EXPLORING THE ROLE OF STIGMATIC EXUDATE IN THE WATER LILY (NYMPHACEAE) POLLINATION MECHANISM USING *N. ampla* (SALISB. DC.)

A Thesis

by

LUIS URIBE

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ABSTRACT

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Water lilies are the only known plant group that uses hyperactive nectar glands to divest pollinators of their pollen. The nectar in *Nymphaea ampla* is thought to contain secondary metabolites that increase pollen deposition possibly by modifying pollinator behavior. This was explored utilizing visitation and fecundity data from the field coupled with survival and behavioral experiments in the laboratory. Replacing nectar with water reduced seed set in *N. ampla* which was attributed to reduced visitation in water-bearing flowers and not to effects on pollinator detention time. Exposure to nectar did not reduce survivability in *Apis mellifera*. Pollen and nectar foragers of *Apis mellifera* extend their proboscis to *N. ampla* nectar in different proportions but the proportions corresponded to the response to 0.1M sucrose in both groups. Neither group drank nectar when presented at the proboscis. Exposure to nectar increased grooming and decreased walking behavior in *Apis mellifera* suggesting physiological action.

DEDICATION

To my parents, Ascencion Uribe and Natalia Uribe: thank you for the support you have given me all these years. I thank my partner Niki Bryan for giving me the drive to persevere, for putting up with the late nights, and for listening patiently as I talked endlessly about everything involving my research. To Xochi Uryan, my precious flower and guiding star.

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CHAPTER I

INTRODUCTION

1.1 Pollination Biology as a Model for the Study of Mutualisms

Seed-plants must move pollen from male to female organs to fertilize ovules in a process known as pollination (Meeuse 1961). Cross-pollination, the movement of pollen between different plants (Mader & Windelspecht 2010), is common in angiosperms because genetic variability provides protection from deleterious alleles and promotes adaptation to new and/or changing environments (Zimmer & Emlen 2013). While gymnosperms typically rely on the wind (anemophily) as a means for pollen transfer, this strategy requires the production of copious amounts of relatively light pollen (Abrahamson 1989). Most angiosperms rely, however, primarily on animal vectors (zoophily, usually by insects) to move pollen from anthers (male) to stigmas (female), one major advantage being the efficiency of animal pollen vectors, which therefore require less investment in pollen production by the plant (Mader & Windelspecht 2010). Consequently, angiosperms have developed mechanisms that prevent self-pollination (Petruzzello 2020).

Self-fertilization can be prevented by immunological means. This mechanism is called self-incompatibility in which “selfing” triggers a response at the cellular level by which female reproductive tissues terminate the growth of pollen tubes from the same flower or plant (Rea & Nasrallah 2008). Abrahamson (1989) notes the importance of spacial separation of stigma and

anthers, referred to as herkogamy, in preventing autogamy. Otherwise, temporal isolation of stamens and pistils (dichogamy) is also an efficient mechanism to avoid self-pollination (autogamy) (Abrahamson 1989). Protandry refers to the precocious release of pollen grains before the floral pistils mature, while protogyny refers to the precocious development of pistil receptivity before stamens of the same flower or plant shed pollen. Generally, one of these mechanisms is enough to limit self-fertilization (Meeuse 2021).

1.2 The Water lilies Nymphaeaceae

Members of the water lily family (Nymphaeaceae) are found in both tropical and temperate regions of the world and are often cultivated for their aesthetic, cultural, and medicinal value (Conard 1906). Evidence for the ritualistic roles of the Nile water lily, *Nymphaea nouchali* DC. are found in Egyptian funerary frescos, ceramics (Emboden 1981, McDonald 2018), and papyri such as the *Book of the Dead* (Bertol et al. 2004). In Sierra Leon, *Nymphaea* flowers are prepared as a decoction and used as a narcotic and sedative (Oliver-Bever 1983). The water lily *Nymphaea ampla* (Salisb.) DC is featured in the Dresden Codex, a Mayan text of calendrical nature that is thought to be a catalogue of divinatory plants used by priests to induce altered states of consciousness (Emboden 1981, 1983). Maya bas-reliefs and ceramics that depict libation and enema rituals using waterlilies also suggest likely pharmacological properties in these plants (de Rios et al. 1974, McDonald & Stross 2012); similar reports of recreational water lily use among contemporary Maya of Mexico lend support to chemical bioactivity (Diaz 1977).

Ecologically, water lilies in the genus *Nymphaea* are sources of pollen for beetles (Coleoptera), flies (Diptera), and bees (Hymenoptera) (Schneider 1981). Of environmental significance, water lilies can be used in the remediation of wetland environments polluted with heavy metals (Song, Huang, & Huang 2016).

The Nymphaeaceae belong to a cluster of primitive flowering-plant group clades that are called the 'basal angiosperms'. Since they have been thriving and evolving since the Early Cretaceous, they provide useful insights into the early developments of plant-animal interactions, and the rapid diversification of angiosperms in the Cretaceous period (Chen 2017). In this respect, water lilies (Nymphaceae) are of particular interest, as they exhibit a wide variety of floral adaptations for pollen vectors (Gandolfo, Nixon, Crepet 2004). The species used in this study, *Nymphaea ampla*, belongs to the most speciose and widespread genus of the family (Wiersema 1988): *Nymphaea*. Five subgenera are generally recognized in the genus: day-flowering Subgenera *Nymphaea, Brachyceras, Anecphya* and night-flowering subgenera *Hydrocallis* and *Lotos* (Conard 1906, Prance & Anderson 1976).

Individual water lily flowers (Nymphaea spp.) typically complete the process of anthesis over a period of three days. Water lilies in the night-flowering *Nymphaea* subgenus *Hydrocallis* feature starchy, thermogenic styles and are considered to be pollinated primarily by nocturnal scarab beetles in the genus *Cyclocephala* (Schneider 1981, Wiersema 1988). *Dipterans,* and *Hymenopterans* are key pollinators of diurnal water lilies, though *Coleopterans* are also known visitors (Robertson 1889, Schneider 1981). A study of the pollination biology of *Nymphaea lotus* from the Northeastern Ivory Coast in Africa reveals floral features including high variability in flower timing, nocturnal thermogenesis, and strong odors that "seem [adapted] to pollination by nocturnal beetles and diurnal bees" (Hirth & Porembski 2003).

1.3 Pollination as Non-symmetric Mutualism

Pollination is generally recognized as a classically mutualistic interaction: a pairwise interaction in which each actor increases the fitness of its mutualist through the exchange of resources or sexual services (Nepi et. al. 2018, Richman 2018, Bertin 1989). Yet incongruent

goals held by each actor in pollinator-plant interactions (e.g., plant produces pollen for reproduction and insects consume pollen for nutrition) allows for the evolution of complex competition strategies that can introduce varying degrees of resource exploitation, whereby one participant in the association develops traits that will maximize its own fitness while still providing a measured degree of benefit to its partner. These interactions are described as quasi-parasitic, exploitive, or non-symmetric mutualisms (Nepi et al. 2018). Compounds present in nectar can alter insect physiology and behavior and are thus regarded as manipulations of animal partners by the plants (Nepi et al. 2018, Wright et al. 2013). A non-symmetric/exploitative pollination scheme of such type appears to have developed in *Nymphaea ampla*.

Nymphaea ampla is a typical day-blooming water lily with three days of anthesis (Caspary 1866 *vide* Wiersema, Novelo, & Bonilla-Barbosa 2008). On the first day of anthesis, flowers feature a fluid trap in the center of a cupular pistil filled with a nectar secreted by hyperactive stigmatic papillae (Wiersema 1988, pers. obs.) [Fig. 1]. When pollinators attempt to land on the upright, slippery and flexible stamens of first-day flowers, they are often forced to slip into a fluid-trap (bath) such that flower might capture their pollen loads to facilitate pollination (Robertson 1889, Hirthe & Porembski 2003). Thousands of pollen grains that are washed off the visitors sink into the watery nectar and deposit themselves on the flower's stigmatic surface. Pollen tubes then emerge from the grains and in short course and fertilize egg cells in thousands of small ovules within the pistil's syncarpous ovaries (Capperino & Schneider 1985).

During day-2 and day-3 of anthesis, the broad, laminar stamens of water lilies turn inwardly, and consequently cover the dry stigmatic surface of the pistil. Their microsporangia (anther sacs) undergo dehiscence and release pollen grains to reward bees or flies and to

eventually fertilize ovules. Visiting insects, whose activities generally focus on the collection of pollen rewards [Fig. 2], continue during the third day of anthesis. At the end of day three, flowers close and begin to submerge themselves underwater by means of a recoiling, fleshy peduncle. A singular berry (often called a fleshy capsule) matures within a few months and eventually dehisces irregularly. Usually, many thousands of floating seeds are released from one capsule (Meeuse & Schneider 1979) the fates of which are now determined by movements of water.

This unique mechanism of pollination has been recognized as a model system of non-symmetric mutualism (Wiersema 1988). While second and third day (functionally male) flowers offer a risk-free nutritive reward in the form of pollen, there is a risk to visitors of non-rewarding first-day flowers, whose aforementioned fluid "baths" in the gynoecial disk often capture substantial portions of the insect's pollen loads (Meeuse & Schneider 1979). The role of nectar in the *Nymphaea* fluid-trap pollination scheme is still poorly understood.

1.4 Water lily nectar

Water lily nectar is very low in sugars, typically containing 1-1.5% solution of hexoses (Meeuse and Schneider 1979), which is thought to be below the detection threshold of bees (von Frish, *vide* Meeuse and Schneider 1979). According to Schmucker (*vide* Meeuse & Schneider, 1979), ions, mostly in the form of chloride salts, are also found to be in higher concentration in water lily nectar than in surrounding pond water. Ions found include boron, calcium, potassium, and magnesium. Ion concentrations, calcium in particular, have been found to be important in pollen germination, while potassium, magnesium, and sodium ions are thought to enhance the effectiveness of calcium (Brewbaker & Kwack 1963).

Various aquatic plant groups are known to alter the surface tension of surrounding water (Hardman 1941), Meeuse and Schneider (1979) demonstrated the presence of surfactants in *N. odorata* nectar and proposed hypothetically that visitors that land in the fluid trap may have their trachea fill with the fluid, which can result in suffocation. However, no chemical structure that can be identified as a surfactant has ever been identified.

Figure 1. First-day Nymphaea ampla *flower with erect stamens surrounding carpellary basin where a number of bees have perished and deposited their pollen loads.*

Figure 2. Second-day flower with inwardly reflexed stamens forming a pollen-dispensing column in the center. Flowers in this stage are mutualistic.

While insects normally escape from the fluid trap with observable effort, bees lose a significant portion of their pollen loads and a substantial percentage of the visitors drown (Conard 1905, pers. obs.), in which case they sacrifice their lives and entire pollen loads to the flower. Observations as far back as 1889 (Robertson) note that repeated dips into the stigmatic nectar can cause drowning (Meeuse & Schneider 1979). Because of the flower's first day parasitic activities, Meeuse and Schneider consider, *Nymphaea* to be exploitative (1979). Fox-Wilson (1937) reported that small amount of liquid present in the *Nymphaea capensis* can result in the drowning of *Eristalis* flies, while Meeuse and Schneider note that the small amount of liquid resulting in the drowning of such a "robust" insect "seems hardly possible" (1979), suggesting some unrecognized property in the liquid that contributes to mortality.

Because small bees exploit mutualisms by favoring male-phase flowers and thus limit pollen transfer (Koski et al 2018), adaptations that increase deliverance of pollen to a flower's stigma may develop, including use of novel toxic secondary metabolites that affect pollinator behavior and physiology (Couvillon et al. 2015). In our preliminary field observations, we noted that bees which fell repeatedly into the trap fluid have difficulty with motor coordination and, if they escape, typically spend a significant amount of time grooming on the flower petals. The observed behavior of bees following repeated exposure to water lily nectar suggests the presence of toxic compounds that induce prolonged grooming behavior and possibly interfere with motor function of visitors, leading to longer detention time within flowers.

