

Effects of experimental warming on plant traits of subalpine wildflower species of Gothic, Colorado

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ABSTRACT

Over the next century the increasing concentration of carbon dioxide (CO₂) and other contributing greenhouse gases is predicted to generate an average increase in temperature 1-6°C (Saavedra et al., 2003; Cleland et al., 2012). The proposed warming climate suggests a common myth of global warming that raising temperatures and CO₂ enrichment will make a greener world. This research has contributed to data on reproductive traits and physiological changes linked to these qualities of various plant species. It was hypothesized that warmed plots may allocate more energy and resources for more competition among other plants within a warmer area. Using warming frames (these frames prevent radiative heat loss therefore allowing them to be slightly warmer) and plots previously set up by David Inouye (Inouye 2008), research was conducted to answer the hypothesis looking at species *Erigeron flagellaris*, *Arenaria congesta*, and *Potentilla gracilis*. Results shows the variables did not differ between treatments for both *Erigeron flagellaris* and *Arenaria congesta*. Height, seed set, and chlorophyll content were all greater in warmed plots for *Potentilla gracilis*. This all suggests a change in plant communities in the near future, species-specific variations, as well as some species (*P. gracilis*) allocating more energy to growth and reproduction in warmed temperatures and less to pollinator attraction.

INTRODUCTION

Climate change has been shown to have a variety of effects on ecological systems across the globe (Price & Waser, 1998; Saavedra et al., 2003; Lambrecht et al., 2006; Inouye, 2008; Cleland et al., 2012). As abiotic dynamics fluctuate in ecosystems, so does the life around it to adjust to the changes. Two of the most prominent biological responses to global climate change are range shifts and changes in phenology (Price & Waser, 1998; Inouye, 2008; Miller-Rushing & Inouye, 2009; Lambert et al., 2010). Over the next century the increasing concentration of CO₂ and other contributing greenhouse gases is predicted to generate an increase in average global temperature ranging from 1-6°C (Saavedra et al., 2003; Cleland et al., 2012). A common idea about global warming is that rising temperatures and CO₂ enrichment will make a greener world (Harte & Harte, 2008). Alternatively, temperature increases may be too rapid and extreme for plants to respond and survive (Snider et al., 2010).

Plants are important elements of ecosystems; their responses to global climate change are capable of affecting pollinators, carbon and nutrient cycling, food webs, and disturbance regimes (Wolkovich et al., 2012). Global warming has also been shown to affect photosynthesis, transpiration, and plant-water potential in some plant species (Lambrecht et al., 2006). Plant phenology has been one of the most useful indicators of the biological effects of climate change, and is conducive to experiments

because plant phenology is sensitive to warming (Wolkovich et al., 2012). Phenology is a good indicator of sensitivity and adaptive capacity; there is a significant amount of data on phenological shifts in response to earlier snowmelt and increasing temperature (Price & Waser, 1998; Inouye, 2008; Cleland et al., 2012). However, little is known about how warming temperatures affect reproductive traits and the physiological processes that are linked to those qualities (Price & Waser, 1998; Lambrecht et al., 2006; Hedhly et al., 2008). Understanding the plasticity of these traits with prevailing temperatures could provide insight into plant ecology and evolution as global warming continues (Hedhly et al., 2008).

Therefore, in this study I focus on various traits associated with plant reproduction in response to temperature in control and warming plots. I examined traits in three subalpine wildflower species in the Colorado Rocky Mountains. To provide possible insight into underlying physiological processes associated with changes in plant traits, photosynthesis and chlorophyll content were also examined. These traits are indicators that help provide insight into how plant species will respond to climate change. Phenology and temperature has been regularly recorded at the Rocky Mountain Biological Laboratory (RMBL), where this study took place. Information about other plant traits can therefore be applied to what is known about plants and phenology and their reactions to warming temperatures, for a more comprehensive understanding of plant responses to climate change. During the course of this study the effects of rising temperatures on plant height, flower size, and quantity of seed production, traits related to plant reproduction and pollinator attraction have all been closely monitored. Because there is so much evidence, we can expect changes in atmospheric CO₂ concentration, temperature, or precipitation to have a direct effect on photosynthesis, respiration, growth, tissue composition (Hughes 2000).

