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TIME, TEMPERATURE AND SPECIES INTERACTIONS IN A DUCKWEED-HERBIVORE MESOCOSM

by

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TIME, TEMPERATURE AND SPECIES INTERACTIONS IN A DUCKWEED-HERBIVORE MESOCOSM

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University of Nebraska, 2010

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Species interactions within a community are impacted by a variety of abiotic factors. Temperature is known to alter population dynamics such that direct and indirect interactions between populations within a community are affected. Here I investigate the effect of temperature change on species interactions within a duckweed-herbivore mesocosm. Multiple communities were constructed, from a single population of duckweed, to two populations of duckweed consumed by aphids. In the one-predator two-prey web we predicted mutually positive indirect effects between duckweed populations during the first generation of growth. As aphid populations respond numerically to more abundant prey, mutually negative and asymmetric indirect effects should occur due to interspecific variation in growth response to temperature. We found direct and indirect interactions varied across time and temperature. Notably, the effects of competition were often asymmetric between duckweed populations. The effects of herbivory were sometimes positive due to the effects of density dependent growth in duckweed populations grown without herbivory. There was also a transient mutually positive indirect effect between duckweed populations at 27°C that did not occur at 19°C. In general, indirect effects between duckweed populations were variable in sign and magnitude across time and temperature.

Chapter 1

Establishing a Duckweed-Herbivore Mesocosm to Examine the Effects of Temperature and Time on Species Interactions in a Diamond Food-Web

Introduction

Species interactions in food-webs are direct, for example predation, or indirect effects resulting from shared interactions with other populations. Abiotic factors such as temperature can alter these interactions, but many questions remain. In a community of two prey under shared predation, do shifts in temperature regime alter indirect interactions? How do these indirect effects change over time? If temperature does have an effect, does this effect fluctuate temporally? I will investigate these questions with an aquatic diamond food-web mesocosm. Here I present the basic work on a system of duckweed species, and associate herbivores, which provides the background for future experiments that will answer questions about temperature and species' interactions.

Duckweed represents a cosmopolitan subfamily of the smallest known angiosperms. Their ubiquitous nature, short generation time and ease of husbandry make them an excellent model system for studying community and population ecology-and for testing existing ecological theory. Much is known about these organisms and their basic distribution, reproduction and morphological characteristics, summarized in two volumes by Landolt (1986). Previous empirical work in ecology has used duckweed as a model species to investigate competitive processes between duckweed and other phototrophic organisms such as Elodea and algae (Roijackers 2004; Szabo 2009). Results from these studies indicate that duckweed growth is affected by these other populations primarily via nitrogen limitation and increased pH. As Elodea populations increase, pH becomes more

acidic, which decreases duckweed population growth. Here I utilize duckweed to answer questions about biotic and abiotic factors which alter community processes.

For these studies, multiple species of duckweed from different geographic locations were used; *Lemna minor* from Memphis, TN and Rochester, NY, *Spirodela polyrhiza* from Lincoln, NE and *Landoltia punctata* from an unknown location. These species varied in multiple phenotypic metrics, including size, palatability to herbivores, root structure and response to temperature. There are numerous interacting organisms in aquatic communities containing duckweed. Multiple insect herbivores are known to feed on these floating plants. Waterlily aphids (*Rhopalosiphum nymphaeae*) reproduce parthenogenetically on duckweed and feed via stylet on phloem nutrients found within the fronds. At larval and adult stages, Duckweed flies (*Lemnaphila scotlandae*) also utilize *L. minor* for nutrition and oviposition sites. These dipterans scrape the surface of the frond, leaving behind parallel grooves in the plant tissue. Female flies lay multiple eggs on the periphery of a single frond of *L. minor*. Larvae then hatch, feed on duckweed tissue and then stay on that frond or relocate to nearby fronds where they eventually pupate and mature to adult form. Here we investigate the basic ecological relationships between these organisms in order to establish a foundation for more complex studies in the future.

For experiment #1 we quantified the effect aphids and flies have individually on duckweed populations. Anecdotal evidence suggested that the impact flies have on duckweed growth is such that multi-generational studies with flies as the herbivore would not be feasible. Eventually, fly populations drive duckweed locally extinct if they are not controlled by a predator, themselves. Aphids seemed like a reasonable alternative;

however it was unclear whether they had a significant effect on duckweed growth. Experiment #1 addressed both of these questions.

To have a functioning diamond food-web it was necessary to establish two separate species of duckweed that are palatable to aphids. We selected *Landoltia punctata*, *Spirodela polyrhiza* and *Lemna minor* as the potential aphid resources. It was also important to establish the relative difference in growth between these species of duckweed in response to temperature. This information would further inform hypotheses about temperature effects on species interactions within the full diamond food-web.

We also investigated the relative preference of aphids when presented two species of duckweed as potential resources, *S. polyrhiza* and *L. minor*. Evidence from this experiment will help form hypotheses and interpret results of future studies where aphids and two duckweed species are interacting. If aphids show a preference for a certain species of duckweed this could explain the sign and magnitude of indirect effects between those duckweed populations.

Lastly, we examined the degree to which duckweed growth is limiting to aphid population growth. If aphid growth is not limited by a given amount of duckweed, then adding more will not result in a significant numerical response in the aphid population. This limitation allows for the possibility of indirect effects between duckweed populations.

Material and Methods

General

Multiple strains of duckweed were used in the following experiments, including *Lemna minor* from Lincoln, NE (40°50’36.72”N, 96°42’0.06”W) and Memphis, TN

(34°57'25.22"N, 90°06'31.56"W), *Landoltia punctata* from Lincoln, NE and *Spirodela polyrhiza* from Lincoln, NE (40°80'68.62"N,-96°68'16.79"W). Each strain's location will be written parenthetically after the genus and species, hereafter. All experimental duckweed populations were grown in 100ml polypropylene cups with Swedish standard duckweed media, under a constant light regime. Fluorescent 40 watt lights, 185 cm in length, were positioned approximately 32.6cm above all experiment units.

Experiment 1: The effect of aphids (*Rhopalosiphum nymphaeae*) and flies (*Lemnaphila scotlandae*) on duckweed (*Lemna minor*) growth

Here I investigated the distinct effect aphids and flies have on duckweed population growth. In this design, experimental units varied in a one-way ANOVA with three treatments. Either 2 large aphids over 3mm in length, two adult flies of undetermined sex, or the control of no herbivore, were placed on the fronds (n=20 per treatment). The duckweed (*Lemna minor*) was collected from a man-made pond in Lincoln, NE. The light cycle consisted of 16 hours of light and 8 hours of dark at a constant temperature of 24C. Each initial population of duckweed consisted of 5 fronds of *Lemna minor*. Duckweed populations were counted by hand 2, 6 and 8 days into the experiment. Each cup was covered by a section of nude Leggs nylon, and placed haphazardly under fluorescent lights.

Only the final duckweed count from day 8 was used in this analysis, because the differences in growth rate between treatments accumulated over time. Furthermore, this avoids the need for analysis of repeated measures. Population growth was calculated using the equation $r=(\ln(N_o)-\ln(N_f))/8$ days. Growth rates for each treatment were fit using maximum likelihood analysis with a Poisson distribution. Likelihood ratio tests

were used to detect significant differences in r among the herbivory treatments. This analysis revealed whether growth was significantly different between populations of duckweed growing with and without herbivory.

Experiment 2: The effect of aphids (*R. nymphaeae*) on duckweed growth (*L. punctata*, *S. polyrhiza* and *L. minor*)

The goal of this experiment was to detect whether aphids significantly lower the growth of multiple species of duckweed. In this 2x3 factorial design each initial population of duckweed consisted of 5 fronds of *L. minor*, *L. punctata* or *S. polyrhiza*. For the herbivore treatment, 2 adult aphids were placed on the fronds. These duckweed populations and experimental apparatus were the same as experiment 1. Duckweed populations were counted by hand 2, 4 and 7 days into the experiment. Experiment 1 showed a time period of one week to be sufficient for significant duckweed growth. Each cup was covered by a section of nude nylon, and placed haphazardly under the lights. Data was analyzed in R using a generalized linear model under an assumed Poisson distribution. While data were plotted across time, only the final count from day 7 was used in the statistical analysis, as the differences in growth rate between treatments accumulated over time. Population growth was calculated using the equation $N(t)=N_o e^{rt}$.

Experiment 3: The Effect of Temperature on Duckweed Growth

Here I quantified the difference in growth for three populations of duckweed across four temperatures. For this experiment populations of *L. minor* (Memphis), *L. minor* (Rochester), *L. punctata* (Memphis) and *S. polyrhiza* (Lincoln). were grown in rooms with a constant temperature of 15°C, 19°C, 22°C and 31°C. Strains of duckweed

were chosen because of genetic and geographic differences, with the expectation that this would maximize differences in growth across temperature. Duckweed populations began at 4-6 fronds. A single HOBO logger was placed in a separate water-filled cup, without duckweed, for each run to measure the ambient temperature at which duckweed populations grew. The experiment was run for 7 days. Growth rates were calculated using the formula $N(t)=N_o e^{rt}$ and values of r among the strains were analyzed using a generalized linear model.

