Look Both Ways: The Influence of Floral Orientation on Feeding Behaviors of the Anna's Hummingbird

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ABSTRACT

Feeding from pendent flowers is much more costly than horizontal flowers. However, in nature there is a large proportion of hummingbird-pollinated pendent flowers. To better understand how the individual decision making impacts pollination efficiency, this paper answers how floral orientation influences the feeding behaviors of the Anna's hummingbird, by examining the differences of handling time, feeding time, and nectar depletion. Handling time was tested in a semi-controlled experiment with two male hummingbirds, where approaches to the feeder were filmed and measured for both horizontal and vertical orientations. Feeding time was tested in an observational experiment with an open access feeder of horizontal or vertical orientation. Nectar depletion was calculated from a controlled binary choice experiment with captive male hummingbirds. Differences in handling time were not statistically significant between orientations. Hummingbirds fed for significantly longer durations and consumed significantly more at horizontal feeders when given the choice between both orientations.

KEYWORDS

Calypte anna, pollination efficiency, handling time, feeding time, nectar consumption

INTRODUCTION

The physical presentation of a flower plays an important role for pollination ecology. Flowers exist in a variety of different colors, shapes, and sizes. Their morphologies are important in the attraction of particular visitors (Vaknin et al. 1996). It is understood that floral attractiveness is not based on a collection of the "best traits", but instead that floral morphologies complexly work together to influence attractiveness (Fenster et al. 2015). The interconnectedness of morphologic traits leads to specific pollination syndromes over evolutionary time, and these syndromes can benefit specific pollinators. Not a lot is understood about pollination syndromes or how certain floral traits work together to improve the attraction. However, it is important to elucidate the influence of traits on the attractiveness of flowers and efficiency of pollination, especially because pollination is such a significant system. It is estimated that global pollination services alone contribute roughly $112 billion in economic value annually (Kearns et al. 1998). Therefore, understanding how pollinators, such as hummingbirds, interact and select their feeding grounds could have larger implications.

Hummingbirds are important pollinators in California. Around 38 species of flowers, spanning 12 families, are hummingbird-pollinated in California (Grant and Grant 1996). This number of species highlights the need of hummingbirds for California pollination. Hummingbird-pollinated flowers vary in size, shape, color, and inflorescence orientation, and these traits are all involved in attraction of pollinators (Vaknin et al. 1996). Hummingbirds are also highly effective pollinators. They can pollinate bee-pollinated flowers as successfully as bees, while the opposite is not true (Castellanos et al. 2003). As hummingbirds are selective pollinators, specific traits may facilitate pollen transfer and increase pollination efficiency (Campbell et al. 1997). These traits that improve pollination efficiency may be selected for in evolutionary time and result in modified pollination syndromes; i.e., "…correlations between plant floral traits and corresponding animal traits, and the apparent strength and generality of such correlations indicate plant-pollinator co-evolution" (Pyke 2019). With time, certain pollination syndromes may have been selected evolutionarily to benefit the hummingbird pollinator.

An important factor influencing attraction to plants for hummingbirds is the energetic cost of visitation. Hummingbirds show a spontaneous preference for red-colored flowers, but when a greater reward is involved, this preference can be reversed (Meléndez-Ackerman et al. 1997). The magnitude of nectar rewards and energetic conservation may underlie many floral preferences of hummingbirds. For example, vertically downward oriented flowers impose a greater energetic cost to visit, which may impede visitation due to a lower net energetic reward (Sapir and Dudley 2013). Experiments have also shown that non-hummingbird pollinators, such as bees, flies, and moths, visit downward facing flowers significantly less frequently than horizontal flowers (Ushimaru et al. 2009; Wang et al. 2014). Additionally, there is reduced pollen transfer from these pollinators when visiting downward facing flowers (Ushimaru et al. 2009; Wang et al. 2014). These findings suggest that insect pollinators do not prefer downward facing flowers, yet interestingly, many hummingbird-pollinated flowers are downward facing.

