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Reproductive Biology of Endangered *Thespesia populnea* in Comparison to its Common Relative *Hibiscus tiliaceus*

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ABSTRACT

Anthropogenic changes, such as deforestation and industrialization, in the world have impacted ecosystems and affected the commonness of many organisms, including plants. Plants sustain life by providing oxygen and sugars through photosynthesis. This project investigated how and why some plant species are less abundant than others by comparing the reproductive biology (including pollination, dispersal, and establishment) of two related species native to Moorea, French Polynesia: the rare *Thespesia populnea* and common *Hibiscus tiliaceus*. Floral visitors, or organisms that visit flowers, frequent *Hibiscus* more often than *Thespesia*. *Hibiscus* is also a more effective disperser than *Thespesia*. However, *Thespesia* has a higher germination rate than *Thespesia*. The presence of man-made rock walls, or walls built along the coastal shoreline to prevent erosion, impact *Thespesia* and *Hibiscus* similarly. These findings will aid in the demonstration of how humans impact parts

INTRODUCTION

As anthropogenic changes have increased, affecting the world and its ecosystems,[1,2] scientists have investigated their impact on species abundance by raising the question: What makes one species less common than another? Deborah Rabinowitz and her colleagues defined three axes of rarity, or the prevalence of species: (1) geographic range, i.e., whether an organism has a larger or smaller range on the map; (2) habitat specificity, i.e., niche breadth, or the diversity of resources and environments under which an organism can survive and reproduce; and (3) local population size, i.e., the number of individuals in a given area.[3] The interactions of these three characteristics provide insight into the different forms of rarity.

Because islands are separated from continents, often in considerable isolation, islands act as natural laboratories, allowing researchers to study organisms in simpler ecosystems to understand how they naturally survive and diversify[4] and provides the foundation to understand ecology and evolution.[4] Thus, because of these unique characteristics, an island environment is a good place to study how the natural habitat impacts rarity.[4] One such place is Moorea, French Polynesia, a high volcanic island in the Society Archipelago.

In Moorea, two native trees, Thespesia populnea and *Hibiscus* tiliaceus, provide a good contrast for addressing rarity. Both trees have important roles to Polynesian culture as they are used in tools, canoe making, fuelwood, clothing, medicine, and decoration.[5,6] Each part of the tree has different uses. For instance, *Hibiscus* flowers can be used in dentistry; Thespesia seeds can be used to treat eczema and to clear sinus infections; and the bark of Thespesia can be applied as a cast for sprain injuries (Valentine Brotherson, personal communication). Despite both belonging to the same family (Malvaceae) and having similar habitats and morphologies, Thespesia is found only along the coast, in increasingly limited places, while *Hibiscus* is distributed throughout the island along an elevational gradient. The comparison of the dispersal and establishment of these two native trees allow us to study the larger question at hand: what impacts an organism's abundance?

The fruit production of the two is quite different, suggesting that the reproductive cycle may have an impact on plant rarity. Thespesia fruit are originally green and small, but over time mature into brown, indehiscent fruits that do not release its seeds. Upon opening the fruit, one can find dozens of large seeds.[5,6] On the other hand, when the fruit of *Hibiscus* tiliaceus is dried, it dehisces, splitting into separate valves that release many tiny seeds.[5,6] Thus, to investigate what makes Thespesia less common, the reproductive cycle (pollination, dispersal, establishment, and development) of *Hibiscus* and Thespesia was compared. A major part of a plant's reproductive cycle is pollination.[7] The type and number of flora visitors, or organisms that interact with flowers, may influence the spread of each species as pollinator activity contributes to plant rarity.[8,9]In fact, Rymer et al. noted a positive correlation between pollinator effectiveness and reproductive success.[10] Moreover, a plant's reproductive cycle depends on its ability to disperse to an appropriate place and germinate. Kunin and Gaston discovered that plants with poorer dispersal abilities were often less common.[11] Thus, dispersal effectiveness is correlated with plant rarity.[12] Lastly, the ability to successfully germinate plays an important role in understanding rarity, so the germination rate of *Hibiscus* and Thespesia as well as the ability to overcome potential obstacles to reach land must be investigated. As sea waters begin to rise due to climate change, rock walls have been built along the coastal shoreline to prevent erosion, creating an unprecedented challenge for plants to reach land and germinate.[13]

In this study, two of the three axes of rarity defined previously were examined: habitat specificity and population size. To understand the habitat specificity of the two trees, the reproductive cycles of Thespesia populnea and *Hibiscus* tiliaceus were compared[7]; specifically, relationships with potential floral visitors, dispersal abilities (fruit or seed drop rates, floating times, and abilities to move across seawalls), and establishment success (germination rates). The entire shore of Cook's Bay was sampled to estimate the population size of both trees. The following hypotheses were tested through both field surveys and laboratory experiments: (1) Floral visitors frequent *Hibiscus* tiliaceus more often than Thespesia populnea. (2) Thespesia populnea is dispersal limited compared to *Hibiscus* tiliaceus with respect to fruit or seed drop rates, floating time in saltwater, and ability to cross seawalls. (3) Thespesia populnea has a lower germination rate than *Hibiscus* tiliaceus. (4) The presence of man-made rock walls[13]impacts the distribution of Thespesia populnea more than the distribution of *Hibiscus* tiliaceus.