Specifically, my research poses to answer a number of questions: How do reproductive traits react to warmer temperatures? A temperature increase may provide an environment where plants make more food, therefore they may be able to allocate resources to either growth (plant height) or reproduction (flower size, seed set). If flowers grow larger in warmed plots, this could reflect pollinator attraction or merely increase reproductive capacity. Allocating more resources to reproduction could lead to increased seed set in warmed plots. Do plants in warmer temperatures produce more chlorophyll and therefore photosynthesize at a higher rate? Is there species-specific variation in how plants respond to warming, or are responses consistent across species? Overall, the goal of this research is to gain a better understanding of how plant traits related to reproduction might change in a warmer world.

METHODS

Study Site

I carried out my research in a series of phenology plots set up previously by David Inouye in the Rocky Mountains of Gothic, Colorado (figure 1; Inouye, 2008). These phenology plots were originally focused on changes in flowering phenology from year to year and have been set up for 40 years (1973-2013). After documenting changes in phenology in relation to snowmelt and temperature, new plots were added alongside the long-term plots in 2010 to better understand how warming affects phenology (Inouye 2008). Plots that are warmed are covered by table-like frames slightly larger than 1m x1m that are slated with plexi-glass along the top. The frames warm the air temperature by 0.5°C during the day and up to 2.0°C at night by preventing radiative heat loss from the plots. Because these warming frames have been in place since 2010, it is likely that plasticity in plant traits will be detectable if present. Both types of plots still monitor phenology. However, I used them to observe flowering, growth, and physiological traits. Samples were taken from the established warming plots. Nonetheless to avoid destructive sampling from the long-term control phenology plots, I created 1m x1m control plots adjacent to the warming plots.

Study species

Three different forb species that reach peak flowering from late June through mid-July were studied in this project. All have been found flowering in abundance in the study plots in past years (2010-2012) and were therefore chosen to be studied in this experiment. *Erigeron flagellaris* (Asteraceae), known as the wandering fleabane daisy, is found in abundance throughout dry rocky terrain in meadow openings among sagebrush. Also found in dry rocky meadows are *Arenaria congesta* (Caryophyllaceae). *Arenaria congesta* thrives in dry rocky soils and tend to grow in small clumps (Darrow 2006). The most abundant forb in this area of the Rocky Mountains is *Potentilla gracilis* (Rosaceae; Elpel 2012). *Potentilla* species thrive in a variety of habitats and are known to be good pioneer species, able to grow in poor soil (Darrow 2006). All three species are animal pollinated. However, *P. gracilis* is also capable of self-pollination.

Study design& field methods

The sample size is the number of plots in which each species is found (ranging from 4 to 6 depending on the species). The occurrence of study species in the warmed plots limited the sample size for each species. The number of plants per plot varies, encompassing natural variation in plant density in the study area.

For each species, up to 30 plants per plot were tagged and observed over the duration of the summer. Single and multiple measurements were taken per plant. Single measurements included each plant's height to the tallest point (measured by ruler in cm), the total number of flowers per plant, and the total number of fruits produced per plant. For *A. congesta*, the number of flowering stems per plant was taken into consideration in analysis, because plants vary greatly in the number of stems. Thus, plants may either alter the number of flowers per stem or stems per plant. I often employed subsampling when multiple measures per plant were necessary to assess plant traits. Up to ten flowers per plant were measured for width in cm, the number of seeds counted per flower, and whether the flowers were able to follow the reproduction cycle into seed success. Fruits and seeds were taken from ten plants per plot and then counted in a lab.