Experiment 4: Aphid foraging preference

Here I quantify differences in aphid location when foraging among two species of duckweed. Separate populations of aphids (*Rhopalosiphum nymphaeae*) were raised on monocultures of *L. minor* or on monocultures of *S. polyrhiza* for a time period of three weeks or more. This method controlled for the effect of previous feeding experience and controlled for maternal effects. Aphid cultures were maintained in round glass dishes filled halfway with Swedish Standard Lemna Media (OECD). These dishes were covered with nude-colored nylon fastened by a rubber band and placed in a growth room under a constant temperature of 20□C.

Polypropylene cups were filled with 100ml of sterile Swedish Standard Lemna Media. 12-15 fronds of *S. polyrhiza* and 16-19 fronds *L. minor* (Rochester) were then placed into each cup. The difference in frond number controlled for the perceived disparity in surface area per frond among the two duckweed species. Duckweed was moved around to form a surface of fronds with a spatially equivalent distribution of both

species. To check the effectiveness of attempted equality in surface area, pictures of each cup were captured using LemnaTec.

Within this 2x2 factorial design one adult aphid, raised on *L. minor* or *S. polyrhiza*, was placed on a frond of either *L. minor* or *S. polyrhiza* (n=25 per treatment combination). Aphids were removed from the monoculture with a small brush and placed onto a frond. Care was taken to make sure the aphid was located on a frond and not in the nutrient solution. The initial duckweed species location of the aphid was recorded. Next the cups were covered with nylon fastened by a rubber band and placed in a growth room. On day 3 I recorded the species of duckweed upon which the original aphid was located. The offspring were counted and their location recorded independently from the parent aphid. After 6 days data was collected again. By then parent aphid offspring were similar in size to the parent aphid so they were recorded as one group in the dataset.

These data were analyzed with a generalized linear model, which included the source duckweed population parent aphids consumed prior to the experiment, the species of duckweed the parent aphid was placed initially and the species of duckweed the aphid was located after a given amount of time.

Experiment 5: Aphid population growth as a function of initial aphid density on *Lemna minor* and *Spirodela polyrhiza*

For this experiment I investigated the effect of aphid density (aphids/fronds) on aphid population growth. Each experimental unit included a polypropylene cup filled with 100ml of Swedish standard Lemna media (n=10). Aphid populations were all initially 1, 3 or 5 and initial duckweed frond number was 3, 5, 7 (n=10) for both *Lemna*

minor and *Spirodela polyrhiza,* which were both present in each cup. The starting densities for each duckweed species were different which compensated for differences in frond size between the two species. The cups were placed in a growth room set at a temperature of 30°C. Temperature was recorded using a HOBO data logger placed in distilled water within a separate plastic cup. Aphid and duckweed counts were done twice a week at days 3, 7, 10 and 14. This amount of time was sufficient for the production of multiple aphid generations. The relationship between aphid population growth and aphid density was analyzed with linear regression for day 3, day 5 and day 8 data with duckweed species combined.

Results

Experiment 1

The growth rates of duckweed with aphids, duckweed with flies and duckweed without an herbivore were 0.15 fronds/day, -0.37 fronds/day and 0.18 fronds/day, respectively (Figure 1). Duckweed growth was significantly reduced by aphid herbivory ($\Delta AIC=8.3$, $p = 0.0013$) and fly herbivory ($p< 0.001$). Fly data departs from the model at day 2. Here duckweed growth is higher than the model predicts, perhaps due to high nutrient amounts relative to day 6 and day 8, and minimal effects of density dependence.

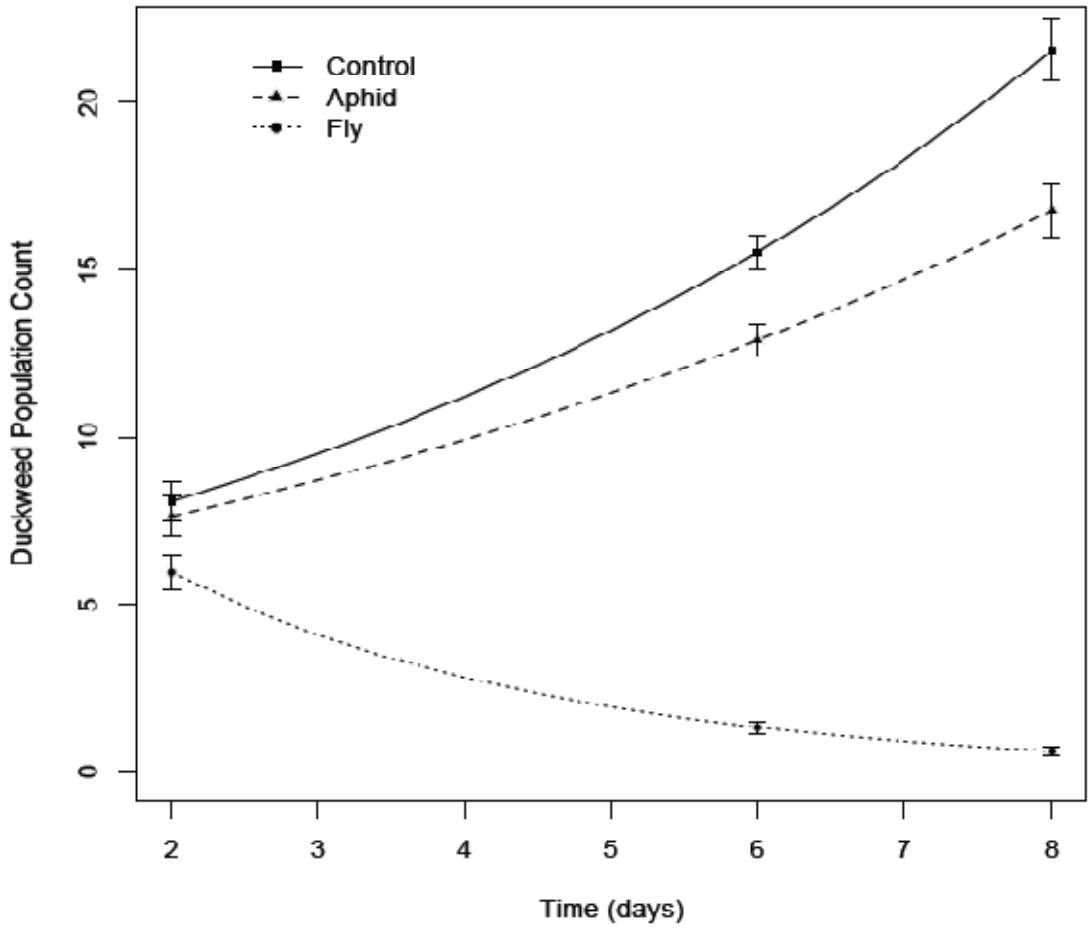


Figure 1: Duckweed growth is significantly reduced by both aphids and flies. Here mean duckweed population count and SEM are plotted at Days 2, 6 and 8. The control, aphid and fly duckweed populations are shown above by the solid, dashed and dotted lines, respectively. The fitted lines were plotted using maximum likelihood analysis.

Experiment 2

By day seven the main effect of aphid herbivory was significantly negative for duckweed population growth of L. *minor*, *S. polyrhiza* and *L. punctata* ($p<0.01$ for all). However, there were no significant interactions among treatments. Each duckweed

species grew slower under herbivory, and there was no significant difference between the effects aphids had on individual duckweed species.

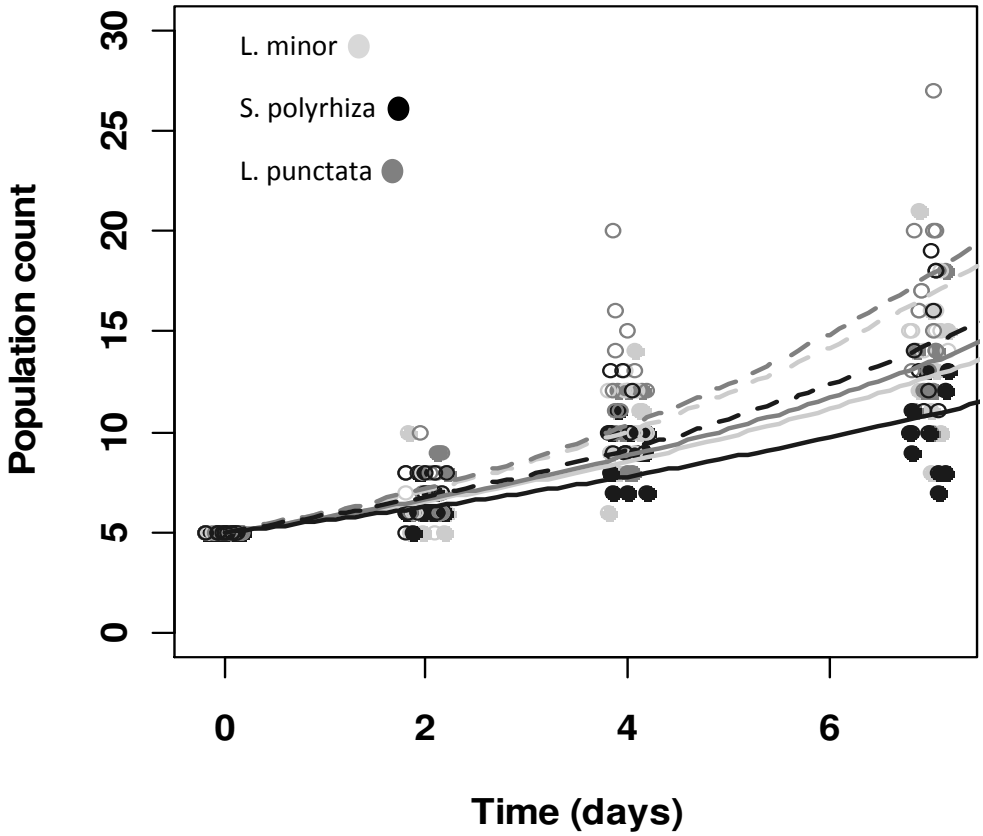


Figure 2: By day 7 aphids significantly lower duckweed growth. Dashed lines represent maximum likelihood estimates of duckweed growth over time without herbivory and solid lines, with herbivory. Closed circles are populations of duckweed grown without herbivory, and open circles, with herbivory.

