

Title: Breakfast of champions: Spatiotemporal variation in the quality of ant nests for bear consumers

Author: Riley Hunter

Co-Author/Advisor: Joshua Grinath

Abstract

Black bears in the Colorado Rocky Mountains forage for western thatching ants as a food source. However, there is little knowledge about the nutritional benefit of ants for black bears, particularly with regard to changes in nutritional value during the ant life cycle. From late spring to mid-summer, thatching ants go through two separate stages of reproduction that greatly influence the composition of individual ant nests. In early summer, the ant colonies produce sexual ants, resulting in large-bodied immature (larvae and pupae) and adult ants (drones and queens) being present in the nest. During mid to late summer, ant colonies produce nonsexual ants, resulting in smaller immature and adult ants (workers). We conducted a study to measure how these nutritional values change from early to late summer, and how the varying phenology of different locations affects these values. We did this by simulating bear attacks on ant nests in two sites with differing phenology in early summer (June) and late summer (July) and collecting samples from the central brood chamber in each nest. We also wanted to see how a prior bear attack on a nest may affect its nutritional value later in the season, which we addressed by comparing nests that were attacked once (in July) vs. nests that were attacked twice (once in June, once in July). The samples were sorted into castes and life stages of ants (adult worker, pupae, queen, or drone) and weighed. These masses were used to approximate food mass per L of nest material for bears and converted into fat content to measure nutritional value. We found that there was significantly greater total mass and fat content in the ant nests in June compared to July. This was driven by large masses of sexual pupae found within the June nests. We found that there was no significant difference in the nutritional value of nests that were previously attacked vs. those that were not previously attacked. This lack of variation suggests that ant colonies can recover quickly from attacks made by bears, and that bears can revisit ant nests as a replenishable source of food. Altogether, these results indicate that seasonality in ant life cycles should be taken into account when evaluating the nutritional value of ants for bears.

Introduction

In ecosystems around the world, bears (Carnivora: Ursidae) are resourceful omnivores known for their diverse diets, which include vegetation, berries, insects, and ungulates (Robbins et al., 2022). In the western U.S., a large portion of the diet of American black bears (*Ursus americanus*) consists specifically of red wood ants, from the *Formica rufa* subgenus group (Bull et al., 2001; Baldwin & Bender, 2009; Stockan & Robinson, 2016). Though bears frequently consume ants, ant nutritional quality for bears is not well understood, particularly variation in nutritional quality across space and time, which can shift with climate change (Deacy et al., 2017).

One species of ant that is common across western North America is *Formica obscuripes*, also part of the *Formica rufa* group (Jurgensen et al., 2005, Stockan & Robinson, 2016). *F. obscuripes*, more commonly known as “thatching” ants, form colonies in prairie and dry forest environments. These colonies take the form of mounded ground nests constructed of small sticks and dried vegetation that contain a brood chamber with a high concentration of immature and adult ants (Jurgensen et al., 2005).

Studies have shown that black bears use these large mounds of condensed ants as a favored food source, particularly in the mountains of Colorado (Baldwin & Bender, 2009; Grinath et al., 2015, Grinath, 2018). Previous experiments have shown that ants are a hearty food source, with a makeup of up to 55% protein (Bull et al., 2001) and approximately 80% unsaturated fatty acids (Abril et al., 2022). Foraging for thatching ants may also be a good way for bears to obtain food without expending too much energy, second only to vegetation browsing (Bull et al., 2001).

Multiple previous studies at the Rocky Mountain Biological Laboratory have been conducted to evaluate bear-ant relationships within a larger trophic cascade (Grinath et al., 2012, Grinath et al., 2015, Grinath, 2018, Grinath, 2021). Here, bears indirectly benefit plants by consuming ants that are mutualists of sap-sucking insects, which provide ants with food in return for protection from predators (Grinath et al., 2015, Grinath, J.B., 2018). One aspect of this food chain that has not been assessed is the nutritional benefits that bears are obtaining from their predation on thatching ants (Pekar & Mantz, 2014). Therefore, this study will help us to better understand this trophic cascade of bears, ants, herbivorous insects, and plants. We are conducting this experiment starting in early June, which is when ant consumption by black bears is at a peak; bears tend to move on to mast and berry plants as they ripen and the fall season approaches (Berry, 1996, Noyce et al., 1997).

