

## Pollination Success and the Implications of Pollinator Decline

Alexandra B. Santas

### ABSTRACT

Habitat loss, environmental degradation, and intensive agricultural methods have all contributed to serious declines in insect-pollinator populations worldwide. Additionally, the effects pollinator decline may have on natural and agricultural landscapes are poorly understood. I grew two sets of branching sunflowers with one group open to pollinator access and one group netted and closed to insect pollinators. After harvesting, I removed, counted and weighed the fully developed seeds to assess the effects of pollinator exclusion on crop yield. I also took a sample of the fully developed seeds from each group to assess germination and fitness of the second generation sunflowers. I found that the exclusion of pollinators caused extremely limited fully developed seed sets per flower head (77.57 open, 7.24 closed,  $p < 0.01$ ) and reduced total fully developed seed weight (0.46 g open, 0.098 g closed,  $p < 0.01$ ). However, the individual weights of seeds were higher in the closed group (8.19 mg open, 19.70 mg closed,  $p < 0.01$ ). I also found that the closed group had a higher rate of successful germination and grew taller than seeds from the open group (1.76 cm open, 4.70 cm closed,  $p < 0.02$ ). Pollen limitation and complementarity help explain the observed declines in crop yield, and energy allotment for reproductive assurance explains the health of the closed second generation. Results suggest that pollinator decline may severely reduce crop yields and limit natural success probability by limiting pollen transfer and fertilization success.

### KEYWORDS

complementarity, pollen limitation, *Apis mellifera*, agroecology, insect-pollination

## INTRODUCTION

Animal pollination is one of the most vital ecosystem services globally with 70% of crop plants being dependent on insect pollination worldwide (Garibaldi et al. 2011). Of insect pollinators, the European Honey bee (*Apis mellifera*) is among the most widely used in agroecosystems, and although it is not native it has been naturalized on all continents except Antarctica (Mortensen et al. 2017). Yet, both wild and managed populations of honey bees, as well as non-honey bee pollinators, have declined in recent years leading to concerns over future deficits in pollination services (Reilly et al. 2020). The reasons behind global pollinator decline are numerous and include habitat loss, habitat fragmentation, pathogen spillover, and land use intensification all of which put considerable strain on managed and wild pollinator populations and make them more vulnerable to other risks like disease or predation (Garibaldi et al. 2011, Szabo et al. 2012).

Managed pollinators are bees, generally, honey bees, that are maintained by beekeepers and brought to agricultural ecosystems to supplement crop pollination (Kremen et al. 2004). Managed species are mainly used on high-intensity industrial farms which have the harshest conditions for the colonies to survive (Weibull and Ostman 2002). These farms have the highest use of fertilizers, pesticides, and large mono-cropped landscapes and make up the majority of farmland in the U.S. (Isaacs et al. 2017). These conditions have led to an average overwintering mortality rate of 26% for managed colonies in North America since 2007 (Osterman et al. 2021). At the same time, there has been a decline in wild pollinator populations due to the industrialization and expansion of agricultural land use. In the last 75 years, populations of *A. mellifera* managed and wild colonies, have declined by 50-70% (Kremen et al. 2004). These rapid declines have led to concerns over the impacts on crop yields, however, yield is only limited when pollen is limited.

In nature, pollen or pollinators, are frequently limited but, the concepts of pollen limitation and complementarity play key roles in understanding the reasons behind this phenomenon (Reilly et al. 2020). Pollen limitation is the theory that insufficient pollen receipt can result in reduced seed or fruit production (Aizen and Harder 2007). Plants must receive both enough pollen to set fruit or seed and to receive pollen ideally from an outcrossed individual to avoid inbreeding depression or other self-crossing risks (Husband and Schemske. 1996). This

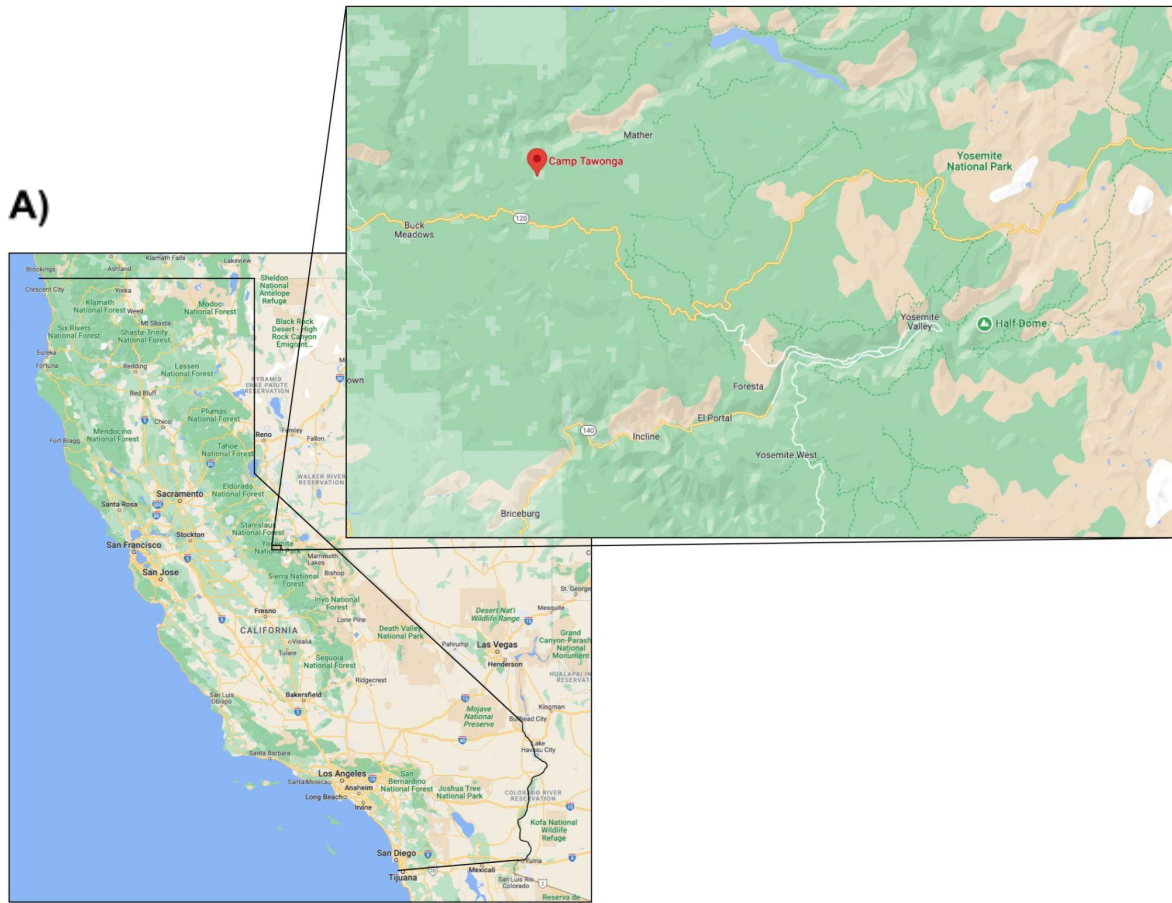
concept suggests some optimum input of pollen, both in quality and quantity, such that the plant produces a maximum output of seed or fruit. Simultaneously, plants and pollinators are co-evolved resulting in increased pollination efficiency. This co-evolution concept is called complementarity: species fit particular niches within an ecosystem and increased biodiversity tends to lead to increased efficiency of ecosystem services like pollination and food production (Hohen et al. 2008). Although the topic of pollen limitation and complementarity has been more extensively studied in natural ecosystems, little is known about their roles in agroecosystems (Isaacs et al. 2017). The potential impact of this decline in pollination services could have unknown repercussions within our food systems.

