Priority effects in experimental populations of the cyanobacterium *Microcystis*

Ineke van Gremberghe,^{1*} Pieter Vanormelingen,¹
Katleen Van der Gucht,¹ Caroline Souffreau,¹
Wim Vyverman¹ and Luc De Meester²

¹Laboratory of Protistology and Aquatic Ecology, Ghent University, Krijgslaan 281 – S8, 9000 Ghent, Belgium.

²Laboratory of Aquatic Ecology and Evolutionary Biology, Katholieke Universiteit Leuven, Charles DeBériotstraat 32, 3000 Leuven, Belgium.

Summary

The arrival order of colonists in developing populations can have a lasting influence on community and population structure, a phenomenon referred to as priority effects. To explore whether such priority effects are important in determining strain composition of populations of the cyanobacterium *Microcystis*, four *Microcystis* strains, isolated from a single lake and differing in functional traits, were grown during 4 weeks in the laboratory in all possible pairwise combinations, with the two strains either inoculated at the same time or with a time lag of 1 week, in the presence or absence of grazing *Daphnia magna*. The relative abundance of strains in the mixtures was assessed using denaturing gradient gel electrophoresis, and the growth rate of each strain in the mixtures was determined for the last 2 weeks of the experiment. We observed strong effects of inoculation order on the final population structure, and these effects were influenced by grazing *Daphnia*. The priority effects were strain-specific and occurred in two directions: some of the strains grew slower while others grew faster when inoculated second compared with when inoculated first. Our results indicate that priority effects may have a profound impact on strain composition of *Microcystis* populations.

Introduction

Community assembly is determined by dispersal constraints, environmental constraints (abiotic factors) and internal dynamics (competition, mutualism, predation and

parasitism) (Belyea and Lancaster, 1999). To become a dominant member of a community, species have to first colonize the habitat and establish themselves, which requires that they are sufficiently well adapted to the local environmental conditions and can deal with the resident community (Leibold *et al.*, 2004; Ricklefs, 2004). Not all species reach a particular habitat at the same time. More specifically, the order of arrival is determined by the regional abundance of the different species, their dispersal capacities, and chance. Lasting effects of the order of arrival of colonists on community structure are termed priority effects (Drake, 1991; Beisner *et al.*, 2003; Schröder *et al.*, 2005). Two mutually non-exclusive mechanisms may be responsible for such priority effects. The first is a purely numerical effect as later colonists have a disadvantage in numbers compared with the first colonists. Carrying capacity may be reached as a consequence of rapid population growth before later immigrants can become abundant, as is exemplified by initial soil exploration and root biomass formation in plants (Körner *et al.*, 2008). Second, early colonists can also alter the environment in a favourable or detrimental way for later colonists. For instance, shading by growing plants can inhibit germination of seeds (Harper, 1961), but can also facilitate the growth of seedlings in semiarid environments by protection against drought, which is often indicated as the 'nurse plant syndrome' (Callaway and Walker, 1997; Maestre *et al.*, 2001; 2003; Brooker *et al.*, 2008). Additionally, unpalatable plants can protect seedlings from herbivory (Brooker *et al.*, 2008).

The occurrence of priority effects, which can be very strong, has been observed in a variety of taxa ranging from protists to plants, insects and amphibians (Morin, 1984; Alford and Wilbur, 1985; Robinson and Dickerson, 1987; Drake, 1991; Blaustein and Margalit, 1996; Sunahara and Mogi, 2002; Louette and De Meester, 2007; Zhang and Zhang, 2007; Körner *et al.*, 2008). In addition, as local conditions may influence competitive interactions among species, they may impact the strength and direction of priority effects. For instance, in aquatic communities, the presence of predators has a large influence on community composition and has been shown to alter priority effects (Morin, 1984; 1999; Louette and De Meester, 2007).