Despite potentially decreased pollination efficiency with insect pollinators, a broad diversity of downward facing flowers are hummingbird-pollinated. In the rainforests of Los Amigos and Puerto Blest in Argentina, over 85% of the hummingbird-pollinated flora was downward facing, compared to less than 35% for insect-pollinated taxa (Aizen 2003). Further in California, some of the 38 species are mainly or solely pollinated by hummingbirds, making them a significant pollinator for these flowers (Grant and Grant 1996). This is a notable find, and while the motivation for pendent flowers may be explained by other factors, such as thermal ecology (van der Kooi et al. 2019), we cannot ignore the disproportionate ratio of hummingbird-pollinated pendent flowers. We must accordingly better understand the high proportion of downward-facing flowers in the hummingbird-pollinated flora by evaluating pollinator behaviors and preferences under controlled circumstances. One such experiment evaluated effects of preference on handling time and feeding time between vertical and horizontal floral orientations for two species of hummingbirds (Castellanos et al. 2004).

However, methods for measurement of handling time and nectar consumption were not specified, and manipulation of live flowers may have altered patterns of nectar production. It would be helpful to run a controlled experiment to more clearly determine if inflorescence orientation is a significant factor that might be evolutionarily selected to increase visitation and pollen transfer via hummingbird pollinators. Further, it would be helpful to expand upon the research of Sapir and Dudley (2013) to explore if increased energetic costs of vertical flower visitation directly impinge on bird behavioral approaches, as well as on rates of nectar consumption.

In this study, I will explore how floral orientation influences hummingbird feeding behaviors, so as to identify potentially significant influences on pollination efficiency. To evaluate this question, I will measure the handling time, defined as how long it takes for a hummingbird to approach a feeder and then actually begin feeding, to assess consequences of floral orientation. I will also measure feeding time at the flower to assess potential differences between floral orientations, as well as the effect on rates of nectar consumption. Lastly, I will create a binary choice experiment to identify if there is a preference for a particular floral orientation. I hypothesize the downward facing flowers will require longer handling times because hummingbirds require postural changes to approach and feed on such flowers, and also use more energy (Sapir and Dudley 2013). Presumably, this effect will cause the hummingbirds to drink more nectar from pendent flowers per visit and also to seek flowers with easier access to nectar.

METHODS

Experimental Set-Up

I conducted both controlled and observational experiments with Calypte anna (Anna's Hummingbird) to observe the impact of floral orientation on hummingbird feeding behaviors, including handling time, feeding time, and orientation preference. During controlled experiments, I worked with four adult male Calypte anna. I studied hummingbirds from the wild on the University of California– Berkeley's campus, located in Berkeley, California. I kept hummingbirds captive for a month during experimentation. I conducted all experiments in a plexiglas cube (0.9 x 0.9 x 0.9 m) with a wood perch. The plexiglas tube was partially covered with mesh camouflage. I offered hummingbirds feeders, or plastic syringes filled with 20% (by weight) sucrose nectar solution. Feeders are suspended in the air by wire and are affixed with artificial flower attachments for visibility and familiarity. Once a hummingbird completed all experiments, I weighed them and released them back into the wild. At any given time during the project, I only held one hummingbird captive.

During the observational study, visitors had access to a feeder affixed with artificial flower attachments. I placed the feeder in a plexiglas cube (0.9 x 0.9 x 0.9 m) with one side open to the wild. Visitors were not controlled, allowing both male and female Calypte anna to feedrom the feeder.

Data Collection

Handling Time

To identify if there is a significant difference in feeding behaviors between vertical and horizontal orientation of flowers, I measured the handling time. I defined handling time as the time a hummingbird is hovering in place while looking at the flower attachment on the feeder but is not feeding. I used a GoPro Hero4 with 29 FPS to capture videos of two captive male hummingbirds as they approached a feeder of either horizontal or vertical orientation. Videos were captured between 10 AM to 4 PM, during active feeding hours, and trials ranged between 20 minutes to 120 minutes. These videos were processed using BORIS and marked to measure the length of handling time.

