

How biotic interactions between *Daphnia magna* and *Vorticella* alter the ability of *Daphnia magna* to respond to abiotic changes

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Abstract

Climate change is altering regional temperatures, forcing species to respond to new environmental challenges. Current research suggests that temperature affects population dynamics of species, altering the timing of species abundance. However, little is known about the combined effects of temperature and interspecific competition. In this study, we examined how temperature and competition with the competitor *Vorticella sp.* affected the population dynamics of the common zooplankton, *Daphnia magna*. We examined how temperature and *Vorticella* both affect *D. magna*'s maximum population size, the timing of population growth, and the final population size. Overall, our results suggest that high temperature and *Vorticella* presence, in combination, can impact the timing of population growth, as well as alter life history characteristics, for *D. magna*. These results suggest that *Vorticella* delay *D. magna*'s population growth only when temperatures are high, and that *Daphnia* respond to high temperatures morphologically through reduction in size. Temperature and *Vorticella* presences did not significantly impact the final population sizes, indicating that although the initial population dynamics are affected by these stressors, final population size was not changed. Because climate change can alter phenological¹ timing of plant and animal species, further insight into the effect of temperature change on population dynamics of zooplankton will benefit scientific understanding as to how key trophic species, such as primary consumers, respond to increased temperature in freshwater aquatic ecosystems.

¹ Referring to the seasonal natural cycles of organisms

Keywords Abiotic and biotic stress, Climate Change, Competition, *Daphnia magna*, Temperature, *Vorticella*

Introduction

Global climate change has led to increased average temperatures across the planet (Hansen et al. 2010; Khalil et al. 2016). Increased temperatures can affect demographic rates related to feeding, fecundity², growth, and time to emergence (Dallas & Ross-Gillespie 2015). They also can alter the phenological timing of plant and animal species, resulting in such effects as decreased nutrient availability, shortened breeding seasons, and a mismatch between population growth and resource availability (e.g., Carvalho and Kirika 2003; Parmesan 2006; Stenseth and Mysterud 2002; Winder and Schindler 2004; Winkler et al. 2002). In addition to the direct effects of changing temperatures on a species, indirect effects may arise through changes in biotic interactions, which may be weaker or stronger depending on how all species in the community respond to changing temperatures (Davis et al 1998). If a species' fitness is already lowered by increased temperatures, competition may further reduce fitness to where the population cannot grow. For many species of interest, temperature tolerance and temperature effects on population growth have been investigated (De Meester et al. 2011; Parmesan 2006; Van Doorslaer et al. 2009). However, the responses to increased temperatures expected in natural populations, which exist in multi-species communities, are less understood. Because a population's ecology cannot be understood without considering both the abiotic and biotic environment they experience, this limits prediction about how a species will respond to climate change. The interaction between temperature and competition can alter species responses.

² The reproductive rate of an organism

Therefore, it is important to examine both how temperature and interspecific interactions affect a species (Walther et al. 2002; Winder and Schindler 2004).

Populations can respond to a changing climate in a variety of ways, including migration to more suitable habitat, evolutionary adaptation to altered conditions, plastic changes, or extinction (Prowse et al. 2009; Gienapp et al. 2008; Stoks et al. 2014). Monitoring shifts in species' distributions has revealed that many populations have migrated in response to changes in local environments (Parmesan 2006). Range shifts occur as the home environment becomes unsuitable for the species. There are many approaches for testing how a species responds to increased temperature. One method focuses on predicting how species distributions will change as temperatures increase (Davis et al. 1998). Through this method, correlations can be drawn between species distributions and environmental conditions. Although this approach can be revealing for temperature-sensitive species, it usually does not incorporate the important effect of species interactions on population dynamics. For instance, it has been shown with *Drosophila*³ species that competition interacts⁴ with temperature, affecting the distribution and abundance of the species (Davis et al. 1998). Species interact in complex trophic networks⁵, and because of this, community-level analysis is highly important to understand the effects of global environmental change (Tylainakis et al. 2008).

Freshwater aquatic species also must contend with restrictions because of their reduced ability to move from a home range due to geographical barriers (Parmesan 2006). Species in freshwater habitats may not be able to respond to large changes in temperature through evolutionary or plastic responses, and instead may migrate horizontally or vertically in the water

³ A genus of fruit fly often used as a model genetic organism

⁴ In statistics, referring to a relationship between two variables where the effect of each variable is not constant on a third variable

⁵ A food web

column seeking out optimal conditions (Brucet et al. 2012; Prowse et al. 2009). Common garden experiments as well as experimental thermal evolution studies have shown that freshwater zooplankton also respond to increased temperature through plastic changes or rapid genetic adaptation both in the presence and absence of competition, predation, or parasitism (Stoks et al. 2013; Van Doorslaer et al. 2009; De Meester et al. 2011). In particular, populations of the zooplankton *Daphnia magna* have been shown to adapt genetically through changes in such characteristics as body size when exposed to changes in temperature in a timeframe of years or even months (De Meester et al. 2011). In another study of *D. magna*, thermal selection⁶ was shown to influence intraspecific competition⁷, increasing competitive ability in populations that were previously exposed to higher temperatures (Van Doorslaer et al. 2009). These studies reinforce the importance of considering both changing temperatures and species interactions (competition, predation, parasitism, mutualism⁸, and commensalism⁹) in combination. Daphniid species play a major role in the food web as a keystone¹⁰ algivore (Weisse 2006). Increased temperatures can result in significant effects on life history characteristics of *Daphnia* as they compete for resources. Therefore, understanding how these zooplankton respond to species interactions and increased temperatures can add important insight into the effects that climate change may have on an *in situ* population, e.g. one that is not able to migrate to new conditions, at least over short time scales.

Over the summer of 2015, we sampled ponds across an elevation gradient of the Front Range of the Rocky Mountains to set up an experimental study designed to analyze the effects of

⁶ An experimental evolution practice in which *Daphnia* clones controlled for home temperature conditions are placed into experimental populations under temperature treatments to analyze selection data

⁷ Referring to competitive interactions between two different species

⁸ A relationship between species where both organisms benefit

⁹ A relationship between two species where one species benefits while not harming the other

¹⁰ An organism that plays an important role in an ecosystem by determining the abundance of many other species

temperature and competition using a natural population. Through the field study, we identified daphniid species and, discovered that in at least two of these ponds a sessile protozoan ciliate in the genus *Vorticella*¹¹ was present and frequently attached to the daphniid species. The complexity of the apparent relationship between daphniids and *Vorticella*—which were not parasitic, but instead epibionts¹² that appeared to compete with daphniids for resources— led us to consider the relationship between *Daphnia magna* and *Vorticella sp.* in a laboratory microcosm.

Research using *Vorticella* is limited due to the difficulty of developing lab cultures (Hyman 1931; Sudo and Aiba 1973). This has led to a gap in understanding the relationship between daphniids and *Vorticella*. Two contrasting relationships have been proposed for their interaction. First, it has been suggested that *Vorticella* compete with *Daphnia* spp. for access to resources (Kankaala and Eloranta 1987; Weisse 2006). Both species feed on algae, bacteria and other microorganisms. Because these ciliates and daphniids have this similar diet, and *Vorticella* frequently attach to *Daphnia* spp., these interactions suggest that *Vorticella* are in direct competition with *Daphnia* spp. For example, *Vorticella* have been shown to reduce food particles around *Daphnia* spp. hosts at a rate of 25 to 33 percent (Kankaala and Eloranta 1987). Alternatively, *Vorticella*, as epibionts, have been suggested to form commensalistic relationships (Aalto et al. 2014). Although *Vorticella* was found to cause a significant reduction in fecundity of *Daphnia* spp. in one study, in another study, no significant reduction in fecundity was observed for *Daphnia* spp. with *Vorticella* attachment (Aalto et al. 2014; Decaestecker et al. 2005). Increased *Vorticella* attachment to daphniid hosts has been associated with individual

¹¹ A stalked, bell-shaped species known to anchor on substrate and other species in the water in order to gain greater access to resources

¹² An organism that lives on the surface of another living organism in a neutralistic or commensalistic relationship

Daphnia spp. size, not the abundance of the *Daphnia* population (Aalto et al. 2014). Therefore, larger *Daphnia* spp. are more susceptible to *Vorticella* attachment, but the population density of *Daphnia* spp. is not correlated with increased *Vorticella* presence (Aalto et al. 2014). As seen from these studies, the relationship of *Daphnia* spp. and *Vorticella* is complex, suggesting that further study is needed to better understand the specific interactions that these species have.

