Clonal structure and depth selection during a *Caullerya mesnili* epidemic in a hybridizing population of the *Daphnia longispina* complex

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Abstract

Daphnia perform Diel Vertical Migration (DVM), a predator-avoidance strategy to migrate towards deeper and colder layers in the water column in the morning and movement to the algae-rich surface layers in the evening. However, individuals performing DVM incur several trade-offs since they might suffer from resource limitation and a slower instantaneous birth rate in deeper depths. DVM patterns may be modified by abiotic factors such as temperature, food concentration or pH and vary among different *Daphnia* species and genotypes. Furthermore, *Daphnia* host a variety of microparasites that might pose an additional factor influencing DVM behaviour. For infected individuals, migration into cooler temperature layers might slow down parasite growth. Moreover, parasites can increase opacity of their hosts. Non-migrating individuals might then be selectively purged from the upper layers by visually hunting predators. With these premises we asked, whether epidemics of the ichthyosporean parasite *Caullerya mesnili* affect or are affected by the DVM behaviour of *Daphnia* in Lake Greifensee, Switzerland by analysing the vertical distribution of *Daphnia* during day and night on two dates. Furthermore, we were interested whether a potential interaction depends on host genotype. We therefore studied the genotypic composition of the integrated population in regular sampling intervals over the course of one year and on a fine-grained vertical resolution during the *Caullerya* epidemic in late summer. Since *Caullerya* infected *Daphnia* migrated equally well as uninfected ones, the findings of this study suggest that *Caullerya* epidemics neither affected nor were affected by the DVM behaviour of *Daphnia*. We observed clonal succession in the lake but could not link this succession to the *Caullerya* epidemic; all except one of the common multilocus genotypes were under-infected. In addition, outbreak and course of this *Caullerya* epidemic seemed to rely mainly on environmental cues. Because this first study only provides a snapshot of time, we hope that further studies will be done to verify our results.

Introduction

Parasite epidemics can strongly influence the dynamics of a host population, causing population cycles (Hudson et al., 1998), maintaining the coexistence of different host genotypes (Freeland, 1983; Park, 1948; Wolinska et al., 2006) or altering the entire community by reducing host and/or predator demography (Dobson & Crawley, 1994). Many host parasite interactions depend on a suitable genotype (host) $\times$ genotype (parasite) combination leading to a genetic arms race which is also known as the 'Red Queen Hypothesis' (van Valen, 1962). Such an arms race with rapid co-evolution of host resistance counteracting parasite virulence might eventually lead to the termination of epidemics (Duffy et al., 2009). Such dynamics, however, do not solely rely on the genetic background of host and parasite and are strongly shaped by environmental perturbations leading to complex epidemic cycles (Liow et al., 2011; Wolinska & King, 2009).

Species of the genus *Daphnia* are a suitable system to study complex epidemic cycles as they are very susceptible to environmental perturbations (Gannon & Stemberger, 1978) and host a variety of microparasites (Ebert, 2005; Green, 1974). Moreover, because of the central role of *Daphnia* in lentic food webs as consumers of primary producers and prey of higher trophic levels (Lampert, 2011) parasite epidemics might have a substantial impact on the entire aquatic community. Therefore, understanding factors affecting *Daphnia* epidemics might be crucial to fully understand dynamics of aquatic food webs.

Onset, termination and magnitude of *Daphnia* parasite epidemics are determined by several intrinsic (e.g. genetic composition of the host population; Mitchell et al., 2004; Wolinska et al., 2004) and extrinsic environmental conditions (e.g. predation, turbulence, temperature or lake productivity; Cáceres et al., 2006; Duffy et al., 2012; Wolinska et al., 2007). Moreover, infection patterns often differ in geographically isolated populations or along a lateral gradient within lakes (Wolinska et al., 2011). However, many lakes experience periodic thermal stratification, resulting in a vertical gradient of temperature, oxygen, light and other physical as well as biological factors such as the distribution of food resources or predators (Boehrer & Schultze, 2008 ; Wetzel, 2001). As a consequence, these factors can drastically vary between different depths of the lake, forming various microenvironments. Such an environmental gradient may represent an additional challenge for aquatic species.

Many zooplankton species perform Diel Vertical Migration (DVM), which is defined as migration towards deeper and colder depths in the morning and movement to the algae-rich surface layers in the evening. Predator avoidance is considered the most important ultimate cause for DVM while light is commonly accepted to be the most important proximate cause (Ringelberg, 1999; Stich & Lampert, 1981; Zaret & Suffern, 1976). DVM patterns may be modified by abiotic factors such as temperature, food concentration or pH (De Meester & Dumont, 1989; Van Uytvanck & De Meester,

1990) and vary among different *Daphnia* species and genotypes (De Meester, 1993; Spaak & Boersma, 2006; Spaak & Ringelberg, 1997; Stich & Lampert, 1981). Migrating and non-migrating individuals might thus experience different selection pressure due to this behaviour, which might mask frequency-dependent selection as suggested by a 'pure' genetic arms race. Moreover, DVM patterns might also affect epidemics as migration to lower depths might on the one hand increase the risk for parasite infections from spores deposited in the sediments (Decaestecker et al., 2002). On the other hand, DVM might also be an adaptive strategy since migration into cooler layers might slow down parasite growth (Schoebel et al., 2011). Moreover, many parasites increase opacity of their hosts and non-migrating *Daphnia* might selectively be purged from the upper layers by visually hunting predators (Wolinska et al., 2011). Finally, highly virulent parasites might impair the DVM behaviour of *Daphnia* since infected individuals might be too weak to migrate (Lampert et al., 2003).