Experiment 3

A 4x4 ANOVA revealed a significant species effect ($p<0.001$) and species by temperature effect ($p=0.05$). A Tukey test, used to determine whether growth rates differed between the duckweed populations in the four temperatures, revealed a

significant difference in growth between *Lemna minor* (Memphis, TN) and *Spirodela polyrhiza* (Lincoln, NE) at 19°C ($p<0.001$) but not at 31°C ($p=0.3$). However, a less conservative pairwise t-test indicated significant difference between L. minor and S. polyrhiza at 31°C ($p=0.02$). These results are contradictory because the Tukey test is a more conservative estimation of significance. At 19°C *S. polyrhiza* (Lincoln) grew at a higher rate while *L. minor* (Memphis) may have grown slightly faster at 31°C. It should be noted, significant algal infection was observed in many of the cups in this experiment.

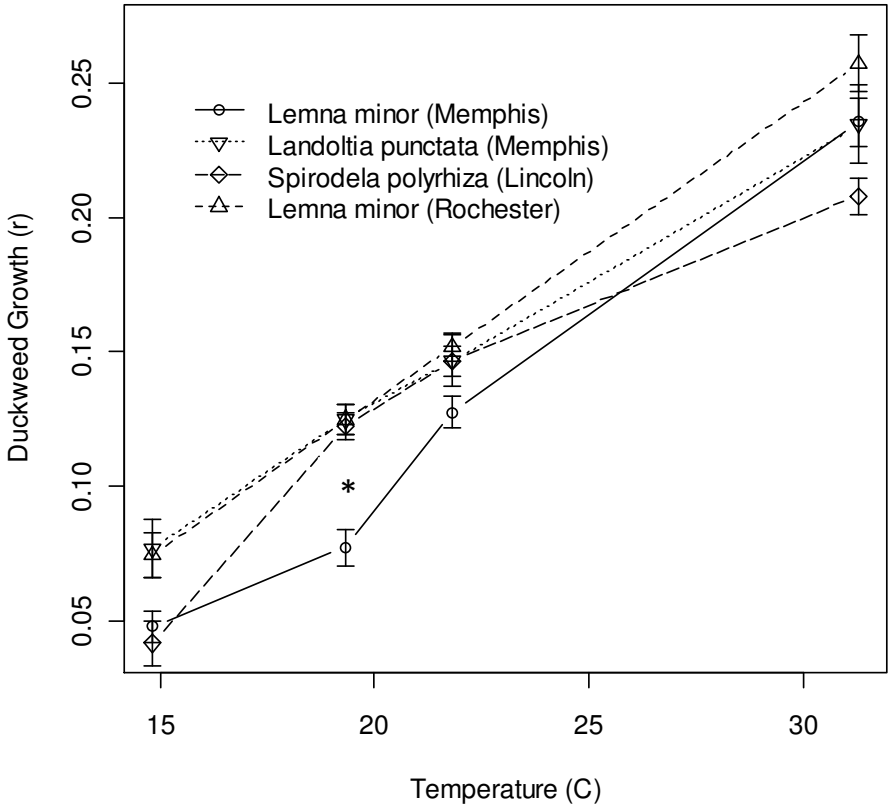


Figure 3: Growth rates are plotted across temperature for four different populations of duckweed. The brackets represent the SEM for all growth rates per temperature.

Experiment 4:

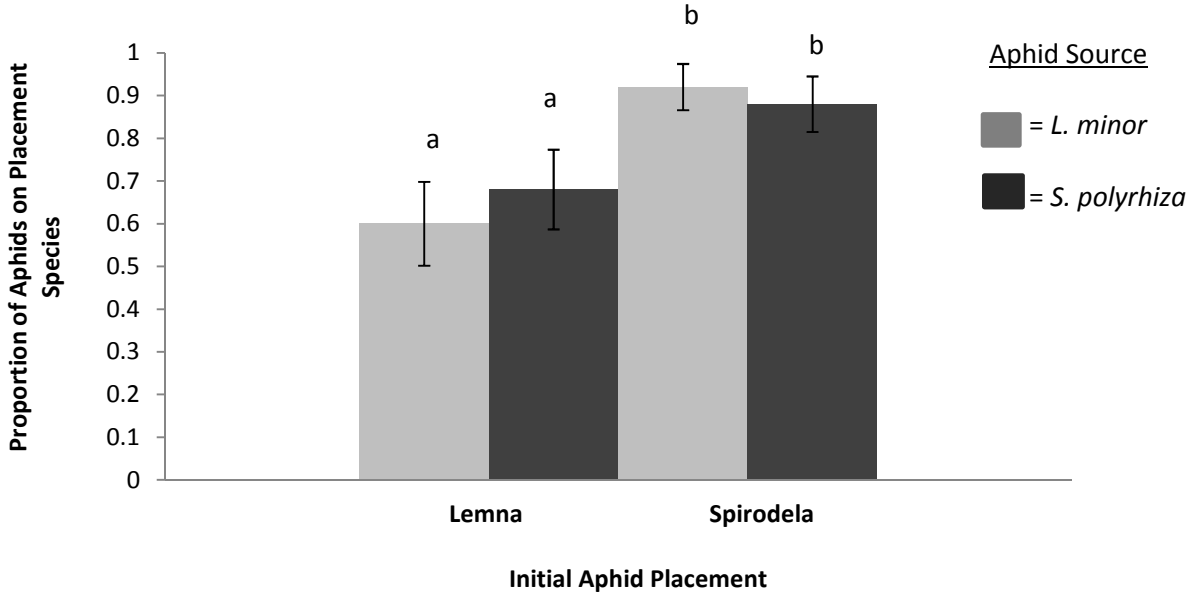
Location data for parent aphids on day 3 was represented by a binomial distribution, while day 3 offspring location, and data on all aphids recorded on day 6, were analyzed using a poisson distribution. Under these two distributions I used a generalized linear model including duckweed species, source and location of duckweed. Adult aphid foraging showed no maternal effect at 3 days. Aphids raised on *L. minor* or *S. polyrhiza* were just as likely to stay on the species of duckweed they were placed, initially ($p>0.05$). However, aphids were more likely to stay on *S. polyrhiza* than they were *L. minor*, regardless of previous feeding experience ($p<0.01$). At day 3 aphid offspring raised, placed and located on *S. polyrhiza* were most numerous, averaging nearly 2.5 aphids ($p<0.01$ compared to all other treatments). However, aphids placed and located on *S. polyrhiza*, that were raised on *L. minor*, were significantly less numerous, indicating a strong maternal effect on aphid fecundity ($p<0.01$). In other words, aphids raised on *S. polyrhiza* produce more offspring. Alternatively, aphids placed and located on *L. minor* showed the opposite maternal effect. Aphids that were raised on *S. polyrhiza* were less numerous than aphids raised on *L. minor* ($p<0.01$). There were no significant maternal effects for aphids placed on *L. minor* and located on *S. polyrhiza*, and vice-versa. However, aphid offspring from adults placed on *S. polyrhiza* and found on *L. minor* were significantly fewer than off-spring placed on *L. minor* and found on *S. polyrhiza*, regardless of the species of duckweed the adult aphid consumed ($p<0.01$).

Aphids raised on *L. minor* showed a significant difference in duckweed species location after 6 days when initially placed on either *L. minor* ($p<0.01$) or *S. polyrhiza* ($p<0.01$) (Figure 6). The aphids tended to stay on the duckweed species they were

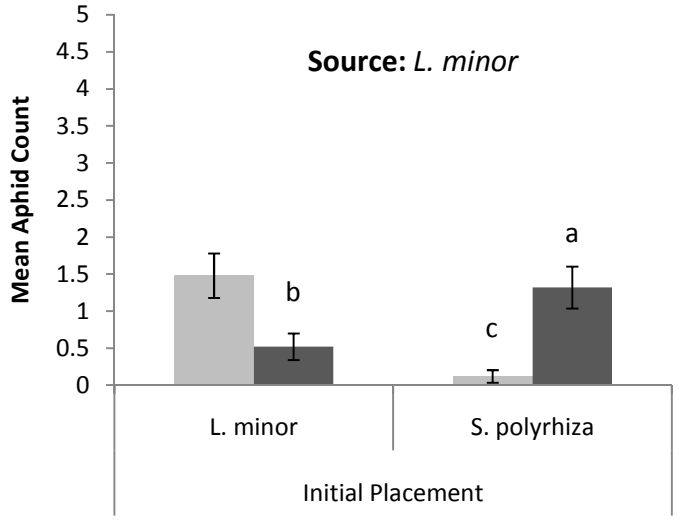
placed, initially. There was also a significant interaction ($p<0.01$), such that aphids placed on *S. polyrhiza* were more likely to stay on *S. polyrhiza* than those placed on *L. minor*. This might be due to *S. polyrhiza* growing larger fronds. Aphids raised on *S. polyrhiza* showed no difference in duckweed species location after 6 days ($p>0.05$) (Figure 6). Aphids placed on *S. polyrhiza* grew significantly faster than aphids placed on *L. minor* ($p<0.01$).