1.5 Plant secondary metabolites in nectar

Plants produce a wide range of secondary metabolites (PSMs) whose adaptive functions in defense against herbivores and pathogens is well established (Wink 1988, Schoonhoven, Loon & Dicke 2005). The presence of these potentially toxic compounds in nectar is quizzical when

considering that the sweet fluid is generally considered a “reward” for pollinators in exchange for their pollen transfer “services”. A chemical analysis of methanolic extracts taken from 31 plant species (n=1535) in the United States found alkaloids and amines to be among the most common secondary chemical classes in pollen and nectar samples (other classes were: flavonoids, terpenoids, chlorogenic acids) (Palmer-Young et. al. 2019). With such variety among and within chemical classes whose interactions are further mediated by ecological context (Gegear 2007), it is not surprising to find a multitude of complex ecological consequences. Secondary metabolites in floral nectar present challenges and opportunities for both interactors whose effects may be mediated by a variety of ecological variables.

The presence of PSMs in nectar can be pleiotropic (Trunz et. al. 2020) or can have adaptive functions. In death camas, toxic compounds exclude generalist pollinators and effectively increase flower constancy by a specialist bee (Cane et al. 2020). Remarkably some PSMs found in nectar such as caffeine and nicotine can affect pollinator cognitive functions, increasing learning or memory (Wright et al. 2013, Mustard 2014, Couvillon et al. 2015, Barrachi et al. 2017). Our combination of field and laboratory experiments indirectly relate the effects of a possibly toxic nectar on insect behavior to the reproductive performance of the water lily, *Nymphaea ampla*.

In studies using toxins with different pharmacological modes of action, more time spent grooming and motor impairments were shown to be characteristic of toxicosis in honey bees (Hurst, Stevenson, Wright 2014). Compounds that bind to sodium-channels (pyrethroids, aconitine & grayanotoxin I) induced honey bees to stay longer in flowers, groom antennae less, and have reduced coordination of their extremities (Oliver et al. 2014). Abdomen dragging and

body curling are other ‘malaise’ behaviors that honey bees may exhibit when exposed to toxins (Hurst, Stevenson, Wright 2014).

The genus *Nymphaea* produces a wide range of secondary phytomolecules and have been proposed as a rich source of novel, pilot molecules for pharmaceuticals (Selvakumari et. al. 2016). In our model water lily, *N. ampla*, aporphine-related compounds, including nymphahaeine, nymphaline, nupharin, α- & β- nupharidine, & quercitin, have been identified in vegetative extracts (Hodge 1956, Diaz 1997, Bertol et. al. 2004). The specific ecological roles of these constituents have yet to be investigated. Moreover, the function of secondary metabolites in flower nectar remains an area of intense study (Stevenson, Nicolson, & Wright 2017, Mustard 2020, Trunz et. al. 2020).

The ecology of water lily pollination features exploitative (non-rewarding) characteristics in first-day flowers, which can either reduce the nutritive pollen loads of their visitors or drown them. The fluid in the trap serves as an important interface in the water lily pollination scheme and may contain secondary compounds that hypothetically enhance pollen deposition effectiveness of insect visitors. If so, this would increase fecundity, and thus relative fitness, as generally measured in relative numbers of successful gametes or seed-set (Zimmer & Emlen 2013).

The primary focus of our research is to probe the ability of water lily nectar to affect pollinator behavior and increase flower fecundity (or individual plant ‘fitness’). We explore this in both field & laboratory experiments using cultivated populations of *Nymphaea ampla* and *Apis mellifera*, respectively.

CHAPTER II

SELF-COMPATIBILITY AND EFFECT OF POLLINATORS

In the first set of field experiments, we sought to answer two key questions that will determine the next set of field experiments i.) is *N. ampla* self-compatible and ii.) do pollinator visitation rates and duration affect seed set.

2.1 Is *Nymphaea ampla* Self-Compatible?

We first determine whether self-incompatibility (self-immunological gamete rejection) prevents self-pollination in *N. ampla*.

2.1.1 Methods

lants used for the experiment were cultivated in artificial ponds in Edinburg, Texas (26°19'N 98°11'W). The treatment period ran for approximately two weeks, during which all available first-day flowers were sampled in order to obtain sufficient sample sizes. To investigate self-incompatibility in *N. ampla*, first-day protogynous flowers (n=64) were pollinated with the flower's own pollen by surgically extracting pollen grains from adjacent closed anthers or otherwise gathering loose pollen grains from early anther dehiscence after 3pm in the outermost stamens. These flowers were bagged before anthesis to prevent pollinator visitation over the course of the 3-day flowering period. Plastic ID tags were wired onto each peduncle for later fruit collection. Following the bagging treatments, fruits were given sufficient time to mature,

after which they were harvested and counted. Seeds that were collected from flowers that were not bagged served as the experiment control.

To collect seeds, individual carpels of the mature fruit were cut open with a scalpel to extract mature seeds. These were carefully separated from the placenta under a dissection microscope and placed into petri dishes. The seeds were then placed in front of a gentle fan to dry for approximately 5-7 days. To estimate seed-set for thousands of small seeds, we randomly selected seeds from 30 fruits and averaged the number of seeds per 0.01 grams. This factor, determined to be 27.1 seeds/0.01 grams, would then be used in the following formula to estimate seed set: $seed\ mass\ from\ fruit\ (g) \times \frac{27.1\ seeds}{0.01g} = estimated\ \#\ of\ seeds$. Estimated seed set was compared between manually-selfed flowers and unmanipulated control flowers using a nonparametric Mann-Whitney mean rank test conducted with SPSS version 27 (IBM, Armonk, NY).

2.1.2 Results

There was no significant difference in estimated seed set between unmanipulated control flowers that were visited naturally and manually-selfed flowers (p=0.5023, CI=95%), indicating that the flowers are, in fact, self-compatible [Fig. 3].

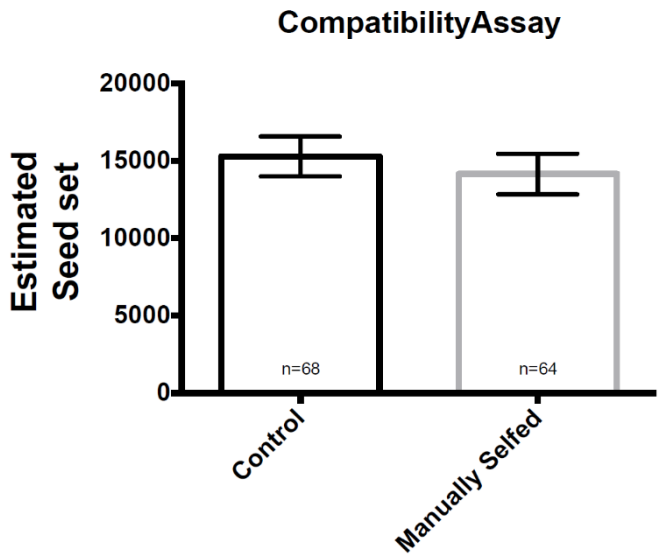

Figure 3: Manually-selfed flowers had estimated seed set similar to unmanipulated, i.e. naturally pollinated flowers (p=0.5023, CI=95%).

2.2 Effect of pollinators

Next, we investigated self-fertilization of *N. ampla* by comparing seed-set of flowers that were open to native pollinators and those that were prevented visitation (i.e., bagged).

2.2.1 Methods

First day flowers of *N. ampla* were sampled for a period of about two weeks in order to obtain sufficient sample sizes. Flowers were covered by a fine-mesh bag designed (n=54) to exclude floral visitors during their first day of anthesis. The bag was secured over the closed flower and around the peduncle early in the morning before the flowers began to open and would remain on the flower until they began to submerge after Day-3. The fruit was then allowed to mature before being collected for seed extraction. Seed extraction and subsequent drying procedure was the same as in the self-compatibility assay (see 2.1.1) The same factor (27.1 seeds/0.01 grams) was used to estimate seed set. Estimated seed set was then compared between unmanipulated (control) flowers and pollinator excluded flowers using non-parametric Mann-Whitney mean rank test.

2.2.2 Results

Bagged flowers had significantly reduced estimated seed set ($p<0.0001$, CI=95%), indicating that flowers are relatively allogamous (outcrossing), but not on account of self-incompatibility [Fig 4].

2.2.3 Conclusion

From the first two assays conducted in the field, we show that our population of *N. ampla* is self-compatible but relies heavily on pollinators to increase seed set. This indicates that *N.*

ampla is highly adapted for outcrossing, which is achieved, ostensibly, through spacial and temporal separation of the reproductive organs (herkogamy and dichogamy, respectively).

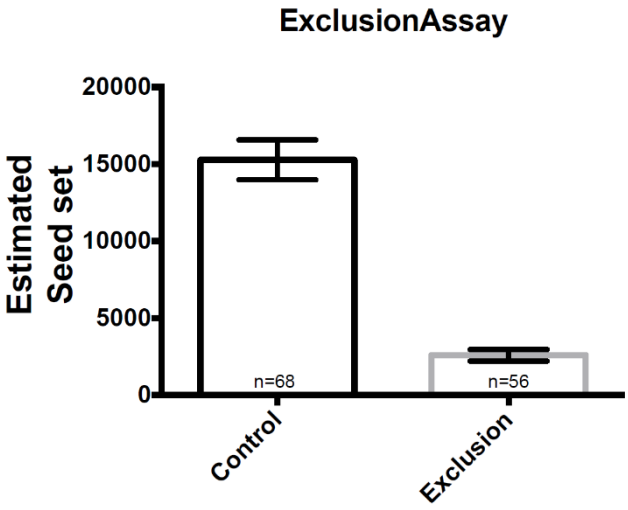

Figure 4: Excluding pollinators from N. ampla *flowers significantly reduced estimated seed set (p<0.0001, CI=95%).*

CHAPTER III

CORRELATIONS AND REGRESSION ANALYSIS

3.1 Association of gynoecium size with flower size

Here we explore associations between floral characteristics and pollinator visitation parameters. First, we examine the association between gynoecium diameter and flower diameter.

3.1.1 Methods

The diameter of the corollas of first-day water lily flowers (cm) were measured 1-2 hours after opening. Gynoecium diameter was measured (mm) across the entire stigmatic region. A Pearson correlation was conducted followed by linear regression analysis using SPSS version 27.

3.1.2 Results

Flower diameter was a strong predictor of gynoecium diameter (Pearson r= 0.857, p=0.000; linear regression R^2=0.735, d.f.=1,75, p<0.000) [Fig. 5].

3.2 Association of nectar volume with gynoecium size

Next, we examined the association of nectar volume with size of the gynoecium.

3.2.1 Methods

Gynoecium diameter of first-day flowers was measured as previously mentioned (see 3.1.1). First-day flowers entering the "water bath" treatment had their nectar removed and

measured using a P-1000 micropipet. Nectar was then aggregated in a Nalgene bottle and frozen for use in laboratory behavior assays. Pearson correlation analysis was conducted followed by linear regression analysis using SPSS version 27.

3.2.2 Results

Nectar volume was strongly predicted by the size of the gynoecium (Pearson r=0.811, p=0.000; linear regression R^2=0.598, d.f.=1,75,p<0.000) [Fig. 6], i.e. larger flowers presented greater volumes of nectar in the fluid trap.

3.3 Association of number of bathing events with gynoecium diameter

We next examined if larger flowers had greater number of bathing events.

3.3.1 Methods

Number of bathing events was determined to have a nonnormal distribution. Associations between number of bathing events and gynoecium diameter (mm) were analyzed using Spearman correlation followed by logarithmic regression using SPSS version 27.

3.3.2 Results

The number of bathing events was only weakly associated with gynoecium diameter (Spearman correlation = 0.335, p=0.006; logarithmic regression, R^2= 0.0.318, d.f.= 1,63, p=0.01) [Fig. 7].

