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Research

Reproductive and physiological responses to simulated climate warming for four subalpine species

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Summary

• The carbon costs of reproduction were examined in four subalpine herbaceous plant species for which number and size of flowers respond differently under a long-term infrared warming experiment.

• Instantaneous measurements of gas exchange and an integrative model were used to calculate whole-plant carbon budgets and reproductive effort (RE).

• Of the two species for which flowering was reduced, only one (*Delphinium nuttallianum*) exhibited higher RE under warming. The other species (*Erythronium grandiflorum*) flowers earlier when freezing events under warming treatment could have damaged floral buds. Of the two species for which flowering rates were not reduced, one (*Helianthella quinquenervis*) had higher RE, while RE was unaffected for the other (*Erigeron speciosus*). Each of these different responses was the result of a different combination of changes in organ size and physiological rates in each of the species.

• Results show that the magnitude and direction of responses to warming differ greatly among species. Such results demonstrate the importance of examining multiple species to understand the complex interactions among physiological and reproductive responses to climate change.

Key words: climate change, *Delphinium*, *Erigeron*, *Erythronium*, *Helianthella*, photosynthesis, reproduction, subalpine.

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Introduction

The impact of ongoing climate change on plant reproduction in high-altitude environments has fundamental implications for species persistence, dispersal, and migration. In high-altitude environments, warmer temperatures advance the timing and rate of snowmelt in the spring and lengthen midsummer periods of low soil water availability (Harte *et al.*, 1995; Inouye *et al.*, 2000). Snowmelt serves as a vital cue to initiate flowering for high-altitude species that emerge and bloom early in the growing season (Holway & Ward, 1965; Walker *et al.*, 1995; Price & Waser, 1998; Inouye *et al.*, 2000; Dunne *et al.*, 2003). Furthermore, correlations between snowpack and reproduction over temporal and spatial snowmelt gradients and in manipulative experiments demonstrate that the timing and abundance of flowering for some species are intimately linked with snowpack depth (Inouye & McGuire, 1991; Galen & Stanton, 1993; Walker *et al.*, 1995; Molau, 1997; Mølgaard & Christensen, 1997; Suzuki & Kudo, 1997; Starr *et al.*, 2000; Heegaard, 2002; Inouye *et al.*, 2002; Dunne *et al.*, 2003; Saavedra *et al.*, 2003; Stinson, 2004; Kudo & Hirao, 2006). While these correlative studies reveal the sensitivity of high-altitude plant reproduction to aspects of climate change, no clear pattern emerges; the response of reproduction to variables associated with climate change is highly variable among species. The mechanisms that

underlie the observed changes in reproduction remain largely unexplained.

An ongoing infrared (IR) warming experiment in a subalpine meadow in the Rocky Mountains of Colorado has enabled observations of multiple consequences of increased infrared forcing for individual plant species as well as ecosystem processes. The warming treatment causes earlier snowmelt in the spring, increases soil temperature, lowers soil moisture content during the growing season, and increases nitrogen (N) mineralization (Harte *et al.*, 1995; Shaw & Harte, 2001). Furthermore, heating has affected plant water potential, thermal acclimation, photosynthesis and transpiration, and biomass accumulation of several plant species, but the direction and magnitude of the responses are highly species-specific (Harte & Shaw, 1995; Loik & Harte, 1996, 1997; Loik *et al.*, 2000; Shaw *et al.*, 2000; DeValpine & Harte, 2001; Saavedra *et al.*, 2003; Loik *et al.*, 2004).

Responses of plant reproduction to IR warming are also species-specific. Most plant species at our study site flower earlier in the season in response to the IR treatment (Price & Waser, 1998; Dunne *et al.*, 2003). Plants in this experiment have been previously grouped into early, middle, and late-season cohorts based on the timing of reproduction (Price & Waser, 1998). Flowering for those species in the early season cohort was tightly linked with the timing of snowmelt, while flowering in the later cohorts was more responsive to other, unidentified cues. The number of flowers produced also varies among species. While some produce fewer flowers in the heated relative to the control plots, others produce more (DeValpine & Harte, 2001; Saavedra *et al.*, 2003). For example, *Erythronium grandiflorum* and *Delphinium nuttallianum*, which belong to the early and middle-season cohorts, respectively, reduce flower production in the IR treatment (Price & Waser, 1998; Saavedra *et al.*, 2003). In contrast, the IR treatment has a negligible to positive effect on flowering rates for *Erigeron speciosus* and *Helanthella quinquenervis*, which flower late in the season (DeValpine & Harte, 2001).

The objective of this study was to examine one possible mechanism for the observed species-specific responses of reproduction to elevated temperatures through a better understanding of the carbon (C) costs of reproduction for each of four different species. Since previous work has demonstrated the species-specific physiological responses to the IR treatment, we hypothesized that these varying responses explain the differential effects of IR warming on flowering rates. More specifically, for species that produce fewer flowers under IR warming, we hypothesized that warming would result in an increase in respiration and/or a decrease in photosynthesis, resulting in greater relative C costs of producing flowers. In contrast, we hypothesized that IR warming effects on gas exchange do not limit the reproduction of those species that did not have reduced flowering rates. While IR warming may simultaneously affect other factors, such as organ development, we limited our analysis to testing one possible effect of IR warming. To test our hypothesis, we examined *E. grandiflorum*, *D. nuttallianum*, *E. speciosus*, and *H. quinquenervis*, because their flowering times span the growing season at our site and their flowering rates respond differently to the IR treatment.

The cost of reproduction in plants is typically defined as reproductive effort (RE), or the relative amount of available C that has been allocated to reproductive tissues (Reekie & Bazzaz, 1987; Bazzaz & Ackerly, 1992). Carbon is the standard currency for estimating RE because it is assumed to be an indirect measure of plant energy balance, which includes the energy required to obtain other resources that may also be limiting to reproduction, such as water or nutrients (Bloom *et al.*, 1985; Reekie & Bazzaz, 1987). Previous work on some of our study species has demonstrated that growth and reproduction of each are limited by a different set of resources (DeValpine & Harte, 2001). Therefore, we used C as a currency to standardize the costs of reproduction across all of the study species. The relative cost of reproduction may increase under warming via an increase in the demand for C from reproductive tissues, a decrease in the C available for allocation, or a combination of both. Carbon demand for reproduction can be altered by changes in reproductive organ size and changes in gas exchange rates from reproductive tissues. Additionally, the availability of resources to allocate toward reproduction may be altered by IR warming. Timing of snowmelt influences patterns of soil moisture availability, which can limit photosynthesis and growth during the growing season in alpine and subalpine areas (Jackson & Bliss, 1984; Walker *et al.*, 1995; Loik *et al.*, 2000). Reduced soil moisture may lower plant water status, resulting in reductions in stomatal conductance and foliar photosynthesis for some species (Loik *et al.*, 2000; Shaw *et al.*, 2000). Ultimately, these combined effects of foliar water stress could reduce net assimilation and the pool of available C to allocate to reproduction in competition with other C demands, such as support of root growth. While some other aspects of climate change (i.e. elevated CO_2, increased nitrogen deposition, altered precipitation) may offset some of these increased costs, we examined only the effects of elevated temperature. In this study, we quantified the annual amount of C allocated to reproduction relative to available C using an integrative C budget model. We examined these costs and the effects of warming on instantaneous foliar gas exchange and water potential in four herbaceous plant species for which flowering responds differently under the IR treatment.

