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Cyanobacteria facilitate parasite epidemics in *Daphnia*

C. Tellenbach,[1,2,8] N. Tardent,[1] F. Pomati,[1,3] B. Keller,[1,4] N. G. Hairston Jr.,[1,5] J. Wolinska,[6,7] and P. Spaak[1,3]

[1]*Department of Aquatic Ecology, Eawag, Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, CH-8600 Switzerland*
[2]*School of Biosciences, University of Birmingham, Birmingham, B15 2TT UK*
[3]*Institute of Integrative Biology, ETH Zurich, Zurich 8092 Switzerland*
[4]*Department of Systematic and Evolutionary Botany, University of Zurich, Zürich 8008 Switzerland*
[5]*Department of Ecology & Evolutionary Biology, Cornell University, Ithaca, NY, USA*
[6]*Department of Ecosystem Research, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Müggelseedamm 301, Berlin 12587 Germany*
[7]*Department of Biology, Chemistry and Pharmacy, Institute of Biology, Freie Universitat Berlin, Königin-Luise-Strasse 1-3, Berlin 14195 Germany*

Abstract. The seasonal dominance of cyanobacteria in the phytoplankton community of lake ecosystems can have severe implications for higher trophic levels. For herbivorous zooplankton such as *Daphnia*, cyanobacteria have poor nutritional value and some species can produce toxins affecting zooplankton survival and reproduction. Here we present another, hitherto largely unexplored aspect of cyanobacteria, namely that they can increase *Daphnia* susceptibility to parasites. In a 12-yr monthly time-series analysis of the *Daphnia* community in Greifensee (Switzerland), we observed that cyanobacteria density correlated significantly with the epidemics of a common gut parasite of *Daphnia*, *Caullerya mesnili*, regardless of what cyanobacteria species was present or whether it was colonial or filamentous. The temperature from the previous month also affected the occurrence of *Caullerya* epidemics, either directly or indirectly by the promotion of cyanobacterial growth. A laboratory experiment confirmed that cyanobacteria increase the susceptibility of *Daphnia* to *Caullerya*, and suggested a possible involvement of cyanotoxins or other chemical traits of cyanobacteria in this process. These findings expand our understanding of the consequences of toxic cyanobacterial blooms for lake ecosystems and might be relevant for epidemics experienced by other aquatic species.

Key words: Caullerya mesnili; *epidemiology; host-parasite interactions; time series analysis; toxins; zooplankton.*

Introduction

The outbreak and spread of epidemics relies importantly on a dynamic interplay between the capacity of the host to fend off the parasite and the ability of the parasite to overcome host resistance. In natural populations, these dynamics are strongly shaped by the external context in which both hosts and their parasites face a multitude of continuous or acute environmental challenges. The strength and direction of the resulting selection pressures by external environmental conditions, as well as their mode of interaction (i.e., synergism, antagonism) varies in time. As a result, these temporally fluctuating processes can lead to seasonal and interannual disease dynamics that make it challenging to predict epidemics (Lafferty and Holt 2003, Altizer et al. 2006, Wolinska and King 2009). A particular environmental state might directly influence the spread of disease if it alters parasite growth, virulence, transmission. and infectivity; or if it either affects host demography, or impairs the host immune system though physiological stress or injury (Lafferty and Holt 2003, Altizer et al. 2006, Marcogliese and Pietrock 2011). The environment might also indirectly affect the spread of disease by altering growth and phenology of other species in the community such as alternate or intermediate hosts, competitors, predators, or prey (de Roode et al. 2011, Paull and Johnson 2014, Penczykowski et al. 2014*a*).

Water fleas (*Daphnia*) often dominate the zooplankton community in lakes and ponds, reaching high population densities. As both primary consumers of phytoplankton and prey for fish, they are thus ecologically important (Lampert 2011, Miner et al. 2012). At the same time, *Daphnia* host a plethora of microparasites of a diversity of taxa, and epidemics are common in natural systems (Green 1974, Ebert 2005, Wolinska et al. 2007). Host-parasite interactions in *Daphnia* are sensitive to fluctuating environmental factors such as temperature (Mitchell et al. 2005, Schoebel et al. 2011), salinity (Hall et al. 2013) or nutrient availability in the water column (Duffy et al. 2012, Decaestecker et al. 2015). In European lakes, epidemics of the gut parasite *Caullerya mesnili* (Ichthyosporea) are especially common (Wolinska et al. 2007, 2011). These epidemics reach a maximum in late summer and early fall (Wolinska et al. 2006, 2011, Tardent et al. 2016). Since *Caullerya* exclusively infects the digestive tract (Lohr et al. 2010), alterations in food

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[8]E-mail: christoph.tellenbach@alumni.ethz.ch

quality of *Daphnia* might also affect *Caullerya* infection success and, consequently, the timing and intensity of epidemics.

An altered food quality often has strong effects on consumer fitness and, consequently, can substantially impact host-parasite interactions (Hall et al. 2009, Aalto et al. 2015). However, the magnitude and direction of how food quality affects host-parasite interactions in *Daphnia* is complex and depends on host and parasite species as well as on *Daphnia* demography (e.g., Frost et al. 2008, Schlotz et al. 2013, Civitello et al. 2015). Food quality is largely determined by the composition and abundance of phytoplankton. Prokaryotic cyanobacteria are phytoplankton species that have poor nutritional value for *Daphnia*, as they contain low levels of poly- and highly unsaturated fatty acids and sterols (von Elert et al. 2003). Some of these fatty acids are, among other functions, precursors of important signaling molecules of immunity processes in invertebrates (Brett and Müller-Navarra 1997, Twining et al. 2016). Furthermore, cyanobacteria may have shapes that mechanically disrupt the feeding apparatus, and they often produce toxins and metabolites that interfere with *Daphnia* metabolism and growth (Rohrlack et al. 2005, Schwarzenberger et al. 2010). Natural *Daphnia* populations have been shown to evolve tolerance mechanisms that ameliorate the effects of dietary cyanobacteria, as shown for *Daphnia galeata* resurrected from different sediment layers representing different eutrophication stages in a large peri-alpine lake (Hairston et al. 1999) and for *Daphnia pulex* from small Chinese ponds and rivers with different trophic states (Jiang et al. 2015). However, cyanobacteria tolerance is costly and might be associated with tradeoffs such as being more vulnerable to starvation for more tolerant individuals (Hairston et al. 2001), or for individuals being more tolerant to cyanobacteria being less resistant to disease.

Little is known about the involvement of cyanobacteria in shaping host-parasite interactions in their grazers. For *Daphnia*, poor food quality, metabolic interference, and toxicity are potential mechanisms by which cyanobacteria might increase the susceptibility to parasites and thus culminate in the outbreak of an epidemic. Here we investigated whether the occurrence of cyanobacteria influences epidemics in the *Daphnia-Caullerya* host-parasite system. Specifically, we conducted a time-series analysis on a 12-yr spanning dataset of plankton dynamics in Greifensee, a peri-alpine lake in northern Switzerland. We then assessed experimentally whether cyanobacteria affect *Caullerya* infections by nutritional or toxic effects, or both; by testing *Daphnia* susceptibility to infection when fed three different cyanobacteria strains of *Microcystis aeruginosa*: (1) a toxic strain isolated from the same lake during a *Caullerya* epidemic, (2) a highly toxic allopatric strain, and (3) a non-toxic knock-out mutant of this latter strain.

