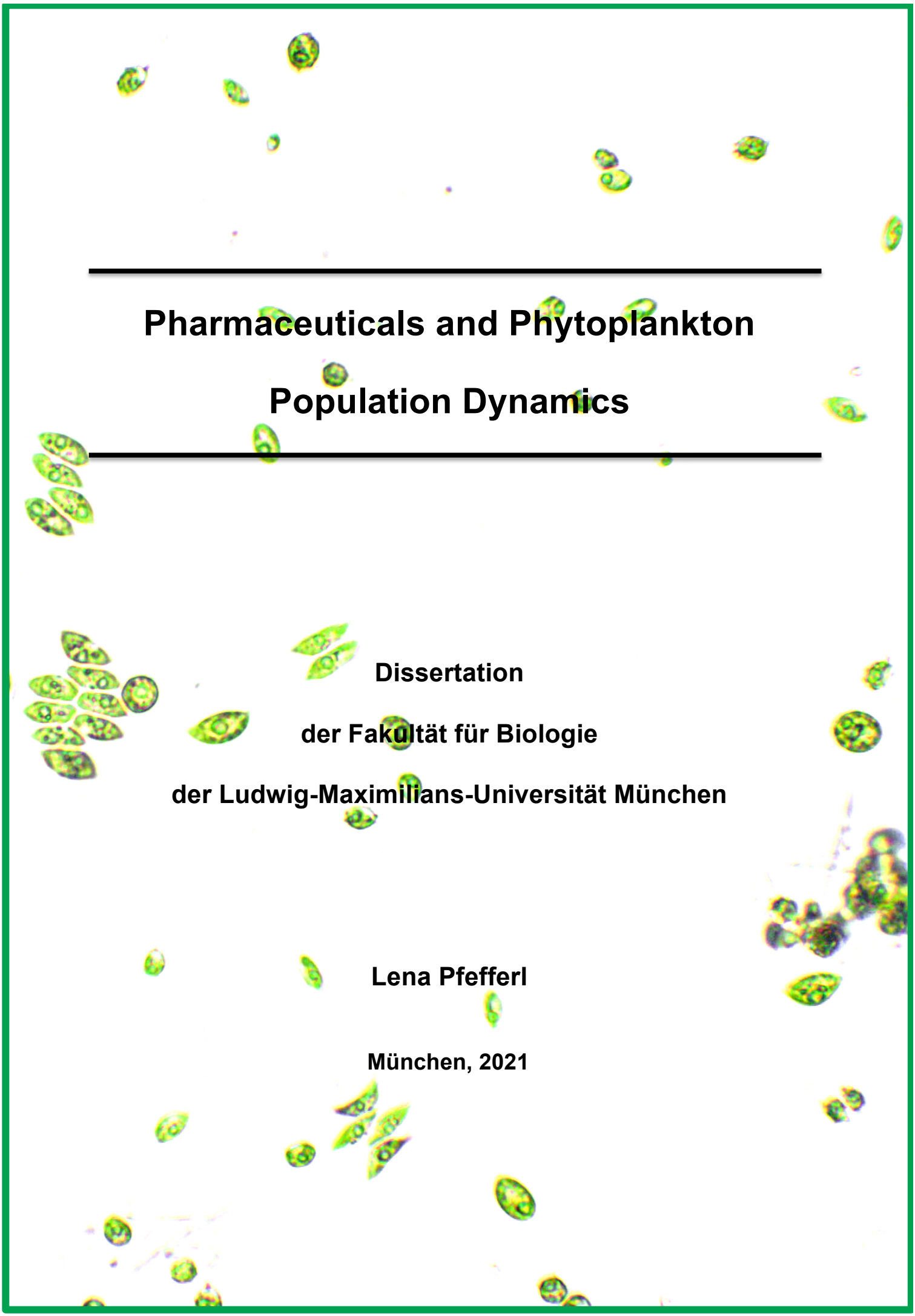

Pharmaceuticals and Phytoplankton Population Dynamics

Dissertation

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ERKLÄRUNG

Ich versichere hiermit an Eides statt, dass meine Dissertation selbständig und ohne unerlaubte Hilfsmittel angefertigt worden ist.

Die vorliegende Dissertation wurde weder ganz, noch teilweise bei einer anderen Prüfungskommission vorgelegt.

Ich habe noch zu keinem früheren Zeitpunkt versucht, eine Dissertation einzureichen oder an einer Doktorprüfung teilzunehmen.

München, den 28.07.2021

Lena Pfefferl

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2. Summary

Aquatic ecosystems and thus algae are globally exposed to pollutions of different types and degrees. One of them is the exposure to an increasing variety of pharmaceuticals. Due to an increasing human population and an ageing society the usage of pharmaceuticals will further increase and thus the potential pollution of aquatic ecosystems. It has already been shown that pharmaceuticals can affect algae in different ways. As algae are primary producers, effects of pharmaceuticals on their dynamics could cause detrimental consequences for food chains and to ecosystem functioning. Already minor changes such as shifts in growth of only one algal species could lead to shifts in community dynamics thereby influencing entire food chain functioning.

My thesis describes how single pharmaceuticals and a mixture of three different pharmaceuticals at environmentally relevant, hence low concentrations affected the biomass production and photosynthetic efficiency of selected algae. Experiments with mono- and polycultures consisting of algal species cultured in the laboratory and with algae from natural assemblages were conducted. Algae showed different dynamics to the three pharmaceuticals, however all three substances affected biomass production and photosynthetic efficiencies. Additionally, the experiments showed that the exposure to a mixture of pharmaceuticals affected the algae in different ways than would have been expected from results where algae were only exposed to single pharmaceuticals. In some cases the effects remain similar but in other cases weaker (antagonistic) or stronger (synergistic) effects were observed. My results also highlight that biotic interactions between algae play an important role when investigating the effects of stressors. Biotic interactions can change the response of single algal species to pharmaceuticals which makes it more difficult to predict responses of algal communities to such stressors from tests with monocultures. Additionally, biodiversity cannot only affect community responses to pharmaceuticals by influencing stability but also by compensatory growth responses.

My results show that with increasing complexity negative or positive effect sizes on specific algae can be compensated on community level depending on the stressor and the algal species. Additionally, the variability of responses was affected by diversity: The coefficient of variance decreased with higher diversity in all pharmaceutical treatments and for all

investigated algal species. Furthermore my study indicated a change in the community structure due to the impact of one of the investigated pharmaceuticals, even at comparably low concentrations.

The effects of biodiversity on community responses to pharmaceutical substances were also seen in experiments with natural phytoplankton, which contrary to laboratory communities share an evolutionary history. The pharmaceutical Carbamazepine changed growth dynamics of phytoplankton communities of three lakes. Diversity manipulations resulted in diversity gradients within each lake community. Within these diversity gradients a significant effect of diversity on the strength of growth responses to Carbamazepine was visible.

In summary my results point towards the need to include more biological complexity in methods and studies analysing effects of pharmaceutical stressors on aquatic communities. Testing monocultures of species in highly controlled environments is necessary to fulfil basic ecotoxicological standards but may not be enough to predict effects of such stressors in a natural environment.

3. Introduction

3.1 Chemical Pollutants in Aquatic Ecosystems

Aquatic ecosystems have to deal with multiple stressors as the following short excerpts of publications show:

“Industrialized animal production—a major source of nutrient and microbial pollution to aquatic ecosystems” (Malin et al. 2003).

“Loss of Biodiversity in Aquatic Ecosystems: Evidence from Fish Faunas” (Moyle and Leidy 1992).

“The Challenge of Micropollutants in Aquatic Systems” (Schwarzenbach et al. 2006)

“Role of bottom sediments in the secondary pollution of aquatic environments by heavy metal compounds” (Linnik and Zubenko 2002).

The main environmental issues aquatic ecosystems have to deal with are global warming, depletion of the ozone layer, species extinction, degradation of habitats and human induced water pollution (Fent 2013). The increasing worldwide contamination of freshwater systems with thousands of industrial and natural chemical compounds is one of the key environmental problems facing humanity. Although most of these compounds are present at low concentrations, many of them raise considerable toxicological concerns, particularly when present as components of complex mixtures (Schwarzenbach et al. 2006).

Water-related diseases are among the most common causes of illness and death, affecting mainly the poor in developing countries. In 2019, nearly two million deaths were caused due to water sanitation hygiene-associated diarrhoeas and some other water/sanitation-associated diseases (schistosomiasis, trachoma, intestinal helminth infections) (UNESCO, UN-Water 2020). The increasing chemical pollution of surface and groundwaters, will worsen the largely unknown long-term effects on aquatic life and on human health. More than one-third of the Earths accessible renewable freshwater is used for agricultural, industrial, and domestic purposes, and most of these activities lead to water contamination (Schwarzenbach et al. 2006).

Currently, 100 000 chemicals out of the approximately 5 million known chemical compounds are in use. Around 500 – 1000 new chemical compounds are introduced annually. Chemicals from industries, households, pesticides and pharmaceuticals from human and veterinary use are released into the environment via different pathways (Fig. 1).

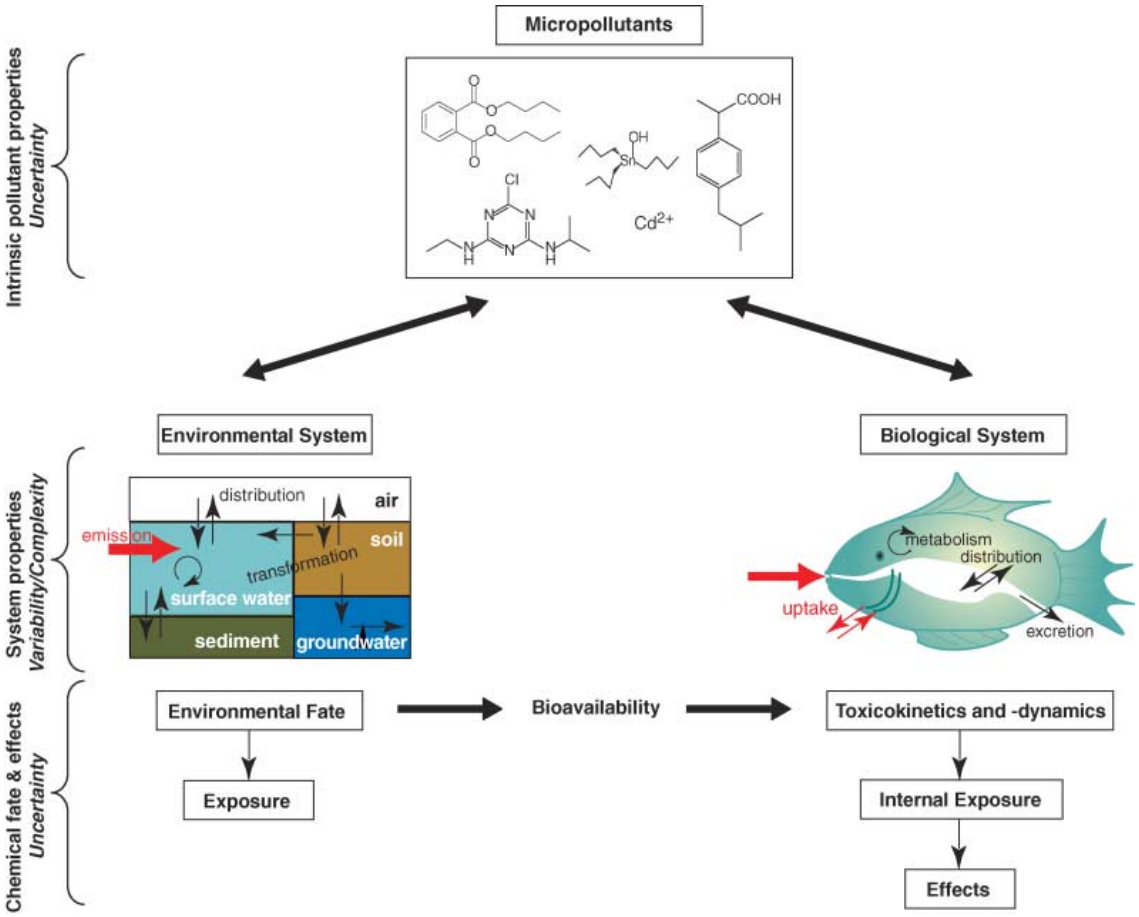


Fig. 1: Chemical compounds can enter the environmental system in various ways. Pollutants interact with environmental and biological systems according to their physicochemical properties and reactivities. Final exposure and risk assessment will always be subject to uncertainty due to inherent variability and complexity of environmental and biological systems (Reference **Fig. 1**: Schwarzenbach et al. 2006).

Some of these compounds have direct or indirect negative impacts on the biotic and abiotic environment. A toxic effect of a compound would be an example for a direct negative

impact, whereas accumulations of chemicals in the soil, in organisms or the disruption of ecological interactions are indirect impacts. These anthropogenic stressors generally cause changes in environmental variables. The effects mostly impact environments negatively and lead to a reduction of biodiversity. The cause of observed reduced biodiversity is therefore mostly the degradation of habitats through anthropogenic stressors (Dudgeon et al. 2006, Reid et al. 2019, WWF 2018). The intensity of the impact of chemicals on ecosystems depends on different factors. Compounds are considered as critical when they are persistent in the environment, when they have a high efficacy with relevant toxicity and if they have a high potential for accumulation in the environment. The pollution is determined by the quantity, the retention time and the characteristic of the pollutant that was introduced.

3.1.1 Multiple stressors

Stressors don't usually act in isolation, but mostly in combination with other stressors. The exposure of organisms and communities to multiple stressors is a well-investigated and important, but highly complex, research topic. The complexity of the interaction of multiple stressors makes it difficult to predict possible effects. There are four main types of interactions. In case of an additive interaction (1), the joint effect is the sum of the effects of each stressor. Another option is the effect of one stressor being dominant over the other stressor (2). Where the stressors strengthen each other's effects, the effects act in a synergistic way (3). The opposite to this synergistic option of interaction is the antagonistic interaction (4), where the stressors weaken each other's effects (Jackson et al. 2016, Schäfer and Piggott 2018).

3.1.2 Pharmaceuticals in Aquatic Ecosystems – Occurrence and Effects

Pharmaceuticals, especially those affecting the hormone system and reproduction (estrogen substances) received awareness in the past (Tiljani 2015). Systematic research started in the 1990's when results were published that showed the existence of pharmaceuticals in local rivers and in sewage treatment plants (STP) (Zuccato et al. 2006). Pharmaceuticals are employed in large amounts in human and veterinary medicines. Pharmaceutical consumption has increased and will continue to do so in the next decades due to a continuously growing population and a demographic shift. Results from studies of the Landesamt für Umwelt Bayern demonstrate that the consumption increased from 6200 t in 2002 to 8000 t in 2012 (Bayerisches Landesamt für Umwelt 2020). Vannini et al. (2011) reported that pharmaceuticals, which are only found at low concentrations in aquatic environments, are able to accumulate via biomagnification in the food chain. The term "biomagnification" refers to the accumulation of a substance in organisms (via direct food intake) along the food chain. The consequences of this observation are as yet unknown. Many investigations have already shown that pharmaceuticals and their metabolites were found in effluents of sewage treatment plant and also in surface waters (Buser et al. 1998, Kolpin et al. 2002, Fent at al. 2006, Van den Beek et al. 2016). Ternes (1998) reported that 32 drugs had been found in German municipal sewage treatment plants.

Pharmaceuticals enter the aquatic environment via excrements in their original form or in metabolites originating from households and hospitals via sewage treatment plants (STP). They are often resistant to biodegradation since metabolic stability is necessary for pharmacological effect. Some of these or their metabolites are also highly water-soluble and therefore the removal in wastewater treatments for such compounds is limited. Veterinary and human pharmaceuticals also enter the aquatic environment by the spreading of manure or sewage sludge on fields for agricultural use. In this way, the pharmaceuticals enter the groundwater. Partially concentrations of some pharmaceuticals found in groundwater were up to 100 ng L^{-1} (Fent 2013; Heberer et al. 2001). The disposal of unused pharmaceuticals contributes to this pollution, but seems to be of minor importance (Heberer 2002). As it is still not possible to remove all of these compounds in conventional

biological treatments, they enter the aquatic environment and the aquatic biota is exposed to them (Dietrich et al. 2002). The elimination efficiency in STPs depends on the substance. For example elimination of Carbamazepine is limited to 5 - 10%, whereas 95 % of cardiac medicines can be eliminated (Fent 2013). An overview of the distribution from pharmaceuticals found in surface waters, groundwater, tap water and/or in drinking water worldwide is shown in Fig. 2.

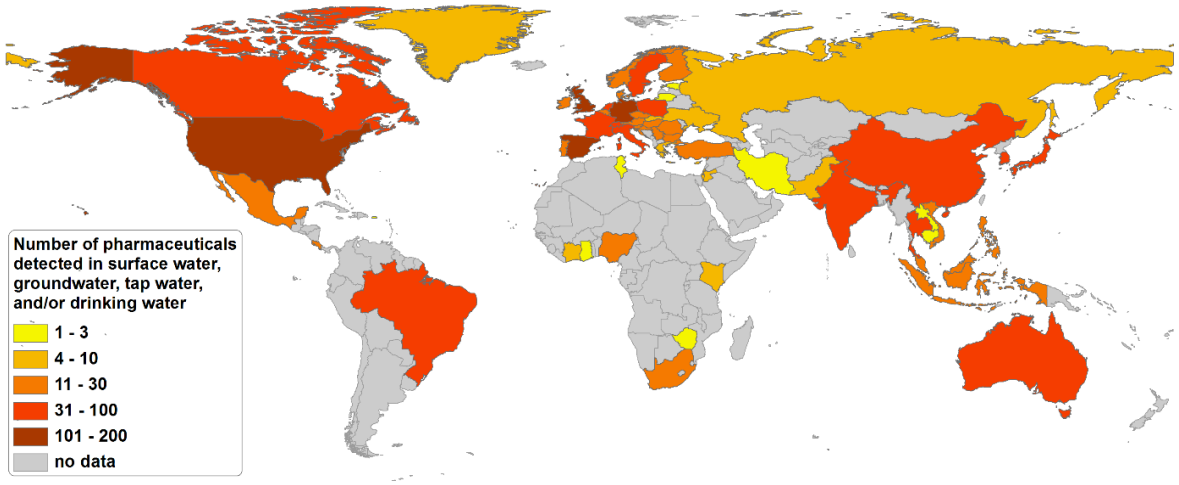


Fig. 2. Number of pharmaceuticals detected in surface waters, groundwater, tap water, and/or drinking water per country. (Van den Beek et al. 2016).

Concentrations found in surface waters range from a few to hundreds of ng L^{-1}. Some pharmaceuticals such as Diclofenac were even found in concentrations of more than 1 µg L^{-1} (Heberer 2002). Depending on their concentrations and toxicity some pharmaceuticals only impose low risk to an aquatic environment, e.g. iopromide, which caused no toxic effects to algae, bacteria or fish at concentrations of 10 g L^{-1} (Steger-Hartmann et al. 1999). Instead other pharmaceuticals such as natural and synthetic sex hormones are known to pose considerable risks (Nash et al. 2004, Crane et al. 2006). Although there is already great knowledge of the occurrence and toxicity of pharmaceuticals in aquatic ecosystems, little is known about the consequences (Zuccatto et al. 2006).

3.1.3 Regulations and Environmental Assessments for Pharmaceuticals

Unexpected negative effects of chemicals in the past led to stricter regulations. Examples are the awareness of effects of DDT to birds, of acid rain and the acidification of water bodies. Also tankship accidents and their devastating consequences to the environment led to a higher awareness of environmental damage resulting from chemical compounds.

In consideration of environmental damage, an increasing environmental awareness laws and regulations have reduced the pollution of ecosystems in the western world. Since 1994 for veterinary and since 1998 for human pharmaceuticals, specific assessments have become necessary when a new pharmaceutical is registered. The use of antibiotics for improving efficiency in agricultural practices has been forbidden within the EU since 2006. In the same year the European Medicines Agency (EMA) published a new guideline that requires an environmental assessment for all new pharmaceuticals. The acute toxicity is assessed with various model organisms from different trophic levels, e.g. bacteria, algae, *Daphnia sp.* and fish.

Normally the results show no high acute toxicity (Fent 2013). As the concentrations of pharmaceuticals in the aquatic environment are constantly present, chronic toxicity tests are also needed. However, compared to acute toxic effects there is much less data available for chronic effects (Fent 2013). Most published aquatic toxicity data and risk assessments for pharmaceuticals are based on short-term acute studies (Henschel et al. 1997 and stated by Crane 2006). The studies of Henschel et al. (1997) also showed that on the basis of acute standard tests alone (algae, *Daphnia sp.* and fish) the full ecotoxic potential of the tested substances would have been underestimated. For example toxic effect on reproduction generally occurred at concentrations, which were one order of magnitude below the acute toxic levels (Richards et al. 2004).

Another issue is, that the available data regarding environmental effects of pharmaceuticals are mostly limited to effects on single-species (Wollenberger et al. 2000; Richards et al. 2004). However, single species communities are extremely rare in nature.

3.2 Phytoplankton Communities

“The great sensitivity of algae to pharmaceuticals is quite worrying, since algae are the basis of many food webs, even slight decreases in algal populations may cause cascading effects at higher trophic levels” (Kümmerer 2009).

About 1,8 millions species, ~ 70 – 80% of all organisms inhabitating our planet, live in aquatic ecosystems (Fent 2013). As more than 70 % of the earth is covered with water, phytoplankton communities and their primary production provide the basis for nearly all aquatic food webs and thus for economically important fish populations. The primary production of phytoplankton represents between a third and a half of the total primary production of the world and therefore it is a significant part of global oxygen and carbon dioxide budgets (Sommer 1994). These remarkable potencies and capabilities of phytoplankton demonstrate its important role for all organisms globally. However, not only a decrease of algal communities may cause problems. Excessive algal production (eutrophication) could also cause expensive problems to the water industries and have deleterious effects upon fisheries and water-based recreation (Reynolds 1993).

Planktonic algae play an enormous role in aquatic ecosystems. Because of their presence in greatly varied microhabitats and their enormous morphological plasticity, they are pioneers in any new biotope. In consequence, algae may also play a role as indicator organisms for current ecological conditions (Mickiewicz and Szafer 2014).

The phytoplankton of both marine and freshwater represents a broad range of taxonomic groups. Many of them differ from each other in their physiological requirements and therefore vary in response to physical and chemical parameters such as light, temperature and nutrient regime (Wetzel 2001). Algal species also differ in their photosynthetic pigments and other biochemical compounds. Due to differences in their morphology, photosynthetic apparatus and resource requirements, they are differently affected by environmental stress factors. A stressor could have an effect on phytoplankton community dynamics when, for example, stress has an influence on even only one specific species. This can result in lower abundances of this species or even in its loss. It is also possible that a stressor has a positive impact on one species and thus this species gains a competitive advantage over other

species. As a consequence the effect of a stressor could affect biodiversity (Pomati et al. 2017, Reid et al. 2019) and community structure (Baho et al. 2019). That higher biodiversity has a positive impact on ecosystem functioning was shown in other investigations and not only for algae (MacArthur, 1955, Tilman 2000, Cardinale et al. 2011, Weisser et al. 2017). All these studies demonstrate that a decreasing diversity of plants and algae will lead to decreases in ecosystem functioning such as indicated by biomass production, resource use efficiency or the stability of ecological processes. As previously mentioned, phytoplankton provides the basis of many aquatic food webs and a shift in their growth and diversity could have tremendous consequences on higher trophic levels like fish.

3.3 Stressors and biotic interactions

When investigating the effects of single and multiple stressors it is important to consider biotic interactions (Germain et al. 2018). Biotic interactions between species, for example competition, could in theory change the effect of a stressor on an organism. An empirical study by Germain et al. (2018) with plant communities showed that the result of competition could depend on environmental conditions. In this study it was observed that in the absence of competition the stressor had no effect on the plant communities.

Biotic interactions would then modify stressor effects. The question arises whether it is then possible to predict the effect of a stressor such as a pharmaceutical on communities from investigations of single species alone. Therefore it is important to collect information of the effect of a stressor on isolated species and of effects of stressors on the same species living in communities. If the effect of the stressor on the isolated species remains unchanged by biotic interactions, it would then be possible to predict stressor effects on communities from investigations of single species. On the contrary, strong impacts of biotic interactions on stressor effects would severely hinder predicting the effects of pharmaceuticals on communities from single species studies.

3.4 Biotic interactions and biodiversity

The number and probability of biotic interactions increase with the complexity of biological communities. A good proxy for the potential number of biological interactions that are possible within a community is the diversity of a community. Higher species diversity would indicate a higher number of potential biological interactions. Biodiversity could therefore interact with stressor effects by potentially increasing the number of biotic interactions. However, biodiversity could affect stressor effects in several more ways. One way would be compensating effects of biodiversity (Thompson and Shurin 2012). Strong effects of a pharmaceutical on for example biomass production of a species could be more easily compensated in highly diverse communities where the chance of including a species much less or not at all sensitive to the pharmaceutical is higher than in low diverse communities. Such a less sensitive species could then use resources that can no longer be utilized by the stress sensitive species and compensate the growth reduction of the more sensitive species. Additionally, a highly diverse community would include more species that could show either positive or negative effect sizes to a stressor such as a pharmaceutical. Thereby positive and negative effects could cancel each other out. The effect of a stressor on net community performance and its coefficient of variation would then decrease with increasing diversity (Schindler et al. 2010, Hector et al. 2010, Schindler et al. 2015). Even if the effects of a stressor on algal species would not be affected by additional biotic interactions the mechanisms described above would still lead to a declining stressor effect on the net performance of a community.

Expected decreases of biological diversity could therefore also potentially increase visible net effects of stressors on communities without changing effects of stressors on individual species *per se*.

3.5 Research Questions

I investigated the effects of different pharmaceuticals as single stressors or combined as multiple stressors (chapter 4.2.1 -4.2.3) on different diverse algal communities in laboratory and in field experiments. The following research questions were addressed:

3.5.1 Question I: How were algal species affected by the investigated pharmaceuticals?

Concentrations of active pharmaceutical ingredients found in the environment are often below acute toxic concentrations. However, not only acute or lethal toxic effects within a short time of exposure are of interest. Even minor effects of a pharmaceutical on one algal species, e.g. through a decrease in photosynthetic performance, can lead to a shift in phytoplankton community structures further on. Therefore, I investigated the effects of Carbamazepine, Ciprofloxacin, Fluoxetine as well as a mixture of these in long-term experiments over three weeks. Selected algal species included representatives from all major algal classes. I investigated effects on two very important phytoplankton traits for population dynamics, photosynthetic performance and biomass production. 1) Photosynthetic performance, which is the physiological base of phytoplankton growth and production. 2) Biomass production, which integrates pharmaceutical effects on a variety of metabolic pathways not necessarily based on photosynthesis. Biomass is a good proxy for community effects of an algal species via resource monopolization and competition and an indicator for the availability of algal species as a food source in food web dynamics (Lampert and Sommer 2007).

3.5.2 Question II: Are algal species impacted differently by pharmaceuticals when treated in polycultures compared to monocultures? Do biotic interactions change the effect of pharmaceuticals? Is there a threshold of diversity above which pharmaceutical effects on phytoplankton species change?

Diversity could play an important role for stressor effects as outlined above (chapter 3.3). Two factors are of importance: Firstly, the impact of biotic interactions and biodiversity on the effect of the pharmaceutical on a single algal species. To answer this question I exposed algal species to the pharmaceuticals and grew them either in monoculture or cultures with increasing diversity. This enabled a comparison of the effect of a pharmaceutical on the same species but grown at conditions with an increasing number of biotic interactions. For example, a strong impact of biotic interactions on the effect of the pharmaceuticals calls data from single species toxicity tests into question to predict community effects.

Secondly, my experiments will additionally allow testing effects of biodiversity on net community performance when exposed to stressors. Comparing biomass production, photosynthesis efficiencies and the coefficient of variation of these parameters at the community level for communities of increasing diversity will allow quantifying such biodiversity effects on communities.

3.5.3 Question III: How does Carbamazepine affect natural algal populations with different diversity?

Carbamazepine is one of the most abundant pharmaceuticals found in aquatic environments (Drewes et al. 2002, Zhang et al. 2008). Its effect on phytoplankton was mostly investigated on monocultures and laboratory strains. However, natural algal populations are much more complex than even highly diverse laboratory algae cultures. Additionally, laboratory algal polycultures are artificially assembled by the investigator and not by ecological and evolutionary forces such as selection, competition etc.. Laboratory cultures usually share no eco-evolutionary histories or strong forces shaping biotic interactions such as the aforementioned selection processes do not operate. Hence, diverse communities in the field, which share an eco-evolutionary history, may react differently than artificially established laboratory cultures. Therefore I investigated the effects of Carbamazepine on natural phytoplankton communities from three lakes which share an evolutionary history and which are exposed to similar natural ecological dynamics. Additionally, these natural phytoplankton communities were also diversity-manipulated by already established methods. The investigation of the effect of Carbamazepine on natural algal populations therefore takes more of the potential interactions of natural populations into account. It is important to analyze whether the effects of pharmaceuticals on diverse (and diversity-manipulated) natural algal communities differ from observed effects on laboratory cultures. If that were the case the question arises whether laboratory communities are best suited to estimate stressor effects in the wild.

4. Materials and Methods

4.1 Test organsims

4.1.1 Laboratory experiments (Research questions I and II)

For the experiments different algae species were used to cover a broad range of common freshwater algal groups. *Scenedesmus obliquus* (Now: *Acutodesmus obliquus* (*Chlorophyta*), *Chroococcus minutus* (*Cyanobacteria*), *Cryptomonas phaseolus* (*Cryptophyta*), *Navicula pelliculosa* (*Bacillariophytina*) and *Peridinium sp.* (*Dinoflagellata*) were selected.

The strains have been obtained from SAG culture collection, Göttingen, Germany. The cultures have been cultivated at identical conditions (light, temperature, medium) since several years in the laboratory. About four weeks before each experiment started aliquots from these cultures were taken to initiate experimental populations. The algae were cultured in WC medium (after Guillard and Lorenzen 1972) in 200 ml cell culture flasks and were kept in a temperature chamber at 20 ± 0.5 °C with 16 h/8 h dark photoperiod regime and a photon flux density PFD = 90 µmol/m^2s^{-1}. All experiments were performed under the same constant temperature and light conditions.

Scenedemsus obliquus (Now: *Acutodesmus obliquus*) (*Chlorophyta*):

Green algae are unicellular or form filamentous colonies. They have mostly two but sometimes also 4 or more flagella. Only a double external membrane encloses the chloroplast, there is no additional fold of the endoplasmic reticulum. They have one or more plastids with pyrenoids for the storage of their assimilation products, mainly starch. The pyrenoid is located in the chloroplast. The chloroplasts contain chlorophyll *a* and *b*. *Chlorophyta* have a typical combination of accessory pigments of several xanthophylls and α-, β- and γ-carotenes. The cell wall of green algae consists of pectin and hemicellulose or cellulose (Van den Hoek et al. 1993, Mickiewicz and Szafer 2014). *Acutodesmus* belongs to the order of *Sphaeropleales* (*Chlorococcales*) (Reynolds 1993, Mickiewicz and Szafer 2014). Species of the genus *Acutodesmus* are abundant in plankton and numerous in an aquatic environment which is rich of nutrients, mainly nitrogen. *Acutodesmus obliquus* was formerly called *Scenedemus obliquus*. Most publications and studies still refer to *Scenedesmus*

obliquus. Therefore and to avoid confusion, it is still called *Scenedesmus obliquus* throughout this thesis.

Chroococcus minutus (*Cyanophyta, Cyanobacteria*):

Blue green algae are found in colonies, as single cells, thalli or filaments. In no phase of the lifecycle flagellate cells are found. Belonging to bacteria, blue-green algae have no nucleus, mitochondria, golgi apparatus, endoplasmic reticulum and no vacuoles enclosed by tonoplasts (Van den Hoek et al. 1993). Unlike eukaryotic photosynthetic plants their photosynthetic pigments are not bound to thylakoids. Instead they are located unbound within the protoplasma. The thylakoids of blue-green algae are not organized in stacks, they lie singly at the same distance to each other and they consist of the pigment chlorophyll *a*. The green chlorophyll is often masked by the blue accessory pigments phycocyanin and allophycocyanin and the red accessory pigment phycoerythrin (Van den Hoek et al. 1993). Their storage product is cyanophyte starch. Their cell wall consists of mucopeptide (Mickiewicz and Szafer 2014). Many blue-green algae are able to produce neurotoxins and secrete them into their aquatic environment. Within the order *Chroococcales*, one example of a family known to produce toxins are species from the family *Microcystis* (*e.g. Microcystis aeruginosa*) (van Apeldoorn et al. 2007). The genus *Chroococcus* belongs to the order *Chroococcales* that is solitary or colonial coccoid (Reynolds 1993). The cells of *Chroococcus* are semicircular (after separation) to circular. Each cell or group of cells is surrounded by a jelly capsule (Van den Hoek et al. 1993). The representatives of the *Chroococcaceae* are found sedentary as well as planktonic, mainly in freshwater. They are important primary producers and many of them cause water blooms while some produce very toxic toxins (*Microcystis*). Some species can be also found in extreme environments like in acid peat water (*Synechococcus elongatus, Synechococcus lividus*) and in thermal springs (Krauter 2010).

Cryptomonas phaseolus (*Cryptophyta*):

Members of the *Cryptophyceae* (the only class of *Cryptophyta*) have two flagellates of different length and are mostly unicellular. A fold of the endoplasmic reticulum encloses their chloroplasts. The chloroplasts contain chlorophyll *a* and c_2. The chlorophyll is masked by different accessory pigments such as phycocyanin, phycoerythrin, α-carotin and xanthopylls (Van den Hoek et al. 1993). *Cryptophyta* have one or two large plastids. Their assimilation product of photosynthesis is starch. *Cryptomonas* belong to the order *Cryptomonadales* (Reynolds 1993). *Cryptophyta* can be found in a wide range of habitats from small and slightly polluted habitats to large eutrophic and oligotrophic lakes.

Navicula pelliculosa (*Bacillariophytina*):

Navicula belongs to the class of *Bacillariophyceae* (diatoms). Diatoms include unicellular and colonial algae and usually have numerous discoid plastids. Each cell is enclosed by a characteristic silica wall that consists of two halves. The cell wall consists mainly of amorphous, polymeric silica, as well as polysaccharide, proteins and fatty substances. The silica shell of *Navicula* consists (as for all diatoms) of the hypotheka (box) and the epitheka (lid). The chloroplasts contain chlorophyll *a,* c_1 and c_2. The chlorophyll is masked by the accessory pigment fucoxanthin (Van den Hoek et al. 1993). Their assimilation products are mainly chrysose and oils (Reynolds 1993). Diatoms represent a large percentage of the phytoplankton of the oceans and contribute to a large extent to the primary production in moderate to cold regions. Diatoms are also found in freshwater systems and in humid and arid soil habitats.

Peridinium sp. (*Dynophyta*):

Dinophyceae are unicellular flagellates, rarely colonial. *Peridinium* is a genus within the order of *Peridinales* which belongs to the class of *Dinophyceae*. Dinophyts have two flagella of different length and orientation. Their flagella are located in a transverse and a longitudinal furrow. They can have numerous plastids or can be colorless. Sometimes their cellulose cell wall is sculptured into plates or they may also be naked. Their assimilation product is starch or oil (Reynolds 1993). A threefold membrane encloses their chloroplasts,

which is not connected to the endoplasmic reticulum. Many *Dinophyta* are heterotrophic and do not have chloroplasts. The color of their chloroplasts is often brown because the green chlorophyll *(a* and c_2) is often masked by β-carotin and a few xanthophylls. There are species among the *Dinophyceae* enclosing endosymbiotic algae that have other accessory pigments (Van den Hoek et al. 1993). *Peridinium* is often a main component of marine plankton but some species can also be found in the freshwater plankton amongst water plants (Krauter 2010).

4.1.2 Field experiments (Research question III)

Beside investigating well defined laboratory strains of algae, it is also of importance to study how pharmaceuticals affect natural phytoplankton communities which are differing in several aspects (complexity, eco-evolutionary history) from laboratory communities. To do this I investigated the effect of Carbamazepine on natural, diversity manipulated phytoplankton communities of three lakes (chapter 4.3.2).

4.2 Investigated Pharmaceuticals

I investigated the effects of Carbamazepine, Fluoxetine and Ciprofloxacin individually and in a combination of a mixture of all three pharmaceuticals on all of the above described algae. These active pharmaceutical ingredients are among the most prescribed pharmaceutical compounds and all of them have already been found in surface waters.

The concentrations of pharmaceuticals for the experiments were based on described concentrations found in surface waters. Treatments included either pharmaceutical substances alone or a mixture of all three pharmaceuticals. The concentration of each pharmaceutical in the mixtures were the same as in the treatments with only one pharmaceutical (see Tab. 1, Tab. 2 and Tab. 3).

4.2.1 Carbamazepine (CBZ)

Carbamazepine has the chemical name 5H-dibenz- (b,f)azepine-5-carboxamide and is a first-generation anticonvulsant drug that has been used to treat partial seizures, trigeminal

neuralgia, manic-depressive illness and explosive aggression for nearly 40 years (Liu et al. 2008).

Carbamazepine is one of the most detected active pharmaceutical ingredients in sewage treatment plants (STPs) (Chen et al. 2006). The metabolites of this pharmaceutical active ingredient are also of environmental concern, especially trans-10,11-dihydro-10,11-dihydroxycarbamazepine (CBZ-diol) which probably has a similar concentration in water bodies to that of its parent drug.

The removal-efficiency of Carbamazepine by degradation and/or retention processes in conventional STPs has been found to be very low and may be attributed to its resistance to biodegradation (Zhang et al. 2008, Ternes 1998).

The occurrence of Carbamazepine has been examined in various water bodies including STP effluents, surface waters, groundwater and drinking water (Zhang et al. 2008). In this context Carbamazepine has been proposed as an anthropogenic marker of sewage contamination in freshwater bodies (Hai et al. 2018).

The deployed concentrations were chosen based on reported concentrations found in surface waters 0.025 - 1.075 µg L^{-1} (Heberer 2002), 0.693 – 1.6 µg L^{-1} in 2015 and 0.550 – 0.9 µg L^{-1} in 2016 (Van den Beek et al. 2016) (Tab. 1). Higher concentrations are likely to be reached due to the persistence of this pharmaceutical substance within the environment.

Tab. 1. Concentrations of Carbamazepine in the respective laboratory and field experiments. The asterisks mark the environmentally relevant concentration in surface waters.

Treatment	NC	C1	C2	C3	C4	C5
Carbamazepine [µg L^{-1}] (laboratory exp.)	-	0.500	1.000*	2.000	4.000	8.000
Carbamazepine [µg L^{-1}] (field exp.)	-	1.000*	8.000			

4.2.2 Fluoxetine (FL)

The antidepressant Fuoxetine [(RS)-N-Methyl-3-phenyl-3-(4-trifluormethylphenoxy) propylamin] is one of the first introduced selective serotonin reuptake inhibitors (SSRI) (Oakes et al. 2010). SSRIs are primarily indicated for depression, but also for compulsive behaviour as well as eating and personality disorders. Because of its mood brightening effect, it has also been used as a lifestyle drug. The patent of the pharmaceutical Prozac expired in 2000. The consequence was that a lot of generics flooded the market. As a result Fluoxetine is one of the most prescribed active pharmaceutical ingredients (API) of SSRIs (Neuwoehner et al. 2009). Fluoxetine is metabolized by cytochrom P-450 isoenzyms to Norfluoxetine (Brooks et al. 2003). This active pharmaceutical ingredient is discharged in STP effluents into surface waters.

This antidepressant and its metabolite Norfluoxetine were identified as specifically toxic toward algae in a quantitative structure–activity-relationship (QSAR) analysis with literature data for algae, Daphnia and fish (Neuwoehner 2009). Neuwoehner et al. (2009) also conclude that Fluoxetine and Norfluoxetine have an effect on the energy budget of algal cells.

Reported environmental concentrations vary widely: Kolpin et al. (2002) investigated samples from 139 U.S. stream sites during 1999-2000. The estimated maximum concentration of Fluoxetine is 0.012 µg L^{-1}. The range of concentrations of Fluoxetine in the study by Schultz and Furlong (2008) was 0.012 – 0.020 µg L^{-1}. The samples were collected from a municipal wastewater-effluent at a metropolitan urban centre and surface water samples collected from a waste-dominated stream. Based on these reported values the concentrations shown in Tab. 2 were used for the treatments.

Tab. 2. Concentrations of Fluoxetine in the respective treatments. The asterisk marks the environmentally relevant concentration in surface waters.

Treatment	NC	C1	C2 *	C3	C4	C5
Fluoxetine [µg L^{-1}]	-	0.006	0.012	0.024	0.048	0.096

4.2.3 Ciprofloxacin (CIP)

Ciprofloxacin, 1-cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid, is an antibiotic that belongs to a group of drugs, which are known as fluoroquinolones. It was detected in surface waters receiving effluents of STPs. It is one of the most abundant residual drugs and therefore of environmental concern (Castiglioni et al. 2006).

Ciprofloxacin is also a metabolite of Enrofloxacin, which is used in veterinary medicine. Concentrations of Ciprofloxacin in STPs were found to be between 0.02 and 0.1 µg L^{-1} in surface waters (Fent 2013). According to these results the concentrations shown in Tab. 3 were used for treatments with Ciprofloxacin.

Tab. 3. Concentrations of Ciprofloxacin in the respective treatments. The asterisk marks the environmentally relevant concentration in surface waters.

Treatment	NC	C1	C2 *	C3	C4	C5
Ciprofloxacin [µg L^{-1}]	-	0.010	0.020	0.040	0.080	0.160

4.3 Experimental setup

4.3.1 Laboratory Experiments (Research question I and II)

For the experiments all 5 algal species in monocultures and eight different polycultures were set up at different diversity treatments (Tab. 4). Three replicates were prepared from each sample. Additionally, diversity replicates in the diversity treatments 2 and 3 were used. For these diversity treatments 3 different combinations of the investigated algal species were prepared.

Tab. 4: The compilation of the diversity treatments with the respective algae species. In diversity treatments 1, the monocultures of each algal species were investigated. Diversity treatments 2 and 3 consisted of three diversity replicates including new species combinations.

Diversity Treatment (Code)	*S. obliquus*	*C. minutuus*	*N. pelliculosa*	*C. phaseolus*	*Peridinum sp.*
1 (1)	monocultures				
2 (2a)			x		x
2 (2b)		x	x		
2 (2c)	x			x	
3 (3a)	x		x		x
3 (3b)		x		x	x
3 (3c)	x	x	x		
4 (4)		x	x	x	x
5 (5)	x	x	x	x	x

All monocultures and polycultures were exposed to Carbamazepine, Fluoxetine, and Ciprofloxacin and to a mixture of all three of these over a period of 21 days. Carbamazepine from Acros Organics (Geel, Belgium, 99%) was dissolved in ethanol (absolute). For

treatments with Fluoxetine and Ciprofloxacin, Fluoxetine HCL CRS and Ciprofloxacin HCL CRS (edqm, 100%) were used. From this solution an appropriate amount was pipetted to cell culture flasks to achieve the different concentrations of the pharmaceuticals. To exclude an impact of the solvent on the test organisms, the solvent was evaporated to dryness before adding medium and test organisms.

For the laboratory experiments the initial algal biovolume was set to be identical for all treatments $2.4*10^6$ fl mL^{-1}. Each species contributed equally to the initial biovolume in the polycultures. The working volume of all treatments was 0.25 L.

To ensure a sufficient nutrient supply and to ensure a constant concentration level of the pharmaceutical, a semi batch culture technique was used. Every 48-72 h 10 % of the total volume was exchanged with fresh WC medium spiked with the respective pharmaceutical in the appropriate concentration.

The pharmaceuticals were added to the mono- and polycultures in five different concentrations as mentioned in the chapters 4.2.1 - 4.2.3. Treatments without the pharmaceutical served as negative controls (NC). Each treatment was set up in three identical replicates (Fig. 3). Additionally three different diversity replicates (different combinations of algae species) were set up for diversity treatments two (according to Tab. 4, diversity treatment code 2a, 2b and 2c) and three (according to Tab. 4, diversity treatment code 3a, 3b and 3c). The experimental design resulted in a total of 234 cultures.

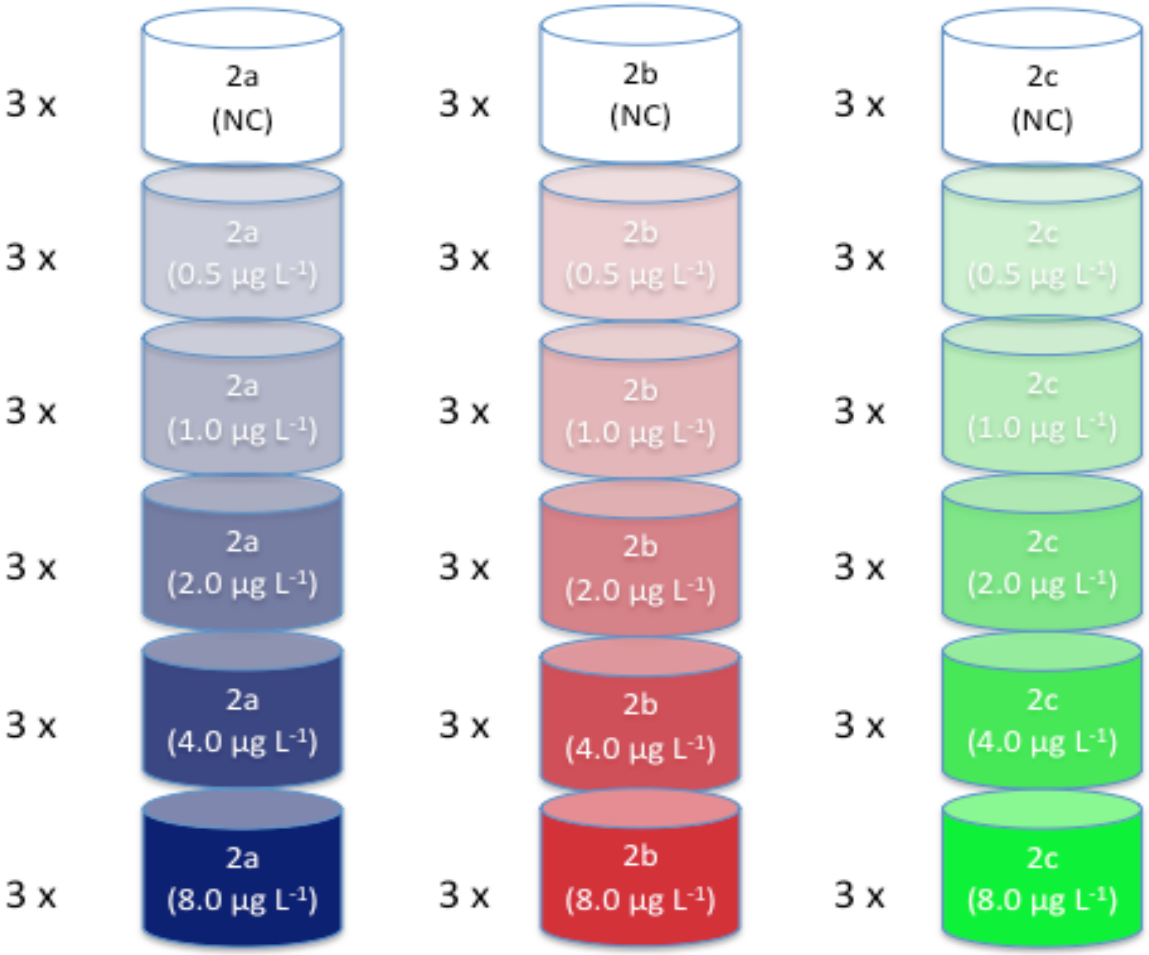


Fig. 3: Overview of experimental set up for a specific polyculture. Shown is an example of a polyculture at the diversity treatment 2 using three different combinations of algae species with NC = negative control; 2a, 2b, 2c = code described in **Tab. 4**.

