Quantifying Pollinator Diversity, Abundance, and Seasonal Changes in Pollinators at Low and High Elevations and Differences in Collection Methods, Southwest Colorado

Lauren Catlin

Dec 12, 2013

Introduction

Bees (Order Hymenoptera) are arguably the most important group of pollinating insects found on the planet, especially because they play an important role in agriculture by pollinating our crops. In the US, over half of the commercially-managed pollinators (mostly honeybees) have disappeared since 1940 with similar declines worldwide and the causes not entirely known (Wenning, 2007). A proposed hypothesis for the decline of these insects has been that of increased global temperatures. Bees are endothermic, and any rise in normal temperatures could be problematic for their thermoregulation. Water loss, droughts and changes in plant phenology would affect bumble bees as well, and heat waves could prematurely wake hibernating queens before adequate flowering resources are available (Rasmont & Iserbyt 2012). Honeybees have been extensively researched because they are economically important to us, but very few studies have been performed for pollinators of non-agricultural ecosystems (Potts, et al 2010). The intention of this study is to help fill this void in information on other pollinating insects by gathering baseline data on pollinators and specifically bumblebees at low and high elevations in southwestern Colorado.

Due to the demands of harsh environmental conditions, pollinator species tend to decrease as altitude increases (Tang 2007), so multiple plant species end up sharing pollinators. When plant species share pollinators in a variable environment, normally competitive situations may change in response to the environment (Kasagi and Kudo 2003). Bartomeus and others (2011) reviewed museum data dating from the 1880s to review long term trends between northeastern North American bees and native plants. The overall findings were that bees parallel the plants they visit in terms of their phenological changes. Most of the species examined were generalists in their interactions, as well. The shifts in bee

and plant phenology were both likely associated with warming climate (Bartomeus et. al. 2011).

Additionally, bees may respond differently to warming changes depending on the elevation at which they reside. High elevations are more subject to dramatic climate change, whereas lower elevation habitats rely less on snow cover and may be more adaptable in response to warming. In a study conducted by Manino and others (2007), it was observed that species adapted to high mountainous regions were generally pushed into even higher altitudes in response to warmer temperatures and that this would lead to distribution fragmentation (Manino 2007). Species able to live at lower elevations like *Bombus mucidus* and *B. monticola* are less subject to risk of extinction due to shrinking habitat than are species like *B. alpinus* and *B. mendax*, the more alpine species (Manino et al. 2007). This might indicate a trend towards environmental selection for species that are more generalist. Other findings have suggested that in habitats at higher elevation, generalist insects like Flies (Order Diptera) predominate and true Hymenopteran pollinators are more abundant at lower elevations (Warren et. al. 1988).

Flies are the second largest group of pollinators on the planet, and they might be more easily described as generalists than bees (Larson 2001). Though numerous fly species are adapted to feed on nectar, they are not quite as efficient at pollinating as bees are. Instead of collecting large amounts of pollen and transferring it from plant to plant (like bees), flies usually just become dusted in pollen as they feed on the flower, and may accomplish some pollination this way (Larson 2001). Because they are second only to bees as pollinators, flies are important to include in this pollinator study.

For this study, the effects of elevation and seasonal changes on pollinator abundance and diversity were examined in May-August 2013. In addition to gathering important baseline data for the currently limited existing research regarding pollinators, the variation of pollinators across an elevation gradient with different growing season temperatures was determined as well as the variation of pollinators at different times in the growing season (early, mid, late). If pollinator species richness

decreased at higher elevations, we expected to see greater bee diversity and abundance at lower elevations. We expected to see more flies in higher elevations due to their generalist nature, and bumblebees (Order Hymenoptera, Family Apidae, and Genus *Bombus*) as the dominant group of true pollinators in the high alpine site.

Methods

To quantify pollinator richness, diversity, and abundance across an elevation gradient, two study sites were chosen based on the following: BLM land ownership, ease of access, no grazing, and no slope being steeper than thirty degrees. The sites identified were the juniper-pinyon woodland of Sale Barn Trailhead, Durango, CO (1,988m) and the alpine tundra of Minnie Gulch, Silverton, CO (3,550m). Sale Barn served as the low elevation site, and Minni Gulch was the high elevation site. Ten plots measuring 10m x 50m ($500m^2$) were established in each study site parallel to the slope, and the plots were spaced 20m apart.