In this study, I ask what the effect of pollinator exclusion might look like on crop yield and plant persistence when compared to unsupplemented wild pollination services to simulate the effects of continuous pollinator decline. I question this on three levels by examining the effects of quantity pollen limitation, quality pollen limitation, and the role of complementarity in pollination effectiveness. I predict that the exclusion of pollinators will depress pollination via limitations compared to natural pollination services. This pollinator limitation would theoretically result in reduced seed set/yield through quantity pollen limitation, reduced seedling vigor through quality pollen limitation, and reduced effects of pollen limitation with higher pollinator species richness.

## METHODS

### Study site and species

This study took place at Camp Tawonga Farm and Garden in the Summer of 2022 (Figure 1). The latitude of the study site is 37.8537° and the longitude is -119.950592°. It is located just outside of Yosemite National Park, USA, and was chosen due to its secluded nature from intensive agriculture and its sustainable, small-scale farming practice. The average temperature highs range between 24.4-42.2 (°C) and lows between 10-22.2 (°C) and summer precipitation of 0-0.5 inches per month (measurements taken from the Jamestown weather station, KMOD, the closest station to Camp Tawonga).



**Figure 1. Figure A is a map of California and the location of Camp Tawonga and B is the Camp Tawonga garden where the sunflowers were planted.**

**Phase 1: Growing and collecting sunflower seeds and pollinator observations***Seed data collection*

I planted two groups of 6 (12 total) branching variety sunflowers (*Helianthus annuus*) after the seedlings germinated in a greenhouse for 6 weeks. After the greenhouse, I transplanted the sprouts into the main bed on July 5th and left them uncovered. Every two weeks I hand-weeded any plants starting to creep into the sunflowers bed too closely. Once the first bloom appeared on August 7th, I covered one set of 6 sunflowers with a mesh bug net (closed to pollination group) and left another group of 6 sunflowers uncovered (open to pollination group). The sunflowers grew for a total of 10 weeks until the majority of blooms had been fully open for 1-2 weeks and ray petals began to wilt away. I then harvested the sunflowers on September 11th in groups, open and closed (netted). Each flower was cut with about 4-6 inches of stem left to group sunflower heads in bunches to hang upside down and dry.

Once the sunflower heads were thoroughly dried, I removed the seeds from the heads by hand. I collected, separated, and counted both partially developed and fully developed seeds for each flower head and placed them in coin envelopes to protect them. I classified and counted partially developed seeds as those whose floret was easily removed and a portion of hardened seed had begun to form but were still light in color. I classified and counted fully developed seeds as those that had fully hardened, turned black or significantly darker, and were swollen (not wrinkled) (Figure 2). I noted flower heads that developed no seeds or suffered predation before harvest. I also weighed the partially developed and fully developed seeds of each flower head separately in grams using a high-precision laboratory scale. To assess just seed weight, I removed the weight of the coin envelope post measurements using the weight of an empty envelope.



**Figure 2: Stages of seed development.** The seed on the far left is what I classified as a fully developed seed, differentiated by having a hardened, dark shell and a swollen size. The three seeds on the left were all counted as partially developed seeds, each has a black, hardened point but lacks a swollen size or completion of the seed shells. Penny is for scale.

### *Pollinator data collection*

During the 48 hours before harvesting, I recorded observations on 6 sunflower heads in the open-pollinated test group to gather data on the number and type of pollinators visiting the sunflowers. I made a series of three observations at various times of day, 9:00-10:30 am, 12:30-2:00 pm, and 3:30-5:00 pm to observe pollinators that differ in their foraging time of day. I only counted pollinators if they landed on the head of the sunflower or landed on another pollinator on the head of that sunflower. Additionally, I identified insect pollinators in the field using the John Muir Laws Sierra Nevada field guide (Laws 2006). I noted observations about the

behavior of the various pollinators regarding interspecies interactions and heavy pollen-laden insects and noted behavior with the duration of sunflower visitation.

## **Phase 2: Growing second-generation and plant vigor observations**

### *Germination data*

This study took place in Berkeley. I randomly sampled 100 fully developed seeds from each open and closed group. I used a random number generator to select which flower head was sampled, and then again used a random number generator to select the grid sections of the flower head from which to select the fully developed seeds. I selected to sample from 2 of the 8 grid sections and had a final sample of 100 seeds from 6 open-to-pollinator flower heads and 100 seeds from 36 flower heads in the closed-to-pollinator group. I then folded groups of 11 to 12 seeds each into paper towels and placed each paper towel into a Petri dish. I rewetted the paper towels when the top layer began to dry. I made observations daily on the status of germination - indicated by the eruption of the tap root.

### *Growth rate and vigor data*

After 14 days, using a random number generator, I randomly selected 15 successfully germinated seeds from each trial group to be planted and observed. During the first two weeks of germination, I collected data every day on the total leaf length, number of leaves, and height of each plant until day 10, then data was gathered every other day. I watered them every 3-5 days when necessary.

## **Statistical Analysis**

To assess the data I removed any flower heads that failed to set at least one fully developed seed. This removal was done under the understanding of the continuous flower output that branching sunflowers have and that these flowers bloomed too closely to harvest to be able to receive pollen and set seeds (Astiz et al. 2011). After removing the data I didn't need to

analyze, I ran independent t-tests between the two groups on seed set and seed weight using RStudio (RStudio Team 2020) and the RCommander package (Fox and Bouchet-Valat 2022). I ran these tests with the untransformed, raw data and with a natural log-transformed set of data to support the validity of the raw data. To determine how seed set affected seed weight for the whole flower head, I evaluated a linear regression model on the two groups with seed set as the explanatory variable and seed weight as the response. In the second phase, I ran another independent t-test to compare the measured height and total leaf length of the two groups (open and closed).

## **RESULTS**

### **Overview of study data**

The 6 plants from each group yielded 129 flower heads from the open group and 217 flower heads from the closed group (Table 1). From those groups, I sampled 75 flower heads each, and after removing flower heads that did not set any fully developed seeds, 23 flower heads from the open group and 38 from the closed group remained. These two groups yielded a total of 1,784 (open) and 275 (closed) fully developed seeds and 6,198 (open) and 8,162 (closed) partially developed seeds. Full raw data set is available in Appendix A. For Phase 2, I then randomly sampled 100 fully developed seeds from each group for the germination test which resulted in 20 successful germinations in the open group and 31 in the closed group. Finally, of those successfully germinated, I randomly selected 15 from each group to further grow and analyze.