Materials and Methods

Field study

Data collection.—The *Daphnia* assemblage of Greifensee, Switzerland – a monomictic, eutrophic (P_{tot} ~50 μg/L) peri-alpine lake with a surface area of 8.5 km^2 and a maximum depth of 33 m – is dominated by *Daphnia galeata* and *D. galeata* × *longispina* hybrids (Keller and Spaak 2004). The entire plankton community has been monitored monthly in a routine sampling program, since 1972 for phytoplankton and 1981 for zooplankton, respectively. Quantitative integrated zooplankton samples were taken from 30 m to the water surface and phytoplankton samples were taken from 20 m to the water surface (details about sampling, see Bürgi et al. 1999). Phytoplankton cell numbers and volumes were counted by the Utermöhl (1958) method. This monitoring program was conducted in combination with a depth-resolved (from 30 to 0 m in 2.5 m increments) physicochemical monitoring program by the Office of Waste, Water, Energy and Air of the Canton of Zurich (AWEL, http://www.awel.zh.ch/).

To study *Daphnia* demography, life-history, taxonomic composition, and parasite epidemics, qualitative and quantitative integrated zooplankton samples (from 30 m to the water surface) were collected biweekly to monthly (depending on season) in a routine field campaign lasting from July 2002 until December 2014 (details about sampling, see Keller and Spaak 2004). No sampling took place between June 2005 and June 2006. Upon collection, these samples were immediately brought to the laboratory and processed within 24 h. Under a dissecting microscope, 80–96 randomly chosen adult *Daphnia* were measured, their sexual stage and fecundity was determined, and they were checked for parasite infections. All samples were always taken at the deepest site of the lake.

Three different parasite taxa infect *Daphnia* in Greifensee, more or less repeatedly each year: an unknown bacterium that infects the haemocoel, an oomycete brood parasite with unknown species designation that attacks only the clutch of gravid females, and the ichthyosporean gut parasite, *Caullerya mesnili* Chatton (Wolinska et al. 2006, Tellenbach et al. 2007). Whereas the former two parasite epidemics break out mainly in spring and late fall (Tellenbach et al. 2007), *Caullerya* epidemics occur in late summer (Wolinska et al. 2006, Tardent et al. 2016) when cyanobacteria are typically most abundant in temperate lakes (Carey et al. 2012), including Greifensee (Bürgi et al. 2003). Since the scope of this study was to investigate the interaction of cyanobacteria with *Daphnia* susceptibility, we narrowed our focus to the *Caullerya* epidemics.

Time series analysis

To explore potential drivers of *Caullerya* epidemics in Greifensee, we investigated the relationship between

parasite prevalence (percentage of individuals infected) and a range of biotic and abiotic lake parameters using time-series analysis. In each case, we calculated cross-correlations for lags 0 and ±1 sample date (i.e., ±30 d because samples were taken monthly). Cyanobacterial cell counts were converted to biomass-density (g/m^3) by assuming cells have the specific gravity of water (Sommer 1981). Cyanobacterial biomass-density was determined for colonial and filamentous forms separately as well as together. Single-celled cyanobacteria were only found occasionally in the routine sampling and were thus attributed to the colonial forms. During the *Caullerya* epidemic in August 2011 in Greifensee, when the experimental organisms were isolated (see ahead), the cyanobacteria community consisted almost entirely of *Microcystis* spp. (96.9%) with a relative biomass of 49.9% of the entire phytoplankton community. Therefore, we did an additional analysis for the *Microcystis* genus only. *Daphnia* demographic responses were assessed using the density (individuals/m^3) of both the entire population and of only the adults. In addition, *Daphnia* population growth rates r (per day), birth rates b (per day) and death rates d (per day) were calculated for pairs of consecutive dates using the Edmondson-Paloheimo egg ratio method (see Appendix S1). Mean temperature (°C) and oxygen concentration (mg/L) of the upper 15 m were used to represent the abiotic environment. Since cyanobacteria are influenced by environmental conditions themselves, we also calculated cross-correlations between cyanobacterial biomass-density and temperature or oxygen, respectively.

Because sampling intervals of the time-series data vary within and among years, data for the individual parameters were smoothed using a nonparametric local polynomial (LOESS) regression. The parameter α that controls the degree of smoothing was adjusted upon visual inspection of the fit for each parameter to assure that data were not over-fitted (α = 0.02 for life-history parameters; r, b, d; α = 0.03 for adult/total *Daphnia* density; α = 0.04 for all other parameters). After smoothing, time-series were re-sampled at regularly spaced intervals. A potential bias in the correlation coefficients caused by arbitrarily chosen values for starting date and re-sampling interval was assessed using heat maps (see Appendix S2). Based on these heat maps, starting date and re-sampling interval were randomly and independently varied within a given range in simulations, and cross-correlations between the different parameters were calculated with the *ccf* function of the R statistical package (R Development Core Team 2013). Each simulation was repeated 999 times and means, standard deviations, and 95% confidence intervals were calculated.

Infection experiment

Isolation and maintenance of Daphnia, *cyanobacteria, and* Caullerya.—During the *Caullerya* epidemic in August-September 2011, we established 23 genetically distinct clonal *Daphnia* lines (based on 10 microsatellite markers, Brede et al. 2006) from egg-bearing asexual females either infected or uninfected by *Caullerya*. *Daphnia* cultures were maintained in filtered lake water (0.45 μm glass fiber filters, Sartorius Stedim AG, Switzerland) at 12°C in a 16:8 light:dark photoperiod. They were fed an equivalent of 1 mg C L^{-1} final concentration of chemostat-grown green algae *Acutodesmus obliquus* (Turpin) Hegewald & Hanagata, cultured in WC medium (Guillard 1975).

Caullerya was isolated on cultures of a long standing *D. longispina* laboratory clone from Lake Constance (denoted BoH7). *Caullerya*-infected animals were collected from Greifensee in August 2011 and immediately homogenized upon arrival in the laboratory. This homogenate was evenly distributed among jars containing BoH7 cultures of all age classes. As soon as an infection became apparent, a single *Caullerya*-infected *Daphnia* individual was moved to fresh, uninfected BoH7 cultures. *Caullerya* cultures were kept at 18°C in the dark, regularly stocked with susceptible juveniles, and medium was replaced as necessary.

A *Microcystis aeruginosa* strain was isolated during the 2011 *Caullerya* epidemic, by transferring 48 single cyanobacterial colonies to individual microtiter plate wells. Each well contained 5 mL WC medium (Guillard 1975) with adjusted pH (~8.5). From these cultures, one pure isolate not contaminated with other phytoplankton species was established and maintained in 250-mL Erlenmeyer flasks containing 150 mL pH-adjusted WC medium. This strain is microcystin-producing, since the presence of the microcystin synthethase A (mcyA) gene was verified by diagnostic PCR using primers mcyA mcyA-Cd 1F and mcyA-Cd 1R (Hisbergues et al. 2003) and the presence of the mcyE gene was verified with mcyE-F2 and mcyE-R4 primers (Rantala et al. 2004). Further, the *M. aeruginosa* strain PCC 7806 (obtained from Pasteur Culture collection), which is known to produce microcystins and which is highly toxic for different *Daphnia* species (Rohrlack et al. 2001), and a non-toxic microcystin-deficient knock-out mutant ΔmcyB of this strain (Dittmann et al. 1997) were included in the experiment. Microcystin production was measured for liquid cultures of the Greifensee and the PCC 7806 strains with the Microcystin ELISA kit (Biorbyt, Cambridge, UK), following the manufacturer's instructions. The concentrations were 696 fg/cell for the Greifensee (MaGr01) strain of *M. aeruginosa*, and 122 fg/cell for the PCC 7806 (MaTox) *M. aeruginosa* strain.

