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Titel der Masterarbeit / Title of the Master's Thesis

„The Gradostat – Development of an experimental Meta-Ecosystem for studying coexistence along environmental gradients"

verfasst von / submitted by

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Abstract:

Most knowledge about resource competition within communities so far is based on homogenous environments, whereas the dynamics in heterogeneous systems still pose a challenge within the field of ecology. In nature, spatially separated habitats are never completely isolated but linked with each other, providing an exchange of biomass, resources, and matter and neighboring habitats may vary in their environmental characteristics such as resource levels. Those systems are referred to as meta-ecosystems.

An approach to experimentally investigate spatially heterogeneous aquatic habitats is the Gradostat, a system with which it is possible to simulate a patchy meta-ecosystem to enhance the understanding about the coexistence and competition of communities in complex aquatic environments. The Gradostat is a Chemostat consisting of three vessels. Each vessel is designed as a Chemostat, with in and outflow. In addition, the chambers are linearly connected through diffusion. By providing medium different in resource concentration a gradient can be created among the connected vessels. This allows niches to form generated by those gradients to observe growth, dispersal of organisms and competition of communities of aquatic phytoplankton.

The aim of this thesis is the further development and enhancement the concept of a Gradostat and its abilities to simulate a meta-ecosystem under laboratory conditions. For this purpose two species phytoplankton communities were grown in nutritionally heterogeneous and homogeneous environments created within the Gradostats and analyzed.

As the achieved results demonstrated very evidently, the expectations for the functionality of the Gradostat system regarding the heterogeneous and homogenous traits of a meta-ecosystem and creating steady states over extended time periods were fulfilled. The experimental data were conform to the simulated datasets regarding the resource diffusions over the vessels creating non-linear gradients over the vessels in the heterogeneous set-up (‘Limitation’) generating a steady state or keeping a homogeneous environment in the ‘Control’ experiment. Also the biomass data show clear differences between the composition of the species communities displaying varying outcomes of competition over the created gradients in the heterogeneous set-up and the gradient free homogeneous set-ups.

Abstract in German/ Zusammenfassung auf Deutsch:

Das meiste Wissen über die Konkurrenz um Ressourcen innerhalb von Gemeinschaften bezieht sich auf homogene Bedingungen, währenddessen stellen die Dynamiken in heterogenen Systemen nach wie vor eine Herausforderung im Studienfeld der Ökologie dar. Unter natürlichen Bedingungen sind räumlich getrennte Lebensräume niemals komplett isoliert voneinander sondern stehen im Kontakt miteinander und ermöglichen einen Austausch von Biomasse, Ressourcen und Stoffflüssen. Benachbarte Habitate können in ihren Umweltbedingungen variieren wie zum Beispiel in ihren Ressourcenverfügbarkeiten. Solche Systeme werden auch als Meta-Ökosysteme bezeichnet.

Ein Ansatz um räumlich heterogene aquatische Habitate experimentell zu studieren ist der Gradostat. Dabei handelt es sich um ein System mit dem es möglich ist uneinheitliche Meta-Ökosysteme zu simulieren um das Verständnis über die Koexistenz und das Konkurrenzverhalten von Gemeinschaften in komplexen aquatischen Lebensräumen zu erweitern. Der Gradostat ist eine Art Chemostat mit drei Kammern wobei jede Kammer als eigener Chemostat gestaltet ist mit einem eigenen Zu- und Abfluss. Zusätzlich sind die Kammern auf lineare Weise miteinander durch Diffusion verbunden. Durch die Versorgung mit Medien, die unterschiedliche Ressourcenkonzentrationen haben, kann ein Gradient über die drei Kammern generiert werden. Durch die so entstandenen Gradienten können sich Nischen bilden und das Wachstum, die Verbreitung der Organismen und das Konkurrenzverhalten der aquatischen Phytoplankton Gemeinschaften untersucht werden.

Das Ziel dieser Arbeit ist die weiterführende Entwicklung und Verbesserung des Konzeptes eines Gradostaten und seinen Eigenschaften zur Simulation von Meta-Ökosystemen unter labormäßigen Bedingungen. Zu diesem Zweck wurden zwei Phytoplankton Arten als Gemeinschaft in selbst designten Gradostaten studiert und analysiert, wobei die Systeme homogene und heterogene Umgebungen durch Steuerung der Nährstoffverteilungen kreierten.

Die ermittelten Resultate konnten eindrucksvoll zeigen, dass die Erwartungen an die Funktionalität eines Gradostaten besonders betreffend der homogenen und heterogenen Eigenschaften eines Meta-Ökosystems und der Erzeugung von ‚steady states' bzw.

Gleichgewichtsphasen über längere Zeiträumen hinweg erfüllt wurden. Die experimentell erhobenen Daten waren konform zu den simulierten Datensets in Bezug auf die Ressourcenverteilungen über die Kammern mittels Diffusion. Im heterogenen Aufbau (‚Limitation') wurden nicht-lineare Gradienten erzeugt und eine Gleichgewichtsphase (‚steady state') generiert bzw. eine homogene Umgebung aufrecht erhalten im Kontrollexperiment (‚Control'). Auch die Daten der Biomasse-Analysen zeigten klare Unterschiede zwischen den Zusammensetzungen der Artengemeinschaften und demonstrierten damit unterschiedliche Auswirkungen der Konkurrenz über die generierten Gradienten im heterogenen System und dem Gradient-freien homogenen System.

Acknowledgement

At this point I would like to thank the people full heartedly who supported und assisted me during the difficult process of this thesis.

I want to express my huge gratitude to my thesis advisor Professor Robert Ptacnik, leader of the research group ‘Aquascale’ and the facility of Wassercluster in Lunz am See in Lower Austria and its staff.

Also my huge thanks and gratitude goes to the people for their assistance and support during that time Lena Eggers, Alexey Ryabov, Christian Preiler, Beate Pitzl, Zsófia Horváth, Radka Ptacnikova, Csaba Vad, Josefa Sommer, Thomas Fleischhacker, Gertraud Steniczka, Ioannis Tsakalakis, Claudia Pezzei, Christian Gröger, Stefan Wöhrer, all people at the Wassercluster in Lunz am See, my family, friends and colleagues.

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1 Introduction

The resource competition theory as formulated by MacArthur (1972), León & Thompson (1975) and Tilman (1980, 1982) is a successful framework for understanding conditions for coexistence and regulation of diversity in natural communities. The concept of resource competition is an important theory for understanding the coherences for regulation of diversity and coexistence in homogeneous and isolated communities. However, those idealized systems don't exist in nature and natural systems differ from idealized systems in important ways: First, spatially separated habitats are never completely isolated but connected to other habitats through dispersal where resources, matter and biomass are exchanged. Second, adjacent habitats may differ in environmental parameters such as resource levels. Therefore as Grover (1997) already depicted resource gradients have an influence on the outcomes within natural systems that varies from uniform systems. The parallel flux of material and organisms therefore including abiotic and biotic exchanges between habitats is conceptualized in the meta-ecosystem concept (Loreau et al. 2003; Leibold et al. 2004).

However, meta-communities (Wilson, 1992; Holt, 1993; Hubbell, 2001; Mouquet & Loreau, 2002) or the more up to date meta-ecosystems (Loreau et al. 2003; Leibold et al. 2004) only focus on the regional level and its spatial flows of biotic components and interspecific interactions like competition and predation, on which the meta-community is focused on, and abiotic matter which the meta-ecosystem concept also includes in its framework and therefore works as an extension to the meta-community concept.

Further enhancements led to the concept of a patchy meta-ecosystem (Tsakalakis *et al*, in prep.) which focus on the different characteristics of the local and regional levels of meta-ecosystems and provides a framework to combines them into one concept.

The concept of a Gradostat firstly mentioned by Lovitt & Wimpenny (1979, 1981) simulates a patchy meta-ecosystem and the further development of its experimental design is the topic of this thesis. Patches with homogenous resource distributions are linearly connected through diffusion under laboratory conditions creating a resource gradient among those patches. The Gradostat is used to link resource competition theory with more realism regarding resource

gradients and connectivity among patches since idealized systems, as they are the approach in theoretical frameworks, don't meet circumstances as found in nature and therefore differ from natural systems. The Gradostat allows to follow growth, dispersal and competition in communities that are exposed to an environment consisting of heterogeneous, connected patches.

In the following chapters the main traits of a patchy meta-ecosystem and the participating frameworks for describing the local and regional aspects are presented.

1.1 Theoretical framework of a patchy meta-ecosystem

The theoretical framework of a patchy meta-ecosystem (Ryabov *et al*, in prep.) primarily combines two important theories about competition: the theory for resource competition in uniform systems as described by Tilman (1980) and the theory for spatially extended systems as formulated by Ryabov and Blasius (2011).

Tilman's resource competition concept for uniform systems describes the resource competition within homogeneous isolated systems. The theory is based on species-specific resource requirements and formulates the R*-rule (Tilman, 1982) which assesses that the consumer who reduces a resource to the lowest level excludes other competitors. The R*-rule also known as the resource ratio hypothesis was tested and experimentally confirmed by Miller (*et al*. 2005).

Ryabov & Blasius approach extends Tilman's concept towards systems consisting of multiple habitats (2011). Their system allows to study interactions between species within spatially structured environments and its effects on distributions of resources therein. Therefore the resource availability and population densities continuously change along the resource gradient. A thorough description of this framework can be found in the chapter 1.3.

An invasion threshold was introduced for spatial systems to predict the outcome of competition by analyzing the maximal resource requirement of an invading species when a resident species is present. The invasion threshold allows a graphic interpretation of the outcome of competition as it is depicted as a curve in the resource graphs. This model leads

to three possible outcomes of competition which are (i) stable coexistence, (ii) competitive exclusion or (iii) bi-stability which describes alternative stable states where species two cannot invade once one species is established.

A patchy meta-ecosystem (Ryabov *et al*, in prep.) has a local and a regional level of coexistence of which each has different traits. The local level describes patches which are homogeneously mixed and Tilman's resource ratio hypothesis (R*-rule) can be applied as it fits to the specifics of a uniform system. The regional level however formulates the connections between patches by dispersal and diffusion of biomass, resources and matter. Therefore the theory of spatially extended systems with invasion thresholds can be applied. Both the interactions happening on local levels have effects on the regional scales and the other way round as the local patches only describe uniform traits without being isolated entities on their own but connected with other patches providing exchange of biomass, matter and energy (see Fig.1.1).

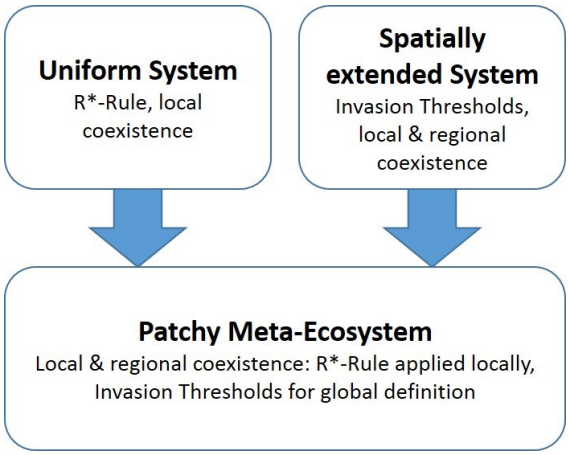

Fig. 1.1: Scheme of theoretical framework for a patchy meta-ecosystem. Characteristics from resource competition in uniform systems with R*-rule (Tilman, 1980) and spatially extended systems with Invasion Thresholds (Ryabov & Blasius, 2011) combined in the patchy meta-ecosystem approach (Ryabov *et al*, in prep.).

1.2 Theoretical review of resource competition in a homogenous system

For a better understanding of the patchy meta-ecosystem concept and the experimental approach of the Gradostat, the theoretical framework of uniform resource competition (Léon & Tumpson, 1975. Tilman, 1980, 1982) is first introduced. Tilman defined the resource ratio hypothesis in 1982, also known as the *R**-rule, as the situation where the growth rate μ equals the mortality rate m. In a Chemostat setup, this is a steady state situation (dilution = *m*). If *R* (= actual resource concentration) > *R** then the biomass is increasing. If R < *R** then the biomass is declining (see Fig.1.2a). This definition is important for the framework of the resource competition theory. The growth rate μ also depends on the concentration of both resources R_1 and R_2 in the way that μ is affected by the resource with the lowest concentration which is best described by Liebig's law of the minimum as: $\mu\ (R_1\ ,\ R_2) = \mu_{max}$ min $(R_1 / H + R_1\ ,\ R_2 / H + R_2)$. Liebig's law of the minimum describes that growth is controlled by the most limiting resource and not by the total amount of resources. *H* describes the half-saturation constant and is used for the definition of the resource adaption of the species. If a resource *R* equals *H* it is set: $\mu(H) = \mu_{max}\ H / (H + H) = \mu_{max} / 2$; *R** is then calculated as following: $R^* = H\ m / (\mu - m)$; therefore *H* is proportional to *R**. The growth rate μ can be limited either by the one or the other resource (R_1 or R_2) of which the limiting resource is described as the essential *R*. For the case that one resource is limited the growth rate μ follows following equation: $\mu\ (R_1\ ,\ R_2) = \mu_{max}\ R_1 / (H + R_1) * R_2 / (H + R_2)$ (see Fig.1.2b).

For describing the minimal concentrations for R_1 and R_2 (when the resource-rate is under dilution) needed for an increase of the biomass the ZNGI (Zero-Net-Growth-Isocline) is introduced (Tilman, 1980; see Fig.1.2c). If the concentrations are below the isoclines the biomass decreases. If, for instance, one resource is above the minimal concentration limit then it is growth limited by the other resource and vice versa. The next important factor is the supply point. If the supply point is above the ZNGI the biomass can grow but if one resource is reduced the biomass is limited and declines. If the biomass grows resource concentrations decrease, therefore the supply point will move towards lower resource concentrations which depends on each species differently. This circumstance is a main reason why the Redfield-Ratio should rather be handled as an average term as experiments within this thesis as well have indicated it. The moving directions of the supply point are shown by the consumption

vectors (Fig.1.2d). The biomass increases over time while the available resources decrease and stabilize when a critical minimal resource concentration is reached (Fig 1.2e). To evaluate how much biomass was produced out of the available resources the maximum growth rate of a species (μ_{max}) and the zero-net-growth (R^*) is needed. The consumption vector will hit the ZNGI at some point marking the supply point because the resources are consumed by the biomass which defines the equilibrium point for that species (see Fig.1.2f). The equilibrium point is also the starting point for the invasion threshold for a second invading species. If the direction of the supply point vector is known, then the balance of growth is known. In the case that a second species invades the consumption vector depicts the border of the supply point and marks where species 2 is still able to invade. The invasion threshold marks the minimal concentrations of resource 1 and resource 2 needed for a successful invasion of species 2 (Fig.1.2f).

For performing an invasion analysis it is firstly assumed that species 1 is resident in an equilibrium, then a species 2 is added and observed if it can invade or not. The figures 1.2g,h describe Tilmans approach to competition-theory (1980). The borders of the area of coexistence are defined by the slopes of the consumption-vectors of both species in figure 1.2g. The other figure 1.2h shows the case of a possible bistability: between the consumptions-vectors no overlap of the areas happens where each species could invade. Therefore coexistence between these two species is not possible. For this outcome there are two possible cases: Firstly if one species is initially in the middle then the other species can't invade. Secondly within bi-stability one species can deplete one resource rendering it impossible for the other species to survive and grow.

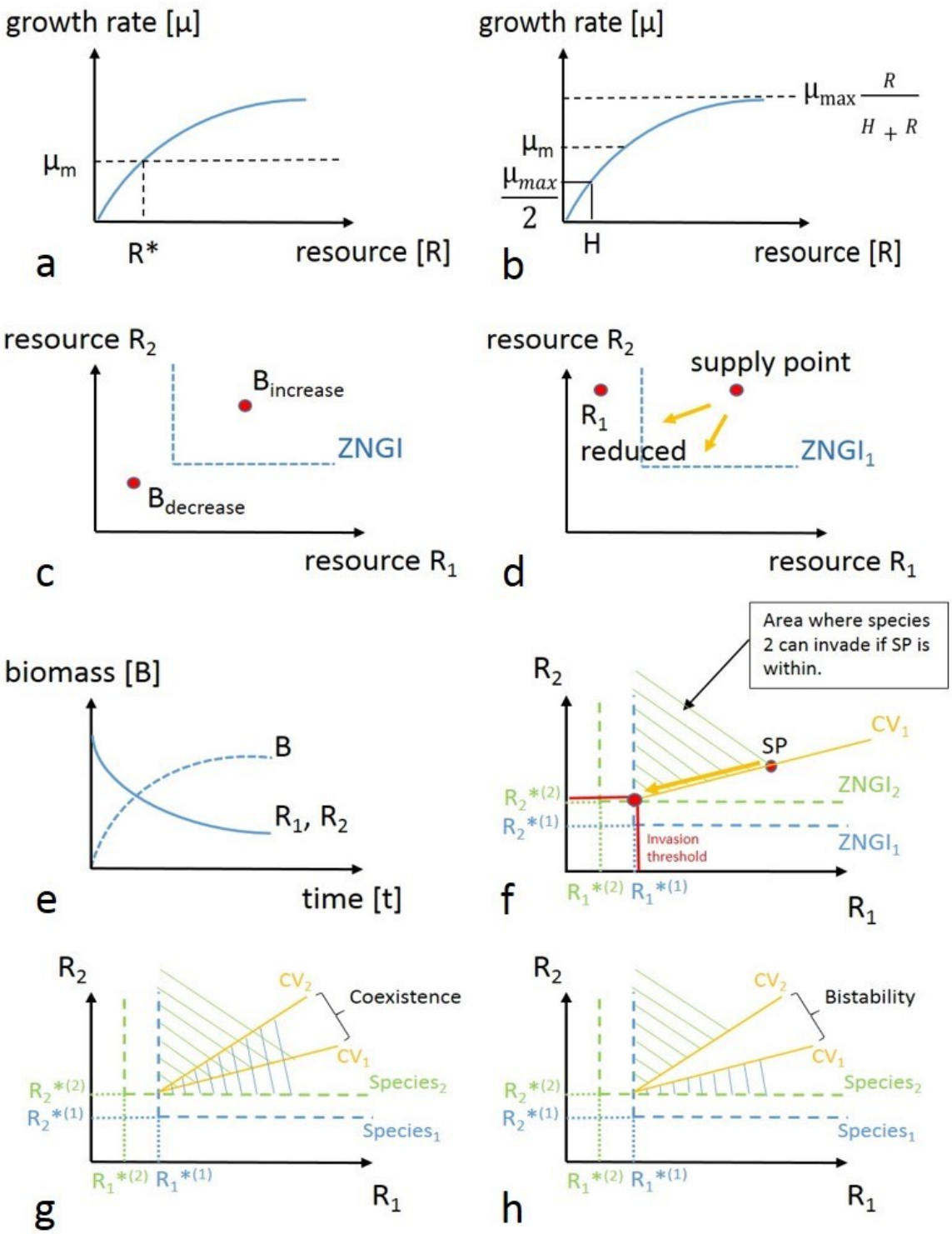

Fig. 1.2: Graphs for the theoretical framework of uniform resource competition (modified after Tilman, 1980. Ryabov & Blasius, 2011). a: Visualization of R*; r = resource, m = mortality rate, R* = zero-net-growth, μ = growth rate; b: Limitation by one resource R; H = half-saturation constant; c: Schematics for critical resource value; ZNGI = Zero-Net-Growth-Isocline; B = biomass; d: Schematic for a supply point, yellow arrows = consumption vectors, ZNGI = Zero-Net-Growth-Isocline, $ZNGI_1$ means ZNGI for species 1; e: Schematics of increase of biomass with decrease of resources over time; f: Schematic of the invasion threshold of species 2; SP = supply point, CV = consumption vector, R = resource, $indices_{1, 2}$ = variables belonging to either species 1 (blue) or species 2 (green), red marking = invasion threshold; g: Schematics for coexistence; CV = consumption vector, R= resource, $indices_{1, 2}$ = variables belonging to either species 1 (blue) or species 2 (green); h: Schematics for bistability; CV = consumption vector, R= resource, $indices_{1, 2}$ = variables belonging to either species 1 (blue) or species 2 (green).

1.3 Theoretical review of resource competition in a heterogeneous system

The theoretical framework for a heterogeneous or spatially extended system was formulated by Ryabov and Blasius (2011). In this approach the influence of a resident species on the resource gradients is crucial for the invasion success of a second species. A spatially continuous system is described as a one-dimensional habitat where the resources were supplied from opposite sides to create gradients as it can be seen for example in the figure 1.3a where the resources light and nutrients mark opposing gradients in a water column. The resource nutrients are supplied from the bottom and the light resource light from the top. As a main point the invasion threshold was proposed which describes the maximal resource requirement for an invader in the case of the existence of a resident species. Therefore the boundaries of this system are created by the availability of the resources which cause a heterogeneous distribution of organisms and resources. For depicting those resource distributions a parametric curve, the 'System State Curve' or SSC (Fig.1.3b) is used. It can be seen as an equivalent to the equilibrium point of the uniform system although it always crosses the ZNGI at two points and the bold part represents the positive net growth and therefore shows the favorable area of the species.

In this case for an invasion analysis it is firstly assumed that species 1 reached the state of equilibrium. The main problem is, that the invasion threshold depends not only on the resource requirements of the resident and invader, but also on the distribution of resources shaped by the resident species. Therefore an invading species is successful if the invasion threshold crosses its ZNGI at two points or more specifically when its resource requirements are below the first order approximation of the invasion threshold which describes a straight dashed line allowing a graphical analysis (Fig.1.3c; Ryabov & Blasius, 2011).

A good example for a spatially extended system is the water column where the resources light and nutrients create two opposing gradients as they are provided from opposite sides, light from the top and nutrients from the bottom. In the scheme of the water-column (Fig.1.3a) it can be seen, that species 1 lives closer to the surface and is therefore more limited by nutrients than species 2 which lives deeper in the water column and is limited by light. They both inhabit different niches within a range where both resources are available at sufficient concentrations

and therefore they can coexist due to their spatial separation. This is not the case within a Chemostat which provides a homogeneous environment. By contrast a Gradostat is an intermediate system between a water column as it incorporates inverse resource gradients and a Chemostat, as locally in each patch the system is homogenous.

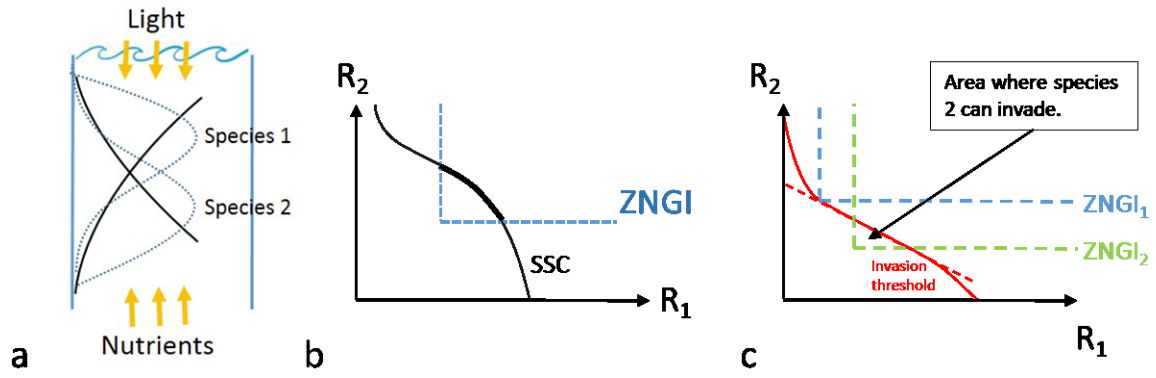

Fig. 1.3: Graphs for the theoretical framework of a spatially extended system (modified after Ryabov & Blasius, 2011). a: Scheme of a water-column, two inverse gradients for light and nutrient availability are created where two competing species (1, 2) inhabit spatially separated niches. b: Scheme of the System State Curve (SSC, favorable area marked bold), ZNGI = Zero-Net-Growth-Isocline. c: Scheme of the invasion threshold of species 2, dashed line is first order approximation of the invasion threshold; $ZNGI_{1/2}$ means ZNGI for species 1 or 2.

1.4 The Gradostat

The concept of the Gradostat was firstly mentioned and developed by Lovitt and Wimpenny (1979, 1981) as a laboratory model to simulate natural microbial ecosystems. They based their Gradostat system on linking Chemostats in series with each other. Chemostats (Monod, 1950; Novick & Szilard, 1950; Smith & Waltman, 1995) are widely used and well-studied within the aquatic ecology and are designed to retain a homogeneous and constant environment. A Chemostat is a vessel which is constantly supplied with fresh medium and also has an outflow at the same rate to keep a distinct volume. Lovitt and Wimpenny (1979, 1981) connected a series of five round glass vessels operating as Chemostats with each other via tubes. Between the vessels solutes were transported in opposite directions for creating opposing gradients simulating a heterogeneous environment for studying microbial growth with steady and non-steady conditions. Tang (1986) developed mathematical principles for the case of a single population within a Gradostat, studies of coexistence of bacterial species (Wimpenny & Abdollahi, 1991) as well as further analyzes of mathematical models for competition within a two-vessel Gradostat were performed (Jäger et. al, 1987; Smith & Tang, 1989, Hofbauer & Joseph, 1994). The mathematical models for competition in cases with more than two vessels (n-vessel or multi-vessel cases) were further developed (Chang & Baltzis, 1989; Smith & Waltman, 1991; Smith et. al, 1991; Hsu & Waltman, 1993). It stands out that generally the system of the Gradostat was mostly theoretically studied with mathematical simulations and less experimentally approached. Even recent papers mostly focus on theoretical frameworks of the Gradostat like Borisov (2015) as he modeled a Gradostat with system simulation software tools or Dong (et al. 2014) and Gaki (et al. 2009) who both focused on models for microbial populations within a Gradostat. Codeço and Grover (2001) deducted an experimental study and operated with a similar Gradostat set-up like Lovitt and Wimpenny (1979, 1981) with five Erlenmeyer flasks connected by tubes and analyzed microbial growth in Chemostats compared to continuous and semi-continuous (with pulsed inflow and periods without inflow) Gradostats. Their findings showed that the possibility of spatial segregation enhances the local diversity in a Gradostat compared to a Chemostat-like system. An issue with the approach with flasks connected by tubes is that the interconnecting tubes should actually be regarded as compartments on their own as they permanently contain a certain amount of fluids and biomass.

In the present thesis the Gradostat is used as a model for a patchy meta-ecosystem, especially as an experimental system to study competition of algae in a patchy meta-ecosystem, and the design was improved to avoid the effects of connecting tubes on the entire system. Instead of using separated glass flasks where tubes can function as additional patches, we designed a compact system with three vessels in an aquarium. The aquarium-shaped Gradostat had two separating walls, creating three compartments which were connected by holes in the walls with each other. Through those diffusion holes the exchange of solutes and biomass was given. At the opposing ends of the Gradostat the resources Phosphorus (P) and Nitrogen (N) were provided which would diffuse through the whole system (Fig.1.4a). In equilibrium or when the Gradostat reaches its steady state the resource ratio would change from one vessel to another creating opposing gradients over all three vessels (Fig.1.4b). Each vessel is a Chemostat and creates a homogenous local patch while all three vessels in linked in series are the heterogeneous regional level. If one species is added it starts to deplete the resources N and P in each vessel. Therefore in equilibrium in one vessel the resources will be depleted under the critical level and in another vessel it is still above the critical level (Fig.1.4c). Also source-sink-dynamics play a role within a Gradostat (Fig.1.4d). The 2nd vessel is the source of biomass of one species and should be higher than the critical level, therefore the net-growth-rate should be positive. The vessel 1 and 3 are the sinks of the biomass, the resource-concentrations will be below the critical levels.

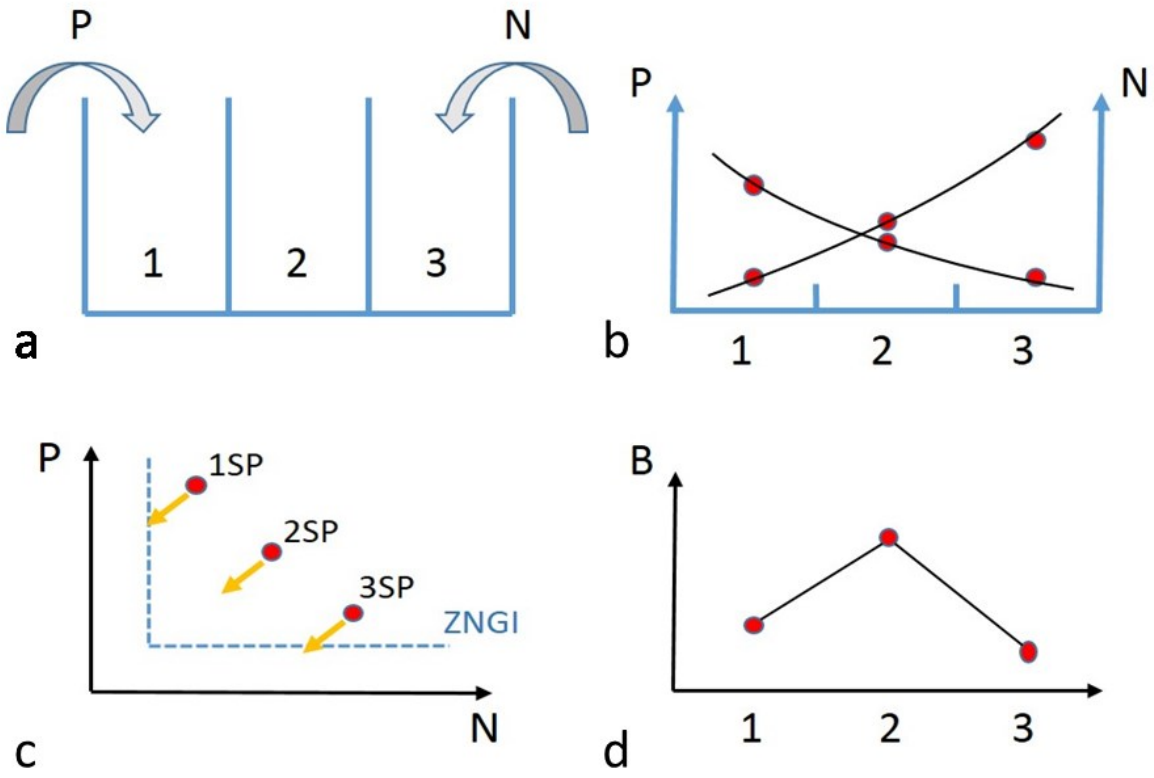

Fig. 1.4: Graphs for theoretical concept of a Gradostat. a: Scheme of a Gradostat with three vessels and Nitrogen and Phosphorus-Input. b: Theoretical distribution of the resources Nitrogen and Phosphorus over the three vessels of the Gradostat. c: Schematics for the depletion of the resources N and P in every vessel of the Gradostat, ZNGI = Zero-Net-Growth-Isocline, SP = Supply Point. d: Schematics for the source-sink-dynamics of one species.

Another important aspect for an operational Gradostat system is the diffusion within the system as well as the in- and outflow of the system. Without an in- and outflow the Gradostat would reach a uniform distribution of the resources within the whole system. The timeframe until it reaches the uniform state depends on the rate of diffusion between the vessels, the higher the rate the sooner it would reach a uniform resource distribution over the whole system since no new resources are provided into this isolated set up (Fig.1.5a). With a permanent in- and outflow for each vessel the resources are constantly renewed and the difference of resource concentrations between the vessels are maintained and a steady state is reached (Fig.1.5b). The result is an open system with a permanent nutrient in- and output as it is the case in natural systems like lakes with rivers or the Baltic Sea. For creating gradients the vessels have different resource distributions as for example the left vessel is provided with resource one and the right vessel with resource two and the vessel in the middle with depleted medium. The vessels are also stirred with air ventilation since they have a rectangular bottom

area for which a magnetic stirrer is unpractical. The so created turbulence also defines the strength of the diffusion between the vessels as it was shown as part of the results within this thesis.

