

EFFECT OF HABITAT STRUCTURE ON REPRODUCTION AND PREY CAPTURE OF A
RARE CARNIVOROUS PLANT, *PINGUICULA LUTEA*

BY

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THESIS

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ABSTRACT

Habitat modification is one of the greatest threats to biodiversity worldwide and the main contributor to the decline of many carnivorous plant species. For carnivorous plants in the Southeast US, including many *Pinguicula* species (butterwort, Lentibulariaceae), habitat modification via altered fire regime has been implicated in their decline. Despite this decline, limited empirical research has been conducted examining the influence of habitat structure on reproduction and prey capture of carnivorous plant species in this region.

The studies described in this thesis aim to address this deficit. Chapter 1 provides a general introduction to carnivorous plants, focusing primarily on *Pinguicula* species. In this chapter, I describe the results of a series of preliminary studies on *Pinguicula lutea* (yellow butterwort) to address gaps in knowledge of its basic biology (e.g., breeding system and prey identification) and to develop sampling protocols (e.g., seed set and prey sampling) for further work. Chapter 2 addresses the impacts of habitat structure (i.e., woody, grassy, and maintained) on reproduction and prey capture for *Pinguicula lutea* in the Florida Panhandle. Lastly, Chapter 3 provides conservation, management, and additional research recommendations for *Pinguicula lutea* and its congeners.

Pinguicula lutea is a carnivorous plant that inhabits fire-dependent longleaf pine savannas of the Southeast US and is listed as threatened in the state of Florida. Based on preliminary studies in 2013, *Pinguicula lutea* is a self-compatible but outcrossing species. Its primary prey are Collembola and small Diptera. In 2014 and 2015 populations were identified occupying three types of habitat structure: maintained (mowed), grassy (dense *Aristida stricta* var. *beyrichiana*), and woody (*Hypericum/Ilex* mix). Reproductive

output was determined by assessing fruit and seed set at each habitat structure. Additionally, prey availability and prey capture were assessed at each habitat structure. In general, habitat structure did not affect reproduction, but did affect the abundance of Collembola, Diptera, and all arthropods combined both in terms of availability and prey capture. Overall, there was a significant difference in total arthropod availability and prey capture among habitat structures where grassy habitats tended to have fewer arthropods available and captured prey than maintained or woody habitats. In addition, arthropod availability and prey capture were higher in 2015 than in 2014. Microclimatic conditions (e.g., light availability) associated with each habitat structure and leaf morphology/physiology could explain the observed arthropod abundance and prey capture patterns. Information from this study will aid in the conservation and management of *Pinguicula lutea*.

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CHAPTER 1: NATURAL HISTORY/POLLINATION BIOLOGY OF *PINGUICULA LUTEA*

Carnivorous plants are unique among angiosperms due to their highly modified leaves capable of capturing and digesting prey. To be considered carnivorous, a plant must be able to trap and digest prey as well as absorb the nutrients from the associated prey capture (Juniper et al. 1989). Phylogenetic evidence suggests that the carnivorous habit has arisen independently six times throughout plant evolutionary history (Ellison and Gotelli 2009). Most likely, this has evolved as a mechanism to obtain essential nutrients that are limited in the soil. This adaptation allows carnivorous plants to thrive in habitats that are otherwise uninhabitable to most plants, including habitats that are nutrient poor and wet (Schnell 2002).

The unique nature of carnivorous plants has fascinated scientists from Charles Darwin to the present. Darwin brought carnivorous plants to the forefront of scientific inquiry by claiming that the Venus flytrap is one of the most wonderful plants in the world and by writing one of the first detailed scientific accounts (Darwin 1875). Since then, studies on plant-insect interactions of carnivorous plants have primarily focused on the novelty of their carnivorous habit. For example, numerous studies are found on trap morphology (Moran et al. 1999; Chin et al. 2010; Mescher and De Moraes 2014), digestion (or breakdown and assimilation of nutrients) (Heslop-Harrison and Knox 1971; Heslop-Harrison and Heslop-Harrison 1981), and prey capture (Ellison and Gotelli 2009; Pavón et al. 2011; Koller-Peroutka et al. 2014; Pavlovič et al. 2014; Bertol et al. 2015). However, with the exception of several pollinator-prey capture conflict studies (Zamora 1999; Anderson and Midgley 2001; Murza et al. 2006; Anderson 2010; Jürgens et al.

2012; Horner 2014; Jürgens et al. 2015) the reproductive ecology (e.g., breeding system and fruit/seed set production) of many of these carnivorous plants has received little attention.

Reproductive ecology studies (i.e., breeding system) have been conducted for only a small subset of the roughly 600 carnivorous plant species worldwide including *Sarracenia flava* (yellow pitcher plant; Schnell 1983), *Sarracenia purpurea* (parrot pitcher plant; Thomas and Cameron 1986; Ne'eman et al. 2006), *Drosera algica* (English sundew; Murza and Davis 2005), *Darlingtonia californica* (California pitcher plant; Meindl and Mesler 2011), *Utricularia* (bladderwort; Jérémie 1989; Taylor 1989; Hobbhahn et al. 2006; Clivati et al. 2014), and *Pinguicula* (butterwort; Molau 1993; García et al. 1994; Zamora 1999). More research is needed at the species-level, as plant breeding systems are often species specific and even closely related species may display a variety of pollination syndromes, breeding systems, or both (Barrett 2010). Studies have also shown considerable interspecific variation occurs in fruit set and seed set for carnivorous species (Zamora et al. 1998).

Work investigating plant-insect interactions is necessary to attain a clear understanding of the basic ecology of these plants. Such information provides the foundation on which more complex ecological questions can be addressed. For carnivorous plants in particular, this information is especially vital as many carnivorous plants are threatened. It is estimated that over half of the 102 carnivorous plant species across seven genera that were evaluated by the International Union for the Conservation of Nature are threatened (Jennings and Rohr 2011). Habitat modification is a major contributor to their decline (Jennings and Rohr 2011). Yet, in many cases we do not

know the basic natural history of a plant well enough to assess how it is being impacted by such habitat alterations. To assess ecological implications of habitat modification at a species-specific level, there first has to be an understanding of the basic biology of the species in question. My research aims to elucidate this basic ecology for a carnivorous species in the genus *Pinguicula* (butterwort; Lentibulariaceae).

Over 100 *Pinguicula* species are found worldwide (Rodondi et al. 2010), all of which have similar vegetative and floral characteristics (Fig. 1.1). *Pinguicula* species have a basal rosette of leaves and an elongated scape with a terminal, showy flower. The leaf surface has stalked and sessile glands capable of trapping and digesting arthropod prey to assimilate nutrients not available in the soil. Flowers are zygomorphic. The corolla consists of five petals that fuse into a tube like structure (the throat) and taper into a nectar spur. The reproductive organs are situated within the throat. Two stamens subtend the flap-like stigma. Only the filaments are visible as the anthers are tucked under the stigma flap (Fig. 1.2). With the exception of species descriptions and species distributions, limited empirical work has been conducted on the *Pinguicula* species of the Southeast United States.

***Pinguicula* Species of the Southeast United States (Subtropical Species)**

There are six *Pinguicula* species native to the Southeast United States: *Pinguicula caerulea* Walter (blueflower butterwort), *Pinguicula ionantha* Godfrey (violet butterwort), *Pinguicula lutea* Walter (yellow butterwort), *Pinguicula planifolia* Chapman (Chapman's butterwort), *Pinguicula primuliflora* Wood and Godfrey (primrose-flowered butterwort), and *Pinguicula pumila* Michaux (small butterwort) (Wunderlin and Hansen

2008). In Florida only *P. pumila* is common. The other five species have received heightened conservation status at the state level (*P. caerulea*, *P. lutea*, *P. planifolia*, and *P. primuliflora*) as well as the federal level (*P. ionantha*) (Wunderlin and Hansen 2008). These species are most identifiable from one another in the field during the reproductive season where floral phenotypic differences can be observed. *Pinguicula ionantha* is highly endemic and restricted to a six county region in the Florida Panhandle. The other *Pinguicula* species vary in their geographic distributions; however, all co-occur within the six county range of *P. ionantha* (Figs. 1.3 and 1.4). This area is the geographic focus of my study.

In the Florida Panhandle, *Pinguicula* species are found in *Pinus palustris* (longleaf pine) savannas. This particular ecosystem includes a wide range of plant community types, from xeric *Pinus-Quercus* scrub to mesic flatwoods and wet savannas, and shrub pocosin to *Taxodium* depressions (Sorrie and Weakley 2006). The longleaf pine savannas are fire-adapted systems (Frost 1998; Platt 1999). Historically, lightning strikes started frequent fires that maintained the understory by removing litter, grasses, and less fire adapted shrubs (Platt 1999) which, in turn, promoted species richness and species turnover (Noss et al. 2015). The combination of heterogeneous plant communities and frequent burn regimes makes this region particularly floristically diverse as well as a center of endemism. However, it is often overlooked as a biological hotspot and has only recently been given this distinction (Noss et al. 2015). This habitat has been drastically reduced to roughly three percent of the 38 million hectares it once occupied largely due to logging and fire suppression (Frost 1993; Frost 2006). Today,

most of the fire-maintained remnants are on military bases, national forests, other public lands, and private reserves (Frost 2006).

Pinguicula lutea can be used as a model system to better understand the basic biology and ecology of these subtropical *Pinguicula* species. Large bright yellow flowers allow it to be easily distinguished from its congeners, all of which have white to purple/blue flowers. Though threatened, this species is locally abundant in the Florida Panhandle where robust populations inhabit areas of varying vegetative structure such as mowed roadside habitats, grassy savanna habitats, and woody scrub habitats. These factors make it an ideal system for the study of a wide range of scientific inquiries from the basic biology of the species to more complex ecological interactions.

I investigated how plant insect interactions during the reproductive season are affected by habitat vegetation structure with an emphasis on pollination and prey capture. In order to do this study, I first needed to investigate the pollination biology of the species and to conduct a survey of the prey community. These preliminary studies conducted in 2013 are described below.

***Pinguicula lutea* Pollination Biology – Preliminary Studies**

Several studies have been conducted on the breeding system of *Pinguicula* species (e.g., Molau 1993; García et al. 1994; Zamora 1999). These studies have shown that most *Pinguicula* species are xenogamous self-compatible, but a few species are autogamous. In the case of *Pinguicula lutea* information on breeding system is not available. Therefore, in 2013 I conducted a breeding system study to determine the pollination biology for *Pinguicula lutea* (Fig. 1.5).

I randomly selected ten reproductive individuals in bud, with petals tightly closed around the reproductive organs at eight sites. Three treatments were performed to assess the potential for autogamy (self pollen deposition in the absence of a pollen vector), self-compatibility (ability to use self pollen), and xenogamy (outcrossing). Buds randomly assigned to the autogamy and self-compatibility treatments were bagged using white 7.6cm x 10.2cm drawstring organza bags. Buds randomly assigned to the xenogamy treatment were marked with thread. Five individuals were assigned to each treatment at each population in the study. Hand pollinations were conducted when the petals began to open, exposing the reproductive organs to potential fertilization. Hand pollination was performed by removing the lower lip of the corolla and using a sharpened, dark colored toothpick to lift the stigma flap and transfer pollen from the anthers to the top of the stigma (receptive side). Once hand pollinations were completed the organza bags were replaced. Individuals across all treatments were monitored weekly for fruit formation. Maturing fruits were collected during the last week in March.

In addition to determining the breeding system for the species, in 2013 I conducted both diurnal pollinator observations and nocturnal pollinator observations of *P. lutea* during the reproductive season (i.e., February to April). However, no insect visitors were recorded using this method. On-line photographs and haphazard observations in the field showed that Hymenoptera do visit *P. lutea* flowers. Therefore, I developed a different method to better assess pollinator visitations. In 2014 and 2015 I used a video recording system to observe pollinators to *P. lutea* (Fig. 1.6). The video recording system used in this study was modeled after the user-built digital video monitoring system as described in Cox et al. (2012). This system was originally designed

for studies examining avian nesting ecology, but was easily adapted for this study. The components of the digital video monitoring system included a digital video recorder (DVR), a battery, and a weatherproof day/night security camera. A BNC cable connected the DVR to the camera. An external monitor was hooked up during initial placement to aid in camera placement. Footage was stored on a four-gigabyte SD card and reviewed in the lab.

Based on this work I was able to determine that *P. lutea* is a self-compatible species. Flower morphology, in particular the position of the anthers and stigma flap, points to the requirement of an insect vector to move pollen from the anthers to the stigma (Fig. 1.7). Pollinator observations and video footage confirmed that cross-pollination can occur in this species as insect visitors were seen foraging at multiple individuals within a population (Fig. 1.8). The main insect visitors of this species are bumblebees (*Bombus spp.*), honey bees (*Apis mellifera*), and carpenter bees (*Xylocopa spp.*).

***Pinguicula lutea* Prey Availability and Prey Capture**

Previous work on *Pinguicula* prey capture and prey availability showed that Diptera and Collembola are the main prey items (Molau 1993; Antor and García 1994; Alcalá and Domínguez 2003; Adler and Malmqvist 2004; Pavón et al. 2011). For *Pinguicula lutea* limited information is available regarding prey capture. Only two published studies are available that provide some information about the prey of this species. Mary Treat, a well-known naturalist from the 19th century, conducted the first study. During the winter of 1875, Treat traveled to Florida and made observation on several *Pinguicula* species. In the particular case of *P. lutea*, she noted the presence of

minute flies on the leaves (Treat 1876). Gibson (1991) conducted the second study and determined that insects with body size above 5 mm can escape from the trap. In this study the insects were not identified to any taxonomic level. No information is available for *Pinguicula lutea* prey availability.