4.3.2 Field Experiment (Research question III)

Lakes

It is important to study how natural algal communities react to pharmaceuticals (here Carbamazepine) in comparison to artificially assembled algae cultured in the laboratory. For analysing the effects of Carbamazepine on various phytoplankton species diversity gradients of natural phytoplankton established within an enclosure experiment were used. The diversity experiment was conducted at the Limnological Fieldstation Seeon of the LMU in

three lakes of different trophic levels in Upper Bavaria. Lake Brunnsee is oligotrophic (nutrient poor), Lake Thalersee is eutrophic (nutrient rich) and Lake Klostersee is meso-oligotrophic. Experiments were carried out in June 2014. Lake Brunnsee (BS, N 47.984170 E 12.436148) is fed by groundwater from subsurface springs with a maximum depth of 18.6 m and a surface area of 5.88 ha. Lake Thalersee has a maximum depth of 7 m and an area of 3.79 ha (TS, N 47.906127 E 12.339043). Lake Klostersee is, with a maximum depth of 16 m and approximately 47 ha of surface area, the largest of the three lakes (KS, N 47.973492 E 12.455118).

Experimental setup

The mesocosms, made of transparent low density polyethylene foil, were installed on rafts located at least 15 m from the shoreline. Mesocosms were cylindrical, 6 m (5m in lake Thalersee, due to its shallowness) deep with a diameter of 0.95 m. So the approximate filling capacity came to 4.2 x 10^3 L (3.5 x 10^3 L). The mescosoms were open to the atmosphere. To exclude mesozooplankton, lakewater was filtered through 250 µm gauze and then filled into the mesocosms. During the following 5 weeks the mesocosms were periodically disturbed (Tab. 5) to establish phytoplankton communities of varoius diversity levels within the mesocosms (Flöder and Sommer 1999, Hammerstein et al. 2017). The experiment consisted of 5 treatments with 2 replicates per lake. To create disturbance, the stratified water column was perturbed for 10 minutes using compressed air that was introduced by a flexible tube at the bottom of the mesocosms.

Tab. 5: Disturbance intervals of mesocosms used in the field experiment.

Treatment	1	2	3	4	5
Disturbances / week	7	3.5	2.33	1.4	1

The cell culture flasks were filled with 0.25 L of water from each of the ten enclosures (i.e. five different treatments, each replicated twice). Two additional lake water samples

(sampled close to the enclosure raft) were taken from each lake, resulting in 12 water samples which were treated with two different concentrations of Carbamazepine (1.0 and 8.0 µg L^{-1}). Controls without Carbamazepine were established for all lakes.

All cultures were kept in a conditioning cabinet (20°C; relative humidity = 60 %; photon flux density PFD = 90 µmol/m^2s) and run as semi-batch cultures for 19 days. Every two to three days, 3 mL of the volume were used for measuring the chlorophyll fluorescence transient (OJIP). Shortly after sampling, the water volume of flasks was adjusted by replacing the sample volume with filtered water (Schleicher & Schuell, glass fibre filters GF6) of the corresponding lake and an appropriate amount of Carbamazepine to maintain nutrient and Carbamazepine concentrations.

4.4 Measurements

Initial estimation of biomass:

The initial biomass estimation of each algae culture was performed by a cell counter and analyser system (CASY Modell TTC, Schärfe System, Germany). For the measurements the cells were suspended in an electrolyte (CASY®ton) and were aspirated through a pore with defined geometry at a constant flow speed. During the measurement process, a pulsed low voltage field was applied to the measuring pore via two platinum electrodes. The electrolyte-filled measuring pore represents a defined electrical resistance. During their passage through the measuring pore, the cells displace a quantity of electrolyte corresponding to their volume (CASY® Cell Counter + Analyser System Model TT, Operator Manual Roche Innovatis AG, 2.3E). This technique was only used during the preparation of the experiment, to set an equal biovolume of all algae at the beginning of the experiments.

Photochemistry parameters:

Every 48-72 hours, photosynthetic parameters of the algae were investigated by measuring the chlorophyll fluorescence transient (OJIP) with an AquaPen device (AquaPen-C AP-C 100; Photon Systems Instruments, Brno, Czech Republic) at a wave length of 450 nm.

The principle behind the chlorophyll fluorescence transient is explained as a consequence of reduction of electron acceptors in the photosynthetic pathway: once PSII absorbs light and Q_A (Plastichinon A) has accepted an electron, it is not able to accept a further one until it has passed the first to a subsequent electron carrier Q_B (Plastichinon B). During this time the reaction centre is ‚closed'. This leads to a corresponding increase in the yield of fluorescence (Maxwell and Johnson 2000).

With this measurement principle it is possible to analyze the fluorescence transient. The results of this analysis provide information about the structure, conformation and function of the photosynthetic apparatus and especially of photosystem II (PSII) (Strasser et al. 2004). A very important parameter that measures the efficiency of PSII photochemistry is Quantum Yield (QY). It measures the proportion of the light absorbed by chlorophyll associated with PSII.

In a dark-adapted sample Quantum Yield is calculated as:

$$QY = \frac{Fv}{FM} \qquad \text{(eq. 1)}$$

F_0: zero fluorescence level

F_M: maximum fluorescence

F_V: $F_V = F_M - F_0$ (maximal variable fluorescence)

When the fluorescence transient of a dark-adapted photosynthetic sample is plotted on a logarithmic time scale fluorescence rise is visible (Fig. 4).

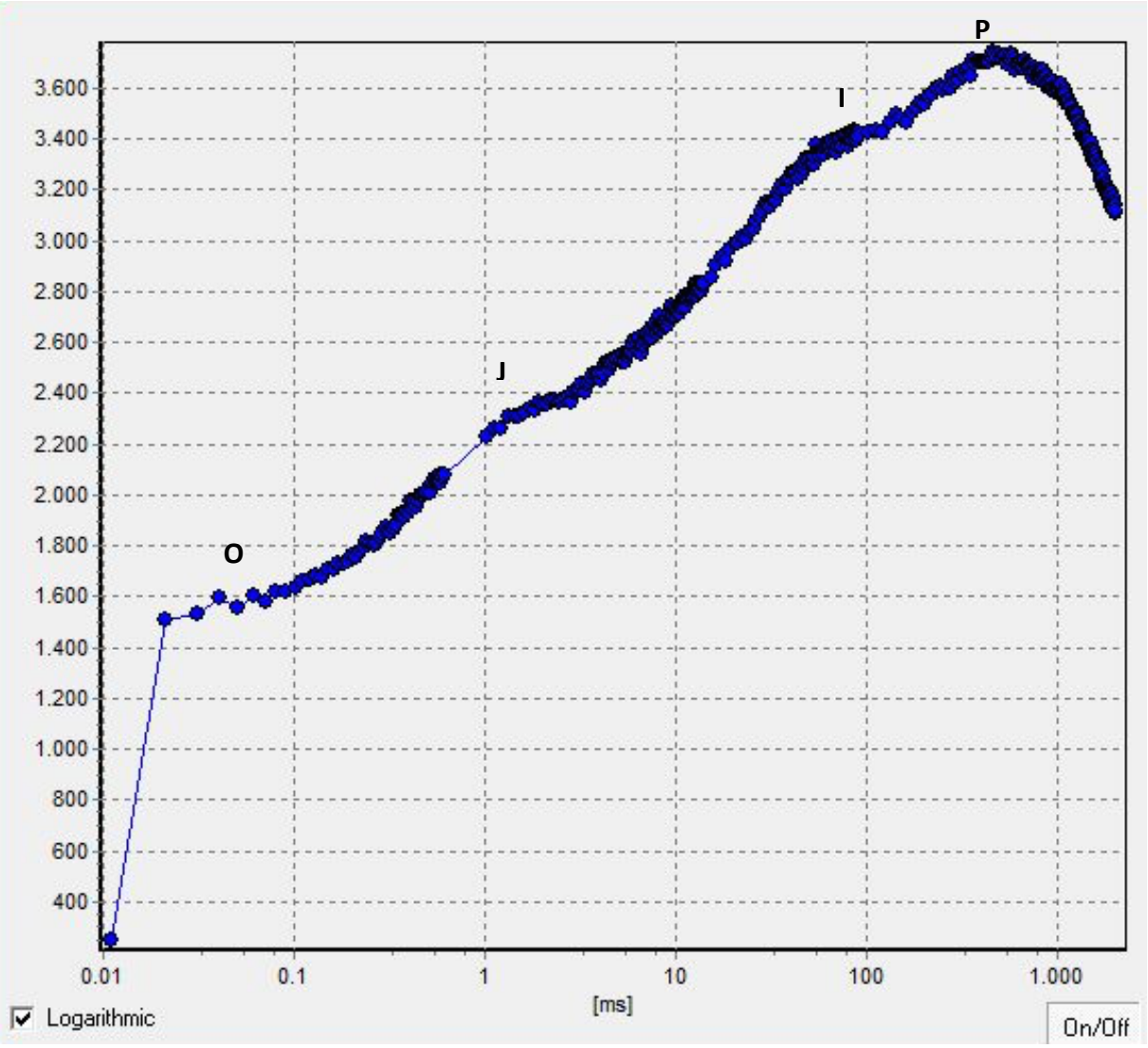


Fig. 4. O-J-I-P fluorescence transient from treatments with 0.02 µg L^{-1} Fluoxetine, diversity treatment 3.

The O-P part reflects the closure of reaction centers. Under high-intensity continuous actinic light the fluorescence rise usually exhibits the steps J and I between the initial O (F0) and the maximum P (FP=FM, the maximal fluorescence intensity). The F0 signal (50µs) is followed by the fluorescence intensities FJ (at 2 ms) and FI (at 30 ms) (Strasser et al. 2004). Hence, the name O-J-I-P arose.

The results of Quantum Yield (efficiency of PS II) are used as an indicator of photosynthetic performance. According to Björkman and Demming 1987, for land plants with two

dimensional leaf structures, values below 0.83 would indicate an exposition to stress. For eukaryotic algal cells of various shape, values below 0.6 usually indicate stress situations. Cyanobacteria usually show even lower values. As part of the OJIP measurement the results of FixArea as Chl *a* specific biomass parameter were used. FixArea corresponds to the area under fluorescence curve between $F_{40\ \mu s}$ und $F_{1\ s}$ with backgrounds substracted (Photon Systems Instruments, 2016). FixArea data can be converted for defined algal groups into chlorophyll *a* (Chl *a*) concentrations as an indirect method of chlorophyll concentration measurement.

4.5 Chemical analyses

The chemical analyses as well as the provision and preparation of the pharmaceuticals were conducted by the department of Prof. Dr. Bracher (Chair of Pharmaceutical/Medicinal Chemistry) at the Faculty of Chemistry and Pharmacy of the Ludwig-Maximilians-University of Munich. A determination of pharmaceutical concentrations during experiments was conducted for Carbamazepine and Fluoxetine. These measurements took place on day 1, 3, 8, 10, 15, 17, and 19 of the experiments to confirm the nominal concentration in each flask. As the determined concentration in the samples for the two pharmaceuticals was found to be adequate, it was assumed, that this would be the same for Ciprofloxacin and for the mixture of the three pharmaceuticals.

4.6 Statistical Analyses and used calculations

Statistical analyses were performed with SigmaPlot 11 (Systat Software 2008). For comparing effects from the different pharmaceutical active ingredients on different algal species I calculated comparable effect sizes. Effect sizes are very useful to demonstrate effects of a treatment in comparison to the control treatment independent of absolute values. I used an effect size calculation according to Osenberg et al. (1997):

$$effect\ size = \ln\left(\frac{mean\ (C_n)}{mean\ (control)}\right) \qquad \text{(eq. 2)}$$

C_n: concentration treatment *n* of the respective algae culture

This specific effect size calculation has the advantage of a clear biological meaning (positive values indicate a positive effect, 0 no effect and negative values a negative effect of a manipulation) and that its statistical distribution is usually normal.

4.6.1 Question I

Two Way Anovas with the factors ‘concentration’ and ‘time’ were conducted to test for general treatment effects and their interactions. Effect sizes (raw data, full crossed) of the pharmaceuticals on FixArea and Quantum Yield of the algal species were used for these analyses. A significant interaction between the factors ‘concentration’ and ‘time’ indicated that the factors were not independent from each other. To further identify statistically significant differences between the different concentrations at defined time points a One Way Anova was performed using the effect sizes (raw data, full crossed) after one week (day 6) and after 22 days (last sampling point). If treatments were significantly different, a post-hoc test (contrast) was used for comparison of treatments with pharmaceuticals versus the control treatments (without pharmaceuticals) and versus day 4 (initial measurement) for the different sampling points in time. The statistical analyses were performed with all investigated concentrations of the pharmaceuticals.

For presenting the results of microscopic counting the mean values and their standard errors were calculated. A One Way Anova was performed using the effect sizes of the abundances (raw data, full crossed) of the respective algae. If treatments were significantly different, a post-hoc test (contrast) was used for comparison of treatments with pharmaceuticals versus the control treatments. The statistical analyses were performed with all investigated concentrations of the pharmaceuticals.

To compare the effects of the single tested pharmaceuticals and the mixture of all pharmaceuticals on algal species, I calculated mean effect sizes for all treatments. By comparing these effect sizes it was possible to estimate if the effect of the mixture of all pharmaceuticals is predictable from the effects induced by the single tested pharmaceuticals. The statistical analyses were perfomed via One Way Anovas using the effect sizes (raw data, full crossed; day 22) of the pharmaceuticals (CBZ: 8.0 µg L^{-1}, CIP: 0.160 µg L^{-1}, FL: 0.096 µg L^{-1}) on FixArea and Quantum Yield of the investigated algal species. If treatments were significantly different, a post-hoc test (contrast) was used for comparison of treatments with pharmaceuticals versus the control treatments.

Additionally, I summarized the different strength of responses of the algal species to the three pharmaceuticals. I therefore performed a Two Way Anova with the factors 'pharmaceutical' and 'algae'. For this analysis I used the effect sizes (raw data, full crossed; day 22) of the pharmaceuticals (CBZ: 8.0 µg L^{-1}, CIP: 0.160 µg L^{-1}, FL: 0.096 µg L^{-1}) on FixArea and Quantum Yield of the investigated algae were used for the statistical analysis.

4.6.2 Question II

Effects of pharmaceuticals on different diverse algae communities:

A One Way Anova and post hoc test (contrast) were performed to investigate differences between the diversity treatments. Effect sizes (raw data, full crossed; day 22) of the pharmecuticals (CBZ: 8.0 µg L^{-1}, CIP: 0.160 µg L^{-1}, FL: 0.096 µg L^{-1}) on FixArea and Quantum Yield of the investigated algal species were used for this statistical analysis. Additionally, the coefficient of variation (CV) was used to investigate if diversity also had an impact on the variability of treatments. For the calculation of the coefficient of variation the raw data of FixArea and Quantum Yield were used. The calculation was performed with Microsoft ® Excel® for Mac 2011.

Effect of pharmaceuticals on different diversity treatments including specific algal species:

Regression analyses with effect sizes (raw data, full crossed; day 22) of the pharmaceuticals (CBZ: 8.0 µg L^{-1}, CIP: 0.160 µg L^{-1}, FL: 0.096 µg L^{-1}) on FixArea of the investigated algal species in the different diverse treatments were performed to investigate if a dependency between the diversity and the effect size of the pharmaceuticals on FixArea of the respective algal species in the diversity treatments exists. If the regression was not significant, I performed a One Way Anova. For the stastical analysis the effect sizes (raw data, full crossed; day 22) of the pharmaceuticals (CBZ: 8.0 µg L^{-1}, CIP: 0.160 µg L^{-1}, FL: 0.096 µg L^{-1}) on FixArea of the algal species were used.

Effect of pharmaceuticals on individual algae species in the different diversity treatments:

Regression analyses with effect sizes (raw data, full crossed; day 22) were performed to investigate if a dependency between the diversity and the effect size of the pharmaceuticals

(CBZ: 8.0 µg L^{-1}, CIP: 0.160 µg L^{-1}, FL: 0.096 µg L^{-1}) on the abundance of the respective algal species in the diversity treatments exists. If the regression was not significant, I performed a One Way Anova with the effect sizes (raw data, full crossed; day 22) of the pharmaceuticals (CBZ: 8.0 µg L^{-1}, CIP: 0.160 µg L^{-1}, FL: 0.096 µg L^{-1}) on the abundance of the algal species.

Effect of pharmaceuticals on community composition:

To achieve a statistical estimate about the similarity of full (5 species) algal communities growing with (CBZ: 8.0 µg L^{-1}, CIP: 0.160 µg L^{-1}, FL: 0.096 µg L^{-1}) and without pharmaceutical substances I performed so called ANOSIM (analysis of similarity) tests. All analyses were performed with PRIMER 7 software. Algal abundances (day 22) were converted to biomass (own data). Data were transformed (log x+1) before analyses; all similarities were based on Bray-Curtis similarity resemblance measures. Visualization of similarities was performed with non-metric Multidimensional Scaling (nMDS) plots (PRIMER 7).

4.6.3 Question III

Linear regression analyses were performed using the effect sizes (raw data, full crossed; day 19) to investigate the effect of the diversity of natural phytoplankton communities on the impact the pharmaceutical Carbamazepine (CBZ: 1.0, 8.0 µg L^{-1}) had on the growth of the respective communities.

5. Results

5.1.1 Question I: How were algal species affected by the investigated pharmaceuticals?

5.1.1.1 Impact of Carbamazepine on algae in monocultures

Scenedesmus obliquus

In the cultures with *Scenedesmus obliquus* Carbamazepine led to an increase of FixArea (a proxy of algal biomass; Fig. 5a) in all treatments. This increase was dose dependent as the mean effect sizes were higher with higher concentrations of Carbamazepine. The mean effect sizes of Carbamazepine on Quantum Yield were around 0 during the entire experiment (Fig. 5b).

a) b)

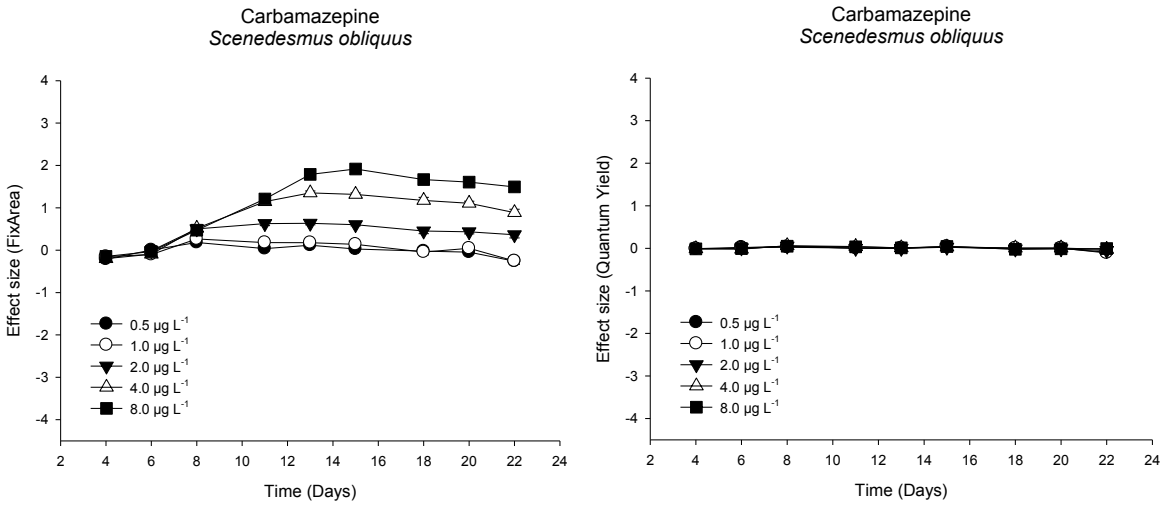


Fig. 5: FixArea (a) and Quantum Yield (b) of *Scenedesmus obliquus* after exposure to different concentrations of Carbamazepine over a period of 22 days. Data expressed in mean effect sizes to the unexposed controls. Error bars represent standard errors.

The result of a Two Way Anova of the FixArea data from *Scenedesmus obliquus* showed, that the factors ‚time', ‚concentration' and the interaction of the two factors ‚concentration

x time' were significant (Tab. 6). The effects of different concentrations of Carbamazepine were time-dependent. The results of a Two Way Anova of Quantum Yield data showed, that the factor 'time' was significant.

Tab. 6: Results of a Two Way Anova including FixArea and Quantum Yield data of *Scenedesmus obliquus* treated with different concentrations of Carbamazepine (n=162); FA=FixArea; QY=Quantum Yield.

Algae species	Source of Variation	DF	F (FA)	P (FA)	F (QY)	P (QY)
S. obliquus	Conc	5	1148.254	<0.001	1.394	0.232
	Day	8	623.966	<0.001	4.647	<0.001
	Conc x Day	40	89.976	<0.001	1.223	0.207

A One Way Anova showed no significant differences in FixArea data between the treatments on day 6 (DF=5; F=0.990; P=0.463). On the last sampling day there were significant differences (DF=5; F=136.414; P<0.001) in the FixArea data found between controls and treatments with Carbamazepine. A post hoc test (Bonferroni t-test, contrast) identified that treatments with 4.0 µg L^{-1} Carbamazepine (t=8.112; P<0.001) and 8.0 µg L^{-1} Carbamazepine (t=19.755; P<0.001) were significantly different to the control.

A One Way Anova identified no significant differences in the Quantum Yield data between the treatments on day 6 (DF=5; F=1.127; P=0.397). At the end of the experiment the differences among the treatments were significant (DF=5; F=6.953; P=0.003). The effect of the treatments with 0.5 µg L^{-1} (t=3.838; P=0.012) and 1.0 µg L^{-1} Carbamazepine (t=4.459; P=0.004) was significantly higher than the control treatments (Bonferroni t-test, contrast).

The results of the microscopic counting showed that similar to the FixArea, the abundance of *Scenedesmus obliquus* increased with higher concentrations of Carbamazepine.

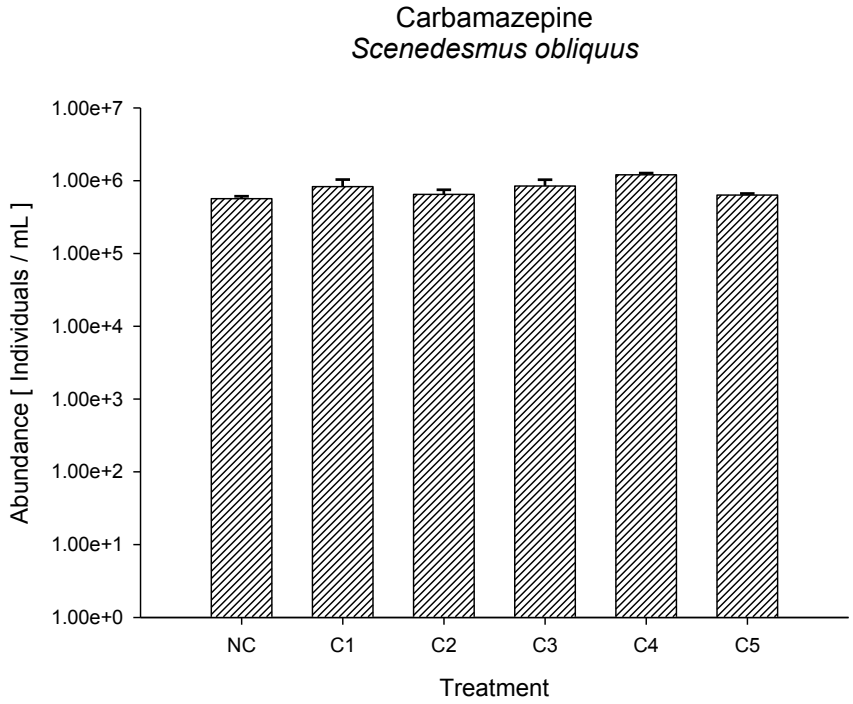


Fig. 6: Mean abundances of *Scenedesmus obliquus* under the influence of Carbamazepine at day 22 of the experiment. Each treatment consisted of 3 replicates. NC: negative control; C1: 0.5 µg L^{-1}; C2: 1.0 µg L^{-1}; C3: 2.0 µg L^{-1}; C4: 4.0 µg L^{-1}; C5: 8.0 µg L^{-1}. Error bars represent standard errors.

The lowest mean abundance of *S. obliquus* was observed in the control treatments. The highest mean abundance was found in treatments with 4.0 µg L^{-1}, the second highest in treatments with 8.0 µg L^{-1} Carbamazepine (Fig. 6). A One Way Anova with the abundances identified significant differences (DF=5; F=3.330; P=0.041) between the treatment groups. A post hoc test (Bonferroni t-test, contrast) showed that the abundance of *Scenedesmus obliquus* was significantly higher in treatments with 4.0 µg L^{-1} Carbamazepine than in the control treatments (t=3.544; P=0.020).

Navicula pelliculosa

The mean effect size of Carbamazepine on FixArea of *Navicula pelliculosa* was around 0 in treatments with concentrations of 0.5 – 4.0 µg L^{-1}. The mean effect size of 8.0 µg L^{-1} Carbamazepine on FixArea was lowest on day 13. From then onwards the mean effect size of Carbamazpeine on FixArea increased up to ~ 2.5. In the other treatments this increase was recognizable from day 20 on. Only a slight effect of Carbamazepine on the Quantum Yield of *Navicula pelliculosa* was observed (Fig. 7 a, b).

a) b)

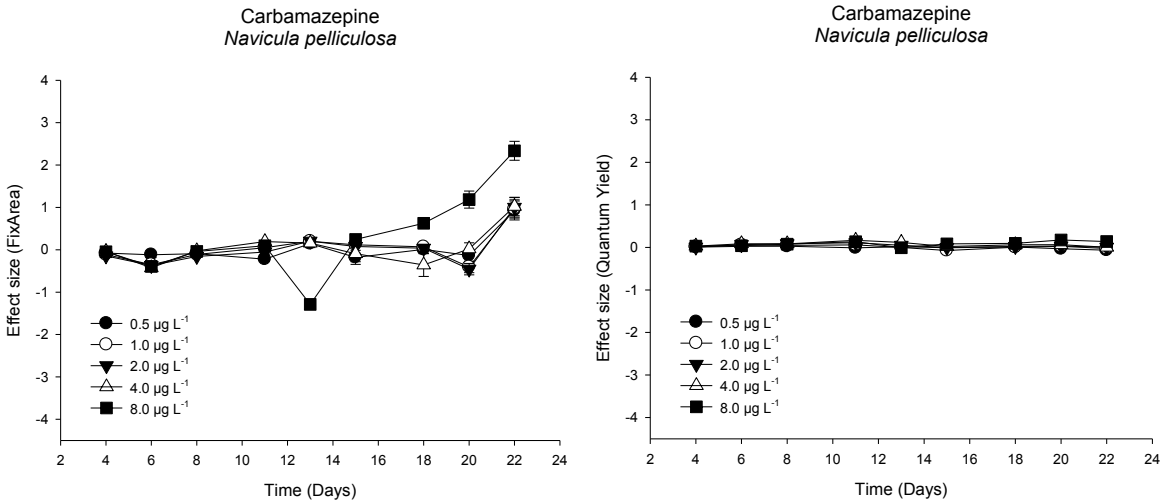


Fig. 7: FixArea (a) and Quantum Yield (b) of *Navicula pelliculosa* after exposure to different concentrations of Carbamazepine over a period of 22 days. Data expressed in mean effect sizes to the unexposed controls. Error bars represent standard errors.

The result of a Two Way Anova with the FixArea and the Quantum Yield data from *Navicula pelliculosa* showed, that the factors ‚time', ‚concentration' and the interaction of the two factors were significant (Tab. 7).

Tab. 7: Results of a Two Way Anova including FixArea and Quantum Yield data of *Navicula pelliculosa* treated with different concentrations of Carbamazepine (n=162); FA=FixArea; QY=Quantum Yield.

Algae species	Source of Variation	DF	F (FA)	P (FA)	F (QY)	P (QY)
N. pelliculosa	Conc	5	20.710	<0.001	50.183	<0.001
	Day	8	44.182	<0.001	25.044	<0.001
	Conc x Day	40	9.155	<0.001	5.386	<0.001

On day 6 (DF=5; F=5.617; P=0.007) significant differences of the FixArea data in treatments were observed. A post hoc test (Bonferroni t-test, contrast) identified that all treatments which were treated with Carbamazepine, except of the lowest dose, were significantly different when compared to the control treatment: 1.0 µg L^{-1} (t=3.472; P=0.023), 2.0 µg L^{-1} (t=3.659; P=0.016), 4.0 µg L^{-1} (t=4.058; P=0.008) and 8.0 µg L^{-1} (t=3.807; P=0.012). On the last sampling day a One Way Anova identified significant differences between the treatments (DF=5; F=31.483; P<0.001). A post hoc test (Bonferroni t-test, contrast) identified that the effect on treatments with 8.0 µg L^{-1} of Carbamazepine was significantly higher (t=10.942; P<0.001) than treatments without Carbamazepine.

A One Way Anova identified no significant differences between the treatments in the Quantum Yield data (DF=5; F=2.145; P=0.129) on day 6. On the last sampling day a One Way Anova (DF=5; F=32.260; P<0.001) identified significant differences. A post hoc test (Bonferroni t-test, contrast) showed that the effect of 0.5 µg L^{-1} (t=3.540; P=0.020) and 8.0 µg L^{-1} (t=8.282; P<0.001) Carbamazepine was significantly different when compared to the control treatments.

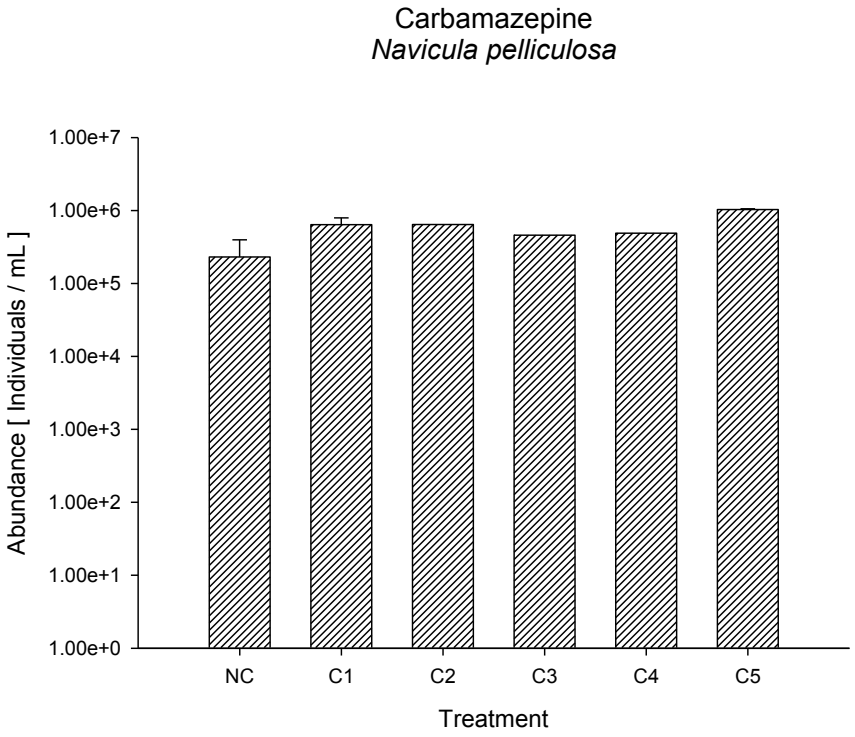


Fig. 8: Mean abundances of *Navicula pelliculosa* under the influence of Carbamazepine at day 22 of the experiment. Each treatment consisted of 3 replicates. Due to a contamination with high numbers of bacteria one replicate from C1, two replicates from C2, C3 and C4 had to be excluded from counting. Therefore it was not possible to calculate the standard error for C2, C3 and C4. NC: negative control; C1: 0.5 µg L^{-1}; C2: 1.0 µg L^{-1}; C3: 2.0 µg L^{-1}; C4: 4.0 µg L^{-1}; C5: 8.0 µg L^{-1}. Error bars represent standard errors.

The results of the microscopic counting showed that *N. pelliculosa* was most abundant in treatments with the highest concentration of Carbamazepine (8.0 µg L^{-1}). The lowest mean abundance of *N. pelliculosa* was found in control treatments (Fig. 8). A One Way Anova with the abundances identified significant differences (DF=2; F=11.642; P=0.013) between the treatment groups. A post hoc test (Bonferroni t-test, contrast) showed that the abundance of *N. pelliculosa* was significantly higher in treatments with 8.0 µg L^{-1} Carbamazepine than in the control treatments (t=4.722; P=0.026).

Peridinium sp.

The mean effect size of Carbamazepine on the FixArea of *Peridinium sp.* was around 0. Carbamazepine had an overall slightly negative effect on the Quantum Yield (Fig. 9 a, b).

a) b)

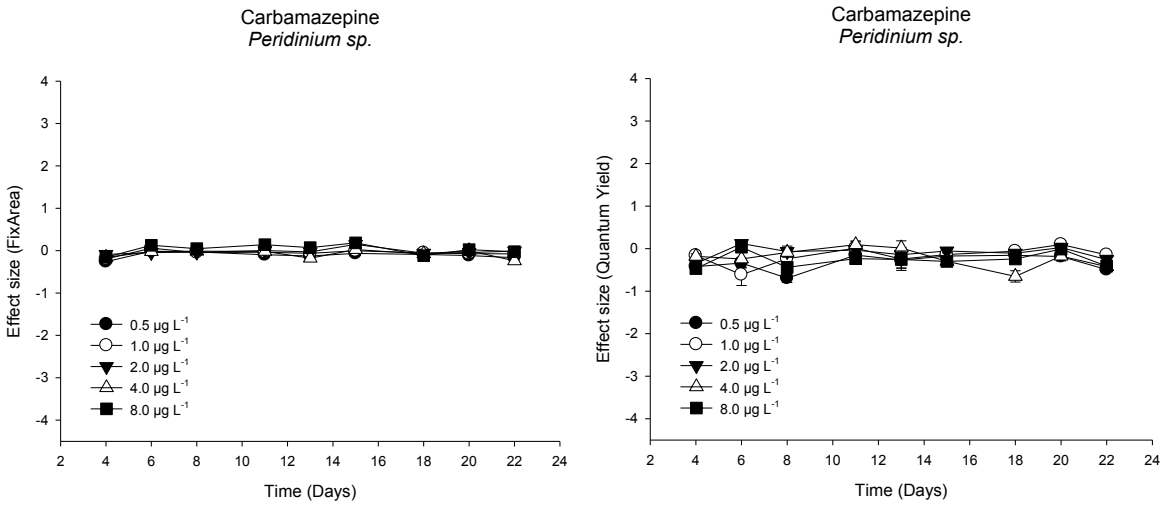


Fig. 9: FixArea (a) and Quantum Yield (b) of *Peridinium sp.* after exposure to different concentrations of Carbamazepine over a period of 22 days. Data expressed in mean effect sizes to the unexposed controls. Error bars represent standard errors.

The Two Way Anova with FixArea and Quantum Yield data from *Peridinium sp.* showed that the factors ‚concentration' and ‚time' each are significant. The interaction of both factors was not significant (Tab. 8).

Tab. 8: Results of a Two Way Anova including FixArea and Quantum Yield data of *Peridinium sp.* treated with different concentrations of Carbamazepine (n=162); FA=FixArea; QY=Quantum Yield.

Algae species	Source of Variation	DF	F (FA)	P (FA)	F (QY)	P (QY)
Perdinium sp.	Conc	5	5,764	<0.001	4.260	0.001
	Day	8	12,374	<0.001	12.113	<0.001
	Conc x Day	40	0,870	0.686	0.639	0.945

A One Way Anova for *Peridinium sp.* showed that on day 6 (DF=5; F1.551; P=0.247) and on day 22 (DF=5; F=2.256; P=0.115) the differences of the FixArea data between the treatments were not significant. Also no significant differences were found on day 6 (DF=5; F= 0.777; P=0.585) and on the last sampling day (DF=5; F=1.893; P=0.169) between the Quantum Yield data. A lysis of *Peridinium sp.* cells was clearly visible in treatments with Carbamazepine (Fig. 10). Due to technical reasons the monocultures of *Peridinium sp.* could not be counted under the microscope.

a) b) c)

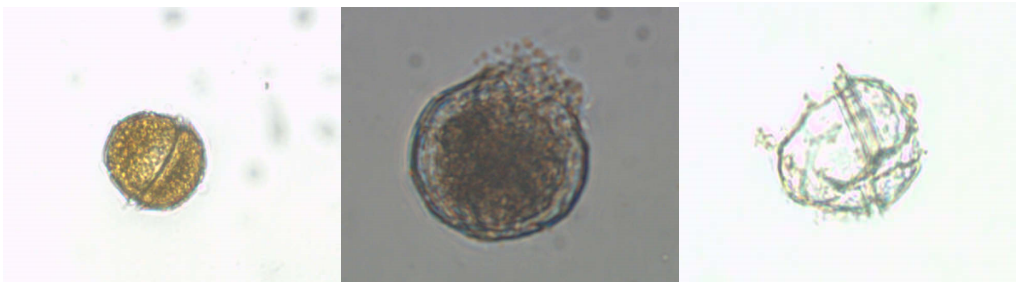


Fig. 10: (a) shows *Perdinium sp.* in treatment without Carbamazepine; (b) shows *Peridinium sp.* In treatment with Carbamazepine: crack of the membrane and elution of the inner part of the algae; (c) shows the empty membrane (photo reference: Eva Theresa Schmidt).

Cryptomonas phaseolus

The two treatments with the highest concentrations of Carbamazepine (4.0 and 8.0 µg L^{-1}) showed slightly negative mean effect sizes on FixArea from day 4 on. Except of the treatments with the lowest concentration of Carbamazepine (0.5 µg L^{-1}), the tested pharmaceutical had a negative, dose dependent effect. The mean effect sizes of Carbamazepine on Quantum Yield were similar: the strongest effects were observed within the treatments with the two highest concentrations of Carbamazepine (Fig. 11 a, b).

a) b)

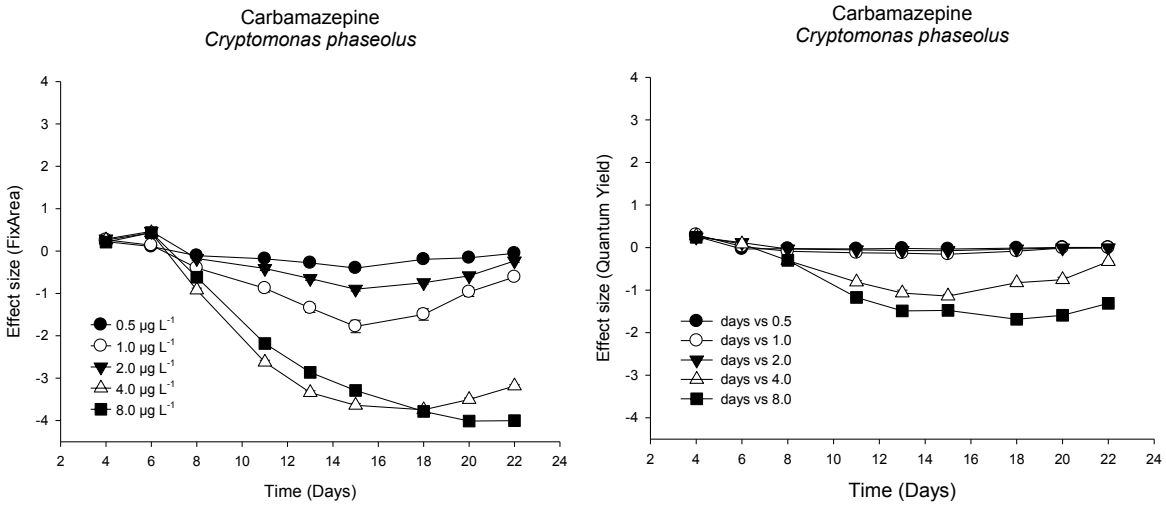


Fig. 11: FixArea (a) and Quantum Yield (b) of *Cryptomonas phaseolus* after exposure to different concentrations of Carbamazepine over a period of 22 days. Data expressed in mean effect sizes to the unexposed controls. Error bars represent standard errors.

The result of a Two Way Anova with the FixArea and Quantum Yield data from *Cryptomonas phaseolus* showed, that the factors ‚time', ‚concentration' and their interaction (concentration x time) were significantly (Tab. 9).

Tab. 9: Results of a Two Way Anova including FixArea and Quantum Yield data from measurements of *Cryptomonas phaseolus* treated with different concentrations of Carbamazepine (n=162). One replicate of Cryptomonas phaseolus from day 6 in the treatment with 8.0 µg L^{-1} was missing (n=161); FA=Fix Area; QY=Quantum Yield.

Algae species	Source of Variation	DF	F (FA)	P (FA)	F (QY)	P (QY)
C. phaseolus	Conc	5	550.099	<0.001	1204.686	<0.001
	Day	8	240.994	<0.001	98.779	<0.001
	Conc x Day	40	43.213	<0.001	67.042	<0.001

A One Way Anova identified that on day 6 the effects of Carbamazepine on the FixArea data were significantly different between the treatments (DF=5; F=10.998; P<0.001). A post hoc test (Bonferroni t-test, contrast) identified that the treatments with 2.0 (t=5.273; P<0.001), 4.0 (t=4.813; P=0.002) and 8.0 µg L^{-1} (t=4.894; P=0.002) Carbamazepine were significantly different compared to the control treatments. A One Way Anova identified significant differences between the groups on the last sampling day (DF=5; F=139.921; P<0.001). A post hoc test (Bonferroni t-test, contrast) identified that all treatments with Carbamazepine, except of 0.5 µg L^{-1}, had significantly lower FixArea values than the control treatments (1.0 µg L^{-1}: t=8.722; P<0.001; 2.0 µg L-1: t=4.037; P=0.008; 4.0 µg L-1: t=18.318; P<0.001; 8.0 µg L-1: t=18.759; P<0.001).

On day 6 significant effects of Carbamazepine on the Quantum Yield of *Cryptomonas phaseolus* were visible (DF=5; H=13.164; P=0.022). A post hoc test (Dunnett's method, contrast) identified no significant differences between the treatments with Carbamazepine compared to the control treatments. On the last sampling day the differences between the treatments were significant (DF=5; F=1034.922; P<0.001) whereas treatments with 4.0 µg L^{-1} and 8.0 µg L^{-1} Carbamazepine were lower than the controls (t=21.544; P<0.001 and t=56.164; P<0.001) which was identified by a post hoc test (Bonferroni t-test, contrast).

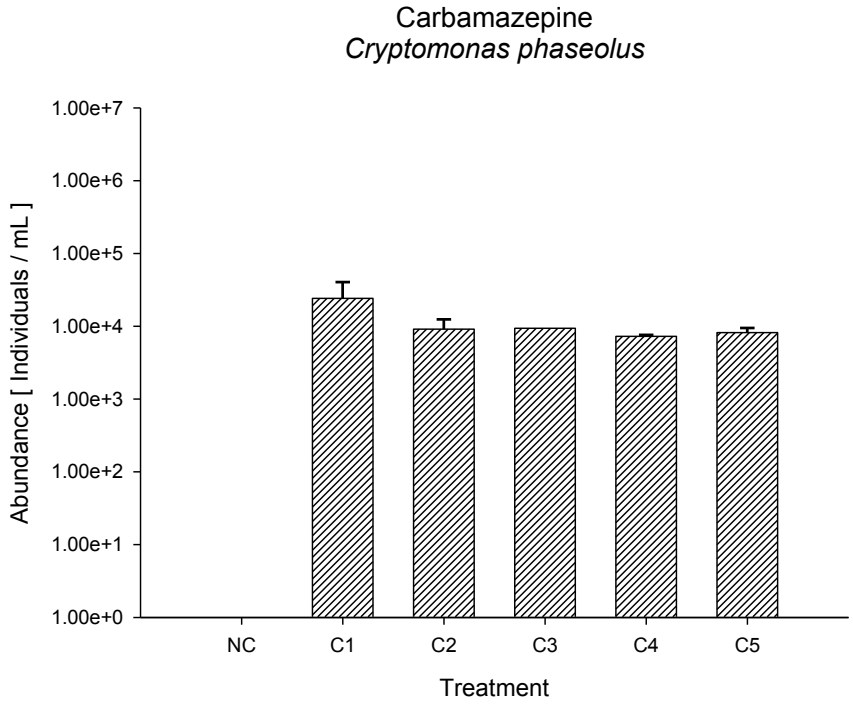


Fig. 12: Mean abundance of *Cryptomonas phaseolus* under the influence of Carbamazepine at day 22 of the experiment. Each treatment consisted of 3 replicates. Due to a contamination with high numbers of bacteria two replicates from NC, one replicate from C2 and two replicates from C3 had to be excluded from counting. Therefore it was not possible to calculate the standard error for C3. NC: negative control; C1: 0.5 µg L^{-1}; C2: 1.0 µg L^{-1}; C3: 2.0 µg L^{-1}; C4: 4.0 µg L^{-1}; C5: 8.0 µg L^{-1}. Error bars represent standard errors.

The results of the microscopic counting showed that *C. phaseolus* was most abundant in treatments with the lowest concentration (0.5 µg L^{-1}) of Carbamazepine (Fig. 12). A One Way Anova with the abundances identified no significant differences (DF=4; H=2.924; P=0.571) between the treatment groups.

Chroococcus minutus

Carbamazepine had an effect on the FixArea of the monocultures of *Chroococcus minutus*. Slight effects were observed in the positive as well as in the negative direction. Overall the treatments with 4.0 and 8.0 µg L^{-1} showed stronger effects on FixArea compared to the other treatments. Carbamazepine had only minor effects on Quantum Yield (Fig. 13 a, b).

a) b)

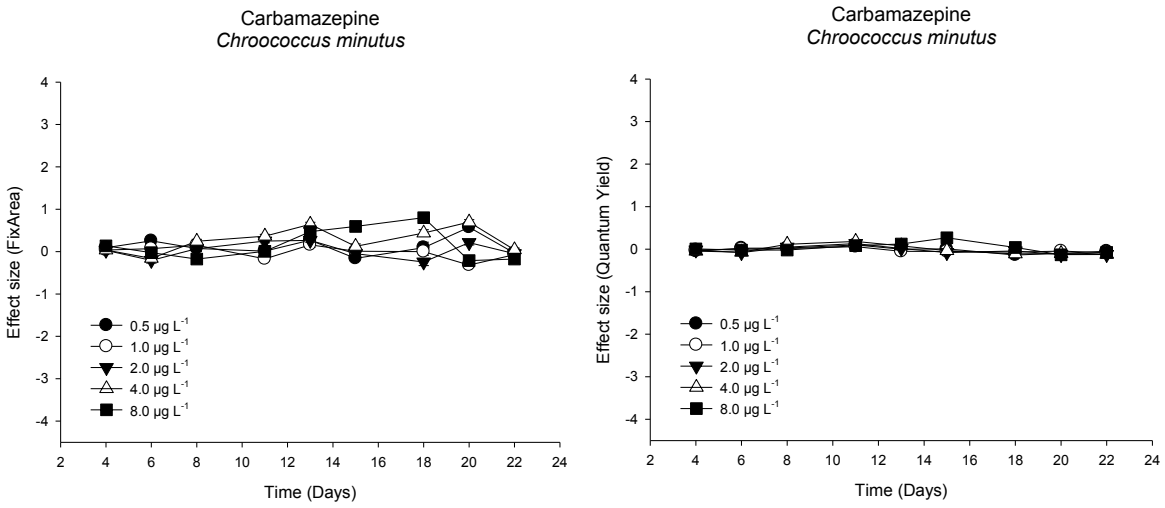


Fig. 13: FixArea (a) and Quantum Yield (b) of *Croococcus minutus* after exposure to different concentrations of Carbamazepine over a period of 22 days. Data expressed in mean effect sizes to the unexposed controls. Error bars represent standard errors.

The result of a Two Way Anova with the FixArea and Quantum Yield data from *Chroococcus minutus* showed, that the factors ‚time', ‚concentration' and the interaction (concentration x time) were significant (Tab. 10).

Tab. 10: Results of a Two Way Anova including FixArea and Quantum Yield data from measurements of *Chroococcus minutus* treated with different concentrations of Carbamazepine (n=162); FA=FixArea; QY=Quantum Yield.