In each plot, nine native bee glycol traps were established (A). Methods were based off of those from the Very Handy Manual: How to Catch and Identify Bees and Manage a Collection (2009). Traps were made with blue, yellow, and white 473 mL plastic cups filled with a 50% water/ 50% glycol solution that were held approximately 30 cm above ground. Cups were held up by a ring made of a 9 cm diameter PVC pipe and a roughly 30 cm tall 2.5 cm diameter PVC pipe. Three traps, one of each color, were placed at 5, 7, and 25 m along the central transect of the plot, and one on each side 3 m away from central traps. Colors were shuffled during placement. The traps were placed five days ahead of collection. There were three collection dates total for each site that were divided between early growing season (May for low elevation, June for high), middle growing season (June for low elevation, July for high), and late growing season (July for low elevation, August for high). For collection, the cups were

emptied into collection jars categorized by study site, date of collection, and plot number. All specimens were examined in the laboratory and identified on bugguide.net or with field guides in the lab.

Although not true pollinators, butterflies (Order Lepidoptera) and beetles (Order Coleoptera) were abundant during collection and included in the study. Insects that were not recurring in the cups were labeled as “other”.

Midway through the first sampling period at the low elevation site, it was observed that insects were being trapped in the PVC tubes that were holding the glycol traps in place. Bees, flies, butterflies, and beetles were crawling down the tubes and becoming trapped at the bottom, where they would perish. This finding was added as another method to the study and insects collected from the tubes were sorted separately from insects collected from the glycol traps (cups). Insects in the tubes were also categorized by study site, date of collection, and plot number. All of the colored traps and their corresponding tubes were pooled for data analyses. Pollinator species richness and abundance were determined after sorting (B) and identifying specimens (C).

To analyze the differences between treatments, a general linear repeated measures analysis (SPSS 18.0) was used. A PC-ORD software (version 5.10, McCune & Mefford 2006) was used to complete NMS analyses. The PERMANOVA test calculated P-values with permutations and also used common ecological distance measure to examine datasets. To identify pollinators that were consistent for particular treatments, indicator-species analysis was used (McCune & Grace 2002).

Results

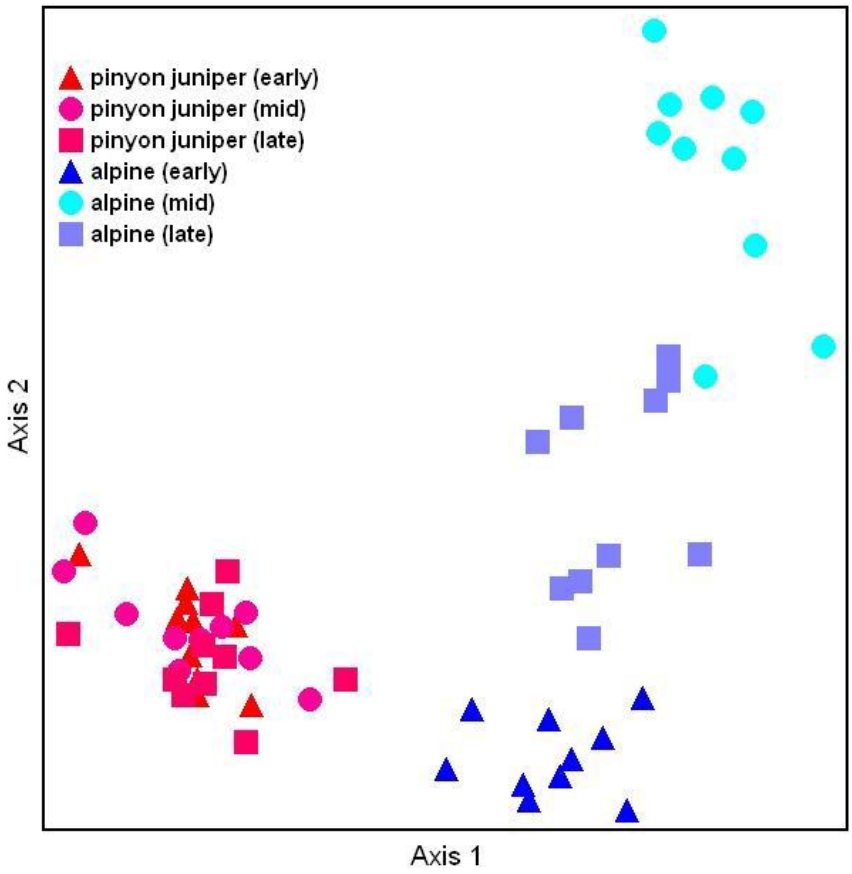

Figure 1. Non-metric multidimensional scaling ordination of the differences in the Order level of pollinators collected in the ten plots per site throughout the early, middle, and late periods of the growing season, Southwest Colorado. (F=133.91, p=0.0002 calculated by PC-Ord, version 5.10) using PERMANOVA. n=10/location/growing season combination.

Table 1. Mean Order richness and Shannon's diversity index for each growing season, Southwest Colorado. n=10/location/growing season combined. There was no significant difference between study sites for diversity or richness.