Due to limited information on prey availability and prey capture for *P. lutea*, surveys were conducted at ten sites in 2013. However, before the prey capture surveys were conducted different techniques were tested to determine the best method to collect prey from *Pinguicula* leaves (Table 1.1). It was determined that white electrical tape could create a “squash” of the leaf surface with no apparent damage to the leaf. This technique proved to be effective for determining prey composition and abundance (Fig. 1.9). Wood rectangles with odorless, colorless, and non-dry glue (Tanglefoot, The Tanglefoot Company, Grand Rapids, Michigan) were used to assess arthropod availability (Fig. 8; see methods for Prey Capture and Arthropod Availability in Chapter 2). Based on the leaf squashes and these wood rectangles, I was able to determine that the main prey captured and available to *Pinguicula lutea* in 2013 were Collembola and small Diptera (Fig. 1.10).

***Pinguicula lutea* Habitat**

In Florida, *Pinguicula lutea* prefers moist to wet sandy-peaty soil in seepage bogs, pine flatwoods and savannas (Godfrey and Stripling 1961). However, site visits to multiple populations in 2013 showed that vegetation structure varied among sites due to the presence or absence of management. *Pinguicula lutea* populations were identified in a gradient of sites ranging from mowed roadside populations to heavily forested

populations. Visually, three habitat structures were identified: maintained (mowed), grassy (dense *Aristida stricta* var. *beyrichiana*), and woody (*Hypericum/Ilex* mix) which mirror the resulting above ground vegetation structure as time since fire (Fig. 1.11). Yet, the effects of habitat structure on pollination and prey capture of *Pinguicula lutea* are unknown.

I set up preliminary studies to assess arthropod availability and prey capture at each of the three habitat structures identified (Fig. 8; see methods for Prey Capture and Arthropod Availability in Chapter 2). In addition, I chose to use populations in sites that represented the extreme of each habitat structure to capture the best and worst habitat conditions for *Pinguicula lutea* persistence. Results of my preliminary study suggested that patterns in arthropod availability and capture among habitat structures exist (Figs. 1.12 and 1.13). In addition, populations in the most heavily wooded habitats (i.e., bare ground, moist, and low light availability) had very small populations with mostly non-reproductive individuals. Although this strongly suggested an impact on reproduction due to very few sites (i.e., two) this extreme habitat structure could not be further explored for a larger scale study.

Summary

I developed field techniques to determine the basic biology and ecology of *Pinguicula lutea*. *Pinguicula lutea* is a self-compatible species that requires a pollen vector. Collembola and small Diptera are the primary prey items. As in the case of many carnivorous plants, *P. lutea* is threatened and habitat modification is a major contributor

to its decline. Chapter 2 focuses on the ecological implications of some of these habitat modifications on the reproduction and prey capture of *P. lutea*.

TABLES

Table 1.1. Techniques tested to determine the best method for prey collection from *Pinguicula lutea* leaves. *Indicates the technique used in my study.

Technique	Outcome
Tweezers	Prey removal is slow and tweezers may leave behind smaller prey items
Moistened Q-Tip	Prey removal is quick, but insects may be damaged during swabbing making identification difficult
*White Electrical Tape	Prey removal is quick and efficient. Tape does not dissolve in alcohol.
Duct Tape	Prey removal is quick and efficient. Tape appears affected by alcohol.
ScotchTape	Prey removal is quick and efficient. Tape appears affected by alcohol.
Travel-sized lint roller	Prey removal is quick and efficient. Tape appears affected by alcohol.

FIGURES



Figure 1.1. All *Pinguicula* species have similar vegetative and floral characteristics including a basal rosette of leaves and an elongated scape with a terminal, showy flower. Some example shown from the Southeast US.



Figure 1.2. Floral morphology of *Pinguicula lutea*. Left: Stigma flap and stamen arrangement in an open flower of *Pinguicula lutea*. Only the filaments are visible as the anthers are tucked under the stigma flap. Right: Dissection of *Pinguicula lutea* flower showing that anther dehiscence is in a transverse form.

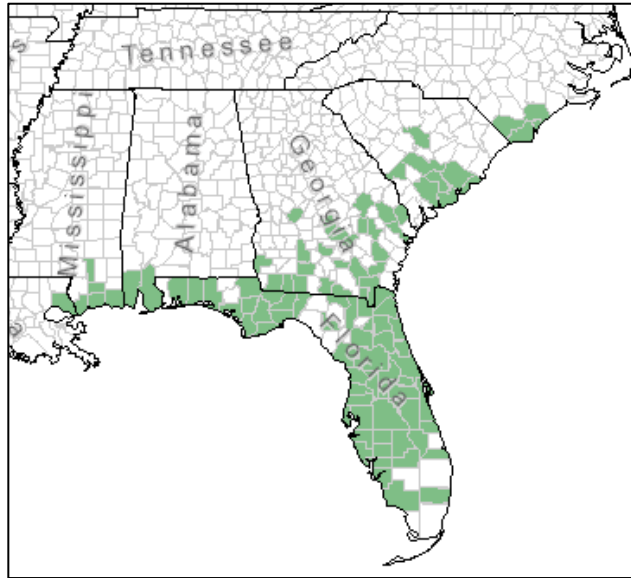


Figure 1.3. United States Department of Agriculture Natural Resources Conservation Service PLANTS Database distribution map for *Pinguicula lutea* (<http://plants.usda.gov/core/profile?symbol=PILU2>). Green polygons indicate counties where vouchered specimens have been collected and white polygons indicate counties where no vouchered specimens have been documented.

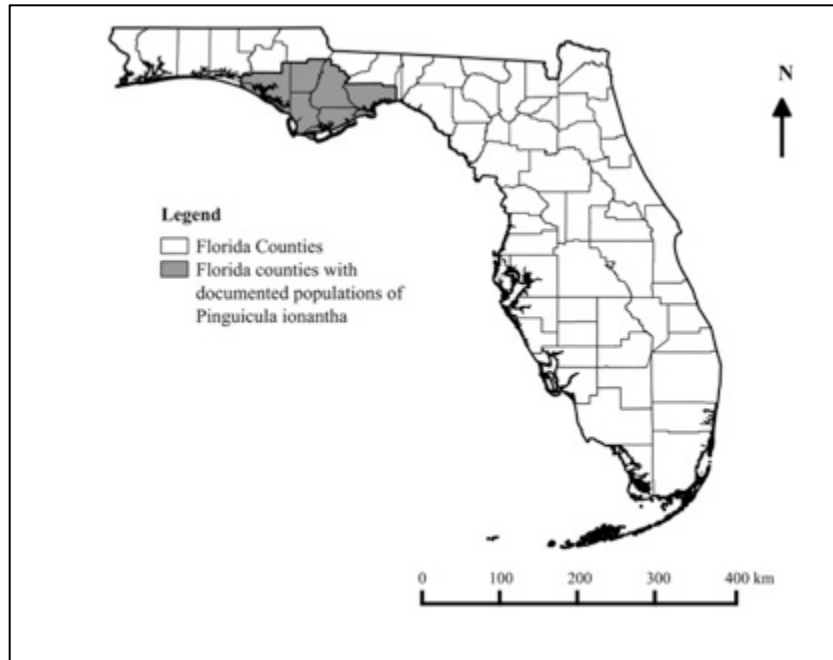


Figure 1.4. Though the six Southeast *Pinguicula* species vary in their geographic distributions, all co-occur within the six county range of *P. ionantha* (gray counties). This area is the geographic focus of my study.



Figure 1.5. Steps to conduct hand pollinations for *Pinguicula lutea* labeled in alphabetical order. a) Flower bud is covered with organza bag, b) bag is removed, c) lower lip of the flower is removed with tweezers to expose the reproductive organs, d and e) pollen is removed from under the stigma using a colored toothpick and then placed on top of the stigma, and f) flower is covered again.

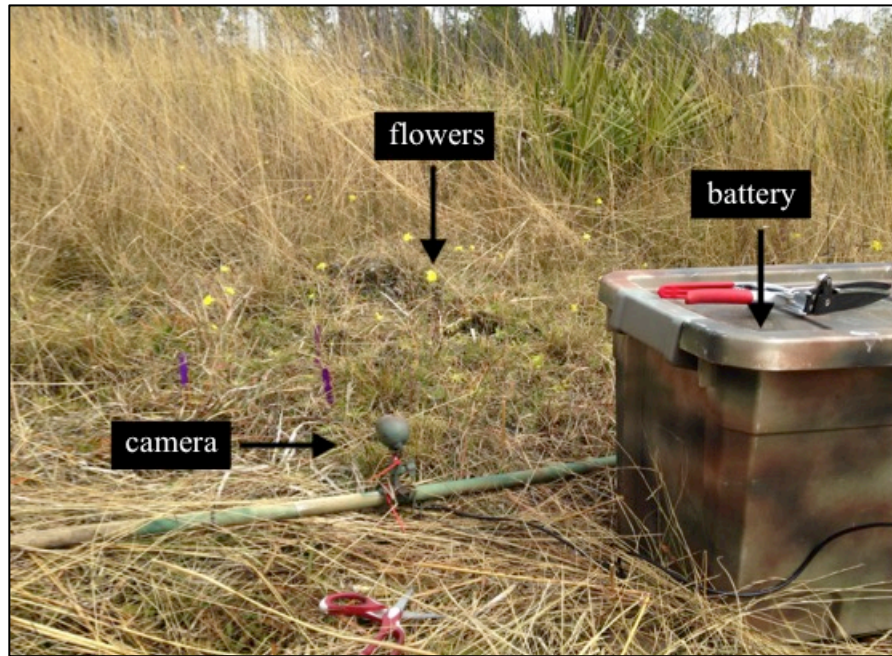


Figure 1.6. Video recording system used to observe pollinator visitation at St. Joseph Bay State Buffer Preserve (Gulf County, Florida) for *Pinguicula lutea* in 2014 and 2015.

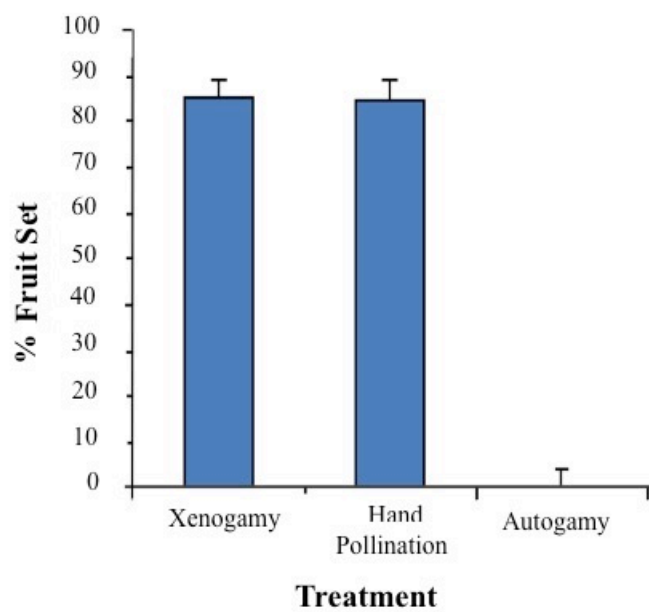


Figure 1.7. Mean % fruit set ($\pm$ SE) for 2013 *Pinguicula lutea* breeding system treatments (ANOVA, $F_{2,18} = 71.187$, $p < 0.001$).

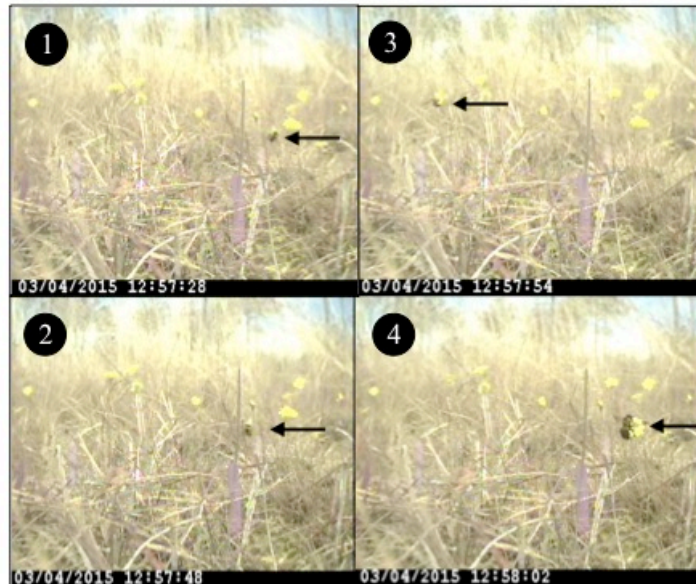


Figure 1.8. Time sequence of pollination event by a carpenter bee (*Xylocopa sp.*) for *Pinguicula lutea* at St. Joseph Bay State Buffer Preserve (Gulf County, Florida). Arrows indicate the pollinator.



Figure 1.9. Left: Sampling method for prey capture (white electrical tape “squash” of the leaf surface with insects) for *Pinguicula lutea* at Tate’s Hell State Forest (Liberty County, Florida). Right: Sampling method for arthropod availability (sticky trap made of wood squares covered with Tanglefoot Tangle-Trap Insect Trap Coating and secured to the ground with a steel groundcover staple).

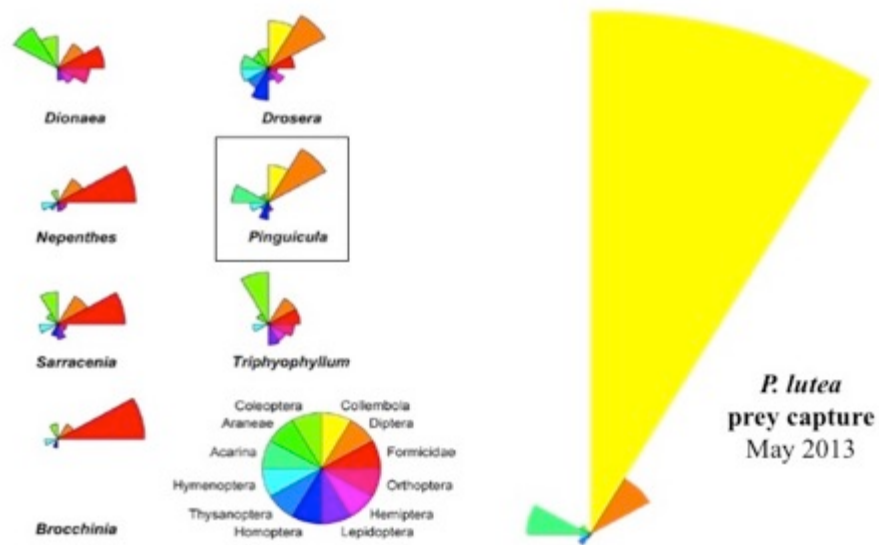


Figure 1.10. Left: Prey spectra of several carnivorous plants (taken from Ellison and Gotelli 2009). Each slice of the pie chart represents the 12 most common prey orders tapped by these. The size of each slice is scaled to the average proportion for the prey taxon it represents (order except for ants—family Formicidae). Right: *Pinguicula lutea* main prey items (i.e., mostly, Collembola and small Diptera) captured in May 2013.