## Phase 1

**Table 1. Key points and information to assist in following the various sampled pools throughout the experiment.** Including the total number of grown flowers between both sets and the number sampled from each. I also included the total number of seeds collected and analyzed through both phases of the experiment.

Phase of Experiment	Attribute	Open	Closed
Phase 1	# of plants originally planted	6	6
	Total # of flower heads collected	129	217
	# of flower heads sampled	75	75
	# of flower heads setting fully developed seeds (analyzed flower heads)	23	38
	# of fully developed seeds from analyzed flower heads	1,784	275
	# of partially developed seeds from analyzed flower heads	6,198	8,162
Phase 2	# of seeds selected for germination	100	100
	# successfully germinated	20	31
	# of germinated seeds selected for further growth	15	15

### *Effect of pollinator exclusion on seed set and weight*

With the exclusion of pollinators from the pollination process I saw a strong difference between open and closed sunflower seed sets (Table 2). I found that the mean number of fully developed seeds in flowerheads excluded from pollination (closed group) was significantly lower (mean=7.24+/-10.48) than the flowerheads open to pollination (mean=77.57 +/- 99.45) with a p-value of 0.0027 (significance at  $p < 0.05$ ). The mean number of total seeds per flower head was also significantly lower in the closed (mean=222.02 +/-108.21) vs. the open group (mean=347.04 +/- 117.55)( $p=0.0001532$ ). However, the two groups showed no significant difference between partially developed seeds ( $p=0.0837$ ).

I also found significant differences in the total weight of fully developed seeds per flower head and the average weight per fully developed seed. The average weight of all of the fully

developed seeds in the open group was 0.46 +/- .59 grams compared to the closed group with an average weight of 0.098 +/- 0.11 grams ( $p=0.0082$ ). However, interestingly, when looking at the weight per seed, the closed group seeds (19.7 +/- 14.17 mg) were over twice as heavy as the open-pollinated seeds (9.19 +/- 6.95 mg) on average. This difference was significant with a p-value of 0.000084.

Because much of these data were skewed, with large standard deviations relative to the means, I also ran the same statistical tests on natural log-transformed data. All measures that were significant in the raw data remained significant in the transformed data (lower half of Table 2).

For each additional seed in the open-to-pollination group there was an average increase in weight by 0.005 g ( $R^2 = 0.7959$ ;  $p = 0.0000000108$ ;  $F = 81.9$ ) (Figure 3). In the closed-to-pollination group, there was an average increase in weight by 0.010 g for every additional seed developed ( $R^2 = 0.8048$ ;  $p = 2.469e-14$ ;  $F = 148.5$ ).

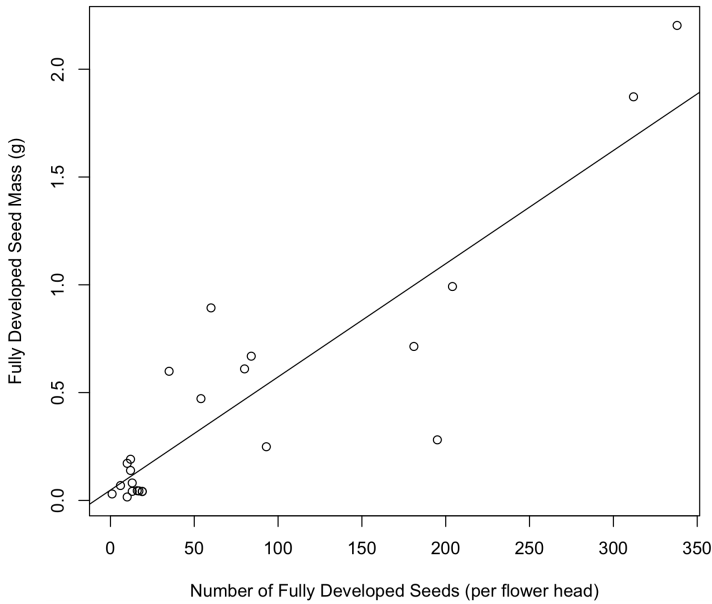
**Table 2a. Comparison of yield data between sunflower heads open and closed to insect pollination excluding sunflower heads that produced no fully developed seeds.** (N(open) = 23) (N(closed) = 38)

Pollination Status		Number Fully Developed Seeds per Flower Head	Number Partially Developed Seeds per Flower Head	Number Total Seeds per Flower Head	Total Mass of All Fully Developed Seeds per Flower Head (g)	Mass Per Fully Developed Seed per Flower Head (mg)
Open (mean +/- SD)		77.57 +/- 99.45	269.48 +/- 121.69	347.04 +/- 117.55	0.46 +/- 0.59	8.19 +/- 6.95
Closed (mean +/- SD)		7.24 +/- 10.48	214.79 +/- 108.40	222.02 +/- 108.21	0.098 +/- 0.11	19.70 +/- 14.17
T-test	t value	-3.3801	-1.7714	-4.1468	-2.8914	4.2341
	df	22.296	42.392	43.545	22.963	57.094
	p-value	0.00266*	0.08368	0.0001532*	0.008243*	0.00008434*

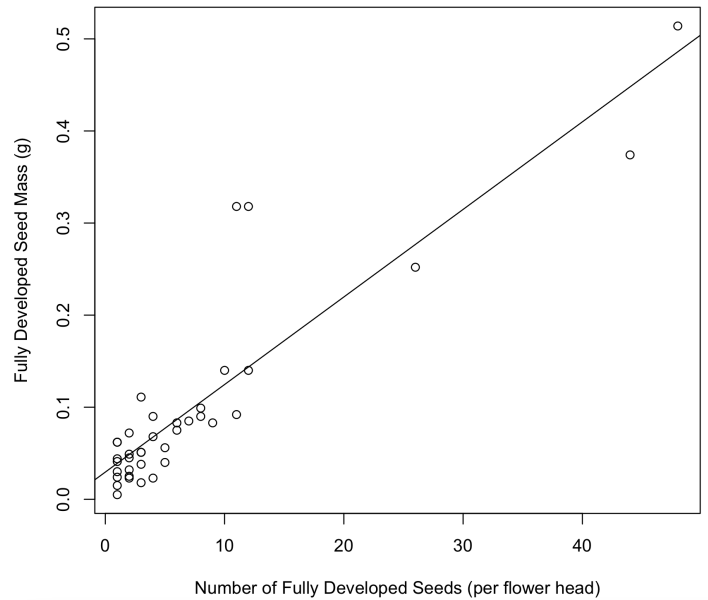
**Table 2b. Same as the above analysis but was performed using natural log-transformed data.**

Pollination Status		Number Fully Developed Seeds per Flower Head	Number Partially Developed Seeds per Flower Head	Number Total Seeds per Flower Head	Total Mass of all Fully Developed Seeds per Flower Head	Mass Per Fully Developed Seed per Flower Head
Open (mean +/- SD)		3.49+/-1.45	5.44 +/-0.69	5.749130 +/- 0.5613181	-1.645913 +/- 1.4461989	1.769261 +/- 0.8540983
Closed (mean +/- SD)		1.365684 +/- 1.062142	5.198605 +/- 0.6636801	5.240579 +/- 0.6490287	-2.768974 +/- 0.9548842	2.773132 +/- 0.6371360
T-test	t value	-6.1	-1.3245	-3.2303	-3.3127	4.8753
	df	36.261	45.303	51.831	33.745	36.831
	p-value	0.000000497*	0.192	0.002149*	0.002211*	0.00002093*

**Open to Pollination**



**Closed to Pollination**



**Figure 3. Crop yield increased with additional successful pollination and development of sunflower seed.** Each point represents one flower head.