Study Site	Growing Season	Order Richness	Shannon's Diversity Index
Pinyon-Juniper	Early	4.6	1.144
	Middle	4.4	1.158
	Late	4.8	1.219
Alpine	Early	4	0.869
	Middle	4.5	0.28
	Late	4.3	0.671

Table 2. Indicator Orders were found only in the high elevation alpine site during the middle growing season. The Monte Carlo test of significance was used to determine Order significance (Indicator value= insect Order frequency x insect Order abundance) and p-value using PC-Ord version 5.10. n=10.

Order	Growing Season	Observed Indicator Value (IV)	p-value
Diptera	Middle	84.6	0.0002
Lepidoptera	Middle	59.6	0.001
Coleoptera	Middle	59.1	0.0022

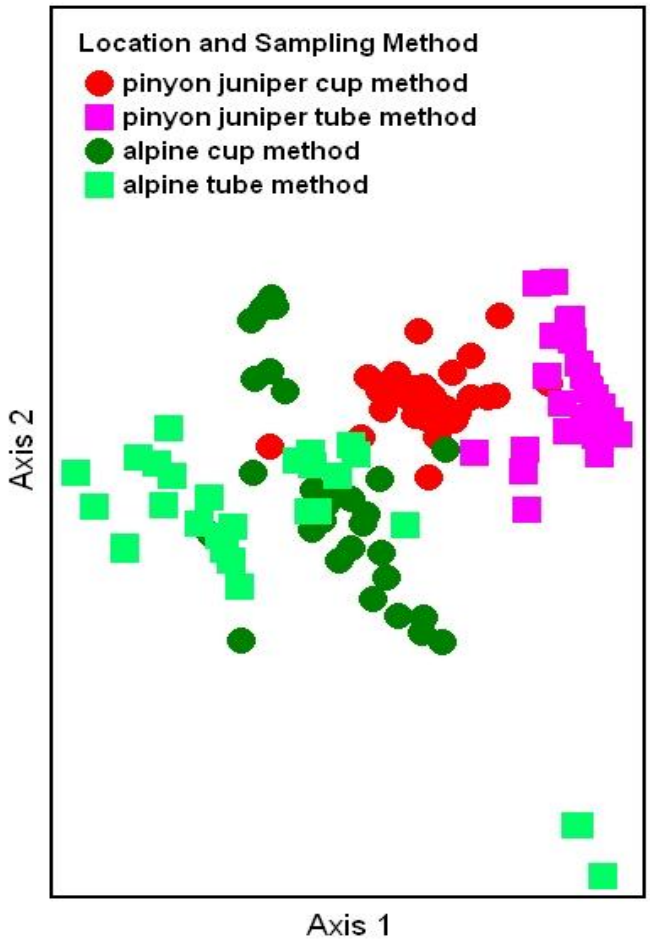

Figure 2. Non-metric multidimensional scaling ordination of the differences in the Order level of insects collected in the plots for different collection methods in each study site, Southwest Colorado. (F=29.238, p=0.0002 calculated by PC-Ord, version 5.10) using PERMANOVA. n=10/location/growing season combined.

Table 3. Mean Order richness and Shannon's diversity index for each method in each study site, Southwest Colorado. n=10/location/growing season combined.

Study Site	Method	Order Richness	Shannon's Diversity Index
Pinyon-Juniper	Cup	4.5	1.201
	Pipe	1.8	0.371
Alpine	Cup	4.1	0.595
	Pipe	2.1	0.449

Table 4. Total insect order counts collected by cup and tube methods throughout the growing season for the low elevation pinyon-juniper site, Southwest Colorado.

	Order									
Growing Season	**Hymenoptera**		**Diptera**		**Lepidoptera**		**Coleoptera**		**Other**	
	Cup	Tube	Cup	Tube	Cup	Tube	Cup	Tube	Cup	Tube
Early	33	125	33	0	14	0	93	1	14	0
Middle	118	29	26	1	24	1	106	10	12	0
Late	89	43	37	3	14	1	53	8	16	6
Total	240	197	96	4	52	2	252	19	42	6

Table 5. Total insect order counts collected by cup and tube methods for the high elevation alpine site throughout the growing season, Durango, Colorado.