Feeding Time

I used a motion-activated video camera to record the duration of feeding (feeding time). A Vikeri Trail Camera with 1520p resolution and 0.1 second trigger speed was set outside of the plexiglas cube. From September 29 to October 7 and on November 2, I recorded clips of hummingbirds feeding at a vertical feeder (-90º angle ± 10º). From October 17 to October 31, I recorded clips of hummingbirds feeding at a horizontal feeder (0º angle ± 10º). During these periods, the camera was continually running, and only recorded a video once it detected motion. I defined feeding time in the footage as the duration in seconds from when the hummingbird's beak was deep enough in the feeder to reach the sucrose solution until when the hummingbird started to move back and away from the feeder.

Nectar Consumption

To identify if there is a significant difference in preference for floral orientation, I measured nectar consumption in a binary choice test. A binary choice experiment tested for preferential hummingbird feeding behavior, measuring the difference in weight (g) of each feeder per trial. I placed two feeders equidistant from the wood perch. One feeder had a horizontal orientation, and the other had a vertical (pendent) orientation. In these binary choice experiments, hummingbirds have a volitional choice to approach the feeders. Syringes are weighed prior to and after a 2-hour trial to calculate the difference in nectar consumption and consumption rate. I swapped feeder positions after 1 hour to eliminate any existing side bias in the individual hummingbird. Each bird repeated this experiment at least five times. I ran experiments every Monday and Wednesday morning at 9:00 AM, when hummingbirds are active and feeding.

Statistical Analysis

To analyze the feeding behaviors data of individual hummingbirds, I used nonparametric Wilcoxon Signed-Rank tests for each experiment. To identify if there is a significant difference between handling times, I compared the handling time data for each orientation with a one-sided paired Wilcoxon test. Similarly, I compared the feeding time data for each orientation with a one-sided Wilcoxon test to determine if there is significant difference between the two orientations. Lastly, I also used a one-sided paired Wilcoxon test to compare the nectar depletion per bird for each orientation. I analyzed these paired Wilcoxon tests using R-Studio with an alpha value of 0.05.

To analyze the data amongst bird data sets, I used a nonparametric Kruskal-Wallis statistical test. By analyzing the variance between the four birds, I determined whether recorded behaviors in individual experimentation was similar amongst all birds or if there was significant variance due to hummingbird identity. To compare the handling time data, I calculated the average handling time for each bird and used the averages in a Kruskal-Wallis statistical test to identify if there is significant variance between the bird data sets. Since feeding time experiments were not controlled for bird age, gender, or identity, all visits were treated as independent and unpaired. Lastly, I used the nectar depletion data for each orientation and to compare between birds in a Kruskal-Wallis statistical test. I analyzed these Kruskal-Wallis tests using R-Studio with an alpha value of 0.05.

RESULTS

Handling Time

Analyses identified statistically non-significant differences in handling time between horizontal and vertical feeders among four individual male hummingbirds. Participants spent an average of 0.21 seconds to approach horizontal feeders and 0.20 seconds to approach vertical feeders. Differentials in time spent to approach and begin feeding at the horizontal and vertical feeders appear different between the two birds tested (Figure 1), but this difference is not significantly different amongst the two birds based on nonparametric statistical analysis.

Feeding Time

Feeding time differentials between horizontal and vertical feeders were statistically significant during the uncontrolled observational experiment. Vertical feeding time was an average of 4.29 seconds in comparison with an average of 5.35 seconds for horizontal feeding. Hummingbirds fed more time at the horizontal (p-value = 0.012). All visits to the feeders were treated as independent and unlinked visitations because bird identity was not controlled for.