Throughout the summer season, the content of ant nests changes across different stages in the life cycle of ant colonies (Stockan & Robinson, 2016); however, it is unclear how this variation impacts the nutritional benefit of ant consumption for bears. In general, red wood ant colonies produce large-bodied sexual ants in the early summer, and then switch to producing smaller-bodied worker ants in mid to late summer as the sexual ants mature and leave the nest to reproduce (Stockan & Robinson, 2016). Ant body size may be an important factor to consider for bear nutrition because larger-bodied ants consist of a smaller overall percentage of indigestible exoskeleton (Stockan & Robinson, 2016) and may have greater nutritional value for bears. Therefore, we may expect that the nutritional value of ant nests for bears will vary over the growing season in relation to the production of different ant castes within nests.

Our research questions are as follows: (Q1) how do ants (*Formica obscuripes*) vary spatially and temporally as a food source for black bears (*Ursus americanus*) in Gunnison County, Colorado? To answer this question, we simulated bear damage to ant nests at two sites and at two times, and then we compared observations of the contents of the ant nests over space and time. In addition, prior work has found that bears will frequently attack individual ant nests multiple times within a growing season, with ants rebuilding their nests between attacks (Grinath, pers. obs.), but the influence of previous bear damage on the contents of ant nests and their nutritional value for bears later in the growing season is unclear. Thus, we further propose to ask: (Q2) how does a previous bear attack influence subsequent ant nest quality for bears and does this vary spatially? Building on the same experiment as above, we answered this question by simulating bear attacks to ant nests twice and comparing the contents of these nests with those that had not been previously attacked. This study can provide insight into the benefits that bears receive from ants and, because black bear consumption of ants can indirectly impact other arthropods and plants (Grinath et al., 2015, Grinath, 2018), the role of bears within their ecological communities.

Methods

Study Site and Nest Selection

To study spatial variation, we manipulated and observed ant nests at two different sites. Our first study site is located in a montane meadow near the town of Almont, Colorado (2669 m elevation; 38° 43'

29.47" N; 106° 48' 48.91" W). The second site is within the meadow surrounding the Snodgrass Trailhead in Mt. Crested Butte, Colorado (2941 m elevation; 38° 55' 14.09" N; 106° 57' 45.16" W). To study temporal variation, we measured and compared ant nests at two different times, June and July, at both sites. All lab work took place at the Rocky Mountain Biological Laboratory in Gothic, Colorado.

To establish our experiments, we first identified and marked ant nests for study at both sites. Nests were selected for study if they did not have any visible indication of recent bear damage, such as the displacement of nest materials. We marked 20 ant nests at our Snodgrass site (Table 1; Figure 1) and 31 nests at Almont (Table 2; Figure 2). Though our intention was to study 20 nests at each site, additional nests were identified at Almont as potential alternates for study because of high bear activity at this site (Grinath, pers. obs.). We then measured the size of each nest (basal area and volume) based on its length, width, and height (in cm). Length was measured as the longest axis of the nest from end to end, and width was measured as the widest part of the nest perpendicular to the axis used to measure length. Height was recorded as the mean of four measurements from four points around the nest to account for the varying slopes and elevations on each side of the nest.

Table 1. Specific ID, coordinates, and measurements of ant nests at the Snodgrass study site.

Snodgrass Site				
Nest ID	Coordinates	Nest Size (LxWxH, cm)	Nest Base Area (cm ²)	Nest Volume (cm ³)
1	(-106.962291, 38.920480)	108 x 90 x 35	9720	340200
2	(-106.962565, 38.920733)	107 x 96 x 49.5	10272	508464
3	(-106.962869, 38.920764)	58 x 52 x 22.5	3016	67860
4	(-106.963083, 38.920754)	79 x 65 x 20	5135	102700
5	(-106.963358, 38.920972)	72 x 67 x 25	4824	120600
6	(-106.963501, 38.921146)	68 x 65 x 26	4420	114920
7	(-106.963985, 38.921192)	62 x 58 x 21.5	3596	77314
8	(-106.964127, 38.921359)	71 x 64 x 18	4544	81792
9	(-106.964293, 38.921400)	55 x 55 x 28	3025	84700
10	(-106.965085, 38.921717)	104 x 74 x 14	7696	107744
11	(-106.965217, 38.921851)	87 x 72 x 19.5	6264	122148
12	(-106.965752, 38.922263)	71 x 63 x 23	4473	102879
13	(-106.966400, 38.922468)	70 x 67 x 16.5	4690	77385
14	(-106.967182, 38.922788)	95 x 91 x 31	8645	267995
15	(-106.967306, 38.922927)	98 x 81 x 18.5	7938	146853
16	(-106.967181, 38.922970)	73 x 69 x 17.5	5037	88147.5
17	(-106.966721, 38.922477)	56 x 46 x 13.5	2576	34776

18	(-106.966773, 38.922461)	98 x 95 x 17.5	9310	162925
19	(-106.966802, 38.922335)	72 x 65 x 23	4680	107640
20	(-106.966505, 38.922143)	77 x 74 x 23.5	5698	133903

Figure 1. GIS image of sampled nests at Snodgrass, labeled with specific Nest ID.