Figure 1.11. From left to right: *Pinguicula lutea* populations in maintained, grassy, and woody habitat structures. In this study, maintained populations were found along roadside corridors where grasses and associated vegetation is maintained via mowing. Grassy populations were dominated by dense wiregrass (*Aristida stricta* var. *beyrichiana*). Woody populations had a mixture of shrubs (*Hypericum/Ilex* mix) and grasses. This vegetation gradient is a proxy for time since fire.

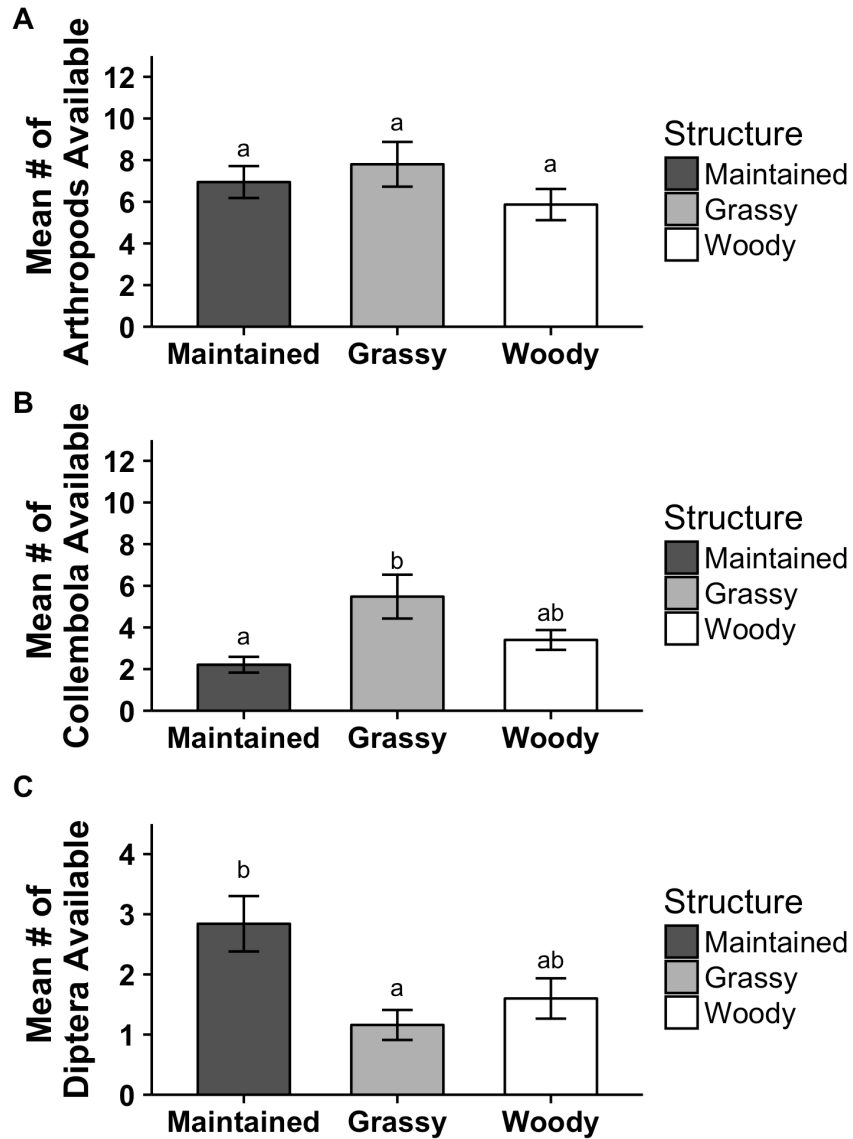


Figure 1.12. Mean ($\pm$ SE) total arthropods available (A. Analysis of Deviance, $\chi^2=5.15$, DF=2, $p=0.35$), Collembola available (B. Analysis of Deviance, $\chi^2=31.71$, DF=2, $p<0.01$), and Diptera available (C. Analysis of Deviance, $\chi^2=16.59$, DF=2, $p<0.01$), for *Pinguicula lutea* across maintained, grassy, and woody habitat structures in May 2013.

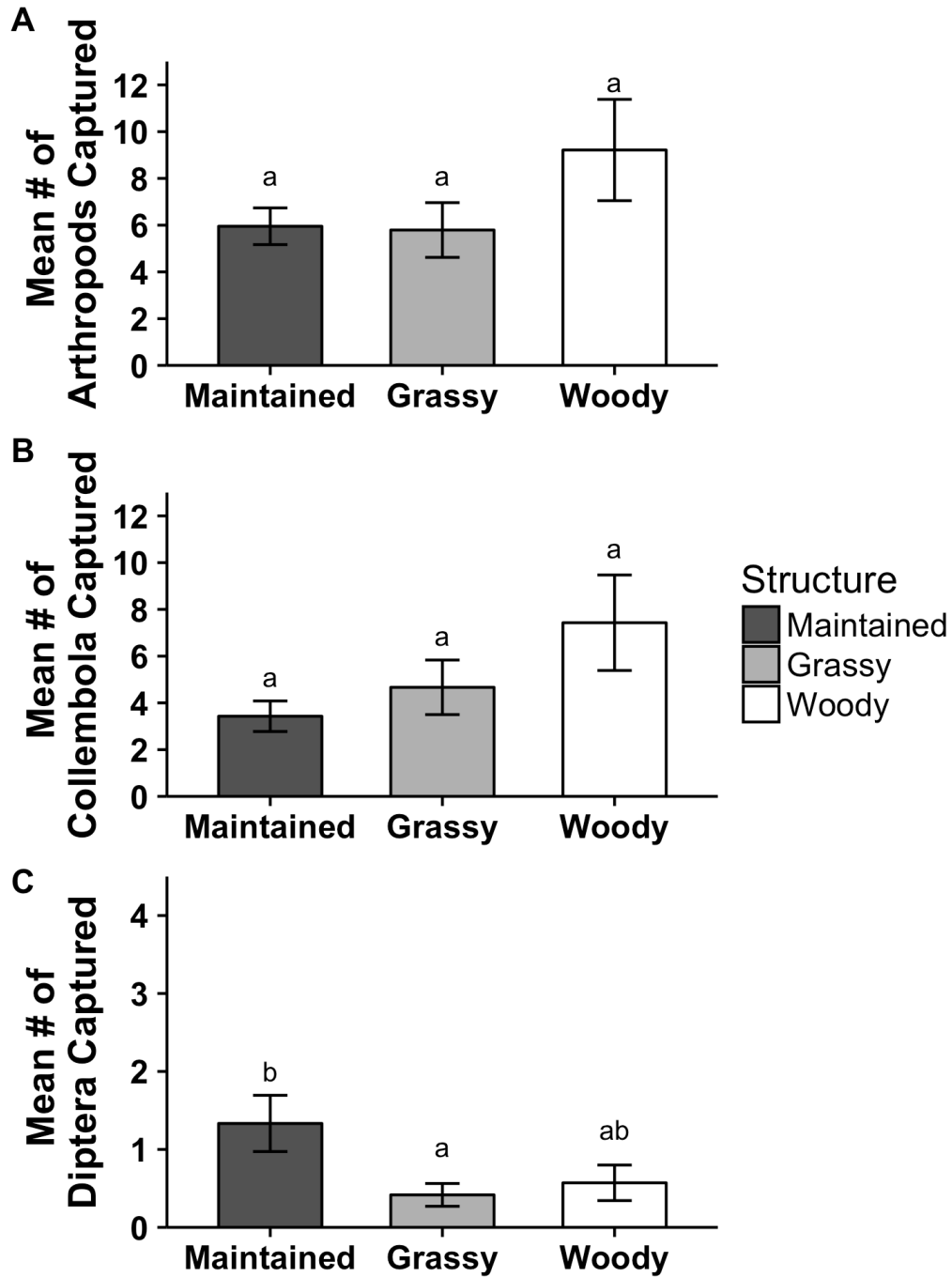


Figure 1.13. Mean ($\pm$ SE) total arthropods captured (A. Analysis of Deviance, $\chi^2=16.72$, DF=2, $p=0.17$), Collembola captured (B. Analysis of Deviance, $\chi^2=26.34$, DF=2, $p=0.10$), and Diptera captured (C. Analysis of Deviance, $\chi^2=12.55$, DF=2, $p=0.17$), for *Pinguicula lutea* across maintained, grassy, and woody habitat structures in May 2013.

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CHAPTER 2: EFFECT OF HABITAT STRUCTURE ON REPRODUCTION AND PREY CAPTURE OF A RARE CARNIVOROUS PLANT, *Pinguicula lutea*

Abstract

Habitat modification is one of the greatest threats to biodiversity worldwide and the main contributor to the decline of many carnivorous plant species. For carnivorous plants in the Southeast United States, including many *Pinguicula* species (butterwort, Lentibulariaceae), habitat modification via altered fire regime has been implicated in their decline. Despite this, limited empirical research has been conducted examining the influence of habitat structural changes on reproduction and prey capture. The objectives of my study are to assess the impacts of habitat structural changes on reproduction and prey capture for *Pinguicula lutea* (yellow butterwort) in the Florida Panhandle.

Pinguicula lutea is a self-compatible, outcrossing, carnivorous plant that inhabits fire-dependent longleaf pine savannas of the Southeast United States and is threatened in the state of Florida. Its primary prey items are Collembola and small Diptera. In 2014 and 2015, 13 populations were identified occupying three different habitat structures: maintained (mowed), grassy (dense *Aristida stricta* var. *beyrichiana*), and woody (*Hypericum/Ilex* mix). Reproductive output was determined by assessing fruit and seed set at each habitat structure. Additionally, prey availability and prey capture were assessed at each habitat structure. In general, habitat structural changes did not affect reproduction, but did affect the abundance of Collembola, Diptera, and all arthropods combined both in terms of availability and prey capture. Arthropod availability and prey capture was taxonomically similar among habitat structures. Prey capture was always a

subset of arthropod availability. Total arthropod availability and prey capture was lowest in grassy habitat structures compared to maintained habitat structures and woody habitat structures. Microclimatic conditions (e.g., light availability) associated with each habitat structure and leaf morphology/physiology could explain the observed arthropod abundance and prey capture patterns. This study is the first comprehensive assessment of plant-insect interactions for *Pinguicula* species of the Southeast US and highlights the importance of habitat quality and management for this understudied group of carnivorous plants.

Introduction

The degree that plant-insect interactions influence an ecosystem is dependent on both biotic and abiotic factors that govern an area and can vary from plant to plant (Zamora 1999). Natural disturbances, such as fire, are often necessary to maintain biological and ecological dynamics in an ecosystem. In the absence of such disturbance (e.g., fire) many fast growing woody species encroach on smaller slower growing plant species (Menges and Kimmich 1996). Such structural and compositional habitat changes may confer site-specific impacts that can alter plant-insect interactions. Studies have shown that foraging behavior of pollinators is affected by habitat changes such as shading (McKinney and Goodell 2010). These changes in foraging behavior can ultimately have a negative impact on reproduction. For plants that depend on insects for both pollination and nutrition such as carnivorous plants, habitat changes resulting from a lack of disturbances could result in a compounded negative impact, such as reduced pollinator visitation and prey capture.

Although only a small fraction of all flowering plants are carnivorous (~ 600 species from 17 genera worldwide; Ellison and Gotelli 2009), their unique life history traits and habitat associations make them ideal systems for addressing general questions in plant population ecology (Ellison et al. 2002), including plant-insect interactions. Carnivorous plants typically live in nutrient poor, wet environments relying on prey capture to provide supplemental nutrients needed for growth and development. In addition, carnivorous plants often have showy floral displays that rise well above the trap of the plant. There is speculation that showy floral displays might be due to pollinator-prey conflict (Zamora 1999), but this trend appears to be consistent for both carnivorous plants in which a natural barrier separates the trapping mechanism from the floral display (i.e., *Utricularia*) as well as for carnivorous plants where no such structural barrier exists (i.e., *Drosera*; Anderson and Midgley 2001).

Some evidence supports the hypothesis that pollination might simply be difficult to achieve in many carnivorous plants. For example, *Drosera* species with shorter scapes received less floral visitors than those with longer scapes (Anderson 2010). This response suggests that carnivorous plants may have evolved elongated scapes to make their floral displays more attractive to potential pollinators. Changes in habitat structure that affect pollinator behavior have been shown to reduce pollinator visitation for some plants (e.g. Herrera 1995), and this response may be the case for some carnivorous plants as well.

Habitat structure that reduces arthropod abundance could also negatively impact carnivorous plants via reduced prey capture. Prey capture is a limiting factor for many carnivorous plants (Thum 1988; Thum 1989; Thorén and Karlsson 1998), so reduced

prey capture may have deleterious effects on carnivorous plant fitness and population growth. For carnivorous plants with passive trapping mechanisms, prey capture is typically driven by arthropod availability in the microhabitat as well as the retention capacity of the trap itself (Zamora 1990; Zamora 1995). Changes in habitat structure can provide microclimatic variation affecting both arthropod availability as well as prey capture. For example, for *Pinguicula vallisneriifolia*, arthropod availability is highest in damp, shaded areas, while retention of prey is highest in sunny, dry area (Zamora 1995; Zamora et al. 1998). However, the degree to which habitat modification affects prey capture in carnivorous plants whose habitats are rapidly changing is unclear.