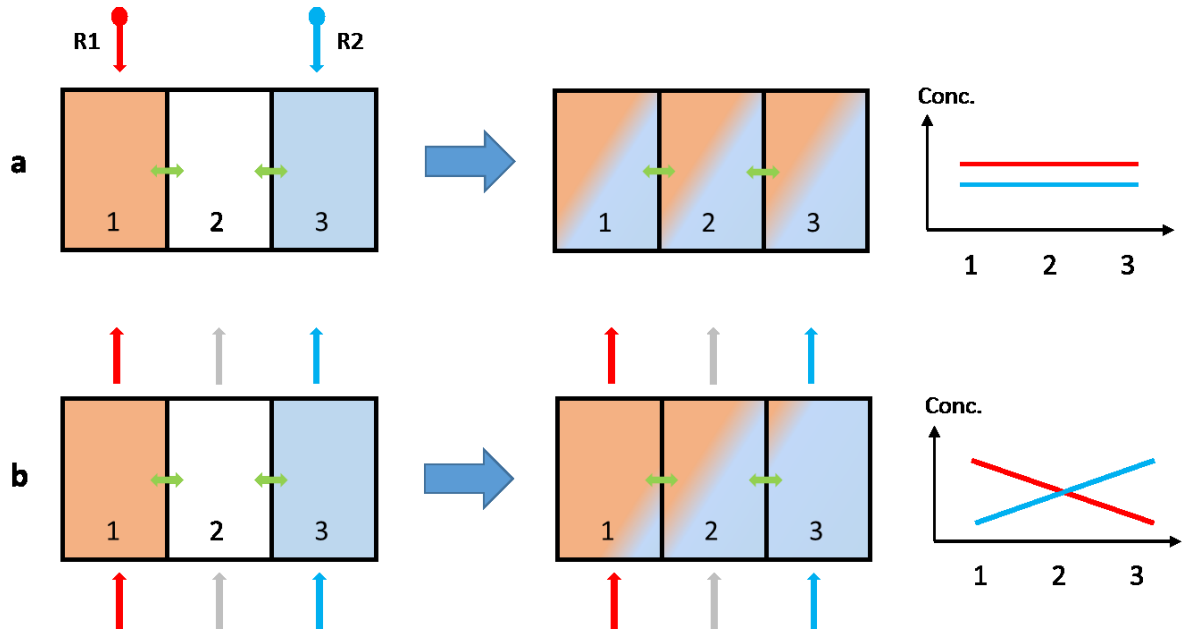

Fig. 1.5: Scheme of the distribution of resources in a Gradostat. a: without permanent inflow and outflow; b: with permanent inflow and outflow into each vessel; red and blue color indicates the two resources R1 (red) and R2 (blue), grey arrow is a permanent supply without resources in the medium; the graphs show the concentration of the resources within the three vessels.

The final set up of the Gradostat (Fig.1.6) is therefore an experimental system to study patchy aquatic meta-ecosystems under steady state: It is built from three vessels connected with each other linearly by diffusion of resources like N/P and organisms. Each vessel is operated as a Chemostat with an in-and outflow which represents the homogeneous local patch of the patchy meta-ecosystem. The provision of medium different in their resource concentrations creates gradients among the vessels trough turbulence. The in- and outflow of the vessels is regulated by a programmable peristaltic pump and they are continuously distributed.

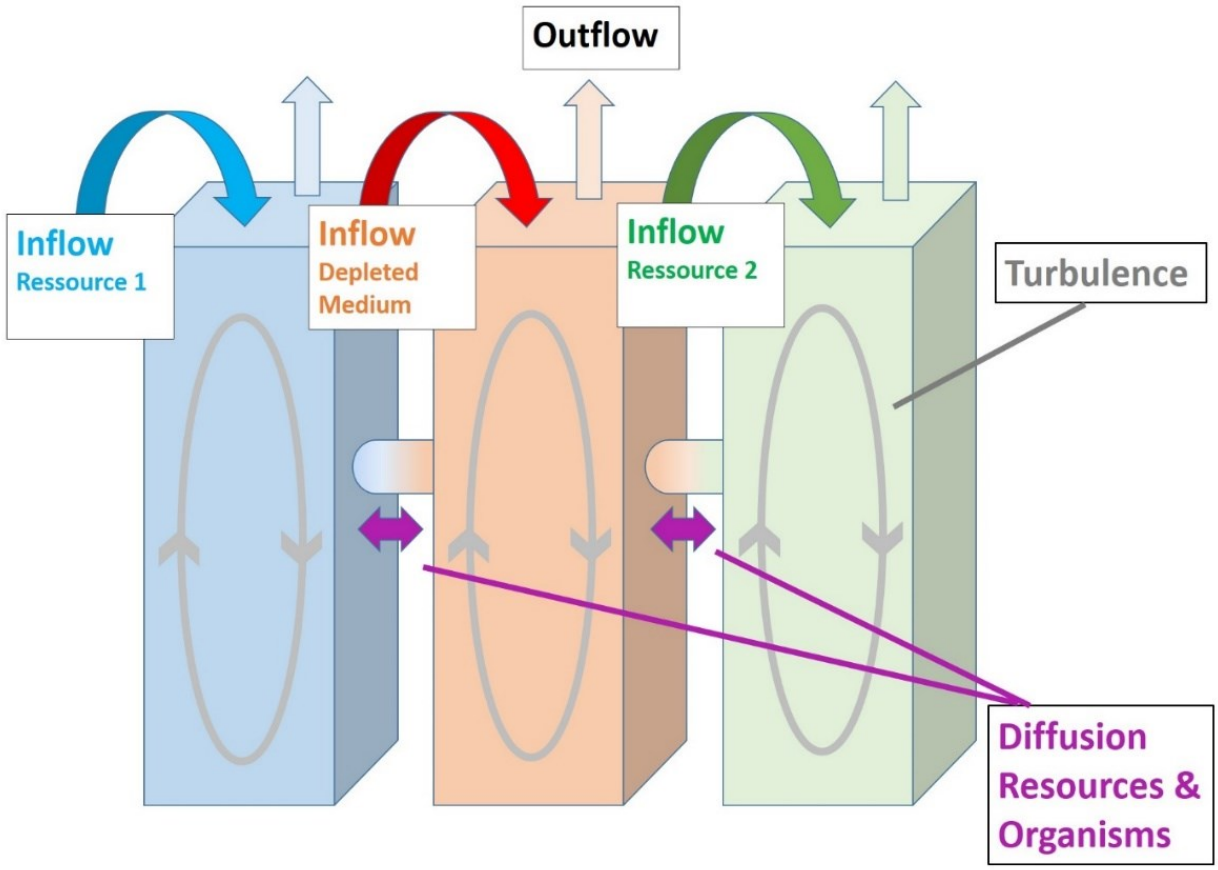

Fig. 1.6: Scheme of a Gradostat with the resource distributions.

2 Material and Methods

2.1 Portrayal of Phytoplankton species

For this thesis seven species of phytoplankton were examined and compared to find two fitting species for a model community to be used within the Gradostat-Experiments. The requirements were to find a species-pair of which one was more thriving on a nitrogen-limited environment and the other on a phosphor-limited surrounding. Three of them were cyanobacteria, *Synechococcus elongatus*, *Microcystis aeruginosa* strain PCC 7806 and strain ΔMCY A-. The strain PCC 7806 of Microcystis *aeruginosa* produces the toxin Microcystin which affects and harms the liver (Falconer et al. 1983) while the other strain ΔMCY A- is nontoxic. The toxicity of the strain PCC 7806 might be a side-effect due to differences within their metabolism probably because of different environmental conditions (Van der Westhuizen & Eloff. 1985; Watanabe & Oishi. 1985). The other species were green algae, *Stichococcus sp.*, *Monoraphidium sp.*, *Scenedesmus sp.* - all coccoid cells without flagellates - and *Chlamydomonas sp.*, an autotroph flagellate.

All species were cultivated in WC-Medium, the limitation of the nutrients were only performed within the experiments. All algae except of the cyanobacteria were morphologically distinguishable under the microscope (Tab.2.1), therefore they could be used together in model communities and contaminations could be rather easily detected.

Species	Properties	>Cell shape
Synechococcus elongatus	cyanobacteria	sphere
Microcystis aeruginosa strain PCC 7806	cyanobacteria	sphere
Microcystis aeruginosa strain ΔMCY A-	cyanobacteria	sphere
Stichococcus sp.	green algae; coccoid without flagellates	cylindric and elongated, sometimes slightly oval
Monoraphidium sp.	green algae; coccoid without flagellates	lunatic body, circular in cross-section
Scenedesmus sp.	green algae; coccoid without flagellates	prolate spheroid
Chlamydomonas sp.	green algae; coccoid with flagellates	Ellipsoid with two flagellates

Tab. 2.1: Overview of the phytoplankton species.

2.1.1 Medium

For the cultivation of the seven phytoplankton species WC-Medium was used after the recipe of Robert R. L. Guillard and Carl J. Lorenzen (1972). Prior autoclaving all ingredients except of $K_2HPO_4*3H_2O$-nutrient solution (P-source), $NaNO_3$-nutrient solution (N-source), micronutrients and the vitamin-solution were added. The reason for this procedure was that else it eventually might flocculate and renders the medium useless. On the basis of this recipe the medium with limited nutrients, one with phosphor and one with nitrogen limitation, were calculated and prepared. Over the course of the experiments it was discovered that the phosphor limited medium was not limited enough whereas the nitrogen-limited medium showed a strong limitation. Therefore for the Gradostat-experiments the nutrient-ratios were changed to reach a stronger limitation especially for phosphor. Therefore the ratio of the WC-

medium was extrapolated to the Redfield-ratio of 16 (16:1 for N:P) since it originally had a ratio of ~9. The ratios for the stronger limited media where then divided by 4 instead by 3 as it was done for the calculations of the previous limitations of the medium.

Medium	**Ratio N:P [m:m]**	**N-source [ml/1000ml]**	**N [µmol]**	**P-source [ml/1000ml]**	**P [µmol]**	**Limitation**
WC	8,97	1	236,53	1	26,37	balanced
WC P-lim.	21,45	1	275,57	0,42	12,82	weak limitation
WC N-lim.	2,40	0,33	93,48	1,25	38,91	strong limitation
WC-redfield	16	1,78	445,65	1	27,01	balanced
WC-r. P-lim.	69,29	1,78	445,65	0,25	6,43	strong limitation
WC-r. N-lim.	3,94	0,45	108,97	1	27,64	strong limitation

Tab. 2.2: Overview of the nutrient-ratios within the media. P-lim. = Phosphor limitation, N-lim. = Nitrogen limitation, WC-r. = WC-redfield.

2.1.2 Evaluation of cell density and biovolume

For evaluating the culture densities and the biovolume the cells were counted and measured. Prior counting the samples were fixed with Lugol. (1% of Lugol from amount of sample). Then the cells were counted either with the Neubauer improved chamber for smaller cells or the Palmer chamber for larger cells like *Chlamydomonas sp* and the cell density per ml was calculated.

With the invert microscope Nikon Eclipse TS100 and the software 'AxioVision' (Re. 4.8.2.0, SP3) the cell size of 20 cells from each sample were measured and the mean biovolumes were calculated according to Hillebrand *et al*. (1999). This was used to be able to compare biovolumes within the vessels of the Gradostat experiments. For further details see supplements S1.

2.2 Nutrient analysis

2.2.1 Phosphorus

The phosphorus samples were prepared and measured modified after the 'Ascorbic Acid Method' (Hansen & Koroleff, 1999) with the Photometer Spectroquant Pharo 300 (MERCK, Software-Version: 1.40, IQ-Lablink-Version: 1.20) at a wavelength of 880nm with a 10 or 50 mm cuvette which depended on the phosphor-concentration within the measured samples. Measurements were done for the Total Phosphorus (TP), the Soluble Reactive Phosphorus (SRP) and Total Particulate Phosphorus (TPP) and the method was adapted for the specific requirements for each type of phosphorus measurement. Also standard rows were measured for the calculation of the concentration of phosphorus.

In all three types of phosphorus measurement Ascorbic acid and Mix reagent for the color-reaction and a stock-solution for the standard rows were needed, only for the TP approach a digestion of the samples was done modified after the 'Persulfate Digestion Method' (Clesceri et al. 1999). The experiments with the Exponential Fed Batch only required measurements for TP, the Gradostat experiments were tested for all three types of phosphorus whereas SRP was only measured for the experiment with a strong nutrients limitation and its control set-up.

TP – Total Phosphor

For the digestion of the samples Peroxodisulfate Solution 5g $K_2S_2O_8$ with 15mL H_2SO_4 4,5M were added and filled up to 100mL with MilliQ. On 4ml of sample in autoclavable flasks 240µL of Peroxidsulfate Solution and on 15mL of Standard 900µL Peroxidsulfate Solution were added and autoclaved at 121°C for 21 minutes.

For the Ascorbic Acid Solution 1g Ascorbic Acid were mixed with 10ml of MilliQ. The Mix Reagent was prepared as following: First 2,5g Ammoniumheptamoylbdate-tetrahydrate were mixed with 31,8ml of MilliQ and 64,2mL of H_2SO_4 4,5M. Then 0,1g Kaliumantimonyltartrate was mixed with 4mL of MilliQ in a second vial and both solutions were mixed together.

For the Stock-Solution 0,439371907g KH_2PO_4 were filled up to 100mL with MilliQ which is an equivalent of 1g of Phosphorus per Liter Solution. The working solution was a dilution of 1:1000 of the stock solution which resembles 1mg Phosphorus per Liter solution. From the

working solution a standard row was prepared with 10 decreasing concentrations of Phosphor.

After autoclaving the samples and standards Ascorbic Acid Solution and Mix Reagents were added, the color reaction needed 2 minutes to finish. On 4,24mL digested sample 64µL Ascorbic Acid Solution and 64µL Mix Reagents were added. On 15,9mL digested Standard were 240µL Ascorbic Acid Solution and 240µL Mix Reagents were added.

Afterwards the samples and standards were measured with a photometer at the wavelength of 880nm within a 10mm cuvette. A dilution of the samples with MilliQ were necessary if the absorption was larger than 0,63.

SRP – Soluble Reactive Phosphor

For the Ascorbic Acid Solution 1g Ascorbic Acid were mixed with 5mL of MilliQ and 5mL of H_2SO_4 4,5M. The Mix Reagent was prepared as following: First 2,5g Ammoniumheptamoylbdate-tetrahydrate were mixed with 25mL of MilliQ and 70mL of H_2SO_4 4,5M. Then 0,1g Kaliumantimonyltartrate was mixed with 4mL of MilliQ in a second vial and both solutions were mixed together. The Stock solution and the standards were the same as in the Total Phosphorus approach. From the working solution a standard row was prepared with 10 decreasing concentrations of Phosphor.

On 10mL of sample 160µL Ascorbic Acid Solution and 160µL Mix Reagent was added and on 15ml of Standard 240µL Ascorbic Acid Solution and 240µL Mix Reagent was added. The color reaction was finished after 2 minutes and the samples as well as the standards were measured with the Photometer at 880nm wavelength in a 10mm cuvette.

TPP – Total Particular Phosphor

10ml of the samples were filtered with acid-washed and muffled GF/F filters and the filters muffled for 10 hours at 550°C.

The Ascorbic acid solution was prepared with 1g Ascorbic acid and 10mL of MilliQ. The Mix Reagent was prepared as following: First 2,7778g Ammoniumheptamoylbdate-tetrahydrate were mixed with 87,1777mL of MilliQ and 22,8222mL of H_2SO_4 4,5M. Then 0,1111g Kaliumantimonyltartrate was mixed with 4,4444mL of MilliQ in a second vial and both solutions were mixed together. The Stock Solution was the same as in the Total Phosphorus

approach but the working solution was diluted with H_2SO_4 4,5M instead of MilliQ to 1:1000 (1mg/L). From the working solution a standard row with 10ml each was prepared with 10 decreasing concentrations of Phosphor.

On the sample-filter 10ml of H_2SO_4 90mMol was added and eluted for 1h in water bath at 95°C and afterwards cooled down to room temperature. Then on the samples and standards 200µL Ascorbic acid and 200µL Mix Reagent were added. The color reaction was finished after 2 minutes and the samples were measured with the Photometer at 880nm in 50mm cuvettes.

2.2.2 Nitrogen

Analysis of dissolved Nitrogen was done with a Continuous Flow Analyzer for Soluble Nitrogen (SN) measured in its compounds NH_{4+}, NO_{3-}, NO_2 and NOx.

2.2.3 Nutrient limitation indicator

NO_3:TP ratios were calculated to track the distribution of the focal nutrients N and P within the Gradostats. The DIN:TP ratio of 3,5:1 was used as reference point for balanced nutrient supply, following limitation bioassay experiments (Ptacnik et al. 2010; Morris and Lewis (1988). Also in this thesis the DIN:TP ratio was shown to be more reliable than the Redfield ratio (see Fig. 3.13, 3.16 & 3.19).

2.2.4 Chlorophyl A

Chlorophyll-a analysis was performed modified after the Method 445 from the Environmental Protection Agency Cincinnati in Ohio (Arar & Collins. 1997).

10ml of samples were filtered on GF/F filers. Chl-a was then extracted in 4ml Aceton 90%. Since the volume was reduced from 10 ml to 4 ml the measured concentration needed to be corrected to the right concentration (Chla a corrected = (Chl a [µg/L] * 4) / 10) and the

corrected values were used in the calibration equation. The values weren't corrected for Pheophytin A. The samples were measured with a Fluorescence Spectrophotometer (F-7000, Hitachi) at 440nm for Excitation and 660nm for Emission.

2.3 Screening of phytoplankton species for species pairings

For operating the Gradostat, two species were needed for cultivating them together. Those species should express different preferences in Phosphorus and Nitrogen uptakes. For choosing fitting species pairs for the experiments the maximum growth rate and their zero net growth R* were investigated.

2.3.1 Maximum Growth Rates

The species-specific maximum growth rate [μ_{max}] was determined for the 6 species by monitoring their rate of change of in chl-a concentration during the exponential growth phase . μ_{max} was calculated from the time intervals where a species showed exponential growth (μ = (ln (T1/T0) / dT , dT = count of days, T = time point [d]; Pirt. 1975; see Fig.2.1):

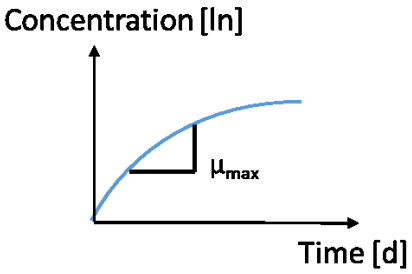

Fig. 2.1: Scheme of the maximum growth-rate [μ] over time and the concentration of biomass (modified after Pirt. 1975).

From each stock culture 15 ml were pipetted into a culture flask with 30ml of WC-medium in 2 replicates and put on full light-intensity like the stock-cultures. Change in biomass was monitored by following the chlorophyll-a concentration over time by in vivo measurements, using a hand-held fluorometer 'AquaPen' (AP 100 (SN-AP-140), Software: Fluoropen 1.0.4.1, Photon Systems Instruments) was used and both wavelengths for the relative content of chlorophyll a and phycoerythrin (450nm and 620nm) were measured for the green algae and Cyanobacteria species. The replicates also were diluted twice over the course of the experiment to prevent a limitation of nutrients and the entering into a 'lag-phase', a limitation of growth when the cell density is getting to high. Therefore the cultures showed three growth

phases. The experiment lasted for 24 days. The rate of doubling per day was 0,69. The dilution was assumed with a factor of 6 (dilution = ln (6/T)).

The maximum growth-rate μ_{max} value was calculated from the relative rate of change in chlorophyll-a concentration during exponential growth. Therefore the logarithm of relative rate of chlorophyll was calculated and graphically plotted with the 'trend line function' in Microsoft excel to derive the regression formula where the slope was taken from which displayed the maximum µ. Alternatively for calculating maximum µ the µ values for each following time point of measurement (µ= ln (T1/T0)/dT) was taken and the maximum µ was calculated as a mean from the µ-values of the timespan of the steepest growth period. For the boxplot calculations all µ values were considered except of the negative ones, which were caused by the dilution steps. From each day the mean of the µ values of the duplicates were calculated and from those the boxplot graphs were created with the program R. For further details see supplements S3.

2.3.2 Exponential Fed Batch

The Exponential Fed Batch (EFB) is a convenient method grow algal cultures under steady-state conditions (Fischer *et al.*, 2014). Medium with nutrients is permanently added proportional to the current volume (without an outflow). This results in a constant dilution rate while the culture medium accumulates (Fig.2.2a). The advantage of this system is that it is easily operated and that a fairly large sample volume can be taken regularly, without disturbing the steady state conditions.

The permanent addition of the medium with a definite amount of nutrients to reach a dilution of 20% per day (factor 0,2) is regulated over a peristaltic pump and a microprocessor, the Arduino, programmed to calculate the time for the pump to run to provide the needed amount of medium correlating to the volume within the flasks (see Supplements S3.2). The tubes were attached to injection needles which were pinned through the cap of the culture flasks to keep the set up as sterile as possible (Fig.2.2b). The growth was monitored with the Fluorometer 'AquaPen' measuring the relative chlorophyll a and phycoerythrin rates and the volume of the flasks regularly set back to prevent the flask of getting too full. As soon as the

growth declined and reached a steady state samples were taken every 2 days for 3 times and frozen for nutrient measurements. Therefore duplicates with 20ml each were taken from every culture flask, filtered with GF/F filters (muffled and acid-washed) and frozen for the Nitrogen (SN – Soluble Nitrogen) and Phosphorus (SRP – soluble reactive phosphorus) measurements.

Three EFB-experiments with different phytoplankton species were performed where they were cultivated either in Nitrogen- or Phosphorus- limited medium within 250ml culture flasks and their growth rates and Nutrients content compared to calculate R* - the zero-net-growth. Limitation of the nutrients means that the Redfield ratio of N:P = 16:1 is shifted into one or other direction of N or P.

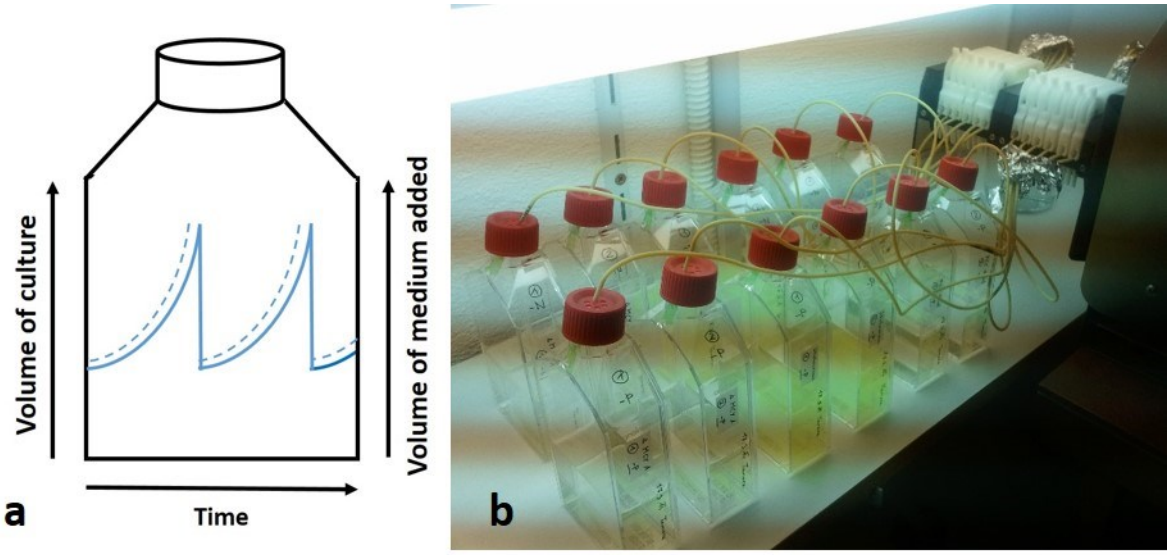

Fig. 2.2: Exponential Fed Batch. a: Scheme of an EFB with a continuous medium supply proportional to the culture volume, sampling sets the volume back to the initial state (modified after Fischer *et al.* 2014); b: Set up of the EFB, 12 culture flasks connected to a peristaltic pump which provided the medium.

EFB 1 with *Microcystis* MCY A- & PCC 7806 and *Stichococcus*:

Of each species *Microcystis aeruginosa* strain MCY A-, *Microcystis aeruginosa* strain PCC 7806 and *Stichococcus* replicates were taken and set up in culture flasks with 50 ml of Medium and 1 ml of culture each (two flasks with N-limited and two flasks with P-limited medium per species). The experiment lasted for 37 days.

A problem occurred in the early stage (after 11 days) of the experiment as both *Microcystis aeruginosa* strains MCY A- and PCC 7806 didn't seem to grow. The culture flasks stayed

completely clear and samples under the microscope didn't show any contaminations with Flagellates but only few cells of *Microcystis aeruginosa*. It could be possible that the cultures didn't start to grow properly due to a stronger lag-phase of the cells and the permanent dilution. Therefore the cultures of *Microcystis aeruginosa* in both N- and P- limited flasks (8 cultures) were exchanged with new culture flasks and a higher concentration of the culture (2ml of each *Microcystis aeruginosa* strain on 50ml Medium). Also the injection needles and caps were changed to new ones.

After 34 days bacteria were found in both medium supply bottles (N- and P-limited WC-Medium) which looked like white flakes. Both bottles were changed and the experiment was kept on. Within the culture flasks of strain MCY A- some little flagellates were found but they seemed to stay at the bacteria flakes, it is possible that they were also already in the original culture of the strain.

EFB 2 with *Synechococcus elongatus*, *Monoraphidium sp.* and *Scenedesmus sp.*:

Of each species *Synechococcus elongatus*, *Monoraphidium sp.* and *Scenedesmus sp.* replicates were taken and set up in culture flasks with 50ml of Medium and 1ml from the *Synechococcus* stock culture, and 0,5ml from the stock cultures of *Monoraphidium* and *Scenedesmus* (two flasks with N-limited and two flasks with P-limited medium per species). The experiment lasted for 29 days.

EFB 3 with *Chlamydomonas sp.*:

From the species *Chlamydomonas sp.* triplicates were taken and set up in culture flasks with 50ml of Medium (WC –N limited Redfield and WC –P limited Redfield Medium) and 1ml from the stock culture (three flasks with N-limited (N1, N2, N3) and three flasks with P-limited medium (P1, P2, P3), dilution 1:50). The experiment lasted for 23 days.

One day after the start of the experiment it was noticed that there was no medium pumped into culture flasks from the supply bottle with the WC-N Redfield limited medium. Therefore the tube of the culture flask N1 was clamped shut and the other two flasks N2 and N3 were manually refilled. The next day the problem was found: the tube of the culture flask N1 was damaged and air was pumped. Therefore no medium could be pumped anymore into the flasks. The tube was repaired and the clamp removed. At the sampling on the 6th day the flask

N1 was sampled first before refilling the missing volume from the time it was separated from the supply bottle due to the ripped tube, it should have been refilled first to achieve a comparable dilution to the other flasks. Afterwards the flasks were sampled all 3 days.

2.3.3 Exponential Fed Batch – Pairing of species

In this experiment two species were incubated together at an equal ratio of biomass to study their competitiveness under N vs P limitation. Three species *Monoraphidium sp.*, *Synechococcus elongatus* and *Scenedesmus sp.* were chosen for that experiment because they exhibited the lowest R* for N and P in the single-species experiments (R*, see Results 3.1.2. 'Monoculture experiments (EFB) to determine R*').

The following two pairings were chosen: *Monoraphidium sp.* with *Synechococcus elongatus* and *Monoraphidium sp.* with *Scenedesmus sp.*. They were incubated in triplicates with N- and P-limited WC medium in 250ml culture flasks in an Exponential Fed Batch set-up as described above in the chapter 2.3.2. 'Exponential Fed Batch' for 35 days at a dilution factor of 0,2.

First the stock cultures were measured with the Photometer (Spectroquant pharo 300, Merck, Software 1.40) at 700nm to determine their turbidity to calculate the amounts of stocks needed to get an equal ratio of biomass from both species (ratio of 1:1) and a dilution of 1:100 from stock, then an inoculum of both species was prepared. Then 12 culture flasks were prepared with 20ml each of Nitrogen and Phosphorus limited medium and the inoculum of species added as triplicates.

During the runtime of the experiment the growth was sampled twice a week and the samples measured with the Fluorometer 'AquaPen' for the relative chlorophyll and phycoerythrin rates, the Photometer (700nm, 10mm cuvette) to track turbidity and cells counted with the Neubauer Improved Chamber and the relative amount to each other calculated. When steady state was reached filtered samples were taken (GF/F-filter) and soluble N and P measured.

It happened a contamination with *Scenedesmus sp.* in the flasks with the pairs of *Monoraphidium sp.* and *Synechococcus elongatus* because it spread via the tubes into all culture flasks.

2.4 Experimental Development of design of the Gradostat

2.4.1 Development of the diffusion holes and turbulence via air-ventilation

The Gradostat is basically an aquarium consisting of three linearly arranged vessels connected with each other via diffusion through holes in the separating walls. Each chamber has an inflow for medium with various nutrient concentrations as well as an outflow. Both inflow and outflow are tubes reaching into each vessel and those tubes are connected with a peristaltic pump controlled by a programmable microcontroller (Arduino UNO). The diffusion between the vessels is driven by turbulence caused by air-ventilation of the vessels.

Medium is supplied to all three vessels at the same rates (Fig.2.3). If nutrient concentration differs among the three vessels, a dynamic equilibrium is reached, resulting from the difference in nutrient concentration in the media and the equilibrating force of diffusion among vessels. They are connected to each other through diffusion to allow nutrients and organisms to diffuse between the vessels. After some time the Gradostat reaches a steady state.

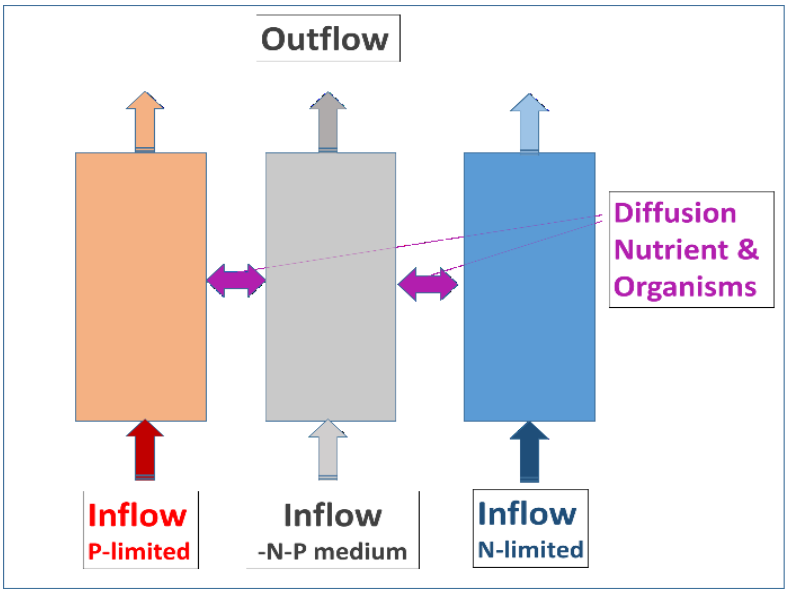

Fig. 2.3: Scheme of a Gradostat with resource distributions. Each vessel functions as a Chemostat and are connected with each other where organisms and nutrients could diffuse between the vessels.