Plants in high-latitude and high-altitude environments have shown varying phenological and physiological responses to simulated infrared warming. However, significant year-to-year variation in flower production and growth within species has made discerning overall patterns complicated (Walker *et al.*, 1995; Henry & Molau, 1997). Our study spanned 3 yr, encompassed species that develop at different times of the growing season and have apparently different responses to IR forcing, and employed an integrative process model to investigate one potential mechanism for altered reproduction in relation to

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temperature change. These combined approaches have enabled us to identify emergent patterns of plant responses to elevated temperature.

Materials and Methods

Site description

We conducted field measurements during 2001–03 in a subalpine meadow at the Rocky Mountain Biological Laboratory (RMBL), located *c.* 10 km north of Crested Butte, CO, USA (38°57.5′N, 106°59.3′W, elevation 2920 m above sea level (masl)). The 3 yr of this study were particularly dry years, with a notable drought occurring in 2002. Vegetation at the site is characteristic of subalpine ecosystems in this region, consisting primarily of grass, forb, and shrub species. In 1990, 10 plots of 3 × 10 m were established perpendicular to an east-facing ridge in the meadow. Above five of the plots, three infrared heaters (Kalglo, Inc. Lehigh, PA, USA), 1.6 m in length, were suspended 1.7 m above the soil surface. The remaining five plots, which alternate with the heated plots, are the control plots. The heaters run continuously and emit 22 W m^{-2} of infrared radiation within the heated plots, a flux that generates surface warming comparable to that predicted from a doubling of atmospheric CO_2 along with associated feedback effects of that doubling, such as increased atmospheric vapor content and convective warming (Ramanathan, 1981; Harte *et al.*, 1995; IPCC, 1996). Shadows cast by the heaters cover less than approx. 0.5% of the plot area for less than one-third of the daytime. The heaters give off no UV radiation and the flux in the near-red is equal to 10^{-6} of solar radiation. The long axis of the plots parallels a natural soil moisture gradient (Harte *et al.*, 1995). The warming has a relatively greater impact on soil moisture and soil temperature in the upper, dry zone of each plot than in the lower, wet zone of the plots (Harte *et al.*, 1995). Further details on the site, climate, and treatment effects appear in Harte *et al.* (1995), Harte & Shaw (1995), and Saleska *et al.* (1999).

Species descriptions

We examined four herbaceous perennial species for this study. These species were selected because of their high frequency in the research plots, widespread geographic presence in the flora of subalpine regions of North America, differing phenology, and contrasting responses of flower production in response to the IR treatment (Price & Waser, 1998; DeValpine & Harte, 2001; Dunne *et al.*, 2003; Saavedra *et al.*, 2003).

Erythronium grandiflorum Pürsh. (Liliaceae; yellow glacier-lily) is an herbaceous perennial geophyte that thrives in meadows and aspen forests from mid- to high elevations throughout much of the western United States (Weber & Wittmann, 2001). It is acaulescent, and flowering plants typically have two opposite leaves and one to two flowers per plant (Thomson *et al.*, 1996). Plants may be several years old before they begin flowering and typically bear only one leaf while in the vegetative condition (Thomson *et al.*, 1996; Loewen *et al.*, 2001). Flowers of *E. grandiflorum* frequently emerge while snow remains around the base of the plant (Hamerlynck & Smith, 1994; Thomson *et al.*, 1996), which, at RMBL, may be mid-April to early June This species typically senesces within 2 months of its emergence (Fritz-Sheridan, 1988; Loewen *et al.*, 2001). The effect of IR treatment on flowering of this species has not been previously studied.

Delphinium nuttallianum Pritzel (Helleboraceae; previously *D. nelsonii*, Nuttall's larkspur) is a widespread herb of meadows, open woodlands, and sagebrush steppe throughout the western United States (Weber & Wittmann, 2001). It produces a racemose inflorescence that produces an average of approximately four flowers per plant (Bosch & Waser, 1999). At RMBL, *D. nuttallianum* typically flowers from late May to mid-June. Previous studies indicate that the warming treatment is associated with reduced flowering rates (Saavedra *et al.*, 2003) and advanced timing of reproduction (Price & Waser, 1998).

Erigeron speciosus (Lindley) de Candolle (Asteraceae; showy fleabane) is a common herb of montane meadows and aspen and spruce-fir forests that produces one to three flowers per stem and has several stems from a single perennial root (Weber & Wittmann, 2001). At RMBL, *E. speciosus* typically flowers throughout July, although foliage typically emerges in early June and develops several weeks before the onset of flowering. Plants may grow to approx. 25 cm in height. Previous studies indicate that the warming treatment is associated with increased proportion of stems flowering for this species in some, but not all, years (DeValpine & Harte, 2001) and significantly advanced timing of reproduction (Dunne *et al.*, 2003).

Helianthella quinquenervis (Hooker) Gray (Asteraceae; aspen sunflower) is a perennial plant of aspen forests and meadows that grows as a rosette for several years before elongated floral stems emerge, sometimes reaching more than 1 m in height (Weber & Wittmann, 2001). It grows from a taproot and produces from one to three flowers per flowering stem. At RMBL, leaves appear soon after snowmelt, but flowering does not begin until early July and may continue into August. Previous studies indicate that the warming treatment has no significant effect on rates of reproduction for this species (DeValpine & Harte, 2001), but it significantly advanced the timing of reproduction (Dunne *et al.*, 2003).

Flower number and parameters of plant size

The total number of flowers produced was counted for each of the species in 2 yr. Within a 0.5 m buffer from the plot edge, the total number of individuals of each species and the number of flowers per individual were counted in 2 yr (2001 and 2003 for *E. grandiflorum* and *D. nuttallianum*; 2002 and 2003 for *E. speciosus* and *H. quinquenervis*). The number

of seeds set per flower was also counted for each species except *H. quinquenervis*, which had very few flowering individuals per plot during the years of this study. Removal of seeds from those flowers would have had a substantial impact on the seed rain into the plots, which we wanted to avoid because of the long-term nature of this research.

Surface areas of whole flowers and fruits were determined using allometric relationships, because minimal plant material could be collected from the plots. First, caliper measurements were made of flower and fruit dimensions on three individuals per plot. Then, surface area was predicted from allometric relationships (see Appendix 1) between caliper measurements of the same dimensions and surface area as measured with a portable leaf area meter (LI-800 A, Li-Cor, Inc., Lincoln, NE, USA). Allometric relationships were developed from plant material collected for water potential measurements and from plants destructively harvested outside the plots. All flowers and fruit that were collected from inside the plots were placed in a 70°C drying oven within 5 h of collection. They were left in the oven for 48 h and mass was measured to the nearest 0.01 g immediately following removal from the oven.