Experimental procedures

The infection experiment was performed with four randomly chosen *Daphnia* clones: originating from two uninfected (Gr06N, Gr21N) and two *Caullerya*-infected founder females (Gr03C, Gr08C), whereas the BoH7 clone, on which *Caullerya* was maintained in culture, was included as a control. The clones from Greifensee are *D. galeata* × *longispina* hybrids whereas the BoH7 clone

is a pure *D. longispina* clone as determined by microsatellite DNA analysis (data not shown). To exclude maternal effects, *Daphnia* clones were cultured for two generations under standardized conditions: 2 weeks prior to the experiment eight juveniles were transferred from the stock cultures kept at 12°C into 200 mL filtered lake water and kept at a 20°C, 18:6 light:dark regime. There were two replicate lines per clone. These lines were fed daily with a non-limiting amount of *A. obliquus* (1 mg C L^{-1}). After 1 week, first-clutch neonates (<24 h) were split to fresh cultures (two individuals per 200-mL jar) under the same conditions, with the medium being exchanged every other day. Of these replicate cultures, second-clutch neonates (<24 h) were pooled and randomly distributed among different experimental units (e.u.). Four different food conditions were applied in the experiment: (1) pure *A. obliquus* as a control (hereafter referred to as AoCont), and three 1:1 mixtures (based on carbon content) of *A. obliquus* with (2) the *M. aeruginosa* strain isolated during a *Caullerya* epidemic in Greifensee (MaGr01), (3) the PCC 7806 *M. aeruginosa* strain (MaTox), and (4) the ΔmcyB *M. aeruginosa* strain (MaMut). All phytoplankton species used in the experiment were grown in 250-mL Erlenmeyer batch cultures in 150 mL WC medium with pH set at ~8.5. Fresh batch cultures were inoculated at weekly intervals with 4.5 mL from the previous week's batch culture, grown for two weeks. Residual WC medium was removed by centrifugation, and the algae were diluted in filtered lake water.

At the start of the experiment, four neonate *Daphnia* (<24 h) of the same clone were placed together in a 50-mL jar containing 40 mL filtered lake water and 1 mg C L^{-1} algal solution of the respective food treatment. On days 2 and 3, experimental animals were fed 0.5 mg C L^{-1}. On day 4, they were transferred into fresh medium, and a homogenized *Caullerya* spore suspension was added to the parasite treatment, while a mock inoculate was added to the control treatment. The parasite and the mock inoculate were produced as follows. One day prior to inoculation, 40 living and five dead infected (uninfected for the mock inoculate) adult *Daphnia* from the BoH7 clone were transferred to 80 mL of filtered lake water and kept overnight without food. On the following day, the animals of each treatment were taken out of the jars, ground up in two 1.5-μL Eppendorf tubes and diluted with 160 μL filtered lake water. The two types of homogenate and the medium in which the animals were kept overnight were equally distributed across the experimental units of the corresponding treatments. The medium was lightly pipetted up and down 3 times per d for 2 consecutive d in order to re-suspend the spores and increase encounter rates of parasitized and experimental animals. No food was provided during this infection period to facilitate spore uptake through filtering. On day 3, all animals were fed with 1 mg C L^{-1}. From then on, *Daphnia* were fed 0.5 mg C L^{-1} daily. In order to maintain as many infective parasite propagules as possible, medium was not exchanged. One jar containing four individuals of the same clone represented one e.u.: 4 (food treatments) × 2 (parasite yes/no) × 5 (clones) × 4 (replicates) resulted in 160 e.u. Experimental individuals were inspected daily for survival and reproduction, and dead adults as well as neonate juveniles were removed. From day 6 on, *Daphnia* were additionally inspected under the microscope for *Caullerya* infections. The experiment was terminated after 22 d.

Statistical analyses

To measure the impact of *Caullerya* on *Daphnia,* we assessed *Caullerya* incidence, defined as a binary variable with values 1 (if at least a single individual was infected per jar) or 0 (if no infection was observed in a respective jar); *Caullerya* prevalence, which is the proportion of infected *Daphnia*; and the number of surviving individuals at a given time-point. *Caullerya* prevalence was analyzed with an ANOVA; *Caullerya* incidence and *Daphnia* survival with binomial generalized linear models (glms). For *Caullerya* incidence and prevalence only *Caullerya*-exposed animals were analyzed and the models therefore included food treatment, clonal identity and the food × clone interaction as terms; for *Daphnia* survival and *Caullerya* exposure the same terms plus all possible interactions with food treatment and parasite were included. Significance of independent variables in the glms was tested with type II Wald χ^2 tests. Residuals were assessed for violations of model assumptions and data were transformed if necessary in each model.

Results

Field study

The robustness of smoothing and re-sampling of the original time-series was verified in simulations (Appendix S2). Time-series analysis revealed a strong correlation between *Caullerya* prevalence and cyanobacteria biomass-density ($\rho = 0.64 \pm 0.009$ 95%-confidence interval [CI]; Table 1; Fig. 1A, B). This association was mainly dependent on the total abundance of cyanobacteria, since the 95% CI of the correlation coefficients for subsets of colonial ($\rho = 0.48 \pm 0.005$) or filamentous cyanobacteria ($\rho = 0.35 \pm 0.010$; Table 1) did not overlap with the 95%-CI of the total cyanobacterial biomass-density. Correlation coefficients for *Microcystis*, the most common genus during the *Caullerya* epidemic in August 2011 were also high when calculated for the same month ($\rho = 0.57 \pm 0.005$) and even higher for the previous month ($\rho = 0.58 \pm 0.009$; Table 1). *Daphnia* demography seemed to play a minor role in affecting and being affected by *Caullerya* epidemics as indicated by absolute values of the correlation coefficients $|\rho|$ smaller than 0.3 for any parameter. Additional potential drivers of *Caullerya* epidemics are dissolved oxygen concentration ($\rho = -0.56 \pm 0.003$) and temperature of the preceding month ($\rho = 0.62 \pm 0.020$). It is not possible to determine if or which of the previously noted factors

Table 1. Mean standard deviation (SD), lower and upper boundary of the 95% confidence intervals for lagged (in days) cross-correlations (ρ) of (A) *Caullerya* prevalence and (B) total cyanobacterial biomass-density with different biotic and abiotic parameters in Greifensee. Negative lag values indicate that the corresponding parameter precedes, and positive lag values that it succeeds.

		Lag (days)	Mean	SD	Lower	Upper
(A) *Caullerya*						
Cyanobacteria biomass-density	Total	0	0.638	0.005	0.629	0.647
		−30	0.556	0.016	0.524	0.588
	Colonial	0	0.479	0.007	0.464	0.493
		−30	0.384	0.016	0.351	0.416
	Filamentous	0	0.349	0.005	0.338	0.359
		−30	0.278	0.010	0.258	0.297
Microcystis spp. biomass-density		0	0.566	0.005	0.556	0.576
		−30	0.582	0.009	0.565	0.600
Daphnia demography	Adult density	0	−0.304	0.007	−0.318	−0.290
		−30	−0.202	0.019	−0.239	−0.165
		30	−0.296	0.011	−0.318	−0.274
	Total density	0	−0.331	0.007	−0.344	−0.318
		−30	−0.220	0.020	−0.259	−0.182
		30	−0.309	0.013	−0.334	−0.284
	r	0	−0.142	0.011	−0.164	−0.120
		−30	−0.224	0.017	−0.258	−0.191
		30	0.021	0.020	−0.018	0.060
	d	0	0.177	0.009	0.160	0.194
		−30	0.251	0.011	0.229	0.273
		30	0.083	0.010	0.064	0.103
	b	0	0.191	0.011	0.170	0.213
		−30	0.285	0.012	0.261	0.310
		30	0.112	0.011	0.090	0.134
Abiotic parameters	Oxygen	0	−0.556	0.001	−0.558	−0.553
		−30	−0.485	0.014	−0.512	−0.457
	Temperature	0	0.457	0.003	0.451	0.463
		−30	0.623	0.010	0.603	0.643
(B) Cyanobacteria						
Abiotic parameters	Oxygen	0	−0.446	0.002	−0.450	−0.443
		−30	−0.361	0.013	−0.386	−0.336
	Temperature	0	0.415	0.002	0.411	0.419
		−30	0.528	0.005	0.518	0.539

(cyanobacteria, oxygen, or temperature of the previous month) were the actual causes of *Caullerya* epidemics. These variables are confounded because cyanobacterial blooms occurred in summer, when temperatures were high and dissolved oxygen was low due to thermal stratification. This was indicated by a negative correlation of cyanobacterial biomass-density with dissolved oxygen ($\rho = -0.45 \pm 0.004$) and a positive correlation with temperature from the preceding month ($\rho = 0.53 \pm 0.010$), which are of the same magnitude as correlations of *Caullerya* prevalence with these two parameters.

