

Characterizing nectar content in wet and dry subalpine meadows during an extreme summer drought

RMBL Student Report 2018

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Abstract

Pollinating bees visit flowers in order to forage for nectar and pollen, their major sources of nutrition. The plant communities that a bee interacts with vary across time and space, as different flower species dominate different habitats. One key driver of the spatial variation in plant communities is soil moisture, which creates distinct wet and dry habitats in the subalpine meadows around the Rocky Mountain Biological Laboratory (RMBL). These different flower species provide different nutritional rewards for visiting bees by producing varying amounts of sugar. Relatedly, flower species have differential responses to drought, and the very dry summer of 2018 represented a significant water challenge to flowering plants. We found that over the course of the season, wet and dry meadows contained the same amount of nectar sugar, based on the 13 species we measured. At certain time points, however, dry meadows contained more nectar owing to higher flower abundances. As expected, we also found species-specific responses to precipitation, with 3 of 4 species increasing their nectar volume and decreasing their nectar concentration once regular summer rains began, ending a period of extreme dryness. In 2 cases, *D. barbeyi* and *M. ciliata*, the volume and concentration responses counteracted each other, meaning that flowers produced the same amount of sugar during the drier and wetter periods. These responses may have implications for bee foraging because highly concentrated nectar in small volumes constitutes a poorer food source for bees, and bees are sensitive to sugar and viscosity cues.

Introduction

The mutualism between pollinating bees and the flowers they visit has enormous functional importance: more than 85% of described plant species are animal-pollinated, and bee species constitute many of the most widespread and effective of these pollinators (Kearns *et al.* 1998, Ollerton *et al.* 2011). Visiting bees acquire nectar and pollen, nutritional rewards that are provided by plants in order to attract vehicles for transporting pollen between conspecific flowers, allowing outcrossing to occur.

Floral nectaries comprise a vital bee resource, providing wild bees with most of the carbohydrates they need to grow and forage (Woodard and Jha 2017). Yet these food rewards are not constant between the species of flower visited by one bee species, or between individual flowers (Pyke 2016). At relevant scales a foraging bee likely experiences a range of very different nectar resources as she moves between meadow patches (Nottebrock *et al.* 2017). At least some of this sugar variation should be predictable, because floral community composition varies with abiotic factors like soil moisture, and even within a similar pollinator syndrome, nectar production tends to vary widely amongst plant species (Nicolson and Thornburg 2007).

Plant-pollinator interactions typically exhibit conditional outcomes: the reward being offered by plants varies both in time and space, so the costs and benefits associated with the interaction are dynamic (Bronstein 1994). As a result, individual events of pollination might show large variation in the reward to bees and plants. Bees, like all pollinators, are under selection to maximize the marginal benefit of each floral visit, that is, maximize net energy intake per unit time (Dreisig 1995). Flowers are under selection to maximize the amount of conspecific pollen transfer, in order to maximize

their reproductive potential (Pyke 2016). This is not, as it often assumed, an arrangement in which the interests of each partner perfectly align. Flowers should produce the least nectar possible for the maximum marginal reproductive gain (Pyke 1991). For their part, the most fit bees should expend as little effort as possible to obtain the highest volumes of sugar-rich nectar and proteinaceous pollen.

Based on these predicted incentives, many bees appear skilled at optimizing their flower foraging strategies. They are capable of dynamic responses to variable floral landscapes, preferring flowers that have more high quality nectar and pollen (i.e. more nutritious flowers), and apparently make good "decisions" about which flower species are on average most worth their effort in a given meadow (Somme *et al.* 2015). Subalpine bees may also not generally travel far: Elliott (2009) suggests that they rarely leave a 100m radius, especially in the early and mid-season periods, when floral resources are generally more than sufficient for colony growth. Foraging efficiency is generally highest when the bees are able to stick to abundant, nearby resources (Dreisig 1995).

However, during periods of aberrantly low floral abundance, e.g. due to phenological advancement or drought, bees may sometimes be faced with low quality resources at the neighborhood scale. There is already some evidence for increases in the frequency of such nutrient challenges in subalpine meadows around the Rocky Mountain Biological Laboratory (RMBL), likely due in part to a warming climate and consequently earlier snowmelts. The advancement of early season flowering peaks has produced a previously unobserved dip in flower abundance around mid-season (Aldridge *et al.* 2011). Additionally, the summer months of 2018 were characterized by an extreme regional drought, with noticeable effects on peak flower abundance compared to prior years (Jane Ogilvie, unpub. data). The ability of flower communities to continue providing sugar resources adequate to attract bee pollinators during these periods of abiotic stress may be a key determinant of floral reproductive success during these periods, and likewise of community resilience.