There were also two significant maternal effects at day 6. Aphids raised on *S. polyrhiza*, placed on *S. polyrhiza* and located on *L.* minor were more numerous than aphids raised on *L. minor*, placed on *S. polyrhiza* and located on *L. minor* ($p<0.01$). In addition, aphids placed and counted on *L. minor*, that were also raised on *L. minor*, were more numerous that aphids placed and counted on *L. minor*, but raised on *S. polyrhiza*.

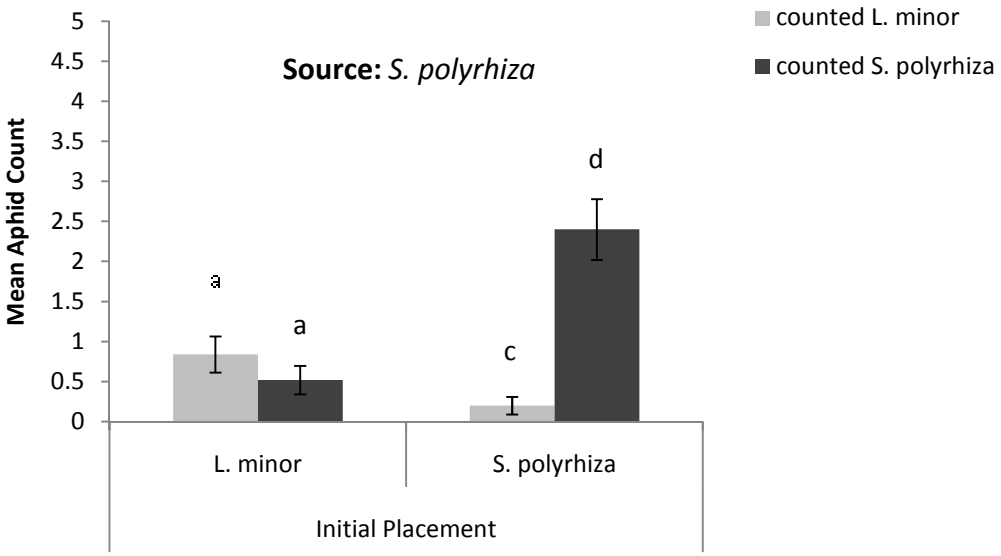
a) Adults (day 3)



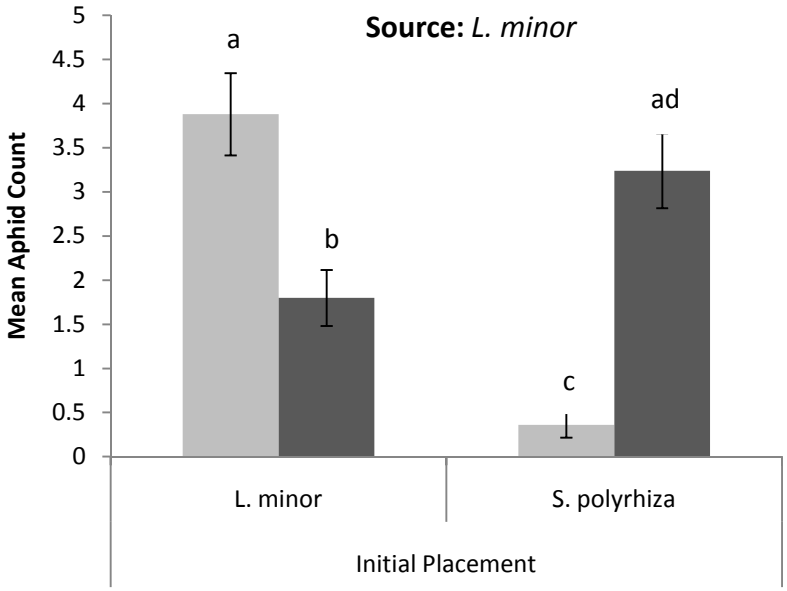
b)


Source: L. minor
Mean Aphid Count
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
b
a
c
L. minor
S. polyrhiza
Initial Placement

c)


counted L. minor
counted S. polyrhiza
Source: S. polyrhiza
Mean Aphid Count
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
a
a
c
d
L. minor
S. polyrhiza
Initial Placement

d)



e)

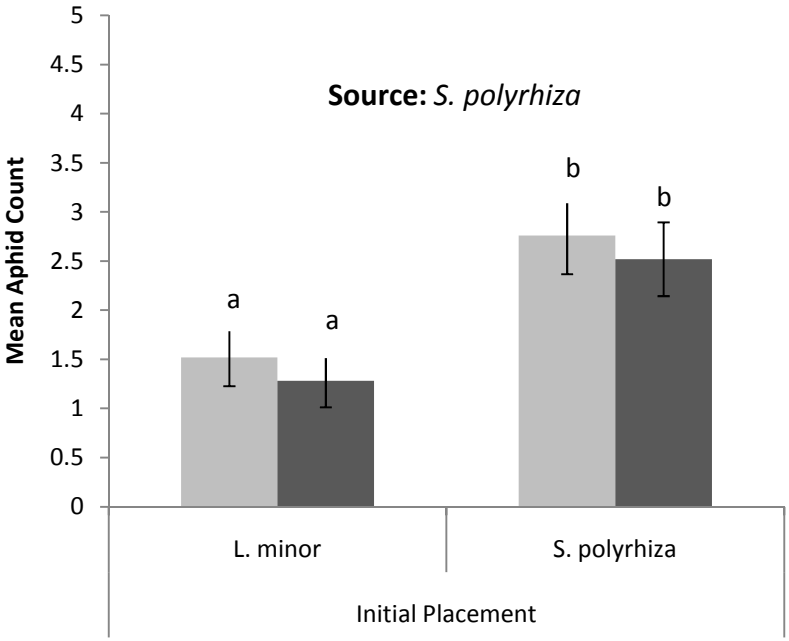


Figure 5: In a) the proportion, with standard error of the mean, of parent aphids found on a given species of duckweed after 3 days is plotted for four combinations of source, initial placement and location. The structure of b) and c) are the same, however, the average count of aphid offspring is plotted, rather than the proportion, after 3 days. In d) and e) the relative abundance of aphids per duckweed species after 6 days is shown.

Experiment 5

Aphid population growth is limited at each density. Furthermore, at this temperature aphid populations grow faster than duckweed populations, making density effects appear more quickly. At all three time intervals aphid populations show density dependent growth patterns (Day3: r=0.32, p<0.001, F-st=43, DF=88; Day 5: r=0.59, p<0.001, F-st=128.3, DF=88; Day 8: r=0.53, p<0.001, F-st=99.82, DF=88).

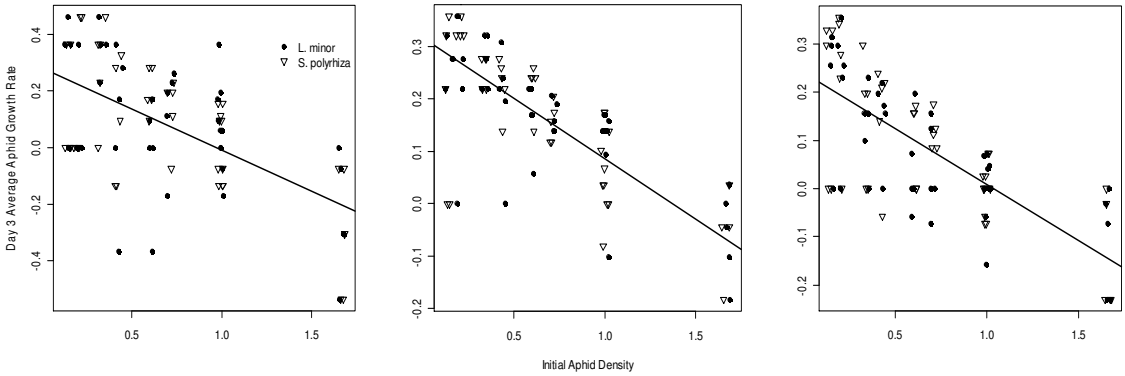


Figure 7: Solid black dots refer to average aphid growth rate at various aphid densities (aphids/frond) after 3, 5 and 8 days, on *L. minor*. Black dots represent average aphid growth rate on *S. polyrhiza*. Linear regression analysis reveals a correlation between initial aphid density and instantaneous aphid growth rate.