The above arguments on the importance of priority effects for community composition may also hold at the

Received 27 February, 2009; accepted 27 May, 2009. *For correspondence. E-mail Ineke.vanGremberghe@UGent.be; Tel. (+32) 9264 85 42; Fax (+32) 9264 85 99. The work was conducted in the Laboratory of Protistology and Aquatic Ecology, Ghent University, Krijgslaan 281 – S8, 9000 Ghent, Belgium.

population level. To the extent that genotypes differ in ecologically important traits, priority effects consisting of both numerical and ecological mechanisms may play a role in populations too, with the strength and direction depending on the local environment. This would add an additional historical component to population structure. Populations of prokaryotes are often characterized by the presence of (functionally) highly divergent strains and differentiation in lineage composition between populations has been attributed to environmental factors (Bronikowski *et al.*, 2001; Bhaya *et al.*, 2007) or geographic isolation (DeChaine *et al.*, 2006). Here, we experimentally investigated whether, in addition to these factors, colonization order might determine the resulting strain composition of prokaryote populations (Fukami *et al.*, 2007).

Microcystis is a common and cosmopolitan cyanobacterium that forms toxic blooms during warm weather conditions in eutrophic water bodies (Chorus and Bartram, 1999; Visser *et al.*, 2005). Naturally occurring *Microcystis* bloom populations are generally composed of several genotypes with different functional traits including growth rate, toxicity and colony morphology (Wilson *et al.*, 2005; 2006; Bañares-España *et al.*, 2006; Kardinaal *et al.*, 2007a). The toxicity of a *Microcystis* bloom is to an important extent determined by the production of the heptapeptide microcystin, which is a potent toxin for eukaryotes, non-ribosomally synthesized by a multifunctional enzyme complex (Börner and Dittmann, 2005). The gene cluster coding for the microcystin synthetase complex consists of 10 microcystin synthetase genes (*mcy*-genes; Tillett *et al.*, 2000). The toxin concentration of a *Microcystis* bloom depends on the strain composition, as not all *Microcystis* strains possess these *mcy*-genes (Janse *et al.*, 2004; Kardinaal *et al.*, 2007a). Consequently, strain composition of a *Microcystis* bloom is expected to have important consequences for other components of the food web through its effect on bloom toxicity (Hansson *et al.*, 2007). Kardinaal and colleagues (2007b) have shown that non-toxic *Microcystis* strains seem to be better competitors for light than toxic *Microcystis* strains. The ability of *Microcystis* to form colonies, which are generally formed by cells clustering together in a mucilaginous sheet, entails advantages such as protection against predation (Fulton and Paerl, 1987), the ability to float and shade unicellular strains, a higher photosynthetic efficiency, and enhanced stress tolerance compared with unicellular strains (Zhang *et al.*, 2007; Wu and Song, 2008). In general, *Microcystis* blooms differ strongly in rDNA ITS genotype composition from each other (Janse *et al.*, 2004; Kardinaal *et al.*, 2007a; I. van Gremberghe *et al.*, unpublished). Little is known about the mechanisms through which this diversity, both within and between lakes, is maintained, although competitive interactions depending on functional traits and grazer-mediated facilitation are likely involved (van

Gremberghe *et al.*, 2009). However, priority effects may also play a role. They may be important during *Microcystis* population assembly in new habitats as well as in seasonal bloom populations that build up each year when conditions become favourable in spring or summer (Ver-spagen *et al.*, 2004).

The aim of the present study was to experimentally test the hypothesis that priority effects among functionally different *Microcystis* strains can influence the strain composition of *Microcystis* populations. Therefore, we investigated priority effects among four naturally co-occurring *Microcystis* strains that differ in growth rate, microcystin production and colony formation in experimental two-strain populations by manipulating the inoculation order of the strains, and determining final population structure and growth rate of the strains. We also tested whether the presence of a grazer changes the strength and direction of priority effects by including a treatment with the large-bodied zooplankton species *Daphnia magna*. Finally, we verified whether the interactions and priority effects observed are in concordance with predictions based on trait differences. Largely based on the observations of a previous experiment in which strain characteristics were studied in monocultures and in competition trials with simultaneous inoculation (van Gremberghe *et al.*, in press), we predicted that first inoculation of a particular strain would not systematically result in dominance of that strain at the end of the experiment. We also predicted that in the presence of *Daphnia*, non-toxic strains may benefit from being inoculated simultaneously with or second to a toxic strain, as they are expected to be facilitated by the protection offered by the toxins produced by their competitors (Rohrlack *et al.*, 1999a). A similar mechanism might work for unicellular versus colonial strains, as colonies hinder *Daphnia* grazing (Rohrlack *et al.*, 1999b).

