The predation paradox: Synergistic and antagonistic interactions between grazing by crustacean predator and infection by cyanophages promotes bloom formation in filamentous cyanobacteria

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Abstract

In this study, we assessed the impact of synergistic/antagonistic interactions between grazing by crustacean predator (Daphnia magna) and infection by cyanophage (Vb-AphaS-CL131) on the population dynamics of the harmful bloom-forming filamentous cyanobacteria Aphanizomenon flos-aquae. We observed synergistic effect of cyanophage infection on D. magna survival and grazing trough the lysis-induced shift in cyanobacteria population structure toward the shorter filaments. However, lysis-mediated- and grazing-enhanced removal of short A. flos-aquae filaments resulted in the dominance of grazing- and infection-resistant A. flos-aquae population. In addition, the presence of D. magna generated a trait-mediated response in the A. flos-aquae population by promoting the aggregation of filaments into colony-like structures. Experiments using temperate A. flos-aquae colony isolates from natural environment demonstrated that colony-embedded A. flos-aquae filaments are insensitive to the addition of both D. magna and cyanophages. Therefore, we propose that interactions between crustacean grazers and cyanophages may promote the emergence of defensive A. flos-aquae genotypes and population traits that eventually lead to bloom formation in aquatic environments.

Predator–prey dynamics between two species is driven by consumptive and non-consumptive interactions. Through the consumptive effect predators directly contribute to prey mortality and, therefore, limit prey proliferation in natural ecosystems, while non-consumptive interactions are associated with changes in prey traits (e.g., morphology or activity) through phenotypic plasticity, and might lead to the emergence of defensive phenotypes that, in turn, change prey vulnerability to predation and increase prey survival rate (Kunert and Weisser 2003; Peckarsky et al. 2008). Therefore, the effect of a particular predator on prey population, on the one hand, and the potential of a prey population to flourish in the environment, on the other, is a function of prey phenotypic variation within the population that, in turn, emerged from complex predatory effects. However, the net outcome of the interactions between a pair of a predator and a prey can be significantly influenced by the appearance of another predator or parasite that relies on the same prey/host population via its synergistic or antagonistic effects (Singh et al. 2004; Friman and Buckling 2012). How such effects between coexisting predators (and parasites) modify interactions between a pair of species (e.g., consumptive vs. non-consumptive interactions), relative distribution of prey phenotypic traits within a population and population dynamics remains poorly understood, especially for the pelagic ecosystems and bloom-forming heterocytous cyanobacteria.

Grazing by crustacean zooplankton and cyanophage-induced lysis significantly contribute to the mortality and population structure of filamentous cyanobacteria (Gerphagnon et al. 2015; Ger et al. 2016), which are important constituents of marine and freshwater ecosystems (Sohm et al. 2011 and references therein; Adam et al. 2016). The roles of grazing and lysis in the population dynamics of these cyanobacteria have usually been examined independently, impeding our understanding of the ecological effects that arise from the interactions of viruses and crustaceans that infect and feed on the same species. These effects can range from neutral to synergistic and antagonistic effects (Huxel 2007; Edeline et al. 2008; Schmidt et al. 2016) and are able to cascade throughout the entire pelagic food web. For example, viral lysis can promote grazing by reducing filament size and abundance, thus helping grazers to overcome prey morphological and density limitations (Martin-Creuzburg et al. 2008). Viral infection can also redirect host metabolism toward the production of fatty acids (Rosenwasser et al. 2008).
2014) as well as reducing cell wall rigidity (Chen et al. 2009), which can facilitate the assimilation of infected filaments by zooplankton (Evans and Wilson 2008). This, in turn, increases the channeling of carbon and energy through the food web to higher trophic levels. Zooplankton can enhance viral transmission and dispersion throughout the host population by the ingestion of virus and its egestion in feces or due to viral absorbance to and segregation from the exoskeleton (Frada and Vardi 2015). Alternatively, expression of some virus-encoded surface proteins during the infection process (Clokie et al. 2006) and the release of toxic or other deleterious intracellular compounds upon viral lysis (Arnold and Koudelka 2014) may have an inhibitory effect on the activity and grazing by zooplankton (Leefvaire and Ten-Hage 2007). It has also been shown that bacterial populations exposed to both ciliates and bacteriophages became more resistant to viral infections compared to populations exposed only to bacteriophages (Örnäla-Odegrip et al. 2015). In addition, viral infections or grazing can promote the production of exopolysaccharides by the host, leading to the formation of bacterial biofilms (Hughes et al. 1998; Gődeke et al. 2011; Scanlan and Buckling 2012), which, in turn, can prevent both infection by viruses and grazing (Mazt et al. 2005; Erken et al. 2011). Collectively, these examples suggest that changes in prey or host traits induced by grazing activity or lysis can affect the overall vulnerability and loss rates of cyanobacterial populations to biotic factors.