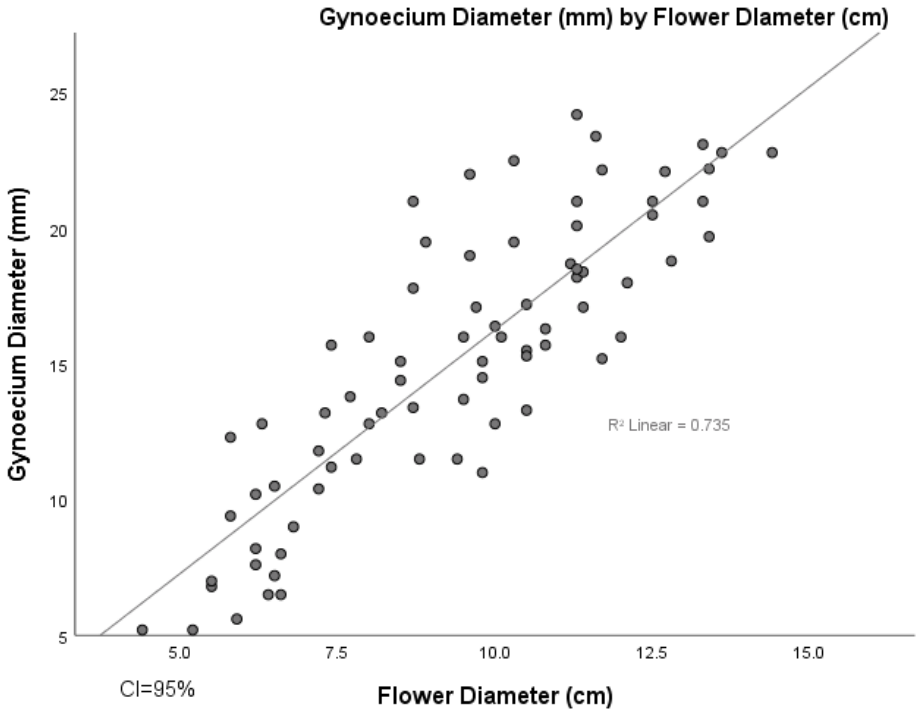

Figure 5. Larger flowers have larger gynoeciums (Pearson r= 0.857, p<0.000, linear regression R^2=0.735, d.f.=1,75, p<0.000).

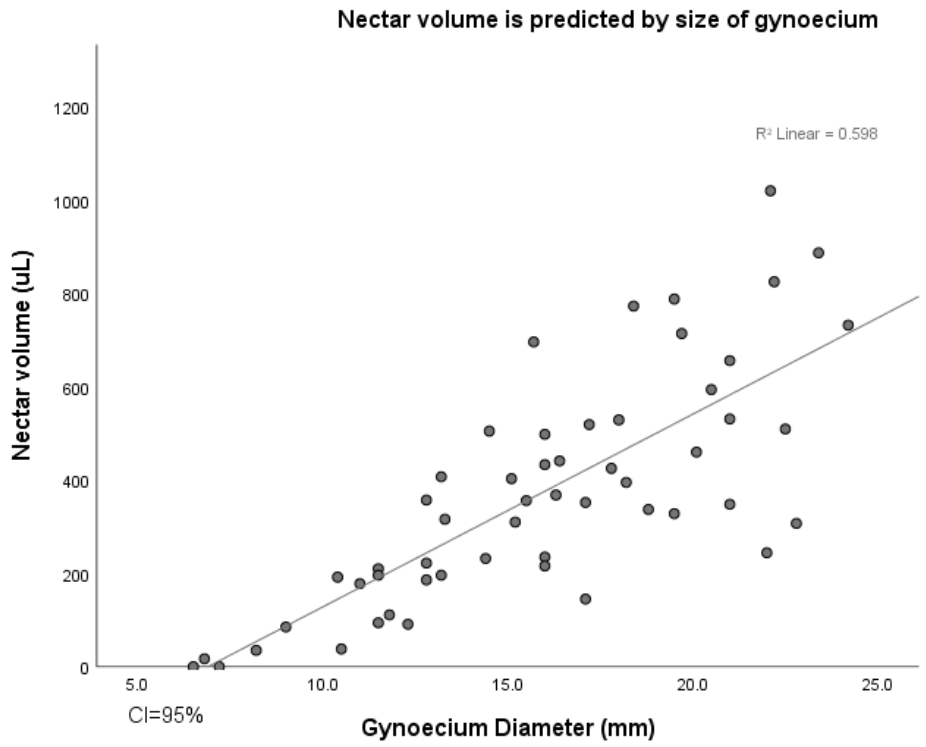

Figure 6. Larger gynoeciums present greater amounts of nectar (Pearson r= 0.811, p<0.000, linear regression, R^2=0.811, d.f.=1,75, p<0.000).

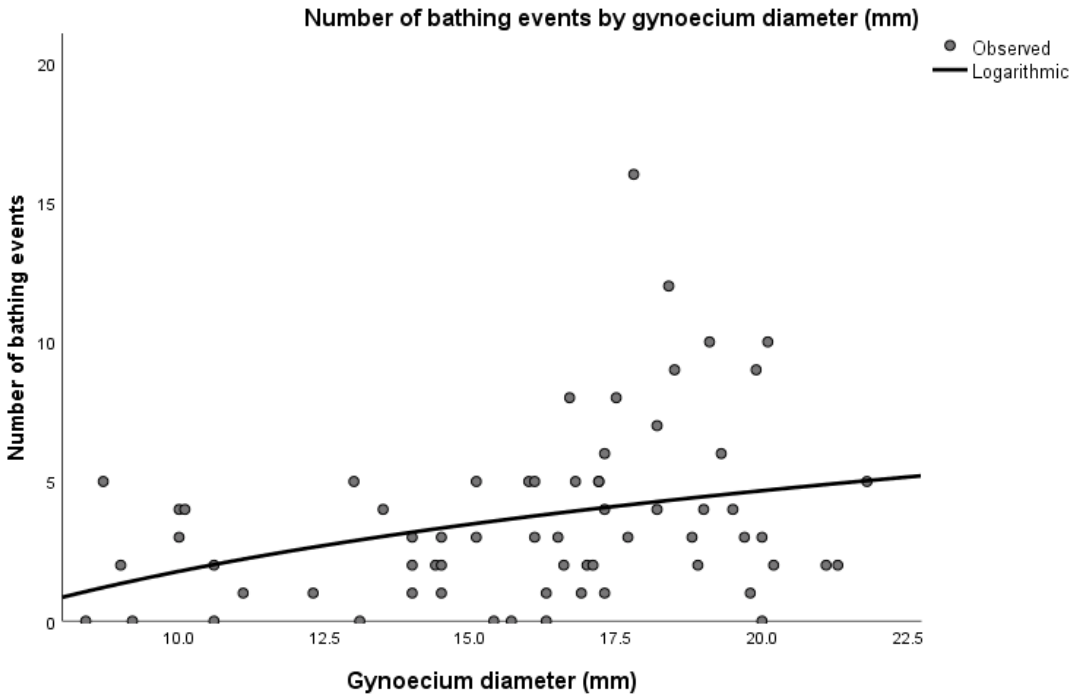

Figure 7. Number of bathing events were weakly, positively correlated with gynoecium size (Spearman's rho= 0.335, p=0.006, logarithmic regression, R^2=0.316, d.f.=1,63,p=0.010)

3.4 Correlating cumulative bathing event time in flower with gynoecium size

Next, the cumulative bathing event time in flowers was related to the size of the gynoecium.

3.4.1 Methods

First-day nectar-bearing flowers were covered with a fine mesh bag until timing of bathing events could be recorded. For 2-3 hours after opening, flowers were observed in fifteen-minute intervals. As flower visitors fell into the nectar bath, a bathing event was registered and time spent in the bath in seconds was recorded using a digital stopwatch. Individual bathing event times were aggregated to form the cumulative bathing time per flower. Associations between the variables were analyzed with Spearman correlation and logarithmic regression using SPSS version 27.

3.4.2 Results

No significant associations were found between cumulative bathing event time in flowers and the size of the gynoecium (Spearman's rho 0.23, p=0.065; logarithmic regression, R^2= 0.199, d.f.=1,63,p=0.111) [Fig.8].

3.5 Correlating number of bathing events and cumulative bathing time per flower to fecundity

Both the number of bathing events and the cumulative duration of bathing events in a flower is expected to correlate positively with seed set. Hypothetically, longer detention times of pollinators within flower increases pollen deposition effectiveness (i.e., seed-set). It should be noted that cumulative duration of bathing events in a flower is not independent of the number of

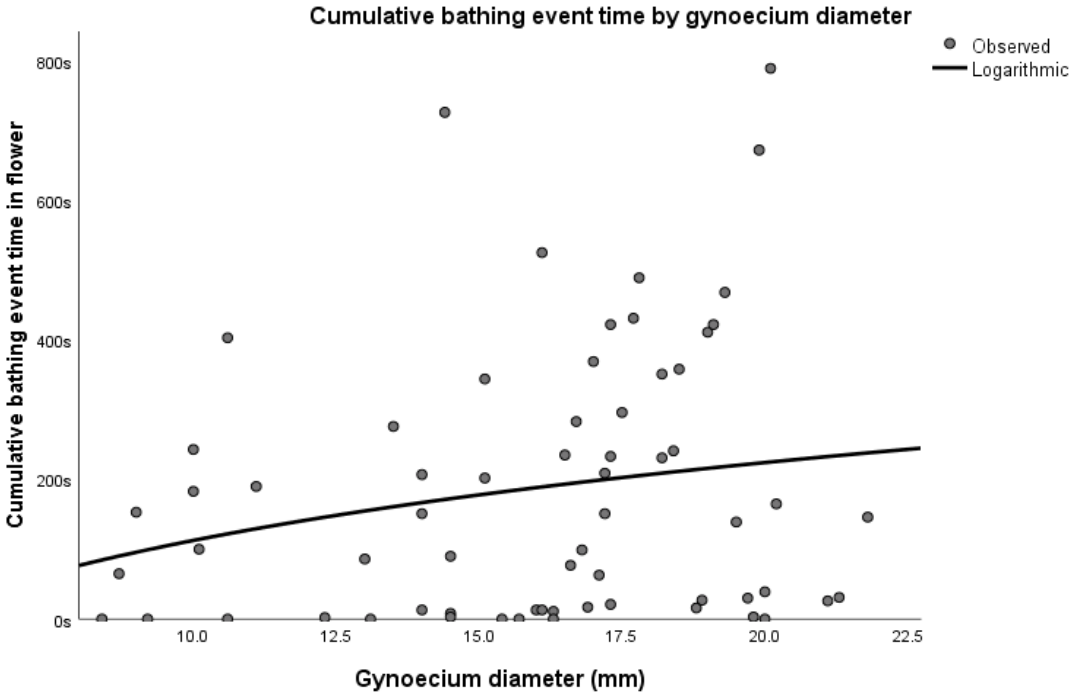

Figure 8. Gynoecium size was not associated with pollinator retention time within the water lily fluid-trap (Spearman's rho 0.23, p=0.065; logarithmic regression, R^2= 0.199, d.f.=1,63,p=0.111).

bathing events, since more bathing events in a flower and will result in increased bathing time regardless of the duration of the individual bathing events.

3.5.1 Methods-Bathing event rates and duration

To investigate the relationship between bathing duration and flower fecundity, the number of bathing events were counted. Concurrently, we measured the time spent by pollinators in nectar-baths of first-day flowers (n=68). Bathing events are defined as direct contact between the pollinator and fluid contained in the stigmatic basin of a water lily flower. Bathing event duration was recorded with a stopwatch in fifteen-minute intervals for the first 2-3 hours of flower (generally from 9:30-noon), at which point pollinator visitation naturally begins to subside. After the 2–3-hour observation period, flowers were covered with a fine mesh bag to prevent additional visitations in Day-1 flowers. Bees that were detained for longer than 15 minutes were considered to have perished in the flower. These points were recorded as 15-minute baths. Associations of fecundity with the number of bathing events and cumulative bathing event time per flower were explored using a Spearman's rho statistical test followed by logarithmic regression using SPSS version 27.