### *Pollinator interactions*

Among pollinator observations in the open group, I noted many interesting behaviors. General trends include different species habitating a flower head at the same time, which resulted in individuals leaving the flower head. This observation is in comparison to several instances of multiple (2 or 3) honey bees sharing one flower head at once. Additionally, some of the honey bees and the resin bee (*Anthidium banningense*) had erratic flight patterns, and simultaneously heavy pollen-loaded legs or abdomens. A table of identified species, pollinators, and others, is included below (Table 4).

Family	Genus/Species	Abundance
Apidae	<i>Apis mellifera</i>	124
	<i>Bombus</i>	1
Megachilidae	<i>Anthidium banningense</i>	1
	<i>Osmia lognaria</i>	1
Halictidae	<i>Lasioglossum sisymbrii</i>	4
Anthophoridae	<i>Anthophara</i>	2
Vespidae	<i>Polistes fuscatus</i>	2
Sphecidae	<i>Chalybion californicum</i>	2
Syrphidae	<i>Eupeodes</i>	11
	<i>Allograpta obliqua</i>	4
Total		152

**Table 4. Insect species visiting sunflower flowers and their total observed abundance.**

## Phase 2

### *Effect of pollinator exclusion on second-generation fitness*

Although sunflowers closed to pollinators produced very few seeds per flower head on average compared to the open group (7.24 vs. 77.57), those seeds were more likely to successfully germinate, with 31 of 100 germinating in the closed group compared to 20 of 100 in the open group (Table 3). The seeds closed to insect pollination also grew significantly taller with an average height of 4.70 +/- 3.91 cm versus the open-to-pollination group at an average height of 1.76 +/- 1.96 cm ( $p=0.017$ ). However, there was not a significant difference in the total leaf length between the two groups ( $p=0.103$ ).

**Table 3: Comparison of fitness data (germination rate, total leaf length, height) between sunflowers open and closed to insect pollination.**

Pollination Status		Number Successfully Germinated (n=100)	Height Per Plant (cm) (n=15) (mean +/- SD)	Total Leaf Length per Plant (cm) (n=15) (mean +/- SD)
Open		20	1.76 +/- 1.96	2.19 +/- 2.44
Closed		31	4.70 +/- 3.91	4.11 +/- 3.65
T-test	t		2.6066	1.6937
	df		20.611	24.44
	p-value		0.01663*	0.103

## DISCUSSION

In this study of the effect of pollinator exclusion on plant reproductive success. I found that in the absence of wild pollinators, flower heads showed a 90% decrease in the number of fully developed seeds and a nearly 80% decrease in total seed mass. However, the average seed weight in the closed group was over twice as high. My study also looked at pollinator exclusion's effect on second-generation plant vigor. Interestingly, I found that more seeds from the closed

group successfully germinated and the second generation from this group was significantly taller. These results support the idea that a decline in wild pollinator populations could have large implications in both natural and agricultural settings.

Two very important concepts are vital in understanding the trends and implications of plant reproductive success or failure. Pollen limitation is the theory that, as a consequence of receiving too few pollen grains, there will be a limitation in the fertilization of potential seeds (quantity limitation). Pollen limitation emphasizes that self-fertilization or mating between related parent plants can also limit seed production due to inbreeding depression or self-aborted seeds (quality limitation) (Aizen and Harder 2007). Complementarity can help explain the effects of pollen limitation. Complementarity is a concept in which different species fill different ecological niches and results in a type of maximum efficiency in resource use for different organisms (Albrecht et al. 2007). These ideas are key in much of the literature that supports my findings and observed trends.

#### *Seed set and weight as a result of pollinator exclusion*

My results are consistent with those seen in several other studies. I found that there were significantly more seeds when flowers were open to pollinators. Degrandi-Hoffman and Chambers (2006) found that in two plantings of self-fertile sunflowers, there was a significant increase overall in seed set between open and bagged sunflower heads. Similarly, Perrot et al. (2019), looking at the field and individual plant yields, found significant yield increases among pollinated sunflowers vs non-insect pollinated sunflowers. Other studies have also shown decreases in per seed mass as a trade-off with increases in seed set; Tamburini et al. (2017) found that increased levels of pollination decreased the weight of 1000 seeds which is consistent with my findings.

These studies, and my own, support the concept of quantity pollen limitation, where seed set can be influenced by the number of pollen grains received. This influence of pollen quantity of seed set is what I predicted to see in my results, but I did not anticipate the weight of individual seeds being heavier in the closed group. Under the principle of quantity pollen limitation, I predicted that the closed group would result in lighter seeds from self-pollination (Aizen and Harder 2007). This self-pollination would, in principle, mean that plants may not

mature all fertilized embryos into fully developed seeds as the closed group would not benefit from the effects of outcrossing. However, resource availability and resource allocation within plants offer possible explanations for my unexpected results (Bierzuchudek 1981). In my experiment, it is possible that the sunflowers in the closed group dedicated more energy to creating additional flower heads and invested more energy into fewer seeds in order to improve the seedlings' chances of survival (Knauer et al. 2021). Heavier seeds are associated with higher energy storage and more nutrient availability to the dispersed seedling upon germination. This increased level of stored energy would guarantee the parent generation the highest likelihood of passing on genetic material under pollen limitation.

### *Interspecific interactions*

During the pollinator observations, I saw many fascinating inter and intraspecific interactions. Over 80% of the pollinators I saw were European honey bees and I saw increased levels of them during the morning hours. I also saw various predators of bees, including a couple of types of wasps but most notably *Polistes fuscatus*, which landed on some sunflowers looking for prey. The most consistent interactions happened on the face of the sunflowers. The majority of the time only one bee was present on a sunflower face at a time. Sometimes two or even three honey bees would land at once. When this happened there was no discernible change in the behavior of the bees. Assuming these bees were from the same colony and shared foraging locations, it makes sense and is consistent with honey bee behavior that they would recognize each other and not leave (Degrandi-Hoffman and Watkins 2000). Occasionally, when a bee or insect of a different species landed alongside a honey bee, the honey bee would quickly fly away, often before the other insect even landed.