The *Daphnia* parasite *Caullerya mesnili* (Chatton, 1907; Green, 1974; Lohr et al., 2010) is an endoparasite which infects the *Daphnia* gut epithelium and forms spore clusters up to 100 µm in diameter, containing 8-20 spores (Bittner et al., 2002; Green, 1974). It is transmitted horizontally and spores are most likely taken up during filter-feeding of *Daphnia* (Bittner et al., 2002). Infected animals are generally heavily impaired, as they grow smaller, produce fewer offspring (or even none at all) and die earlier (Lass & Bittner, 2002; Lohr et al., 2010; Wolinska et al., 2006).

In Lake Greifensee, Switzerland, *Daphnia* and their parasites have been monitored regularly over the last decade. Previous work showed that the coexisting species of the *D. longispina* complex and their interspecific hybrids in Greifensee differ in prevalence, and that those differences change among epidemics (Wolinska et al., 2006; Wolinska et al., 2004). Because *Caullerya* strongly affects the fitness of its *Daphnia* host, we hypothesized that it will influence the ability of *Daphnia* to migrate vertically. Infected animals might not have the energy to migrate in the water, which means that they would not show a DVM behaviour. Furthermore, since both DVM and parasite prevalence differs among *Daphnia* taxa and genotypes, we hypothesized that more common clones prior to the epidemic should be more strongly infected than less common ones and that the migration behaviour of infected clones should be mostly impaired.

With these premises we asked, whether *Caullerya* epidemics affect or are affected by the DVM behaviour of *Daphnia* in Greifensee and whether this interaction depends on host genotype. We therefore compared by means of microsatellite markers the genetic composition of the *Daphnia* population over the course of a *Caullerya* epidemic in 2013 and analysed the vertical distribution of *Caullerya* infected and uninfected *Daphnia* during day and night at the peak of the epidemic.

Methods

Sampling

Lake Greifensee (N 47°21', E 8°41') is a eutrophic monomictic peri-alpine lake in Switzerland with a maximum depth of 32 m that is stratified during summer. The *Daphnia* community in Greifensee consists of different taxa of the *D. longispina* species complex and their interspecific hybrids (Bürgi et al., 1985; Spaak et al., 2001). Quantitative samples of the whole water column (30 – 0 m) to estimate *Daphnia* density and demography were collected in bi-weekly to monthly intervals (depending on season) with a 95 µm closing net (12 cm in diameter each; Bürgi, 1983) between January and December 2013 (Table S1). Three replicated net hauls from three different sites in the central basin of the lake (one net haul per site) were pooled to adjust for sampling error. To track the density of *Daphnia* in 2013, all sexes and age classes of *Daphnia* were counted. Qualitative random zooplankton samples were collected over the entire water column (30 - 0 m) in monthly intervals from May to December 2013 using a 250 µm net (17.5 cm). Depending on the *Daphnia* density in the lake one to three replicated net hauls were pooled. From these samples 96 adult female *Daphnia* were randomly selected for genotyping.

In addition to the routine samples over the entire water column, quantitative samples to study DVM of infected and uninfected *Daphnia* were taken on 29 August and 3 September 2013 at noon and midnight for 6 distinct depth layers (30–20 m, 20–10 m, 10–7.5 m, 7.5–5 m, 5–2.5 m, and 2.5–0 m) using the Bürgi closing net (Bürgi 1983). During this study, the lake was clearly stratified with the thermocline decreasing sharply around 10 m (see the webpage from the Office of Waste, Water, Energy and Air of the Kanton of Zurich, http://www.hw.zh.ch/chemie/see/02_2013.pdf). Oxygen was mostly abundant from the surface to approximately 5 m depth and decreased with increasing depth. Hence, the two uppermost layers corresponded to the epilimnion, the layers 7.5–5 m and 10–7.5 m corresponded to the metalimnion while the layers below (30–20 m and 20–10 m) corresponded to the hypolimnion. In the laboratory, their infection status was noted using a Leica binocular dissecting microscope using a magnification of 25×. Qualitative samples to study the vertical clonal distribution and to measure life-history traits were taken on 29 August at noon and midnight for the same depths as the quantitative data. On these two dates, samples were taken at the deepest part of the lake (32m depth; N 47°21.2496, E 8°40.52568) using a 250 µm closing net (17.5 cm in diameter). Details about the qualitative and quantitative depth samples can be found in Tables S3 and S4.

Samples were transported to the laboratory immediately upon collection and processed: Quantitative depth samples were concentrated to a volume of 40 ml, preserved in 99% ethanol and subsamples thereof counted to estimate the number of *Daphnia* per litre and the proportion of infected individuals (see Table S2 for details). *Daphnia* densities for the depth-resolved samples were weighted by depth volume of Greifensee to get an estimate abundance of *Daphnia* per depth. Only adult female *Daphnia* were considered. Qualitative samples were examined for parasites and the

number of eggs in the brood pouch was counted under a microscope at 50× magnification and, as for the quantitative depth samples, only adult female *Daphnia* were considered. From these samples 40 random ('random sample') and an additional subset of 40 *Caullerya*-infected female adult *Daphnia* ('infected sample') were chosen if possible. All individuals were put into 96 well microtiter plates containing 10 µl of nanopure H₂O and were frozen at -80 °C for further genetic analyses.