We report here an experimental study that addresses the synergistic/antagonistic effects of a crustacean predator (Daphnia magna) and a viral parasite (cyanophage Vb-AphaS-CL131; hereafter CL131; Šulčius et al. 2015c) grazing and infecting the same species of filamentous cyanobacteria Aphanizomenon flos-aquae (Šulčius et al. 2015a) that cause harmful blooms in temperate fresh and brackish water ecosystems (Šulčius et al. 2015b). We performed a short-term (12 d) laboratory survival experiments in which D. magna and CL131 cyanophages were added to A. flos-aquae cultures either separately or in combination (simultaneously or consecutively). We followed the changes in cyanobacterial population density, growth and size structure as well as trait response of the cyanobacterium when either D. magna or cyanophages was added, thereby allowing an assessment of adaptive plasticity-based microbial interactions. We discuss the possible implications of the synergistic/antagonistic effects between grazers and viruses in terms of cyanobacterial population and bloom dynamics.

**Material and methods**

**Experimental organisms and culture conditions**

**A. flos-aquae strain**

The clonal, non-toxic strain used in the present study, A. flos-aquae 2012/KM1/D3, was isolated from surface water collected from the Curonian Lagoon (N 55°30′, E 21°15′) in the south-eastern part of the Baltic Sea (Šulčius et al. 2015a,b). It was cultured in modified AF-6 medium (Anderson 2005) without the addition of a nitrogen source under a 14/10-h light-dark cycle and at a light intensity of approximately 120 µmol m⁻² s⁻¹ using cool, white fluorescent illumination (Philips TL-D 36W/840) at 20°C.

**D. magna strain**

*D. magna* used in the experiments was obtained from the Daphtoxit kit F magna biotest kit (MicroBioTests, Belgium). *D. magna* was hatched from ephippia and cultured for several weeks prior to the commencement of grazing experiments in 500 mL glass Erlenmeyer flasks (Schott Duran, Mainz, Germany) containing 300 mL standard freshwater medium (prepared according to the manufacturer’s instructions) maintained at the same temperature and light conditions as *A. flos-aquae* cultures. During culture, *D. magna* was fed with Crucigeniella apiculata (2013/SPE1/B7) culture grown in MWC medium (Guillard and Lorenzen 1972), obtained from the Collection of pure cultures of algae and cyanobacteria at Nature Research Centre (Vilnius, Lithuania) (Koreiviene et al. 2016). *D. magna* had been pre-adapted to *A. flos-aquae* by feeding them with short filaments (≤ 40 µm; ~ 2 x 10⁷ filaments mL⁻¹ final concentration) for at least five generations. Prior to the start of the experiment, *D. magna* was acclimated for a 24 h period of gut-emptying. During the entire experiment, daily records of *D. magna* survival and reproduction were made. Filament ingestion was investigated using an epifluorescence inverted microscope (Nikon T1 Eclipse).

**Cyanophage**

Cyanophage Vb-AphaS-CL131 (CL131) was isolated from the Curonian Lagoon (Lithuania) using *A. flos-aquae* strain 2012/KM1/D3 as a host organism (Šulčius et al. 2015c). CL131 is a large (capsid size of ~ 97 nm, tail length ~ 361 nm, genome size ~ 113 kb) siphovirus with an lytic infection cycle of ~ 36 h. Prior the experiment, CL131 was propagated using exponential growth phase *A. flos-aquae* cultures under the same conditions as those described above.