Results

Detailed information on the growth rate, microcystin production and colony formation of the four *Microcystis* strains is given in van Gremberghe and colleagues (in press), but a summary is also given in the experimental procedures. T1 and T2 are toxic strains (they produce microcystins), whereas NT1 and NT2 are non-toxic. NT2 and T2 form colonies, with NT2 only doing so in the presence of grazers.

Figure 1 shows the relative abundances of the strains after 4 weeks of growth. Pronounced differences in strain composition in relation to inoculation order were often present, with strains generally having a higher relative abundance when inoculated first in the absence of grazing. This positive effect was, however, often reduced in the presence of *Daphnia*. When inoculated at the same

Fig. 1. The relative abundances of the four *Microcystis* strains in the mixed populations as determined by DGGE after 4 weeks of growth. T = toxic strain, NT = non-toxic strain. (0): the two strains inoculated at the same time; (1): the upper strain inoculated first and (2): the upper strain inoculated second in the absence or presence of predation. Error bars indicate the standard error of the mean ($n = 3$). Asterisks above the bars of the (1) or (2) treatment indicate significant differences in relative abundance compared with the (0) treatment, and asterisks between bars indicate significant differences between the relative abundances of the (1) and (2) treatments (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

time, strain T1 was generally a weaker competitor than the other strains. However, when T1 was inoculated first, it became dominant or co-dominant at the end of the experiment in both predation treatments. When inoculated second in the absence of grazers, T1 was almost outcompeted in most mixtures. In contrast, in the presence of grazers this strain became dominant when inoculated after NT1 and co-dominant when inoculated after NT2 (Fig. 1). T2 was generally a good competitor when

inoculated at the same time with a competing strain, and when inoculated first, it strongly dominated the final population. Even when inoculated after T1 or NT2, T2 was dominant to co-dominant at the end of the experiment. However, when inoculated after NT1, T2 was only dominant in the presence of predation (Fig. 1). NT1 benefited from being inoculated first in the absence of predation in combination with NT2, and dominated the final population in these treatments. It was, however, largely outcompeted

Table 1. ANOVA table on the effects of inoculation order, competing strain and predation treatment on the growth rate (day 14 to day 28) of the four studied *Microcystis* strains in mixed populations.

	d.f.	Mean square	F	P	Post hoc inoculation	NP	P
Effect T1							
Inoculation order (I)	1	0.248	141.670	< 0.001	T2	0.435	0.033
Competing strain (C)	2	0.237	135.481	< 0.001	NT1	< 0.001	0.028
Predation (P)	1	0.357	204.343	< 0.001	NT2	0.023	0.047
I*C	2	0.278	159.120	< 0.001			
I*P	1	0.527	301.669	< 0.001			
C*P	2	0.307	175.889	< 0.001			
I*C*P	2	0.284	162.231	< 0.001			
Effect T2							
Inoculation order (I)	1	0.007	6.360	0.019	T1	0.188	0.021
Competing strain (C)	2	0.002	1.380	0.271	NT1	0.403	0.136
Predation (P)	1	0.007	6.575	0.017	NT2	0.465	0.495
I*C	2	0.002	1.928	0.167			
I*P	1	0.002	1.759	0.197			
C*P	2	0.001	1.096	0.350			
I*C*P	2	7.66E-5	0.070	0.932			
Effect NT1							
Inoculation order (I)	1	0.329	377.165	< 0.001	T1	0.224	0.069
Competing strain (C)	2	0.981	1122.758	< 0.001	T2	0.023	< 0.001
Predation (P)	1	0.174	198.729	< 0.001	NT2	< 0.001	< 0.001
I*C	2	1.765	2020.700	< 0.001			
I*P	1	0.265	303.738	< 0.001			
C*P	2	0.196	223.865	< 0.001			
I*C*P	2	0.157	179.440	< 0.001			
Effect NT2							
Inoculation order (I)	1	0.033	15.407	0.001	T1	0.827	0.115
Competing strain (C)	2	0.209	97.905	< 0.001	T2	0.890	< 0.001
Predation (P)	1	0.023	10.850	0.003	NT1	0.051	< 0.001
I*C	2	0.130	60.793	< 0.001			
I*P	1	0.008	3.813	0.063			
C*P	2	0.105	49.289	< 0.001			
I*C*P	2	0.177	82.960	< 0.001			

