodiversity, species composition, and biomass. Neither of the algal was able to exclude a resident species. Based on these findings and my results, further studies on the impact of GMM are indicated in two directions. First, studies on the direct effects of the released recombinant proteins on natural species, as also suggested by a publication dealing with risk assessments of GMM (Henley et al. 2013). Second, experiments on the changing effects of the nutritional composition of algae due to genetically modifications (e.g., protein content, fatty acids or other biochemical composition) of the GMM have to be conducted for a more detailed understanding of the effects of the genetical modification on the biomass composition and, thereby, the food quality of GMM.

The increased research on GMM being of major interest for a variety of biotechnical compounds has to be accompanied by detailed research addressing how accidental released GMM would perform in nature and how they would potentially affect ecosystem dynamics.

"*One of the most challenging tasks of ecology is to derive from the characteristics of individuals the principles that regulate higher-level characteristics such as diversity"*

Lampert and Sommer 2007

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7. Appendix

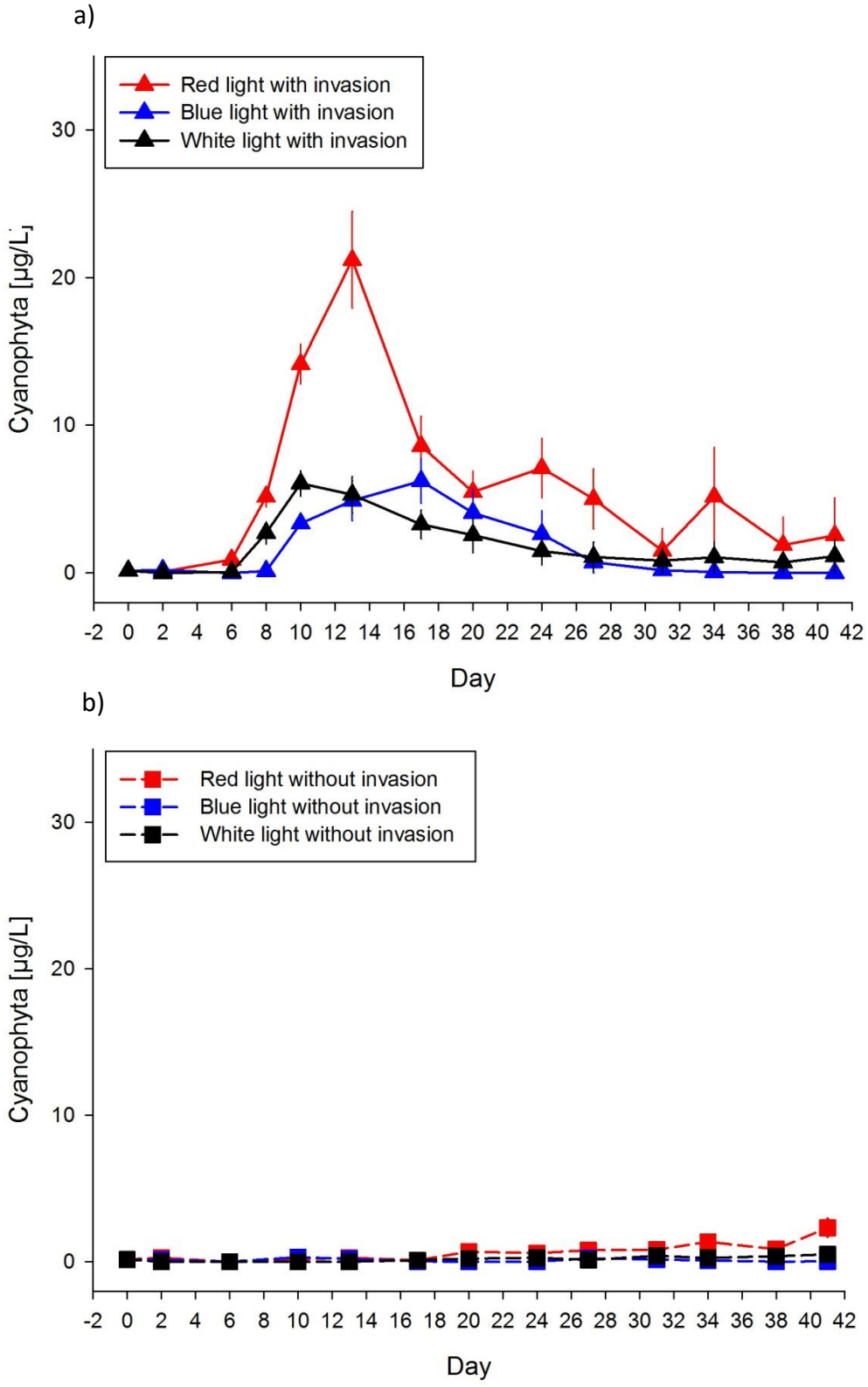

Figure S1: Cyanophyta abundances in phytoplankton communities with (a) and without (b) the addition of potential invasive *Anabaena cylindrica* over the time course of the experiment.