This study asked how interspecific competition and increased temperatures affect *D. magna* population dynamics. *D. magna* tolerates temperatures between 10 to 30 °C, but achieves highest fitness between 18 and 22°C (Ebert 2005; De Meester et al. 2011). With this understanding, and under the assumption that *Vorticella* is a competitor for resources, we predicted that: [1] *D. magna* raised at a low temperature (19°C) would have larger population sizes than *D. magna* raised at a high temperature (25°C), [2] *D. magna* not cultured with *Vorticella* (V-) would have larger population sizes than *D. magna* raised in the presence of *Vorticella* (V+), and [3] temperature treatment and *Vorticella* presence would interact, so that when cultured at 25°C with *Vorticella*, *D. magna* population sizes would be strongly reduced (Fig. 1).

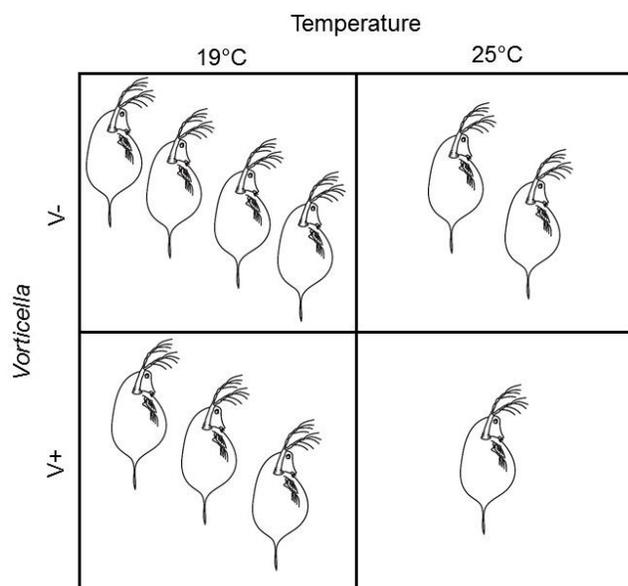


Figure 1. A qualitative depiction of the predictions about the abundance of *D. magna* for the competition experiment. *D. magna* raised at a low temperature (19°C) without *Vorticella* were predicted to sustain the highest population size for the duration of the experiment.

Methods

Natural History of *Daphna magna*

D. magna was selected for this project because of its role as a model organism (De Meester 2011) and because of the importance of *D. magna* to freshwater food webs (Nevalainen et al. 2014). As a model organism, *D. magna* reproduces easily in a laboratory setting and has been studied in a wide array of experiments (Wagner 2004; Bernot et al. 2006; Vandenbrouck 2011), including those aimed at understanding climate change impacts (Van Doorslaer et al. 2009; De Meester 2011). This species can also reproduce asexually and has a short lifespan, allowing us to more easily observe population dynamics of multiple generations over a short period of time. Finally, because *D. magna* typically reproduce sexually during seasonal changes,

the production of males and ephippia¹³ are a good indicator of stress conditions (Bernot et al. 2006).

Daphnia magna Culture

D. magna used for these experiments were cultured in the lab, raised at 19°C [Carolina Biological Supply Company, Burlington, NC]. *D. magna* individuals were placed in three liters of spring water with pebbles, 1 cm in diameter, lining the floor of the tank. Food was prepared by adding 200 mL of spring water to 0.4 grams of a mixture of dried algae and baker's yeast. Two milliliters of this mixture was then added to the culture twice a week. Species in the mixture were spirulina (*Arthrospira platensis*) [Vitamin Shoppe, Inc., Secaucus, NJ], *Chlorella pyrenoidosa* [The Vitamin Shoppe, North Bergen, NJ], and *Saccharomyces cerevisiae* [ACH Food Companies, Inc., Memphis, TN]. In addition to feeding, the water level was maintained through periodic replacement of a proportion of medium with spring water. A small population of *D. magna* was selected to test viability in the media to be used in the *Vorticella* competition experiment.

Vorticella sp. Culture

Because of the difficulties involved in culturing *Vorticella* in the lab, during the months of June and July of 2015, *Vorticella* sp. [Carolina Biological Supply Company, Burlington, NC] were cultured in multiple media types, as suggested in the literature, to test for the media type that would produce lasting laboratory populations (Hyman 1931; Suido and Aiba 1972). These cultures included media consisting of cereal grass, protist pellets with wheat seeds, timothy hay,

¹³ Winter eggs or diapause eggs

wheat seed [Carolina Biological Supply Company, Burlington, NC], or wheat grass [Amazing Grass, Newport Beach, CA] (Hyman 1931) (Table 1).

Concentration	Media	Measurement
High	Cereal	5.00 g
High	Protist Pellet with Wheat Seeds	0.44 g
High	Timothy	2.00 g
High	Wheat Grass	4.00 g
High	Wheat Seed	20 grains
Low	Cereal	2.50 g
Low	Protist Pellet with Wheat Seeds	0.22 g
Low	Timothy	1.00 g
Low	Wheat Grass	2.00 g
Low	Wheat Seed	10 grains

Table 1. *Vorticella* culture concentrations. All media were prepared with 1000 mL of spring water and boiled.

Each media type was prepared, mixed with spring water and boiled to sterilize the contents. For each type, high and low concentrations were prepared for each type of medium (cereal grass, protist pellets with wheat seeds, timothy hay, wheat seed, and wheat grass) (Table 1). Each type of medium was then inoculated with the bacteria, *Bacillus cereus* and *Serratia marcescens* DI [Carolina Biological Supply Company, Burlington, NC]. *Vorticella* was then added to 20 400 mL containers of each of the 10 types of media for low and high concentrations and temperatures of 19°C and 25°C. Population growth in each of these monocultures was recorded for every second day over two weeks. Cereal grass, timothy hay, and wheat grass had the highest population growth over this time, and were considered further. For these types, we then replaced 10% of the media in half the replicates, and 90% of the media in the other half. All treatments, including these additional treatments for cereal grass, timothy hay, and wheat grass were monitored for population growth for an additional four days. The low concentration wheat

grass medium with a 10% media replacement had the greatest population growth for the *Vorticella* under the temperature conditions (Table 2). Thus, wheatgrass with 10% replacement was used for the *D. magna* and *Vorticella* competition experiments.

Temperature	Media	Concentration	Day Maximum	% Media Replaced (Week 2)	Maximum Population	End Day	Final Population
19	Cereal	High	12	10	1026	12	1026
19	Protist Pellet	High	12	NA	98	12	98
19	Timothy	High	12	90	338	12	338
19	Wheat Grass	High	12	10	640	12	640
19	Wheat Seed	High	12	NA	0	12	0
19	Cereal	Low	12	10	399	12	399
19	Protist Pellet	Low	12	NA	0	12	0
19	Timothy	Low	12	90	88	12	88
19	Wheat Grass	Low	12	10	1354	12	1354
19	Wheat Seed	Low	12	NA	0	12	0
25	Cereal	High	9	NA	273	12	90
25	Protist Pellet	High	10	NA	16	12	0
25	Timothy	High	4	NA	384	12	166
25	Wheat Grass	High	7	NA	238	12	130
25	Wheat Seeds	High	12	NA	2	12	12
25	Cereal	Low	9	NA	187	12	22
25	Protist Pellet	Low	12	NA	0	12	12
25	Timothy	Low	3	NA	245	12	0
25	Wheat Grass	Low	4	NA	112	12	20
25	Wheat Seed	Low	10	NA	8	12	0

Table 2. *Vorticella sp.* population sizes achieved at high and low concentrations of medium at 19°C and 25°C. Wheat grass at 19°C with a 10% replacement had the largest population size.

Daphnia magna and *Vorticella sp.* Competition

Experimental set-up

The *D. magna* and *Vorticella* competition experiment began in July 2015. The experiment included two temperature treatments, 19°C and 25°C. Additionally, populations of *D. magna* were cultured with *Vorticella* (V+) or without *Vorticella* (V-). Eight replicates of each

treatment combination were established for a total of thirty-two populations (Fig. 2). Replicates were cultured in 400 mL containers, containing 100 mL of the low concentration wheat grass medium and 300 mL of spring water. This medium was inoculated with bacterial species (as above) 48 hours prior to use. Each competitive microcosm was started with fifteen individuals from the *D. magna* source culture (e.g. from 19°C). The sizes and number of adults and juvenile *D. magna* were randomly selected. To each V + replicate we also added 0.5 mL of well mixed *Vorticella* culture (19°C, low concentration wheat grass). We calculated that this was equivalent to 1354 *Vorticella* individuals based on the source culture densities (Table 2).