Algae species	Source of Variation	DF	F (FA)	P (FA)	F (QY)	P (QY)
C. minutus	Conc	5	20.484	<0.001	5.256	<0.001
	Day	8	105.711	<0.001	30.087	<0.001
	Conc x Day	40	8.634	<0.001	4.541	<0.001

The results of the One Way Anova identified significant differences for FixArea data between the treatments on day 6 (DF=5; F=3.144; P=0.048). A post hoc test (Bonferroni t-test, contrast) identified no significant differences between the treatments with Carbamazepine compared to the control treatments. A One Way Anova identified no significant differences for FixArea data between the treatments on day 22 (DF=5; F=3.316; P=0.651). There were no significant differences found in the Quantum Yield data on day 6 (DF=5; F=1.046, P=0.435) or on day 22 (DF=5; F=1.689; P=0.212).

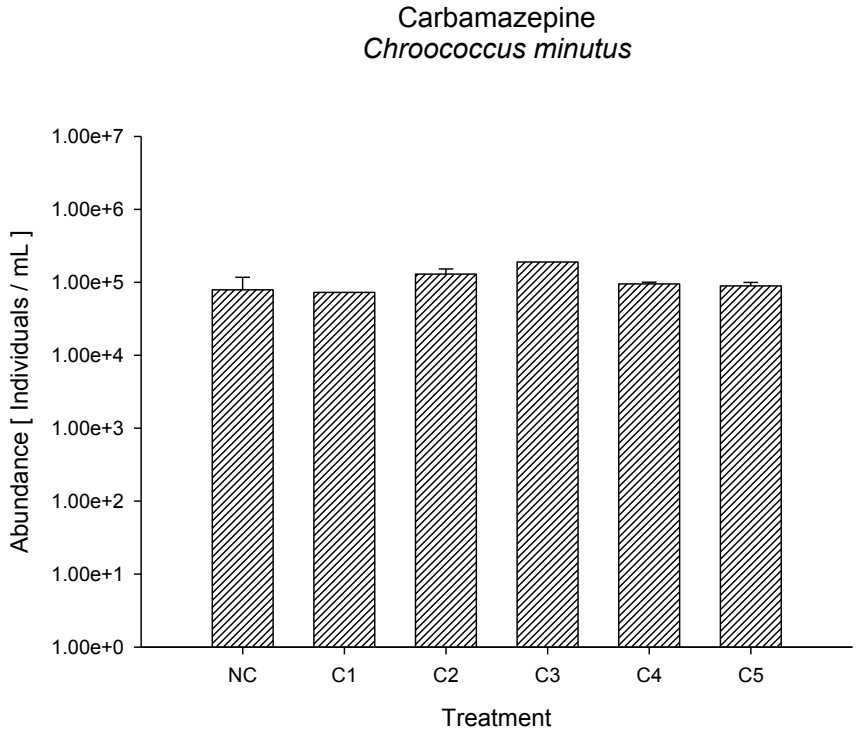


Fig. 14: Mean abundance of *Chroococcus minutus* under the influence of Carbamazepine at day 22 of the experiment. Each treatment consisted of 3 replicates. Due to a contamination with bacteria two replicates from C1, two replicates from C3 and one replicate from C4 and C5 had to be excluded from counting. Therefore it was not possible to calculate the standard error for C1 and C3. NC: negative control; C1: 0.5 µg L^{-1}; C2: 1.0 µg L^{-1}; C3: 2.0 µg L^{-1}; C4: 4.0 µg L^{-1}; C5: 8.0 µg L^{-1}. Error bars represent standard errors.

Up to a concentration of 2.0 µg L^{-1} Carbamazepine an increase in the mean abundance of *Chroococcus minutus* was observable. The addition of 4.0 and 8.0 µg L^{-1} led to a decrease in the mean abundance of *C. minutus* and abundances were comparable to the control treatments and the treatments with 1.0 µg L^{-1} (Fig. 14). A One Way Anova with the abundances identified no significant differences (DF=5; H=5.628; P=0.344) between the treatment groups.

5.1.1.2 Impact of Ciprofloxacin on algae in monocultures

Scenedesmus obliquus

The effects of Ciprofloxacin on FixArea of *Scenedesmus obliquus* were slightly positive with higher concentrations of Ciprofloxacin compared to the control. On the last day of the experiment a negative mean effect size was observed for treatments with 0.010 µg L^{-1} Ciprofloxacin. The effects of Ciprofloxacin on Quantum Yield were around 0 (Fig. 15 a, b).

a) b)

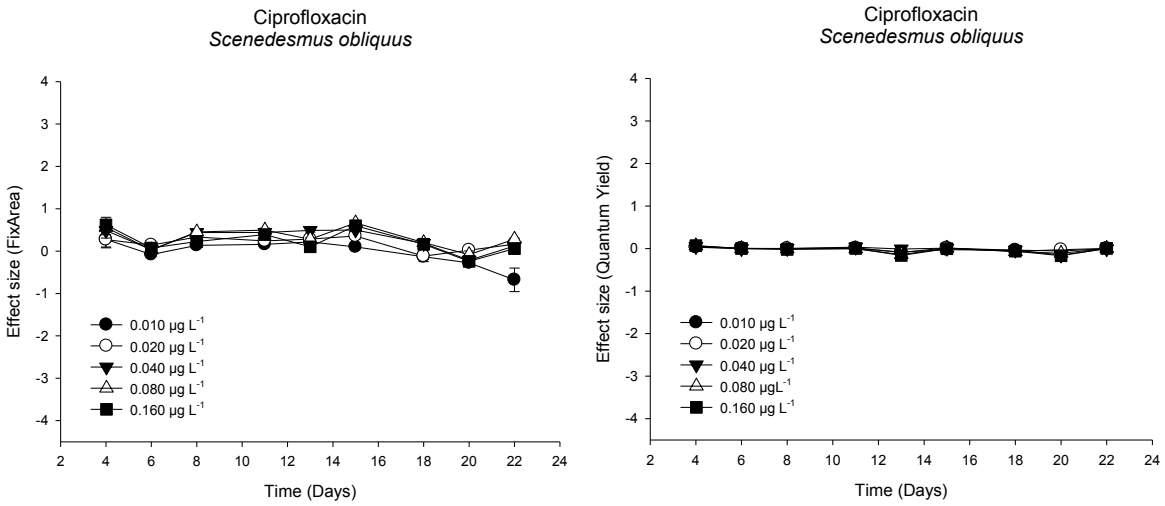


Fig. 15: FixArea (a) and Quantum Yield (b) of *Scenedesmus obliquus* after exposure to different concentrations of Ciprofloxacin over a period of 22 days. Data expressed in mean effect sizes to the unexposed controls. Error bars represent standard errors.

The result of a Two Way Anova with the FixArea data from *Scenedesmus obliquus* showed, that the factors ‚time', ‚concentration' and the interaction (concentration x time) were significant. The results with the Quantum Yield data showed, that the factor 'time' was significant (Tab. 11).

Tab. 11: Results of a Two Way Anova including FixArea and Quantum Yield data of *S. obliquus* treated with Ciprofloxacin (n=162); FA=FixArea; QY=Quantum Yield.

Algae species	Source of Variation	DF	F (FA)	P (FA)	F (QY)	P (QY)
S. obliquus	Conc	5	10.064	<0.001	1.416	0.224
	Day	8	131.074	<0.001	10.167	<0.001
	Conc x Day	40	1.927	0.004	1.251	0.182

On day 6 (DF=5; F=1.914; P=0.166) there were no significant differences for the FixArea data identified by a One Way Anova. On the last sampling day no significant differences were found (DF=5; F=2.549; P=0.086) by a One Way Anova.

The results of a One Way Anova also showed no significant effect of Ciprofloxacin on the Quantum Yield values between the treatments on day 6 (DF=5; F=0.222; P=0.946) and day 22 (H=7.185; DF=5; P=0.207).

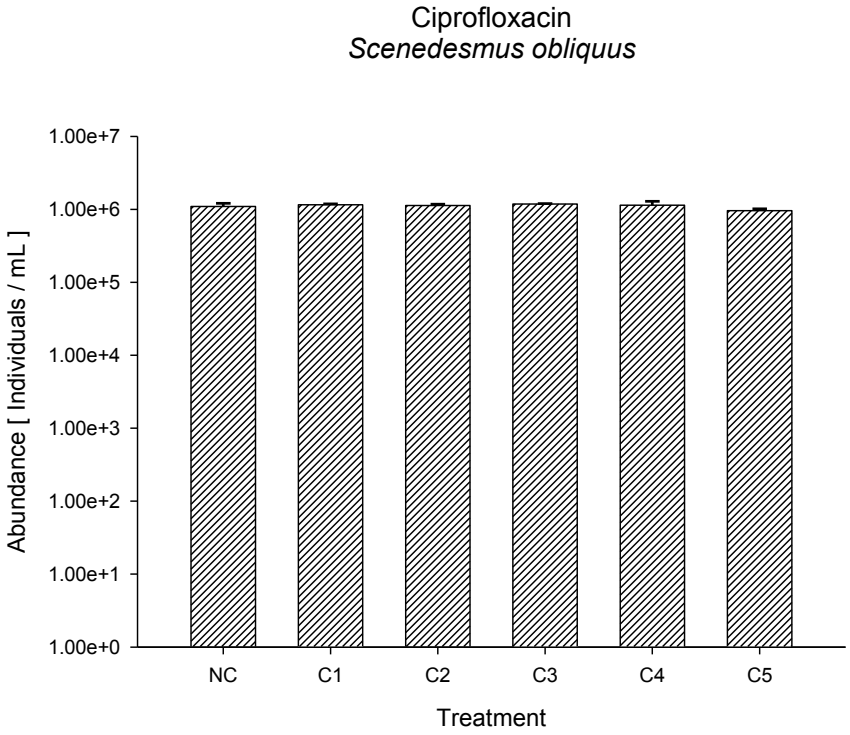


Fig. 16: Mean abundance of *Scenedesmus obliquus* under the influence of Ciprofloxacin at day 22 of the experiment. Each treatment consisted of 3 replicates. NC: negative control; C1: 0.010 µg L^{-1}; C2: 0.020 µg L^{-1}; C3: 0.040 µg L^{-1}; C4: 0.080 µg L^{-1}; C5: 0.160 µg L^{-1}. Error bars represent standard errors.

The mean abundance of *Scenedesmus obliquus* was not negatively affected by concentrations ranging from 0.010 to 0.080 µg L^{-1} Ciprofloxacin. In treatments with 0.160 µg L^{-1} a decrease in the mean abundance was recognizable (Fig. 16). A One Way Anova with the abundances identified no significant differences (DF=5; F=0.881; P=0.522) between the treatment groups.

Navicula pelliculosa

From day 6 to day 13 positive effects of Ciprofloxacin on the FixArea became visible. Strongest effects were seen with 0.040 µg L^{-1}. After that the effect of Ciprofloxacin on FixArea became negative (Fig. 17 a).

a) b)

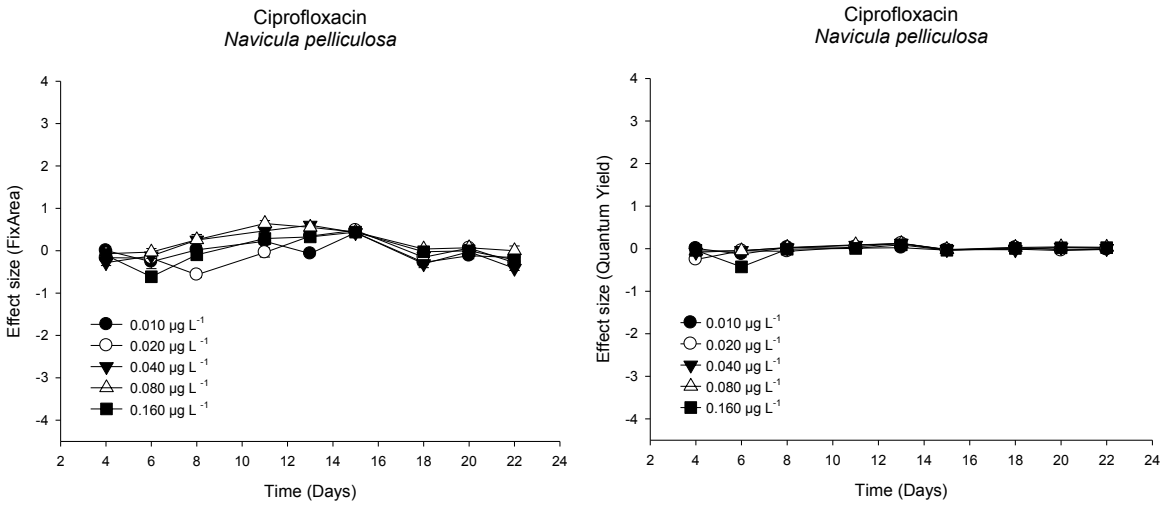


Fig. 17: FixArea (a) and Quantum Yield (b) of *Navicula pelliculosa* after exposure to different concentrations of Ciprofloxacin over a period of 22 days. Data expressed in mean effect sizes to the unexposed controls. Error bars represent standard errors.

The strongest effect of Ciprofloxacin on Quantum Yield was observed within the highest concentration (0.0160 µg L^{-1}) at day 6. After this the initial negative effect of Ciprofloxacin on Quantum Yield effect sizes turned into positive and approached 0 (Fig. 17 b).

The result of a Two Way Anova with the FixArea and Quantum Yield data from *Navicula pelliculosa* showed, that the factors ‚time', ‚concentration' and the interaction (concentration x time) were significant (Tab. 12).

Tab. 12: Results of a Two Way Anova including FixArea and Quantum Yield data of *N. pelliculosa* treated with Ciprofloxacin (n=162); FA=FixArea; QY=Quantum Yield.

Algae species	Source of Variation	DF	F (FA)	P (FA)	F (QY)	P (QY)
N. pelliculosa	Conc	5	5.000	<0.001	2.984	0.015
	Day	8	236.142	<0.001	68.136	<0.001
	Conc x Day	40	2.175	<0.001	2.054	0.002

A One Way Anova identified no significant differences in the FixArea data on day 6 (DF=5; F=1.231; P=0.353) or on day 22 (DF=5; F=1.566; P=0.243). The addition of Ciprofloxacin did not lead to significant differences in the Quantum Yield values on day 6 (DF=5; F=3.061; P=0.052) nor on day 22 (DF=5; F=2.761; P=0.069), which was shown by a One Way Anova.

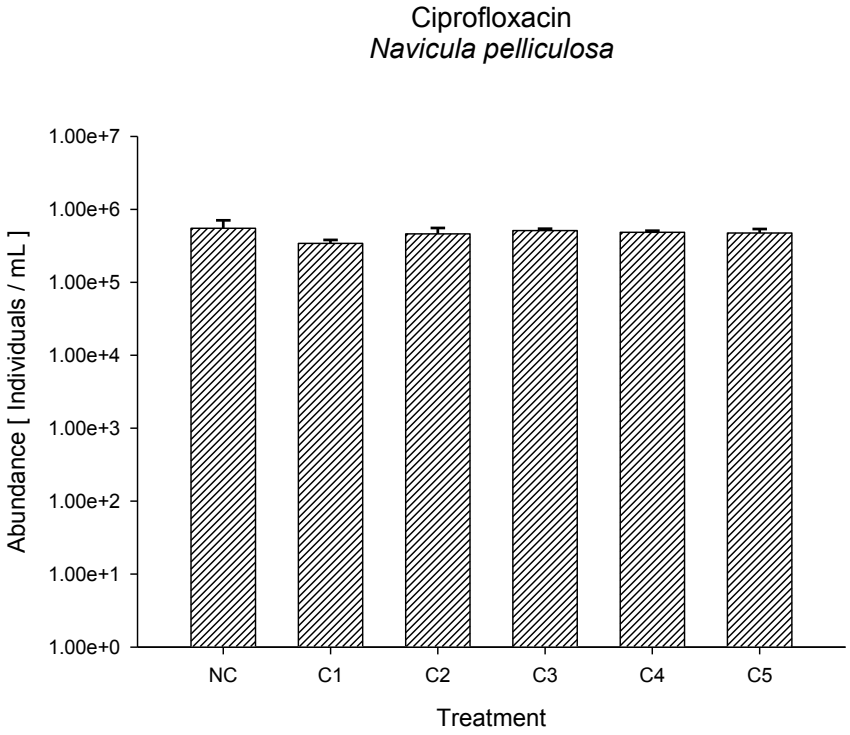


Fig. 18: Mean abundance of *Navicula pelliculosa* under the influence of Ciprofloxacin at day 22 of the experiment. Each treatment consisted of 3 replicates. NC: negative control; C1: 0.010 µg L^{-1}; C2: 0.020 µg L^{-1}; C3: 0.040 µg L^{-1}; C4: 0.080 µg L^{-1}; C5: 0.160 µg L^{-1}. Error bars represent standard errors.

Compared to the control treatment a slight decrease in the mean abundance in all treatments with Ciprofloxacin was seen (Fig. 18). A One Way Anova with the abundances identified no significant differences (DF=5; F=0.725; P=0.617) between the treatment groups.

Peridinium sp.

Over the time course of the experiment monocultures of *Peridinium sp.* got less dense. Therefore a sample was taken to investigate the cultures under the microscope. The monocultures of *Peridinium sp.* showed a similar response (lysis) such as seen when exposed to Carbamazepine (Fig. 10). The mean effect sizes of Ciprofloxacin on the FixArea and Quantum Yield were around 0 in the *Peridinium sp.* cultures during the experiment (Fig. 19 a, b).

a) b)

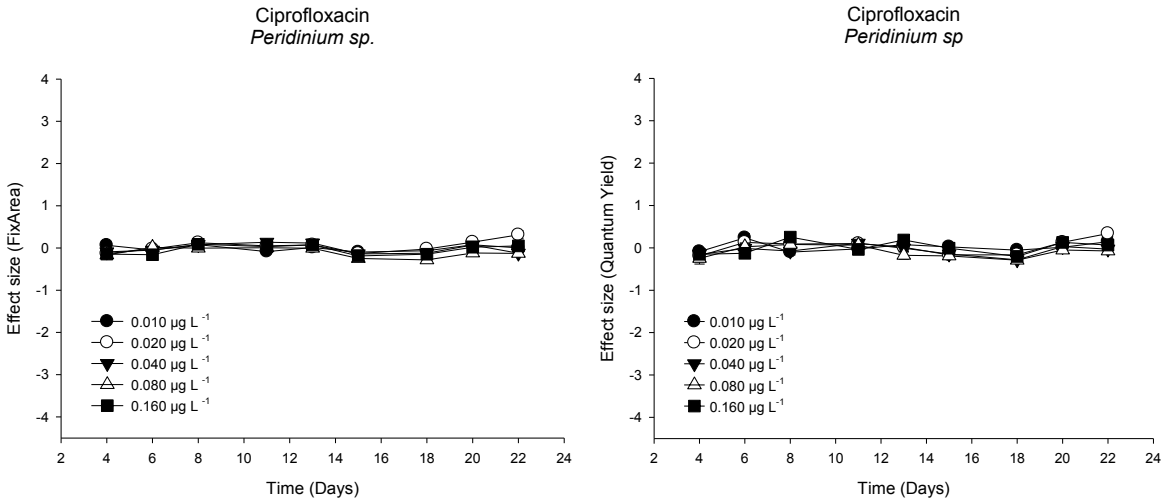


Fig. 19: FixArea (a) and Quantum Yield (b) of *Peridinium sp.* after exposure to different concentrations of Ciprofloxacin over a period of 22 days. Data expressed in mean effect sizes to the unexposed controls. Error bars represent standard errors.

The results of the Two Way Anova showed that the significant effects of the different concentrations of Ciprofloxacin on the FixArea and the Quantum Yield values were dependent on the factor time (Tab. 13).

Tab. 13: Results of a Two Way Anova including FixArea and Quantum Yield data of *Peridinium sp.* treated with Ciprofloxacin (n=162); FA=FixArea; QY=Quantum Yield.

Algae species	Source of Variation	DF	F (FA)	P (FA)	F (QY)	P (QY)
Perdinium sp.	Conc	5	5.259	<0.001	2.605	0.029
	Day	8	61.142	<0.001	8.630	<0.001
	Conc x Day	40	1.773	0.011	1.161	0.270

There were no significant differences in the FixArea data found on day 6 (DF=5; F=1.584; P=0.238) by a One Way Anova. On day 22 a One Way Anova identified significant differences in the data (DF=5; H=11.433; P=0.043). In comparison to the control group no significant differences were identified by a post hoc test (Dunnett's method, contrast).

A One Way Anova was performed to identify significant differences at days 6 and 22 in the Quantum Yield data. On day 6 no significant differences were found (DF=5; F=1.416; P=0.287). A One Way Anova identified significant differences in the values (DF=5; F=3.147; P=0.048) on day 22 for the Quantum Yield values. Compared to the control group there were no significant differences found by a post hoc test.

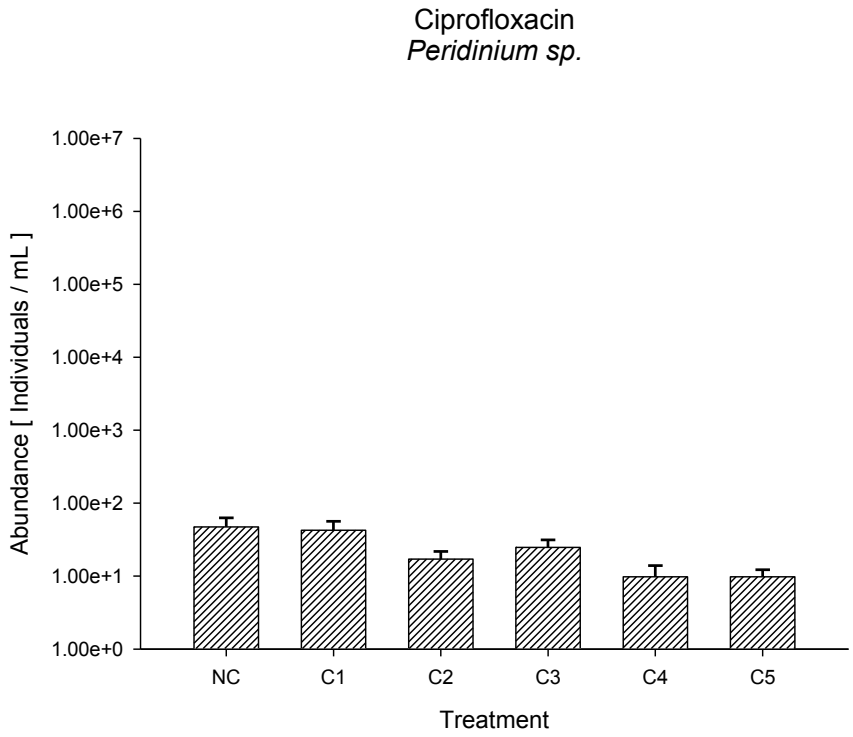


Fig. 20: Mean abundance of *Peridinium sp.* under the influence of Ciprofloxacin at day 22 of the experiment. Each treatment consisted of 3 replicates. NC: negative control; C1: 0.010 µg L^{-1}; C2: 0.020 µg L^{-1}; C3: 0.040 µg L^{-1}; C4: 0.080 µg L^{-1}; C5: 0.160 µg L^{-1}. Error bars represent standard errors.

The visual observation that the cultures got less dense when exposed to Ciprofloxacin was also reflected in the results of microscopic counting of the monocultures with *Peridinium sp*. (Fig. 20). With the exception of the concentration of 0.040 µg L^{-1} Ciprofloxacin, the mean abundance of *Peridinium sp.* decreases with higher concentrations. A One Way Anova with the abundances identified no significant differences (DF=5; F=3.007; P=0.055) between the treatment groups.

Cryptomonas phaseolus

Ciprofloxacin had a positive effect on the FixArea in the first two weeks compared to the control and to the lowest concentration. From day 15 onwards this pattern switched. Treatments of all concentrations were negatively affected and this effect was stronger in treatments with higher concentrations (Fig. 21 a).

a) b)

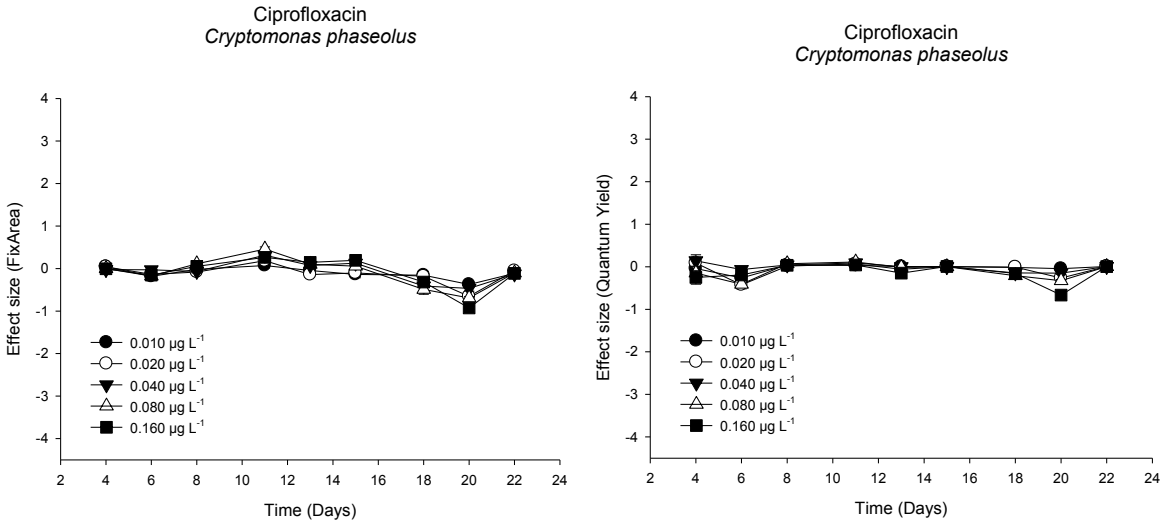


Fig. 21: FixArea (a) and Quantum Yield (b) of *Cryptomonas phaseolus* after exposure to different concentrations of Ciprofloxacin over a period of 22 days. Data expressed in mean effect sizes to the unexposed controls. Error bars represent standard errors.

The mean effect sizes of Ciprofloxacin on the Quantum Yield (Fig. 21 b) were in the first and in the last week negatively impacted by Ciprofloxacin. In the second week the mean effect sizes on Quantum Yield were around 0. A Two Way Anova showed significant effects of concentration and time, both factors also interacted in a significant way (Tab. 14).

Tab. 14: Results of a Two Way Anova including FixArea and Quantum Yield data of *C. phaseolus* treated with Ciprofloxacin. Two replicates on day 20 of the control and one replicate in the treatment with 0.010 µg L^{-1} were missing (n=159); FA=FixArea; QY=Quantum Yield.

Algae species	Source of Variation	DF	F (FA)	P (FA)	F (QY)	P (QY)
C. phaseolus	Conc	5	3.895	0.003	8.520	<0.001
	Day	8	340.418	<0.001	221.775	<0.001
	Conc x Day	40	2.408	<0.001	3.125	<0.001

There were significant differences of the FixArea found between treatments on day 6 (DF=5; F=5.094; P=0.010). A post hoc test (Bonferroni t-test, contrast) identified that the FixArea data were significantly different with concentrations of 0.010 µg L^{-1} (t=3.759; P=0.014), 0.080 µg L^{-1} (t=3.598; P=0.018) and 0.160 µg L^{-1} (t=3.158; P=0.041) compared to the control treatments. On the last sampling day the differences between the treatments were not significant (DF=5; F=1.170; P=0.379).

On day 6 a One Way Anova identified significant differences in the Quantum Yield data (DF=5; F=4.328; P=0.018). A post hoc test (Bonferroni t-test, contrast) identified that treatments with 0.020 and 0.080 µg L^{-1} Ciprofloxacin were significantly different than the control treatments (t=3.516; P=0.021 and t=3.615; P=0.018). On the last sampling day no significant differences in the Quantum Yield data were found by a One Way Anova (H=3.073; DF=5; P=0.689).

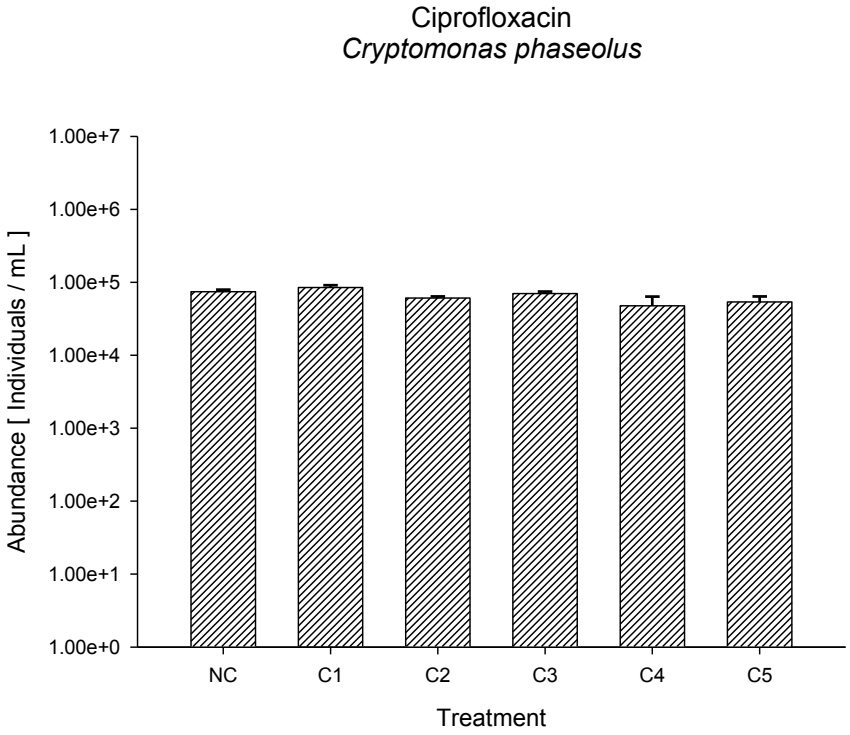


Fig. 22: Mean abundance of *Cryptomonas phaseolus* under the influence of Ciprofloxacin at day 22 of the experiment. Each treatment consisted of 3 replicates. NC: negative control; C1: 0.010 µg L^{-1}; C2: 0.020 µg L^{-1}; C3: 0.040 µg L^{-1}; C4: 0.080 µg L^{-1}; C5: 0.160 µg L^{-1}. Error bars represent standard errors.

The highest mean abundance of *C. phaseolus* was found in the treatments with the lowest Ciprofloxacin concentration (0.010 µg L^{-1}) and in the control treatments. All other treatments showed lower mean abundances. Monocultures that were exposed to the two highest concentrations (0.080 and 0.160 µg L^{-1}) reached the lowest mean abundances (Fig. 22). A One Way Anova with the abundances identified no significant differences (DF=5; F=2.423; P=0.097) between the treatment groups.

Chroococcus minutus

It has to be mentioned that in the monocultures of *Chroococcus minutus* a contamination with *Scenedesmus obliquus* occurred. Therefore the mean effect sizes of Ciprofloxacin on FixArea and Quantum Yield of *Chroococcus minutus* could have been influenced by this contamination.

a) b)

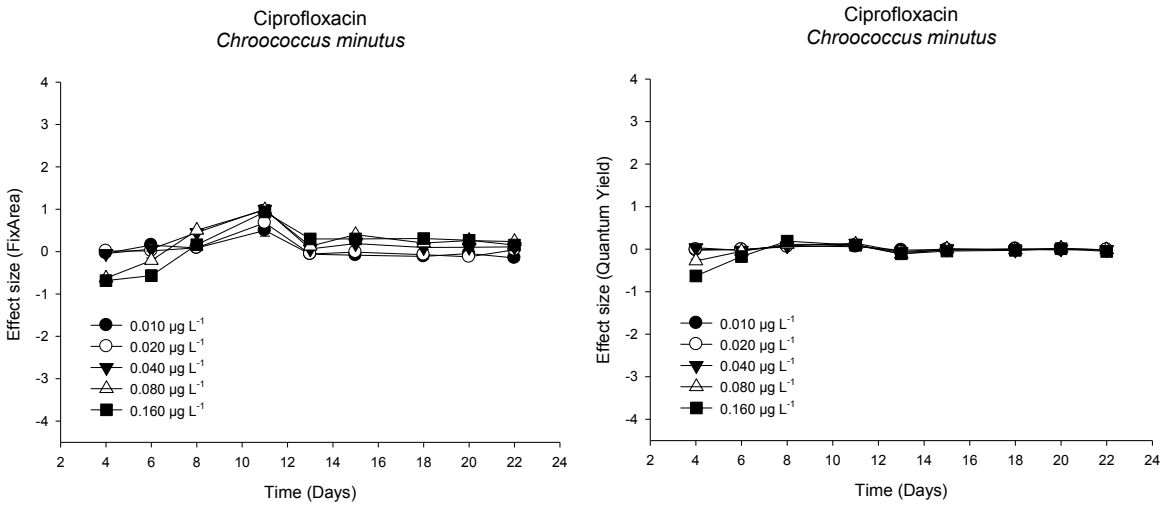


Fig. 23: FixArea (a) and Quantum Yield (b) of *Chroococcus minutus* after exposure to different concentrations of Ciprofloxacin over a period of 22 days. Data expressed in mean effect sizes to the unexposed controls. Error bars represent standard errors.

Ciprofloxacin had a positive effect on FixArea, up to a mean effect size of ~1.0 in the first 11 days. This was visible in treatments with 0.040, 0.080 and 0.160 µg L^{-1} of Ciprofloxacin. In the following the effect decreased and a slight dose dependent effect was observable with less negative impact with lower concentrations of Ciprofloxacin. The two highest concentrations of Ciprofloxacin (0.080 and 0.160 µg L^{-1}) affected the Quantum Yield until day 6. Afterwards the mean effect sizes of Ciprofloxacin on Quantum Yield were around 0 (Fig. 23 a, b).

The result of a Two Way Anova with the FixArea and the Quantum Yield data from *Chroococcus minutus* showed, that the factors ‚time', ‚concentration' and the interaction of the two factors were significant (Tab. 15).

Tab. 15: Results of a Two Way Anova including FixArea and Quantum Yield data of *C. minutus* treated with Ciprofloxacin (n=162); FA=FixArea; QY=Quantum Yield.

Algae species	Source of Variation	DF	F (FA)	P (FA)	F (QY)	P (QY)
C. minutus	Conc	5	32.719	<0.001	8.478	<0.001
	Day	8	829.591	<0.001	425.026	<0.001
	Conc x Day	40	4.058	<0.001	6.277	<0.001

A One Way Anova identified significant differences in the FixArea data between the treatments (DF=5; F=5.523; P=0.007). A post hoc test (Bonferroni t-test, contrast) identified that the treatments with the highest concentration (0.160 µg L^{-1}) were significantly different than the control treatments (t=3.263; P=0.034) on day 6. At the last sampling day significant differences in the FixArea data between the treatments were identified. A post hoc test (Bonferroni t-test, contrast) identified that treatments with 0.080 µg L^{-1} Ciprofloxacin were significantly different from the control treatments (t=3.761; P=0.014).

Neither on day 6 (DF=5; H=6.031; P=0.303) nor on day 22 (DF=5; H=10.716; P=0.057) significant differences in the Quantum Yield data were found between the treatments (DF=5; H=10.716; P=0.057) by a One Way Anova.

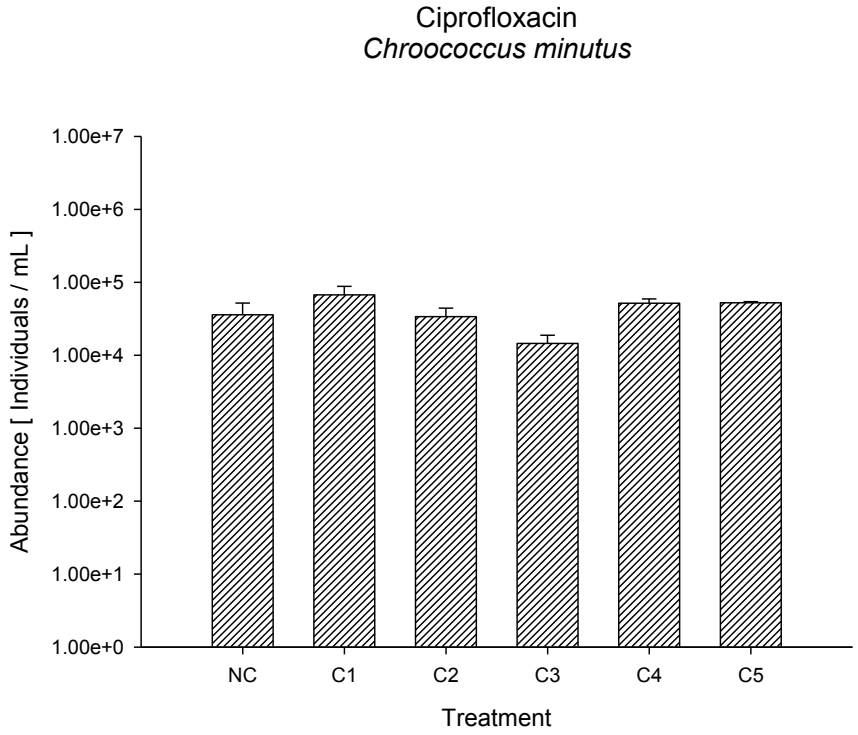


Fig. 24: Mean abundance of *Chroococcus minutus* under the influence of Ciprofloxacin at day 22 of the experiment. Each treatment consisted of 3 replicates. NC: negative control; C1: 0.010 µg L^{-1}; C2: 0.020 µg L^{-1}; C3: 0.040 µg L^{-1}; C4: 0.080 µg L^{-1}; C5: 0.160 µg L^{-1}. Error bars represent standard errors.

Ciprofloxacin had a slightly negative effect on the mean abundances of *Chroococcus minutus* (Fig. 24). A One Way Anova with the abundances identified no significant differences (DF=5; F=2.335; P=0.106) between the treatment groups.

5.1.1.3 Impact of Fluoxetine on algae in monocultures

Scenedesmus obliquus

One replicate of the treatments with 0.048 µg L^{-1} was spilled at the first measurement (day 4). For that reason this replicate was excluded from analyses. Therefore *n* for final analyses was 154 instead of 162.

The effect of Fluoxetine on the FixArea increased with experimental duration. The highest concentration of Fluoxetine (0.096 µg L^{-1}) had the largest effect on FixArea of *S. obliquus*. The mean effect sizes of Fluoxetine on Quantum Yield were around 0 (Fig. 25 a, b).

a) b)

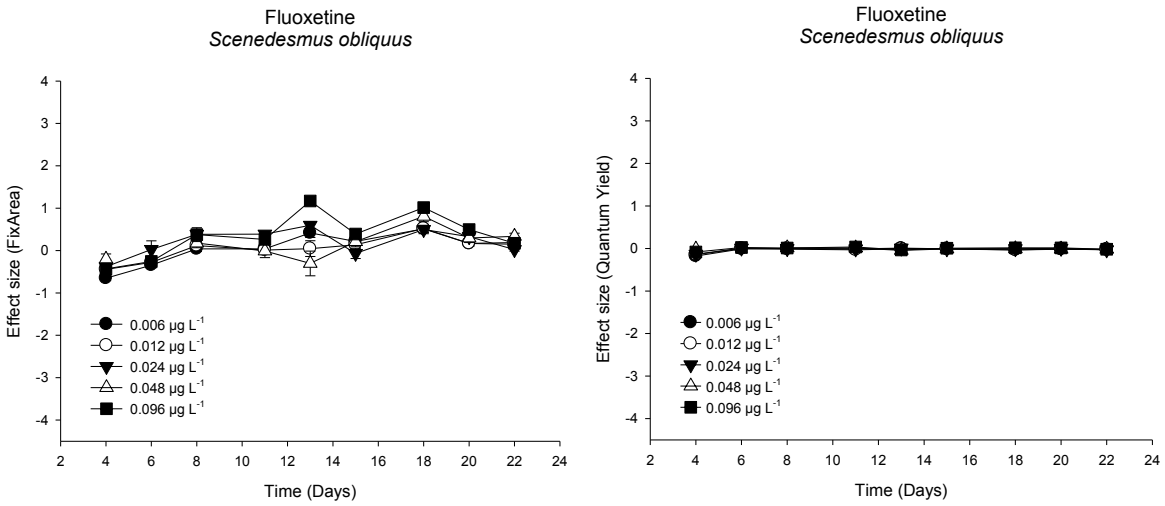


Fig. 25: FixArea (a) and Quantum Yield (b) of *Scenedesmus obliquus* after exposure to different concentrations of Fluoxetine over a period of 22 days. Data expressed in mean effect sizes to the unexposed controls. Error bars represent standard errors.

A Two Way Anova showed significant effects of concentration and time for FixArea and Quantum Yield, in both cases also the interaction between the two factors were significant (Tab. 16).

Tab. 16: Results of a Two Way Anova including FixArea and Quantum Yield data of *S. obliquus* treated with Fluoxetine (n=154); FA=FixArea; QY=Quantum Yield.

Algae species	Source of Variation	DF	F (FA)	P (FA)	F (QY)	P (QY)
S. obliquus	Conc	5	14.053	<0.001	4.280	0.001
	Day	8	226.487	<0.001	40.717	<0.001
	Conc x Day	40	2.253	<0.001	2.870	<0.001

A One Way Anova identified no significant differences in the FixArea data between the treatments on day 6 (DF=5; F=0.584; P=0.712) and also not on day 22 (DF=5; F=1.055; P=0.431). The addition of Fluoxetine did not lead to significant differences in the Quantum Yield data between the treatments on day 6 (DF=5; H=6.061; P=0.300) nor on day 22 (DF=5; F=2.549; P=0.086).

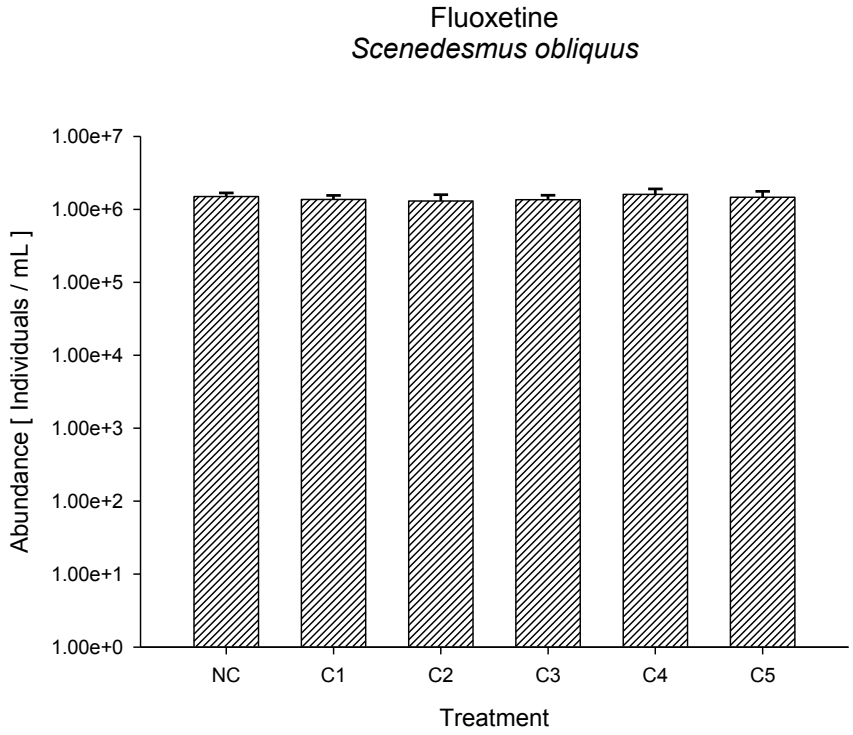


Fig. 26: Mean abundance of *Scenedesmus obliquus* under the influence of Fluoxetine at day 22 of the experiment. Each treatment consisted of 3 replicates. NC: negative control; C1: 0.006 µg L^{-1}; C2: 0.012 µg L^{-1}; C3: 0.024 µg L^{-1}; C4: 0.048 µg L^{-1}; C5: 0.096 µg L^{-1}. Error bars represent standard errors.

Microscopic countings showed that the mean abundance of *Scenedesmus obliquus* was not negatively affected by Fluoxetine (Fig. 26). A One Way Anova with the abundances identified no significant differences (DF=5; F=0.223; P=0.946) between the treatment groups.

Navicula pelliculosa

The highest effects of Fluoxetine on the FixArea were observed in treatments with 0.024 and 0.048 µg L^{-1} Fluoxetine until day 13. In the second half of the experiment the mean effect sizes were around 0. Effects of Fluoxetine on the Quantum Yield were observed on day 6 where concentrations of 0.024 µg L^{-1} Fluoxetine had a positive effect and concentrations of 0.048 and 0.096 µg L^{-1} Fluoxetine had slight negative effects on the Quantum Yield of *Navicula pelliculosa*. After day 11 the mean effect sizes of all treatments were around 0 (Fig. 27 a, b).

a) b)

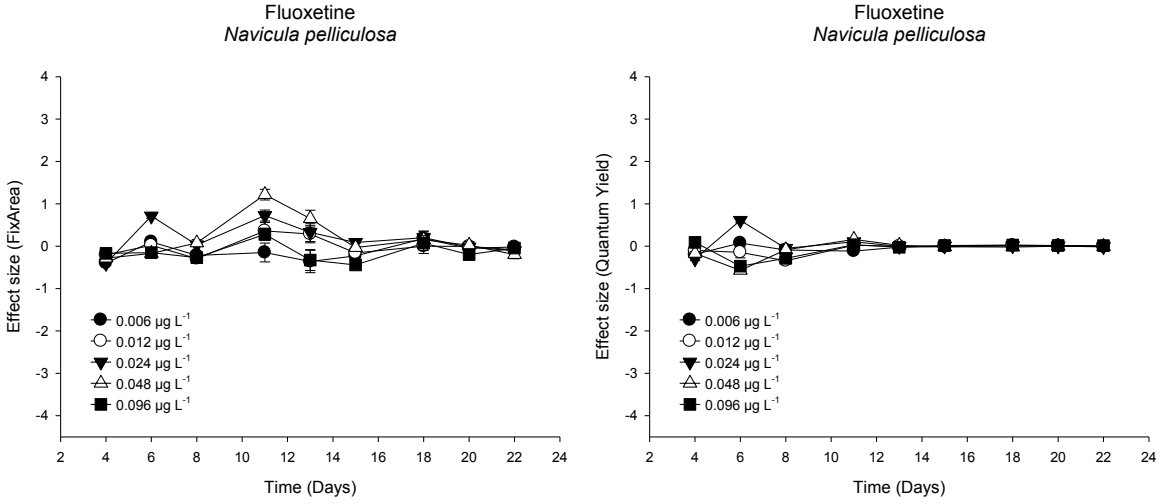


Fig. 27: FixArea (a) and Quantum Yield (b) of *Navicula pelliculosa* after exposure to different concentrations of Fluoxetine over a period of 22 days. Data expressed in mean effect sizes to the unexposed controls. Error bars represent standard errors.

A Two Way Anova showed significant effects of the factors concentration and time for FixArea. The interaction between the two factors was not significant. Significant effects of the factors concentration and time were found in the Quantum Yield data for the factors concentration and time and also their interaction was significant (Tab. 17).

Tab. 17: Results of a Two Way Anova including FixArea and Quantum Yield data of *N. pelliculosa* treated with Fluoxetine (n=162); FA=FixArea; QY=Quantum Yield.

Algae species	Source of Variation	DF	F (FA)	P (FA)	F (QY)	P (QY)
N. pelliculosa	Conc	5	2.506	0.035	4.382	0.001
	Day	8	266.729	<0.001	355.541	<0.001
	Conc x Day	40	1.254	0.180	6.492	<0.001

A One Way Anova identified that there were significant differences in the data of the FixArea between the treatments on day 6 (DF=5; F=20.270; P<0.001). A post hoc test (Bonferroni t-test, contrast) identified significant differences in treatments with 0.024 µg L^{-1} (t=7.357; P<0.001) compared to the control treatments. On day 22 there were no significant differences between the treatments (DF=5; F=0.315; P=0.894) found by a One Way Anova.