Nectar Consumption

Overall hummingbirds consumed more nectar from the horizontal feeder than the vertical feeder (Figure 1). This difference in nectar consumption as a response to floral orientation was statistically significant among the four male hummingbirds tested. Hummingbirds consumed an average of 0.90 grams more sucrose solution at the horizontal feeder. The greatest difference in consumption was bird "4", consuming an average of 1.94 grams more solution from the horizontal feeder than the vertical feeder. These differences are present in all bird participants (p-value = 0.5014) based on a Kruskal-Wallis statistical analysis. Birds 1, 2, and 3 all had a singular trial in which more nectar was consumed from the vertical feeder (Figure 4), opposing the expected results.

DISCUSSION

There is a gap in knowledge considering that there is an apparent disadvantage of pendent flowers, but there are a large number of hummingbird-pollinated pendent flowers. This research looks into how orientation impacts the feeding behaviors of birds. Handling time is non-significantly different between vertical and horizontal feeders. Feeding time is significantly longer in horizontal feeding than vertical feeding. There is a significant preference for horizontal feeding through depletion differentials. These findings are important because they explain individual-level decision making, which impacts larger pollination behaviors, in-turn having a greater impact on local speciation and possible impacts for co-evolution of flowers and their pollinators.

The lack of significant difference in vertical handling time in comparison with horizontal handling time may be a result of competing influences. In *Calypte anna*, hovering is significantly more costly than forward flight (Clark and Dudley 2010). This may support the hypothesis that vertical orientations result in longer handling times since the orientation requires more hovering time, which is more energetically costly. Effectively, the entire approach of a vertical flower requires an individual to hover. However, horizontal flowers allow for forward flight for a portion of the approach to the flower, then relies on hovering. The awkward and strained approach for vertical flowers may contribute to the increased time to approach and begin feeding. However there is a slight benefit for vertical flowers as hummingbirds are not inhibited by the angle of approach (Fenster et al. 2009). Horizontal flowers require that hummingbirds approach at a specific angle since feeding can not be completed from the sides or back of the flower. This may support the hypothesis that horizontal orientations result in longer handling times since the approach to the flower is more limited.

The longer horizontal feeding time between the vertical and horizontal orientations may be explained by what Carpenter et al. observed in *Calypte anna* feeding strategies. Hummingbirds were more likely to have different feeding strategies as a result of the role they played as defending or intruding a floral nectar source (Carpenter et al. 1991). Intruding birds were likely to consume more because they did not have a consistent nectar source and therefore drank larger crops, even at the cost of more efforted flight (Carpenter et al. 1991). This strategy contrasts greatly to that of defending birds. Defending birds drink more optimally: smaller volumes at a higher frequency. In the case of this research paper, birds played more of the role of defending birds. They were given a consistent source of nectar without the competition found in nature. This might be evidence that birds see vertical flowers as more of a stable food source or there is greater comfort associated with drinking. It is possible that vertical flowers are not as disadvantageous as previous thought. However, birds in captivity tend to drink larger crops than in the wild due to a higher reliance on flight and greater competition (Carpenter et al. 1991). While tested birds were not held captive, it is possible that this increase in food crop is a result of experimental design rather than indicative of feeding behaviors.

There is a lower metabolic cost to feed from horizontal flowers (Sapir and Dudley 2013). Feeding from vertical flowers requires a different body position, including dorsal head flexion (Sapir and Dudley 2013). Metabolic rates determined from measuring respiratory gasses revealed a significantly higher cost when feeding at vertical flowers (Sapir and Dudley 2013). In addition to the lower cost of forward flight (Clark and Dudley 2010), this may be the explanation for the observed significant preference for horizontal orientations.

Limitations and Future Directions

This research is limited as it lacks some consistency experimentally within itself. Handling time and orientation preference are both conducted in controlled experiments with birds in captivity, which help to control confounding factors but do not have the greatest external validity. Feeding time however is highly representative of what happens in nature, but there is little control to the visitors at the feeder. Additionally, this experiment is limited only to *Calypte anna* captured from UC Berkeley's campus. Extending findings to other species or birds from other locations may not be possible.