2.4.1.1 Air ventilation

A permanent turbulence was required within each vessel of the Gradostat for keeping the algae in suspense and to assure homogenous distribution of resources within each vessels. This task was solved by pumping air into each vessel with an aquarium air-pump (Air pump 100, Eheim, Type: 3701010 100L/h; 3,5Watt; 230V; 50Hz; 2 H_{max} mbar) to reach a steady circulation in the vessels without causing too many turbulences at the same time. Initial experiments showed that the turbulence created by air-ventilation had a bigger influence on the diffusion-rates than the diameter of the diffusion-holes themselves. In order to regulate the intensity of bubbling as precise as possible, bubble counters (CO2-Bubble counter, Pro Flora Count, SBL GmbH) and larger buffer-bottles were added to the set up (Fig.2.4) to be able to track the continuity and strength of the air-ventilation within the Gradostat. The Buffer-bottle was used to ensure a more constant air-pressure for the bubble counters as it was feared that the air pump couldn't be reliable enough for keeping up a consistent air-flow.

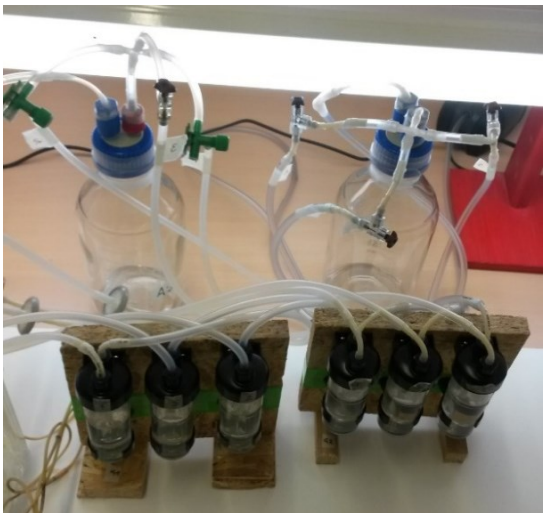

Fig. 2.4: Set-up for air-ventilation. Bottles for air-reservoir and the two sets of bubble counters connected with two aquarist air-pumps.

The glass tubes and tips could be autoclaved for sterilization and connected to an air-filter set before the bubble-counters. The glass tube with the tip was placed close to the edge of the short side of the vessel close to the bottom where the air-bubbles could dwell up and cause a steady circulation within the vessel (Fig.2.5).

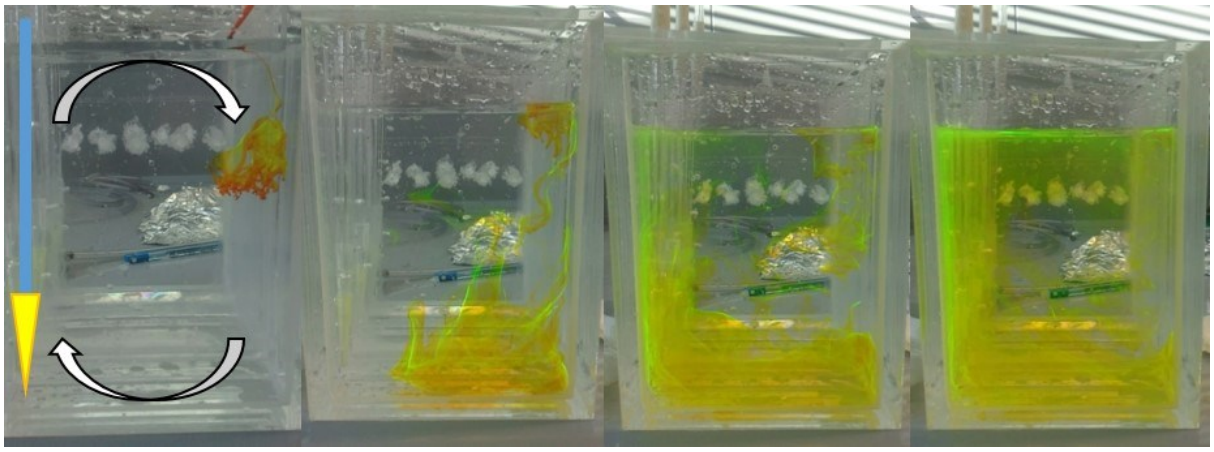

Fig. 2.5: Turbulence within the Gradostat. Circulation of the water column within one vessel driven by air ventilation visualised with the Fluorophor Uranin in 4 time-shots. The glass tube with the tip is visualised on the left side.

The photograph (Fig.2.6) shows the actual set-up of the Gradostat experiment. It was run as duplicates where both Gradostats were supplied with the same medium bottles and one peristaltic-pump. Each Gradostat had an own air-pump, a buffer-bottle to enhance the continuity of the air-pressure and three bubble-counters which were connected with each vessel of the Gradostat (see also Fig.2.7).

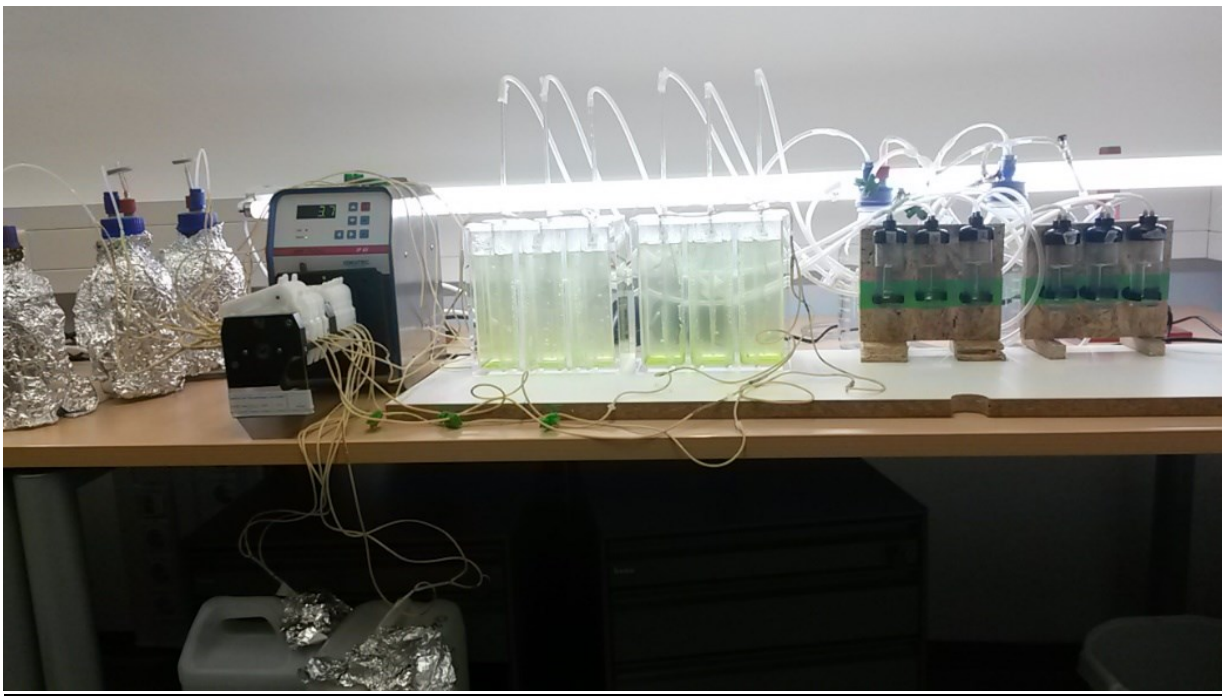

Fig. 2.6: Picture of the final Gradostat set-up. From left to right: 3 bottles with the media, peristaltic pump, two Gradostats, 2 sets of bubble counters for the air ventilation. Under the pump are located the waste-bottles for the outflow medium.

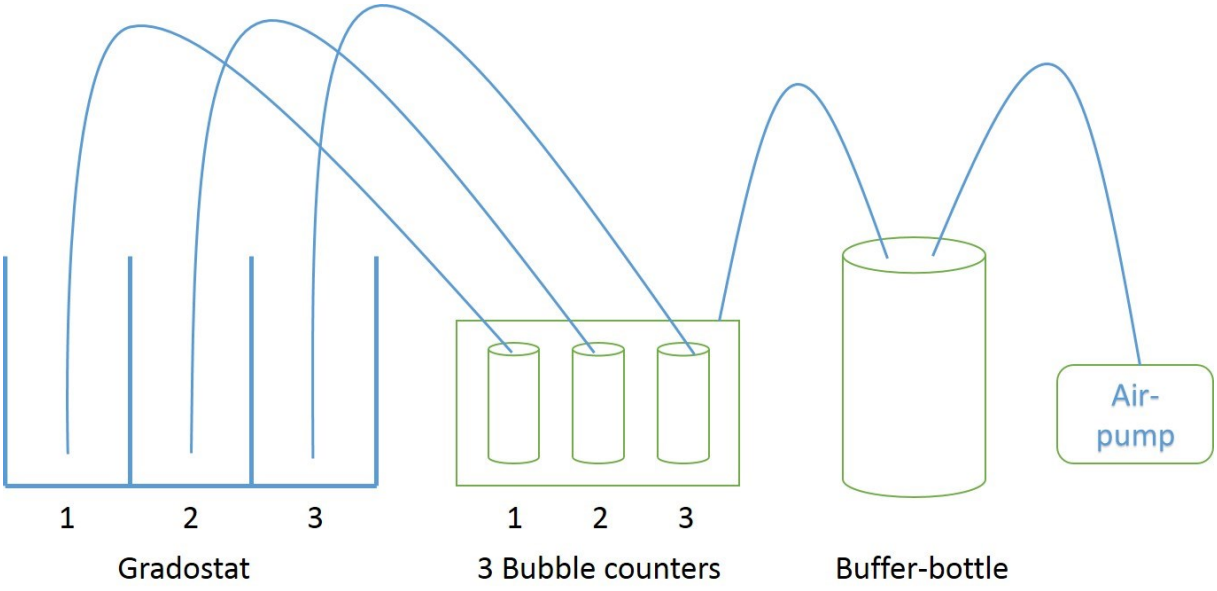

Fig. 2.7: Scheme of the air ventilation of the Gradostat. It contains (from right to left) air-pump, buffer-bottle, 3 bubble counters and the Gradostat.

2.4.1.2 Development of the diffusion-holes

The diffusion holes are necessary to allow a flow of nutrients and organisms between the vessels. The challenge was how large it needed to be and where to locate it to get a diffusion-rate between 0,1 and 0,4. Many tests with different hole-sizes and strengths of air-ventilations for the turbulence were made before it was settled to 5mm of diameter and an air-bubble rate of 160 bubbles per minute which were controlled via the bubble-counters for each chamber. It also needed to be made sure that the vessels were sealed against each other otherwise an uncontrolled flow would have happened.

For testing the diffusion-rates the fluorescence dye Uranin (Excitation/Emission: 490/515nm) was used and its spread over the whole Gradostat monitored over several days for each setting. The Gradostat was filled with tap water, the air-ventilation with the lid installed and being let run for a while with the circulating air-ventilation and then the bubbles adjusted with the bubble-counters. 30µl of concentrated Uranin-solution was always pipetted into the middle vessel and the diffusion between the vessels was daily monitored. For the measurements of the fluorescence the ‘AquaPen’ was used with the wavelength of 450nm.

The diffusion-rate per hour was calculated out of the measurements and multiplied with 24 to get the rate of diffusion per day. Following hole-sizes were tested: 3mm, 2mm, 2,5mm, 3,5mm, 4mm and 5mm.

For a better understanding of how much the diffusion was affected by the turbulence within the vessels six experiments were performed with various bubble counts per minute and the diffusion hole size of 5mm: from these four experiments were done at 160 bubbles per minute, one experiment with 210 and one with 110 bubbles per minute. Four experiments at 160 bubbles per minute were done because 160 bubbles showed to be an adequate count, since 210 bubbles were very hard to count and 110 bubbles seemed to cause a rather weak turbulence which seemed insufficient for homogenous distribution of suspended particles like algal cells. The Gradostat was set-up with the bubble counters and Uranin added to the middle vessel, the fluorescence monitored with the Fluorometer 'AquaPen' over several days and the diffusion coefficient calculated out of the standardized fluorescence values of each vessel over the time period of the experiments (4-5 days of runtime).

A problem of these first set ups was the fact that sampling volume from one vessel caused a unidir3ectional flow from adjacent vessels, which was unavoidable since all three vessels couldn't be sampled at the exact same time. For the subsequent experiments with the Gradostat a better solution was found for this problem and sampling was performed with the help of the peristaltic pump and a script for the Arduino which made it possible to sample two Gradostats (therefore 6 chambers) at the same time with the same amount of volume.

2.4.2 Quantification of resource distribution within the Gradostat

In order to assess the gradient in resource distribution emerging when providing resources at the two outer vessels, I performed tracer experiments, involving salt and Uranine. The results of these experiments were compared to a mathematical simulation, provided by A Ryabov (Univ Oldenburg).

For the two resources Nitrogen and Phosphorus medium labeled with Uranin (fluorescence dye) and NaCL were provided to the two outer vessels of the Gradostat and the resulting diffusion was monitored over 10 days. Resource 1 (Uranin, 35,3 µL in 1L A.dest.) was

distributed to vessel 1 (left vessel), resource 2 (Salt, 10 g/L A.dest.) was distributed to vessel 3 (right vessel). The set-up started with A.dest. in all vessels permanently providing the resources to implement a dilution-rate of 0.2^{-day}. The simulation of the dispersal of resources and the calculation of dilution and diffusion-coefficient were performed computationally with an R-script (A. Ryabov. unpublished) and GNU-Octave (Eaton *et al.* 2011) with Odepkg (Treichel & Corno. 2013). Uranin was measured with the Fluorometer 'AquaPen' and the salinity was measured via the conductivity (in µS) of the sample. Every day 20 ml were sampled from each chamber with the help of the sampling program for the peristaltic pump and afterwards refilled to original volume via the exponential fed batch program while clamping shut the outflow tubes.

On the 4^{th} day of the experimental runtime a problem happened with the tubes which didn't seem to have a big effect on the experiment especially when comparing the resource distributions of day 4 and 5 no major differences could be seen. It happened that the Gradostat lost volume and the waterline sank just 1cm above the diffusion-holes but still leaving them underwater. The tubes for the outflow were not properly attached while the volume after sampling was refilled via a program for the exponential fed batch to reach the original volume of 850ml per chamber. They were still immersed within the Gradostat which led to a loss of volume due to gravity. Immediately after the problem was found medium was added to the Gradostat via the speed-run function of the peristaltic pump to bring the diffusion holes 1cm deeper under the waterline to minimize a possible quicker resource exchange between the chambers when the diffusion holes would be closer to the circulative turbulence in the water column. After that the program for the EFB-batch was reestablished for reaching the original volume-level. On the next day no samples were taken but the conductivity sensor directly dunked into the Gradostat for measurement. On the following days normal sampling was performed again for the measurements.

2.5 Experiments with the Gradostat

This chapter describes the preparations for the experiments performed with the Gradostat, the species pairings of the phytoplankton species as chosen in the previous experiments. Three experiments were done with two Gradostats run as duplicates and varying species and nutrient parameters. The first experiment 'Invasion' contained the community with the species *Monoraphidium sp.* and *Synechococcus elongates*. This setup was invaded by *Chlamydomonas sp.* It was run without a nutrient gradient therefore all vessels contained the same nutrient ratio and were supplied with WC-Redfield medium therefore it had a homogenous resource distribution. The second experiment 'Limitation' included a nutrient gradient over the three chambers of the Gradostats where the left vessel was supplied with phosphor limited medium, the middle one with medium without phosphor and nitrogen and the right vessel with nitrogen limited medium which led to a heterogeneous resource distribution. The community contained the species *Monoraphidium sp.* and *Chlamydomonas sp.* The third experiment was the control set-up to the second experiment and had no gradient over the three vessels as they were all supplied with WC-Redfield medium with a balanced N and P ratio again providing a homogenous resource distribution. The species paring was the same as in the second experiment with *Monoraphidium sp.* and *Chlamydomonas sp.*.

Overview of the three experiments with the main specifications:

1 **Invasion under homogenous resource distribution:**

Species: *Monoraphidium sp.*, *Synechococcus elongatus*, invaded by *Chlamydomonas sp.*

Limitation: WC-Redfield medium in all three vessels

2 **Limitation under heterogeneous resource distribution:**

Species: *Monoraphidium sp.*, *Chlamydomonas sp.*

Limitation: left vessel: WC-Redfield-P-Limitation, middle vessel: medium without N & P, right vessel: WC-Redfield-N-Limitation

3 **Control under homogenous resource distribution:**

Species: *Monoraphidium sp.*, *Chlamydomonas sp.*

Limitation: WC-Redfield Medium in all three vessels

2.5.1 General Preparations

The main vessel of the Gradostat could not be autoclaved. For sterilization the Gradostat and its components were submersed in an acid-bath for several days, while the tubes for the medium, and the GF/F-filter holders for the air-ventilation all were autoclaved. Afterwards the Gradostat was rinsed with A.dest. and put into a drying closet at 60°C until dry. Then the Gradostat was filled with the medium without N and P at the clean-bench and for the heterogeneous resource distribution N&P were extra added into each vessel after the set-up was installed on its final place to avoid mixing due to movement of the basin. For the control setup or the homogenous resource experiment the Gradostat was filled with the medium + nutrients (WC-Redfield medium) at the clean bench since all three vessels contained the same medium and then moved to the set-up, every vessel had 800 ml of start-volume (therefore a total of 2100 ml of medium in the whole Gradostat). At the set-up the Gradostat was attached to the air-ventilation and the bubble counters were set to 160 bubbles per minute, regularly controlled and readjusted if necessary over the course of the experiment.

For inoculation with two species, comparable densities were reached by diluting the stock cultures to comparable turbidities (photometric measurements). Furthermore, the effective density was app. 1/50 compared to the stock cultures, ensuring that both algae could reach exponential growth after inoculation. Then the stock cultures were measured for their density at 700nm in 10mm cuvettes and the amount calculated needed to reach the photometric value of 1 ('needed amount of stock culture' : 1 = 16 : 'measured photometer-value'). Out of those calculations an inoculum for the Gradostat of both species mixed together was prepared and the required amount per vessel was pipetted directly into each vessel of the Gradostat after the set-up was installed and in operation with peristaltic pump (Arduino script for the Gradostat in Supplements S2.2.2.) and air-ventilation. Two Gradostats were operated and monitored as duplicates in every experiment.

<u>Sampling:</u>

Sampling a Gradostat without disturbing the steady state posed a challenge. Sampling one vessel would have resulted in flow from the other vessels, effectively disturbing the diffusion

rates. Therefore the peristaltic pump and extra tubes were employed to be able to sample all three vessels simultaneously, therefore minimizing any unwanted suctions and distortions to the interconnected system. During the ongoing experiment the Gradostat was regularly sampled and 21 ml taken from each vessel via a "sampling program" (script in Supplements S2.2.3) for the Arduino and sampled via autoclaved tubes placed in each vessel. The peristaltic pump pumped from each vessel the same amount of sample. This sampling method had the advantage that each vessel could be sampled simultaneously without risking an artificial diffusion due to the gravitational impact of a lower volume in one vessel since all vessels were connected through the diffusion holes. The Gradostat was sampled twice a week and the samples measured for the relative amount of chlorophyll with the Fluorometer 'AquaPen' to measure the growth rates until steady state and some of the sample was fixed with Lugol for cell-counting. The rest of the sample was frozen for total phosphor and nitrogen measurements. After sampling the volume was refilled with an adapted script following Exponentially Fed Batch dilution (Fischer et al. 2014) with the outflow tubes clamped shut.

At the end of the experiment when the Gradostat reached a steady state it was sampled every two days for three times with 53 ml of sample per vessel. The samples were measured with the 'AquaPen', some amount of the sample was fixed with Lugol for cell-counting and biovolume-calculation, 20 ml of the sample was frozen for total N & P measurements and three times 10 ml were filtered with GF/F filters for PRP and Chlorophyll measurements. Twice 10 ml was frozen each for soluble N & P measurements.

2.5.2 Experiment 1: Invasion under homogenous resource distribution

In this experiment every vessel was filled with medium with the same nutrient-ratio (WC-Redfield medium without $NaSiO_3$, due to a flocculation in the stock) and the species growth in each vessel was monitored. The experiment lasted for 19 days.

Two species *Monoraphidium sp.* and *Synechococcus elongatus* were used in the inoculum of a biomass ratio 1:1 calculated out of the turbidity measurement with the Photometer at 700 nm in a 10 mm cuvette and the same amount of inoculum added to every vessel of the Gradostat duplicates.

16 ml of *Monoraphidium sp.* and 28,1 ml of *Synechococcus elongatus* was needed to reach a dilution of the rate for inoculum to medium of 1:50 in a vessel with 800 ml as starting volume (44,1 ml of algae inoculum + 755,9 ml of medium per vessel).

After 8 days of the running experiment a problem occurred with one 2 liter medium supply bottle and the tube. As a consequence it wasn't pumped enough medium into the Gradostats whereas the filling level of both Gradostats lowered just above the diffusion holes. All outflow tubes were clamped shut to refill the Gradostat and no samples were taken. The affected vessel was the right vessel in both Gradostats. Moreover a contamination happened with *Chlamydomonas sp.* where both stock cultures of *Monoraphidium* and *Synechococcus elongatus* were affected.

2.5.3 Experiment 2: Limitation under heterogeneous resource distribution

Since *Chlamydomonas* was found in both stock cultures of *Synechococcus elongatus* and *Monoraphidium* as a contamination (very low density in the *Monoraphidium* stock and higher density in the *Synechococcus* stock) it was decided to use the species pair of *Chlamydomonas* and *Monoraphidium* as model community for this experiment, as contamination with *Chlamydomonas* seemed unavoidable. The right vessel contained a strong limitation of Phosphor (WC-Redfield-P-Limitation), the middle vessel had no nutrients (WC-Medium without N & P) and the left vessel contained a strong limitation of Nitrogen (WC-Redfield-N-Limitation).

An inoculum of both species with a biomass ratio 1:1 calculated out of the turbidity measurement with the Photometer at 700 nm in a 10 mm cuvette was prepared similar to exp. 1 and the same amount added in every vessel filled with medium of the Gradostat duplicates to reach a dilution rate of 1:50 for inoculum to medium. 16,9 ml of *Chlamydomonas sp.* and 6,6 ml of *Monoraphidium sp.* stock cultures were needed to reach a dilution of 1:50 in one vessel with 800 ml of total volume (23,5 ml algae and 776,5 ml medium).

After 4 days the second Gradostat G2 showed a higher filling level as G1, the inflow tubes for G2 were clamped shut and the outflow tubes were clamped with a higher pressure to the

pump. Before sampling the water line of G2 was brought back to the same level as G1 by running the pump on a faster speed manually.

On the 18th day it happened that both Gradostats were nearly run over due to a problem with the outflow tubes but the content of each vessel didn't brim over into the other vessel. Therefore the experiment was terminated.

2.5.4 Experiment 3: Control – homogenous resource distribution

This experiment was the control set-up for experiment 2 with a homogenous distribution with WC-Redfield medium in all vessels. The species used for the model community were *Monoraphidium sp.* and *Chlamydomonas sp.*.

An inoculum of both species with a biomass ratio 1:1 calculated out of the turbidity measurement with the Photometer at 700 nm in a 10 mm cuvette was prepared and the same amount added in every vessel (800ml) of the Gradostat duplicates to reach a dilution rate for inoculum to medium of 1:50.

30,361 ml of *Chlamydomonas sp.* and 23,7 ml of *Monoraphidium sp.* stock cultures were needed to reach a dilution rate of 1:50 in one vessel with 800 ml of total volume (54,1 ml algae and 745,93 ml medium). The experiment lasted for 30 days.

After putting the set-up into operation it occurred, that two of the tips of the air-ventilation system where the air was going out into the Gradostat didn't work correctly although they were previously tested. They didn't let pass the bubbles properly causing a too weak turbulence. Therefore the attached bubble counters needed to be adjusted to a stronger level to compensate it. Following tips were affected: middle vessel in Gradostat 1, the bubbles were pumped out weaker but continuously, right vessel in Gradostat 2, it only released bubbles in bursts.

After 15 days G1 had a lower filling level as G2, probably because one clamp on the pump got loose for one of the inflow tubes (Gradostat 1, vessel 1) leading to a lower pumping pressure on it one day before. The clamp was reattached and at the next day after sampling both Gradostats were put back to the same filling line by pumping off volume from G2, afterwards

the exponential fed batch program was used to redistribute the missing volume simultaneously into both Gradostats.

The night before the 25th day the Exponential Fed Batch program was run again with outflow tubes clamped shut to redistribute the volume missing from sampling the days before. In the morning the program for keeping a stable volume in the Gradostat was reintroduced but its duration time was only set to 5 hours. Therefore the pump stood still for the next two days (weekend) and no medium was pumped in and out of the Gradostat.

On the 28th day the air-ventilation tip of the left vessel in Gradostat 2 made problems by letting no air passing through. The bubble counter needed to be turned on to a strong pressure to free the tip and letting bubbles pass. Therefore in Gradostat 2 two tips (left and right vessel) weren't exactly adjustable, whereas the vessel in the middle was adjusted to 160 bubbles per minute.

At the end of the experiment small flagellates with a size similar to *Monoraphidium sp.* were found in both Gradostats, the visual impression was that G1 contained more Flagellates than G2.

3 Results

3.1 Screening for suitable phytoplankton species (R*)

3.1.1 Maximum Growth Rates

In order to assess maximum growth rates, the cyanobacteria *Microcystis aeruginosa* strain ΔMCY A- and PCC 7806, *Synechococcus sp.* and the green-algae *Stichococcus sp.*, *Monoraphidium sp.* and *Scenedesmus sp.* were all grown as monocultures in batch cultures. In order to keep algae at exponential growth, culture volume was diluted once a certain biomass concentration was reached, which happened twice during the course of the experiment. Therefore three growth peaks can be observed which are represented here as linear increase in the log-transformed graphs (Fig. 3.1) and the growth rates of the duplicates were similar. The μ_{max}-values were calculated from the most linear slope from the log-transformed data which represents the steepest growth (see supplements Tab.S3.1 & Tab.S3.2). Both species *Monoraphidium sp.* and *Scenedesmus sp.* showed similarly high growth rates with μ_{max} is 1,2 for *Monoraphidium sp.* and 1,1 for *Scenedesmus sp.* followed by *Stichococcus sp.* (μ_{max} = 0,6) and *Synechococcus elongatus* (μ_{max} = 0,4; Fig.3.2). Interestingly, from the two strains of *Microcystis aeruginosa,* MCY A- reached higher growth rates than PCC 7806 with μ_{max} = 0,3, which only had a very weak growth rate with μ_{max} = 0,1.

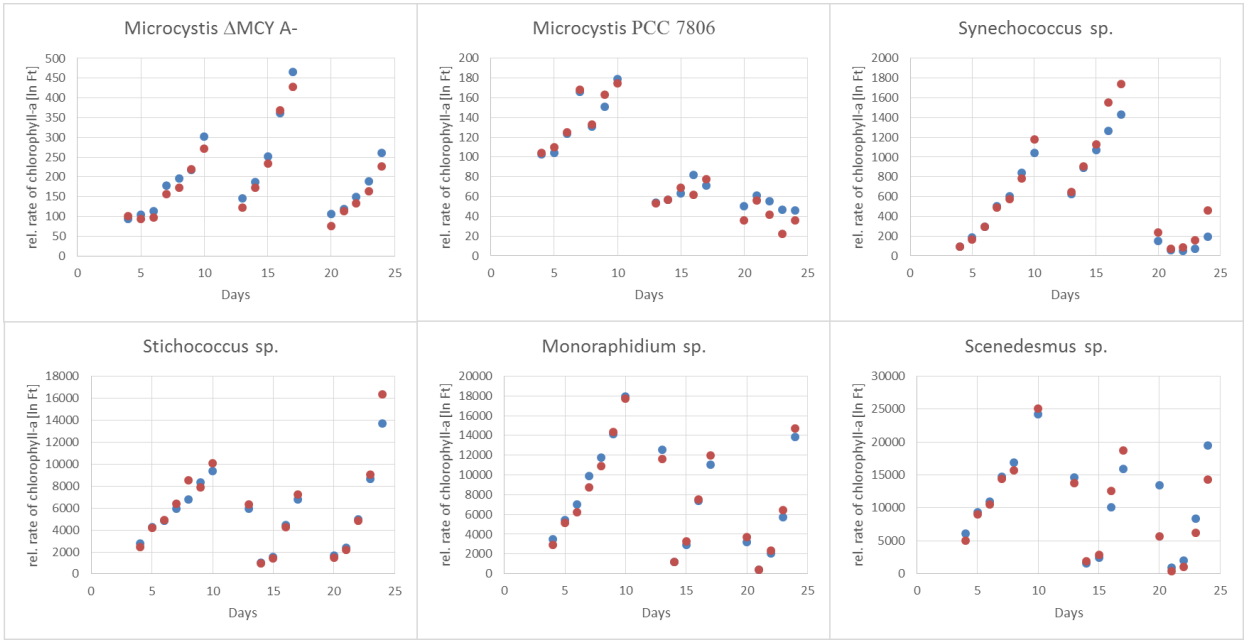

Fig. 3.1: Growth curves of phytoplankton species. Patterns of growth rates of the six phytoplankton species *Microcystis* ΔMCY A- and PCC 7806, *Synechococcus sp.*, *Stichococcus sp.*, *Monoraphidium sp.*, *Scenedesmus sp.* and their duplicates (colored red and blue), logarithm of the relative chlorophyll-a concentration measured as ΔFt (auto fluorescence). The μ_{max} was calculated from the most linear slope of the log-transformed dataset (see Supplements Tab.S3.1).

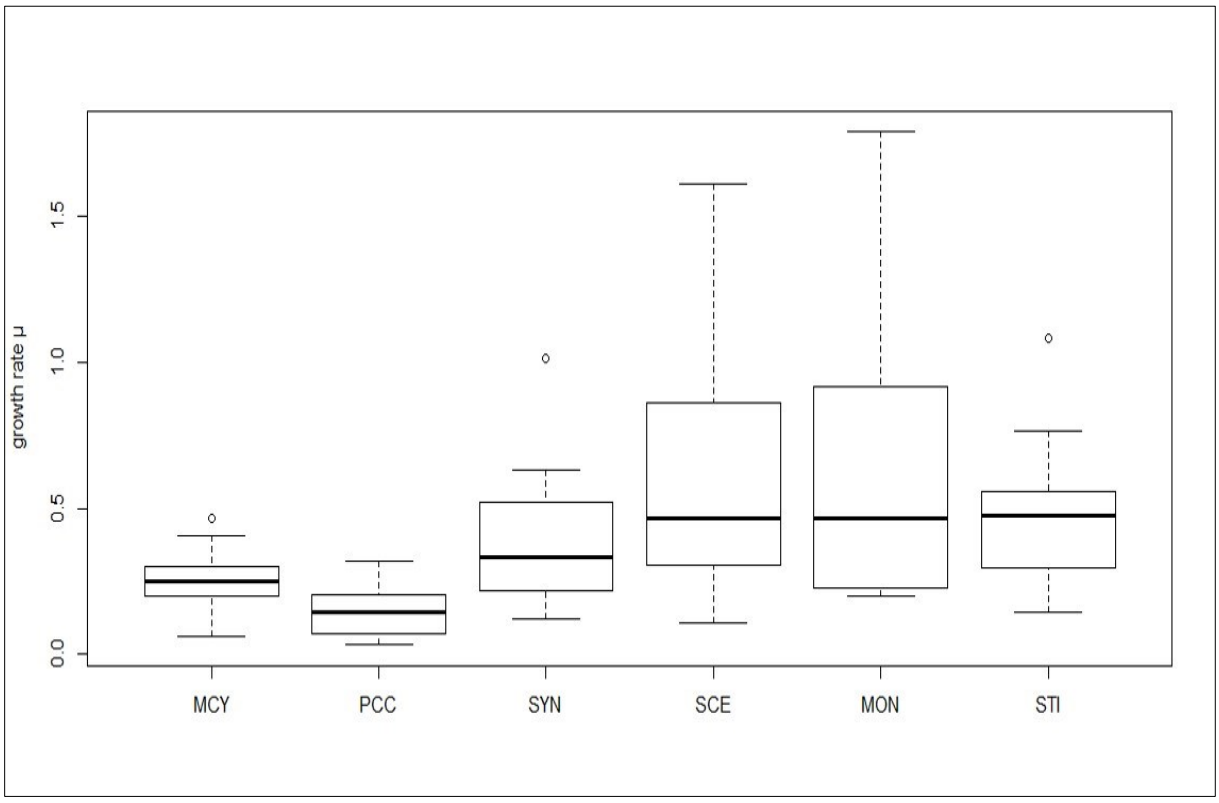

Fig. 3.2: Box-plot of growth rates [μ] from six phytoplankton species. Growth rates [μ] calculated from chlorophyll-a measurements over time, from all six species *Microcystis* MCY A- (MCY), *Microcystis* PCC 7806 (PCC), *Synechococcus elongatus* (SYN), *Scenedesmus sp.* (SCE), *Monoraphidium sp.* (MON) and *Stichococcus sp.* (STI). Values were calculated from the mean of the μ-values of the duplicates over the course of the experiment (see supplements Tab.S3.2). Negative μ values were not considered.