A One Anova identified significant differences between the treatments on day 6 in the Quantum Yield data (DF=5; F=14.906; P<0.001). A post hoc test (Bonferroni t-test, contrast) identified that the Quantum Yield data in treatments with 0.024 µg L^{-1} Fluoxetine were significantly different to the control treatments (t=4.967; P=0.002). Also on day 22 a One Way Anova identified significant differences in the Quantum Yield data between the treatments (DF=5; H=11.857; P=0.037). The post hoc test (Dunnett's method, contrast) identified no significant differences between the Quantum Yield data of the treatments with Fluoxetine compared to the control treatments.

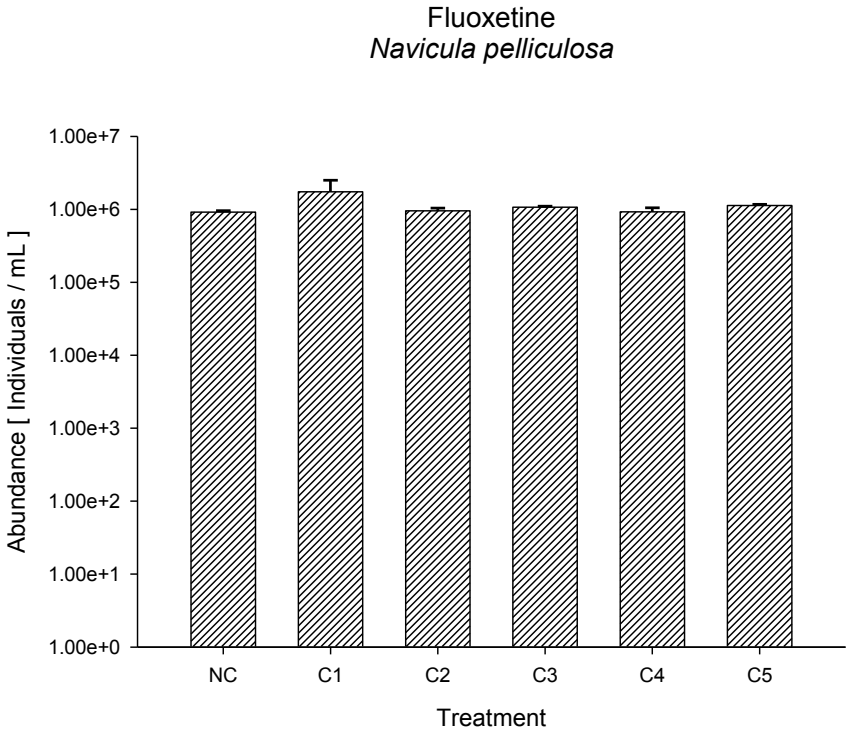


Fig. 28: Mean abundance of *Navicula pelliculosa* under the influence of Fluoxetine at day 22 of the experiment. Each treatment consisted of 3 replicates. NC: negative control; C1: 0.006 µg L^{-1}; C2: 0.012 µg L^{-1}; C3: 0.024 µg L^{-1}; C4: 0.048 µg L^{-1}; C5: 0.096 µg L^{-1}. Error bars represent standard errors.

No clear effect of Fluoxetine on the mean abundance of *Navicula pelliculosa* was observed (Fig. 28). A One Way Anova with the abundances identified no significant differences (DF=5; F=0.986; P=0.466) between the treatment groups.

Peridinium sp.

The addition of Fluoxetine led to mean effect sizes on the FixArea around 0. No strong impacts of Fluoxetine on FixArea and Quantum Yield were observable (Fig. 29 a, b).

a) b)

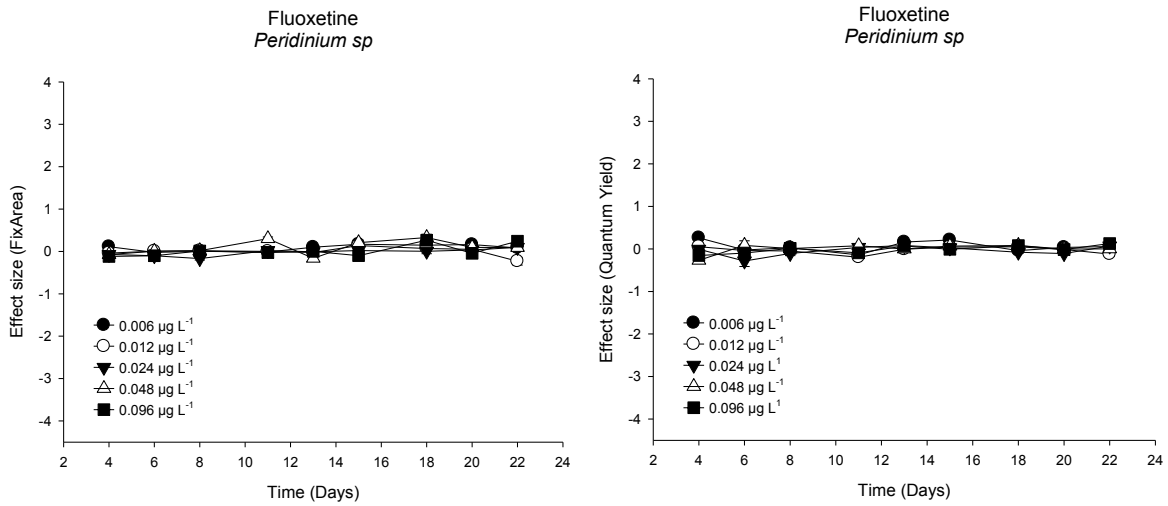


Fig. 29: FixArea (a) and Quantum Yield (b) of *Peridinium sp.* after exposure to different concentrations of Fluoxetine over a period of 22 days. Data expressed in mean effect sizes to the unexposed controls. Error bars represent standard errors.

A Two Way Anova showed significant effects of the factors concentration and time for FixArea and Quantum Yield, in both cases also the interaction between the two factors was significant (Tab. 18).

Tab. 18: Results of a Two Way Anova including FixArea and Quantum Yield data of *Peridinium sp.* treated with Fluoxetine (n=162); FA=FixArea; QY=Quantum Yield.

Algae species	Source of Variation	DF	F (FA)	P (FA)	F (QY)	P (QY)
Perdinium sp.	Conc	5	3.567	0.005	3.012	0.014
	Day	8	102.891	<0.001	64.259	<0.001
	Conc x Day	40	1.762	0.011	2.306	<0.001

A One Way Anova identified no significant differences on day 6 in the values of FixArea data (DF=5; F=2.120; P=0.133) between treatments. The same was visible at day 22 (DF=5; F=1.791; P=0.189). Neither on day 6 (DF=5; F=0.708; P=0.628) nor on day 22 (DF=5; F=2.009; P=0.149) significant differences between treatments were found for Quantum Yield data by a One Way Anova.

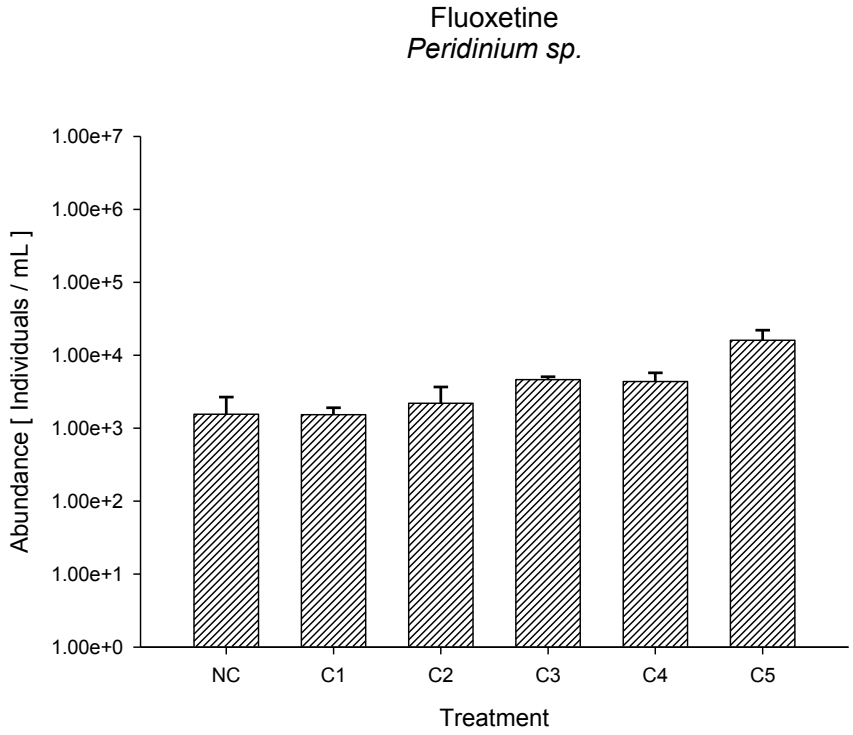


Fig. 30: Mean abundance of *Peridnium sp.* under the influence of Fluoxetine at day 22 of the experiment. Each treatment consisted of 3 replicates. NC: negative control; C1: 0.006 µg L^{-1}; C2: 0.012 µg L^{-1}; C3: 0.024 µg L^{-1}; C4: 0.048 µg L^{-1}; C5: 0.096 µg L^{-1}. Error bars represent standard errors.

The results of the microscopic counting showed that Fluoxetine had an impact on the mean abundance of *Peridinium sp.*. With higher concentrations of Fluoxetine the mean abundances increased (Fig. 30). A One Way Anova with the abundances identified no significant differences (DF=5; H=10.310; P=0.067) between the treatment groups.

Cryptomonas phaseolus

The effect of Fluoxetine on the FixArea of *Cryptomonas phaseolus* increased over the entire duration of the experiment. The mean effect sizes of Fluoxetine on Quantum Yield were around 0 (Fig. 31 a, b).

a) b)

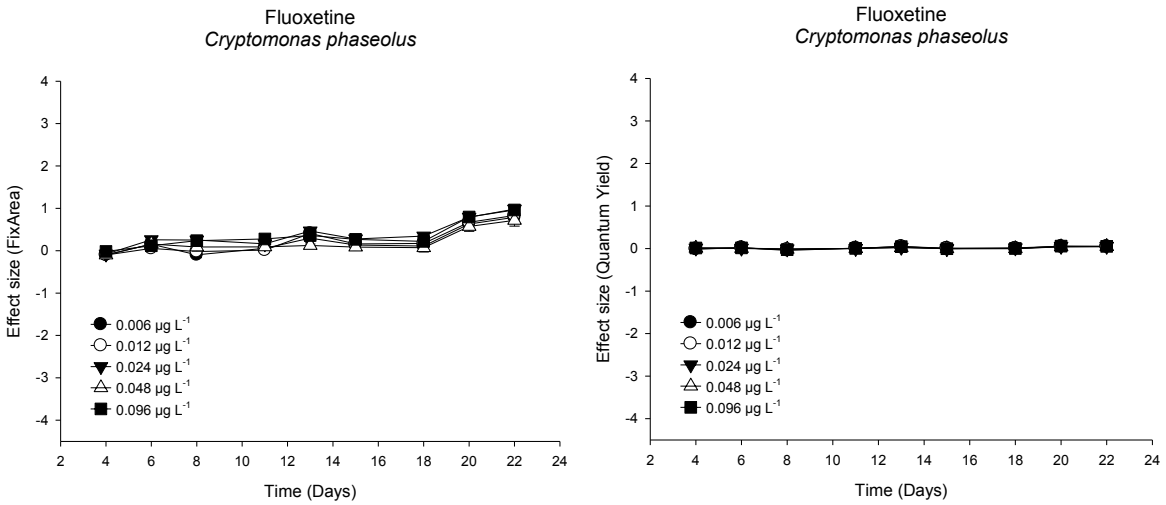


Fig. 31: FixArea (a) and Quantum Yield (b) of *Cryptomonas phaseolus* after exposure to different concentrations of Fluoxetine over a period of 22 days. Data expressed in mean effect sizes to the unexposed controls. Error bars represent standard errors.

The Two Way Anova identified significant effects of the factors concentration and time and their interaction on FixArea. For Quantum Yield significant effects of time were observed and a significant interaction between the factors ‘concentration’ and ‘time’ (Tab. 19).

Tab. 19: Results of a Two Way Anova including FixArea and Quantum Yield data of *C. phaseolus* treated with Fluoxetine (n=162); FA=FixArea; QY=Quantum Yield.

Algae species	Source of Variation	DF	F (FA)	P (FA)	F (QY)	P (QY)
C. phaseolus	Conc	5	26.044	<0.001	1.816	0.116
	Day	8	201.487	<0.001	134.344	<0.001
	Conc x Day	40	2.924	<0.001	0.684	<0.001

A One Way Anova showed no significant differences for FixArea data between the treatments (DF=5; F=0.984; P=0.466) on day 6. At the last sampling day significant differences in the FixArea data between the treatments were found (DF=5; H=11.807; P=0.038). A post hoc test (Dunnett's method, contrast) identified that treatments with 0.024 µg L^{-1} Fluoxetine (q'=2.829; P<0.05) and 0.096 µg L^{-1} Fluoxetine (q'=2.753; P<0.05) were significantly different compared to the control treatments.

On day 6 no significant differences were found (DF=5; H=8.603; P=0.126) in the Quantum Yield data between the treatments by a One Way Anova. On day 22 significant differences were found (DF=5; F= 9.747; P<0.001) between the treatments for the Quantum Yield values. A post hoc test (Bonferroni t-test, contrast) identified that treatments with 0.006 µg L^{-1} Fluoxetine (t=4.874; P=0.002), 0.012 µg L^{-1} Fluoxetine (t=5.139; P=0.001), 0.024 µg L^{-1} Fluoxetine (t=5.774; P<0.001), 0.048 µg L^{-1} Fluoxetine (t=5.086; P=0.001) and 0.096 µg L^{-1} Fluoxetine (t=5.774; P<0.001) were significantly different to the control treatments.

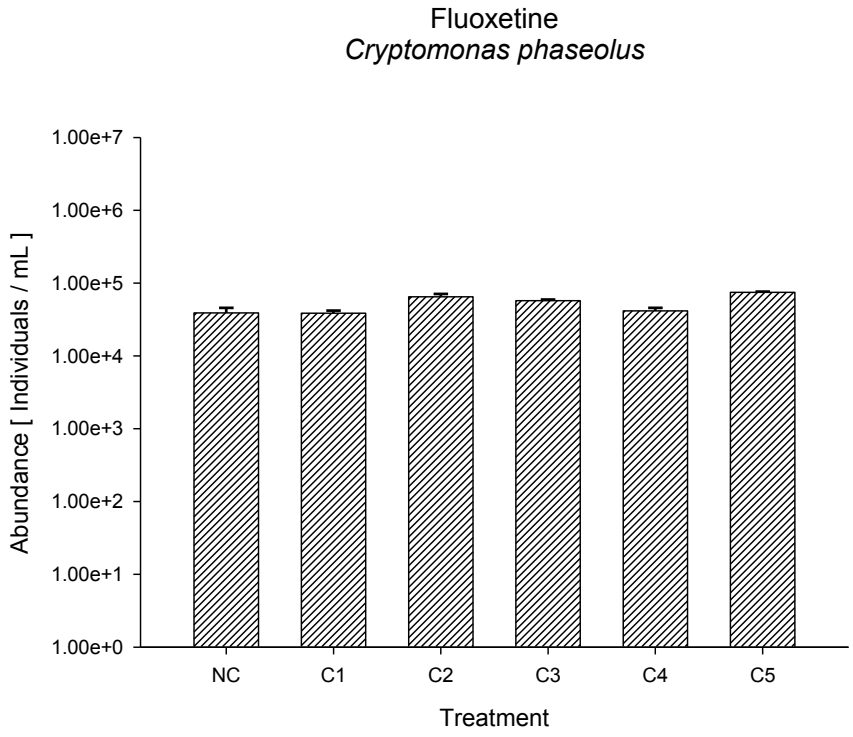


Fig. 32: Mean abundance of *Cryptomonas phaseolus* under the influence of Fluoxetine at day 22 of the experiment. Each treatment consisted of 3 replicates. NC: negative control; C1: 0.006 µg L^{-1}; C2: 0.012 µg L^{-1}; C3: 0.024 µg L^{-1}; C4: 0.048 µg L^{-1}; C5: 0.096 µg L^{-1}. Error bars represent standard errors.

The lowest mean abundance of *Cryptomonas phaseolus* was found in treatments with the lowest concentrations of Fluoxetine. With higher concentrations the mean abundance increased (Fig. 32). A One Way Anova with the abundances identified significant differences (DF=5; F=11.502; P<0.001) between the treatment groups. A post hoc test (Bonferroni t-test, contrast) showed that the abundance of *Cryptomonas phaseolus* was significantly higher in treatments with 0.012 µg L^{-1} Fluoxetine (t=4.050; P=0.008) and in treatments with 0.096 µg L^{-1} Fluoxetine than in the control treatments (t=5.611; P<0.001).

Chroococcus minutus

The effect of Fluoxetine on FixArea of *C. minutes* fluctuated over the experimental duration. From day 6 to day 8 the mean effect sizes of Fluoxetine on FixArea were between 0.5 and 1.0, except the mean effect sizes of Fluoxetine on FixArea in treatments with the highest concentrations of Fluoxetine. From day 15 to day 20 negative mean effect sizes of Fluoxetine on FixArea were observed. At the end of the experiment the mean effect sizes of Fluoxetine on FixArea were between 0.5 and -0.5 (Fig. 33 a).

a) b)

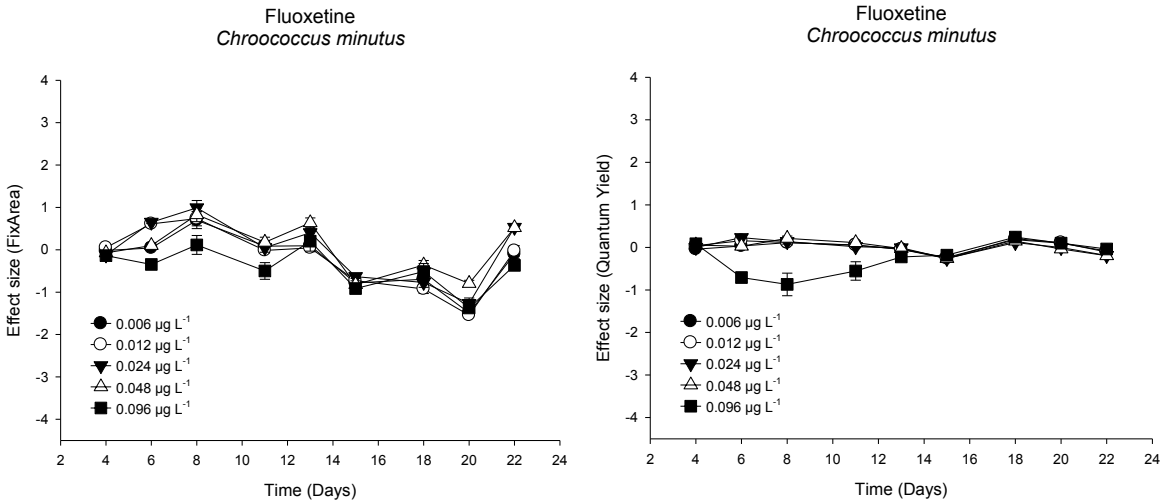


Fig. 33: FixArea (a) and Quantum Yield (b) of *Chroococcus minutus* after exposure to different concentrations of Fluoxetine over a period of 22 days. Data expressed in mean effect sizes to the unexposed controls. Error bars represent standard errors.

In the first half of the experiment the highest concentration of Fluoxetine showed a negative mean effect size of Fluoxetine on Quantum Yield whereas in all other treatments the mean effect size of Fluoxetine on Quantum Yield was around 0 (Fig. 33 b).

The Two Way Anova identified significant effects of the factors concentration and time for FixArea and Quantum Yield data and significant interactions between the factors 'concentration' and 'time' (Tab. 20).

Tab. 20: Results of a Two Way Anova including FixArea and Quantum Yield data of *C. minutus* treated with Fluoxetine (n=162); FA=FixArea; QY=Quantum Yield.

Algae species	Source of Variation	DF	F (FA)	P (FA)	F (QY)	P (QY)
C. minutus	Conc	5	16.304	<0.001	5.641	<0.001
	Day	8	43.568	<0.001	4.476	<0.001
	Conc x Day	40	7.465	<0.001	3.405	<0.001

A One Way Anova identified significant differences in the FixArea data between the treatments (DF=5; F=40.482; P<0.001). A post hoc test (Bonferroni t-test, contrast) identified significant differences between treatments with 0.012 µg L^{-1} (t=7.726; P<0.001) and 0.024 µg L^{-1} Fluoxetine (t=8.344; P<0.001) compared to the control treatments on day 6. A One Way Anova identified significant differences on day 22 between treatments. A post hoc test (Bonferroni t-test, contrast) identified that treatments with 0.024 (t=3.271; P=0.033) and 0.048 µg L^{-1} Fluoxetine (t=3.268; P=0.034) were significantly different compared to the control.

A One Way Anova identified significant differences for Quantum Yield between the treatments (DF=5; H=13.786; P=0.017) on day 6. A post hoc test (Dunnett's method, contrast) identified no significant differences between the treatments with Fluoxetine and the control treatments. On the last sampling day a One Way Anova identified significant differences between the treatments (DF=5; F=3.418; P=0.038). Treatments with 0.024 and 0.048 µg L^{-1} Fluoxetine were significantly different compared to the control treatments (t=3.174; P=0.040 and t=3.249; P=0.035; post hoc Bonferroni t-test, contrast).

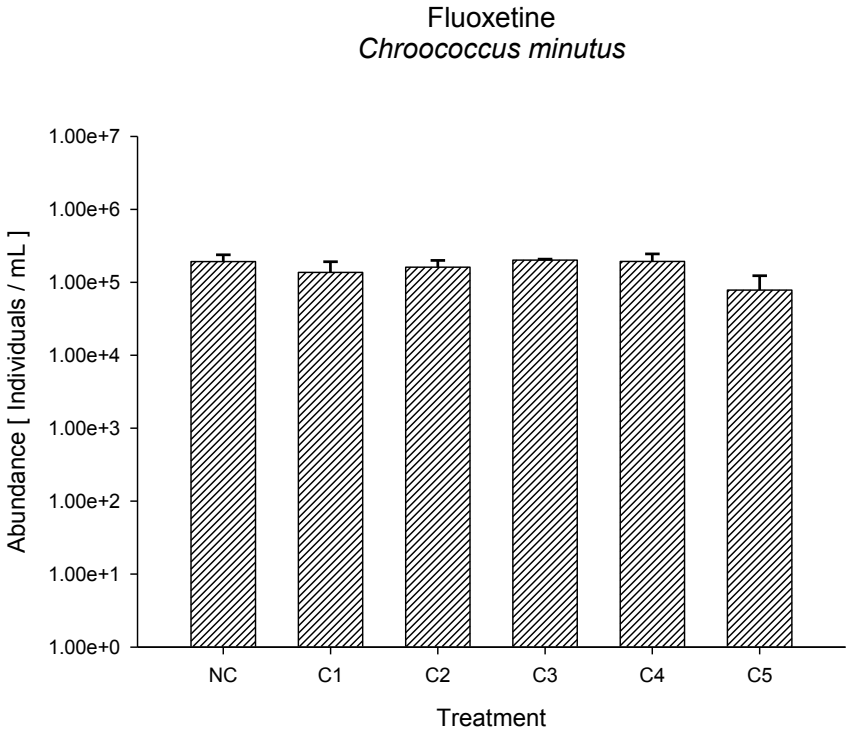


Fig. 34: Mean abundance of *Chroococcus minutus* under the influence of Fluoxetine at day 22 of the experiment. Each treatment consisted of 3 replicates. NC: negative control; C1: 0.006 µg L^{-1}; C2: 0.012 µg L^{-1}; C3: 0.024 µg L^{-1}; C4: 0.048 µg L^{-1}; C5: 0.096 µg L^{-1}. Error bars represent standard errors.

The microscopic counting showed that Fluoxetine led to a slight decrease in the mean abundances of *Chroococcus minutus* in some treatments (Fig. 34). A One Way Anova with the abundances identified no significant differences (DF=5; F=1.193; P=0.369) between the treatment groups.

5.1.1.4 Impact of a mixture of Carbamazepine, Ciprofloxacin and Fluoxetine on algae in monocultures

This set of experiments was conducted to see if effects of combined pharmaceuticals were different from effects of the single active pharmaceutical ingredients.

Scenedesmus obliquus

The value from one replicate of the C4 treatments was missing on day 20. Therefore *n* for final analyses was 161 instead of 162.

The mixture had in general positive effects on FixArea. The highest concentration of the mixture had the highest impact. The mean effect sizes of the mixture on the Quantum Yield were around 0 (Fig. 35 a, b).

a) b)

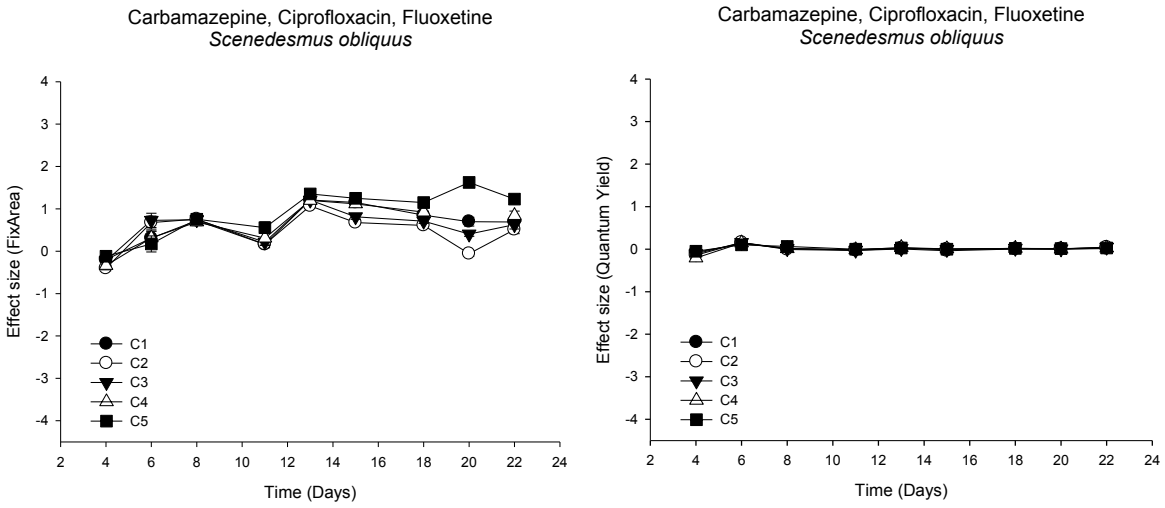


Fig. 35: FixArea (a) and Quantum Yield (b) of *Scenedesmus obliquus* after exposure to different concentrations of a mixture of Carbamazepine, Ciprofloxacin and Fluoxetine over a period of 22 days. Data expressed in mean effect sizes to the unexposed controls. Error bars represent standard errors.

The Two Way Anova identified significant effects of the factors concentration and time for FixArea and for the factor time within the Quantum Yield data. The Two Way Anova identified a significant interaction between the factors 'concentration' and 'time' for the FixArea and Quantum Yield values (Tab. 21).

Tab. 21: Results of a Two Way Anova including FixArea and Quantum Yield data of *S. obliquus* treated with a mixture of Carbamazepine, Ciprofloxacin and Fluoxetine (n=161); FA=FixArea; QY=Quantum Yield.

Algae species	Source of Variation	DF	F (FA)	P (FA)	F (QY)	P (QY)
S. obliquus	Conc	5	93.534	<0.001	1.468	0.206
	Day	8	290.460	<0.001	59.521	<0.001
	Conc x Day	40	12.659	<0.001	2.085	0.002

A One Way Anova identified no significant differences on day 6 in the data of the FixArea between the treatments (DF=5; F=2.633; P=0.079). On the last sampling day significant differences were found (DF=5; F=10.920; P<0.001). The post hoc test (Bonferroni t-test, contrast) identified significant differences at the last sampling day in the FixArea values in treatments with C1 (P=0.05), C4 (P=0.012) and with C5 (P<0.001) compared to the control treatments.

On day 6 a One Way Anova identified significant differences in the Quantum Yield values in treatments. A post hoc test (Bonferroni t-test, contrast) delivered that C2 (t=3.730; P=0.014), C3 (t=3.681; P=0.016), C4 (t=3.408; P=0.026) were significantly different to the control. On day 22 no significant differences were found by a One Way Anova (DF=5; F=1.901; P=0.168).

Effects of the mixture and the single tested (Fig. 36) pharmaceuticals on the FixArea and the Quantum Yield of *Scenedesmus obliquus* were different. A One Way Anova identified significant differences between the effect sizes of the different pharmaceutical treatments on FixArea (DF=3, F=86.252; P<0.001). A post hoc test (Bonferroni t-test, contrast) identified significant differences between treatments with the mixture and treatments with Ciprofloxacin (t=10.539, P<0.001) and between the mixture and Fluoxetine (t=9.522, P<0.001).

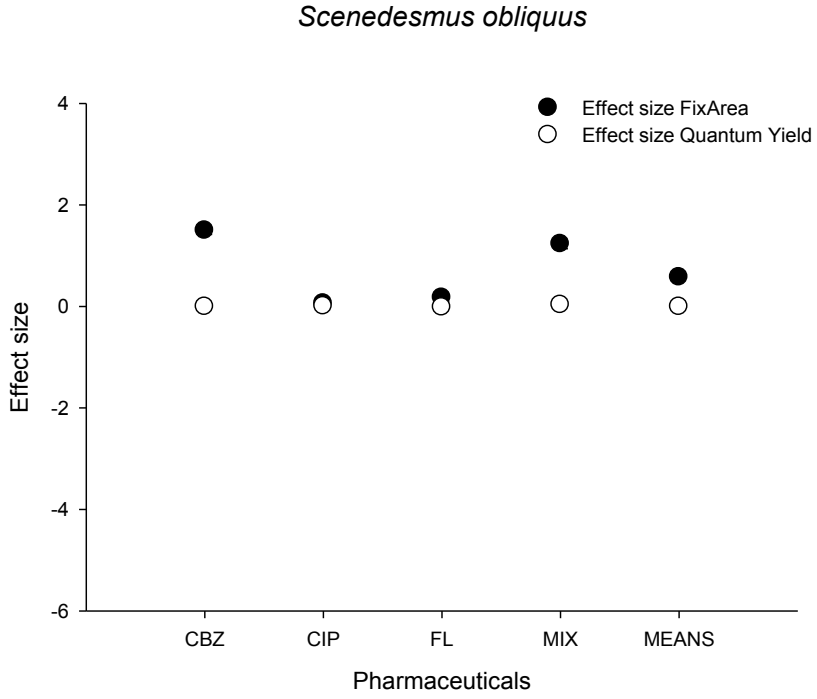


Fig. 36: Comparison of the effects of each single and combined tested pharmaceutical (CBZ: Carbamazepine, CIP: Ciprofloxacin, FL: Fluoxetine, MIX: mixture of CBZ, CIP and FL, MEANS: means of effect sizes of CBZ, CIP and FL) on the FixArea and on the Quantum Yield of *Scenedesmus obliquus*. Results represent the mean effect sizes from day 22 of the experiment from treatments with highest concentration of the investigated pharmaceuticals (CBZ: 8.0 µg L^{-1}, CIP: 0.160 µg L^{-1}, FL: 0.096 µg L^{-1}). Error bars represent standard errors.

A One Way Anova identified significant differences between the effect sizes of the different pharmaceutical treatments on Quantum Yield (DF=3, F=8.100; P<0.001). A post hoc test (Bonferroni t-test, contrast) identified significant differences between treatments with the

mixture and with Fluoxetine (t=4.737, P<0.001) and between the mixture and Carbamazepine (t=3.500, P=0.004).

Navicula pelliculosa

The effect of the mixture on the FixArea was reaching a size up to 1 on day 11 and 13. From day 15 on the mean effect sizes of the mixture on the FixArea of *Navicula pelliculosa* were negative (up to -2) and even lower except of the highest concentration. An effect of the mixture on Quantum Yield was observable for the highest concentration on day 8 and 11 (Fig. 37 a, b).

a) b)

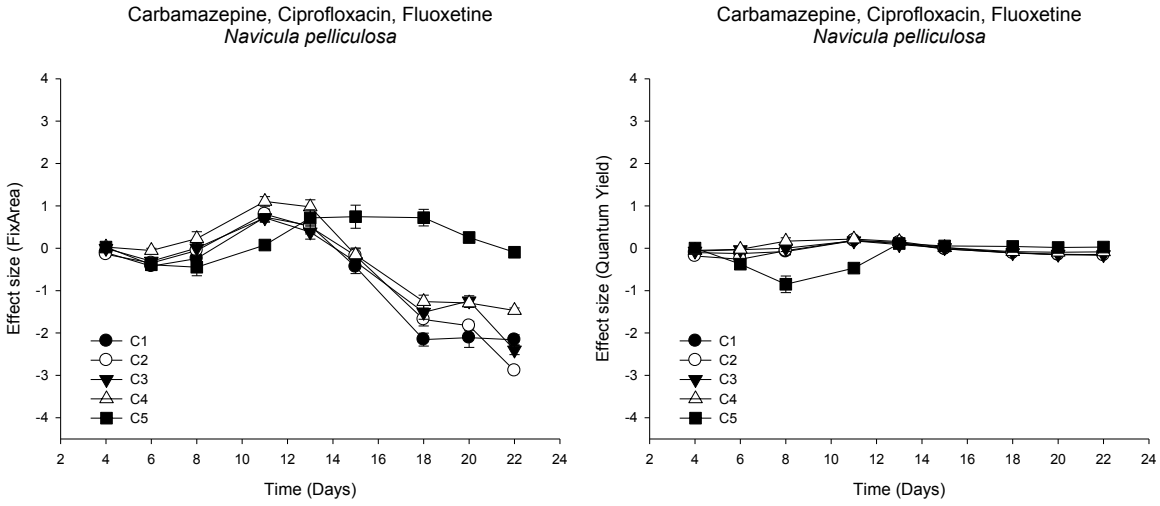


Fig. 37: FixArea (a) and Quantum Yield (b) of *Navicula pelliculosa* after exposure to different concentrations of a mixture of Carbamazepine, Ciprofloxacin and Fluoxetine over a period of 22 days. Data expressed in mean effect sizes to the unexposed controls. Error bars represent standard errors.

The differences in the FixArea and the Quantum Yield data were found to be significant for the factors concentration and time. The Two Way Anova also identified a significant

interaction between the factors ‘concentration’ and ‘time’ for the FixArea and Quantum Yield values (Tab. 22).

Tab. 22: Results of a Two Way Anova including FixArea and Quantum Yield data of *N. pelliculosa* treated with a mixture of Carbamazepine, Ciprofloxacin and Fluoxetine (n=162); FA=FixArea; QY=Quantum Yield.

Algae species	Source of Variation	DF	F (FA)	P (FA)	F (QY)	P (QY)
N. pelliculosa	Conc	5	58.910	<0.001	5.463	<0.001
	Day	8	65.047	<0.001	54.626	<0.001
	Conc x Day	40	13.966	<0.001	5.507	<0.001

A One Way Anova identified significant differences on day 6 in the FixArea data (DF=5; F=3.847; P=0.026). A post hoc test (Bonferroni t-test, contrast) identified that the treatments C1 were significantly different compared to the control treatments (t=3.301; P=0.032; Bonferroni t-test, contrast). At the last sampling day significant differences in the FixArea data in treatments were found by a One Way Anova (DF=5; H=15.690; P=0.008). A post hoc test (q’=3.135; P<0.050; Dunnett’s method, contrast) identified that treatments with C1 were significantly different compared to the control treatments.

On day 6 no significant differences in Quantum Yield values were found by a One Way Anova (DF=5; F= 1.815; P=0.184) in treatments with a mixture of the pharmaceuticals. On the last sampling day significant differences were found by a One Way Anova (DF=5; F= 117.709; P<0.001). A post hoc test (Bonferroni t-test, contrast) identified that the treatments C1 (t=13.383; P=<0.001), C2 (t=15.094; P<0.001), C3 (t=13.953; P<0.001) and C4 (t=7.988; P<0.001) were significantly different compared to the control treatments, except of the treatments with the highest concentrations (t=2.749; P=0.088).

Effects of the mixture and the single tested (Fig. 38) pharmaceuticals on the FixArea and the Quantum Yield of *Navicula pelliculosa* were different. A One Way Anova identified

significant differences between the effect sizes of the different pharmaceutical treatments on FixArea (DF=3, F=92.155; P<0.001). A post hoc test (Bonferroni t-test, contrast) identified significant differences between Carbamazepine and the mixture (t=12.661, P<0.001).

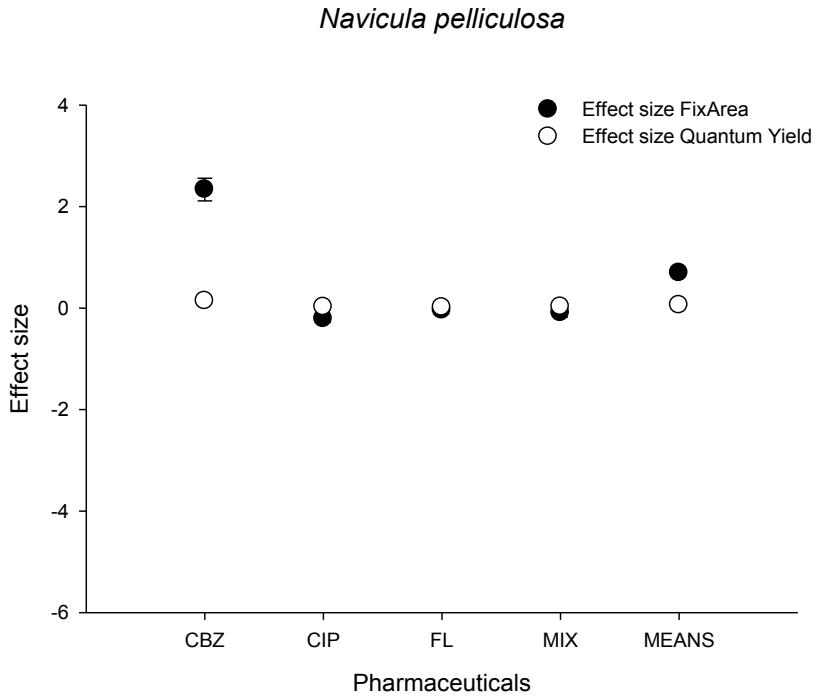


Fig. 38: Comparison of the effects of each single and combined tested pharmaceutical (CBZ: Carbamazepine, CIP: Ciprofloxacin, FL: Fluoxetine, MIX: mixture of CBZ, CIP and FL, MEANS: means of effect sizes of CBZ, CIP and FL) on the FixArea and on the Quantum Yield of *Navicula pelliculosa*. Results represent the mean effect sizes from day 22 of the experiment from treatments with highest concentration of the investigated pharmaceuticals (CBZ: 8.0 µg L^{-1}, CIP: 0.160 µg L^{-1}, FL: 0.096 µg L^{-1}). Error bars represent standard errors.

A One Way Anova identified significant differences between the effect sizes of the different pharmaceutical treatments on Quantum Yield (DF=3, F=62.112; P<0.001). A post hoc test (Bonferroni t-test, contrast) identified significant differences between Carbamazepine and the mixture (t=10.511, P<0.001).

Peridinium sp.

Effects of the pharmaceutical mixture on FixArea were lowest between day 11 and 15 in all treatments but in general around 0. The mean effect sizes of the mixture on Quantum Yield were fluctuating over the course of time around 0. In all treatments a small peak in the mean effect sizes of the mixture on Quantum Yield on day 6 was observable (Fig. 39 a, b).

a) b)

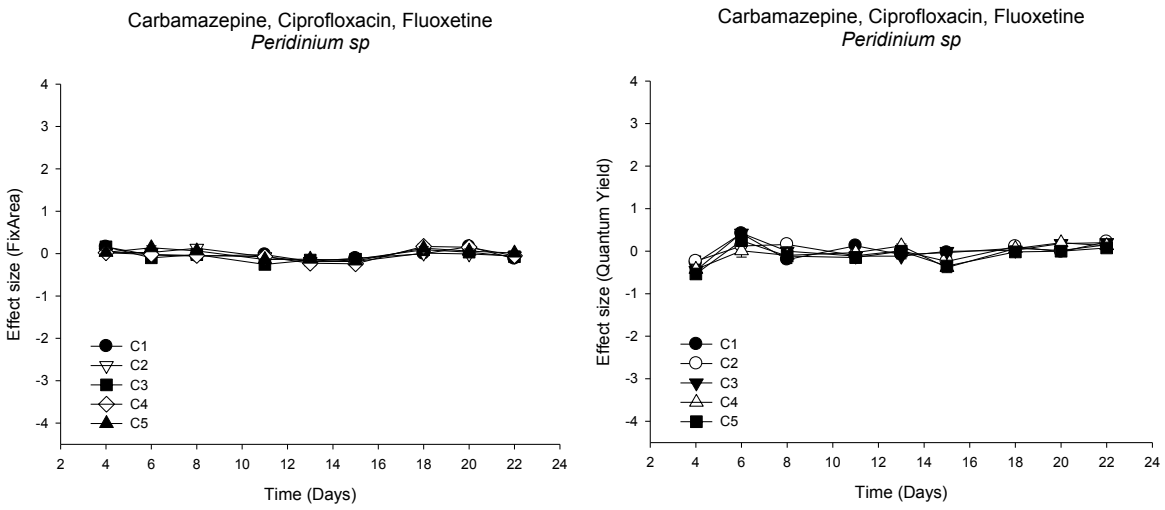


Fig. 39: FixArea (a) and Quantum Yield (b) of *Peridinium sp.* after exposure to different concentrations of a mixture of Carbamazepine, Ciprofloxacin and Fluoxetine over a period of 22 days. Data expressed in mean effect sizes to the unexposed controls. Error bars represent standard errors.

The Two Way Anova showed no significant effects of the concentrations on FixArea and Quantum Yield, but significant effects of time. Interactions between concentration and time were significant for the FixArea values (Tab. 23).

Tab. 23: Results of a Two Way Anova including FixArea and Quantum Yield data of *Peridinium sp.* treated with a mixture of Carbamazepine, Ciprofloxacin and Fluoxetine (n=162); FA=FixArea; QY=Quantum Yield.

Algae species	Source of Variation	DF	F (FA)	P (FA)	F (QY)	P (QY)
Peridinium sp.	Conc	5	0.928	0.466	0.853	0.515
	Day	8	82.729	<0.001	8.808	<0.001
	Conc x Day	40	1.695	0.017	0.895	0.647

A One Way Anova showed no significant differences between the treatments for FixArea (day 6: DF=5; F=0.982; P=0.467; day 22: DF=5; F=0.955; P=0.482) nor for Quantum Yield on day 6 (DF=5; F=1.014; P=0.451) and on day 22 (DF=5; F=0.888; P=0.519).

Effects of the mixture and the single tested pharmaceuticals on the FixArea and the Quantum Yield of *Peridinium sp.* were different (Fig. 40). A One Way Anova identified significant differences between the effect sizes of the different pharmaceutical treatments on FixArea (DF=3, F=13.005; P<0.001). A post hoc test (Bonferroni t-test, contrast) identified significant differences between Fluoxetine and the mixture (t=4.916, P<0.001).

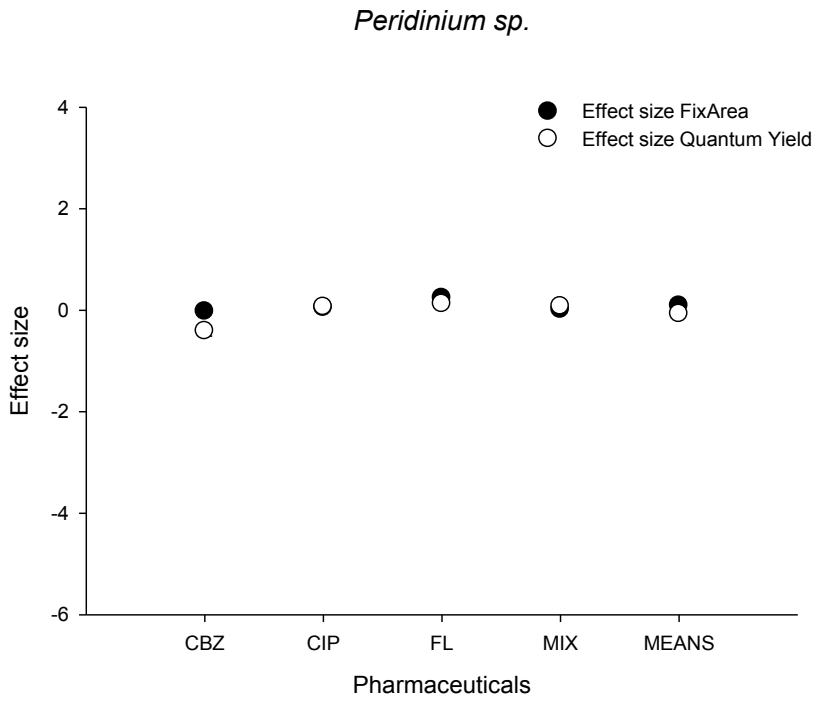


Fig. 40: Comparison of the effects of each single and combined tested pharmaceutical (CBZ: Carbamazepine, CIP: Ciprofloxacin, FL: Fluoxetine, MIX: mixture of CBZ, CIP and FL, MEANS: means of effect sizes of CBZ, CIP and FL) on the FixArea and on the Quantum Yield of *Peridinium sp.*. Results represent the mean effect sizes from day 22 of the experiment from treatments with highest concentration of the investigated pharmaceuticals (CBZ: 8.0 µg L^{-1}, CIP: 0.160 µg L^{-1}, FL: 0.096 µg L^{-1}). Error bars represent standard errors.

A One Way Anova identified significant differences between the effect sizes of the different pharmaceutical treatments on Quantum Yield (DF=3, F=14.654; P<0.001). A post hoc test (Bonferroni t-test, contrast) identified significant differences between the mixture and Carbamazepine (t=5.248, P<0.001).

Cryptomonas phaseolus

The mixture had generally a positive effect on the FixArea. In the beginning a slightly negative effect was observed in C4 and C5 treatments. The mean effect sizes of the mixture on FixArea were increasing over time in all treatments. The mean effect sizes in C4 treatments approached 0 at the end of the experiment. In the other treatments mean effect sizes up to 2 were observed. Mean Effect sizes of the mixture on the Quantum Yield were around 0 at the beginning and increased slightly in a positive direction until the end of the experiment (Fig. 41 a, b).

a) b)

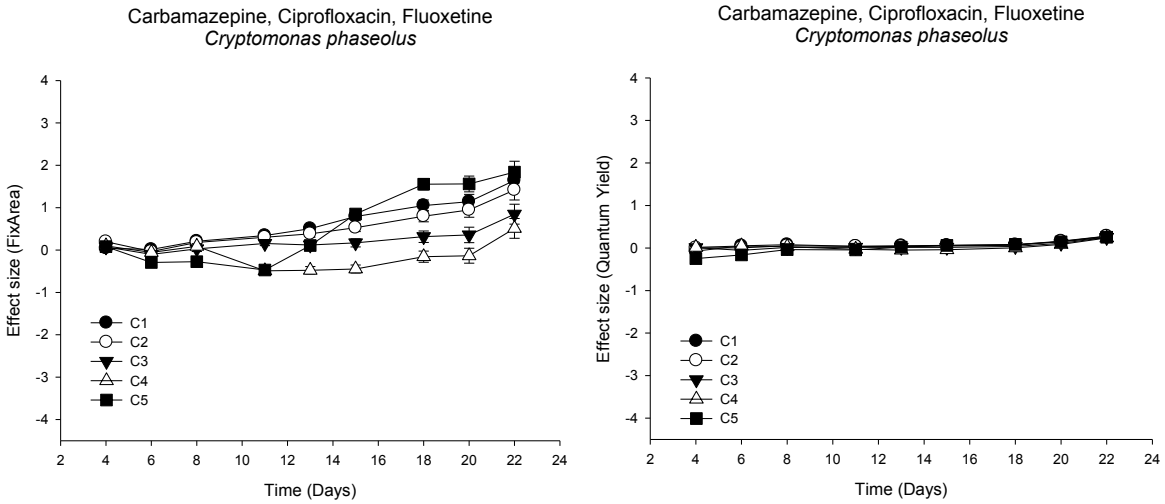


Fig. 41: FixArea (a) and Quantum Yield (b) of *Cryptomonas phaseolus* after exposure to different concentrations of a mixture of Carbamazepine, Ciprofloxacin and Fluoxetine over a period of 22 days. Data expressed in mean effect size to the unexposed controls. Error bars represent standard errors.