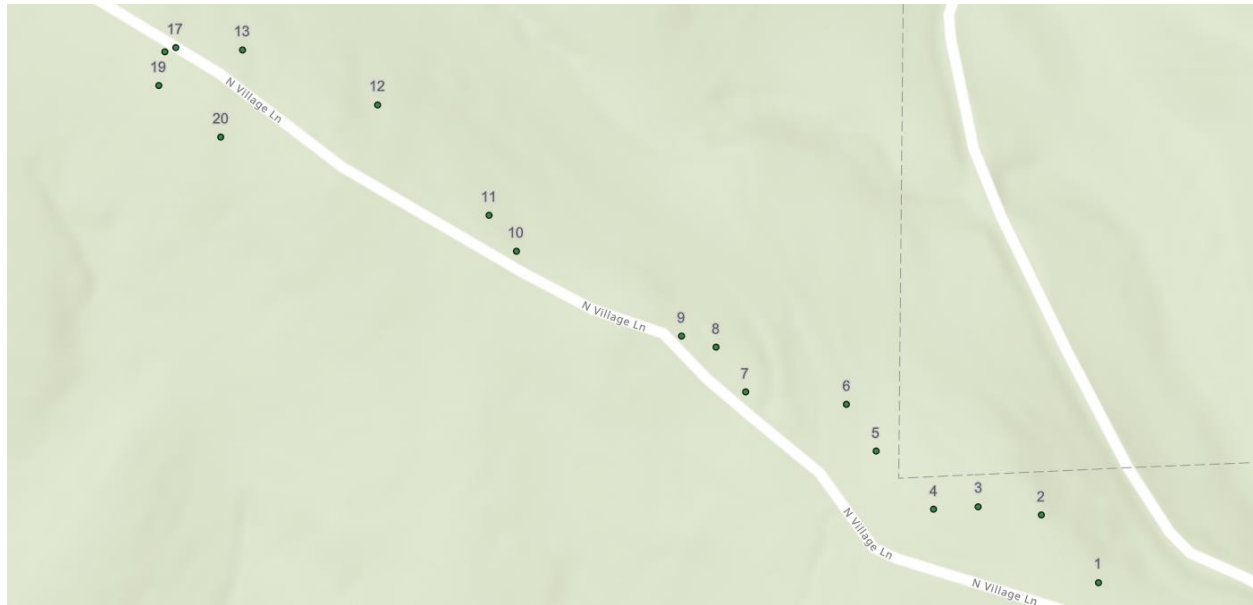
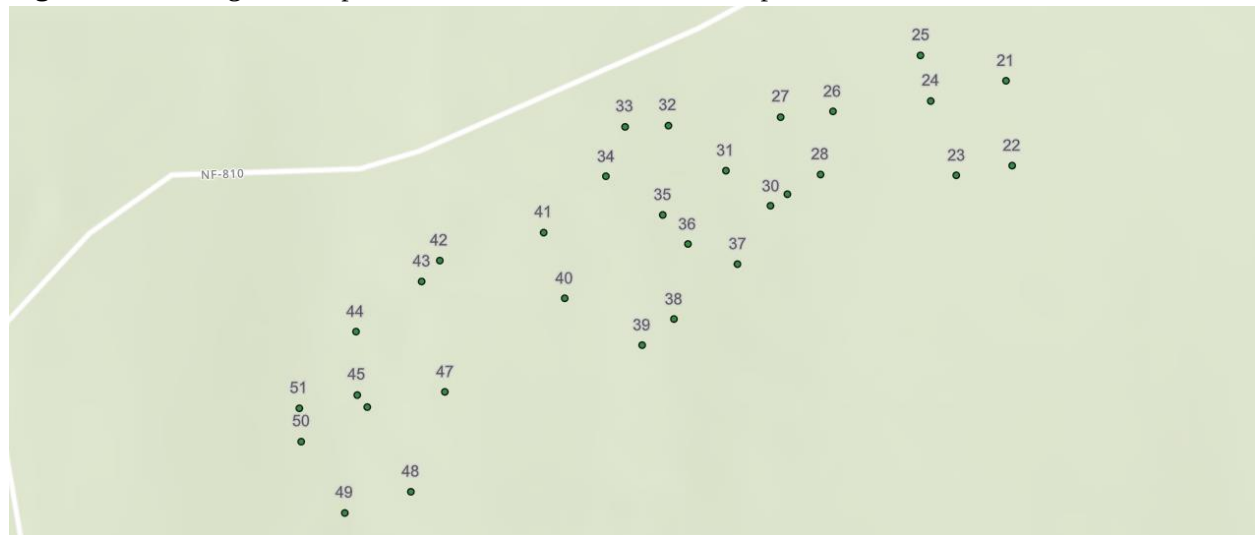


Table 2. Specific ID, coordinates, and measurements of ant nests at the Almont study site.

