

**The effects of climate change on plant traits and fruiting
phenology of *Delphinium nuttallianum*.**

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Introduction

Ecologists agree that both plant genetics and environmental factors contribute to plant phenological patterns. Typically, plant phenological events, including date of flowering and fruiting, occur approximately the same time each growing season as dictated by the plant's genetics, which is likely related to adaptation to environmental variation. However, abiotic cues for plant communities may be changing because increasing greenhouse gases such as carbon dioxide are predicted to warm the earth by several degrees Celsius in the next few decades. Many models of climate change also predict variable changes in precipitation (Price and Waser, 1998). This increase in earth temperature and change in precipitation may cause a phenological shift in plant lifecycles, for which there is already evidence (e.g. Fitter et al. 2002). Increasing temperatures may also affect plant traits that are important for fitness, such as flower production. Subalpine communities may demonstrate effects of climate change with an advance in the timing of snowmelt, onset of plant growth, sexual reproduction, increase in nectar production, and changes in fruit set (Price and Waser, 1998). Long-term experimental studies simulating climate change effects on plant communities in subalpine high-altitude environments have been conducted for years in the Colorado Rocky Mountains; however, many of these studies suggest that the effects of climate change on plant ecology will be species-specific (Saavedra et al., 2003).

Therefore, in this study I chose to focus on the effects of experimental warming and watering on plant traits and phenology of *Delphinium nuttallianum* (dwarf larkspur). The

wildflower *D. nuttallianum*, a member of the Ranunculaceae family, has been the subject of previous experimental warming and supplemental watering studies (Price and Waser, 1998; Saavedra et al. 2003; Zimmerman, 1983). Saavedra et al. (2003) found that with increased temperatures, the abundance of *D. nuttallianum* plants decreased as did the number of flowers per plant. However, these experimental warming treatments increased temperatures at study sites with electrical heaters suspended 2 meters above the ground, which also increased soil temperature and decreased soil moisture. The experimental warming treatments used in this study did not change either aspect of soil and represents a less extreme model of climate change. Additionally, I examined the effect of supplemental watering and warming on *D. nuttallianum* nectar production and plant traits. Zimmerman (1983) studied the effects of supplemental watering on nectar production and found that an increase in watering increases the amount of nectar produced by flowers. Zimmerman (1983) provided only one level of water treatments to plants, but in my study I provided plots with two levels of water supplementation. I hypothesized that plants have a limit to increased nectar production in response to watering. It is my goal to report the effects of experimental warming frames and watering treatments on the number of flowers, fruit production, fruiting phenology, nectar volume, plant height, number of aborted flowers, total number of flowers, and survival of *D. nuttallianum* to understand how species may alter important traits related to fitness in response to climate change. I hypothesized that warming frames and supplemental watering will cause a phenological shift in *D. Nuttallianum* and alter many of the plant traits.

Methods

Study Site

Observations and measurements of *D. Nuttallianum* were made in plots established by D.W. Inouye in 1973 at Rocky Mountain Biological Laboratory (RMBL) in Gothic, CO (38°57.5'N, 106°59.3'W, 2900 m a.s.l.), which is located in the East River valley of the West Elk Mountains. The plots are located in a rocky, dry meadow of natural vegetation dominated by perennial angiosperms.

Study Species

Delphinium nuttallianum is an herbaceous perennial flowering soon after snowmelt in dry montane meadows. Flowers bloom for about six days on a vertical raceme and mature in sequence from the bottom to the top of the plant (Zimmerman, 1983). This variance in flower maturity also causes a nectar gradient in the plant, with flowers on the bottom producing more nectar. Nectaries are located in two nectar spurs, which protrude from the back of the dark blue flower (Zimmerman, 1983). There is no resorption by nectaries and each flower produces nectar at a constant rate. Each plant is vegetatively active for only 3-5 weeks and is a major source of food for broad tailed hummingbirds and bumblebee queens, which serve as the plant's major pollinators (Inouye and Miller-Rushing, 2009). While timing of snowmelt is an important factor in determining the first date of flowering in *D. nuttallianum*, temperature also explains a significant amount of variation in

its flowering phenology (McKinney & Inouye, unpublished), and will also likely be important for fruiting phenology.