METHODS

STUDY SITES

The study was based at the University of California, Berkeley Richard B. Gump Research Station, located in Cook's Bay on the island of Moorea, French Polynesia (17°29'25.92"S, 149°49'34.30"W) (Fig. 1). The project included both field work and laboratory work and was conducted September 2021 to November 2021.

The species of interest were the rare Thespesia populnea and the common *Hibiscus* tiliaceus. Photographs of Thespesia fruit, flower, and leaf and *Hibiscus* seed, flower, and leaf are presented in Appendix A (Appendix A, Fig. 5). Voucher specimens of both species were deposited in the University and Jepson Herbaria, UC Berkeley for permanent record.

STATISTICAL ANALYSIS

All data collected in this study was analyzed using R,14 as implemented in RStudio.[15] All statistical tests used an $\alpha = 0.05$ level.

POLLINATION

Five different, randomly selected *Hibiscus* and Thespesia trees were observed between 8:00 AM and 10:30 AM for 1-2 hours on five different days. The trees were counted around the Gump Research Station and given a number. Then, a random number generator was used to select five trees to conduct a pollinator-vegetation survey. Date, time of observation, the tree number, type of floral visitor (bird, bee, wasp, skink, or butterfly), and number of visitors present were recorded.

A Paired Wilcoxon Signed Rank Test was performed on whether the visitor-day distribution of *Hibiscus* is different from the visitor-day distribution of Thespesia.

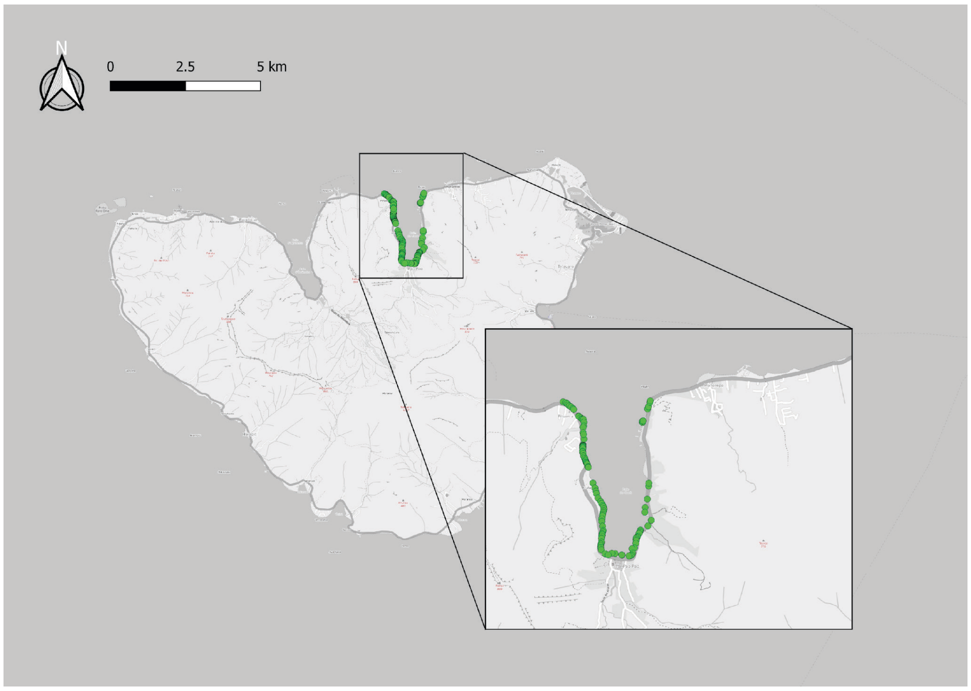

Figure 1: Sites sampled in this study around Cook's Bay.

DISPERSAL EFFECTIVENESS OF FRUITS AND SEEDS

To study the dispersal effectiveness of *Hibiscus* seeds and Thespesia fruits: (1) the fruit or seed drop rate was calculated, (2) the number of seeds present in dried *Hibiscus* flowers within 1.83 meters from the ground were counted to understand the method of *Hibiscus* seed dispersal, (3) the average length of time that the seeds and fruits can float in saltwater over a five week period was recorded, and (4) the average number of *Hibiscus* seeds and Thespesia fruits that crossed over a mock rock wall when under wave simulation was calculated.

A shade cloth was placed under two *Hibiscus* and two Thespesia trees. The shade cloth was periodically checked (morning, afternoon, and night) for a month to count the number of seeds and fruits that dropped onto the cloth. Additionally, the weather conditions (presence of wind and/or rain) as well as the start and end time of the recording was noted. The drop rate (per day per tree) of *Hibiscus* seeds and Thespesia fruits over a time interval was recorded (Fig. 2). A 95% confidence interval was used to capture the true rate of fruits and seeds dropped on the ground, on the assumption that the droppings of fruits and seeds follow the Poisson Arrival Process.[16]

For *Hibiscus* flowers within 1.83 meters from the ground, the number of seeds in the dried flowers were recorded. Then, a Mann-Whitney test was run to determine the mean dispersal amount of *Hibiscus* is greater than 0.