Foliar gas exchange measurements

Instantaneous measurements of photosynthesis (A), stomatal conductance to water vapor (g_s), and transpiration (E) were measured approximately every 2 h on leaves of one plant in each of the plots from approx. 07:00 to 18:00 h Mountain Standard Time (MST) using a portable infrared gas analyzer LI-6400 (Li-Cor). Temperature and photosynthetically active radiation (PAR) within the cuvette were maintained at ambient values and [CO_2] was held at 36 Pa. Leaf-to-air vapor pressure deficit (VPD) was calculated from measurements of leaf temperature made during gas exchange measurements along with measurements of air temperature and relative humidity simultaneously recorded at a nearby (< 100 m) meteorological station (Fig. 1). Leaf water potential (Ψ) was measured simultaneously with gas exchange measurements using a Scholander-type pressure chamber (PMS Instruments, Corvallis, OR, USA) at predawn (05:00 h) and again at mid-afternoon (14:00 h) on five leaves from both the control and heated plots. Plants used for measurements were randomly selected from those that were at approximately similar phenological stages within each species. These measurements were made at least twice during each of the distinct phenological stages within a year for each of the species, for a minimum of eight sets of measurements per species over the entire experiment. For both *E. grandiflorum* and *D. nuttallianum*, these stages were the flowering and fruiting stages. For *E. speciosus* and *H. quinquenervis*, the stages were vegetative (when only foliar tissues had developed) and reproductive. We measured photosynthetic capacity by measuring rates of A in relation to varying internal leaf CO_2 concentration (C_i), or A/C_i curves. The A/C_i curves were measured with the LI-6400 on one individual in each plot during each of the developmental stages, with the same frequency and selection criteria as above. During all measurements, PAR was held at approx. 1500 μmol m^{-2} s^{-1} using a red-blue LED. All measurements were made when ambient temperatures were between *c.* 15 and 23°C and VPD was less than *c.* 1.2 kPa. Photosynthesis was measured and C_i was calculated three times at 10 s intervals at each of the following cuvette [CO_2] values: 10, 20, 30, 40, 60, 80, 100, and 150 Pa. The maximum photosynthetic rate under saturating light and optimal ambient conditions (A_{max}) was calculated using nonlinear regression between A and cuvette [CO_2]. The maximum rate of carboxylation (Vc_{max}), and the maximum rate of electron transport (J_{max}) were calculated from the A/C_i curves following Harley *et al.* (1992). Measured parameters were adjusted to a common temperature of 20°C following Bernacchi *et al.* (2001).

Measurements of R_d at night were made on leaves, flowers, and fruit every 2 h from approx. 1.5 h before sunset to approx. 2 h after sunrise twice per year for each species. Shadows cast by nearby mountains increase the period of 'night' light intensities at the plots, as indicated by measured irradiance values at the nearby meteorological station. These measurements

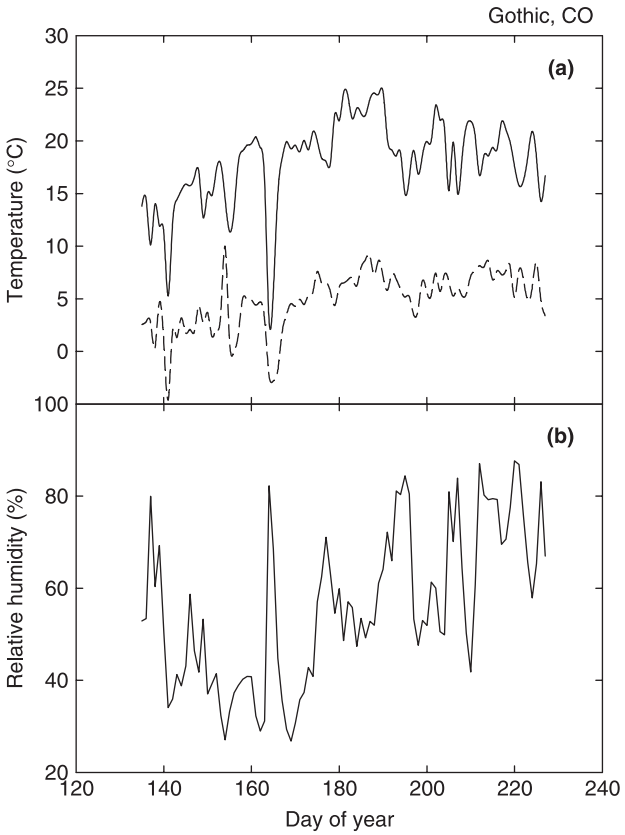


Fig. 1 Daily maximum (solid line) and minimum (dashed line) temperature (a) and average daytime relative humidity (b) measured at Gothic, CO, and used for parameterizing the carbon models in this study. Day 140 = May 20.

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may overestimate respiration because of the gasket effect on CO_2 diffusion while measuring low rates of gas exchange (Pons & Welschen, 2002).

Reproductive effort and carbon budget model

We calculated RE for each of the species, where RE is defined as the total amount of C diverted from vegetative tissues into reproductive tissues (Reekie & Bazzaz, 1987; Bazzaz & Ackerly, 1992). The equation for RE is:

$$RE = (B_r + R_{(flower+fruit)})/(P_{net} + TNC) \quad \text{Eqn 1}$$

(B_r, biomass of all reproductive tissues; $R_{(flower+fruit)}$, total net respiration from all reproductive tissues; P_{net}, annual net photosynthesis of the plant; TNC, total nonstructural carbohydrate stored in and available for translocation from root and shoot tissues (variables and inputs used in the model for RE are listed in Table 1)). All values were expressed in g C.

We estimated B_r on three individuals per plot by making caliper measurements on flowers and fruit, as described above, and predicting mass with allometric relationships (Appendix 1) between these dimensions and biomass developed on plants outside the plots. Biomass values were converted to g C by using the average [C] of flowers and fruit of each species.

To calculate $R_{(flower+fruit)}$, we used measured surface areas, measured night CO_2 flux, and temperature values measured at the meteorological station (Fig. 1). Daytime values of reproductive respiration were calculated as 70% of measured respiration in the dark (see rationale later, under description of leaf respiration). The temperature response of the respiration measurements was calculated using an energy of activitaion Arrhenius-type function (Lloyd & Taylor, 1994). The sum of all daily respiration values was calculated to estimate $R_{(flower+fruit)}$ over the entire period of flower and fruit development.

We used a photosynthesis model previously customized by one of the authors in order to determine P_{net} and RE for each species (McDowell & Turner, 2002). First, average daily values of g_s were calculated for each of the species according to Monteith (1995) by developing a linear regression between diurnal measurements of E made with the LI-6400 with values of VPD calculated from temperature and relative humidity recorded at a nearby meteorological station:

$$1/E = 1/a(VPD) + b \quad \text{Eqn 2}$$

This regression was used to extrapolate the maximum value of g_s (g_{max}), which is equal to a, and the maximum value of E (E_{max}), which is $1/b$. Daily values of g_s for H_2O were calculated as follows:

Table 1 Definitions and sources for parameters used in the model calculating reproductive effort (RE)

Variable	Definition	Units	Source
Gas exchange			
A	Net assimilation	μmol m^{-2} s^{-1}	Calculated (Eqn 4)
E	Transpiration	mmol m^{-2} s^{-1}	Measured
g_s^{daily}	Daily stomatal conductance	μmol m^{-2} s^{-1}	Calculated from VPD and E measurements (Eqn 3)
J_{max}	Maximum rate of electron transport	μmol m^{-2} s^{-1}	Calculated from A/C_i curve measurements
P_{net}	Annual net photosynthesis	g C	Calculated from A and R_d over the growing season
R_d	Dark respiration	μmol m^{-2} s^{-1}	Measured
$R_{(flower+fruit)}$	Respiration of reproductive tissues	g C	Calculated from temperature and measurements of floral and fruit dark respiration
R_L	Respiration in light	μmol m^{-2} s^{-1}	Calculated as 70% R_d
TNC	Total nonstructural carbohydrates	g C	Measured from roots as described in text
V_c	Carboxylation rate of Rubisco	μmol m^{-2} s^{-1}	Calculated from Vc_{max}, J_{max}, g_s, PAR, T, VPD, $[CO_2]$, $[O_2]$, leaf area, and model constants
Vc_{max}	Maximum rate of carboxylation	μmol m^{-2} s^{-1}	Calculated from A/C_i curve measurements
V_o	Oxygenation rate of Rubisco	μmol m^{-2} s^{-1}	Calculated from Vc_{max}, J_{max}, g_s, PAR, T, VPD, $[CO_2]$, $[O_2]$, leaf area, and model constants
Plant size			
B_r	Reproductive biomass	g C	Calculated from allometric equations in Appendix 1
Leaf area		cm^2	Calculated from allometric equations in Appendix 1
Environment			
Altitude		masl	Obtained from survey records and used to calculate oxygen and CO_2 concentrations of air
Day length	Length of day during which there was suitable PAR for net assimilation	s	Calculated from PAR measurements at meteorological station
PAR	Photosynthetically active radiation	μmol m^{-2} s^{-1}	Measured at meteorological station
T	temperature	°C	Measured at meteorological station
VPD	Leaf-to-air vapor pressure deficit	kPa	Measured at meteorological station

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$$g_s^{daily} = g_{max}/[1 + g_{max}(VPD/E_{max})] \quad \text{Eqn 3}$$

using an average daytime VPD (Fig. 1). The values of g_s^{daily} for H_2O were then divided by 1.6 to account for the difference in diffusivity between H_2O and CO_2.

