

Research Article

The invasion success of the cyanobacterium *Cylindrospermopsis raciborskii* in experimental mesocosms: genetic identity, grazing loss, competition and biotic resistance

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Editor's note:

This study was first presented at the special session on aquatic invasive species at the 33rd Congress of the International Society of Limnology (SIL) (31 July – 5 August 2016, Torino, Italy) (<http://limnology.org/meetings/past-sil-congress/>). This special session has provided a venue for the exchange of information on ecological impacts of non-native species in inland waters.

Abstract

The potentially toxic, invasive cyanobacterium *Cylindrospermopsis raciborskii*, originating from sub-tropical regions, has spread into temperate climate zones in almost all continents. Potential factors in its success are temperature, light and nutrient levels. Grazing losses through zooplankton have been measured in the laboratory but are typically not regarded as a factor in (failed) invasion success. In some potentially suitable lakes, *C. raciborskii* has never been found, although it is present in water bodies close by. Therefore, we tested the invasive potential of three different isolates introduced into natural plankton communities using laboratory mesocosm experiments under three grazing levels: ambient zooplankton densities, removal of large species using 100 µm mesh and a ca. doubling of large species. Three *C. raciborskii* isolates originating from the same geographic region (North-East Germany) were added separately to the four replicates of each treatment and kept in semi-continuous cultures for 21 days. Two isolates disappeared from the mesocosms and were also not viable in filtered lake water indicating that the lake water itself or the switch from culture medium to lake water led to the decay of the inoculated *C. raciborskii*. Only one out of the three isolates persisted in the plankton communities at a rather low level and only in the treatment without larger zooplankton. This result demonstrates that under potentially suitable environmental conditions, top-down control from zooplankton might hamper the establishment of *C. raciborskii*. Non-metric multidimensional scaling showed distinct variation in resident phytoplankton communities between the different grazing levels, thus differential grazing impact shaped the resident community in different ways allowing *C. raciborskii* only to invade under competitive (= low grazing pressure) conditions. Furthermore, even after invasion failure, the temporary presence of *C. raciborskii* influenced the phytoplankton community.

Key words: alien species, Cyanobacteria, competitive resistance, consumptive resistance, herbivory, harmful algae, microbial invasion

Introduction

Besides many prominent and obvious examples of biological invasions in freshwater systems, a number of less visible invasions occur and may cause serious problems (Litchman 2010). These microbial invasions

are much less studied, with only a few exceptions. One microbial invader that has received a lot of attention is the cyanobacterium *Cylindrospermopsis raciborskii* (Woloszynska) Seenayy et Subba Raju, that has spread from tropical to temperate regions worldwide (for reviews see Padisák 1997; Sukenik

et al. 2012; Antunes et al. 2015; Wilk-Wózniak et al. 2016) with a number of negative effects on water quality such as bloom formation, decreased water transparency and toxin production (Sukenik et al. 2015).

Typical habitats in temperate regions are shallow eutrophic lakes that have a well-mixed water column throughout the season or are at least periodically mixed (Mehner et al. 2010). A number of ecological and physiological traits have been assigned to explain its invasion success including a high intraspecific phenotypic plasticity (Padisák 1997; Bonilla et al. 2012; Soares et al. 2013; Bolius et al. *in press*), high phosphorus uptake rates (Istvánovics et al. 2000; Burford et al. 2006), adaptation to warmer temperatures (e.g. Briand et al. 2004; Mehner et al. 2010; Bonilla et al. 2016), N-fixation (Saker and Neilan 2001; Spröber et al. 2003; Moisaner et al. 2012) and the presence of akinetes (Wiedner et al. 2007; Mehner et al. 2014). However, none of these traits is unique for *C. raciborskii* but common for some other cyanobacteria and/or eukaryotic phytoplankton. Besides these bottom-up factors, *C. raciborskii* may also suffer from herbivory despite its filamentous form which typically reduces edibility for zooplankton (Lampert 1987). However, cladocerans (Soares et al. 2009a; Panosso and Lüring 2010) and copepods (Rangel et al. 2016) but also rotifers (Soares et al. 2010; Sperfeld et al. 2010; Kâ et al. 2012) have been shown to ingest and/or even suppress *C. raciborskii* in small-scale laboratory experiments. This suggests that a combination of traits in relation to environmental conditions drives the invasion success of *C. raciborskii*. In the past, potential reasons or factors responsible for the invasion success of *C. raciborskii* have been derived from studies on physiology, life history or genetics. Experimental studies under near-natural conditions in a community context are lacking, although needed to elaborate which traits under what kind of environmental conditions are responsible for invasion success.