Almont Site				
Nest ID	Coordinates	Nest Size (L x W x H, cm)	Nest Base Area (cm ²)	Nest Volume (cm ³)
21	(-106.813600, 38.724640)	65 x 56 x 23	3640	83720
22	(-106.813586, 38.724496)	60 x 51 x 27	3060	82620
23	(-106.813709, 38.724479)	82 x 73 x 24	5986	143664
24	(-106.813764, 38.724605)	67 x 63 x 19.5	4221	82309.5
25	(-106.813787, 38.724683)	70 x 64 x 24	4480	107520
26	(-106.813977, 38.724588)	90 x 84 x 30.5	7560	230580
27	(-106.814092, 38.724578)	90 x 66 x 31	5940	184140
28	(-106.814004, 38.724480)	84 x 68 x 34.5	5712	197064
29	(-106.814077, 38.724447)	102 x 75 x 25	7650	191250
30	(-106.814114, 38.724427)	103 x 75 x 28.5	7725	220162.5
31	(-106.814211, 38.724487)	101 x 97 x 34.5	9797	337996.5

32	(-106.814337, 38.724564)	85 x 74 x 19	6290	119510
33	(-106.814431, 38.724561)	98 x 78 x 20	7644	152880
34	(-106.814473, 38.724478)	106 x 90 x 24	9540	228960
35	(-106.814349, 38.724412)	94 x 80 x 30.5	7520	229360
36	(-106.814294, 38.724362)	105 x 84 x 29.5	8820	260190
37	(-106.814186, 38.724328)	76 x 64 x 28	4864	136192
38	(-106.814324, 38.724234)	100 x 78 x 24	7800	187200
39	(-106.814394, 38.724190)	110 x 81 x 26	8910	231660
40	(-106.814563, 38.724270)	72 x 67 x 23	4824	110952
41	(-106.814609, 38.724382)	73 x 67 x 24	4891	117384
42	(-106.814835, 38.724334)	67 x 49 x 22.5	3283	73867.5
43	(-106.814875, 38.724298)	62 x 52 x 16.5	3224	53196
44	(-106.815018, 38.724213)	42 x 33 x 10	1386	13860
45	(-106.815015, 38.724105)	86 x 77 x 26	6622	172172
46	(-106.814994, 38.724085)	47 x 37 x 17.5	1739	30432.5
47	(-106.814824, 38.724111)	91 x 72 x 22.5	6552	147420
48	(-106.814899, 38.723941)	87 x 80 x 23.5	6960	163560
49	(-106.815043, 38.723904)	90 x 75 x 21	6750	141750
50	(-106.815138, 38.724026)	90 x 76 x 21.5	6840	147060
51	(-106.815142, 38.724082)	88 x 72 x 19	6336	120384

Figure 2. GIS image of sampled nests at Almont, labeled with specific Nest ID.



Nest Manipulation

This study consists of two experiments based on simulated bear attacks to the same set of ant nests. To investigate Q1, we compared nest contents at both sites in June and July (observational experiment), and for Q2, we compared nests at both sites in July that were or were not previously attacked (manipulative experiment). To simulate bear attacks on ant nests, we followed a protocol based on natural bear damage to nests in Almont, where it is estimated that approximately 1 L of material is removed from the central brood chamber area of a nest during an attack, which generally takes place prior to dawn (Grinath, pers. obs.; Figure 3).



Figure 3. Camera-trap photo of bear attacking an ant nest at our site in Almont, Colorado.

The details of nest manipulation are important to evaluate these ant-bear interactions from the point of view of the bear. To simulate bear damage to a nest, we visited nests just prior to dawn and used a trowel to remove the top of the nest. Then we removed 1 L of contents from the brood chamber area of the nest into a 1 L measuring cup, including nest materials and ant pupae and adults of multiple castes (potentially). These samples were then transferred to pre-labelled ziplock plastic bags and placed in coolers with ice packs before then being transferred to a freezer to dispatch the ants.

To assign ant nests to different experimental groups, we first listed the ant nests at each site according to size (basal area) and divided the lists into pairs of nests with similar size (the Almont list was trimmed to cover the same range of sizes as the Snodgrass list). Within each pair, we then used a random number generator to assign nests to temporal treatments in two groups: nests that were attacked in both June and July, and nests that were only attacked in July. This resulted in sample sizes of $n = 10$ for each experimental group, with a similar range of ant nest sizes represented in each experimental group. We conducted the June bear attack simulations on June 20 at Almont and June 22 at Snodgrass; July simulations were conducted three weeks later on July 11 at Almont and July 13 at Snodgrass.