Nectar production is a plastic trait and as such should respond to abiotic stress: higher levels of soil moisture, for instance, are thought to release plants from water limitation and allow production of greater nectar volumes (e.g. Wyatt *et al.* 1992, Carroll *et al.* 2001). Low soil moisture levels, in contrast, could result in lower concentrations of nectar due to depressed photosynthetic rate (Phillips *et al.* 2018). This evidence is more mixed, however, and photosynthetic failure may be more relevant during the most extreme water shortages. Water is also one of the primary drivers of plant distributions, and in the RMBL area, water availability contributes to the existence of subjectively defined "wet" and "dry" characteristic floral communities (Weltzin *et al.* 2003; pers. obs.). In these meadows, floral species identities and abundances are quite different, meaning that floral resource availability may also be consistently different between habitats. Wetter habitats may be less water-limited, and so might produce more, higher quality nectar (Carroll *et al.* 2001). In contrast, at some points of the growing season, dry

meadows contain higher numbers of flowers than wet meadows, meaning that in principle they could harbor higher community-level sugar mass (Jane Ogilvie, unpub. data). These differences in floral resources suggest that the availability of bee-accessible nutrients may differ both spatially and temporally between wet and dry meadows. Similarly, during drought, unique species-specific responses in nectar traits could lead to changes in the nutrient landscape compared to a non-drought year.

We ask two questions: how do community-level floral nectar volume and concentration differ between wet and dry subalpine meadows, and how did extreme drought alter nectar volumes and concentrations? To address the first question we estimated flower abundances and average sugar mass per flower to obtain community-level sugar content over the course of the growing season. To address the second, we analyzed nectar samples obtained for four species both during and after a period of intense water limitation, an unplanned natural experiment made possible by the drought. We hypothesized that flower communities in wet meadows would produce higher volumes of nectar with the same mass of sugar (resulting in lower concentrations), because of release from water limitation. We also expected that during drier time frames, there would be lower volumes of nectar and lower sugar mass produced, due to water stress and the consequent depression of photosynthetic efficiency.

Materials & Methods

Our study focused on the nectar available to *Bombus* species in 6 adjacent pairs of wet and dry meadows in areas North and South of the Rocky Mountain Biological Laboratory. The six sites were called: "Stormy Pass" located 3 miles up-valley from Gothic, "Avery Wash" located 1.5 miles up-valley from Gothic on Trail 401, "Gothic Spires" located 0.75 miles up-valley from Gothic on Trail 401, "Copper Creek" located in the Research Meadow near Gothic, "Frogs Pond" located down-valley of Gothic, and "Rosy Hills" located 1 mile down-valley of Gothic. Each of the six sites are separated by over 1 km, and are consequently unlikely to have overlap in pollinator communities.

Established in 2015 by Jane Ogilvie, the 6 meadows sites have had 100 meters of transects spread through both wet and dry meadows, as well as in aspen forest. Every 5–7 days during April through August, we counted the number of currently open flowers of bee-visited species within 0.5 meters of the transect lines. We used these counts as estimates of the number of open flowers of each species for a given week.

When sampling nectar volume and concentration, we used separate-but-similar meadows nearby these 6 focal sites (*Fig. 1*). We chose not to use the focal meadows for nutritional sampling to avoid impacting ongoing pollinator network observations in those sites. In order to exclude pollinators and other nectar consumers, we covered

flowers with bridal veil bags approximately 24-hrs before sampling nectar in most cases. We did not remove nectar before bagging, and consequently our measurements include both the standing crop at time of bagging plus volume produced in 24-hrs. We used Drummond Microcaps (size 1 μ L, 2 μ L, 5 μ L, or 10 μ L depending on species) to remove nectar from flowers, and converted distance travelled up the tube into volume. For the asters *Helimeris multiflora* and *Erigeron speciosus*, a microcentrifuge was used to spin out the nectar from the flowers for 1 min at 2400rpm and 2000rpm respectively. Nectar concentration (sugar percent, °Bx) was measured with Eclipse low-volume hand refractometers, and converted to grams sugar per grams solution as described in Bolten *et al.* (1979).

We gathered nectar from the following 13 species: *Mertensia ciliata*, *Vicia americana*, *Delphinium nuttallianum*, *Lathyrus leucanthus*, *Hydrophyllum fendlerii*, *Delphinium barbeyi*, *Helianthella quinquenervis*, *Ipomopsis aggregata*, *Chamaenerion angustifolium*, *Aconitum columbianum*, *Campanula rotundiflora*, *Helimeris multiflora*, and *Erigeron speciosus*. We sampled between 15 and 40 times for each species, including some measurements from flowers on the same individual in order to assess within-individual variation. For asters, nectar volume was measured on a per-inflorescence basis, but for all other flowers on a per-flower basis. We attempted to gather nectar from flowers of roughly the same age whenever possible, based on physical condition of the petals, the presence of pollen, and the number of other mature flowers on an inflorescence. For all nectar samples under 0.15 μ L, the sample was discarded as unreadable by hand refractometer (and likely not reflective of the actual production rate of the flower). Community sugar content was determined using species-specific average sugar mass per flower multiplied by number of flowers open in the site.

During our nectar content experiment, a severe drought affected much of Southern Colorado, including our study sites (US Drought Monitor, NIDIS). By chance, for 4 species of flower we sampled nectar both during an extended period of very low average precipitation and during the beginning of more regular rains. The "dry" period of 05/31/18 - 07/07/18 included only one measurable rain event, if a fairly large one (25.91mm accumulation on 06/17/18); however, our measurements for this "dry" period did not begin until 06/26/18, so none of the sampled flowers were open during that single rain event. In contrast, the "wet" period beginning 07/09/18 experienced regular brief rainfall events, with many days showing measurable accumulation (*Figs. 2a-b*).