	Order									
Growing Season	**Hymenoptera**		**Diptera**		**Lepidoptera**		**Coleoptera**		**Other**	
	Cup	Tube	Cup	Tube	Cup	Tube	Cup	Tube	Cup	Tube
Early	18	1	156	9	40	28	4	0	9	0
Middle	97	3	6640	79	298	30	34	0	11	1
Late	55	10	928	126	120	34	0	10	16	2
Total	170	14	7724	214	458	92	38	10	36	3

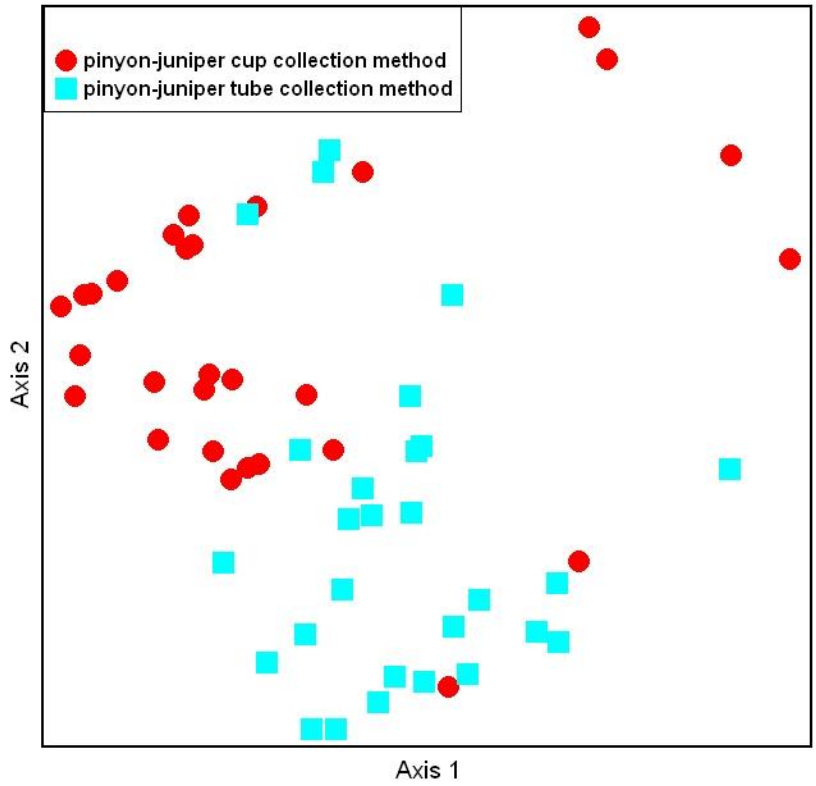

Figure 3. Non-metric multidimensional scaling ordination of the differences in the family level of Hymenoptera collected by each method in the ten plots per site throughout the growing season in the low elevation pinyon-juniper site, Durango, Colorado. (F=12.992, p=0.0002 calculated by PC-Ord, version 5.10) Using PERMANOVA n=10/growing season/method combined.

Table 6. Indicator Families for both collection methods in the low elevation pinyon-juniper site, Durango, Colorado. The Monte Carlo test of significance was used to determine family significance (Indicator value= Family frequency x Family abundance, n=10) and p-value using PC-Ord version 5.10. n=10/growing season/method combined.

Family	Method	Observed Indicator Value (IV)	p-value
Megachilidae	Tube	66.1	0.0002
Halictidae	Cup	67.6	0.0004
Apidae	Tube	50.4	0.0004

Table 7. Mean Hymenoptera Family richness and Shannon's diversity index for each method in the low elevation site, Durango, Colorado. n=10/growing season/method combined.

Method	Family Richness	Shannon's Diversity Index
Cup	2.6	0.668
Tube	2.8	0.806

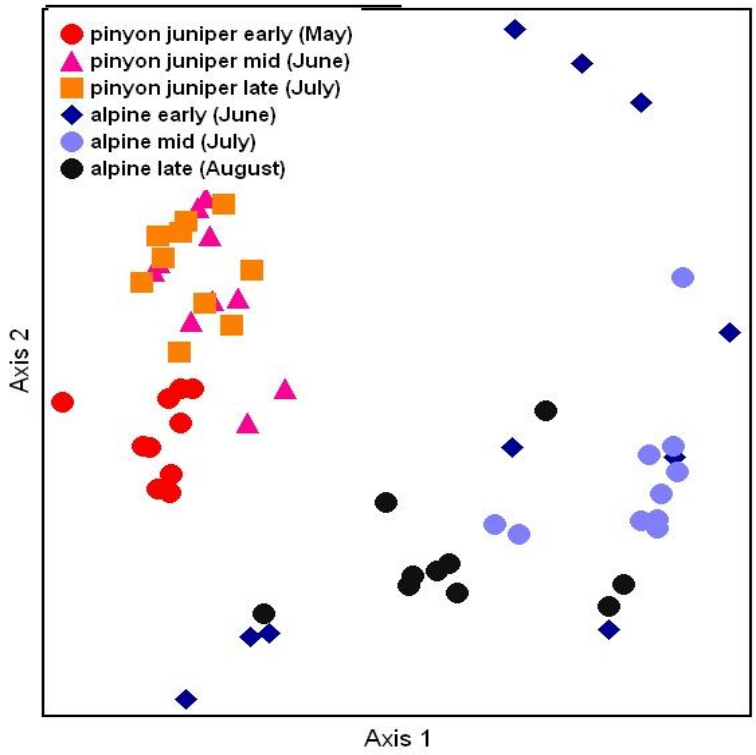

Figure 4. Non-metric multidimensional scaling ordination of the differences in the Family level of Hymenoptera collected in both methods in the ten plots per site throughout the growing season at the low and high elevation sites, Southwest Colorado. (F=11.103, p=0.0002 calculated by PC-Ord, version 5.10) using PERMANOVA. N= 10/location/growing season combination.