An experiment was performed to determine how long seeds and fruits can float in saltwater. Twenty Thespesia fruits, twenty Thespesia seeds, and twenty *Hibiscus* seeds were floated in running saltwater circulating in a cement tank with a mesh top. The number of seeds and fruits that floated, sank, or went missing were recorded. Seeds and fruits that had not touched the bottom of the tank were labeled as "floating"; seeds and fruits that had touched the bottom of the tank and remained at the bottom were labeled as "sunk"; seeds and fruits that had disappeared from the tank and could not be found were labeled as "missing". In the process of running this experiment, one Thespesia fruit was destroyed and consequently removed from the experiment.

A Fisher's Exact Test for Count Data on the number of floating, sunk, and missing fruits and seeds among Thespesia seeds and Thespesia fruits was used to test the hypothesis that the distribution of floating, sunk, and missing fruits and seeds were equally distributed between the two. A Fisher's Exact Test for Count Data on the number of floating, sunk, and missing fruits and seeds among Thespesia seeds and *Hibiscus* seeds was used to test the hypothesis that the

$q[p, d]$ is th pth quantile of the χ^2-distribution with d degrees of freedom.

$$\text{Confidence Interval: } \left(\frac{1}{2} q\left[\frac{\alpha}{2}, 2\lambda\right], \frac{1}{2} q\left[\frac{1-\alpha}{2}, 2(\lambda+1)\right] \right)$$

$$\text{Significance Level: } \alpha = 0.05$$

$$\text{Observed Drop Rate: } \lambda = 0.587 \text{ drops/day (Thespesia)}, 0.870 \text{ drops/day (Hibiscus)}$$

Figure 2: Drop rate of *Thespesia* and *Hibiscus* with 95% confidence interval calculation.

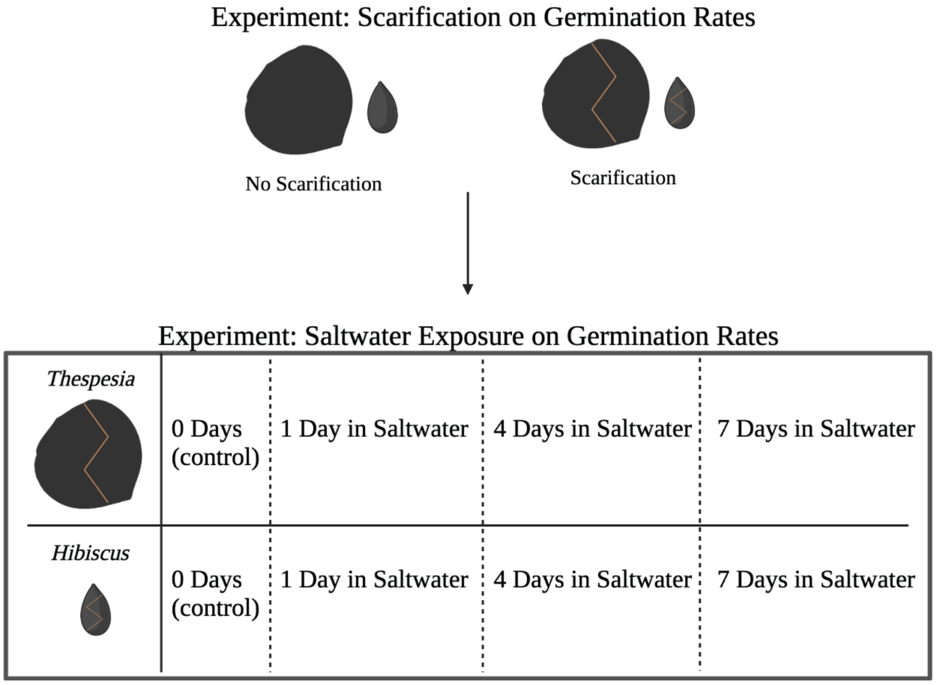

Figure 3: Germination Experiments on *Thespesia* and *Hibiscus*.