The Two Way Anova identified significant effects of the factors concentration and time and significant interactions of both factors on FixArea and Quantum Yield (Tab. 24).

Tab. 24: Results of a Two Way Anova including FixArea and Quantum Yield data of *C. phaseolus* treated with a mixture of Carbamazepine, Ciprofloxacin and Fluoxetine (n=162); FA=FixArea; QY=Quantum Yield.

Algae species	Source of Variation	DF	F (FA)	P (FA)	F (QY)	P (QY)
C. phaseolus	Conc	5	83.403	<0.001	10.178	<0.001
	Day	8	136.405	<0.001	107.000	<0.001
	Conc x Day	40	14.265	<0.001	2.838	<0.001

A One Way Anova identified a significant difference in the FixArea data on day 6 (DF=5; F=7.852; P=0.001). A post hoc test (Bonferroni t-test, contrast) identified that the treatments with the highest concentration compared to the control treatments were significantly different (t=5.103; P=0.001). On the last sampling day a significant difference on FixArea values (DF=5; H=15.082; P<0.05) was identified. A significant difference between the lowest (q'=2.677; P<0.05) and the highest (q'=2.829; P=<0.05) concentration treatments compared to the control treatments was identified with a post hoc test (Dunnett's method, contrast).

A One Way Anova showed a significant difference in the Quantum Yield values between treatments (DF=5; F6.051; P=0.025). A post hoc test identified (Bonferroni t-test, contrast) that on day 6 treatments with the highest concentration were significantly different from control (t=3.433; P=0.025). On day 22 a post hoc test (Dunnett's method, contrast) identified significant differences between C1 and control treatments (q'=3.174; P<0.05) and C2 (q'=2.715; P<0.05) treatments compared to the control treatments.

The effects of the single tested pharmaceuticals differed from the effect of the mixture (Fig. 42). A One Way Anova identified significant differences between the effect sizes of the different pharmaceutical treatments on FixArea (DF=3, F=326.924; P<0.001). A post hoc test (Bonferroni t-test, contrast) identified significant differences between treatments with the

mixture and Carbamazepine (t=28.993, P<0.001), between the mixture and Ciprofloxacin (t=9.689, P<0.001) and between the mixture and Fluoxetine (t=4.356, P<0.001).

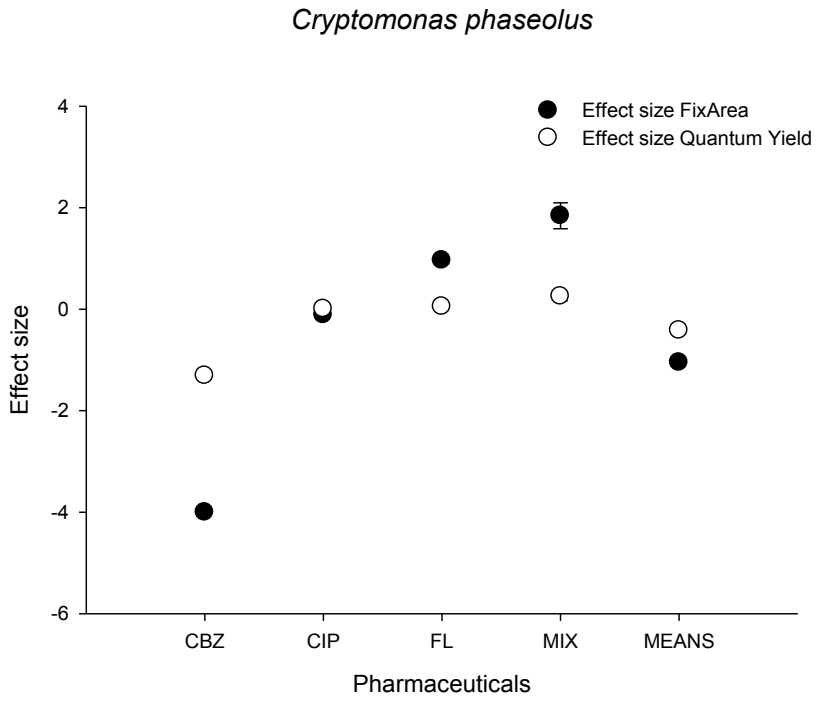


Fig. 42: Comparison of the effects of each single and combined tested pharmaceutical (CBZ: Carbamazepine, CIP: Ciprofloxacin, FL: Fluoxetine, MIX: mixture of CBZ, CIP and FL, MEANS: means of effect sizes of CBZ, CIP and FL) on the FixArea and on the Quantum Yield of *Cryptomonas phaseolus*. Results represent the mean effect sizes from day 22 of the experiment from treatments with highest concentration of the investigated pharmaceuticals (CBZ: 8.0 µg L^{-1}, CIP: 0.160 µg L^{-1}, FL: 0.096 µg L^{-1}). Error bars represent standard errors.

A One Way Anova identified significant differences between the effect sizes of the different pharmaceutical treatments on Quantum Yield (DF=3, F=224.374; P<0.001). A post hoc test (Bonferroni t-test, contrast) identified significant differences between treatments with the mixture and Carbamazepine (t=23.166, P<0.001), between the mixture and Ciprofloxacin (t=3.700, P=0.002) and between the mixture and Fluoxetine (t=2.976, P=0.017).

Chroococcus minutus

The mean effect sizes of the mixture on FixArea fluctuated between positive and negative mean effect sizes (around -0.5 and 0.5). The effects of the mixture of the pharmaceuticals on Quantum Yield were also fluctuating between 0.2 and -0.2 and around 0 at day 22 (Fig. 43 a, b).

a) b)

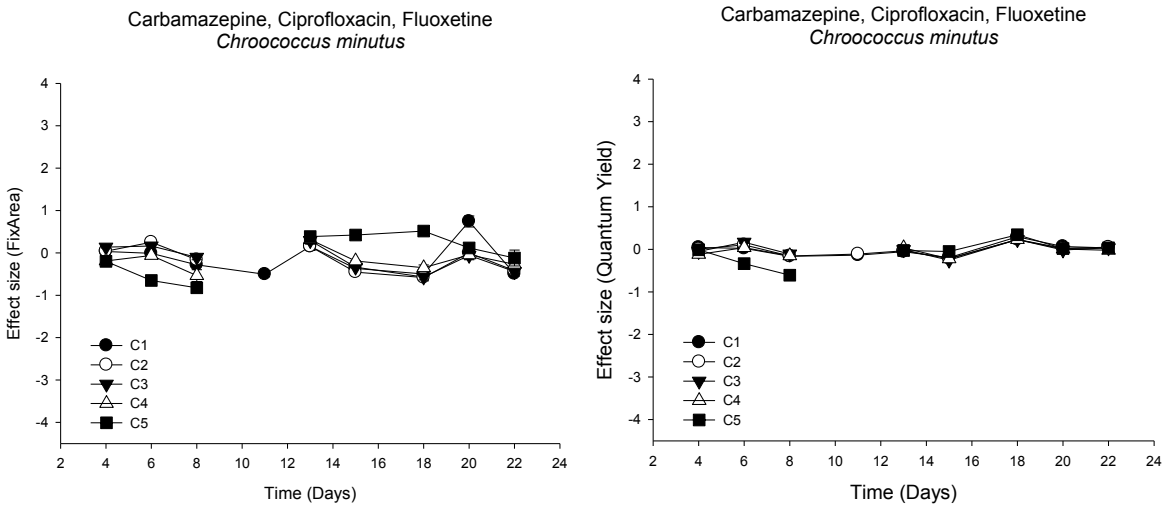


Fig. 43: FixArea (a) and Quantum Yield (b) of *Chroococcus minutus* after exposure to different concentrations of a mixture of Carbamazepine, Ciprofloxacin and Fluoxetine over a period of 22 days. Data expressed in mean effect size to the unexposed controls. Error bars represent standard errors.

The Two Way Anova showed that the factors concentration and time resulted in significant differences for FixArea and Quantum Yield values. Also the interaction between the factors 'concentration' and 'time' were statistically significant (Tab. 25).

Tab. 25: Results of a Two Way Anova including FixArea and Quantum Yield data of *C. minutus* treated with a mixture of Carbamazepine, Ciprofloxacin and Fluoxetine (n=144). The data from day 11 were not included in the calculation as there were missing values and a calculation of the interaction between the factors 'concentration' and 'time' would not have been possible otherwise. FA=FixArea; QY=Quantum Yield.

Algae species	Source of Variation	DF	F (FA)	P (FA)	F (QY)	P (QY)
C. minutus	Conc	5	15.867	<0.001	5.001	<0.001
	Day	8	122.836	<0.001	31.495	<0.001
	Conc x Day	40	9.828	<0.001	10.387	<0.001

A One Way Anova found significant differences in the FixArea values (DF=5; F=11.884; P<0.001) between treatments on day 6. A post hoc test (Bonferroni t-test, contrast) identified that the FixArea data in the treatments with the highest concentration was significantly different compared to the control treatments (t=4.464; P=0.004). On day 22 there were no significant differences found in FixArea values (DF=5; H=8.977; P=0.110).

A One Way Anova found significant differences in the Quantum Yield values (DF=5; F=17.172; P<0.001) between treatments on day 6. A post hoc test (Bonferroni t-test, contrast) identified that the Quantum Yield values on day 6 were significantly different in the treatments with the highest concentration (t=5.229; P=0.001) and in C3 treatments (t=3.328; P=0.030) compared to the control treatments. On day 22 no significant differences were found in the Quantum Yield data (DF=5; F=0.740; P=0.608) in the different treatments compared to the control treatments.

The effects of the single tested pharmaceuticals on *Chroococcus minutus* differed from the effect of the mixture (Fig. 44). A One Way Anova identified significant differences between the effect sizes of the different pharmaceutical treatments on FixArea (DF=3, F=3.051; P=0.043). A post hoc test (Bonferroni t-test, contrast) identified no significant differences between the mixture and the single tested pharmaceuticals. A One Way Anova identified no significant differences between the effect sizes of the different pharmaceutical treatments on Quantum Yield (DF=3, F=2.705; P=0.062).

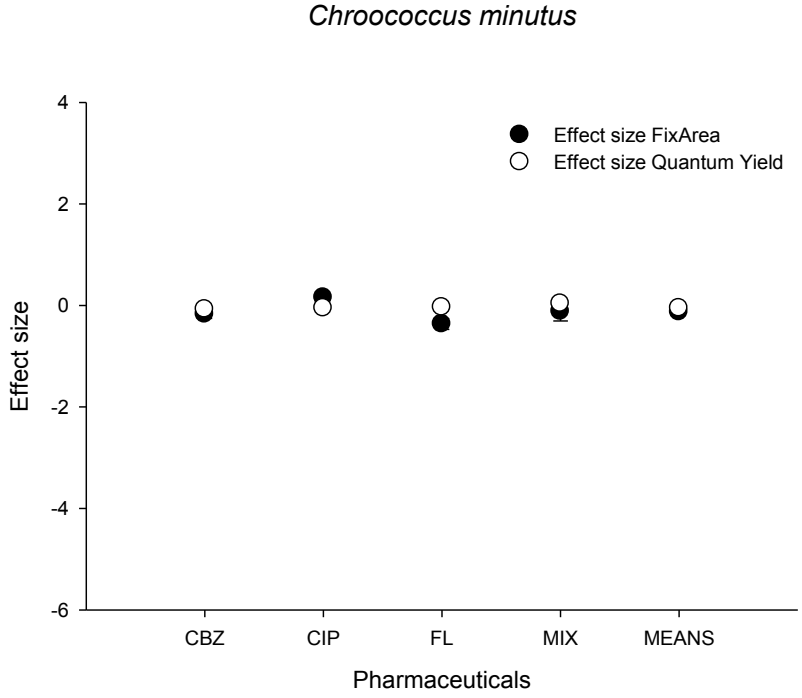


Fig. 44: Comparison of the effects of each single and combined tested pharmaceutical (CBZ: Carbamazepine, CIP: Ciprofloxacin, FL: Fluoxetine, MIX: mixture of CBZ, CIP and FL, MEANS: means of effect sizes of CBZ, CIP and FL) on the FixArea and on the Quantum Yield of *Chroococcus minutus*. Results represent the mean effect sizes from day 22 of the experiment from treatments with highest concentration of the investigated pharmaceuticals (CBZ: 8.0 µg L^{-1}, CIP: 0.160 µg L^{-1}, FL: 0.096 µg L^{-1}). Error bars represent standard errors.

5.1.1.5 Comparison of the effects caused by the different pharmaceutical treatments on algal species

In the previous chapters I investigated the effects of the pharmaceuticals Carbamazepine, Ciprofloxacin, Fluoxetine and of the mixture of all three of them. Here I want to summarize the different strength of responses of my investigated algal species to the three pharmaceuticals. I therefore performed a Two Way Anova with the factors 'pharmaceutical' and 'algae'.

The effect of the different pharmaceutical on FixArea depends on which algal species is present. There is a significant interaction between the two factors 'pharmaceutical' and 'algae' (Tab. 26; P<0.001).

Tab. 26: Results of Two Way ANOVA for the effect of Carbamazepine (8.0 µg L^{-1}), Ciprofloxacin (0.160 µg L^{-1}), Fluoxetine (0.096 µg L^{-1}) and the mixture (CBZ: 8.0 µg L^{-1}, CIP: 0.160 µg L^{-1}, FL: 0.096 µg L^{-1}) on FixArea values (day 22) and the investigated algal species: *S. obliquus*, *C. minutus*, *C. phaseolus*, *N. pelliculosa*, *Peridinium sp.*. Asterisks indicate significant differences.

FixArea	DF	F	P
Pharmaceutical	3	34.931	<0.001*
Algae	4	63.371	<0.001*
Pharmaceutical x algae	12	169.718	<0.001*

The effect of the different pharmaceutical on Quantum Yield depends also on which algal species is present. There is a significant interaction between the two factors 'pharmaceutical' and 'algae' (Tab. 27, P<0.001).

Tab. 27: Results of Two Way ANOVA for the effect of Carbamazepine (8.0 µg L^{-1}), Ciprofloxacin (0.160 µg L^{-1}), Fluoxetine (0.096 µg L^{-1}) and the mixture (CBZ: 8.0 µg L^{-1}, CIP: 0.160 µg L^{-1}, FL: 0.096 µg L^{-1}) on Quantum Yield values (day 22) and the investigated algal species: *S. obliquus*, *C. minutus*, *C. phaseolus*, *N. pelliculosa*, *Peridinium sp.*. Asterisks indicate significant differences.

Quantum Yield	**DF**	**F**	**P**
Pharmaceutical	3	118.570	<0.001*
Algae	4	35.317	<0.001*
Pharmaceutical x algae	12	66.413	<0.001*

These results show that effects of the three pharmaceuticals on biomass production (FixArea) and the efficiency of the PS II (Quantum Yield) are critically dependent on the exposed algal species. The detailed results of the respective pairwise post hoc test are attached in the appendix (Tab. 37 + Tab. 38).

5.1.2 Question II: Are algal species impacted differently by pharmaceuticals when treated in polycultures compared to monocultures? Do biotic interactions change the effect of pharmaceuticals? Is there a threshold of diversity above which pharmaceutical effects on phytoplankton species change?

5.1.2.1 Effects of pharmaceuticals on different diverse algae communities

5.1.2.1.1 Carbamazepine

Differences in the effect sizes of Carbamazepine (8.0 µg L^{-1}) on FixArea and Quantum Yield between monocultures and more diverse treatments were seen after 22 days (Fig. 45). Statistical analyses of the effect sizes of Carbamazepine on FixArea by a One Way Anova revealed significant differences between the diversity treatments (DF=4, H=16.216, P=0.003). A post hoc test (Dunn's method, contrast) identified significant differences between the monocultures and diversity treatments 3 (Q=2.701; P<0.05). Statistical analyses of the effect sizes of Carbamazepine on Quantum Yield by a One Way Anova revealed no significant differences between the diversity treatments (DF=4, H=2.064, P=0.724).

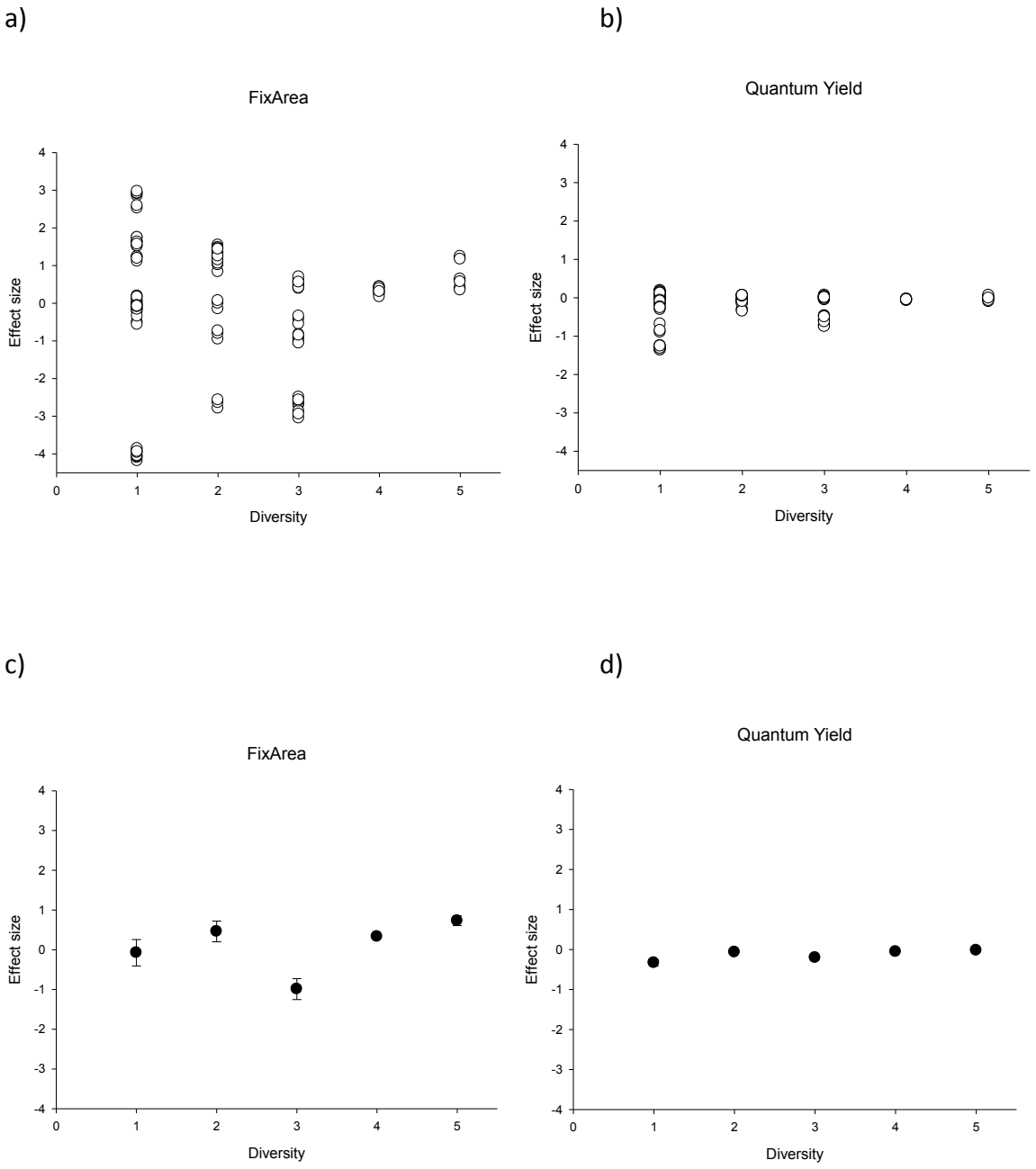


Fig. 45: Effect sizes of Carbamazepine (8.0 µg L^{-1}) on FixArea (day 22) of diversity treatments; b: on Quantum Yield (day 22) of diversity treatments; c: data as in a) plotted as mean +/- S.E.; d: data as in b) plotted as mean +/- S.E. Diversity treatment 1 includes the values of all monocultures and their replicates ($N_{(diversity\ 1)}$ = 45; $N_{(diversity\ 2)}$ = 27; $N_{(diversity\ 3)}$ = 27; $N_{(diversity\ 4)}$ = 9; $N_{(diversity\ 5)}$ = 9; $N_{(total)}$ = 117).

The coefficient of variation (CV) of the FixArea and Quantum Yield values was lowest in treatments with the diversity treatment 5 when exposed to the highest concentration of Carbamazepine (Tab. 28).

Tab. 28: Coefficient of variation (CV) of FixArea and Quantum Yield values of each diversity treatment. $N_{(diversity\ 1)}$ = 15; $N_{(diversity\ 2)}$ =9; $N_{(diversity\ 3)}$ = 9;$N_{(diversity\ 4)}$ = 3;$N_{(diversity\ 5)}$ = 3. The measurements are from the last day (day 22) and from treatments with the highest concentration of Carbamazepine (8.0 µg L^{-1}).

	Diversity 1	Diversity 2	Diversity 3	Diversity 4	Diversity 5
CV % (FixArea)	142,00	122,87	90,11	6,07	3,83
CV % (Quantum Yield)	53,43	22,79	28,06	0,82	3,76

5.1.2.1.2 Ciprofloxacin

Diversity influenced the effects of Ciprofloxacin on FixArea and Quantum Yield (Fig. 46 a, b, c, d). A One Way Anova identified significant differences of the effect sizes of Ciprofloxacin on FixArea between the diversity treatments (DF=4, H=16.208, P=0.003). A post hoc test (Dunn's method, contrast) found significant differences between the diversity treatments 4 and monocultures (Q=2.596; P<0.05) and between treatment 2 and the monocultures (Q=2.916; P<0.05). A One Way Anova identified significant differences of the effect sizes of Ciprofloxacin on Quantum Yield between the diversity treatments (DF=4, H=10.645, P=0.031). A post hoc test (Dunn's method, contrast) identified no significant differences compared to the control treatments.

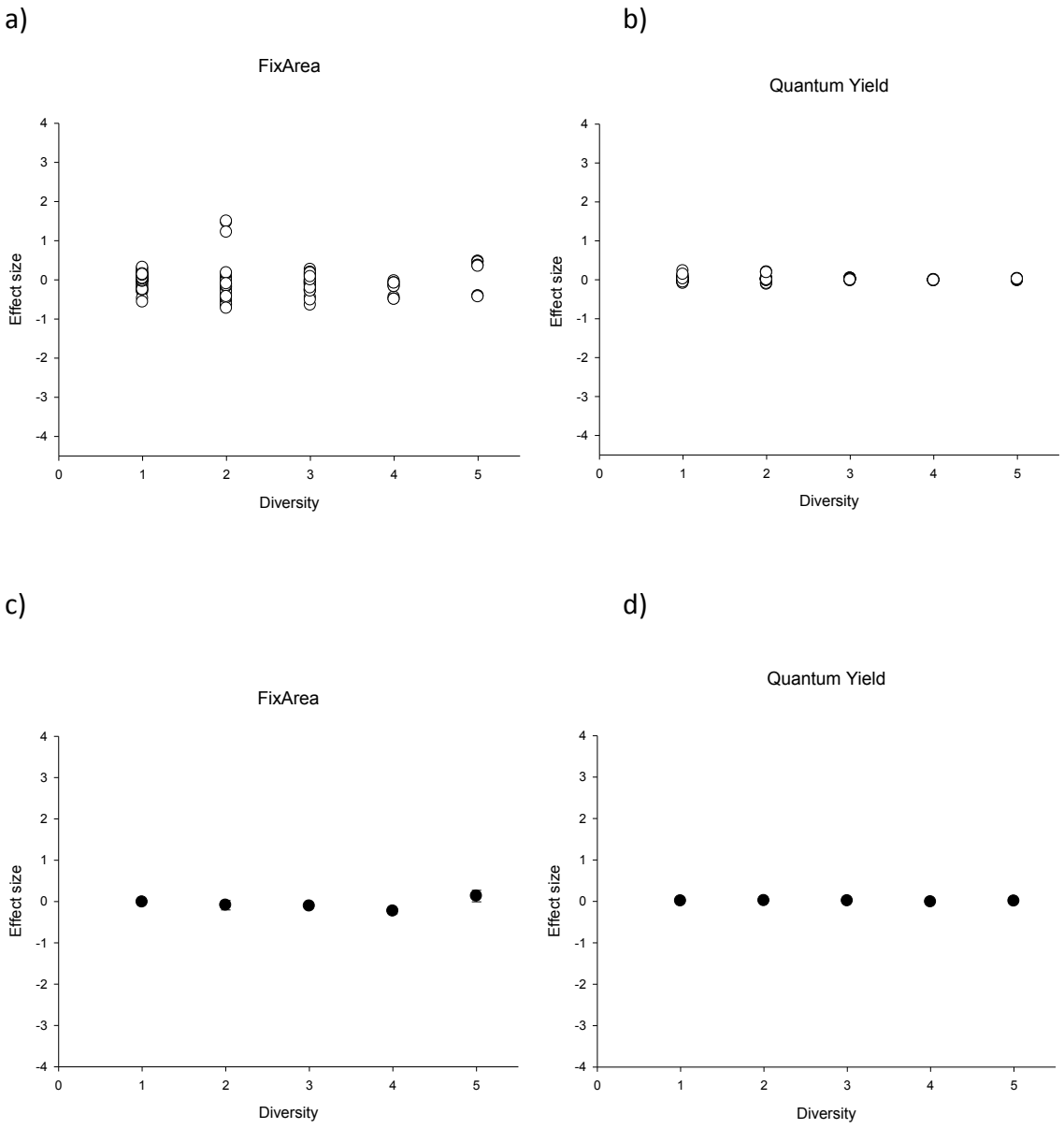


Fig. 46: Effect sizes of Ciprofloxacin (0.160 µg L^{-1}) on FixArea (day 22) of diversity treatments; b: on Quantum Yield (day 22) of diversity treatments; c: data as in a) plotted as mean +/- S.E.; d: data as in b) plotted as mean +/- S.E. Diversity treatment 1 includes the values of all monocultures and their replicates ($N_{(diversity\ 1)}$ = 45; $N_{(diversity\ 2)}$ = 27; $N_{(diversity\ 3)}$ = 27; $N_{(diversity\ 4)}$ = 9; $N_{(diversity\ 5)}$ = 9; $N_{(total)}$ = 117).

The coefficient of variation of the FixArea values as well as of the Quantum Yield values decreased with higher diversity (Tab. 29).

Tab. 29: Coefficient of variation (CV) of FixArea and Quantum Yield values of each diversity treatment. $N_{(diversity\ 1)}$ = 15; $N_{(diversity\ 2)}$ =9; $N_{(diversity\ 3)}$ = 9; $N_{(diversity\ 4)}$ = 3; $N_{(diversity\ 5)}$ = 3. The measurements are from the last day (day 22) and in treatments with the highest concentration of Ciprofloxacin (0.160 µg L^{-1}).

	Diversity 1	Diversity 2	Diversity 3	Diversity 4	Diversity 5
CV % (FixArea)	68,59	59,16	22,55	2,96	1,22
CV % (Quantum Yield)	26,69	9,49	1,60	0,77	0,58

5.1.2.1.3 Fluoxetine

Diversity influenced the effect of Fluoxetine on FixArea and Quantum Yield (Fig. 47 a, b, c, d). A One Way Anova found significant differences of the effect sizes on the FixArea between the diversity treatments (DF=4, H=19.920, P=<0.001). A post hoc test (Dunn's method, contrast) identified significant differences between diversity treatments 4 and the monocultures (Q=3.228; P<0.05) and between diversity treatments 2 and the monocultures (Q=2.886; P<0.05). A One Way Anova found significant differences of the effect sizes of Fluoxetine on the Quantum Yield between the diversity treatments (DF=4, H=32.975, P=<0.001). A post hoc test (Dunn's method, contrast) identified significant differences between the monocultures and diversity treatments 3 (Q=5.055; P<0.05).

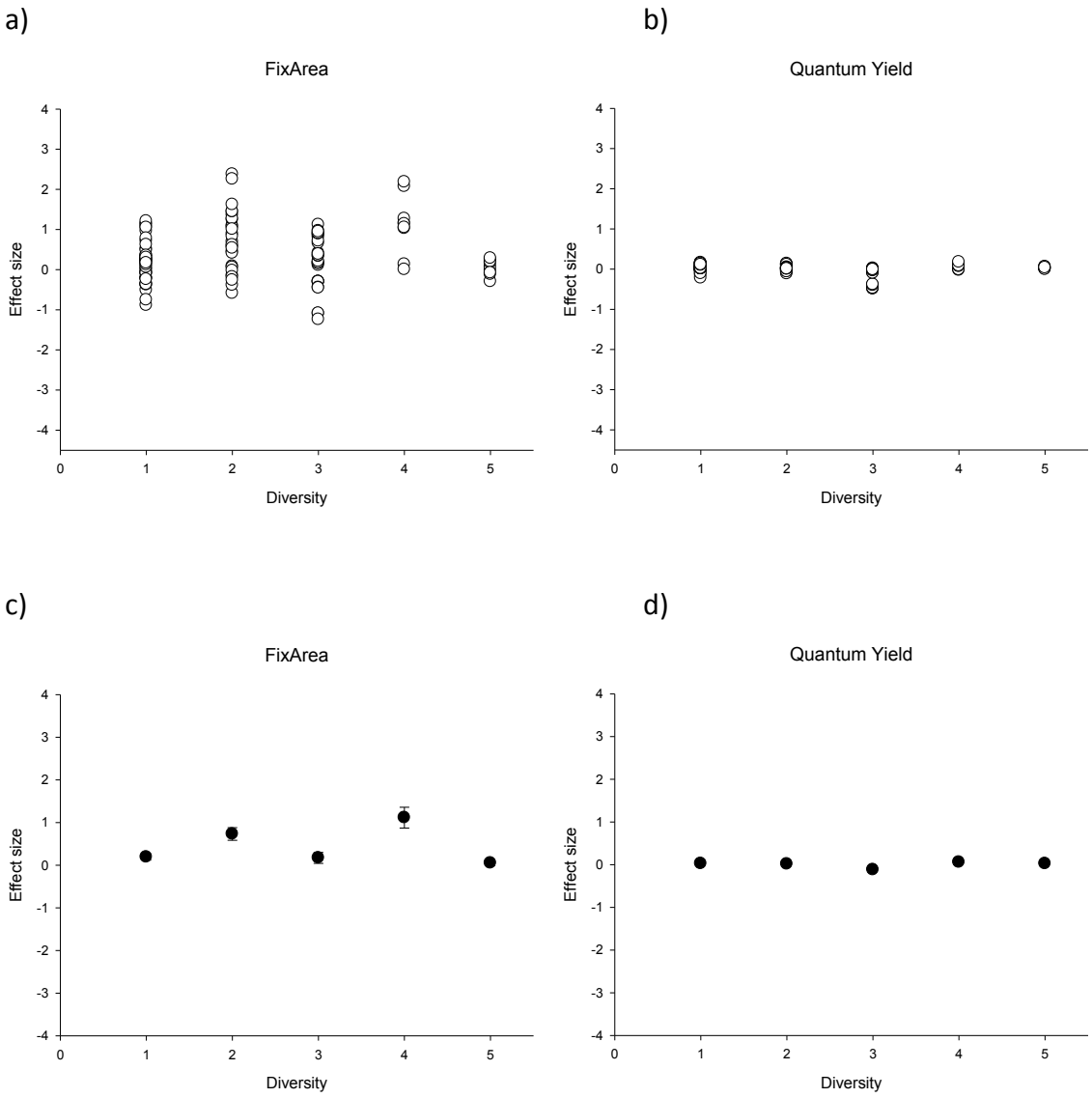


Fig. 47: Effect sizes of Fluoxetine (0.096 µg L^{-1}) on FixArea (day 22) of diversity treatments; b: on Quantum Yield (day 22) of diversity treatments; c: data as in a) plotted as mean +/- S.E. ; d: data as in b) plotted as mean +/- S.E. Diversity treatment 1 includes the values of all monocultures and their replicates ($N_{(diversity\ 1)}$ = 45; $N_{(diversity\ 2)}$ = 27; $N_{(diversity\ 3)}$ = 27; $N_{(diversity\ 4)}$ = 9; $N_{(diversity\ 5)}$ = 9; $N_{(total)}$ = 117).

The coefficient of variation of the FixArea and Quantum Yield values decreased with higher diversity in treatments with the highest concentration of Fluoxetine (Tab. 30).

Tab. 30: Coefficient of variation (CV) of FixArea and Quantum Yield values of each diversity treatment. $N_{(diversity\ 1)}$ = 15; $N_{(diversity\ 2)}$ =9; $N_{(diversity\ 3)}$ = 9; $N_{(diversity\ 4)}$ = 3; $N_{(diversity\ 5)}$ = 3. The measurements are from the last day (day 22) and in treatments with the highest concentration of Fluoxetine (0.096 µg L^{-1}).

	Diversity 1	Diversity 2	Diversity 3	Diversity 4	Diversity 5
CV % (FixArea)	97,61	97,37	52,13	49,44	18,23
CV % (Quantum Yield)	22,43	13,87	22,86	6,03	1,21

5.1.2.1.4 Mixture of Carbamazepine, Ciprofloxacin and Fluoxetine

The effect of diversity reduced the impact of the pharmaceutical mixture on FixArea and Quantum Yield (Fig. 48). A One Way Anova identified significant differences of the effect sizes of the mixture on the FixArea between the diversity treatments (DF=4, H=23.490, P=<0.001). A post hoc test (Dunn's method, contrast) identified significant differences between diversity treatments 3 and the monocultures (Q=3.573; P<0.05). A One Way Anova found significant differences of the effect sizes of the mixture on the Quantum Yield between the diversity treatments (DF=4, H=24.272, P=<0.001). A post hoc test (Dunn's method, contrast) identified significant differences between diversity treatments 5 and the monocultures (Q=2.731; P<0.05) and between diversity treatments 2 and the monocultures (Q=4.006; P<0.05).

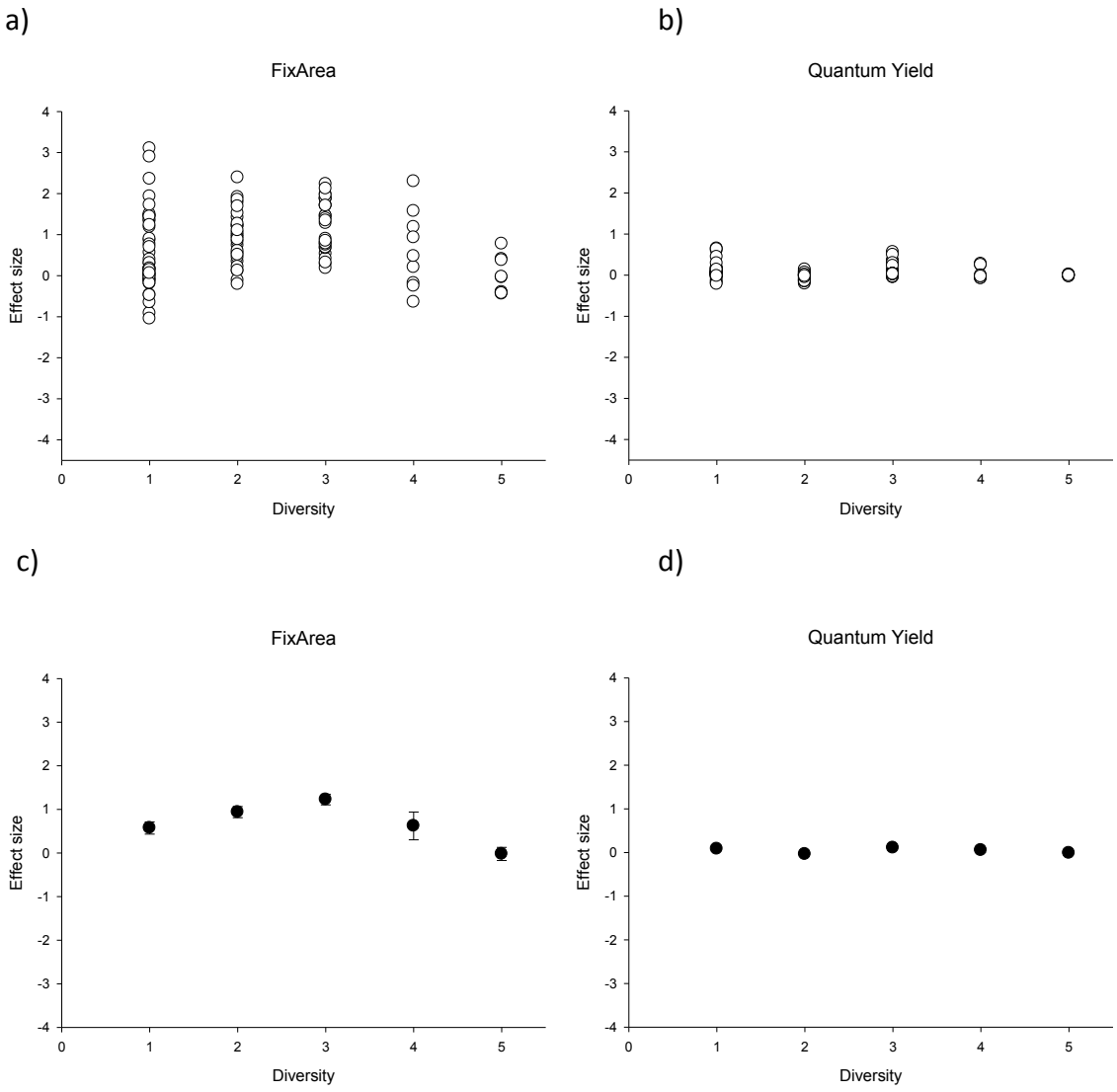


Fig. 48: Effect sizes of Carbamazepine (8.0 µg L^{-1}), Ciprofloxacin (0.0160 µg L^{-1}) and Fluoxetine (0.096 µg L^{-1}) on FixArea (day 22) of diversity treatments; b: on Quantum Yield (day 22) of diversity treatments; c: data as in a) plotted as mean +/- S.E.; d: data as in b) plotted as mean +/- S.E. Diversity treatment 1 includes the values of all monocultures and their replicates ($N_{(diversity\ 1)}$ = 45; $N_{(diversity\ 2)}$ = 27; $N_{(diversity\ 3)}$ = 27; $N_{(diversity\ 4)}$ = 9; $N_{(diversity\ 5)}$ = 9; $N_{(total)}$ = 117).

The coefficient of variation of the FixArea and Quantum Yield values showed a decrease with higher diversity after exposure to a mixture of the pharmaceuticals Carbamazepine, Ciprofloxacin and Fluoxetine in the highest concentrations (8.0, 0.160 and 0.096 µg L^{-1}; Tab. 31).

Tab. 31: Coefficient of variation (CV) of FixArea and Quantum Yield values of each diversity treatment. $N_{(diversity\ 1)}$ = 15; $N_{(diversity\ 2)}$ =9; $N_{(diversity\ 3)}$ = 9;$N_{(diversity\ 4)}$ = 3;$N_{(diversity\ 5)}$ = 3. The measurements are from the last day (day 22) and in treatments with the highest concentration of Carbamazepine, Ciprofloxacin and Fluoxetine (8.0; 0.160 and 0.096 µg L^{-1}).

	Diversity 1	Diversity 2	Diversity 3	Diversity 4	Diversity 5
CV % (FixArea)	99,78	108,53	73,74	58,17	51,02
CV % (Quantum Yield)	33,14	16,07	32,08	1,63	1,37

5.1.2.2 Effect of pharmaceuticals on different diversity treatments including specific algal species

This chapter describes and compares the effect sizes of the pharmaceuticals on FixArea for each monoculture and all diversity treatments in which the respective monoculture was included.

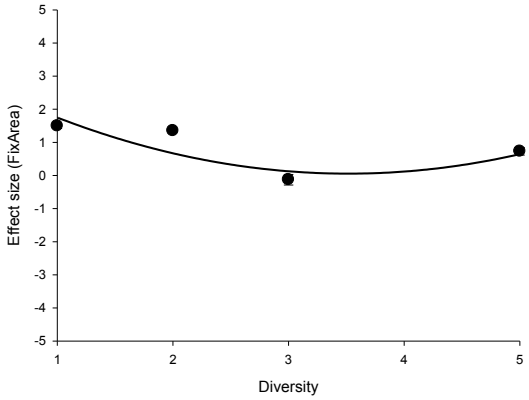
5.1.2.2.1 Carbamazepine

The effect of Carbamazepine on the FixArea of *Scenedesmus obliquus* and *Navicula pelliculosa* in communities was influencend by diversity (Fig. 49 a, b). A regression (quadratic) revealed a significant dependency between the diversity and the effect size of Carbamazepine on FixArea of *Scenedesmus obliquus* in diversity treatments: $y= 3.3651 - 1.8821*x + 0.2676*x^2$, $R^2=0.5004$, $P<0.001$. A regression (quadratic) revealed a significant dependency between the diversity and the effect size of Carbamazepine on FixArea of *Navicula pelliculosa* in diversity treatments: $y= 4.4311 - 2.8866*x + 0.4395*x^2$, $R^2=0.3866$, $P<0.001$.

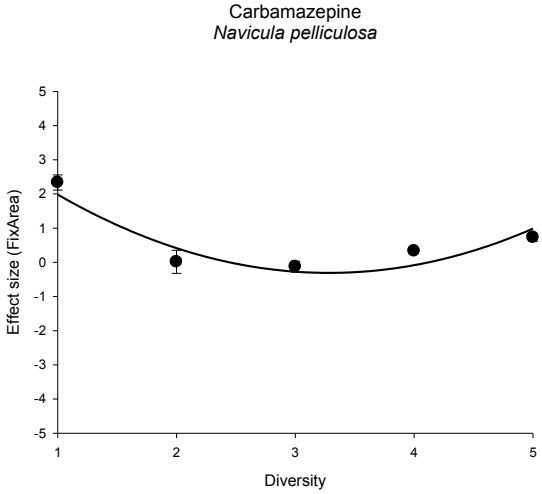
Diversity influenced the effect of Carbamazepine on the FixArea of *Peridinium sp.* in treatments (Fig. 49 c). A regression (quadratic) revealed a significant dependency between the diversity and the effect size of Carbamazepine on FixArea of *Peridinium sp*. in diversity treatments: $y= 1.6303 - 1.4199*x + 0.2472*x^2$, $R^2=0.1104$, $P=0.0507$.

Carbamazepine affected the FixArea of *Cryptomonas phaseolus* in monocultures more strongly than the more diverse treatments. The lowest effect was observed in diversity treatment 4 (Fig. 49 d). A regression (linear) revealed a significant dependency between the diversity and the effect size of Carbamazepine on FixArea of *Cryptomonas phaseolus* in diversity treatments: $y= -3.3937 + 0.844*x$, $R^2=0.3191$, $P<0.001$.

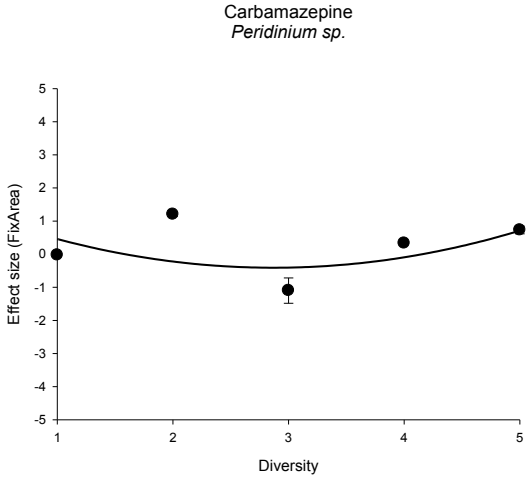
a)


Carbamazepine
Scenedesmus obliquus
Effect size (FixArea)
Diversity

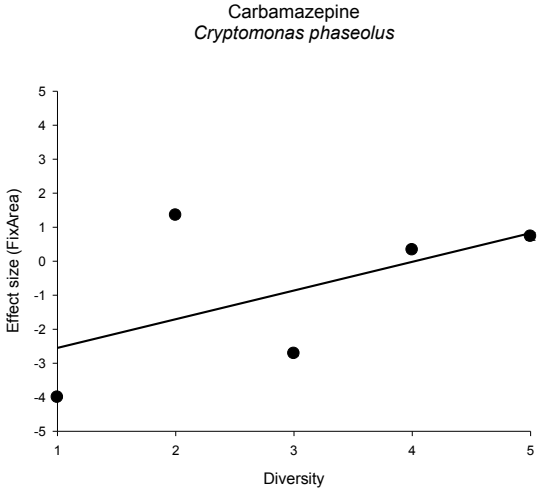
b)


Carbamazepine
Navicula pelliculosa
Effect size (FixArea)
Diversity

c)


Carbamazepine
Peridinium sp.
Effect size (FixArea)
Diversity

d)


Carbamazepine
Cryptomonas phaseolus
Effect size (FixArea)
Diversity

e)

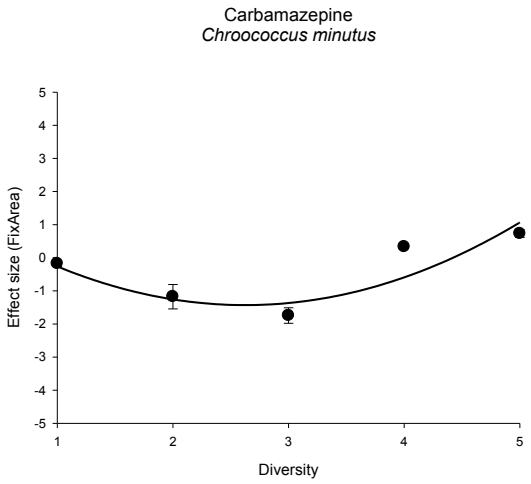


Fig. 49: Effects of Carbamazepine (8.0 µg L^{-1}) on FixArea of algae species in monoculture and the different diversity treatments on day 22. **a:** *Scenedesmus obliquus*; **b:** *Navicula pelliculosa*; **c:** *Peridinium sp.*; **d:** *Cryptomonas phaseolus;* **e:** *Chroococcus minutus*. Diversity treatment 1 includes the values of all monocultures and their replicates ($N_{(diversity\ 1)}$ = 9; $N_{(diversity\ 2)}$ = 9; $N_{(diversity\ 2;\ N.pelliculosa)}$ = 18; $N_{(diversity\ 3)}$ = 18; $N_{(diversity\ 4)}$ = 9; $N_{(diversity\ 5)}$ = 9). Data expressed in mean effect size to the unexposed controls. Error bars represent standard errors. Regression was performed with effect sizes (raw data, full crossed) of Carbamazepine on FixArea of the algae. Solid line: significant on < 5% level.

The effect of Carbamazepine on FixArea of *Chroococcus minutus* was around 0 in the monocultures. In diversity treatments 2 and 3 the FixArea was affected negatively. In diversity treatments 4 and 5 the effect of Carbamazepine on the FixArea of *Chroococcus minutus* was positive and had an effect size around 1 (Fig. 49 e). A regression (quadratic) revealed a significant dependency between the diversity and the effect size of Carbamazepine on FixArea of *Chroococcus minutus* in diversity treatments: $y = 1.6194 - 2.3227*x + 0.4424*x^2$, R^2=0.4910, P<0.001.