These observations are consistent with several studies that have found that higher species diversity contributes to increased pollination through a mechanism called niche complementarity (Albrecht et al. 2007). Additionally, a study by Greenleaf and Kremen (2012) found that non-apis wild bees increase the pollination efficiency of honey bees by about 5 times in hybrid sunflowers. The study found that with low wild bee abundance, honey bees pollinated about 3 seeds per visit on average but with increasing wild bee abundance that increased to up to 15 seeds per visit. It was documented in Greenleaf and Kremen's (2012) study that interspecific

interactions increased the frequency of honey bee transfer between sunflowers. This increase can be attributed to the idea of niche complementarity which is well reflected in a study done by Albrecht et al. (2007). In which they compared different levels of species richness and examined pollination efficiency. Finding that up to a certain level, there were significant increases in fruit and seed set with increasing species richness especially when moving from one to three different functional groups of species. Although direct measurements of pollination efficiency were not possible in my study, the observations I made were consistent with the literature on the implications of increased species richness and offer insight to crop pollination on agricultural fields.

#### *Second generation vigor as result of pollinator exclusion*

I found no studies looking at the effects of pollinator exclusion on second-generation seed vigor. However, I did find studies that looked at the correlation between seed weight and vigor. A literature review performed by Ambika et al. (2014), references an unaccessible study by Nagaraju that showed larger seed size significantly improved plant vigor in sunflowers. Larger seeds were more likely to germinate and grew taller compared to medium and small seeds (Nagaraju 2001). Similarly, a study done by Hocking and Steer (1989) found that sunflower seedlings emerging from small seeds were significantly shorter than those of large seeds, regardless of nitrogen levels, within 14 days of emergence. This finding aligns with the well-supported relationship that heavier seeds have more nutrients stored in the endosperm, which act as an energy source for seedlings before they can perform photosynthesis, leading to higher seedling vigor.

#### *Limitations and future directions*

My study had a number of important limitations. In my open vs closed analysis, I only looked at sunflowers, so my results may not be generalizable to other species. I focused my analysis on total observations in the open and closed groups, versus analyzing the original individual plant stocks, which limited the types of analyses that I was ultimately able to do. Additionally, due to time and resource limitations, I was not able to perform as many pollinator



observations as preferable. This limited the scope of conclusions I was able to make on my observations as well as the reliability of my observations. In future studies, I would refine the methods and expand the number of pollinator observations made in similar research. Similarly, in future studies, I would recommend an increased sample size for the second-generation seedling vigor test. The relationship between pollen limitation and second-generation seedling vigor has not been studied extensively and the scope of this experiment was again limited by resources and resulted in a limited sample size.

### *Broader implications*

Looking back at the overarching goal of this project, I aimed to examine the implications of pollinator decline in agricultural and natural landscapes. I found roughly an 80% increase in crop yield under open pollination vs closed pollination when accounting for total floral output. This crop yield increase implies that not only are pollinators vital to sunflower pollination, but that wild pollinators alone can significantly increase successful pollination. Additionally, my observations are consistent with the theory that increased species diversity of pollinators can contribute to increased pollination efficiency in agricultural settings. The broader implication of this study in a natural setting suggests that pollinator decline doesn't have a significant effect on second-generation seedling vigor. However, the results of this study do suggest that in natural settings pollinator decline can greatly reduce the probability of successful seedling dispersal and thus reduce natural plant populations and their survivability.

## **ACKNOWLEDGEMENTS**

Thank you to many wonderful people who helped me throughout the process of writing my senior thesis. Thank you to Camp Tawonga and to Morgan Smith for allowing me to run my experiment on their farm and garden and for mentoring me as I learned about how to take care of my sunflowers. Thank you to Ellen Simms and the Simms Lab for allowing me to use their equipment to measure data and for being an amazing example of undergraduate support at UC Berkeley. Thank you to Patina Mendez for advising me in this project and being a mentor to not just me, but the entire Environmental Science undergraduate program. And finally, thank you to

my friends and family for being my biggest cheerleaders and for always supporting me in my academic and personal pursuits.

## REFERENCES

- Aizen, M. A., and L. D. Harder. 2007. Expanding the Limits of the Pollen-Limitation Concept: Effects of Pollen Quantity and Quality. *Ecology* 88:271–281.
- Albrecht, M., D. Kleijn, N. M. Williams, M. Tschumi, B. R. Blaauw, R. Bommarco, A. J. Campbell, M. Dainese, F. A. Drummond, M. H. Entling, D. Ganser, G. Arjen de Groot, D. Goulson, H. Grab, H. Hamilton, F. Herzog, R. Isaacs, K. Jacot, P. Jeanneret, M. Jonsson, E. Knop, C. Kremen, D. A. Landis, G. M. Loeb, L. Marini, M. McKerchar, L. Morandin, S. C. Pfister, S. G. Potts, M. Rundlöf, H. Sardiñas, A. Sciligo, C. Thies, T. Tscharntke, E. Venturini, E. Veromann, I. M. G. Vollhardt, F. Wäckers, K. Ward, D. B. Westbury, A. Wilby, M. Woltz, S. Wratten, and L. Sutter. 2020. The effectiveness of flower strips and hedgerows on pest control, pollination services and crop yield: a quantitative synthesis. *Ecology Letters* 23:1488–1498.
- Albrecht, M., B. Schmid, Y. Hautier, and C. B. Müller. 2012. Diverse pollinator communities enhance plant reproductive success. *Proceedings of the Royal Society B: Biological Sciences* 279:4845–4852.
- Ambika, S., V. Manonmani, and G. Somasundar. 2014. Review on Effect of Seed Size on Seedling Vigour and Seed Yield. *Research Journal of Seed Science* 7:31–38.
- Ashman, T.-L., T. M. Knight, J. A. Steets, P. Amarasekare, M. Burd, D. R. Campbell, M. R. Dudash, M. O. Johnston, S. J. Mazer, R. J. Mitchell, M. T. Morgan, and W. G. Wilson. 2004. Pollen Limitation of Plant Reproduction: Ecological and Evolutionary Causes and Consequences. *Ecology* 85:2408–2421.
- Astiz, V., L. A. Iriarte, A. Flemmer, and L. F. Hernández. 2011. Self-compatibility in modern hybrids of sunflower (*Helianthus annuus* L.) fruit set in open and self-pollinated (bag isolated) plants grown in two different locations. *Helia* 34:129–138.
- Bierzychudek, P. 1981. Pollinator Limitation of Plant Reproductive Effort. *The American Naturalist* 117:838–840.
- Bohart, G. E. 1972. Management of Wild Bees for the Pollination of Crops. *Annual Review of Entomology* 17:287–312.
- Colling, G., C. Reckinger, and D. Matthies. 2004. Effects of pollen quantity and quality on reproduction and offspring vigor in the rare plant *Scorzonera humilis* (Asteraceae). *American Journal of Botany* 91:1774–1782.