T = toxic strain, NT = non-toxic strain. Post hoc LSD tests for inoculation order are presented (*P*-values) separately for each competing strain in the absence of predation (NP) and the presence of predation (P). Significant *P*-values are in bold.

when inoculated after NT2. In the presence of predation, NT1 was outcompeted by NT2 in all inoculation order treatments (Fig. 1).

As the relative abundances of the strains at the end of the experiment might in part reflect differences in the amount of time the strains could grow, we also calculated the growth rate for each strain in the mixed populations from day 14 to day 28, and determined that priority effects occur when there is a significant inoculation order effect on growth rate, following Louette and De Meester (2007) (Table 1, Fig. 2). Our experiment revealed significant priority effects in 12 out of 24 tests. In some cases, the priority effect was very strong, from a clear positive to a clear negative growth (T1 in combination with NT1 and NT1 in combination with NT2 in the absence of grazing; NT1 in combination with NT2 and NT2 in combination with T2 in the presence of grazing) or vice versa (NT1 in combination with T2 in the presence of grazing). In most of these cases, arriving first or second made the difference between dominating the final population or being outcompeted. We observed negative as well as positive priority effects in the mixed populations. Significant nega-

tive effects of the first colonizers on the later arrivals were detected in five out of 24 tests (three in the absence of predation and two in the presence of predation), whereas positive effects of the first on the second colonizers were detected in seven cases. Most of these facilitative interactions (six out of seven cases) occurred in the presence of grazers. Below, we discuss the observed priority effects in detail for each strain separately (Table 1, Fig. 2).

For T1, a significant effect of inoculation order, competing strain and predation treatment on the growth rate was detected as well as significant interaction effects between these factors (Table 1). Inoculation order significantly influenced growth rate of T1 in five out of six mixtures. The growth rate of T1 was significantly lower when inoculated after NT1 or NT2 compared with when inoculated before these strains in the absence of predation, to such an extent that the growth rate of T1 was negative when inoculated after NT1, and zero when inoculated after NT2. In the presence of predation, the growth rate of T1 was higher when inoculated second compared with when inoculated first in all three combinations (Table 1, Fig. 2). For T2, there was a significant effect of inoculation order

Fig. 2. The growth rate of the four *Microcystis* strains from day 14 to day 28 in the mixed populations. T = toxic strain, NT = non-toxic strain. The competing strain is indicated on the x-axis, the first bar indicates the growth rate when the strain is inoculated before the competing strain, the second bar indicates the growth rate when the strain is inoculated after the competing strain. Growth rates in the absence of predation are in black and growth rates in the presence of predation in grey. Error bars indicate the standard error of the mean ($n = 3$). Asterisks indicate a significant effect of inoculation order on the growth rate (* $P < 0.05$; *** $P < 0.001$).