This serendipitous experiment allowed us to test the widely-held hypothesis that flowering plants plastically respond to soil moisture levels, by comparing the nectar of the following species before and after the arrival of seasonal rains: *Helianthella quinquenervis*, *Mertensia ciliata*, *Delphinium barbeyi*, and *Ipomopsis aggregata*. For *D. barbeyi* and *H. quinquenervis*, the same meadows were sampled before and after the rains, although the individuals were different. For *I. aggregata* and *M. ciliata*, no

flowering individuals remained in the before meadows by the time of the rain, so different meadows were used. All samples were obtained by NAM except for *I. aggregata* before the rain, which are courtesy of Nickolas Waser.

Groups were then compared using Welch's *t*-tests in R (v. 3.5.1).

Results

Community Differences: Wet vs. Dry

Across the 6 sites, the wet and dry habitats exhibited no detectable differences in nectar content, when taking into account the entire season (Welsh's *t*-test, $t_{6.97} = 0.89$, $p = 0.4$)(Fig. 3).

During mid-season (June 4-6), however, dry meadows contained marginally significantly more total sugar mass (196.56 mg dry vs. 28.12 mg wet, $t_{5.38} = 2.29$, $p = 0.06$)(Fig. 4a). This trend was similar, but less pronounced, during late-season (July 24-26) (180.69 mg dry vs. 58.48 mg wet, $t_{5.9} = 1.83$, $p = 0.12$)(Fig. 4b).

Responses to Rainfall

Nectar volumes in two out of four species increased significantly after rainfall: *D. barbeyi* (0.59 ± 0.45 μ L before vs. 1.70 ± 1.40 μ L after, $t_{31.3} = 4.14$, $p = 0.0002$) and *M. ciliata* (0.22 ± 0.16 μ L before vs. 0.58 ± 0.50 μ L after, $t_{23.0} = 3.01$, $p = 0.006$)(Table 1, Fig. 5a). Reported error is 1 SD.

Nectar concentrations fell significantly in all four species: *D. barbeyi* (1.10 ± 0.06 mg/ μ L before vs. 0.44 ± 0.12 mg/ μ L after, $t_{41.9} = 25.6$, $p = 0$), *M. ciliata* (1.03 ± 0.06 mg/ μ L before vs. 0.36 ± 0.10 mg/ μ L after, $t_{24.8} = 24.1$, $p = 0$), *H. quinquenervis* (1.10 ± 0.06 mg/ μ L before vs. 0.64 ± 0.33 mg/ μ L after, $t_{11.5} = 4.4$, $p = 0.0009$), and *I. aggregata* (0.37 ± 0.10 mg/ μ L before vs. 0.29 ± 0.10 mg/ μ L, $t_{31.9} = 2.94$, $p = 0.006$)(Table 1, Fig. 5b).

Nectar sugar mass did not change significantly for any of the four species: *D. barbeyi* (0.73 ± 0.54 mg before vs. 0.78 ± 0.60 mg after, $t_{52.4} = 0.36$, $p = 0.7$), *M. ciliata* (0.22 ± 0.16 mg before vs. 0.22 ± 0.16 mg after, $t_{27.4} = 0.01$, $p = 0.9$), *H. quinquenervis* (0.40 ± 0.23 mg before vs. 0.28 ± 0.19 mg after, $t_{6.7} = 0.99$, $p = 0.4$), and *I. aggregata* (0.60 ± 0.32 mg before vs. 0.51 ± 0.27 mg after, $t_{27.4} = 0.93$, $p = 0.4$)(Table 1, Fig. 5c).

Discussion

Based on our estimates, the wet and dry subalpine meadows contained the same amount of sugar over the course of the season (Fig. 3). It is fairly unsurprising that this coarse-

scale measure would not show differences between the communities in nectar rewards. On shorter, weeks-length time scales, however, some differences emerge. There are hints that with slightly higher power, we would have seen significantly more nectar in the dry meadows than the wet (*Figs. 4a-b*). This pattern is driven primarily the fact that of the 13 species we measured, the species with highest floral abundances are most commonly found in dry meadows: during mid-season, these are *D. nuttallianum* and *L. leucanthus*, and during late-season *H. multiflora* and *E. speciosus*.

These results emphasize the importance of measuring on the appropriate time scale: here, the weeks-long scale is probably more relevant to an individual foraging bee than whole-season differences. The fact that the possible higher nectar mass in dry meadow effect is driven by so few species is somewhat troubling: this suggests that if we missed even a couple crucially important mid- or late-season species, our results could have been drastically altered. Based on our transect data, there are several highly abundant species we did not obtain estimates for that could fill this role, including *Potentilla pulcherrima*, *Claytonia lanceolata*, and *Mertensia fusiformis*. The latter two dominate the early-season species, a time frame for which we have no good data for at all. For this reason, 13 species is likely too few to draw strong conclusions about the progression of sugar availability in the two habitats over the course of the season. In future experiments using this methodology, estimates would be greatly bolstered by including all the abundant, bee-visited species.