3.1.2 Monoculture experiments (EFB) to determine R*

The growth curves (Fig.3.3) of the seven species show that all species reached a steady state. As it was observed before in the experiment where maximum growth rates were determined, the strain PCC 7806 of *Microcystis aeruginosa* showed decreased growth and even a decline after 30 days which was not the case for the other strain ΔMCY A^-. *Monoraphidium sp.* displayed a visibly increased growth in phosphor limited medium, this was also the case for *Scenedesmus sp.* although the difference was smaller.

Especially the species *Monoraphidium sp.*, *Synechococcus elongatus*, *Scenedesmus sp.* and *Chlamydomonas sp.* had low R* (Fig.3.4) which indicated that they had better advantages for competing and eventually dominating in resource restricted environments. Only the R* of *Monoraphidium sp.* was found above the N:P ratio of 8,97:1, the ratio of the balanced WC-Medium, displayed as a green dotted lineto serve as an orientation in the figure 3.4 and indicates higher abilities to compete under N-limited conditions. The R* of all the other species were found under the N:P ratio which indicated better competitive abilities in P-limited environments. *Microcystis aeruginosa* PCC 7806 showed the highest R* and therefore probably the lowest ability for growing under limited N and P conditions. The data for the N- and P- concentrations for the graphical R*-analysis are found in the supplements (table S3.3). According to the previous results two model communities each comprising of two species (*Monoraphidium*/*Synechococcus* and *Monoraphidium*/*Scenedesmus)* were chosen for subsequent Exponential Fed Batch experiments under limited N & P conditions.

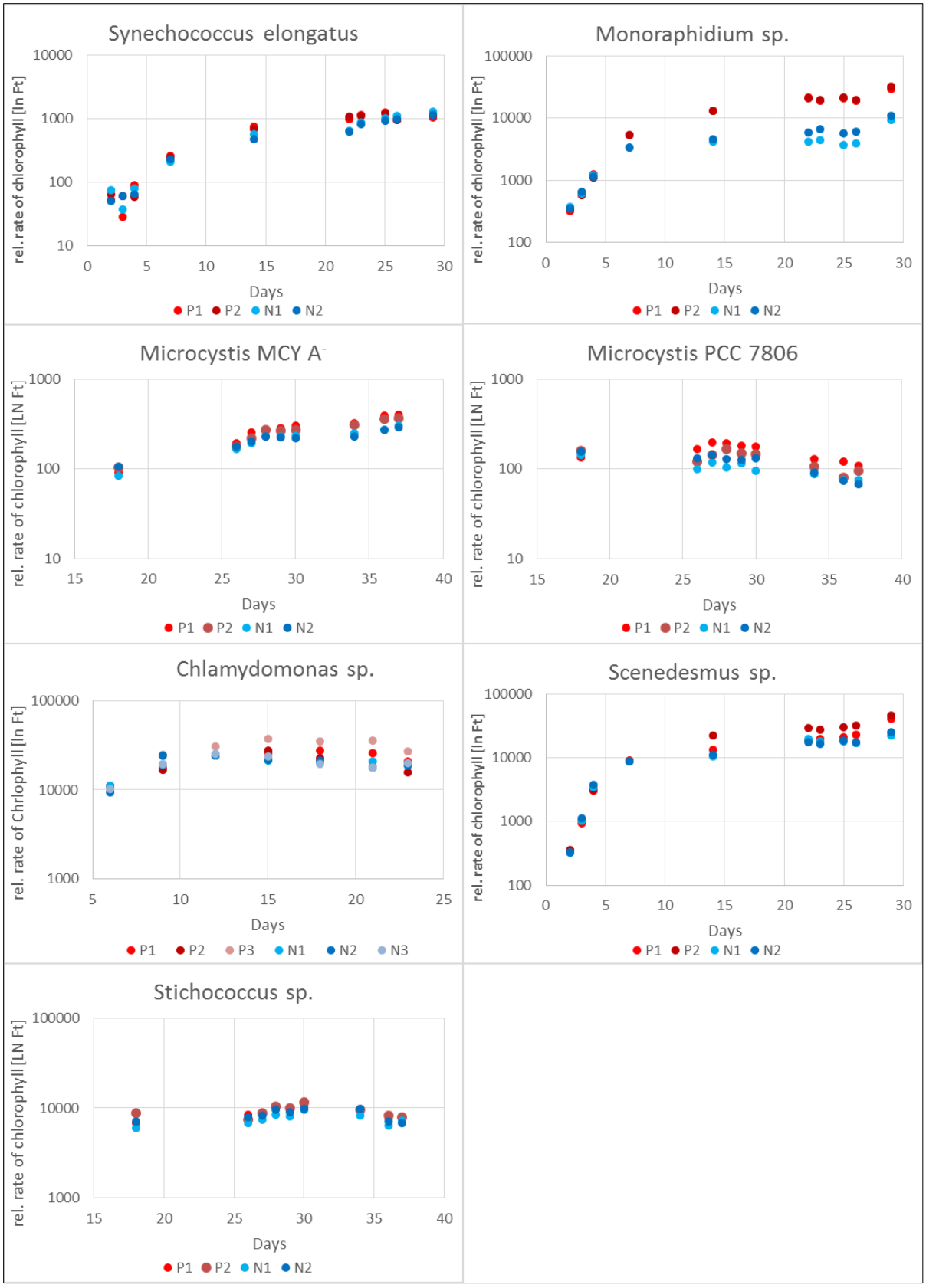

Fig. 3.3: Growth curves of all six phytoplankton species in N- & P-limited medium. *Microcystis* strains MCY A- and PCC 7806, *Stichococcus sp., Synechococcus elongatus*, *Monoraphidium sp.* and *Scenedesmus sp.* in duplicates, *Chlamydomonas sp.* in triplicates for each limitation; P is Phosphorus-limited medium and N is Nitrogen-limited medium; logarithm of the relative rate of chlorophyll [LN Ft] portrayed on y-axis.

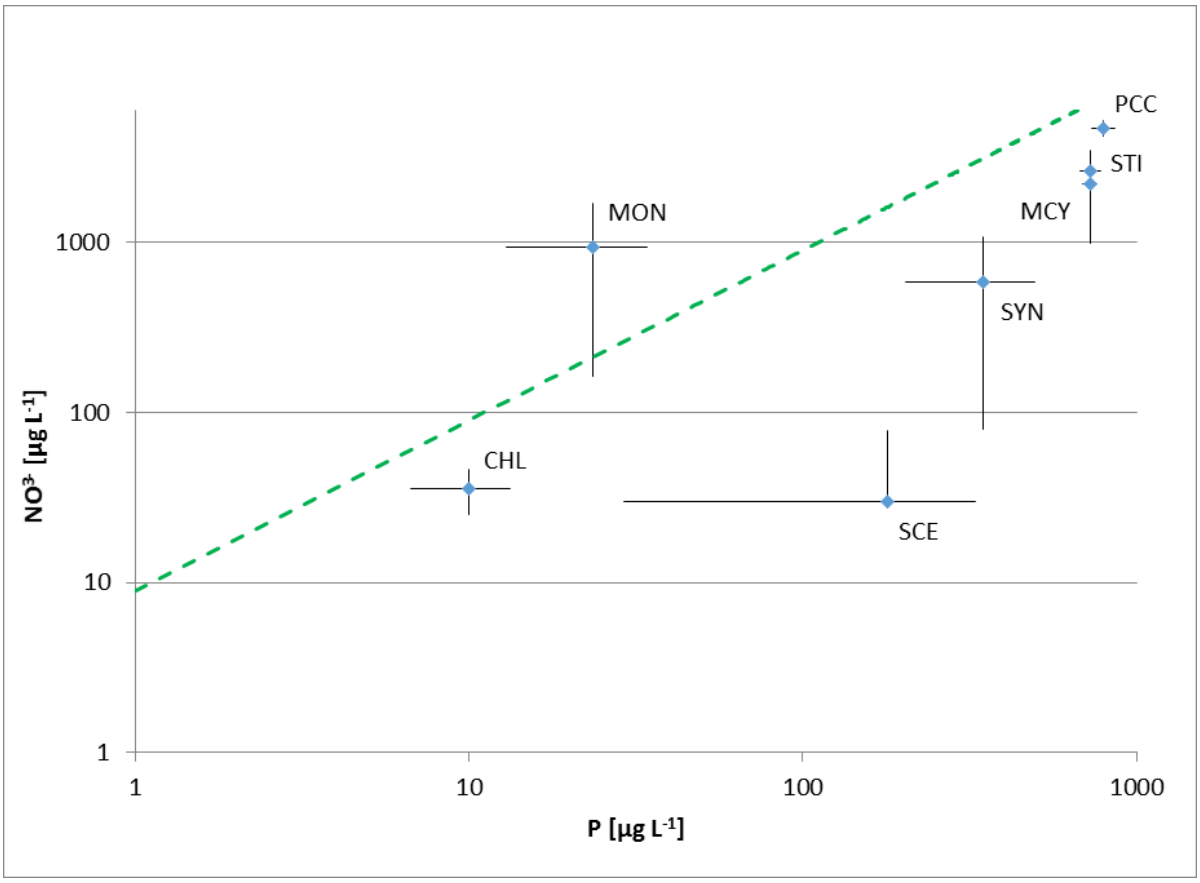

Fig. 3.4: R* of all six phytoplankton species. Species: *Microcystis* strains MCY A- (MCY) and PCC 7806 (PCC), *Stichococcus. sp.* (STI), *Synechococcus elongatus* (SYN), *Monoraphidium sp.* (MON), Chlamydomonas sp. (CHL) and *Scenedesmus sp.* (SCE); R* calculated from the mean of all three time points of N/P measurements from samples taken after EFB reached steady state. The green-dotted line corresponds to the N:P ratio 8,97:1 in the WC medium. Species above that line compete better in N-limitation, species below it compete better in P-limitation. N/P data can be found in the Supplements (S3.2).

3.1.3 Pairwise test of species (EFB) under N/P limitation

Based on the analysis of R* under N and P limitation (Fig.3.4.), *Monoraphidium sp.*, *Synechococcus elongatus* and *Scenedesmus sp.* were chosen as best candidates for the competition experiment (with *Monoraphidium sp.* having higher requirements for Nitrogen and *Synechococcus elongatus* and *Scenedesmus sp.* higher requirements for Phosphorus). In a subsequent pairwise EFB experiment, I tested the performance of the two potential species pairs *Monoraphidium sp.* with *Synechococcus elongatus* and *Monoraphidium sp.* with *Scenedesmus sp.* Within the Exponential Fed Batch set-ups the species were pairwise incubated under N/P limitation where the species pairs were *Monoraphidium sp.* / *Synechococcus elongatus* and *Monoraphidium sp.* / *Scenedesmus sp.*. The final biovolumes

indicated that *Monoraphidium sp.* and *Synechococcus elongatus* would form a more appropriate species community since *Synechococcus elongatus* showed a large increase in growth under P-limitation and *Monoraphidium sp.* was more successful under N-limitation. This pattern was even more obvious during the later stages of the experiment. *Scenedesmus sp.* seemed to be the species with the highest competitive ability; it even managed to grow through the supply tubes into the culture flasks with the *Monoraphidium/Synechococcus* communities inside. *Scenedesmus sp.* obtained a high biomass under both N and P limitation and outgrew *Monoraphidium sp.*; especially in P-limited Medium was *Scenedesmus sp.* very successful (Fig. 3.5). On the other hand, *Monoraphidium sp.* proved to be much stronger under P-limitation in competition with *Synechococcus elongates,* whereas under N-limitation it reached a similar biovolume as *Synechococcus elongatus*. Therefore *Scenedesmus sp.* was dismissed as second potential species pairing partner, and the Gradostat experiment was set up with the two species community *Monoraphidium sp./Synechococcus elongatus*.

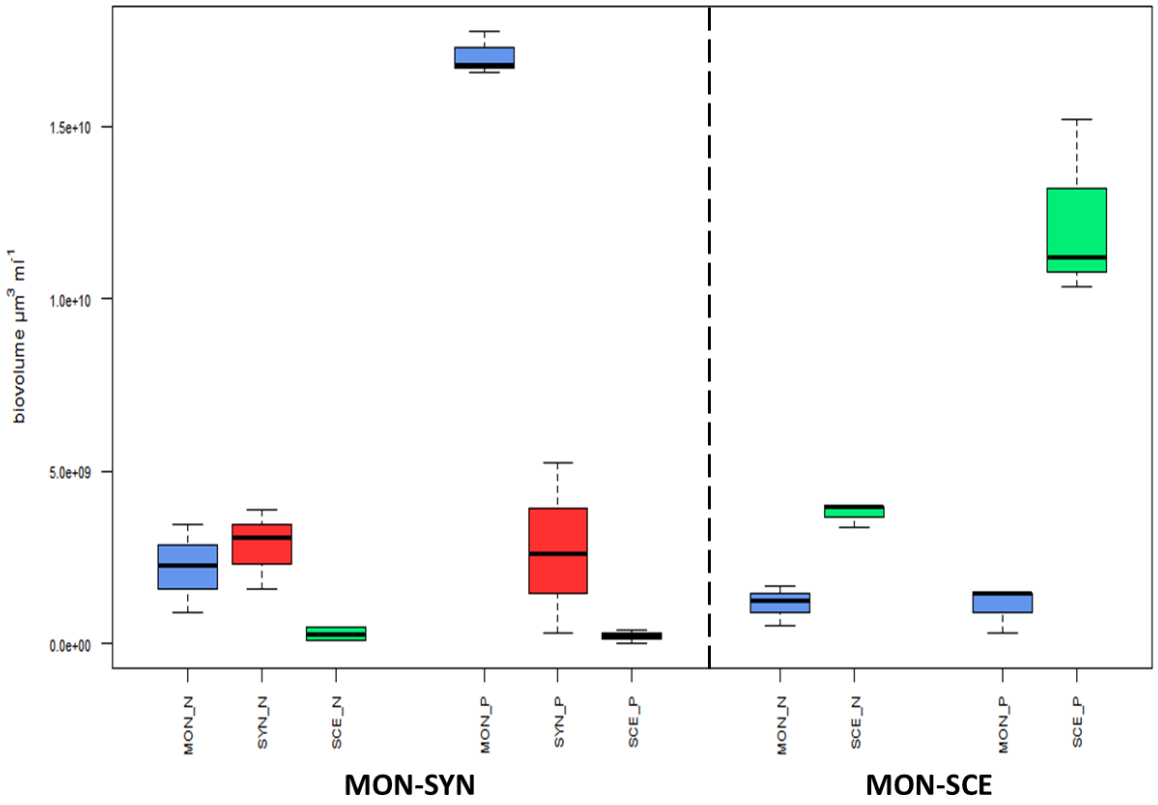

Fig. 3.5: Biovolumes [µm³ ml^{-1}] of the pairwise incubated species in comparison. Biovolumes calculated from the last 3 time points for the species pairs *Monoraphidium sp.* / *Synechococcus elongatus* (MON-SYN; MON in blue, SYN in red) and *Monoraphidium sp./Scenedesmus sp.* (MON-SCE, SCE in green) incubated in either N- or P-limited WC-Redfield Medium: the mean was calculated out of the triplicates.

3.2 Development of the Gradostat

3.2.1 Controlling diffusion through hole diameter and water turbulence

3.2.1.1 Comparison of strong and weak turbulence

Testing different strengths of water turbulence and diameters of the diffusion holes proved that the strength of turbulence had a larger impact on diffusion of resources than size of the diffusion holes. Diffusion rate of Uranin between the vessels of the Gradostat with 3 mm in diameter sized diffusion holes; one experiment performed with strong and another with a weak turbulence. Stronger turbulence caused for a higher diffusion of Uranin among the vessels (Fig. 3.6.).

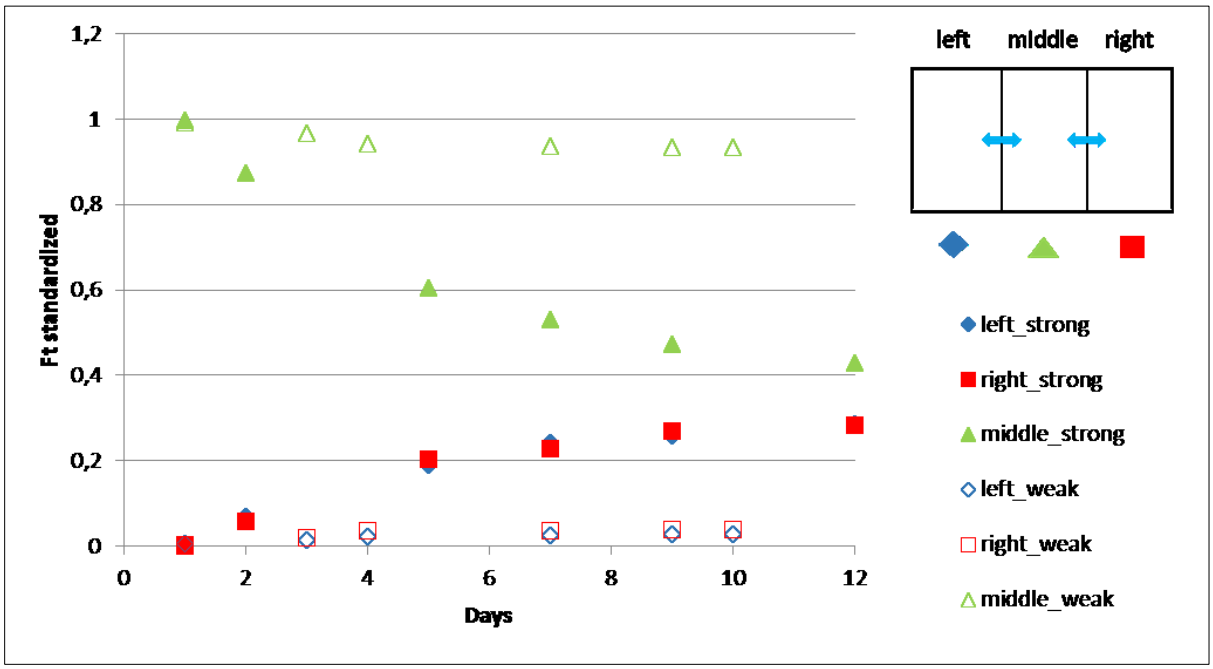

Fig. 3.6: Graph of relationship between turbulence and diffusion. Displayed at the example of strong and weak (~1 bubble/second) turbulence with 3mm diffusion holes; diffusion of Uranin measured in all three vessels (left - rhombus, middle - triangle and right - square). Strong turbulence is marked with closed symbols, weak turbulence with open symbols. In the upper right corner a scheme of the Gradostat is displayed.

3.2.1.2 Relationship between turbulence and diffusion

In Fig. 3.7 the correlation between the water turbulence and the diffusion coefficient can be observed. The diffusion coefficient of the passage between the left and middle vessel had an explained variance of R^2=0,768 and of the middle-right passage a value of R^2=0,928. Those findings indicate that the strength of the water turbulence driven by an increased air ventilation has an impact on the size of the diffusion coefficient and can be used to adjust the diffusion rate (Fig. 3.7).

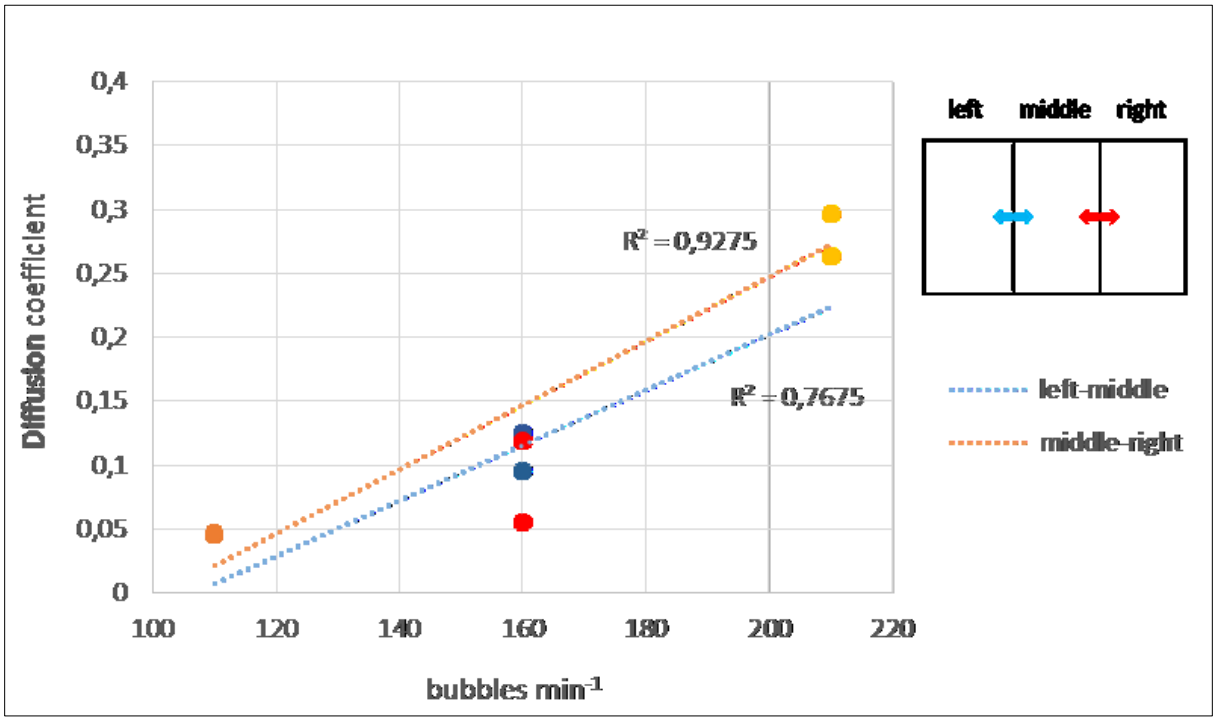

Fig. 3.7: Relationship between turbulence and diffusion with trendlines. The water turbulence was caused by air bubbles [count bubbles min^{-1}]; the diffusion coefficient was calculated for the passage left /middle vessel and the middle/right vessel. The dotted lines are linear trend lines, blue for left-middle and red for middle-right trend line. The pairwise colored data-dots are the two diffusion coefficients left-middle and middle-right from four experiments performed at 110, 160 and 210 bubbles min^{-1}. In the upper right corner a scheme of the Gradostat with the two diffusion holes for the vessels is illustrated.

3.2.2 Quantification of resource distribution within the Gradostat

Salt and uranine were used as tracers to simulate the diffusion of two nutrients in the Gradostat. In this experiment, medium enriched with uranine and salt were supplied to the two outer vessels, while medium without stain was supplied to the central vessel, and concentration of uranine and salt were monitored by Aquapen & conductivity measurements. The distribution of the resources Salt and Uranin reached a steady state with distinctive concentrations of the resources in each vessel creating a stable equilibrium among the connected vessels (Fig.3.8). The left vessel (vessel 1) where the Salt was provided showed the highest salt concentration, the right vessel (vessel 3) the lowest concentration whereas the middle vessel (vessel 2) where only aqua dest. was supplied had a salt concentration in between. The same also accounted to the other direction for the resource Uranin which was provided to the right vessel (vessel 3). The diffusion of the resources across the system corresponded well with a computational simulation (Fig.3.9). After some time a balance of the resources set in steady state was maintained within the vessels. The lower the diffusion rate the higher was the concentration of the resource within the distinct vessels due to a decreased diffusion. Two different diffusion rates were maintained due to a different strength in turbulence: Vessel 1 – Vessel 2: 0,13 (Uranin, max = 0,7), Vessel 2 – Vessel 3: 0,45 (Salt: Max = 0,5).

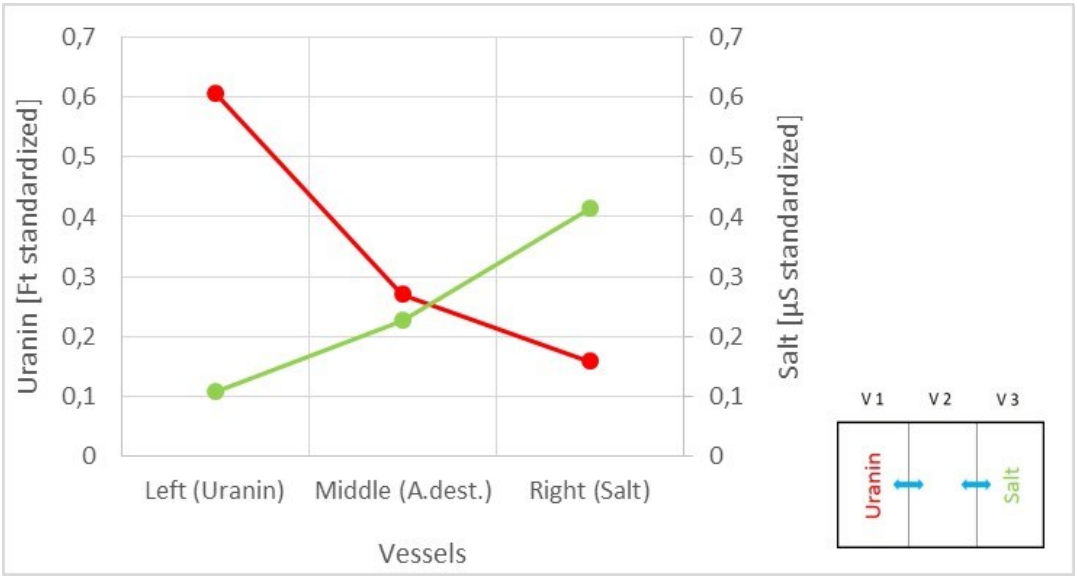

Fig. 3.8: Distribution of resource concentration at steady state. Mean of the measured values of the last 6 days (4^{th} to 9^{th} day); red = Uranin, green = Salt, V = Vessels. It can be observed that the Gradostat reached a steady state, where a consistent resource gradient was established among the connected vessels. In the lower right corner a scheme of the Gradostat with the two diffusion holes for the vessels is illustrated.

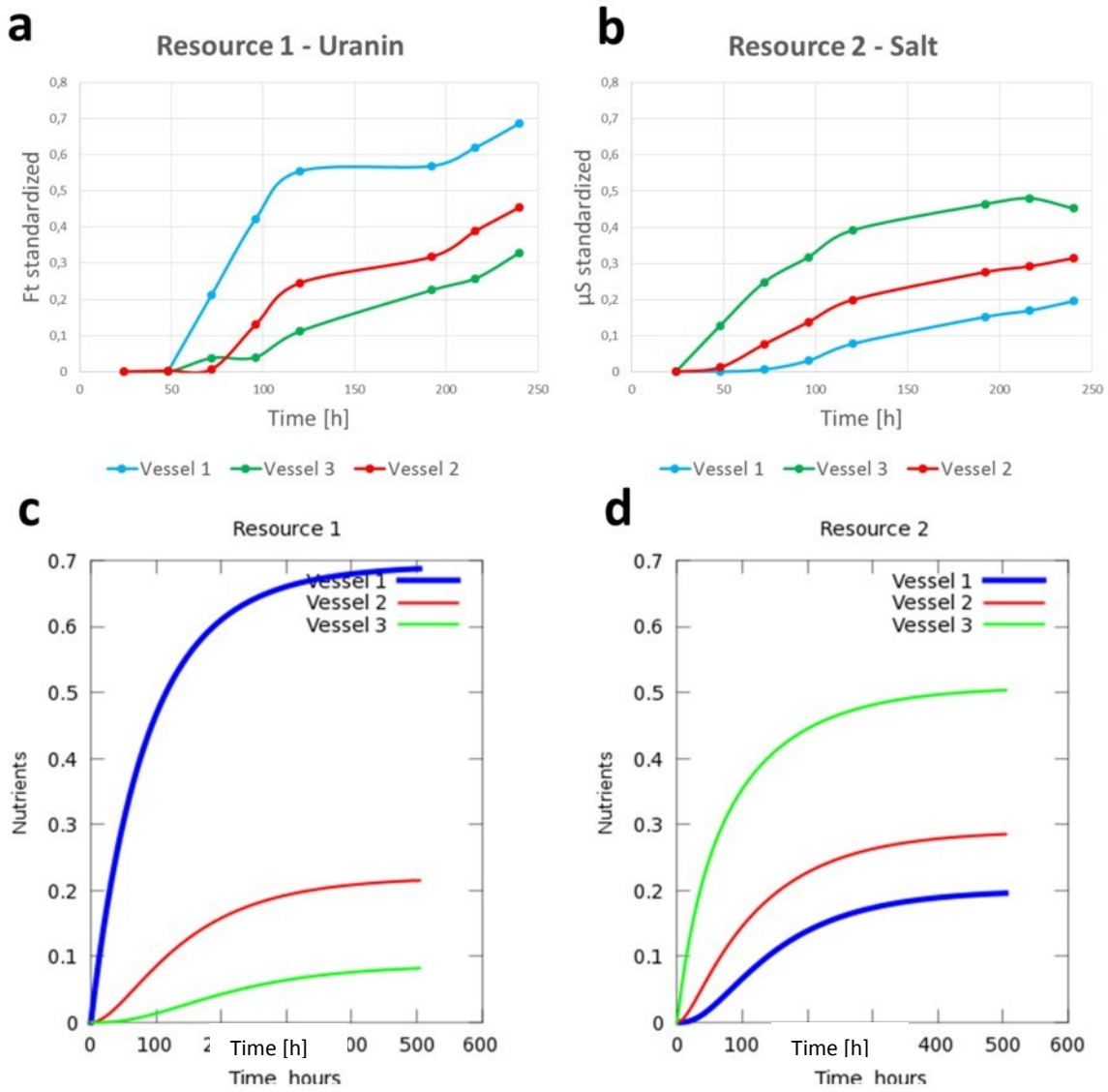

Fig. 3.9: Quantification of resource distribution in the Gradostat: Comparison of experimental with simulated data. a: Distribution of Uranin: 35,3 µL L^{-1} A.dest. in vessel 1, Ft - Proxy of fluorescence; b: Distribution of Salt: NaCl 10 g L^{-1} A.dest. in vessel 3; c: Simulation of Uranin distribution with diffusion rate = 0,13; d: Simulation of Salt distribution with diffusion rate = 0,45; vessel 2 provided with A.dest. Left vessel = vessel 1, middle vessel = vessel 2, right vessel = vessel 3. Time [h] measured in hours.