Biotic resistance, i.e. when biotic interactions prevent an invasion event, can be due to strong competition (competitive resistance) or predation (consumptive resistance). For freshwater systems, consumptive resistance is often more important than competitive resistance (Alofs and Jackson 2014). Such invader-environment relations can also be influenced by the genotype of the invader. It is a general notion, also known as the paradox of invasion (Roman and Darling 1997) that the inoculum of an invasive species consists only of one or very few genotypes. Despite the resulting impoverished gene pool, some species are extremely successful invaders. Thus, a specific genotype might be much more successful than

another one of the same species, at least under certain environmental conditions (Marinho et al. 2013; Burford et al. 2016). To test this, we performed a laboratory experiment using natural water from a lake where *C. raciborskii* has not previously been found (data from local water authorities). In particular, we tested the hypothesis that a) the invasion success of *C. raciborskii* is strain-dependent and b) whether invasion success might be hampered by competitive or consumptive resistance. Therefore, we tested the invasion success of three different genotypes of *C. raciborskii* in natural plankton communities in which we manipulated zooplankton biomass at three levels.

Methods

The three cultures of *C. raciborskii* genotypes used in this study were kept in a modified Woods Hole medium WC with a concentration of 80 $\mu\text{g PO}_4\text{-P L}^{-1}$ and a pH of 8, buffered with 2 mM HEPES. All genotypes originated from lakes in North-East Germany: 26D9 was isolated from Rangsdorfer See, 52°17'19"N; 13°24'14"E, (Mehner et al. 2010; Ramm et al. 2012), ZIE 11 from Zierker See, 53°21'41"N; 13°02'09" E, (Haande 2008; Sperfeld et al. 2010; Mehner et al. 2010) and MEL 07 from Melangsee, 52°09'40"N; 13°59'18"E. DNA-fingerprinting revealed that these are indeed different genotypes and their growth rates measured under laboratory semi-continuous turbidostat conditions differed: 0.26 d^{-1} (26D9), 0.21 d^{-1} (ZIE 11) and 0.36 d^{-1} (MEL 07) (Boliu et al. *in press*). All genotypes were negatively tested for the cyanobacteria toxin cylindrospermopsin (using Liquid Chromatography-Mass Spectrometry, Haande et al. 2008 and C. Wiedner, *pers. comm.*) They differed significantly in length and diameter: ZIE11 had the shortest filaments with a length of 62 $\mu\text{m} \pm 37\mu\text{m}$ (SD) and a width of 1.56 μm ; strain 26D9: length 99 $\mu\text{m} \pm 36 \mu\text{m}$ (SD), width 1.89 μm ; MEL07: length 266 $\mu\text{m} \pm 136 \mu\text{m}$, width 1.84 μm ($n = 50$ each).

A mixed epilimnetic water sample from 1 to 2 m depth was taken from eutrophic Lake Glindower See using a 3.5 L Ruttner-type sampler on August 14th 2014. From this sample, relevant limnological parameters were determined and the same water was used to set-up the experiment. On the site, a vertical depth profile was taken using a multi-parameter probe (Idronaut, Italy) measuring temperature, pH, O_2 -saturation, O_2 -concentration and conductivity. Vertical light penetration of photosynthetically active radiation (400–700 nm) was measured using an underwater spherical light sensor (Li-Cor 193) corrected for changes in surface irradiance using a second sensor measuring in air.

For chlorophyll-*a* analysis, subsamples were filtered on GF/C glass fibre filters (Whatman) and chlorophyll-*a* was extracted over night with hot ethanol. Chlorophyll-*a* concentration was determined using a fluorometer (TD-700 Turner Design) calibrated with laboratory standards. Total phosphorus was determined using the molybdate-method after digestion with sulfuric acid and potassium persulfate for 20 min at 120 °C in an autoclave.