Table 8. Mean Hymenoptera Family richness and Shannon's diversity index for the growing seasons in both sites, Southwest Colorado. n=10/location/growing season combined. There was a significant difference between study sites for richness and diversity.

Study Site	Growing Season	Family Richness	Shannon's Diversity Index
Pinyon-Juniper	Early	4.4	1.15
	Middle	4.7	1.163
	Late	4.2	1.015
Alpine	Early	1.6	0.304
	Middle	2.6	0.767
	Late	3.1	0.912

Table 9. The Monte Carlo test of significance was used to determine Hymenoptera Family significance in both study sites throughout the growing season, Southwest Colorado. (Indicator value= Family frequency x Family abundance) and p-value using PC-Ord version 5.10. n=10/location/growing season combined.

Family	Study Site	Growing Season	Observed Indicator Value (IV)	p-value
Tenthredinidae	Alpine	Middle	79	0.0002
Ichneumonidae	Alpine	Middle	57.3	0.0002
Apidae	Pinyon-Juniper	Early	48.4	0.0004
Megachilidae	Pinyon-Juniper	Early	59.8	0.0002
Halictidae	Pinyon-Juniper	Middle	46.5	0.0002

Table 10. Total counts for *Bombus* specimens collected by both methods throughout the growing season at the high elevation alpine study site, Southwest Colorado.

	Growing Season		
Species	**Early**	**Middle**	**Late**
B. communis	0	0	2
B. mixtus	2	0	1
B. flavifrons	0	3	3
B. balteatus	4	0	4
Bombus spp.	0	0	1
Total	6	3	11

Table 11. Estimations of percent of plants flowering at each study site throughout the growing season.

	Growing Season		
Study Site	**Early**	**Middle**	**Late**
Pinyon-Juniper	25%	10%	<10%
Alpine	25%	>75%	40%

Significant differences for pollinator diversity and abundance across the growing season and elevation gradient and differences found in the two collection methods were observed. When analyzed to the Order level, differences were seen between the two study sites as well as throughout the growing season for the alpine site ($n=10$, $p<0.05$) (Fig. 1). The most notable observation was that there were thousands more Diptera (flies) found in the alpine site than Hymenoptera (bees, wasps, sawflies) (Table 5). Notable differences between pollinator Orders were also found in collection methods for both study sites (Tables 4 and 5, Fig. 2). In the pinyon-juniper site, more bees were collected via the tube method in the early collection period (table 4). No significant differences were found for Order pollinator richness or diversity between the two study sites throughout the growing season (Table 1). Only in the alpine site during the middle growing season were indicator Orders of pollinators (Diptera, Lepidoptera, Coleoptera) observed ($n=10$, $p<0.05$) (table 2). Hymenoptera was not an indicator Order. Order richness and diversity did, however, differ between collection methods for both the pinyon-juniper site and the alpine site (Table 3). Higher Order richness and diversity was observed in the cup collection method for both sites.

When insects from the Order Hymenoptera were analyzed to the Family level, more differences in pollinator diversity and abundance were observed. More Hymenoptera were found for both collection methods (cup or pipe) in the pinyon-juniper site than the alpine site (Table 4). There were differences in Hymenoptera Families collected by the different methods ($n=10$, $p<0.05$) (Fig. 3). In the pinyon-juniper site, two indicator Hymenoptera Families (Megachilidae and Apidae) were found for the tube collection method, and one indicator Family (Halictidae) was found for the cup collection method for the combined growing season ($n=10$, $p<0.05$) (Table 6). No notable differences in Family richness or diversity were observed between the collection methods in the pinyon-juniper site for the combined growing season (Table 7).

When comparing the two study sites, significant differences for Hymenoptera Families throughout the growing season were observed ($n=10$, $p<0.05$), and communities of Hymenopteran Families shifted more throughout the growing season at the alpine site than at the pinyon-juniper site (fig. 4). There was a significant difference for Family richness and diversity between the two study sites (table 8). For the entire growing season, Family richness and diversity was greater in the pinyon-juniper site than in the alpine site. Different indicator Families were found in pinyon-juniper site as well as the alpine site ($n=10$, $p<0.05$) (Table 9). No indicator Families were found in the late growing season. For the alpine site, instead of bees, Tenthredinidae (sawflies) and Ichneumonidae (parasitic wasps) were the indicator Families. The indicator Families (Apidae, Megachilidae, and Halictidae) for the pinyon-juniper site were all bees (Table 9).