Pinguicula lutea Walter (yellow butterwort; Lentibulariaceae) is a self-compatible, outcrossing species endemic to the Southeast United States and is threatened in the state of Florida. Though threatened, robust populations can be found in the longleaf pine savannas of the Florida Panhandle. Prior research indicates that this habitat is very sensitive to burn regimes, requiring a burn cycle every two to three years to maintain ecosystem dynamics (Kesler et al. 2008). For *Pinguicula* species of the Southeast U.S., fire plays a crucial role in reducing tall above-ground vegetation (Hermann 1995). In the absence of fire, more aggressive vegetative species displace *P. lutea*. This displacement has been suggested as the reason for its decline (Gulledge et al. 2011); however, little empirical work exists to support this claim. The passive sit-and-wait trap and need for pollination might make *Pinguicula* species acutely sensitive to changes in insect communities due to habitat alteration. If changes in the composition of neighboring vegetation reduce the movement or abundance of arthropods, *P. lutea* could experience reduced pollination and prey capture.

The objective of this study is to determine if habitat structure affects plant-insect interactions for *Pinguicula lutea* as they relate to reproduction and prey capture. Since the cause of species decline in this area has been largely attributed to changes in habitat structure I expect that 1) fruit set and seed set will be greatest in areas where the surrounding vegetation is low and flowers are most visible to potential pollinators, 2) prey capture will be highest in populations where the vegetation is low and the basal rosette is most likely to be exposed to higher levels of light intensity than populations where the vegetation is higher which may shade the basal rosette, and 3) prey capture, as a passive trapping technique, will follow the ambient abundance of insect prey across maintained, grassy, and woody habitat structures.

Methods

Species Description

Pinguicula lutea is a perennial herb with a basal rosette of carnivorous, ovate leaves. Individual leaves lie flat against the soil surface or curve slightly upward. Leaf margins may roll upward creating a V-shape from tip to base (Godfrey and Stripling 1961; Legendre 2000). Leaves are studded with trichomes capable of producing sticky mucilage to trap prey and digestive enzymes to break down and assimilate nutrients (Heslop-Harrison and Heslop-Harrison 1981). This mechanism of prey capture is aptly referred to as a passive trap, as the plant consumes items that happen to land and stick to its leaves. In the case of *Pinguicula lutea* it has been determined that Collembola and Diptera are the primary prey type captured by the leaves. In the Florida Panhandle *Pinguicula lutea* blooms mainly from February – April. Plants bear one to several

flowers, each of which is borne singly on an elongated scape. The main pollinators of this species are bumblebees (*Bombus spp.*), honey bees (*Apis mellifera*), and carpenter bees (*Xylocopa spp.*).

Study Site

I conducted my fieldwork in a four county region in the Florida Panhandle (Bay, Gulf, Franklin, and Liberty; Fig. 2.1), an area historically dominated by longleaf pine savanna communities. The disruption of fire regimes and anthropogenic changes has resulted in the rapid decline of this community type (Van Lear et al. 2005). Currently, most of this community type has largely been appropriated to federal or state managed lands. Common management practices across both federal and state lands include mechanical removal of woody species, stand thinning, mowing, and prescribed fire. However, the frequency at which these management practices are employed can vary between management units.

In 2014 and 2015, I chose 13 sites and assigned each to one of three habitat structures: maintained (mowed), grassy (dense *Aristida stricta* var. *beyrichiana*), and woody (*Hypericum/Ilex* mix). Some, but not all, sites were studied in both years (Table 2.1). Some sites do not necessarily represent distinct populations, as some populations included more than one habitat structure. For example, maintained sites were commonly mowed roadside populations. However, these populations often extended past the mowed corridor and into surrounding vegetation, which was grassy or woody and created two habitats based on distinct microclimatic conditions. Zamora (1990) and Zamora et al.

(1998) used similar site classifications when a single population represented different ecological scenarios.

Structural Analysis

At each site, I established a 20m transect where the site appeared to be the most representative of the overall habitat structure, nearest to the middle of the population. I placed five 1m² quadrats on alternating sides at 5m intervals along the transect (0m left, 5m right, etc.). I considered all vegetation rooted inside the quadrat for the structural analysis. Additionally, dead vegetation was only classified as litter if it was no longer rooted. This is an important distinction for this study since the wiregrass often had both new and old (dead) growth on the same plant. Within each quadrat, I visually ranked percent cover into a cover class (0-6) using a traditional Daubenmire scale for six components of structure: graminoids, woody, forbs, bare ground, litter, ground cover (Daubenmire 1959). In many cases, the total percent cover in a quadrat is greater than 100. This observation reflects the various strata of the components in which different structural components may inhabit overlapping vertical space.

In addition to these six components, I also recorded vegetation height for each quadrat as it is thought that vegetation height might be an important factor influencing *P. lutea*. Vegetation height was sampled at five points, the corners and the middle of each quadrat. These numbers were averaged to get the mean vegetation height for each quadrat. Vegetation height was recorded in centimeters.

Light Availability

In addition to vegetation cover and height, light availability was measured to assess differences among habitat structures. In 2014 and 2015, light availability, measured as photosynthetically active radiation (PAR; $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), was recorded using a six-sensor line quantum handheld meter (MQ-306, Apogee Instruments, Utah, USA). All readings were taken between 11am and 2pm EST on sunny cloudless days. One full sun reading was recorded at each site and served as the “ambient light reading” and was used as the maximum available light to compare to light at ground level. The middle of all quadrats were marked and used as the midpoints for the sensor for all light readings. Two diagonal readings and two perpendicular readings were taken (making a star shape) at the ground level against the soil surface, and averaged, for each quadrat.

Fruit Set

In February of 2014 and 2015, I randomly selected 10 buds (one bud per individual) per site. Only buds in which the corolla was still tightly rolled around the reproductive organ of the flower were used to ensure that none of the selected individuals were pollinated yet. I marked the scape of each bud with thread and monitored for evidence of fruit formation. In April, those individuals that produced mature fruit were collected for seed set analysis. For the purpose of data analysis, I defined fruit set as the number of marked individuals pollinated with visible signs of fruit formation (i.e. fruit present or absent).

Seed Set

For each year, I assessed seed set by determining the proportion of fully developed seeds to the total number of ovules per fruit. Fully developed seeds were considered to be dark brown to black and larger than ovules. I used a digital counting method to count both seeds and ovules due to their extremely small size and large number. I dissected individually collected fruits, separating all seeds and ovules from the chaff. All seeds and ovules were removed, placed on a white background (to improve contrast), and spread as evenly as possible. The seeds and ovules of each individual fruit were then photographed using a Nikon D40 camera (Nikon, Thailand).

I extracted seed and ovule counts from each photograph using ImageJ v1.47. I manually counted all seeds by identifying them individually. Counted seeds were marked with a colored dot and numbered to ensure accuracy. To count the ovules accurately, each image required processing. Images were converted to 32 bit black/white with a threshold of 48.00, 186. Resulting photos were then made into a binary image, and the particles were counted based on pixel size. All objects with an area of ten pixels or smaller were considered to be ovules. I verified accuracy of the digital method with hand counts.

Prey Capture and Arthropod Availability

I assessed prey capture and arthropod availability during the reproductive season (February-April) in 2014 and 2015 at maintained, grassy, and woody sites. In both years of this study all sites mirror those used in the fruit set and seed set component of this project. At each site, I haphazardly selected five reproductive individuals (i.e. flower buds present).

Selected individuals were surveyed every two weeks during peak flowering (late February-early April) for a total of three survey periods.

Surveys consisted of two-day intervals in which the fully open most distal leaf with prey was selected by placing small colored toothpicks on either side of the leaf. Because *Pinguicula* traps begin digesting prey within a few hours, leaves were not cleared of prey prior to sampling, as digestive glands may only be activated once (Heslop-Harrison and Knox 1971). After 48 hours I revisited each leaf and took a squash of the leaf surface, removing arthropod prey from the leaves. While other prey-related *Pinguicula* studies have collected leaves to identify prey (Zamora 1990; Zamora 1995; Alcalá et al. 2010), I was limited to methods that do not impact the plant due to the threatened status of *P. lutea*. For this reason the squash of the leaf surface was conducted with white electrical tape allowing for prey to be removed in a single press of the leaf. The tape with prey was placed into an 8ml plastic vial filled with 70% ethyl alcohol. All sampling was conducted during sunny days where there was no risk of precipitation washing prey from the leaf surface. Arthropods were identified to the order level and abundance was determined for each order. Since some taxa made up the majority of the total prey capture, I reported total prey abundance, Collembola prey abundance, and Diptera prey abundance.

To assess how prey capture at each site compared to the arthropods available at those sites I created artificial passive traps by coating thin wood rectangles (5.1cm x 7.6cm x 0.6cm) with odorless, colorless, non-dry glue (Tanglefoot, The Tanglefoot Company, Grand Rapids, Michigan). This method has been used successfully in other *Pinguicula* sp. studies (Antor and García 1994; Zamora 1995; Alcalá and Domínguez 2003). One artificial trap was secured to the ground next to a corresponding individual identified for prey capture at

the beginning of a sampling period and collected after two days. Artificial traps were brought back to the lab where arthropods were identified to the order level and abundance was determined for each order. As with prey capture, some taxa comprised the majority of the total arthropods available. Only total arthropods, Collembola, and Diptera are reported.

Statistical Analysis

All data were analyzed in R version R 3.2.2 GUI 1.66 (R Core Team 2015). The following packages were used when appropriate: ggplot2, plyr, reshape2, plotrix, car, multcomp, and vegan (Lemon 2006; Wickham 2007; Hothorn et al. 2008; Wickham 2009; Fox and Weisberg 2011; Wickham 2011; Oksanen et al. 2015).

Structural analysis

I placed each site into one of three habitat structures based on the observed structure in the field and verified the structures using non-metric multidimensional scaling (NMDS) implemented with the vegan package (Oksanen et al. 2015). I compared the structural composition for all sites in both 2014 and 2015 using the six evaluated components of habitat structure as well as vegetation height (Table 2.1).

Light availability

I used a generalized linear model to test how light availability at ground level differs among habitat structures. The dependent variable, light availability, was modeled with a Gaussian distribution. The two independent variables were habitat structure and year (2014 vs. 2015). Assumptions of normality of residuals and equal variance were

met. I compared the difference in the residual variance for the null model, which included light availability and tested the difference against an F distribution. I followed this analysis with a Tukey's *post hoc* test to determine which treatment means were statistically significant from each other, where tests were conducted separately within each year in cases of a significant interaction. Means and standard errors are reported.

Fruit set

I used a generalized linear model to test how fruit set differs among habitat structures. The dependent variable, fruit set, was modeled with a binomial distribution (i.e. fruit present or absent). I tested the significance of the two independent variables, habitat structure and year (2014 vs. 2015). Visual inspection of the normality of the residuals and equal variance appeared appropriate. I compared the difference in the residual variance for the null model, which included fruit set and tested the difference against a χ^2 distribution. Means and standard errors are reported.

Seed set

I used a generalized linear model to test how fruit set differs among habitat structures. The dependent variable, seed set, was modeled with a Gaussian distribution. The two independent variables were habitat structure and year (2014 vs. 2015). Though residuals did not meet the normality assumption, the sample size was large enough to negate this problem (Gotelli and Ellison 2004). The data had equal variance. I compared the difference in the residual variance for the null model, which included seed set and tested the difference against an F distribution. Means and standard errors are reported.

Prey capture

I used a generalized linear model to test the relationship between habitat structure and abundance of arthropod prey captured. The dependent variables, abundance of total arthropods, Collembola, and Diptera, were modeled with a quasi-Poisson distribution (due to over dispersion) for maintained, grassy, and woody habitat structures. The model included two independent variables, habitat structure and year (2014 vs. 2015), as well as the interaction term between the independent variable. Visual inspection of the normality of the residuals and equal variance appeared appropriate. I compared the difference in the residual variance for the null model with an analysis of deviance test, by testing the difference against a χ^2 distribution. I followed this with a Tukey's *post hoc* test as referenced above. Means and standard errors are reported.

Arthropod availability

I used a generalized linear model to test the relationship between habitat structure and abundance of arthropod prey available. The dependent variables, abundance of total arthropods, Collembola, and Diptera, were modeled with a quasi-Poisson distribution for maintained, grassy, and woody habitat structures. I tested the significance of the two independent variables, habitat structure and year (2014 vs. 2015), with a generalized linear model that included an interaction term between each independent variable. Though assumptions of normality and equal variance were not statistically met, visual inspection of the normality of the residuals and equal variance appeared appropriate. I compared the difference in the residual variance for the null model with an analysis of

deviance test, by testing the difference against a χ^2 distribution. I followed this analysis with a Tukey's *post hoc* test as referenced above. Means and standard errors are reported.

Results

Structural Analysis

Table 1 summarizes the six components of structure: vegetation height, graminoids, woody, forbs, bare ground, litter, and ground cover for each site per year. Compositional differences were found in maintained, grassy, and woody habitat structures. These compositional differences were defined in the NMDS ordination using the six components of structure as well as vegetation height ($K=2$, stress=0.143).

Maintained habitats had the most compositional variation, while the woody habitats had the least compositional variation (Figs. 2.2 and 2.3). Woody habitats are clustered near the bottom left where the woody and litter criteria are. Grassy habitats cluster at the top left where the bare ground and graminoids components are. Maintained habitats appear to be less clustered along the y-axis, but are well clustered along the x-axis.