Discussion

Here, we present the initial experiments that were designed to inform and guide future studies about the temporal effect of temperature on species interactions within simple food-webs. These experiments quantified previously unknown details about numerous strains of duckweed and its herbivores. There is a significant difference in growth rate between *L. minor* and *S. polyrhiza* across temperature. Both aphids and flies have a negative effect on duckweed growth, however flies reduce duckweed growth to a greater extent than aphids. Furthermore, aphids feed on multiple species of duckweed,

while flies only consumed *L. minor* in the experiments. Furthermore, aphid movement and foraging preference is affected by the species of duckweed it consumes. Aphids that fed previously on *S. polyrhiza* are more likely to leave the frond they were placed initially, whereas aphids that fed on *L. minor* were less likely to migrate to another frond. Lastly, aphid population growth is density dependent across all aphid/duckweed ratios, a condition that facilitates indirect effects between duckweed populations.

Experiment 1: The effect of aphids (*Rhopalosiphum nymphaeae*) and flies (*Lemnaphila scotlandae*) on duckweed (*Lemna minor*) growth

The larvae of duckweed flies were previously observed living in and around fronds of *L. minor* (Landolt 1986). However, prior to this experiment, the effects of adult flies and aphids on duckweed growth had not been described empirically. Results show that both aphids and flies reduce duckweed growth significantly. Duckweed populations under fly herbivory grew positively at day 2. However, subsequent data collection showed that duckweed populations decreased under fly herbivory after day 2. One possible explanation for this positive growth is that the effects of flies had yet to accumulate by day 2. The duckweed growth up until day 2 was a result of nutrient acquisition and metabolism that occurred prior to the addition of flies. It is also likely that media nutrient amounts were relatively high at this point. These two factors promoted positive growth of duckweed under fly herbivory. Whereas duckweed under fly herbivory eventually went extinct, duckweed under aphid herbivory had a positive, although reduced, growth rate. Therefore aphid populations can respond numerically to increased amounts of duckweed, thus enabling apparent competition to occur between duckweed populations. In addition, aphids have a generation time similar to duckweed, of

approximately one week. Adult aphids produce off-spring and continue to feed on duckweed, producing multiple generations in a lifetime. Therefore, aphids are better suited for multigenerational studies of species interactions with duckweed species.

Experiment 2: The effect of aphids (*R. nymphaeae*) on duckweed growth (*L. punctata*, *S. polyrhiza* and *L. minor*)

Aphids feed on multiple species of duckweed; having negative effects on each. Although these effects are not likely to be equivalent, it is only necessary that aphids negatively impact both species of duckweed to facilitate indirect effects.

Experiment 3: The Effect of Temperature on Duckweed Growth

Two of these duckweed species, *Lemna minor* (Memphis) and *Spirodela polyrhiza* (Lincoln), differ in growth rate at 19° C and may begin to differ at 30°C. However, these populations were grown in the presence of algae, whereas future studies will use axenic duckweed cultures. There were inevitably differences in algae population size across cups and perhaps differences in the effect of algae on duckweed growth across species. Thus, the absence of algae could enhance or eliminate the mean difference in growth between duckweed populations under different temperatures depending on the precise difference in the algae's effect on the co-occurring duckweed populations. In combination with experiment 2, these results suggest that aphids and these two species of duckweed will work well to understand the effect of temperature regime on short and long term indirect effects between prey populations in a diamond food-web.

There seems to be a trend towards a larger difference in growth at higher temperatures between *S. polyrhiza* (Lincoln) and *L. minor* (Memphis), which is not surprising given the average climate of Memphis, TN and that of Lincoln, NE; locations where temperatures differ by an average of 5°C during the summer months. This difference may have facilitated regional adaptation of duckweed growth to the local temperature regime.

Experiment 4: Aphid foraging preference

Aphid distribution is effected by past feeding experience. Aphids reared on populations of *L. minor* were more likely to stay on the species of duckweed they were initially placed. However, aphids reared on *S. polyrhiza* were more likely to travel to other fronds. This result is contrary to a study by McLean et al. (2009) in which aphids expressed a strong foraging preference for the maternal host plant. Aphid populations also grow more quickly on *S. polyrhiza*, which suggests aphids are healthier and more robust to spend energy on movement, whereas aphids on *L. minor* are relatively undernourished, and have less energy for movement.

These results suggest that aphids raised on *S. polyrhiza* would better suit a fully functioning diamond food-web in which the consumer feeds readily on both resource populations. However, it is important to note that during experimentation subsequent aphid generations will likely alter their movement as they find themselves on *L. minor* or *S. polyrhiza*. We predict the effects of the duckweed upon which they were raised prior to experimentation will become less significant in comparison with the current species of duckweed they are exploiting.

Experiment 5: Aphid population growth as a function of initial aphid density on *Lemna minor* and *Spirodela polyrhiza*

Our results reveal that at all but the lowest aphid densities, aphid population growth is sub-maximal. This result is not surprising given previous research on aphid population dynamics. Dib et al. (2010) has shown that populations of the rosy apple aphid (*Dysaphis plantaginea*) exhibit strong density dependence both in the presence and absence of a predator. In our study, it is likely that as aphid density increased the amount of duckweed nutrients available per aphid decreased, as well as the amount of space aphids could occupy per frond. Thus, duckweed amounts will always be limiting to aphid growth outside transient conditions where aphids exist at very low densities. This relationship between aphid density and growth rate raises the probability that apparent competition will occur between prey in this system. For apparent competition to occur aphids must respond numerically to greater amounts of duckweed, thereby increasing the negative effect they have on the other species of duckweed.

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CHAPTER 2

Increased Temperature Produces Transient Mutually Positive Indirect Effects between Prey under Shared Predation

Introduction

Indirect interactions are measured as the effects populations have on each other via an intermediate population in a given food-web. As food webs grow in complexity, indirect interactions become more abundant relative to direct interactions (Holt 1977). Much research, both empirical and theoretical, has examined species interactions between two prey populations under shared predation (Leibold 1996; Abrams 1998; Brassil 2006; Stap 2008). A food-web of this structure is the simplest in which indirect effects can take place. Theory predicts a variety of indirect interactions between shared prey, from mutually negative effects, to mutually positive effects (Abrams 1996). Furthermore, these indirect effects can be trait mediated or density mediated (Abrams 1995). Both the detection and importance of trait mediated and density mediated indirect effect has recently been discussed heavily in the literature (Mouritsen 2008; Souza 2008; Veen 2009; Veen 2005; Luttbeg 2003; Werner 2003; Okuyama 2007). In addition, temperature is known to impact indirect interactions within tri-trophic food chains (Barton et al., 2009). However, it is unknown what effect temperature can have on either type of indirect effect between prey in a one-predator two-prey web, or how this effect might change over time. Whereas previous work on temperature and indirect effects focused on a tri-trophic food chain of terrestrial populations inhabiting a climate of naturally varying

temperature, we present a study of indirect effects over multiple generations of population growth in a food-web of two species under shared predation.

Microcosm experiments can be a powerful tool for studying the ecological effects of climate change (Benton et al., 2007). For studies of community ecology model systems are amenable to rapid data collection and precise treatment manipulation. The work presented here continues a line of ecological research that utilizes a model system to investigate the effects of climate change on community processes. This system of organisms has been used in the past to study basic questions in community ecology. Included are two duckweed species, *Lemna minor* and *Spirodela polyrhiza,* both of which are consumed by the Waterlily aphid, *Rhopalosiphum nymphaeae*.

Here I construct multiple communities, from a single population of duckweed, to two populations of duckweed consumed by aphids. In the one-predator two-prey web we predict mutually positive indirect effects between duckweed populations during the first generation of growth. These are predicted to result from a dispersal effect in the aphid population. However, as aphid populations respond numerically to more abundant prey, mutually negative and asymmetric indirect effects should occur due to interspecific variation in growth response to temperature. The duckweed population that grows faster should have a greater negative effect, via apparent competition, on the other duckweed population. This should cause a negative-zero or negative-negative indirect interaction between prey. However, as aphid populations cycle there is the potential for positive indirect effects between duckweed populations, but it is unknown how this interaction could be affected by temperature.

Furthermore, aphid movement is known to vary with diet (see chapter 1). Aphids feeding on *L. minor* are more likely to stay on *L. minor*, whereas aphids feeding on *S. polyrhiza* are more likely to disperse. Aphid population growth is also greater on *S. polyrhiza* than it is on *L. minor*. The interaction between duckweed species and aphid growth/movement creates the potential for trait-mediated indirect effects, which could vary with temperature, as well.

This experiment builds upon work in community ecology aimed to further understand direct and indirect effects that occur between interacting populations in simple food-webs under different temperatures. By tracking aphid movement and quantifying population growth of all interacting populations we present a comprehensive analysis of species interactions within simple food-webs under two temperature regimes.

Materials and Methods

Here 14 replicates of each food-web were grown under two different temperatures, 19 □ C and 27 □ C. There were 20 different treatment combinations that varied among three factors, aphid presence or absence, duckweed species and initial density, and temperature. Cups contained 2 aphids or no aphids. Duckweed populations were combined in a trimmed response surface design (Inouye 2001). Amounts per cup were 8-11 fronds of *S. polyrhiza*, 3-6 fronds of *S. polyrhiza*, 5-8 fronds of *L. minor*, 11-14 fronds of *L. minor* or 5-8 fronds of *L. minor* along with 3-6 fronds of *S. polyrhiza*. The starting density of duckweed was doubled to understand the relative effects of interspecific and intraspecific competition. One HOBO data-logger, programmed to record light intensity and water temperature every two minutes, was placed in each of two bins per in 100ml of nutrient solution and covered with nylon. Actual temperatures

were calculated from the experiment by averaging all temperatures recorded by the two loggers separately in each room, and then averaging those numbers together.