Infection experiment

In total, *Daphnia* became infected in 30 out of 80 experimental units (jars) in the parasite treatments (i.e., 69 of 320 exposed animals became infected). Spore clusters first became visible 9 d post-infection in the BoH7 clone, and 11 d post-infection in the other clones. The clone BoH7, on which *Caullerya* was isolated and maintained, was the most susceptible and highly infected in all food treatments (Table 2). In contrast, fewer infections occurred in the other four clones that were isolated during a *Caullerya* epidemic in Greifensee, as visible by lower *Caullerya* incidence and prevalence (Table 2). Cyanobacteria increased the susceptibility to *Caullerya* of all *Daphnia* clones, as shown by a significant food effect on *Caullerya* incidence and prevalence and by the absence of any significant food × clone interaction (Table 3). Most infections occurred in the MaGr01 treatment, followed by the MaTox treatment (Table 2). This was further confirmed in significant pairwise comparisons with a Tukey's HSD test, where *Caullerya* prevalence of the MaGr01 treatment was significantly higher

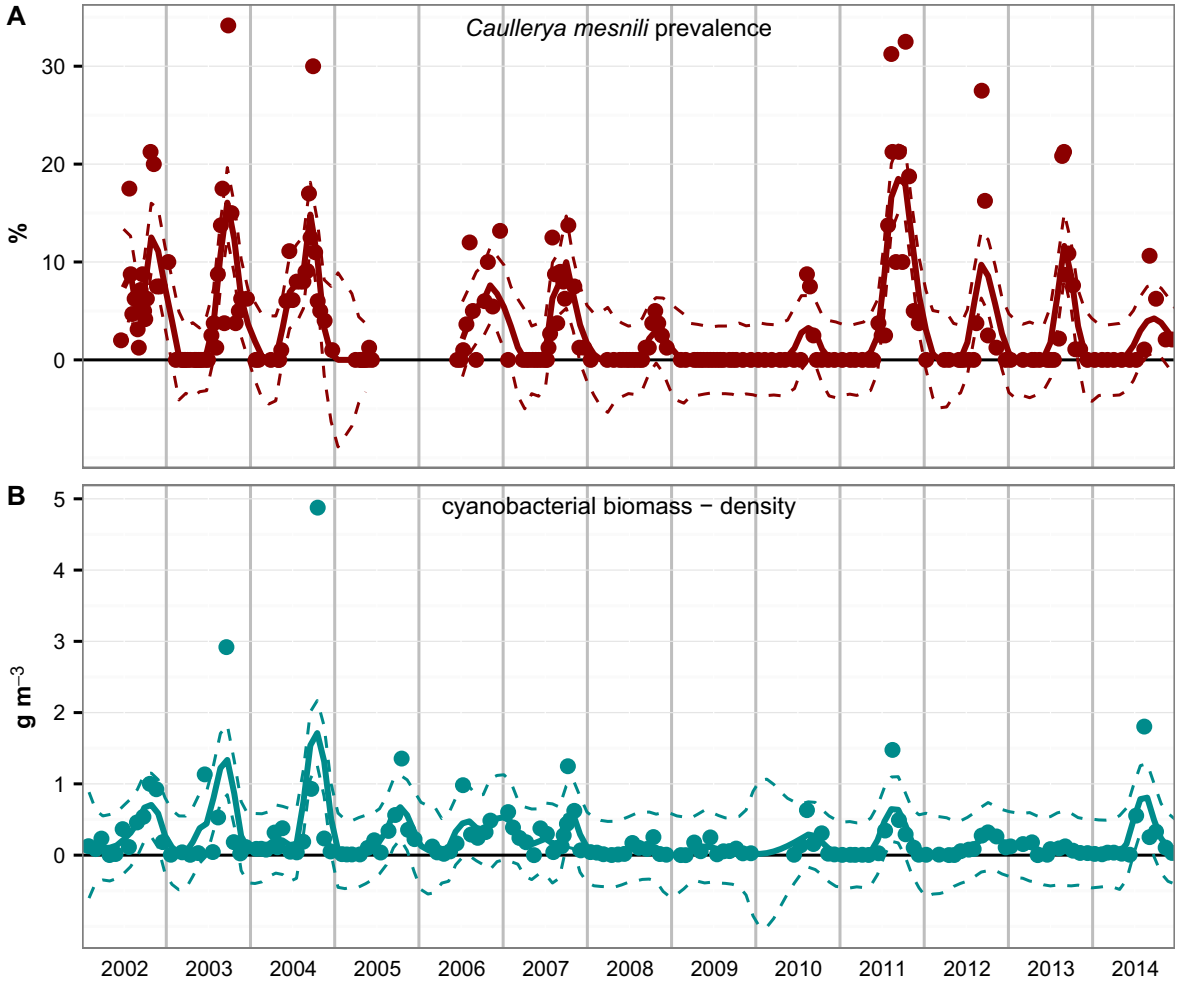

FIG. 1. Prevalence of *Caullerya mesnili* (A) and biomass-density of cyanobacteria (B) in Greifensee (January 2002–December 2014). Symbols indicate the original values, solid lines the fitted values, and dashed lines the boundaries of the 95%-confidence interval. (Colour figure can be viewed at wileyonlinelibrary.com.)

TABLE 2. (A) Incidence of *Caullerya* (i.e., the number of jars where at least one individual became infected) and (B) mean ± standard deviation (SD) of *Caullerya* prevalence in five *Daphnia* clones fed the green alga *Acutodesmus obliquus* (AoCont) or one of three *Microcystis aeruginosa* strains: a toxin (MaTox), a non-toxin producing (MaMut) laboratory mutant, and a natural strain (MaGr01). Each row represents a single food treatment and columns indicate the different *Daphnia* clones in the infection experiment. There were four jars in total per treatment.