Experimental Design

To simulate the effect of climate change on *D. nuttallianum*, five 1m by 1m plots of meadow containing *D. nuttallianum* plants were covered with plexiglass warming frames. These warming frames mimic climate change by increasing mean temperatures by 2 °C throughout the night and by approximately 0.5 °C during the day. Plots without plexiglass warming frames received two levels of supplementary water treatments – three plots received the amount of water recorded in the wettest July and three plots doubled that amount, in addition to natural rainfall. As a control, five meadow plots containing *D. nuttallianum* were not covered by plexiglass warming frames and were not watered. This design was already set-up as part of a long-term study of wildflower phenology, and the warming and watering treatments were established in early summer 2010. Over the course of the study, I observed changes in *D. nuttallianum* regarding the following dependent variables: height at flowering, total number of flowers per plant, fruiting phenology, number of bud abortions, and plant survival (total stem and leaf senescence prior to fruiting). These variables were selected to assess several stages of the plant life cycle that may be affected by climate change.

Nectar was collected from six separate experimental plots because flowers were destroyed during collection and were unable to progress fully through their reproductive stages. Five plants were underneath warming frames; six plants received watering

treatment one, six plants received watering treatment two, and four plants served as a control.

Phenological Measurements

I tagged and monitored 124 *D. nuttallianum* plants in the rocky meadow plots: 25 plants in the warming plots, 21 plants in the single watering treatment plots, 9 plants in the double watering treatment plots, and 69 plants in control plots. Variation in plant number reflects natural abundance in the plots. These plants were observed two times per week from June 29th -July 29th, 2011 as each individual proceeded through six phenological stages. These stages included: unopened buds (stage 1), flowers (stage 2), old flowers (stage 3), initiated fruit (stage 4), expanding fruit (stage 5) and dehiscent fruit (stage 6). The individuals were given a “phenological score” by averaging the stages present on the plant each day of observation. Observations ended when all fruits on a plant had dehiscent. Many plants senesced before fruiting (did not “survive”) or were eaten by herbivores over the course of the experiment, and these plants did not continue to be observed and were recorded as “dead” or “eaten.” In addition, I used an T-Test to compare the “average timing of reproduction” which is defined as the Julian date at which plants reached a phenological score at or near 3.5.

Plant Trait Measurements

Nectar measurements were taken from flower nectaries three times at weekly intervals from flowers in designated nectar experimental plots. These nectaries replenish

nectar lost to pollinators every 24 hours, so individuals were bagged 24 hours in advance before nectar was collected. At least five flowers from each nectar plot manipulation were chosen every week. The amount of nectar found in the flowers 24 hours later was measured by slitting the two nectar spurs and collecting the nectar in a 5 μ L microcapillary tube (Zimmerman, 1983). Then, a ruler was used to measure the amount of nectar in the tube in millimeters and the height of the plants. Volume was then calculated (Kearns and Inouye, 1993).

The height of *D. nuttallianum* in the observational phenology plots were measured in centimeters using a ruler at the third week of the study when plants had reached maximum growth. Flower number, number of flower abortions, and survival were recorded by sight the third week of the study. Height, flower number and number of abortions were also counted on plants that had senesced.

Analysis

An ANOVA test was used to compare the means of each treatment for plant traits that are described by a continuous distribution: number of flowers per plant, aborted buds per plant, and height. The two watering treatments were combined to increase sample size and statistical power; there were 21 plants given the first treatment and 9 plants given the second watering treatment. Preliminary analysis revealed that there were no significant trait differences between watering treatments. Thus, trait analysis comparisons are between only three treatments (warming, combined watering, and control). First, each response variable was averaged by plot to avoid pseudoreplication. Then, an ANOVA was

used to compare the means of each treatment. For survival, I took a random sample of 25 out of the 68 control plots to make the number of plants per treatment more even. Survival is a binomial response variable (0 or 1), so I used a X^2 test to compare the probability of survival in each treatment compared to the control.

To analyze the phenological scores I used simple linear regression. Phenological scores were first averaged over all plants in each plot and census date. Time (Julian Day) was the explanatory variable and phenological score was the response variable for regression analysis. I used an ANCOVA to test for evidence of different slopes among treatments. Phenological score was the response variable, and treatment and Julian Day and their interaction were predictor variables. The interaction between treatment and Julian Day tested for an effect of Treatment on the relationship between Julian Day and phenological score. Then I performed a separate regression for each treatment and compared the slopes to see if they were different.

To analyze nectar production, an ANOVA was used to compare the means of each treatment. Then, an ANCOVA was used to test if treatment co-varies with plant height. In this analysis, the two watering treatments were also combined. All of the above analyses were performed in JMP (SAS Institute, North Carolina).

Results

Effect of experimental warming and supplemental watering on plant height, total flowers, aborted buds, and plant survival.