- Degrandi-Hoffman, G., and M. Chambers. 2006. Effects of Honey Bee (Hymenoptera: Apidae) Foraging on Seed Set in Self-fertile Sunflowers (*Helianthus annuus* L.). *Environmental Entomology* 35:1103–1108.
- DeGrandi-Hoffman, G., and J. C. Watkins. 2000. The foraging activity of honey bees (*Apis mellifera*) and non—*Apis* bees on hybrid sunflowers (*Helianthus annuus*) and its influence on cross—pollination and seed set. *Journal of Apicultural Research* 39:37–45.
- Garibaldi, L. A., I. Steffan-Dewenter, C. Kremen, J. M. Morales, R. Bommarco, S. A. Cunningham, L. G. Carvalheiro, N. P. Chacoff, J. H. Dudenhöffer, S. S. Greenleaf, A. Holzschuh, R. Isaacs, K. Krewenka, Y. Mandelik, M. M. Mayfield, L. A. Morandin, S. G. Potts, T. H. Ricketts, H. Szentgyörgyi, B. F. Viana, C. Westphal, R. Winfree, and A. M. Klein. 2011. Stability of pollination services decreases with isolation from natural areas despite honey bee visits: Habitat isolation and pollination stability. *Ecology Letters* 14:1062–1072.
- Garibaldi, L. A., I. Steffan-Dewenter, R. Winfree, M. A. Aizen, R. Bommarco, S. A. Cunningham, C. Kremen, L. G. Carvalheiro, L. D. Harder, O. Afik, I. Bartomeus, F. Benjamin, V. Boreux, D. Cariveau, N. P. Chacoff, J. H. Dudenhoffer, B. M. Freitas, J. Ghazoul, S. Greenleaf, J. Hipolito, A. Holzschuh, B. Howlett, R. Isaacs, S. K. Javorek, C. M. Kennedy, K. M. Krewenka, S. Krishnan, Y. Mandelik, M. M. Mayfield, I. Motzke, T. Munyuli, B. A. Nault, M. Otieno, J. Petersen, G. Pisanty, S. G. Potts, R. Rader, T. H. Ricketts, M. Rundlof, C. L. Seymour, C. Schuepp, H. Szentgyorgyi, H. Taki, T. Tschardtke, C. H. Vergara, B. F. Viana, T. C. Wanger, C. Westphal, N. Williams, and A. M. Klein. 2013. Wild Pollinators Enhance Fruit Set of Crops Regardless of Honey Bee Abundance. *Science* 339:1608–1611.
- Greenleaf, S. S., and C. Kremen. 2006. Wild bees enhance honey bees' pollination of hybrid sunflower. *Proceedings of the National Academy of Sciences* 103:13890–13895.
- Hevia, V., J. Bosch, F. M. Azcárate, E. Fernández, A. Rodrigo, H. Barril-Graells, and J. A. González. 2016. Bee diversity and abundance in a livestock drove road and its impact on pollination and seed set in adjacent sunflower fields. *Agriculture, Ecosystems & Environment* 232:336–344.
- Hocking, P. J., and B. T. Steer. 1989. Effects of seed size, cotyledon removal and nitrogen stress on growth and on yield components of oilseed sunflower. *Field Crops Research* 22:59–75.
- Hoehn, P., T. Tschardtke, J. M. Tylianakis, and I. Steffan-Dewenter. 2008. Functional group diversity of bee pollinators increases crop yield. *Proceedings of the Royal Society B: Biological Sciences* 275:2283–2291.
- [https://entnemdept.ufl.edu/creatures/misc/BEES/euro\\_honey\\_bee.htm#:~:text=European%20races%20of%20Apis%20mellifera,on%20all%20continents%20except%20Antarctica.](https://entnemdept.ufl.edu/creatures/misc/BEES/euro_honey_bee.htm#:~:text=European%20races%20of%20Apis%20mellifera,on%20all%20continents%20except%20Antarctica.) (n.d.). .
- Husband, B. C., and D. W. Schemske. 1996. Evolution of the Magnitude and Timing of Inbreeding Depression in Plants. *Evolution* 50:54–70.

- Isaacs, R., N. Williams, J. Ellis, T. L. Pitts-Singer, R. Bommarco, and M. Vaughan. 2017. Integrated Crop Pollination: Combining strategies to ensure stable and sustainable yields of pollination-dependent crops. *Basic and Applied Ecology* 22:44–60.
- Jadhav, J. A., K. Sreedevi, and P. R. Prasad. (n.d.). Insect pollinator diversity and abundance in sunflower ecosystem:8.
- Knauer, A. C., H. Kokko, and F. P. Schiestl. 2021. Pollinator behaviour and resource limitation maintain honest floral signalling. *Functional Ecology* 35:2536–2549.
- Kremen, C., N. M. Williams, R. L. Bugg, J. P. Fay, and R. W. Thorp. 2004. The area requirements of an ecosystem service: crop pollination by native bee communities in California: Area requirements for pollination services to crops. *Ecology Letters* 7:1109–1119.
- Muhammad, W. 2021. Pollinator Community of Sunflower (*Helianthus annuus* L.) and its Role in Crop Reproductive Success. *Asian Journal of Agriculture and Biology*.
- Osterman, J., M. A. Aizen, J. C. Biesmeijer, J. Bosch, B. G. Howlett, D. W. Inouye, C. Jung, D. J. Martins, R. Medel, A. Pauw, C. L. Seymour, and R. J. Paxton. 2021. Global trends in the number and diversity of managed pollinator species. *Agriculture, Ecosystems & Environment* 322:107653.
- Oz, M., A. Karasu, I. Cakmak, A. T. Goksoy, and Z. Metin. (n.d.). Effects of honeybee (*Apis mellifera*) pollination on seed set in hybrid sunflower (*Helianthus annuus* L.):7.
- Parker, F. D. 1981. Sunflower Pollination: Abundance, Diversity and Seasonality of Bees and Their Effect on Seed Yields. *Journal of Apicultural Research* 20:49–61.
- Pellmyr, O. 1992. Evolution of insect pollination and angiosperm diversification. *Trends in Ecology & Evolution* 7:46–49.
- Perrot, T., S. Gaba, M. Roncoroni, J.-L. Gautier, A. Saintilan, and V. Bretagnolle. 2019. Experimental quantification of insect pollination on sunflower yield, reconciling plant and field scale estimates. *Basic and Applied Ecology* 34:75–84.
- Reilly, J. R., D. R. Artz, D. Biddinger, K. Bobiwash, N. K. Boyle, C. Brittain, J. Brokaw, J. W. Campbell, J. Daniels, E. Elle, J. D. Ellis, S. J. Fleischer, J. Gibbs, R. L. Gillespie, K. B. Gundersen, L. Gut, G. Hoffman, N. Joshi, O. Lundin, K. Mason, C. M. McGrady, S. S. Peterson, T. L. Pitts-Singer, S. Rao, N. Rothwell, L. Rowe, K. L. Ward, N. M. Williams, J. K. Wilson, R. Isaacs, and R. Winfree. 2020. Crop production in the USA is frequently limited by a lack of pollinators. *Proceedings of the Royal Society B: Biological Sciences* 287:20200922.
- Tahir, M. H. N., and S. S. Mehdi. (n.d.). Evaluation of Open Pollinated Sunflower (*Helianthus annuus* L.) Populations Under Water Stress and Normal Conditions.
- Tamburini, G., F. Lami, and L. Marini. 2017. Pollination benefits are maximized at intermediate nutrient levels. *Proceedings of the Royal Society B: Biological Sciences* 284:20170729.