Microsatellite analysis

DNA was isolated according to the HotSHOT protocol (Truett et al., 2000) using 50 µl of lysis and 50 µl neutralization buffers. Ten microsatellite markers (Brede et al., 2006) were amplified in a multiplex polymerase chain reaction (multiplex PCR). To the reverse primers a so-called non-specific 'PIGtail' sequence (GTTTCTT) was added to the 5'-end (Brownstein et al., 1996) and forward primers were fluorescently labelled; all primers were obtained from Microsynth AG (Balgach, Switzerland). PCR reactions contained 1.5 µl extracted DNA, 5.5 µl Multiplex PCR Mastermix (Qiaagen, Hilden, Germany), equimolar concentrations of forward and reverse primers (0.3 µM SwiD15, SwiD10, SwiD1 and DaB10_14; 0.4 µM SwiD4, SwiD14, SwiD2 and SwiD5; 0.5 µM Dp512 and 0.6 µM SwiD12) and were filled up to a final volume of 11.5 µl with nanopure H₂O. The PCR cycling parameters included initial denaturation at 95 °C for 15 min, followed by 32 cycles at 94 °C for 30 s, with an annealing temperature of 54 °C for 90 s, and elongation at 72 °C for 60 s followed by a final elongation at 60 °C for 30 min. PCR products were diluted 1:10 with nanopure H₂O, shaken for two minutes and subsequently spun down. 0.5 µl of the dilution were mixed with 9.75 µl HiDi (Life Technologies, Carlsbad, CA, USA) and 0.25 µl GeneScan500 LIZ size standard (Life Technologies, Carlsbad, CA, USA) and loaded into the ABI Genetic Analyzer 3130xl (Life Technologies, Carlsbad, CA, USA). Fragment analysis results were scored with the STRand software (Toonen & Hughes, 2001) and binned in R (R Core Team, 2013) using the library MSatAllele (Alberto, 2013). Individuals were identified as multi-locus genotypes (MLGs), and retained if no more than two markers were missing. The exact number of genotyped animals can be found in Tables S3 and S4.

Data analyses

Depth selection of infected and uninfected Daphnia

The effective number of *Daphnia* per depth was analysed using a generalized linear mixed effects modelling (GLMM) approach using the quantitative depth sample: We began by fitting a GLMM with binomial link function, in which the count of *Caullerya*-infected and uninfected individuals was expressed as a function of the fixed effects of depth (6 levels) and time of day (day or night samples), and the two-way interaction between these two factors. Sampling date (29 August or 3 September)

was included as random factor. Model simplification was performed by serially removing terms and checking their significance with type II Wald χ^2 tests, starting with the two-way interaction, followed by removing the fixed terms. Because p-values given by this procedure are approximate, only the p-value's order of magnitude was considered. Therefore model terms were considered to be statistically significant when their removal led to p-values lower than 0.01 (Zuur, 2009).

Depth selection, DVM, and infection status of MLGs

We tested whether multilocus genotypes (MLG) differed with regard to their depth selection during day and night, and whether these behaviours differed in association with infection status, using a generalized linear modelling (GLM) approach.

To model depth selection, we considered the “day” and “night” samples separately. For each of these samples, we expressed the *Daphnia* count as a function of the fixed effects of depth (6 levels), whether the sample was randomly drawn or only infected individuals were considered (“random” or “infected” samples), the specific MLG (day n = 49, night n = 40), and all interactions possible between those factors, using a Poisson link function. In this model, differential depth selection by different MLGs would be indicated by a significant interaction between MLG and depth. Whether this depth selection of MLGs was different between random and infected samples would be indicated by a significant three-way interaction between MLG, depth, and sample type. To investigate DVM, we combined the day and night samples, and refit the model above including a “daytime” term and all interactions. DVM *per se* would be indicated by an interaction between depth and daytime, while differential DVM by MLGs would be indicated by an interaction between depth, daytime, and MLG.

Significance of the above model terms was investigated via sequential analysis of deviance. Each term was added in order, starting with the main effects, followed by two-way interactions, and ending with higher-order interactions. For each additional term, the associated reduction of deviance was taken as a χ^2 test statistic, and a p-value was generated by comparing that test statistic against a distribution with degrees of freedom equal to one less than the number of term levels. Because the significance of terms in some part could depend on their order of addition to the model, we re-fit the models with each possible order of terms. This shuffling did not affect the significance of any model terms.

As a complement to the above MLG-based test of depth selection, we tested whether the *Daphnia* exhibited depth-based population structure using Discriminant Analysis of Principal Components (DAPC) as implemented in the R package *adegenet* (Jombart, 2008). This method first transforms the microsatellite data using Principal Components Analysis, which ensures that variables are not correlated, and then applies discriminant functions to the transformed data in an attempt to

minimize within-group variance while maximizing between-group variance. The groups we attempted to discriminate were the depth levels. We separately tested the infected and random samples to determine whether infected animals exhibit different vertical structure, and performed these tests for both the day and night samples. In each case, we performed the test with maximum sensitivity to population structure, that is, depth of origin. Therefore, in the first step (dimension reduction), we derived all Principal Components up to a threshold of 99% explained variance, and in the second step (discriminant analysis), we applied as many possible discriminant functions to those PC-scores as we could, which in this case was one fewer than the number of depth layers. Because animals with the same MLG appeared in several depth layers, we included all sampled animals in this analysis and did not, for example, only include one member of each MLG per layer. Detectable vertical genetic structure would result in assignment of individual animals to their depths of origin, while the absence of structure leads to assignment probability evenly split among depth layers. We considered an animal to be correctly assigned if it was assigned to its source layer with posterior probability > 0.95.