Although not an indicator for the alpine site, Apidae was the most abundant bee family found at the site. All Apidae collected from the high elevation alpine site were of the genus *Bombus* (bumblebees) (Table 10). The most abundant bumblebees collected were *Bombus flavifrons* and *Bombus balteatus*.

Overall, fewer species of flowering plants were seen at the pinyon-juniper site than at the alpine site (Table 11).

Discussion

We found that pollinator abundance and diversity does change with elevation. The most significant finding was that at the lower elevation pinyon-juniper site, Hymenoptera were the most abundant pollinators throughout the entire growing season. Hymenoptera Family richness and diversity was also greater at the pinyon-juniper site than the high elevation alpine site. At our alpine site, we found that Dipterans were extremely dominant. This is consistent with findings from a similar study where lowland environments were predominantly pollinated by Hymenoptera and habitats of higher

altitude were pollinated more by Diptera and Lepidoptera (Warren et. al. 1988). Warren and others (1988) analyzed data from various locations in Utah and focused on two sites, one at low elevation and one at higher elevation. The authors provided several possible explanations for the abundance of Hymenopterans at low elevations and Dipterans and Lepidopterans at high elevations. Dipterans are generalists and can thrive in a broad range of environments, like cold alpine meadows (Larson 2001). A possible explanation for why bees are found more frequently at lower than high elevation is the special design that bees possess in regard to pollination. Bees are the most efficient insects when it comes to manipulating flowers to gain access to nectar and pollen (Collins et. al. 1983). Open flowers were far more abundant in higher elevations in the Utah study than complex flowers (Warren et. al. 1988), which would favor generalist pollinators like Dipterans, which do not possess special pollinating adaptations like Hymenopterans do. This could also be an explanation for our findings. Flowers, especially open flowers, were underrepresented at the pinyon-juniper site but extremely abundant in the alpine site (Table 11).

We found unique indicator families for each study site. Apidae (honeybees, bumblebees, etc.) and Megachilidae (mason bees) were indicators for the early growing season at the low elevation study site, when the most flowering was observed. These findings were expected as they are all bees, which are the most abundant pollinators in lower elevations. Megachilidae and Halictidae (sweat bees) are solitary bees, and solitary bees typically collect pollen from a selection of few plants (Strickler 1979). Apidae are eusocial bees, those that live socially in colonies, and typically collect pollen from a broad range of plants (Strickler 1979). This could be a possible explanation for why more solitary bee families were found at the low elevation site, where there wasn't such a wide diversity of flowering plant species.

Tenthredinidae (sawflies) and Ichneumonidae (parasitic wasps) were the indicator Hymenopterans for the high elevation site. This was not expected as neither are true pollinating insects.

Ichneumonidae, in fact, do not pollinate as they are parasitic and feed on host insect tissue (Bartlett 2004). Tenthredinidae, the sawflies, are herbivorous and are often found on flowers (DiTerlizzi 2005). This is consistent with other findings where in the Scottish Mountains and Swiss Alps, Tenthredinids were very abundant (Mani 1968).

Although Apidae was not an indicator family for the high elevation study site, we chose to include data regarding the bumblebees because they were the predominant bee genera that we observed in the alpine. This can be explained by bumblebees' unique adaptations that allow them to survive in colder environments. They are most often covered by a thick "fuzzy" coat of hair that reduces convective heat loss by over 50%, and they can activate thoracic muscles during flight to keep a body temperature that can be 20°C above ambient temperature (Lundberg 1980). In addition, by being eusocial bees they live in colonies. Larger numbers of individuals living in the colony contribute to keeping a warm nest. We found four species of bumblebees in the alpine. *Bombus flavifrons* and *Bombus balteatus* were the most common at the high elevation site. *Bombus flavifrons* is fairly common throughout the Western United States and is found in most habitats. *Bombus balteatus* is unique to high altitude environments in the Western United States and is classified as rare (Koch et. al. 2012). *Bombus balteatus* possesses unique adaptations to survive in cold environments such as a relatively large body size and thick hair coverage.