Measurements in correlation with physiological changes that might be linked to reproductive traits were limited to *P. gracilis* due to the small leaf size in the other two species. Using a chlorophyll meter, SPAD502, we were able to attain a rating of chlorophyll content (Spectrum Technologies, Inc, Aurora, IL). Measurements that come from the meter produce relative indexed SPAD meter values that are proportional to the chlorophyll content present in the leaf (Ling et al., 2011). Three leaflets per plant in each plot were tested for chlorophyll content amount then averaged. The values were defined by Konica Minolta, and indicate a relative amount of chlorophyll content present in plant leaves (Spectrum Technologies Inc.). Photosynthetic rate was measured in a similar way, using a Li-Cor infrared gas analyzer; subsamples were taken at each plot (Li-Cor Biosciences, Lincoln, NE). Leaflets were individually clamped into a chamber that measures the amount of CO₂ that flows through the plant. Once there was a stable reading, photosynthetic capacity was logged three times then averaged, using μmol of CO₂/m²/sec. This process was repeated for three plants per plot. The photosynthetically active radiation (PAR), time, and flow of CO₂ through the Li-Cor were also measured during the time of photosynthetic activity. The PAR was set to match ambient conditions because we wanted to compare photosynthesis under field conditions. The CO₂ flow rate was set at 400 ppm. Because not all leaves were the same size or did not fill the chamber that measures CO₂ capacity, a recalculation was done to attain more accurate photosynthetic rates. Each leaflet measured was taken from the plants, and cut to the size of the area of the chamber (the part of the leaf that was measured). The true area of the leaf within the chamber was found using computer program ImageJ, then photosynthetic capacity was adjusted to control for variation in leaf area. The leaf area was divided by six (the area in cm² of the Li-Cor chamber) then multiplied by the original photosynthetic rate found.

Pollinator Observations

I conducted pollinator observations to assess whether the warming frames alter pollinator behavior or rates of foraging, which could in turn affect plant reproduction. To keep the study unbiased, pollinator observations were conducted on sunny days above 15.5°C during the day between 10AM-3PM when most pollinators are active. These data on pollinator visitation allows me to assess whether the warming frames had unintended effects on pollinator visitation to plants. If differences in plant reproductive success (fruit and seed set) were found between treatments, then the pollinator visitation was also compared between treatments.

Statistical analysis

The mean values of plant traits were compared between treatments and t-tests were used to compare each plant trait between treatments, unless response variables violated assumptions (normally distributed response variable and equal variance between treatments). If normality was violated, nonparametric statistics were used to compare responses between treatments. If variances were not equal between the treatments, I used a Welch t-test to account for unequal variance.

RESULTS

Erigeron flagellaris

The height ($t=-1.3322$, $p=0.25$; figure 2), the total number of flowers per plant ($t=1.2857$, $p=0.2679$; figure 3), and the average flower width ($t=-1.4652$, $p=0.2167$; figure 4) all did not differ between the warmed and control treatments. *Erigeron flagellaris* seed set showed a similar trend where there was no difference between treatments ($t=-1.4123$, $p=0.2307$; figure 5).

Arenaria congesta

The height of plants per plot did not differ between treatments ($t=-0.269$, $p=0.797$; figure 6). The total number of flowers ($p=0.486$, Wilcoxon rank sum test; figure 7), the number of stems ($t=0.550$, $p=0.602$; figure 8), and the flower width ($t=-0.133$, $p=0.899$; figure 9) per each plot did not differ between treatments. Seed set in *A.congesta* also did not differ between treatments ($p=0.8857$, Wilcoxon rank sum test; figure 10).

Potentilla gracilis

Potentilla gracilis plants were taller in warmed plots relative to the controls ($t=-2.5404$, $p=0.02935$; figure 11). Using a Wilcoxon rank sum test, the total *P. gracilis* flowers per plot ($p=0.31$; figure 12) and flower width ($p=0.48$; figure 13) did not differ between treatments. Though there are slight observational differences in the seed set of *p. gracilis*, overall the seed sets from the warmed treatment was marginally greater than seed set from controlled treatments ($t=-1.9401$, $p=0.08107$;

figure 14). Analyzing the content of chlorophyll showed that there tends to be more chlorophyll in plants under warming frames than in the controls ($p=0.0043$, Wilcoxon rank sum test; figure 15). However, photosynthetic capacity between treatments did not differ ($t=-0.4052$, $p=0.6879$; figure 16).

Discussion

Overall, many of the results did not differ between treatments. This could lead to a number of possibilities. Some plants with short generation times may be able to adapt *in situ* as microevolutionary change (Hughes 2003). If some species are not able to respond with the changes in the appropriate way they are subject to become unsynchronized which can lead to endangerment or extinction (Inouye, 2008; Hedhly et al., 2008; Burkle et al., 2013). To avoid extinction some plants here may be changing just enough to avoid huge impacts on their populations. Though *P. gracilis* is adapting to live productively in warmer temperatures, it is possible it may out-compete other species in the same area to change species distributions. The reproductive phrase in plants provides the plant with an opportunity to adapt to environmental changes (Hedhly et al., 2008). Seed set in *P. gracilis* was marginally significant under the warming frames. This along with the higher growth under warming frames suggests results are consistent with its ability to self-pollinate as *P. gracilis* did not allocate as much resources to traits associated with pollinator attraction.