The experiment was set up with one control, C, treatment which contained the ambient plankton composition and to which no *C. raciborskii* was added. The same water with *C. raciborskii* addition was termed Z^N. Then, we manipulated the zooplankton biomass and composition by sieving water through 100 µm mesh representing the Z-treatment. The Z⁺-treatment was created by gently pumping out 50% of the original lake water from a container through a 12 cm wide plastic tube covered with a 100 µm mesh. The remaining water contained roughly double the concentration of larger zooplankton but similar densities of smaller zooplankters such as rotifers and nauplii (see results). For all treatments 800 mL of water was added to 1 L Erlenmeyer flasks in quadruplicate. To the three treatments Z⁻, Z^N and Z⁺, we added each of the three isolates of *C. raciborskii* with a biomass of 109 µg carbon L⁻¹ to separate sets of flasks. For the calculation of the carbon content, we determined the biovolume by multiplying the cumulative length of the filaments by their width and then converted the biovolume into carbon by using the conversion function from Rocha and Duncan (1985). Depending on the length and diameter of the filaments, this biomass represents ca. 900 (Mel07), 2450 (26D9) and 6000 (ZIE11) filaments per mL. This density was chosen because it is much lower than the filament density that is considered to affect cladoceran filtering and reproduction and would allow consumption by crustaceans (Dawidowicz et al. 1988; Gliwicz 1990). The total amount of 40 flasks (3 strains × 3 zooplankton levels × 4 replicates plus 4 flasks without *C. raciborskii* addition serving as controls for all strains) were kept in a climate chamber at ambient temperature (22 °C), a light intensity of 130 µmol photons m⁻² s⁻¹ photosynthetic active radiation and a light:dark cycle (15:9 hrs) for 21 days. Every 3 days, a 150 mL sample was taken, fixed with Lugol's iodine and the volume was substituted with sterile lake water (collected at the starting day of the experiment) filtered through 0.2 µm cellulose acetate filter (Sartorius). Before sampling, the flasks were gently shaken to homogenize the distribution of the plankters, thus zooplankton was removed with the phytoplankton. From these samples the phytoplankton biomass and composition was

determined for days 3, 12 and 21 using an inverted microscope (Thalheim Optik, Jena) and standard algal counting procedures (Utermöhl 1958). To calculate the biovolume of non-filamentous species the number of cells/colonies was multiplied by their volume calculated from cell/colony measurements and approximation to geometric shapes (Utermöhl 1958). The biovolume was then converted into carbon content using the formula of Rocha and Duncan (1985). For the 26D9-Z⁺ and 26D9-Z^N-treatment, we also determined the biomass of *C. raciborskii* on day 18 to determine the day of disappearance (see below). The length of the filaments of strain 26D9 was measured again at the end of the experiment in the treatment where *C. raciborskii* persisted (n = 30 in each replicate) using an inverted microscope, (Zeiss Axiovert, Jena). The zooplankton biomass at the beginning was determined after fixation with Lugol's iodine using an inverted microscope (Thalheim Optik, Jena) and size of the animals was converted into carbon content using formulas of Telesh et al. (1998) for rotifers and Bottrell et al. (1976) and Watkins et al. (2011) for crustaceans. The experiment was terminated after 21 days, when a mass development of the diatom *Navicula* sp. occurred. Since *Navicula* sp. is a typical benthic form and not a representative member of the plankton community, we assume it first grew on the wall/bottom of the flasks and was then mixed into the water. A similar observation was made in lake enclosures after ca. 10 days (Weithoff et al. 2000). We regard this as an artefact and present the data for day 21 without them.

To compare the phytoplankton assemblages, we performed non-metric multi-dimensional scaling (NMDS) using the function metaMDS in R. NMDS is a non-parametric (using the rank order of elements) ordination technique that is a mathematic-statistical tool to display similarities among n numbers of samples in a multi-dimensional space. We used the biomass data for each species and treatment as input data (double-standardized, Wisconsin-standardisation). To better visualize and compare between treatments, we calculated 95% confidence ellipsoids. Using this technique, we can compare all treatments and in particular the treatments with the same ambient zooplankton densities that differ in the temporary presence of *C. raciborskii* i.e. it is possible to detect an effect of a failed invasion on the resident community. To compare total phytoplankton biomass among treatments at the end of the experiment, the absolute biomass of *C. raciborskii*, and its relative contribution to total phytoplankton, were subject to the non-parametric Kruskal-Wallis test, because of inhomogeneity of variances.