Next, average instantaneous rates of photosynthesis (μmol m^{-2} s^{-1}) were calculated for each day for each of the species based on the model of Farquhar *et al.* (1980) for daytime net assimilation, where:

$$A = V_c - 0.5V_o - R_L \quad \text{Eqn 4}$$

(V_c and V_o, are the carboxylation and oxygenation rates of Rubisco). These parameters were calculated from g_s^{daily}, values of Vc_{max}, and J_{max} calculated from the measured A/C_i curves, measured values of R_d, estimates of whole-plant leaf area, measurements of photosynthetically active radiation and temperature measured at the meteorological station, the concentration of [CO_2] and [O_2] in the atmosphere, and biochemical constants from Woodrow & Berry (1980), which were modified in DePury & Farquhar (1997). We calculated R_L as 70% of measured R_d, which is a proportion based on average reported ratios between R_L and R_d (Atkin *et al.*, 2000, 2006; Tissue *et al.*, 2002). Owing to errors associated with using Q_{10} values to calculate the temperature response of respiration rates over a broad range of temperatures (Amthor, 1989; Ryan *et al.*, 1994; Tjoelker *et al.*, 2001), the temperature response of R_L was calculated using an Arrhenius-type equation for the energy of activation, as described by Lloyd & Taylor (1994). The temperature responses of Vc_{max} and J_{max} were calculated following Bernacchi *et al.* (2001). Since Vc_{max}, J_{max}, and g_s changed with the phenological stages, the model was run separately for each of these stages described earlier. Daily values of net C exchange were calculated as the sum of A over all daylight hours except for approx. 2 h following sunrise and 1.5 h before sunset (which was a timeframe determined based upon measured irradiance values from the meteorological station) minus temperature-corrected R_d. For all species except *E. speciosus*, rates were scaled to estimated whole-plant leaf area because, owing to the architecture of these species, all leaves received full irradiance throughout the day. For *E. speciosus*, we used our previously published light response curves (Loik *et al.*, 2000) and an estimate that 70% of the upper canopy received full sunlight to calculate our daily A values. P_{net} is the sum of all of these daily values.

We measured TNC values of plant tissues to estimate the amount of nonstructural carbohydrates translocated from vegetative to reproductive tissues. To prevent damage to plants in the experimental plots, TNC values were measured on plants collected from outside the experimental plots. We assumed that the TNC values for these plants were representative of those in both the control and heated plots. There is extensive evidence that formation of reproductive tissues and seeds in high-elevation plant species and spring ephemerals such as *Erythronium* is not strongly influenced by the amount of stored TNC in roots (Wyka, 1999; Lapointe, 2001; Meloche & Diggle, 2003; Kelijn *et al.*, 2005; Monson *et al.*, 2006). Furthermore, in a study in which high-altitude plants were transplanted to warmer, lower elevations, the concentration of carbohydrates in the roots increased with warmer temperatures while the mass of the roots decreased, resulting in no net change in the mean amount of stored TNC available for translocation (Scheidel & Bruelheide, 2004). However, these reported results may be confounded by a decline in moisture availability at the low elevation sites. Therefore, our assumption that TNC values of plants collected outside of the plots were representative of both the control and treatment plants is valid. Five plants were collected for each species during each of the developmental stages, coinciding with measurements of leaf gas exchange in the plots. Root, leaf, and floral/fruit tissues were separated, dried, ground to a fine powder with a ball grinder, and analyzed for TNC following Tissue & Wright (1995). The contribution of shoot and root TNC toward reproduction was calculated as the reduction in these values observed during the reproductive period. This estimate is the maximum potential contribution of root and shoot TNC toward reproduction given that some of the root and shoot TNC may be allocated to other functions rather than reproduction.

Data analyses

Repeated-measures ANOVA was used to examine differences between the control and heated plots for flower number, using year as the time variable. Our diurnal data were also analyzed with repeated-measures ANOVA, using hour as the time variable. Analysis of covariance was used to test for differences among model input parameters, using replicates within and between seasons as a covariate. Student's *t*-tests were used to compare leaf nitrogen, plant size measures, and model outputs. Assumptions of homogeneity of variance and normality were tested with plots of the data and residuals. For all analyses, $\alpha = 0.05$ was used.

Results

Effects of warming on flowering

Over the years of this study, we observed that the warming treatment was associated with reduced numbers of flowers for *E. grandiflorum* and *D. nuttallianum*, increased flowers for *Erigeron*, and had no effect on flowering of *H. quinquenervis* (Table 2). *H. quinquenervis* was the only species for which the effect of the IR treatment differed between years, where in the first year there was essentially no effect of warming on flowering, while, in the second year, flowering increased in the warming plots. These results were the same irrespective of whether the total number of flowers per plot or the proportion of stems flowering was used for comparison.

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Table 2 The average percentage change in flower production under the warming treatment relative to the controls

	Change in flower number (%)	Significance of change[a]	Year × treatment
Erythronium grandiflorum	–28.7	$F_{1,20} = 8.71$, $P = 0.01$	$F_{1,20} = 2.66$, $P = 0.08$
Delphinium nuttallianum	–48.9	$F_{1,20} = 7.51$, $P = 0.03$	$F_{1,20} = 0.63$, $P = 0.75$
Erigeron speciosus	+39.9	$F_{1,14} = 4.44$, $P = 0.05$	$F_{1,14} = 0.22$, $P = 0.64$
Helianthella quinquenervis	+2.5	$F_{1,16} = 3.88$, $P = 0.06$	$F_{1,16} = 4.98$, $P = 0.04$

[a]*F*-values are from repeated-measures ANOVA.

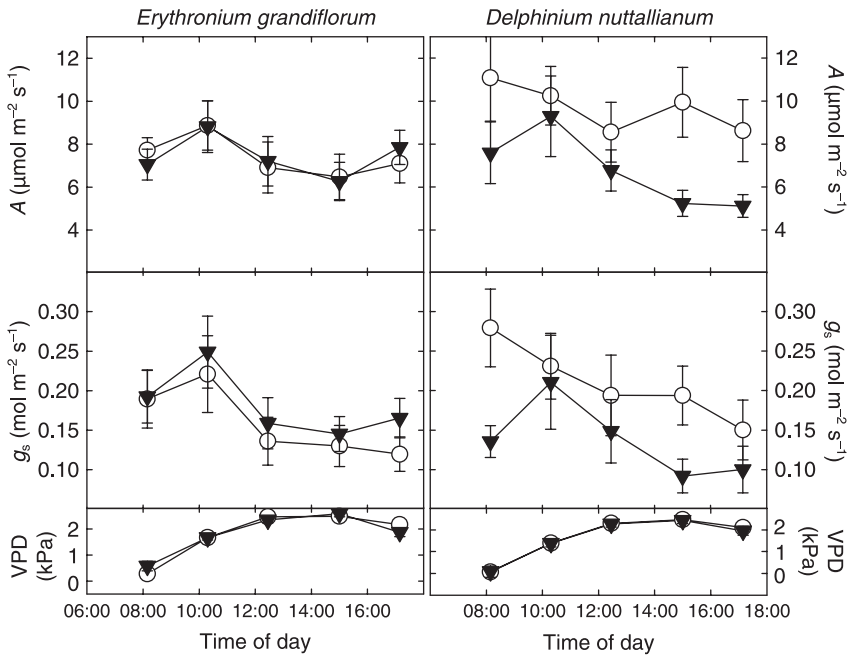


Fig. 2 The average diurnal course of photosynthesis (*A*), stomatal conductance (g_s), and leaf vapor pressure deficit (VPD) for *Erythronium grandiflorum* and *Delphinium nutallianum*. Control, circles; heated, triangles.