The results of my research support the idea that warming effects are species specific (Loik & Harte, 2006; Lambrecht et al., 2006; Hedhly et al., 2008). While the traits measured in this study did not differ between treatments for *Arenaria congesta*, there were differences in some traits for *Potentilla gracilis*. Thus, these plant traits did not react the same way to experimental warming. As plants react with plasticity to live in a warmer world, plant communities may also change (Loik & Harte, 1996). My results support this idea because of the variations that were attained from the results. *Erigeron flagellaris* and *A. congesta* seem to be adapting just enough to get by, whereas *P. gracilis* is adapting with what could be more success. Some plants will adapt more quickly than other and may out-compete other species.

However, we do know that something is changing with the mild temperature difference; as there were some considerable differences in height, seed set, and chlorophyll content of *P. gracilis*. *Potentilla gracilis* seems to be investing more in plant height (growth) and seed set (reproduction) in warmed plots, and not in pollinator attraction (flower with and flowers per plant). This is consistent with its ability to self-pollination. Chlorophyll content was higher in warmed treatments, consistent with the idea that rising temperatures and CO₂ enrichment will make a greener world. Yet there was no

difference of photosynthetic capacity between treatments. While increasing temperature and CO₂ are thought to extensively affect photosynthesis and respiration more so than other physiological traits (Hedhly et al., 2008), photosynthesis in *P. gracilis* might be limited by water-use efficiency, CO₂ concentration in the air, or another factor that we did not quantify in this study. Prasad (2003) performed a study on *Arachis hypogaea* L. (peanut) focusing on leaf photosynthesis and reproductive traits at ambient and elevated CO₂ levels at optimal temperatures. The research found that doubling CO₂ increased leaf photosynthesis as well as seed yields (Prasad et. al., 2003). However, more data on other species may be advantageous to support this hypothesis.

Though beneficial, the warming frames may have had unintended effects. The frames were built to warm the air beneath it, but they may be manipulating soil temperature as well. Changing the soil temperature could be affecting the soil microbe communities living with roots and nutrient availability. Another possible unintended effect could include the plexi-glass slated on top. Once in a while there is condensation that appears along the top, and this may aid in trapping moisture and provide more water availability to the warmed plots. There is also a possibility that the frames may provide some shade to the plants below it; though unlikely an analysis of the PAR taken during the time photosynthetic capacity would be beneficial to check for any unwanted variables.

A future study could include looking at abundant species in the area that have larger leaves, in order to test the relationship between chlorophyll content and photosynthetic capacity in multiple species to compare how the different plants are reacting to the experimental warming. It would be interesting to add a respiration study into future research. If the temperature is raised under the warming frames by up to 2.0°C at night, it would be interesting to see if the flow of CO₂ (respiration) was affected any different by a larger temperature change. Different traits could also be added: an original pollen count before pollinators arrive would be an interesting addition to this research. Chemical composition and metabolism of pollen has been shown to be affected by high temperatures, and though dramatic effects are not expected there may be a reduced available amount of pollen limiting the number of sired seeds (Hedhly et al., 2008). A closer look on seed set, as well as maybe the addition of looking at the success of the seeds from warmed plots versus controls may be a beneficial addition to the research. Another good addition could be a coupled CO₂-warming factorial experiment. One could look at the effects of the variables of warming and CO₂ on plants on their own, the results of warming and CO₂ together, and comparing the results to a control.

Plants are important attributes to ecosystems and the way they respond to climate change can lead to biotic variables that run those ecosystems. They affect pollinators, food webs, and disturbance

regimes (Wolkovich et al., 2012). Now that there are preliminary data on traits coupled with reproduction it can be combined with what we already know about phenology to better understand how areas may change with global warming. We can expect changes in temperature or atmospheric CO₂ to have a direct effect on growth and photosynthesis as seen in my research, as well as past studies (Hughes 2000). As temperature continues to rise, we can expect plant communities to be changing in the near future since reproduction and linked physiological processes are beginning to respond.