Species Identification

MLGs were assigned to species using information from our panel of reference genotypes made up of 57 *D. galeata*, 30 *D. longispina*, 31 *D. cucullata*, and 49 *D. galeata x longispina* hybrids drawn from lakes covering much of western Europe north and south of the Alps (Möst, 2013). The reference genotypes were clustered using Discriminant Analysis of Principal Components (DAPC), in which we retained Principal Components explaining 80% of the variance, and derived 3 discriminant functions. These functions were then applied to the MLGs collected during this study.

Fecundity analysis

Differences in fecundity among parasitized and non-parasitized *Daphnia* as well as fecundity-dependent depth selection were analysed with a non-parametric Wilcoxon rank sum test since fecundity data were strongly zero-inflated.

Results

The *Caullerya mesnili* epidemic started in late July 2013 and reached a maximum between late August and early September in Greifensee (Fig. 1). The epidemic ceased in mid-October, but infected animals were found until the end of the year.

During the diurnal study at the end of August and early September, a stable thermal stratification existed in the lake with oxygen levels of $<1 \text{ mg l}^{-1}$ at depths below 5 m. The analysis of the vertical distribution showed that both infected and uninfected *Daphnia* performed diel vertical migration (DVM). However, the DVM pattern did not differ between infected and uninfected *Daphnia* (GLMM, $\chi^2 = 1.23$, $\text{df} = 3$, $p > 0.05$); both were mostly abundant in deeper depths (~50% from 7.5 – 5 m) during the day and equally distributed in the three uppermost layers (~30% each) during the night (Fig. 2). The two deepest layers (30-20 and 20-10) contained less than $0.01 \text{ Daphnia l}^{-1}$ during day and night and are therefore not visible in Figure 2.

In the qualitative routine samples a total of 649 individuals were genotyped. Exact numbers per date can be found in Table S4. We detected 67 MLGs in total from which eight reached at least once an abundance of more than 15% from June until December (Fig. 3). One of these MLGs (MLG83) dominated the population throughout the summer and decreased in dominance prior to the onset of the *Caullerya* epidemic. Three MLGs (MLG31, MLG46, MLG54) dominated the population at the beginning of the epidemic in August and disappeared at the end of the epidemic in October. In contrast, MLG66 became abundant during and three MLGs (MLG18, MLG27, MLG61) after the epidemic. MLG18 dominated the population after the epidemic for about a month but reached similar levels as the other three MLGs thereafter.

In the qualitative depth sample a total of 533 individuals were genotyped (461 without the whole water column samples). All of the *Daphnia* we collected were identified as hybrids between *D. galeata* and *D. longispina* with posterior probability > 0.95 . Exact numbers per depth can be found in Table S3, and were used to construct a generalized linear model with Poisson link function. The full models can be found in Tables S6-S8; model terms of interest are presented here. Different MLGs did not show significant differences in depth selection during the day (GLM, depth x MLG interaction, $\chi^2 = 31.3$, $\text{df} = 40$, $p = 0.84$) or during the night ($\chi^2 = 31.9$, $\text{df} = 52$, $p = 0.98$), nor in their migration pattern (GLM, MLG x depth x daytime interaction, $\chi^2 = 24.4$, $\text{df} = 24$, $p = 0.44$). Furthermore, depth selection did not differ between random and *Caullerya*-infected samples either during the day (GLM, depth x MLG x sample type interaction, $\chi^2 = 1.04$, $\text{df} = 4$, $p = 0.90$) or at night ($\chi^2 = 0.73$, $\text{df} = 6$, $p = 0.95$). Therefore, MLG data were aggregated across all depths for random population and *Caullerya* samples (Table 1). Most clones were under-infected, which means that they were more common in the random population samples than in the non-random *Caullerya* samples. The most common MLG (MLG83) prior to the epidemic was under-infected during the *Caullerya* epidemic at the end of August. The pattern for MLGs that were common during the epidemic was not consistent; with some clones being over- and some clones being under-infected.

As found on the clonal level, we did not detect any genetic structure between different depths despite the extremely permissive DAPC settings (Fig. 4). Different depth levels showed only

slight separation, and few individuals could be assigned to their source depth. In the Random samples, only 4 of 147 individual animals collected during the day were correctly assigned to their source depth, and only 2 of 144 from the night sample. This poor assignment was similar in the infected samples: assignment was correct for only 3 of 92 daytime and 6 of 78 nighttime animals. All of the above correctly assigned individuals were of different genotypes, with the exception that two individuals of the same genotype were correctly assigned to their layer of origin in the random daytime samples.

In general, *Caullerya*-infected females were almost entirely castrated with only 17 out of 685 (2.5%) individuals collected during the year having at least one egg, compared to 784 of 1745 (44.9%) in the random samples. This discrepancy also accounts for the vertical distribution of *Caullerya*-infected *Daphnia* (Fig. 5). Therefore, no meaningful statistical analysis of fecundity was possible to investigate the vertical distribution of *Caullerya*-infected females. In contrast, fecund females in the population samples showed a significantly distinct diurnal migration behaviour compared to less fecund females as shown in a non-parametric Wilcoxon rank sum test for all depths ($W = 20217.5$, p -value = 0.050) as well as for the upper 10 m ($W = 725$, p -value = 0.021), indicating that fecund females resided at lower depths during the day than non-fecund ones.