3.3 Community experiments with the Gradostat

In this chapter the results of three Gradostat experiments with phytoplankton model communities are presented. Each experiment was performed in duplicates (Gradostat 1 (G1) and Gradostat 2 (G2)) and comprised an inoculum of two phytoplankton species as model community. The first experiment 'Invasion under homogenous resource distribution' was performed without a resource gradient and describes the invasion of a highly competitive third phytoplankton species into a two-species community. The second and third experiment were setup with a resource gradient (Experiment 2 – Limitation: heterogeneous resource distribution) and without a gradient (Experiment 3 – Control: homogenous resource distribution) the same two-species community. The results comprise cell counts, biovolumes and resource quantifications in order to follow the distributions and ratio of species and resources among all three vessels of the Gradostat.

3.3.1 Experiment 1 - Invasion under homogenous resource distribution

Here are the results provided for the first experiment with the Gradostat and a model community originally consisting of *Monoraphidium sp.* and *Synechococcus elongatus* and the invasion of *Chlamydomonas sp.*. All vessels were supplied with WC-Redfield medium without limitation and therefore without a resource gradient.

The visual timeline of the development of the Gradostat duplicates (Fig. 3.10) displays the photographed vessels at different stages during the experiment (4 days, 8 days, 11 days and 15 days). After 8 days (Fig.3.10b) it can be observed that the left and middle chamber in Gradostat 1 (G1) are brighter in the green coloration than Gradostat 2 (G2) whereas G2 was generally darker colored than G1. It can only be speculated if the problem with the medium supply in the right chamber of both Gradostats could be the reason. After 15 days (Fig. 3.10d) G1 showed a similar green color in all three chambers. This was expected due to the same nutrient ratio in each chamber. Only G2 seemed to be very bright in the right chambers although the other two were similarly dark colored.

The invasion of the species *Chlamydomonas sp.* was discovered after 11 days of operating the Gradostat and the cell counts of *Chlamydomonas sp.* were stable at a magnitude of 10^2 to 10^3 cells ml^{-1} (Fig.3.11) in comparison to the counts of *Monoraphidium sp.* with ~10^7 cells ml^{-1} and *Synechococcus elongatus* with about 10^8 to 10^9 cells ml^{-1}. The cell counts of *Monoraphidium sp.* declined in growth after the 11^{th} day and even started to decrease which was not the case for *Synechococcus elongatus*. The cell densities were comparable in both Gradostats.

The results of the biovolumes in comparison of both Gradostats (Fig. 3.12) show that *Chlamydomonas sp.* had in overall low biovolumes in all vessels. The biovolume of *Synechococcus elongatus* was reduced in those vessels where the biovolume of *Chlamydomonas sp.* was increased. This could be observed in both Gradostats. In both Gradostats G1 and G2 the lowest biovolume of *Chlamydomonas sp.* were found in the right vessel as well as the highest biovolumes *Synechococcus elongatus*. In G1 the differences for the biomass of *Chlamydomonas sp.* between the vessels were smaller than in G2, where the biovolumes of *Chlamydomonas sp.* peaked stronger in the middle vessel. In G1 the biovolume of *Synechococcus elongatus* was higher than the biovolume of *Monoraphidium sp.*, in G2 the other way around was the case and *Monoraphidium sp.* was slightly increased in all three vessels, especially in the right vessel it peaked strongly. The proportional biovolumes (Tab. S4.1 in Supplements) showing the percentage of the biovolumes in each vessels to the total biovolume in the Gradostat which gives a percentage ratio of the means of the vessels MON:SYN:CHL = 32,556 : 67,439 : 0,005 % in G1 and 61,155 : 38,830 : 0,015 % in G2. The proportional biovolumes of all three taxa were very homogenous in all three vessels in G1. The percentage shift of *Monoraphidium sp.* and *Synechococcus elongatus* in G2 is attributed to the right vessel, where the biovolumes of both were highly increased (right vessel G2: MON = 37,770 %, SYN= 21,880 %, CHL=0,002%; right vessel G1: MON = 11,671 %, SYN = 26,603 %, CHL = 0,001 %; percentage of the total biovolume of the whole Gradostat) due to a low biovolume of *Chlamydomonas sp.*. *Synechococcus elongatus* was strongly decreased at around 8% in the left and middle vessel where *Chlamydomonas sp.* had higher biovolumes.

In Fig. 3.13 the NO_3:TP ratios as mean over the whole runtime of the experiment display that the distribution of NO_3 and TP was similar and homogeneous in all three vessels in both Gradostats G1 and G2 as expected from the set-up as the same resource concentrations were provided in each vessel. The Gradostat G1 showed a lightly higher concentration of NO_3 and

TP as Gradostat G2. Furthermore the NO_3:TP ratios of all vessels of both Gradostats aligned far better with the DIN:TP ratio N:P=3,5:1 depicted as green dotted line (Ptacnik *et al*. 2010) and not with the Redfield ratio 16:1 (Redfield, 1934) shown as red dotted line in the graph. The results of the statistical analyses (Tab. 3.1) showed no significant differences between the resource ratios and the vessels (p = 0,873).

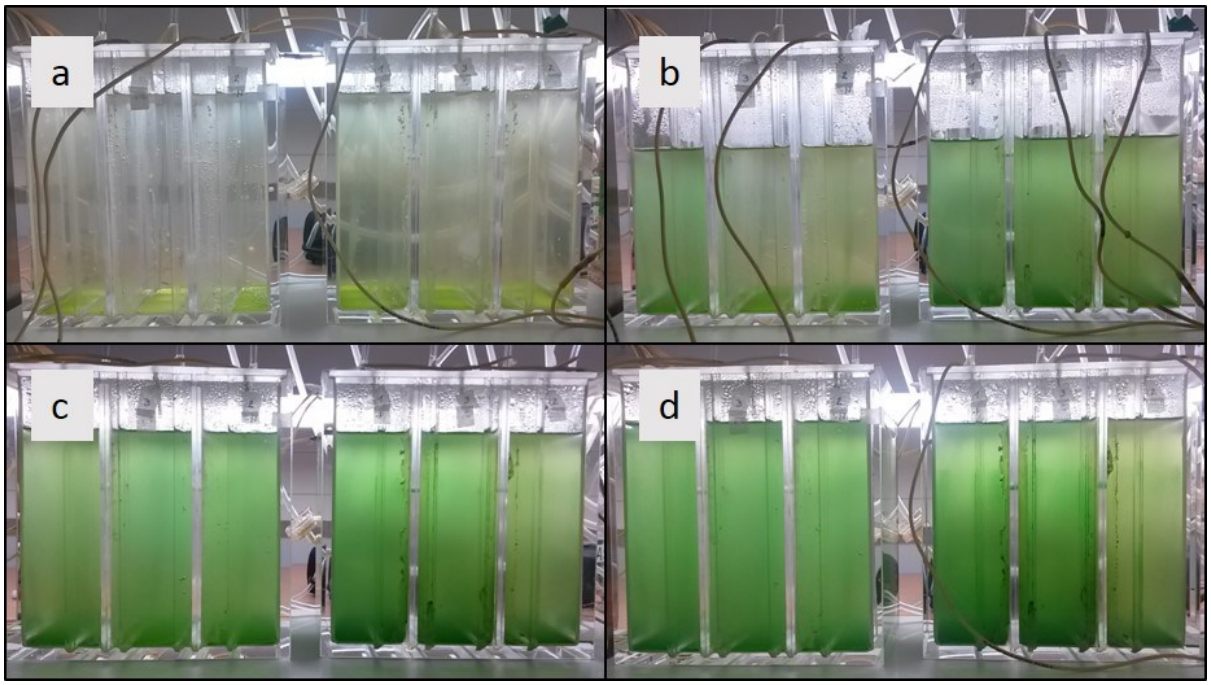

Fig. 3.10: The set-up with the Gradostat duplicates G1 (left) and G2 (right). a: after 4 days, b: after 8 days, c: after 11 days, d: after 15 days.

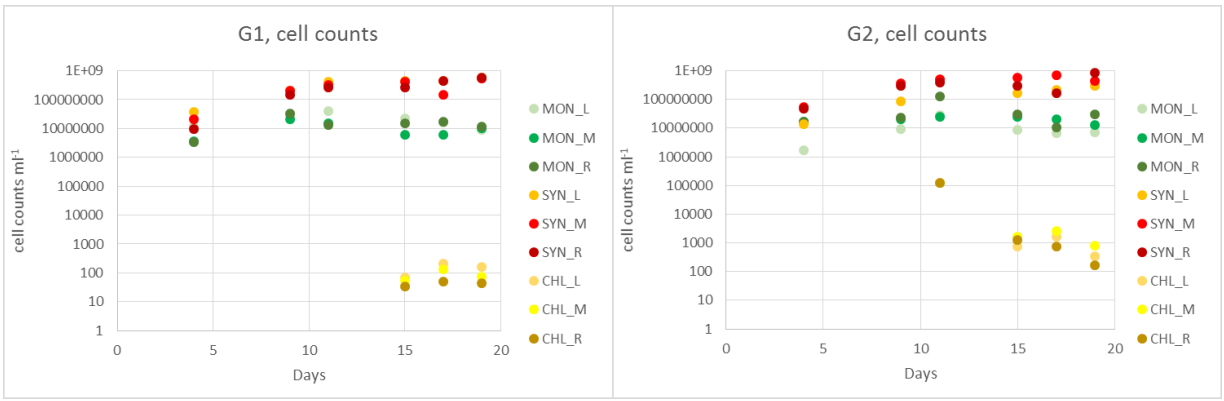

Fig. 3.11: Cell counts of Experiment 1 – Time course of cell densities of the three algal species in exp 1 in both Gradostat vessels. Cell counts ml^{-1} in both Gradostats G1 (left) and G2 (right); *Monoraphidium sp.* (MON, green), *Synechococcus elongatus* (SYN, red) and *Chlamydomonas sp.* (CHL, yellow); y-axis is log-transformed.

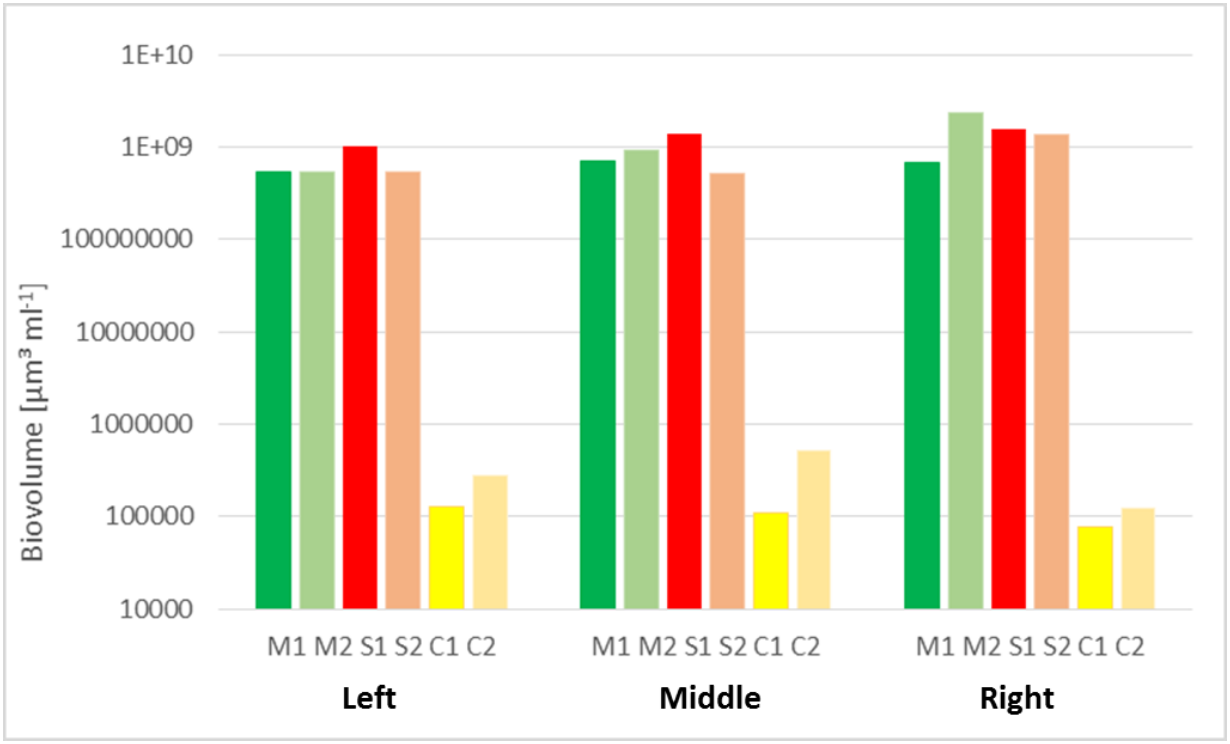

Fig. 3.12: Biovolumes [µm³ ml^{-1}] of Experiment 1 – Invasion under homogeneous resource distribution. Gradostat duplicates G1 (1, intense color) and G2 (2, lighter color) from day 19 (last day) of experiment; *Monoraphidium sp.* (M, green), *Synechococcus elongatus* (S, red) and *Chlamydomonas sp.* (C, yellow); y-axis is log-transformed.

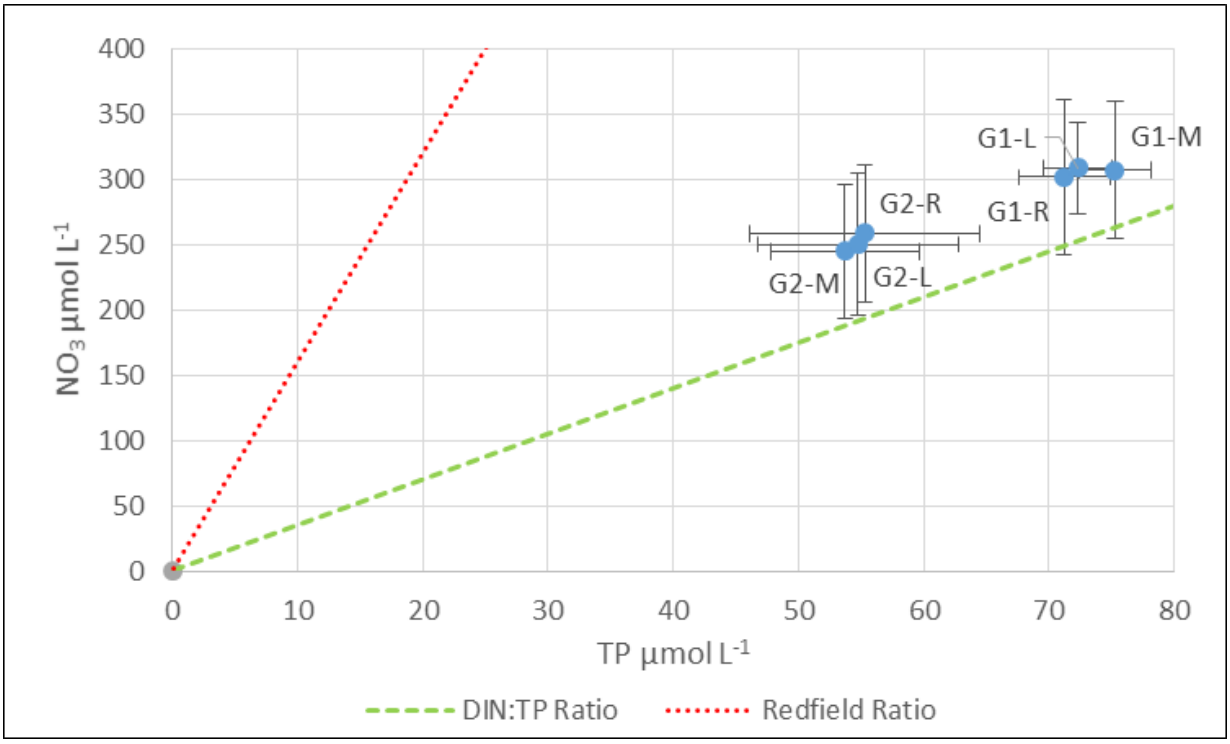

Fig. 3.13: NO_3:TP Ratio of Experiment 1 – Invasion under homogeneous resource distribution. Gradostat duplicates G1 and G2 with the vessels R-right, M-middle and L-left as mean over the whole course of the experiment 1 'Invasion'; the error indicators for x- and y-lab indicate the standard deviations. The red trend line marks the Redfield ratio N:P = 16:1, the green trend line marks the DIN:TP ratio 3,5:1.

ANOVA	Df	Sum Sq	Mean Sq	F-Value	Pr (>F)
Limitation	2	0,02246	0,01123	0,142	0,873
Residuals	3	0,23776	0,07925		

Tab. 3.1: Results of a one-way ANOVA analysis for NO_3:TP Ratio of Experiment 1 – Invasion under homogeneous resource distribution. Two one-way ANOVA-Analyses of the NO_3:TP ratio, testing for differences among vessels (left, middle and right. Significance codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 ' ', 1.

3.3.2 Experiment 2 – Limitation – heterogeneous resource distribution

In the second Gradostat experiment with a community consisting of *Monoraphidium sp.* and *Chlamydomonas sp.* was exposed to a gradient from N to P-limitation. This was achieved by supplying P- (left vessel) and N-limited (right vessel) WC-Redfield medium at the two outer vessels, and replete medium to the central vessel of the Gradostat.

The cell counts (Fig. 3.14) of both Gradostats show that the biomass behaved similarly in both Gradostats. The cell counts for *Chlamydomonas sp.* with the range of 10^2-10^3 cells ml^{-1} were much lower than those of *Monoraphidium sp.* which were about 10^8 cells ml^{-1}. *Chlamydomonas sp.* decreased over the course of the experiment especially in the Phosphorus limited vessels to 10^1 cells ml^{-1} where it had the largest decline. It has to be noticed that the cell size of *Chlamydomonas sp.* was much larger than the cell size of *Monoraphidium sp.*. Moreover *Chlamydomonas sp.* developed different cell sizes in limited medium (Fig. 3.15) where it expressed smaller cell sizes in the right vessel with the N-limitation and in the middle vessel where no nutrients were provided. On the contrary *Monoraphidium sp.* didn't show any major differences in cell size under limited conditions. Therefore the biovolumes was calculated for each vessel extra for *Chlamydomonas sp.* and *Monoraphidium sp.*. This was not necessary for the control set up of Experiment 3 'Control' with a nutrient balanced WC-Redfield medium in all vessels.

The NO_3:TP ratios differed very strongly among the three vessels within each Gradostat, but these differences were comparable among the two replicates (Fig. 3.16). As expected the NO_3:TP ratios fitted to both balanced middle vessels, where no N and P was supplied but were provided with N and P by diffusion from both adjacent N- and P-limited vessels, very comparable to the balanced DIN:TP ratio (3,5:1). The NO_3:TP ratios of the P-limited vessels

were found to lie above and the NO_3:TP ratios of the N-limited vessels below the DIN:TP ratio depicted in the graph as green dotted line. The Redfield ratio (red dotted line) showed to be an insufficient indicator as it didn't fit to the measured balanced nutrients distribution of the middle vessels in comparison to the DIN:TP ratio. The statistical analyses (Tab. 3.2) confirmed the variation between the vessels with a strong significance ($p = 0{,}00934$). The post-hoc analysis (Tab. 3.3) showed the strongest variations and high significance to be found between the P-limited and N-limited vessels ($p = 0{,}0095036$) followed by the P-limited vessels and the balanced middle vessels ($p = 0{,}0190161$). Interestingly the balanced middle vessels compared to the N-limited vessels resulted in a low variation ($p = 0{,}3501788$) although they seem different in the graph (Fig. 3.16). The NO_3:TP ratios were calculated as a mean over the whole runtime of the experiment.

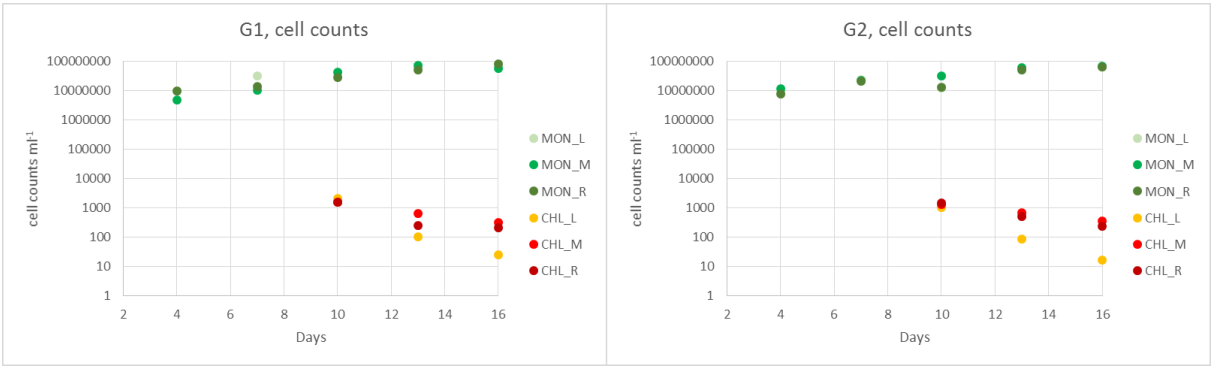

Fig. 3.14: Cell counts of Experiment 2 – Limitation – heterogeneous resource distribution. Cell counts per ml in both Gradostats G1 and G2; *Monoraphidium sp.* (MON), *Chlamydomonas sp.* (CHL); L= left vessel (P-limitation), M=middle vessel (no resources), R=right vessel (N-limitation).

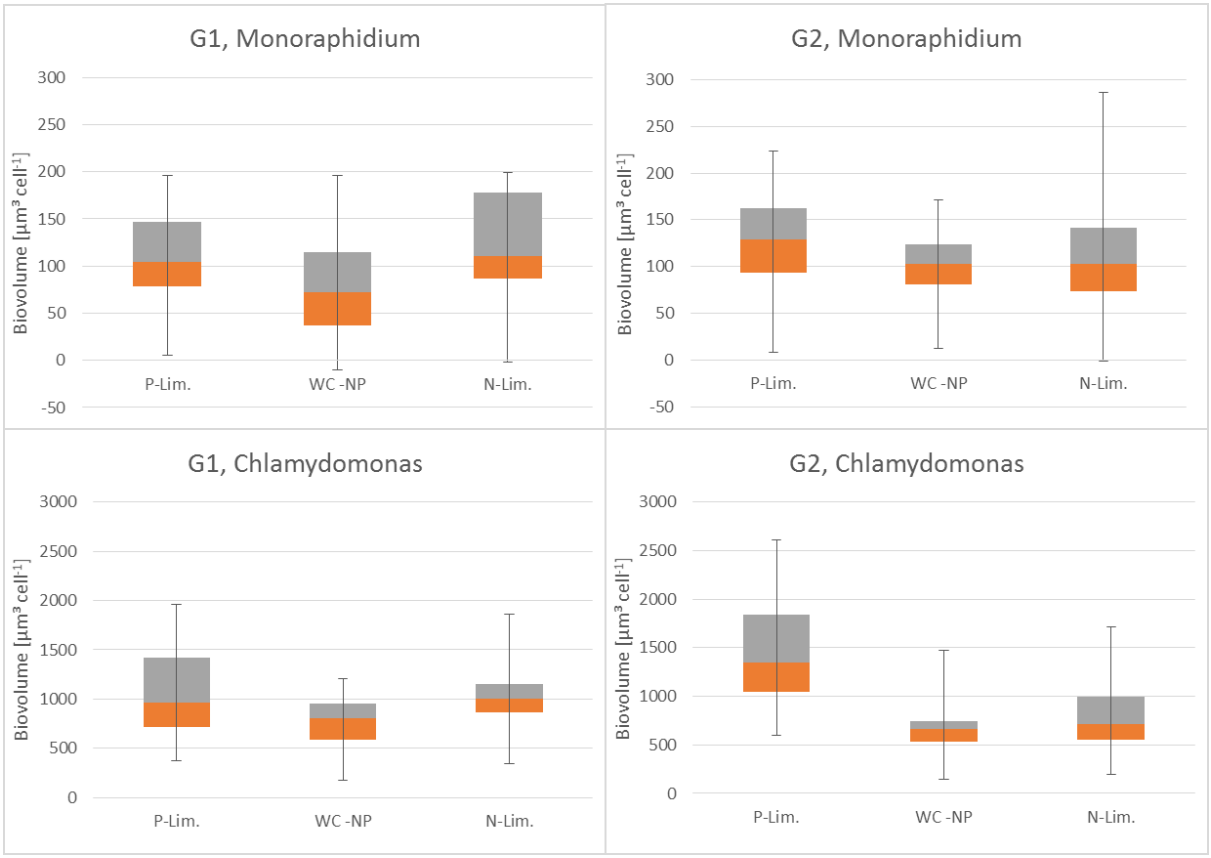

Fig. 3.15: Biovolumes of Monoraphidium sp. and Chlamydomonas sp. in comparison for both Gradostats. Measurements taken from 20 cells each and its biovolumes calculated to distinguish effects of N/P-limitation on cell size. P-Lim. = left vessel, WC –NP = middle vessel, N-Lim. = right vessel.

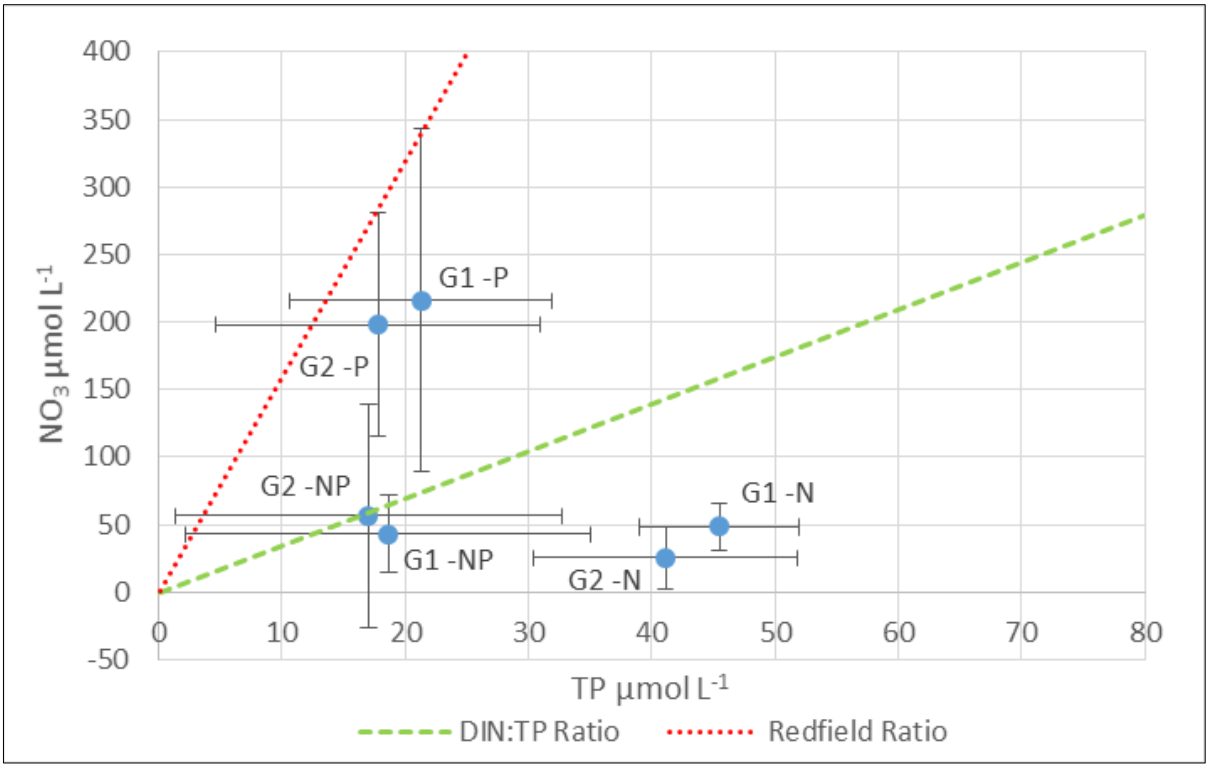

Fig. 3.16: NO3:TP Ratio of the Gradostat duplicates G1 and G2. (-P) P-limitation in left vessel, (-N) N-Limitation in right vessel and (-NP) without added N/P in middle vessel; Calculation as mean over the whole course of the experiment 'Limitation'; the error indicators for x- and y-lab indicate the standard deviations. The red trendline indicates the Redfield ratio N:P = 16:1, the green trendline indicates the balanced DIN:TP ratio 3,5:1.

ANOVA	**Df**	**Sum Sq**	**Mean Sq**	**F-Value**	**Pr (>F)**
Limitation	2	192,04	96,02	32,33	0,00934 (**)
Residuals	3	8,91	2,97		

Tab. 3.2: Results of a one-way ANOVA analysis for NO_3:TP Ratio of Experiment 2 – Limitation under heterogeneous resource distribution. Two one-way ANOVA-Analyses of the NO_3:TP ratio correlating with the left, middle and right vessels Significance codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 ' ', 1.

Tukey HSD	**diff**	**lwr**	**Upr**	**P adj**
NP-N	2,867625	-4,334297	10,06955	0,3501788
P-N	13,175303	5,973381	20,37723	0,0095036
P-NP	10,307678	3,105755	17,50960	0,0190161

Tab. 3.3: Results of the post-hoc Tukey HSD test. Multiple comparison of means, 95% family-wise confidence level, ANOVA results of the NO_3:TP ratios analyzed.

3.3.3 Experiment 3 – Control – homogenous resource distribution

The third experiment was performed as a control for experiment 2, i.e. *Monoraphidium sp.* and *Chlamydomonas sp.* Exposed to a homogenous resource environment. Therefore every vessel was provided with the same nutrient concentration (WC-Redfield medium).

The cell counts (Fig. 3.17) of both Gradostats showed that again both Gradostats had comparable growth-patterns in all vessels and reached a steady state. Interestingly *Chlamydomonas sp.* had higher cell counts between 10^3 and 10^4 cells per ml than in the previous experiment with counts between 100 and 1000 cells per ml (Fig. 3.14) with the limited nutrient conditions where the growth also started to decline in favor for an increased growth of *Monoraphidium sp.* reaching around 10^7 cells per ml. Here the cell count of *Monoraphidium sp.* was not increasing and seemed to be stable at 10^6 cells per ml along with *Chlamydomonas sp.* in both Gradostats.

Also for this experiment the cell sizes and volumina of each vessel in both Gradostats were determined to evaluate if the sizes might differ between the vessels and the Gradostats. This was not the case as it can be taken from the boxplots (Fig. 3.18) which was expected since the same medium was provided into every vessel. Therefore the biovolumina per ml didn't need to be calculated for each vessel separately.

The results of the NO_3:TP ratios calculated as mean over the runtime of the experiment show the homogenous and even distribution of the resources NO_3 and TP in all vessels in both Gradostat-duplicates (Fig. 3.19). All ratios of the vessels were clustered together and as the graph also displays is that the DIN:TP ratio of 3,5:1 is more appropriate for the measured resource ratios than the Redfield ratio. The statistical analyses support the graphical analysis as there were no significant differences between the vessels (p=0,78).