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Figure 1: The two sites used in my research; the upper rocky meadow consists of four warming frames and six control plots. *Erigeron flagellaris* and *Arenaria congesta* were found here. The other site, lower wet meadow, was the research site for *Potentilla gracilis*. Six warming frames and six control plot were used in this area.

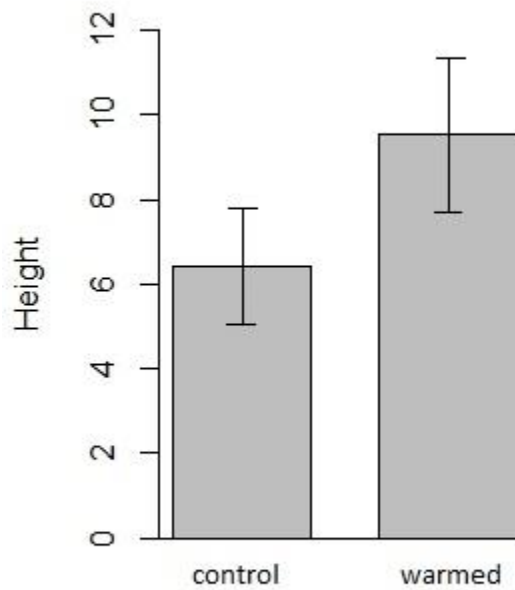


Figure 2: The height of *E. flagellaris* did not differ between warmed treatments and the control ($t=-1.3322$, $p=0.2536$).

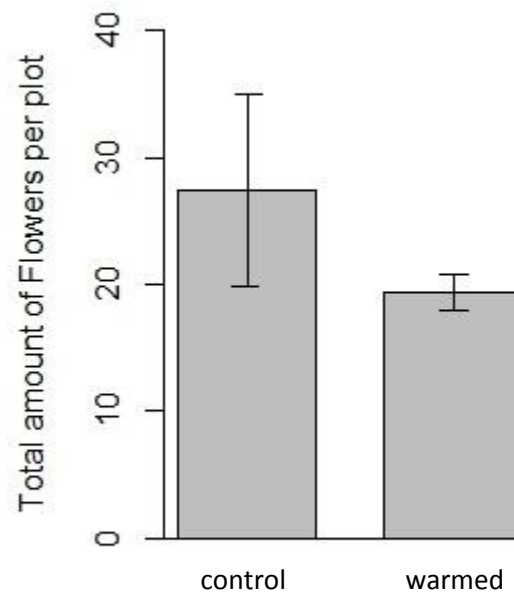


Figure 3: The amount of flowers per plant for *E. flagellaris* did not differ between treatments ($t=1.2857$, $p=0.2679$).

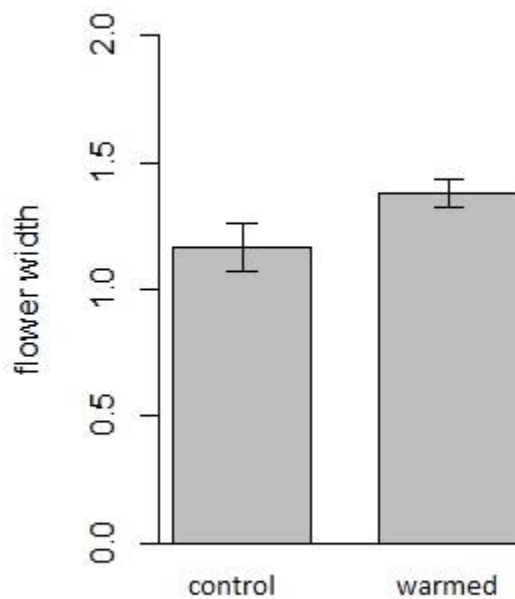


Figure 4: The width of the flowers for *E. flagellaris* did not differ between warmed treatments and controls ($t=-1.4652$, $p=0.2167$).