Discussion

As found for other European lakes, interspecific hybrids between *Daphnia galeata* and *D. longispina* performed diel vertical migration (DVM) in Greifensee (e.g. De Meester et al., 1995; Ringelberg, 2010; Schwenk & Spaak, 1995; Stich & Lampert, 1981), which is a common predator-avoidance strategy in zooplankton (Stich & Lampert, 1981). However, *Daphnia* performing DVM to avoid fish predation incur several trade-offs since the migration activity is energetically costly (Ringelberg, 2010) and because they might suffer from resource limitation in deeper depths. In those depths algal productivity is low, and, due to the cooler temperatures, the egg development time will also be longer, resulting in a slower instantaneous birth rate (Ringelberg, 2010; Stich & Lampert, 1981). We hypothesized that parasitism should further aggravate the situation for infected *Daphnia* which should therefore cease migration due to overly high costs. However, contrary to our expectations, *Caullerya* infected *Daphnia* migrated equally well as uninfected ones, as shown on the population as well as on the genotype level. Although DVM was not impaired by *Caullerya* infections, *Daphnia* suffered high costs as apparently infected individuals were almost entirely castrated, which is in accordance with previous studies that showed that *Caullerya* infected *Daphnia* lifespan and fecundity were considerably reduced (Bittner et al., 2002; Lohr et al., 2010). Another trade-off of migrating *Daphnia* that might incur is risking a higher chance of infection from parasite spores deposited in the sediments (Decaestecker et al., 2002). Firstly, since infected and uninfected *Daphnia*

migrated equally well this hypothesis is not substantially supported by our data. Moreover, the sediment as source of infection is unlikely for taxa of the *D. longispina* complex in Greifensee as the population resided in the upper 7.5 m whilst the maximum depth of the lake is 32 m. Since the thermocline was around 10 m, we can also refute the hypothesis that migration to deeper layers is an adaptive behaviour to slow down parasite growth.

We further found clonal succession in Greifensee during the period of observation, but this succession did not primarily seem to be influenced by or influence the *Caullerya* epidemic since all except one of the common MLGs were rather under- than over-infected, which is contradictory to a host-parasite arms race, where parasites should evolve higher virulence towards more common genotypes (van Valen, 1962). Nevertheless, these findings are in accordance with those of previous studies on *Caullerya* epidemics in the *D. longispina* complex (Wolinska et al., 2004; Wolinska & Spaak, 2009; Yin et al., 2012) and it was suggested that this phenomenon arises from “snapshot” sampling at different points of a co-evolutionary cycle (Wolinska et al., 2008). However, our study was too short to address this hypothesis. Moreover, random host genotype selection might be advantageous for a parasite under fluctuating environmental conditions (Gandon, 2002; Gandon et al., 1998). Therefore, outbreak and course of *Caullerya* epidemics seem to be driven by a suitable combination of fluctuating environmental conditions such as food quality and quantity and host density (Lange et al., 2014). Indeed, *Caullerya* infection patterns are modified by temperature as shown in experiments in the laboratory (Schoebel et al., 2011). Further support comes from field studies on other *Daphnia* parasites that suggest that the outbreak and course of epidemics is strongly dependent on abiotic conditions such as temperature (Duffy et al., 2009) or lake productivity (Wolinska et al., 2007) as well as biotic conditions such as host density and presence of other parasites (Tellenbach et al., 2007). Other mechanisms, especially environmental fluctuations and heterogeneity are considered to play an important role in this maintenance of genetic variation within and between species, too (Chesson & Huntly, 1997; Sommer, 1984), and it was shown that different *Daphnia* genotypes differ in their response to various environmental stressors, such as oxygen (Weider & Lampert, 1985), food quality (Brzeziński & von Elert, 2007; Weider et al., 2005) or temperature (Carvalho & Crisp, 1987). Depending on the combination of stressors (i.e. depending on the temporal and spatial structure) different genotypes may dominate the population until the combination of these stressor changes, leading to clonal succession.

Finally, egg bearing females in Greifensee resided at lower depths than less fecund females during the *Caullerya* epidemic in late autumn 2013. This finding is in line with previous studies (Duncan et al., 1993; Winder & Spaak, 2001). Since migrating *Daphnia* face a trade-off in being at risk of predation at higher depths and suffering from starvation at lower depths an alternative strategy “better dead than unfed” has been suggested with epilimnetic non-migrators that have a higher

reproductive rate (Huntley & Brooks, 1982). Our results further suggest that *Caullerya* infections do not alter these different strategies.

Conclusions

This is one of the first studies investigating the interaction between the DVM behaviour of zooplankton and parasite epidemics, even though it has been known for a while that parasites are common in zooplankton populations of large lakes. The findings of this study show that *Caullerya* epidemic neither did affect nor was affected by the DVM behaviour of *Daphnia*. Furthermore, outbreak and course of *Caullerya* epidemics seem to rely strongly on environmental cues. Because of our limited number of samples, this study provides only a snapshot of how the interaction between parasite epidemics and DVM behaviour of *Daphnia* might affect each other and, hence, further studies on a finer resolved temporal and spatial scale are required to disentangle factors influencing this interaction, which will ultimately lead to a better understanding of their impact on population dynamics.