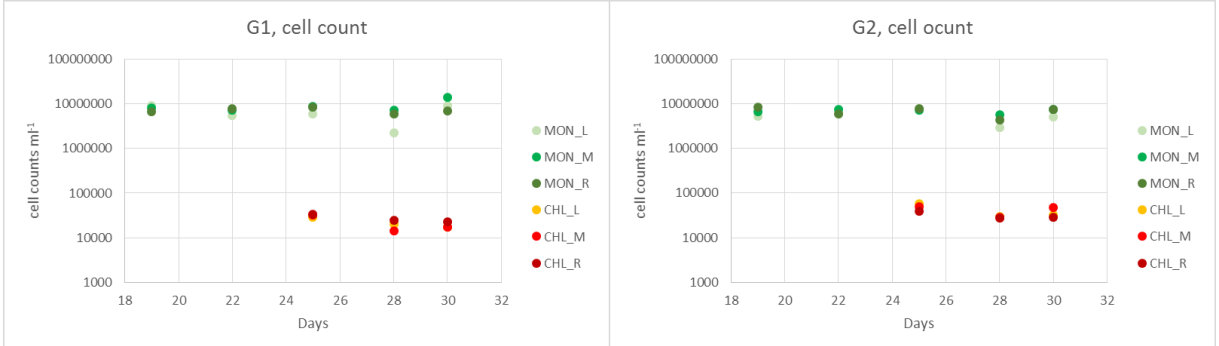

Fig. 3.17: Cell counts of experiment 3 – 'Control'. Cell counts ml^{-1} in both Gradostats G1 and G2; *Monoraphidium* (MON, green), *Chlamydomonas* (CHL, red).

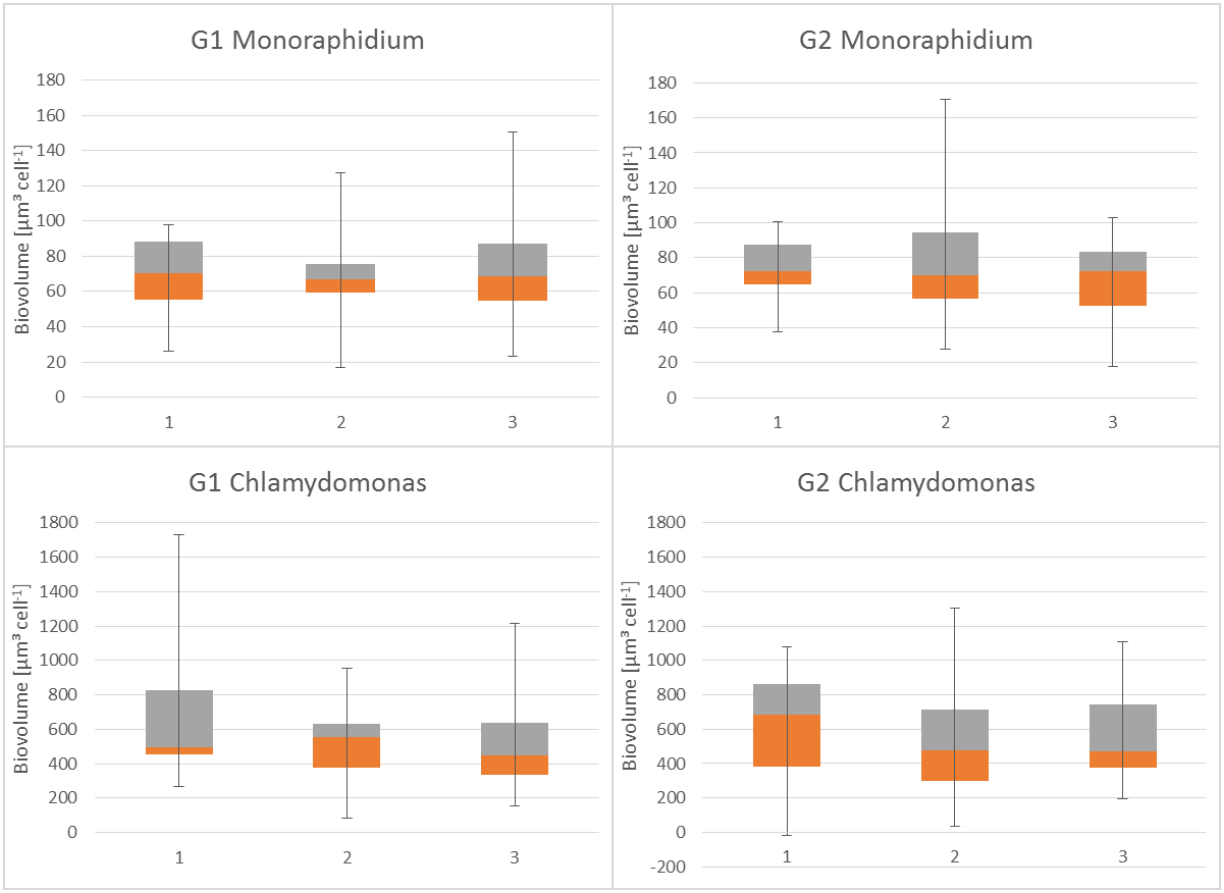

Fig. 3.18: Boxplot for the biovolumes of the cells of Monoraphidium and Chlamydomonas in comparison for both Gradostats. Measurements taken from 20 cells each and biovolumes calculated to analyze effects on cell size. 1^= left vessel, 2 = middle vessel, 3 = right vessel.

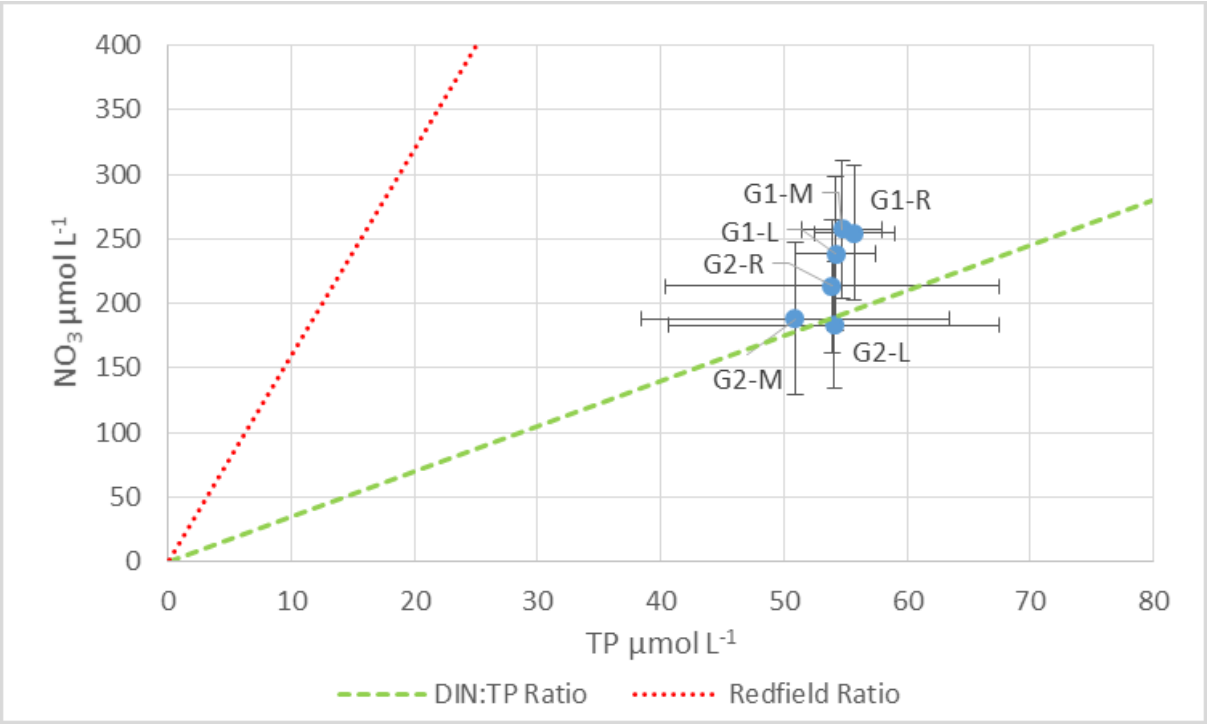

Fig. 3.19: NO3:TP Ratio of the Gradostat duplicates G1 and G2. The vessels R-right, M-middle and L-left were taken as mean over the whole course of the experiment 'Control'; the error indicators for x- and y-lab indicate the standard deviations. The red trendline indicates the Redfield ratio N:P = 16:1, the green trendline the DIN:TP ratio 3,5:1, which should be indicative for co-limitation.

ANOVA	**Df**	**Sum Sq**	**Mean Sq**	**F-Value**	**Pr (>F)**
Limitation	2	0,1688	0,08441	0,27	0,78
Residuals	3	0,9365	0,31218		

Tab. 3.4: Results of a one-way ANOVA analysis for NO_3:TP Ratio of Experiment 3 - 'Control'. Analysis of the NO_3:TP ratio correlating with the left, middle and right vessels and the Gradostat-duplicates (G1, G2). Significance codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 ' ', 1.

3.3.4 Analysis and Comparison of the Gradostat Experiments 2 (Limitation) and 3 (Control)

In order to compare the resource distributions of the dissolved nutrients N & P between experiments, the concentrations of dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP) of all three vessels of the Gradostat duplicates in both experiments were determined which allows conclusions about the established or not established resource gradients within the Gradostat systems. The analysis of the biovolumes provides indications about the impacts of the resource gradients on the overall growth and the biomass-ratios of the two species *Monoraphidium sp.* and *Chlamydomonas sp.* to display the varying outcome of competition between each other over an established resource-gradient (Limitation) and not established resource-gradient as in a homogenous distribution (Control) in the Gradostats.

3.3.4.1 Resource distribution of soluble N (SN) and P (SRP)

When analyzing the resource distributions of dissolved N (DIN) and P (SRP) among the three vessels (left, middle, right) of the two Gradostats G1 and G2 from the experiments 2 (Limitation) and 3 (Control) (Fig. 3.20), then the high concentrations of nitrogen were noticed. But still a resource-gradient within both Gradostats was established and kept up over the course of the experiment. The data also show that the concentration of nitrogen in the Gradostat 1 of the limited set-up was higher (N=196,484 µmol L^{-1}) in the right vessel with the phosphorus limitation then it was in G2 (N = 130,818 µmol L^{-1}). It also should be considered that the WC-Medium with limited phosphorus content had a concentration of N of 445,574 µmol L^{-1}. Therefore the biomass did consume and reduce the nitrogen strongly. Also the resource distributions for the control-set up displayed that nutrient distribution of N and P was evenly among all three vessels and no gradient was established. Interestingly, the first Gradostat showed a higher N-concentration as the second Gradostat although both contained similar biovolumes (Fig. 3.21).

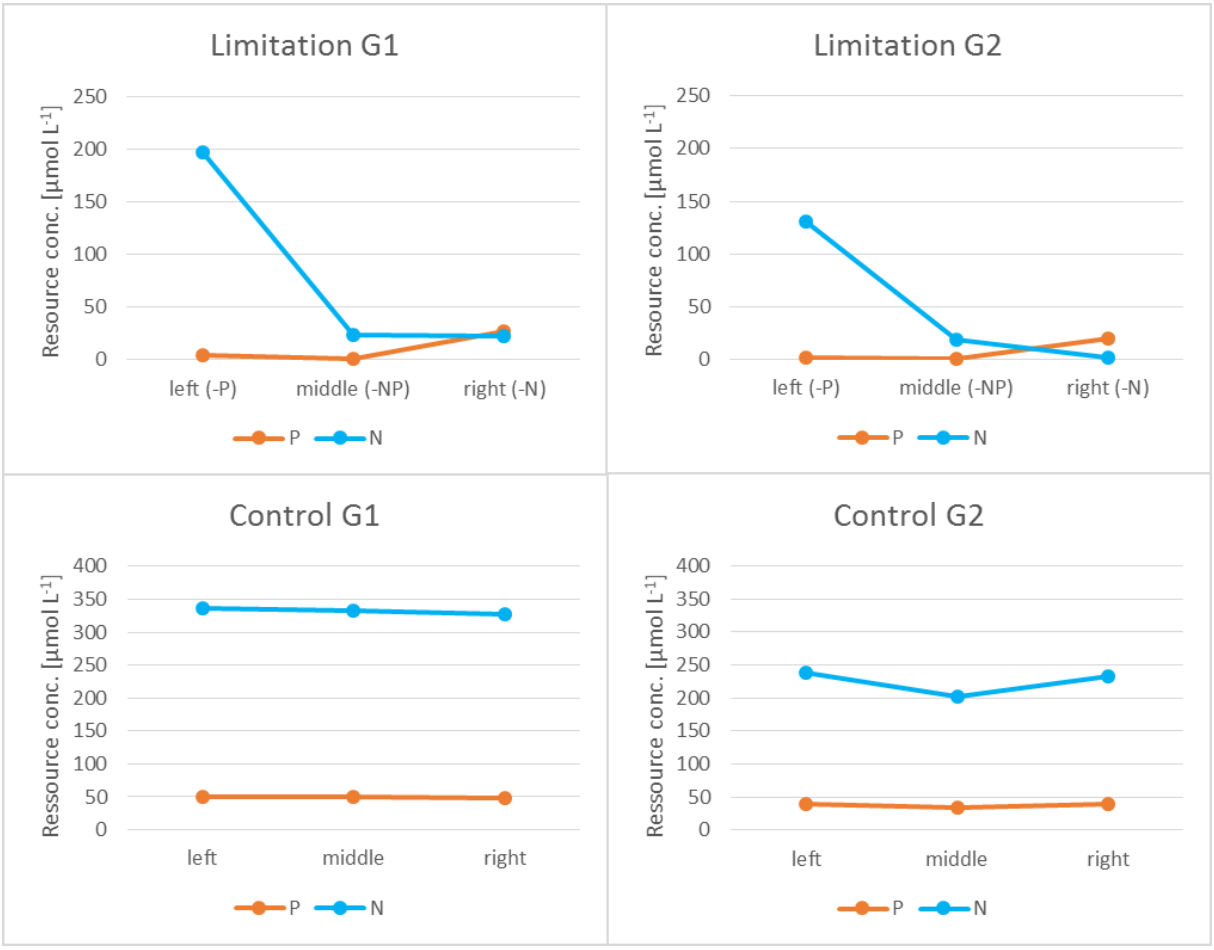

Fig. 3.20: Resource distributions of soluble N (DIN) and P (SRP). Distribution over the three vessels (left, middle, right) of the Gradostat from the experiments 2 (Limitation) and 3 (Control). Data were taken from exp. 2 from the 16th day and from Exp. 3 from the 28th day (data in Supplements Tab.S4.4).

3.3.4.2 Analysis and comparison of the biovolumes

The biovolumes of the two species *Monoraphidium sp.* and *Chlamydomonas sp.* were calculated for the overall growth and the biomass ratios of both species to each other. The competition over the resources phosphorus and nitrogen one time over a gradient (Limitation) and the other time over a uniform distribution in all vessels (Control) was investigated.

The total sum of biovolumes of both species together was about 18,4 times higher in the 2nd set-up 'Limitation' as in the 3rd set-up 'Control' (see Supplements S4.2). In the 'control' set-up the total biovolumes were very similar in both Gradostat duplicates (mean = 1,606E+09 µm³ ml^{-1}, G1 = 1,722E+09 µm³ ml^{-1}; G2 = 1,490E+09 µm³ ml^{-1}), whereas in the limited set-up the first

Gradostat-duplicate G1 differed lightly from G2 and had the highest overall biovolume (G1 = 3,618E+10 µm³ ml^{-1}, G2 = 2,302E+10 µm³ ml^{-1}).

In all three vessels in both Gradostats within the experiment 'Control' the biovolumes of *Chlamydomonas sp* were more than two orders of magnitude higher (around 10^7 µm³ ml^{-1}; Fig. 3.21) than in the experiment under limited conditions (around 10^4 to 10^5 µm³ ml^{-1}). In the control set-up *Monoraphidium sp.* had a biomass of round 10^9 µm³ ml^{-1} and in the limited set-up with round 10^{10} µm³ ml^{-1}. Therefore in the control set-up the biovolumes of *Monoraphidium sp.* was lower and the overall biovolumes of *Chlamydomonas sp.* was higher as under limited conditions.

Further analyses showed that in the second experiment 'Limitation' *Chlamydomonas sp.* had the lowest biovolume in the left vessel under P-limitation although the largest cell sizes were also measured there (Fig. 3.15). The highest biovolumes of *Chlamydomonas sp.* were in the middle vessel where no nutrients were supplied. Also the result of the statistical analysis (Tab. 3.5b) showed a strong significance for the differences of *Chlamydomonas sp.* between the vessels (p = 0,00353). The data suggest that *Monoraphidium sp.* would outgrew *Chlamydomonas sp.* in the P-limited environment, therefore *Monoraphidium sp.* profited the most under P-limitation. The highest biovolume of *Monoraphidium sp.* was found under N-limitation in the right chamber of both Gradostats but the differences of the biovolumes between the vessels were generally low which also was the results of the statistical analysis (p = 0,498, Tab. 3.5a). In the third experiment 'Control' the biovolumes of each *Monoraphidium sp.* and *Chlamydomonas sp.* were very similar between the vessels as expected and also the statistical test didn't result in a significant difference (Tab. 3.5c & d).

When looking at the ratios of the biovolumes of Monoraphidium sp. to Chlamydomonas sp. in each vessel of both Gradostat-duplicates (Fig. 3.22) a clear distinction between the limited and control set-up can be observed. The ratios of the control-experiment were more bulked and closer to the 1:1 ratio depicted as green dotted line in the figure. It displays the lower biovolumes of *Monoraphidium sp.* (MON) and the higher biovolumes of *Chlamydomonas sp.* (CHL) as the ratios had a range of 13,0 to 62,5 for MON:CHL (see Tab. S4.3 in the Supplements). The ratios of the duplicate vessels of the limited set-up showed a clear difference between the resource limitations of N and P and was more on the side of *Monoraphidium sp.*. Especially the ratios under P-limitation were visibly separated from the other vessels and stood close

together (MON:CHL = 290 518,0 for G1 and 320 990,4 for G2). The ratio of the vessels under N-limitations differed from each other (MON:CHL = 124 102,5 for G1 & 43 415,7 for G2), the ratio of the 2nd Gradostat-duplicate G2 was close to the ratios of the vessels without resource input (-NP) whereas G1 had a stronger ratio shift towards *Monoraphidium sp.*. The ratios of the NP-limited vessels were also very congruent (MON:CHL = 31 944,6 for G1 & 33 069,5 for G2).

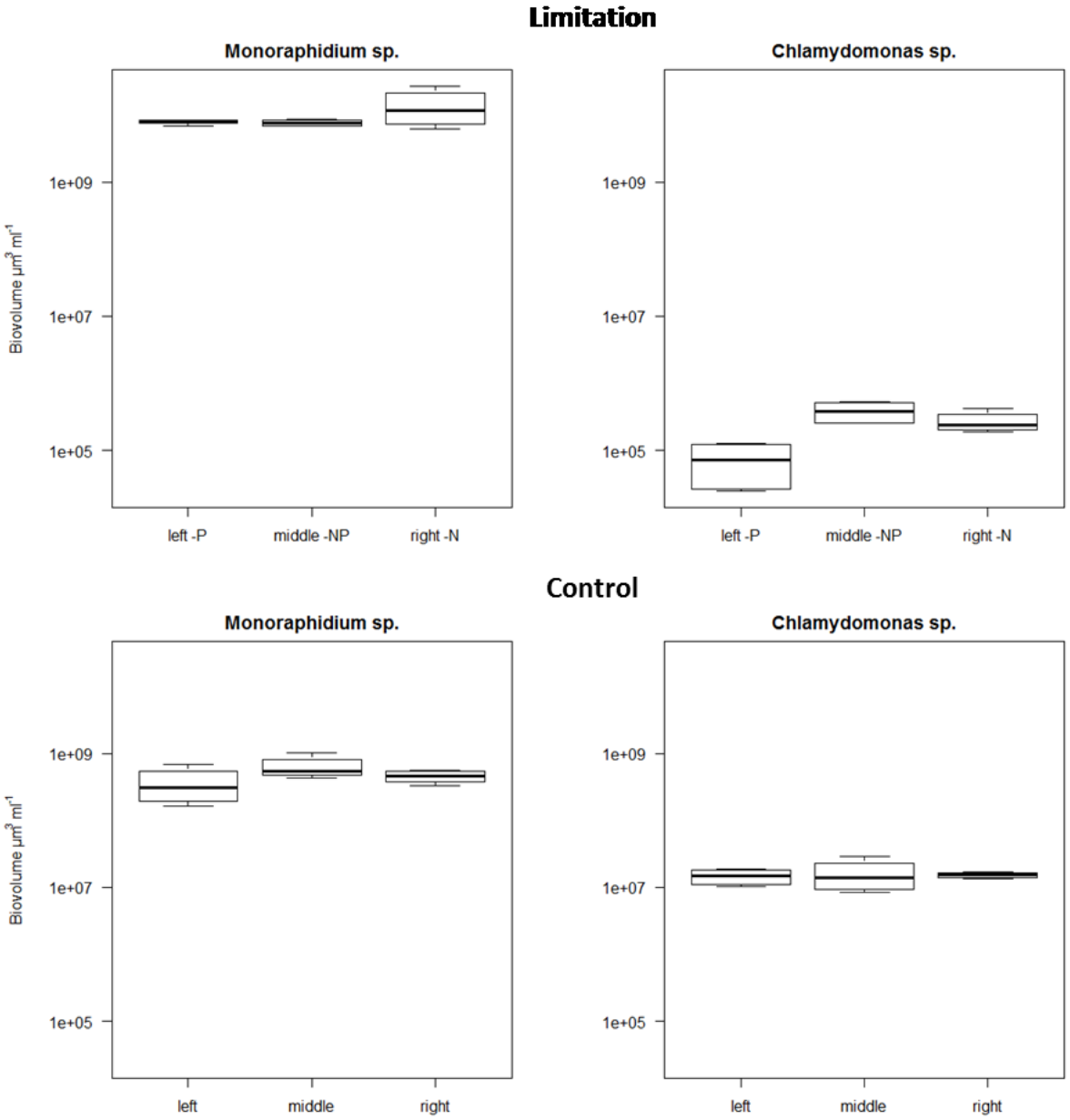

Fig. 3.21: Comparison of biovolumes from Monoraphidium sp. and Chlamydomonas sp.. Biovolumes [μm^3 ml^{-1}] taken from the two last time points of the duplicates of G1 and G2 for each vessel from the Experiment 2 (Limitation, day 13 and 16) and Experiment 3 (Control, day 28 and 30); y-axis is log-transformed.

ANOVA	Df	Sum Sq	Mean Sq	F-Value	Pr (>F)
Limitation	2	5,668e+19	2,834e+19	0,887	0,498
Residuals	3	1,777e+20	5,923e+19		

Tab.3.5a: Results of a one-way ANOVA-Analysis for the comparison of biovolumes of *Monoraphidium sp.* in Experiment 2 – "Limitation". Analysis of the biovolumes correlating with the left, middle and right vessels. Data taken from the last two time points. Significance codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 ' ', 1.

ANOVA	Df	Sum Sq	Mean Sq	F-Value	Pr (>F)
Limitation	2	9,906e+10	4,953e+10	63,19	0,00353 **
Residuals	3	2,351e+09	7,838e+08		

Tab.3.5b: Results of a one-way ANOVA-Analysis for the comparison of biovolumes of *Chlamydomonas sp.* in Experiment 2 – "Limitation". Analysis of the biovolumes correlating with the left, middle and right vessels. Data taken from the last two time points. Significance codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 ' ', 1.

Tukey HSD	diff	lwr	upr	p adj
NP-N	109149,9	-7840,083	226139,82	0,0597360
P-N	-201077,8	-318067,778	-84087,87	0,0113556
P-NP	-310227,7	-427217,646	-193237,74	0,0032464

Tab.3.5c: Results of the post-hoc Tukey HSD test for the one-way ANOVA-Analysis in Tab.3.5.b. Multiple comparison of means, 95% family-wise confidence level, Analysis of one-way ANOVA for the comparison of biovolumes of *Chlamydomonas sp.* in Experiment 2 – "Limitation" (see Tab.3.5.b).

ANOVA	Df	Sum Sq	Mean Sq	F-Value	Pr (>F)
Limitation	2	7,812e+16	3,906e+16	2,638	0,218
Residuals	3	4,442e+16	1,481e+16		

Tab.3.5c: Results of a one-way ANOVA-Analysis for the comparison of biovolumes of *Monoraphidium sp.* in Experiment 3 – "Control". Analysis of the biovolumes correlating with the left, middle and right vessels. Data taken from the last two time points. Significance codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 ' ', 1.

ANOVA	Df	Sum Sq	Mean Sq	F-Value	Pr (>F)
Limitation	2	2,084e+12	1,042e+12	0,025	0,975
Residuals	3	1,246e+14	4,155e+13		

Tab.3.5d: Results of a one-way ANOVA-Analysis for the comparison of biovolumes of *Chlamydomonas sp.* in Experiment 3 – "Control". Analysis of the biovolumes correlating with the left, middle and right vessels. Data taken from the last two time points. Significance codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 ' ', 1.

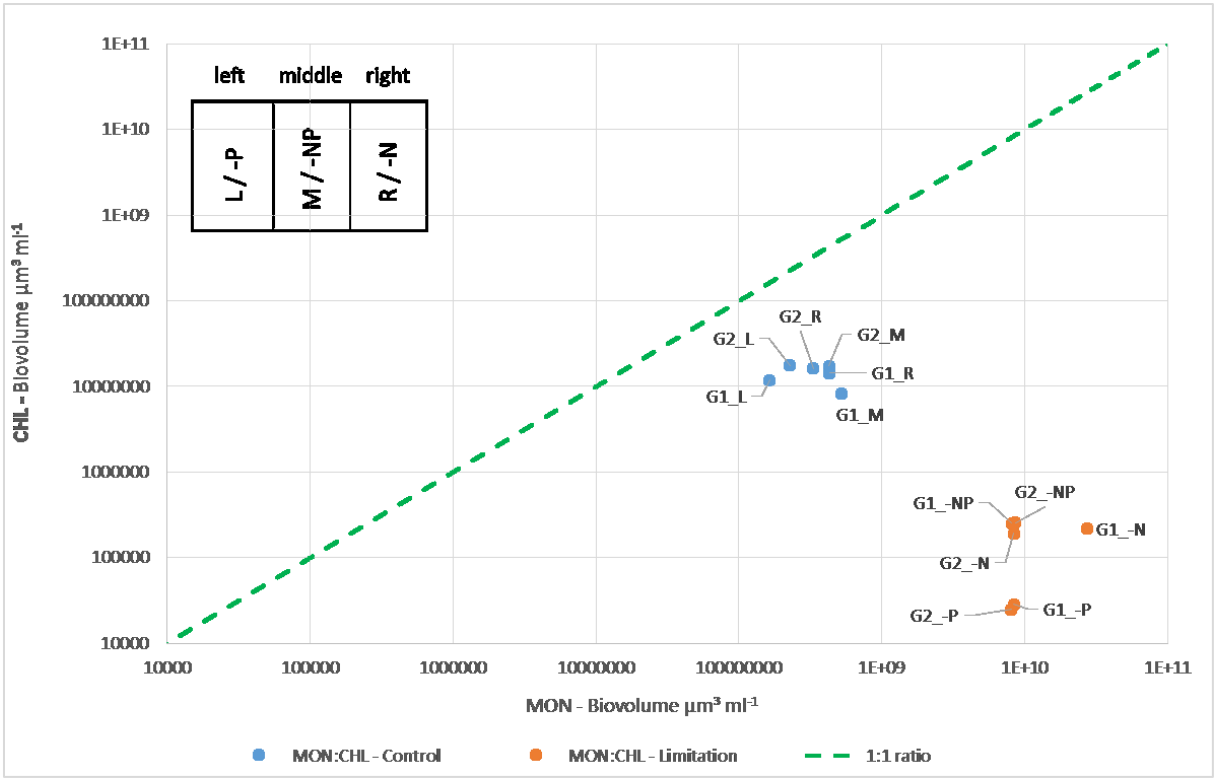

Fig. 3.22: Ratios of biovolumes of Monoraphidium sp. (MON) and Chlamydomonas sp. (CHL). Data from the last time point of measurement of both experiments (Exp. 2 'Limitation' – red; Exp. 3 'Control' – blue), Gradostat-duplicates (G1,G2) and vessels (L = left, M = middle, R = right for Exp. 2 – Control; -P = P-limitation, -NP = no resource input, -N = N-limitation for Exp. 3 – Limitation); 1:1 Ratio depicted by green dotted line. Table of the ratios in Supplements (Tab.S4.2.2).

4 Discussion

The goal of this thesis was to develop and to test an experimental Gradostat setup, which allows empirical experiments of resource competition along resource gradients. The set up was tested with real phytoplankton communities under homogenous and heterogeneous resource conditions. For that purpose several possible candidates of phytoplankton species were screened for their resource requirements to find suitable taxa for the communities. The results were compared to simulated datasets as well. Although the work with the set-ups and experiments faced some difficulties, the implementation overall worked well and compared very well to analytical simulations.

4.1 Screening suitable phytoplankton taxa for their resource requirements (R^*)

The aim of the screening was to find a species pair where one species is a better competitor for N, and the other for P. The screening involved a number of strictly photoautotrophic phytoplankton taxa that were available in the culture collection of the AquaScale group (Chlorophyta & Cyanoprokaryota). The resource concentration for zero net growth (R*) was assessed for both nitrogen and phosphorus limitation. In addition, I assessed the maximum growth rates (μ_{max}) under non-limiting conditions. The screening involved the chlorophytes *Monoraphidium sp.*, *Stichococcus sp.*, *Scenedesmus sp.*, and *Chlamydomonas sp.*, and the cyanoprokaryotes *Synechococcus elongatus* and two *Microcystis aeruginosa* strains, PCC 7806 (toxic) and ΔMCY A- (non-toxic).

In the comparison of the growth rates of all taxa (Fig. 3.2) *Monoraphidium sp.* and *Scenedesmus sp.* showed the highest growth rates followed by *Chlamydomonas sp.*. In an Exponential Fed Batch experiment (EFB) four species were screened for their R* with regard to N and P limitation: *Monoraphidium sp.*, *Scenedesmus sp.*, *Chlamydomonas sp.* and *Synechococcus elongatus* as they had the lowest R*. *Monoraphidium sp.* was the only species, which showed high competitive abilities in nitrogen limited medium as its R* was situated on the nitrogen side of the N:P ratio of 8,97:1 (Fig. 3.4; N:P ratio visualized as green dotted line).

In the pairwise EFB-tests under N/P limitation *Scenedesmus sp.* was clearly the strongest competitor under both N and P limitation, hence this taxon was not an option. It was a too strong competitor in comparison to the other species as it outgrew *Monoraphidium sp.* and even managed to invade the *Monoraphidium sp.*/*Synechococcus elongatus* communities via the supply tubes in the EFB for the pairwise tests under N/P limitation (Fig. 3.5).

The toxic freshwater cyanobacterium *Microcystis aeruginosa* strain PCC 7806, which produces the toxin Microcystin, was the weakest competitor of all species as it had lowest growth rates in nitrogen and phosphor limited environments and also exhibited the highest R*- which indicates that this taxon needs higher concentrations of nutrients for growth. This was not the case for the non-toxic strain ΔMCY A- showing good growth rates under nutrient limited conditions. These results suggest that *Microcystis aeruginosa* strain PCC 7806 could rather be adapted to nutrient rich environments. This matches results by Frangeul (et.al. 2008) where the genome of PCC 7806 was examined and a genetic basis for eutrophic fresh water habitats were found.

Finding a suitable species pairings proved to be a challenge since the R* for N and P correlated with most taxa as they mostly showed to be higher competitive under P-limited conditions (see Fig. 3.4). It would have been beneficial if more species could have been analyzed for their competing properties with the outlook to find species pairs who show stronger differentiation in competition traits regarding N & P limitation for the purpose of this thesis. More different set-ups could have been tested with the Gradostat and different species pairs could have been compared. Though unfortunately this would have gone far beyond the scope of this thesis, the AquaScalegroup also only had a limited culture collection which limited the possibilities.