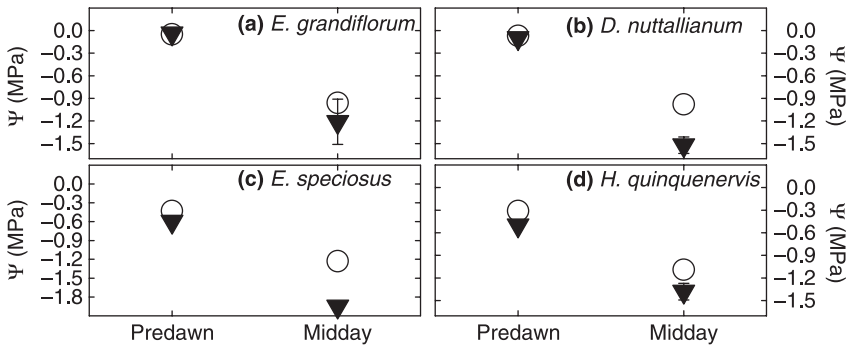


Fig. 3 Predawn and midday water potential (Ψ) for *Erythronium grandiflorum* (a), *Delphinium nutallianum* (b), *Erigeron speciosus* (c) and *Helianthella quinquenervis* (d). Note the different scales for each species. Control, circles; heated, triangles.

Effects of warming on foliar physiology

Diurnal measurements of foliar *A* and g_s reveal different patterns for each of the species. For *E. grandiflorum*, the warming treatment had no significant effect on *A* or g_s (Fig. 2; $F = 0.64$, $P = 0.63$ and $F = 0.31$, $P = 0.58$ for *A* and g_s, respectively). Under both treatments, g_s declined as VPD increased. The warming treatment also did not affect VPD ($F = 0.003$, $P = 0.96$). Similarly, predawn and midday Ψ values were similar between the treatments (Fig. 3; $t = 1.47$, $P = 0.10$ and $t = 0.57$, $P = 0.29$ for predawn and midday, respectively).

Photosynthesis and g_s (Fig. 2; $F = 4.11$, $P = 0.05$ and $F = 11.15$, $P = 0.003$, respectively) were significantly reduced in the heated relative to the control plots for *D. nuttallianum*. Both measures declined as VPD increased during the day. However, VPD remained similar between the treatments

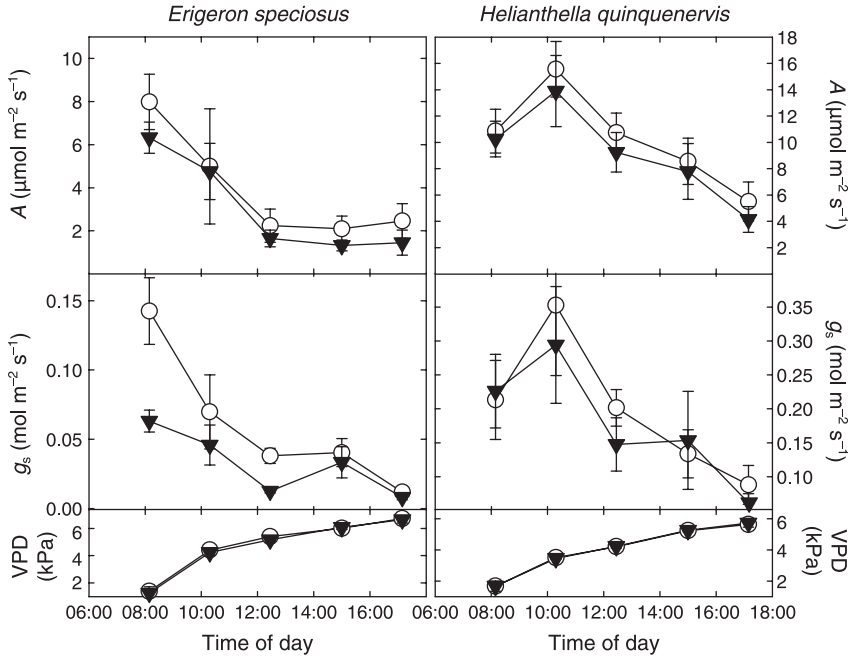


Fig. 4 The average diurnal course of photosynthesis (*A*), stomatal conductance (g_s), and vapor pressure deficit (VPD) for *Erigeron speciosus* and *Helianthella quinquenervis*. Note the different scales for each species. Circles, control; triangles, heated.

($F = 0.5$, $P = 94$). Predawn Ψ was statistically similar between the treatments (Fig. 3; $t = 2.0$, $P = 0.058$), but midday Ψ was significantly lower in the heated plots (Fig. 3; $t = 3.21$, $P = 0.04$).

For *E. speciosus*, *A* was similar between the treatments, but g_s was reduced in the heated relative to the control plots (Fig. 4; $F = 1.89$, $P = 0.20$ and $F = 6.52$, $P = 0.03$ for heated and control plots, respectively). Therefore, for a given value of g_s, *A* was higher in the heated plots relative to the controls. Under both treatments, g_s declined over the course of the day, as VPD increased. VPD was similar between the treatments ($F = 0.04$, $P = 0.94$). Predawn and midday Ψ were both lower in the heated relative to the control plots (Fig. 3; $t = 2.27$, $P = 0.03$ and $t = 2.33$, $P = 0.05$, respectively).

Diurnal measurements of *H. quinquenervis* showed similar *A* and g_s rates in both the control and heated plots (Fig. 4; $F = 1.17$, $P = 0.31$ and $F = 1.11$, $P = 0.33$, respectively). As with the other species, g_s was responsive to increasing VPD under both treatments. VPD was similar between the treatments ($F = 0.008$, $P = 0.96$). Predawn and midday Ψ values were lower in the heated plots relative to the controls (Fig. 3; $t = 2.01$, $P = 0.05$ and $t = 3.07$, $P = 0.001$, respectively).

Measurements of A/C_i curves and the calculations of photosynthetic capacity and respiration from these curves also revealed that each of the species responds differently to the warming treatment. The most pronounced effects were observed for *D. nuttallianum* and *E. speciosus*, both of which showed a reduction in Vc_{max} and an increase in R_d in the heated plots during at least part of their development (Fig. 5; Table 3). Interestingly, the only significant between-year interaction term was that for Vc_{max} of *D. nuttallianum* ($F = 2.5$, $P = 0.04$). The heating treatment appeared to have little effect on the photosynthetic capacity or on R_d of *E. grandiflorum* and *H. quinquenervis* (Fig. 5; Table 3).

Effects of warming on plant size and on costs of reproduction

Plants had lower total leaf area in the warming treatment relative to control plots. Both leaf area and floral area were reduced for most of the species in the heated plots relative to the controls (Table 4). The flower area values shown are the whole-plant floral area, but the area of individual flowers (or inflorescences of *E. speciosus* and *H. quinquenervis*) was also reduced in the warming treatment.