Every Monday and Friday nutrient replacement was conducted. This protocol avoids nutrient limitation which causes yellowing fronds and lowers duckweed growth rates. Distilled water was first added to each cup, bringing it back to 100ml of fluid. Then 30ml was removed with a sterile pipette and 30ml of nutrient solution added to every cup. Cups were then placed in a random location within the bin, and the bin placed back in the appropriate growth room.

Data collection involved counting frond number in each cup at the end of every 7 days. A frond was determined to be any independent round formation, regardless of size. Frond counts were done within ImageJ® on jpeg images of each cup taken by a Canon Powershot A710. The entire experiment ran for 21 days. The dry weight of a subset of duckweed and aphid populations was also taken at the conclusion of the experiment.

Average growth rates (r) were then calculated using the function "$(\ln(N_f)-\ln(N_i))/7$" where N_f is the final number of fronds after 7, 14 and 21 days and N_i is the initial number of fronds. Data analysis began with multiple ANOVA for 19°C and 27°C at 7, 14, 21 days in which the interactions between the presence and absence of herbivory were crossed with the presence and absence of competition. All significant results are reported (Table 1), along with the estimated magnitude ("mag") of effect size. When reporting effects of competition, herbivory and apparent competition the initial density for each duckweed species will be denoted parenthetically after the species name.

To calculate the effect of competition at a given time and temperature the average instantaneous growth for a population of duckweed, grown alone, was subtracted from

the average growth in the presence of another population of duckweed. The effect of herbivory was the difference in average growth in the presence and absence of an herbivore. Indirect effects between duckweed populations were calculated as the average growth in the diamond food-web minus the average growth under herbivory, minus the average growth under competition, plus the average growth alone. In other words, we assume the effects of competition and herbivory operate similarly in the diamond food-web, and that these effects are additive. Thus, any difference between duckweed growth (r) in the diamond food-web and duckweed growth alone, after the presumed effects of herbivory and competition are subtracted, is considered positive or negative apparent competition.

Results

Data from this experiment reveal interactions that are species specific, temporally dynamic and temperature dependent. The effects of competition were more prevalent at the higher temperature, whereas herbivory effects were significant across temperature. However, at the last time step the effects of herbivory become positive due to aphid population cycling. Apparent competition between duckweed populations was the most variable species interaction within this experiment. At 19°C *S. polyrhiza* experienced negative apparent competition until day 21when effects became insignificant. At this temperature *L. minor* did not experience apparent competition until days 14 and 21 when it grew faster via positive indirect effects from *S. polyrhiza* populations. Duckweed populations also experienced these effects at the higher temperature, however at 14 days apparent competition was mutually positive. A pairwise t-test of growth rates revealed

that *L. minor* grew faster than *S. polyrhiza* across both temperatures (19°C, p<0.01 & 27°C, p<0.01) (Figure 1).

Aphid population dynamics played a crucial role in determining the strength and direction of indirect effects throughout the experiment (Figure 6-7). At 19°C after 7 there were significantly more aphids on *S. polyrhiza* than *L. minor* (p=0.002), however there was no significant difference in the number of aphids on either duckweed species at day 14 or day 21 (p<0.01). At 27°C there were more aphids on *S. polyrhiza* than *L. minor* after 7 and 14 days (p=0.05), however aphid distribution was approximately even across duckweed species at day 21.

DW	Density	Temp	Day	p Comp	p Pred	p AC	Comp Mag	Pred Mag	AC Mag
S	Low	19	7	**0.04**	0.549	**<0.001**	-0.00283	-0.0529	0
L	Low	19	7	2.60	**<0.001**	0.65	-0.00233	-0.04423	0.011869
L	High	19	7	0.07	**<0.001**	0.35	0.002034	-0.03987	0.00554
S	Low	19	14	0.90	**<0.001**	**0.05**	-0.01314	-0.0529	-0.10408
L	Low	19	14	0.58	**<0.001**	0.09	-0.0182	-0.05085	0.036366
L	High	19	14	0.15	**<0.001**	**0.006**	-0.015858	-0.04851	0.01786
S	Low	19	21	**0.001**	**0.02**	0.13	-0.0122	0.02954	1.00E-08
L	Low	19	21	**<0.001**	**<0.001**	**<0.001**	-8.16E-02	-1.37E-02	7.32E-02
L	High	19	21	**<0.001**	**0.02**	**<0.001**	-8.16E-02	-1.27E-02	7.63E-02
S	Low	27	7	0.22	**0.02**	**0.008**	0.00376	-0.04252	-0.02519
L	Low	27	7	0.25	**<0.001**	0.47	-2.37E-02	-7.66E-02	4.19E-02
L	High	27	7	0.63	**<0.001**	**0.005**	3.02E-03	-4.99E-02	1.05E-02
S	Low	27	14	**0.002**	**<0.001**	**<0.001**	0	-0.09529	0.05534
L	Low	27	14	**<0.001**	0.44	**<0.001**	-1.23E-01	-7.23E-02	9.68E-02
L	High	27	14	**<0.001**	**<0.001**	**<0.001**	-7.92E-02	-2.90E-02	5.84E-02
S	Low	27	21	**<0.001**	**0.03**	**0.04**	-0.00289	0.02995	0.002256
L	Low	27	21	**0.04**	**<0.001**	0.753	-3.40E-02	1.02E-01	-3.5E-02
L	High	27	21	**<0.001**	**<0.001**	0.07	-1.80E-02	1.18E-01	-5.9E-03

Table 1: Starting from the left the column heading "Species" refers to the duckweed species and is denoted by an "S" for *S. polyrhiza* or an "L" for *L. minor*. The second column describes the starting density of duckweed. The third column is the water

temperature in °C. The next column is the day of data collection. The next three columns contain p-values generated from a 2x2 ANOVA (presence or absence of herbivory and competition) which denote whether competition, herbivory or apparent competition altered duckweed growth significantly for a given duckweed species, at a specific time, in a certain temperature. P-values in red are significant. The last three columns contain the magnitude of effect sizes.