**A. flos-aquae colonies**

Temperate isolates of *A. flos-aquae* colonies (Fig. 1) were collected from the central part of the Curonian Lagoon on 01 September 2014, during *A. flos-aquae* bloom conditions. A water sample was obtained from the surface layer (0-0.3 m below air-water interface) using a nylon plankton net with a mesh size of 150 µm. A concentrated sample was transported to the laboratory within 4 h of collection and transferred to a 1 L glass cylinder which was kept in a growth chamber under the conditions described above for *A. flos-aquae* strain 2012/KM1/D3. After 60 min of incubation, colonies of *A. flos-aquae* migrated to the upper layer of the cylinder and formed a dense cyanobacterial mat. These colonies were collected and washed with ultra-filtered (100,000 kDa PES filters, Sartorius, Germany) lagoon water and sterile AF-6 medium until most of the single filaments, protists and other phytoplankton species were removed (based on
microscopic evaluation; data not shown). After washing, A. flos-aquae colonies were transferred to the culture medium using microcapillary tubes and left for 20 d to acclimatize in the growth chambers. During this period colonies were monitored daily for changes in morphology, zooplankton, and phytoplankton contamination as well as for unspecific lysis. The number of filaments counted for 100 μm length of A. flos-aquae colony ranged from 24 to 312. Filament length within colonies varied from 70.7 μm to 307.7 μm.

**Experimental design and manipulations**

All treatments were incubated for 12 d at the culture conditions described above. The initial conditions at the start of the experiments are given in Table 1. Using A. flos-aquae filaments (A_F) we set up the following treatments: (1) control treatment containing only A. flos-aquae filaments (A_FC); (2) A. flos-aquae culture with addition of CL131 cyanophages (A_FV); (3) A. flos-aquae culture with addition of D. magna (A_FG); (4) A. flos-aquae culture to which CL131 and D. magna were added consecutively (A_FV + G); (5) A. flos-aquae culture to which cyanophages and D. magna were added simultaneously (A_F(V + G)).

Cyanobacterial inoculum was derived from late exponential phase cultures and added to 50 mL Erlenmeyer flasks (Schott Duran, Mainz, Germany) containing nitrogen-free AF6 medium at a density of \(10^3\) filaments mL\(^{-1}\) (± 0.6 × 10^3 filaments mL\(^{-1}\)) to prevent density-limitation effect on D. magna grazing (Sahuquillo et al. 2007). Filaments of A. flos-aquae were measured at the onset of the experiment (\(n = 100\)) and these ranged in length from 25 nm to 425 nm (mean 118.5 μm ± 77.6 μm; mode = 95.0 μm). In the viral treatments (A_FV, A_FV + G, A_F(V + G), A_F(V + G)), CL131 cyanophages were added to cultures of cyanobacteria at the multiplicity of infection (MOI) of \(~ 1\) (Šulčius et al. 2015c). In grazing treatments (A_FG, A_FV + G, A_F(V + G), A_FG, A_F(V + G)), 10 similarly sized individuals of adult D. magna, taken from stock of acclimated cultures, were used. In treatments with temperate A. flos-aquae isolates, 30 differently sized colonies per treatment were used. All treatments had two replicates.

**Sampling and measurements**

Subsamples (1 mL) for determination of A. flos-aquae filament length and abundance were taken every 24 h, fixed.
with acidified Lugol’s iodine solution (to give a final concentration of ~ 1%) and kept in the dark at +4°C until ready for analysis. Filaments of *A. flos-aquae* were examined under a light microscope (Nikon Ti) using the Utermöhl scoring technique (Utermöhl 1958) at ×20 magnification, with a minimum of 100 filaments being measured for each time point. The biomass of *A. flos-aquae* (expressed as carbon (C) μg mL⁻¹) was estimated by multiplying filament abundance by the mean filament volume. The apparent growth rate (μ, d⁻¹) was calculated from the slope of the linear regression of natural-log transformed cell biomass vs. incubation time.