The remaining components for calculating the costs of reproduction included respiration from reproductive tissues and available TNC from root and shoot tissues. Respiration rates of flowers and fruit, when standardized to a common temperature, were similar between the treatments (Table 4). Only *E. grandiflorum* and *E. speciosus* showed significant contributions of root and leaf TNC to reproduction ($t = 3.8$, $P = 0.003$ and $t = 3.1$, $P = 0.007$, respectively). For *E. grandiflorum*, approx. 3.7% of leaf and root TNC were translocated to reproduction. Using estimates of plant biomass for each of the treatments, estimated TNC contributions to reproduction were 0.7×10^{-3} g C per flower + fruit in the control plots and 0.5×10^{-3} g C per flower + fruit in the heated plots. For *E. speciosus* roots approx. 4.0% of leaf and root TNC were translocated to reproduction. Using estimates of plant biomass, this contribution is equivalent to approx. 0.003 g C per flower + fruit in the control plots and approx.

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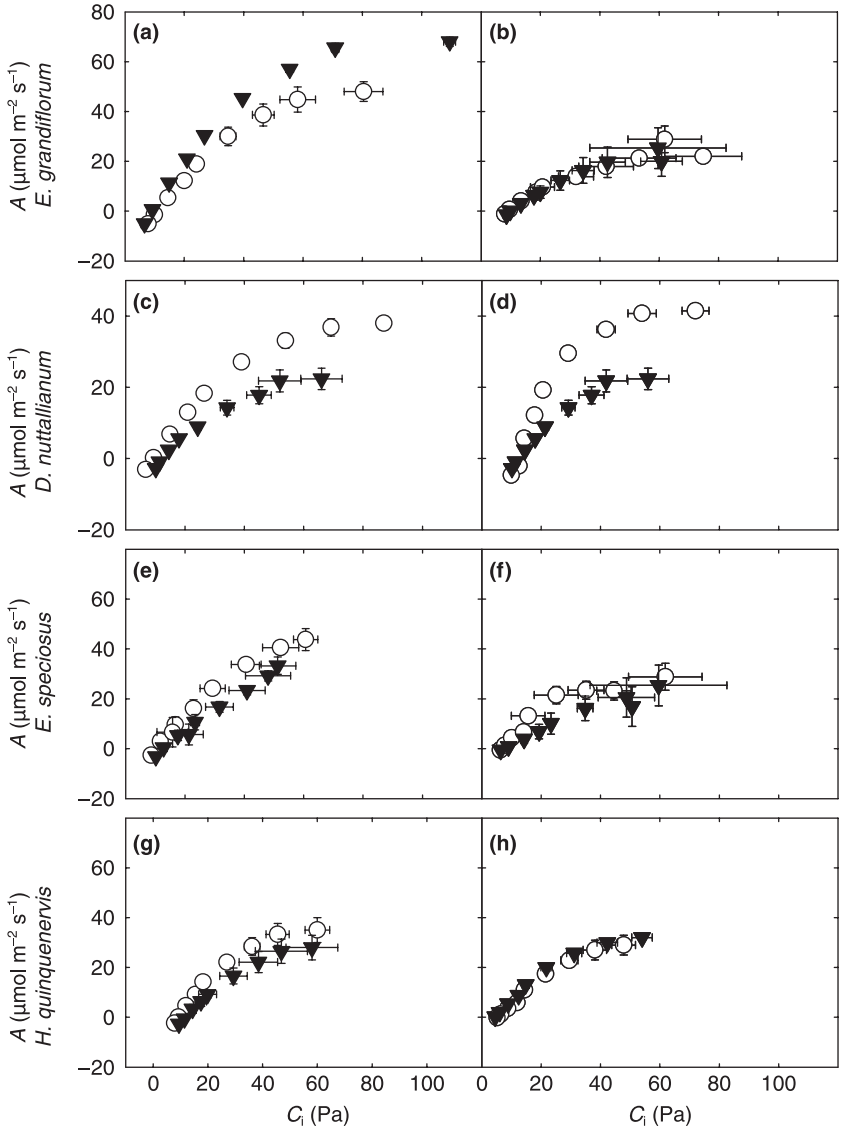


Fig. 5 Average A/C_i curves for *Erythronium* ($n = 13$ for each curve) and *Delphinium* ($n = 20$ for each curve) during the flowering (a, c) and fruiting stages (b, d), and for *Erigeron* ($n = 16$ for each curve) and *Helianthella* ($n = 20$ for each curve) during the vegetative (e, g) and reproductive stages (f, h). Control, circles; heated, triangles. Points are means ± 1 SE.

0.002 g C per flower + fruit in the heated plots. The other two species did not show a significant contribution of TNC to reproduction.

The species-specific effects of the warming treatment on leaf physiology, $R_{(\text{flower+fruit})}$, and plant size produced different patterns of RE for each of the species in response to the warming treatment. RE was not affected by the warming treatment for either *E. grandiflorum* or *E. speciosus* ($t = 1.58$, $P = 0.07$ and $t = 0.82$, $P = 0.21$, respectively; Table 4). However, RE was increased for both *D. nuttallianum* and *H. quinquenervis* ($t = 1.86$, $P = 0.04$ and $t = 1.90$, $P = 0.04$, respectively; Table 4).

Seed production per plant was significantly reduced for *E. speciosus* ($t = 2.7$, $P = 0.02$) and *D. nuttallianum* ($t = 3.2$, $P = 0.02$), but was not affected in *E. grandiflorum* ($t = 0.44$, $P = 0.33$). It is not clear whether changes in seed production were the result of fewer ovules, reduced pollination visits, or increased abortion of fertilized ovules.

Discussion

Our data support the hypothesis that warming affects respiratory and photosynthetic inputs into reproductive effort for two of the four species in this study. The C costs of reproduction were increased by warming for one species for which flower number was reduced (*D. nuttallianum*), but not for the other (*E. grandiflorum*). For *E. speciosus*, which did not exhibit reduced reproduction under warming, the costs of reproduction were not relatively greater in the heated plots relative to the controls. However, RE was greater under IR warming for *H. quinquenervis*, for which flowering rates were not affected by warming. The mechanisms underlying these different responses vary with each species. We consider the diversity of these responses to IR warming to be notable, as they highlight the complexity of linkages between physical forcing, physiology, and reproduction.

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Table 3 Model parameters calculated from A/C_i curves (μmol m^{-2} s^{-1}) standardized to a common temperature (20°C) and leaf nitrogen values (%) during each of the developmental stages