To elucidate other influences on feeding time, a controlled experiment in captivity should study the feeding time of adult male Anna's hummingbirds. This would improve comparability with the other findings within this research paper. Additionally, it could be interesting to have an observational study of hummingbirds with access to vertical and horizontal flowers.

Broader Implications

These individual behaviors are important since many floral species in California are hummingbird pollinated, meaning individuals will be able to impact the local flora (Grant and Grant 1996). This also has implications for the speciation events in California that can be shown on a smaller scale (Grant and Grant 1967). Grant and Grant found that local hummingbird migrations had an impact on the seasonal location of hummingbird-pollinated flowers in California (1967). Observing these individual-level behaviors can inform local feeding trends, which may have larger impacts across the country or the world.

These findings are also important in adding to the conversation of co-evolution and directionality of pollination syndromes that favor ornithophily (bird pollination) over entomophily (insect pollination). There have been a variety of literature that identify these shifts in directionality, divergence, and convergence in floral species that parallel their pollination sources (Thomson, James D. 2008). Hummingbird bills and flower traits match each other to benefit the hummingbird pollinator and could lead to highly specialized flower-pollinator syndromes (Rico-Guevara et al. 2021). Understanding these individual behaviors may elucidate the influences in larger trends, such as trait-matching and speciation, which will shape pollination trends as more hummingbirds are preferred as pollinators and other insects are shut out.

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APPENDIX A

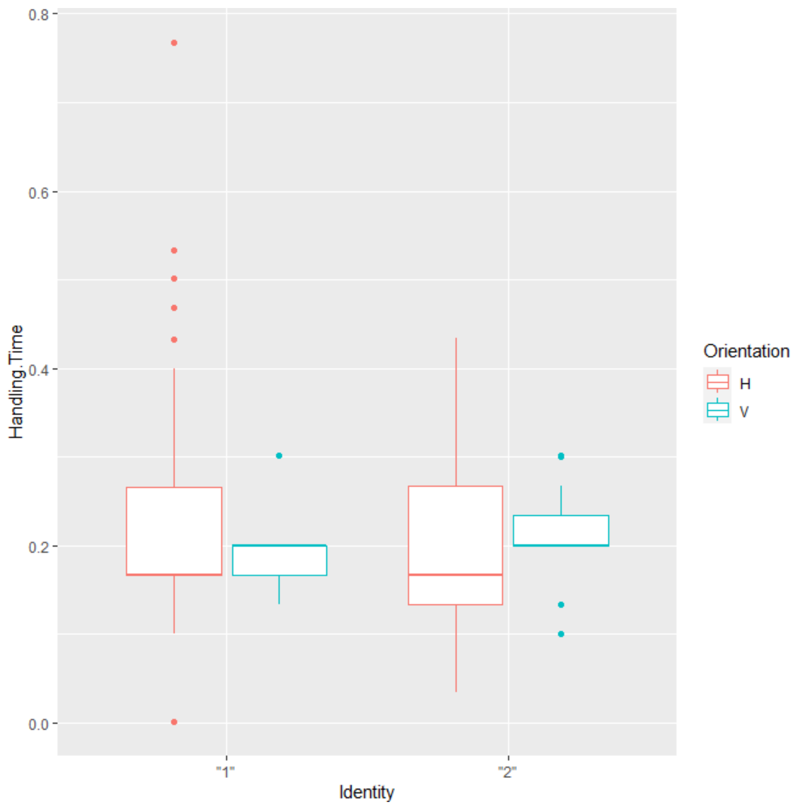

Figure 1. Handling Time in Seconds for Each Feeder Orientation. Box and whisker plot comparison for handling time between two male hummingbirds tested and sorted by orientation.