Nest Sorting and Data Collection

After the nest samples were sufficiently frozen (2-3 days), they were transferred from the plastic bags into paper bags for drying. These bags were labeled with the corresponding Nest ID, and then placed into the drying oven for three days at 65°C to remove all moisture from the samples. After drying to constant weight, each sample was hand-sorted into different components.

For our nest-sorting procedure, we started by removing large pieces of nesting material from each 1 liter sample, specifically those meeting or exceeding 2.5 centimeters in length; this prevented large fragments of material from taking up unnecessary volume when we next took a 250 mL sub-sample of the sifted nesting material. We then used both 4,000 micron and 2,000 micron sieves to further sift through this sub-sample to separate large and fine materials. We then removed all ant biomass from the sub-sample using tweezers, which we sorted into categories. We classified ants as (a) pupae, (b) adult workers, (c) virgin queens (not mated, with wings), and (d) drones. Each category was stored in a separate plastic bag with its own label and color code.

After sorting each nest, we measured the dry mass (in grams) of each ant group per nest sub-sample. We then multiplied our values by four to estimate values in units of mass per 1 L of brood chamber material. Using published estimates of the fat value of red wood ants (e.g. Sundstrom, 1995; Sorvari & Hakkarainen, 2009), we then calculated fat content for the nest components and for entire nests, including separate conversions for each caste.

Table 3. Fat percentage conversions for each ant caste.

Ant Caste	Fat Content %	Source
Adult Worker	9.5%	(Sorvari & Hakkarainen, 2009)
Worker Pupae	9.5%	(Sorvari & Hakkarainen, 2009)
Adult Queens	15.15%	(Sundstrom, 1995)
Adult Drones	8.6%	(Sundstrom, 1995)
Sexual Pupae	11.875%	(Sundstrom, 1995)

Analyses

We analyzed our data using two-way ANOVA in R v4.2.2 ('glms' function in the 'nlme' package). To address Q1, we compared the fat content, total mass, and pupal mass for total ants per nest across space (Almont vs. Snodgrass), time (June vs. July), and the interaction of space and time. To address Q2, we compared the fat content, total mass, and pupal mass for total ants per nest across space (Almont vs. Snodgrass), previous attack (present vs. absent), and the interaction of space and previous attack. Results were considered significant if $P \leq 0.05$.

Mass to Nutrition Conversion

To get an idea of the nutritional value that bears are getting from these ants, aside from food volume, we decided to convert the nest masses into percentage of fat content. Recent studies show that bears have evolved to prefer high fat food sources as opposed to high protein sources (Robbins et al., 2022). Thus, fat content adequately represents a portion of the nutritional value that bears are obtaining from the ants. We used studies surrounding other similar redwood ant species to make conversions of biomass to fat percentage. We found one study stating that the mean fat content of *Formica aquilonia* worker ants is 9.5% (Sorvari & Hakkarainen, 2009). This value was applied to the masses of adult worker ants and worker pupae. Another study compared the fat content between monogynous and polygynous male and female *Formica truncorum* ants. For mature males, the average fat content was 8.6%, so this value was applied to the drone masses within each sample. For mature females, the average fat content was 15.15%, so this value was applied to the queen masses within each sample (Sundstrom, 1995). To quantify sexual pupae fat content, we took an average between the male and female values and applied 11.875% to the mass values.

Results

Table 3. Question (1) 2-way ANOVA results.

Response Variable	Site		Month		Interaction	
	χ^2	<i>P</i>	χ^2	<i>P</i>	χ^2	<i>P</i>
Fat Content	0.313	0.576	8.872	0.003	0.4067	0.524
Total Mass	0.739	0.390	5.432	0.020	0.489	0.484
Pupal Mass	0.232	0.630	5.665	0.017	0.432	0.511

Table 4. Question (2) 2-way ANOVA results.

Response Variable	Site		Previous Attack		Interaction	
	χ^2	<i>P</i>	χ^2	<i>P</i>	χ^2	<i>P</i>
Fat Content	0.0061	0.938	0.406	0.524	0.148	0.701
Total Mass	0.017	0.898	0.373	0.541	0.126	0.722
Pupal Mass	1.588	0.208	0.007	0.934	0.124	0.724

Regarding Question 1, we found significant results for the temporal comparison (June vs. July) regarding fat content, total mass, and pupal mass (Table 3). For all three response variables, we found that

June had higher values than July (Figure 4-6). However, there were no significant differences in response variables between study sites, and there were no significant interactions between space and time.