5.1.2.2.2 Ciprofloxacin

A regression revealed no significant dependency between the diversity and the effect size of Ciprofloxacin on FixArea of *Scenedesmus obliquus* in diversity treatments. Therefore I performed a One Way Anova, which identified significant differences between the diversity treatments including *Scenedesmus obliquus* (DF=3, F=3.020, P=0.041). A post hoc test (Bonferroni t-test, contrast) identified no significant differences between the effect sizes of Ciprofloxacin on FixArea of *Scenedesmus obliquus* in monocultures and in diversity treatments (Fig. 50 a).

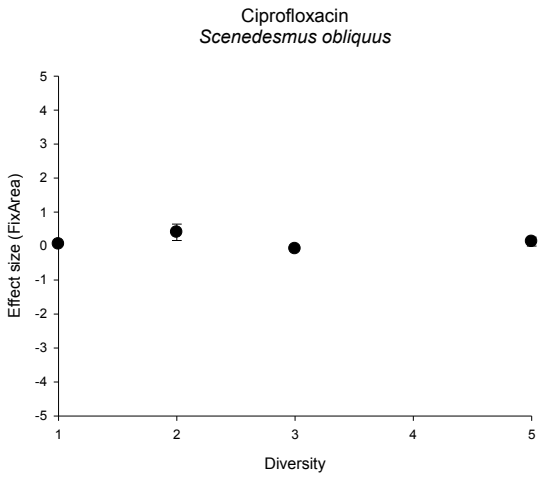
A regression revealed no significant dependency between the diversity and the effect size of Ciprofloxacin on FixArea of *Navicula pelliculosa* in diversity treatments. A One Way Anova identified significant differences between the diversity treatments including *Navicula pelliculosa* (DF=4, F=6.290, P<0.001). A post hoc test (Bonferroni t-test, contrast) identified significant differences between the effect sizes of Ciprofloxacin on FixArea of *Navicula pelliculosa* in monoculture and diversity treatments 5 (P=0.017) (Fig. 50 b).

A regression revealed no significant dependency between the diversity and the effect size of Ciprofloxacin on FixArea of *Peridinium sp.* in diversity treatments. A One Way Anova identified significant differences between the diversity treatments including *Peridinium sp.* (DF=4, F=6.057, P<0.001). A post hoc test (Bonferroni t-test, contrast) identified significant differences between the effect sizes of Ciprofloxacin on FixArea of *Peridinium sp.* in monocultures and diversity treatments 2 (P=0.003) (Fig. 50 c).

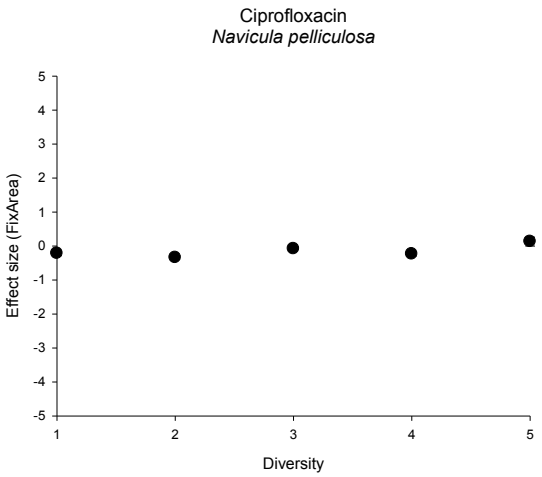
A regression revealed no significant dependency between the diversity and the effect size of Ciprofloxacin on FixArea of *Cryptomonas phaseolus* in diversity treatments. A One Way Anova identified significant differences between the diversity treatments including *Cryptomonas phaseolus* (DF=4, F=3.406, P=0.017). A post hoc test (Bonferroni t-test, contrast) identified no significant differences between the effect sizes of Ciprofloxacin on FixArea of *Cryptomonas phaseolus* in diversity treatments and the monocultures (Fig. 50 d).

Diversity influenced the effect of Ciprofloxacin on the FixArea of *Chroococcus minutus* in treatments (Fig. 50 e). A regression (quadratic) revealed a significant dependency between the diversity and the effect size of Ciprofloxacin on FixArea of *Chroococcus minutus* in diversity treatments: $y= 0.5323 - 0.5053*x + 0.0844*x^2$, $R^2=0.2217$, P=0.0017.

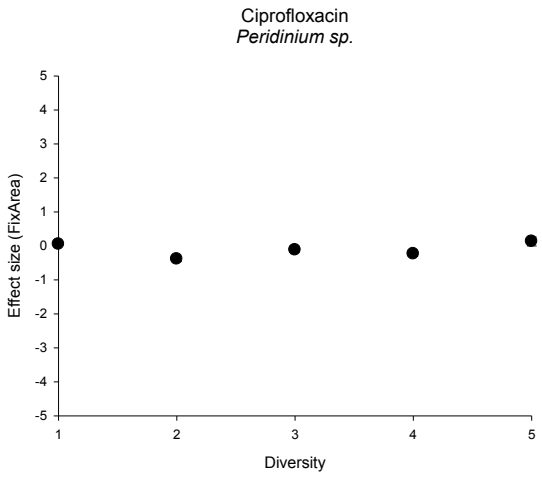
a)



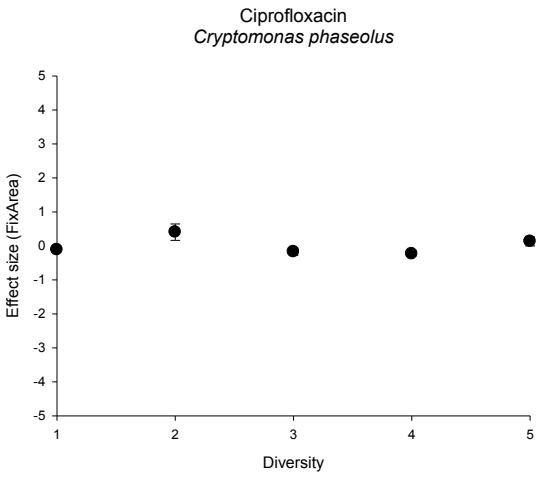
b)



c)



d)



e)

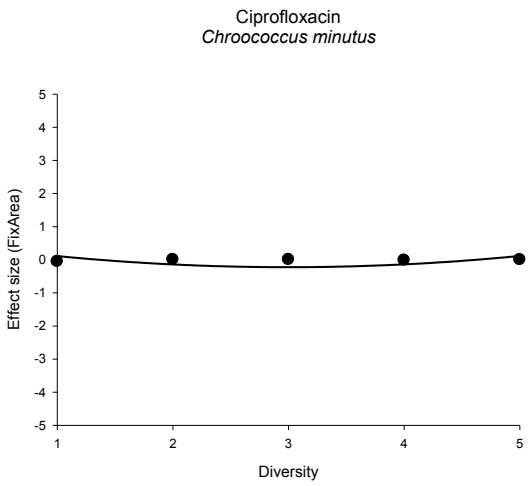
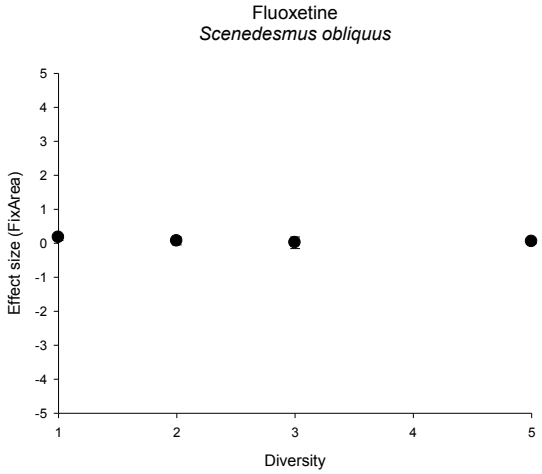


Fig. 50: Effects of Ciprofloxacin (0.160 µg L^{-1}) on FixArea of algae species in monoculture and the different diversity treatments on day 22. **a:** *Scenedesmus obliquus*; **b:** *Navicula pelliculosa*; **c:** *Peridinium sp.*; **d:** *Cryptomonas phaseolus;* **e:** *Chroococcus minutus*. Diversity treatment 1 includes the values of all monocultures and their replicates ($N_{(diversity\ 1)}$ = 9; $N_{(diversity\ 2)}$ = 9; $N_{(diversity\ 2;\ N.pelliculosa)}$ = 18; $N_{(diversity\ 3)}$ = 18; $N_{(diversity\ 4)}$ = 9; $N_{(diversity\ 5)}$ = 9). Data expressed in mean effect size to the unexposed controls. Error bars represent standard errors. Regression was performed with effect sizes (raw data, full crossed) of Ciprofloxacin on FixArea of the algae. Solid line: significant on < 5% level.

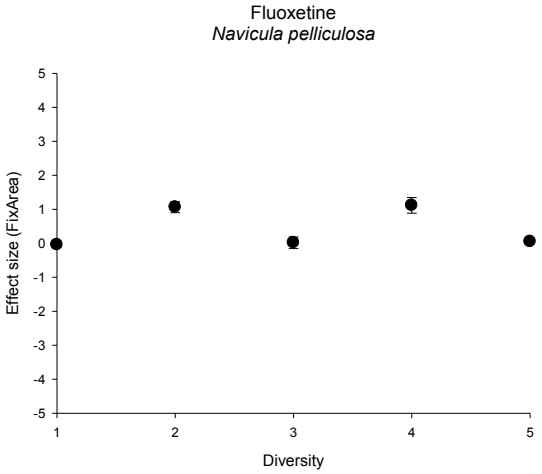
5.1.2.2.3 Fluoxetine

The diversity of a community including *Scenedesmus obliquus* did not affect the mean effect size of Fluoxetine on FixArea. As *Scenedesmus obliquus* was not present in diversity treatment 5, no data is available for this diversity treatment (Fig. 51 a). A regression revealed no significant dependency between the diversity and the effect size of Fluoxetine on FixArea of *Scenedesmus obliquus* in diversity treatments. A One Way Anova identified no significant differences between the diversity treatments (DF=3, F=0.177, P=0.912).

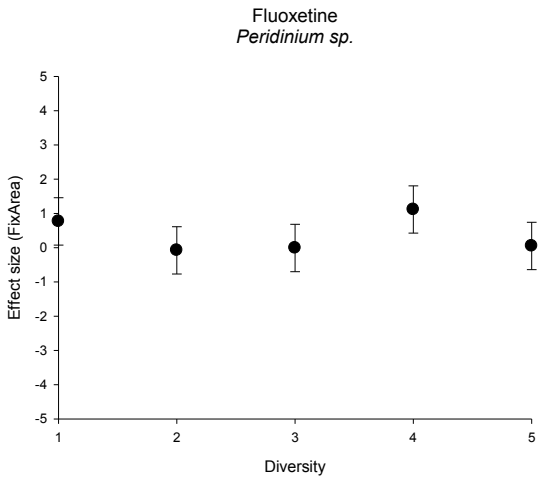
a)



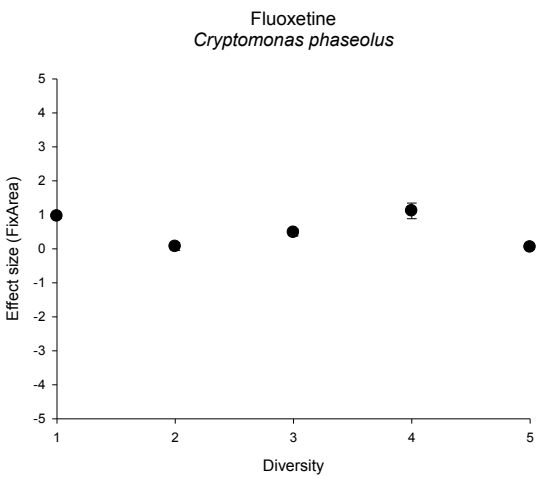
b)



c)



d)



e)

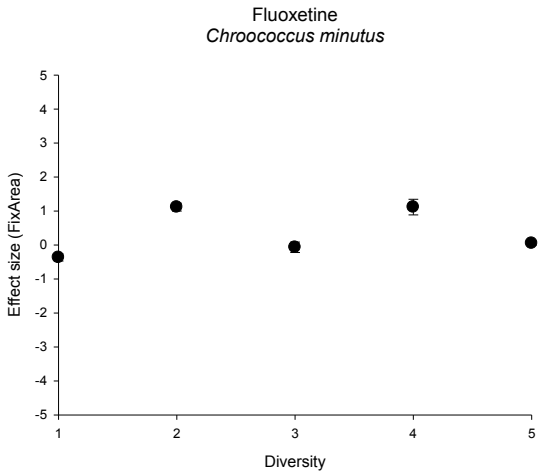


Fig. 51: Effects of Fluoxetine (0.096 µg L^{-1}) on FixArea of algae species in monoculture and the different diversity treatment on day 22. **a:** *Scenedesmus obliquus*; **b:** *Navicula pelliculosa*; **c:** *Peridinium sp.*; **d:** *Cryptomonas phaseolus;* **e:** *Chroococcus minutus*. Diversity treatment 1 includes the values of all monocultures and their replicates ($N_{(diversity\ 1)}$ = 9; $N_{(diversity\ 2)}$ = 9; $N_{(diversity\ 2;\ N.pelliculosa)}$ = 18; $N_{(diversity\ 3)}$ = 18; $N_{(diversity\ 4)}$ = 9; $_{(diversity\ 5)}$ = 9). Data expressed in mean effect size to the unexposed controls. Error bars represent standard errors. Regression was performed with effect sizes (raw data, full crossed) of Fluoxetine on FixArea of the algae.

The mean effect sizes of Fluoxetine on FixArea of *Navicula pelliculosa* in diversity treatments were around 0 in the monocultures and in the diversity treatments 3 and 5. The mean effect sizes of Fluoxetine on FixArea of *Navicula pelliculosa* were between 1 and 2 in the monocultures and in the diversity treatments 2 and 4 (Fig. 51 b). A regression revealed no significant dependency between the diversity and the effect size of Fluoxetine on FixArea of *Navicula pelliculosa* in diversity treatments. A One Way Anova identified significant differences between the diversity treatments (DF=4, F=11.244, P<0.001). A post hoc test (Bonferroni t-test, contrast) identified significant differences between the effect sizes of Fluoxetine on FixArea of *Navicula pelliculosa* in monocultures and diversity treatments 2 (P<0.001) and 4 (P<0.001).

The mean effect sizes of Fluoxetine on FixArea of *Peridinium sp.* in diversity treatments were around 0 in the monocultures and in the diversity treatments 3 and 5. The mean effect sizes of Fluoxetine on FixArea of *Peridinium sp.* were between 1 and 2 in the monocultures

and in the diversity treatments 2 and 4 (Fig. 51 c). A regression revealed no significant dependency between the diversity and the effect size of Fluoxetine on FixArea of *Peridinium sp.* in diversity treatments. A One Way Anova identified significant differences between the diversity treatments including *Peridinium sp.* (DF=4, F=6.848, P<0.001). A post hoc test (Bonferroni t-test, contrast) identified significant differences between the effect sizes of Fluoxetine on FixArea of *Peridinium sp.* in monocultures and diversity treatments 2 (P=0.014) and 4 (P=0.004).

The mean effect sizes of Fluoxetine on FixArea of *Cryptomonas phaseolus* in diversity treatments were around 1 in the monocultures and in diversity treatments 4. The mean effect sizes of Fluoxetine on FixArea of treatments including *Cryptomonas phaseolus* were around 0 in the diversity treatments 2, 3 and 5 (Fig. 51 d). A regression revealed no significant dependency between the diversity and the effect size of Fluoxetine on FixArea of *Cryptomonas phaseolus* in diversity treatments. A One Way Anova identified significant differences between the diversity treatments (DF=4, F=12.701, P<0.001). A post hoc test (Bonferroni t-test, contrast) identified significant differences between the effect sizes of Fluoxetine on FixArea of *Cryptomonas phaseolus* in monocultures and diversity treatments 5 (P<0.001) and 2 (P<0.001).

The mean effect sizes of Fluoxetine on FixArea of *Chroococcus minutus* in diversity treatments were around 0 in the monocultures and in the diversity treatments 3 and 5. The mean effect sizes of Fluoxetine on FixArea of *Chroococcus minutus* were between 1 and 2 in the monocultures and in the diversity treatments 2 and 4 (Fig. 51 e). A regression revealed no significant dependency between the diversity and the effect size of Fluoxetine on FixArea of *Chroococcus minutus* in diversity treatments. A One Way Anova identified significant differences between the diversity treatments including *Chroococcus minutus* (DF=4, F=16.387, P<0.001). A post hoc test (Bonferroni t-test, contrast) identified significant differences between the effect sizes of Fluoxetine on FixArea of *Chroococcus minutus* in monocultures and diversity treatments 4 (P<0.001) and 2 (P<0.001).

5.1.2.2.4 Mixture of Carbamazepine, Ciprofloxacin and Fluoxetine

Mean effect sizes of the mixture on FixArea of *Scenedesmus obliquus* in diversity treatments were reduced with higher diversity treatment (Fig. 52). As *S. obliquus* was not present in diversity treatment 5, no data is available for this diversity treatment. A regression (quadratic) revealed a significant dependency between the diversity and the effect size of the mixture on FixArea of *Scenedesmus obliquus* in diversity treatments: $y= 1.1145+0.2429*x - 0.0945*x^2$, $R^2=0.5102$, $P<0.001$.

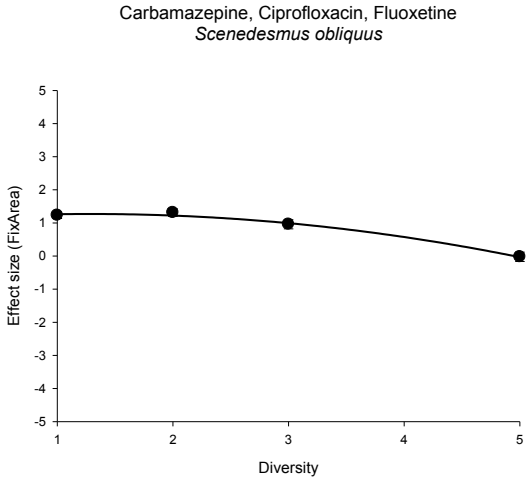
Mean effect sizes of the mixture on FixArea of *Navicula pelliculosa* in diversity treatments were higher in diversity treatments 2, 3 and 4. Diversity treatment 5 was comparable to the monocultures (Fig. 52 b). A regression (quadratic) revealed a significant dependency between the diversity and the effect size of the mixture on FixArea of *Navicula pelliculosa* in diversity treatments: $y= -1.3230 + 1.5253*x - 0.2547*x^2$, $R^2=0.2989$, $P<0.001$.

Mean effect sizes of the mixture on FixArea of *Peridinium sp.* in diversity treatments were higher in diversity treatments 2, 3 and 4. Diversity treatments 5 were comparable to the monocultures (Fig. 52 c). A regression (quadratic) revealed a significant dependency between the diversity and the effect size of the mixture on FixArea of *Peridinium sp.* in diversity treatments: $y= -1.4767 + 1.9256*x - 0.3322*x^2$, $R^2=0.4353$, $P<0.001$.

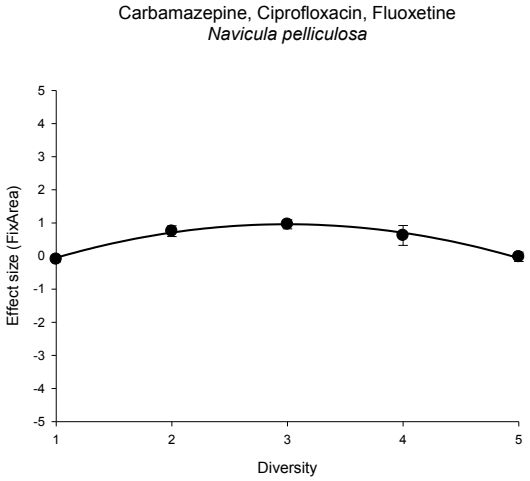
Mean effect sizes of the mixture on FixArea of *Cryptomonas phaseolus* in diversity treatments was reduced with higher diversity treatments (Fig. 52 d). A regression (linear) revealed a significant dependency between the diversity and the effect size of the mixture on FixArea of *Cryptomonas phaseolus* in diversity treatments: $y=2.427 – 0.441*x$, $R^2=0.774$, $P=0.049$.

Mean effect sizes of the mixture on FixArea of *Chroococcus minutus* in diversity treatments were higher in diversity treatments 2, 3 and 4. Diversity treatments 5 were comparable to the monocultures (Fig. 52 e). A regression (quadratic) revealed a significant dependency between the diversity and the effect size of the mixture on FixArea of *Chroococcus minutus* in diversity treatments: $y = -1.6318 + 1.7998*x - 0.2961*x^2$, $R^2=0.4335$, $P<0.001$.

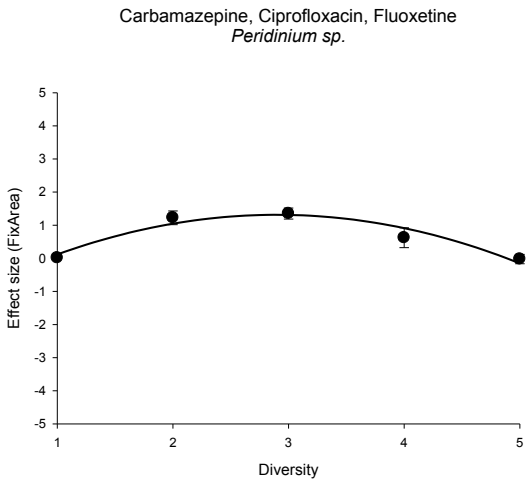
a)



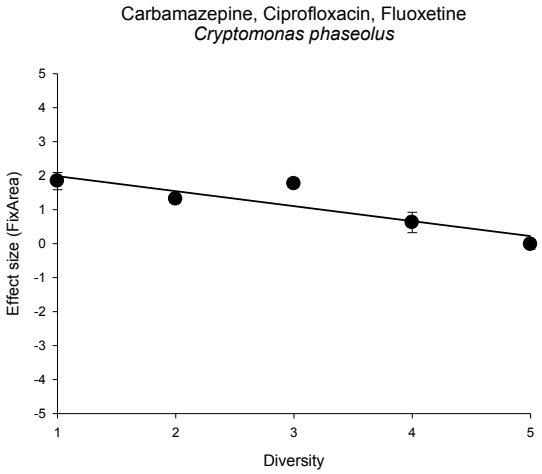
b)



c)



d)



e)

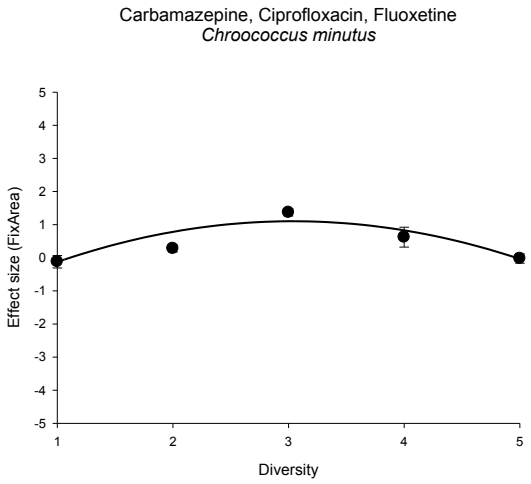


Fig. 52: Effects of Carbamazepine, Ciprofloxacin and Fluoxetine (8.0, 0.160, 0.096 µg L^{-1}) on FixArea of algae species in monoculture and the different diversity treatments on day 22. **a:** *Scenedesmus obliquus*; **b:** *Navicula pelliculosa*; **c:** *Peridinium sp.*; **d:** *Cryptomonas phaseolus;* **e:** *Chroococcus minutus*. Diversity treatment 1 includes the values of all monocultures and their replicates ($N_{(diversity\ 1)}$ = 9; $N_{(diversity\ 2)}$ = 9; $N_{(diversity\ 2;\ N.pelliculosa)}$ = 18; $N_{(diversity\ 3)}$ = 18; $N_{(diversity\ 4)}$ = 9; $N_{(diversity\ 5)}$ = 9). Data expressed in mean effect size to the unexposed controls. Error bars represent standard errors. Regression was performed with effect sizes (raw data, full crossed) of pharmaceutical mixture on FixArea of the algae. Solid line: significant on < 5% level.

5.1.2.3 Effect of pharmaceuticals on individual algae species in the different diversity treatments

Whereas the previous two chapters describe effects on pharmaceuticals on summary parameters of different diverse communities this chapter describes and compares the effect size of the pharmaceuticals on the mean abundance of the respective algae species in monoculture and the different diversity treatments.

5.1.2.3.1 Carbamazepine

A diversity mediated effect of Carbamazepine on the abundance of *Scenedesmus obliquus* was observed in treatments including 2 other algae species and in treatments with 4 different algae species. The effect size was negative in diversity treatment 3 and positive in diversity treatment 5 (Fig. 53 a). *S. obliquus* was not used in diversity treatments 4, therefore no data is available. A regression (quadratic) revealed a significant dependency between the diversity and the effect size of Carbamazepine on the abundance of *Scenedesmus obliquus* in the diversity treatments: $y= 2.8271 - 2.7036*x + 0.4528*x^2$, $R^2=0.2603$, P=0.0028.

In diversity treatments 3 and 5 with *Navicula pelliculosa* no algae of this species were finally found by microscopic counting (Fig. 53 b). A regression (quadratic) revealed a significant dependency between the diversity and the effect size of Carbamazepine on the abundance of *Navicula pelliculosa* in the diversity treatments: $y= 7.2426 - 6.3026*x + 1.1082*x^2$, $R^2=0.2400$, P=0.0108.

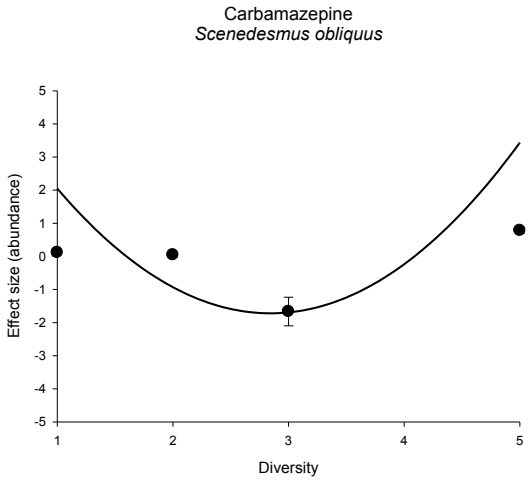
The monocultures with *Peridinium sp.* were not analyzed (loss of samples). Carbamazepine had in general a negative effect on the abundance of *Peridinium sp.*, which was less pronounced in diversity treatments 4. In diversity treatment 5 no *Peridinium sp.* individuals were found (Fig. 53 c).

In the control treatments of the monocultures of *Cryptomonas phaseolus* algae were completely overgrown by bacteria and no effect size was calculated. In diversity treatments 2 and 5 no cells of *Cryptomonas phaseolus* were found. From diversity treatments 3 to 4 the

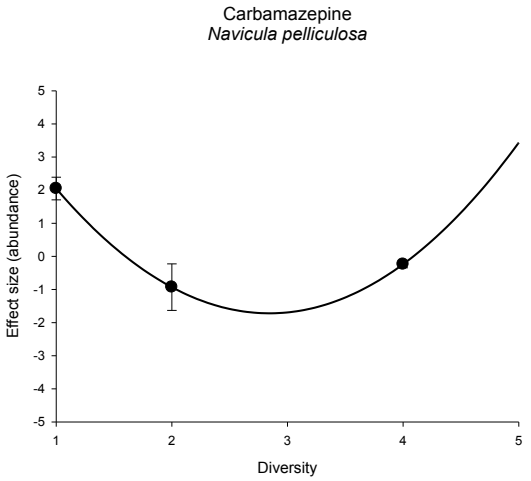
negative effect of Carbamazepine on the abundance of *Cryptomonas phaseolus* was reduced (Fig. 53 d).

In monocultures and in treatments with 4 different algae species the mean effect size of Carbamazepine on the abundance of *Chroococcus minutus* was around 0. In diversity treatments 3 and 4 the effect of Carbamazepine on the abundance of *Chroococcus minutus* was negative (Fig. 53 e). A regression (linear) revealed a trend of a dependency between the diversity and the effect size of Carbamazepine on the abundance of *Chroococcus minutus* in diversity treatments: y= 1.6305 - 0.4029*x, R^2=0.0542, P=0.1113.

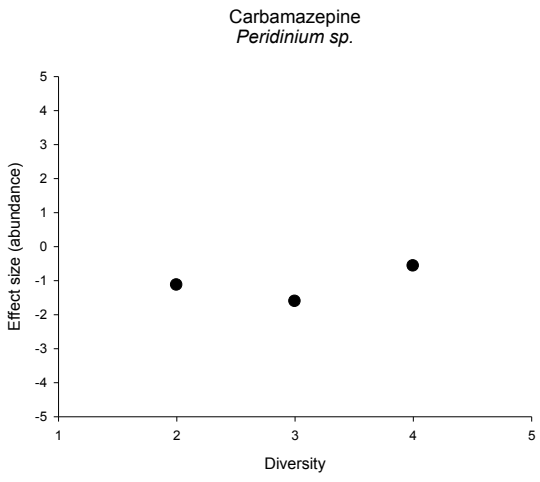
a)



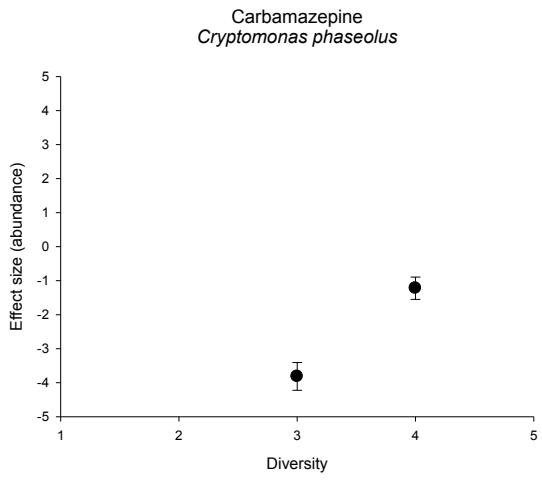
b)



c)



d)



e)

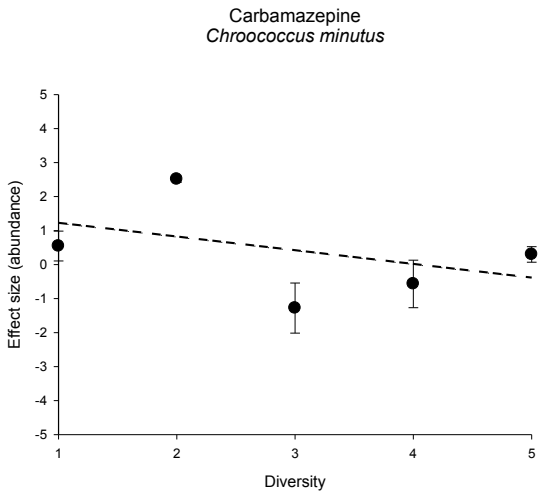


Fig. 53: Effects of Carbamazepine (8.0 µg L^{-1}) on individual algal species abundances grown in mono- and polyculture on day 22. **a:** *Scenedesmus obliquus* ($N_{(diversity\ 1)}$ = 9; $N_{(diversity\ 2)}$ = 6; $N_{(diversity\ 3)}$ = 18; $N_{(diversity\ 5)}$ = 9); **b:** *Navicula pelliculosa* ($N_{(diversity\ 1)}$ = 9; $N_{(diversity\ 2)}$ = 18; $N_{(diversity\ 4)}$ = 9); **c:** *Peridinium sp.* ($N_{(diversity\ 2)}$ = 2; $N_{(diversity\ 3)}$ = 2; $N_{(diversity\ 4)}$ = 2); **d:** *Cryptomonas phaseolus* ($N_{(diversity\ 3)}$ = 9; $N_{(diversity\ 4)}$ = 9)*;* **e:** *Chroococcus minutus* ($N_{(diversity\ 1)}$ = 6; $N_{(diversity\ 2)}$ = 9; $N_{(diversity\ 3)}$ = 18; $N_{(diversity\ 4)}$ = 9; $N_{(diversity\ 5)}$ = 9). Data expressed in mean effect size to the unexposed controls. Error bars represent standard errors. Regression was performed with effect sizes (raw data, full crossed) of Carbamazepine on the abundance of the algae. Solid line: significant on < 5% level. Dashed line: significant on < 10% level.

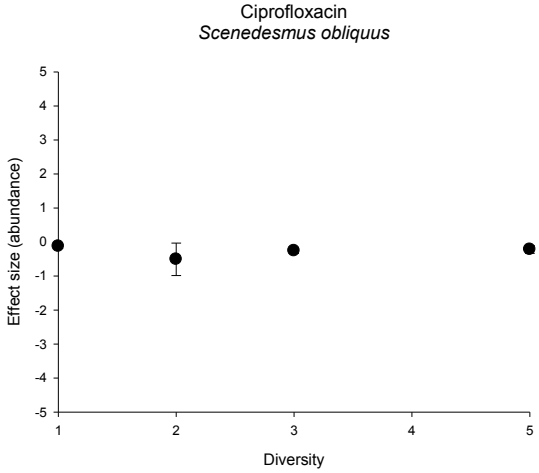
5.1.2.3.2 Ciprofloxacin

The mean effect sizes of Ciprofloxacin on the abundances of *Scenedesmus obliquus* were around 0 in all diversity treatments. *Scenedesmus obliquus* was not used in diversity treatment 4, therefore no data is available (Fig. 54 a). A regression revealed no significant dependency between the diversity and the effect size of Ciprofloxacin on the abundance of *Scenedesmus obliquus* in the diversity treatments. A One Way Anova identified no significant differences of the effect size of Ciprofloxacin on the abundances of *Scenedesmus obliquus* between the diversity treatments of (DF=3, F=0.439, P=0.726).

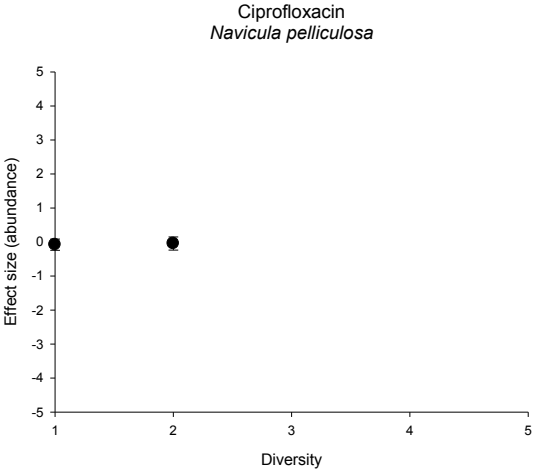
The mean effect sizes of Ciprofloxacin on the abundance of *Navicula pelliculosa* were around 0 in monocultures and in treatments with one additional algae species. In treatments with diversity treatments 3, 4 and 5 no cells of *Navicula pelliculosa* were found (Fig. 54 b). A regression revealed no significant dependency between the diversity and the effect size of Ciprofloxacin on the abundance of *Navicula pelliculosa* in the diversity treatments. A One Way Anova identified no significant differences of the effect size of Ciprofloxacin on the abundances of *Navicula pelliculosa* between the diversity treatments (DF=1, F=1.739, P=0.206).

The effect of Ciprofloxacin on the abundance of *Peridinium sp.* was lower within treatments with 1, 2 or 3 additional algae species. In diversity treatments with 4 additional algae species a high positive effect of Ciprofloxacin on the abundance of *Peridinium sp.* was observed (Fig. 54 c). A linear regression was significant: $y=-3.0621 + 1.1970*x$, $R^2= 0.7135$, $P<0.001$.

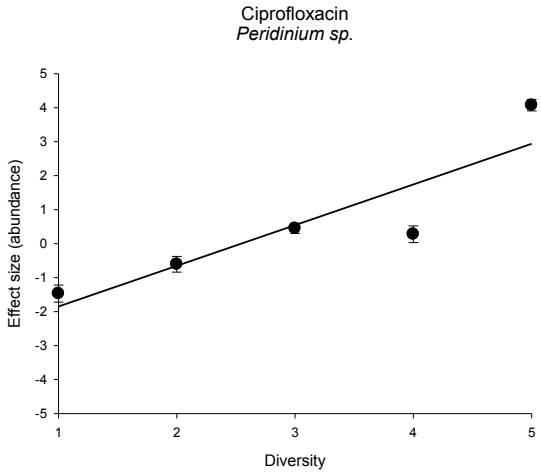
a)


Ciprofloxacin
Scenedesmus obliquus
Effect size (abundance)
Diversity

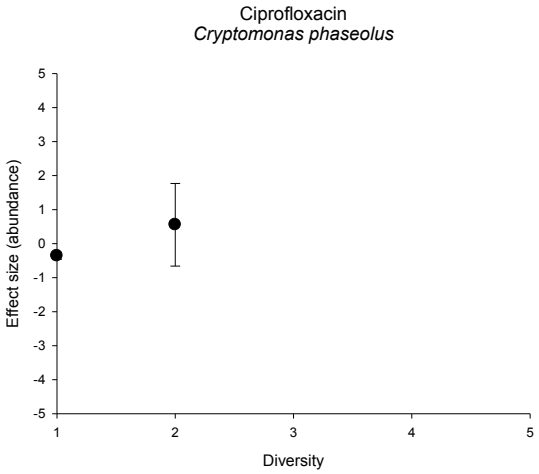
b)


Ciprofloxacin
Navicula pelliculosa
Effect size (abundance)
Diversity

c)


Ciprofloxacin
Peridinium sp.
Effect size (abundance)
Diversity

d)


Ciprofloxacin
Cryptomonas phaseolus
Effect size (abundance)
Diversity

e)

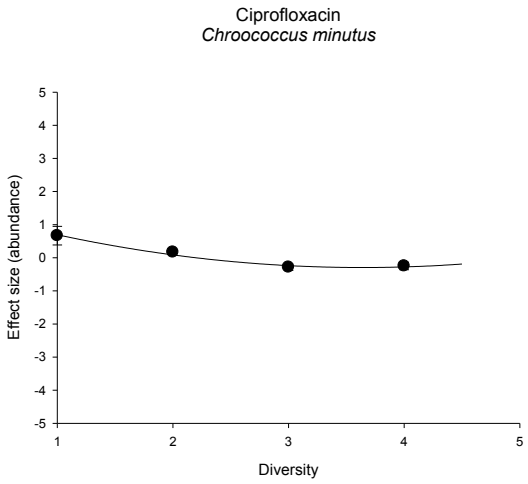


Fig. 54: Effects of Ciprofloxacin (0.160 µg L^{-1}) on individual algal species abundances grown in mono- and polyculture on day 22. **a:** *Scenedesmus obliquus* ($N_{(diversity\ 1)}$ = 9; $N_{(diversity\ 2)}$ = 9; $N_{(diversity\ 3)}$ = 15; $N_{(diversity\ 5)}$ = 9); **b:** *Navicula pelliculosa* ($N_{(diversity\ 1)}$ = 9; $N_{(diversity\ 2)}$ = 18); **c:** *Peridinium sp.* ($N_{(diversity\ 1)}$ = 9; $N_{(diversity\ 2)}$ = 9; $N_{(diversity\ 3)}$ = 12; $N_{(diversity\ 4)}$ = 9; $N_{(diversity\ 5)}$ = 9); **d:** *Cryptomonas phaseolus* ($N_{(diversity\ 1)}$ = 9; $N_{(diversity\ 2)}$ = 9)*;* **e:** *Chroococcus minutus* ($N_{(diversity\ 1)}$ = 9; $N_{(diversity\ 2)}$ = 9; $N_{(diversity\ 3)}$ = 15; $N_{(diversity\ 4)}$ = 9). Data expressed in mean effect size to the unexposed controls. Error bars represent standard errors. Regression was performed with effect sizes (raw data, full crossed) of Ciprofloxacin on the abundance of the algae. Solid line: significant on < 5% level.

The mean effect size of Ciprofloxacin on the abundance of *Cryptomonas phaseolus* was higher in treatments with 1 additional algae species than in the monocultures. In the diversity treatments 3, 4 and 5 no more cells of *Cryptomonas phaseolus* were found on day 22 (Fig. 54 d). A regression revealed no significant dependency between the diversity and the effect size of Ciprofloxacin on the abundance of *Cryptomonas phaseolus* in diversity treatments. A One Way Anova identified no significant differences of the effect sizes of Ciprofloxacin on the abundances of *Cryptomonas phaseolus* between the diversity treatments (DF=1, F=0.498, P=0.491).

The mean effect size of Ciprofloxacin on the abundance of *Chroococcus minutus* was slightly above 0 and was around 0 in treatments with 1, 2 and 3 additional algae species. In treatments with 4 additional algae species no cells of *Chroococcus minutus* were found (Fig. 54 e). A regression (quadratic) revealed a significant dependency between the diversity and

the effect size of Ciprofloxacin on the abundance of *Chroococcus minutus* in diversity treatments: $y= 1.5870 -1.0341*x + 0.1421*x^2$, $R^2=0.3798$, $P<0.001$.

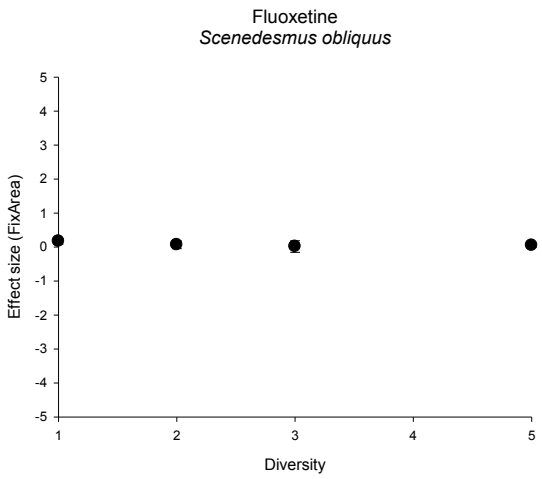
5.1.2.3.3 Fluoxetine

The mean effect size of Fluoxetine on the abundance of *Scenedesmus obliquus* in all treatments was around 0 at day 22. The effect size was not influenced by diversity. A regression revealed no significant dependency between the diversity and the effect size of Fluoxetine on the abundance of *Scenedesmus obliquus* in diversity treatments. A One Way Anova identified no significant differences between the diversity treatments (DF=3, F=2.212, P=0.101). *S. obliquus* was not used in diversity treatments 4, therefore no data is available (Fig. 55 a).

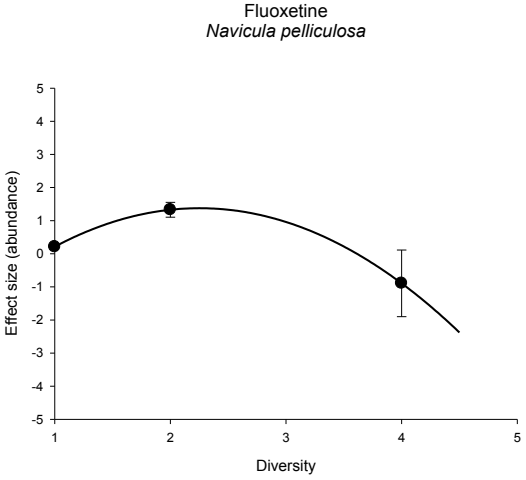
The mean effect size of Fluoxetine on the abundance of *Navicula pelliculosa* was around 0 for the monocultures. In treatments with 1 additional algal species a positive effect on the abundance of *Navicula pelliculosa* was observed. In treatments with 3 additional algae species the effect was slightly smaller but negative (Fig. 55 b). A regression (quadratic) revealed a significant dependency between the diversity and the effect size of Fluoxetine on the abundance of *Navicula pelliculosa* in diversity treatment: $y= -2.3898 + 3.3444*x - 0.7425*x^2$, $R^2=0.2371$, $P=0.0115$.

The mean effect size of Fluoxetine on the abundance of *Peridinium sp.* was around 2 in the monocultures. In the diversity treatments with 1 and 2 additional algal species the mean effect sizes of Fluoxetine on the abundance of *Peridinium sp.* were around 0 and slightly above 0 in treatments with 3 and 4 additional algal species (Fig. 55 c). A regression (quadratic) revealed a significant dependency between the diversity and the effect size of Fluoxetine on the abundance of *Peridinium sp.* in diversity treatments: $y= 3.7824 – 2.6806*x + 0.4406*x^2$, $R^2=0.4685$, $P<0.001$.

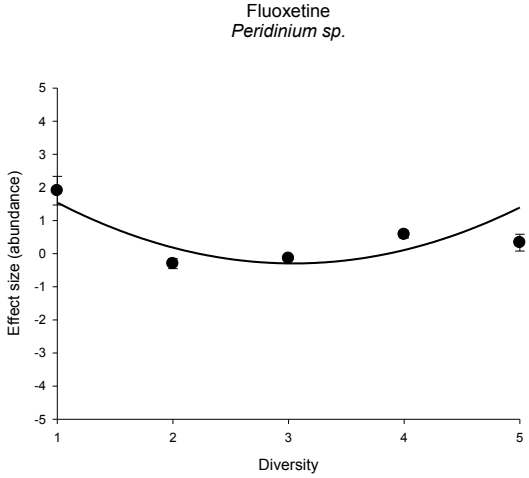
a)


Fluoxetine
Scenedesmus obliquus
Effect size (FixArea)
Diversity

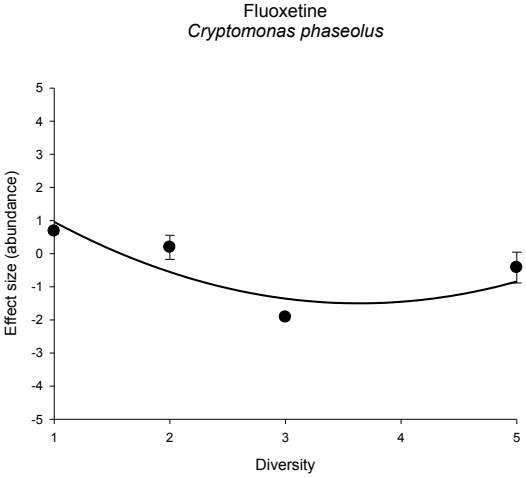
b)


Fluoxetine
Navicula pelliculosa
Effect size (abundance)
Diversity

c)


Fluoxetine
Peridinium sp.
Effect size (abundance)
Diversity

d)


Fluoxetine
Cryptomonas phaseolus
Effect size (abundance)
Diversity

e)

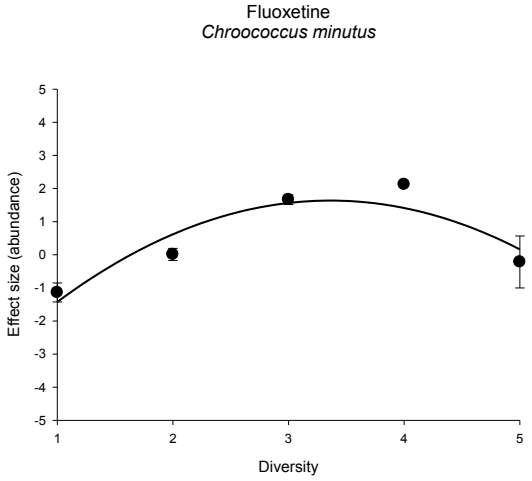


Fig. 55: Effects of of Fluoxetine (0.096 µg L^{-1}) on individual algal species abundances grown in mono- and polyculture day 22. **a:** *Scenedesmus obliquus* ($N_{(diversity\ 1)}$ = 9; $N_{(diversity\ 2)}$ = 9; $N_{(diversity\ 3)}$ = 9; $N_{(diversity\ 5)}$ = 9); **b:** *Navicula pelliculosa* ($N_{(diversity\ 1)}$ = 9; $N_{(diversity\ 2)}$ = 18; $N_{(diversity\ 4)}$ = 9); **c:** *Peridinium sp.* ($N_{(diversity\ 1)}$ = 6; $N_{(diversity\ 2)}$ = 9; $N_{(diversity\ 3)}$ = 9; $N_{(diversity\ 4)}$ = 9; $N_{(diversity\ 5)}$ = 2); **d:** *Cryptomonas phaseolus* ($N_{(diversity\ 1)}$ = 9; $N_{(diversity\ 2)}$ = 9; $N_{(diversity\ 3)}$ = 9; $N_{(diversity\ 5)}$ = 6)*;* **e:** *Chroococcus minutus* ($N_{(diversity\ 1)}$ = 9; $N_{(diversity\ 2)}$ = 9; $N_{(diversity\ 3)}$ = 9; $N_{(diversity\ 4)}$ = 6; $N_{(diversity\ 5)}$ = 6). Data expressed in mean effect size to the unexposed controls. Error bars represent standard errors. Regression was performed with effect sizes (raw data, full crossed) of Fluoxetine on the abundance of the algae. Solid line: significant on < 5% level.