	BoH7	Gr06N	Gr21N	Gr03C	Gr08C
	+	+	+	+	+
(A) *Caullerya* incidence					
AoCont	4	0	0	1	0
MaMut	4	0	0	0	0
MaTox	4	1	0	1	1
MaGr01	4	1	3	2	3
(B) *Caullerya* prevalence					
AoCont	0.88 ± 0.14	0.00 ± 0.00	0.00 ± 0.00	0.06 ± 0.12	0.00 ± 0.00
MaMut	0.69 ± 0.24	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
MaTox	0.88 ± 0.25	0.06 ± 0.12	0.00 ± 0.00	0.06 ± 0.12	0.06 ± 0.12
MaGr01	0.94 ± 0.12	0.12 ± 0.25	0.31 ± 0.24	0.12 ± 0.14	0.25 ± 0.20

TABLE 3. Generalized linear models testing for the effect of food (phytoplankton strain), parasite, *Daphnia* clone as well as all possible interactions on *Caullerya* incidence, prevalence, and *Daphnia* survival at days 10 and 22 (end) of the experiment; $df_{(res)}$ = (residual) degrees of freedom, $F = F$ statistics, χ^2 = Type II Wald's χ^2.

	df	*Caullerya* incidence	*Caullerya* prevalence	*Daphnia* survival (Day 10)	*Daphnia* survival (End)
		χ^2	F (df_{res} = 60)	χ^2	χ^2
Food	3	10.5*	8.2***	29.2***	35.3***
Parasite	1	–	–	3.9*	4.7*
Clone	4	51.0***	96.6***	1.8	18.9***
f × p	3	–	–	11.9**	6.3‡
f × c	12	6.3	0.7	28.7**	42.0***
p × c	4	–	–	8.4‡	9.2‡
f × p × c	12	–	–	8.8	6.9

***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; ‡$P < 0.1$.

in the other food treatments (Appendix S3: Table S1, for each pairwise comparison, $|t|$ was >3.1 and $P < 0.05$). No significant interactions occurred among the other food treatments. Moreover, the BoH7 clone had a significantly higher prevalence than the other clones (Appendix S3: Table S1, $|t| > 15.6$, $P < 0.001$), whereas there were no significant differences among the other clones. *Daphnia* mortality was considerably higher for *Caullerya*-exposed *Daphnia* in the MaTox treatment, with most individuals dying before any infection became apparent, i.e., before day 10 (Fig. 2). This higher mortality in the MaTox treatment was reflected in a significant food × parasite interaction, as calculated for day 10 (Table 3), which was also supported by significant pairwise comparisons in a Tukey's HSD test where mortality was higher in the MaTox treatment for *Caullerya*-exposed *Daphnia* than unexposed ones (Appendix S3: Table S3, $|t| = 3.3$, $P < 0.05$) and mortality was higher in the MaTox treatment compared to the other treatments for *Caullerya*-exposed *Daphnia* (Appendix S3: Table S3, $|t| > 1.7$, $P < 0.05$). At the end of the experiment (day 22) food stress outweighed parasite harm as mortality caused by *Caullerya* was the same in all food treatments, as indicated by a nonsignificant food × parasite interaction (Table 3). However, mortality of *Caullerya*-exposed animals was still higher than mortality of unexposed animals (Table 3). Furthermore, there was a significant food × clone interaction for mortality at day 10 and at the end of the experiment (Table 3). There was no indication of a tradeoff between *M. aeruginosa* tolerance and *Caullerya* resistance, since there appears to be a positive relationship between mean *Caullerya* prevalence and mean mortality in absence of *Caullerya* for the four Greifensee clones in the MaGr01 treatment (Fig. 3).

DISCUSSION

Interannual variation of epidemics is common and cycles often depend on complex interactions of different biotic and abiotic environmental factors. With a time-series approach, we observed that the seasonal abundance of cyanobacteria and the outbreak of *Caullerya mesnili* epidemics in *Daphnia longispina-galeata* population of Greifensee are coupled. This correlative evidence, suggesting that cyanobacteria influence the *Daphnia–Caullerya* interaction, was supported by the laboratory infection experiment, where *Caullerya* infections occurred more frequently in *Daphnia* fed with *Microcystis aeruginosa*, than in those fed with the green alga *Acutodesmus obliquus*, which is a more suitable food species than cyanobacteria (Lampert 1987, von Elert et al. 2003).

Even though the Eubacteria phylum Cyanobacteria includes morphologically distinct filamentous, colonial, and single-celled taxa, a higher correlation of total cyanobacterial biomass-density suggests that an influence on the *Daphnia-Caullerya* interaction is primarily caused by characteristics other than morphology that are common across the phylum of Cyanobacteria (e.g., food quality, toxicity or both). Moreover, high correlations of *Microcystis* spp. biomass-density with *Caullerya* prevalence suggest a particularly close association of these species with *Caullerya* epidemics in the field. The results of the infection experiment further indicate that the production of microcystins and other cyanobacterial metabolites might be an important factor in increasing *Daphnia* susceptibility for *Caullerya*. A significantly higher number of *Caullerya*-infected *Daphnia* was found for hosts fed with the *M. aeruginosa* Greifensee strain, compared with *Daphnia* fed the PCC 7806 strain, which had a five times lower microcystin content, or for *Daphnia* fed with the knock-out mutant strain that is unable to synthesize microcystin. However, cyanobacteria produce different structural microcystin congeners that differ in toxicity (Blom and Jüttner 2005), and with the ELISA kit we were not able to distinguish among different congeners. It is thus possible that the Greifensee strain mainly produces a less toxic form, whilst the PCC 7806 strain is known to produce the highly toxic microcystin-LR form (Dittmann et al. 1997). A significantly higher *Daphnia* mortality for this latter strain before day 10 indicates that toxicity synergistically exacerbated effects of *Caullerya* virulence