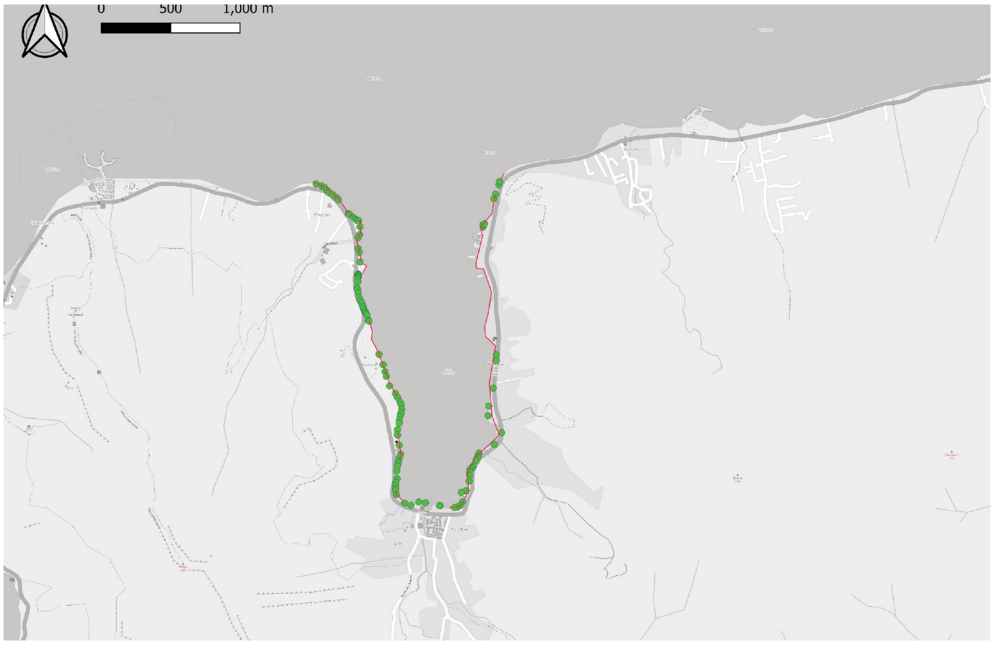

Figure 4: Sites sampled in this study around Cook's Bay with the location of man-made rock walls.

distribution of floating, sunk, and missing fruits and seeds were not equally distributed between the two. A Fisher's Exact Test for Count Data on the number of floating, sunk, and missing fruits and seeds among Thespesia seeds and *Hibiscus* seeds was used to test the hypothesis that the distribution of floating, sunk, and missing fruits and seeds were not equally distributed between the two.

Lastly, a wave simulation experiment was performed to measure the ability of twenty *Hibiscus* seeds and twenty Thespesia fruits to cross over a model rock wall. To simulate waves, a brick was placed orthogonal to the bottom of the tank and pushed downward. There were six treatments, each representing a tank filled with seawater to a different degree (37.5% filled, 41.7%, 45.8%, 50.0%, 58.3%, and 66.7%). The experiment was repeated ten times for each treatment and the number and species of seeds and fruits that crossed over or stuck to the wall was recorded. The data was divided into six groups based upon the amount of seawater. Then, a Wilcoxon Signed Rank Test was applied to each of the six datasets, comparing the crossing and/or sticking abilities of *Hibiscus* seeds and Thespesia fruits.

GERMINATION AND ESTABLISHMENT

To study how scarification affects germination rate, thirty Thespesia seeds and fifteen *Hibiscus* seeds were either scarred or left intact (control). Both groups were rinsed with freshwater and dried before a scar was made by rubbing sandpaper once on the skin of the seed. Then, the seeds were placed in damp filter paper until the seeds germinated (Fig. 3). A Chi Squared Test of Homogeneity was used to test the hypothesis that scarification affects the germination rates of both Thespesia and *Hibiscus*.

To study how the length of saltwater exposure affects the germination rate, two hundred *Hibiscus* seeds and two hundred Thespesia fruits were equally divided into a control group (no saltwater exposure) or into one of three experimental groups (1-day saltwater exposure, 4-day saltwater exposure, and 7-day saltwater exposure) (Fig. 3). After one of these four treatments, Thespesia fruits and *Hibiscus* seeds were rinsed with freshwater, then dried. Next, Thespesia fruits were carefully broken open to extract the seeds. Then, every seed was briefly scarred by rubbing sandpaper on the outer layer of the seed.

Fisher's Exact Tests for Count Data were used to test the hypothesis that the average length of saltwater exposure on germination rates will differ among the control group and the three experimental groups (1-day, 4-day, 7-day) for each species.

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I kayaked around the coast of Cook's Bay, starting from (-17.48488, -149.8293) and ending at (-17.48473, -149.81685), to map the location and number of Thespesia and *Hibiscus* within three meters from shore and to map the location of man-made rock walls (Fig. 4). For each section with and without rock walls, the number of Thespesia and *Hibiscus* were counted. Then, a Welch two-sample t-test was run to compare the number or species of trees in the presence of rock walls with those from non-rock wall sections. A Welch two-sample t-test was also used to compare the total number of Thespesia and *Hibiscus* present in Cook's Bay.

RESULTS

POLLINATION

The Paired Wilcoxon Signed Rank Test showed that the visitor-day distribution of *Hibiscus* is different from the visitor-day distribution of Thespesia: there is a statistically significant (p=0.01189) difference, indicating that the null hypothesis is rejected. The average difference between visitors of *Hibiscus* was higher than visitors of Thespesia (Appendix B, Fig. 6A).

DISPERSAL EFFECTIVENESS OF FRUITS AND SEEDS

Thespesia's drop rate (fruits per day per tree) is λ=27/46 while *Hibiscus*'s drop rate is λ=40/46. The 95% Confidence Intervals for the drop rate of Thespesia trees and *Hibiscus* trees are (0.386808, 0.8539909) and (0.6212301, 1.184101), respectively. Because, the observed drop rate for *Hibiscus* trees does not appear in the 95% confidence interval for the drop rate of the Thespesia trees, and vice versa, the difference in drop rates between the two species is statistically significant (Appendix B, Fig. 6B). *Hibiscus* had a higher average drop rate than Thespesia.