We found unique results from collecting pollinators using the glycol traps as well as the PVC pipes that held up the cups. For the most part, more pollinators were collected in the cups, as we expected, although there was no significant difference between Hymenoptera Family richness and diversity between the collection methods. The sweetness of the glycol and the varying colors of the cups were intended to attract flying pollinators (Droege 2009). However, the tube collection method during the early growing season at the low elevation site collected far more pollinators (specifically bees) than in the cups (125 compared to 33). We speculated that maybe the bees were attracted to the

long white tubes as they would be to a particularly large corolla of a flower. It might be more of a nesting preference, at least for the mason bees (Hymenoptera, Megachilidae). Mason bees naturally live in holes and will sometimes build nests in the stalks of reeds. Gardeners use artificial "nesting tubes" made from cardboard to attract the bees (Delaplane and Mayer 2000). These nesting tubes are placed horizontally, however, and the cardboard is rough enough to provide traction for the bees to crawl into and out of. Once bees fell into our PVC pipes, we assume they became trapped and could not crawl up the smooth surface of the pipe. According to Delaplane and Mayer (2000), gardeners are encouraged to place their artificial nests in the early springtime, before the bees build their nests. It is possible that more bees were becoming trapped in the PVC pipe during the early growing season (May) in the low elevation site because that is closest to when nests were being sought and built.

Conclusion

Because current research on wild pollinators and particularly alpine pollinators is limited in the San Juan Mountains, we hope our findings will provide more baseline data regarding these insects. Most of our observations were consistent with the data that already exists, especially when comparing pollinators across an elevation gradient. Our modification of the glycol trap/ PVC pipe combination could be useful for bee collection at lower elevation sites in the early growing season when bees are building nests. The glycol trap method was also particularly effective at the high elevation site where weather was unpredictable and surveying methods for pollinator data collection were not so effective. Some traps were damaged by marmots at the high elevation site. Placing plumber's putty between the cup and the pipe could hold the cups in the pipe more securely.

Literature Cited

Bartlett, T. (2004, February 16). Family Ichneumonidae. *BugGuide*.

Bartomeus, I., Ascher, J. S., Wagner, D., Danforth, B. N., Colla, S., Kornbluth, S., and Winfree, R. (2011). Climate-associated phonological advances in bee pollinators and bee-pollinated plants. PNAS 108: 20645-20649.

Collins, P. D., Harper, K. T. & Pendleton, B. K. 1983. Comparative Life History and Floral Characteristics of Desert and Mountain Floras in Utah. Great Basin Nat., 43: 385-393.

Delaplane, K. S., and Mayer, D. F. 2000. *Crop Pollination by Bees*. New York, NY: CABI Publishing.

DiTerlizzi, T. (2005, March 18). Family Tenthredinidae. *BugGuide*.

Droege, S. 2009. The Very Handy Manual: How to Catch and Identify Bees and Manage a Collection.

Kasagi, T. and Kudo, G. (2003). Variations in Bumble Bee Preference and Pollen Limitation among Neighboring Populations: Comparisons between Phyllodoce caerulea and Phyllodoce aleutica (Ericaceae) along Snowmelt Gradients. American Journal of Botany 90: 1321-1327

Koch, J., Strange, J., and Williams, P. (2012). *Bumblebees of the Western United States*. Pollinator Partnership.

Larson, B. M., Kevan, P. G., and Inouye, D. W. (2001). Flies and Flowers: taxonomic diversity of anthophiles and pollinators. Canadian Entomologist 133: 439-465.

Lundberg, H. 1980. Effects of weather on foraging-flights of bumblebees (Hymenoptera, Apidae) in a subalpine/alpine area. Holarctic Ecology, 3: 104-110.

Mani, M. S. (1968). *Ecology and Biogeography of High Altitude Insects*. North Virginia: Dr. W. Junk N. V. Publishers.

Manino, A., Patetta, A., Porporato, M., Quaranta, M., Intoppa, F., Piazza, M. G., Frilli, F. (2007). Bumblebee Distributions in High Mountains and Global Warming. Redia: 125-129.

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., Kunin, W. E. (2010). Global Declines: trends, impacts, and drivers. Trends in Ecology and Evolution 25: 345-351.

Strickler, K. 1979. Specialization and Foraging Efficiency of Solitary Bees. Ecology, 60: 998-1009.

Tang, Y., Xie, J., and Sun, H. (2007). Pollination Ecology of *Pedicularis Muscoides* H. L. Li subsp. *Himalayca* Yamakzaki from Alpine Areas of Western Sichuan, China. Arctic, Antarctic, and Alpine Research 39: 481-487.

Warren, S. D., Harper, K. T. & Booth, G. M. 1988. Elevational Distribution of Insect Pollinators. The American Midland Naturalist, 120: 325-330.

Wenning, R. J. (2007). The Status of Pollinators. Integrated Environmental Assessment and Management 3: 309.

Appendix A: Field Methods for Using Glycol Traps

Glycol traps are useful tools for collecting native bees over an extended period of time. They are composed of plastic cups suspended roughly 12 inches above ground level by a PVC pipe. The cups are removable from the pipe for easy specimen collection.