and predation treatment on growth rate (Table 1). The growth rate of T2 was significantly higher when inoculated after T1 compared with when inoculated before T1 in the presence of predation (Table 1, Fig. 2). For NT1, a significant effect of inoculation order, competing strain and predation treatment on growth rate was found as well as significant interaction effects between these factors (Table 1). Inoculation order significantly influenced growth rate of NT1 in four out of six mixtures. The growth rate of NT1 was higher when inoculated after T2 compared with when inoculated before T2. This was especially clear in the presence of grazing, where the growth rate of NT1 was negative when inoculated before T2. When inoculated after NT2, the growth rate of NT1 was lower (even negative) compared with when inoculated before NT2 in both the absence and presence of grazing (Table 1, Fig. 2). Also for NT2, a significant effect of inoculation order, competing strain and predation treatment on growth rate was detected as well as significant interaction effects between the factors, except for the interaction between inoculation order and predation treatment

(Table 1). Inoculation order significantly influenced growth rate of NT2 in two out of six mixtures. In the presence of predation, the growth rate of NT2 was lower (even negative) when inoculated after T2 compared with when inoculated before T2, whereas the growth rate of NT2 was higher when inoculated after NT1 compared with when inoculated before NT1 (Table 1, Fig. 2).

Discussion

Our results highlight the importance of priority effects in determining strain composition of *Microcystis* populations. Inoculation order was found to be important in determining growth rate in half of the mixtures. A time lag of just 1 week had pronounced qualitative implications. The observation that growth rates are negative when inoculated second (four cases) or first (one case) clearly indicates that the observed priority effects have long-lasting consequences and the dominance patterns of the competing strains in these mixtures are not a temporary phenomenon. These observations are in concordance with a

growing number of studies that detect important priority effects in a wide range of taxa (e.g. Drake, 1991; Shorrocks and Bingley, 1994; Fukami *et al.*, 2007; Louette and De Meester, 2007; Körner *et al.*, 2008). Strikingly, we observed as many positive as negative priority effects. In agreement with the observations of Kardinaal and colleagues (2007b), this implies that a numerical advantage of a *Microcystis* strain does not systematically result in the final dominance of that strain. Overall, negative effects were very strong, involving a shift from positive to negative or zero growth in all cases, whereas positive effects were weaker except in one case. Thus, positive effects of arriving after a time lag of 1 week are common, but tend to have less impact than negative effects associated with being second. Most priority effects reported in literature are negative (e.g. Drake, 1991; Shorrocks and Bingley, 1994; Louette and De Meester, 2007; Körner *et al.*, 2008). However, some studies on communities of amphibians (Alford and Wilbur, 1985) and coral reef fish (Almany, 2003) also detected a facilitative effect of first colonizing taxa on later colonizing taxa. In plant communities, resident plants can also protect seedlings from drought (Maestre *et al.*, 2001; 2003; Brooker *et al.*, 2008) and taxa arriving first may protect others arriving later against herbivory (Brooker *et al.*, 2008).

The population structure of the different mixtures observed at the end of the experiment is largely in line with a priori predictions based on information on toxin production and colony formation combined with their impact on competitive strength (see also van Gremberghe *et al.*, in press). When the two toxic strains are combined, T2 dominates when inoculated first and T1 takes little advantage of being inoculated before T2. This is likely the result of T2 producing colonies, giving it an additional competitive advantage over T1 when it is inoculated first. When the two non-toxic strains are combined in the absence of predation, a similar asymmetry is seen: when inoculated together, NT1 is a better competitor than NT2, but NT2 takes more advantage of being first. In the presence of predation, NT2 always dominates because it is better protected against grazing thanks to colony formation. Here we see a striking facilitation effect: NT1 is not outcompeted when inoculated after NT2, in contrast to when inoculated before NT2. In the presence of grazing, it is disadvantageous for NT1 to arrive before NT2 compared with arriving together or after NT2, because it is readily eaten in the absence of NT2 and can take profit of the protection offered by NT2 colonies hindering grazing. In the presence of predation, it seems that it is better for non-toxic strains to be inoculated simultaneously with or second to toxic strains than being the first colonizer. There is a negative effect of being first compared with the control for non-toxic strains in three (two significant for NT1 and one borderline non-significant for NT2) out of four combi-