3.5.2 Results

We find a weak association between estimated seed-set and the number of bathing events (Spearman's r=0.285, p=0.018; logarithmic regression, d.f.=1,58, p=0.102) [Fig. 9] and cumulative duration of bathing events within flower nectar traps (Spearman's rho= 0.279, p=0.021; logarithmic regression, d.f.=1,58, p=0.087) [Fig. 10].

3.5.3 Summary

As expected, fecundity increases with higher numbers of bathing events (i.e. visitations attended by water baths). The number of bathing events is related to the cumulative duration of bathing events in flowers, as more bathing events will inherently lead to more bathing time in flowers and increased pollen transfer. However, number of bathing events does not correlate precisely with accumulated bathing times, insofar as a single 10-minute bath may well account for more time than 5 individual baths that endure for 10 seconds each. What remains to be explored is whether fecundity is affected by longer individual bathing events, which we hypothesize might conceivably be affected by secondary metabolites in *Nymphaea* nectar. To shed light on behavioral changes of pollinators following baths observed in the field and to explore the potential modification of detention time to increase fecundity, we experimentally compare fecundity of flowers with natural nectar baths to manipulated flowers containing a water bath.

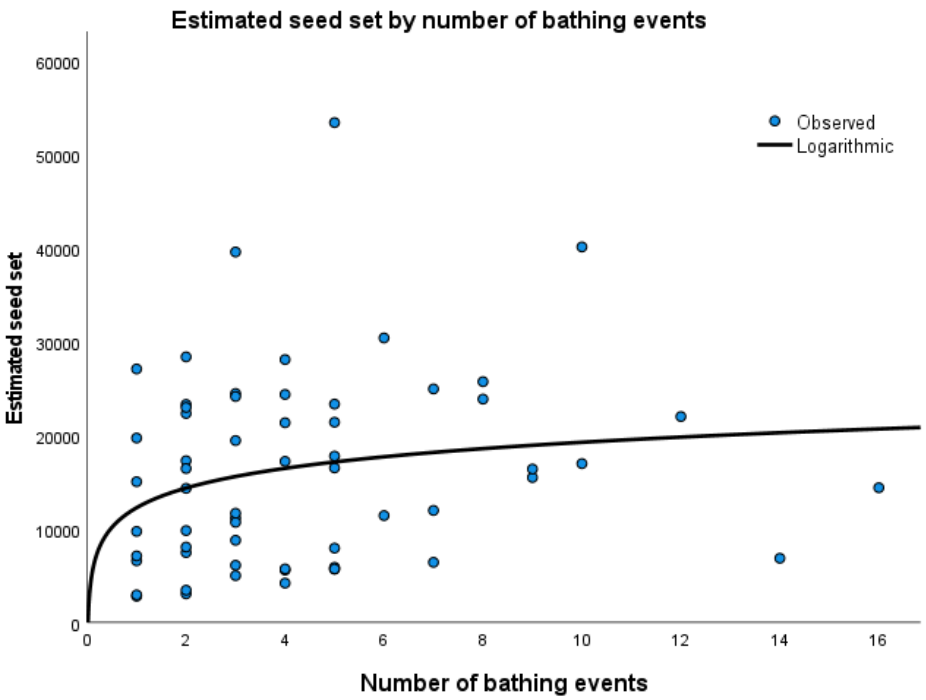

Figure 9. Seed estimation was weakly correlated with number of bathing events (Spearman's rho=0.285, p=0.018; logarithmic regression, d.f.=1,58, p=0.087).

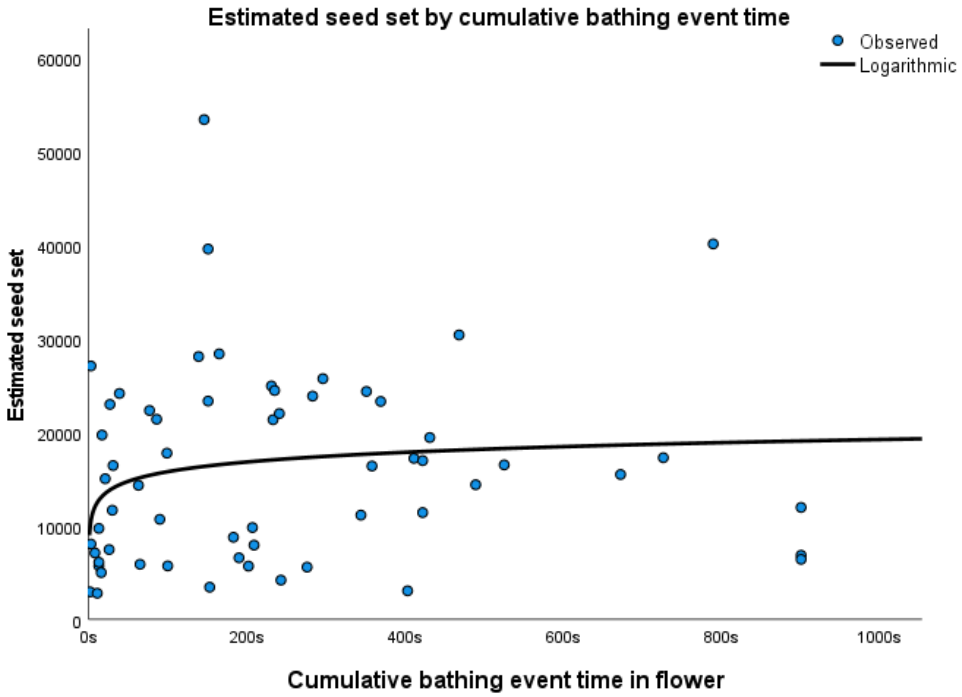

Figure 10: Seed set estimation was weakly correlated with cumulative bathing event time in flower (Spearman's rho= 0.279, p=0.021; logarithmic regression, d.f.=1,58, p=0.087).

CHAPTER IV

NECTAR VS. WATER IN 1ST DAY FLOWER FLUID TRAP

4.1 Is water equal to nectar in promoting fecundity?

In the next set of field experiments we explore whether distilled water is equal to nectar baths in promoting fecundity in *N. ampla*. These experiments are designed to compare ways in which the chemical constitution of water lily nectar might enhance floral function of gamete exchange. Since seed-set is limited by pollen deposition, and pollen deposition events are affected by the number of bathing events in first day flowers and also by the duration of bathing events, we hypothesize that nectar plays a role in the latter factor by extending the duration of pollinator baths. Judging from cursory observations of lethargic bee behavior after a nectar bath, we hypothesize that nectar properties lead to behavioral modification of pollinators and cause them to remain longer in the bath to the benefit of the reproductive success of the plant. We test whether or not nectar promotes fecundity by comparing seed set between flowers containing natural nectars and those whose nectar has been swapped with distilled water.

4.1.1 Methods

Nectar from first-day flowers was removed from the gynoecium's stigmatic basin with a glass dropper, and then replaced by equivalent amounts of distilled water: our "water treatment" group. Seed set of the water treatment group was then compared to those of natural nectar-containing flowers. All first day flowers were sampled for a period of about two weeks. Fruit

from these flowers were collected and seeds were extracted following the same procedure used in the self-compatibility and pollinator exclusion assays (see 2.1.1). A nonparametric Mann-Whitney U test was used to determine significant differences in estimated seed-set distributions.

4.1.2 Results

Estimated seed set was significantly greater in control flowers that contained nectar in comparison to flowers that had their nectar replaced with distilled water (Kruskal-Wallis for unequal sample sizes, $n_{nectar}=65$, $n_{water}=69$, $p=0.001$, CI=95%) [Fig. 11].

4.1.3 Summary

From our seed set estimations we conclude that water is unequal and less efficient than nectar in promoting seed set. This observation does not reveal the specific mechanistic or chemical cause of this result. For example, nectar properties may well be affecting fecundity by increasing pollen germination; or nectar might be impacting rates of pollinator visitation or length of nectar baths. In the next section, we examine differences in seed-set of bathing events in terms of number of visitations and duration in relation to flowers that contain nectar vs. distilled water.

4.2 Comparing cumulative bathing event time per flower between nectar and water-bearing flowers

In order to assess the potential role of floral nectar on affecting the retention time of pollinators in waterlilies, we quantified differences between the cumulative bathing times per flower with respect to nectar-bearing vs. water-bearing flowers

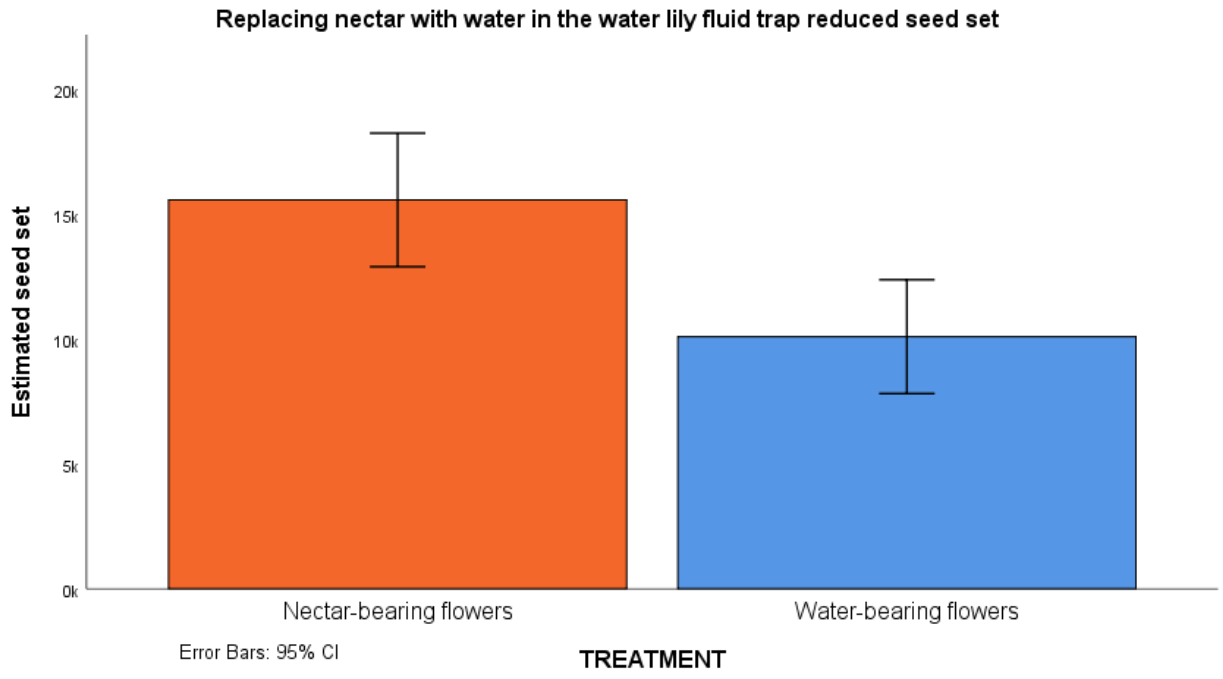

Figure 11. Swapping water with nectar reduced seed set in N. ampla *flowers (Kruskal-Wallis for unequal sample sizes, nnectar=65, nwater=69, p=0.001, CI=95%)*

4.2.1 Methods

Flowers containing water and nectar were observed for 2-3 hours after opening in the morning. We recorded the length of bathing events in treated (with distilled water) and non-treated (nectariferous) flowers, after which we bagged the flowers to prevent further visitations. Data was recorded with stopwatches in fifteen-minute intervals (see chapter 3.1.1).