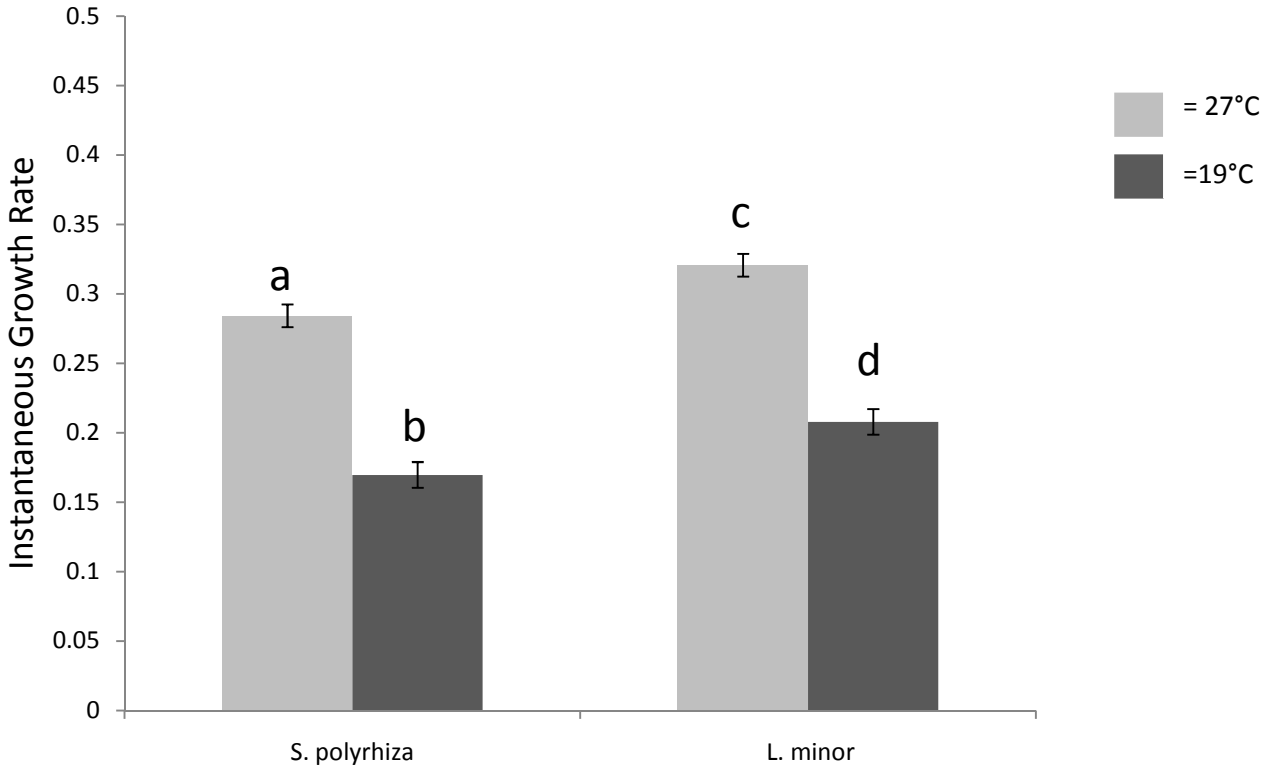
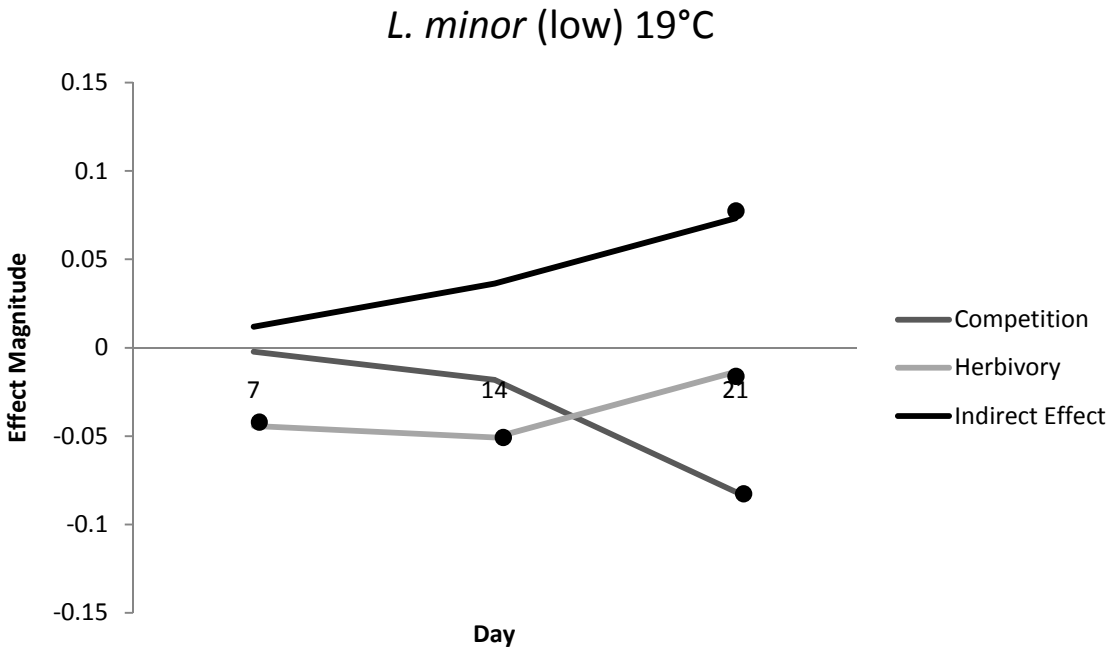
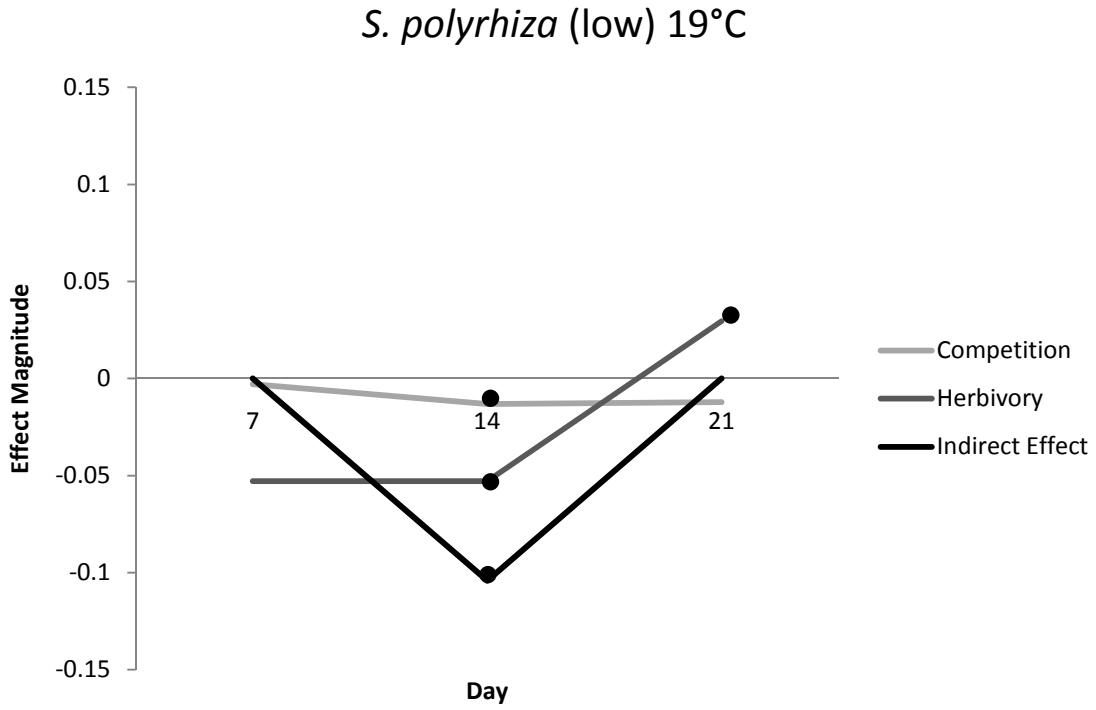
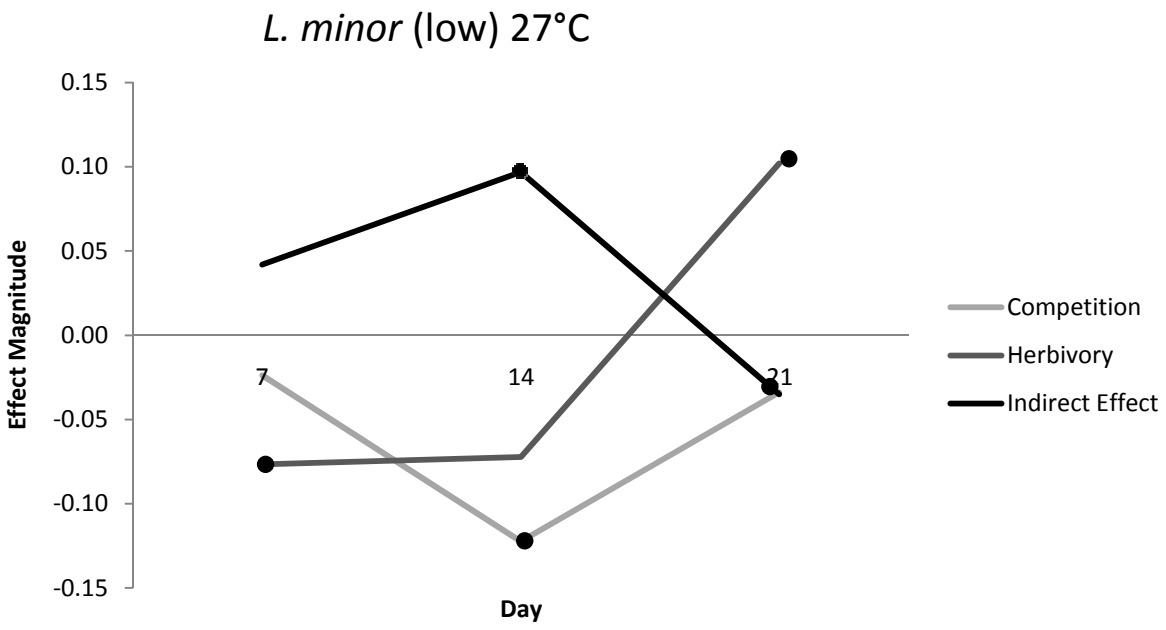
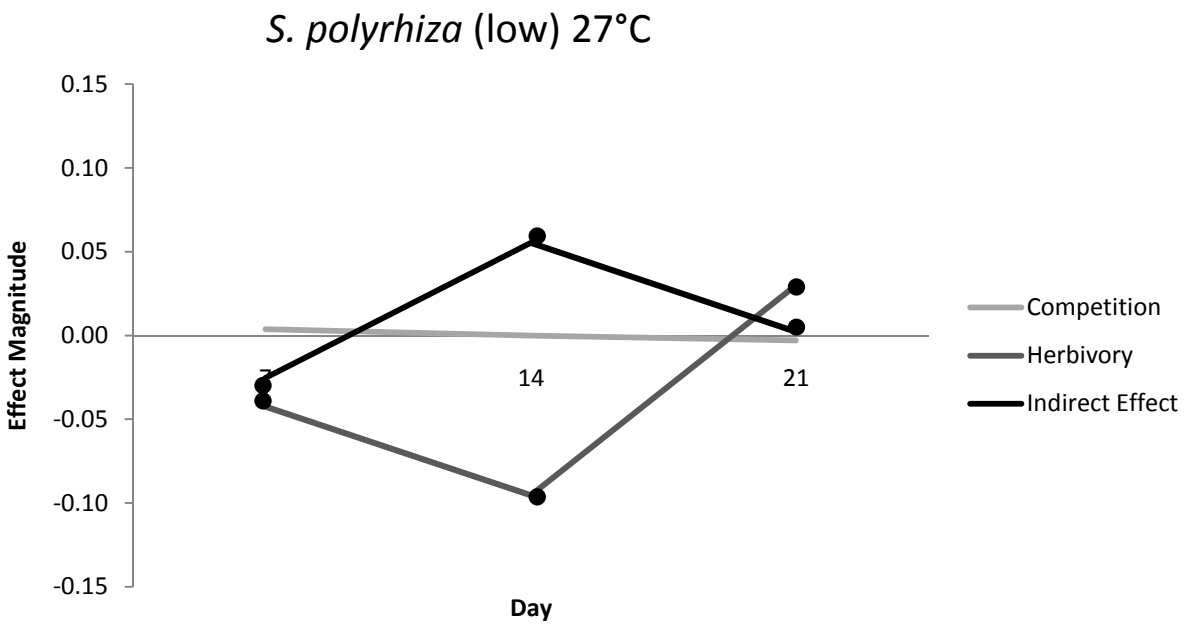


Figure 1: Here the instantaneous growth is plotted for two species of duckweed, *S. polyrhiza* and *L. minor*. Black bars represent growth rates under 27°C and grey bars, under 19°C. Standard error of the mean is displayed for all data.