There was no significant difference in plant height, total number of flowers, or number of aborted buds among the three treatments (Figure 1). The Chi Squared test, which was used to compare survival among treatments, indicated increased survival of *D. nuttallianum* in the first watering treatment versus the control ($\chi^2 = 8.52$; $p = 0.0035$). Additionally, there is a significant difference in survival between the second watering treatment and the control ($\chi^2 = 15.791$; $p = .0001$). There was no difference in survival between the warming and control treatments ($\chi^2 = 0.35$; $p = .56$).

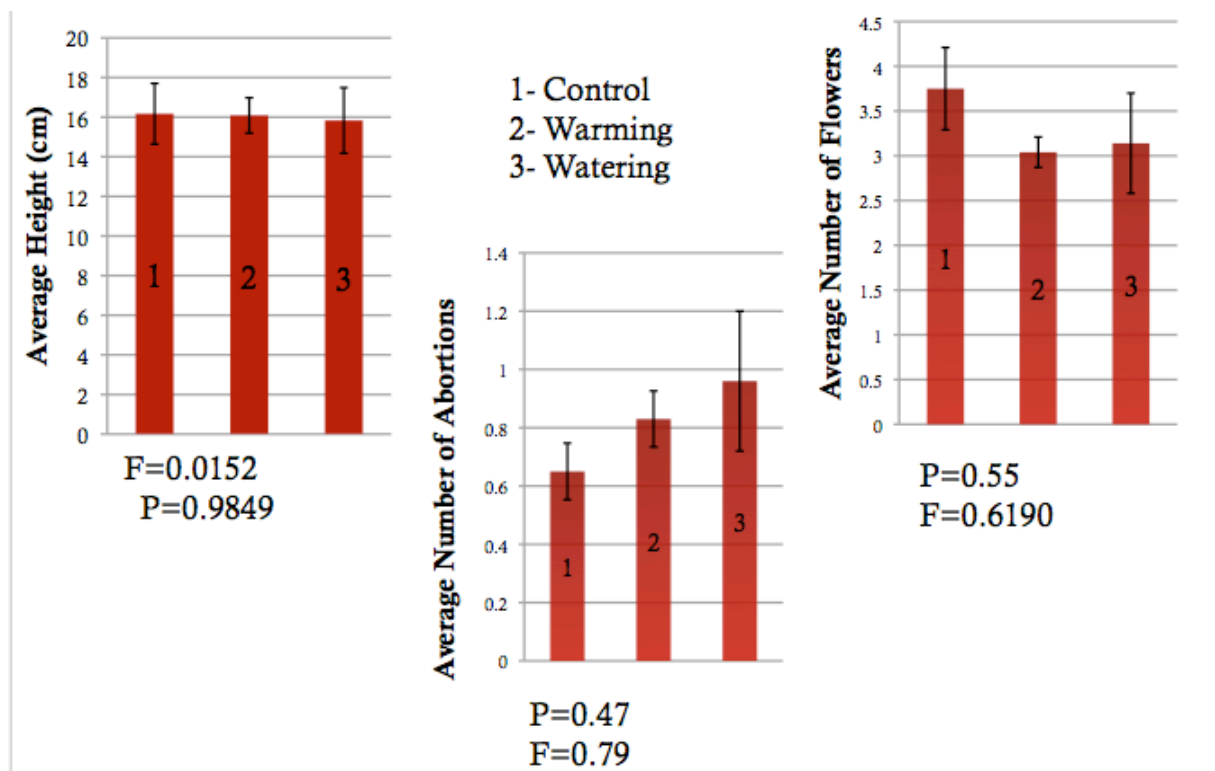


Figure 1 – Graphs demonstrating the results of the ANOVA test comparing the means of the treatment for the plant traits of average height, average number of abortions, and average number of flowers.

Effect of experimental warming and supplemental watering on flowering and fruiting phenology.

As expected, the relationship between phenology score and Julian day were highly significant (Table 1). The ANCOVA indicated that the effects of treatment on the relationship between phenology score and date were marginally significant. Therefore, I compared the slope of each treatment shown in table 1 with the control. The slopes were not significantly different between control versus warming ($t=0.62$; $p>0.05$), control versus watering treatment one ($t=1.15$; $p>0.05$), or control versus watering treatment two ($t=-1.59$; $p>0.05$). However, if a regression is made with the average phenological score of the plants that survived the duration of the study, then it can be seen that from days 199 to 210, warming plots had a higher average phenological score than watering plots or control plots (Fig. 2). Figure 2 also suggests that watering treatment II has a delayed flowering and fruiting phenology compared to the control. The comparison of average timing of reproduction showed that watering II had significantly delayed reproduction compared to control ($t=2.54$; $p=0.39$).