Table S1. Detailed results of two-way ANOVA and *post hoc* Tukey test for the effect of light (r = red light, b = blue light, w = white/full spectrum light) and invasion (y = with invasion, n = without invasion) on chlorophyll-*a* content of phytoplankton communities. All values expressed are p-values. Asterisks indicate statistically significant differences ($p \leq 0.05$).

Day	Effect of Light on Chl a	Effect of Invasion on Chl a	Interaction between Light and Invasion	Post-hoc Analysis of Effect of Light on Chl a	Comparisons of each treatment with all other treatments
6	<0.01*	<0.05*	0.87	red-blue: <0.01* white-blue: 0.09 white-red: 0.27	r:n-b:n 0.17 w:n-b:n 0.78 b:y-b:n 0.94 r:y-b:n 0.02* w:y-b:n 0.11 w:n-r:n 0.79 b:y-r:n 0.54 r:y-r:n 0.76 w:y-r:n 0.99 b:y-w:n 0.99 r:y-w:n 0.16 w:y-w:n 0.61 r:y-b:y 0.08 w:y-b:y 0.38 w:y-r:y 0.90
10	0.03*	0.93	0.07	red-blue: 0.99 white-blue: 0.05* white-red: 0.04*	r:n-b:n 0.93 w:n-b:n 0.03* b:y-b:n 0.43 r:y-b:n 0.95 w:y-b:n 0.41 w:n-r:n 0.13 b:y-r:n 0.90 r:y-r:n 0.99 w:y-r:n 0.89 b:y-w:n 0.53 r:y-w:n 0.12 w:y-w:n 0.55 r:y-b:y 0.88 w:y-b:y 1.00 w:y-r:y 0.86

20	0.66	0.11	0.92	red-blue: 0.69 white-blue: 0.99 white-red: 0.73	r:n-b:n 0.99 w:n-b:n 0.99 b:y-b:n 0.86 r:y-b:n 0.52 w:y-b:n 0.92 w:n-r:n 0.99 b:y-r:n 0.99 r:y-r:n 0.82 w:y-r:n 0.99 b:y-w:n 0.95 r:y-w:n 0.68 w:y-w:n 0.98 r:y-b:y 0.99 w:y-b:y 0.99 w:y-r:y 0.95
24	0.28	0.56	0.24	red-blue: 0.77 white-blue: 0.26 white-red: 0.59	r:n-b:n 0.65 w:n-b:n 0.88 b:y-b:n 0.92 r:y-b:n 0.99 w:y-b:n 0.28 w:n-r:n 0.99 b:y-r:n 0.99 r:y-r:n 0.86 w:y-r:n 0.97 b:y-w:n 0.99 r:y-w:n 0.98 w:y-w:n 0.83 r:y-b:y 0.99 w:y-b:y 0.79 w:y-r:y 0.47
41	0.47	0.07	0.58	red-blue: 0.45 white-blue: 0.88 white-red: 0.74	r:n-b:n 0.96 w:n-b:n 0.99 b:y-b:n 0.56 r:y-b:n 0.98 w:y-b:n 0.98 w:n-r:n 0.86 b:y-r:n 0.20 r:y-r:n 0.67 w:y-r:n 0.70 b:y-w:n 0.75 r:y-w:n 0.99 w:y-w:n 0.99 r:y-b:y 0.92 w:y-b:y 0.89 w:y-r:y 0.99

Table S2: Detailed results of two-way ANOVA and *post hoc* Tukey test for the effect of light (r = red light, b = blue light, w = white/full spectrum light) and invasion (y = with invasion, n = without invasion) on Diversity (H') of phytoplankton communities. All values expressed are p-values. Asterisks indicate statistically significant differences ($p \leq 0.05$).