Light Availability

In 2014 and 2015 mean light availability was greatest in maintained habitat structures and lowest in grassy and woody habitat structures (Fig. 2.4). While there was no significant effect of year ($F_{1,21}=0.44$, $p=0.51$), there was a significant effect of habitat structure ($F_{4,19}= 6.82$, $p<0.01$). The interaction between year and habitat structure was not

significant ($F_{2,19} = 0.23$, $p = 0.79$). In both years, roughly only a quarter of ambient light was available at ground level for grassy habitat structure types and woody habitat structure type, whereas maintained habitat structures had more than twice as much light available. In 2014, maintained sites had an average of $57.4\% \pm 9.1$ ($\pm$ standard error), while grassy and woody sites had an average of $26.3\% \pm 7.2$ and $27.2\% \pm 8.0$, respectively. Similarly, in 2015, maintained sites had an average of $67.7\% \pm 7.8$, while grassy and woody sites had an average of $27.2\% \pm 6.6$ and $28.0\% \pm 7.6$, respectively. A Tukey's *post-hoc* test shows significant differences between maintained and grassy sites as well as maintained and woody sites. However, grassy and woody sites were not significantly different from each other.

Fruit Set and Seed Set

Mean fruit set was high across maintained, grassy, and woody habitat structures in 2014 and 2015 (Fig. 2.5). In 2014 fruit set across all habitat structures ranged from $55.0\% \pm 16.6$, to $72.5\% \pm 8.5$. In 2015 fruit set across all habitat structures ranged from $70.0\% \pm 12.9$ to $78.0\% \pm 3.7$. No significant differences in fruit set were found between years (Analysis of Deviance, $\chi^2 = 2.66$, $DF = 1$, $p = 0.10$) or among habitat structures (Analysis of Deviance, $\chi^2 = 1.42$, $DF = 2$, $p = 0.49$), and the interaction between year and habitat structure was not significant (Analysis of Deviance, $\chi^2 = 2.23$, $DF = 2$, $p = 0.33$).

Similarly, mean seed set was high across maintained, grassy, and woody habitat structures in both survey years (Fig. 2.6). Seed set ranged from $84.0\% \pm 2.5$ to $89.4\% \pm 1.7$ in 2014 and from $81.1\% \pm 1.6$ to $86.2\% \pm 1.5$ in 2015. There was no significant effect of

year ($F_{3,260}=2.45$, $p=0.06$), habitat structure ($F_{2,261}=2.76$, $p=0.06$), and the interaction between year and habitat structure ($F_{5,258}= 2.01$, $p=0.08$).

Arthropod Availability

Total arthropods

Overall, the total arthropod availability was 50% greater in 2015 than 2014. However, this increase was not the same across all three habitat structures. Populations in the maintained habitat structure exhibited the most dramatic increase in arthropod availability with nearly twice as many arthropods available in 2015 than 2014. Populations in the grassy habitat structure and the woody habitat structure increased by 21% and 37% respectively (Fig. 2.7A). Significant differences were found between years (Analysis of Deviance, $\chi^2=178.99$, $DF=1$, $p<0.001$) as well as among habitat structures (Analysis of Deviance, $\chi^2=140.45$, $DF=2$, $p=<0.001$), and the interaction between year and habitat structure was significant (Analysis of Deviance, $\chi^2=30.65$, $DF=2$, $p=0.02$).

In 2014, the maintained habitat structure had the most arthropods available at 8.8 ± 0.7 , followed by the woody structure with a mean of 8.6 ± 0.7 total arthropods available, and the grassy habitat structure with a mean of 7.1 ± 0.9 (Fig. 2.7A).. However, the differences in arthropod availability were not significant across habitat structures (Analysis of Deviance, $\chi^2=10.18$, $DF=2$, $p=0.34$)

In 2015, the maintained habitat structure had the most arthropods available at 16.2 ± 1.0 , followed by the woody structure with a mean of 11.8 ± 0.8 total arthropods available, and the grassy habitat structure with a mean of 8.6 ± 0.7 (Fig. 2.7A). The differences in arthropod availability were significant across habitat structures (Analysis

of Deviance, $\chi^2=90.46$, $DF=2$, $p<0.001$). A Tukey's *post-hoc* test shows significant differences between maintained, grassy, and woody habitat structures

Collembola

Collembola availability was 131% greater in 2015 than 2014. This increase was largely due to the 315% increase between years in maintained habitat structures. Woody habitat structures showed a 124% increase while grassy habitat structures showed a 34% increase (Fig. 2.7B). The interaction between year and habitat structure was significant (Analysis of Deviance, $\chi^2=114.74$, $DF=2$, $p<0.0001$). Also, significant differences were found between years (Analysis of Deviance, $\chi^2=458.14$, $DF=1$, $p<0.001$), but not among habitat structures (Analysis of Deviance, $\chi^2=6.02$, $DF=2$, $p=0.58$).

In 2014, the grassy habitat structure had the most Collembola available at 5.2 ± 0.9 , followed by the woody structure with a mean of 3.9 ± 0.6 Collembola available, and the maintained habitat structure with a mean of 2.4 ± 0.3 . The differences in Collembola availability were significant across habitat structures (Analysis of Deviance, $\chi^2=63.45$, $DF=2$, $p<0.0001$). A Tukey's *post-hoc* test shows significant differences between maintained and grassy sites, while woody sites were not significantly different from either maintained or grassy sites (Fig. 2.7B).

In 2015, the maintained habitat structure had the most Collembola available at 10.9 ± 0.9 , followed by the woody structure with a mean of 8.7 ± 0.7 Collembola available, and the grassy habitat structure with a mean of 7.0 ± 0.7 . The differences in arthropod availability were significant across habitat structures (Analysis of Deviance, $\chi^2=57.32$, $DF=2$, $p<0.0001$). A Tukey's *post-hoc* test shows significant differences between

maintained and grassy sites, while woody sites were not significantly different from either maintained or grassy sites (Fig. 2.7B).

Diptera

Diptera availability was 32% lower in 2015 than in 2014. A decrease in Diptera availability was exhibited in maintained, grassy, and woody habitat structures (Fig. 2.7C). In 2014 the grassy habitat structure had the lowest Diptera availability at 0.9 ± 0.2 and the maintained habitat structure had the highest Diptera availability at 5.4 ± 0.6 . In 2015 the grassy habitat structure had the lowest Diptera availability at 0.8 ± 0.2 and the maintained habitat structure had the highest Diptera availability at 4.5 ± 0.4 . Significant differences were found between years (Analysis of Deviance, $\chi^2=22.134$, DF=1, $p=0.002$), and among habitat structures (Analysis of Deviance, $\chi^2=374.67$, DF=2, $p<0.001$). However, there was not a significant interaction between year and habitat structure (Analysis of Deviance, $\chi^2=5.28$, DF=2, $p=0.34$). A Tukey's *post-hoc* test shows significant differences between maintained, grassy sites, and woody sites.

Prey Capture

Total arthropods

Total number of arthropods captured was 99% greater in 2015 than 2014. An increase in total arthropods captured was exhibited in maintained, grassy, and woody habitat structures (Fig. 8A). In 2014 the grassy habitat structure had the least total number of arthropods captured at 1.8 ± 0.3 and the woody habitat structure had the most total number of arthropods captured at 3.5 ± 0.4 . Similarly, in 2015 the grassy habitat structure

had the least total number of arthropods captured at 3.0 ± 0.3 and the maintained habitat structure had most total number of arthropods captured at 6.2 ± 0.6 . Significant differences were found between years (Analysis of Deviance, $\chi^2=181.13$, $DF=1$, $p < 0.001$), and among habitat structures (Analysis of Deviance, $\chi^2=109.1$, $DF=2$, $p < 0.001$). However, there was not a significant interaction between year and habitat structure (Analysis of Deviance, $\chi^2=14.32$, $DF=2$, $p=0.10$). In 2014, a Tukey's *post-hoc* test indicates significant differences between maintained and woody sites as well as grassy and woody sites. However, maintained and grassy sites were not significantly different from each other. In 2015, a Tukey's *post-hoc* test indicates significant differences in grassy sites and maintained sites and grassy sites and woody sites; however, maintained site and woody sites were not significantly different from each other.

Collembola

Number of Collembola captured was 160% greater in 2015 than 2014. Though this increase was exhibited in all three of the habitat structures, the difference between years was much larger in maintained habitat structures and woody habitat structures than in grassy habitat structures (Fig. 2.8B). Maintained habitat structures showed a 334% increase between years, while the grassy habitat structures and woody habitat structures showed a 101% increase and a 107% increase respectively. Significant differences were found between years (Analysis of Deviance, $\chi^2=226.94$, $DF=1$, $p < 0.001$), among habitat structures (Analysis of Deviance, $\chi^2=116.18$, $DF=2$, $p < 0.001$), and interaction between year and habitat structure (Analysis of Deviance, $\chi^2=25.75$, $DF=2$, $p=0.01$).

In 2014, the woody habitat structure had the most Collembola captured at 2.4 ± 0.3 , followed by the maintained structure with a mean of 1.1 ± 0.1 Collembola captured, and the grassy habitat structure with a mean of 1.0 ± 0.2 . The differences in Collembola prey capture were significant across habitat structures (Analysis of Deviance, $\chi^2=50.50$, $DF=2$, $p < 0.0001$). A Tukey's *post-hoc* test shows significant differences between maintained and woody sites as well as grassy and woody sites. However, maintained and grassy sites were not significantly different from each other (Fig. 2.8B).

In 2015, the woody habitat structure had the most Collembola prey captured at 4.9 ± 0.6 , followed by the maintained structure with a mean of 4.6 ± 0.5 Collembola captured, and the grassy habitat structure with a mean of 2.0 ± 0.2 . The differences in Collembola prey capture were significant across habitat structures (Analysis of Deviance, $\chi^2=91.43$, $DF=2$, $p < 0.0001$). A Tukey's *post-hoc* test shows significant differences between maintained and grassy sites as well as grassy and woody sites. However, maintained and woody sites were not significantly different from each other (Fig. 2.8B).

Diptera

Number of Diptera captured was similar in both 2014 and 2015 (Fig. 2.8C). In 2014 the grassy habitat structure had the least Diptera captured at 0.3 ± 0.2 and the maintained habitat structure and the most Diptera captured at 0.9 ± 0.2 . Similarly, in 2015 the grassy habitat structure had the least Diptera captured at 0.4 ± 0.2 and the maintained habitat structure and the most Diptera captured at 1.1 ± 0.2 . The interaction between year and habitat structure was not significant (Analysis of Deviance, $\chi^2=9.99$, $DF=2$, $p=0.19$). No significant difference was found between years (Analysis of Deviance, $\chi^2=0.01$,

DF=1, $p=0.96$); however, there was a significant difference among habitat structures (Analysis of Deviance, $\chi^2=39.83$, DF=2, $p=0.001$) in 2015. A Tukey's *post-hoc* test shows significant differences between maintained sites and grassy sites and maintained sites and woody sites. Grassy sites and woody sites were not significantly different from each other. The interaction between year and habitat structure was not significant (Analysis of Deviance, $\chi^2=9.99$, DF=2, $p=0.19$).

Discussion

Habitat structure affected some, but not all, plant-insect interactions for *Pinguicula lutea* as they relate to reproduction and prey capture. In this study, habitat structure did not affect any measured aspect of the pollination ecology (i.e. fruit set, seed set) of *P. lutea*. In contrast, habitat structure affected prey capture, most likely due to its affect on arthropod availability. *Pinguicula lutea* in grassy habitat structures captured consistently fewer prey than those in maintained and woody habitat structures in both survey years. Plants in woody habitats captured the most prey, while maintained habitats had the most variable prey capture. The number of Collembola captured was the primary driver of these trends.

Fruit Set and Seed Set

Several studies have reported that changes in habitat structure can affect fruit set and seed set due to increased herbivory in shaded habitats or pollinator avoidance of shaded habitats (Herrera 1995; Louda and Rodman 1996; Chi and Molano-Flores 2015). In the case of *P. lutea*, habitat structure did not affect fruit set and seed set. This finding

did not support the prediction that encroachment and shading would reduce fruit set and seed set compared to more open and sunny habitats. For plants of reproductive age, those that produced a flower had equal opportunity for pollination regardless of habitat structure. Similarly, pollinated individuals had high seed set regardless of the habitat structure. These results are inconsistent with Zamora (1999), who found that for *P. vallisneriifolia* fruit set and seed set were lower in shaded habitats compared to sunny habitats.

The lack of a significant effect of habitat structure on reproduction could be heavily influenced by *P. lutea* breeding system and flower longevity. *Pinguicula lutea* is an obligate outcrosser and one visit from a potential pollinator may be enough for successful pollination (Chapter 1). Furthermore, individual flowers can remain open for 21 days (B. Molano-Flores personal communication), which may help ensure the likelihood of a pollination event even if visitation is infrequent. When a pollination event does occur, seed set is uniformly high across all habitat structures types, suggesting an efficient pollination system. Similar results have been found for other *Pinguicula* species. Work conducted by Molau (1993), García et al. (1994), and Alcalá and Domínguez (2003) have reported high fruit set and seed set for *P. alpina*, *P. longifolia*, and *P. moranensis*, respectively. In addition, these studies also have reported that open flowers can persist between 8-27 days.

Prey Capture and Arthropod Availability

Habitat structure affected prey capture and arthropod availability. In both survey years general patterns in prey capture tended to mirror patterns in arthropod availability

at maintained, grassy, and woody habitat structures, with captured arthropods comprising a subset of total arthropods available. However, number of Collembola captured primarily drove the observed trends. *Pinguicula lutea* in grassy habitat structures captured consistently fewer or similar, but not more individuals than those in maintained and woody habitat structures in both survey years.

These trends in arthropod availability and prey capture are broadly consistent with other *Pinguicula* species studied. For example, three alpine *Pinguicula* species, *P. alpina*, *P. villosa*, and *P. vulgaris* showed similar patterns in prey capture where Collembola and Diptera accounted for the majority of captured prey (Karlsson et al. 1987). However, not all *Pinguicula* species favor the same prey (Karlsson et al. 1994). In other studies, Diptera were observed to be the main prey items of various *Pinguicula* species (Antor and García 1994; Alcalá and Domínguez 2003; Adler and Malmqvist 2004; Pavón et al. 2011). Several studies examining arthropod availability and capture report an overrepresentation of certain taxa of captured prey compared to arthropods available (Karlsson et al. 1987; Antor and García 1994; Zamora 1995; Alcalá and Domínguez 2003). While it has been suggested that leaf color or size may attract prey, other *Pinguicula* studies attribute the differences to environmental variation (i.e. abiotic conditions) as well as capacity of mucilage to retain prey (Zamora 1995; Alcalá and Domínguez 2003).