The number of the infectious cyanophages was determined by the most-probable-number (MPN) assay modified from Suttle and Chan (1993) and Saitoh et al. (2003). Subsamples were diluted 10-fold from 10⁻¹ to 10⁻¹⁰, and 50 μL aliquots of each dilution were inoculated with 200 μL (~ 5 × 10⁶ cells mL⁻¹) of an exponentially growing *A. flos-aquae* culture in a 96-well plate and incubated at 20°C for 7 d, as described by Sulčius et al. (2015c).

**Statistical analysis**

Data from two technical replicates were pooled together prior to comparison of treatments. The differences in filament length and width were then compared between treatments over the time course of experiment using a repeated-measures ANOVA (RM-ANOVA) with treatments (Aمنازل C, A المنزل G, A المنزل V, A المنزل V + G and A المنزل (V + G)) as a within-subjects factor. In case of significant RM ANOVA, Tukey HSD post hoc test was used for multiple pairwise comparisons to identify which treatments are significantly different from each other. To comply with assumptions of normality and homogeneity of variance (tested using Kolmogorov–Smirnov and Levene’s test, respectively) the RM-ANOVA analysis was performed on log10-transformed data. The statistical significance of the differences between the apparent growth rates (μ) for population growth (log vs. stationary; A منزل C and A منزل G treatments), infection and recovery (A منزل V, A منزل V + G and A منزل (V + G) treatments) phases between and within the different treatments, was estimated by comparing the slopes of the regression lines using t-tests. The distinction between the population log and stationary growth phases in A منزل C and A منزل G treatments has been made from biomass changes over the time course of experiment (Fig. 2c,f) and based on RM ANOVA analysis for filament length using sampling days as a within subject factor. The infection and recovery phases in A منزل V, A منزل V + G, and A منزل (V + G) treatments correspond to *A. flos-aquae* biomass changes from the start of the experiment to its minimum values at ~ 7 d post-infection and then its subsequent increase until the end of experiment, respectively. In addition, the

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**Fig. 2.** Changes in filament abundance, length, and population biomass of *A. flos-aquae* under different experimental conditions. *A Manor C* - control treatment containing only *A. flos-aquae* filaments, *A منزل V* - *A. flos-aquae* with addition of CL131 cyanophages, *A منزل G* - *A. flos-aquae* with addition of *D. magna*, *A منزل V + G* - *A. flos-aquae* in which CL131 and *D. magna* were added consecutively, *A منزل (V + G)* - *A. flos-aquae* in which cyanophages and *D. magna* were added simultaneously.
changes in cyanophage titers were used to separate between infection and recovery phases (Fig. 5). All statistical analyses were performed with Statistica 10 software (StatSoft, U.S.A.) with a $p$ value $< 0.05$ considered as significant.

Results

Treatments using single A. flos-aquae filaments

Control treatment (A$_c$C)

In the control treatment containing A. flos-aquae filaments (A$_c$C; Fig. 2), population growth followed a regular pattern of increase in filament number (Fig. 2a) and subsequent population biomass (Fig. 2c). During the log phase (first 7 d of the experiment) the estimated growth rate for the A. flos-aquae population was 0.63 d$^{-1}$, and diminished to 0.04 d$^{-1}$ when population reached the stationary phase. This was accompanied by a significant reduction in the average filament length ($F = 7.2, p < 0.01, df = 11$), which changed from 123.72 $\mu$m (SD = 5.89 $\mu$m) to 96.08 $\mu$m (SD = 3.71 $\mu$m) in the log and stationary phase, respectively (Fig. 2b).