	A_{max} (μmol m^{-2} s^{-1})	Vc_{max} (μmol m^{-2} s^{-1})	J_{max} (μmol m^{-2} s^{-1})	R_d (μmol m^{-2} s^{-1})	Leaf N (%)
Erythronium grandiflorum[a]					
Flowering					
Control	28.4 (2.2)	84.5 (7.7)*	168.0 (20.0)	2.9 (1.3)	5.03 (0.48)
Heated	26.7 (1.1)	134.7 (19.8)*	198.0 (20.0)	3.1 (1.4)	4.58 (0.29)
Fruiting					
Control	8.9 (1.3)	88.6 (14.2)	168.8 (30.2)	5.1 (2.4)	3.25 (0.19)
Heated	6.6 (2.0)	86.3 (5.1)	228.0 (13.0)	6.2 (2.8)	3.05 (0.34)
Delphinium nuttallianum[a]					
Flowering					
Control	21.6 (3.8)*	115.9 (19.9)	245.8 (27.9)	3.3 (0.9)	2.74 (0.28)
Heated	15.8 (2.9)*	88.0 (12.6)	223.9 (23.8)	3.0 (0.6)	2.96 (0.28)
Fruiting					
Control	14.4 (2.7)*	123.8 (9.9)*	223.3 (24.0)	3.0 (0.9)*	3.64 (0.32)
Heated	10.6 (2.7)*	96.7 (15.4)*	219.6 (9.9)	6.5 (1.8)*	3.24 (0.29)
Erigeron speciosus[a]					
Vegetative					
Control	17.1 (1.7)*	72.0 (5.4)	181.0 (43.8)	2.2 (0.5)	4.52 (0.10)
Heated	10.6 (1.6)*	67.9 (5.4)	216.9 (16.0)	3.4 (1.1)	3.79 (0.30)
Reproductive					
Control	14.2 (4.0)	65.1 (9.0)*	178.6 (24.7)	1.3 (0.2)***	3.47 (0.11)*
Heated	6.6 (2.5)	32.7 (3.4)*	122.0 (1.1)	3.0 (0.1)***	2.99 (0.15)*
Helianthella quinquenervis[a]					
Vegetative					
Control	12.7 (1.4)	62.7 (9.3)	180.2 (38.3)	3.3 (0.4)	5.03 (0.26)
Heated	11.1 (1.9)	56.7 (15.5)	156.6 (50.8)	3.4 (0.4)	4.77 (0.21)
Reproductive					
Control	9.2 (0.8)	56.5 (7.4)	179.3 (48.3)	0.5 (0.2)	4.17 (0.12)**
Heated	10.3 (1.6)	51.8 (4.0)	212.9 (32.3)	0.8 (0.1)	3.42 (0.14)**

Values are means (± 1 SE).
*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ based on ANCOVA for all measures except N, which is based on *t*-tests.
[a]For each stage and treatment, $n = 13$ for *E. grandiflorum*, $n = 20$ for *D. nuttallianum*, $n = 16$ for *E. speciosus*, and $n = 20$ for *H. quinquenervis*.

Table 4 Whole-plant leaf and flower area, vegetative biomass, reproductive respiration rates (standardized to a common temperature, 20°C), and the calculated values of reproductive effort (RE)

	Leaf area (cm^2)	Vegetative biomass (g)	Flower area (cm^2)	R_{flower} (μmol m^{-2} s^{-1})	R_{fruit} (μmol m^{-2} s^{-1})	RE g C $(g\ C)^{-1}$
Erythronium grandiflorum[a]						
Control	55.6 (1.3)	0.13 (0.001)	9.4 (0.8)*	1.7 (0.6)	1.0 (0.1)*	0.11 (0.001)
Heated	56.6 (2.6)	0.13 (0.001)	7.0 (1.0)*	1.6 (0.1)	0.7 (0.1)*	0.09 (0.001)
Delphinium nuttallianum[a]						
Control	17.6 (1.8)**	0.37 (0.003)	37.5 (3.9)***	1.0 (0.07)*	2.4 (0.6)	0.82 (0.04)*
Heated	10.6 (2.0)**	0.33 (0.03)	17.8 (2.8)***	1.2 (0.06)*	2.3 (0.7)	0.98 (0.12)*
Erigeron speciosus[a]						
Control	35.6 (1.4)*	0.50 (0.002)***	1.1 (0.2)**	1.1 (0.1)	1.1 (0.2)	0.22 (0.01)
Heated	29.5 (2.4)*	0.43 (0.005)***	0.4 (0.1)**	1.5 (0.2)	1.7 (0.5)	0.19 (0.02)
Helianthella quinquenervis[a]						
Control	223.5 (40.7)*	4.04 (0.5)*	29.2 (2.6)**	4.7 (0.9)	2.2 (0.5)	0.26 (0.05)*
Heated	123.4 (25.5)*	2.67 (0.33)*	16.9 (2.9)**	5.4 (2.2)	2.6 (0.6)	0.37 (0.04)*

Values are mean (± 1 SE).
*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ based on paired *t*-tests.
[a]$n = 15$ for all treatments.

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Flowering rates

The reduction of flower number between the warmed and control plots was apparent for both *E. grandiflorum* and *D. nuttallianum*, while flower production by *E. speciosus* increased in the warmed plots. On average, flowering rates of *H. quinquenervis* appeared unaffected by the warming treatment, but this was likely the result of a significant regional drought in 2002, when plants in both treatments produced very few flowers. In 2003, flower production by *H. quinquenervis* in the heated plots was greater than in the controls. The results for each of these species are comparable to previously observed patterns (DeValpine & Harte, 2001; Saavedra *et al.*, 2003). Although there are no pretreatment data on flower numbers per plot, it appears unlikely that these differences were remnants of initial conditions. The date of snowmelt explains a large fraction of the variance in flower numbers and above-ground growth from plot to plot and from year to year (Harte, 2001; D. W. Inouye & J. Harte, unpublished). One of the most pronounced effects of the warming treatment is earlier snowmelt timing (Harte *et al.*, 1995). Therefore, it is likely that the observed patterns of flower numbers are largely explained by the effect of the warming treatment on the timing of snowmelt. Furthermore, the timing of snowmelt has also proved to be an important variable affecting flowering rates for many species growing in high-latitude and high-altitude settings under both natural and manipulated snowpacks (Inouye & McGuire, 1991; Galen & Stanton, 1993; Mølgaard & Christensen, 1997; Heegaard, 2002; Stinson, 2004; Kudo & Hirao, 2006). However, the relative importance of snowmelt can vary with the time of year at which plants emerge (Price & Waser, 1998; Keller & Körner, 2003; Kudo & Hirao, 2006).

Reproductive effort

The RE for *D. nuttallianum* was significantly increased by IR treatment because of a combination of both reduced foliar photosynthesis and increased $R_{(\text{flower+fruit})}$. In fact, RE, which typically lies in the range 0.10–0.30 for most plant species, was particularly high for this species. Since RE is an estimate of the proportion of available C that is allocated to reproduction, it is apparent that plants in the heated plots simply have no more C available to allocate to the production of additional flowers. However, it is not clear from our results whether the observed changes in photosynthesis were due directly to the IR warming or indirectly to other, simultaneously changing factors such as soil moisture availability. Because this species has the smallest and most shallow roots of those in this study, it would have limited capacity to store or gain access to deeper water sources. In a previous study, abortion of floral buds in *D. nuttallianum* increased under the IR treatment (Saavedra *et al.*, 2003). Plants frequently abort floral buds when under water stress (Stephenson, 1981).

There was no evidence for increased costs of reproduction associated with IR warming for *E. grandiflorum*, a species for which initiation of growth and development is tightly linked with timing of snowmelt (Fritz-Sheridan, 1988; Hamerlynck & Smith, 1994). In fact, one aspect of photosynthetic capacity ($Vc_{\max}$) was enhanced by the warming treatment, perhaps because warming may lead to more optimal temperatures for biochemical activity. This enhanced $Vc_{\max}$, along with smaller flower and fruit size, led to somewhat reduced RE in the heated plots relative to the controls.

One alternative explanation for decreased reproduction for *E. grandiflorum* in the warming plots was increased exposure of plants to freezing temperatures. Because snow melts approx. 2 wk earlier in the heated plots, plants in those plots are exposed to more early spring freezing events than plants in the control plots. Snow cover on the control plots may provide better insulation from low night-time temperatures compared with any extra warmth the heaters may have provided. Although foliar tissues of *E. grandiflorum* recover rapidly following freezing (Germino & Smith, 2001; but see Loik *et al.*, 2004), floral tissues appear quite sensitive to temperature (Thomson *et al.*, 1994; Price & Waser, 1998). Loewen *et al.* (2001) found that populations of *E. grandiflorum* at high-elevation sites in British Columbia produced proportionately fewer flowers than low-elevation populations. Furthermore, although vegetative individuals generally have one leaf, the high-elevation sites had a high occurrence of two-leaf, nonflowering individuals. The authors hypothesized that the two-leaf individuals had aborted floral buds because of the less than favorable temperatures at higher elevations (Loewen *et al.*, 2001). Earlier onset of flowering in response to a climate warming experiment was also associated with a higher frequency of freezing damage for flowers of *Papaver radicatum*, another high-altitude species (Mølgaard & Christensen, 1997).