For *Pinguicula* species, arthropod availability and prey capture have been shown to be directly influenced by abiotic conditions associated with the microhabitat (e.g. light availability, temperature, humidity; Zamora 1990, 1995; Karlsson et al. 1994; Zamora et al. 1998; Alcalá and Domínguez 2003). For example, Zamora (1995) examined

differences in arthropod availability in *P. vallisneriifolia* growing in a gradient of light regimes finding an inverse relationship between light availability and arthropod availability. Prey capture followed patterns in light availability among habitat structure types. While Zamora (1995) also documented a significant decrease in light availability from open, shaded, and heavily shaded sites, my study found an equally drastic reduction in light availability in both grassy and woody habitat structures compared to maintained habitat structures. However, the patterns in light availability did not reflect the pattern of prey capture among these habitats which suggests that other aspects of the habitat influence prey capture for *P. lutea*. For example, grassy habitats tended to have a relatively homogenous vegetation structure dominated by wiregrass, whereas woody habitats had more of a heterogeneous vegetation structure dominated by woody shrubs. Vegetation structure may have more of a direct effect on arthropod availability and prey capture.

Abiotic conditions may also impact the morphology and physiology of the trapping mechanism (e.g. leaf morphology, mucilage volume, mucilage viscosity) for carnivorous plants, influencing prey capture and digestion. For example, Zamora (1995) and Zamora et al. (1998) found that leaves of *Pinguicula* species growing in sunny habitats exhibit a greater degree of leaf rolling along the margins, had smaller and more pointed leaves, and produced more viscous mucilage than those in shaded habitats. Yet, the pattern of mucilage production and retention at the sites where Zamora did his work are more complex. Mucilage production was greatest at the semi-shaded sites, followed by the sunny sites, and least at the shaded sites. However, prey retention was highest at sites with the most light availability and lowest in sites with the least light availability.

Though I did not directly measure morphological and physiological characteristics for *P. lutea*, field observations suggest that the same patterns are observed in *P. lutea*. More viscous mucilage along with a greater degree of leaf rolling in maintained habitat structures could help explain why *P. lutea* in this habitat structure captured a greater number of Diptera than in grassy and woody habitat structures.

Although annual differences were not the main focus of my study, I did observe differences between years in arthropod availability and prey capture. Yearly and seasonal prey capture and availability have been reported for carnivorous plants (Murza and Davis 2005; Horner et al. 2012), including *Pinguicula* species (Zamora 1995; Pavón et al. 2011). In the present study, arthropod availability varied between years to the extent that the sites with the highest prey availability in 2014 had roughly the same amount of prey as the sites with the lowest prey availability in 2015. This observed yearly variation may be the result of differences in weather patterns influencing arthropod emergence in the study sites.

To what extent does prey capture matter for *Pinguicula lutea* fitness? Carnivorous plants derive some of their nutrients from arthropod prey and this can lead to higher growth and fecundity. Studies that have examined the effects of supplemental feeding on carnivorous plant growth and reproductive output have found a positive response to additional feeding for *Pinguicula* species (Zamora et al. 1997) as well as for other *Drosera* species (Thum 1988, Schulze and Shulze 1990, Karlsson and Pate 1992). However, the degree to which nutrients derived from prey capture actually benefit carnivorous plants is often species specific and may be difficult to resolve. Additional

research is needed to better understand the benefits of prey capture on the growth and reproduction of *Pinguicula lutea*.

Conclusion

Pinguicula lutea grows in habitats that range from sites managed to keep vegetation short, sites with abundant wiregrass, and sites with woody encroachment. This series of habitats roughly represents a post-disturbance successional regime for the longleaf pine ecosystem where *P. lutea* grows. In the absence of fire, open, maintained sites become grassy and are eventually invaded by woody vegetation. However, the pattern of both pollinator and prey interactions with *P. lutea* does not follow this order. Pollination was uniformly high across the three habitat structures. Maintained and woody sites had the highest arthropod availability and prey capture, and grassy sites had the lowest or similar, but never highest.

Grassy habitat structure types appear to be the least favorable for *P. lutea*. Though there was no difference in fruit and seed set, individuals in this habitat structure caught less prey than in maintained and woody structures. Though it appears that *P. lutea* can persist in woody habitats structures, it may have more to do with a heterogeneous vegetation matrix where microclimatic conditions provide patches of more suitable habitat (i.e. patches of sunlight). As these sites become woodier, (e.g. woody sites used in our preliminary study, Chapter 1) populations can no longer be sustained. Adherence to a frequent burn regime (every 2-3 years) will suppress the abundance of grasses and prevent woody encroachment.

To conclude, this study has determined that habitat structure affected some aspects of *Pinguicula lutea* plant-insect interactions. Habitat structure did not affect fruit set and seed set. However, habitat structure did affect prey capture and arthropod availability. In grassy habitats less prey were captured than in maintained and woody habitats in both survey years. The results of this study suggest that when changes in habitat structure do occur as the result of presence or absence of habitat management, pollinator visitation is minimally impacted, but impact on prey capture is more severe. Additional research is needed to determine to what extent the observed prey capture differences matter for *Pinguicula lutea* fitness as the relationship between prey capture and plant fitness is complex, and more work is needed to show the extent to which carnivorous plants benefit from increased prey capture.

TABLES

Table 2.1. List of 2014 and 2015 *Pinguicula lutea* study sites referenced by their site codes and habitat structures along with the associated values for each component used to determine structure (vegetation height, graminoids, woody, litter, forbs, ground cover, and bare ground).

Year	Site	Habitat Structure	Veg Height (cm)	Cover Class (% Cover) averaged by range of cover midpoint					
				Graminoids	Woody	Litter	Forbs	Ground Cover	Bare Ground
2014	ANF 181	Maintained	42.56	80.5	12.5	15	33	10	10
2014	ANF OCM	Maintained	10.32	85	4.5	28.5	10	2.5	12.5
2014	ANF WLM	Maintained	20.20	29	17	52.5	19.5	2.5	19
2014	THSF 65-1	Maintained	17.28	76	2.5	47.5	24	10	5
2014	THSF 65-2M	Maintained	5.16	33	4.5	28.5	43	29	12
2014	SJBP PL	Grassy	35.44	95	7	2.5	12.5	0.5	5
2014	SJBP SH2	Grassy	43.32	97.5	9.5	10	7	11.5	7.5
2014	TAFB DRF	Grassy	35.52	88	1	2.5	12.5	0	21.5
2014	ANF 180	Woody	37.20	57	43	67	12.5	1.5	2.5
2014	ANF ABE	Woody	35.52	14.5	52.5	24	26	9.5	33
2014	ANF OCE	Woody	50.64	59.5	43	33.5	14.5	2	7.5
2014	ANF WLE	Woody	33.92	47.5	24	33	19.5	2.5	15
2014	THSF 65-2E	Woody	55.24	38	43	52	24	4	5
2015	ANF 106	Maintained	3.56	57	2.5	71	24	2.5	2
2015	ANF 365	Maintained	3.72	75.5	2	72	33	2.5	2
2015	ANF OCM	Maintained	2.16	83	2.5	29	5	1.5	7.5
2015	THSF 65-1	Maintained	13.08	85	12.5	42.5	33	7.5	7
2015	THSF 65-2M	Maintained	2.08	62	2	62	52.5	14.5	5
2015	ANF ABW	Grassy	32.34	21.5	47	71	17	5	7.5
2015	ANF WLG	Grassy	21.69	85	1	15	37.5	0.5	15
2015	SJBP PL	Grassy	33.08	95	3	7.5	12.5	0	5
2015	SJBP SH2	Grassy	61.11	85	5	37.5	3.5	3.5	15
2015	ANF ABE	Woody	31.84	95	6	7.5	19.5	0.2	2.5
2015	ANF OCE	Woody	46.84	50.9	54.5	56.5	5	2.5	10
2015	ANF WLE	Woody	42.13	29	52.5	42.5	33	2.5	17
2015	THSF 65-2E	Woody	62.90	62	45.5	42.5	15	0.9	3.5

FIGURES

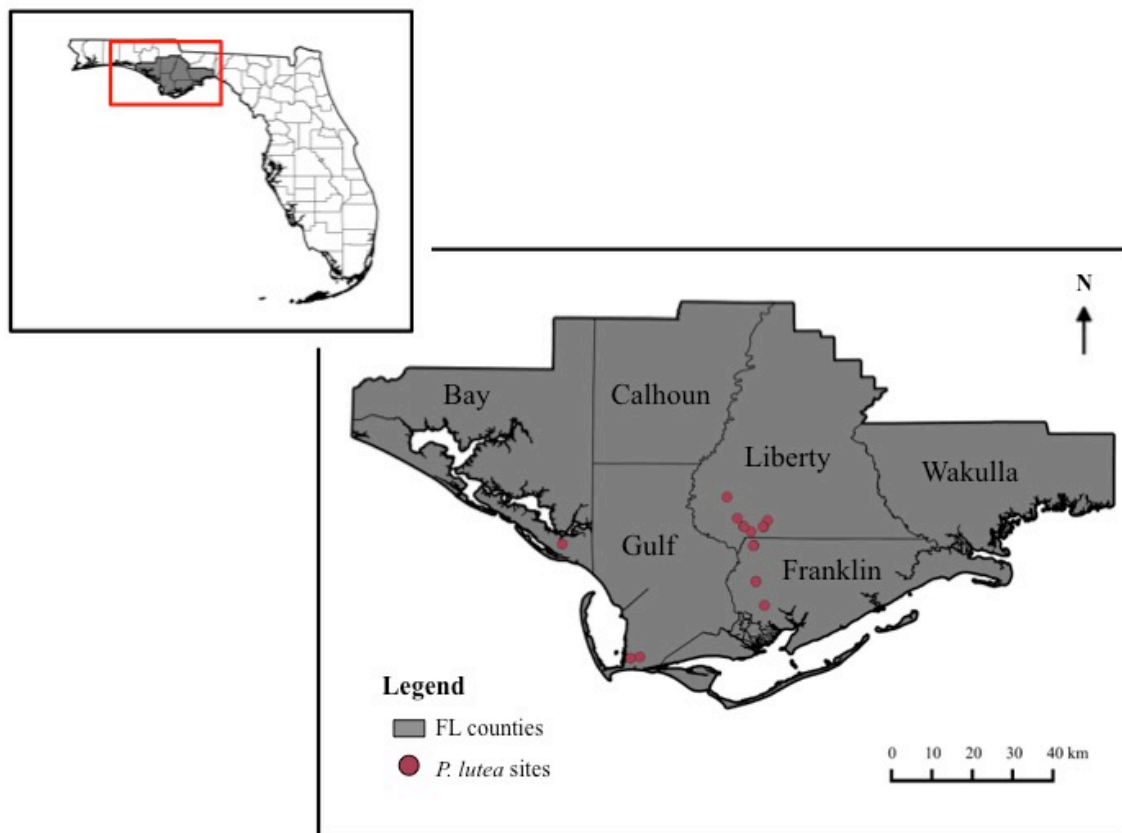


Figure 2.1. Map of all *Pinguicula lutea* sites sampled in 2014 and 2015 in the Florida Panhandle, US.

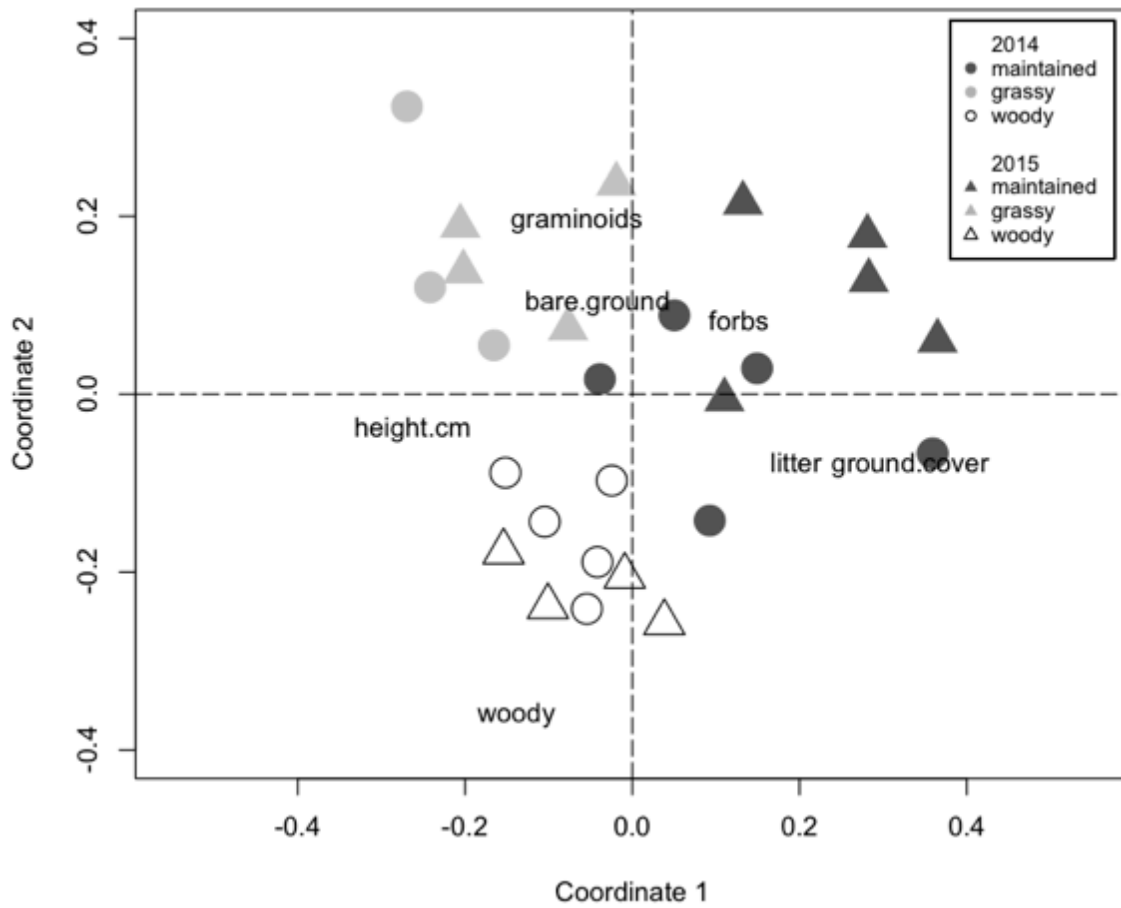


Figure 2.2. Non-metric multidimensional scaling (NMDS) for all *Pinguicula lutea* sites sampled. Habitat structures are maintained (dark gray), grassy (light gray) and woody (white). Sites were sampled in 2014 (circles) and 2015 (triangles) (n=26). The structures are clearly separated in the ordination using the six components of structure and vegetation height. K=2, stress=0.143.