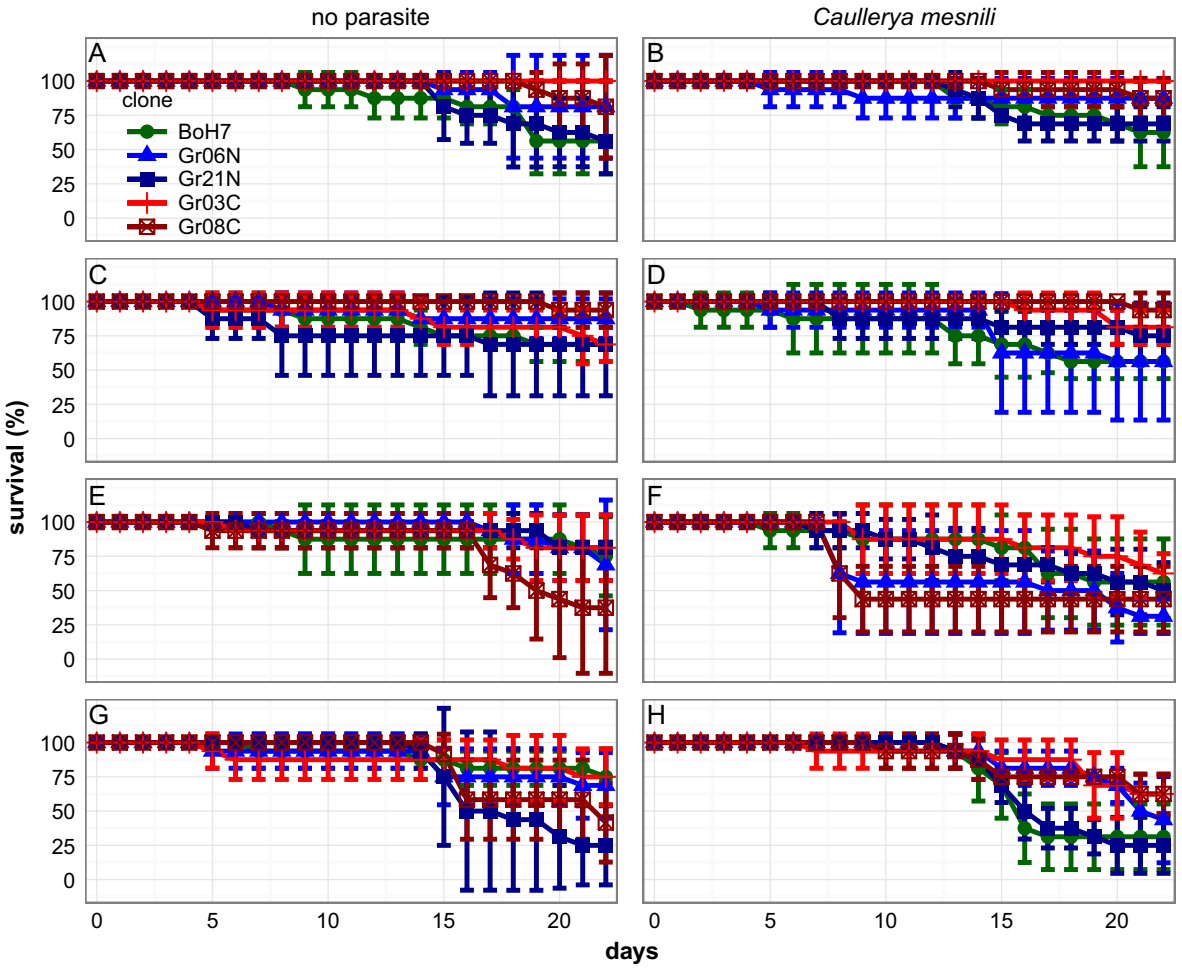

FIG. 2. Percentage (mean ± standard deviation) of surviving *Daphnia* inoculated without (left column) and with *Caullerya* (right column). *Daphnia* were fed with the green alga *Acutodesmus obliquus* (A, B) and one of three *Microcystis aeruginosa* strains: a non-toxin (C, D) and a toxin-producing laboratory strain (E, F), and a natural strain (G, H). Different colors indicate different *Daphnia* clones. *Daphnia* clones, *Caullerya* and the natural strain of *Microcystis* were all isolated from Greifensee during the *Caullerya* epidemic in August 2011. (Colour figure can be viewed at wileyonlinelibrary.com.)

leading to host death before any infection became apparent. However, although the PCC 7806 strain and its knock-out mutant ΔmcyB are genetically nearly identical, they considerably differ in their transcriptomic and metabolic profiles since microcystins can bind to proteins (Zilliges et al. 2011, Makower et al. 2015). Therefore, any apparent strain effect might as likely be caused by other cyanobacterial metabolites as by microcystins. This has been shown for a non-toxic *M. aeruginosa* strain that prevented infections of the white fat cell disease agent in *D. magna* in infection assays (Coopman et al. 2014). In plate growth assays, this particular strain lowered *Escherichia coli* growth suggesting antibiotic effects of cyanobacterial metabolites other than microcystins (Coopman et al. 2014).

Equally, cyanobacteria might minimize the spread of infection due to a lower nutritional value causing *Daphnia* to grow more slowly and to have lower feeding-rates. Therefore, spore uptake and transmission get minimized (Penczykowski et al. 2014*b*, Aalto et al. 2015). In contrast, a low nutritional value can also increase parasite infections (Frost et al. 2008, Schlotz et al. 2013, Pulkkinen et al. 2014, Civitello et al. 2015), which might be due to a lowered host resistance (Aalto et al. 2015, Twining et al. 2016). For *Caullerya,* poor food conditions might be advantageous as shown in a previous study, where *Caullerya* infections were higher in *D. galeata* fed *A. obliquus* grown under low phosphorus conditions (Schoebel et al. 2010). In the present study, dietary effects might potentially explain a higher mortality in *Daphnia* fed with the PCC 7806 strain, if the Greifensee strain was more nutritious (e.g., in terms of fatty acids, Dalsgaard et al. 2003, Bi et al. 2014).

We further assessed other factors such as temperature, oxygen and host demography as potential triggers of *Caullerya* epidemics. The correlational time-series

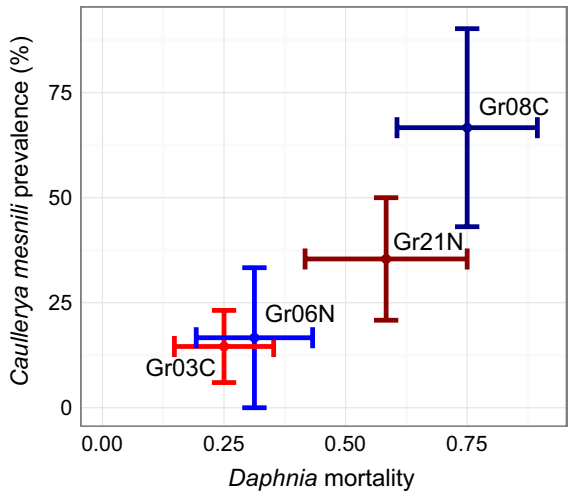

FIG. 3. Maximum *Caullerya* prevalence (mean ± standard error of the mean) vs. mortality (mean ± SEM) of *Daphnia* clones not inoculated with *Caullerya* and fed the Greifensee *Microcystis aeruginosa* strain. Clone names are annotated next to the respective symbol. *Daphnia* clones, *Caullerya* and the natural strain of *Microcystis* were all isolated from Greifensee during the *Caullerya* epidemic in August 2011. (Colour figure can be viewed at wileyonlinelibrary.com.)

analysis showed that temperature from the previous month correlated in the same order of magnitude with *Caullerya* prevalence as did total cyanobacterial biomass-density. *Daphnia* genotype-dependent temperature-sensitivity of *Caullerya* infections was previously shown in a laboratory study (Schoebel et al. 2011). However, the results were contradictory with the present data, since five out of six clones were more strongly infected at 12°C than at 20°C (Schoebel et al. 2011). A potential explanation is that a direct influence of temperature on *Caullerya* infections in the field was less pronounced than the impact of temperature on the spread of infection in the laboratory, where no additional temperature-sensitive stressors (e.g., growth-promotion of cyanobacteria) could interfere. Finally, *Daphnia* demography was only moderately associated with *Caullerya* epidemics. A moderately positive correlation with death-rate from the previous month indicates that mortality was generally higher in years where *Caullerya* epidemics were high. This suggests that higher epidemics occurred in years when *Daphnia* were generally more stressed. These results are in accordance with a study of 11 water reservoirs, where *Daphnia* demography played a subordinate role in *Caullerya* epidemics (Wolinska et al. 2011). *Daphnia* demography should also be affected by cyanobacteria due to their harmful effects. Using the same statistical approach, correlation coefficients of cyanobacterial biomass-density with different *Daphnia* demography parameters were very similar to correlation coefficients of *Caullerya* prevalence with *Daphnia* demography (data not shown). This might indicate that *Daphnia* demography is affected by cyanobacteria, *Caullerya*, and their interaction.

Coping with multiple stressors is associated with allocating resources among different specific defense pathways related to each individual stressor (Lafferty and Holt 2003). This might result in a tradeoff in the handling of one stressor vs. another (e.g., predator-induced defense vs. parasite-resistance; Yin et al. 2011). In the present study, however, the opposite holds true: clones sensitive to cyanobacteria also suffered more strongly from *Caullerya*. Thus, cyanobacteria and *Caullerya* might together exert strong selection pressure on less tolerant *Daphnia* genotypes in natural populations. Such a synergistic interaction between cyanobacteria and parasites and perhaps some of the constraints on the trait combinations among genotypes as documented by Ellner (2013) might explain why tolerance for cyanobacteria evolved so rapidly in the *D. galeata* population in Lake Constance. Specifically, tolerance persisted even after re-oligotrophication, when cyanobacteria substantially decreased in abundance (Hairston et al. 2001), probably because parasites (including *Caullerya*) remained common after re-oligotrophication (Bittner 2001). *Caullerya* resistance and cyanobacterial tolerance would seem to be tied by a constraint. Moreover, tolerance for cyanobacteria not only depends on the concentration of cellular microcystins and secondary metabolites, but also on the *Daphnia* clone as well as the cyanobacterial strain (Lemaire et al. 2012). Therefore, tolerance underlies a strong genotype × genotype (G×G) interaction. Accordingly, we found a G×G interaction with respect to survival as represented by a significant food × clone interaction, but none for an increased susceptibility of *Daphnia* to *Caullerya*. This suggests that cyanobacteria lower the threshold of infection of the population as a whole, but with clonal variation underlying the population mean response.