The effect of Fluoxetine on the abundance of *Cryptomonas phaseolus* was around 0 in the monocultures and in treatments with 1 additional algal species. In diversity treatments 3 a negative effect size of Fluoxetine on the abundance of *Cryptomonas phaseolus* of around -2 was observed. In diversity treatments 4 no cells of *Cryptomonas phaseolus* were found on the last day with the highest concentration of Fluoxetine. In diversity treatments 5 the effects of Fluoxetine on the abundance of *Cryptomonas phaseolus* were smaller and between 0 and -1 (Fig. 55 d). A regression (quadratic) revealed a significant dependency between the diversity and the effect size of Fluoxetine on the abundance of *Cryptomonas phaseolus* in diversity treatments: $y= 3.1791 – 2.5741*x + 0.3540*x^2$, R^2=0.4812, P=0.0001.

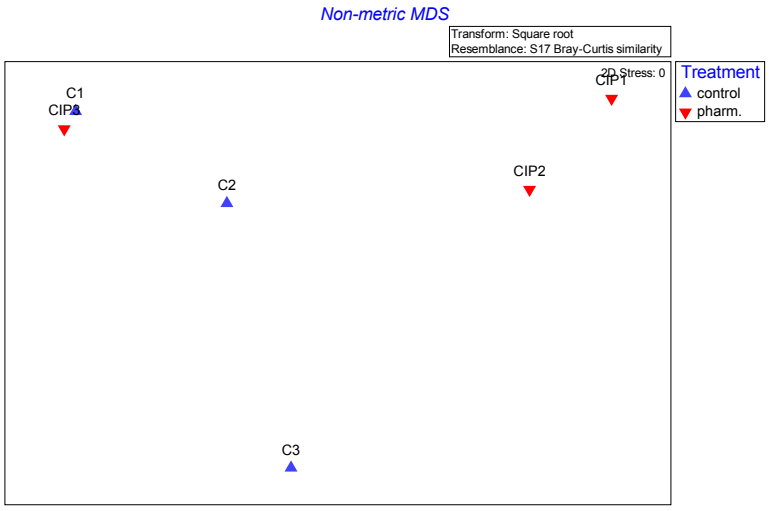
The effect of Fluoxetine on the abundance of *Chroococcus minutus* was around -2 in the monocultures (Fig. 55 e). In diversity treatments 2 the effect was smaller with mean effect

sizes of Fluoxetine on the abundance of *Chroococcus minutus* around 0. In the diversity treatments 3 and 4 the effects were higher with effect sizes around 2. In diversity treatments 5 the mean effect size of Fluoxetine on the abundance of *Chroococcus minutus* was around 0. A regression (quadratic) revealed a significant dependency between the diversity and the effect size of Fluoxetine on the abundance of *Chroococcus minutus* in diversity treatments: $y= -4.5693 + 3.6918*x – 0.5491*x^2$, $R^2=0.5430$, $P<0.0001$.

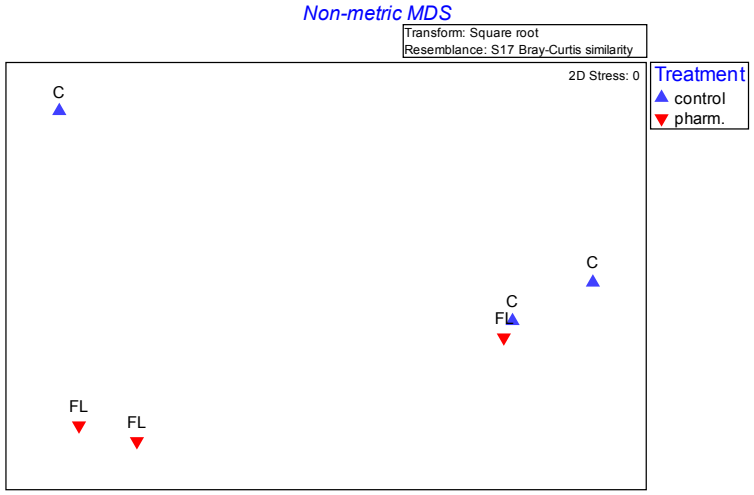
5.1.2.4 Effect of pharmaceuticals on community composition

My pharmaceutical treatments were very low in comparison to other studies, even in the highest concentrations (CBZ: 8.0 µg L^{-1}, CIP: 0.160 µg L^{-1}; FL: 0.096 µg L^{-1}). The similarity (Bray-Curtis) of the community composition between pharmaceutical treatments and controls were still around 70 to 80%. ANOSIM analyses resulted in non-significant effects of Ciprofloxacin and Fluoxetine on community composition. Whereas differences were visible, large variation between replicates did probably not allow to detect small differences between controls and treatments. Treatments with Carbamazepine showed larger differences in community composition compared to controls and the significance level of an ANOSIM analysis was at 10%, indicating a clear trend ($R = 0.963$; $p <0.1$; Fig. 56).

a)


Non-metric MDS
Transform: Square root
Resemblance: S17 Bray-Curtis similarity
2D Stress: 0
Treatment
control
pharm.
C1
CIP1
CIP2
C2
C3

b)


Non-metric MDS
Transform: Square root
Resemblance: S17 Bray-Curtis similarity
2D Stress: 0
Treatment
control
pharm.
C
C
FL
FL
FL

c)

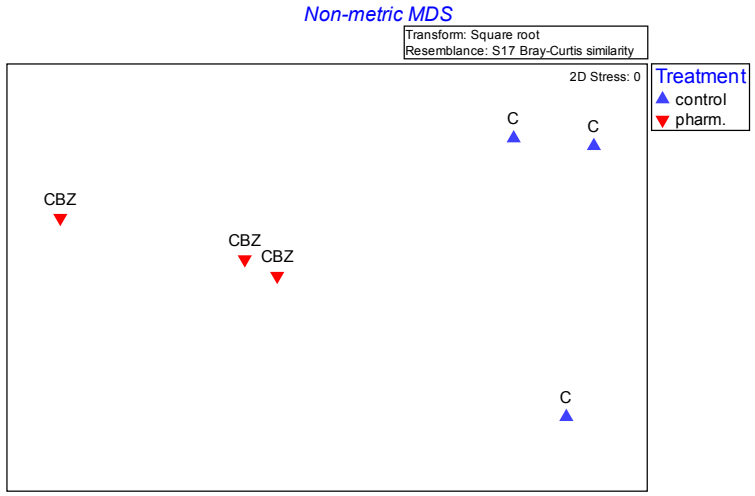


Fig. 56: Non Metric Multidimensional Scaling (MDS) plots showing the similiarity between the community composition of control treatments and treatments with **(a)** Ciprofloxacin (0.096 µg L^{-1}), **(b)** Fluoxetin (0.160 µg L^{-1}) and **(c)** Carbamazepine (8.0 µg L^{-1}) on day 22.

5.1.3 Question III: How does Carbamazepine affect natural algal populations with different diversity?

I investigated the impact of Carbamazepine on plankton communities from three different lakes. The communities were experimentally manipulated in their phytoplankton diversity. The parameters of interest were phytoplankton Quantum Yield and FixArea (a proxy for biomass).

In phytoplankton communities from Lake Klostersee the effect size of Carbamazepine on FixArea changed from a negative effect to a positive effect with increasing diversity in both treatments with low (1.0 µg L^{-1}) and high (8.0 µg L^{-1}) concentrations of Carbamazepine (Fig. 57). A linear regression identified a trend (< 10%) between effect size of Carbamazepine on FixArea and diversity in treatments with low concentrations of Carbamazepine (P=0.068, R^2= 0.143) whereas a significant dependency (<5%) between effect size of Carbamazepine on FixArea and diversity was found in treatments with high Carbamazepine concentrations (y=-1.033 + 1.468*x; P= 0.045, R^2= 0.170).

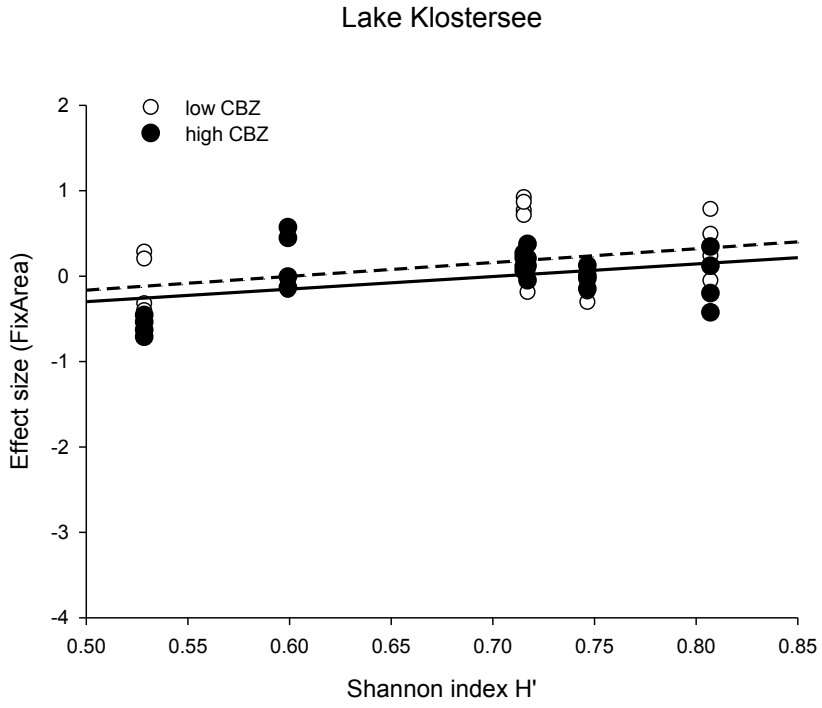


Fig. 57: Relationship between the effect of Carbamazepine on FixArea and phytoplankton diversity (Shannon index H') for Lake Klostersee for a low (1.0 µg L^{-1}) and a high (8.0 µg L^{-1}) concentration of Carbamazepine (day

19). For statistical analysis regressions (linear) were performed (n=24 for each concentration treatment). Solid line: significant on < 5% level. Dashed line: a trend on < 10% level.

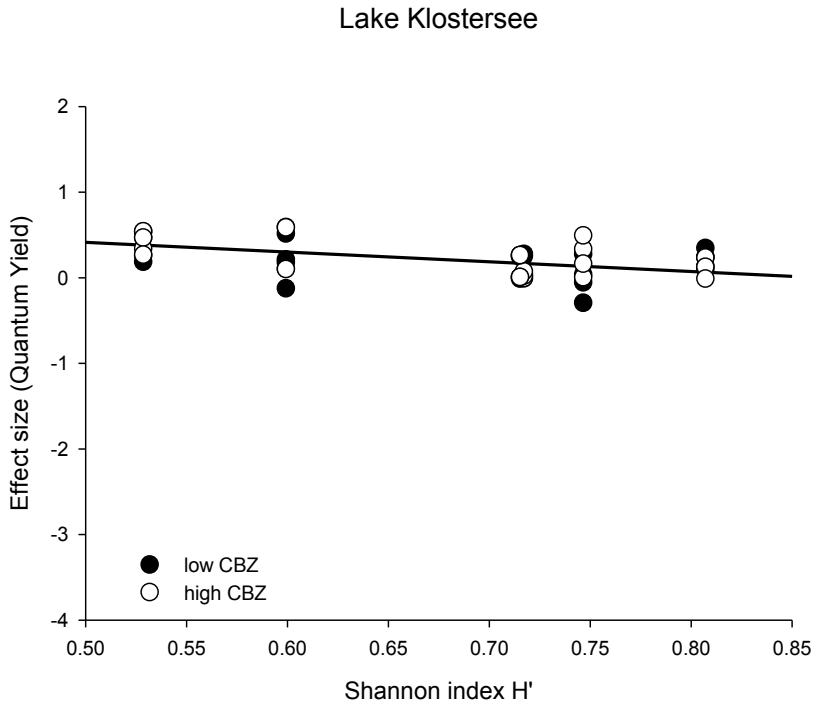


Fig. 58: Relationship between the effect of Carbamazepine on Quantum Yield and phytoplankton diversity (Shannon index H') for Lake Klostersee for a low (1.0 µg L^{-1}) and a high (8.0 µg L^{-1}) concentration of Carbamazepine (day 19). For statistical analysis regressions (linear) were performed (n=24 for each concentration treatment). Solid line: significant on < 5% level.

In treatments with a low concentration of Carbamazepine (1.0 µg L^{-1}) no significant relationship between the effect size of Carbamazepine on the Quantum Yield and diversity was identified (linear regression: P=0.107, R^2= 0.114). In treatments with 8.0 µg L^{-1} Carbamazepine a significant relationship between diversity and the effect of Carbamazepine on Quantum Yield was observed (y=0.983 - 1.137*x; P=0.006, R^2=0.292). With increasing diversity of phytoplankton community effects of Carbamazepine on the photosynthetic activity of PSII got lower (Fig. 58).

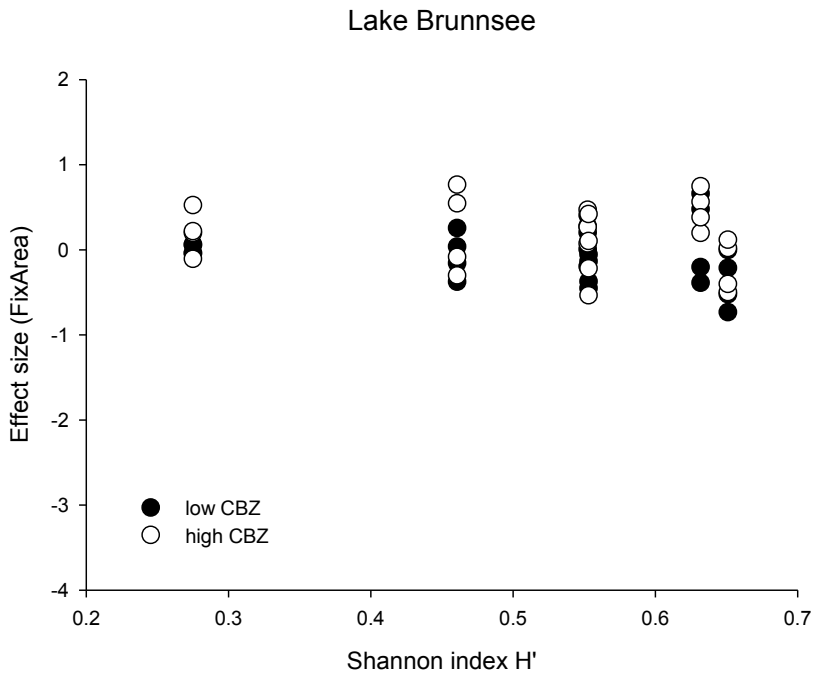


Fig. 59: Relationship between the effect of Carbamazepine on FixArea and phytoplankton diversity (Shannon index H') for Lake Brunnsee for a low (1.0 µg L^{-1}) and a high (8.0 µg L^{-1}) concentration of Carbamazepine (day 19). For statistical analysis regressions (linear) were performed (n=24 for each concentration treatment).

A linear regression identified no significant relationship between the effect size of Carbamazepine on the FixArea and diversity (1.0 µg L^{-1}: P=0.481, R^2= 0.0228; 8.0 µg L^{-1}: P= 0.603, R^2= 0.0125) (Fig. 59).

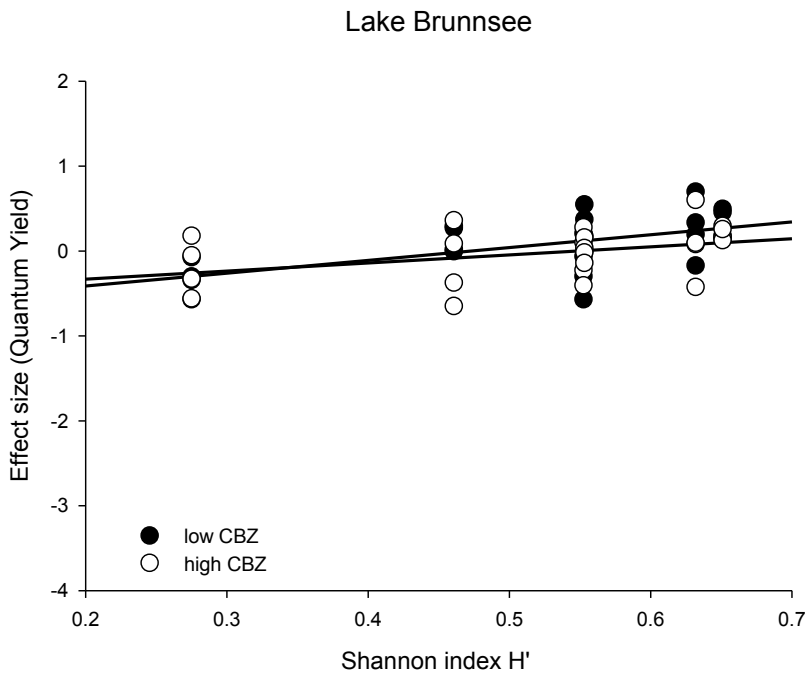


Fig. 60: Relationship between the effect of Carbamazepine on Quantum Yield and phytoplankton diversity (Shannon index H') for Lake Brunnsee for a low (1.0 µg L^{-1}) and a high (8.0 µg L^{-1}) concentration of Carbamazepine (day 19). For statistical analysis regressions (linear) were performed (n=24 for each concentration treatment). Solid line: significant on < 5% level.

For Lake Brunnsee, a linear regression indicated a significant influence of diversity on the effect size of Carbamazepine on the Quantum Yield in treatments with 1.0 and 8.0 µg L^{-1} Carbamazepine (1.0 µg L^{-1}: y=-0.715+1.512*x; P= 0.003, R^2= 0.331; 8.0 µg L^{-1}: y=-0.521+0.951*x; P= 0.058, R^2= 0.154). With increasing diversity of phytoplankton communities the effects of Carbamazepine on the photosynthetic efficiency of PS II got lower (Fig. 60).

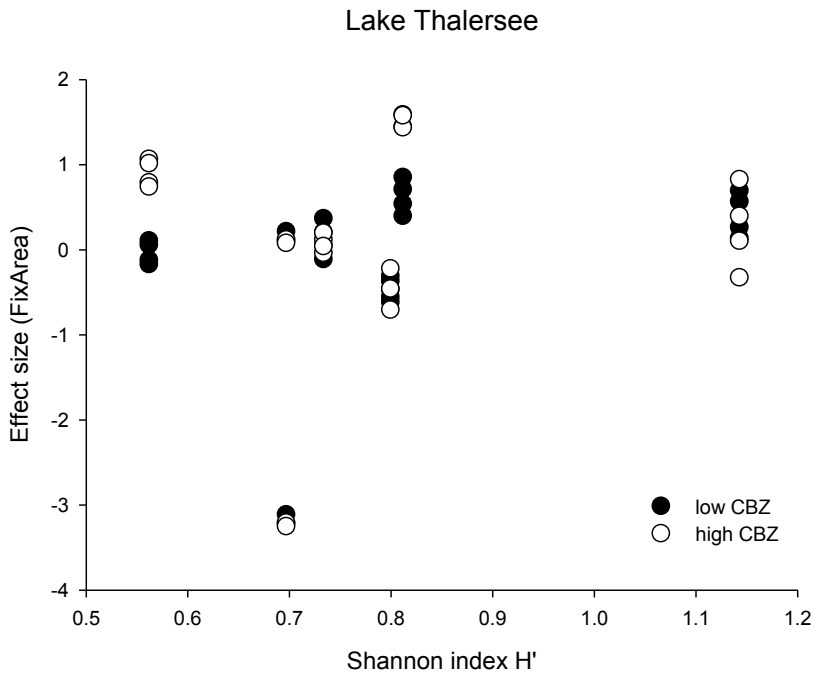


Fig. 61: Relationship between the effect of Carbamazepine on FixArea and phytoplankton diversity (Shannon index H') for Lake Thalersee for a low (1.0 µg L^{-1}) and a high (8.0 µg L^{-1}) concentration of Carbamazepine (day 19). For statistical analysis regressions (linear) were performed (n=24 for each concentration treatment).

For Lake Thalersee no effect of the diversity on the effect of Carbamazepine on FixArea was observed. A linear regression found neither a trend nor a significant relationship between the effect size of Carbamazepine on FixArea and diversity (1.0 µg L^{-1}: P= 0.182, R^2= 0.0796; 8.0 µg L^{-1}: P= 0.853, R^2= 0.00159) (Fig. 61).

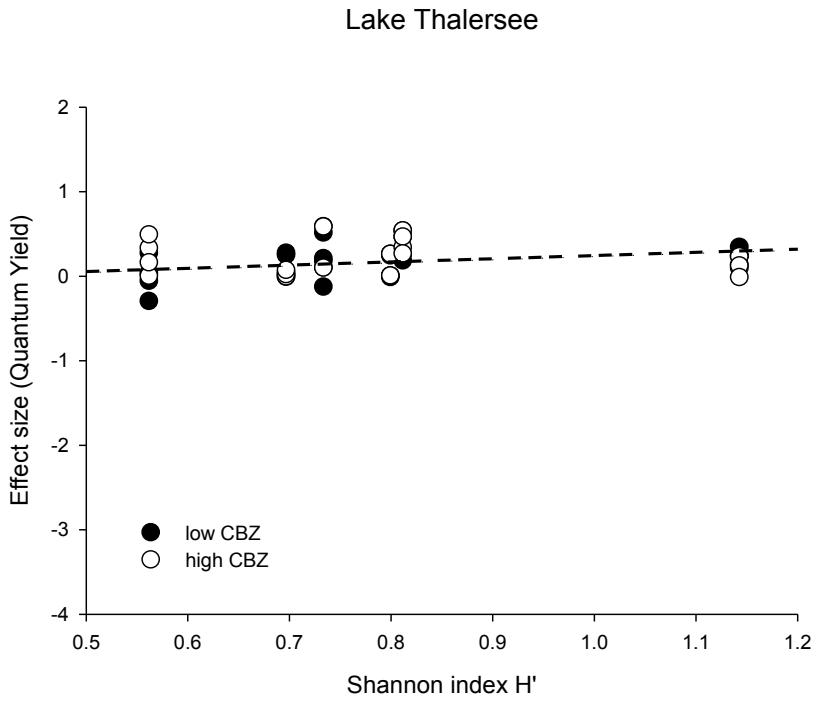


Fig. 62: Relationship between the effect of Carbamazepine on Quantum Yield and phytoplankton diversity (Shannon index H') for Lake Thalersee for a low (1.0 µg L^{-1}) and a high (8.0 µg L^{-1}) concentration of Carbamazepine (day 19). For statistical analysis regressions (linear) were performed (n=24 for each concentration treatment). Dashed line: significant on < 10% level.

In treatments with a high concentration of Carbamazepine (8.0 µg L^{-1}) no significant relationship between the effect size of Carbamazepine on the Quantum Yield and diversity was identified (linear regression: P= 0.485, R^2= 0.022). A linear regression identified a trend (< 10%) between diversity and the effect size of Carbamazepine on Quantum Yield in treatments with low concentrations of Carbamazepine (P= 0.101, R^2= 0.117; Fig. 62).

6. Discussion

I will discuss how my results help to answer the main research questions described within the introduction. I will evaluate how the different pharmaceuticals affected two important ecological functions of phytoplankton (biomass production / chlorophyll production, photosynthetic efficiency). The important new aspect is that I investigated the effects of pharmaceuticals on algae grown in monoculture and in different diverse polycultures. Thereby I was able to analyse whether biotic interactions influence effects of pharmaceuticals on algae and whether diversity *per se* plays a role for phytoplankton community responses to pharmaceuticals.

6.1.1 Question I: How were algal species affected by the investigated pharmaceuticals?

Due to a rising human population it is assumed that the need for pharmaceuticals will increase correspondingly. In consequence the concentrations of pharmaceuticals in surface waters will also increase. Concentrations of pharmaceuticals found in environmental systems are often below acute toxic concentrations. However, not only strong acute toxic effects, which occur after a short period of time of exposure to a pharmaceutical, or strong chronic toxic effects, which occur after a longer period of time of exposure to a pharmaceutical, are of interest. Also weak (minor) effects on one single algal species, e.g. through a decrease in photosynthetic performance, can change competitive abilities and thereby lead to a shift in phytoplankton community structure.

In my first research question I therefore investigated the impact of Carbamazepine, Ciprofloxacin, Fluoxetine and a mixture of all three pharmaceuticals on selected algae species representing major algal groups. I could show that the effects of pharmaceuticals are dependent on both, the pharmaceutical and the exposed algal species.

6.1.1.1 Carbamazepine

The exposure of algae to Carbamazepine had an impact on the efficiency of the photosystem II, their chlorophyll *a* levels and their abundance in the respective treatments. I found that the effects of Carbamazepine varied in strengths and characteristics. The impact of the pharmaceutical varied between algal species.

Carbamazepine has antiepileptic properties. It stabilizes hyperagitated nerve membranes, inhibits repeated discharges and reduces the synaptic spread of excitatory impulses. Among others the effects are attributed to the blockage of sodium channels in nerve cells. The mode of action in humans is not yet fully explained. Carbamazepine is processed in the liver via the cytochrome P450 enzyme system. According to Miazek and Brozek-Pluska (2019) Carbamazepine is a human anticonvulsant for which anti-algal activity is also documented.

Carbamazepine was reported to be growth inhibitory to various microalgal strains (Miazek and Brozek-Pluska 2019). In my study Carbamazepine affected the investigated algal species differently (Tab. 32).

Scenedesmus obliquus (*Chlorophyta*)

Monocultures of *Scenedesmus obliquus* showed an increase in chlorophyll *a* (measured by the proxy FixArea) in treatments with the three highest concentrations compared to the control. It seemed that this increase is dose dependant as the values were higher with higher concentrations of Carbamazepine. As the interaction-factor 'concentration x time' was significant, the effects of Carbamazepine on chlorophyll *a* production depended on the duration of exposure. A decline in the efficiency of the PSII in the treatments was also observed. The duration of exposure to Carbamazepine had a significant impact on the PSII Quantum Yield of *Scenedesmus obliquus*.

Zhang et al. (2012) found that Carbamazepine inhibited the growth and chlorophyll content of *Scenedesmus obliquus* and *Chlorella pyrenoidosa* significantly when exposed to a concentration of 1 mg L^{-1}. In contrast, Andreozzi et al. (2002) showed that Carbamazepine had no significant effect on the green algae *Pseudokirchneriella subcapitata* and *Ankistrodesmus braunii*. Andreozzi et al. (2002) also observed no accumulation of Carbamazepine within the algal cells. The authors assumed that Carbamazepine was taken up by the cells and entered into biochemical metabolic processes without going into biochemical detail. The uptake and utilization of complex biochemical compounds is for example reported by Gross and Schnarrenberger (1995) who observed, that the red algae *Galdieria sulphuraria* grew on a wide range of rare sugar-alcohols. In contrast, Jos et al. (2003) reported that the growth of the green algae *Chlorella vulgaris* was inhibited by Carbamazepine in a concentration dependent way. The authors recommended that Carbamazepine should be classified as "R52/53 Harmful to aquatic organisms and may cause long-term adverse effects in the aquatic environment" according to the European legislation on the classification and labelling of chemicals (92/32/ECC).

Miazek and Brozek-Pluska (2019) reported that among other pharmaceuticals Carbamazepine has the ability to alter antioxidant enzyme activity. Zhang et al. (2012) found

that Carbamazepine triggers an increase in catalase (CAT) and superoxide dismutase (SOD) activity in *Scenedesmus obliquus* and *Chlorella pyrenoidosa*. A study of Xiong et al. (2016) showed a decrease of SOD activity and an increase in CAT activity with higher concentrations of Carbamazepine. Compared to my study (8.0 µg L^{-1} at most), Zhang et al. (2012) and Xiong et al. (2016) used much higher concentrations of Carbamazepine (200 mg L^{-1}), which could be the reason for the differences in response strength reported in their studies and the study I performed.

Navicula pelliculosa (*Bacillariophytina*)

With exception of the last day of the experiment Carbamazepine had a slightly negative effect on chlorophyll *a* levels. The Quantum Yield decreased only at the end of the experiment, with exception of the highest concentration of Carbamazepine.

Ferrari et al. (2004) reported that diatoms were the most sensitive to Carbamazepine within their investigated algal species, which included the green algae *Pseudokirchneriella subcapitata*, the diatom *Cyclotella meneghiniana* and the blue-green algae *Synechococcus leopolensis*.

Claessens et al. (2013) found that Carbamazepine had moderate acute toxicity towards the *diatom Phaeodactylum tricornutum*. The authors investigated the response of the marine *diatom Phaeodactylum tricornutum* in a 72-hour growth inhibition test. The results showed no immediate risk for acute toxic effects at Carbamazepine concentrations found in Belgian marine waters.

Peridinium sp. (Dynophyta)

The microscopic investigation of the *Peridinium sp.* cultures showed that the algae died after three to seven days (lysis) under the influence of Carbamazepine at all five concentrations. Hence, in comparison to the other investigated algae species the interaction of the factors ‚concentration' and ‚time' on effects of Carbamazepine was not significant. Contrary to Carbamazepine treatments, all replicates of the control treatments showed normal growth of *Peridinium sp*.. An allelochemical effect as described by Fistarol et al. 2004 where dinoflagellate cells suffered from algal released substances can be ruled out as I also observed the mortality by lysis within the monocultures. Therefore it seems that a metabolite of Carbamazepine or Carbamazepine itself leads to the observed cell lysis of the *dinoflagellate*. The exact mechanism behind the observed lysis of *Peridinium sp.* is unknown. There is evidence that algizide bacteria can result in an efficient lysis of *Peridinium*, for example Kang et al. (2008) described that over 90% of *Peridinium bipes* cells degraded within 8 days after inoculation with two bacterial strains. The authors identified extracellular substances released by bacteria as the reason behind the observed lysis. However, it is still open to which extend direct effects of the pharmaceuticals or indirect effects of bacterial populations or their excretions are the reason behind the observed lysis of *Peridinium sp.* in my study, which has to be resolved in future experiments.

Cryptomonas phaseolus (*Cryptophyta*)

The *cryptomonade Cryptomonas phaseolus* showed a strong decrease in Quantum Yield due to exposure to Carbamazepine. Additionally, a significant difference in biomass production was found in the three highest used concentrations of Carbamazepine compared to the control. The interaction of the factors 'concentration' and 'time' was significant, hence effects of Carbamazepine were dependent on the duration to exposure. Up to a concentration of 2.0 µg L^{-1} only a slight growth inhibition of *Cryptomonas phaseolus* was observed. Above a concentration of 4.0 µg L^{-1} a considerably stronger growth inhibition was observed. It seems that between concentrations of 2.0 µg L^{-1} and 4.0 µg L^{-1} a threshold value exists up to which *Cryptomonas phaseolus* can tolerate the drug Carbamazepine to some extent. If this threshold value was exceeded, there was a sharp increase in growth

inhibition. I could not find any published data describing responses of *Cryptomonas phaseolus* to exposure with Carbamazepine, my results are the first describing these effects.

Chroococcus minutus (*Cyanophyta, Cyanobacteria*)

No significant differences in chlorophyll *a* or on the Quantum Yield between Carbamazepine treatments and controls could be detected in the experiments with *Chroococcus minutus*. Thus, Carbamazepine had no growth-influencing effect on this algal species. These results are interesting as I was able to show that Carbamazepine affected the other tested algae. The other algae belong to eukaryotes whereas *Chroococcus minutus* is the only prokaryote. Therefore the differences in the physiology and the cell structure of eukaryotes and prokaryotes could be the underlying reason for the different mode of action.

Inhibition of growth of *Spirulina platensis*, a planktonic blue–green algae, were shown by Wang et al. (2020) at Carbamazepine concentrations over 1 mg L^{-1}. They observed an increase of the content of lipids, carbohydrates, chlorophylls, carotenoids and of their SOD and CAT activities with concentrations ranging from 1 to 25 mg L^{-1} Carbamazepine.

As already mentioned, Zhang et al. (2012) also observed this effect of Carbamazepine for *Scenedesmus obliquus* and *Chlorella pyrenoidosa* and Wang et al. (2020) interpreted their results as a similar protective mechanism towards Carbamazepine toxicity as found in eukaryotic microalgae cells. The authors also found that higher concentrations (50-100 mg L^{-1}) of Carbamazepine led to a decrease in the photosynthetic activity. Interestingly, they observed a fast recovery of the growth rate and photosynthetic activity when Carbamazepine was removed.

6.1.1.2 Ciprofloxacin

Quinolones, a class of fluoroquinolones, chemically have a bactericidal effect by inhibiting the bacterial DNA gyrase (National Center for Biotechnology Information 2020). The effects of antibiotics on microalgae are mainly attributed to the inhibition and interference of the pathways involved in protein synthesis and photosynthetic metabolism in the chloroplast. Some of these mechanisms can affect the photosynthetic electron transport chain, the activities of photosystems I and II, and the biosynthesis of proteins and pigments.

Krajcovic et al. (1989) showed that Ciprofloxacin amongst other quinolones, strongly affected the chloroplasts of *Euglena gracilis*. They found that DNA-gyrase inhibitors were responsible for this effect. A detailed description of this mode of action was not explained. Brain et al. (2009) identified that the plastid replication and DNA gyrases in plants are targets for fluoroquinolones. As the structure of chloroplasts in plants and green algae are similar, the results of Brain et al. (2009) could also be valid for green algae.

Scenedesmus obliquus (*Chlorophyta*)

My results show that Ciprofloxacin had no significant effect on the Quantum Yield and on algal growth and thereby abundance. Negative effects of Ciprofloxacin on algal abundance were only seen at the highest concentrations but these differences were not significant. Halling-Sørensen et al. (2000) observed toxicity of Ciprofloxacin towards the green algae *Selenastrum capricornutum* with an EC_{50}= 2.97 mg L^{-1}. Xiong et al. (2017) investigated the toxicity of Ciprofloxacin to the green algae *Chlamydomonas mexicana* and the ability of algae to remove Ciprofloxacin from wastewater. The concentration of Ciprofloxacin used in their study was higher than usually found in aquatic environments (2 – 100 mg L^{-1}). They observed a protective mechanism of the algae cells:

The exposure to toxic contaminants can lead to an over-generation of reactive oxygen species (ROS) including superoxide radicals (O_2^-), hydroxyl radicals ($^{\bullet}OH$) and hydrogen peroxide (H_2O_2) in microalgal cells. These strong oxidation properties can damage cellular organelles by peroxidizing polyunsaturated fatty acids (PUFA). Xiong et al. (2017) investigated that with higher concentrations of Ciprofloxacin the Malondialdehyde (MDA)

content in microalgal cells indeed increased. MDA is an aldehyde and a representative product of PUFA. They described that photosynthetic organisms react to antibiotics by increasing their anti-oxidative defense mechanisms, diminishing the effects of reactive oxygen species as described above. Superoxide dismutase (SOD) is such an anti-oxidative enzyme and scavenges free radicals. The SOD activity in *C. mexicana* gradually increased with increasing the Ciprofloxacin concentrations (Xiong et al. 2017).

Navicula pelliculosa (Bacillariophytina)

A slight, but not significant decrease in the abundance of *Navicula pelliculosa* was observed when exposed to Ciprofloxacin. The effect of Ciprofloxacin on chlorophyll *a* production fluctuated and decreased towards the end of the experiment. No effects of Ciprofloxacin on the Quantum Yield were observed.

Hagenbuch and Pinckney (2012) investigated the influence of Ciprofloxacin on the diatoms *Cylindrotheca closterium* and *Navicula ramosissima*. They observed a slight toxicity. The authors also investigated the impact of a mixture of antibiotics (Ciprofloxacin, Lyncomycin and Tylosin). The effects of the antibiotics on the individual algal species were different and therefore some species gained a competitive advantage over others.

Wilson et al. (2003) reported a significant impact of Ciprofloxacin on the diatoms *Synedra sp.* and *Navicula sp.*. They observed an increase of *Synedra sp.* at lower concentrations (0,012 and 0,12 µg L^{-1}). On the contrary in my experiments, a decrease in the abundance of *Navicula sp.* at 0,12 µg L^{-1} of Ciprofloxacin was observed in one out of two experiments.

There is only a limited amount of toxicity studies about effects of antibiotics and especially Ciprofloxacin on diatoms. Results from Guo et al. (2016) with different antibiotics (Trimethoprim, Lyncomycin and Tylosin) showed a growth inhibition in the diatoms *Navicula pelliculosa* and *Phaeodactylum tricornutum*.

Peridinium sp. (Dynophyta)

I observed that chlorophyll *a*, the Quantum Yield and the abundance (cells) of *Peridinium sp.* decreased with higher concentrations of Ciprofloxacin, however these differences were not

significant. Similar as for Carbamazepine, there are only few studies available that describe effects of Ciprofloxacin on dinoflagellates.

Niu et al. (2019) investigated effects of Norfloxacin on the dinoflagellate *Prorocentrum lima* at environmentally relevant concentrations (0, 10 and 100 ng L^{-1}). Like Ciprofloxacin, Norfloxacin belongs to the class of fluorquinolones. The authors observed that the growth of the dinoflagellate was even stimulated. Most of the toxins produced by dinoflagellates are polyketides in their origin (Rein and Snyder 2006) and many of the polyketides were rated as antibacterial drugs (Choudhary et al. 2017). Their assumption was that dinoflagellates are less sensitive to antibiotics because the nature of their allelopathic toxins is similar to that of antibacterial drugs.

Their results do not correspond to the results of my research. A possible explanation as to why Ciprofloxacin negatively affected *Peridinium sp.* could also include associated bacterial consortia. The so called “holobiont” for example would include bacterial cells on the surface of algae that could be important for vitamin production, for example Vitamin B12 (Croft et al. 2005) or other resource uptake related processes. Hence, an effect of the antibiotic harming such bacterial associates would affect the growth of algae in a negative way.

Cryptomonas phaseolus (Cryptophyta)

The abundance of *Cryptomonas phaseolus* slightly decreased when exposed to Ciprofloxacin concentrations ranging from 0.020 to 0.160 µg L^{-1}. Chlorophyll *a* and Quantum Yield also decreased with exposure to Ciprofloxacin over the duration of the experiment. I did not find literature describing effects of Ciprofloxacin on *Cryptomonas phaseolus*, *Cryptomonodales* or *Cryptophpyta* in general. One assumption is that similar to *Peridinium sp.*, bacteria supplying essential compounds to the algae, such as for example vitamin B12 (Haines and Guillard 1974, Croft et al. 2005, Akduman et al. 2020), were strongly supressed by the antibiotic Ciprofloxacin.

Chroococcus minutus (Cyanophyta, Cyanobacteria)

The abundance and the efficiency of the PS II of *Chroococcus minutus* were not heavily affected by Ciprofloxacin. A significant effect of Ciprofloxacin on chlorophyll *a* was observed at the end of the experiment at the highest concentration of Ciprofloxacin (0.080 µg L^{-1}).

Beside positive facilitation effects of bacteria such as described above one could also assume opposing negative effects of bacteria by being very efficient competitors for phosphorus (P) (Vadstein et al. 1988). Bacteria present in the cultures were killed as Ciprofloxacin acts as a broad-spectrum antibiotic against numerous bacteria. Therefore the phosphorus for which the algae and bacteria usually compete is now more easily accessible to the algae, which in turn could thereby increase their growth.

Halling-Sørensen et al. (2000) observed a different effect of Ciprofloxacin on the cyanobacteria *Microcystis aeruginosa* than my study showed for cyanobacteria: a strong toxicity towards the cyanobacteria *Microcystis aeruginosa* with EC_{50}s ranging from 5 to 60 µg L^{-1} Ciprofloxacin was observed.

As *Microcystis aeruginosa* is a prokaryote it is not unexpected that the toxicity of the antibiotic was strong. A similar observation as done by Halling-Sørensen et al. (2000), was made by Robinson et al. (2005). They investigated the toxicity of Ciprofloxacin to *M. aeruginosa* (EC_{50}: 7.9 µg L^{-1}) and *P. subcapitata* (EC_{50}: 18.700 µg L^{-1}) and observed that *M. aeruginosa* was more sensitive. Like Halling-Sørensen et al. (2000), Robinson et al. (2005) also used relatively high concentrations for the test. The highest concentration (100 µg L^{-1}) they used was based on concentrations (3-87 µg L^{-1}; 0.7-124 µg L^{-1}) that were found in hospital wastewaters in Germany (Hartmann et al. 1998, 1999), which are therefore much higher than the concentrations found in most natural aquatic ecosystems.

Ebert et al. (2011) studied the toxicity of two antibiotics of the fluorquinolone class (Enrofloxacin and Ciprofloxacin) to the blue green algae *Anabaena flos-aquae* amongst others. The authors observed strong toxicity of both antibiotics with EC_{50} values of 10,2 µg L^{-1} (for Ciprofloxacin) and 173 µg L^{-1} (for Enrofloxacin).

The above-mentioned studies all used very high concentrations of antibiotics. This could be one reason for differing results compared to my thesis where very low concentrations (typically found in natural water bodies) were used. In my thesis I investigated no significant effects on chlorophyll *a* or on Quantum Yield of the investigated algae (Tab. 32).

Chroococcus minutus was more sensitive to the exposure of Ciprofloxacin than the eukaryote *Scenedesmus obliquus* (Fig. 15, Fig. 23). Similar results comparing effects of Ciprofloxacin on prokaryote and eukaryote algal species were also observed in the studies of Halling-Sørensen et al. (2000), Ebert et al. (2011) and Robinson et al. (2005).

6.1.1.3 Fluoxetine

The antidepressant Fluoxetine is one of the first introduced selective serotonin reuptake inhibitors (SSRI) (Oakes et al. 2010). SSRIs are primarily indicated for depression, but also for compulsive behaviour as well as eating and personality disorders. The main effect of Fluoxetine is to inhibit the uptake of serotonin from the synaptic space. This mode of action leads to a prolonged serotonin action. In addition, Fluoxetine has direct effects on the serotonin receptors 5-HT2C of the central nervous system. In high doses, Fluoxetine can also inhibit the reuptake of norepinephrine.

Munoz-Bellido et al. (2000) discussed the effects of Fluoxetine on microorganisms. They suggested that it inhibits efflux pumps, as it acts as a cell pump inhibitor. Another suggestion was that it acts on basic metabolic processes like the biosynthesis of important structural compounds of microorganisms, e.g. slime synthesis.

Scenedesmus obliquus (Chlorophyta)

Fluoxetine affected the chlorophyll *a* production and the abundance of *Scenedesmus obliquus* in a positive direction. The observed differences between the treatments were not significant. Bi et al. (2018) quantified a NOEC (No Observed Effect Concentration) of 40.2 µg L^{-1} for *Scenedesmus obliquus* and a LOEC (Lowest Observed Effect Concentration) of 80.4 µg L^{-1} for *Scenedesmus quadricauda*. These values are considerably higher than the highest concentration used in my experiments.

The mechanisms of the toxicity of Fluoxetine to algal species are not known for certain. To a certain extent, Fluoxetine may have a disruptive effect on the cell membrane protein binding processes (Bi et al. 2018, DeLorenzo and Fleming 2008).

Neuwoehner et al. (2009) investigated the toxicity of Fluoxetine to different algal species. They concluded that Fluoxetine does not act on the photosystem II of algae. Based on their findings they assumed that Fluoxetine and Norfluoxetine are able to interact with membranes and disturb the membrane-protein interfaces in a nonspecific way.

El-Bassat et al. (2012) measured an oxidative stress in the two green algae *Chlorella vulgaris* and *Ankestrodesmus falcatus* when exposed to Fluoxetine. Their results showed a reduced SOD and CAT activity and an enhanced lipid peroxidation level.

Brooks et al. (2003) observed cell deformities in *Pseudokirchneriella subcapitata* (green algae) when treated with 13.6 and 27.2 µg L^{-1} Fluoxetine. However, the authors also could not explain how Fluoxetine resulted in such cell deformities.

Navicula pelliculosa (Bacillariophytina)

In treatments with the diatom *Navicula pelliculosa* negative and positive effects of Fluoxetine on chlorophyll *a* production were observed but it seemed not to be dependent on the concentration of Fluoxetine. Abundance and Quantum Yield were slightly increased with the addition of the pharmaceutical. All observed effects were not significantly different from the control treatments.

Contrary to my results, Petersen et al. (2014) observed a growth inhibiting effect of the antidepressant Fluoxetine on *Skeletonema pseudocostatum*. The observation is also supported by Minguez et al. (2014, 2018) reporting toxicity of Fluoxetine on the diatom *Skeletonema marinoi*. With an EC_{50} at 43 µg L^{-1}, they used relatively high concentrations and this could be an explanation for the divergent results compared to my thesis (0.006 – 0.096 µg L^{-1}).

Peridinium sp. (Dynophyta)

The exposure of *Peridinium sp.* to Fluoxetine led to an increase in abundances of this algal species. Chlorophyll *a* was also mostly positively affected by the pharmaceutical. One reason for this could be that bacteria were able to utilize Fluoxetine and therefore became more abundant. In this case the mixotroph *Peridinium sp.* (Jones et al. 2009) would have had more bacteria as food source and therefore its abundance increased. Due the mixotrophic and osmotrophic properties of *Peridinium sp.* it could also be possible that *Peridinium sp.* metabolized Fluoxetine directly. For Quantum Yield positive and negative effects of Fluoxetine were observed, but not in a dose-dependent way.

There is no literature describing effects of Fluoxetine on *Peridinium sp.* or dinoflagellates in general. A described theory is that melatonin can protect algal cells from toxic effects of Fluoxetine, as melatonin efficiently guards against oxidative stress (Galano et al. 2011). The presence of the hormone melatonin can be observed in a wide range of species including dinoflagellates (Murch and Saxena 2002).

Cryptomonas phaseolus (Cryptophyta)

Fluoxetine showed positive effecs on chlorophyll *a* production and Quantum Yield. The abundance of *Cryptomonas phaseolus* also increased significantly with the addition of Fluoxetine. *Cryptomonas phaseolus* was the only algal species which was significantly affected by Fluoxetine (Tab. 32). Such growth enhancing effects as seen in my experiments could be attributed to two reasons. First, the direct uptake of Fluoxetine or a metabolite by *Cryptomonas* could serve as an additional carbon source for growth. Second, *Cryptomonas* is well known to be an effective mixotroph (bacterivor) algal species (Tranvik 1989, Katechakis et al. 2005). If Fluoxetine was used by bacteria as a food source, mixotrophic nutrition and growth of *Cryptomonas phaseolus* could be enhanced, potentially increasing its abundance. Biodegradation of Fluoxetine by bacteria is known, for example the bacterial strain *Labrys portucalensis* is reported to use Fluoxetine as food source in a highly efficient way (Moreira et al. 2014). However, there is no literature describing effects of Fluoxetine on *Cryptomonas phaseolus*, *Cryptomonodales* or *Cryptophpyta* in general.

Chroococcus minutus (Cyanophyta, Cyanobacteria)

The impact of Fluoxetine on cultures of *Chroococcus minutus* showed no clear pattern. The chlorophyll *a* production as well as Quantum Yield fluctuated over time. Abundances within different treatments also did not reveal a specific pattern and no dose dependent effects were observed. Experiments performed by the company Eli Lilly and Company (2005), showed that Fluoxetine hydrochloride had a minimum inhibitory concentration of 250 mg L^{-1} to *Nostoc sp.*. The cyanobacteria *Nostoc sp.* belongs to the order *Nostocales* whereas *Chroococcus minutus* is a member of the order *Chroococcales*. However, taxonomic differences between cyanobacteria are not known yet to strongly affect impacts of pharmaceuticals on important metabolic pathways. I would therefore assume that inhibitory concentrations for *Chroococcus minutus* would be within the same order of magnitude as for *Nostoc sp.*. Considering the results from the company Eli Lilly and Company (2005) the tested environmental relevant concentrations I used in my study (0.006 – 0.096 µg L^{-1}) were much lower (several order of magnitudes) than the identified minimum inhibitory concentration in the study of the company Eli Lilly and Company (2005; 250 mg L^{-1}).