Based on the rainfall results, plants in our population thought to be experiencing more severe water stress generally made lower volumes of highly concentrated nectar. We did not take soil moisture measurements as part of this experiment, but argue that the extremity of the drought suggests that the beginning of summer rains almost certainly altered the water status of these plants. The beginning of rain was linked with a significant increase in volume for all species but *I. aggregata*, and *H. quinquenervis*, and a significant decrease in concentration for all four species. *H. quinquenervis* was sampled during the very beginning of the rainfall events and may have not had time to fully respond to the rain when we began taking our putative "post-rain" measurements, because the later sampling events showed large, twofold increases in volume similar to the other two species. *I. aggregata* is thought to be highly drought tolerant, with growth and reproduction seeming to continue largely unaffected during extreme drought, at least in the absence of herbivory (Levine and Paige 2004). Our results are consistent with this, as nectar production in *I. aggregata* had the smallest responses to drought of any species.

In the cases of *D. barbeyi* and *M. ciliata*, the inverse responses of volume and concentration cancelled each other out in terms of total sugar mass; that is, flowers produced the same amount of sugar during both the drier and wetter period, just packaged in different amounts of water. Our results are broadly consistent with the

hypothesis that water shortages can limit the volume of nectar a plant is able to produce (corroborating Wyatt *et al.* 1992, Carroll *et al.* 2001, and others). On the other hand, the constant sugar production of all species measured is not consistent with the other hypothesis that water shortages lead to lower sugar concentrations in nectar by depressing photosynthetic efficiency. Phillips (2018) and Carroll (2001) found a similar constancy in nectar sugar production during artificially imposed droughts in several related species. It may be that the water limitation on sugar production does not commonly occur in our 4 species, but it is difficult to say conclusively whether this effect would hold for other plant species.

Even if the total mass of sugar present in a nectar source was the same during the worst phase of drought and after the rains began, changes in volume are likely to have significant effects on pollinator foraging efficiency, and potentially on foraging preferences. Bumble bees lap up nectar with their hairy tongues, and their efficiency depends on the rate of tongue movement and the volume obtained per lap (Harder 1986). Nectar that is highly concentrated is more viscous, and eventually the energy benefits imparted by highly concentrated sugar rewards are outweighed by the marginal costs to foraging efficiency at higher concentrations (Heyneman 1983). Harder (1986) found that bumble bees were able to forage with maximum efficiency on a $\mu\text{L/s}$ basis using nectar of 0-40% sucrose concentration, but that beyond this concentration the rate of nutrient acquisition began to drop steeply. This effect could be due to a combination of decreased effectiveness of capillary action when fluids are viscous and difficulty in removing the stickier nectar from the tongue quickly when it is retracted into the mouth (Harder 1986). Consistent with the hypothesis that this decreased foraging efficiency is driven by viscosity and not sugar content, honeybees fed artificial nectar at a constant 20% sucrose concentration were found to strongly prefer less viscous solutions (Nicolson *et al.* 2013).

With this evidence in mind, it is likely that the extremely high nectar concentrations we observed during the driest part of the drought constituted a suboptimal food supply for the bees, even though some flowers were able to provide the same mass of sugar throughout. Interestingly, all the early- and mid-season species measured responded similarly to the very dry conditions, except for *I. aggregata*. This resulted in a "compression" effect in which much of the natural interspecific variation in nectar quality was reduced, as most species produced very small volumes of highly concentrated 70-80% sucrose nectar (around $1\text{mg}/\mu\text{L}$).

Our results indicate that flower species may converge on producing the same nectar rewards during times of severe water stress, unless they are highly drought tolerant (as with *I. aggregata*). The consequences of this nectar quality compression could be difficult to predict. Bumble bee species occupy niches that are linked to both tongue length (longer nectar tubes impose a morphological limit on which species can

effectively forage at them) and to flower specialization, whose proximate causes are not well understood (e.g. long-tongued bees do not visit all the long-tubed flowers) (Goulson and Darvill 2004). As available nectar resources become similar between species, one expectation may be expansions in diet breadth.

Notably, our study focused only on concentration of simple sugars (sucrose equivalents) and volume. This is an incomplete picture from the perspective of visitors: nectar contains many other solutes, including amino acids and lipids, which bees and other pollinators probably both depend on for nutrition and use as cues (Nicolson and Thornburg 2007). In spite of this limitation, it is our hope that a description of how sugar is distributed in the meadows of the upper East River Valley will help future researchers understand patterns in plant-pollinator ecology.

Many interesting questions emerge from the trends we observed. Do bees alter their foraging behavior when severe drought alters nectar quality in species-specific ways? Do they forage optimally? Does intensity of interspecific competition alter foraging strategies or diet breadth? Similarly, we might ask whether the plants in our study were behaving optimally: did producing the same amount of sugar but packaging it in smaller volumes of water maximize reproductive success? I predict negative consequences for bee foraging efficiency, but potentially no difference in fruit set in flowers: more bee transit between flowers during nutrient shortages and longer stays at each flower due to more viscous nectar could balance out any declines in bee populations. Because nectar lies at the interface between pollinator and plant, and is both heritable and highly plastic, avenues of future questions touch on many areas of evolutionary ecology; and because of the ubiquity and functional importance of the plant-pollinator relationship, these questions have far-reaching implications.