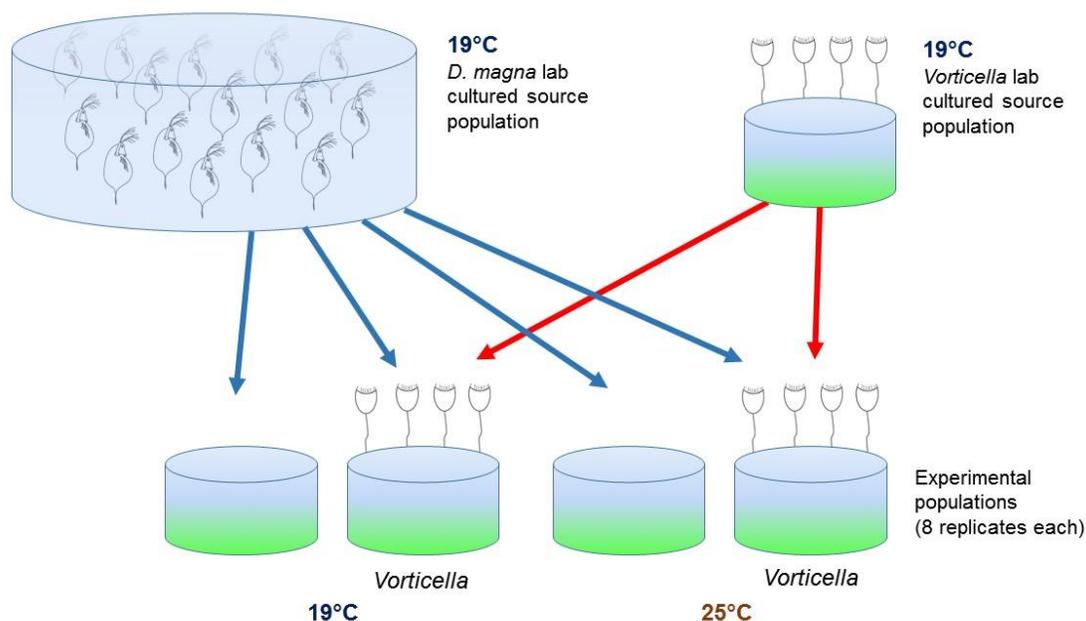


Figure 2. Diagram of experimental set up. *Vorticella* and *D. magna* source populations were cultured at 19°C and then placed into one of four treatments.

Data collection

We counted the populations of *Vorticella* and *D. magna* in each microcosm three times a week for the first four weeks, followed by two times a week for three weeks, and finally once per

week for the remaining time. This counting schedule allowed us to examine transient dynamics during the early stages of growth, as well as to measure the long term dynamics. During the first four weeks, we added 0.5 mL of food (as above in the section on *D. magna* cultures) to the microcosms. We replaced 10% of the media in each replicate with 40 mL of fresh media weekly. Ehippia were collected and counted from the replicates at the end of the experiment to analyze stress conditions for *D. magna* in relation to temperature and *Vorticella* presence.

Daphnia magna Individual Measurements

At the end of the competition experiment, (October, 2015), we randomly selected *D. magna* individuals from each treatment to be measured. Both adults and juvenile individuals isolated for the follow-on fecundity experiment (as described below) were photographed. The magnification was noted for each sample for calibration, and measurements were calibrated using a micro ruler under the same magnification. Measurements were then recorded using ImageJ [<http://imagej.nih.gov/ij/>]. Body measurements were length from eye to the end of the tail spine, width, eye diameter, and tail spine length.

Additionally, individual juvenile *D. magna* from the competition experiment were randomly selected for measurement of fecundity. Juveniles from each of the original four treatments were placed in a 100 mL container containing the same media, and home temperature of 19°C or 25°C. A total of 128 individuals were isolated, with eight individuals sampled from four randomly selected competition experiment microcosms. For each juvenile we recorded date of first brood and the number of offspring in their first brood.

Daphnia magna behavior in the presence of *Vorticella* sp.

In December of 2015, we conducted an experiment to observe the effects of *Vorticella sp.* on *D. magna* swimming behavior. Paired tests containing either substrate with *Vorticella sp.* or substrate without *Vorticella sp.* were set up in two small petri plates. Each plate held a fixed amount of medium, and was placed on a numbered grid containing numbers one through twenty-eight. If the plate intersected a square that was too small, this was included as part of another square (Fig. 3).

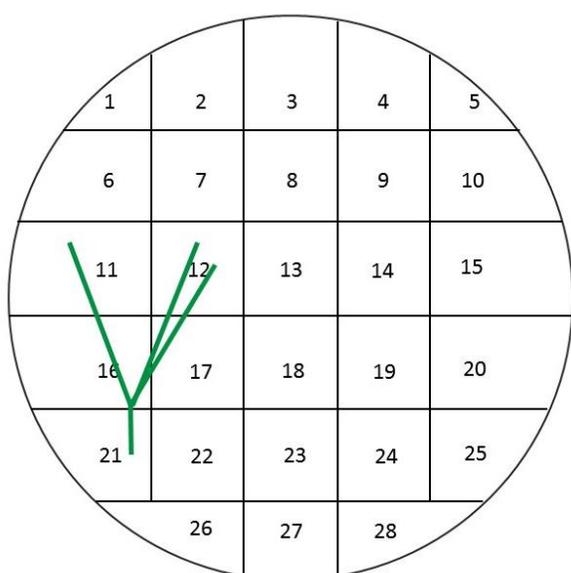


Figure 3. *D. magna* behavior trials arena. A petri plate was divided into 28 squares to record movement within the plate. Green substrate is shown in squares 11, 12, 16, 17, and 21.

Each *D. magna* individual was placed in one of the two plate types and given a 1 minute warm up time to adjust to the new environment. The order of plate type, i.e. substrate with *Vorticella sp.* or substrate without *Vorticella sp.*, was randomized for each *D. magna* trial. After the 1 minute warm up period, *D. magna* individuals were timed for 1 minute swimming during which, we recorded how often the individual entered each grid cell. The same individual was then moved to the other type of petri plate, where the 1 minute warm up followed by a 1 minute

trial was repeated. This was replicated using 10 different *D. magna* individuals, where each moved in the plate with *Vorticella sp.* and the plate without *Vorticella sp.*

Statistical Analysis

For the *D. magna* and *Vorticella sp.* competition data we used linear models to analyze differences in population dynamics between treatments. We used four response variables as summary measures of population dynamics: maximum population size, the day on which the population was largest, and the final population size. For each of these response variables, we tested for independent effects of temperature and *Vorticella* and their interaction. We assured that the assumptions of normality and homoscedasticity held for these analyses, by examining the distribution of the response variables and checking residual plots for each model.

Assumptions held for the data for maximum population size and the day on which the population was largest. The final population size data was transformed using a log transformation to improve normality. To analyze the difference in ephippia production by the end of the competition experiment, we tested the effect of the temperature and *Vorticella* treatments and their interaction using a linear model. We also tested for an effect and interaction of these two variables on body size measurements using linear models and conducted a PCA to consider the multivariate changes in body size. Finally, the *D. magna* behavior data were plotted for each substrate-containing cell to determine if activity differed between trials with *Vorticella* and without *Vorticella*. A linear model was used to confirm the results. All data analyses were performed using the R statistical computing language [R package version 3.1.3, libraries: effects, plotrix, and vegan].

Results

Daphnia magna and *Vorticella* sp. Competition

We used statistical analyses to ask how the interaction between *Vorticella* and *D. magna* affected *D. magna* population dynamics at a typical temperature observed in nature, and at a higher temperature. *D. magna* population dynamics for each temperature and *Vorticella* treatment followed a pattern of an early population peak, followed by a decline, and then a stabilizing period (Fig. 4). This is consistent with the known dynamics of *Daphnia* (Adoteye et al. 2015; Cauchie et al. 2000; Smith 1963). *D. magna* tends to exceed carrying capacity after a period of fast growth, after which density stabilizes.