The Mann-Whitney test was used to determine that the mean seed dispersal amount of *Hibiscus* is greater than 0. The null hypothesis that the mean seed dispersal amount is equal to 0 was rejected. A 95% confidence interval of the true median was [0, 2], indicating that, on average, most or all the *Hibiscus* seeds disperse, leaving 0-2 seeds remaining (Appendix B, Fig. 6C). This test demonstrates that *Hibiscus* is an effective seed disperser.

There is no difference in the distribution of floating, sunken, and missing Thespesia fruits and Thespesia seeds (p= 0.2351). There is a difference in the distribution of floating, sunken, and missing Thespesia fruits and *Hibiscus* seeds (p=2.742e-08). There is a difference in the distribution of floating, sunken, and missing Thespesia and *Hibiscus* seeds (p=4.055e-07)(Appendix B, Fig. 6D).

The Wilcoxon Signed Rank Test indicated that, on average, *Hibiscus* seeds stuck on or crossed over the wall more often than Thespesia fruits.

GERMINATION AND ESTABLISHMENT

The Chi-Squared Test of Homogeneity showed that the distribution of germinated seeds when scarred is different from the distribution of germinated seeds without a scar (p=1.706e-07) (Appendix B, Fig. 6E).

A Fisher's Exact Test for Count Data found a statistically significant difference in the germination rates of Thespesia seeds in the control and experimental groups (p=0.0006465). The germination rates of Thespesia seeds differed when they were exposed to saltwater under varying time lengths. A Fisher's Exact Test for Count Data found no statistically significant difference in the germination rates of *Hibiscus* seeds in the four groups (p=0.2557). The germination rates of *Hibiscus* seeds did not differ when they were exposed to various time lengths of saltwater. A Fisher's Exact Test for Count Data found a statistically significant difference in the germination rates of Thespesia and *Hibiscus* seeds in the control and experimental groups (p=0.0013407499032238). The germination rates of Thespesia seeds were, on average, higher than that of *Hibiscus* seeds among the four groups.

A Fisher's Exact Test for Count Data found that there was no statistically significant difference in the germination rates of Thespesia seeds and *Hibiscus* seeds after being exposed to saltwater for one day (p=0.271415383029334). The germination rates of Thespesia and *Hibiscus* seeds did not differ after one day of saltwater exposure. A Fisher's Exact Test for Count Data found that there was no statistically significant difference in the germination rates of Thespesia seeds and *Hibiscus* seeds after being exposed to saltwater for four days (p=0.268660207522161). The germination rates of Thespesia and *Hibiscus* seeds did not differ after four days of saltwater exposure. A Fisher's Exact Test for Count Data found that there was a statistically significant difference in the germination rates of Thespesia seeds and *Hibiscus* seeds after being exposed to saltwater for seven days (p=0.0347310446394649). The germination rate of Thespesia seeds, on average, was higher than that of *Hibiscus* seeds after seven days of saltwater exposure.

RELATIONSHIP WITH MAN-MADE ROCK WALLS

There was no statistically significant difference between the presence or absence of rock walls and the number of trees of either species (p=0.82) (Appendix B, Fig. 6F).

DISCUSSION

This study examined two of the three Rabinowitz axes of rarity (habitat specificity and population size) to help understand the relative rarity of Thespesia as compared to *Hibiscus*3. The two trees' relationships with visitors, their dispersal abilities, and establishment success were compared. Fruit or seed drop rates, floating times, and their abilities to cross over a replica rock walls were observed to study their relative dispersal abilities. To study population size, the entire shore of Cook's Bay was sampled, where the distribution of Thespesia and *Hibiscus* under the presence or absence of rock walls was studied.

Hibiscus had more visits of potential pollinators and a higher variety of visitors than Thespesia. This difference may in part account for the lower abundance of Thespesia. This finding supports the idea that the type and number of pollinators may influence the spread of each species8, and that there is a positive correlation between pollinator effectiveness and reproductive success10. Since this study did not include pollinator observations, follow-up studies should examine whether effective pollen movement was occurring by each type of visitor, including studying night pollinator visits to understand the overall plant-pollinator relationships of Thespesia and *Hibiscus*. For instance, Scopece et al. showed that night pollinators, like moths, were most effective at pollinating Agarista revoluta, a coastal flowering plant.17

The relative rarity of Thespesia may also be influenced by its poorer dispersal abilities11. This study showed that, as compared to *Hibiscus*, Thespesia has a lower fruit drop rate, shorter floating time, and a weaker ability to cross over a rock wall. The differences in *Hibiscus* and Thespesia fruit morphology may play a role in their dispersal effectiveness: Thespesia fruit are indehiscent and do not release its seeds, while *Hibiscus* fruit dehisces when dry, releasing its seeds.5,6 *Hibiscus* seeds are small and light, allowing many modes of dispersal.