nations, and the effect is stronger for NT1 than for NT2. The latter can be interpreted as being the result of NT2 being somewhat protected against grazing because it forms colonies. Strain NT1 does not produce toxins nor produces colonies, and performs poorly when inoculated first in the presence of grazers. However, when inoculated first in the absence of grazers, NT1 outcompeted T1, probably because the non-toxic NT1 is a better competitor for light than the toxic T1 (Kardinaal *et al.*, 2007b). In the case of T2 and NT2, there is a strong bias towards dominance of T2 in the presence and absence of predation, a pattern that we cannot explain from our knowledge on trait variation among these strains, and possibly reflects interactions through additional functional traits of these strains, such as the production of allelopathic substances (Oberhaus *et al.*, 2008). Overall, priority effects are stronger when colony forming strains are inoculated before and weaker when these strains are inoculated after unicellular strains because of the added competitive advantage offered by colony formation. For microcystin production, the opposite pattern was seen. This is in accordance with Kardinaal and colleagues (2007b), who showed that the non-toxic strains they studied were better competitors for light than the toxic strains. Furthermore, the cost of being inoculated second seems lower for toxic or colony forming strains in the presence of grazing than in the absence of grazing when they are combined with strains that are vulnerable to grazing (non-toxic, unicellular strains). Finally, in the presence of grazers, non-toxic or unicellular strains benefit from being inoculated simultaneously or second rather than prior to a toxic or colony forming strain, as they are facilitated by the protection offered by the toxins or large colonies of their competitors.

As we worked with only a few strains that all differ in combinations of traits, further experiments are needed to investigate whether the patterns we observed for these specific strains can be generalized. In addition, it is clear that other environmental factors in addition to zooplankton grazing, such as nutrient concentrations and N:P ratios, might influence the strength and direction of priority effects. There is a need for further experimental research to explore the impact of priority effects under these varying environmental conditions. Furthermore, we do not claim to have information on all traits that may be relevant to the observed interactions. It is, however, striking that the observed patterns are in line with a priori predictions based on the two key traits considered. The priority effects we observed were so strong that they may make the difference between a toxic and non-toxic *Microcystis* population. Especially in the absence of grazers, inoculation order in combinations of toxic and non-toxic strains has a pervasive effect on the degree to which toxic strains dominate in the population. Strikingly, in the presence of grazers, simultaneous colonization of toxic and non-toxic

strains leads to the lowest relative abundances of toxic strains.

Our study showed that priority effects can differ profoundly in the absence and presence of the large-bodied cladoceran *D. magna*. The mechanism by which predation impacts priority effects in our experiment is twofold. First, by reducing competitive interactions, a tendency for negative priority effects is reduced by the presence of grazers. Similarly, Morin (1984) showed that negative effects of early odonate breeders on late breeders occurred only in the absence and not in the presence of fish. Predators can eliminate competitive priority effects by reducing the abundance of preceding species and increasing resource levels for late arrivals and/or reducing the abundance of late species and lowering the demand for remaining resources (Morin, 1987). Louette and De Meester (2007) also showed the importance of the presence of a predator on the occurrence of priority effects in experiments with three large cladoceran zooplankton species. Priority effects were reduced or alternative priority effects occurred due to selective predation that influenced competitive interactions between the cladoceran species. The second mechanism by which grazing *Daphnia* in our experiment caused strong inoculation order effects is by their strong impact on strains that are not protected against grazing when they are inoculated first. The fact that unprotected *Microcystis* strains perform better when inoculated simultaneously with protected strains than when inoculated first, is similar to the protection against herbivory of palatable plants by unpalatable plants (Callaway *et al.*, 2005; Graff *et al.*, 2007; Brooker *et al.*, 2008).