Figure 4. Question (1) fat content comparison.

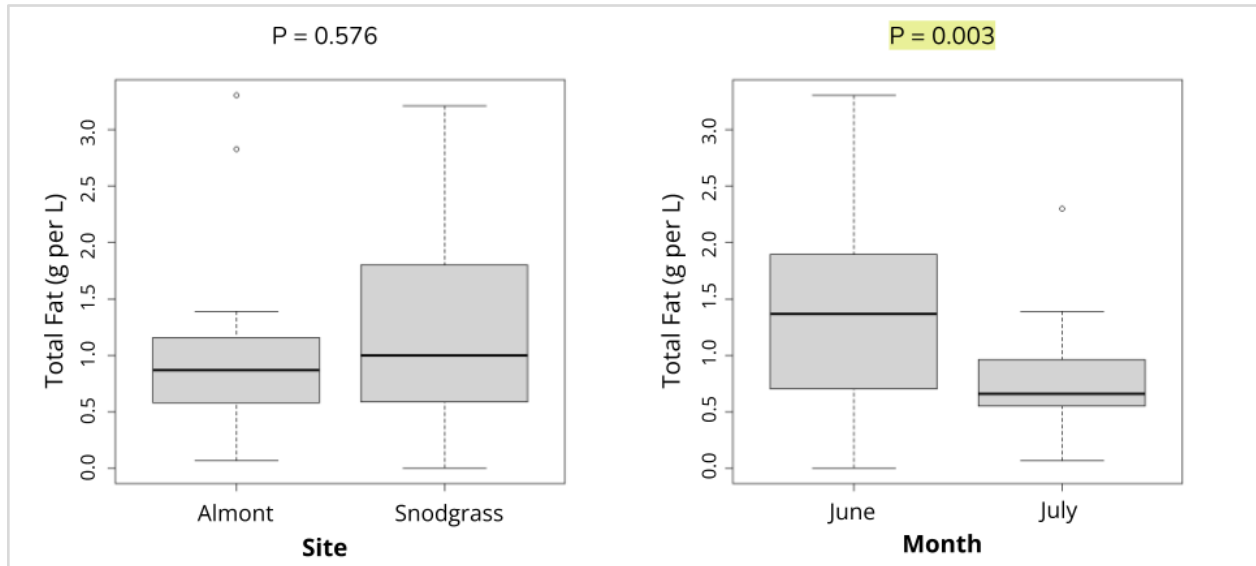


Figure 5. Question (1) total mass comparison.