Furthermore, because *Hibiscus* fruits dehisce and release its seeds, there is a higher amount of *Hibiscus* propagules released compared to Thespesia fruits, contributing to a difference in drop rate. Though both Thespesia fruits and *Hibiscus* seeds float well and could reach distant destinations, the small size of *Hibiscus* seeds allows them to move more easily and to be scattered across various locations. Lastly, Thespesia fruits were unable to disperse over an obstacle, while *Hibiscus* seeds often can cross over an obstacle. This finding further suggests that *Hibiscus* seeds are more effective in dispersing than their Thespesia counterparts, providing an explanation to why *Hibiscus* is more commonly found throughout Moorea than Thespesia.

While *Hibiscus* is a more effective disperser than Thespesia, Thespesia may be more effective at germination, contrary to hypothesis number 4. This difference in germination rates could explain why, in certain regions of the coast, there are many clusters of Thespesia. Furthermore, in this study, scarification conditions that affect germination rates of *Hibiscus* and Thespesia seeds were observed. Future studies could focus on finding the optimal temperature, humidity, and other environmental factors for germination post scarification.

Lastly, along the shore of Cook's Bay, there was no significant difference in Thespesia and *Hibiscus* frequency, regardless of whether rock walls were present or absent. During a survey of Cook's Bay, some of the Thespesia trees may have been planted in privately-owned residences which may have confounded the results of the statistical test. Additional surveys noting whether the tree species was found in private residences are needed to determine if rock walls significantly impact the establishment of Thespesia and *Hibiscus* in its native environment and whether humans play a role in their distribution.

In summary, while Thespesia and *Hibiscus* share similar external morphological features and are closely related, *Hibiscus* has more effective dispersal and establishment mechanisms than Thespesia, offering a likely explanation as to why *Hibiscus* is more abundant than Thespesia. However, because Thespesia has a higher germination rate than *Hibiscus*, it still manages to maintain some population around the island. The results from this study will aid in conservation efforts, such as demonstrating how humans impact parts of the plant reproductive cycle, thus influencing their abundance and proneness to extirpation.

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APPENDIX A: Species Identification

Figure 5A: *Hibiscus* flower.

Figure 5B: *Hibiscus* leaf and fruit with seeds inside.

Figure 5E: The relative size of a *Hibiscus* seed (left) and a mature *Thespesia* fruit (right).

Figure 5C: *Thespesia* flower.

Figure 5D: *Thespesia* fruit and leaf.

Figure 5: Comparing the seeds and flowers of Thespesia and Hibiscus.

APPENDIX B: Data Analysis

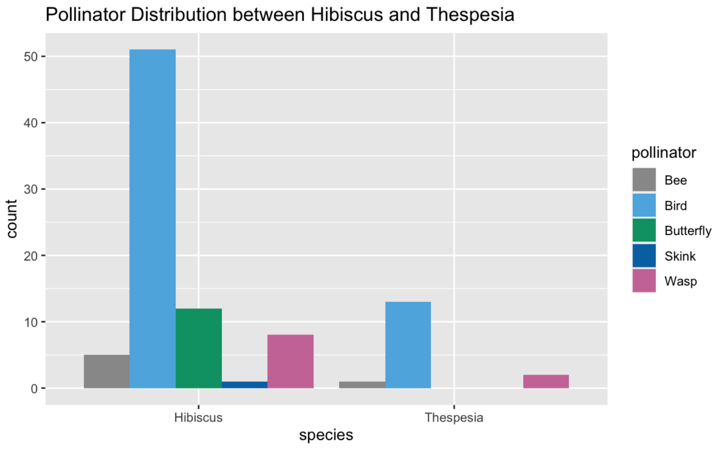

Figure 6A: Bar Graph of the Distribution of Pollinators between *Thespesia* and *Hibiscus*.

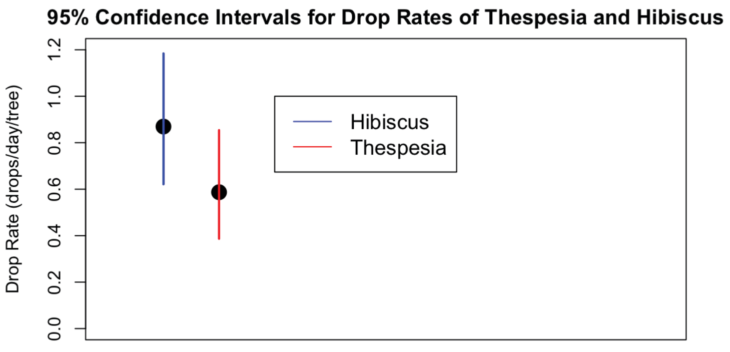

Figure 6B: A graph of the 95% Confidence Intervals for the drop rate of *Thespesia* and *Hibiscus*.