Results

The conditions in the lake on the day of sampling can be characterized as follows: the water column was stratified, exhibiting a thermocline at 5 m depth. In the epilimnion, the temperature was 22 °C, oxygen concentration was 8 mg L⁻¹ and the pH was 8.26. Below 5 m to the bottom at 6.85 m, temperature decreased to 16 °C, O₂-concentration to 3 mg L⁻¹ and pH to 7.5. The theoretical euphotic depth, calculated as 1% of surface irradiation was at 7 m. Epilimnetic total phosphorus concentration was 84 µg L⁻¹ and chlorophyll-*a* concentration was 12 µg L⁻¹.

The zooplankton community was made up of roughly equal amounts of rotifers (most abundant species, in descending order of biomass: *Pompholyx sulcata*, *Keratella cochlearis*, *Trichocerca* spp. and *Gastropus* sp.), nauplii and larger zooplankton (*Daphnia galeata/cucullata* complex and *Acanthocyclops* sp.) (Figure 1, top). The removal of the larger zooplankton by 100 µm mesh worked well for the larger individuals since they were absent after filtering, the small ones (rotifers and nauplii) remained unaffected. The phytoplankton community was dominated by cryptophytes (*Cryptomonas* sp.). Dinophytes (*Peridinium* sp. and *Ceratium hirundinella*) were subdominant and of lesser importance were chlorophyceae, prymnesiophyceae, synurophyceae and bacillariophyceae (Figure 1, bottom). Only very few cyanobacteria filaments were found, belonging to the genera *Anabaena*, *Aphanizomenon* and *Limnothrix*; neither *Cylindrospermopsis* nor *Microcystis* were detected. The zooplankton manipulation had a negligible direct effect on phytoplankton composition, as total biomass and relative contribution of the main groups were very similar among treatments (Figure 1, bottom).

Two out of the three *C. raciborskii* genotypes were not able to invade the mesocosms. They disappeared within a few days after addition. The test of whether the inoculum survives the transfer from artificial medium into filtered lake water showed that the two genotypes were also not viable in filtered lake water. Thus, the invasion failure holds without any biological interactions. Only genotype 26D9 persisted and only in the Z⁻-treatment. Its average filament size increased to 239 ± 221 µm (SD). Over the time course of the experiment, phytoplankton biomass declined and the composition changed and differed among treatments. The highest phytoplankton biomass was found in the Z⁻-treatments (Figure 2). After a considerable decline within the first three days, the biomass of *C. raciborskii* diverged between treatments and only in the Z⁻-treatment was establishment of *C. raciborskii* at a low level found