Equipment needed:
Plastic cups (473 mL) (as many as you need traps)
1" diameter PVC pipe (about one foot per trap)
5" diameter PVC pipe (about one inch per trap)
Screws (no longer than one inch)
Propylene glycol (about 200 mL per trap)
Water (about 200 mL per trap)
Dish soap
Gallon jugs
Collection jars
Small net

To assemble the support for each trap, PVC pipes should be cut to the appropriate lengths. Then, using a screwdriver and one metal screw, the 5" diameter pipe piece can be attached to the top inch of the 1" diameter piece. It is recommended for the supports to be built prior to entering the field. The traps are plastic cups filled with a 50:50 solution of propylene glycol: water. Plastic cups should be painted white, blue, and yellow to attract bees. Before entering the field, mix one half gallon of water with one half gallon of glycol, and add a squirt of unscented dish soap to the jug (this makes the surface tension weaker, so bees are more effectively trapped in the liquid). Multiple jugs can be mixed this way, depending on how many traps you are setting up.

In the field, place the PVC pipe supports in relatively soft soil. The deeper you can push them into the ground, the less likely they will be tipped over. Place a plastic cup in the holder, and make sure that the pipe hasn't been pushed so far into the ground that the cup is no longer suspended. Fill the cup ¾ full with your glycol/water solution. This completed trap should be left out in the field for three to five days to allow for adequate bee collection but to prevent extensive evaporation.

When ready to collect, bring enough collection jars to account for how you would like to separate your data (per trap, per plot, per habitat, etc.). Also, bring empty gallon jugs to collect excess glycol. Remove large insects that you might discard later. Pour a small amount of the glycol in the trap into a collection jar. Next, hold the net over the gallon jug and pour the rest of the glycol and insects that were in the trap into the net while collecting the glycol in the jug as it pours out. Empty the insects in the net into the collection jar, and close and label the jar. Repeat for all traps. Keep empty cups that were used as traps for additional trap set ups later. Insects may remain suspended in glycol in the collection jar for a week or two before sorting.

Appendix B: Lab Method for Sorting Pollinators

Equipment needed:
Sorting tray
Larval forceps
Glass vials
Ethanol
Cardstock

Once you have collected all your specimens, bring them to the lab for sorting. Select one collection jar to begin with. Prepare a sorting surface- a plastic tray or even plastic cutting board will work. Using larval forceps, sort insects by visual identification to the Order level. Be sure to work at one collection jar at a time and do not pour out all of your samples at once (unless you have only one jar). It is easiest to begin with the largest pollinators like Lepidoptera and Orthoptera. Grasp each insect from the "start" pile (from the collection jar) to an "Order" pile on one section of your sorting tray. On a sheet of paper, mark tallies for insects from each order. If you are not saving insects that are not Hymenopterans, you may discard after counting. Keep the insects of the order Hymenoptera (and other Orders you wish to sort to the Family level) separated and place in a vial of ethanol. Cut a small rectangle of cardstock to label with pencil and place inside the vial.

When ready to sort Hymenopterans to the Family level, pour out the vial into a small jar. Using larval forceps and your sorting tray, lay out one vial of insects at a time so that you can observe differences. Sort Hymenopterans by visual characteristics such as size, hair color, presence of pollen basket, antennae type, etc. It is better to over-separate than to group together insects that might possibly not be related. It is likely that you will separate insects that end up belonging to the same family. Keep like insects in vials of ethanol until ready to identify.

Appendix C: Lab Method for Insect Identification

Equipment needed:
Larval forceps
Paper towels
Dissection scope
Lamp
Digital camera
Computer
Field guide for Hymenopterans or other insects in your area

To identify insects, you may want to use *http://bugguide.net/node/view/15740* or *http://www.discoverlife.org/.*

For identification through *http://bugguide.net/node/view/15740*, dry your specimen of choice of ethanol. With larval forceps, place the specimen on a paper towel and allow to air dry. Place the specimen under a dissection scope and lamp. Focus the scope and, using a digital camera, photograph the specimen through the lens. You will have to create an account on the website, and uploading instructions are available after registering.

For identification through *http://www.discoverlife.org/*, select "ID nature guides", then follow the series of links to your insect of choice.

Appendix D: Lab Method for Drying and Pinning Bees

Equipment needed:
Mason jar
Dish soap
Insect pins
Pinning block
Foam (inside pinning box)

Empty specimens from ethanol vials and individually wash each specimen in soapy tap water in a mason jar. To wash, drop each bee in the water, place the lid on the jar, and invert the jar several times. Remove the bee and place in clean tap water to rinse. Using insect pins and a pinning block, penetrate the mid thorax of the bee so the bee is about ¾ up the length of the pin. Pin each bee onto foam board to be suspended and air dried. As bees dry, use the end of another pin to "fluff" hairs away from the body to prevent matting.