4.2.2 Results

Cumulative visitation time was greater in nectar-bearing flowers than flowers with distilled water (Kruskal-Wallis test for unequal sample sizes, $n_{nectar}=68$, $n_{water}=76$, $p=0.005$, CI=95%) [Fig. 12].

4.2.3 Summary

Cumulative visit time was determined to be greater in nectar-bearing flowers, but this observation is not independent of bathing event number or duration of individual bathing events. The contribution of these two components is explored in more detail in the next sections.

4.3 Do water baths affect the length of bathing events?

Differences in the number of bathing events between nectar-bearing and distilled water-bearing flowers are examined to determine contribution to greater cumulative bathing event time in nectar flowers.

4.3.1 Methods

Bathing event data gathered during nectar vs. water fecundity assays (see chapter 3.1.1) was analyzed using a nonparametric Kruskal-Wallis test for unequal sample sizes to determine significant differences in number of bathing events between nectar and water flowers.

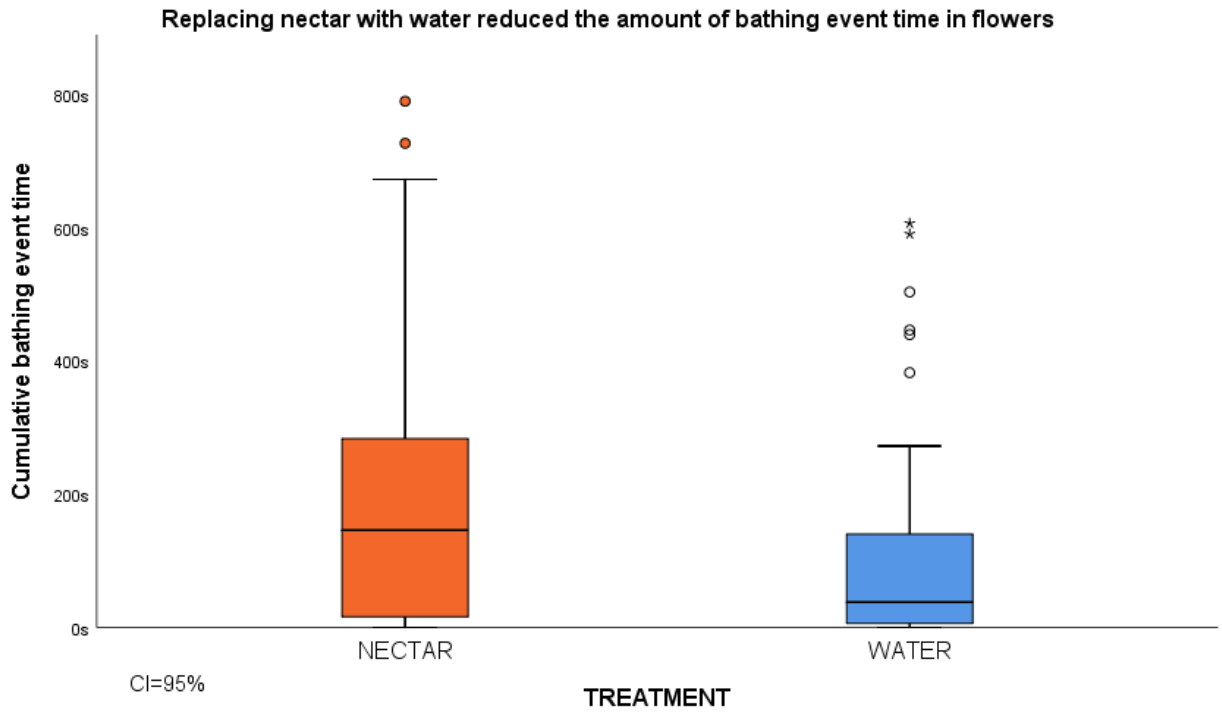

Figure 12. Swapping nectar with distilled water reduced the cumulative bathing even time in flowers (Kruskal-Wallis test for unequal sample sizes, nnectar=68, nwater=76, p=0.005, CI=95%).

4.3.1 Results

Mean rank number of bathing events was greater for nectar-bearing flowers than in water-bearing flowers (Kruskal-Wallis for unequal sample sizes, n_{nectar}=60, n_{water}= 65, $p<0.000$, CI=95%) [Fig. 13].

4.3.3 Summary

Greater bathing event rates in nectar flowers suggest that natural nectar (thought to contain sugars below the perception threshold of bees (Meeuse & Schneider 1979)) contains chemical attractants that possibly "lure" pollinators more efficiently than distilled water. The duration of individual bathing events in nectar and water baths in flowers is compared in the following section.

4.4 Are bathing events longer in nectar-bearing flowers?

Longer individual bathing events in nectar-bearing flowers in comparison to those observed in water-bearing flowers would support the hypothesis that nectar baths modify pollinator behavior by increasing time spent in the flower: ostensibly because this would increase pollen deposition.

4.4.1 Methods

During the course of observations in the nectar-water swap assays, the durations of a total of 440 bathing events were recorded by using a stopwatch in fifteen-minute intervals over a 2–3-hour period (see chapter 3.3.1). Potential differences in relative flower fecundity were analyzed by using a Welsh's t-test for unequal sample sizes.

4.4.2 Results

There was not a significant difference in the mean detention times between nectar-bearing and water-bearing flowers (Welsh's t-test for unequal sample sizes, n_{nectar}=294, n_{water}=146, d.f.=1,438, p=0.583) [Fig. 14].

4.4.3 Summary

Greater cumulative bathing time in nectar-containing flowers is affected by a greater number of bathing events, supporting the role of nectar as attractant or "lure" in the water lily pollination mechanism. This likely contributes to greater seed set in nectariferous flowers. There was no statistically significant difference in pollinator detention time within nectar and water baths in the field. We conclude that field studies do not support our hypothesis that nectar extends detention time by modifying pollinator behavior. However, a more detailed analysis of pollinator behavior *in situ* between nectar and water baths is required to rule out behavioral modification action post-nectar exposure in the water lily fluid trap.

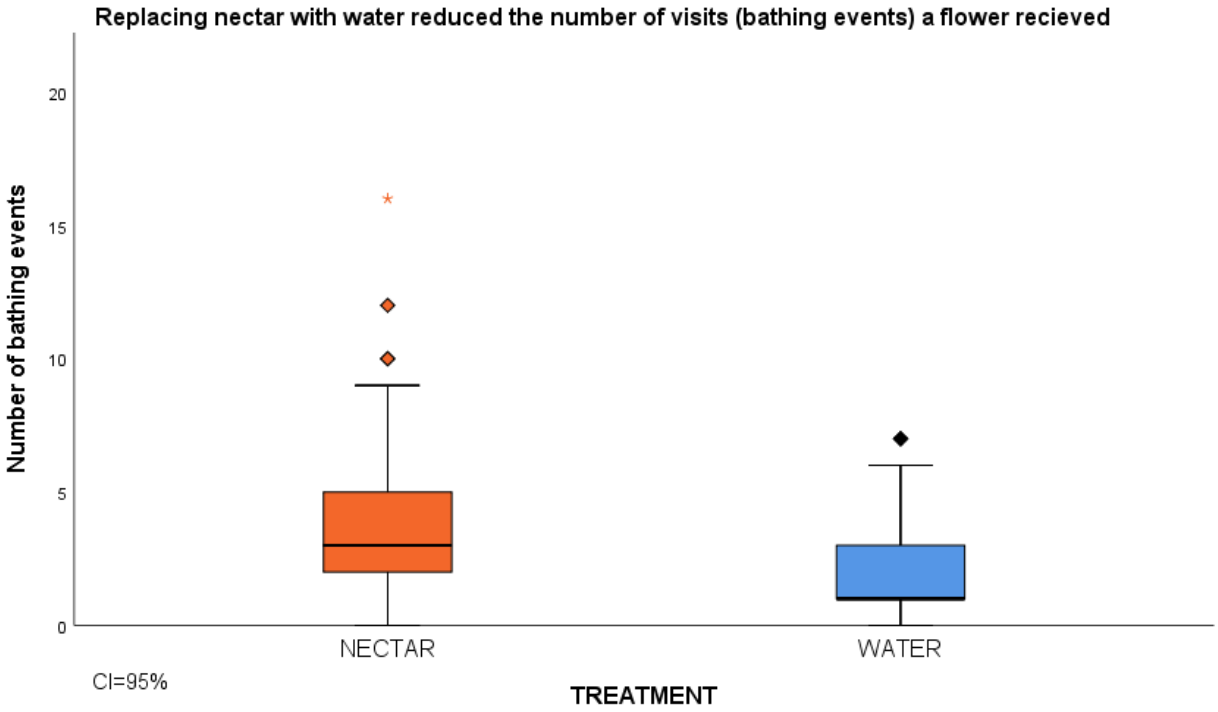

Figure 13. Swapping nectar with distilled water reduced the number of bathing events a flower received (Kruskal-Wallis for unequal sample sizes, nnectar=60, nwater= 65, p<0.000, CI=95%).

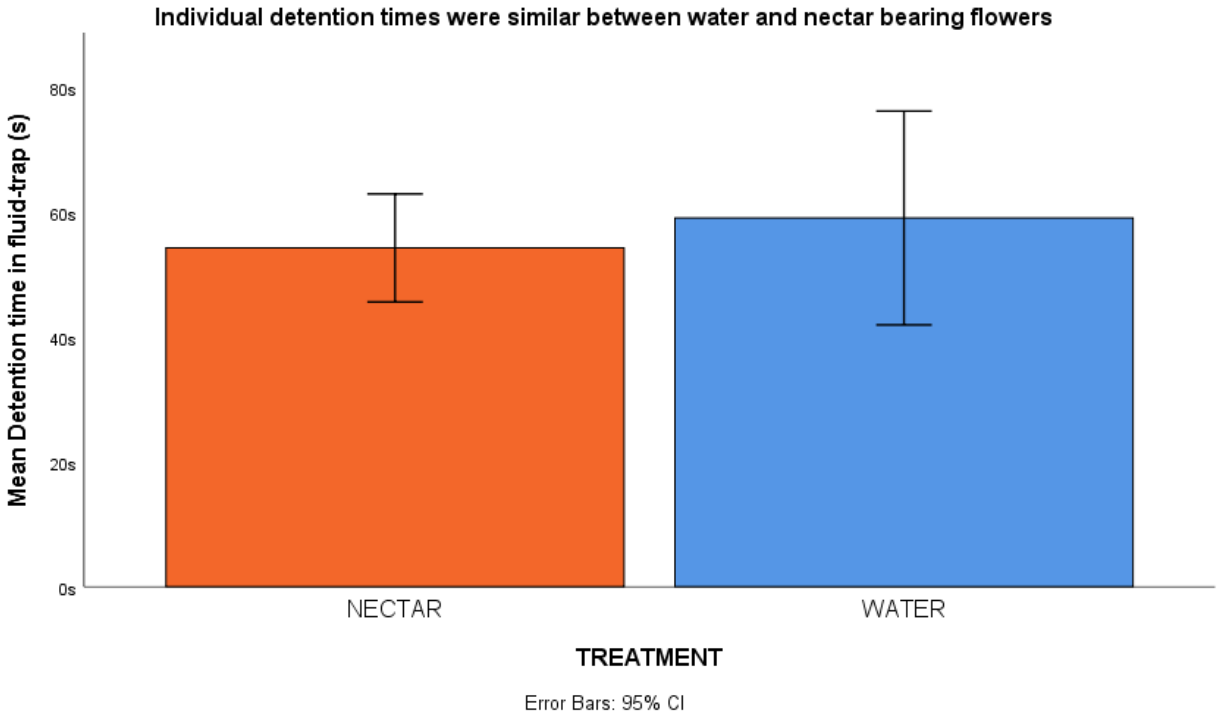

Figure 14. Swapping nectar with distilled water did not reduce the detention time of pollinators in the flower's fluid trap (Welsh's t-test for unequal sample sizes, nnectar=294, nwater=146, d.f.=1,438, p=0.583).