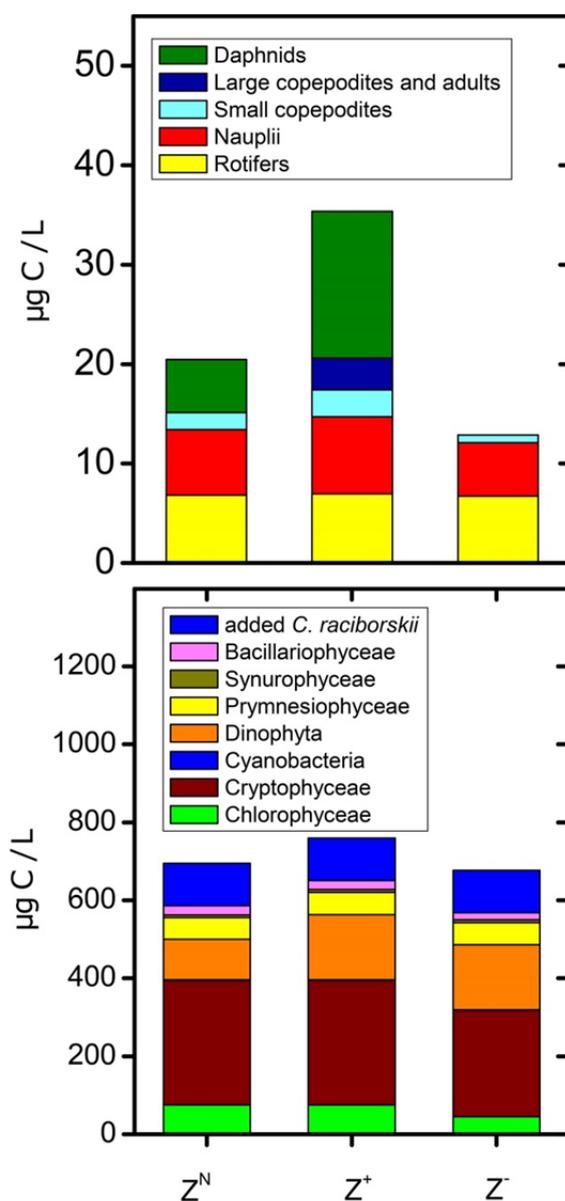


Figure 1. Biomass and composition of zooplankton (top) and phytoplankton (bottom) at the beginning of the experiment. Note, the Control and the Z^N-treatment differ only in the addition of *C. raciborskii*.

(Figure 3, $p = 0.02$, Kruskal-Wallis test). Comparing the phytoplankton community structure using NMDS, it became apparent that the four treatments continuously diverged according to the different zooplankton levels (Figure 4) and the individual flasks from each treatment formed separate clusters at the end of the experiment.

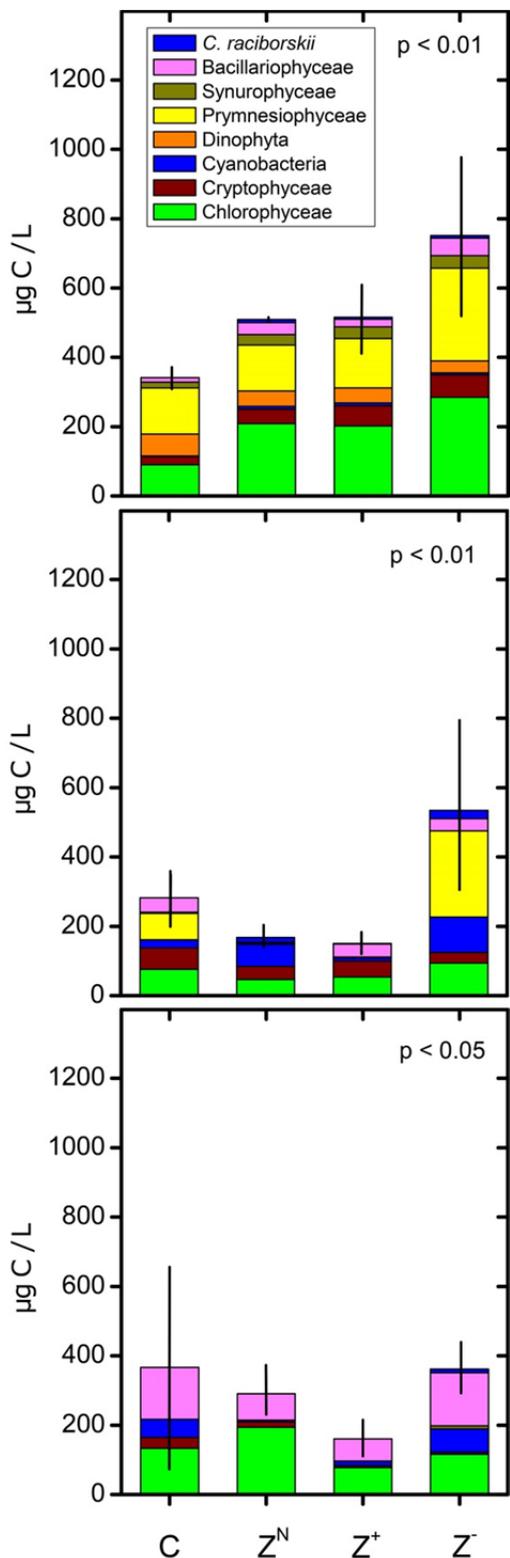


Figure 2. Phytoplankton biomass and composition at day 3 (top), 12 (middle) and 21 (bottom) from the treatments with the strain 26D9 and the control. Mean values of four replicates, error bar denote standard deviation of the total biomass, p values from nonparametric Kruskal-Wallis test.