Table 1. Simple linear regression of phenological score (using average score of each plot on each census day) for each treatment, with Julian day as the predictor variable.

Treatment	Slope	F Ratio	R ²	P Value
Control	0.13± 0.0006	441	0.91	$p<.0001$
Watering I	0.12 ±.0063	370.1	0.94	$p<.0001$
Watering II	0.144 ±.0064	5.13	0.95	$p<.0001$
Warming	0.125±.0054	538.6	0.94	$p<.0001$

Table 2. ANCOVA of the effects of treatment on the relationship between phenology score and Julian Day.

	P value	F Ratio
Treatments	.0001	15.951
Julian Day	.0001	1710.815
Treatment*Julian Day	P=0.086	2.24

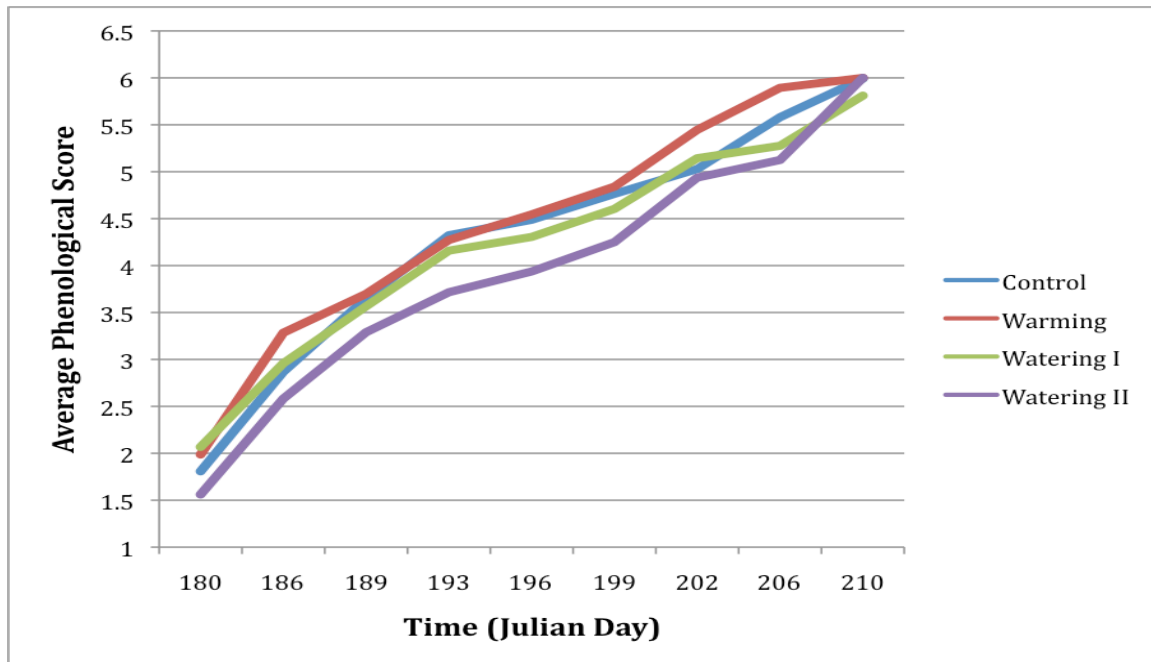


Figure 2 – A linear regression graphing all plants in four treatments that survived the duration of the study. From days 199 to 210 the *D. Nuttallianum* plants underneath the warming frames had higher average phenological scores than the control and watered plots.

Effect of experimental warming and supplemental watering on nectar production.

The ANCOVA test indicated that there was no significant difference in nectar production across the three treatments, and no effect of height on nectar production. (F ratio=0.0790, p= 0.9711).

Discussion

Global warming is an immediate concern for the flora of high-elevation subalpine ecosystems. The consequences of increased temperature may include a shift in plant phenology, decreased floral abundance, and increased fruit set (Price and Waser, 1998). However, in order to gauge the effects of climate change on plant communities, species-specific studies must be performed. In this study, I investigated the effects of climate change on the early-flowering plant *Delphinium nuttallianum* by increasing temperatures with plexiglass warming frames and administering two levels of supplementary watering.