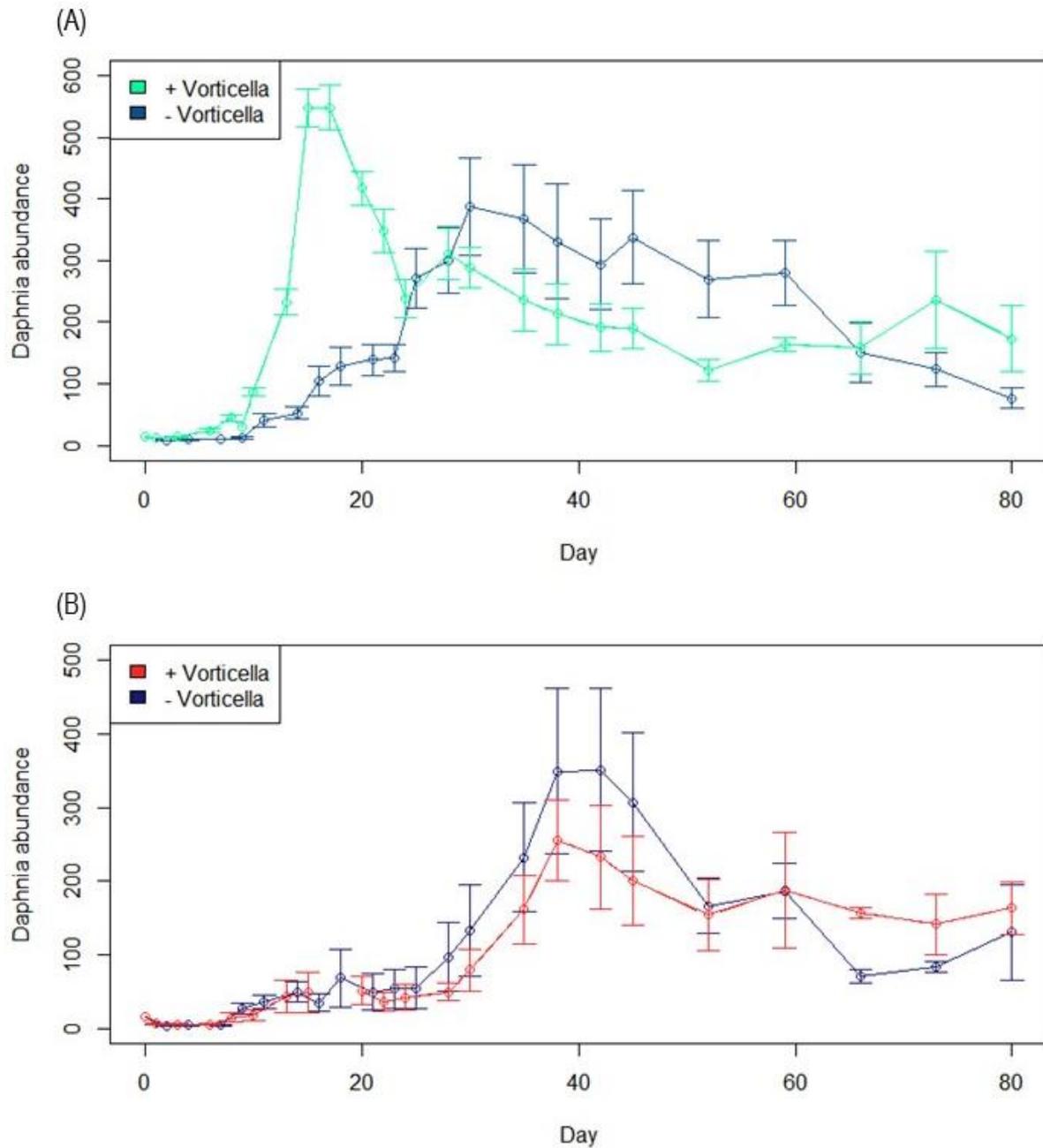


Figure 4. (A) Change in population sizes of *D. magna* over 80 days when cultured at 19°C with (green) and without *Vorticella* sp. (blue). (B) Change in population sizes of *D. magna* over 80 days when cultured at 25°C, with (green) and without *Vorticella* sp. (blue). Points are means across 8 replicate populations. Error bars represent standard errors.

Both temperature and *Vorticella* presence affected the day at which the maximum population of *D. magna* was reached (Table 3): most striking was the significant interaction between temperature and *Vorticella* presence (Fig. 5). Populations raised at 25°C with *Vorticella* reached their maximum population size with an average delay of 13.5 days (+/- 3.3 days) ($p < 0.01$). The effect of temperature alone was to result in a slightly shorter time to maximum population size (on average 4.88 days, $p = 0.04$). The effect of *Vorticella* alone was also to produce a slightly shorter time to maximum population size (on average 5.13 days, $p = 0.03$).

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	15.25	1.631	9.351	<<0.01
Temperature (25°C)	-4.88	2.306	-2.114	0.04358
<i>Vorticella</i> (Yes)	-5.125	2.306	-2.222	0.03453
Temperature (25°C) and <i>Vorticella</i> (Yes)	13.500	3.262	4.139	<0.01

Table 3. Linear model results for the effects of temperature, *Vorticella* and their interaction on the day on which *D. magna* populations reached their maximum. (Adjusted R-squared: 0.3466; F-statistic: 6.482 on 3 and 28 DF, p-value: 0.001804)

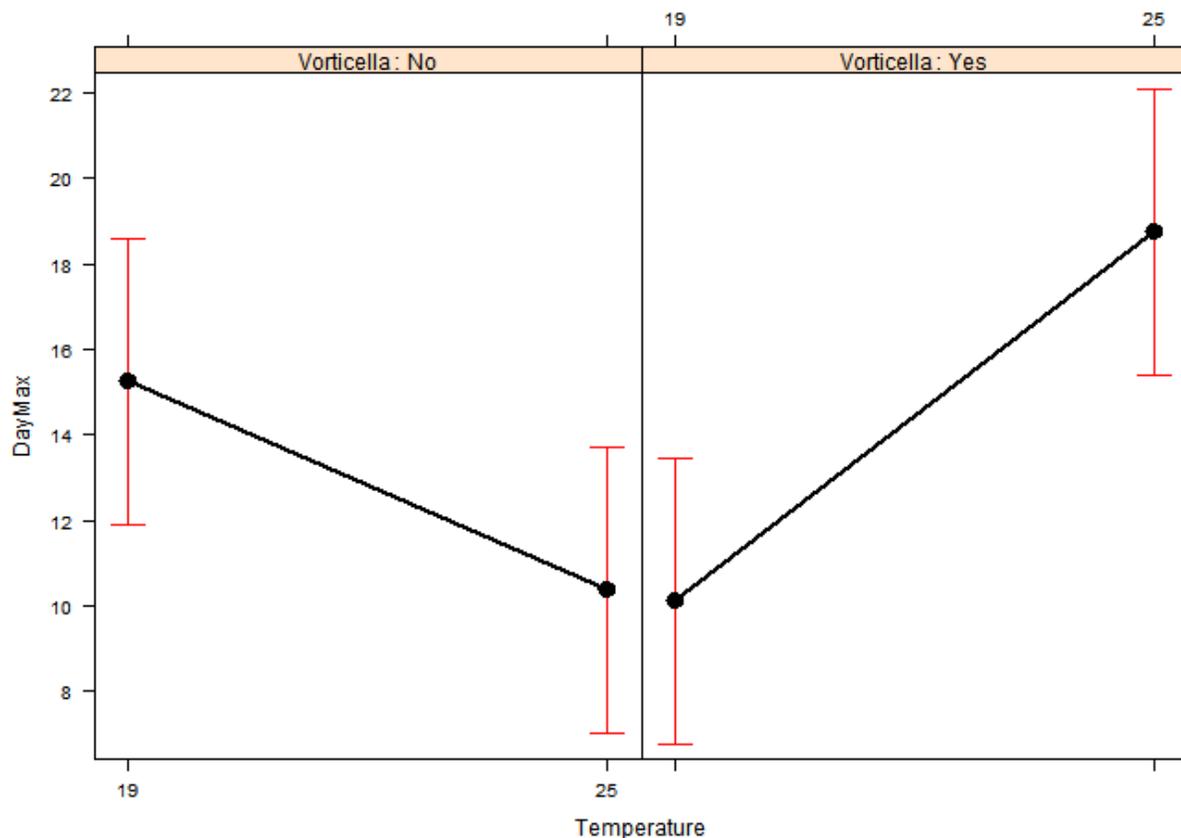


Figure 5. Interaction plot for the model in Table 3, showing the combined effects of temperature and *Vorticella* presence on the timing of *D. magna* maximum population size. Error bars show 95% confidence intervals.

The maximum population size did not vary significantly with the experimental treatments of temperature or presence of *Vorticella* (Table 4), although there was a weak trend of decreased maximum population size of *D. magna* at 25°C. The highest maximum population observed was 780, for a culture raised at 19°C without *Vorticella* present.

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	498.75	67.9	7.346	<<0.01
Temperature (25°C)	-171.12	96.02	-1.782	0.0856
<i>Vorticella</i> (Yes)	91.25	96.02	0.95	0.3501
Temperature (25°C) and <i>Vorticella</i> (Yes)	-110.13	135.8	-0.811	0.4242

Table 4. Linear model results for the effects of temperature, *Vorticella* and their interaction on maximum population size of *D. magna*. (Adjusted R-squared: 0.2258; F-statistic: 4.013 on 3 and 28 DF, p-value 0.01706)

By the final day of the experiment (day 80) the mean size of the *D. magna* populations were much lower than their maximum observed sizes, and were on average 63 individuals across all treatments (Table 5). There was no significant change in final population size, regardless of temperature or *Vorticella* treatments.