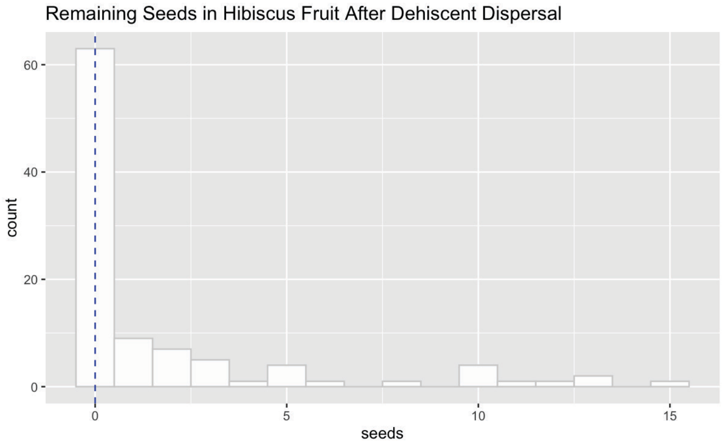

Figure 6C: A histogram of the number of seeds found in dried *Hibiscus* flowers. The blue dotted line represents the median of the histogram.

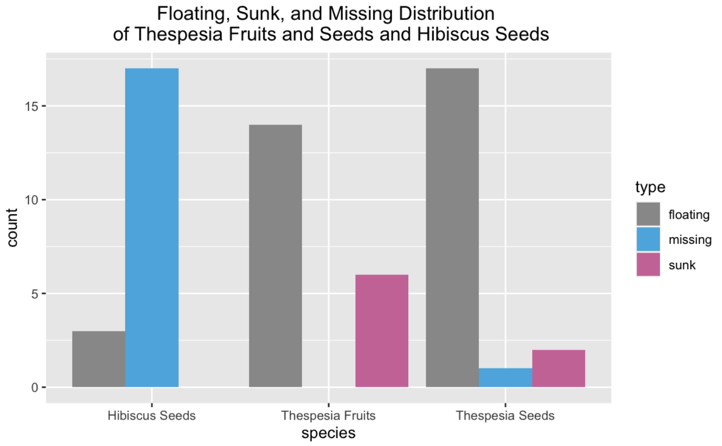

Figure 6D: Bar Graph of the Distribution of Floating, Missing, and Sunk *Thespesia* fruits and seeds and *Hibiscus* seeds.

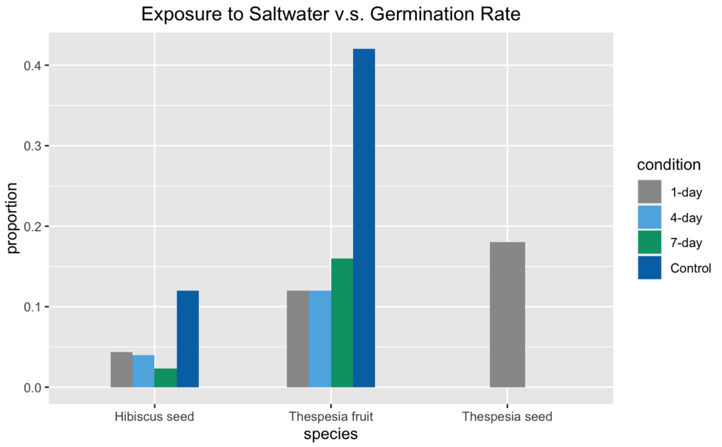

Figure 6E: Bar Graph of the Control Group and Four Experimental Groups (1-day, 4-day, 7-day, and 1-day *Thespesia* seed soaked in saltwater).

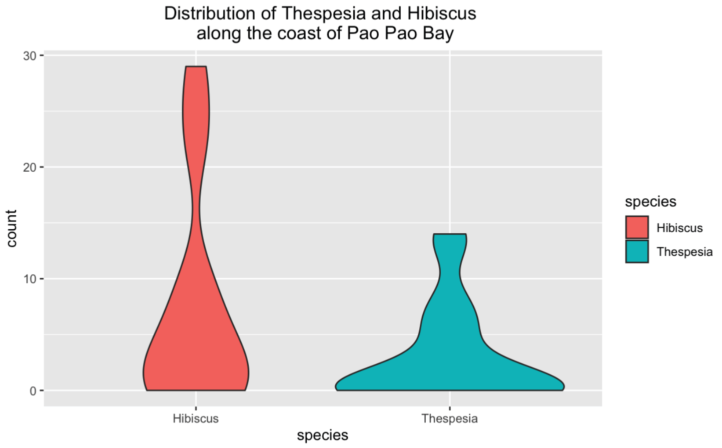

Figure 6F: Violin Plot of *Thespesia* and *Hibiscus* distributions in the presence and absence of rock walls.

Figure 6: Data analysis results from each section of my experiment: pollination, dispersal effectiveness, germination and establishment, interaction with man-made rock walls.