D. magna treatment (A$_c$G)

There was no treatment (D. magna) effect on the filament length ($F = 320.6, p < 0.01, df = 4$; HSD $p > 0.05, df = 4$) and growth rate ($t = 0.31, p > 0.05, df = 2$) of A. flos-aquae population (Fig. 2e). During the log phase the growth rate of A. flos-aquae was 0.54 d$^{-1}$, and became reduced to 0.03 d$^{-1}$ 7 d after the start of the experiment. However, differently from the control (A$_c$C) treatment, changes in the growth rate coincided with structural changes to the A. flos-aquae population, in which filaments began to aggregate into colony-like structures (Fig. 3). These structures increased in length and width during the last few days of incubation (Fig. 3a–d). The aggregation of filaments also resulted in the lower abundance (Fig. 2d) and biomass (Fig. 2f) of free floating (non-aggregated) filaments of A. flos-aquae by the end of experiment.

No D. magna were seen grazing on the filaments of A. flos-aquae. It would appear that filaments of A. flos-aquae had an adverse effect on the filtration appendages of D. magna, preventing active feeding and resulting in evacuation of the gut during incubation experiments (Fig. 4a,b). Consequently, the growth and reproduction of D. magna became suppressed, and no egg production was observed. Only $\sim$ 35% (3 and 4 of 10 in each replicate) of Daphnia survived to the end of experiment.

Cyanophage treatments (A$_c$V)

The effect of infection and lysis on the filament length was significant ($F = 320.6, p < 0.01, df = 4$), and differed from the control and all other treatments (HSD $< 0.01$ for all multiple pairwise comparisons). In the A$_c$V treatment, the growth rate of the culture was also significantly suppressed compared with the control (A$_c$C) and grazing (A$_c$G) treatments. During the infection phase (first 6 d of the experiment), the calculated growth rate for A. flos-aquae was $-0.22$ d$^{-1}$. Over this period, the mean length of A. flos-aquae filaments were reduced by $\sim 75\%$ (Fig. 2b) and changed from 115.7 $\mu$m (SD = 43.7 $\mu$m, mode = 85 $\mu$m) to 30.1 $\mu$m (SD = 13.7 $\mu$m, mode = 25 $\mu$m). Due to lysis-induced filament breakage, their abundance began to increase (from the initial numbers of $7.82 \times 10^3$ filaments mL$^{-1}$) on the second day following infection, reaching a maximum on day 5 ($1.89 \times 10^4$ filaments mL$^{-1}$).
filaments mL$^{-1}$) (Fig. 2g). The massive lysis of the cyanobacterial population occurred between days 5 and 7, when both the length and the number of the filaments (decreased by 4.4 times to 4.29 × 10$^3$ filaments mL$^{-1}$) diminished synchronously (Fig. 2g–h). These observations were consistent with changes in the titer of infectious viruses in the A$_f$V treatment (Fig. 5). Seven days following cyanophage infection, the total cell abundance dropped to 21.6% of the initial value. The remaining population of A. flos-aquae began to recover both in terms of filament length (Fig. 2h) and density (Fig. 2g). The growth rate of the recovering A. flos-aquae population was 0.44 d$^{-1}$, and this did not differ significantly ($t=0.98$, $p>0.05$, df = 2) from the growth rate of the control population (A$_f$C) during log phase.

Cyanophage and D. magna treatments (A$_f$V + G and A$_f$(V + G))

Both treatments had a significant effect on the growth and filament length ($F=370.6$, $p<0.01$, df = 4), and were different between each other and compared to other treatments (HSD $p<0.01$ for all multiple pairwise comparisons). However, significant differences were also observed between treatments depending on the sequence employed for the addition of CL131 and Daphnia (Fig. 2). In A$_f$V + G treatment, the growth rate of A. flos-aquae was suppressed ($-0.25$ d$^{-1}$), and both the length and the number of filaments diminished dramatically 7 d post-infection (Fig. 2j,k). During this period, the average filament length was reduced by 59% and changed from 116.5 μm (SD = 63.3 μm; mode = 97 μm) to 47.8 μm (SD = 31.2 μm; mode = 30 μm). The filament abundance was reduced by 24.6% (Fig. 2j), while the total cell numbers dropped to 29.9% of their initial value. D. magna, which was added on the 6$^{th}$ day of the experiment, significantly reduced the growth rate of the recovering population ($0.20$ d$^{-1}$) compared with both control (A$_f$C; 0.63 d$^{-1}$) and virus treatments (A$_f$V; 0.44 d$^{-1}$), indicating that D. magna was able to graze on the short filaments. Consequently, the average length of the filaments had recovered faster compared to virus treatments (A$_f$V) ($t=25.8$, $p<0.01$, df = 2), and by the end of incubation had exceeded the initial values (Fig. 2k). All D. magna survived the entire course of the experiment, even though no egg production was detected (data not shown).