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	4.1502	0.6736	6.161	<0.01
Temperature (25°C)	-1.31	0.9526	-1.374	0.18
<i>Vorticella</i> (Yes)	0.1732	0.9526	0.182	0.857
Temperature (25°C) and <i>Vorticella</i> (Yes)	1.0396	1.3472	0.772	0.447

Table 5. Log transformed linear model results for the effects of temperature, *Vorticella* and their interaction on final population size for *D. magna*. (Adjusted R-squared: 0.000886; F-statistic: 1.009 on 3 and 28 DF, p-value 0.4034)

Vorticella abundances showed rapid fluctuations and larger variability than that of *D. magna* populations (Fig. 6). *Vorticella* dynamics typically fluctuate to a greater extent because of their shorter life cycle and time to reproduce (Finley and Williams 1955; Reid 1969; Zingel and Nõges 2010). *Vorticella* numbers appeared low during periods of high *D. magna* population sizes, but appeared to increase as *D. magna* populations began to decline. *Vorticella* attachment to *D. magna* was also monitored, with attachment only occurring early in the experiment (Fig. 6). Attachment rates of *Vorticella* were extremely low in samples of *D. magna* collected later in the experiment.

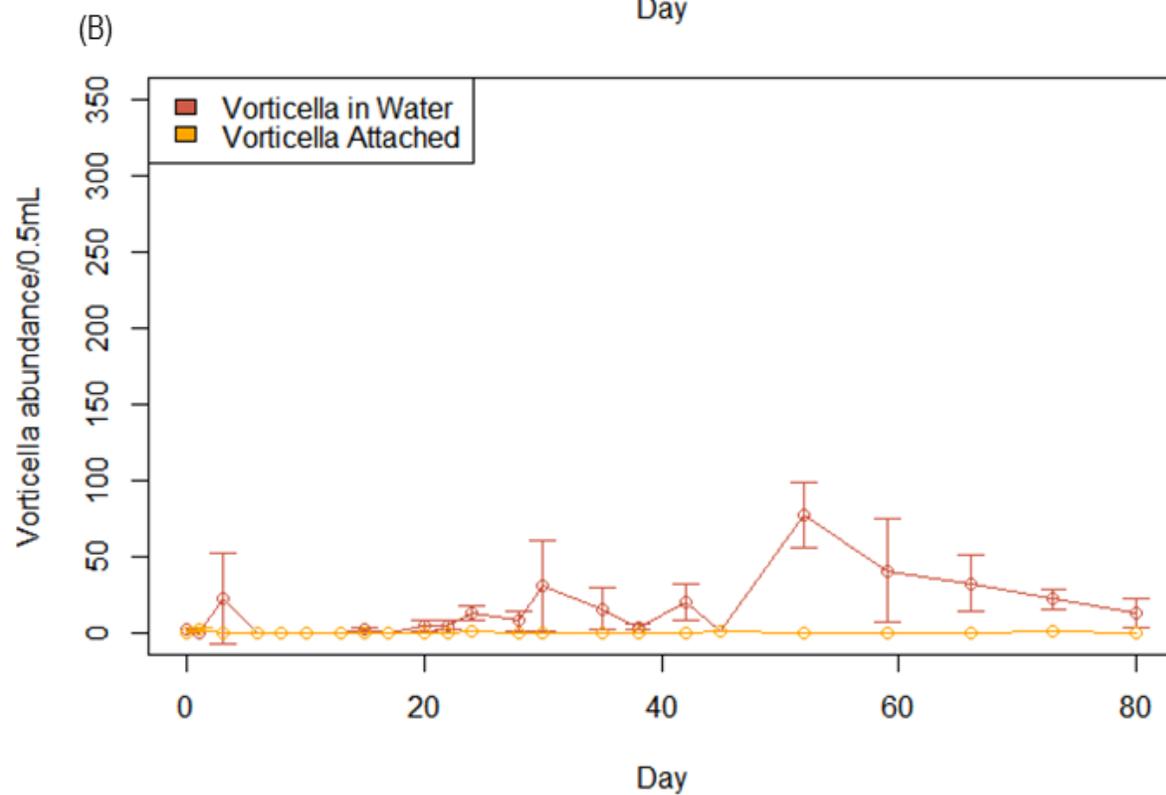
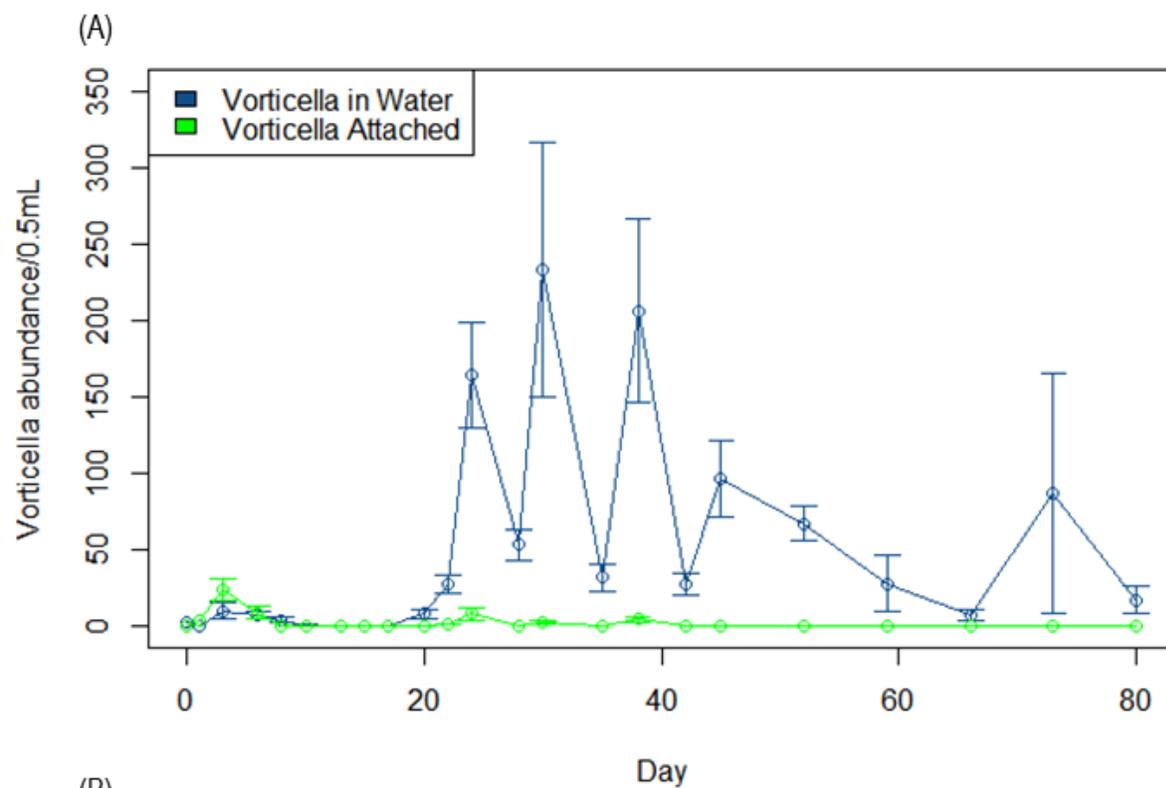


Figure 6. (A) *Vorticella* mean population sizes for *Vorticella* free-living in media (blue) and *Vorticella* attached to *D. magna* (green), over 80 days when cultured with *D. magna* at 19°C. (B) *Vorticella* mean population sizes for *Vorticella* free-living in media (red) and *Vorticella* attached to *D. magna* (orange), over 80 days when cultured with *D. magna* at 25°C. Error bars represent standard errors.

There were differences in the mean number of ephippia produced between temperature treatments at the end of the experiment (Fig. 7). To control for the possible confounding effect of maximum population size across the treatments, we included maximum population size as a covariate. There were about 102 (+/- 32) fewer ephippia produced at 25°C compared to 19°C (p-value: <0.01, Adjusted R-squared: 0.58, 27 DF) (Table 6). There were also about 79 more ephippia (+/- 31) present when *D. magna* was raised with *Vorticella* (p-value: 0.017, Adjusted R-squared: 0.58, 27 DF). There was no significant interaction and no significant effect of maximum population size on ephippia produced.