The present study shows for the first time a link between cyanobacteria stress and *Caullerya* epidemics in *Daphnia.* Overall, our results indicate that cyanobacteria blooms play an important role in the persistence of *Caullerya* epidemics in *Daphnia* populations by increasing host susceptibility and thus lowering infection thresholds. Our work further shows an involvement of cyanotoxins in increasing *Daphnia* susceptibility to *Caullerya*, but it also suggests that other factors such as nutritional value of the individual cyanobacteria strains, form, and concentration of toxin congeners, and the production of other metabolites may additionally be important for successful parasite development and spread. In the future, *Caullerya* epidemics might become even more pronounced due to raising summer temperatures that may increase the probability of cyanobacterial blooms (Carey et al. 2012). This study illustrates the value of long-term time-series observations for fully addressing the consequences of environmental fluctuations and global change on the spread of diseases in aquatic environments. Finally, it also highlights a hitherto largely overlooked role of cyanobacteria in natural aquatic systems – namely as stress agents that might facilitate epidemics.

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Literature Cited

Aalto, S. L., E. Decaestecker, and K. Pulkkinen. 2015. A three-way perspective of stoichiometric changes on host–parasite interactions. Trends in Parasitology 31:333–340.

Altizer, S., A. Dobson, P. Hosseini, P. Hudson, M. Pascual, and P. Rohani. 2006. Seasonality and the dynamics of infectious diseases. Ecology Letters 9:467–484.

Bi, R., C. Arndt, and U. Sommer. 2014. Linking elements to biochemicals: effects of nutrient supply ratios and growth rates on fatty acid composition of phytoplankton species. Journal of Phycology 50:117–130.

Bittner, K. 2001. Parasitismus bei *Daphnia* im Bodensee. Diss Univ Konstanz, 2001, Konstanz.

Blom, J. F., and F. Jüttner. 2005. High crustacean toxicity of microcystin congeners does not correlate with high protein phosphatase inhibitory activity. Toxicon 46:465–470.

Brede, N., A. Thielsch, C. Sandrock, P. Spaak, B. Keller, B. Streit, and K. Schwenk. 2006. Microsatellite markers for European *Daphnia*. Molecular Ecology Notes 6:536–539.

Brett, M., and D. Müller-Navarra. 1997. The role of highly unsaturated fatty acids in aquatic foodweb processes. Freshwater Biology 38:483–499.

Bürgi, H. R., C. Heller, S. Gaebel, N. Mookerji, and J. V. Ward. 1999. Strength of coupling between phyto- and zooplankton in Lake Lucerne (Switzerland) during phosphorus abatement subsequent to a weak eutrophication. Journal of Plankton Research 21:485–507.

Bürgi, H. R., H. Bührer, and B. Keller. 2003. Long-term changes in functional properties and biodiversity of plankton in lake Greifensee (Switzerland) in response to phosphorus reduction. Aquatic Ecosystem Health and Management 6:147–158.

Carey, C. C., B. W. Ibelings, E. P. Hoffmann, D. P. Hamilton, and J. D. Brookes. 2012. Eco-physiological adaptations that favour freshwater cyanobacteria in a changing climate. Water Research 46:1394–1407.

Civitello, D. J., R. M. Penczykowski, A. N. Smith, M. S. Shocket, M. A. Duffy, and S. R. Hall. 2015. Resources, key traits, and the size of fungal epidemics in *Daphnia* populations. Journal of Animal Ecology 84:1010–1017.

Coopman, M., K. Muylaert, B. Lange, L. Reyserhove, and E. Decaestecker. 2014. Context dependency of infectious disease: the cyanobacterium *Microcystis aeruginosa* decreases white bacterial disease in *Daphnia magna*. Freshwater Biology 59:714–723.

Dalsgaard, J., M. St. John, G. Kattner, D. Müller-Navarra, and W. Hagen. 2003. Fatty acid trophic markers in the pelagic marine environment. Advances in Marine Biology 46: 225–340.

de Roode, J. C., R. M. Rarick, A. J. Mongue, N. M. Gerardo, and M. D. Hunter. 2011. Aphids indirectly increase virulence and transmission potential of a monarch butterfly parasite by reducing defensive chemistry of a shared food plant. Ecology Letters 14:453–461.

Decaestecker, E., D. Verreydt, L. De Meester, and S. A. J. Declerck. 2015. Parasite and nutrient enrichment effects on *Daphnia* interspecific competition. Ecology 96:1421–1430.

Dittmann, E., B. A. Neilan, M. Erhard, H. Von Döhren, and T. Börner. 1997. Insertional mutagenesis of a peptide synthetase gene that is responsible for hepatotoxin production in the cyanobacterium *Microcystis aeruginosa* PCC 7806. Molecular Microbiology 26:779–787.

Duffy, M. A., J. H. Ochs, R. M. Penczykowski, D. J. Civitello, C. A. Klausmeier, and S. R. Hall. 2012. Ecological context influences epidemic size and parasite-driven evolution. Science 335:1636–1638.

Ebert, D. 2005. Ecology, epidemiology, and evolution of parasitism in *Daphnia*. National Center for Biotechnology Information (US), Bethesda (MD).

Ellner, S. P. 2013. Rapid evolution: from genes to communities, and back again? Functional Ecology 27:1087–1099.

Frost, P. C., D. Ebert, and V. H. Smith. 2008. Responses of a bacterial pathogen to phosphorus limitation of its aquatic invertebrate host. Ecology 89:313–318.

Green, J. 1974. Parasites and epibionts of Cladocera. The Transactions of the Zoological Society of London 32:417–515.

Guillard, R. R. L. 1975. Culture of phytoplankton for feeding marine invertebrates. Pages 29–60 *in* W. L. Smith and M. H. Chantey, editors. Culture of marine invertebrate animals. Springer US, Boston, MA.

Hairston, N. G., W. Lampert, C. E. Caceres, C. L. Holtmeier, L. J. Weider, U. Gaedke, J. M. Fischer, J. A. Fox, and D. M. Post. 1999. Lake ecosystems: rapid evolution revealed by dormant eggs. Nature 401:446.

Hairston, N. G. Jr, C. L. Holtmeier, W. Lampert, L. J. Weider, D. M. Post, J. M. Fischer, C. E. Caceres, J. A. Fox, and U. Gaedke. 2001. Natural selection for grazer resistance to toxic cyanobacteria: evolution of phenotypic plasticity? Evolution 55:2203–2214.

Hall, S. R., C. J. Knight, C. R. Becker, M. A. Duffy, A. J. Tessier, and C. E. Cáceres. 2009. Quality matters: resource quality for hosts and the timing of epidemics. Ecology Letters 12:118–128.

Hall, M., A. Vettiger, and D. Ebert. 2013. Interactions between environmental stressors: the influence of salinity on host–parasite interactions between *Daphnia magna* and *Pasteuria ramosa*. Oecologia 171:789–796.

Hisbergues, M., G. Christiansen, L. Rouhiainen, K. Sivonen, and T. Börner. 2003. PCR-based identification of microcystin-producing genotypes of different cyanobacterial genera. Archives of Microbiology 180:402–410.