In A$_f$(V + G) treatment, in which both CL131 and Daphnia were added simultaneously at the beginning of the experiment, the observed pattern of A. flos-aquae dynamics was similar to that of A$_f$V and A$_f$V + G treatments (growth restriction → lysis → recovery; Fig. 2m–o). The growth rate for this treatment was $-0.31$ d$^{-1}$, similar to those obtained for A$_f$V ($-0.22$ d$^{-1}$) and A$_f$V + G ($-0.25$ d$^{-1}$) treatments. However, unlike A$_f$V and A$_f$V + G treatments, the overall average length of A. flos-aquae filaments (mean = 115.8 μm, SD = 59.8 μm; mode = 89 μm, range = 107.9–128.8 μm), did not change significantly (Day 1 to Day 5) until the onset of a massive lysis episode (on Day 6; Fig. 2n), remaining significantly higher (HSD $p<0.05$ for all multiple pairwise comparisons for within the group effects (days of incubation)) compared with A$_f$V (Fig. 2h) and A$_f$V + G (Fig. 2k) treatments. During the recovery phase (Day 8–12), the average length of A. flos-aquae filaments was not significantly different ($t=0.81$, $p>0.05$, df = 2) to that observed for A$_f$V + G treatment (Fig. 2k), but significantly differed ($t=9.6$, $p<0.05$, df = 2) from the value obtained for A$_f$V (Fig. 2h). During the recovery phase, the increase in the number of A.
flos-aquae filaments (Fig. 2m) was not significantly different from that observed for the A_{V}+G treatment (Fig. 2j), and furthermore, was lower than for the virus only (A_{V}) treatment (Fig. 2g). It is important to note, that no filament aggregation was observed for the A_{V}(V + G) treatment. Like the A_{V}+G treatment, all D. magna survived the course of the entire experimental period, and no eggs were produced.

Treatments using A. flos-aquae colonies

In order to test the effects of cyanophage infection and grazing by D. magna on the colony-aggregated A. flos-aquae filaments, we undertook laboratory experiments using temporal A. flos-aquae colony isolates (see Material and methods). During the 12 d incubation experiments, no changes to colony size and filament abundance within the aggregates were observed, neither for control (A_{C}C), virus (A_{V}V) nor D. magna (A_{C}G) treatments, including A_{C}(V + G) (data not shown). D. magna was not able to graze on colonial A. flos-aquae, and this resulted in 60% and 55% D. magna mortality in A_{C}G and A_{C}(V + G) treatments, respectively. The number of infectious viruses in the A_{C}V and A_{C}(V + G) treatments remained stable over the course of experiment (Fig. 5), indicating that neither infection nor adsorption to the surface of the colonies had occurred.

Discussion

In this study, we examined how interactions between crustacean zooplankton and cyanophages can affect the population dynamics of a harmful, filamentous cyanobacterium. Firstly, we demonstrated the different effects of D. magna and cyanophages on the structure of the A. flos-aquae population. D. magna alone was not able to graze on the relatively long A. flos-aquae filaments (A_{G} treatment), hence its consumptive effect on A. flos-aquae population was rather negligible. However, the presence of D. magna has generated trait changes in A. flos-aquae by promoting filament aggregation (Fig. 3). Viral infection and lysis had consumptive effect by reducing population density and significantly changing filament size distribution (A_{V}, Fig. 2g–i). We further showed that viral lysis had a synergistic effect on D. magna, changing the interactions between D. magna and A. flos-aquae from non-consumptive to consumptive, that is preventing the clogging of D. magna filtration system by generating short-length filaments, eventually enabling Daphnia to graze on A. flos-aquae (A_{V}+G, Fig. 2j,k; A_{V}(V + G), Fig. 2m,n). Finally, we observed that colony - embedded A. flos-aquae filaments are resistant to grazing by D. magna. These observations have significant implications for bloom dynamics and the role of biotic factors in controlling cyanobacterial proliferation in natural aquatic ecosystems.