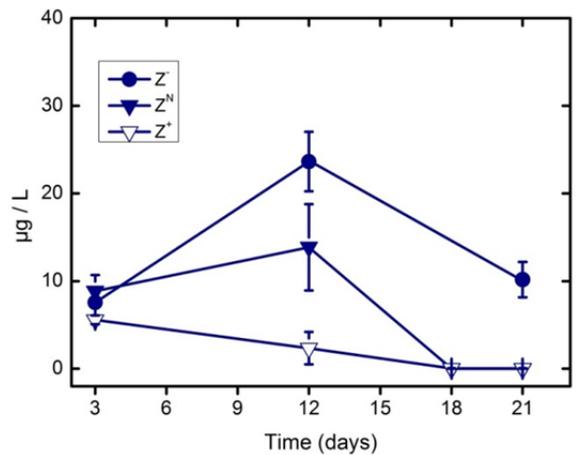


Figure 3. Time course of the biomass of *C. raciborskii* strain 26D9 at day 3, 12 and 21 after addition (mean, ± standard error).

Discussion

Transfer effects

Two out of three genotypes did not withstand the transfer into the lake water, whether this was sterile filtered water or included the whole plankton community. There are two potential reasons. The chemical composition of the water itself may have been harmful to the two genotypes causing cell death. Although theoretically possible, we do not believe this is a reasonable explanation. Lake Glindower See is part of a larger watershed without any geological or geochemical peculiarities. The pH at the day of sampling was 8.26, close to the pH of the stock culture and way below the upper end of the suitable pH range of cyanobacteria. The more likely explanation is that the transfer itself caused harm to *C. raciborskii* simply because of an abrupt change in environmental conditions. The cells were probably not able to adapt fast enough to this change. A gradual stepwise exchange of medium through lake water weeks prior to the experiment might have overcome the rapid disappearance of *C. raciborskii* after addition to lake water. Twenty months after this experiment such a stepwise procedure was successfully used to culture all genotypes in filtered lake water. However, when spreading into new geographic areas, changes in water characteristics from one lake to another likely occur and coping with such changes acts as a selective force in invasion success.

Food web and grazing impact

A common notion is that zooplankton, and crustacean plankton in particular, cannot control cyanobacteria blooms (Lampert 1987; Ger et al. 2014).

Instead they themselves are negatively affected by cyanobacteria because of the filamentous or colonial organisation which strongly reduces ingestability, the potential for toxin production, or because of low nutritional value e.g. the lack of essential polyunsaturated fatty acids (Ger et al. 2014). However, when community grazing pressure is high and the biomass of cyanobacteria is low, top-down control may be possible. A number of studies have shown that diverse zooplankton species are able to ingest *C. raciborskii* e.g. copepods (Rangel et al. 2016), daphnids (Soares et al. 2009b; Panosso and Lüring 2010) and rotifers (Soares et al. 2010; Sperfeld et al. 2010). Direct comparisons of the susceptibility to zooplankton grazing between *C. raciborskii* and other nostoclean cyanobacteria are rare, but it appears that *C. raciborskii* is as susceptible, or slightly more susceptible, to grazing than other cyanobacteria (Panosso et al. 2003; Kâ et al. 2012). In our experiments, *C. raciborskii* only successfully invaded the lake water community without larger crustacean zooplankton. The rotifer density at the time of the inoculum of *C. raciborskii* was similar in all treatments. Thus, only after release from predation by larger crustaceans was *C. raciborskii* able to compete with the resident community.

The experimental set-up, in particular the preparation of the different zooplankton treatments, focusses mainly on the starting conditions. Whereas the removal of the large zooplankters was fully successful, i.e. no large specimens passed the mesh; the doubling of large but rare crustaceans bears some inaccuracies due to stochastic uncertainties, so that in some treatments large specimens were perhaps under- or overrepresented. During the subsequent, regular sampling process, few large individuals might have been removed disproportionately. Thus, the main differences between the zooplankton treatments occurred at the beginning and potentially levelled off towards the end of the experiment. Therefore, the differences in invasion success in our experiment are most likely because of the different initial conditions.