CHAPTER V

LABORATORY STUDIES-SURVIVAL AND BEHAVIOR

To build upon the field studies, a series of assays were conducted in the laboratory to i) gauge the antennal and appetitive responsiveness of honey bees (*Apis mellifera)* to *Nymphaea ampla* (Salisb. D. C.) nectar ii) determine if exposure to *N. ampla* nectar is directly lethal to *A. mellifera* and iii) investigate behavioral changes in *A. mellifera* induced by exposure to *N. ampla* nectar.

5.1 Proboscis Extension Response (PER)

In insects, olfactory chemoreceptors are located on the antennae and the palps of the mouthparts (Klowden 2013). Honey bees will extend their proboscis following stimulation of the antennae with a sugar solution of sufficient concentration. A proboscis extension assay was employed using nectar and known sucrose solutions of varying concentration to compare sucrose and *N. ampla* nectar response thresholds in *Apis mellifera.*

5.1.1 Methods

A modified sucrose response threshold assay was used to compare sensitivity of *A. mellifera* to *N. ampla* nectar and sucrose solutions (Mustard et al. 2012, 2019). Water lily exudate was gathered from first-day blooms from the aforementioned site in Edinburg, TX and an additional site in Harlingen, TX (26°13'N, 97°78' W) where the plants are grown in artificial ponds. The nectar was kept frozen in a Nalgene bottle for storage and removed from the

refrigerator and allowed to warm to room temperature before being used in experiments. Samples of nectar are aggregated from several *N. ampla* flowers.

Carniolan subspecies of *Apis mellifera* were gathered from an onsite hive outside the laboratory in the Life and Health Sciences Building, University of Texas-Rio Grande Valley, Brownsville (25°53'N 97°29'W). Foraging, adult bees were captured individually in glass vials from colony entrances as bees returned to the hive. Pollen foragers were identified by the presence of pollen on their corbiculae. Returning bees without pollen were assumed to be nectar foragers. Captured bees were cooled at 4°C until immobile for harnessing.

Plastic tubes (approx. 5mm diameter x 20 mm length), derived from cut plastic drinking straws, were used to fashion harnesses. The bee was slipped headfirst into the tube until the head protruded. The head was then gently pushed over the edge of the tube and we ensured the proboscis was free from the restraint before securing the bee with a strip of duct tape placed between the head and thorax. After mobility was restored, bees were fed a 1M sucrose solution until satiated, placed in a plastic container, and allowed to acclimate to laboratory conditions for 24 hours before beginning the assay.

Nymphaea ampla nectar and sucrose solutions (0.01M, 0.03M, 0.1M, 0.3M and 1M) were removed from refrigeration, put into glass dropper pipettes, and allowed to warm to room temperature before being used in the assay. Acclimated pollen and nectar foragers were removed from plastic container and initially fed water to satiation; this was done to ensure that any response to test solutions was not driven by thirst. We allowed 15 minutes after water feeding to begin the response assay. A drop of test solution from the tip of a glass pipette dropper was touched to both antennae on the honey bee to elicit the proboscis extension response. The test began with *N. ampla* nectar and continued with sucrose solutions of increasing concentration

(0.01, 0.03, 0.1, 0.3, 1M). Water was used between test solutions to rule out positive responses to mechanical stimulation. Five minutes elapsed between each antennal stimulation.

A positive response (i.e. bee extended proboscis following antennal stimulation) was coded as 1 while negatives were coded as 0. Twenty-six pollen foragers and 33 nectar foragers were used in the PER assay. Data analysis were conducted using SPSS version 27 or GraphPad Prism version 6 (La Jolla, CA). Distributions were analyzed using nonparametric Cochran's Q test for related samples.

5.1.2 Results

Nymphaea ampla nectar elicited a response in 42% of tested nectar foraging bees (n=33) a proportion statistically similar to those responding to 0.1 M sucrose solution at 54% (Cochran's Q test, d.f.=5, p=0.319). Seventy-six percent (76%) of pollen foragers responded to *N. ampla* nectar, a statistically similar proportion to those responding to 0.1 M sucrose solution at 84% (Cochran's Q test, d.f.=5, p=0.507) [Fig. 15].

5.1.3 Summary

The proportion of nectar foragers differed from pollen foragers in their response to *N. ampla* nectar (42% and 76% nectar response, respectively). Interestingly, both nectar and pollen foragers responded to *N. ampla* nectar in proportions similar to 0.1 M sucrose solutions despite having different perception thresholds. Surveys of sugar concentration in tropical water lilies conducted by Schmucker in the 1930s (vide Meeuse & Schneider 1979) report low concentrations of sugar at 1-2%. Schmucker reported that insects are attracted by olfactory stimuli. Our study supports this claim and suggests that *Apis mellifera*, pollen foragers in particular, will be attracted to the nectar in the *N. ampla* fluid trap of first day flowers. However,

it must be stated that *A. mellifera* individuals are rarely seen entering first-day flowers of *N. ampla*.

5.2 Proboscis Sensitivity Drink Assay

Honey bees have gustatory receptors on their proboscis and antennae, they may extend their proboscis but choose not to drink when presented with test solution (Mustard et al. 2019). The exploitative nature of the *Nymphaea* pollination syndrome suggests that nectar does not attract pollinators by virtue of its nourishment but acts primarily as an attractant or "lure" for the fluid-trap of first-day flowers. Here we test whether hungry honey bees will drink *N. ampla* nectar.

5.2.1 Methods

To determine if honey bees will drink *N. ampla* nectar, a modified drink assay was conducted (Mustard et al. 2012). Bees were harnesses as above (see 5.1.1) and fed 1 M sucrose solution to satiation before being allowed to acclimate to laboratory conditions for 24 hours. Bees were given water to satiation fifteen minutes before the start of the assay. The subject's antennae were stimulated with 1 M sucrose solution to elicit proboscis extension before being presented with 0.6 μL of test solution at the tip of the proboscis. Each bee was presented test solution in three trials. We presented nectar in the first trial, followed by water in the second, and finally 1 M sucrose solution in the third. Bees that were injured or did not drink any of the solutions were removed from the data set. A score of one (1) was recorded when the bees drank the entire drop of test solution presented to them; a score of zero (0) denoted refusal to drink the drop. Binary data was analyzed using nonparametric related-samples McNemar's test.

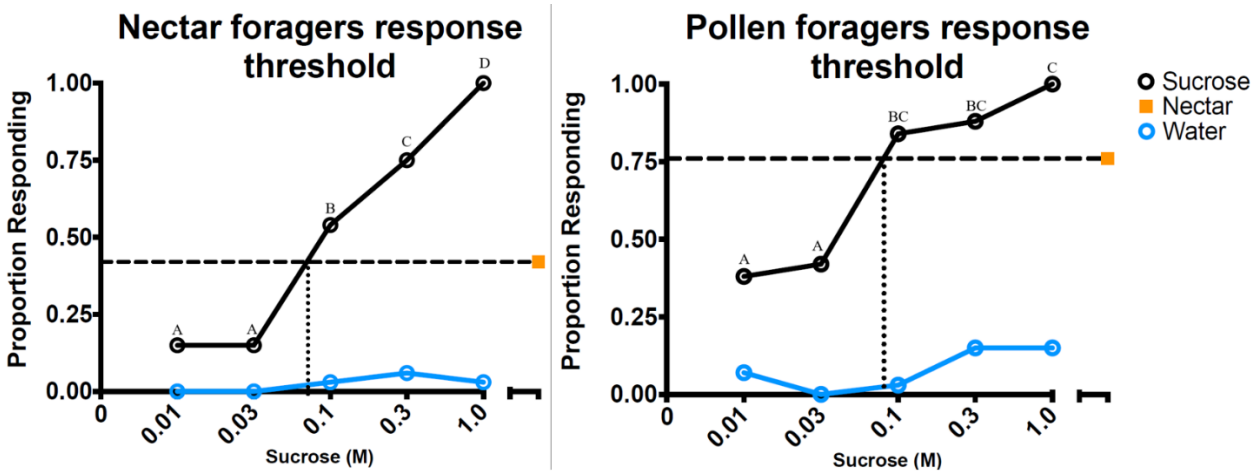

Figure 15. Proportion responding to Nymphaea ampla *nectar was similar to response to 0.1M sucrose solution in both nectar (n=33) and pollen (n=26) foragers. Letters above the proportions for each treatment display statistical levels (Cochran's Q test, d.f.=5, CI=95%)*

5.2.2 Results

Two of the 57 bees surveyed (3.5%) drank the nectar when presented at the proboscis. More specifically, none of nectar foragers drank the liquid (n=31) and 2 of the 26 pollen foragers surveyed drank the nectar. Related-samples McNemar's test revealed significantly different distributions between *N. ampla* nectar and 1 M sucrose consumption in *A. mellifera* (d.f.=1, n=57, p<0.000, CI=95%)

5.2.3 Summary

We conclude that *Apis mellifera* does not find *Nymphaea ampla* nectar appetitive when presented at the proboscis.

5.3 Exposure survival assay

Small alkali bees that frequent first day *N. ampla* flowers occasionally perish inside the fluid trap. A number of causes of mortality have been proposed including asphyxiation by volatiles (Plachon 1853 *vide* Meeuse and Schneider 1979, Delpino 1869 *vide* Meeuse and Schneider 1979), harmful confinement (Bacon 1874), and drowning (Robertson 1889, Conard 1905). Here we test whether exposure to *N. ampla* nectar reduces survivability in *A. mellifera*.

5.3.1 Methods

Carniolan subspecies of *Apis mellifera* were gathered from an onsite hive outside the laboratory in the Life and Health Sciences Building, University of Texas-Rio Grande Valley, Brownsville (25°53'N 97°29'W). Bees were captured individually in glass vials and cooled at 4˚ C until immobilized. The bees were harnessed using a strip of hook-and-loop tape with

dimensions approximately 4cm x 3cm and two smaller strips about 0.5cm x 4cm. The harness was designed to allow test fluid to reach the bee without the subject being completely submerged, to allow for the subject to be dried following exposure, and for rapid release of the bee into the observation chamber in the behavioral assay (next subchapter). The immobilized bees were laid on their dorsal side upon the larger section of hook-and-loop tape, after which the two smaller strips are used to secure the bee by placing one strip over the body of the bee between the thorax and abdomen the other between head and thorax, underneath mandible to allow free movement of proboscis and antennae [Fig. 16]. The harnessed bees were then mounted onto a plexiglass board using additional hoop-and-loop tape for handling during experiments. Once all the bees were mounted, they were set upright (with the board nearly vertical) for feeding. Bees were fed 1 M sucrose solution to satiation. Three (3) mL of fluid was added to wells in a six-well plastic cell culture dish. This amount was used as it allowed the test fluids to contact the subject without completely submerging them. The level of liquid is marked on the plastic well and maintained at this depth throughout each trial with additional liquid as necessary. Bees were randomly assigned to one of seven treatment groups that were exposed to *N. ampla* nectar for 1, 10, or 20 minutes, water for 1 or 20 minutes, or a 'no exposure' control group. After immersion period, the bees are removed, wicked dry by placing a paper towel at each corner of the harness for a 3-5 seconds and replaced onto the mounting board for the remainder of the experiment where they were fed 1 M sucrose to satiation daily and deaths were recorded each day for one week. Kaplan-Meier survival curves were constructed for each treatment group. Differences in the survival distribution were determined with a log rank test.

5.3.2 Results

Analysis revealed no statistically significant increase in mortality between the treatments (Kaplan-Meier followed by Mantel-Cox Log rank test (X^2=2.402, d.f.=5, p=0.791).