Therefore for the Gradostat experiments the two species pairings of *Monoraphidium sp.* with *Synechococcus elongatus* and *Monoraphidium sp.* with *Chlamydomonas sp.* were chosen. For both species pairings *Monoraphidium sp.* was taken because of its good competitive abilities under nitrogen limited conditions.

4.2 Development of the Design of the Gradostat

The initial idea for the design of the Gradostat as it was used in this thesis was to keep the connecting links between the vessels as small as possible to avoid creating additional niches between the vessels. Previous Gradostat experiments often used bottles as vessels connected by tubes to allow diffusion between the vessels. Those approaches were easier in the handling since the bottles could be easily disconnected for probing, but those intermediate tubes would actually describe distinctive compartments of their own which weren't considered. Therefore the vessels were designed as chambers in an aquarium where a hole in the wall was the connection for diffusion which meant no intermediate compartments between the vessels were created. This design also required some considerations concerning handling and sterility which are described in the following paragraphs.

It was found to be the best to split the set-ups of the Exponential Fed Batch and the Gradostat in functional parts, which could be handled and sterilized individually as their own 'closed systems' before being assembled to one large set-up. Therefore for example the medium bottles with the attached tubes and in the case of the EFB with the culture flasks were one such unit, the air ventilation tubes with the air-filter a second unit. Finally, the sealed Gradostat filled with medium was the third. These units could be separately autoclaved or acid washed and assembled each under sterile conditions like a Clean Bench. One compromise on the question of sterility was the assembly of the three entities at the non-sterile work space in the laboratory. The holes of the Gradostat for inserting tubes were also protected and only opened to connect the tubes for the in- and outflow of the medium. The tubes were then clamped to the peristaltic pump. The glass air tubes with the air filters were already attached within the Gradostat under sterile conditions and only needed to be connected to the air pumps and the buffer bottle. Although lots of thought and effort was taken to maintain sterility, contamination of small flagellates happened at the end of the 3rd experiment 'Control'. A possible issue was, that different tubes had to be inserted into the vessels to be able to sample the Gradostat. For sampling an own set of tubes was used, which were autoclaved prior and afterwards each usage. It was meant, that the tubes connected to the medium bottles didn't need to be reconnected for sampling purposes as those extra manipulations of the system would have meant a much higher risk of contamination since the

tubes would have needed to be dismantled from its as 'closed systems' designed units for that purpose. Since the Gradostat couldn't be moved, as moving would have caused unwanted and uncontrolled turbulence and mixing of the different vessels as they are connected with each other, the sampling had to take place at the non-sterile work-space which also was a compromise in handling and sterility. The safest way would have been to run the system at a sterile location but due to limited resources a Clean Bench or a similar sterile work space couldn't be permanently occupied for months to come. But still it should also be considered that the contamination only occurred after about nearly a month of running, handling and periodically sampling the Gradostats and also only in one out of three experiments at the non-sterile workspace (the other contamination in Experiment 1 was an issue with a stock culture). Therefore the efforts for maintaining sterility over those prolonged time periods and the approach for pre-assembled isolated units were shown to be rather successful.

Considering the issues with sterility the choice of the material Acryl glass for the Gradostat vessel needs to be reevaluated. On the one side it is a highly suitable material for realizing own designs of vessel-shapes with holes etc. in them as it is easy to work with it without risking to break it. On the other side it only could be acid washed as the material wouldn't withstand the heat of autoclaving or the contact of ethanol, which caused irreparable damage like fissures and ruptures within the acryl glass. The choice of normal glass would have made the handling easier for sure although for glass designs a glasser or aquarium builder would have been needed.

A crucial requirement for the implementation of a Gradostat is to maintain a constant passive diffusion rate among adjacent vessels (while medium inside a given vessel should be homogenously mixed). For testing the diffusion, I used a fluorescent dye as tracer (Uranine), which easily could be monitored by a Fluorometer, plus salt in one experiment. The adjustment of the diffusion rate first focused on adjusting the hole size, with a variation in hole size from 1-5 mm. First experiments then revealed overall a low correspondence between hole size and diffusion rate, while it was found that the turbulence induced by bubbling each vessel (for mixing medium in each vessel) had a strong impact on the diffusion. (Fig. 3.7). Therefore the focus was laid on ways to regulate the turbulence and bubble counters were installed to be better able to set, monitor and adjust the strength of the air ventilation in the vessels. Although with many modifications to the air-ventilation system a perfectly stable air-

ventilation could not be achieved as several issues occurred during the runtime of the experiments. A possibly more reliable way to create turbulence within each vessel would be the usage of magnetic stirrers but this would have gone beyond the capacities for this thesis and also would have required major adaptations to the system. Therefore regular monitoring and readjusting of the air-ventilation via the bubble counters was considered an appropriate way to maintain an as-constant-as-possible diffusion rate.

The experiment for testing the distribution of resources over the Gradostat with Uranin and Salt as resource substitutes supplied on each side of the Gradostat was very promising. Since bubble counters were not installed yet at this stage and the air-distribution couldn't be that precisely monitored. The results demonstrated very nicely that the resources were dispersed over the vessels (Fig. 4.2.a-b) as predicted by the computer simulations (Fig. 4.2.c-d) and a consistent resource gradient over the system was established as the Gradostat reached a steady state. As expected the concentrations of the substitutes decreased from the vessel where they were supplied and therefore had the highest concentration to a minimum on the other end of the Gradostat and vice versa. In agreement with the spatially extended system model by Ryabov A. & Blasius B. (2011), the resulting gradient was non-linear. The gradients remained stable during the runtime of the experiment and corresponded well to the computer simulations. I could also demonstrate that reducing the hole size caused a steeper resource gradients among connected vessels due to reduced diffusion rate. When different turbulence settings with turbulence being switched on/off in different vessels was simulated and the outcome of the resource distribution was calculated it shows that even a failed turbulence in one to two vessels doesn't mean a breakdown of the gradients but merely a shift of the resource ratios and increased concentration of the resource in the vessels with no turbulence (Fig. 4.1.a-d). This might suggest that the factor turbulence actually doesn't need to be overvalued since it is a closed system. As long as the turbulence is given at a steady rate it is more important to reach a diffusion rate within the scope of the needed parameters of the diffusion coefficient. Therefore to reach a consistent diffusion rate and dispersal of resources a uniform turbulence (air-venting) is needed in all vessels which could be obtained with f.ex. bubble-counters as it was later done for the Gradostat-experiments with biomass.

Fig.4.1.a

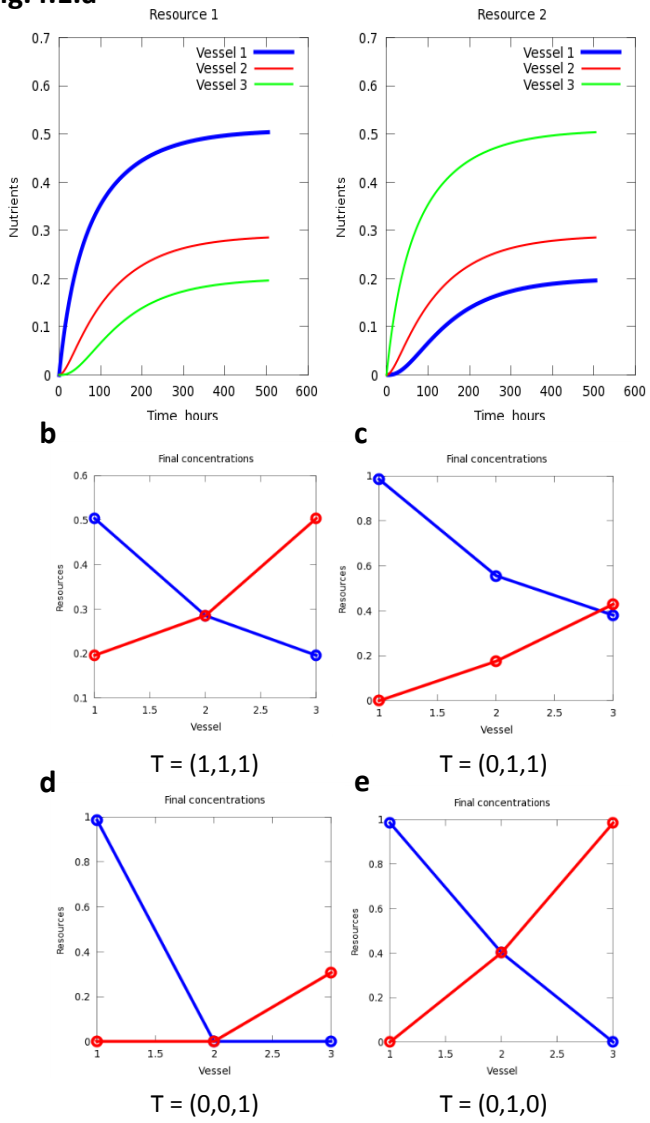

Fig. 4.1: Simulation of turbulence and resource distribution.

Fig.4.2.a

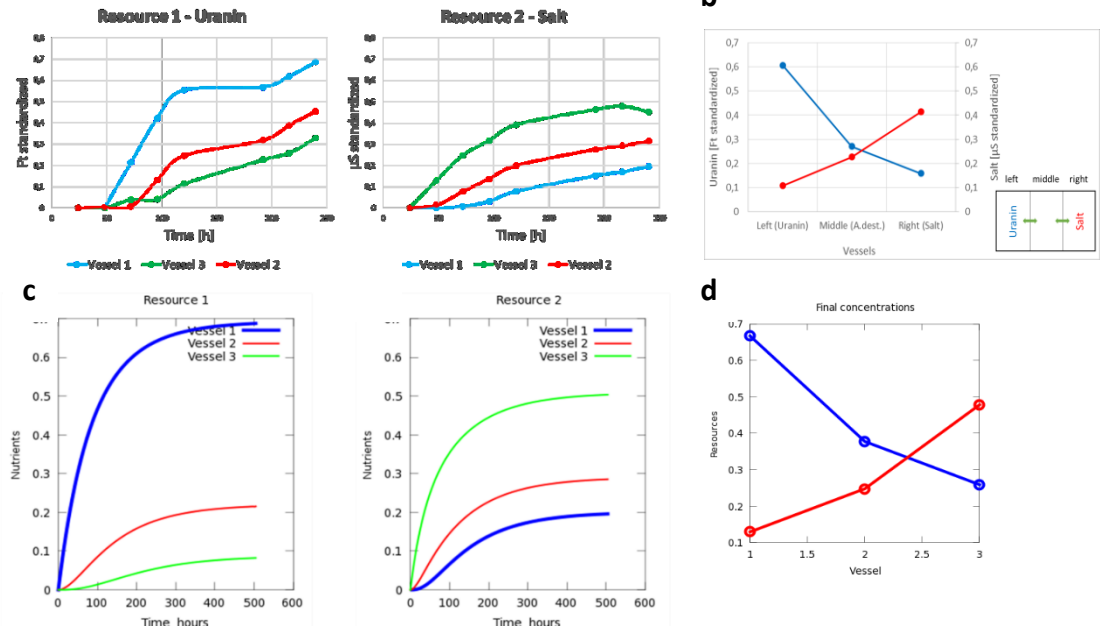

Fig. 4.2: Comparison of resource distribution and diffusion rate with simulated and measured data.

Fig. 4.1: Simulation of turbulence and resource distribution. a: Simulation of distribution of two resources at diffusion rate 0,45; b: simulated resource concentration of two resources with equal turbulence (T) in all vessels (T = (1,1,1)); c: sim. res conc. when turbulence is off in left vessel (T = (0,1,1)); d: no turbulence in left and middle vessel (T = (0,0,1)); e: sim. res. conc. when turbulence is on in the middle vessel (T = (0,1,0)).

Fig. 4.2: Comparison of resource distribution and diffusion rate, simulated and measured data. a: Distribution of Uranin in vessel 1 (blue), A.dest. in vessel 2 (red), Salt in vessel 3 (green); b: resource concentration at steady state, blue = Uranin, red = Salt; c: Simulation of Uranin distribution with diffusion rate = 0,13 and simulation of Salt distribution with diffusion rate = 0,45; d: simulated resource concentration at steady state, blue = Uranin, red = Salt; Left vessel = vessel 1, middle vessel = vessel 2,

4.3 Resource competition in homogenous and heterogeneous environments (Gradostat experiments)

4.3.1 Experiment 1 - Two-species community with invasion (homogenous resource distribution)

This experiment was conducted under homogenous resource conditions involving the two previously chosen taxa *Monoraphidium sp.* & *Synechococcus sp.* and two Gradostat systems with three vessels each. Therefore no nutrient gradient was created as the WC-Redfield medium was equally supplied to all vessels. The development of the species growth in each vessel was monitored as well for differences between the vessels. It was expected to find similar growth patterns in all vessels due to the lack of a nutrient gradient. A green flagellate, *Chlamydomonas sp.*, became conspicuous from day 15 in all vessels.

Firstly the results of the NO_3 and TP measurements confirmed that no gradient of nutrients was established and NO_3 and TP were evenly distributed over all three vessels in both Gradostats although the Gradostat G2 had overall lower concentrations of NO_3 and TP than G1. Therefore the accident with the medium supply in the early stage of the experiment didn't have a noticeable impact on the distribution of nutrients between the vessels. The resource ratio DIN:TP = 3,5:1 as suggested in the paper from Ptacnik (*et al.* 2010) to be the best indicator for in situ N:P limitation of chemical monitoring data of phytoplankton was compared to the well-established Redfield Ratio N:P = 16:1 (Redfield, 1934). Indeed the DIN:TP ratio was far more fitting for the dataset than the Redfield ratio and aligned well to the balanced resource ratios since non-limited medium was supplied.

Analyzing the results of the biovolume (Fig.3.12) it seemed as the invasion of *Chlamydomonas sp.* caused a decline of growth for both taxa *Synechococcus elongatus* and *Monoraphidium sp.* and even negatively influenced both taxa in growth. The biovolume of *Chlamydomonas sp.* were 4 - 5 magnitudes lower as *Synechococcus elongatus* and remained relatively stable although the densities were slightly higher in Gradostat G2. A similar pattern was also the case in the Exponential Fed Batch where *Scenedesmus sp.* invaded into the model community flasks of *Monoraphidium sp.* and *Synechococcus elongatus* and also could have had negative effects on growth of *Monoraphidium sp.*.

As already stated the biomass of *Chlamydomonas sp.* was in overall low in all vessels in both Gradostats but where its biomass was increased the biomass of *Synechococcus elongatus* was reduced. What can be noticed is that the vessel with the lowest biomass of *Chlamydomonas sp.* also had the highest biomass of *Synechococcus elongatus* and vice versa. This also explains the strong growth of *Monoraphidium sp.* in the right vessel of G2 where the stronger abundance of *Chlamydomonas sp.* had a reduced growth of *Synechococcus elongatus* as cause. *Chlamydomonas sp.* had the lowest biovolume in the right vessel and a generally 'weakened' *Synechococcus sp.* in its abundance in the whole Gradostat. This could have left enough room for *Monoraphidium sp.* to prosper and grow, creating a patch for *Monoraphidium sp.* through productivity and growth trade-off between *Synechococcus elongatus* and *Chlamydomonas sp.*.

All in all the differences as observed in the cell concentrations, growth rates and biovolumes between the species only varied slightly between the vessels and demonstrates a community of two similarly strong species (*Monoraphidium sp.* and *Synechococcus elongatus*). The intervention of a highly competitive invasive species (*Chlamydomonas sp.*) seemed to had an impact on only one of both species, *Synechococcus elongatus*, where trade-offs in growth could be observed creating possible advantages for the second resident species *Monoraphidium sp.*.

4.3.2 Analysis and Comparison of the Experiments 2 (Limitation – heterogeneous resource distribution) and 3 (Control – homogenous resource distribution)

In this chapter the results of the 2nd experiment 'Limitation – heterogeneous resource distribution' and the 3rd experiment 'Control – homogenous resource distribution' are discussed and compared with each other. Both experiments were conducted with the species pair *Monoraphidium sp.* and *Chlamydomonas sp.*.

Beginning with the second experiment 'Limitation' in which a resource gradient was successfully established by creating a Phosphorus limited environment in the left vessel and Nitrogen limited environment in the right vessel while the middle vessel was supplied with an N- and P- free medium. The highest concentration of NO_3 was found in the right P-limited

vessel to the lowest concentration in the left N-limited vessel and the other way round for TP. Those results were also visible in the DIN:TP ratio analysis since all three limitations showed strong differentiations: the ratios of the balanced middle vessels which were supplied with N & P solely by diffusion from the flanking N- and P-limited vessels fitted as expected to the DIN:TP ratio 3,5:1. The other ratios of the limited vessels were shifted once to the DIN side as for the P-limited vessels and once to the TP side in case of the N-limited vessels and allowed a clear distinction between the limitations. Comparing the results with the Redfield ratio the data showed that it was far on the P-limited side and didn't align with the balanced ratios as it also was the case in the first experiment.

The communities in the two replicated Gradostats developed similarly concerning the growth curves and cell counts allowing a good comparison of both. The cell counts of *Chlamydomonas sp.* declined the strongest in the left vessel with the P-limited medium in both Gradostats. At the same time, the cell size of *Chlamydomonas sp.* peaked for both Gradostats in the P-limited vessel, differences were clearly visible in that case. *Monoraphidium sp.* didn't show many differences in cell counts and cell size between the vessels in both Gradostats as there were no clear patterns to distinguish although weak tendencies for smaller cell sizes in the nutrient depleted middle vessels can be seen. In the Exponential Fed Batch experiments *Monoraphidium sp.* displayed an extended growth in a P-limited environment and a lower R* for P as *Chlamydomonas sp.* which might explain the decline of *Chlamydomonas sp.* in the P-limited left vessel and also its larger cell size as a trade off because of the competition for the P since larger cells might offer an advantage by having larger maximum uptake rates and being able to store more nutrients than smaller cells (Edwards *et al.* 2011).

The third experiment 'Control' was provided with the Nitrogen and Phosphorus balanced WC-medium in all vessels creating an evenly distributed nutrient concentration over all three vessels in both Gradostats which was also visible in the NO_3 and TP data. The results of the NO_3:TP ratios turned out to be clustered together and close to the DIN:TP ratio as expected since both Gradostats were similarly distributed with the same WC-Redfield medium as it was also the case in the first experiment. It was not intended to create a nutrient-gradient in the Gradostats but an even and balanced distribution in all vessels, the statistical analysis supported that no significant differences in nutrient concentrations were found between the vessels.

Judging from the cell counts in both Gradostats the replicates behaved very similar in this experiment. The cell counts were very congruent over the time curse in all vessels as well as the cell sizes didn't differ as expected for an ident medium supply in all vessels. Interestingly *Chlamydomonas sp.* had higher cell counts between 10^3 and 10^4 cells ml^{-1} than in the previous experiment 2 'Limitation' with the limited nutrient conditions showing counts between 10^2 and 10^3 cells ml^{-1}. There the growth started to decline in favor for an increased growth of *Monoraphidium sp.* reaching around 10^7 cells ml^{-1} in the left and right vessels with nutrient limitation. The middle vessels were more evenly distributed with N and P by diffusion from the neighboring N- and P- limited vessels and the cell counts of *Chlamydomonas sp.* remained the most stable there in both replicates. In this experiment 3 with balanced nutrient distribution in all vessels the cell count of *Monoraphidium sp.* was not increasing and seemed to be stable at 10^6 cells ml^{-1} along with the counts of *Chlamydomonas sp.* in both Gradostats. Comparing those cases where a balanced N- and P- distribution was provided to the vessels it indicates that *Chlamydomonas sp.* showed a higher resilience and a more stable growth under nutrient balanced conditions in competition with *Monoraphidium sp.* which had better competitive abilities in an N- and P- limited environment.

4.3.3 Resource distribution and biovolumes of Experiment 2 'heterogeneous' and 3 'homogenous'

in both experiments - 2 'Limitation' and 3 'Control' - the resource distributions were tracked by monitoring concentrations of dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP) within the three vessels of both Gradostat duplicates and compared. Those data are the main source for evaluating if and how much a resource gradient could be established over the three connected vessels within the Gradostat. SRP was chosen over TP in this case because the main focus lied on the analysis of the dispersal patterns of the dissolved resources over the system and not on the N/P limitation of the phytoplankton for which the DIN:TP ratio is recommended by Morris and Lewis (1988) as well as by Ptacnik (*et al.* 2010). Therefore the biomass proportion was left out.

Firstly the data show that in the experiment 2 'Limitation' gradients from a high to a low concentration over the three vessels in both Gradostats for SRP and in reverse for DIN were

established and could be maintained over the period of the experiment (Fig. 3.20). Those gradients created by the diffusion of the resources between the vessels showed non-linear patterns like it is predicted by the model of Ryabov and Blasius (2011) for spatially extended systems which expresses a non-linear change of resource concentrations over the system. Also the species have an effect on shaping the resource distributions over the system as it is more closely examined within the biovolume analysis. In the experiment 3 'Control' the resource concentration were similar in all vessels and showed as expected a homogenous distribution over the system without generating gradients.

Secondly for the higher nitrogen values it needs to be considered that the supply medium contained higher nitrogen concentrations. It was noticed that G2 had lower nitrogen concentrations than G1 in both experiments. The overall biovolumes of both species together were comparably similar in both Gradostats in both experiments (Exp.1. – 'Limitation' and Exp.2 – 'Control'). The resource supply happened from the same supply bottles with the same tubes over one peristaltic pump as one bottle supplied the same vessel in both Gradostats – for example one bottle supplied both left vessels of G1 and G2 to ensure a as maximum as possible conformity for the resource supply in both Gradostats. Although all tubes were tested for the desired medium supply rate right before every experiment some deviation may have occurred during the experiment due to the permanent mechanical exposure on the material which could have an effect on the amount of resource supply into the vessels.

For further insights into the influences of an established gradient of a heterogeneous distribution ('Limitation') or a homogenous distribution ('Control') of the two resources nitrogen and phosphorus on possible varying outcomes of competition between the two species *Monoraphidium sp.* and *Chlamydomonas sp.* the biovolumes of both species were analyzed.

Comparing the plots of the biovolumes (Fig. 3.21) of both species from the end stage of both experiments the differences in the biovolumes in the experiment 2 'Limitation' in relation to the experiment 3 'Control' stood out. The overall biovolumes of *Chlamydomonas sp.* were distinctly higher in the 'Control' set-up and led there to decreased biovolumes of *Monoraphidium sp.*. Between the vessels no differences between the biovolumes of both species could be determined which was expected in the control set-up since all vessels in both Gradostats were supplied with the same resource ratios. Also the inoculums were checked of

which the turbidity ratio of both species was calculated to be a 1:1 ratio and the amount of the stock cultures to reach approximately 1/50 of the volume of the vessel for each species. In the case of the experiment 'Control' more volume of the stock cultures was needed per vessel because lower turbidities were measured as in the experiment 'Limitation' but the added amounts were adequately calculated to equal the turbidity ratios of 1:1. Therefore the volume ratio of CHL:MON in 'Control' was 1,28 and in 'Limitation' 2,56 due to the different measured turbidities and required amounts to reach a turbidity ratio of 1:1 for both species. When only considering the volume it would suggest that *Chlamydomonas sp.* would have had a head start in the 'Limitation' set-up which was not the case as the biovolume data show. This suggests that *Chlamydomonas sp.* had an effect on the growth of *Monoraphidium sp.* and performed more successfully when the nutrients ratios were balanced as it was in the experiment 'Control'.

In the experiment 2 'Limitation' it was expected that the resource gradients would cause stronger differences in the biovolumes ratios between the vessels but only small differences could be seen within the biovolumes of *Monoraphidium sp..* The biovolume was a bit increased in the right N-limited vessel in comparison to the other two vessels. The most pronounced difference for the biovolumes of *Chlamydomonas sp.* was to be observed in the P-limited vessel where it declined over the last days. There the lowest biovolume content and the largest cell sizes of *Chlamydomonas sp.* were measured whereas the biovolumes in the other two vessels were comparable to each other. The temporal trend of biovolumes suggested that *Monoraphidium sp.* might have outcompeted *Chlamydomonas sp.* on the long run under P-limitation, though some immigration from the adjacent vessels probably would have prevented complete exclusion of *Chlamydomonas sp.*. Changes in cell size may reflect either an adjustment to the environment as surface:volume ratio affects nutrient uptake and cell size (Lewis. 1976) and colony formation affects susceptibility to grazing (Van Donk *et al*. 2011). Alternatively, a change in size may also be related to imbalanced nutrition under resource limitation for example to accommodate surplus carbon if cell division is constrained by lack of N and/or P.

The analysis of the ratios of the biovolumes of *Monoraphidium sp.* and *Chlamydomonas sp.* as depicted in Fig. 3.22 of the set-up with N/P-limitation and of the control set-up show that they were clearly separated from each other as expected: different resource distributions

(heterogeneous – 'Limitation' and homogenous – 'Control') would induce different outcomes of competition causing different biovolume ratios between the involved species. The biovolume ratios of the vessels in the homogenous set-up of experiment 3 'Control' were closer to the 1:1 ratio as well as more cumulated. There were no clear distinctions between the ratios of the vessels since all vessels were provided with the same balanced resource concentrations resulting in a similar outcome of competition in each vessel which was expected. It was also observed in the biovolume plots (Fig. 3.21) that *Chlamydomonas sp.* had higher growth rates under balanced conditions which is also indicated in the closer approach to the 1:1 ratio in comparison to the heterogeneous set-up. The ratios of the heterogeneous experiment 2 'Limitation' resulted in clear differences between the vessels under N- and P-limitation. The ratios of the P-limited vessels were congruent and clearly separated from the other vessel-ratios as the biovolumes of *Chlamydomonas sp.* were distinctively lower. Also the ratios of the NP-limitation were found to be very close together. The two ratios of the vessels with N-limitation varied as one ratio was situated close to the NP-limitations (Gradostat 2 – G2) while the second ratio had a higher biovolume content of *Monoraphidium sp.* (Gradostat 1 – G1). A possible explanation is a discrepancy in the N-concentrations between both Gradostats that occurred in both experiments and was described in the chapter 3.3.4.1 of the results. Gradostat 2 showed lower N-concentrations than Gradostat 1 in both experiments though it had no effect for the P-limited vessels where the varying N-supply would have had the highest impact. It even was the case that both biovolume ratios of the P-limited vessels were very congruent to each other due to the restriction of P. But it can be seen in the figure 3.20. that the N-content in the N-limited vessel of G1 was higher as in G2 where it was reduced nearly to zero. Since *Monoraphidium sp.* performed better with higher N-concentration this circumstance could have been an advantage as it can be seen in the higher biovolumes of *Monoraphidium sp.* in this vessel compared to G2 since N was the limiting factor. This issue didn't seem to have an effect on the homogenous nutrient-balanced 'Control' set up though.

In summary as seen within the heterogeneous experiment *Chlamydomonas sp.* showed rather strong differences between the limitations regarding cell-counts, biovolumes and cell-sizes. This was not that strongly expressed by *Monoraphidium sp.* where only rather weak tendencies like slightly lower cell-sizes in the nutrient depleted middle vessel or the higher biovolumes in the N-limited vessel could be distinguished and interpreted. Therefore it should also be considered that the species pairing of *Chlamydomonas sp.* and *Monoraphidium sp.*

was not the optimal pairing regarding their differences within N- & P- limited environments and preferably stronger differing species pairings should be tested. This could explain the weaker tendencies of Monoraphidium sp. and the rather similar concentrations of organisms. Also comparing it with the homogeneous set-up Monoraphidium sp. seemed to be the overall stronger competitor regarding limited environments which might also explain the rather similar concentrations of organisms.

Concluding of the outcome of both experiments the data suggested that *Chlamydomonas sp.* had an effect on the growth of *Monoraphidium sp.* and performed better when enough of both nutrients nitrogen and phosphorus were available as it was the case in the homogenous experiment 'Control'. It even invaded another homogenous set-up which implies being successful compared to the resident taxa (Experiment 1). Between the vessels no differences between the biovolumes of both species could be determined and *Chlamydomonas sp.* also reached higher biovolumes as in the other heterogeneous limited set-up where it was far less successful. It even was threatened to be outgrown by *Monoraphidium sp.* especially in the P-limited environments where it expressed compensations the nutrient restrictions with measurably larger cell sizes.

4.4 Assessment of the experimental results with the theoretical framework

How do the results of the experimental Gradostat fit with Ryabov's and Blasius (2011) model of a patchy meta-ecosystem to describe competition over continuously changing resource concentrations in spatially extended environments? The resource competition model of a patchy meta-ecosystem fuses the uniform habitat theory of Tilman (1980, 1982) with the spatially variable theory (Hsu & Waltman 1993, Smith & Waltman 1995, Wu et al. 2004). Therefore in a spatially variable habitat with two resources coexistence can be generated through both the trade-off by usage of the two resources (uniform habitat theory) and the trade-off by growth and therefore productivity (spatially variable theory) since denser cell concentrations of a species have a bigger influence on resource distributions.

The aim of the experimental Gradostat is to inhibit both main traits of a meta-ecosystem as described by Leibold (*et al.* 2004) and Holyoak (*et al.* 2005) to be homogenous on a local scale and at the same time expressing a heterogeneous 'patchy' environment on a regional level. The homogeneous environment within the local 'patch' is approached by the resource ratio theory of Tilman (1980, 1982). The heterogeneous environment as it is described by Ryabov A. & Blasius B. (2011) within their model of a spatial system was created by expressing a resource gradient where the resource reaches its highest concentration on the side where it is continuously supplied. Its concentration decreases then as it diffuses into the other vessels and has its lowest concentration in the vessel the furthest away from the supply point. Therefore several vessels connected together display the regional scale. The turbulence in each vessel contributes to a well-mixed and therefore homogenous concentration within each vessel displaying the local level.

As the achieved results demonstrated very evidently, the expectations for the functionality of the Gradostat system regarding the heterogeneous and homogenous traits of a meta-ecosystem and creating steady states over extended time periods were fulfilled. The experimental data were conform to the simulated datasets regarding the resource diffusions over the vessels creating non-linear gradients over the vessels in the heterogeneous set-up ('Limitation') generating a steady state or keeping a homogeneous environment in the 'Control' experiment. Also the biomass data show clear differences between the composition

of the species communities displaying varying outcomes of competition over the created gradients in the heterogeneous set-up and the gradient free homogeneous set-ups.

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List of figures

Acknowledgement: I have tried to trace all owners of the image rights and to acquire their consent for the usage of the images within this thesis. If a copyright infringement might become apparent I request for reporting it to me!

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Supplements

S1. Cell counts and biovolumes

S1.1. Cell counts

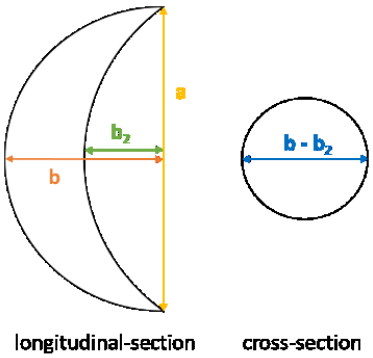
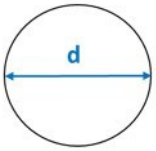
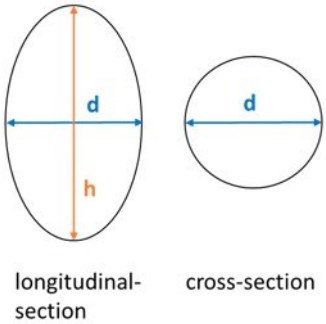
For counting the cells two counting chambers were used, the Neubauer Improved Chamber for the smaller species and higher cell densities and the Palmers Chamber for larger species and/or lower cell densities. Prior counting the samples were fixed with Lugol (1% on amount of sample), on 15 µL Lugol 1.5ml sample were pipetted and then stored in the fridge. To minimize counting errors as far as possible a minimum of 400 cells per species were counted of each sample.