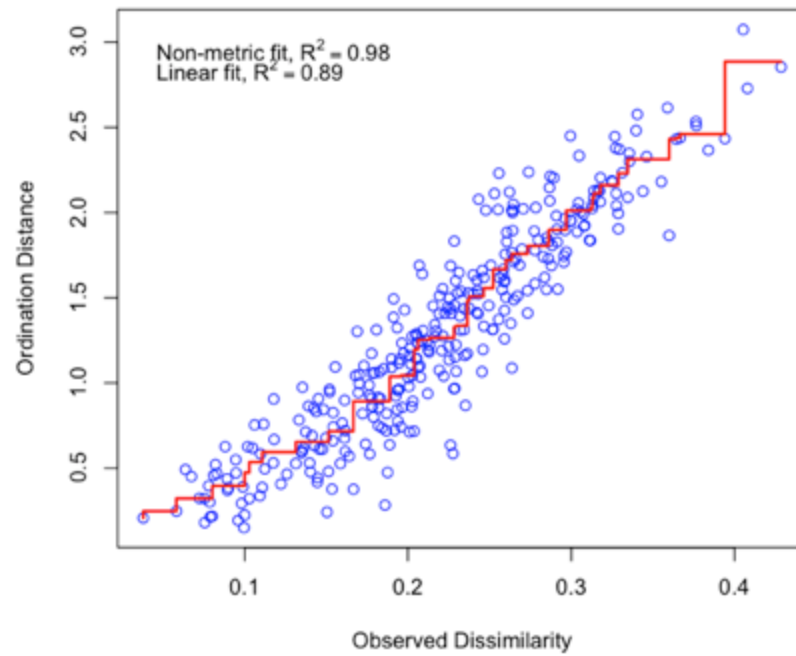


Figure 2.3. Sheppard's plot displaying the appropriateness of the NMDS ordination for the observed habitat structure data. The close relationship between observed dissimilarity and ordination distance indicates a strong fit.

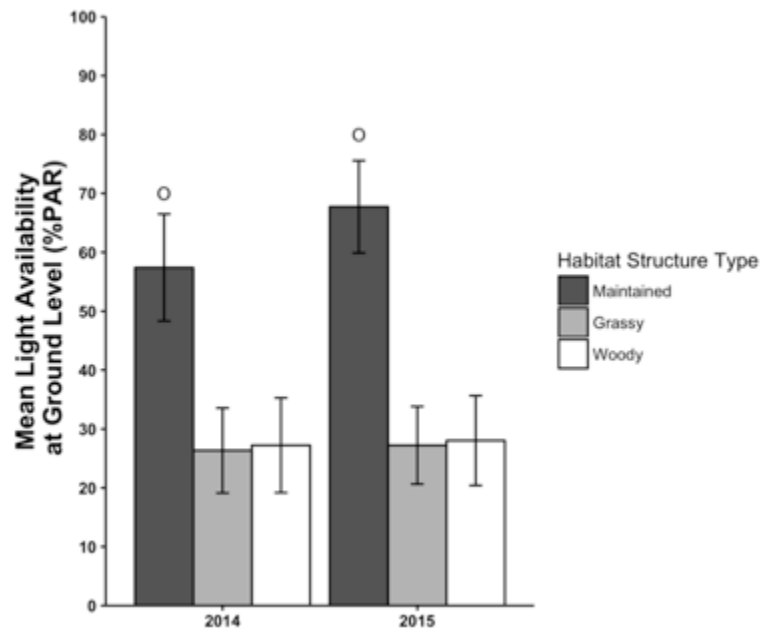


Figure 2.4. Mean ($\pm$ SE) light availability (%PAR) in 2014 and 2015 at ground level for *Pinguicula lutea* in maintained, grassy, and woody habitat structures. Open circles indicate significant treatments within each year.

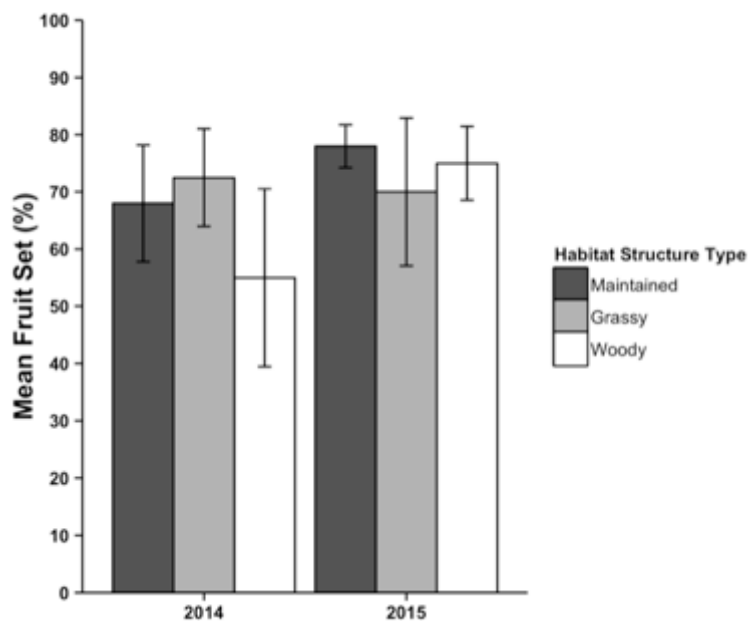


Figure 2.5. Mean ($\pm$ SE) fruit set for *Pinguicula lutea* in 2014 and 2015 for maintained, grassy, and woody habitat structures.

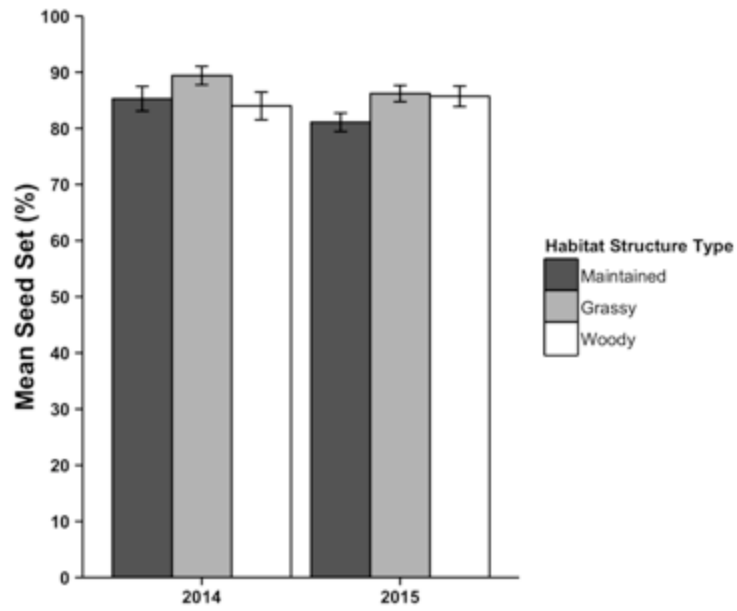
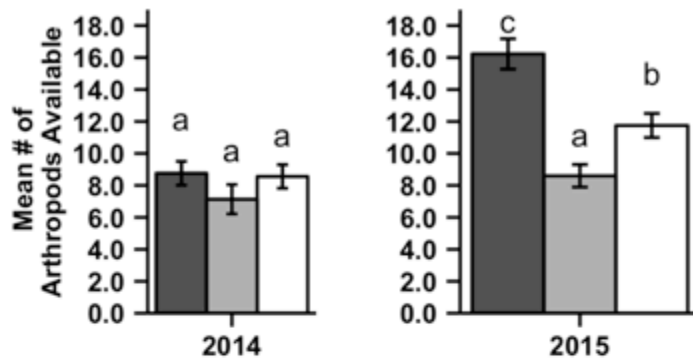
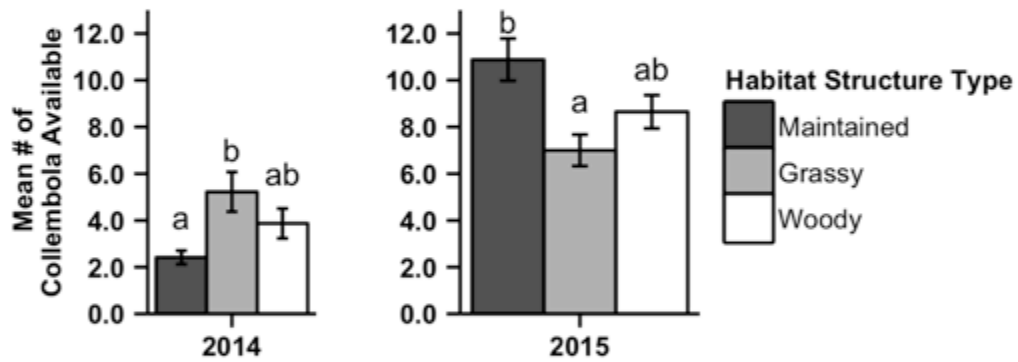


Figure 2.6. Mean ($\pm$ SE) seed set for *Pinguicula lutea* in 2014 and 2015 for maintained, grassy, and woody habitat structures.

A



B



C

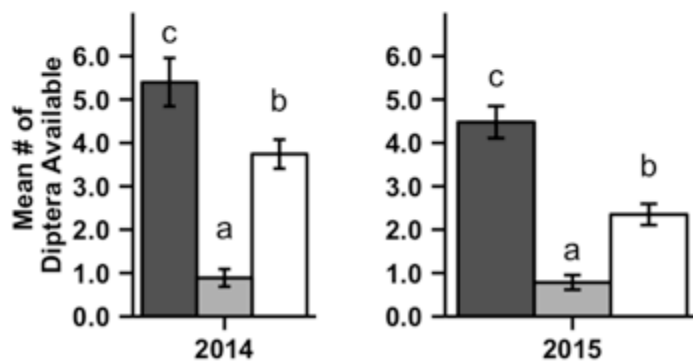
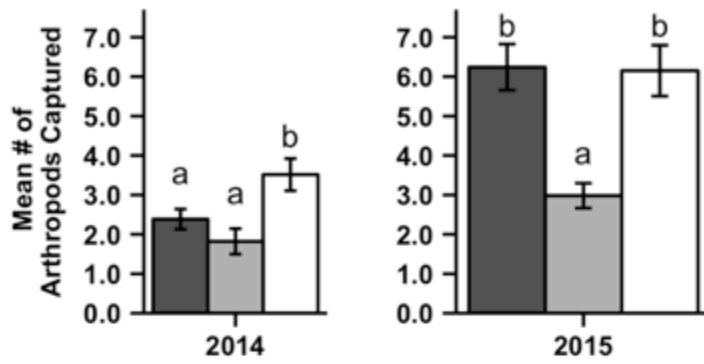
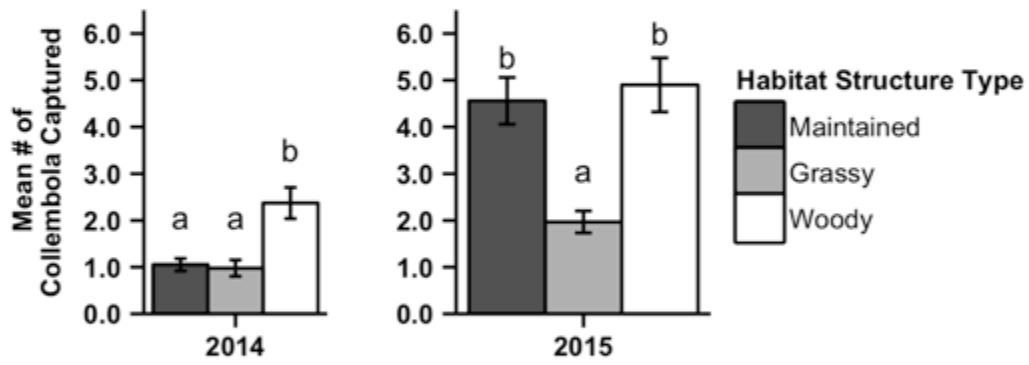


Figure 2.7. Mean ($\pm$ SE) arthropod availability for *Pinguicula lutea* across maintained, grassy, and woody habitat structures in 2014 and 2015.

A



B



C

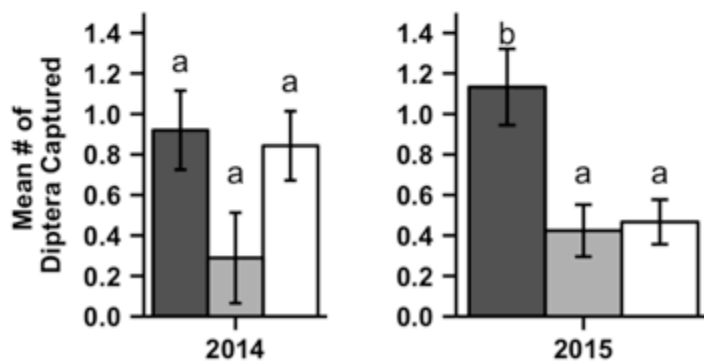


Figure 2.8. Mean ($\pm$ SE) prey capture for *Pinguicula lutea* across maintained, grassy, and woody habitat structures in 2014 and 2015.