- Todesco, M., N. Bercovich, A. Kim, I. Imerovski, G. L. Owens, Ó. Dorado Ruiz, S. V. Holalu, L. L. Madilao, M. Jahani, J.-S. Légaré, B. K. Blackman, and L. H. Rieseberg. 2022. Genetic basis and dual adaptive role of floral pigmentation in sunflowers. *eLife* 11:e72072.
- Venjakob, C., A.-M. Klein, A. Ebeling, T. Tschardtke, and C. Scherber. 2016. Plant diversity increases spatio-temporal niche complementarity in plant-pollinator interactions. *Ecology and Evolution* 6:2249–2261.
- Weibull, A.-C., and Ö. Östman. 2003. Species composition in agroecosystems: The effect of landscape, habitat, and farm management. *Basic and Applied Ecology* 4:349–361.
- Young, A. G., L. M. Broadhurst, and P. H. Thrall. 2012. Non-additive effects of pollen limitation and self-incompatibility reduce plant reproductive success and population viability. *Annals of Botany* 109:643–653.

## APPENDIX A: RAW DATA

FLOWER	STATUS	PREDATION	NUM_FULL	NUM_PARTIAL	NUM_TOTAL	PERCENT_FULL	MASS_FULL (g)	MASS_FULL_PERSEED(mg)	MASS_PARTIAL (g)	MASS_PARTIAL_PERSEED (mg)
1	CLOSED	no	26	274	300	8.67	1.5647	9.70	1.79	1.75
2	CLOSED	no	0	0	0	0.00	0	0.00	0.00	0.00
3	CLOSED	yes	1	50	51	1.96	1.3743	61.80	1.46	2.86
4	CLOSED	yes	2	26	28	7.14	1.3448	16.15	1.37	2.33
5	CLOSED	no	0	28	28	0.00	0	0.00	1.39	2.75
6	CLOSED	no	3	139	142	2.11	1.35	12.50	1.52	1.51
7	CLOSED	yes	2	219	221	0.90	1.361	24.25	1.72	1.87
8	CLOSED	no	48	108	156	30.77	1.826	10.70	1.51	1.81
9	CLOSED	no	8	289	297	2.69	1.412	12.44	1.90	2.02
10	CLOSED	no	0	0	0	0.00	0	0.00	0.00	0.00
11	CLOSED	no	44	160	204	21.57	1.6867	8.50	1.58	1.66
12	CLOSED	no	4	64	68	5.88	1.3351	5.65	1.40	1.33
13	CLOSED	no	4	351	355	1.13	1.3803	16.95	1.69	1.08
14	CLOSED	yes	1	100	101	0.99	1.3272	14.70	1.49	1.78
15	CLOSED	yes	0	82	82	0.00	0	0.00	1.51	2.40
16	CLOSED	no	2	350	352	0.57	1.3843	35.90	1.82	1.45
17	CLOSED	no	0	96	96	0.00	0	0.00	1.49	1.82
18	CLOSED	no	2	232	234	0.85	1.3351	11.30	1.79	2.05
19	CLOSED	no	0	26	26	0.00	0	0.00	1.38	2.55
20	CLOSED	no	1	119	120	0.83	1.3363	23.80	1.53	1.82
21	CLOSED	no	3	163	166	1.81	1.3633	16.93	1.55	1.48
22	CLOSED	yes	0	159	159	0.00	0	0.00	1.48	1.07
23	CLOSED	no	0	219	219	0.00	0	0.00	1.59	1.26
24	CLOSED	no	1	241	242	0.41	1.3741	61.60	1.70	1.59
25	CLOSED	no	3	101	104	2.88	1.3304	5.97	1.54	2.26
26	CLOSED	no	6	302	308	1.95	1.3877	12.53	1.71	1.33
27	CLOSED	yes	0	93	93	0.00	0	0.00	1.56	2.66
28	CLOSED	yes	9	144	153	5.88	1.3953	9.20	1.55	1.62
29	CLOSED	no	0	44	44	0.00	0	0.00	1.41	2.14
30	CLOSED	no	11	207	218	5.05	1.4042	8.34	1.59	1.33
31	CLOSED	no	1	308	309	0.32	1.3569	44.40	1.73	1.37
32	CLOSED	no	0	35	35	0.00	0	0.00	1.35	1.09
33	CLOSED	no	0	92	92	0.00	0	0.00	1.45	1.50
34	CLOSED	no	0	83	83	0.00	0	0.00	1.44	1.57
35	CLOSED	no	5	244	249	2.01	1.3522	7.94	1.62	1.27
36	CLOSED	no	3	391	394	0.76	1.4236	37.03	1.91	1.53
37	CLOSED	no	1	377	378	0.26	1.3428	30.30	1.93	1.63
38	CLOSED	no	11	397	408	2.70	1.6304	28.90	1.99	1.71
39	CLOSED	no	0	98	98	0.00	0	0.00	1.43	1.24
40	CLOSED	yes	1	149	150	0.67	1.3171	4.60	1.55	1.57
41	CLOSED	no	0	83	83	0.00	0	0.00	1.44	1.55
42	CLOSED	no	0	299	299	0.00	0	0.00	1.79	1.59
43	CLOSED	no	0	63	63	0.00	0	0.00	1.40	1.43
44	CLOSED	no	0	231	231	0.00	0	0.00	1.52	0.88
45	CLOSED	no	0	134	134	0.00	0	0.00	1.53	1.63
46	CLOSED	no	0	115	115	0.00	0	0.00	1.50	1.66
47	CLOSED	no	5	366	371	1.35	1.3682	11.14	1.86	1.50