Day	Effect of Light on Diversity (H)	Effect of Invasion on Diversity (H)	Interaction between Light and Invasion	Post-hoc Analysis of Effect of Light on Diversity (H)	Comparisons of each treatment with all other treatments
6	<0.01*	<0.01*	<0.01*	red-blue: 0.98 white-blue: <0.01* white-red: <0.01*	r:n-b:n <0.01* w:n-b:n 0.27 b:y-b:n 0.76 r:y-b:n 0.02* w:y-b:n 0.04* w:n-r:n <0.01* b:y-r:n 0.01* r:y-r:n <0.01* w:y-r:n <0.01* b:y-w:n 0.03* r:y-w:n 0.59 w:y-w:n 0.87 r:y-b:y <0.01* w:y-b:y <0.01* w:y-r:y 0.99
10	0.41	<0.01*	0.43	red-blue: 0.98 white-blue: 0.43 white-red: 0.54	r:n-b:n 0.91 w:n-b:n 0.56 b:y-b:n 0.90 r:y-b:n 0.52 w:y-b:n 0.93 w:n-r:n 0.97 b:y-r:n 0.38 r:y-r:n 0.14 w:y-r:n 0.44 b:y-w:n 0.12 r:y-w:n 0.04* w:y-w:n 0.17 r:y-b:y 0.98 w:y-b:y 0.99 w:y-r:y 0.96

20	0.66	0.11	0.92	red-blue: 0.69 white-blue: 0.99 white-red: 0.73	r:n-b:n 0.99 w:n-b:n 0.99 b:y-b:n 0.86 r:y-b:n 0.52 w:y-b:n 0.92 w:n-r:n 0.99 b:y-r:n 0.99 r:y-r:n 0.82 w:y-r:n 0.99 b:y-w:n 0.95 r:y-w:n 0.68 w:y-w:n 0.98 r:y-b:y 0.99 w:y-b:y 0.99 w:y-r:y 0.95
24	0.28	0.56	0.24	red-blue: 0.77 white-blue: 0.26 white-red: 0.59	r:n-b:n 0.65 w:n-b:n 0.88 b:y-b:n 0.92 r:y-b:n 0.99 w:y-b:n 0.28 w:n-r:n 0.99 b:y-r:n 0.99 r:y-r:n 0.86 w:y-r:n 0.97 b:y-w:n 0.99 r:y-w:n 0.98 w:y-w:n 0.83 r:y-b:y 0.99 w:y-b:y 0.79 w:y-r:y 0.47
41	0.47	0.07	0.58	red-blue: 0.45 white-blue: 0.88 white-red: 0.74	r:n-b:n 0.96 w:n-b:n 0.99 b:y-b:n 0.56 r:y-b:n 0.98 w:y-b:n 0.98 w:n-r:n 0.86 b:y-r:n 0.20 r:y-r:n 0.67 w:y-r:n 0.70 b:y-w:n 0.75 r:y-w:n 0.99 w:y-w:n 0.99 r:y-b:y 0.92 w:y-b:y 0.89 w:y-r:y 0.99

8. Manuscripts and author contributions

8.1 Manuscripts

From my dissertation, the following manuscripts resulted:

Stockenreiter, M., **Buchberger, F**., Stibor, H. (2017): Light spectrum shifts affect community assembly in freshwater phytoplankton. *Freshwater Science*, in revision.

Addressing the research question: *Can changes in the light spectrum, naturally accompanying changes in mixing depth, influence phytoplankton communities?*

Buchberger F., Stockenreiter M.: Unsuccessful invaders structure a natural freshwater phytoplankton community. *Ecosphere*, in revision.

Addressing the research question: *Are phytoplankton species from different taxonomic groups able to invade a natural phytoplankton community and, if not, do they still influence the resident community?*

Guan L., **Buchberger F**., Stockenreiter M.: Natural plankton community response on invasion pressure under different light conditions. In prep. for *Journal of Plankton Research*

Addressing the research question: *How far do anthropogenic changes interact in affecting natural phytoplankton communities and can these interactions affect higher trophic levels?*

Buchberger F., Stockenreiter M., Neusius D., Nickelsen J., Stibor H.: Life history effects on *Daphnia magna* fed with different strains of *Chlamydomonas reinhardtii*. In prep. for *Applied Ecology*

Addressing the research question: *Can genetically modified microalgae (GMM) for biotechnological purposes affect consumers from higher trophic levels when invading plankton communities?*

8.2 Author contributions

Stockenreiter M. and Stibor H. planned the experiment. Buchberger F. conducted the experiment and analyzed the data. All authors wrote the manuscript.

Buchberger F. and Stockenreiter M. designed the experiment and wrote the manuscript. Buchberger F. conducted the experiment and analyzed the data with the advice of Stockenreiter M.

Buchberger F. and Stockenreiter M. designed the experiment. Guan L. and Buchberger F. conducted the experiment and analyzed the data. All authors wrote the manuscript.

Buchberger F. and Stockenreiter M. planned the experiment. Buchberger F. and Neusius D. conducted the experiment. Buchberger F. analyzed the data and wrote the manuscript with the advice of Stockenreiter M., Stibor H. and Nickelsen J.