In conclusion, our experiment shows pronounced and strain-specific priority effects on the strain composition in *Microcystis* populations, which are strongly influenced by grazers. Whereas the interactions among *Microcystis* strains seem to be predictable from their ecology, priority effects will introduce a strong element of stochasticity in the strain composition of natural *Microcystis* populations.

Experimental procedures

Isolation and genotypic characterization of *Microcystis* strains

As the taxonomy of *Microcystis* is still largely based on morphology and does not correspond to genetic features (Otsuka *et al.*, 2001), we used the 16S-23S rDNA ITS sequence to distinguish *Microcystis* genotypes from each other irrespective of morphospecies identification (Janse *et al.*, 2004). Four *Microcystis* rDNA ITS types (GenBank accession numbers: FJ830618–FJ830621), isolated from lake Leeuwenhof (Ghent, Belgium) and differing in important functional traits (growth rate, microcystin production and colony formation; see van Gremberghe *et al.*, in press) were used in the experiment. Strains T1 and T2 are toxic (microcystin concentration

measured by enzyme-linked immunosorbent assay in monoculture in absence of grazing in pg per ng C: 17.9 for T1 and 2.8 for T2) while NT1 and NT2 are non-toxic (no *mcys*-genes and no microcystins detected). T2 has a lower monoculture growth rate than the other three strains and forms colonies in both the absence and presence of grazing *Daphnia*. T1 and NT1 have a lower monoculture growth rate than NT2 (growth rates of strains in monoculture in absence of grazing in day⁻¹: 0.212 for T1, 0.187 for T2, 0.213 for NT1 and 0.227 for NT2). The latter strain forms only colonies in the presence of grazing while T1 and NT1 never form colonies. The two toxic strains T1 and T2 have a strongly negative influence on the survival of *D. magna* individuals, and the *Daphnia* individuals seem to graze more easily on unicellular than on colonial strains (van Gremberghe *et al.*, in press).

Experimental design

Before the start of the experiment, the four *Microcystis* strains were grown in WC medium (Guillard and Lorenzen, 1972; but without pH adjustment) at a temperature of 23°C and a light intensity of 120 µmol photons m⁻² s⁻¹ (Light Dark cycle: 12 h:12 h). Culture conditions were the same throughout the study. Exponentially growing cells were used as inocula for the experiment. The four *Microcystis* strains were grown in triplicate in all possible combinations of two strains, either inoculated at the same time or with a time lag of 1 week (both directions) in 200 ml Erlenmeyer flasks during 4 weeks. All treatments were grown with and without addition of four individuals of *D. magna*. This design resulted in a total of 6 different mixtures × 3 inoculation sequences × 2 grazing treatments × 3 replicate observations = 108 experimental units. All cultures were shaken by hand and their position randomized daily. Cultures without *D. magna* ('no predation', 'NP') were inoculated at a total density of 50 000 cells per ml, whereas the cultures with *D. magna* ('predation', 'P') were inoculated at a total density of 500 000 cells per ml. We used two different inoculation densities because a pilot experiment indicated that this resulted in comparable densities towards the end of the experiment (third and fourth week) when the interactions are expected to be the strongest and most samples were taken (see also van Gremberghe *et al.*, in press). Inoculation at low and similar densities in both predation treatments in the pilot experiment resulted in the near-extermination of the *Microcystis* populations in the grazing treatment and thus widely different densities at the end of the experiment, reflecting the strong potential of *Daphnia* to prevent *Microcystis* population development. This procedure implies that the total cell density at the end of the experiment cannot be compared between predator treatments. Yet, to the extent that densities in the final period of the experiment are similar across predation treatments, we can directly compare growth rates during that period (see below). Within each mixed population, all strains had the same initial relative abundance (two times 25 000 cells per ml for cultures without *Daphnia* and two times 250 000 cells per ml for cultures with *Daphnia*). The *Daphnia* individuals were obtained from a multiclonal population and clones were isolated from several water bodies to make sure that the observed effects of grazing represent an average effect of *Daphnia* on *Microcystis*. *Daphnia* was grown in the laboratory feeding on