Jiang, X., H. Liang, Y. Chen, X. Xu, and D. Huang. 2015. Microgeographic adaptation to toxic cyanobacteria in two aquatic grazers. Limnology and Oceanography 60:947–956.

Keller, B., and P. Spaak. 2004. Nonrandom sexual reproduction and diapausing egg production in a *Daphnia* hybrid species complex. Limnology and Oceanography 49:1393–1400.

Lafferty, K. D., and R. D. Holt. 2003. How should environmental stress affect the population dynamics of disease? Ecology Letters 6:654–664.

Lampert, W. 1987. Feeding and nutrition in *Daphnia*. Pages 107–141 *in* R. H. Peters and R. De Bernardi, editors. Daphnia. Memorie Dell'instituto di Idrobiologia, Pallanza.

Lampert, W. 2011. Daphnia: development of a model organism in ecology and evolution. International Ecology Institute, Oldendorf/Luhe, Germany.

Lemaire, V., S. Brusciotti, I. van Gremberghe, W. Vyverman, J. Vanoverbeke, and L. De Meester. 2012. Genotype × genotype interactions between the toxic cyanobacterium *Microcystis* and its grazer, the waterflea *Daphnia*. Evolutionary Applications 5:168–182.

Lohr, J. N., C. Laforsch, H. Koerner, and J. Wolinska. 2010. A *Daphnia* parasite (*Caullerya mesnili*) constitutes a new member of the Ichthyosporea, a group of protists near the animal-fungi divergence. Journal of Eukaryotic Microbiology 57:328–336.

Makower, A. K., J. M. Schuurmans, D. Groth, Y. Zilliges, H. C. P. Matthijs, and E. Dittmann. 2015. Transcriptomics-aided dissection of the intracellular and extracellular roles of microcystin in *Microcystis aeruginosa* PCC 7806. Applied and Environmental Microbiology 81:544–554.

Marcogliese, D. J., and M. Pietrock. 2011. Combined effects of parasites and contaminants on animal health: parasites do matter. Trends in Parasitology 27:123–130.

Miner, B. E., L. De Meester, M. E. Pfrender, W. Lampert, and N. G. Hairston. 2012. Linking genes to communities and ecosystems: *Daphnia* as an ecogenomic model. Proceedings of the Royal Society B: Biological Sciences 279: 1873–1882.

Mitchell, S. E., E. S. Rogers, T. J. Little, and A. F. Read. 2005. Host-parasite and genotype-by-environment interactions: temperature modifies potential for selection by a sterilizing pathogen. Evolution 59:70–80.

Paull, S. H., and P. T. Johnson. 2014. Experimental warming drives a seasonal shift in the timing of host-parasite dynamics with consequences for disease risk. Ecology Letters 17:445–453.

Penczykowski, R. M., S. R. Hall, D. J. Civitello, and M. A. Duffy. 2014*a*. Habitat structure and ecological drivers of disease. Limnology and Oceanography 59:340–348.

Penczykowski, R. M., B. C. P. Lemanski, R. D. Sieg, S. R. Hall, J. Housley Ochs, J. Kubanek, and M. A. Duffy. 2014*b*. Poor resource quality lowers transmission potential by changing foraging behaviour. Functional Ecology 28:1245–1255.

Pulkkinen, K., M. Wojewodzic, and D. Hessen. 2014. Phosphorus limitation enhances parasite impact: feedback effects at the population level. BMC Ecology 14:29.

R Development Core Team. 2013. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rantala, A., D. P. Fewer, M. Hisbergues, L. Rouhiainen, J. Vaitomaa, T. Borner, and K. Sivonen. 2004. Phylogenetic evidence for the early evolution of microcystin synthesis. Proceedings of the National Academy of Sciences of the United States of America 101:568–573.

Rohrlack, T., E. Dittmann, T. Börner, and K. Christoffersen. 2001. Effects of cell-bound microcystins on survival and feeding of *Daphnia* spp. Applied and Environmental Microbiology 67:3523–3529.

Rohrlack, T., K. Christoffersen, E. Dittmann, I. Nogueira, V. Vasconcelos, and T. Borner. 2005. Ingestion of microcystins by *Daphnia*: Intestinal uptake and toxic effects. Limnology and Oceanography 50:440–448.

Schlotz, N., D. Ebert, and D. Martin-Creuzburg. 2013. Dietary supply with polyunsaturated fatty acids and resulting maternal effects influence host - parasite interactions. BMC Ecology 13:41.

Schoebel, C. N., J. Wolinska, and P. Spaak. 2010. Higher parasite resistance in *Daphnia* populations with recent epidemics. Journal of Evolutionary Biology 23:2370–2376.

Schoebel, C. N., C. Tellenbach, P. Spaak, and J. Wolinska. 2011. Temperature effects on parasite prevalence in a natural hybrid complex. Biology Letters 7:108–111.

Schwarzenberger, A., A. Zitt, P. Kroth, S. Mueller, and E. Von Elert. 2010. Gene expression and activity of digestive proteases in *Daphnia*: effects of cyanobacterial protease inhibitors. BMC Physiology 10:6.

Sommer, U. 1981. The role of r-selection and K-selection in the succession of phytoplankton in Lake Constance. Acta Oecologica-Oecologia Generalis 2:327–342.

Tardent, N., C. Tellenbach, P. Turko, and P. Spaak. 2016. Clonal structure and depth selection during a *Caullerya mesnili* epidemic in a hybridizing population of the *Daphnia longispina* complex. Hydrobiologia. doi:10.1007/s10750-015-2632-3.

Tellenbach, C., J. Wolinska, and P. Spaak. 2007. Epidemiology of a *Daphnia* brood parasite and its implications on host life-history traits. Oecologia 154:369–375.

Twining, C. W., J. T. Brenna, N. G. Hairston, and A. S. Flecker. 2016. Highly unsaturated fatty acids in nature: what we know and what we need to learn. Oikos 125:749–760.

Utermöhl, H. 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. Mitteilungen Internationale Vereinigung für theoretische und angewandte Limnologie 9:1–38.

von Elert, E., D. Martin-Creuzburg, and J. R. Le Coz. 2003. Absence of sterols constrains carbon transfer between cyanobacteria and a freshwater herbivore (*Daphnia galeata*). Proceedings of the Royal Society of London Series B-Biological Sciences 270:1209–1214.

Wolinska, J., and K. C. King. 2009. Environment can alter selection in host–parasite interactions. Trends in Parasitology 25:236–244.

Wolinska, J., K. Bittner, D. Ebert, and P. Spaak. 2006. The coexistence of hybrid and parental *Daphnia*: the role of parasites. Proceedings of the Royal Society B: Biological Sciences 273:1977–1983.

Wolinska, J., B. Keller, M. Manca, and P. Spaak. 2007. Parasite survey of a *Daphnia* hybrid complex: host-specificity and environment determine infection. Journal of Animal Ecology 76:191–200.

Wolinska, J., J. Seda, H. Koerner, P. Smilauer, and A. Petrusek. 2011. Spatial variation of *Daphnia* parasite load within individual water bodies. Journal of Plankton Research 33: 1284–1294.

Yin, M., C. Laforsch, J. N. Lohr, and J. Wolinska. 2011. Predator-induced defense makes *Daphnia* more vulnerable to parasites. Evolution 65:1482–1488.

Zilliges, Y., J.-C. Kehr, S. Meissner, K. Ishida, S. Mikkat, M. Hagemann, A. Kaplan, T. Börner, and E. Dittmann. 2011. The cyanobacterial hepatotoxin microcystin binds to proteins and increases the fitness of *Microcystis* under oxidative stress conditions. PLoS ONE 6:e17615.

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