Taking the dilution rate of 18.75% every three days (150 mL sample from 800 mL volume) into account, the more or less constant biomass of *C. raciborskii* from day 3 to day 21 is a result of positive growth. Comparing *C. raciborskii* biomass with the biomass of other filamentous cyanobacteria from laboratory experiments, an inhibition of zooplankton in our experiment is very unlikely: the biomass in our experiment was much lower than that in the experiments of Gliwicz (1990) and Dawidowicz et al. (1988) and below a level where negative effects on *Daphnia* growth were detected. However, the critical cyanobacteria biomass for zooplankton is most likely

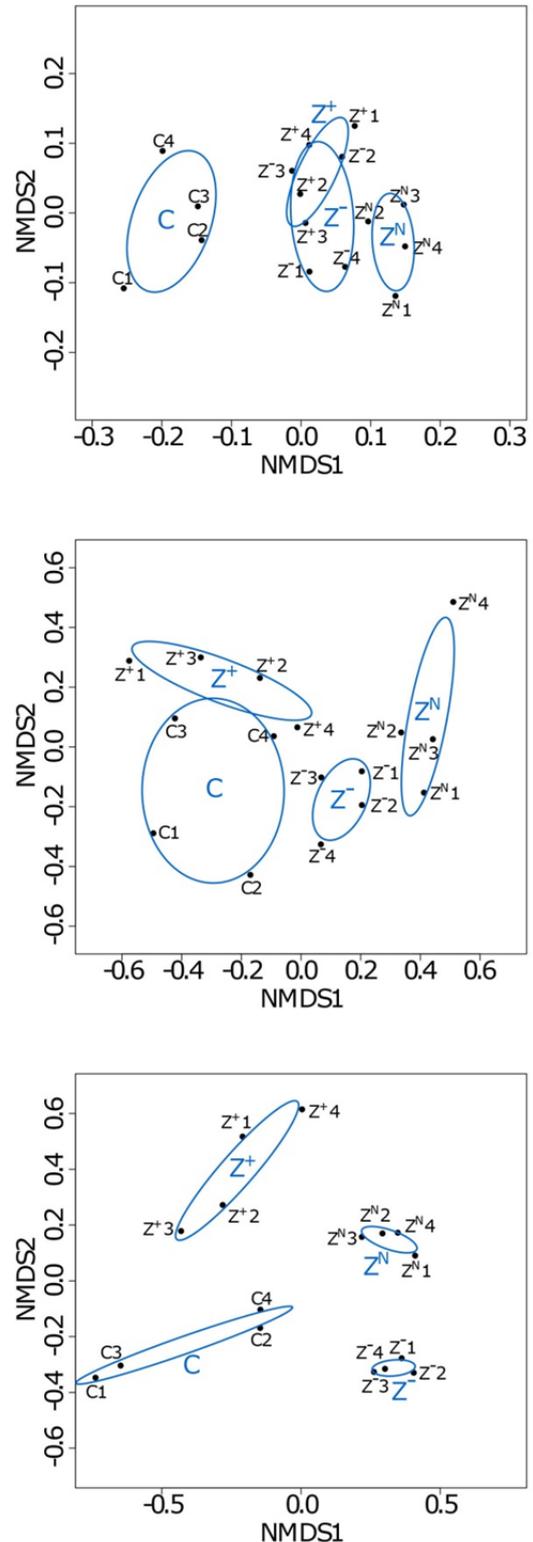


Figure 4. Ordination of all 16 individual mesocosms based on the phytoplankton biomass using non-metric multi-dimensional scaling (NMDS). Day 3 (top), day 12 (middle) and day 21 (bottom) from the treatments with 26D9 and the control. The ellipsoids indicate the 95% confidence area.

species- or even strain- specific and both cyanobacteria and their consumer have the potential for adaptation (Ger et al. 2016). Shorter filaments can be grazed more efficiently than longer ones (Rangel et al. 2016). In the Z-treatment, *C. raciborskii* had longer filaments at the end than in the beginning, although almost the whole spectrum of lengths was represented. This increase in average filament length might be due to slightly selective grazing on shorter filaments or on physiological changes over the three-week experimental period (e.g. Soares et al. 2013, Yamamoto and Shiah 2014). Field data for *C. raciborskii* and zooplankton suggest a complex relationship. In a tropical reservoir, high biomass of *C. raciborskii* was accompanied by high biomass of rotifers and copepods; the latter have been shown to break filaments during feeding activities making them accessible for other plankters (Bouvy et al. 2001). However, in another tropical reservoir, copepods and *Daphnia gessneri* increased after development of *C. raciborskii* (Soares et al. 2009b). These results and other studies on zooplankton-cyanobacteria interactions suggest that at low cyanobacteria biomass and sufficient edible quality food, zooplankton such as daphnids and rotifers (Weithoff and Walz 1995; Soares et al. 2009b) may use both food sources for substantial growth.