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CHAPTER 3: CONSERVATION AND MANAGEMENT RECOMMENDATIONS FOR *PINGUICULA LUTEA* AND ITS CONGENERS

Roughly 70 percent of subtropical grasslands and savannas worldwide are degraded due to suppression of natural fire regimes (Shlisky et al. 2007). In spite of this degradation, these ecosystems are some of the most floristically diverse in the world. In the United States, these areas are best exemplified in the longleaf pine (*Pinus palustris*) savannas of the Southeast US. The longleaf pine savannas are fire-adapted systems (Frost 1998; Platt 1999). Historically, lightning strikes started frequent fires that maintained the understory by removing litter, grasses, and less fire adapted shrubs (Platt 1999) which, in turn, promoted species richness and species turnover (Noss et al. 2015). The combination of heterogeneous plant communities and frequent burn regimes makes this region particularly floristically diverse as well as a center of endemism. This habitat has been drastically reduced to roughly three percent of the 38 million hectares it once occupied largely due to logging and fire suppression (Frost 1993; Frost 2006). In addition to the overall loss of this habitat, there are 16 federally threatened or endangered plant species (Van Lear et al. 2005) and roughly 200 more considered rare at some level (Walker 1993) found in longleaf pine savannas. Several of these rare plant species are carnivorous.

The Florida Panhandle is the epicenter for carnivorous plant diversity. In Florida, there are 32 carnivorous plant species from five families. Thirty of these species are present in the Panhandle (Wunderlin and Hansen 2008) including all six *Pinguicula* species endemic to the Southeast US. Habitat loss and degradation have been implicated in their decline. Five of the six species have received heightened conservation status, including *P. lutea* which is threatened at the state level. Research (see Chapters 1 and 2)

on *P. lutea* provides biological insight that is directly applicable to conservation and management issues for this species as well its congeners.

Beyond the traditional recommendations of habitat protection and prevention of harvesting for *Pinguicula* species, this chapter will focus on additional conservation and management recommendations that state and federal agencies can implement for *P. lutea* and its congeners within the Florida Panhandle. Also, additional opportunities to conduct research are presented.

Monitoring

The Florida Natural Areas Inventory is tasked with documenting federally listed species. However, state threatened species, such as *P. lutea*, are not documented with the same rigor (Gulledge et al. 2011). There are no defined population assessments or databases in which to record element occurrences for this species. The *P. lutea* populations that I identified for my research (see Chapters 1 and 2) were the result of talking to land managers and plant enthusiasts as well as accessing mostly old herbarium records (Wunderlin and Hansen 2008). I suggest using a more intensive approach to monitoring *P. lutea* and its congeners including documenting new populations and incorporating them into an interagency database. Within this database, associated information on population size and demography as well as other observations, such as site conditions to evidence of herbivory, may easily be added to each accession. Population data along with long-term demographic plots can then be used to develop count-based and/or matrix based population viability analysis (PVA) to predict the future status of any *Pinguicula* population of conservation concern and assess the effectiveness of different management approaches. A similar approach was taken by Kesler et al.

(2008) who generated a PVA to determine fire frequency and season of burn that resulted in the maximum population growth rate for *P. ionantha*.

Habitat Management

As previously noted, the longleaf pine savannas of the Southeast US are fire-adapted systems, and fire suppression has caused noticeable changes in vegetation structure (i.e., more litter, grasses, and invasive shrubs). Though it is widely agreed that fire plays a crucial role in ecosystem maintenance, the most appropriate burn regimes have been debated. However, in the Florida Panhandle dendrochronologically dated fire scars provide direct evidence indicating that pre-European settlement fires occurred most frequently in 2 to 3 year intervals and always occurred during the growing season (April-August) when cloud to ground lightning strikes are most prevalent (Huffman 2006). Presently, fire suppression has caused noticeable changes in vegetation structure (i.e., more litter, grasses, and invasive and/or woody vegetation).

In areas that have been severely fire suppressed, mechanical removal (e.g. mowing, roller-chopping, or hand clearing) or chemical removal of woody vegetation may be required prior to burning. In some cases there may even be a more substantial effect of restoration than burning alone or in conjunction with other techniques (Huffman and Werner 2000). However, mechanical and chemical removal practices should not be seen as a substitute for frequent fire. Even when these practices are used in conjunction with prescribed fire, effectiveness is variable (Menges and Gordon 2010; Platt et al. 2015) and should be implemented only if tested first on a small scale.

For *Pinguicula lutea* I suggest a burn regime of every 2-3 years to maintain above ground vegetation. In areas that have been severely fire suppressed mechanical removal of woody vegetation may be required prior to burning. Although my project did not directly explore effects of fire on *Pinguicula lutea*, a 1-2 year prescribed burn regime has been suggested for *P. ionantha* (Kesler et al. 2008) as it maximized population growth. Based on my research (Chapters 1 and 2), prescribed fire during the growing season should not negatively impact *P. lutea* once fruit formation and seed dispersal has occurred (mid April). However, before a specific burn season can be recommended for this species additional studies should be conducted.

Research

Federal and state agencies (e.g., USFW, FFS, BLM) within the Florida Panhandle have expressed the need for more information concerning the *Pinguicula* species of the region and have provided funding for ecological research. Recently, studies examining demography, population genetics, seed bank dynamics, and seed germination have been conducted for *P. ionantha* (Kesler et al. 2008, Molano-Flores et al. 2014; Molano-Flores et al. 2015). Also, seed germination studies have been conducted for *P. lutea*, *P. planifolia*, and *P. pumila* and some demographic data has been gathered for *P. planifolia* (Molano-Flores et al. 2014; Molano-Flores et al. 2015). This research is among the most comprehensive work of its kind for *Pinguicula* species in the Southeast US. Nevertheless, additional research is needed to address many aspects of their biology and ecology.

In the particular case of *P. lutea* further investigation into the effects of habitat structure on reproductive output (i.e., fruit set and seed set) should be explored further. For, example, the work present in Chapter 2 did not factor in the ratio of number of reproductive individuals to non-reproductive individuals in a given population. Flowering is heavily influenced by light availability (Alcalá and Domínguez 2003), so populations that are more heavily shaded may in fact produce fewer flowers than population that are less heavily shaded. In 2013, I found no evidence of flower production for *P. lutea* in heavily woody encroached sites (Chapter 1). Therefore, the overall total flower number and fruit/seed set could be reduced in shaded populations, which could indirectly influence plant-insect interactions. Also, the photosynthetic costs associated with botanical carnivory (Givnish et al. 1984; Benzing 1987; Ellison and Gotelli 2001) should be explored under different habitat structures.

In addition, a survey should be conducted at multiple times throughout the year to assess seasonality in arthropod availability and prey capture abundance and if seasonal variation influences growth and/or reproduction. Karlsson et al. (1994) found seasonal variation of prey captured for three subarctic *Pinguicula* species and experimental additions of prey have been shown to influence reproduction (Zamora et al. 1998). Also, while overall numbers of prey capture matter, prey items can also be assessed in terms of their relative contribution to overall nutrient content. This aspect might be particularly important for *P. lutea*, as the size difference between Collembola and Diptera is great and nutrient content of these prey items increases proportionately (Karlsson et al. 1987). Lastly, while conducting my study I noticed signs of herbivory, specifically of

reproductive structures (florivory and fructivory; Fig. 3.1). A study should be conducted to determine if such herbivory has any impact the reproductive output of *P. lutea*.

Furthermore, due to the heightened conservation status for these species, research should also focus on the development of *ex-situ* collection for all rare *Pinguicula* species. Collection of seeds and development of additional plant propagation protocols will assist in the conservation and preservation of these rare plants. Already, propagation protocols are available for several *Pinguicula* species that could be used or modified for Southeast US *Pinguicula* species (Gonçalves et al. 2008; Saetiew et al. 2011; Grevenstuck and Romano 2012; Di Martino et al. 2014). Any *ex-situ* collections for *Pinguicula* species should be in conjunction with botanical gardens in Florida such as Bok Tower Gardens and Fairchild Tropical Botanic Garden and the International Carnivorous Plant Society. These collections can assist with reintroduction efforts at restored and/or managed sites.

New Technology and Tools

Interest is growing in habitat suitability modeling with a wealth of resources to fill that interest (Elith and Leathwick 2009). Habitat suitability models allow researchers and land managers to assess habitat quality for a species within a particular area. For example, in GIS these models relate suitability to raster based-layers land cover, topographic features or disturbances. Many permutations of habitat suitability maps can be produced quickly and efficiently. Models for *Pinguicula lutea* could be easily created using a modified version of occurrence data and varying combinations of environmental layers, allowing the model to be more easily updated as new population occurrences are documented or more fine scale environmental parameters become available.

While habitat suitability modeling has generally been considered for species with very large geographic distributions, it is increasingly being used as a tool to map species with smaller geographic distributions. For example, I created a habitat suitability map for the federally endangered, endemic *Pinguicula ionantha* (Molano-Flores et al. 2015), and a similar type of map can be created for *Pinguicula lutea*. In the future refined versions for *Pinguicula* species of the Southeast US will be possible as both finer scale data and standardized regional parameters become available.

The use of technology that will facilitate the assessment of *P. lutea* suitable habitat such as LiDAR (*Light Detection and Ranging*, a remote sensing method) should be implemented. For example, in the United Kingdom this technology has been used successfully for the assessment of upland habitats which harbor unique biodiversity, are of cultural value and provide sense of wilderness (Kincey and Challis 2010). In Florida, this and other remote sensing methods have been successfully used for vegetation mapping in the Everglades (Zhang 2014) and to assess tree demography, plant competition, and fuel and fire characteristics in longleaf pine savannas (Loudermilk et al. 2011).

Lastly, in addition to habitat suitability maps, easy to use tools that have been developed to determine how plants will respond to climate change should be used. Based on climate change reports, Florida will be highly impacted by sea level rise, warmer temperatures, and more severe weather patterns (Ingram et al. 2013). I recommend the use of NatureServe's tool Climate Change Vulnerability Index as a first step to assess the impact of climate change on *P. lutea* and its congeners. This tool uses elements of occurrence and biological information in combination with precipitation and temperature

data to assess the impact of climate change. This type of assessment has been conducted for other rare plants (Anacker et al. 2013; Baty et al. 2015) and rare Florida animals (Dubois et al. 2011).

Conclusion

The conservation, management, and research suggestions provided in this chapter for *P. lutea* and its congeners, the preliminary research on reproductive ecology and prey capture presented in Chapter 1 and the results associated with the role that habitat structure is playing on the reproductive ecology and prey capture of *P. lutea* (Chapter 2) will aid state and federal agencies in the Florida Panhandle to allocate resources to better protect these carnivorous plants and the habitat where they live.

FIGURES

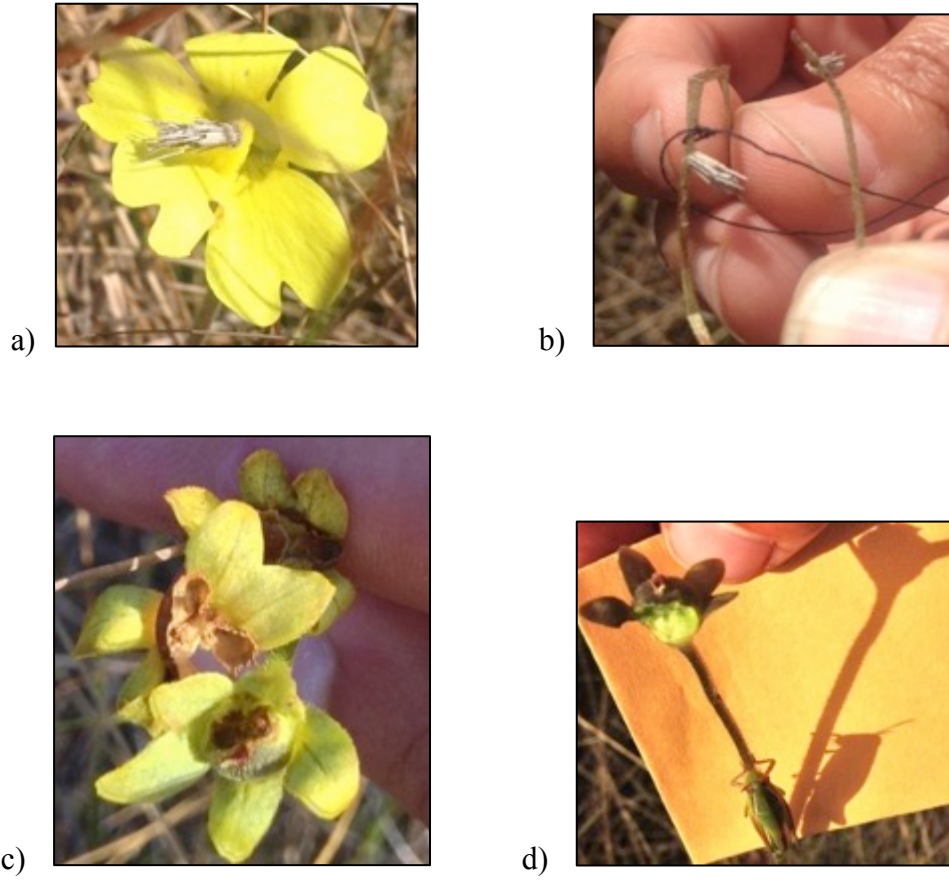


Figure 3.1. Evidence of herbivore damage to *Pinguicula lutea* flowers (a and b) and fruits (c and d) at St. Joseph Bay State Buffer Preserve (Gulf County, Florida).

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