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Tables

Table 1. Frequency^a of the most common multilocus genotypes (MLG) in random population and non-random *Caullerya*-infected samples from 29 August 2013.

MLG ^b	Random sample	<i>Caullerya</i> sample	Total	$\log_2(R/C)^c$
54	43	73	116	0.8
83	71	3	74	-4.6
18	47	18	65	-1.4
41	35	2	37	-4.1
31	28	6	34	-2.2
67	19	12	31	-0.7
66	12	14	26	0.2
68	12	9	21	-0.4
78	10	7	17	-0.5
64	7	4	11	-0.8
25	3	4	7	0.4
47	3	3	6	0
81	6		6	
65	3	2	5	-0.6
61	3		3	
27	1	1	2	0
46	1		1	

^aData were pooled over depths as there were no differences in DVM behaviour.

^bNumbers in bold indicate MLGs that attain a certain degree of dominance throughout the year; therefore, the last three clones were added though they were rare on this particular sampling date.

^cLogarithm to the base 2 of the frequency ratio of random (R) and *Caullerya*-infected (C) samples; positive values indicate an over-, negative values an under-infection.

Figure Legends

Figure 1: Changes in *Daphnia* density (grey area) and *Caullerya* prevalence (black line) in the entire water column of Greifensee from January to December 2013.

Figure 2. Distribution of *Caullerya* infected (black) and uninfected (light grey) *Daphnia* during day and night from quantitative samples taken in August and September 2013. The proportions of infected and uninfected individuals sum to 1 each, during day and night.

Figure 3: Changes in population dominance by different MLGs from May to December 2013 (random samples). For clarity only clones which reached at least once a proportion of 15% or higher are shown. In total, 67 MLGs were detected during this period. See Table S4 for more details.

Figure 4: Discriminant analysis of principal components (DAPC) based on microsatellite data with depth of origin as a prior. Dots represent group centroids with respect to the first two functions (LD1, LD2), and lines connect group centroids to individual *Daphnia* scores. Day and night samples are left and right, while random and infected samples are top and bottom

Figure 5. Vertical distribution of *Daphnia* fecundity in population and *Caullerya*-infected samples on August 29 2013 during daytime (grey) and night (black); numbers in brackets indicate the number of individuals per sample. Fecund females in the population samples showed a significantly distinct diurnal migration behaviour compared to less fecund females as shown in a non-parametric Wilcoxon rank sum test for all depths ($W = 20217.5$, $p\text{-value} = 0.050$) as well as for the upper 10 m ($W = 725$, $p\text{-value} = 0.021$).

List of supplementary Tables (see: Electronic_Supplement_Tables_S1_S8)

Table S1_Quantitative Routine Samples

Sampling and counting details of the quantitative routine samples from January to December 2013. The sampled volume was concentrated to 40 ml from which a subsample was counted and *Daphnia* per litre were calculated. All life stages of *Daphnia* were considered (female, male, juvenile).

Table S2_Quantitative Depth Samples

Sampling and counting details of the quantitative depth samples from 29 August 2013 and 03 September 2013 during day and night. The sampled volume was concentrated to 40 ml from which a subsample was counted, infection status noted and *Daphnia* per litre were calculated. Only adult female *Daphnia* were considered.

Table S3_Qualitative Depth Samples

Sampling details of the qualitative depth samples from 29 August 2013 during day and night. From the sampled volume 80 *Daphnia* (40 random, 40 *Caullerya* infected) were sampled and genotyped. Only adult female *Daphnia* were considered and only animals with no more than 2 missing markers were used for the analysis

Table S4_Qualitative Routine Samples

Genotyping details of the qualitative routine samples from May to December 2013. Only adult female *Daphnia* were considered and only animals with no more than 2 missing markers were used for the analysis

Table S5_Data Set_Analyses

Data sets used for the individual analyses.

Table S6_Day_model

Testing for depth selection during the day. Analysis of deviance table for a GLM (binomial link) in which *Daphnia* counts are expressed as a function of depth layer, random vs. infected sample, MLG, and all interactions. Differential depth selection by MLGs is indicated by an interaction between layer and MLG, differential depth selection by infected animals is given by the random X layer interaction, and finally the layer X random X MLG interaction indicates whether MLGs vary in their depth selection based on infection status.

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Table S7_night_model

Testing for depth selection during the night. Analysis of deviance table for a GLM (binomial link) in which *Daphnia* counts are expressed as a function of depth layer, random vs. infected sample, MLG, and all interactions. Differential depth selection by MLGs is indicated by an interaction between layer and MLG, differential depth selection by infected animals is given by the random X layer interaction, and finally the layer X random X MLG interaction indicates whether MLGs vary in their depth selection based on infection status.