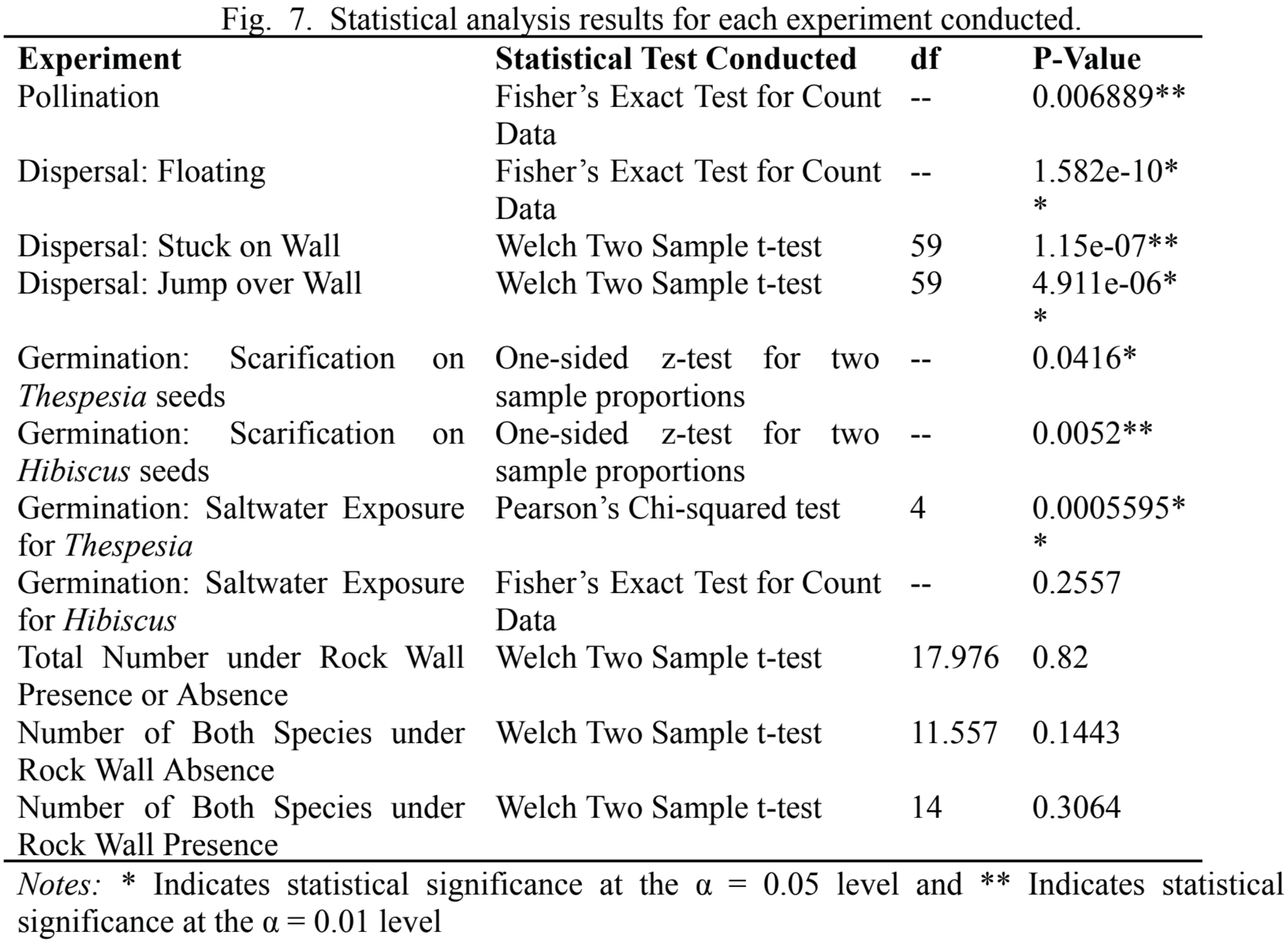

Fig. 7. Statistical analysis results for each experiment conducted.

Experiment	Statistical Test Conducted	df	P-Value
Pollination	Fisher's Exact Test for Count Data	--	0.006889**
Dispersal: Floating	Fisher's Exact Test for Count Data	--	1.582e-10**
Dispersal: Stuck on Wall	Welch Two Sample t-test	59	1.15e-07**
Dispersal: Jump over Wall	Welch Two Sample t-test	59	4.911e-06**
Germination: Scarification on *Thespesia* seeds	One-sided z-test for two sample proportions	--	0.0416*
Germination: Scarification on *Hibiscus* seeds	One-sided z-test for two sample proportions	--	0.0052**
Germination: Saltwater Exposure for *Thespesia*	Pearson's Chi-squared test	4	0.0005595**
Germination: Saltwater Exposure for *Hibiscus*	Fisher's Exact Test for Count Data	--	0.2557
Total Number under Rock Wall Presence or Absence	Welch Two Sample t-test	17.976	0.82
Number of Both Species under Rock Wall Absence	Welch Two Sample t-test	11.557	0.1443
Number of Both Species under Rock Wall Presence	Welch Two Sample t-test	14	0.3064

Notes: * Indicates statistical significance at the α = 0.05 level and ** Indicates statistical significance at the α = 0.01 level

Figure 7: Statistical analysis of the results from each section of my experiment: pollination, dispersal effectiveness, germination and establishment, interaction with man-made rock walls.