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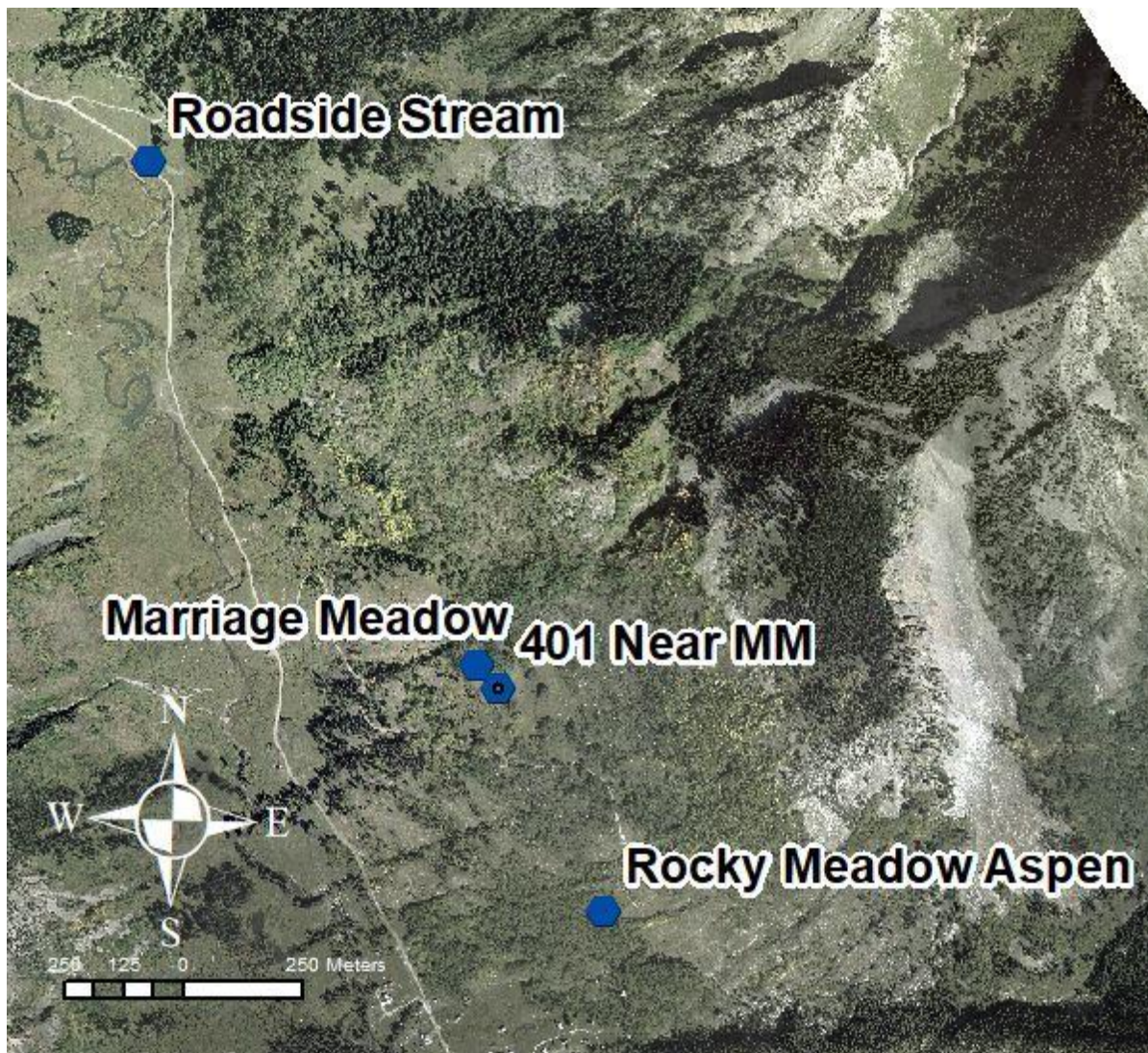
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Tables and Figures

Table 1: Nectar volume, concentration, and mass before and after rains for *D. barbeyi* ($N = 36, 30$), *M. ciliata* ($N = 14, 19$), *H. quinquenervis* ($N = 8, 14$), and *I. aggregata* ($N = 21, 35$).

Species	Volume Before (μl)	Volume After (μl)	p	Concentration Before (mg/μl)	Concentration After (mg/μl)	p	Sugar Mass Before (mg)	Sugar Mass After (mg)	p
<i>Delphinium</i>	1.59 ± 0.45	1.70 ± 1.40	0.0002	1.10 ± 0.06	0.44 ± 0.12	0	0.73 ± 0.54	0.78 ± 0.60	0.7
<i>Mertensia</i>	0.22 ± 0.16	0.58 ± 0.50	0.006	1.03 ± 0.06	0.36 ± 0.10	0	0.22 ± 0.16	0.22 ± 0.16	0.9
<i>Helianthella</i>	0.36 ± 0.22	0.53 ± 0.48	0.3	1.10 ± 0.06	0.64 ± 0.33	0.0009	0.40 ± 0.23	0.28 ± 0.19	0.4
<i>Ipomopsis</i>	1.76 ± 1.07	1.93 ± 1.26	0.6	0.37 ± 0.10	0.29 ± 0.10	0.006	0.60 ± 0.32	0.51 ± 0.27	0.4

Fig. 1: Map of the four sites from which nectar was collected. Courtesy of RMBL.