Table S8_combined_model

Testing for DVM. Analysis of deviance table for a GLM (binomial link) in which *Daphnia* counts are expressed as a function of depth layer, random vs. infected sample, MLG, daytime, and all interactions. DVM in general is given by the layer X daytime interaction, and differential DVM by MLGs is given by the interaction between layer, daytime, and MLG. Whether these behaviours differ between random and infected animals is given by the addition of the "random vs. infected" term to the above interactions

Figure 4

Figure 5

Table S1

Date	Net hauls	Mesh size (µm)	Sampled volume (l)	Concentrated volume (ml)	Counted subsamples (ml)	<i>Daphnia</i> in subsample	<i>Daphnia</i> l ⁻¹ lake
1/9/2013	3	95	1017.88	40	6	182	1.19
2/15/2013	3	95	1017.88	40	4	13	0.13
3/4/2013	3	95	1017.88	40	6	3	0.02
4/12/2013	3	95	1017.88	60	4	21	0.31
4/24/2013	3	95	1017.88	60	4	73	1.08
5/7/2013	3	95	1017.88	80	4	486	9.55
5/14/2013	3	95	1017.88	150	4	385	14.18
6/17/2013	3	95	1017.88	80	4	283	5.56
7/1/2013	3	95	1017.88	100	4	270	6.63
7/16/2013	3	95	1017.88	80	4	167	3.28
8/7/2013	3	95	1017.88	100	4	517	12.70
8/21/2013	3	95	1017.88	60	4	299	4.41
9/3/2013	3	95	1017.88	40	4	299	2.94
9/17/2013	3	95	1017.88	40	4	575	5.65
10/7/2013	3	95	1017.88	60	4	327	4.82
10/17/2013	3	95	1017.88	60	4	588	8.66
11/7/2013	3	95	1017.88	60	4	812	11.97
12/9/2013	3	95	1017.88	60	4	400	5.89

Table S2

Layer	Daytime	Date	Net hauls	Mesh size (μm)	Sampled volume (l)	Concentrated volume (ml)	Counted subsamples (ml)	<i>Daphnia</i> per subsample	<i>Daphnia</i> l ⁻¹	<i>Caullerya</i> prevalence (%)	<i>Daphnia</i> per layer	<i>Daphnia</i> per layer * 10 ⁸	infected counted	uninfected counted
2.5-0	day	9/3/2013	3	250	360.79	40	5	118	2.6165	27.12	5.41E+10	540.56	32	86
5-2.5	day	9/3/2013	3	250	360.79	40	3	162	5.9868	24.07	1.18E+11	1184.49	39	123
7.5-5	day	9/3/2013	3	250	360.79	40	3	291	10.7541	32.99	2.01E+11	2005.64	96	195
10-7.5	day	9/3/2013	5	250	601.32	40	9	90	0.6652	41.11	1.13E+10	113.42	37	53
20-10	day	9/3/2013	5	250	2405.28	40	40	21	0.0087	80.95	4.16E+08	4.16	17	4
30-20	day	9/3/2013	5	250	2405.28	40	40	4	0.0017	75	3.96E+07	0.40	3	1
2.5-0	night	9/3/2013	3	250	360.79	40	3	156	5.7651	39.1	1.19E+11	1191.07	61	95
5-2.5	night	9/3/2013	3	250	360.79	40	4	173	4.7950	20.23	9.49E+10	948.69	35	138
7.5-5	night	9/3/2013	5	250	601.32	40	3	275	6.0977	24	1.14E+11	1137.22	66	209
10-7.5	night	9/3/2013	5	250	601.32	40	40	157	0.2611	24.2	4.45E+09	44.52	38	119
20-10	night	9/3/2013	5	250	2405.28	40	40	6	0.0025	66.67	1.19E+08	1.19	4	2
30-20	night	9/3/2013	5	250	2405.28	40	40	14	0.0058	78.57	1.39E+08	1.39	11	3
2.5-0	day	8/29/2013	1	250	120.26	40	12	317	8.7862	9.78	1.82E+11	1815.23	31	286
5-2.5	day	8/29/2013	1	250	120.26	40	11	314	9.4943	10.19	1.88E+11	1878.44	32	282
7.5-5	day	8/29/2013	1	250	120.26	40	9	341	12.6019	9.09	2.35E+11	2350.26	31	310
10-7.5	day	8/29/2013	1	250	120.26	40	40	30	0.2495	30	4.25E+09	42.53	9	21
20-10	day	8/29/2013	1	250	481.06	40	40	5	0.0104	20	4.95E+08	4.95	1	4
30-20	day	8/29/2013	1	250	481.06	40	40	2	0.0042	0	9.91E+07	0.99	0	2
2.5-0	night	8/29/2013	1	250	120.26	40	9	239	8.8324	13.81	1.82E+11	1824.78	33	206
5-2.5	night	8/29/2013	1	250	120.26	40	6	166	9.2020	18.07	1.82E+11	1820.61	30	136
7.5-5	night	8/29/2013	1	250	120.26	40	6	130	7.2064	25.38	1.34E+11	1343.99	33	97
10-7.5	night	8/29/2013	1	250	120.26	40	40	41	0.3409	34.15	5.81E+09	58.13	14	27
20-10	night	8/29/2013	1	250	481.06	40	40	5	0.0104	40	4.95E+08	4.95	2	3
30-20	night	8/29/2013	1	250	481.06	40	40	4	0.0083	25	1.98E+08	1.98	1	3