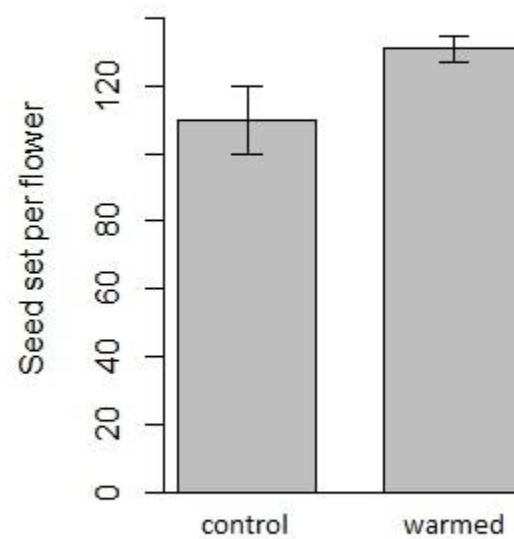


Figure 5: The seeds set of *E. flagellaris* did not differ between treatments ($t=-1.4123$, $p=0.2307$).

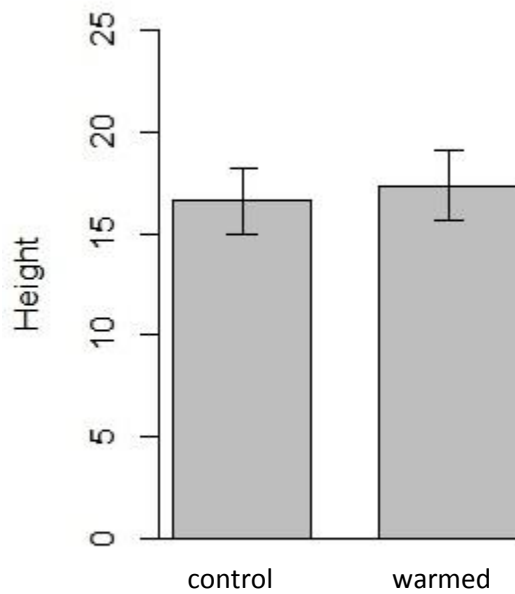


Figure 6: In *Arenaria congesta*, the plants of different treatments did not differ in height ($t=0.2688$, $p=0.7971$).

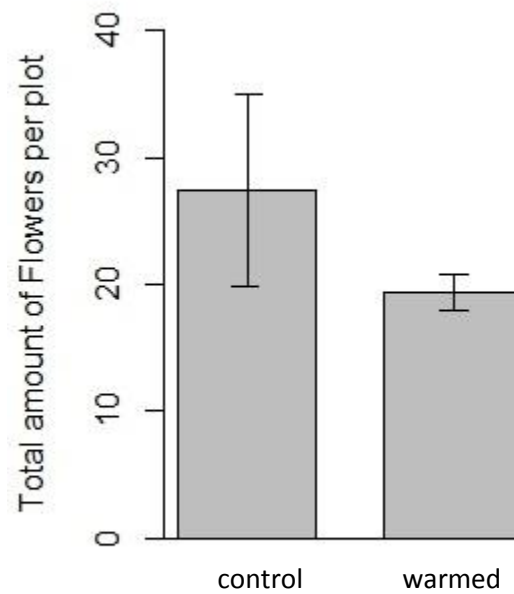


Figure 7: The number of flowers in *A. congesta* does not differ between treatments ($p=0.4857$, Wilcoxon rank sum test).

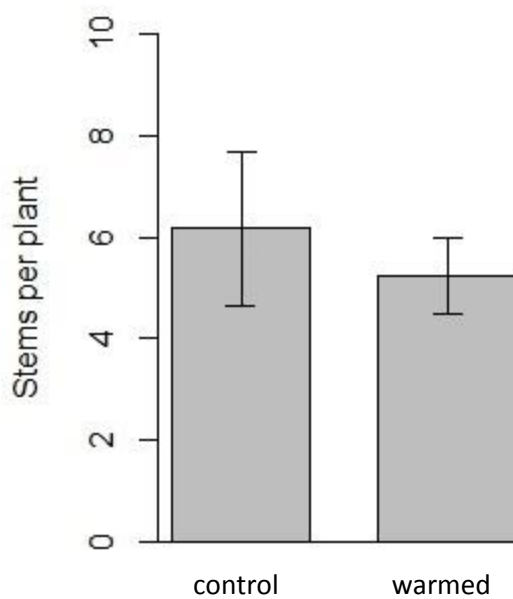


Figure 8: The number of stems in *A. congesta* does not differ in between the warmed treatment and the control ($t=0.5504$, $p=0.6019$).