5.3.3 Summary

Kaplan- Meier analysis following survival experiments suggest that no compound within *N. ampla* nectar is directly lethal to our model species *Apis mellifera,* although it should be recognized that *A. mellifera* is not endemic to the neotropics of North America where *N. ampla* grows naturally. Future studies should utilize the small alkali bees that frequent diurnal *Nymphaea* in place of, or in addition to, *A. mellifera*.

Figure 16. Apis mellifera harnessed using three strips of hook-and-loop tape. This design allows for the bee to be exposed to test liquid without submerging the bee and for handling pre- and post-treatment.

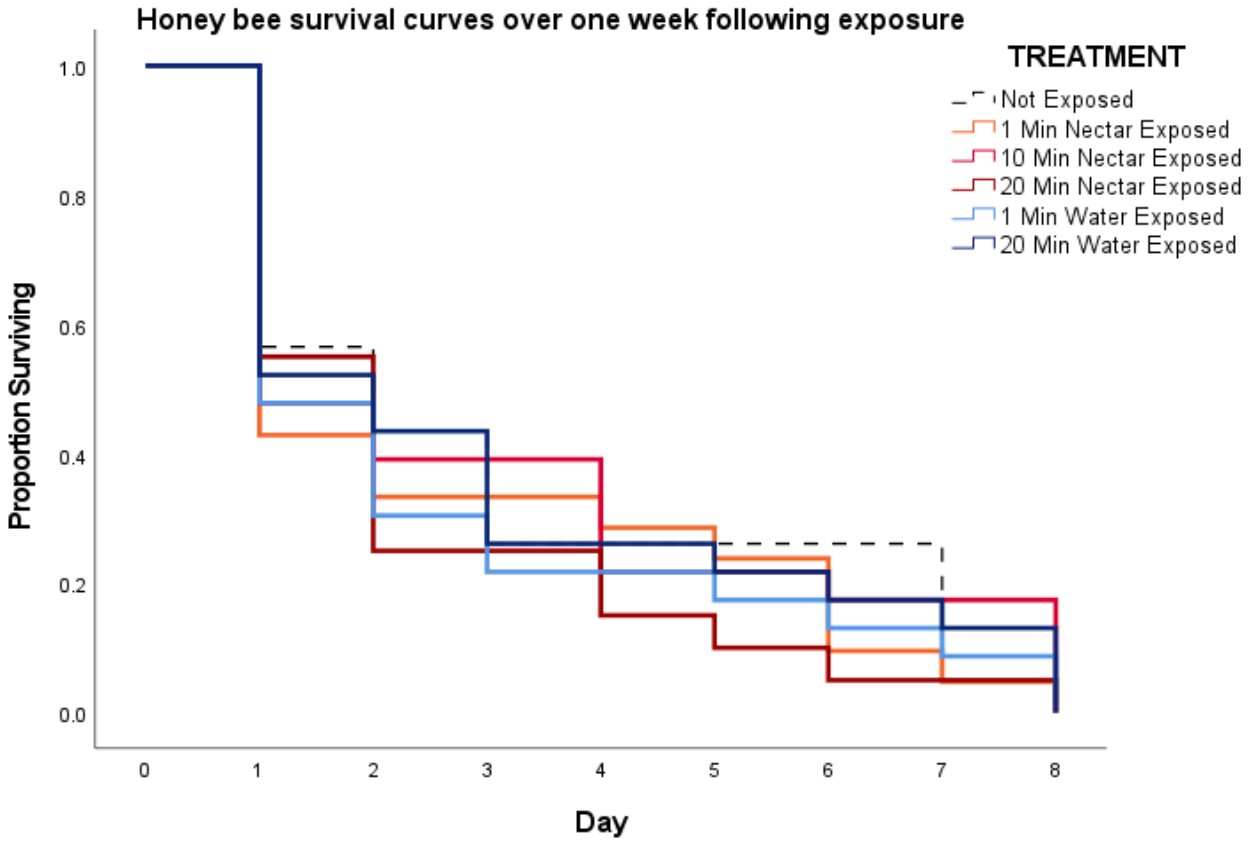

Figure 17. Kaplan-Meier, Log rank survival analysis did not show significant differences in survival between treatments X2=2.402, d.f.=5, p=0.791.

5.4 Behavior following exposure to *N. ampla* nectar

Preliminary observations in the field noted that bees spent significant time grooming on the petals after escaping the fluid trap. In addition to the loss of pollen load and energy spent to escape the nectar, the *N. ampla* fluid trap imparts another tax on benefactors in the form of time lost both within the flower and in grooming. From the previous assays we conclude that bees are attracted by the nectar but will not drink it, presumably due to the low sugar concentration. While not directly lethal, behavioral changes elicited by secondary compound present in *N. ampla* nectar could reasonably contribute to drowning. Some behaviors can serve as indicators of motor impairment, for example, increased time spent upside down may convey difficulties with

coordination. In the next assay, we determine if exposure to *N. ampla* nectar elicits behavioral changes in *Apis mellifera*.

5.4.1 Methods

To determine the effects of exudate on pollinator behavior, honeybees were harnessed with hook-and-loop tape (see 5.3.1), fed 1 M sucrose solution to satiation, and allowed to acclimate to laboratory conditions for about 24 hours. On the day of the experiment, bees were again fed to satiation 30 minutes prior to start of exposure treatments. The bees were randomly assigned to one of the following seven treatment groups: 'no exposure' control, water exposure (1, 5, or 10 minutes), or *N. ampla* nectar exposure (1, 5, or 10 minutes). Bees were immersed in 3 mL of test fluid (nectar or distilled water) in a plastic well according to their assigned treatment. Following exposure, bees were wicked dry and transferred to an observation chamber composed of a clear, six-centimeter diameter petri dish with lid, wherein harnesses were removed, and behaviors were recorded using Observer Noldus behavioral software which allows behaviors to be quantified by mean bout lengths and behavior duration as percent of total observation time (10 minutes). The behaviors we observed were walking, grooming leg, grooming antennae, grooming proboscis, flipped on their back (upside down), attempted flying, and wing fanning.

Significant differences in mean bout length and the percent of interval spent by the bee in walking, grooming, and upside-down behavior were compared between nectar and water exposed bees for similar treatment times with nonparametric Mann-Whitney U-tests or independent samples Kruskal-Wallis tests on SPSS version 27.

5.4.2 Results

Variables in this assay are related, as less time spent in one activity means more time spent in a different activity. Walking behavior was significantly decreased in nectar-exposed bees when compared to water-control bees in the one-minute treatment [Fig. 18]. Bees that were exposed to *N. ampla* nectar had significantly increased time spent grooming in the 1-minute treatment when compared to water exposure (coinciding with less time spent walking) (Mann Whitney U test, $p<0.05$), although Mann-Whitney U tests did not show significance in the 5 and 10 minute treatments [Fig. 19].

Analysis of mean bout length revealed statistically significant increase in total grooming behavior following 1 minute nectar exposure compared similar exposure duration to distilled water (independent samples T-test, d.f.=20, p=0.039). In 5 minute exposure times, walking behavior was significantly reduced in bees exposed to nectar (independent samples T-test, d.f.=20, p=0.035) [Fig. 20].

There were no statistical differences in upside down behavior between treatments in either percent of interval (independent samples Kruskal-Wallis test, d.f.=6, p=0.533, CI=95%) [Fig. 21] or mean bout length (independent samples Kruskal-Wallis test, d.f.=6, p=0.311, CI=95%) [Fig. 22].

5.4.3 Summary

Decreased walking and increased grooming behaviors in bees exposed to nectar suggests physiological action. Grooming behavior has been attributed to malaise resulting from exposure to toxic compound but may also result from the bee needing to clean surfactants or irritants from the cuticle. Interestingly, walking and grooming bout lengths were roughly equal in water

exposure treatments but differed in nectar exposed bees, again suggesting an additional effect by nectar though we do not determine whether this effect is due to physiological or surfactant action.

Contrary to our suppositions, exposure to *N. ampla* nectar did not appear to impair motor skills as evidenced by the absence of statistical differences in upside down behavior between exposure treatments.

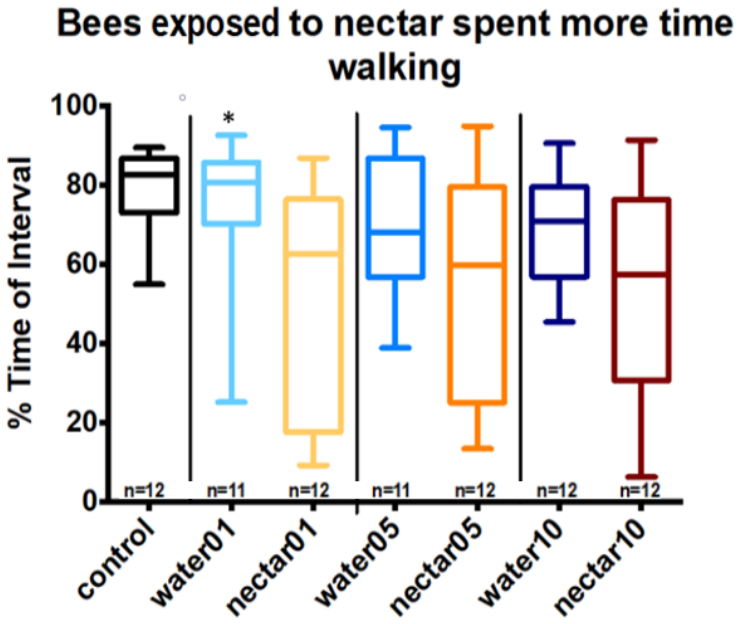

Figure 18. Mean percent of 10 minute interval spent in walking behavior. Notice the greater variability in nectar exposed bees. Asterisks indicate significance within same treatment time using Mann-Whitney U test ($p<0.05$)

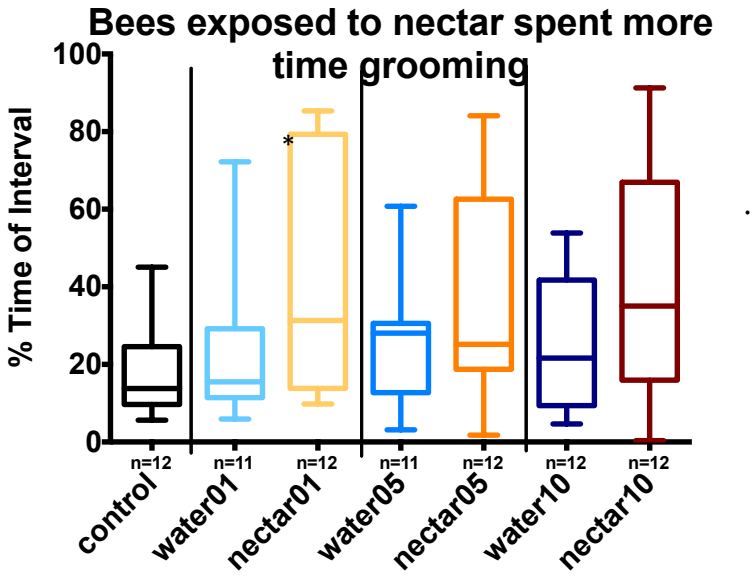

Figure 19. Bees exposed to nectar spent more time in malaise behavior (grooming) & exhibited greater variability in behavior. Asterisks indicate significance within same treatment time using Mann-Whitney U test ($P < 0.05$).