Figs. 2a-b: At Gothic, CO, (a) cumulative precipitation and (b) daily accumulation for the month of July 2018, in mm. Courtesy of Billy Barr.

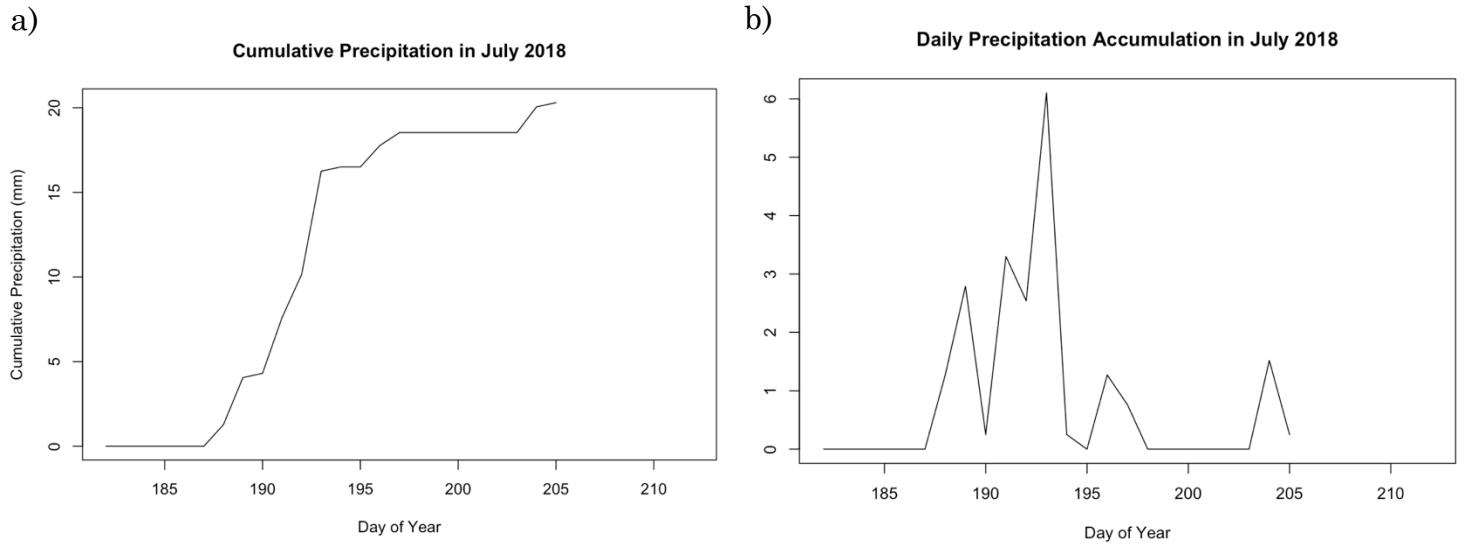
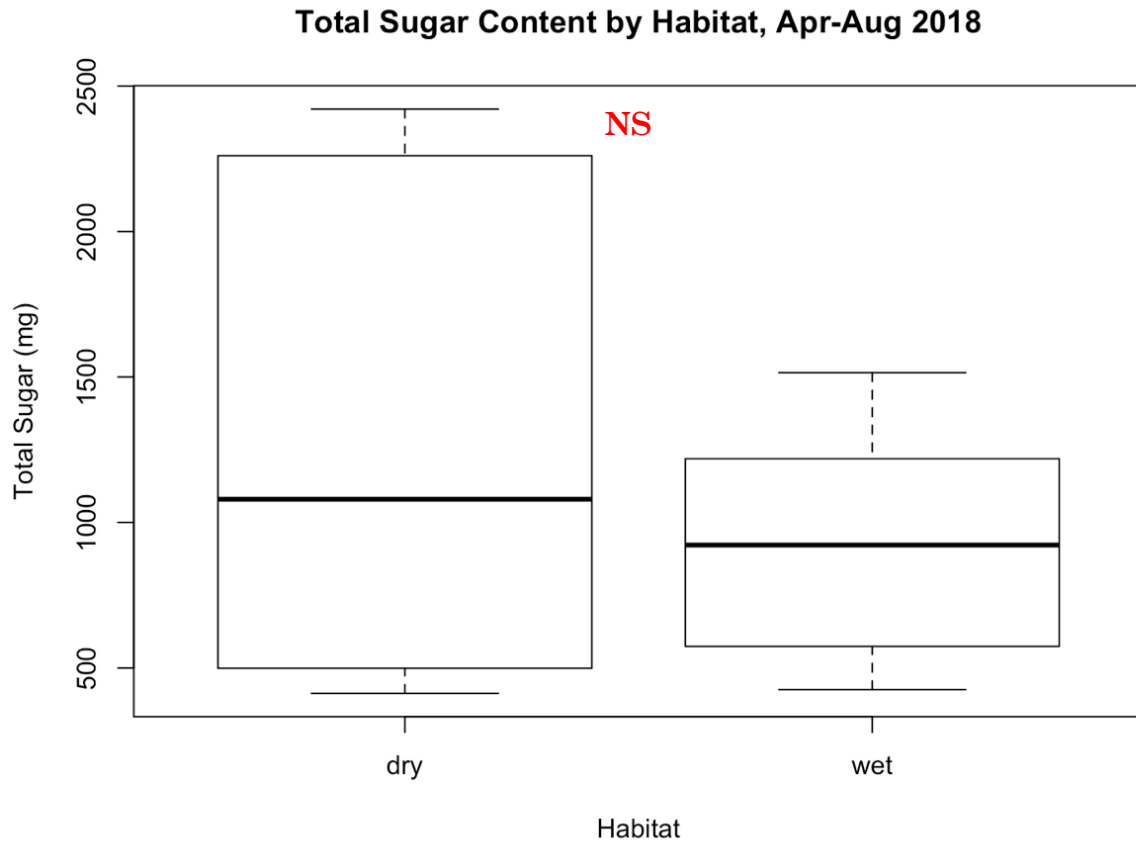
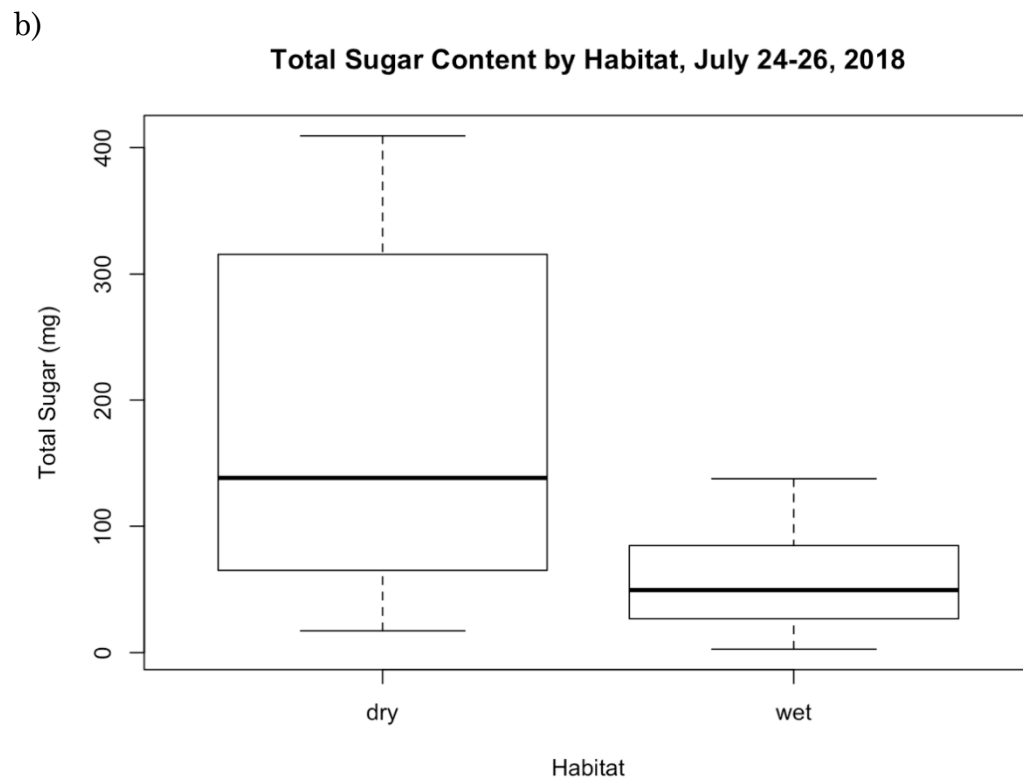
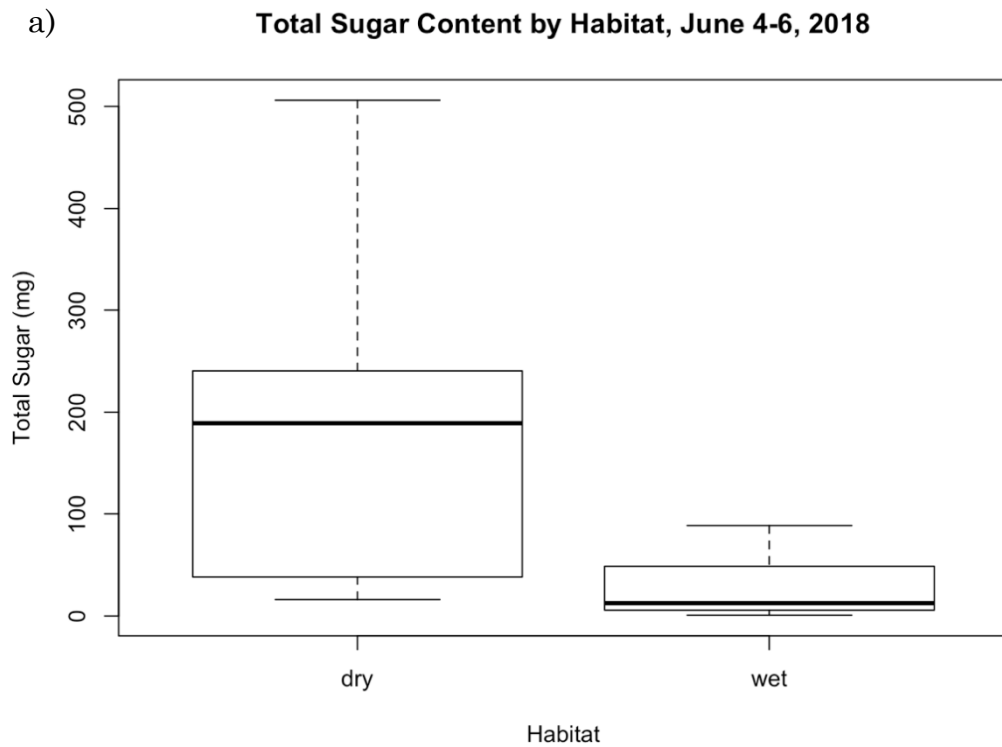