Competition

In general, when top-down control is low, bottom-up forces such as exploitative competition gain in importance. Our results suggest that *C. raciborskii* has a higher chance of invading plankton communities when grazing pressure is low. Either a high growth rate or a high nutrient uptake rate is necessary to outcompete other species. The growth rates of *C. raciborskii* in general (e.g. Istvánovics et al. 2000; Soares et al. 2013; Yamamoto and Shiah 2014) and the three genotypes used in our experiment (Mehnert et al. 2010; Bolijs et al. *in press*) are not extraordinarily high. They compare with those measured from other filamentous cyanobacteria with a tendency for comparably higher growth of *C. raciborskii* at higher temperatures (> 25 °C). Compared to growth rates for e.g. *Cryptomonas* spp., the dominant species at the start of the experiment, *C. raciborskii* growth rates are low (Gervais 1997). Cryptophytes are typically abundant in temperate lakes and exhibit peak abundances in transient environmental conditions (Stewart and Wetzel 1986) and/or when nutrient pulses occur (Weithoff et al. 2000, 2001), but they suffer from herbivory, even by small species such as rotifers. In our study they disappeared towards the end of the experiment and

were replaced by less edible chlorophytes and diatoms. An alternative advantageous strategy is to take up nutrients faster than competitors and thus monopolize the nutrients for later growth (Schmidtke et al. 2010). A high phosphorus uptake affinity has been shown for *C. raciborskii* (Istvánovics et al. 2000) and this might be key to its invasion success. A high storage capacity compensates for lower growth rates compared to competitors and allows for prolonged growth phases under nutrient depleted conditions. Since we have no data on the concentrations of available nutrients over the course of the experiment, we may only speculate about the role of exploitative competition for the invasion of *C. raciborskii* in our experiment. However, with the high total phosphorus level in the experimental lake water and the comparably low total phytoplankton biomass and chlorophyll concentration, the competitive abilities of *C. raciborskii* might have been of minor importance.

Consumptive vs competitive resistance

Native communities can prevent biological invasions either through competitive or consumptive resistance, or a combination of both. For freshwater systems, consumptive resistance has been found to be more important than competitive resistance (Alofs and Jackson 2014), potentially because trophic cascades are stronger in aquatic systems than in terrestrial ones, and in particular because top-down control of producer biomass is stronger (Cyr and Pace 1993). This might be due to a lower proportion of wooden biomass in aquatic system than in terrestrial. According to Alofs and Jackson (2014), for aquatic plants, arthropods and molluscs, consumptive resistance was found. In our experiments too, consumptive resistance appeared to be the driving factor, since only the release of grazing pressure from large crustaceans allowed *C. raciborskii* persistence. Overall, the relative share of the invading *C. raciborskii* to total phytoplankton was rather low, and fast growth or replacement of resident species cannot be expected after a shift to a new biotic and abiotic environment within the short time frame of three weeks. One may only speculate about long-term success, but persistence over three weeks, despite regular dilution, exhibits at least the potential for long-term establishment. When communities are resistant to invasions, they are not necessarily unaffected by the temporary occurrence of an invader. Case (1995) demonstrated in a modelling study that a resident community can be temporarily invaded by a species that is outcompeted some time afterwards. The temporary presence, however, changed the environmental/competitive conditions in such a way that after the

disappearance of the invader the resident community was changed (i.e. a legacy effect) and species might even go extinct. The resident phytoplankton communities from the control and the Z^N -treatment (same zooplankton densities) at the end of our experiment differed, revealed by the NMDS, although *C. raciborskii* had gone extinct. Since the NMDS is a non-parametric method, ranks of abundance instead of absolute values are used to calculate the ordination of the samples. Along NMDS-axis 1, the rank of larger flagellates such as *Cryptomonas* and *Chlamydomonas* tended to decrease and along NMDS-axis 2 the rank of filamentous forms and pennate diatoms tended to increase, leading to the separation of the four treatments. With our multi-species experiment, we cannot pinpoint the underlying mechanism, but we hypothesize that such a legacy effect exists. To conclude, we found support for both of our hypotheses: a) the invasion success of *C. raciborskii* was strain-dependent, and b) invasion success was driven by the degree of herbivory. *C. raciborskii* benefitted from grazing release.

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