The Neubauer Improved Chamber has the smallest grid fitting for counting the more dense cultures and smaller cells. It contains 16 squares with 50 µm side length each and every square is partitioned into 16 smaller squares with 156.25 µm² each. The depth of the chamber is 100 µm. The chamber was counted with the microscope Leica DM 750. The cell density was then calculated as followed: ml = (counted cells*1000 mm^3) / (count of smallest squares * width of of square unit² * depth of chamber) *or* ml = (counted cells*10^{12}) / (156,25*100*count of smallest squares).

In the Palmers Chamber 50µl of sample were counted and if the cell density was too high it was diluted. The Palmers chamber was preferably used for larger species like *Chlamydomonas* and/or if the cell density of the species was too low for the Neubauer Improved Chamber and the minimal count of 400 cells couldn't be reached. The Palmers chamber was counted under the Invert-Microscope Nikon Eclipse TS100.

S1.2. Biovolumes

With the invert microscope Nikon Eclipse TS100, the camera AxioCam MRc5 and the software 'AxioVision' (Re. 4.8.2.0, SP3) photos of the cells were taken and the cell size measured with the integrated measuring tools of the software. The volumes were then calculated out of the taken measures with formulas according to the cell-shapes taken from Hillebrand (*et. al.* 1999). From each sample 20 cells were measured and the mean-value calculated. This was used to compare biovolumes within the chambers of the Gradostat experiments.

Species	Anatomical shape
Monoraphidium sp. has a lunatic body and is circular in cross-section, therefore the shape remembers those of a banana. The Volume of the cell was calculated as following: $V = (b - b_2)^2 / 8 * (2b - (b - b_2) + a) * (pi^2 / 6 + 1)$	
Synechococcus elongatus' anatomical shape is the one of a sphere and the volume is calculated as following: $V = pi / 6 * d^3$	
Scenedesmus sp. has a prolate spheroid shape and its biovolumes is calculated as following: $V = pi/6 * d^2 * h$	

Tab. S1.1: Anatomical shapes and formulas for biovolumes.

S2. Peristaltik-Pump and Arduino

The Peristaltik-Pump is a main component of the experimental Gradostat-set-up because it regulates the in- and outflow in each vessel of the Gradostat and also the sampling of the Gradostats were performed with it. It was operated by a programmed microcontroller – the Arduino Uno.

S2.1. Peristaltik-Pump

For most of the experiments the Peristaltik Pump BVP-Process IP 65, Ismatec (Unit of IDEX-Corporation) was used except for one Exponential Fed Batch experiment with the species *Chlamydomonas sp.* (see chapter 2.3.2 Exponential Fed Batch) where the Peristaltik Pump Ecoline, Ismatec (Unit of IDEX-Corporation) was used. Both pumps are working comparably.

The speed and the direction of rotation are adjustable. The pump gives off heat over its metallic casing for cooling the machine.

Each tube has a „stopper“, which protects it from being dragged through the pump. The stoppers were manually glued onto the tubes with instant glue after the autoclaving process because the glue couldn’t resist the heat and pressure of the autoclave. The tubes themselves need to be suited for autoclaving, they shouldn’t deform and need to be inert since the medium is transported over a huge surface within the tubes. The delivery rate of the pump also depends of the inner diameter of the used tube.

Evaluation of the delivery rate at different pump speeds for the BVP-Process IP 65 pump:

At first it needed to be evaluated how much of water (Aqua dest. with a density of 1) can be pumped in a certain time within a tube with 0.51mm diameter. For the measurement 5 tubes/replicates were taken and with the same tension clamped onto the peristaltic pump and be ensured that the tubes were filled with water through letting the pump running until the water started to drop out at the other end of the tube to start with similar conditions. Then empty epis were weighed and the time measured with a stop watch while filling them with the pump at a certain speed. This was repeated at 5 different rotational speeds (50, 25,

10, 5, 1). Then the difference of the weight of the empty and full epis were calculated, the deviations calculated and mean and standard deviation assessed.

The delivery rates of the tubes shouldn't differ between each other more than maximum 3%. It was also estimated if the differences between the delivery rates might vary at different rotational speeds of the pump.

	tube 1		**tube 2**		**tube 3**		**tube 4**		**tube 5**	
pump-speed	mean	stdabw	mean	stdabw	mean	stdabw	mean	stdabw	mean	stdabw
50	1,601	0,081	0,372	0,116	0,620	0,091	2,542	0,149	0,692	0,149
25	1,325	0,180	0,605	0,112	0,159	0,072	1,300	0,310	0,421	0,092
10	0,756	0,058	0,754	0,062	0,089	0,175	0,325	0,068	0,375	0,175
5	0,631	0,076	0,714	0,098	0,101	0,098	0,357	0,060	0,289	0,126
1	0,478	0,250	0,901	0,210	0,218	0,164	0,310	0,142	0,521	0,192

Tab. S1.2: Mean & standard-deviation [in %] of the deviations of the delivery rate of a pump for the tubes with 0.51 mm diameter.

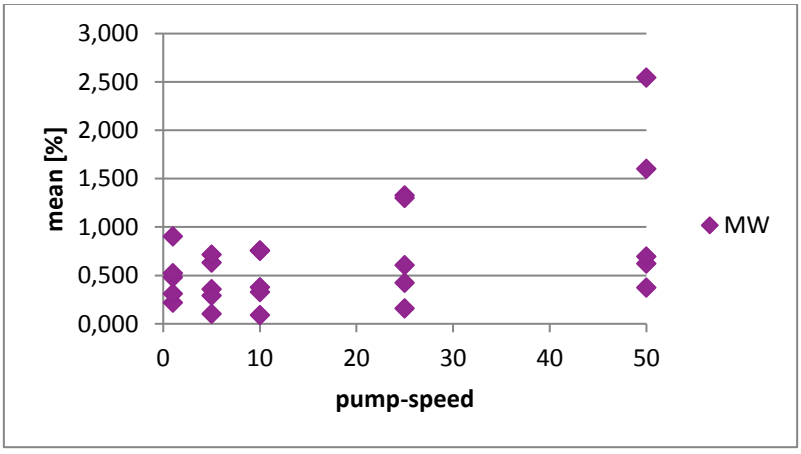


Fig. S1.1: Mean of the deviations of the delivery rate of a pump [%].

As it can be visualized in the figure S1.1 the deviations of the delivery rates of the pump are very small especially in the lower areas of the rotational speed of the pump (speeds of 5 to 10 (pump speed 10 = 3 ml h^{-1}, pump speed 30 = 9,5 ml h^{-1}, pump speed 180 = 51.6 ml h^{-1}; Linearity of the pump speed with $y = 0,2839x + 0,5459, R^2 = 0,9998$)) and are increasing with higher speeds. That means with increasing speed the pump is getting more inaccurate.

S2.2. Arduino

The Arduino UNO is a microprocessor with an open source self-programmable software. It was connected to the peristaltic pump and controlled the rotational speed and the time the pump was running (as being switched on) or switched off. Three scripts were written with the Software Arduino 1.0.5-r2 (Banzi M. *et al.* 2014) for the needs of an Exponential Fed Batch, sampling of the Gradostat and keeping the volume of the Gradostat at a certain level.

The scripts were designed to calculate the time the pump needed to be running in order to pump the required volumes so that the pump was running a certain amount of time each hour to have a consistent transport of volume over a certain time course. Each hour was a cycle where the pump had a certain amount of time running and the rest of the time pausing so that it was pumping every hour and provided a consistent distribution of resources to the experiment. The calculations for the on- and off cycles of the pump in order to pump the required volumes was performed with a loop which is defined as one hour per cycle. The scripts were able to perform the calculations of the required volumes based on the volume within the experiment (Gradostat or the EFB), the dilution factor per day, the capacity of the pump per hour in ml and the amount of time in hours the pump should be running.

Please note the pump itself didn't set its speed with ml h^{-1} but with an intern speed measuring. Moreover the Arduino has an intern speed measure too which also varies from the speed that is displayed on the pump. Therefore the speed couldn't be taken from the pump display directly. Therefore the value of the variable "set_pump_capacity" in ml h^{-1} needs to be coherent to the intern pump speed "outp_pmw_speed_pump", which needs to be acquired via testing first. The first variable is needed to calculate the volumes and time of activation of the pump and the second variable is the actual speed of the pump which the Arduino uses to signal the pump. For accuracy I proceeded to test it by measuring the pumped volumes before each set-up.

The times it was running was about 3-5 days, after which it needed to be sampled or in the case of the EFB the volume within the EFB needed to be set back since the added volume would fill the bottles. For example the pump speed of time had a capacity of 3.0 ml $h^{-1.}$.The scripts were tested via simulation on the online tool 'jsfiddle' (www.jsfiddle.net) where the output of the scripts could be displayed and weighting the provided volume and calculating

the transfer of ml h^{-1}. The time for a run was also estimated with the online page jsfiddle.com where the program was simulated and the outputs could be displayed.

S2.2.1. Script for the Exponential Fed Batch

This script added the required volume to an Exponential Fed Batch based on the start volume.

```
int outp_pmw_speed_pump = 5;
int outp_220v = 7;
int outp_run_backwards_pump = 8;

// Insert your data into the "set_#"-variables:
double set_volume_bottles = 40;  // set start-volume of your experiment/bottles in ml
double set_dilution_factor_day = 0.2;  // set factor for dilution per day
double set_pump_capacity = 3.4;  // set capacity of pump in ml h-1
int set_runtime_hours = 168; // set runtime of experiment until sampling/ volume reset, only full hours

// Don't change following variables:
int count = 1;
long pump_on_time = 1;   // time that pump is running in ms
long pump_off_time = 1;  // time that pump is off
double vol_increase = 1;    // amount of volume that is added per hour by the pump

void setup() {
  pinMode(outp_pmw_speed_pump, OUTPUT);
  pinMode(outp_220v, OUTPUT);
  pinMode(outp_run_backwards_pump, OUTPUT);
}

int runtime_hours = set_runtime_hours + 1;
```

```
void loop()  // 1 Loop = 1 hour
{
 if (count < runtime_hours)
 {
  vol_increase = (set_volume_bottles * set_dilution_factor_day) / 24; //amount of volume added per hour
  set_volume_bottles = set_volume_bottles + vol_increase; // amount of current volume
  pump_on_time = (long) ((vol_increase / set_pump_capacity) * 60 * 60 * 1000);   // pump on in ms
  pump_off_time = 3600000 - pump_on_time; // defines time where pump is off in ms
  analogWrite(outp_pmw_speed_pump, 10);     // insert fitting speed of pump for your "set_pump_capacity" value, pump has an intern speed measure which needs to be acquired through testing, turns the Pump on (f.ex. capacity of 3.4 ml h-1 has a speed of 10 in this script, on the pump display the outp_pmw_speed_pump = 8 would be depicted as value 1).
  delay(pump_on_time);
  analogWrite(outp_pmw_speed_pump, 0);   // switches pump off
  delay(pump_off_time);
  count = count + 1;  // counts the hours = loops
 }
 else
 {
  analogWrite (outp_pmw_speed_pump, 0);
  delay(3600000);
 }
}
```

S2.2.2. Script for the Gradostat to keep the Volume

This program was an adapted version of the Exponential Fed Batch script to only provide the amount of medium needed to keep the dilution rate at a certain stable volume.

```
int outp_pmw_speed_pump = 5;
int outp_220v = 7;
int outp_run_backwards_pump = 8;

// Insert your data into the "set_#"-variables:
double set_volume_bottles = 800;  // set start-volume of your cultures
double set_dilution_factor_day = 0.2;  // set factor for dilution per day
double set_pump_capacity = 9.5;  // set capacity of pump in ml h-1
int set_runtime_hours = 168; // set only full hours

// Don't change following variables:
int count = 1;
long pump_on_time = 1;   // time that pump is running in ms
long pump_off_time = 1;  // time that pump is off
double vol_increase = 1;    // amount of volume that is added per hour by the pump

void setup() {
  pinMode(outp_pmw_speed_pump, OUTPUT);
  pinMode(outp_220v, OUTPUT);
  pinMode(outp_run_backwards_pump, OUTPUT);
}

int runtime_hours = set_runtime_hours + 1;

void loop()  // 1 Loop = 1 hour
{
  if (count < runtime_hours)
```

```
 {
  vol_increase = (set_volume_bottles * set_dilution_factor_day) / 24;  //amount of volume added per hour
  pump_on_time = (long) ((vol_increase / set_pump_capacity) * 60 * 60 * 1000);   // pump active in ms
  pump_off_time = 3600000 - pump_on_time; // defines time where pump is off
  analogWrite(outp_pmw_speed_pump, 30); // insert fitting speed of pump for your "set_pump_capacity" value, pump has an intern speed measure which needs to be acquired through testing, turns the Pump on (f.ex. capacity of 3.0 ml h-1 has a speed of 10).
  delay(pump_on_time);
  analogWrite(outp_pmw_speed_pump, 0); // switches pump off
  delay(pump_off_time);
  count = count + 1; // counts the hours = loops
 }
 else
 {
  analogWrite (outp_pmw_speed_pump, 0);
  delay(3600000);
 }
}
```

S2.2.3. Script for sampling the Gradostat

Sampling was done with the peristaltic pump to simultaneously withdraw the same amount of volume from each vessel. This program calculated the needed runtime of the pump for extracting the sample-volume. Afterwards the volume was refilled with an adapted version of the first program for the Exponential Fed Batch.

```
int outp_pmw_speed_pump = 5;
int outp_220v = 7;
int outp_run_backwards_pump = 8;

// Insert your data into the "set_#"-variables:
double set_volume_bottles = 800;  // set start-volume of your cultures in ml
double set_volume_sample = 32; // set volume of amount to be sampled in ml
double set_dilution_factor_day = 0.2;  // set factor for dilution per day
double set_pump_capacity = 51.6;  // set capacity of pump in ml h-1
int set_runtime_hours = 1; // only full hours!

// Don't change following variables:
int count = 1;
long pump_on_time = 1;   // time that pump is running in ms
long pump_off_time = 1;  // time that pump is off
double vol_increase = 1;    // amount of volume that is added per hour by the pump

void setup() {
  pinMode(outp_pmw_speed_pump, OUTPUT);
  pinMode(outp_220v, OUTPUT);
  pinMode(outp_run_backwards_pump, OUTPUT);
}
int runtime_hours = set_runtime_hours + 1;

void loop()  // 1 Loop = 1 hour
```

```
{
 if (count < runtime_hours)
 {
  pump_on_time =  ((set_volume_sample / set_pump_capacity) * 60 * 60 * 1000);  // pump on in ms
  pump_off_time = 3600000 - pump_on_time; // defines time where pump is off
  analogWrite(outp_pmw_speed_pump, 180); // insert fitting speed of pump for your "set_pump_capacity" value, pump has an intern speed measure which needs to be acquired through testing, turns the Pump on (f.ex. capacity of 3.0 ml h-1 has a speed of 10).
    delay(pump_on_time);
    analogWrite(outp_pmw_speed_pump, 0);   // switches pump off
    delay(pump_off_time);
  }
 else
 {
  analogWrite (outp_pmw_speed_pump, 0);
  delay(3600000);
 }
}
```

S3. Screening for suitable phytoplankton species

S3.1. Maximum Growth Rates

Here are the tables with the data used for calculating the graphs for the maximum growth rates μ_{max} of the six phytoplankton species *Monoraphidium sp.* (MON), *Scenedesmus sp.* (SCE), *Stichococcus sp.* (STI), *Synechococcus elongatus* (SYN) and *Microcystis aeruginosa* strain ΔMCY A- (MCY) and PCC 7806 (PCC). Of each species duplicates were maintained and the chlorophyll a levels measured to obtain the growth rate μ. Then the strongest periods of growth were taken (marked green in table S3.1) to determine the maximum growth rate μ_{max}. The maximum growth rate μ_{max} for each species was calculated as mean from the μ of the steepest growth phases of the duplicates (tab. S3.2).

Species	MCY				PCC				SYN			
Duplicates	1		2		1		2		1		2	
Day	Chl a (LN Ft)	µ	Chl a (LN Ft)	µ	Chl a (LN Ft)	µ	Chl a (LN Ft)	µ	Chl a (LN Ft)	µ	Chl a (LN Ft)	µ
4	4,533		4,605		4,635		4,644		4,511		4,543	
5	4,644	0,112	4,533	-0,073	4,644	0,010	4,700	0,056	5,220	0,709	5,100	0,557
6	4,727	0,083	4,575	0,042	4,820	0,176	4,828	0,128	5,684	0,463	5,680	0,580
7	5,182	0,454	5,050	0,475	5,112	0,292	5,124	0,296	6,219	0,535	6,188	0,508
8	5,278	0,096	5,153	0,103	4,875	-0,237	4,890	-0,234	6,400	0,182	6,360	0,171
9	5,380	0,102	5,394	0,240	5,017	0,142	5,094	0,203	6,735	0,334	6,666	0,306
10	5,710	0,331	5,606	0,212	5,187	0,170	5,165	0,071	6,952	0,217	7,071	0,405
13	4,977	-0,734	4,812	-0,794	3,989	-1,198	3,970	-1,194	6,436	-0,516	6,477	-0,594
14	5,231	0,254	5,153	0,341	4,043	0,054	4,043	0,073	6,796	0,360	6,812	0,335
15	5,529	0,298	5,455	0,302	4,143	0,100	4,234	0,191	6,978	0,183	7,029	0,217
16	5,889	0,359	5,908	0,453	4,407	0,264	4,127	-0,107	7,141	0,163	7,347	0,318
17	6,142	0,253	6,059	0,151	4,263	-0,144	4,357	0,230	7,268	0,127	7,460	0,114
20	4,663	-1,479	4,331	-1,728	3,912	-0,351	3,584	-0,773	5,024	-2,244	5,464	-1,997
21	4,771	0,107	4,727	0,397	4,111	0,199	4,025	0,442	4,060	-0,963	4,304	-1,160
22	5,004	0,233	4,898	0,170	4,007	-0,104	3,738	-0,288	3,951	-0,109	4,419	0,115
23	5,242	0,238	5,094	0,196	3,850	-0,157	3,091	-0,647	4,304	0,353	5,075	0,656
24	5,565	0,323	5,421	0,327	3,829	-0,022	3,584	0,492	5,273	0,969	6,133	1,058
Species	SCE				MON				STI			
Duplicates	1		2		1		2		1		2	
Day	Chl a (LN Ft)	µ	Chl a (LN Ft)	µ	Chl a (LN Ft)	µ	Chl a (LN Ft)	µ	Chl a (LN Ft)	µ	Chl a (LN Ft)	µ
4	8,719		8,518		8,148		7,990		7,924		7,803	
5	9,139	0,420	9,108	0,590	8,595	0,447	8,545	0,554	8,356	0,432	8,341	0,538
6	9,303	0,163	9,261	0,152	8,859	0,265	8,741	0,196	8,480	0,124	8,506	0,165
7	9,601	0,298	9,574	0,314	9,200	0,341	9,074	0,333	8,693	0,213	8,769	0,263
8	9,732	0,131	9,658	0,084	9,374	0,175	9,298	0,224	8,826	0,133	9,050	0,281
9					9,558	0,183	9,570	0,272	9,026	0,200	8,971	-0,079
10	10,097		10,131		9,795	0,238	9,782	0,212	9,148	0,122	9,220	0,249
13	9,587	-0,510	9,531	-0,600	9,440	-0,356	9,360	-0,422	8,687	-0,461	8,753	-0,467
14	7,352	-2,235	7,532	-2,000	7,070	-2,370	7,105	-2,255	6,925	-1,763	6,871	-1,882
15	7,781	0,428	7,942	0,411	7,972	0,903	8,089	0,984	7,328	0,403	7,263	0,392
16	9,221	1,440	9,436	1,494	8,908	0,936	8,926	0,836	8,401	1,074	8,355	1,093
17	9,674	0,453	9,836	0,401	9,311	0,402	9,391	0,466	8,820	0,418	8,888	0,533
20	9,501	-0,173	8,642	-1,195	8,067	-1,244	8,216	-1,175	7,435	-1,384	7,313	-1,575
21	6,846	-2,655	5,989	-2,653	5,903	-2,165	5,914	-2,303	7,769	0,334	7,697	0,384
22	7,588	0,742	6,966	0,977	7,623	1,721	7,766	1,852	8,514	0,745	8,481	0,784
23	9,036	1,448	8,738	1,772	8,652	1,029	8,766	1,000	9,064	0,550	9,108	0,627
24	9,875	0,840	9,568	0,831	9,537	0,886	9,598	0,832	9,528	0,464	9,701	0,593

Tab S3.1: Values of chlorophyll a (logarithm of Ft) and µ used to calculate the maximum growth rates. Six phytoplankton species *Monoraphidium sp.* (MON), *Scenedesmus sp.* (SCE), *Stichococcus sp.* (STI), *Synechococcus elongatus* (SYN) and *Microcystis aeruginosa* strain ΔMCY A- (MCY) and PCC 7806 (PCC) were analyzed. The green marked values are the growth rates µ of which the maximum growth rate μ_{max} were calculated.

Species	MCY	PCC	SYN	SCE	MON	STI
μ_{max}_mean	0,302	0,092	0,435	1,101	1,220	0,652
μ_{max} _STDEV	0,014	0,001	0,014	0,130	0,012	0,029

Tab.S3.2: Maximum growth rate μ_{max}. Calculated as mean (and standard deviation) from the µ of the steepest growth phases of the duplicates for each of the six phytoplankton species *Monoraphidium sp.* (MON), *Scenedesmus sp.* (SCE), *Stichococcus sp.* (STI), *Synechococcus elongatus* (SYN) and *Microcystis aeruginosa* strain ΔMCY A- (MCY) and PCC 7806 (PCC)

S3.2. Monoculture Experiments (EFB) to determine R*

For estimating R* via graphical analysis within the figure 3.4 the nutrient concentrations from the N- and P limited cultures were measured and the mean and standard deviations calculated from three time points and from the triplicates of all six species *Monoraphidium sp.* (MON), *Scenedesmus sp.* (SCE), *Stichococcus sp.* (STI), *Synechococcus elongatus* (SYN) and *Microcystis aeruginosa* strain ΔMCY A- (MCY) and PCC 7806 (PCC).

Species	**P [µg L^{-1}]**		**NO_3^- [µg L^{-1}]**	
	mean	**stdev**	**mean**	**stdev**
MCY	724,683	34,017	2216,736	1240,227
MON	23,614	10,717	933,883	770,885
PCC	795,649	65,022	4683,925	493,147
SCE	178,789	149,741	29,633	49,140
STI	726,965	52,915	2630,173	396,565
SYN	348,088	144,859	580,008	500,533
CHL	10,036	3,320	35,711	10,737

Tab.S3.3: Mean and standard deviation of the N and P measurements of three time points and the triplicates for R* calculation. Six phytoplankton species tested: *Monoraphidium sp.* (MON), *Scenedesmus sp.* (SCE), *Stichococcus sp.* (STI), *Synechococcus elongatus* (SYN) and *Microcystis aeruginosa* strain ΔMCY A- (MCY) and PCC 7806 (PCC).

S4. Community experiments with the Gradostat

S4.1. Experiment 1 – Two species community with invasion (homogenous)

In this experiment a community of *Monoraphidium sp.* and *Synechococcus elongatus* with the invasion of a third species *Chlamydomonas sp.* was analyzed. To identify shifts between the ratios of the three species the proportion of the biovolumes in percent of each species within each vessel the Gradostat duplicates were calculated and set in a ratio.

[%]	vessel	MON	SYN	CHL
Gradostat 1	left	9,046	17,544	0,002
	middle	11,839	23,292	0,002
	right	11,671	26,603	0,001
Gradostat 2	left	8,615	8,752	0,004
	middle	14,770	8,198	0,008
	right	37,770	21,880	0,002
mean vessels	G1	10,8521	22,4795	0,0018
	G2	20,3850	12,9435	0,0049
stdev vessels	G1	1,5661	4,5842	0,0004
	G2	15,3673	7,7446	0,0032
% total biov.	G1	32,5562	67,4386	0,0053
	G2	61,1550	38,8304	0,0146

Tab. S4.1: Proportional biovolumes in percent [%] from the total biovolume per Gradostat. Depicted for each vessel and as mean and standard deviation for each Gradostat (G1 and G2 for the duplicates). MON = *Monoraphidium sp.*, SYN = *Synechococcus elongatus*, CHL = *Chlamydomonas sp.,* biov = biovolume.

S4.2. Experiment 2 & 3 - Limitation and Control

Graphical depiction of the total sum of biovolumes of *Monoraphidium sp.* and *Chlamydomonas sp.* in both Gradostat duplicates. To estimate the difference between both experiments the mean of the total biovolumes of both duplicates in both experiments was calculated and the ratio to each other defined. The total biovolume of the 2nd Experiment 'Limitation' was about 18,4 times higher than in the 3rd experiment 'Control'.

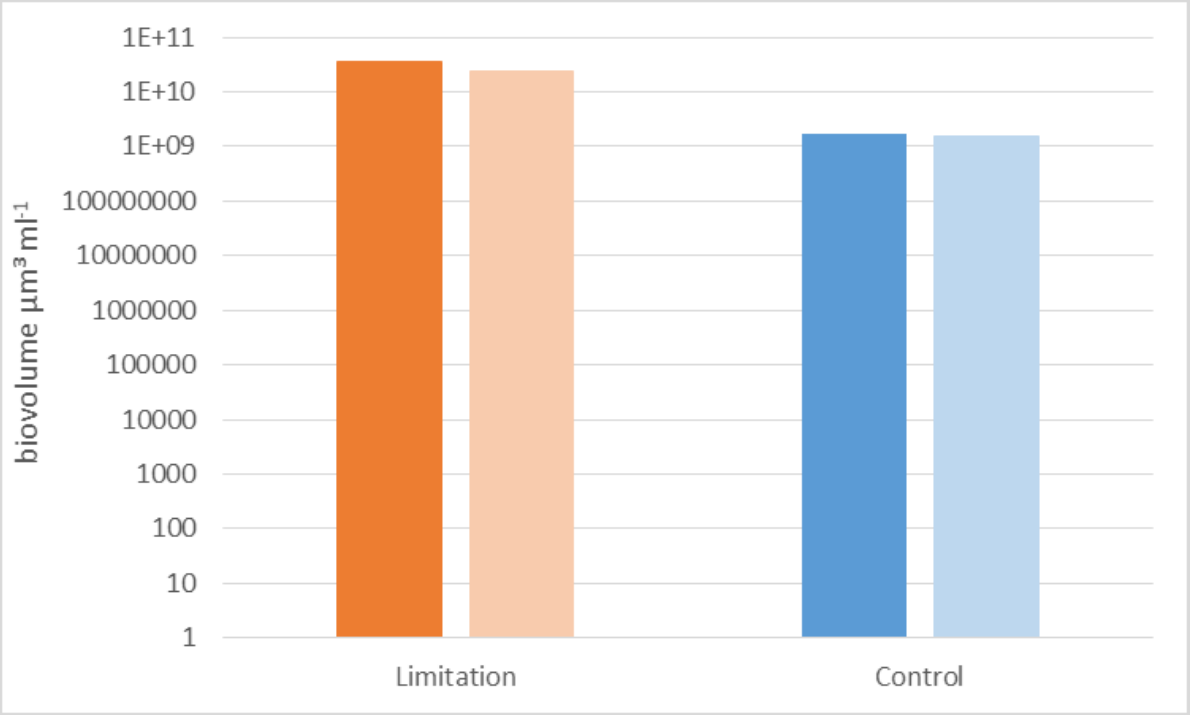


Fig.S4.2: Total sum of biovolumes of *Monoraphidium sp.* and *Chlamydomonas sp.* in both Gradostat duplicates G1 (left column, strong color) and G2 (right column, light color) as mean of the last two time points of both experiments Limitation (red) and Control (blue); y-axis log-transformed.

To identify shifts between the ratios of the species *Monoraphidium sp.* and *Chlamydomonas sp.* the proportion of the biovolumes in percent of each species within each vessel the Gradostat duplicates were calculated and set in a ratio.

MON:CHL [µm³ ml⁻¹]	Control	Limitation
G1_L	13,857	290518,005
G1_M	62,520	31944,653
G1_R	29,872	124102,528
G2_L	12,968	320990,397
G2_M	24,601	33069,468
G2_R	20,492	43415,699

Tab.S4.3: Biovolume ratios of *Monoraphidium sp.* (MON) and *Chlamydomonas sp.* (CHL) of each vessel (L-left, M-middle, R-right) of each Gradostat-duplicate (G1, G2) of the homogenous 'Control' and heterogeneous 'Limitation' experiments. Ratios calculated of MON:CHL in µm³ ml^{-1}.

Limitation, day 16		**µmol/L**		**Control, day 28**		**µmol/L**
G1	**P**	**N**		**G1**	**P**	**N**
left (-P)	3,81641732	196,484438		**left**	49,2488891	336,026447
middle (-NP)	0,41109078	23,4510563		**middle**	50,2329443	333,059184
right (-N)	27,2844688	22,0883728		**right**	48,4493442	327,060152
G2				**G2**		
left (-P)	1,79534556	130,81761		**left**	38,7317988	237,945493
middle (-NP)	0,47686531	18,5228189		**middle**	34,5495641	202,499597
right (-N)	20,0492711	1,84647637		**right**	38,7010471	231,930334

Tab.S4.4: DIN:TP data for the Fig.3.20 in the chapter 3.3.4.1.

S5. Equipment and Machines

Microscopes

Invert-microscope:

Nikon Eclipse TS100

Camera: AxioCam MRc5

Software: AxioVision Re. 4.8.2.0, SP3

AxioVS40 V 4.8.2.0, Copyright 2006-2010, Carl Zeiss MicroImaging GmbH

Microscope:

Leica DM 750

Counting chambers:

Neubauer improved chamber

Palmer chamber

Fluorometer:

AquaPen

AP 100 (SN-AP-140), Photon Systems Instruments

Software: FluorPen 1.0.4.1, Photon Systems Instruments, Copyright 1996-2006 PSI, Ltd.

Photometer:

Spectroquant pharo 300, MERCK

Software-Version: 1.40

IQ-Lablink-Version: 1.20

CleanBench:

Safemate 1.2, BioAir

Fluorescence Spectrophotometer:

F-7000, Hitachi

F-7000 v. Inula

Software: UVprobe

Pumps:

Peristaltik Pump: BVP-Process IP 65, Ismatec (Unit of IDEX-Corporation)

Peristaltik Pump: Ecoline, Ismatec (Unit of IDEX-Corporation)

Arduino:

Arduino UNO

Software: Arduino 1.0.5-r2

Bubble counter:

CO2-Bubble counter, Pro Flora Count, SBL GmbH

Dosage on 50 L: 4° dKH: 5 bubbles/min

10 dKH: 12 bubbles/min

Air pump:

Air pump 100, EHEIM

100L/h; 3,5Watt; 230V; 50Hz; 2 Hmax m

Tubes:

- Tubes used for Peristaltik pump: PharMed BPT, ID: 0,51mm, wall: 0,90 mm, ISMATEC (IDEX-Corporation)
- Transparent tube: Rotilabo-PTFE-Tube, diameter inside 2mm, wall 3mm; ROTH
- Big transparent tube: Rotilabo-Silikontube, diameter inside 4mm, wall 6mm; ROTH Reference: 07053905Fi; SC0339 – 9523
- Fat tube: : PharMed BPT, ID: 2,54 mm, wall 0,85 mm, ISMATEC (IDEX-Corporation) Reference: 07053917Fi; SC0352 – 9536

Time switch:

MZ 20, Brennenstuhl