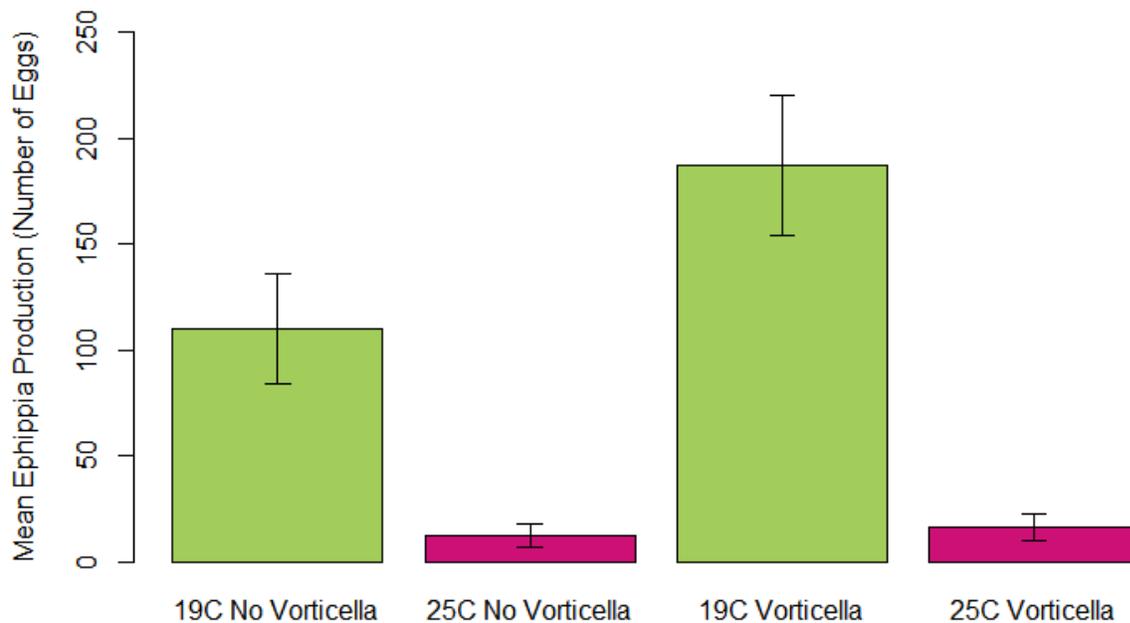


Figure 7. Mean ehippia production for each temperature and *Vorticella* treatment. Error bars show standard errors.

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	120.1959	37.05178	3.244	0.00313
Temperature (25°C)	-101.33	32.31776	-3.135	0.00411
<i>Vorticella</i> (Yes)	78.84255	31.11738	2.534	0.0174
Maximum Population	-0.02019	0.06028	-0.335	0.74022
Temperature (25°C) and <i>Vorticella</i> (Yes)	-75.0987	43.8194	-1.714	0.09802

Table 6. Linear model results for *D. magna* ehippia production for temperature, *Vorticella*, maximum population, and the interaction between temperature and *Vorticella*. (Adjusted R-squared: 0.5689; F-statistic: 11.23 on 4 and 27 DF, p-value: <<0.01)

These results suggest that both increased temperature and *Vorticella* can alter *D. magna* population dynamics in relation to timing of the population maximum. However, increased temperature and *Vorticella* presence did not significantly decrease maximum population size, or the final population size of *D. magna*. Temperature and *Vorticella* did independently increase the ehippia produced, with more ehippia produced at 19°C or with *Vorticella* present.

Daphnia magna and *Vorticella* Individual Measurements

To understand the relationship between temperature or *Vorticella* presence and *D. magna* life history characteristics, we analyzed individual body measurements. Measurements of body length from eye to spine, width, and spine length showed significant effects of the interaction between temperature and *Vorticella* presence. Eye diameter was not significantly affected by the interaction between temperature and *Vorticella* presence. Because size measurements were highly correlated (e.g., for length and width 0.709), we report here results for length only. Body length was significantly affected by increased temperature and *Vorticella* presence (p-value: <<0.001, Adjusted R-squared: 0.19, 139 DF) (Table 7). *D. magna* were on average smaller

(reduction of $301 \mu\text{m} \pm 51 \mu\text{m}$) at 25°C as compared to 19°C (Fig. 8). *Vorticella* presence reduced this difference, but the interaction of *Vorticella* presence and increased temperature still significantly affected the body length of *D. magna* (p-value: <0.01 , Adjusted R-squared: 0.19, 139 DF). PCA results confirm the correlation among body measurements (Fig. 9). As PC1 increases, length and eye diameter also increase. At increased temperature (25°C), *D. magna* have a longer tail spine, and are smaller.

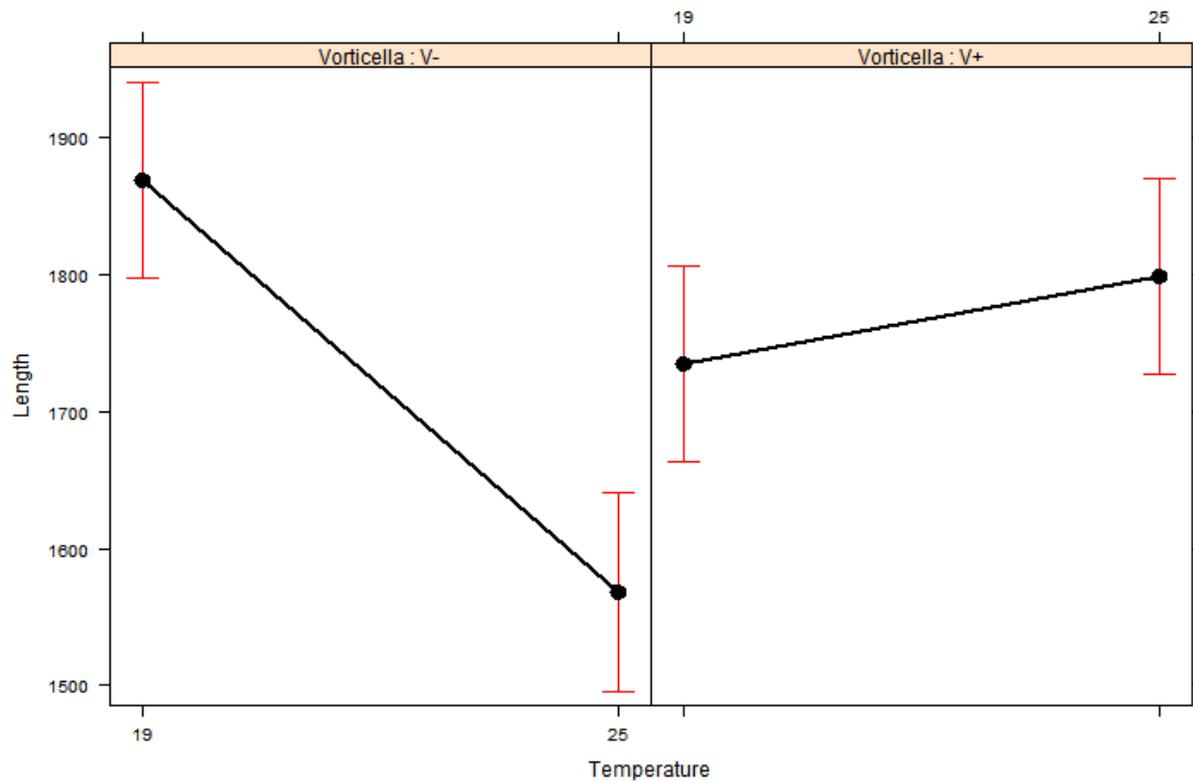


Figure 8. Interaction plot for the model in Table 7, showing the interaction of temperature and the presence of *Vorticella* on *D. magna* body length. Error bars show 95% confidence intervals.

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	1869.04	36.13	51.728	<<0.01
Temperature (25°C)	-300.63	51.46	-5.842	<<0.01
Vorticella (Yes)	-134.24	51.1	-2.627	<0.01
Temperature (25°C) and Vorticella (Yes)	364.62	72.52	5.028	<0.01

Table 7. Linear model results for *D. magna* body length from eye to tail spine for temperature, Vorticella, and the interaction between temperature and Vorticella. (Adjusted R-squared: 0.1946; F-statistic: 12.43 on 3 and 139 DF, p-value: <<0.01)