Table S3

Layer	Daytime	Date	Net hauls	Mesh size (μm)	Sampled volume (l)	Individuals sampled	Individuals genotyped*
2.5-0	day	8/29/2013	3	250	360.79	80	30
5-2.5	day	8/29/2013	3	250	360.79	80	76
7.5-5	day	8/29/2013	3	250	360.79	80	62
10-7.5	day	8/29/2013	5	250	601.32	66	63
20-10	day	8/29/2013	5	250	2405.28	NA	NA
30-20	day	8/29/2013	4	250	1924.22	8	8
30-0	day	8/29/2013	3	250	360.79	80	33
2.5-0	night	8/29/2013	3	250	360.79	80	64
5-2.5	night	8/29/2013	3	250	360.79	80	54
7.5-5	night	8/29/2013	5	250	601.32	80	49
10-7.5	night	8/29/2013	5	250	601.32	80	45
20-10	night	8/29/2013	5	250	2405.28	7	4
30-20	night	8/29/2013	4	250	1924.22	7	6
30-0	night	8/29/2013	3	250	360.79	80	39

*with no more than two missing markers

Table S4

Date	Individuals sampled	Individuals genotyped	Mesh size (μm)
5/14/2013	96	58	250
6/17/2013	96	76	250
7/16/2013	96	73	250
8/21/2013	96	86	250
9/17/2013	96	91	250
10/17/2013	96	92	250
11/7/2013	96	86	250
12/9/2013	96	87	250

Table S5

Type of analysis	Data set
Depth distribution of infected and uninfected <i>Daphnia</i>	Quantitative Depth
MLG population	Qualitative Depth
MLG over time	Qualitative Routine
<i>Daphnia</i> density over time	Quantitative Routine
<i>Daphnia</i> fecundity	Qualitative Depth

Table S6

Model Term	Df	Deviance	Residual Df	Resid. Dev	Pr(>Chi)	Of Interest?	How?	Eco. Interpretation
NULL	NA	NA	116	2.49E+02	NA			NULL
Layer	5	1.11E+01	111	2.37E+02	4.87E-02			Counts differ by Layer
random	1	5.42E-04	110	2.37E+02	9.81E-01			None
mlg	48	1.69E+02	62	6.89E+01	2.55E-15			Counts differ by MLG
Layer:random	4	4.05E+00	58	6.48E+01	4.00E-01	YES	Infection by Depth	Counts by Layer differ between Infected and Random Samples
Layer:mlg	40	3.13E+01	18	3.35E+01	8.36E-01	YES	Depth Selection	Counts per layer differ by MLG
random:mlg	11	2.85E+01	7	5.01E+00	2.69E-03			Counts per MLG differ between Infected and Random Samples
Layer:random:mlg	7	5.01E+00	0	1.55E-15	6.59E-01	YES	Inf. by depth by MLG	MLG difference per Layer differs between Infected and Random Samples

Response variable: Daphnia Counts

Data Subset: Day Samples

Table S7

Model Term	Df	Deviance	Residual Df	Resid. Dev	Pr(>Chi)	Of Interest?	How?	Eco. Interpretation
NULL	NA	NA	124	1.49E+02	NA			NULL
Layer	6	11.406378	118	1.38E+02	0.076600025			Counts differ by Layer
random	1	9.419391	117	1.28E+02	0.002147029			None
mlg	39	74.499522	78	5.39E+01	0.000530613			Counts differ by MLG
Layer:random	4	3.001891	74	5.09E+01	0.557508954	YES	Infection by Depth	Counts by Layer differ between Infected and Random Samples
Layer:mlg	52	31.935032	22	1.89E+01	0.987177472	YES	Depth Selection	Counts per layer differ by MLG
random:mlg	11	12.767548	11	6.17E+00	0.308789537			Counts per MLG differ between Infected and Random Samples
Layer:random:mlg	11	6.169888	0	-5.72E-27	0.861791562	YES	Inf. by depth by MLG	MLG difference per Layer differs between Infected and Random Samples

Response variable: Daphnia Counts

Data Subset: Day Samples

Table S8

Model Term	Df	Deviance	Residual Df	Resid. Dev	Pr(>Chi)	Of Interest?	How?	Eco. Interpretation
NULL	NA	NA	241	3.99E+02	NA			NULL
Layer	6	17.244726	235	3.82E+02	8.42E-03			None
Daytime	1	1.347182	234	3.81E+02	2.46E-01			None
mlg	66	224.977855	168	1.56E+02	3.20E-19			Counts differ by MLG
random	1	3.038418	167	1.53E+02	8.13E-02			None
Layer:Daytime	5	11.807708	162	1.41E+02	3.75E-02	YES	Total DVM	Counts per Layer differ between Day and Night Samples
Layer:mlg	71	37.733277	91	1.03E+02	1.00E+00			None
Daytime:mlg	16	12.551058	75	9.07E+01	7.05E-01			None
Layer:random	4	6.786221	71	8.39E+01	1.48E-01			None
Daytime:random	1	2.41431	70	8.15E+01	1.20E-01			None
mlg:random	16	37.945055	54	4.35E+01	1.54E-03			Count per MLG differ between Random and Infected Samples
Layer:Daytime:mlg	24	24.429292	30	1.91E+01	4.37E-01	YES	DVM by MLG	Counts of each MLG per layer differ between Day and Night Samples
Layer:Daytime:random	4	2.664834	26	1.64E+01	6.15E-01			Per layer count differences between Day and Night are different in Infected and Random Samples
Layer:mlg:random	20	13.709846	6	2.70E+00	8.45E-01			None
Daytime:mlg:random	3	1.404602	3	1.30E+00	7.04E-01			None
Layer:Daytime:mlg:random	3	1.299257	0	9.55E-15	7.29E-01			None

Response variable: Daphnia Counts
Data Subset: Combined Day and Night Samples