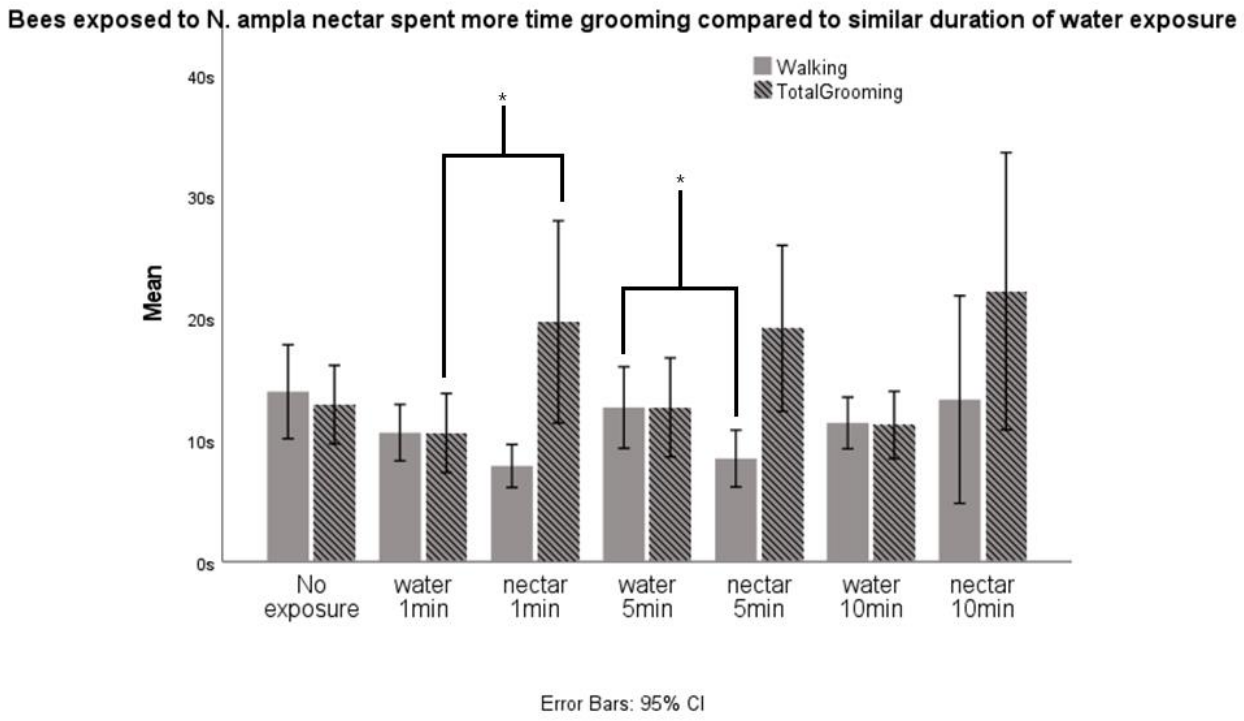

Figure 20. Grooming bout length was significantly increased in bees exposed to nectar for 1 minute (independent samples T-test, d.f.=20, p=0.039). Walking behavior was significantly decreased in bees exposed to nectar for five minutes compared to water exposure of similar time (independent samples T-test, d.f.=20, p=0.035). Asterisks show significantly differences within similar exposure time groups

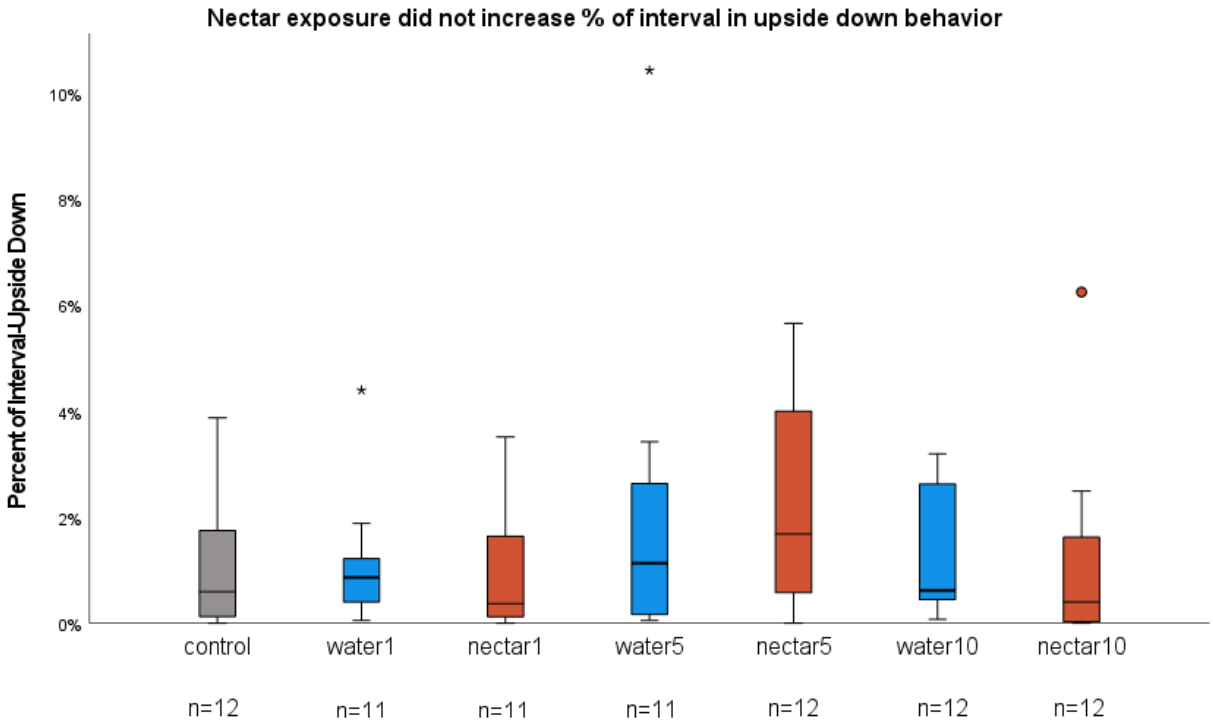

*Figure 21. Nectar exposed bees had similar % of interval in upside down behavior as water exposed and unexposed bees (Independent samples Kruskal-Wallis test, d.f.=6, p=0.533, CI=95%).*Scale reduced to enlarge boxplots, asterisks denoted extreme values.*

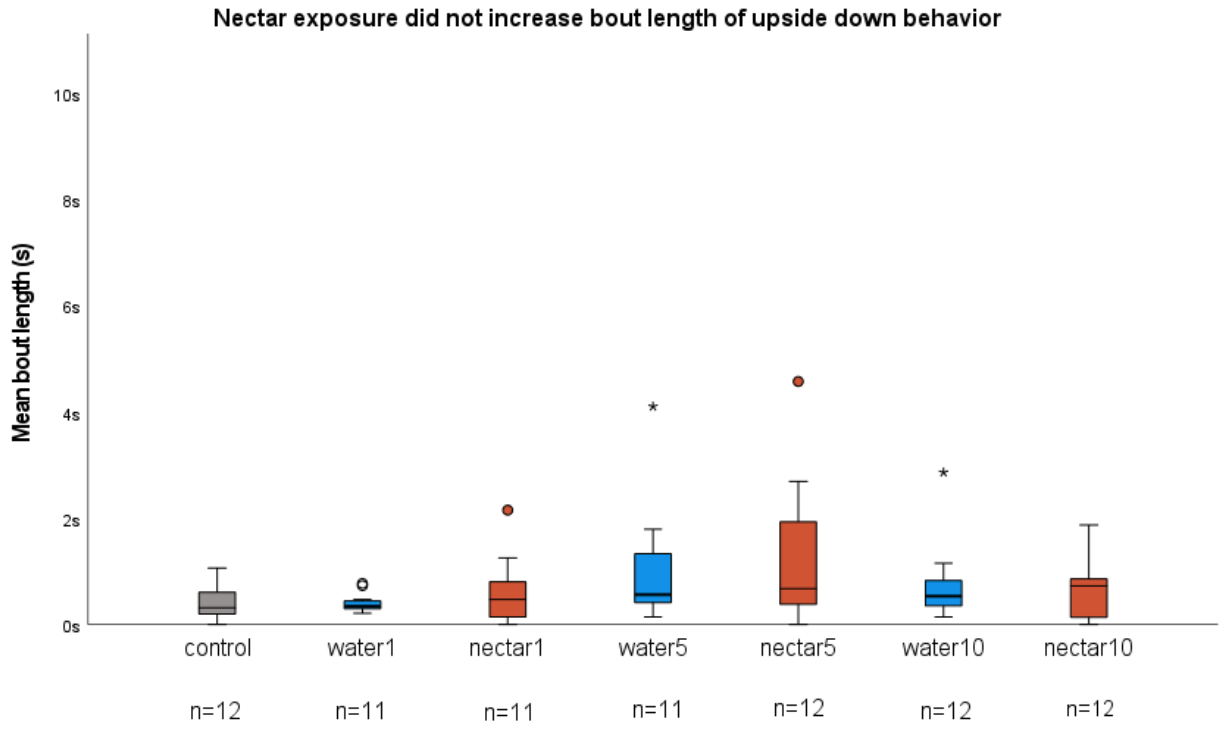

*Figure 22. Nectar exposed bees had similar mean bout lengths as water exposed bees and unexposed bees (independent samples Kruskal-Wallis test, d.f.=6, p=0.311, CI=95%). *Scale reduced to enlarge boxplots, asterisks and circles denote extreme values.*

CHAPTER VI

CONCLUSION

Water lilies “occupy a critical evolutionary space” that can help illuminate the mechanisms in the development and evolution of angiosperms (Chen et al. 2017). *Nymphaea* is a large, cosmopolitan genus of basal angiosperms with a variety of pollen vectors and a complex pollination scheme and thus, demonstrates potential as a model for studying adaptive radiation from beetle to bee-and-fly pollination as well as related changes in phytochemistry (Meeuse & Schneider 1979).

Our field studies first examined autogamy and how insect-vectors contributed to seed set. In line with current literature, our population of *N. ampla* is capable of autogamy as evidenced by the high mean estimated seed set in manually-selfed flowers similar to unmanipulated, naturally pollinated flowers (Conard 1905, Wiersema 1988). However, flowers experimentally isolated from pollinators had significantly reduced estimated seed set indicating that although flowers are capable of self-pollination, they have developed “striking[ly] sophisticat[ed]” features that favor outcrossing (Meeuse and Schnieder 1979). Our experiments confirm that our population of *N. ampla* is adapted to xenogamous or geitonogamous pollination. Therefore, pollen availability limits reproductive success in a self-compatible species of water lily that relies on outcrossing for increased seed set, thereby creating selective pressure for features that promote xenogamy and geitonogamy such as dichogamous flowers found in two distinct stages of sexual function.

In comparing water-bearing flowers to natural, nectar-bearing flowers, our analysis displayed significant reduction in fecundity of water-bearing flowers. Further analysis pointed to decreased pollinator recruitment, which hints at the presence of attractant VOCs in a non-nutritive nectar.

Our studies in the laboratory using *Apis mellifera* reveal a strong antennal response to *N. ampla* nectar (especially in pollen foragers). However, the bees do not find the nectar appetitive when presented at the proboscis. These findings deepen the exploitative nature of the water lily. Exposure to *N. ampla* nectar did not decrease survivability in *Apis mellifera* in laboratory experiments. However, behavioral changes following exposure such as increased grooming and decreased walking suggest possible bioactive compounds or surfactants as previously proposed by Meeuse and Schneider (1979). The honey bee is not endemic to the neotropics where N. ampla grows naturally and is considerably larger than the small halictid bees that frequent water lily flowers. Future survival and behavioral experiments may benefit from utilizing the native bees that are naturally exposed to the exudate in the water lily fluid trap.

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BIOGRAPHICAL SKETCH

Luis Uribe has earned a Master's of Science degree in Biology from the University of Texas Rio Grande Valley in 2021 and an Associates of Physics degree from Texas State Technical College in 2016. As a graduate student in the Plant Systematics and Ethnobotany laboratory, Luis developed a strong interest in historical and current environmental management systems and philosophies. Luis has worked with various governmental and non-profit organizations including the United States Department of Agriculture (USDA) and the Audubon Society and volunteers with local Texas Master Naturalist chapters. He continues to quest for unconventional solutions to environmental problems, looking at historical and emerging landscape management practices in order to develop integrated strategies that will benefit both ecosystems and industries in his native Rio Grande Valley.

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