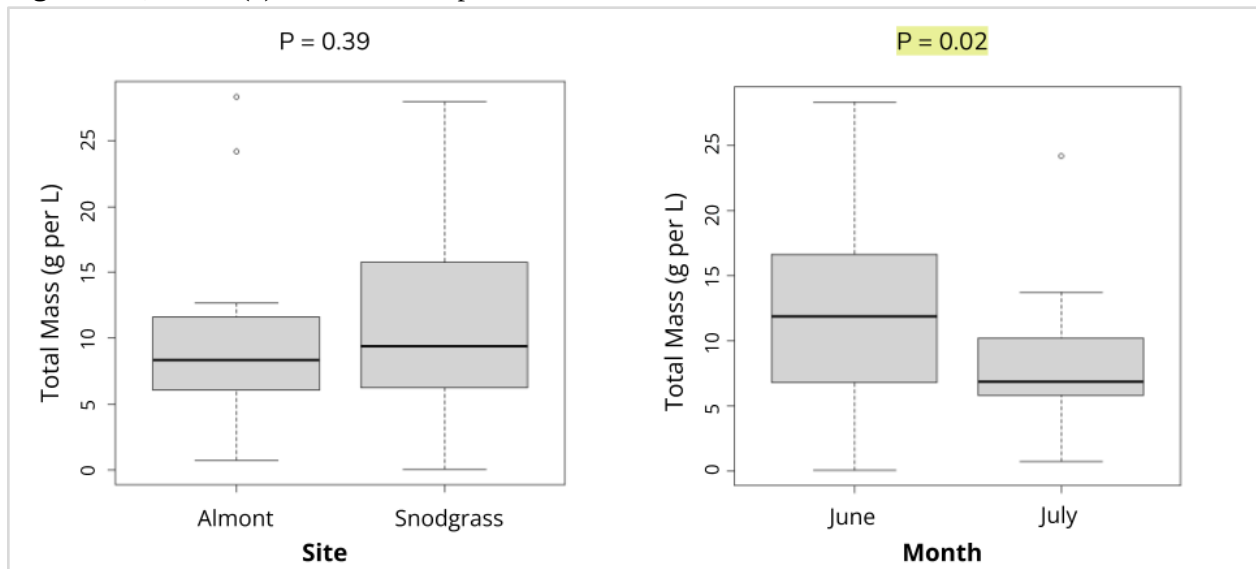
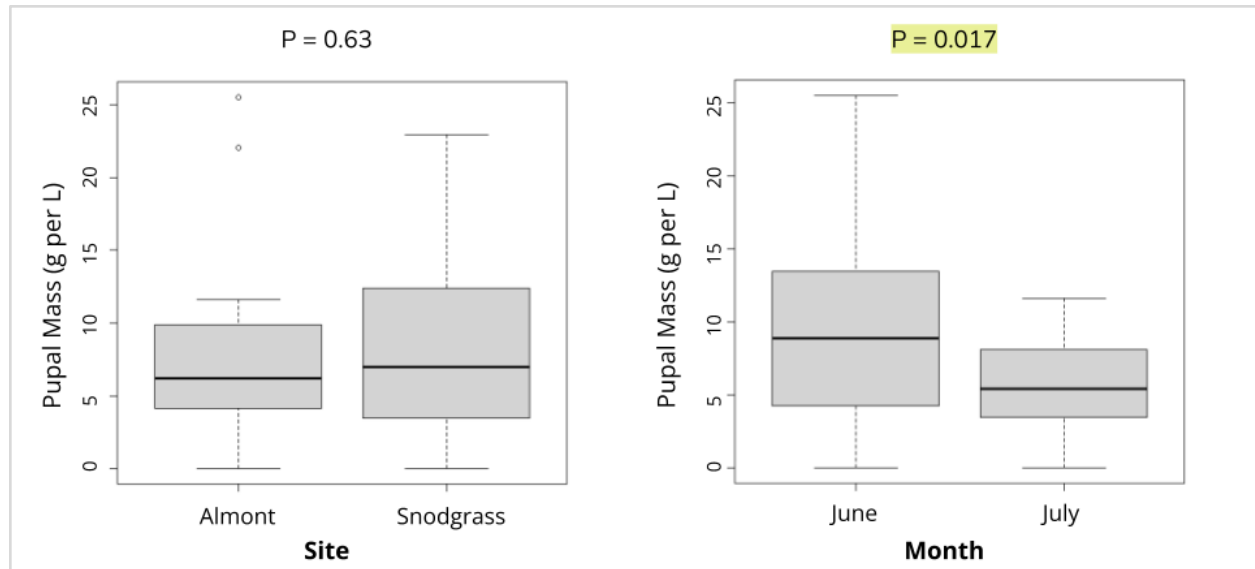


Figure 6. Question (1) pupal mass comparison.



Regarding Question 2, overall we found no significant differences between treatment groups (Table 4). There was no significant difference regarding previous attacks for fat content, total mass, or pupal mass. Nests that were attacked twice did not have mass or fat content values that were significantly any less than nests that were attacked only once. Also, site and the interaction between site and previous attack were not significant for fat content, total mass, or pupal mass.

Discussion

For our first question, we wanted to know how western thatching ants vary across space and time as a food source for black bears. We found that, over space, there was no significant difference in the nutritional value of ants between our two study sites (Snodgrass and Almont). Over time, we found that there was a significantly greater nutritional value for ant nests that were sampled in June, rather than nests sampled in July. There was a greater fat content in the ants sampled in June; this greater fat content was driven by a greater total mass per nest, and this was driven by the proportionally greater mass of pupae. The majority of ant nests sampled in June contained sexual pupae, which are generally larger in size than workers. As of now, we can assume that the large amounts of sexual pupae that are present in ant nests in June are what are driving the nutritional value of the nests. This indicates that ant life cycles should be considered when estimating the nutritional value of ants for bears.

For our second question, we found no significant results. This means that there was no difference in fat content, total mass, or pupal mass between nests that were previously attacked and those that were not. This suggests that attacked ant colonies are capable of recovering at a faster rate than expected, which has positive implications for both ants and bears. The ants can survive and continue thriving as a working colony, even after suffering notable damage by a foraging bear. In turn, the bear can rely on ant nests as a continuous food source that does not diminish after each forage.

Conclusion

We have learned that the reproductive cycle of western thatching ants affects nest composition and the potential nutritional value of ants for bears. In particular, we can expect the nutritional quality of ant nests to be greater earlier in the summer, when ant colonies are producing large-bodied sexual ants. We have also learned that a previous attack on an ant nest does not have significant repercussions on the nutritional value of the nest later in the summer.