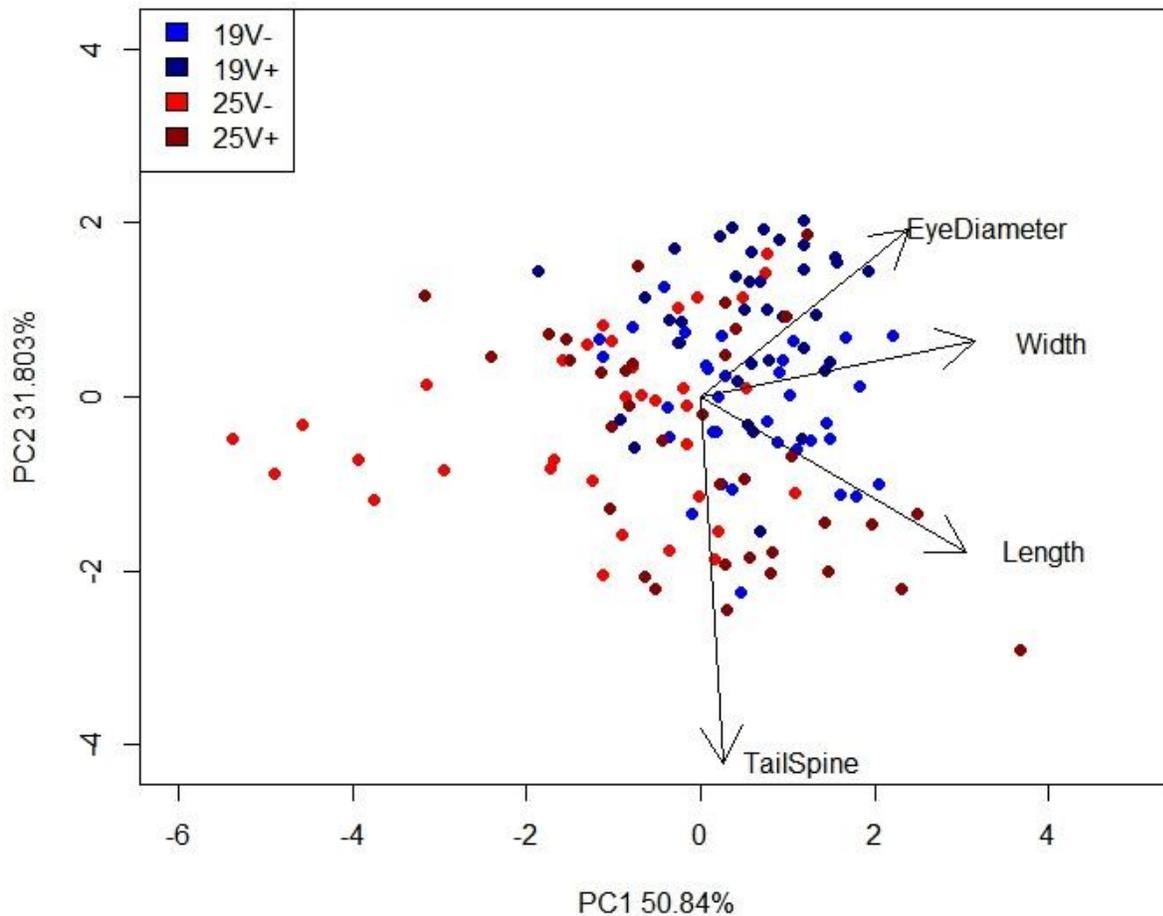


Figure 9. PCA results (for PC axis 1 and PC axis 2) describing the variation in *Daphnia* adult body size across treatments sampled from the competition experiment. Explained variance is shown on the axis labels. (The arrows show how the body measurements load onto the two PCA axes. The colors indicate which treatment combination each individual *Daphnia* came from, i.e. temperature and Vorticella treatment type.)

Juvenile *Daphnia* were raised following the competition experiment and the number of offspring in their first brood was measured. Here, the number of offspring was not significantly affected by temperature, *Vorticella*, or their interaction (Table 8).

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	4.9375	0.6664	7.409	0.0000
Temperature (25°C)	-1.06	0.9285	-1.136	0.2610
<i>Vorticella</i> (Yes)	0.4625	0.9581	0.483	0.6310
Temperature (25°C) and <i>Vorticella</i> (Yes)	2.0188	1.4078	1.434	0.157

Table 8. Linear model results for *D. magna* number of offspring at first brood for temperature, *Vorticella*, and the interaction between temperature and *Vorticella*. (Adjusted R-squared: 0.05245; F-statistic: 2.07 on 3 and 55 DF, p-value: 0.1147)

These results indicate that increased temperature and *Vorticella* presence can alter *Daphnia* phenotype, for body length, width, and spine length measurements. However, temperature and *Vorticella* presence did not affect eye diameter or the number of offspring at first brood.

Daphnia magna behavior in the presence of *Vorticella* sp.

To further understand the effect that *Vorticella* may have on *Daphnia*, we looked at the swimming behavior of *Daphnia* in the presence of *Vorticella* (Fig. 10). A review of the data from the ten sets of swimming trials revealed no pattern of behavior for *Daphnia* in the presence of *Vorticella*. The number of times that *Daphnia* visited cells that contained either substrate without *Vorticella* or substrate with *Vorticella* was not significantly different (Table 9).

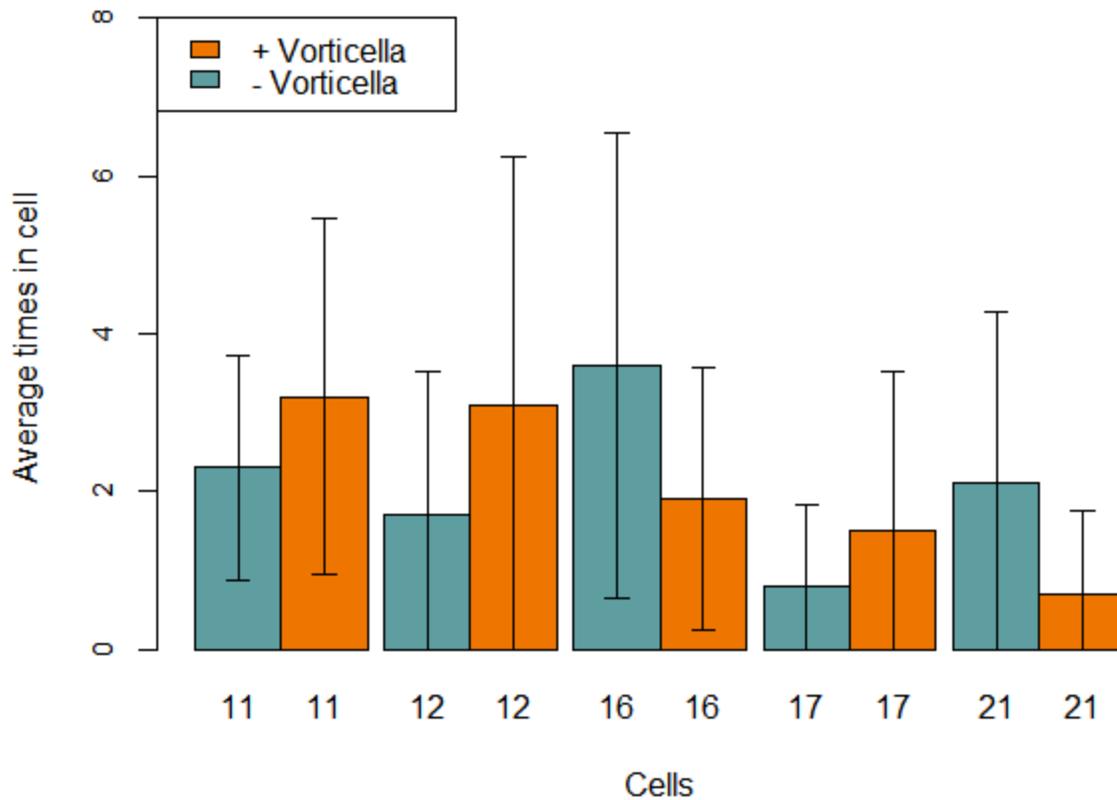


Figure 10. Behavior trials of *Daphnia* with and without *Vorticella*. Bars show the average number of visits to each of these cells, across the behavioral trials (10 individuals). Substrate containing *Vorticella* (orange) or no *Vorticella* (green-blue) were placed in cells 11, 12, 16, 17, and 21 of the petri plate grid.

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	4.9375	0.6664	7.409	<<0.01
<i>Vorticella</i> (Yes)	<<0.01	0.7296	0	1.00

Table 9. Linear model results for *D. magna* behavior with and without *Vorticella*. (Adjusted R-squared: -0.0102; F-statistic: <<0.001 on 1 and 98 DF, p-value: 1)

Discussion

Our study analyzed the effects of temperature and interspecific interactions on the population dynamics of *D. magna* by examining changes in the timing of the maximum population size, the maximum population size observed, and the final population size. Interspecific interactions and warming conditions acted synergistically, such that the time at which a population reached its maximum size depended on the combination of the two variables of *Vorticella* presence and temperature experienced. The effects of these variables were, therefore, not additive. Despite these early differences in dynamics, the final populations were similar across all treatments. Because delayed timing of population growth in a natural population can have important consequences for the species if the delay results in a mismatch between natural cycles in resource abundance, further study is required to understand the relevance of this result for natural populations. For example, studies show that changing temperatures and changing competitive interactions (such as through immigrant species) have produced declines in natural populations of *Daphnia longispina* and *Daphnia pulex* (Nevalainen et al. 2013). One important result from our study was that although early dynamics were significantly affected by temperature and the presence of *Vorticella*, the final population sizes were comparable across all treatments. Studies have shown that *D. magna* and other Cladoceran species can respond to increased temperatures, through genetic and plastic responses (De Meester et al. 2011; Van Doorslaer et al. 2007). *Daphnia* are able to cope with high temperatures through heat shock protein (HSP) production, which allows them to tolerate a larger range of temperatures (Mikulski et al. 2011).