APPENDIX B

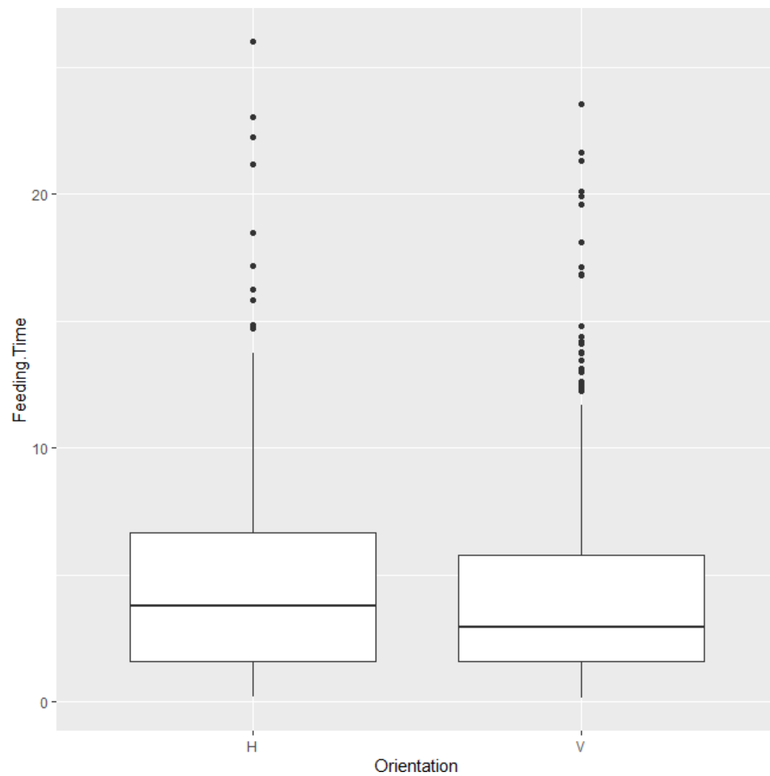

Figure 2. Feeding Time in Seconds for Each Feeder Orientation. Box and whisker plot comparison for handling time between horizontal and vertical orientations. Each visit was treated as an independent and unpaired visitation to the feeder.