The grazing by D. magna had no consumptive effect on A. flos-aquae filament abundance and length (A_{G}, Fig. 2d,e). Moreover, microscopic analysis showed that the filtration appendages of D. magna were heavily immobilized by A. flos-aquae filaments (Fig. 4a,b), resulting in evacuation of the gut and death of D. magna. Thus, the lack of a significant consumptive effect of D. magna on A. flos-aquae is likely to promote cyanobacterial proliferation in natural environments leading to a negative feedback in terms of D. magna growth and reproduction. In aquatic ecosystems, development of filamentous cyanobacteria can be further promoted by selective exclusion of A. flos-aquae from the crustacean diet (Epp 1996), the increased availability of an alternative food source for D. magna and the top-down control of D. magna by fish predation. Nevertheless, the presence of D. magna generated a trait-mediated response in the A. flos-aquae population by promoting filament aggregation (Fig. 3). Zooplankton-induced colony formation is one of the adaptive response strategies of many cyanobacteria and algae to an increased risk of predation (Lurling and Van Donk 1997; Van Donk et al. 1999; Yang et al. 2006). Although A. flos-aquae colonies resembling those found in natural environments (Fig. 1) were not formed in our experiments, such phenotypic plasticity-based changes in the population (Fig. 3) may have significant implications for the establishment of A. flos-aquae blooms, and thus community structure and energy flows within the pelagic food web (Peckarsky et al. 2008; Schmitz et al. 2010). The aggregation into colonies, or colony-like structures, of filamentous nitrogen-fixing cyanobacteria may increase their proliferation (Van Grembergh et al. 2009; Mello et al. 2012), not only in terms of reducing grazing pressure as a result of the size mismatch between predator and prey (Hambright et al. 2001), but also because of higher rates of nitrogen and carbon fixation in aggregated colonies compared with single filaments (Ploug et al. 2010). It may also increase buoyancy-controlled vertical movement of A. flos-aquae colonies enabling them to respond more efficiently to changing environmental conditions (e.g., light availability). In addition, filament aggregation may also have an indirect effect on A. flos-aquae population structure and community composition by enhancing prey-switching to non-colonial cyanobacteria and algae, thus leading to bloom formation by aggregated filaments. Finally, filament aggregation resulted in the lower abundance of free-floating filaments (Fig. 2d; compared to the control (AFC) treatment), which in natural environment may adversely influence the rate of successful viral infections. Thus, collectively, the results indicate that both the nature (average filament length) and adaptive response (filament aggregation) of filamentous A. flos-aquae help them to evade D. magna control. Consequently, this may increase the potential of A. flos-aquae to form a bloom.

Cyanophage lysis has a strong consumptive impact on A. flos-aquae population by significantly reducing population density and the average length of the filaments (A_{V}, Fig. 2g,h). A reduction in filament abundance and shift in filament size toward relatively small filaments (A_{V}, Fig. 2h) can increase their vulnerability to grazing (Oberhaus et al. 2007;
Martin-Creuzburg et al. (2008) and may have a negative impact on their metabolic activity, including rates of CO₂ and N₂ fixation (Chan et al. 2004; Rosenwasser et al. 2014; Puxty et al. 2016 and authors unpublished results). However, no complete lysis of A. flos-aquae was observed, suggesting that resistance to cyanophage infection may be present or develop in A. flos-aquae population. This, however, suggest that infection influences the frequency distribution of resistant and susceptible genotypes, by selecting mutants with reduced (or no) susceptibility to infection (Williams 2013), eventually leading to the emergence of infection-resistant filaments able to recover the initial population abundance within a few days (AFG; Fig. 2g).