D. magna previously adapted to higher temperatures do typically respond better to higher temperature conditions than those not initially exposed to these conditions (De Meester et al. 2011); the absence of pre-adaptation in our experiment may explain the slight effect of

temperature on the maximum population sizes (which were slightly smaller at the higher temperature), and the higher extinction rates for the 25°C treatment. Because all initial populations were sourced from 19°C temperature conditions, *D. magna* in the higher temperature treatments had to rapidly respond to the temperature condition or face extinction. We found that *D. magna* in the 25°C treatment were smaller by the end of the experiment than those in the 19°C treatment, although we cannot differentiate between changes driven by phenotypic plasticity versus those driven by selection. Typically, higher temperatures result in a reduced body size for *D. magna* (Maceda-Veiga et al. 2015), possibly indicating the optimization of traits for high temperature environments. However, the general result that *D. magna* were able to respond to warm temperatures has significant implications that may extend to competitive outcomes (Van Doorslaer et al. 2009). Populations can decline when they are unable to respond to changing temperatures and changing competitive interactions, such as through immigrant species. This has been seen in natural populations of *Daphnia longispina* and *Daphnia pulex* as climate change affects regional temperatures (Nevalainen et al. 2013). In this long-term observational study, community turnover occurred where daphniids were replaced by *Chydorus cf. sphaericus*¹⁴ early in the study, followed by colonization of *Bosmina*¹⁵. Changes in community structure were attributed to climate warming, resulting in increased distribution of *Bosmina* which then outcompeted the local daphniid populations.

Fecundity in *D. magna* can be negatively affected by increased temperature (Maceda-Veiga et al. 2015; Garbutt et al. 2014). Temperatures of 26°C to 29°C have been shown to result in high mortality, and reduced fecundity as a result of stress from increased temperature, including higher oxygen demands (Burns 1969). Our study saw no significant decline in

¹⁴ A small cladoceran with a spherical carapace

¹⁵ A genus in the order Cladocera

fecundity as a result of increased temperature or from the presence of *Vorticella*. Given the lack of effect on the final population sizes seen in this study, and the lack of effect on fecundity, this may indicate that slightly higher (and so more stressful) temperatures would have been necessary to see observable impacts on fecundity.

Larger *Daphnia* spp. often reduce other cladoceran populations, through high filter rates and chemical signaling (Burns 1969; Burns 2000), but some smaller species can outcompete the juveniles of larger daphniids through their specialized feeding on smaller cell sizes of food in the water (Neill 1975). Size may be an important factor in the relationship between *D. magna* and *Vorticella*. Both juvenile *D. magna* and the smaller *Vorticella* feed on smaller particles in the water, resulting in direct competition. On the other hand, larger *Daphnia* have more surface area that may result in more area for attachment of epibionts and potentially reduced fecundity due to higher loads of epibionts (Stirnadel and Ebert 1997). We found that *D. magna* populations tended to reach their maximum size faster when they co-occurred with *Vorticella*. This may suggest a facilitative relationship between the species at 19°C. In addition, we noted that, in contrast to the cultures without *Vorticella* at 25°C, in which adult *D. magna* were much smaller, when *Vorticella* was present at 25°C *D. magna* sizes were much larger. This might indicate character displacement to minimize competition, despite the expectation that *D. magna* usually decrease in size at increased temperatures (Maceda-Veiga et al. 2015).

The possible facilitative relationship as seen through our study can be explained through several potential factors. First, if *D. magna* and *Vorticella* have slightly different resource usage, apparent competition between algae may attribute to the positive relationship seen in our study (Burns 1969). *Daphnia* spp. developmental rates are affected by biotic environmental factors. Faster development of *D. magna* can reduce competition with other species when because

chemical signals reinforce the competitive advantage for *D. magna* (Burns 2000). *Vorticella* attachment in the early stages of the experiment may have possibly signaled *D. magna* to develop faster. This might also increase the shedding of carapaces along with those *Vorticella* attached, thus reducing their impact on *D. magna*. In our experiment, *Vorticella* attachment to *D. magna* was high only early in the experiment. Attachment of *Vorticella* can impact swimming behavior, leading to reduced fitness if the number of epibionts becomes too high to manage (Aalto et al. 2014). However, if the extent of attachment of *Vorticella* is low enough, *D. magna* may experience little effect on their ability to access resources or on their reproductive output (Havens 1993; Jansen et al. 2010).

Behavior trials, designed to understand possible chemical signaling from *Vorticella*, showed no evidence of *D. magna* avoidance or attraction to *Vorticella* when horizontal swimming behavior was observed. This suggested no evidence of changed in horizontal swimming behavior. Still, further analysis of vertical behavior in the presence of *Vorticella* may be necessary in order to strengthen this result. Chemical signals are important in some interspecific interactions. In particular, *Daphnia* avoid predator kairomones¹⁶ through vertical migration to lower depths (Dodson and Hanazato 1995; Bernot 2006; Vandenbrouck et al. 2011). Conditions for olfactory cues for intraspecific crowding and most competitors (only *D. pulex* has been shown to effect behavior of *D. magna*) have shown no change in behavior of *D. magna* (Sereni and Einum 2015; Roozen and Lüring 2001). The behavior results of our study, along with those from the competition experiment suggest that *D. magna* are not affected by *Vorticella* presence at low temperatures. Despite direct competition for access to food, this study suggests that *Vorticella* presence at 19 °C results in a facilitative relationship (Aalto et al. 2014; Kankaala

¹⁶ A semiochemical that is emitted by an organism mediating interspecific interactions in a way that benefits an individual of a different species that receives it, and harms the species emitting it

and Eloranta 1987). Only is when temperature is increased that this relationship changes to that of competition (Heugens et al. 2006; Reid 1969).

By the end of the competitive experiment, ephippia were present in replicates from all treatments. Typically, the production of males and subsequently ephippia are indicative of stress conditions. Although response to environmental cues can be species specific, ephippia are seen as a result of predation, limited food or seasonal changes in temperature, particularly during the fall (Bernot et al. 2006). In this experiment, more ephippia were produced by *D. magna* raised at 19°C rather than at 25°C. Ephippia production can be important because it introduces genetic diversity to a population through sexual reproduction. Without males, natural populations of clonal individuals would be at a disadvantage in responding to changes in the environment (Dodson and Hanazato 1995). Because of this, it may be that the higher production of ephippia seen in *Daphnia* raised at 19°C is indicative of a natural population cycle brought on by population growth rather than stress from *Vorticella* presence (Heckmann et al. 2008). Additionally, more males were seen earlier in 25°C treatments, and several populations went functionally extinct in populations where males became the only individuals present. This suggests the increased temperature was a cue for sexual reproduction in these populations. However, with 15 individuals in starting populations, the presence of males earlier on had a detrimental effect on population survival, ultimately leading to extinctions before more ephippia could be produced.

There were also changes in *D. magna* population dynamics dependent on both *Vorticella* presence and temperature. There is evidence for reductions in *D. magna* population growth rates resulting from many different sources of stress, including low pH conditions, competition, high temperatures, and reduced resource availability (Dodson and Hanazato 1995). Conversely,

cladoceran species have been shown to be better competitors under stress conditions brought on by a warming climate (such as from crowding, temperature or limnological regime shifts) (Burns 2000; Feniova et al. 2013; Nevalainen et al. 2014). In our study, *D. magna* responded to competition and increased temperature through a delay in population growth (possibly due to a delay in reproductive timing or developmental delay). Although there was a clear, but transient effect of having *Vorticella* present, temperature more strongly impacted the outcome of *D. magna* population size. *D. magna* populations eventually became similar in size at 25°C compared to those in the 19°C treatment. However, a delay such as this could have broader implications for survival, since it could result in a mismatch between *Daphnia* abundance and algal blooms (Stenseth and Mysterud 2002; Wagner and Benndorf 2007).

Conclusions

Observational studies play an important role in understanding species interactions. When organisms are studied in a home environment, natural behaviors can be seen in populations as they interact with their surroundings (Kankaala and Eloranta 1987; Aalto et al. 2014). However, observational studies have suggested contrasting relationships between *Daphnia* and *Vorticella* that have clouded understanding of their true relationship (Aalto et al. 2014; Kankaala and Eloranta 1987). Therefore, an experimental study with controlled variables can shed more light on this complex relationship. As seen from the results of our study, increased temperature and *Vorticella* presence impact the timing of population growth, but does not impact the maximum population numbers of *D. magna* or the final population size. *D. magna* is equipped to respond to these stressors despite direct competition for resources. However, in natural populations, this

delay can result in a mismatch in *Daphnia* and resource abundance. Understanding how resource limitations in conjunction with temperature and competition would help add understanding as to how *Daphnia* would respond to a scenario of natural climate warming (Neill 1975). Therefore, future research into resource limitation is needed to untangle the complexity of this relationship.

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