APPENDIX C

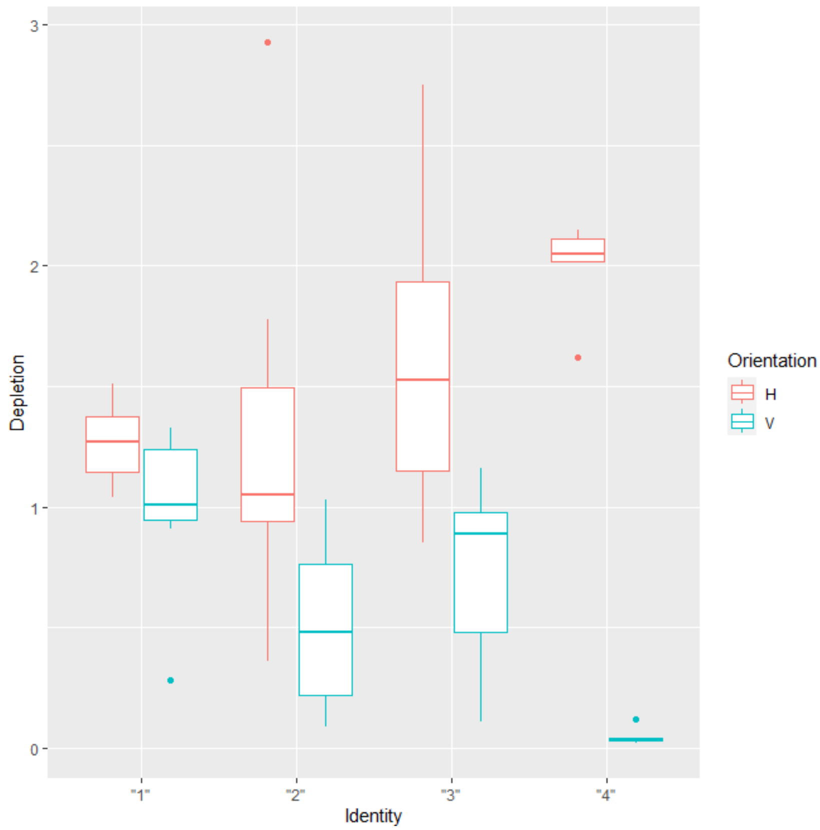

Figure 3. Nectar Depletion in Grams for Each Feeder Orientation. Box and whisker plot comparison for nectar depletion between four male hummingbirds tested and sorted by orientation.

APPENDIX D

Hummingbird Identity	Delta Mass [Horizontal (-) Vertical Depletion (g)]	Significance (p-value)
"1"	0.27	0.03906
"2"	0.81	0.02344
"3"	0.89	0.04688
"4"	1.94	0.01563

Table 1. Delta Mass of Nectar Depletion in Grams for Each Hummingbird Tested. Table displays the delta mass of nectar depletion, horizontal depletion minus vertical depletion, and the alpha values from the Wilcoxon signed rank test. Alpha values smaller than 0.05 are considered statistically significant.

APPENDIX E

Hummingbird Identity	Horizontal Feeder Starting Weight (g)	Horizontal Feeder End Weight (g)	Nectar Consumed (g)	Vertical Feeder Starting Weight (g)	Vertical Feeder End Weight (g)	Nectar Consumed (g)	Delta Mass [Horizontal (-) Vertical Depletion (g)]
"1"	6.19	5.08	1.11	6.27	5.36	0.91	0.2
"1"	6.46	5.28	1.18	6.25	5.24	1.01	0.17
"1"	6.37	5.33	1.04	6.2	5.04	1.16	-0.12
"1"	6.32	4.98	1.34	6.1	4.77	1.33	0.01
"1"	6.25	4.74	1.51	6.12	5.84	0.28	1.23
"1"	6.26	4.85	1.41	6.41	5.09	1.32	0.09
"1"	6.59	5.32	1.27	6.77	5.79	0.98	0.29
"2"	16.48	13.55	2.93	17.76	17.28	0.48	2.45
"2"	17.29	16.36	0.93	17.03	16	1.03	-0.1
"2"	17.72	16.67	1.05	16.9	16.08	0.82	0.23
"2"	18.36	16.58	1.78	18.29	18.2	0.09	1.69
"2"	17.23	16.28	0.95	17.87	17.73	0.14	0.81
"2"	19.01	18.65	0.36	19.55	19.25	0.3	0.06
"2"	18.68	17.47	1.21	17.68	16.97	0.71	0.5
"3"	18.08	16.01	2.07	17.43	16.27	1.16	0.91
"3"	18.27	15.52	2.75	18.86	18.75	0.11	2.64
"3"	17.55	16.02	1.53	17.63	16.76	0.87	0.66
"3"	18	16.97	1.03	18.53	17.53	1	0.03
"3"	18.02	17.17	0.85	18.21	17.3	0.91	-0.06

“3”	18.17	16.65	1.52	17.84	17.49	0.35	1.17
“4”	18.07	16.02	2.05	18.23	18.21	0.02	2.03
“4”	17.58	15.47	2.11	18.01	17.98	0.03	2.08
“4”	17.75	16.13	1.62	18.54	18.42	0.12	1.50
“4”	17.66	15.64	2.02	18.29	18.26	0.03	1.99

Table 2. Raw Nectar Depletion in Grams Data for Each Hummingbird Tested. Table displays the raw data from the nectar depletion experiment. Highlighted boxes show individual trials where more nectar was depleted from the vertical feeder than from the horizontal feeder.