L. minor (low) 19°C
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
Effect Magnitude
7
14
21
Day
Competition
Herbivory
Indirect Effect


S. polyrhiza (low) 19°C
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
Effect Magnitude
7
14
21
Day
Competition
Herbivory
Indirect Effect





Figures 2-5: Effect magnitude and statistical significance is plotted over time for both populations of duckweed in both experimental temperatures. Black circles signify an effect that was significantly different from zero.

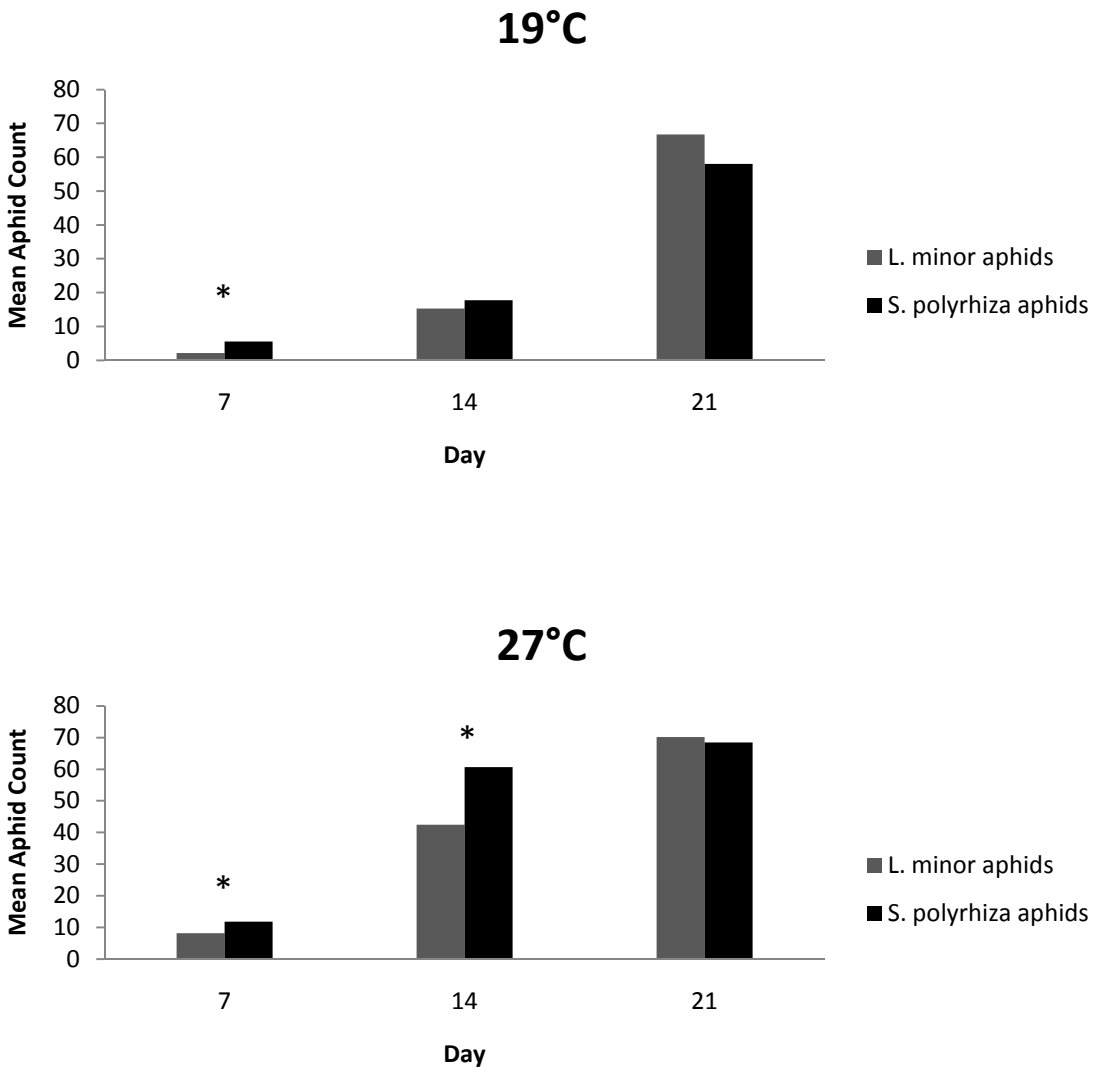


Figure 6-7: Mean aphid count per duckweed species is shown for each day. Asterisks denote significant differences between the average number of aphids on either species of duckweed on a given day.

Discussion

Most experimental results were consistent with a priori predictions. The effects of herbivory were negative at both temperatures, although at higher temperatures populations of duckweed grew faster under herbivory than they did when grown alone by

day 21. At this time step the negative effects of density dependence decreased duckweed growth more than herbivory. Populations of *S. polyrhiza* grew at approximately the same rate in the presence or absence of *L. minor* at all times and temperatures other than day 14, whereas *L. minor* growth was significantly reduced in the presence of *S. polyrhiza*.

Indirect effects differed across time and temperature, producing (0,0), (0,-) and (+,0) interactions for *L. minor* and *S. polyrhiza*, respectively, at 19°C and (0,-), (+,+) and (0,+) interactions at 27°C. The positive indirect effect experienced by *L. minor* at day 21 is likely the net result of aphids feeding on *S. polyrhiza* preferentially during the first two weeks of experimentation. I argue that aphid distribution at the previous time step offers more explanatory power regarding indirect effects than aphid distribution at the time step in question. By day 21, the aphids are located on *L. minor* more often; however these effects have not yet accumulated, thus resulting in a positive indirect effect. *S. polyrhiza* experiences a transient negative indirect effect at day 14, likely resulting from aphids foraging on *S. polyrhiza* more often than *L. minor*. At 27°C indirect effects occurred earlier, as suspected. The negative-zero interaction measured at 19°C occurred at day 7, rather than day 14, a result of higher growth rates due to increased temperature. At day 14 the growth rate of both populations of duckweed was higher than expected. One possible explanation is that herbivory pressure was decreased per duckweed population, thus outweighing the negative effects of resource competition. Supporting this logic are two pieces of evidence. First, *S. polyrhiza* experienced negligible effects of competition at this time step, and aphids foraged preferentially on *S. polyrhiza* within the diamond food-web.

Intraspecific competition among duckweed varied with density across time and treatment. Results indicating positive effects of herbivory suggest that aphid foraging increases per capita duckweed growth. The aphids mitigate intraspecific competition by lowering population density. By doing so, populations under herbivory grow faster than populations grown alone, which reach carrying capacity at an earlier time step. Thus, the net effect of aphid herbivory on duckweed population growth is the relative magnitude of the direct negative effect on growth, via reduction in phloem nutrients, and the indirect positive effect on growth, resulting from decreased intraspecific competition between individual duckweed fronds. This same relationship is also relevant when measuring other species interactions, such as interspecific competition and apparent competition. Any population that alters the density of another population directly, via predation, or indirectly, via resource competition or apparent competition, may also alter the effects of intraspecific competition on that population, as well. Therefore, one must exercise caution when interpreting the indirect effects presented here.

Theoretical work by Holt et al. (1994) put forth simple rules for predicting the outcome of indirect interspecific competition in a diamond food-web. These rules resemble classic R* competition theory where the prey species that exploits resources to a level below that of the other prey species will enjoy a competitive advantage. This insight provides an alternate explanation for the indirect effects described above. It is likely that *S. polyrhiza* and *L. minor* differ in resource use, but unknown whether these differences are great enough to alter indirect interactions. Aphid feeding location and resource use likely interact to create the community dynamics we have quantified.

Nutrients were collected after the experiment was complete and will be analyzed in the near future.

The extent to which the above results contribute to previous research on trait and density mediated indirect effects is uncertain. Does uneven aphid distribution across duckweed species, resulting from a foraging preference, create trait mediated indirect effects? If it does, how can we separate the simultaneous effects of aphid density? For this study to mesh with the literature on trait and density mediated indirect effects it is possible that aphids facilitate indirect effects between prey that are simultaneously trait and density mediated.

The reality of climate change provides an impetus for ecologists to study the effects of temperature on community dynamics. The results of this study will shed light on the potential impact of temperature on short and long term species interactions in a simple food-web of two prey under shared predation. Although the food-webs constructed and monitored for this study were highly simplified, there were still non-intuitive results. Furthermore, this study may increase our understanding of trait mediated and density mediated indirect effects, or at least provide a commentary on the dichotomy of trait and density mediated effects.

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