Our results also demonstrate a synergistic effect, that of viral lysis on grazing by D. magna and its survival throughout a study period characterized by the generation of relatively short length filaments (A_V + G, Fig. 2k; A_G(V + G), Fig. 2n). First, no clogging of D. magna filtration appendages was observed for A_V + G and A_G(V + G) treatments (Fig. 4c). In aquatic ecosystems, this would not only increase the survival rate of D. magna, but would also allow it to feed successfully on other species. Second, as indicated by differences in the changes to filament abundance and length for A_V + G and A_G(V + G) treatments compared with A_V, D. magna successfully ingested short A. flos-aquae filaments (Fig. 4c), resulting in the survival of all D. magna throughout the entire course of these treatments. Consequently, we propose that as long as cyanophage lysis predominates over the non-consumptive effect of D. magna on the A. flos-aquae population, D. magna may benefit from viral infections.

Paradoxically, however, the synergistic effect of viral lysis on D. magna grazing led to a dramatic increase in the average filament length of A. flos-aquae (A_V + G, Fig. 2k; A_G(V + G), Fig. 2n), even though it delayed the recovery of A. flos-aquae population density (A_V + G, Fig. 2j; A_G(V + G), Fig. 2m). The removal of short filaments, which emerged as a result of viral lysis, by D. magna grazing not only selects for both grazing- and virus-resistant filaments, but at the same time have an indirect positive effect by removing competitors within a recovering A. flos-aquae population and by favoring the growth of relatively long filaments, which has been shown to fix atmospheric nitrogen more efficiently than their short counterparts (Chan et al. 2004). Consequently this may enhance A. flos-aquae proliferation and bloom development in the natural aquatic environments.

Finally, from the perspective of predator–prey interactions, colony formation is an important defensive trait that may prevent both viral infections and grazing (Hamm et al. 1999; Jacobsen et al. 2007). In pelagic environments, blooms of A. flos-aquae mostly consist of filaments aggregated into colonies. If colony aggregation can be induced by the non-consumptive effect of D. magna, as our study suggests, and has been demonstrated in other studies for other cyanobacteria and algae (Lampert et al. 1994; Yang et al. 2006), then biological control of filamentous cyanobacteria by zooplankton grazing is negligible, including bloom development and collapse stages. The inability of cyanophages to infect A. flos-aquae colonies in our experiments could indicate that either A. flos-aquae colonies were dominated by the resistant genotypes or that filament aggregation may, in addition to grazing, provide a protection against viral infections for sensitive yet colony-embedded filaments. For example, experimental and modelling studies suggest that aggregation of infection-sensitive cells into colonies protects them from viral attacks and lysis, and that probability of successful viral infections decrease as colony size increases (Ruordij et al. 2005; Jacobsen et al. 2007). In addition, our preliminary data show that both the relative abundance of resistant genotypes and the exopolysaccharide matrix that surrounds the colony-embedded filaments play a role in resistance of A. flos-aquae colonies (unpublished data). However, the inability of cyanophages to attach and infect A. flos-aquae colonies in our study (Fig. 5), implies that a relatively greater number of single A. flos-aquae filaments will be removed from the pelagic zone by viral lysis, thus indirectly promoting the formation of colony-embedded A. flos-aquae population. The lack of top-down control of cyanobacterial colonies would also affect the flow of materials and energy within the ecosystem, since most of the biomass produced would be lost from the pelagic zone due to increased sedimentation upon bloom collapse (Lürling and Van Donk 2000).

To conclude, the outcome of interactions between a pair of species is determined by their density and specific traits, which, however, can change as a result of the appearance of a third species. We show here how the consumptive interactions between cyanophages and cyanobacteria can modify their interactions with D. magna via the synergistic effect of viral lysis. We also propose that the non-consumptive effect of grazers might lead to colony formation and that this, in turn, prevents cyanobacteria from being grazed and potentially from being infected by viruses. Considering that crustacean grazers and cyanophages co-occur in natural aquatic environments, this study advances our understanding of how synergistic and antagonistic effects between crustacean zooplankton, represented by D. magna, and CL131 cyanophages can modify the population dynamics and bloom formation of filamentous cyanobacteria.

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Conflict of Interest

None declared.