Fig. 3: Total sugar mass by habitat across the 6 focal sites from April 22 - July 26, 2018. Total sugar mass (mg) is estimated by multiplying per-flower sugar mass estimates by floral abundance in each habitat for each week.

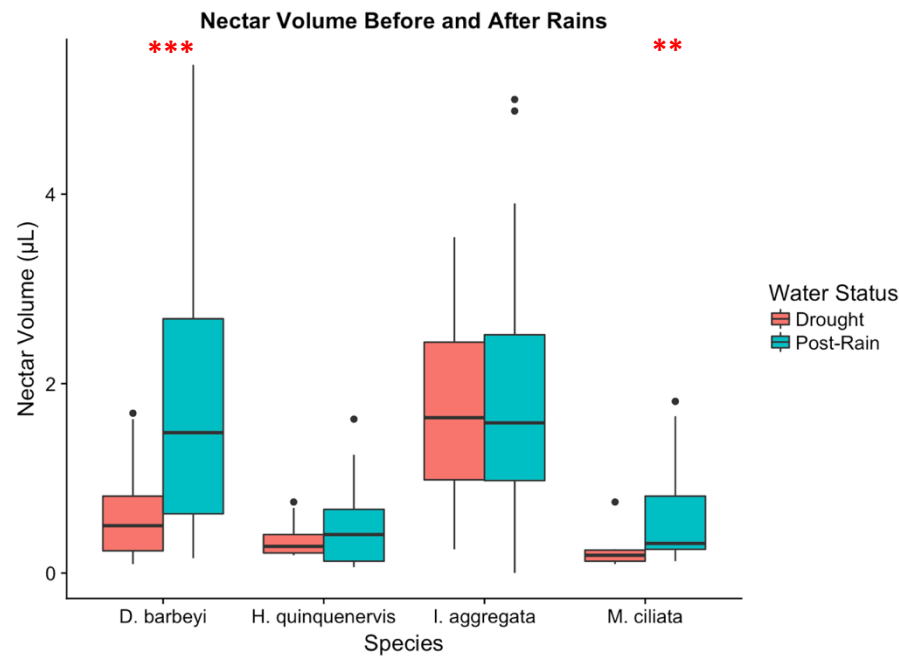


Figs. 4a-b: Total sugar mass by habitat across the 6 focal sites from (a) June 4-6, 2018 and (b) July 24-26, 2018. Total sugar mass (mg) is estimated by multiplying per-flower sugar

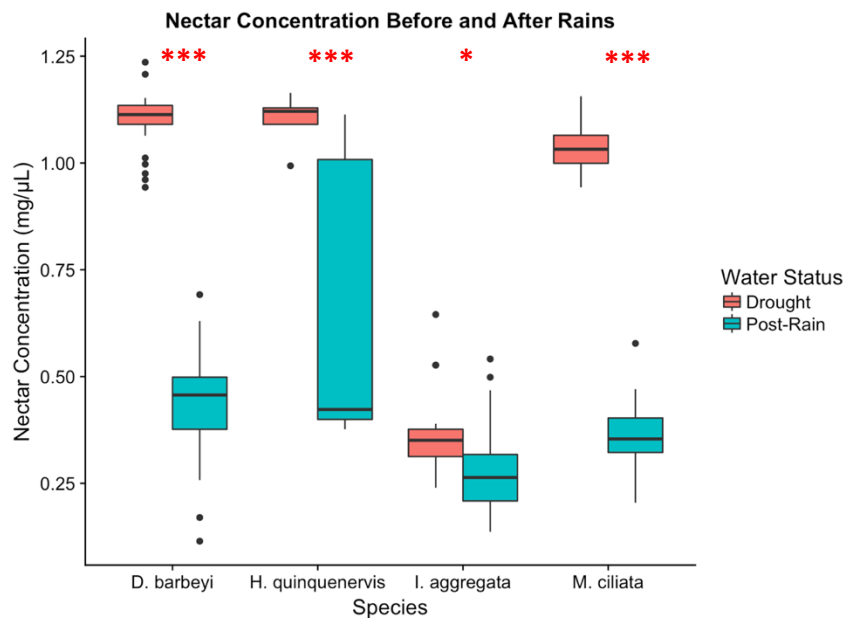


mass estimates by floral abundance in each habitat for each week.
Figs. 5a-c: (a) Nectar volume (μL), *(b)* Concentration ($\text{mg}/\mu\text{L}$), and *(c)* mass of sugar (g) per-flower for the four species sampled before and after regular summer rains began. N for each are reported in Table 1 above.

a)



b)



c)