6.1.1.4 Effects of combined pharmaceuticals

An enormous number of pharmaceuticals from different drug classes used in human and veterinary medicine are released into aquatic environments (Heberer et al. 2001, Heberer 2002, Kasprzyk-Hordern et al. 2009, Blair et al. 2015). Hence, organisms in the environment are not exposed to single pharmaceuticals such as in most toxicity tests but to a mixture of pharmaceuticals and their metabolites (Petersen et al. 2014, Geiger et al. 2016, Xin et al. 2020). For example, concentrations of 56 active pharmaceutical ingredients and seven of their metabolites in effluents from 50 US sewage treatment plants were detected (Kostich et al. 2014).

Several studies (Cleuvers 2003, Backhaus et al. 2011, Hagenbuch and Pinckney 2012, Geiger et al. 2016, Bi et al. 2018, Minguez et al. 2018) revealed that the combination of

pharmaceuticals show different results when compared to results from singly tested pharmaceuticals because of interactions of the substances.

Interactions can be additive, dominant, synergistic or antagonistic. In case of an additive interaction, the joint effect is the sum of the effects of each stressor. Another option is the effect of one stressor being dominant over the other stressor. A synergistic interaction means that the effect of two substances together is greater than the sum of their separate effect at the same dosage. Opposite to that an antagonistic interaction means, that the effect of two substances is actually less than the sum of the individual effects of the two substances (Jackson et al. 2016, Schäfer and Piggott 2018).

One potential scenario could be that a combination of pharmaceuticals (stressors) has an impact on several vital functions of algal cells because each pharmaceutical has a different mode of action and/or target cell organelles. This could lead to an elimination of all tested algal species. Another possible scenario could be that the combination of different pharmaceuticals led to the mutual elimination of their effects. None of these scenarios became apparent within my study. However effects of a mixture of Carbamazepine, Ciprofloxacin and Fluoxetine differed from effects of single tested pharmaceuticals.

Scenedesmus obliquus (Chlorophyta)

A mixture of Carbamazepine, Ciprofloxacin and Fluoxetine had different effects on the chlorophyll *a* amount of *Scenedesmus obliquus* than it would have been expected by the sum of the single substance mean effect sizes (Fig. 36). The effect caused by the mixture was synergistic. The mixture also had different effects on the efficiency of the PS II of *Scenedesmus obliquus* than it would have been expected by the sum of the single substance mean effect sizes. The effect caused by the mixture was synergistic. The effects of the mixture of pharmaceuticals on the chlorophyll *a* production of *Scenedesmus obliquus* were significantly different then the effects caused by Ciprofloxacin and Fluoxetine. Ciprofloxacin and Fluoxetine obviously did not mitigate the positive effects of Carbamazepine on the chlorophyll *a* production.

Navicula pelliculosa (Bacillariophytina)

A mixture of Carbamazepine, Ciprofloxacin and Fluoxetine had different effects on the chlorophyll *a* amount and on the efficiency of the PS II of *Navicula pelliculosa* than it would have been expected by the sum of the single substance mean effect sizes (Fig. 38). The effect caused by the mixture was antagonistic compared to the effects caused by the single substances. The effects of the mixture of pharmaceuticals on the chlorophyll *a* production and of the efficiency of the PSII of *Navicula pelliculosa* were significantly different than the effects caused by Carbamazepine. The positive effects of Carbamazepine on the chlorophyll *a* production and of the efficiency of the PSII were obviously reduced by Ciprofloxacin and Fluoxetine, so that no effects of the mixture were observed at the end of the experiment anymore.

Peridinium sp. (Dynophyta)

A mixture of Carbamazepine, Ciprofloxacin and Fluoxetine had different effects on the chlorophyll *a* amount of *Peridinium sp.* than it would have been expected by the sum of the single substance mean effect sizes (Fig. 40). The effect caused by the mixture was antagonistic. The effect of the mixture on the Quantum Yield of *Peridinium sp.* was similar to the effects caused by the single substances, but the effect of the mixture was positive whereas the effect of the sum of the single substance mean effect sizes was negative.

By analyzing the effects caused by the single tested and combined pharmceuticals on the chlorophyll *a* production and the efficiency of the PSII of *Peridinium sp.* significant differences were found (Tab. 32). The observed lysis of algal cells of *Peridinium sp.* in treatments with single addition of Carbamazepine or Ciprofloxacin was not observed when *Peridinium sp.* was exposed to the mixture of all three pharmaceuticals. It seems that the addition of Fluoxetine blocked strong effects of Carbamazepine and Ciprofloxacin on *Peridinium sp.* lysis out.

Cryptomonas phaseolus (Cryptophyta)

A mixture of Carbamazepine, Ciprofloxacin and Fluoxetine had different effects on the chlorophyll *a* amount of *Cryptomonas phaseolus* than it would have been expected by the sum of the single substance mean effect sizes (Fig. 42). The effect caused by the mixture was synergistic. The effect of the mixture on the Quantum Yield of *Cryptomonas phaseolus* was different than it would have been expected by the sum of the single substance mean effect sizes. The effect on the efficiency of the PS II caused by the mixture was antagonistic.

On the one hand, the chlorophyll *a* production and the efficiency of the PSII of *Cryptomonas phaseolus* were significantly negatively affected by Carbamazepine. On the other hand, when *Cryptomonas phaseolus* was exposed to a mixture of Carbamazepine, Ciprofloxacin and Fluoxetine these effects changed significantly towards a positive effect size of the mixture on the chlorophyll *a* production and the efficiency of the PSII which led to an increase of both (Tab. 32). An explanation could be, that the negative effects of Carbamazepine and the positive effects of Fluoxetine on the efficiency of PS II balanced each other out. Regarding the chlorophyll *a* production the positive effect of Fluoxetine remained, indicating that the effect of Fluoxetine within the mixture was dominant over the effect of Carbamazepine.

Chroococcus minutus (Cyanophyta, Cyanobacteria)

The effect of the mixture of all three pharmaceuticals had similar effects on the chlorophyll *a* amount of *Chroococcus minutus* than it would have been expected by the sum of the single substance mean effect sizes (Fig. 44). The effect of the mixture on the Quantum Yield of *Chroococcus minutus* was different than it would have been expected by the sum of the single substance mean effect sizes. The effect on the efficiency of the PS II caused by the mixture was synergistic.

Tab. 32: Significant effect of Carbamazepine (8.0 µg L^{-1}), Ciprofloxacin (0.160 µg L^{-1}), Fluoxetine (0.096 µg L^{-1}) and combined pharmaceuticals on chlorophyll *a* and on Quantum Yield of the investigated algal species. (Chl *a* = chlorophyll *a*; QY=Quantum Yield; **+**: positive effect; **0**: no effect; **-**: negative effect, *****: lysis of algal cells observed). Significances were extracted from the Two Way Anova results.

	Carbamazepine		**Ciprofloxacin**		**Fluoxetine**		**Mixture**	
	Chl *a*	**QY**	**Chl *a***	**QY**	**Chl *a***	**QY**	**Chl *a***	**QY**
S. obliquus	+	0	0	0	0	0	+	0
C. minutus	0	0	+	0	0	0	+	+
C. phaseolus	-	-	+	0	+	+	+	+
N. pelliculosa	+	+	0	0	0	0	0	0
Peridinium sp.	0*	0*	0*	0*	0	0	0	0

These results clearly show that the effect of the mixture of the three pharmaceuticals on algal traits was specifically dependent on the respective algal species and different than it would have been expected from the effects caused by the individual single pharmaceuticals.

Yang et al. (2008) investigated growth-inhibiting effects of different antibacterial substances and their mixtures on the green algae *Pseudokirchneriella subcapitata*. In a mixture containing Ciprofloxacin and Norfloxacin, they observed synergistic effects. Additionally, they performed an experiment using a mixture of 12 antibacterial substances to test the effects of this mixture on the growth of *Pseudokirchneriella subcapitata* at concentrations of 0.001, 0.01, 0.1, 1, and 10 µg L^{-1} for each compound. Algal growth was significantly inhibited (23% inhibition) at a concentration of 0.1 µg L^{-1} for each substance. This is very interesting as growth inhibition of *Pseudokirchneriella subcapitata* by Triclosan (included in the mixture) was observed at concentrations of 0.2 µg L^{-1}. This result suggests that antibacterial compounds in the aquatic environment, although at relatively low concentrations, may still cause toxic effects on algae due to the combined action of antibacterial mixtures (Yang et al.

2008). At concentrations of 1 or 10 µg L^{-1}, antibacterial mixtures showed antagonistic effects as indicated by the lower inhibition rates than the corresponding expected inhibition rates from single substances alone. The concentrations of Ciprofloxacin (0.006 – 0.096 µg L^{-1}), which I used for my experiments, were in the range of the study of Yang et al. (2008). Whereas I tested a different mixture of pharmaceuticals, I also observed a similar synergistic effect of pharmaceuticals on the growth of the green algae *Scenedesmus obliquus*.

Vannini et al. (2011) monitored the average growth, metabolism, DNA damage and protein production of *Pseudokirchneriella subcapitata* exposed to pharmaceuticals. They used a mixture of various pharmaceuticals containing also Carbamazepine (0.033 µg L^{-1}) and Ciprofloxacin (0.026 µg L^{-1}). The authors observed an increase of chlorophyll *b* and quantitative changes in the occurrence of proteins involved in metabolism and photosynthesis, which shows that already very low concentrations at ng L^{-1} levels of each component could cause effects when these substances are combined. In contrast to my study, Vannini et al. (2011) did not compare their described effects of pharmaceutical mixtures with effects from single tested pharmaceuticals. However, similar to my study they showed that effects of pharmaceuticals could already be caused by „natural“ concentrations found in aquatic environments when pharmaceuticals act in combination.

In studies with a mixture of Triclosan (0 – 2000 µg L^{-1}) and Fluoxetine (0 - 1280 µg L^{-1}), antagonistic and additive effects on the growth rate were observed in experiments with seven algal cultures (i.e. *Scenedesmus obliquus*), whereas synergistic effects were not observed (Bi et al. 2018). I observed synergistic effects on the biomass production of *Scenedesmus obliquus*. However, taking all investigated algal species into consideration I found more antagonistic than synergistic effects.

Similarly, Minguez et al. (2018) observed a significant growth inhibition of the diatom *Skeletonema marinoi* when exposed to a combination of nine different antidepressants. At concentrations of 4.1 µg L^{-1} of Fluoxetine they observed an inhibition of 5 % and at 138.5 µg L^{-1} Fluoxetine a growth inhibition of 80%. However, when the algae were exposed to a combination of all nine tested antidepressants, growth inhibition of 5 % was already observed at concentrations of 0.5 µg L^{-1} Fluoxetine and 80% growth inhibition at 4.5 µg L^{-1}

Fluoxetine. Hence, when using the combination of pharmaceuticals an order of magnitude lower concentration is needed compared to when using a single given pharmaceutical.

The results from the studies described above (Yang et al. 2008, Vaninni et al. 2011, Bi et al. 2018, Minguez et al. 2018) point towards an important quantitative aspect how pharmaceutical mixtures can affect algal species in a different way than single substances. Even if the concentrations of the individual parts of the mixture are below their known concentrations where effects are expected from tests of the individual pharmaceuticals, their combined action induces effects at much lower concentrations. The reported no effect concentrations from tests of individual pharmaceuticals may therefore not reflect “no effect concentrations” in the presence of other pharmaceuticals. My experiments show that it depends on the pharmaceutical and on the algal species if the effects of the mixture on chlorophyll *a* and the efficiency of the PSII are stronger than the effect of the single tested pharmaceuticals, indicating lower “no effect concentrations” (Tab. 33). Therefore the combination effects of stressors should be considered in the risk assessments of pharmaceutical substances. A wide range of possible combinations of pharmaceuticals make this evaluation very complex and time consuming but necessary, as studies described above (Yang et al. 2008, Vaninni et al. 2011, Bi et al. 2018, Minguez et al. 2018) have shown.

Tab. 33: Mean effect sizes of single tested pharmaceuticals on chlorophyll *a* and Quantum Yield on algae in monocultures compared to the mean effect sizes of a mixture of pharmaceuticals on chlorophyll *a* and Quantum Yield in monocultures (day 22). Pharmaceuticals: Carbamazepine: 8.0 µg L^{-1}, Ciprofloxacin: 0.160 µg L^{-1}, Fluoxetine: 0.096 µg L^{-1}; Mixture: 8.0 µg L^{-1} Carbamazepine, 0.160 µg L^{-1} Ciprofloxacin, 0.096 µg L^{-1} Fluoxetine; Chl *a* = chlorophyll *a*; QY=Quantum Yield; **√**: effect of mix is weaker; **x**: effect of mixture is stronger).

	Carbamazepine		**Ciprofloxacin**		**Fluoxetine**	
	Chl *a*	**QY**	**Chl *a***	**QY**	**Chl *a***	**QY**
S. obliquus	√	x	x	x	x	x
C. minutus	√	√	√	√	√	√
C. phaseolus	√	√	x	x	x	x
N. pelliculosa	√	√	√	x	x	x
Peridinium sp.	√	√	√	x	√	√

6.1.2 Question II: Are algal species impacted differently by pharmaceuticals when treated in polycultures compared to monocultures? Do biotic interactions change the effect of pharmaceuticals? Is there a threshold of diversity above which pharmaceutical effects on phytoplankton species change?

Within the first part of my thesis I investigated and analysed the response of five individual algal species to single and combined stressors, in my case pharmaceuticals. However, ecological systems consist of complex community structures with numerous amounts of interacting populations of different species. Therefore, it is important to gain more knowledge which impact stressors have on more complex systems than for example individual monocultures of algae. I therefore investigated the effect of pharmaceuticals on polycultures of different complexity, which also allowed quantifying how the diversity of a culture and biotic interactions influenced the effect of pharmaceuticals on algal growth and physiology.

The effect of single or multiple stressors on organisms has been mostly investigated and quantified in very simple systems, not taking into account the importance of biotic interactions for the outcome of such effects (Vasque et al. 2014, Brooks and Crowe 2018, Germain et al. 2018). Hence, organisms will never experience stressors without simultaneously interacting with other organisms and species in the wild. Knowledge about whether and how strong such biotic interactions affect responses to stressors is therefore an urgent and important task.

My experiments allowed quantifying the role of biotic interactions for responses to stressors in a rigorous and quantifiable way. Additionally, I also investigated whether the complexity of biotic interactions matters; doing this by experimentally increasing the diversity of algal competitors for resources within my experimental setup.

This experimental strategy allowed quantifying two aspects. First, the role of biotic interactions for stressor effects *per se* and second the role of diversity (and thereby the number of potential interactions) for stressor effects on population and community level.

My experiments increased the diversity of algal species that were exposed to the different pharmaceuticals from single species monocultures to polycultures with five species. All monocultures and different diverse polycultures were replicated including all five species. Thereby it was possible quantifying the average effect of the pharmaceuticals on a mixture of these five species either growing alone, growing in combinations of two species and up to all five species together. For example, if the mean effect of a pharmaceutical on all five algal species grown in monoculture does not differ from the effect of the pharmaceutical on the polycultures of these five species one would not assume large importance of biotic interactions for stressor effects. The response of the community would then be predictable from the response of the individual members of the community when growing alone.

Additionally, I can compare the average of effects on all five algae species grown in monocultures with the average effect when algae were grown in combinations of two, three and four algal species. Thereby effects of diversity could be quantified, meaning whether some threshold of complexity exists above which effects of biotic interactions might be visible. Biotic interactions would be of importance, because they would seriously hinder predicting the behaviour of a complex community to a stressor from knowledge of how the individual members of the community reacted to the stressor.

My results showed that it depends on the pharmaceutical substance whether the presence of other algae, and thereby biotic interactions, resulted in different effects of the pharmaceutical substances on algal communities than on monocultures. Tab. 34 summarizes whether the effects of a pharmaceutical on polycultures were similar (effect size 0) compared to mean effects on monocultures or not (stronger effects than observed in monocultures or weaker effects than observed in monocultures). It seems that the effects of Carbamazepine and Ciprofloxacin on the photosynthetic efficiency of PS II were not affected by biotic interactions whereas the effects on biomass production (measured by the proxy FixArea) were clearly affected by biotic interactions for all investigated pharmaceuticals. Hence, it seems that effects of pharmaceuticals on biomass production measured in monocultures cannot be easily used to predict the effects of the pharmaceuticals on biomass production in polycultures of the same species.

Tab. 34: Significant effects (mean effect sizes) of pharmaceuticals on chlorophyll *a* and Quantum Yield on algae in polycultures compared to the effects (mean effect sizes) of pharmaceuticals on chlorophyll *a* and Quantum Yield in all monocultures (day 22). Pharmaceuticals: Carbamazepine: 8.0 µg L^{-1}, Ciprofloxacin: 0.160 µg L^{-1}, Fluoxetine: 0.096 µg L^{-1}; Chl *a* = chlorophyll *a*; QY=Quantum Yield; **0**: no difference in the effect between mono- and polycultures; **√**: weaker effect; **x**: stronger effect).

Carbamazepine		**Ciprofloxacin**		**Fluoxetine**		**Mixture**	
Chl *a*	**QY**	**Chl *a***	**QY**	**Chl *a***	**QY**	**Chl *a***	**QY**
x	0	x	0	x	x	x	√

These above-discussed analyses of biotic interactions on community level were further supported by results from microscopic countings, allowing quantifying the effects of pharmaceuticals on a single algal species growing in monoculture or different diverse polycultures. Different effect sizes of a pharmaceutical on a single algal species grown alone or in company with other algae would also indicate biotic effects on stressor responses. Contrary to the community analyses described above (which tested whether it is possible to predict community responses from responses of the individual members of the community), this analyses allowed to test whether the importance of biotic interactions for stressor effects differed between the investigated algae species. Such differences cannot be quantified from community analyses of biomass production or photosynthetic efficiency.

My results show that the influence of the presence of additional algal species on the effects of pharmaceuticals on an individual algal species depends on the respective algal species and the pharmaceutical (Tab. 35). A striking pattern that can be seen is that diversity (the number of additional algal species) obviously influenced the effects of the pharmaceuticals most often in a unimodal way. In most cases (Tab. 35) a quadratic regression gave the best fit. This means that diversity obviously had negative and positive effects on the stressors impact on biomass production and the efficiency of the PSII.

Potential mechanisms that could explain such a unimodal pattern are for example that the addition of a second species would suddenly result in strong interspecific competition. The

addition of more species would potentially increase the potential number of interactions and thereby also increase the probability of negative effects on strong competitors from other introduced species; this effect should become stronger with increasing diversity (Tilman 1981, Begon et al. 2006). Hence, increasing diversity could therefore initially result in a strong effect on the impact of a pharmaceutical on an individual species by adding interspecific competition. Interspecific competition for resources by a strong competitor could be seen as an additional stressor. However, the effect may become smaller further on as with more additional species more interactions are possible and strong competitors may themselves be affected by the additionally added species (Goudard and Loreau 2008). However, whereas my experiments showed clearly strong effects of diversity and biotic interactions, my experimental setup was not designed to investigate the detailed mechanisms behind the observed relationships between diversity and the effects of pharmaceuticals on individual algal species. Future experiments have to explore the observed unimodal relationships in more detail.

Tab. 35: Impact of diversity on the effect of Carbamazepine (8.0 µg L^{-1}), Ciprofloxacin (0.160 µg L^{-1}) and Fluoxetine (0.096 µg L^{-1}) on the abundance of algal species. **+**: effect increased linearly with higher diversity; **-**: effect decreased linearly with higher diversity; **0**: no directed effect; *****: quadratic dependency between diversity and effect size of pharmaceuticals on abundance of algae; **n.a.:** data not available (contaminated monocultures, no presence in monocultures or polycultures).

	Carbamazepine		**Ciprofloxacin**		**Fluoxetine**		**Mixture**	
	Chl *a*	**Abundance**	**Chl *a***	**Abundance**	**Chl *a***	**Abundance**	**Chl *a***	**Abundance**
S. obliquus	*	*	0	0	0	0	*	n.a.
C. minutus	*	-	*	*	0	*	*	n.a.
C. phaseolus	-	n.a.	0	n.a.	0	*	-	n.a.
N. pelliculosa	*	*	0	n.a.	0	*	*	n.a.
Peridinium sp.	*	n.a.	0	+	0	*	*	n.a.

My experiments allowed also a third important analyses, to quantify the role of diversity *per se* for stressor effects on communities of increasing complexity. For example, investigating differences of how a stressor affected a community with a specific algal species grown alone or with one, two and four additional algal species allowed quantifying whether effects of a stressor on the single algae could be compensated by growth responses of the other algae. If such mechanism would operate one would assume that negative or positive effect sizes on a single species grown in monoculture would be moving towards zero effect sizes with increasing diversity. My results showed that this mechanism was only observed within cultures including *Scenedesmus obliquus* and *Cryptomonas phaseolus* when exposed to Carbamazepine (Fig. 49 a, d) and the pharmaceutical mixture (Fig. 52 a, d). In these cases negative or positive effect sizes on algae were compensated on community level with increasing complexity. However, in most cases compensatory effects were not found (Tab.

36). As most of the significant relationships between diversity and effect size of pharmaceuticals on Chl *a* of algae followed a quadratic model (Tab. 35), positive or negative effects observed in monocultures got obviously weaker in polycultures, but still existed.

Tab. 36: Comparison of the effect of Carbamazepine (8.0 µg L^{-1}), Ciprofloxacin (0.160 µg L^{-1}) and Fluoxetine (0.096 µg L^{-1}) and their mixture on Chl *a* of communities of the same species growing with one, two, three and four more algae species versus monocultures. **0**: no difference in the effect; **√**: weaker effect; **x**: stronger effect; **-**: not applicable.

	Carbamazepine				**Ciprofloxacin**				**Fluoxetine**				**Mixture**			
No. of added algae species	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Scenedesmus obliquus	0	√	-	√	0	0	-	0	0	0	-	0	x	√	-	√
Chroococcus minutus	x	x	x	x	x	x	x	0	x	0	x	√	x	x	x	0
Cryptomonas phaseolus	√	√	√	√	0	x	0	x	√	√	0	√	√	0	√	√
Navicula pelliculosa	√	√	√	√	0	0	0	x	x	0	x	0	x	x	x	0
Peridinium sp.	x	x	x	x	x	0	0	0	√	√	x	√	x	x	x	0

Such compensatory effects of diversity as described above could be visible on response parameters such as biomass or photosynthesis but also on the variability of responses (measured by the coefficient of variation of responses) along a biodiversity gradient. Diversity should largely dampen such variability (Ives and Hughes 2002, Thébault and Loreau 2005). My results clearly show that the variability of responses to pharmaceuticals was indeed affected by diversity. The coefficient of variance decreased with higher diversity in all pharmaceutical treatments and for all investigated algae species (Tab. 28, Tab. 29, Tab.

30, Tab. 31.). In a highly diverse community it is more likely that species could show either positive or negative effect sizes to a stressor such as a pharmaceutical. Positive and negative effects could somehow cancel each other out and the effect of a stressor on net community performance and its coefficient of variation would then decrease with increasing diversity (Boyer et al. 2009, Schindler et al. 2010, Schindler et al. 2015, Harrison et al. 2020).

Furthermore my study showed that even in robust and not very complex laboratory communities pharmaceuticals could result in a change in community structure. Concentrations of my pharmaceutical treatments were comparably low, even at the highest concentrations of pharmaceuticals. However, even such low concentrations of Carbamazepine, Ciprofloxacin and Fluoxetine (CBZ: 8.0; CIP: 0.160, FL: 0.096 µg L^{-1}) resulted in small but measurable effects on community structure. Hence, large species abundance variations (as usual in community analyses) make analyses often less robust and only effects of Carbamazepine on community composition were found to be significant at a 10% level.

The observed change in the community structure due to Carbamazepine is not surprising as a disturbance of a community by stressors can affect the performance of each species in a different way, depending on their stress tolerance (Townsend et al., 1997, Flöder and Sommer, 1999, Hammerstein et al. 2017). My results showed that *Navicula pelliculosa*, *Peridinium sp.* and *Cryptomonas phaseolus* were stronger affected by the pharmaceutical Carbamazepine than *Scenedesmus obliquus* and *Chroococcus minutus* (Fig. 53 b, c, d). The results from the monocultures showed that for example *Scenedesmus obliquus* benefited from Carbamazepine (Fig. 5 a), whereas *Cryptomonas phaseolus* (Fig. 11 a) was negatively affected in their photosynthetic performance.

In an experimental study, Wilson et al. (2003) observed a reduction of algal species richness and shifts in community structure when algal communities were exposed to Ciprofloxacin. In my results I observed no significant shift in community structure when algae were exposed to Ciprofloxacin. Wilson et al. (2003) used similar concentrations of Ciprofloxacin, but instead of using natural algal communities I used selected laboratory algal strains for my experiments, which could explain differences between the studies.

Several studies (Wilson et al. 2003, Baho et al. 2019) showed that algae species were affected differently by pharmaceuticals even at low but environmentally relevant concentrations. If one algal species is inhibited in growth by the stressor, more resources are available for other algal species. This could lead to a better growth and so higher biomass of these algal species (compensatory growth) (Flöder et al. 2010, Franco et al. 2017). Hence, even if there was a strong effect of the stressor on one algal species, the compensatory growth of other algal species could result in similar final biomass concentrations such as in control treatments. Therefore, the measure of a biomass proxy alone is not meaningful in risk assessments of stressor effects on communities without also characterizing community composition. A change in community structure could have severe impacts on food chain dynamics and ecosystem functioning (Flecker and Townsend 1994, Balvanera et al. 2006, Winder and Jassby 2011).

6.1.3 Question III: How does Carbamazepine affect natural algal populations with different diversity?

All the analyses described above were done with artificially established laboratory communities. Only this approach allows full control over species composition and diversity of algal communities. However, as pointed out in the introduction, laboratory communities do not share an evolutionary history such as communities in the wild. Laboratory polycultures are usually assembled randomly and/or constrained by the availability of laboratory strains, whereas selective forces shape the community assembly of natural communities. Strength and direction of biotic interactions within natural communities shaped by environmental parameters might therefore be different. Such selective forces act at each time point a community interacts. Hence, also the selective processes and biotic interactions (competition) in the past shape the composition and dynamics of communities. Currently existing communities can be seen as the result of such past events (Sommer and Worms 2002, Grace and Tilman 1990). Strong competition in the past may have led to niche differentiation and thereby reduced biotic interactions (Connell 1980). Hence, the so-called "ghost of competition past" is additionally a strong factor shaping communities which will not be acting in artificially assembled laboratory cultures. The same number of similar species in laboratory communities may therefore show stronger interactions compared to the same number of species originating from natural phytoplankton communities. Additionally, natural communities are constantly exposed to a large number of species which are dispersing into their habitats and trying to invade. Hence, not only successful invaders but also unsuccessfully invading species contribute to community dynamics by interactions during the invasion process (Miller et al. 2009, Buchberger and Stockenreiter 2018). Finally, beside negative interactions such as competition also cooperation can play a role in the evolutionary history shaping communities as a study of Jousset et al. (2013) demonstrated. The authors investigated how the evolutionary history affects the emergence and spread of defectors in bacterial communities of varying diversity and phylogenetic relatedness. Their study showed that evolutionary relationships could predict

the stability of cooperation, as cooperation is more stable in closely related communities. The question is, if this is also found within algal communities. Certainly, facilitation is a further example of positive interaction, which influences the shape of phytoplankton communities (Bruno et al. 2003, Krichen et al. 2019).

From all the points described above it becomes clear that natural phytoplankton communities may respond differently to stressors than comparable artificially assembled laboratory communities. I therefore also investigated experimentally diversity manipulated phytoplankton communities from three lakes. Such experimentally manipulated natural phytoplankton communities allow investigating a subset of different diverse communities originating from the same initial phytoplankton community (Hammerstein et al. 2017). Analyses of these experiments are therefore comparable with the above described “third” analyses of laboratory communities (chapter 6.1.2), where subsets of different diverse communities from the same initial species pool were analysed. One would expect that increasing complexity could also result in a decreasing effect size (coming towards zero) of stressors on natural phytoplankton communities as compensatory effects could play a role. In two of three lakes it was observed that with an increasing diversity of phytoplankton community, effects of Carbamazepine on the photosynthetic activity of the PSII got lower (Fig. 58, Fig. 60). This was not observed for the production of chlorophyll *a*. A possible explanation for that observation could be that other factors such as for example nutrient limitation by micro- or macronutrients, was affecting the algae in their more complex processes of biomass production. Taking an approach using natural communities from different lakes allowed analysing, whether “natural polycultures” of algae differ from laboratory assembled polycultures in their response to Carbamazepine. Firstly, my diversity manipulations allow comparing diversity effects in natural versus laboratory communities. Beside the fact of differences between laboratory and natural algal assemblages described above, natural communities are often also more complex in terms of species richness. Secondly, my approach allowed to study directed natural diversity gradients. Laboratory gradients of diversity are assembled by replacing or removing available species whereas in natural communities the loss of stress sensitive or rare species is often the main reason for reduced diversity (Moyle and Leidy 1992, Flöder and Sommer 1999, Sih et al. 2011). By

mimicking such processes (loss of stress sensitive species) in my experiments it was possible to analyse whether diversity shaped by eco-evolutionary processes acts differently than randomly created laboratory diversity gradients interacting with pharmaceuticals and algal communities.

Effects of pharmaceuticals were larger in laboratory monocultures and cultures including two and three algal species than effects observed for natural algal assemblages including usually more than 30 species. The effect sizes of Carbamazepine on chloropyll *a* production and on the efficiency of the PS II were however similar between laboratory communities including four and five algal species and natural communities. These comparisons of effect sizes would indicate that at higher diversities results from laboratory and natural communities become more similar. Comparing grassland communities Jochum et al. (2020) also showed that results from diversity experiments with randomly selected communities are comparable to results from non-randomly, natural communities. The authors compared data from the so-called "Jena experiment" (Weisser et al. 2017), the largest and longest-running (15 years) grassland experiment and a 7 yearlong grassland experiment (Tilman et al. 2001) with real-world grassland plant communities (Fischer et al. 2010) and semi-natural grasslands which were close to the Jena experiment.

My experiments point towards a threshold of three species in laboratory cultures above which effects became similar between artificial and natural algal communities. Further investigations would be necessary to strengthen this assumption. The existence of such a threshold would need further support by experiments including more communities and pharmaceuticals, but potentially would have a large impact on future investigations of such effects of pharmaceuticals. It would probably make it possible to predict effects of pharmaceutical stressors on natural, diverse, communities by investigating defined laboratory communities including a comparably low number of algal species. Such laboratory communities would additionally allow to investigate effects on all individual species of the communities which is not possible with complex natural communities. A potential reason for such an observed fast "saturation" of biodiversity effects could be based on the relative contribution of each species added to a community. While the relative

contribution to an increase in diversity is largest going from a monoculture to a community with two species (100%), it is already only 50 % by adding one more additional species; the relative contribution of every new species decreases with community richness.

My field experiments also included diversity manipulations. A general concern is that a loss of diversity will affect ecosystem functioning (MacArthur, 1955, Tilman 2000, Cardinale et al. 2011, Weisser et al. 2017). Also the resistance against stressors could be reduced with declining diversity and effects of stressors could therefore become larger (Thompson and Shurin 2012). However I could not see such a general pattern in my field data. Whereas the diversity manipulations affected the strength of responses of natural communities to Carbamazepine, it was not necessarily in a way that a reduction of diversity always increased effect sizes of Carbamazepine on biomass production or photosynthetic efficiencies. Hence, whereas not all field experiments showed an increasing stressor effect with reduced diversity it was still observed in communities from two of the investigated lakes.

Different strength and directions of effects of diversity on the responses of phytoplankton to Carbamazepine between natural phytoplankton communities were however to be expected. My laboratory data showed clear evidence that the composition of polycultures largely influenced their response to pharmaceuticals. My investigated lakes are different in their nutrient and light regime (chapter 4.3.2) with resulting large differences in phytoplankton community composition (Lampert and Sommer 2007). It seems that in both, laboratory and field communities the community composition had a stronger effect on responses to pharmaceuticals than diversity *per se*. A reduction of diversity resulted in a stronger effect of Carbamazepine on the chlorophyll *a* production in half of the laboratory assemblages and in one of the three investigated lakes. In the experiments with algal communities from natural assemblages the observed relationship between diversity and the effect of the pharmaceutical was linear. On the contrary, in experiments with randomly selected laboratory algal species the relationship of diversity and the effects of the pharmaceutical followed mostly a unimodal model. The differences in the relationships could probably be explained by absolute and relative differences in the gradient of diversity

between experiments with natural communities and laboratory communities as already described above.

I could not find strong support from my field studies that a diversity loss results in a stronger overall effect of Carbamazepine on chlorophyll *a* production or the efficiency of the PSII. Beside the above mentioned different community composition an additional reason could have been that the loss of diversity resulting from my disturbance manipulations (chapter 4.3.2) was too low to result in significant effects. The initial diversity of my natural assemblages was much higher compared to polycultures in my laboratory experiments. Hence, my experimental manipulations of field communities resulted in reductions of diversity but not in very low diversities including only one or just a few species. Recent studies (Craven et al. 2018, Le Bagousse-Pinguet et al. 2019) showed that more facets of diversity than species richness and abundance (taxonomic diversity) have to be considered when investigating the relationship with ecosystem functioning. Recent results (Le Bagousse-Pinguet et al. 2019) indicate that a large variety of diversity attributes are needed to maximize multiple ecosystem functions. Additionally, diversity is not the only driver of ecosystem functioning and a large number of other environmental parameters will also affect species dynamics and performances (Van der Plas 2019).

However, my results showed clearly, that in addition to experiments with fully controlled laboratory communities, investigations on natural algal assemblages are important too. Numerous biotic and abiotic factors play an important role in shaping responses of phytoplankton communities to pharmaceuticals. These factors can usually not all be considered in laboratory experiments using laboratory algal strains, which were cultivated for years in a highly controlled and stable laboratory environment. Highly controlled laboratory microcosms have the advantage that they are easily reproducible, but as they are usually conducted at low complexity, it is not always possible to upscale results from such experiments to accurately predict the impact of stressors on natural ecosystems. There is a general trade-off between naturalness and the control of experiments. The more the experimental systems correspond to reality, i.e. an actual ecosystem, the more difficult it becomes to repeat the experiments and achieve reproducible results (Fent 2013, Vasquez et

al. 2014). Hence, my results showed that laboratory experiments could potentially predict the effect of pharmaceuticals of natural communities if the laboratory communities had a certain degree of complexity. My approach gives first evidence that including biodiversity into highly controlled laboratory experiments to study effects of pharmaceuticals on algal communities could allow obtaining results that could also be used to predict effects on natural communities. In summary, both, my experiments with algae from natural phytoplankton assemblages and with laboratory cultures brought clear evidence that diversity influenced the effects of pharmaceuticals on algal communities. It is not easily possible to predict the effects of pharmaceuticals on diverse polycultures from experiments with monocultures. However, natural diversity effects and laboratory diversity effects on the impact of pharmaceuticals on algal communities were similar. Hence, future analyses of the effects of pharmaceutical stressors on aquatic communities must take care of such effects and include the factor diversity into laboratory study designs.

7. References

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8. Abbreviations

API: Active pharmaceutical ingredient

CAT: Catalase

CBZ: Carbamazepine

Chl *a*: Chlorophyll *a*

CIP: Ciprofloxacin

EC_{50}: The concentration of a drug that gives half-maximal response (half maximal effective concentration)

EMA: European Medicines Agency

FA: FixArea

FL: Fluoxetine

MDA: Malondialdehyde

PUFA: Peroxidizing polyunsaturated fatty acids

PS: Photosystem

QSAR: Quantitative structure–activity-relationship

QY: Quantum Yield

ROS: Reactive oxygen species

SOD: Superoxid dismutase

SSRI: Selective serotonin reuptake inhibitors

STP: Sewage treatment plant

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11. Appendix

Tab. 37: Detailed results of Two Way ANOVA post hoc test (Bonferroni t-test, all pairwise) for the effect of **(a)** Carbamazepine (CBZ) (8.0 µg L^{-1}), **(b)** Ciprofloxacin (CIP) (0.160 µg L^{-1}), **(c)** Fluoxetine (FL) (0.096 µg L^{-1}) and **(d)** the mixture (MIX) (CBZ: 8.0 µg L^{-1}, CIP: 0.160 µg L^{-1}, FL: 0.096 µg L^{-1}) on FixArea values (day 22) and the investigated algal species: **(e)** *S. obliquus*, **(f)** *C. minutus*, **(g)** *C. phaseolus*, **(h)** *N. pelliculosa*, **(i)** *Peridinium sp.*. Asterisks indicate statistically significant differences ($p \leq 0.05$).

a)

Carbamazepine	**t**	**P**	**P<0.05**
N. pelliculosa vs. *C. phaseolus*	40.504	<0.001	Yes*
N. pelliculosa vs. *C. minutus*	16.052	<0.001	Yes*
N. pelliculosa vs. *Peridinium sp.*	15.084	<0.001	Yes*
N. pelliculosa vs. *S. obliquus*	5.376	<0.001	Yes*
S. obliquus vs. *C. phaseolus*	35.128	<0.001	Yes*
S. obliquus vs. *C. minutus*	10.676	<0.001	Yes*
S. obliquus vs. *Peridinium sp.*	9.708	<0.001	Yes*
Peridinium sp. vs. *C. phaseolus*	25.420	<0.001	Yes*
Peridinium sp. vs. *C. minutus*	0.968	1.000	No
C. minutus vs. *C. phaseolus*	24.452	<0.001	Yes*

b)

Ciprofloxacin	**t**	**P**	**P<0.05**
C. minutus vs. *N. pelliculosa*	2.352	1.000	No
C. minutus vs. *C. phaseolus*	1.705	1.000	No
C. minutus vs. *Peridinium sp.*	0.670	1.000	No
C. minutus vs. *S. obliquus*	0.612	1.000	No
S. obliquus vs. *N. pelliculosa*	1.741	1.000	No
S. obliquus vs. *C. phaseolus*	1.093	1.000	No
S. obliquus vs. *Peridinium sp.*	0.0579	1.000	No
Peridinium sp. vs. *N. pelliculosa*	1.683	1.000	No
Peridinium sp. vs. *C. phaseolus*	1.035	1.000	No
C. phaseolus vs. *N. pelliculosa*	0.648	1.000	No

c)

Fluoxetine	**t**	**P**	**P<0.05**
C. phaseolus vs. *C. minutus*	8.498	<0.001	Yes*
C. phaseolus vs. *N. pelliculosa*	6.426	<0.001	Yes*
C. phaseolus vs. *S. obliquus*	5.053	<0.001	Yes*
C. phaseolus vs. *Peridinium sp.*	4.602	0.002	Yes*
Peridinium sp. vs. *C. minutus*	3.896	0.027	Yes*
Peridinium sp. vs. *N. pelliculosa*	1.824	1.000	No
Peridinium sp. vs. *S. obliquus*	0.451	1.000	No
S. obliquus vs. *C. minutus*	3.445	0.138	No
S. obliquus vs. *N. pelliculosa*	1.373	1.000	No
N. pelliculosa vs. *C. minutus*	2.073	1.000	No

d)

Mixture	t	P	P<0.05
C. phaseolus vs. *C. minutus*	12.521	<0.001	Yes*
C. phaseolus vs. *N. pelliculosa*	12.365	<0.001	Yes*
C. phaseolus vs. *Peridinium sp.*	11.670	<0.001	Yes*
C. phaseolus vs. *S. obliquus*	3.902	0.027	Yes*
S. obliquus vs. *C. minutus*	8.619	<0.001	Yes*
S. obliquus vs. *N. pelliculosa*	8.462	<0.001	Yes*
S. obliquus vs. *Peridinium sp.*	7.768	<0.001	Yes*
Peridinium sp. vs. *C. minutus*	0.851	1.000	No
Peridinium sp. vs. *N. pelliculosa*	0.694	1.000	No
N. pelliculosa vs. *C. minutus*	0.157	1.000	No

e)

Scenedesmus obliquus	**t**	**P**	**P<0.05**
CBZ vs. CIP	9.178	<0.001	Yes*
CBZ vs. FL	8.456	<0.001	Yes*
CBZ vs. MIX	1.697	1.000	No
MIX vs. CIP	7.481	<0.001	Yes*
MIX vs. FL	6.760	<0.001	Yes*
FL vs. CIP	0.722	1.000	No

f)

Chroococcus minutus	**t**	**P**	**P<0.05**
CIP vs. FL	3.335	0.201	No
CIP vs. CBZ	2.110	1.000	No
CIP vs. MIX	1.749	1.000	No
MIX vs. FL	1.586	1.000	No
MIX vs. CBZ	0.360	1.000	No
CBZ vs. FL	1.226	1.000	No

g)

Cryptomonas phaseolus	**t**	**P**	**P<0.05**
MIX vs. CBZ	37.334	<0.001	Yes*
MIX vs. CIP	12.477	<0.001	Yes*
MIX vs. FL	5.609	<0.001	Yes*
FL vs. CBZ	31.725	<0.001	Yes*
FL vs. CIP	6.868	<0.001	Yes*
CIP vs. CBZ	24.857	<0.001	Yes*

h)

Navicula pelliculosa	**t**	**P**	**P<0.05**
CBZ vs. CIP	16.295	<0.001	Yes*
CBZ vs. MIX	15.535	<0.001	Yes*
CBZ vs. FL	15.205	<0.001	Yes*
FL vs. CIP	1.090	1.000	No
FL vs. MIX	0.330	1.000	No
MIX vs. CIP	0.760	1.000	No

i)

Peridinium sp.	**t**	**P**	**P<0.05**
FL vs. CBZ	1.702	1.000	No
FL vs. MIX	1.459	1.000	No
FL vs. CIP	1.231	1.000	No
CIP vs. CBZ	0.472	1.000	No
CIP vs. MIX	0.229	1.000	No
MIX vs. CBZ	0.243	1.000	No

Tab. 38: Detailed results of Two Way ANOVA post hoc test (Bonferroni t-test, all pairwise) for the effect of **(a)** Carbamazepine (CBZ) (8.0 µg L^{-1}), **(b)** Ciprofloxacin (CIP) (0.160 µg L^{-1}), **(c)** Fluoxetine (FL) (0.096 µg L^{-1}) and **(d)** the mixture (MIX) (CBZ: 8.0 µg L^{-1}, CIP: 0.160 µg L^{-1}, FL: 0.096 µg L^{-1}) on Quantum Yield values (day 22) and the investigated algal species: **(e)** *S. obliquus*, **(f)** *C. minutus*, **(g)** *C. phaseolus*, **(h)** *N. pelliculosa*, **(i)** *Peridinium sp.*. Asterisks indicate statistically significant differences ($p \leq 0.05$).

a)

Carbamazepine	**t**	**P**	**P<0.05**
N. pelliculosa vs. *C. phaseolus*	26.373	<0.001	Yes*
N. pelliculosa vs. *C. minutus*	3.958	0.022	Yes*
N. pelliculosa vs. *Peridinium sp.*	10.006	<0.001	Yes*
N. pelliculosa vs. *S. obliquus*	2.650	1.000	No
S. obliquus vs. *C. phaseolus*	23.724	<0.001	Yes*
S. obliquus vs. *C. minutus*	1.309	1.000	No
S. obliquus vs. *Peridinium sp.*	7.356	<0.001	Yes*
Peridinium sp. vs. *C. phaseolus*	16.368	<0.001	Yes*
C. minutes vs. *Peridinium sp.*	6.047	<0.001	Yes*
C. minutus vs. *C. phaseolus*	22.415	<0.001	Yes*

b)

Ciprofloxacin	**t**	**P**	**P<0.05**
N. pelliculosa vs. *C. minutus*	1.378	1.000	No
C. phaseolus vs. C. minutus	1.036	1.000	No
Peridinium sp. vs. C. minutus	2.171	1.000	No
S. obliquus vs. C. minutus	1.056	1.000	No
N. pelliculosa vs. *S. obliquus*	0.322	1.000	No
S. obliquus vs. *C. phaseolus*	0200	1.000	No
Peridinium sp. vs. *S. obliquus*	1.116	1.000	No
Peridinium sp. vs. *N. pelliculosa*	0.793	1.000	No
Peridinium sp. vs. *C. phaseolus*	1.136	1.000	No
N. pelliculosa vs. *C. phaseolus*	0.342	1.000	No

c)

Fluoxetine	**t**	**P**	**P<0.05**
C. phaseolus vs. *C. minutus*	1.618	1.000	No
C. phaseolus vs. *N. pelliculosa*	0.715	1.000	No
C. phaseolus vs. *Scenedesmus*	1.294	1.000	No
Peridinium sp. vs. *C. phaseolus*	1.327	1.000	No
Peridinium sp. vs. *C. minutus*	2.945	0.706	No
Peridinium sp. vs. *N. pelliculosa*	2.041	1.000	No
Peridinium sp. vs. *S. obliquus*	2.620	1.000	No
S. obliquus vs. *C. minutus*	0.325	1.000	No
N. pelliculosa vs. *S. obliquus*	0.579	1.000	No
N. pelliculosa vs. *C. minutus*	0.904	1.000	No

d)

Mixture	t	P	P<0.05
C. phaseolus vs. *C. minutus*	3.985	0.019	Yes*
C. phaseolus vs. *N. pelliculosa*	4.115	0.012	Yes*
C. phaseolus vs. *Peridinium sp.*	3.215	0.300	No
C. phaseolus vs. *S. obliquus*	4.078	0.014	Yes*
C. minutus vs. *S. obliquus*	0.0924	1.000	No
S. obliquus vs. *N. pelliculosa*	0.0372	1.000	No
Peridinium sp. vs. *S. obliquus*	0.863	1.000	No
Peridinium sp. vs. *C. minutus*	0.771	1.000	No
Peridinium sp. vs. *N. pelliculosa*	0.900	1.000	No
C. minutus vs. *N. pelliculosa*	0.130	1.000	No

e)

Scenedesmus obliquus	**t**	**P**	**P<0.05**
MIX vs. FL	0.870	1.000	No
MIX vs. CBZ	0.643	1.000	No
MIX vs. CIP	0.446	1.000	No
CIP vs. FL	0.424	1.000	No
CIP vs. CBZ	0.197	1.000	No
CBZ vs. FL	0.227	1.000	No

f)

Chroococcus minutus	**t**	**P**	**P<0.05**
MIX vs. CBZ	2.044	1.000	No
MIX vs. CIP	1.594	1.000	No
MIX vs. FL	1.287	1.000	No
FL vs. CBZ	0.757	1.000	No
FL vs. CIP	0.307	1.000	No
CIP vs. CBZ	0.450	1.000	No

g)

Cryptomonas phaseolus	**t**	**P**	**P<0.05**
MIX vs. CBZ	28.444	<0.001	Yes*
MIX vs. CIP	4.543	0.002	Yes*
MIX vs. FL	3.654	0.066	No
FL vs. CBZ	24.790	<0.001	Yes*
FL vs. CIP	0.889	1.000	No
CIP vs. CBZ	23.901	<0.001	Yes*

h)

Navicula pelliculosa	**t**	**P**	**P<0.05**
CBZ vs. FL	2.298	1.000	No
CBZ vs. CIP	2.130	1.000	No
CBZ vs. MIX	2.044	1.000	No
MIX vs. FL	0.254	1.000	No
MIX vs. CIP	0.0861	1.000	No
CIP vs. FL	0.167	1.000	No

i)

Peridinium sp.	**t**	**P**	**P<0.05**
FL vs. CBZ	9.749	<0.001	Yes*
FL vs. CIP	1.080	1.000	No
FL vs. MIX	0.887	1.000	No
MIX vs. CBZ	8.862	<0.001	Yes*
MIX vs. CIP	0.193	1.000	No
CIP vs. CBZ	8.669	<0.001	Yes*

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