Plant Survival

The results of this experiment indicate that if climactic changes produce an increased amount of yearly rainfall, then *Delphinium nuttallianum* has an increased chance of surviving the duration of its growing season. If more plants remain vegetatively active throughout the season, then the amount of food available to pollinators increases. The amount of fruits should also increase in this scenario, which may have positive effects on population maintenance in *D. nuttallianum*. Additionally, there is some evidence that increased rainfall may have overall positive effects on *D. nuttallianum*.

Nectar Production

As the primary reward for pollinators, nectar distribution affects pollinator movement in plant populations. Therefore, these movements affect pollen dispersal and mating patterns in plant populations (Kearns and Inouye, 1994). The amount of nectar production was relatively similar across all three treatments. This diverges from many nectar volume studies that indicate that increased rain or supplemental watering has a

positive effect on nectar volume in *D. nuttallianum* plants (Zimmerman, 1983). My results may have been confounded by many factors, including “lucky hits,” or flowers that contain a disproportionately larger volume of nectar than other flowers in the same climactic conditions (Kearns and Inouye, 1994). Additionally, the summer of 2011 was an especially wet summer, so *D. nuttallianum* nectar production may not have been limited by rainfall. Therefore, the supplementary watering may not have increased nectar production because the plants already received enough natural rainfall.

Plant Traits

Many experimental warming studies find evidence for the effects of climate change on plant traits such as number of flower abortions and total flower production in *D. nuttallianum*. My study did not find evidence for such changes, and this may perhaps be explained by *Delphinium*’s ability to pre-form buds one or two years in advance (Saavedra et al. 2003). Because the treatments were established in 2010, the effects of the warming frames and watering treatments on the number of flowers may become evident during the next growing season, if plants mostly pre-form buds two years in advance. However, the direction of the effect of warming on flower number is consistent with previous work, exhibiting fewer flowers per plant (not significant) in warming compared to control plots. Additionally, plant height may also be predetermined by abiotic factors such as past nutrient availability, photoperiod, or the severity of winter temperatures. In their experimental warming treatment, Saavedra et. al (2003) reported a decrease in flower abundance in warming plots over the course of a four years. However, Saavedra used warming lights to increase temperature, which consequently decreased soil moisture. The

warming frames in this study only increased air temperature. If the soil does not dry out, the warming frames may not have an influence on plant traits, even over the course of many summers. This difference in results between passive and active warming is important to consider when interpreting the effects of experimental manipulations of temperature on plant traits.

Phenological Changes

The highly significant relationship between phenological score and Julian Day shows that *D. nuttallianum* progressed through the expected phenological phases and also suggests that the bi-weekly censuses were an accurate representation of phenology. The slight trend for plants to exhibit advanced fruiting phenology underneath the warming frame may indicate the consequences global warming may have on *D. nuttallianum* and is consistent with past research (e.g. Price and Waser 1998). A comparison of average timing of reproduction showed that watering II had significantly delayed reproduction compared to controls. In addition, although the slopes were only marginally different from one another, watering II showed a general delay in phenology compared to the control. This result is interesting because most observational work suggests an advancement of flowering phenology with climate change (Fitter and Fitter 2002). Taken together with the effect of warming, *D. nuttallianum* may experience phenological delays early in the season, whereas the effects of warming and watering may cancel one another out later in the season. These results support the creation of a new treatment for future work, warming plus watering, to better understand the effects of climate change on wildflower phenology.

It is important to note that this study has limited ability to accurately mimic climate change because climate variables were manipulated independently. For example, snowmelt is an important phenological cue in montane through alpine habitats (insert citation), but this study does not manipulate snowmelt because the goal was to isolate effects of warming and watering on flowering phenology. Advanced snowmelt is likely a direct effect of increased air temperature in subalpine environments, and snowmelt has a strong effect on the flowering phenology of early blooming wildflowers such as *D. nuttallianum* (Price and Waser, 1998).

Conclusions

The results of this experiment demonstrate the importance of species-specific studies testing the effects of experimental warming on sub-alpine wildflowers. Studies executing a variety of warming methods to simulate differing models of climate change, such as plexiglass warming frames that increase air temperature versus warming lights that decrease soil moisture will be necessary to observe the broad spectrum of plant responses to different types of warming. Although generalities might be made about the phenological and plant trait changes of wildflowers due to climate change, studies on individual species should continue in order to better inform such generalities and to understand variation in species responses to climate change. Additionally, warming experiments and watering treatments should be conducted over a series of summers to properly evaluate some of the *D. nuttallianum* trait responses in light of pre-formation of flower buds. Interestingly, the results of this study suggest that warming and relatively large increases in precipitation may have opposing effects on plant phenology and success.

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