9. Personal notes

9.1 Curriculum vitae

Name:	Felicitas Buchberger
Date of birth:	13.03.1986
Place of birth:	Starnberg, Germany
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Academic education

Since 01/ 2014	**Ludwig-Maximilians-University, Munich, Germany** Dissertation: „Plankton community dynamics and anthropogenic changes “ Supervisor: Prof. Dr. Herwig Stibor
10/ 2011 – 10/ 2013	**Ludwig-Maximilians-University, Munich, Germany** Master of Science in Evolution, Ecology and Systematics Thesis: “Analysis of predator mediated changes in gene expression in *Triops*- and fish-induced *Daphnia magna* – a qPCR study” Supervisors: Prof. Dr. Christian Laforsch Prof. Dr. Justyna Wolinska
10/ 2008 –07/ 2011	**Ludwig-Maximilians-University, Munich, Germany** Bachelor of Science in Biology Thesis: “Multiple Stressoren bei *Daphnia magna*: Räuber, Parasiten und Ihre Auswirkungen auf die life history” Supervisors: Prof. Dr. Christan Laforsch, Prof. Dr. Justyna Wolinska
09/ 2003 – 06/ 2007	**Gymnasium (grammar school), Kolleg St. Matthias Wolfratshausen, Germany,** A-levels

Grant

08/2016	LMU Travel grant, for the participation at the SIL conference in Turin, Italy; provided by the “Ludwig-Maximilians-University graduate center Munich, Germany

Workshops

05/ 2014	"Multivariate statistics in R", Seeon, Ludwig Maximilians-University Munich, Germany (Dr. Robert Ptacnik)
02/ 2012	"Leading and Promoting Discussions", Sprachraum, Ludwig-Maximilians- University Munich, Germany (C. Hübner)
09/ 2012	"qPCR course", Bioline, Ludwig-Maximilians-University Munich (Dr. S. Hawkins)

Additional skills

Languages	German: native language English: fluent Latin: Latinum Old Greek: Greacum
Computing	MS Office, Cell P, Amira
Statistic programs	SPSS, Sigma Plot, R

9.2 Presentations

Talks

Buchberger, F., Stockenreiter, M., Stibor, H. 2016. Silent visitors – Lasting effects of non-successful invaders on freshwater phytoplankton community dynamics. SIL conference Turin, Italy.

Stockenreiter, M., **Buchberger, F.**, Stibor, H. 2016. Light spectrum shifts affect community assembly in perialpine and prealpine lake phytoplankton. SIL conference Turin, Italy.

Buchberger, F., Rabus, M., Rusek, J., Wolinska, J., Laforsch, C. 2013. Analysis of predator mediated changes in gene expression in fish- and *Triops*-induced *Daphnia magna* –A qPCR study. Conference, Evolution, Ecology and Systematics –Masterprogram, Munich, Germany.

Poster

Stockenreiter, M., **Buchberger, F.**, Stibor, H. 2016. Light spectrum shifts affect assembly in prealpine lake phytoplankton. DGL & SIL Tagung, Vienna, Austria.

Buchberger, F., Stockenreiter, M., Stibor, H. 2015. Effects of non-successful invaders (ghost species) on phytoplankton community dynamics. Fresh Blood for Fresh Water Meeting, Mondsee, Austria.

Buchberger, F., Heß, M. 2012. Histology and 3D microanatomy of a dwarf male of *Scalpellum scalpellum* (Cirripedia, Crustacea). Conference, Evolution, Ecology and Systematics – master program, Munich Germany.

Buchberger, F., Rabus, M., Laforsch, C. 2012. *Triops*-induced hidden defenses in *Daphnia magna*. Conference, Evolution, Ecology and Systematics – master program, Munich Germany.

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11. Declaration

Eidesstattliche Erklärung

Ich versichere hiermit an Eides statt, dass die vorgelegte Dissertation von mir selbständig und ohne unerlaubte Hilfe angefertigt ist.

München, den 22.11.2017 .....

Felicitas Buchberger

(Unterschrift)

Erklärung

Hiermit erkläre ich, *

☒ dass die Dissertation nicht ganz oder in wesentlichen Teilen einer anderen Prüfungskommission vorgelegt worden ist.

☒ dass ich mich anderweitig einer Doktorprüfung ohne Erfolg **nicht** unterzogen habe.

☐ dass ich mich mit Erfolg der Doktorprüfung im Hauptfach .....

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☐ dass ich ohne Erfolg versucht habe, eine Dissertation einzureichen oder mich der Doktorprüfung zu unterziehen.

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*) Nichtzutreffendes streichen