48	CLOSED	no	3	267	270	1.11	1.3639	17.13	1.68	1.36
49	CLOSED	yes	8	178	186	4.30	1.4021	11.20	1.62	1.75
50	CLOSED	no	0	232	232	0.00	0	0.00	1.78	2.03
51	CLOSED	yes	7	249	256	2.73	1.3973	12.11	1.73	1.68
52	CLOSED	no	0	75	75	0.00	0	0.00	1.44	1.72
53	CLOSED	no	0	73	73	0.00	0	0.00	1.41	1.36
54	CLOSED	yes	0	23	23	0.00	0	0.00	1.39	3.29
55	CLOSED	no	10	147	157	6.37	1.4528	14.03	1.60	1.98
56	CLOSED	no	0	240	240	0.00	0	0.00	1.61	1.25
57	CLOSED	no	0	128	128	0.00	0	0.00	1.48	1.33
58	CLOSED	yes	0	23	23	0.00	0	0.00	1.40	3.87
59	CLOSED	no	0	439	439	0.00	0	0.00	1.88	1.29
60	CLOSED	no	0	47	47	0.00	0	0.00	1.40	1.87
61	CLOSED	no	0	37	37	0.00	0	0.00	1.38	1.92
62	CLOSED	no	12	154	166	7.23	1.4529	11.70	1.65	2.16
63	CLOSED	yes	6	209	215	2.79	1.3956	13.85	1.71	1.90
64	CLOSED	no	0	271	271	0.00	0	0.00	1.76	1.65
65	CLOSED	no	0	84	84	0.00	0	0.00	1.49	2.06
66	CLOSED	no	0	121	121	0.00	0	0.00	1.42	0.90
67	OPEN	yes	60	185	245	24.49	2.2052	14.88	2.37	5.71
68	OPEN	no	181	158	339	53.39	2.0267	3.95	1.61	1.86
69	OPEN	yes	0	41	41	0.00	0	0.00	1.40	2.02
70	OPEN	no	312	101	413	75.54	3.1844	6.00	1.52	2.08
71	OPEN	no	0	58	58	0.00	0	0.00	1.42	1.85
72	OPEN	no	93	353	446	20.85	1.5611	2.67	1.63	0.91
73	OPEN	yes	0	26	26	0.00	0	0.00	1.40	3.38
74	OPEN	no	0	216	216	0.00	0	0.00	1.43	0.52
75	OPEN	no	19	356	375	5.07	1.354	2.18	1.55	0.66
76 (B)	OPEN	no	0	274	274	0.00	0	0.00	1.46	0.55
77	OPEN	no	0	123	123	0.00	0	0.00	1.41	0.79
78	OPEN	yes	0	61	61	0.00	0	0.00	1.42	1.79
79	OPEN	yes	0	73	73	0.00	0	0.00	1.43	1.67
80	OPEN	no	0	28	28	0.00	0	0.00	1.41	3.39
81	OPEN	no	0	190	190	0.00	0	0.00	1.45	0.71
82	OPEN	no	13	211	224	5.80	1.3931	6.20	1.67	1.68
83	OPEN	no	0	0	0	0.00	0	0.00	0.00	0.00
84	OPEN	no	0	19	19	0.00	0	0.00	1.37	2.86
85	OPEN	no	84	241	325	25.85	1.9811	7.96	1.75	1.80
86	OPEN	no	195	348	543	35.91	1.5932	1.44	1.74	1.22
87	OPEN	no	6	277	283	2.12	1.3816	11.52	1.41	0.35
88	OPEN	yes	0	298	298	0.00	0	0.00	1.68	1.23
89	OPEN	no	0	104	104	0.00	0	0.00	1.48	1.64
90	OPEN	no	0	0	0	0.00	0	0.00	0.00	0.00
91	OPEN	no	0	0	0	0.00	0	0.00	0.00	0.00
92 (D)	OPEN	no	35	327	362	9.67	1.9111	17.10	2.64	4.06
93	OPEN	no	0	117	117	0.00	0	0.00	1.50	1.62
94	OPEN	no	0	33	33	0.00	0	0.00	1.43	3.61

95	OPEN	no	0	128	128	0.00	0	0.00	1.40	0.69
96	OPEN	no	0	52	52	0.00	0	0.00	1.40	1.62
97	OPEN	no	0	135	135	0.00	0	0.00	1.40	0.65
98	OPEN	no	0	0	0	0.00	0	0.00	0.00	0.00
99	OPEN	no	204	184	388	52.58	2.3049	4.86	1.64	1.78
100 (F)	OPEN	no	0	307	307	0.00	0	0.00	1.45	0.44
101	OPEN	no	1	357	358	0.28	1.3421	29.60	1.47	0.45
102	OPEN	no	0	0	0	0.00	0	0.00	0.00	0.00
103	OPEN	no	0	0	0	0.00	0	0.00	0.00	0.00
104	OPEN	no	0	231	231	0.00	0	0.00	1.38	0.29
105	OPEN	no	0	162	162	0.00	0	0.00	1.38	0.39
106	OPEN	no	80	76	156	51.28	1.9225	7.63	1.47	2.09
107	OPEN	no	0	326	326	0.00	0	0.00	1.80	1.49
108	OPEN	no	0	89	89	0.00	0	0.00	1.40	0.97
109	OPEN	no	0	162	162	0.00	0	0.00	1.44	0.78
110	OPEN	no	0	39	39	0.00	0	0.00	1.36	1.12
111	OPEN	no	0	82	82	0.00	0	0.00	1.34	0.28
112	OPEN	no	0	0	0	0.00	0	0.00	0.00	0.00
113	OPEN	no	0	0	0	0.00	0	0.00	0.00	0.00
114	OPEN	no	338	167	505	66.93	3.5156	6.52	1.49	1.07
115	OPEN	no	19	384	403	4.71	1.3539	2.18	1.58	0.70
116	OPEN	no	13	406	419	3.10	1.3546	3.24	1.68	0.89
117	OPEN	no	0	269	269	0.00	0	0.00	1.39	0.28
118	OPEN	no	0	251	251	0.00	0	0.00	1.36	0.21
119	OPEN	no	0	0	0	0.00	0	0.00	0.00	0.00
120	OPEN	no	0	0	0	0.00	0	0.00	0.00	0.00
121	OPEN	no	10	255	265	3.77	1.4848	17.23	2.27	3.76
122 (E)	OPEN	no	10	414	424	2.36	1.3283	1.58	1.50	0.46
123	OPEN	no	16	386	402	3.98	1.3578	2.83	1.54	0.60
124	OPEN	no	0	200	200	0.00	0	0.00	1.35	0.21
125	OPEN	no	0	259	259	0.00	0	0.00	1.42	0.41
126	OPEN	no	0	0	0	0.00	0	0.00	0.00	0.00
127	OPEN	no	12	442	454	2.64	1.5032	15.89	2.43	2.53
128 (C)	OPEN	no	0	230	230	0.00	0	0.00	1.36	0.22
129	OPEN	no	17	396	413	4.12	1.3568	2.61	1.56	0.62
130	OPEN	no	0	389	389	0.00	0	0.00	1.58	0.68
131	OPEN	no	0	315	315	0.00	0	0.00	1.39	0.24
132	OPEN	no	0	0	0	0.00	0	0.00	0.00	0.00
133	OPEN	no	0	0	0	0.00	0	0.00	0.00	0.00
134	OPEN	no	0	0	0	0.00	0	0.00	0.00	0.00
135	OPEN	no	54	150	204	26.47	1.7845	8.74	1.81	3.34
136	OPEN	no	12	24	36	33.33	1.4517	11.60	1.38	2.67
137 (A)	OPEN	no	0	304	304	0.00	0	0.00	1.42	0.34
138	OPEN	no	0	58	58	0.00	0	0.00	1.35	0.61
139	OPEN	no	0	310	310	0.00	0	0.00	1.41	0.31
140	OPEN	no	0	0	0	0.00	0	0.00	0.00	0.00
141	OPEN	no	0	0	0	0.00	0	0.00	0.00	0.00

142	CLOSED	yes	12	393	405	2.96	1.6305	26.50	2.13	2.08
143	CLOSED	no	0	219	219	0.00	0	0.00	1.64	1.51
144	CLOSED	no	1	331	332	0.30	1.3538	41.30	1.67	1.09
145	CLOSED	yes	0	311	311	0.00	0	0.00	1.81	1.60
146	CLOSED	no	4	229	233	1.72	1.4022	22.43	1.70	1.68
147	CLOSED	yes	0	282	282	0.00	0	0.00	1.59	0.99
148	CLOSED	yes	2	95	97	2.06	1.3579	22.70	1.50	1.94
149	CLOSED	no	0	151	151	0.00	0	0.00	1.55	1.60
150	CLOSED	yes	2	39	41	4.88	1.3375	12.50	1.42	2.87