Scenedesmus obliquus. Before adding *Daphnia* to the *Microcystis* cultures, they were starved for at least 3 h. During the experiment, the *Daphnia* individuals were systematically replaced by new individuals in order to keep densities the same across cultures. Replacements were done after 3 days during the first week, after 2 days during the second week and after 1 day during the last 2 weeks. The replacements were consistent across all cultures. *Daphnia* survival was low in some of the cultures, especially from the third week of the experiment onwards (see also van Gremberghe *et al.*, in press). To determine *Microcystis* cell density, a weekly subsample (1 ml) was taken from each culture and the optical density was measured immediately at 750 nm with a spectrophotometer (Shimadzu UV-1601). Samples for DNA extraction (2 ml) were taken after 2 and 4 weeks and stored at -20°C .

Sample analyses

The methods used for DNA extraction, PCR and denaturing gradient gel electrophoresis (DGGE), are described by van Gremberghe and colleagues (in press) and based on the protocol of Janse and colleagues (2003; 2004). The DGGE band intensity was used to monitor the relative abundance of the *Microcystis* strains as it was shown previously to accurately reflect relative strain abundance (van Gremberghe *et al.*, in press). The relative abundances of the strains in the populations were determined after 2 and 4 weeks of growth of the first strain. Cell densities of the different strains in the mixtures were estimated by converting optical density into cell density using standard curves in combination with the relative abundance of each strain as determined by DGGE (van Gremberghe *et al.*, in press). Based on these data, the growth rate during the last 2 weeks of the experiment was determined for each strain in the different mixtures using the formula: $[\text{Ln}(\text{cells ml}^{-1})_{t_2} - \text{Ln}(\text{cells ml}^{-1})_{t_1}] / (t_2 - t_1)$ with $t_1 = \text{day 14}$ and $t_2 = \text{day 28}$. As shown in van Gremberghe and colleagues (in press), growth curves of the monocultures using weekly density measurements showed an exponential increase of *Microcystis* cell density over the full 4 week period, confirming that the growth rate was exponential during the whole experiment. For the cultures with *Daphnia*, only the net growth rate was determined (= cell growth rate minus grazing rate).

Statistical analyses

For each strain and predation treatment separately, two-way analysis of variance (ANOVA) was used to test for significant differences in relative abundance (arcsine of square root transformed relative abundances) at the end of the experiment among mixtures that differ in inoculation order and competing strain. Post hoc least significant difference (LSD) tests adjusted by Bonferroni correction were performed for each competing strain separately to test for significant differences in relative abundances of strains inoculated at the same time and inoculated with a time lag, and between first inoculation and second inoculation. Only the results of the post hoc tests are shown.

Prior to further statistical analysis, we tested whether population densities on day 14 were similar across predation treat-

ments for the samples in which strains were inoculated with a time lag using three-way ANOVA (factors: predation, population and inoculation). As no significant main effect of predation on population density on day 14 was observed ($F = 0.592$; $P = 0.445$), we included predation as a factor in our analyses on growth rate. Removing predation from the analyses outlined below and performing ANOVA on the two predation treatments separately does not change our conclusions on the strength and direction of priority effects. For each strain separately, three-way ANOVA was used to test for significant differences in growth rate (day 14 to day 28) among mixtures that differ in inoculation order, competing strain and predation treatment. Post hoc LSD tests were performed to test for significant differences in growth rate in relation to inoculation order for each competing strain separately, in the presence and absence of predation. We conclude that (long-lasting) priority effects occur when there is a significant inoculation order effect on growth rate, indicating that early colonists influence the establishment of later arrivals (Louette and De Meester, 2007). By using growth rate data of the last 2 weeks of the experiment, we assure that differences in growth rates are not merely the result of the first colonist being inoculated in an empty habitat and thus having a better scope for growth. At that time, all strains were present in the populations for at least 1 week, and population densities were similar across treatments.

All statistical analyses were performed in SPSS 15 (SPSS, Chicago, USA).

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