These findings are important to understanding bear diets, but this study only gives us a glimpse of the nutritional quality of ants as a food source for bears. Moving forward, we could take this study in multiple directions. One aspect we could assess further is a more detailed analysis of the composition of the ant nests. For example, what percentage of queens, drones, workers, etc. are making up the nests throughout the summer? We could also take nest size into further consideration, which sometimes (not always) relates to age of nests. We could use nest size as a covariate in our analyses, and possibly use historical data (Grinath, as per obs) to estimate nest age. Another important step moving forward would be to make other nutritional conversions such as protein and carbohydrate content. Additional work is needed to understand the spatial and temporal variation of ants as a food source for bears and the contribution of ants to bear nutrition.

References

- Abril, S., Pinzón, M., Hernández-Carrión, M., & Sánchez-Camargo, A. d. P. (2022). Edible Insects in Latin America: A Sustainable Alternative for Our Food Security. *Frontiers in Nutrition (Lausanne)*, 9, 904812. <https://doi.org/10.3389/fnut.2022.904812>
- Baldwin, R. A., & Bender, L. C. (2009). Foods and nutritional components of diets of black bear in Rocky Mountain National Park, Colorado . *Canadian Journal of Zoology*, 87(11), 1000-1008. <https://doi.org/10.1139/Z09-088>
- Berry, M. (1996). Black bear (*Ursus americanus*) autumnal use of foothills riparian habitats. *City of Boulder, Open Space Department & Division of Mountain Parks, Boulder, Colorado*.
- Bull, E. L., Torgersen, T. R., & Wertz, T. L. (2001). The importance of vegetation, insects, and neonate ungulates in black bear diet in northeastern Oregon. *Northwest Science.*, 75(3), 244-253.
- Deacy, W. W., Armstrong, J. B., Leacock, W. B., Robbins, C. T., Gustine, D. D., Ward, E. J., Erlenbach, J. A., & Stanford, J. A. (2017). Phenological synchronization disrupts trophic interactions between Kodiak brown bears and salmon. *Pnas*, <https://doi.org/10.1073/pnas.1705248114>
- Grinath, J. B., Inouye, B. D., Underwood, N., & Billick, I. (2012). The indirect consequences of a mutualism: comparing positive and negative components of the net interaction between honeydew-tending ants and host plants. *The Journal of Animal Ecology*, 81(2), 494-502. <https://doi.org/10.1111/j.1365-2656.2011.01929.x>
- Grinath, J. B., Inouye, B. D., & Underwood, N. (2015). Bears benefit plants via a cascade with both antagonistic and mutualistic interactions. *Ecology Letters*, 18(2), 164-173. <https://doi.org/10.1111/ele.12396>
- Grinath, J. B. (2018). Short-term, low-level nitrogen deposition dampens a trophic cascade between bears and plants. *Ecology and Evolution*, 8(22), 11213-11223. <https://doi.org/10.1002/ece3.4593>
- Grinath, J. B. (2021). Chronic, low-level nitrogen deposition enhances abundances of ant-protected herbivores inhabiting an imperiled foundation species. *Acta Oecologica (Montrouge)*, 110, 103706. <https://doi.org/10.1016/j.actao.2021.103706>
- Jurgensen, M. F., Storer, A. J., & Risch, A. C. (2005). Red wood ants in North America. *Annales Zoologici Fennici*, 42(3), 235-242.
- Noyce, K. V., Kannowski, P. B., & Riggs, M. R. (1997). Black bears as ant-eaters: seasonal associations between bear myrmecophagy and ant ecology in north-central Minnesota. *Canadian Journal of Zoology*, 75(10), 1671-1686. <https://doi.org/10.1139/z97-794>
- Pekár, S., & Mayntz, D. (2014). Comparative analysis of the macronutrient content of Central European ants (Formicidae): Implications for ant-eating predators. *Journal of Insect Physiology*, 62, 32-38. <https://doi.org/10.1016/j.jinsphys.2014.01.008>

- Robbins, C. T., Christian, A. L., Vineyard, T. G., Thompson, D., Knott, K. K., Tollefson, T. N., Fidgett, A. L., & Wickersham, T. A. (2022). Ursids evolved early and continuously to be low-protein macronutrient omnivores. *Scientific Reports*, 12(1), 15251. <https://doi.org/10.1038/s41598-022-19742-z>
- Sorvari, J., & Hakkarainen, H. (2009). Forest Clear-Cutting Causes Small Workers in the Polydomous Wood Ant *Formica aquilonia*. *Annales Zoologici Fennici*, 46(6), 431-438. <https://doi.org/10.5735/086.046.0604>
- Stockan, J. A., & Robinson, E. J. H. (2016). *Wood Ant Ecology and Conservation*. Cambridge University Press.