For *E. speciosus*, RE was not significantly different between the treatments. Although *E. speciosus* had reduced photosynthetic capacity and g_s and increased R_d, thereby decreasing the potential pool of available C, floral heads were substantially smaller in the warming treatment so that the overall relative C costs were not increased. The results of foliar gas exchange are consistent with previous observations for this species (Loik *et al.*, 2000). For *H. quinquenervis*, on the other hand, there was a significant increase in RE because of slight changes in photosynthesis under the warming treatment. However, unlike *D. nuttallianum*, RE for *H. quinquenervis* was in the typical range for most plant species and was low enough that it may not have limited plant growth and survival. The onset of reproduction for both species was advanced by almost 2 wk in the heated plots. Advanced onset of growth and reproduction has been reported for many species exposed to elevated temperatures (Henry & Molau, 1997; Mølgaard & Christensen, 1997; Suzuki & Kudo, 1997; Starr *et al.*, 2000). Interestingly, predawn water potential values measured during flowering in the heated plots were similar to those measured during

flowering in the control plots, which occurred 2 wk later (data not shown). A previous experiment with these species identified that water was a limiting resource to biomass production by both of these species (DeValpine & Harte, 2001). However, since we did not separate the effects of IR warming from those of simultaneously changing soil moisture, we cannot conclude whether advanced flowering in these species enabled them to take advantage of greater soil moisture availability.

The advanced phenological development of *E. speciosus* and *H. quinquenervis* was associated with smaller plant size and reduced leaf nitrogen. Species that develop early in the spring, such as *E. grandiflorum* and *D. nuttallianum*, develop and reproduce rapidly, relying on below-ground stored reserves to initiate growth (Hamerlynck & Smith, 1994). In contrast, both *E. speciosus* and *H. quinquenervis* grow for several weeks before the onset of reproduction, during which time they accumulate both C and nitrogen. Other experiments that have observed advanced phenology in association with experimental warming have also observed that few species appeared to be able to take advantage of the potential for a lengthened growing season in terms of enhanced growth (Henry & Molau, 1997; Mølgaard & Christensen, 1997; Suzuki & Kudo, 1997; Starr *et al.*, 2000). Reduced leaf nitrogen content has also been observed among several (Henry & Molau, 1997; Suzuki & Kudo, 1997), but not all species (Suzuki & Kudo, 1997; Starr *et al.*, 2000) exposed to climate warming experiments.

An additional cost of earlier flowering that is difficult to estimate is the disruption of temporal synchronization between the plant and its pollinators. Unless the phenology of the pollinators for these species is similarly advanced as temperature increases, pollination and seed set may be reduced. Furthermore, research conducted in nearby areas in Colorado has documented that floral buds of *E. speciosus* and *H. quinquenervis* are particularly susceptible to frost damage (D. W. Inouye, unpublished).

While were limited in examining only the effects of IR warming on plant physiology and reproduction, it is clear that IR warming produces complex responses within and among species. Our results highlight the importance of including multiple species in studies of plant responses to climate change. Models of plant community shifts and of ecosystem processes in response to climate change often operate under the assumption that species within a particular habitat will behave similarly. However, under past climate change, we have observed that co-occurring species did not shift ranges as a group (Davis, 1989). Categorizing plants into groups, such as functional types or phenological groups, is an approach gaining increased support in models of vegetation change in response to climate change (Neilson *et al.*, 2005). Our data highlight the importance of studying species-level responses to aspects of climate change in order to understand the range of climate change effects better.

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Appendix 1

Equations for predicting area and mass of flowers, fruit, and leaves are detailed in this section.

Erythronium grandiflorum

Flower area = $N(-0.14 + 0.06L)$ ($R^2 = 0.25$, $P = 0.38$)

(N, the number of petals per flower; L, average length of the petals).

Mass = $(6.34 \times 10^{-18}) + (0.038 \times \text{area})$ ($R^2 = 0.99$, $P < 0.0001$)

Capsule area = $-3.191 + 0.17H + 0.29W$ ($R^2 = 0.94$, $P < 0.001$)

(H, average fruit height; W, average fruit width).

Mass = $(1.11 \times 10^{-17}) + (0.033 \times \text{area})$ ($R^2 = 0.99$, $P < 0.0001$)

Individual leaf area = $8.53 + 0.16 \times \text{length}$ ($R^2 = 0.91$, $P = 0.008$)

Mass = $(8.64 \times 10^{-17}) + (0.02 \times \text{area})$ ($R^2 = 0.99$, $P < 0.0001$)

Delphinium nuttallianum

Flower area = $-3.648 + 0.15D + 0.18W + 0.089H$
($R^2 = 0.50$, $P = 0.048$)

(D, corolla depth; W, corolla width; H, corolla height).

Mass = $(-1.4 \times 10^{-17}) + (0.005 \times \text{area})$ ($R^2 = 0.99$, $P < 0.0001$)

Capsule area = $(2 \times L_1 \times W_1) + (2 \times L_2 \times W_2) + (2 \times L_3 \times W_3)$

(L_i, length of each of the three sections of the capsule; W_i, width of each of the three sections of the capsule).

Mass = $(-4.7 \times 10^{-18}) + (0.005 \times \text{area})$ ($R^2 = 0.99$, $P < 0.0001$)

Individual leaf area = $-3.27 + 0.23L_n + 0.34L_l$
($R^2 = 0.96$, $P = 0.02$)

(L_n, number of lobes on leaf; L_l, average length of lobes on leaf).

Mass = $(7.5 \times 10^{-16}) + (0.063 \times \text{total area})$
($R^2 = 0.99$, $P < 0.0001$)

Erigeron speciosus

Inflorescence area = $(3 \times 10^{-7}) * (D_{avg}^{4.87})$
($R^2 = 0.99$, $P = 0.002$)

(D_{avg}, the average of two perpendicular measurements of inflorescence diameter).

Floral mass = $(6.73 \times 10^{-18}) + (0.03 \times \text{area})$
($R^2 = 0.99$, $P < 0.0001$)

Fruit mass = $(0.85 \times 10^{-17}) + (0.03 \times \text{area})$
($R^2 = 0.99$, $P < 0.0001$)

Individual leaf area = $0.16 \times \text{length} + 8.53$
($R^2 = 0.60$, $P = 0.02$)

Total mass = $(-6.3 \times 10^{-18}) + (0.02 \times \text{total area})$
($R^2 = 0.99$, $P < 0.0001$)

Helianthella quinquenervis

Inflorescence area = $0.730 + 0.6(D_1 \times D_2)$
($R^2 = 0.90$, $P < 0.0001$)

(D_1 and D_2, two perpendicular measurements of inflorescence diameter).

Floral mass = $(7.53 \times 10^{-18}) + (0.03 \times \text{area})$
($R^2 = 0.99$, $P < 0.0001$)

Fruit mass = $(1.15 \times 10^{-17}) + (0.04 \times \text{area})$
($R^2 = 0.99$, $P < 0.0001$)

Individual leaf area = $0.2083 \times \text{length}$
($R^2 = 0.68$, $P = 0.0007$)

Total mass = $(-5.5 \times 10^{-17}) + (0.02 \times \text{total area})$
($R^2 = 0.99$, $P < 0.0001$)

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