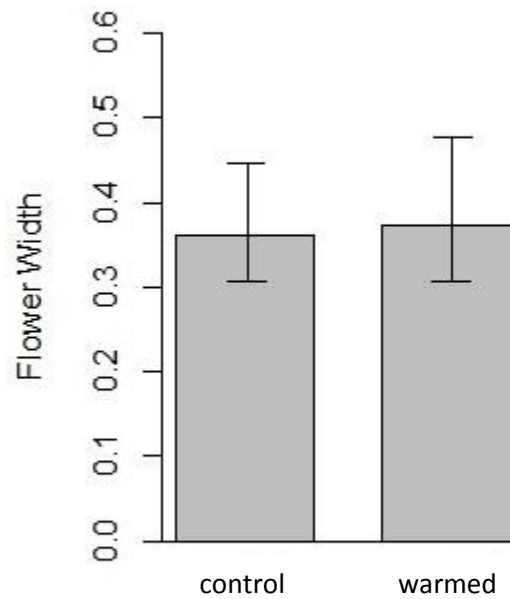


Figure 9: The width of the flowers between treatments were nearly identical in *A. congesta* ($t=-0.1328$, $p=0.8987$).

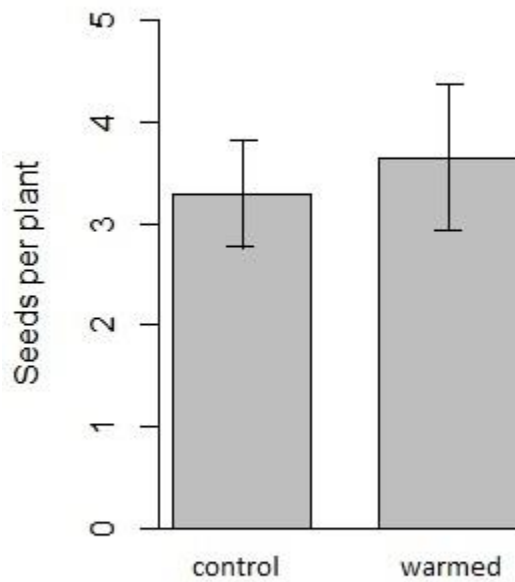


Figure 10: *Arenaria congesta* seed set did not differ between seed set ($p=0.8857$, Wilcoxon rank sum test).

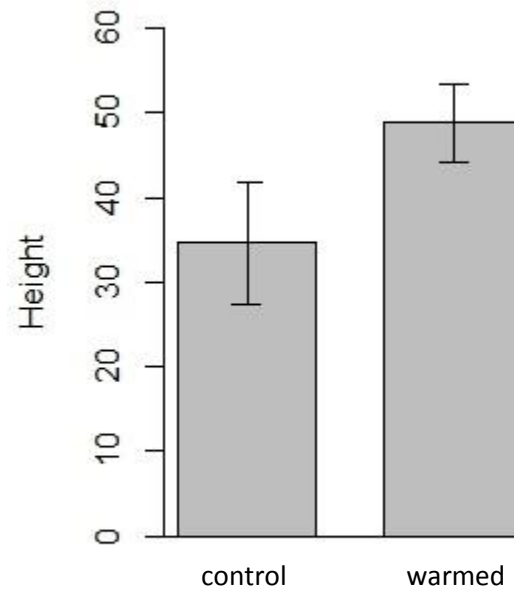


Figure 11: Plants in the warmed treatment were taller than ones in control treatments ($t=-0.1328$, $p=0.02935$).

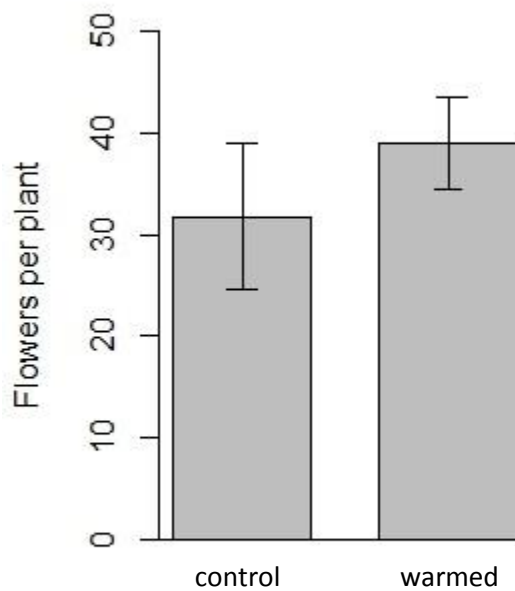


Figure 12: The number of *P. gracilis* flowers per plant did not differ between treatments ($p=0.3095$, Wilcoxon rank sum test).

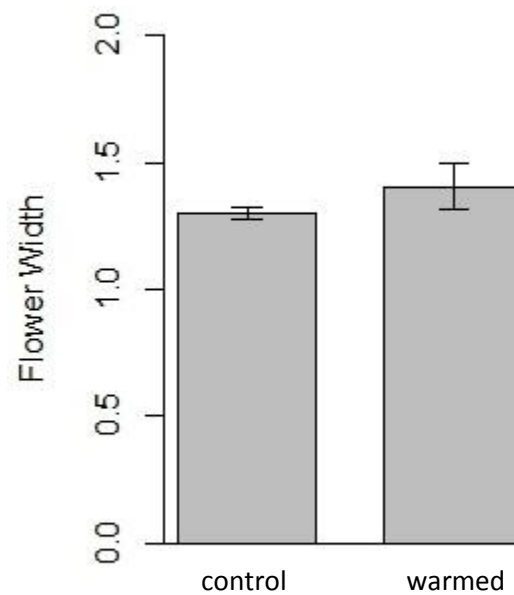


Figure 13: The width of *P. gracilis* flowers did not differ between treatments ($p=0.4848$, Wilcoxon rank sum test).

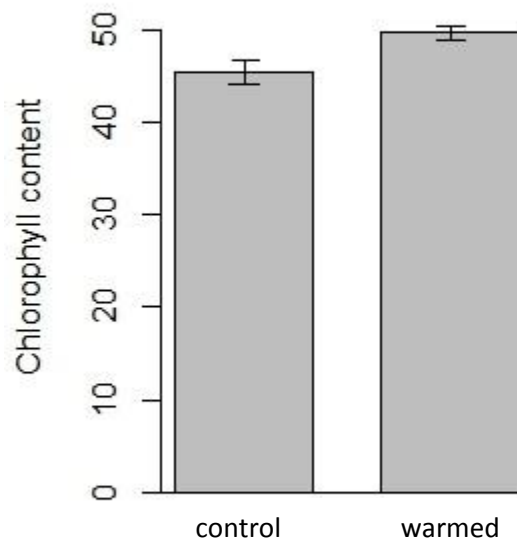


Figure 14: Chlorophyll content in *P. gracilis* leaflets of warmed treatments were higher than in controlled treatments ($p=0.0043$, Wilcoxon rank sum test).

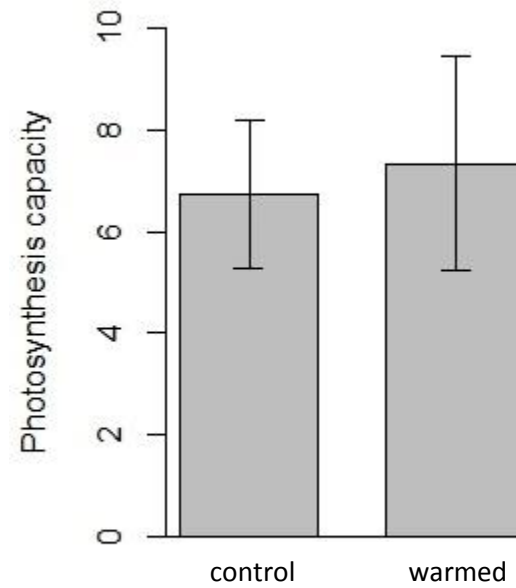


Figure 15: In *P. gracilis*, the photosynthetic rates between treatments did not differ ($t=-0.4052$, $p=0.6879$).